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Effect of the previous summer crops and different levels of nitrogen, phosphorus and potassium on wheat crop productivity and its components

Abomarzoka , E.A*. and Hamadny M. KhA.**

*Crop Physiology Res. Dep. - Field Crops Research Institute- Agric. Res. Center, Egypt

**Crop intensification Res. Dep.- Field Crops Research Institute- Agric. Res. Center, Egypt

ABSTRACT

Two field experiments were conducted in the Experimental Farm of Sakha Agricultural Research Station during 2014/15 and 2015/16 growing seasons. to study the effect of preceding summer crops (i.e. soybean, maize cotton and sunflower), N fertilizer rates (i.e. 45,60 and 75kg N fed.$^{-1}$), phosphorus(0 and 15 kg P_2O_5fed.$^{-1}$) and potassium (0 and 24 kg K_2SO_4 fed.$^{-1}$) as well as 2 doses of N fertilizer were used.

The objective of the present study was to investigate the effect of different previous crops and different levels of N , P and K fertilization splitting on wheat growth , yield and its components on yield and yield components of wheat CV Gemmeiza 11. The experimental design was strip – plot design in three replications , where crops (soybean, maize cotton and sunflower), were laid out in the vertical strip – plot ,while the N , P and K fertilization treatments :

1- 45 kg N + 0 kg P_2O_5 + 0 kg K_2SO_4 fed^{-1}.,
2- 45kgN+15.5kgP_2O_5+0kgK_2SO_4fed^{-1}.,.
3- 45kgN+15.5kgP_2O_5+24kgK_2SO_4fed^{-1}.,
4- 60kgN+0kgP_2O_5+0kgK_2SO_4fed^{-1}.,
5- 60kgN+15.5kgP_2O_5+0kgK_2SO_4fed^{-1}.,
6- 60kgN+15.5kg P_2O_5 + 24 kg K_2SO_4 fed^{-1}.,
7- 75kgN+0kgP_2O_5+0kgK_2SO_4 fed^{-1}.,
8- 75kgN+15.5kgP_2O_5+0kgK_2SO_4fed^{-1}
9- 75 kg N + 15.5 kg P_2O_5 + 24 kg K_2SO_4 fed^{-1}

Laid out in the horizontal strip– plots of two seasons . The main results could the summarized as follows :-

Growing wheat by the preceding soybean crop were significantly effected on concentration from chlorophyll a and chlorophyll b and flag leaf area , plant height , number of spikes / m^2 , number of spiklet / spike, grain weight / spike, 1000 - grain weight as well as grain and straw yield fed^{-1}. and attribute percentage from nitrogen , phosphorus and potassium of grains were higher on growing sequence cotton , maize and sunflower in most studied characters.

Wheat preceded by soybean markedly surpassed those preceded by maize or cotton and sunflower in growth, yield and its components by raising nitrogen fertilizer rate up to 75 kg N fad^{-1}.with 15 .5 kg P_2O_5 and 24 kg K_2SO_4fed^{-1}. Resulted in a significant increased in all studied characters of wheat in the two seasons. The interaction between the studied treatments showed that the highest grain yield was obtained when wheat plants grown after soybean and adding 75 kg N fed^{-1}maxing 15.5 kgP_2O_5 fed^{-1} and 24 K_2SO_4fed^{-1} in the both seasons compared anther treatments under maize or cotton and sunflower on growth, yield and its attributing of wheat .

Key words:

INTRODUCTION

Population growth intensifies food problems in developing countries, which requires improvement in quantity and quality of agricultural products as well as fertilizer use. Unbalanced use of chemical fertilizers reduces soil fertility and quality of crops (Malakooti and Gheybi, 2003). Wheat (*Triticum aestivum L.)* is the most important cereal and forage crop all over the world and it is considered the main food crop in Egypt as in many other countries of the world. Furthermore, wheat is considered the most important winter crop, because its grains are the main food for the urban and rural societies and its straw is a very important fodder for animal feed, especially during summer. The domestic wheat production in 2013 season was estimated by 8.7 million tones, whereas the Egyptian national consumption of wheat is about 17.7 million tons in 2013; there is a great gab between the consumption and production (USDA, 2013). Egypt imports above five million tones of wheat grains. Unless domestic wheat production increases annually, the deficit will increase, due to the increase birth rate (2%) and present per capita consumption estimated by about 200 kg year^{-1} (USDA, 2013). Increasing wheat production could be possible via two ways: horizontal expansion through increasing the cultivated area with wheat and vertical expansion through the development of new cultivars having the high potentiality and subsequently implementing the proper cultural practices (Ragab 2011). Egypt utilizes fertilizers at an accelerating rate, due to various factors such as the increase in the cropped area, raising the rate of fertilizer application for various crops and the depletion of the High Dam. Consequently, Egypt is considered to be a heavy user of chemical fertilizers, especially N fertilizers then P and K fertilizers. Soil fertility continues to decline because of combined effects of increasing pressure for land use for crop production, inadequate compensation of nutrients exported and lack of nutrients management. Therefore, the objective of this study was to quantify the previous summer crops on the productivity of wheat under dif-

ferent levels of nitrogen, phosphorus and potassium fertilizers for wheat (cv. Gemmeiza 11) production under North Delta region, Egypt.

MATERIALS AND METHODS

This work was carried out in Sakha Agricultural Research Station during 2014/15 and 2015/16 growing seasons. to study the effect of preceding summer crops (i.e. soybean, maize cotton and sunflower) , N fertilizer rates (i.e. 45,60 and 75 kg N / fed.), phosphorus (0 and 15 kg P_2O_5 fed^{-1}) and potassium (0 and 24 kg K_2SO_4 fed^{-1}) as well as 2 doses of N fertilizer were used. The objective of the present study was to investigate the effect of previous different summer crops and splitting different levels of N , P and K fertilization on wheat growth , yield and its components CV Gemmeiza 11.

The experimental design was strip- plot design in three replications . Each previous crops were evaluated in a separate experiment . Where (i.e. maize , cotton , soybean and sunflower) were laid-out in the vertical strip - plots , while the N P and K fertilization treatments were laid-out in the horizontal - strip plots . N P and K fertilization treatments were repeated with each previous crops during the two growing seasons were detected as follows:

A - Vertical strip - plots includes four treatments:

1- Maize. 2– Cotton .

3 – Soybean . 4 - Sunflower

B - Horizontal strip - plots includes nine treatments:

1- 45 kg N + 0 kg P_2O_5 + 0kg K_2SO_4 fed^{-1}, (T1).
2- 45kgN+15.5kgP_2O_5+0kg$K_2SO_4$$fed^{-1}$., (T2).
3- 45kgN+15.5kgP_2O_5+24kg$K_2SO_4$$fed^{-1}$., (T3).
4- 60kgN+0kgP_2O_5+0kg$K_2SO_4$$fed^{-1}$., (T4).
5- 60kgN+15.5kgP_2O_5+0kg$K_2SO_4$$fed^{-1}$., (T5).
6- 60kgN+15.5kg P_2O_5 + 24 kg K_2SO_4 fed^{-1}., (T6).
7- 75kgN+0kgP_2O_5+0kgK_2SO_4 fed^{-1}., (T7).
8- 75kgN+15.5kgP_2O_5+0kg$K_2SO_4$$fed^{-1}$ (T8).
9- 75 kg N + 15.5 kg P_2O_5 + 24 kg K_2SO_4 fed^{-1} (T9).

All another cultural practices for both maize and soybean production were under taken as recommended. sunflower May 15th and 25 th May through 2010 and 2011 seasons, respectively. The size of each Horizontal strip - plots was 10.5 m^2 (3.5m length and 3.0 m wide). Concerning the previous summer crops, , cotton seeds took place in April 15th , 19th in the first and second seasons , respectively . Sunflower was planted on April 26th and 28th through 2014 and 2015 seasons, respectively. . Maize and soybean were planted at the same sowing dates May 20th and 25th in the first and second seasons, respectively. Grain of wheat were sown in November 20th and 24 in the two seasons at a rate of 60kg /fed. Nitrogen in the form of urea (46%N).Normal cultural practices for soybean ,cotton, maize and sunflower crops were normally adopted .for wheat Nitrogen fertilizer rate was split into two doses: the first dose (50%) before the 1stirrigation (27 days from sowing), the 2nd dose (50%) before the 2nd irrigation 56 days from sowing). On the other hand, both of P one dose before the 1st irrigation and K fertilizers were applied into two doses: the first dose (50%) before the 1st irrigation (27 days from sowing), the 2nd dose (50%) before the 2nd irrigation 56 days from sowing). All agricultural practices of wheat crop such as: tillage, irrigation and fertilization were done in the experiment as practical by farmers.

Soil Analysis

Samples of soil were collected from surface layer (0-30 cm) after harvesting summer crops in the two growing seasons. The samples were analyzed for estimating nitrate according to Kjeldahel method as described by Jackson (1958),available P according to Olsen ***et al***.(1965) and K was determined by flame photo metrically using E.E.L. flame photometer as mentioned. The field soil was clay in texture and available N,P and K are presented in Table (1).

Table (1): Available N, P and K (ppm) and pH of soil after soybean, cotton, maize and sunflower in 2014/2015 and 2015/2016 seasons

Preceding crops	2014/2015				2015/2016			
	Available nutrients (ppm)			pH	Available nutrients (ppm)			pH
	N	P	K		N	P	K	
soybean	41	8.7	559	8.3	46	8.4	587	8.7
cotton	37	8.1	523	8.1	43	8.2	562	8.6
maize	34	7.8	508	7.9	41	7.9	534	8.4
sunflower	31	7.7	493	7.7	38	7.6	528	8.1

The measured traits in both seasons.

At the end of heading stages, the determination of photosynthetic pigments in flag leaf. The total chlorophyll pigments were determined according to Wettesteien (1957),ten guarded plants were random from each sub-plot to determine the following characters:

1- Flag leaf area (cm^2).The flag leaf area was calculated using the following equation of Palaniswamy and Gomez (1974).

Leaf area (L.A) = K (Lw).

Where : L = leaf length, w = Maximum width of the leaf and K = factor of 0.75.

2- Plant height (cm). The average height of ten randomly chosen plants from each plot and measured from ground level to the spike tip, excluding owns.

At harvest, ten guarded plants of one square meter of each sub-plot were taken at random to estimate the following characters :

1- Number of spikes/m^2 counted in randomly chosen 1 m^2 in each plot .
2 - Spike length (cm).
3- Number of spikelet's /spike.

Table (2): flag leaf concentration from chlorophyll a , chlorophyll b, flag leaf area and plant height as affected by preceding summer crops , N, P and K rates in 2014/2015 and 2015/2016 seasons

Treatments	Chlorophyll a		Chlorophyll b		Flag leaf area		Plant height	
	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
Proceeding crops								
Soybean	3.424	3.801	1.872	2.056	26.35	27.93	106.98	116.08
Cotton	3.325	2.532	1.818	1.709	26.35	23.59	105.47	113.18
Maize	2.829	2.456	1.598	1.521	23.48	22.66	102.23	107.89
Sun flower	2.63	2.39	1.351	1.428	22.04	21.65	101.46	105.47
F. test	*	*	*	*	*	*	*	*
LSD at 0.05	0.158	0.52	0.082	0.162	1.9	2.9	0.88	1.99
Treatments								
1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	2.921	2.707	1.516	1.547	23.32	22.97	96.94	105.13
2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	2.933	2.717	1.586	1.59	23.6	23.46	102.46	108.4
3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	2.961	2.735	1.611	1.617	23.93	23.71	103.57	110.22
4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	3	2.781	1.648	1.659	23.93	23.83	104.77	111.15
5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	3.059	2.785	1.728	1.708	24.16	24.12	105.47	111.42
6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	3.08	2.826	1.728	1.732	24.32	24.23	105.95	111.95
7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	3.124	2.836	1.734	1.749	24.49	24.41	106.57	111.98
8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	3.217	2.854	1.75	1.786	24.69	24.57	108.96	112.91
9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	3.245	2.952	1.775	1.788	24.77	24.78	110.14	114.08
F-test	*	*	*	*	*	*	*	*
LSD at 0.05	1.969	2.627	0.072	0.122	3.48	2.423	1.97	2.63
Interaction (pxt)								
LSD at 0.05	NS	NS	NS	NS	NS	NS	NS	NS

* and N.S indicate P< 0.05 not significant, respectively.

4- Number of grains /spike.

5- Grain weight /spike (g). It was estimated from 10 randomly chosen main spikes from each plot .

6- 1000-grain weight (g). Average weight of 1000-grain randomly taken from each plot.

7- Grain yield. Weight of grains harvested from each plot converted to ardab (Ardab =150 kg).

8- Straw yield .It was calculated by sub Thresher grain yield from the total yield for each plot and converted to ton/fed.

The obtained data were statistically analyzed using the SAS program and LSD test at the 5% level of probability was used to compare the treatments means according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

A-Effect of preceding summer crops :

Data in Table (2, 3 and 4)show the effect of preceding summer crops on growth, yield and yield components of wheat during 2014/2015 and 2015/2016 seasons. Results clear that the all estimated characters were significantly affected by the preceding crops. The averages of flag leaf concentration from chlorophyll a and chlorophyll b and flag leaf area, plant height ,number of spikes/m²,spike length, number of spikelet's/spike, number of grains /spike, grain weight/spike,1000-grain weight as well as grain and straw yields /fed. Were higher when the previous crop was soybean .Cotton as summer preceding crops came in the second rank after soybean in this concern. On the other hand, the values of aforementioned traits . were lower when wheat was preceding by .sunflower. Grain yield perfeddan of wheat grown after soybean was 2016 and 20.67ardab/fed. in the first and second seasons, respectively .The increase in wheat grain yield when planted after soybean may be attributed to the high basal doses of N, P and K which were applied to soybean and to the reserve high levels of soil fertility after soybean harvesting (Table 2) because this crop has deeply roots under soil in comparison with cotton, maize chained sunflower. Similar results were reported by El-Douby (1997) and Seif El-Nasr and Zahran (1998).In general, those results revealed that values that values of wheat characters grown after soybean were superior to those after cotton, maize and sunflower. The cultivation of wheat after harvest leguminous crops distinct from plants grown after the other crops, this is due to increased soil content of carbohydrate and nitrogen ratio of carbon and lower the pH and thus have graven-

Table (3): Number of spikes/m², number of spikelet's/spike, number of grains /spike, grain weight/spike,1000-grain weight (g) as affected by preceding summer crops , N, P and K rates in 2014/2015 and 2015/2016 seasons

Treatments	Number of spikes/ m²		Number of spike-let's/spike		Weight of grain/ spike		Weight of 1000 grains	
	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
Proceeding crops								
Soybean	442.03	411.92	85.54	82.83	3.51	3.56	48.05	46.04
Cotton	411.15	377.72	81.5	79.04	3.341	3.35	44.05	42.71
Miaze	386.32	365.12	79.25	71.33	2.83	3.08	40.88	34.6
Sun flower	341.82	341.81	62.71	62.96	2.78	2.61	35	32
F. test	*	*	*	*	*	*	*	*
LSD at 0.05	5.968	5.507	1.9	2.903	0.224	0.16	0.52	1.38
Treatments								
1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	375.92	358.1	69.17	66	2.8	2.82	39.68	36.45
2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	383.82	365.6	73.58	70.92	2.97	2.95	40.14	37.59
3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	387.57	370.07	76.75	73.58	3.01	3.03	40.82	38.33
4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	390.5	374.65	77.67	74.67	3.08	3.09	41.88	38.78
5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	394.32	377.57	78.5	75.83	3.12	3.22	42.34	39.03
6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	401.07	381.57	79.42	76.42	3.22	3.28	42.65	39.74
7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	394.27	371.87	77.89	75.98	3.2	3.31	41.32	38.8
8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	401.17	383.1	80.92	76.67	3.31	3.38	44.35	40.22
9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	404.32	384.77	82	78.25	3.38	3.44	44.78	40.62
F-test	*	*	*	*	*	*	*	*
LSD at 0.05	7.703	7.957	2.66	4.201	0.456	0.241	0.84	1.74
Interaction (pxt)								
LSD at 0.05	15.408	17.14	4.32	7.402	0.812	0.582	1.681	3.47

* and N.S indicate P< 0.05 not significant, respectively.

ing in addition various mineral fertilizers of nitrogen, phosphorus and Potassium, making it safe to use grains as food for humans and reduce the cost of production.

were lower when wheat was preceding by .sun-flower. Grain yield per feddan of wheat grown after soybean was 2016 and 20.67ardab/fed. in the first and second seasons, respectively .The increase in wheat grain yield when planted after soybean may be attributed to the high basal doses of N,P and K which were applied to soybean and to the reserve high levels of soil fertility after soybean harvesting (Table2) because this crop has deeply roots under soil in comparison with cotton, maize chained sunflower. Similar results were reported by El-Douby (1997) and Seif El-Nasr and Zahran (1998).In general, those results revealed that values that values of wheat characters grown after soybean were superior to those after cotton, maize and sunflower. The cultivation of wheat after harvest leguminous crops distinct from plants grown after the other crops, this is due to increased soil content of carbohydrate and nitrogen ratio of carbon and lower the pH and thus have graven-ing in addition various mineral fertilizers of nitrogen, phosphorus and potassium, making it safe to use grains as food for humans and reduce the cost of production.

B-Effect of nitrogen, phosphorus and potassium fertilization rates:-

Data presented in Table 2,3 and 4 show the effect of NPK fertilizer rates on some characters of growth, yield and yield components of wheat plants in the two seasons. N, P and K fertilizer rates had significant effect on all the studied characters. Increasing N,P and K up to 75kg N+15.5kg P_2O_5+24 kg K_2SO_4 significantly increased flag leaf concentration from chlorophyll a and chlorophyll b and flag leaf area ,plant height, number of spikes/m², spike length, number of spikelet's/ spike, number of grains /spike, grain weight/spike,1000-grain weight as well as grain and straw yields /fed. in both seasons.

The increase in plant height may be due to the stimulation of cell division and internodes elongation by addition of NPK fertilizers .Increasing NPK rates from (45,60 and75 N kg/fed.), (0 to 15.5 kg P_2O_5/fed.) and (0 to 24 kg K_2SO_4 /fed.). and (0 to 24 kg K_2SO_4).16.608,

Table (4): Grain yield (ardab/fed) and Straw yield (t /fed). as affected by preceding summer crops ,N,P and K rates in 2014/2015 and 2015/2016 seasons

Treatments	Grain yield (ardab/fed)		Straw yield (t /fed)	
	2014/15	2015/16	2014/15	2015/16
		Proceeding crops		
Soybean	21.45	20.59	3.79	3.54
Cotton	18.68	17.738	3.39	3.3
Miaze	17.46	16.98	3.1	2.81
Sun flower	16.1	15.56	2.69	2.61
F. tests	*	*	*	*
LSD at 0.05	0.558	1.15	0.108	0.403
		Treatments		
1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	17.67	16.62	3.05	2.96
2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	17.84	16.97	3.12	2.99
3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	18.1	17.17	3.17	3.02
4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	18.22	17.62	3.18	3.06
5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	18.54	18.03	3.24	3.08
6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	18.68	18.23	3.29	3.1
7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	18.37	17.95	3.27	3.05
8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	19.04	18.39	3.42	3.13
9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	19.36	18.48	3.44	3.19
F-test	*	*	*	*
LSD at 0.05	0.602	0.759	0.18	0.427
		Interaction (pxt)		
LSD at 0.05	1.38	1.519	0.361	0.655

* and N.S indicate P< 0.05 not significant, respectively.

16.739, 17.107, 18.316 , 18.808 , 19.348 , 19.686 18.316 and 19.908 ardab /fed. in the first season and from 16.685 ,17.008 ,17.228,17 .897 ,18.262 ,18.506 , 18.547 and 18.801 ardab / fed . in the second season , respectively . The increase in grain yield per Fadden with increasing nitrogen , phosphorus and potassium rates may be attributed to the role of nitrogen in activation the growth and hence increasing yield components i.e. number of spikes /m^2, number of grains / spike, spike weight and 1000-grain weight .These results are in good accordance with those reported by Abd Allah ***et al***.(1999) , Amer (2005) , Andersion (2008) and Rosolem and Calonego (2013) . Preceding crops significantly influenced Nutrient concentration content of grain wheat (%) (Table 5) . The lowest and highest N % , P % and K % content obtained for continuous wheat grown after soybean (2.315 and 2.233 %) , respectively. While , there was significances different between other preceding crops .

Interaction effect :

The interaction between preceding summer crops and NPK fertilizer rates had significant effects on number of spikes/m^2 ,1000 grain weight, grain and straw yields in the both seasons. Show the data in Tables (6 and 7) .The highest means of those aforementioned characters were obtained when wheat plants were grown after soybean and adding 75 kg N+15.5 kg P_2O_5 + 24 kg K_2SO_4 / fed. On contrary, the lowest values were obtained from the plants were grown after sunflower and adding 45 kg N + $_0$ P+ $_0$K / fed . The addition of N-fertilizer increased N and K concentration and hence uptake of wheat plants growth (tillering, booting and maturity). Furthermore, the N concentration in grain and straw of wheat significantly increased by increased added N. N uptake by wheat straw was significantly increased with increasing N-levels for the same treatment of 75 kg N , 15.5 kg P , 24 kg K in the first and second seasons, respectively. Similar results were obtained by many researchers who found that grain yield was increased by application of mineral NPK fertilization as reported by El-Kholy (2000) , Abd-Hadi (2004),Chen ***et al***.(2004), Zhao ***et al***. (2006), Tanaka ***et al.*** (2007), Erocli ***et al. (***2008), Rieger ***et al***. (2008), Blanco ***et al***. (2009). Fertilize wheat nitrogen, phosphorus and potassium mineral increases Article carbohydrates and photosynthetic efficiency of plants wheat and work to increase the composition of carbohydrates resulting from the increased representation of the process of breathing (demolition), and all this to the activity of potassium inside cells. Kanani (1996). Also, data indicated that this

Table (5): Nutrient concentration in grain wheat (%) as affected by preceding summer crops , N ,P and K rates in 2014 / 2015 and 2015 / 2016 seasons

Treatments	Nutrient concentration in grain (%)					
	N%		P%		K%	
	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
			Proceeding crops			
Soybean	2.329	2.242	0.269	0.213	0.518	0.494
Cotton	2.033	1.924	0.232	0.204	0.472	0.457
Miaze	1.887	1.835	0.197	0.185	0.442	0.379
Sun flower	1.742	1.68	0.184	0.165	0.376	0.376
F. tests	*	*	*	*	*	*
LSD at 0.05	1.269	1.154	0.453	0.318	1.028	0.984
			Treatments			
1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	1.908	1.795	0.209	0.182	0.424	0.392
2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	1.926	1.83	0.211	0.187	0.429	0.405
3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	1.954	1.855	0.214	0.188	0.433	0.41
4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	1.982	1.917	0.219	0.19	0.448	0.421
5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	2.002	1.962	0.22	0.192	0.455	0.429
6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	2.026	1.98	0.229	0.196	0.466	0.439
7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	2.029	1.971	0.227	0.194	0.468	0.439
8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	2.061	1.989	0.223	0.196	0.469	0.442
9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	2.091	1.995	0.232	0.202	0.475	0.465
F-test	*	*	*	*	*	*
LSD at 0.05	1.621	1.257	0.687	0.558	1.324	1.025
			Interaction (pxt)			
LSD at 0.05	1.83	1.719	0.859	0.861	1.628	1.255

* and N.S indicate P< 0.05 not significant, respectively.

increase was more pronounced in the presence of phosphate and potassium fertilizers than its absence. Concerning potassium, it is an important factor for synthesis of amino acids and proteins from ammonium ions as well as for plants growing in solution enriched in ammonium ions that accumulate in them. Fischer ***et al***. (2002), Montemuro ***et al***. (2006), Wang ***et al***. (2007), Riedell ***et al***. (2009) and Heiazi ***et al***. (2010).

Economic Return:

Table 8 showed that the highest of total and net returns values of wheat crop was obtained by growing wheat crop after soybean and using 75 kg Nfad^{-1}. with 15.5 kg P_2O_5 and 24 kg K_2O / fed^{-1}. gave the highest values of total income and values are (L.E. 11392.42 and 11875.4 / fed) in the first and second seasons ,respectively, compared with the other treatments in both seasons , due to increase soil N, p and k in addition to the improvement of soil fertility after soybean crop.

Prices of main products are that of 2014 / 2015: L.E. 411 for ardab of wheat, L.E. 632 for ton of straw and 2015 / 2016 : L.E. 420 for ardab of wheat, L.E. 640 for ton of straw .

CONCLUSION

Generally, planting wheat after harvest Leguminous crop (soybean) and using 75 kg Nfad-1. with 15.5 kg P2O5 and 24 kg K2O / fed-1. gave the highest values of wheat growth , yield and its components and therefore gave the highest values of total income of the farmers and led to decreasing in the amount of mineral fertilizer added and the product is the security of health on human and animal health and environmental due to the low percentage of its mineral fertilizer (NPK) proportion in wheat grain and straw .

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Table (6): Number of spikes/m², 1000-grain weight (g) , grain yield (ardab / fed.) and straw yield (t / fed.) as affected by the interaction between summer preceding crops and NPK fertilizer rates

Charac-ters	Treatments	Number of spikes/m²		Weight of 1000 grains gm		Grain yield (Ardab / fed)		Straw yield (Ton /fed)	
		2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
Soybean	1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	426. 70	384. 70	45.5	44.87	20.47	18.21	3.517	3.503
	2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	430	390. 70	45.78	45.25	20.88	19.21	3.563	3.517
	3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	432	398.3	45.94	45.28	21.25	19.54	3.658	3.52
	4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	433	406.3	47.76	45.61	21.09	20.39	3.662	3.52
	5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	443	416	47.39	45.64	21.6	20.91	3.775	3.527
	6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	450.3	424	48.29	46.16	21.61	21.45	3.801	3.547
	7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	452	425.3	48.9	46.19	21.83	21.68	3.805	3.548
	8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	454	428	50.79	47.22	21.94	21.89	4.188	3.566
	9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	457.3	434	52.11	48.16	22.38	22.04	4.192	3.693
Cotton	1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	393.3	372	43.56	37.84	17.84	17.4	3.263	3.153
	2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	403	377	43.6	41.3	18.06	17.43	3.337	3.163
	3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	409.3	377	43.75	42.3	18.29	17.65	3.377	3.183
	4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	411.7	379	43.99	43.13	18.29	17.73	3.377	3.32
	5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	412.3	379.3	44.04	43.63	18.66	17.95	3.4	3.36
	6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	419.3	379.3	44.14	44.09	18.87	18.02	3.45	3.367
	7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	406.9	364.2	43.21	42.96	17.96	17.24	3.384	3.309
	8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	421.3	380	45.06	44.47	19.9	18.07	3.47	3.437
	9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	423.3	382. 70	45.16	44.74	20.29	18.16	3.51	3.493
Maize	1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	365. 70	357. 70	38.93	33.16	17.03	16.66	2.893	2.733
	2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	370.3	362. 70	39.42	33.3	17.05	16.82	3.027	2.777
	3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	371	367	40.29	33.58	17.21	16.87	3.037	2.797
	4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	371.3	367.3	41.05	34.23	17.44	16.92	3.07	2.823
	5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	375	368	41.78	34.43	17.63	17.1	3.09	2.827
	6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	380. 70	369	41.83	35.81	17.7	17.11	3.177	2.85
	7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	374.2	364	38.24	34.09	17.62	16.98	3.164	2.726
	8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	382. 70	368. 70	43.18	36.26	17.73	17.17	3.22	2.877
	9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	386	371. 70	43.27	36.62	17.79	17.21	3.247	2.9
sunflower	1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	340.3	318	30.73	29.95	15.35	14.21	2.543	2.46
	2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	341.7	332	31.77	30.52	15.38	14.42	2.58	2.513
	3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	346	338	33.32	32.16	15.65	14.65	2.617	2.603
	4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	359	346	34.72	32.18	16.07	15.44	2.623	2.613
	5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	360	347	36.18	32.42	16.28	16.16	2.733	2.63
	6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	360.3	354	36.35	32.93	16.54	16.37	2.767	2.67
	7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	354.2	344	34.96	31.97	16.08	15.92	2.735	2.648
	8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	360. 70	346. 70	38.37	32.96	16.62	16.44	2.823	2.67
	9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	360. 70	350. 70	38.6	32.97	16.99	16.51	2.85	2.693
	F-test	*	*	*	*	*	*	*	*
	LSD at 0.05	15.408	17.14	1.681	3.47	1.38	1.519	0.361	0.255

* and N.S indicate P< 0.05 not significant, respectively.

Table (7): Nitrogen % , P % and K % on grain wheat as affected by the interaction between summer preceding crops and NPK fertilizer rates

Characters	Treatments	N %		P %		K %	
		2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
Soybean	1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	2.211	1.967	0.246	0.21	0.487	0.48
	2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	2.255	2.075	0.249	0.211	0.49	0.484
	3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	2.295	2.11	0.256	0.211	0.492	0.484
	4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	2.333	2.258	0.264	0.211	0.511	0.488
	5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	2.334	2.317	0.266	0.213	0.517	0.494
	6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	2.37	2.358	0.293	0.222	0.558	0.515
	7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	2.364	2.341	0.288	0.218	0.549	0.509
	8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	2.388	2.377	0.266	0.213	0.523	0.494
	9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	2.417	2.38	0.293	0.214	0.543	0.505
Cotton	1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	1.927	1.879	0.221	0.196	0.466	0.405
	2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	1.95	1.882	0.221	0.2	0.467	0.442
	3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	1.975	1.906	0.223	0.203	0.468	0.453
	4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	1.975	1.915	0.232	0.203	0.471	0.461
	5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	2.015	1.939	0.235	0.204	0.471	0.467
	6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	2.038	1.946	0.236	0.207	0.472	0.472
	7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	2.078	1.943	0.238	0.205	0.471	0.466
	8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	2.149	1.952	0.241	0.208	0.482	0.476
	9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	2.191	1.961	0.246	0.21	0.483	0.479
Maize	1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	1.839	1.799	0.191	0.174	0.417	0.355
	2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	1.841	1.817	0.194	0.182	0.422	0.356
	3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	1.859	1.822	0.196	0.182	0.431	0.359
	4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	1.884	1.827	0.198	0.184	0.439	0.366
	5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	1.904	1.847	0.198	0.185	0.447	0.368
	6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	1.912	1.848	0.2	0.191	0.448	0.383
	7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	1.911	1.846	0.197	0.186	0.453	0.379
	8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	1.915	1.854	0.201	0.193	0.462	0.388
	9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	1.921	1.859	0.203	0.195	0.463	0.392
sunflower	1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	1.658	1.535	0.178	0.148	0.329	0.32
	2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	1.661	1.557	0.181	0.151	0.34	0.327
	3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	1.69	1.582	0.182	0.157	0.344	0.357
	4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	1.736	1.668	0.183	0.157	0.372	0.344
	5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	1.758	1.745	0.184	0.164	0.387	0.347
	6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	1.786	1.768	0.187	0.166	0.389	0.352
	7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	1.765	1.756	0.187	0.164	0.402	0.349
	8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	1.795	1.776	0.187	0.169	0.411	0.353
	9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	1.835	1.783	0.189	0.171	0.413	0.353
	F-test	*	*	*	*	*	*
	LSD at 0.05	1.83	1.719	0.859	0.861	1.628	1.255

* and N.S indicate P< 0.05 not significant, respectively.

Table (8). Economic return as affected by the preceding summer crops, N fertilizer rates and their interactions during the two growing seasons, 2014/2015 and 2015/2016

Char-acters	Treatments	2014/15			2015/16		
		Grain yield	Straw	total income	Grain yield	Straw	total income
Soybean	1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	8413.17	2213.89	10627.07	7648.2	2250.88	9899.08
	2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	8581.68	2222.74	10804.42	8068.2	2280.32	10348.52
	3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	8733.75	2224	10957.76	8206.8	2344.32	10551.12
	4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	8667.99	2225.27	10893.26	8782.2	2414.72	11196.92
	5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	8877.6	2229.06	11106.66	9009	2433.92	11442.92
	6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	8881.71	2241.7	11123.41	9399.6	2681.6	12081.2
	7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	8910.48	2242.33	11152.82	8563.8	2341.12	10904.92
	8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	9017.34	2253.71	11271.05	9168.6	2433.92	11602.52
	9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	9058.44	2333.97	11392.42	9193.8	2681.6	11875.4
Cotton	1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	7332.24	2062.21	9394.456	7308	2017.92	9325.92
	2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	7422.66	2108.98	9531.644	7320.6	2024.32	9344.92
	3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	7517.19	2134.26	9651.454	7413	2037.12	9450.12
	4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	7517.19	7446.6	10627.07	2134.26	2124.8	9899.08
	5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	7669.26	7539	9651.454	2148.8	2150.4	9571.4
	6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	7755.57	7568.4	9818.06	2180.4	2154.88	9689.4
	7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	7085.64	7543.2	9935.97	2138.68	2117.76	9723.28
	8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	8178.9	7589.4	9224.32	2193.04	2199.68	9660.96
	9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	8339.19	7627.2	10371.94	2207.57	2246.4	9789.08
Maize	1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	6999.33	6997.2	10546.77	1828.37	1749.12	9873.6
	2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	7007.55	7064.4	8827.7	1913.06	1777.28	8746.32
	3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	7073.31	7085.4	8920.61	1919.38	1790.08	8841.68
	4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	7167.84	7106.4	8992.69	1940.24	1806.72	8875.48
	5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	7245.93	7182	9108.08	1952.88	1809.28	8913.12
	6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	7274.7	7186.2	9198.81	2007.86	1824	8991.28
	7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	7241.82	7131.6	9282.56	1999.64	1744.64	9010.2
	8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	7287.03	7211.4	9241.46	2035.04	1841.28	8876.24
	9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	7311.69	7228.2	9322.07	2052.1	1856	9052.68
sunflower	1- 45kg N+0 P_2O_5+ 0 K_2SO_4/fed	5840.31	6447	9363.79	1554.72	1627.52	9084.2
	2- 45kg N+15.5P_2O_5+ 0 K_2SO_4/f	5926.62	6459.6	7395.03	1588.21	1651.2	8074.52
	3- 45kg N+15.5P_2O_5+ 25 K_2SO_4/fed	6021.15	6573	7514.83	1653.94	1665.92	8110.8
	4- 60kg N+0 P_2O_5+0 K_2SO_4/fed	6345.84	6749.4	7675.09	1657.73	1672.32	8238.92
	5- 60kg N+15.5P_2O_5+0 K_2SO_4/fed	6641.76	6837.6	8003.57	1727.25	1683.2	8421.72
	6- 60kg N+15.5P_2O_5+ 24 K_2SO_4/fe	6797.94	6875.4	8369.01	1748.74	1708.8	8520.8
	7- 75kgN+ 0 P_2O_5+ 0 K_2SO_4/fed	6608.88	6686.4	8546.68	1728.52	1694.72	8584.2
	8- 75kg N+15.5 P_2O_5+0 K_2SO_4/fed	6830.82	6904.8	8337.4	1784.13	1708.8	8381.12
	9- 75kg N+15.5 P_2O_5+ 24K_2SO_4/fed	6982.89	6934.2	8614.95	1801.2	1723.52	8613.6

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الملخص العربى

تأثير المحاصيل الصيفية السابقة ومستويات مختلفة من النيتروجين والفوسفور والبوتاسيوم على إنتاجية محصول القمح ومكوناته

السيد عبد المقصود ابومرزوقة[١] – محمد خالد حمدنى عامر[٢]

[١] قسم بحوث فسيولوجيا المحاصيل– معهد بحوث المحاصيل الحقلية – مركز البحوث الزراعية– مصر

[٢] قسم بحوث التكثيف المحصولي – معهد بحوث المحاصيل الحقلية – مركز البحوث الزراعية– مصر

أجريت تجربتان حقليتان بمزرعة محطة البحوث الزراعية بسخا خلال الموسمين الزراعيين ٢٠١٥/٢٠١٤ و٢٠١٦/٢٠١٥م لدراسة تأثير المحاصيل الصيفية السابقة(فول صويا– قطن– ذرة شامية – دوار الشمس) ومعدلات سمادية من كلا النتيروجين (٤٥ و ٦٠ و ٧٥ كجم ن/فدان) والفوسفور (صفر و ١٥ كجم فو٢أ٥/فدان) والبوتاسيوم (صفر و٤٥ كجم بو٢كب ا٤/فدان). و قد نفذت التجربة قى تصميم القطع الشريطية (الشرائح المتعامدة) فى ثلاث مكررات حيث كانت محاصيل(فول صويا– القطن– الذرة الشامية – دوار شمس) فى الشرائح العمودية , ووضعت معاملات التسميد النيتروجينى و الفوسفاتى و البوتاسى فى الشرائح الافقية فى الموسمين كالتالى: (١) التسميد بـ٤٥ كجم ن+ بدون فوسفور+ بدون بوتاسيوم /فدان . (٢) التسميد بـ٤٥ كجم ن+ ١٥,٥ كجم فوسفور+ بدون بوتاسيوم /فدان. (٣) التسميد بـ٤٥ كجم ن+ ١٥,٥ كجم فوسفور+٢٤ كجم بوتاسيوم /فدان. (٤) التسميد بـ٦٠كجم ن + بدون فوسفور+ بدون بوتاسيوم /فدان .(٥) التسميد بـ٦٠كجم ن + ١٥,٥ كجم فوسفور+ بدون بوتاسيوم /فدان. (٦) التسميد بـ٦٠كجم ن + ١٥,٥كجم فوسفور+ ٢٤ كجم بوتاسيوم / فدان. (٧) التسميد بـ٧٥كجم ن+ بدون فوسفور+ بدون بوتاسيوم /فدان. (٨) التسميد بـ٧٥كجم ن+١٥,٥ كجم فوسفور + بدون بوتاسيوم /فدان. (٩) التسميد بـ٧٥كجم ن+١٥,٥كجم فوسفور+٢٤ كجم بوتاسيوم /فدان , على النمو والمحصول ومكوناتة لصنف قمح جميزة ١١ وقد اطهرت النتائج المتحصل عليها ما يلي:–

١– زراعة القمح عقب محصول فول الصويا كان له تاثير معنوى على تركيز صبغات التمثيل الضوئى (كلوروفيل أ، كلوروفيل ب) فى ورقة العلم ومساحتها ؛وطول النبات وعدد السنابل /م٢ وعدد السنيبلات/ سنبلة ووزن حبوب السنبلة ووزن الالف حبة وكذلك محصول الحبوب والقش/ فدان والنسب المئوية لكلا من النيتروجين والفوسفور والبوتاسيوم فى الحبوب كان متفوقا على المنزرع عقب قطن أو ذرة شامية أو دوار شمس فى كل الصفات المدروسة.

٢– أدت زيادة فى معدل السماد النيتروجيني ٧٥ كجم ن مع اضافة ١٥,٥ كجم فوسفور+٢٤ كجم بوتاسيوم للفدان الى زيادة معنوية فى جميع الصفات المدروسة خلال موسمى الدراسة.

٣– أوضح التفاعل بين عاملى الدراسة ألى أنمحصول الحبوب للفدان كان اعلى مع القمح المنزرع عقب فول صويا والمعامل بـ٦٠ كجم نيتروجين+١٥,٥ كجم فوسفور+٢٤ كجم بوتاسيوم للفدان مقارنة بباقى المعاملات خلال موسمى الدراسة.

و قد اوضحت الدراسة أن أفضل المعاملات عند زراعة القمح عقب فول صويا مع التسميد بـ٦٠ كجم نيتروجين+١٥,٥ كجم فوسفور+ ٢٤ كجم بوتاسيوم أعطت أعلى القيم لنمو القمح والمحصول ومكوناته ، بالمقارنة بالقمح المنزرع عقب قطن أوذرة شامية أو دوار الشمس وأعطت بالتالي أعلى القيم من إجمالي الدخل للمزارع وأدت إلى انخفاض كمية الأسمدة المعدنية المضافة و محصول الحبوب الناتج أمن على صحة الإنسان والحيوان والبيئة ويرجع ذلك إلى انخفاض نسبة الأسمدة المعدنية في حبوب القمح والتبن و ذلك تحت ظروف التجربة فى منطقة شمال الدلتا.

Effect of Previous Winter Crops Under Different Tillage Systems and Nitrogen Fertilizer Rates on Maize Yield and its Attributes

Hamdany Amer M.Kh.
Crop Intensification Research Department, Field Crops Research Institute, A.R.C., Egypt.

ABSTRACT

The present investigation has been conducted at Gemmiza Agricultural Experiments and Research Station, Agricultural Research Station, El- Gharbia governorate, during 2013/2014 and 2014/2015 seasons to study the effect of preceded winter crops , tillage system and Nfertilizer rates and yield components on yield / maize (cross T.W.C Giza 353) to decrease nitrogen (N) fertilizer inputs of maize crop and increase net income. The treatments were the combinations among three factors; the first factor was the preceded winter crops (berseem , faba bean and wheat), the second factor was tillage systems (no tillage 'T_1', chisel plowing 'T_2', moldboard plowing 'T_3' and combined of chisel + moldboard 'T_4' and the third factor was N fertilizer rates (70, 85 and 100 kg N /fed). The experimental design was split split plot design arrangement with three replications. The data indicated that maize grain yield /plant grain yield / fed and 100 – kernel weight were increased significantly after harvest of legume crops berseem and faba bean compared to those grown after wheat harvest. The fourth tillage system (chisel + moldboard) improved soil properties which reflected positively on maize grain yield and its attributes. Increasing N fertilizer rates from 70 to 100 kg N/ fed resulted in significant increments in maize grain yield and its attributes. Most of the interactions affected significantly maize grain yield and its attributes. The preceded winter crops x tillage systems x N fertilizer rates affected significantly 100 – kernel weight. Nitrogen use efficiency (NUE) ranged from 112.65 to 239.58 kg grain yield / kg nutrient applied in the first season and from 178.17 to 285.60 kg grain yield / kg nutrient applied in the second season. Growing maize crop after berseem cutting and using combined of chisel + moldboard with the application of 100 kg N/ fed decreased N inputs of maize by 16.66 % of maize recommended N fertilizer rate and increased net return for farmers.

Keywords: Maize yield, Winter field crops, tillage systems, NUE, net return.

INTRODUCTION

It is known that nitrogen (N) is an essential element required for successful plant growth. Once organic fertilizers are applied to soils and mineralization begins, inorganic N is released and absorbed by plants. There are close relationships between the excessive application of N fertilizers and environmental problems (Gastal and Lemaire, 2002). Accordingly, excess N is lost in ionic or gaseous form through leaching, volatilization and denitrification. If nitrate is not absorbed by plant roots, it is carried away by runoff or leaches into the soil along with water (Tamme *et al.*, 2009). However, the rate of mineralization is controlled by several factors, including agricultural management, microorganism, soil properties, temperature and water content (Fan and Li, 2010).

Moreover, crop residues are known to affect soil physical properties (Hulugalle *et al.*, 1986), soil biological activity (Tian *et al.*, 1993) and availability of nutrients (Asghar *et al*., 2006). Certainly, crop residue is a good source of nutrients in many agr-ecosystems for sustainable crop production and environment (Blanco-Canqui and Lal, 2009). Hence, soil physical properties are related directly to crop residues, especially bulk density, porosity, water sorptivity and aggregation dictates the water infiltration characteristics of the soil (Shaver, 2010). Consequently, tillage practices influences significantly soil physical characteristics and crop productivity (Kahlon, 2014).

Accordingly, integration of crop sequence and tillage system could be related to soil nutrient availability where there are strategic food crops such as berseem (*Trifolium alexandrinum* L.), faba bean (*Vicia faba*) and wheat (*Triticum aestivum* L.) grown during the winter season in Egypt. On the other hand, demand for the maize grains in the Egyptian market is intensively increasing, it is a strategic crop during the summer season and it is used for human consumption, animal and poultry feeding and industrial purposes. Although, maize plant development is strongly dependent on the availability of N in the soil and the efficiency of N utilization for biomass production and yield (Sonnewald, 2012), however, maize cultivated area reached about 1673517 fad in 2014 (Bulletin of Statistical Cost Production and Net Return, 2015). In this concern, Abdel-Wahab *et al.* (2016) found that growing maize with using 100% of the recommended mineral N fertilizer rate after harvest of the legume crops had the highest studied traits of maize compared to the other treatments. Therefore, the main objective of the present research was to decrease N inputs of maize crop by using the suitable cropping sequence and tillage system.

MATERIAIS AND METHODS

This investigation was carried out in Gemmiza Agricultural Experiments and Research Station, El-Gharbia Governorate, during two successive seasons (2013/2014 and 2014/2015) to decrease N inputs of maize crop by the suitable preceding crop and tillage system to increase net income. Some chemical properties have been recorded in Table (1) after harvest the winter crops and before sowing maize crop. These analyses were done by Environment, Water and Soil Research Institute, A.R.C. Methods of mechanical and chemical analysis were described by Chapman and Pratt (1961).

Table (1). Some chemical properties of the experimental soil after harvest of winter field crops under different tillage systems in the two growing seasons

Crop	Preceding	N (ppm)		P (ppm)		K (ppm)	
		2014	2015	2014	2015	2014	2015
Berseem	T_1	35.84	34.94	16.37	15.96	288.80	281.58
	T_2	40.45	39.44	19.42	18.93	313.05	305.22
	T_3	36.73	35.81	18.20	17.75	310.50	302.73
	T_4	41.44	40.40	18.34	17.87	273.79	266.94
Faba bean	T_1	30.34	29.58	19.08	18.60	308.28	300.57
	T_2	29.64	28.90	18.68	18.21	305.85	298.20
	T_3	31.75	30.96	18.74	18.27	304.93	297.30
	T_4	33.99	33.14	19.68	19.18	306.52	298.86
Wheat	T_1	26.30	25.63	19.21	18.72	310.90	303.12
	T_2	30.27	29.51	19.48	18.99	314.28	306.42
	T_3	34.36	33.50	19.86	19.36	314.25	306.39
	T_4	35.22	34.33	19.50	19.01	309.99	302.24

T_1= no tillage T_2= chisel plowT_3= moldboard plowT_4= moldboard + chisel plow

This experiment included 36 treatments which were the combinations of three preceding winter crops (berseem cv. Helaly, faba bean cv. Giza 716 and wheat cv. Gemmiza 11), four tillage systems (T_1= No-tillage, T_2 = Chisel plow, T_3 = Moldboard plow and T_4 = Combined of moldboard plow + chisel plow) and three N fertilizer rates (70, 85 and 100 kg/fad).

Furrow irrigation was the irrigation system in the area. Berseem seeds were sown on October 8th and 10th, faba bean seeds were sown on November 10th and 12th and wheat grains were sown on November 16th and 19th in 2013 and 2014 seasons, respectively. All cultural practices of growing all the tested crops were applied as recommended in the region. Recommended culture of maize was conducted by growing maize after berseem cutting and using T_2 system with 120 kg N/ fed. Accordingly, this study included three rates of N fertilizer without the recommended N fertilizer rate of maize (120 kg N/ fed) under clay soil conditions according to Habtegebrial *et al.* (2007) who mentioned that level of applied N can be reduced under suitable tillage. Although the biological N fixation by the legume crops should be considered, however there was no way to determine the amount of N derived from fixation and absorption from the soil in this experiment.

The treatments were laid out in split split plot design with three replicates. The winter field crops were randomly assigned to the main plots, tillage systems were allocated in sub-plots and N fertilizer rates were devoted to sub sub plots. The area of sub sub plot was 10.5 m^2, each plot consisted of 5 ridges, and each ridge was 3.0 m in length and 0.7 m in width.

The studied traits

A- Soil properties

After harvest of the winter field crops, soil samples were taken after each crop from each plot for the following soil measurements:

1- Bulk density: It was calculated by dividing the soil weight in grams by soil volume in cubic centimeter (ASTM, 1980). Bulk density = soil weight (g)/ soil volume (cm^3) x 100.

2- Soil porosity : soil porosity space percentage was estimated according to ASTM (1980) by the following formula:

$$\text{Soil porosity} = \frac{P_s - P_b}{P_s} \times 100$$

Where : P_s = Real density (g/cm^3) and P_b = Bulk density (g/cm^3) .

B – Field crops :

1 – Maize crop

Ten plants were chosen at random from each sub sub plot to estimate the following traits: Plant height (cm), ear height (cm), ear leaf area , ear length (mm), ear diameter (cm), number of rows/ear, number of grains/ row, 100 – kernel weight (gm),, grain yield /plant (g) and grain yield / fed (ardab) was determined from the weight of grains adjusted to 14% moisture of the three inner ridges of each sub subplot and converted to ardab per feddan (one ardab = 140 kg).

2 – The preceding winter field crops

Forage yield of berseem (ton) was determined from the weight of each plot and converted to tons per faddan. Also, Seed yield of faba bean (ardab) was determined from the weight of each plot and converted to ardab per feddan (one ardab = 155 kg). Finally, grain yield of wheat (ardab) was determined from the weight of each plot and converted to ardab per feddan (one ardab = 150 kg).

Nitrogen use efficiency (NUE).

NUE for each treatment was determined using the agronomic efficiency (AE) and partial factor productiv-

ity (PFP) indices (Dobermann, 2007): AE = $(Y - Y_o)/F$ and PFP = (Y_o / F) + AE, where F = amount of (fertilizer) nutrient applied (kg/ fed); Y = Crop yield with applied nutrients (kg/ fed) and Y_o = crop yield (kg/ fed) in a control treatment with application of 60 kg N/ fed. A basic assumption was that N uptake is the same in fertilized and unfertilized plots. This assumption was made with a caution since soil N transformations and root development may differ between fertilized and unfertilized plots (Weber and Day, 1996 and Brye *et al*., 2002).

Economic Return

Forage yield of berseem and faba bean seeds, as well as, wheat and maize grains prices presented by (Bulletin of Statistical Cost Production and Net Return, 2015) were used. Net return was calculated as follows: Net return = total costs – total return according to the preceding winter field crop and N fertilizer rates of maize.

Statistical analysis

The data for each experiment were then analyzed by MSTAT software for comparison of the mean values and the two seasons by L.S.D. test at 5% level. Response equations were calculated according to Snedecor and Cochran (1998).

RESULTS AND DISCUSSIONS

1 – Soil properties

A – Effect of the preceding winter field crops on soil bulk density

The residual effects of legume crops decreased soil bulk density than that by wheat residues in the two growing seasons (Table 2). The residual effect of the legume crops especially berseem decreased soil weight to soil volume which could be reflected positively on electrical conductivity of the soil and thereby soil nutrient dynamics. In this concern, Eichler-Löbermann *et al*. (2008) mentioned that there was positive influence of legume crops on the physical and chemical properties of soil.

Accordingly, it is likely that the preceding winter field crops expressed soil physical, chemical and biological measurements on a volumetric basis for soil quality assessment and comparisons between management systems. These results are similar to those obtained by Yang *et al.* (2005) who indicated that planting alfalfa could reduce soil bulk density. Also, Liu *et al.* (2006) showed that application of green manure could reduce soil bulk density and pH, as well as, increase soil organic matter and soil microbes in a tobacco field.

B – Effect of the preceding winter field crops on soil porosity

The results in Table (2) indicate that the residual effects of legume crops improved soil porosity than those by wheat residues. These data suggest that the preceding legume crops especially berseem improved soil structure and soil porosity which furnished a favorable environment in the soil. This finding imply that the preceding winter field crops appeared to have the strongest effect on soil nutrient dynamics. Soil porosity may cause restrictions to root growth, and poor movement of air and water through the soil (Arshad *et al.*, 1996). These results are in agreement with those observed by Yang *et al.* (2005) who reported that planting alfalfa could enhance soil porosity.

Table (2). Some physical properties of the experimental soil after harvest of winter field crops under different tillage systems in the two growing seasons.

Preceding Crop		2014 season		2015 season	
		Bulk density (g/cm^3)	Porosity (%)	Bulk density (g/cm^3)	Porosity (%)
Berseem	T1	1.205	52.42	1.180	53.66
	T2	1.217	53.45	1.149	55.00
	T3	1.210	55.00	1.132	56.00
	T4	1.182	56.00	1.122	57.66
L.S.D. 5%		0.143	1.880	0.104	2.080
Faba bean	T1	1.285	48.00	1.231	51.88
	T2	1.258	51.00	1.212	54.00
	T3	1.249	51.40	1.192	55.00
	T4	1.133	51.81	1.182	55.66
L.S.D. 5%		0.133	1.780	0.105	1.140
Wheat	T1	1.334	46.00	1.290	50.00
	T2	1.295	48.00	1.218	50.66
	T3	1.285	49.20	1.275	51.00
	T4	1.213	49.30	1.170	51.33
L.S.D. 5%		0.112	1.285	0.116	N.S.

C – Effect of tillage systems on soil bulk density

Differences in soil bulk density between tillage systems were significant for faba bean in the first season and for wheat in the two growing seasons (Table 2). The lowest soil bulk density was recorded ($P \leq 0.05$) by using T_4 system followed by T_3 system then T_2 system, meanwhile T_1 system recorded the highest values after harvest of the preceding winter field crops in the two growing seasons. In this concern, Al-Janobi and Al-Suhaibani (1998) applied the proposed model by Harrigan and Rotz (1995) to disk harrows, moldboard plows, disk plows and chisel plows; they found that the specific drafts measured were less than those predicted for disk harrow implements. They attributed the difference to different soil condition, shapes and sizes of the tested disk harrow. However, specific draft for the moldboard plow and the chisel plows were very close to the predicted values. The present results are in parallel with those obtained by Gommaa and El-Douby (2002) who revealed that no tillage had higher soil bulk density than conventional tillage.

D–Effect of tillage systems on soil porosity

The results in Table (2) show that the soil porosity had significantly effected by tillage systems in the two growing seasons except soil porosity after wheat harvest in the second season. The lowest values of soil porosity were associated with T_1 system followed by T_2 system then T_3 systems, meanwhile T_4 system recorded the highest percentage *($P \leq 0.05$)* of soil porosity in the two growing seasons. These results are in agreement with those reported by Gommaa and El-Douby (2002) who concluded that tillage systems can influence soil physical condition since they reduce its compactness and also improve mechanical disturbance of soil compared with no tillage.

2 –Maize grain yield and its attributes

A – Effect of the preceding winter field crops

Grain yield/plant, 100 – kernel weight and grain yield/ fed were affected significantly by the preceding winter field crops in the two growing seasons, meanwhile plant and ear heights, ear leaf area , ear length and diameter, number of rows/ear and number of grains/row were not affected (Table 3). Grain yield /plant, 100 – kernel weight and grain yield / fed were increased *($P \leq 0.05$)* after berseem cutting compared to those by wheat harvest. Maize grain yield / fed after berseem cutting was increased by 30.92 and 30.34 % in the first and second seasons, respectively, compared to that by wheat harvest. These data could be due to legume crops especially berseem improved N soil availability (Table 1) that enhanced efficiency of photosynthetic process of maize crop and reflected on the accumulation of dry matter per plant. These findings indicate that growth and development of different parts of maize plant may be acclimated positively after berseem cutting and/or faba bean harvest which improved the translocation of water and nutrients between root and leaves of the plant than those after wheat harvest.

These results could be attributed to berseem and faba bean as legume crops had important role in enriching the soil fertility with N (Table 1) and organic matter, as well as, its positive effect of their residues on the physical (Table 2), chemical and biological traits of the soil. It is expected that this improvement had a great ecological importance in decreasing intra–specific competition between maize plants for soil N use through biological N fixation by the legume crops. Wheat increased bulk density of the experimental soil and hence there was a negative effect on soil porosity percentage that could led to imbalance in the electrical conductivity and water content of the experimental soil before maize sowing, especially Liu and Shan (2003) found that high soil mechanical resistance caused by increased bulk density or lowered soil water content reduced maize plant size. Moreover, it is known that wheat had longer duration and high capability to absorb more soil nutrients and thereby adverse effects on maize yield **(**Zohry, 2005). In this concern, Javanmard (2015) investigated that the bulk density content was significantly higher in control soil than in legume crop treatments.

B –Effect of tillage systems

All the studied traits of maize were affected significantly by the tillage systems in the two growing seasons, meanwhile number of rows/ear was not affected (Table 3). T_4 system recorded the highest values of plant and ear heights, ear leaf area , ear length and diameter, number of grains/row, grain yield/plant, 100 – kernel weight and grain yield / fed followed by T_3 system then T_2 system, mean while the opposite trend was true for T_1 system. These results show that grain yield / fed was increased ($P \leq 0.05$) over T_1 system by 32.21, 20.25 and 13.90% in the first season and 31.89, 19.72 and 13.19 % in the second season for T_2 , T_3 and T_4, respectively. Accordingly, it is expected that growth and development of maize plant with using T_1 system had not enough period to grow and competition for climatic and edaphic conditions compared to the others. These results could be due to T_4 system enhanced the mineralization of soil organic C and N by crop residues, disrupting soil aggregates, and increasing aeration (Tangyuan *et al.*, 2009). Similar results were obtained by Selim and El–Sergany (1995) who found that grain yield of maize was increased with tillage system compared with no-tillage system.

C – Effect of N fertilizer rates

Plant and ear heights, ear leaf area , ear length and diameter, number of grains/row, grain yield/plant, 100 – kernel weight and grain yield / fed were affected significantly by N fertilizer rates in the two growing seasons, meanwhile, number of rows/ear was not affected (Table 3). It is observed that plant and ear heights were increased by increasing N fertilizer rate of maize from 70 to 100 kg N / fed. Increasing N fertilizer rate from 70 to 100 kg N /fed increased *($P \leq 0.05$)* plant height by 6.60 % in the first season and 6.62 % in the second season. Also, increasing N fertilizer rate up to 100 Kg N / fed increased ear height by 10.85% in the first season and 9.51 % in the second season. The increase in ear height could possibly be ascribed to the fact that N increased plant growth regulators that resulted in more nodes and internodes of the plant. Similarly, **Ayub *et al.* (2003)** reported that high N application increased plant height and ear leaf area of maize plant.

Also, there was growth disadvantage of maize crop when it received 70 or 85 kg N / fed than that received 100 kg N / fed. Increasing N fertilizer rate up to 100 kg N/ fed increased ear leaf area , ear length and diameter, number of grains/row, grain yield /plant, 100 – kernel weight and grain yield / fed. However, number of rows/ ear seems to be affected greatly by the genetic resources than edaphic resources. In this respect, Moraditochaee *et al.* (2012) showed that the application of N fertilizer was non-significant on number of rows /ear.

Obviously, the highest rate of N fertilizer led to largely balance in plant–to–plant competition for climatic and edaphic environmental conditions that enhanced ear leaf area for convert more solar energy to chemical energy and more translocation of photosyntheses metabolites to the sink (ears) compared with the others. Consequently, yield attributes of maize were increased

Table (3). Effect of the preceding winter field crops, tillage systems, N fertilizer rates and their interactions on maize grain yield and its attributes in the two growing seasons, 2013/2014 and 2014/2015

Treatments	Traits	Plant height (cm)								Ear height (cm)							
		2013/2014 season				2014/2015 season				2013/2014 season				2014/2015 season			
		N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean
Berseem	T1	246.63	246.63	261	251.42	241.7	241.7	256.42	246.6	131.27	131.32	141.66	134.75	128.64	128.69	139.54	132.29
	T2	253.01	258	262.66	257.89	247.95	252.92	257.7	252.85	134.58	138.83	144.33	139.24	132.31	136.25	142.16	136.9
	T3	252.84	266.23	266.33	261.8	247.89	260.9	262.33	257.04	134.37	148.33	149.27	143.99	131.99	146.1	146.28	141.45
	T4	254.91	268.84	274.33	266.02	249.81	264.3	270.21	261.44	135.56	156.66	160.33	150.85	132.85	153.98	157.92	148.25
Mean		251.84	259.92	266.08	259.28	246.83	254.95	261.66	254.48	133.94	143.78	148.89	142.2	131.44	141.25	146.47	139.72
Faba bean	T1	240.42	245.1	260.61	248.71	235.61	240.19	255.78	243.86	127.85	128.78	141.64	132.75	125.3	126.21	138.88	130.13
	T2	245.33	257.41	261	254.58	241.65	252.26	257.08	250.33	129.68	138.33	142.33	136.78	127.09	136.05	140.19	134.44
	T3	251.66	262.66	262.96	259.09	247.78	258.72	258.72	255.07	134	145.36	145.85	141.73	131.68	142.45	142.93	139.02
	T4	251.66	268.33	271.66	263.88	247.89	263.46	267.59	259.64	134.33	153.53	157.12	148.32	131.89	150.46	154.31	145.55
Mean		247.26	258.37	264.05	256.56	243.23	253.65	259.79	252.22	131.46	141.5	146.73	139.89	128.99	138.79	144.07	137.28
Wheat	T1	233.06	247.31	255.12	245.16	228.4	242.37	250.02	240.26	124.75	131.64	137.33	131.24	120.73	129.01	135.27	128.33
	T2	239.08	247.95	255.78	247.6	234.3	242.99	250.66	242.65	123.2	131.89	138.09	131.06	122.25	129.25	135.33	128.94
	T3	239.08	249.53	257.41	248.67	234.3	244.54	252.26	243.7	125.06	132.33	138.18	131.85	122.56	129.86	135.41	129.27
	T4	249.53	258.08	260.33	255.98	244.54	254.13	255.4	251.35	132.51	139.84	141	137.78	130.34	136.69	138.61	135.21
Mean		240.18	250.71	257.16	249.35	235.38	246	252.08	244.49	126.38	133.92	138.65	132.98	123.97	131.2	136.15	130.43
Average of T1		240.03	246.34	258.91	248.42	235.23	241.42	254.07	243.57	127.95	130.58	140.21	132.91	124.89	127.97	137.89	130.25
Average of T2		245.8	254.45	259.81	253.35	241.3	249.39	255.14	248.61	129.15	136.35	141.58	135.69	127.21	133.85	139.22	133.42
Average of T3		247.86	259.47	262.23	256.52	243.32	254.72	257.77	251.93	131.14	142	144.43	139.19	128.74	139.47	141.54	136.58
Average of T4		252.03	265.08	268.77	261.96	247.41	260.63	264.4	257.48	134.13	150.01	152.81	145.65	131.69	147.04	150.28	143
Average of N fertilizer rates		246.42	256.33	262.43	255.06	241.81	251.53	257.84	250.39	130.59	139.73	144.75	138.35	128.13	137.08	142.23	135.81
L.S.D. 5% of preceding winter field crops (W)					N.S.				N.S.				N.S.				N.S.
L.S.D. 5% of tillage systems (T)					5.52				5.42				2.71				2.66
L.S.D. 5% of N fertilizer rates (N)					4.14				4.06				1.47				1.44
L.S.D. 5% of W × T					N.S.				N.S.				2.9				2.85
L.S.D. of W × N					N.S.				N.S.				N.S.				N.S.
L.S.D. 5% of T × N					8.16				8.01				2.9				2.85
L.S.D. 5% of W × T × N					N.S.				N.S.				N.S.				N.S.
Recommended culture of maize					262.15				265.66				157.16				155.58

T_1= no tillage T_2= chisel plow T_3= moldboard plow T_4= moldboard + chisel plow
N_1 = 70 kg N/fed N_2 = 85 kg N/fed N_3 = 100 kg N/fed

Table (3). Continued.

Treatments \ Traits		Ear leaf area (cm²)								Ear length (cm)							
		2013/2014 season				2014/2015 season				2013/2014 season				2014/2015 season			
		N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean
Berseem	T1	1156.81	1175.62	1360	1230.81	1126.81	1145.62	1346.2	1206.21	17	17.24	19.8	18.01	16.95	17.09	19.65	17.89
	T2	1260	1346.4	1370	1325.46	1249.08	1324.32	1360	1311.13	18.48	19.14	20.06	19.22	18.33	18.99	19.89	19.07
	T3	1250	1425.6	1425.6	1367.06	1240	1405.6	1405.6	1350.4	18.33	21.12	21.12	20.19	18.28	20.96	20.96	20.06
	T4	1279.08	1440	1510	1409.69	1250	1430	1500	1393.33	18.49	21.33	21.66	20.49	18.34	21.28	21.61	20.41
Mean		1236.47	1346.9	1416.4	1333.25	1216.47	1326.38	1402.95	1315.26	18.07	19.7	20.66	19.47	17.97	19.58	20.52	19.35
Faba bean	T1	940.5	990	1356.3	1095.6	930	970	1336.3	1078.76	16.83	16.83	19.66	17.77	16.7	16.7	19.61	17.67
	T2	1000	1300	1366.2	1222.06	990	1290	1350	1210	17	19	20	18.66	16.95	18.95	19.89	18.59
	T3	1237.5	1380	1420.15	1345.88	1220	1370	1390.15	1326.71	18.15	20.06	20.37	19.52	18.01	19.95	20.2	19.38
	T4	1247.4	1440	1494.9	1394.1	1227.4	1430	1471.56	1376.32	18.18	21.33	21.45	20.32	18.02	21.28	21.28	20.19
Mean		1106.35	1277.5	1409.38	1264.41	1091.85	1265	1387	1247.94	17.54	19.3	20.37	19.07	17.42	19.22	20.24	18.96
Wheat	T1	884.07	1185.03	1287	1118.7	854.07	1155.03	1258.48	1089.19	15.98	17.49	18.66	17.37	15.85	17.35	18.62	17.27
	T2	930.6	1217.7	1288.48	1145.59	910.5	1192.65	1267	1123.38	15.98	17.55	18.81	17.44	15.85	17.4	18.65	17.3
	T3	940	1222.65	1297.89	1153.51	910.6	1197.7	1267.89	1125.39	16.61	17.66	18.81	17.69	16.47	17.62	18.66	17.58
	T4	1230	1354.32	1354.32	1312.88	1217.5	1324.32	1326.4	1289.4	17.86	19.33	19.47	18.88	17.71	19.28	19.32	18.77
Mean		996.16	1244.92	1306.92	1182.67	973.16	1217.42	1279.94	1156.84	16.6	18	18.93	17.84	16.47	17.91	18.81	17.73
Average of T1		993.79	1116.88	1334.43	1148.36	970.29	1090.21	1313.66	1124.72	16.6	17.18	19.37	17.71	16.5	17.04	19.29	17.61
Average of T2		1063.53	1288.03	1341.56	1231.04	1049.86	1268.99	1325.66	1214.83	17.15	18.56	19.62	18.44	17.04	18.44	19.47	18.31
Average of T3		1142.5	1342.75	1381.21	1288.82	1123.53	1324.43	1354.54	1267.5	17.69	19.61	20.1	19.13	17.58	19.51	19.94	19.01
Average of T4		1252.16	1411.44	1453.07	1372.22	1231.63	1394.77	1432.65	1353.01	18.17	20.66	20.86	19.89	18.02	20.61	20.73	19.78
Average of N fertilizer rates		1112.99	1289.77	1377.56	1260.1	1093.82	1269.6	1356.63	1240.01	17.4	19	19.98	18.79	17.28	18.9	19.85	18.67
L.S.D. 5% of preceding winter field crops (W)					N.S.				N.S.				N.S.				N.S.
L.S.D. 5% of tillage systems (T)					34.17				34.04				0.88				0.58
L.S.D. 5% of N fertilizer rates (N)					34.42				34.4				0.58				0.58
L.S.D. 5% of W × T					67.87				67.84				N.S.				N.S.
L.S.D. of W × N					N.S.				N.S.				N.S.				N.S.
L.S.D. 5% of T × N					67.87				67.84				N.S.				N.S.
L.S.D. 5% of W × T × N					N.S.				N.S.				N.S.				N.S.
Recommended culture of maize					1484.17				1469.03				21.42				21.36

T_1= no tillage T_2= chisel plowT_3= moldboard plowT_4= moldboard + chisel plow
N_1 = 70 kg N/fed N_2 = 85 kg N/fed N_3 = 100 kg N/fed

Table (3). Continued.

Treatments \ Traits		Ear diameter (mm)								Number of rows/ear							
		2013/2014 season				2014/2015 season				2013/2014 season				2014/2015 season			
		N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean
Berseem	T1	38.56	39.18	45.33	41.02	38.23	38.85	45.19	40.75	13.53	14.52	14.59	14.21	13.3	13.41	14.39	13.7
	T2	42	44.88	45.66	44.18	41.89	44.54	45.55	43.99	13.93	14	14.59	14.17	13.82	13.96	14.39	14.05
	T3	41.66	47.52	47.52	45.56	41.56	47.16	47.16	45.29	13.93	14.59	14.59	14.37	13.82	14.48	14.48	14.26
	T4	42.63	48	50.33	46.98	42.27	47.88	50.2	46.78	13.93	14.66	14.66	14.41	13.82	14.63	14.63	14.36
Mean		41.21	44.89	47.21	44.43	40.98	44.6	47.02	44.2	13.83	14.44	14.6	14.29	13.69	14.12	14.47	14.09
Faba bean	T1	31.35	33	45.21	36.52	31.25	32.75	44.87	36.29	13.26	13.33	14	13.53	13.16	13.3	13.96	13.47
	T2	33.33	43.33	45.54	40.73	33.25	44.54	45.22	41	13.33	14	14.59	13.97	13.3	13.96	14.39	13.88
	T3	41.25	46	47.33	44.86	40.94	45.88	46.93	44.58	14.52	14.59	14.59	14.56	13.74	14.39	14.48	14.2
	T4	41.58	48	49.83	46.47	41.26	47.88	49.45	46.19	13.93	14.59	14.66	14.39	13.74	14.48	14.63	14.28
Mean		36.87	42.58	46.97	42.14	36.67	42.76	46.61	42.01	13.76	14.12	14.46	14.11	13.48	14.03	14.36	13.95
Wheat	T1	29.46	39.5	42.9	37.28	29.21	39.16	42.57	36.98	13.2	13.6	13.93	13.57	13.08	13.82	13.82	13.57
	T2	31.02	40.59	42.94	38.18	30.78	40.28	42.58	37.88	13.2	13.93	13.93	13.68	13.08	13.49	13.82	13.46
	T3	31.33	40.75	43.26	38.44	31.08	40.4	42.89	38.12	13.26	13.93	14	13.73	13.16	13.96	13.96	13.69
	T4	41	45.14	45.14	43.76	40.89	44.76	44.76	43.47	13.93	14	14	13.97	13.74	13.96	13.96	13.88
Mean		33.2	41.49	43.56	39.41	32.99	41.15	43.2	39.11	13.39	13.86	13.96	13.73	13.26	13.8	13.89	13.65
Average of T1		33.12	37.22	44.48	38.27	32.89	36.92	44.21	38	13.33	13.81	14.17	13.77	13.18	13.51	14.05	13.58
Average of T2		35.45	42.93	44.71	41.03	35.3	43.12	44.45	40.95	13.48	13.97	14.37	13.94	13.4	13.8	14.2	13.8
Average of T3		38.08	44.75	46.03	42.95	37.86	44.48	45.66	42.66	13.9	14.37	14.39	14.22	13.57	14.27	14.3	14.04
Average of T4		41.73	47.04	48.43	45.73	41.47	46.84	48.13	45.48	13.93	14.41	14.44	14.26	13.76	14.35	14.4	14.17
Average of N fertilizer rates		37.09	42.98	45.91	41.99	36.88	42.83	45.61	41.77	13.66	14.14	14.34	14.04	13.47	13.98	14.24	13.89
L.S.D. 5% of preceding winter field crops (W)					N.S.				N.S.				N.S.				N.S.
L.S.D. 5% of tillage systems (T)					1.13				1.13				N.S.				N.S.
L.S.D. 5% of N fertilizer rates (N)					1.14				1.14				N.S.				N.S.
L.S.D. 5% of W × T					2.26				2.24				N.S.				N.S.
L.S.D. of W × N					N.S.				N.S.				N.S.				N.S.
L.S.D. 5% of T × N					2.26				2.24				N.S.				N.S.
L.S.D. 5% of W × T × N					N.S.				N.S.				N.S.				N.S.
Recommended culture of maize					49.63				48.88				14.63				14.59

T_1= no tillage T_2= chisel plow T_3= moldboard plow T_4= moldboard + chisel plow
N_1 = 70 kg N/fed N_2 = 85 kg N/fed N_3 = 100 kg N/fed

Table (3). Continued.

Treatments \ Traits		Number of grains/row								Grain yield/plant (g)							
		2013/2014 season				2014/2015 season				2013/2014 season				2014/2015 season			
		N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean
Berseem	T1	34.28	34.6	39.2	36.02	33.98	34.3	38.9	35.72	126.14	127.67	169.17	140.99	122.42	124.54	165.02	137.32
	T2	36.86	38	39.85	38.23	36.55	37.9	39.55	38	154.94	166.71	174.93	165.52	150.37	161.79	170.26	160.8
	T3	36.33	42.04	42.36	40.24	36.24	41.68	42	39.97	149.47	183.15	184.68	172.43	145.8	178.66	179.23	167.89
	T4	36.86	42.79	43.66	41.1	36.55	42.47	43.55	40.85	155.6	194.16	221.77	190.51	151.01	188.43	216.33	185.25
Mean		36.08	39.35	41.26	38.89	35.83	39.08	41	38.63	146.53	167.92	187.63	167.36	142.4	163.35	182.71	162.81
Faba bean	T1	32.34	32.66	38.8	34.6	32.06	32.42	38.47	34.31	116.05	118.01	169.07	134.37	112.95	114.87	164.57	130.79
	T2	33.33	37.56	39.45	36.78	33.25	37.28	39.11	36.54	123.71	165.73	169.76	153.06	120.41	160.84	165.6	148.95
	T3	35.6	40	40.66	38.75	35.33	39.9	40.56	38.59	143.63	176.19	180.61	166.81	140.11	170.99	176.19	162.43
	T4	35.66	42.46	43.33	40.48	35.57	42.14	43.22	40.31	147.52	186.32	200.03	177.95	143.59	181.35	195.13	173.35
Mean		34.23	38.17	40.56	37.65	34.05	37.93	40.34	37.43	132.72	161.56	179.86	158.04	129.26	157.01	175.37	153.88
Wheat	T1	29.75	34.62	37.19	33.85	29.49	34.36	36.87	33.57	111.3	128.61	157.3	132.4	108.02	124.82	153.11	128.65
	T2	30.38	34.95	37.24	34.19	30.15	34.69	36.96	33.93	114.39	130.09	158.63	134.37	111.15	126.62	154.74	130.83
	T3	31.66	35.25	37.24	34.71	31.58	34.95	36.96	34.49	114.4	134.8	163.92	137.7	111.6	131.21	159.09	133.96
	T4	35.33	38	38.33	37.22	35.24	37.9	38.23	37.12	135.56	167.79	168.53	157.29	131.56	163.68	164.04	153.09
Mean		31.78	35.7	37.5	34.99	31.61	35.47	37.25	34.77	118.91	140.32	162.09	140.44	115.58	136.58	157.74	136.63
Average of T1		32.12	33.96	38.39	34.82	31.84	33.69	38.08	34.53	117.83	124.76	165.18	135.92	114.46	121.41	160.9	132.25
Average of T2		33.52	36.83	38.84	36.39	33.31	36.62	38.54	36.15	131.01	154.17	167.77	150.98	127.31	149.75	163.53	146.86
Average of T3		34.53	39.09	40.08	37.9	34.38	38.84	39.84	37.68	135.83	164.71	176.4	158.98	132.5	160.28	171.5	154.76
Average of T4		35.95	41.08	41.77	39.6	35.78	40.83	41.66	39.42	146.22	182.75	196.77	175.24	142.05	177.82	191.83	170.56
Average of N fertilizer rates		34.03	37.74	39.77	37.18	33.83	37.49	39.53	36.95	132.72	156.6	176.52	155.28	129.08	152.31	171.94	151.11
L.S.D. 5% of preceding winter field crops (W)					N.S.				N.S.				6.27				6.12
L.S.D. 5% of tillage systems (T)					2.03				2.02				8.35				8.12
L.S.D. 5% of N fertilizer rates (N)					1.07				1.06				6.96				6.77
L.S.D. 5% of W × T					2.11				2.1				13.73				13.36
L.S.D. of W × N					N.S.				N.S.				N.S.				N.S.
L.S.D. 5% of T × N					2.11				2.1				13.73				13.36
L.S.D. 5% of W × T × N					N.S.				N.S.				N.S.				N.S.
Recommended culture of maize					42.93				42.62				216.47				212.68

T_1= no tillage T_2= chisel plowT_3= moldboard plowT_4= moldboard + chisel plow
N_1 = 70 kg N/fed N_2 = 85 kg N/fed N_3 = 100 kg N/fed

Table (3). Continued.

Treatments \ Traits		100 – kernel weight (g)								Grain yield/fed (ardab)							
		2013/2014 season				2014/2015 season				2013/2014 season				2014/2015 season			
		N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean
Berseem	T1	27.57	28.21	31.26	29.01	27.29	27.8	30.8	28.63	13.52	13.79	21.2	16.17	12.86	13.24	20.46	15.52
	T2	29.61	30.45	31.54	30.53	29.17	29.92	30.92	30	18.66	20.77	22.23	20.55	17.85	19.89	21.4	19.71
	T3	29.28	31.96	32.21	31.15	28.85	31.44	31.58	30.62	17.69	23.7	23.97	21.78	17.03	22.9	23	20.97
	T4	29.75	33.23	34.59	32.52	29.45	32.58	33.91	31.98	20.78	25.67	30.6	25.68	17.96	24.64	29.63	24.07
Mean		29.05	30.96	32.4	30.8	28.69	30.43	31.8	30.3	17.66	20.98	24.5	21.04	16.42	20.16	23.62	20.06
Faba bean	T1	26.98	27.13	31.2	28.43	26.58	26.6	30.59	27.92	11.72	12.07	21.19	14.99	11.17	11.51	20.38	14.35
	T2	27.24	30.18	31.38	29.6	26.98	29.7	30.91	29.19	13.09	20.59	21.31	18.33	12.5	19.72	20.57	17.59
	T3	28.85	31.73	31.92	30.83	28.56	31.42	31.49	30.49	16.64	22.46	23.25	20.78	16.01	21.53	22.46	20
	T4	28.85	32.57	33.57	31.66	28.56	32.09	32.91	31.18	17.34	24.27	26.72	22.77	16.64	23.38	25.84	21.95
Mean		27.98	30.4	32.01	30.13	27.67	29.95	31.47	29.69	14.69	19.84	23.11	19.21	14.08	19.03	22.31	18.47
Wheat	T1	23.01	28.42	29.81	27.08	22.78	28.09	29.51	26.79	10.87	13.96	19.09	14.64	10.29	13.28	18.34	13.97
	T2	23.53	28.57	30	27.36	23.29	28.15	29.58	27	11.42	14.23	19.32	14.99	10.84	13.61	18.63	14.36
	T3	25.25	28.82	30.17	28.08	24.88	28.26	29.59	27.57	11.43	15.07	20.27	15.59	10.92	14.43	19.4	14.91
	T4	28.82	30.52	30.6	29.98	28.26	30.15	30.15	29.52	15.2	20.96	21.09	19.08	14.49	20.22	20.29	18.33
Mean		25.15	29.08	30.14	28.12	24.8	28.66	29.7	27.72	12.23	16.05	19.94	16.07	11.63	15.38	19.16	15.39
Average of T1		25.85	27.92	30.75	28.17	25.55	27.49	30.3	27.78	12.03	13.27	20.49	15.26	11.44	12.67	19.72	14.61
Average of T2		26.79	29.73	30.97	29.16	26.48	29.25	30.47	28.73	14.39	18.53	20.95	17.95	13.73	17.74	20.2	17.22
Average of T3		27.79	30.83	31.43	30.01	27.43	30.37	30.88	29.56	15.25	20.41	22.49	19.38	14.65	19.62	21.62	18.63
Average of T4		29.14	32.1	32.92	31.38	28.75	31.6	32.32	30.89	17.77	23.63	26.13	22.51	16.36	22.74	25.25	21.45
Average of N fertilizer rates		27.39	30.14	31.51	29.68	27.05	29.68	30.99	29.24	14.86	18.95	22.51	18.77	14.04	18.19	21.69	17.97
L.S.D. 5% of preceding winter field crops (W)					0.93				0.92				1.12				1.09
L.S.D. 5% of tillage systems (T)					0.62				0.61				1.49				1.45
L.S.D. 5% of N fertilizer rates (N)					0.73				0.72				1.24				1.21
L.S.D. 5% of W × T					N.S.				N.S.				2.45				2.38
L.S.D. of W × N					N.S.				N.S.				N.S.				N.S.
L.S.D. 5% of T × N					N.S.				N.S.				2.45				2.38
L.S.D. 5% of W × T × N					2.49				2.46				N.S.				N.S.
Recommended culture of maize					34.04				33.82				29.81				28.94

T_1= no tillage T_2= chisel plowT_3= moldboard plowT_4= moldboard + chisel plow
N_1 = 70 kg N/fed N_2 = 85 kg N/fed N_3 = 100 kg N/fed

by increasing N fertilizer rate from 70 to100 kg N/ fed. A similar trend in yield differences across N rates has been reported by **Lawrence *et al.* (2008)**. These results could be due to the highest N fertilizer rate increased the strength of physiological source such as ear leaf N content **(El-Shamy *et al*., 2015)** that contributed greatly in photosynthetic process during maize growth and development. Consequently, it is expected that growth and development of maize crop was affected negatively by decreasing N fertilizer rate which reflected on growth resources such as soil N and water and converted to the lowest growth regulators and dry matter accumulation **(Abdel-Wahab *et al*., 2016)**.

D – The interaction between the preceding winter field crops and tillage system

The interaction effects of the preceding winter field crops x tillage systems on maize yield and its attributes were significant for all the studied traits of maize crop except ear length, number of rows/ear and 100 – kernel weight in both seasons as shown in Table (3). Growing maize crop after berseem cutting with using T_4 system had the highest values of ear heigh , ear leaf area , ear diameter, number of grains /row and grain yield per plant and grain yield per fed , meanwhile the lowest values of these characters were recorded by growing maize crop after wheat harvest with T_1 system. These results could be attributed to the legume crop integrated positively with T_4 system to allow good aeration, rapid infiltration of water, easy plant root penetration, and good water drainage, as well as, providing good conditions for soil micro-organisms to thrive before maize sowing. These data reveal that there was effect *($P \leq 0.05$)* of the preceding winter field crops x tillage system on ear heigh , ear leaf area , ear diameter, number of grains /row and grain yield per plant and grain yield per fed **.**

E – The interaction between the preceding winter field crops and N fertilizer rates

The interaction effects of the preceding winter field crops x N fertilizer rates were not significant for all studied traits of maize crop in both seasons as shown in Table (3). These data show that each of these two factors act independently on all the studied traits of maize crop meaning that the preceding winter field crops responded similarly ($P > 0.05$) to N fertilizer rates.

F – The interaction between tillage system and N fertilizer rates

The interaction effects of tillage systems x N fertilizer rates on maize yield and its attributes were significant for all studied traits of maize crop except ear length, number of rows/ear and 100 – kernel weight in both seasons as shown in Table (3). The results show that the highest values of plant and ear heights, ear leaf area , ear diameter, number of grains /row and grain yields per plant and per fed were recorded by using T_4 system with 100 kg N/ fed, meanwhile the opposite trend was observed by using T_1 system with 70 kg N / fed. Similar results were observed by Mahdi Al-Kaisi* and David Kwaw- Mensah (2007) they found that tillage x N rate had a significant effect on plant N uptake. These data show that each of these factors act dependently on some traits of maize meaning that N fertilizer rates responded differently *($P \leq 0.05$)* to tillage system for plant and ear heights, ear leaf area , ear diameter, number of grains/ row, grain yield/plant and grain yield / fed. These results are in accordance with those obtained by Sangoi *et al.* (2007) who indicated that there was no significant effect of the interaction between soil tillage and N management system for the evaluated variables of maize crop during the three experimental years.

G – The interaction between the preceding winter field crops, tillage system and N fertilizer rates

The interaction effects of the preceding winter field crops x tillage systems x N fertilizer rates on maize yield and its attributes were not significant for all studied traits of maize crop except 100 – kernel weight in both seasons as shown in Table (3). Growing maize crop after berseem cutting with using T_4 system and 100 kg N/ fed recorded the highest 100 – kernel weight, meanwhile the lowest 100 – kernel weight was obtained by growing maize after wheat harvest with using T_1 system and 70 kg N/ fed. These data show that each of these factors act dependently on plant 100 – kernel weight meaning that the preceding winter field crops responded differently *($P \leq 0.05$)* to tillage systems and N fertilizer rates for 100 – kernel weight.

N use efficiency (NUE)

NUE values of 40–80 kg/kg are usual with values > 60 kg/kg for NUE being common in well–managed systems or at low levels of N use, or at low soil N supply (Doberman, 2007). Partial factor productivity (NUE–PFP) expressed as crop yield per unit of N applied (Roberts 2008) are indicative of the degree of economic and environmental efficiency in use of nutrient inputs.

In this study, NUE ranges from 112.65 to 239.58 kg grain yield per kg nutrient applied in the first season and from 178.17 to 285.60 kg grain yield per kg nutrient applied in the second season (Figure 1). Maize crop after wheat harvest and using T_1 system with 70 kg N / fed had the lowest ($P \leq 0.05$) NUE compared to that of the other treatments. These results could be due to legume crops especially berseem enhanced N soil availability (Table 1) that interacted with the highest N fertilizer rate of maize to compensate 16.6 % N loss under Egyptian conditions which reflected on values of NUE in the two growing seasons. N use efficiency may be affected by crop species, soil type and application rate of N fertilizer (Halverson and Wienhold, 2001). Each kilogram of N applied to the soil increased grain yield, depending on the N management system and growing season (Sangoi *et al.*, 2007).

Similar results were obtained by Constantin *et al.* (2010) who showed that the role of legume crops in the reduction of N losses from the cropping system by adsorbing it from the soil and transferring to the following crop.

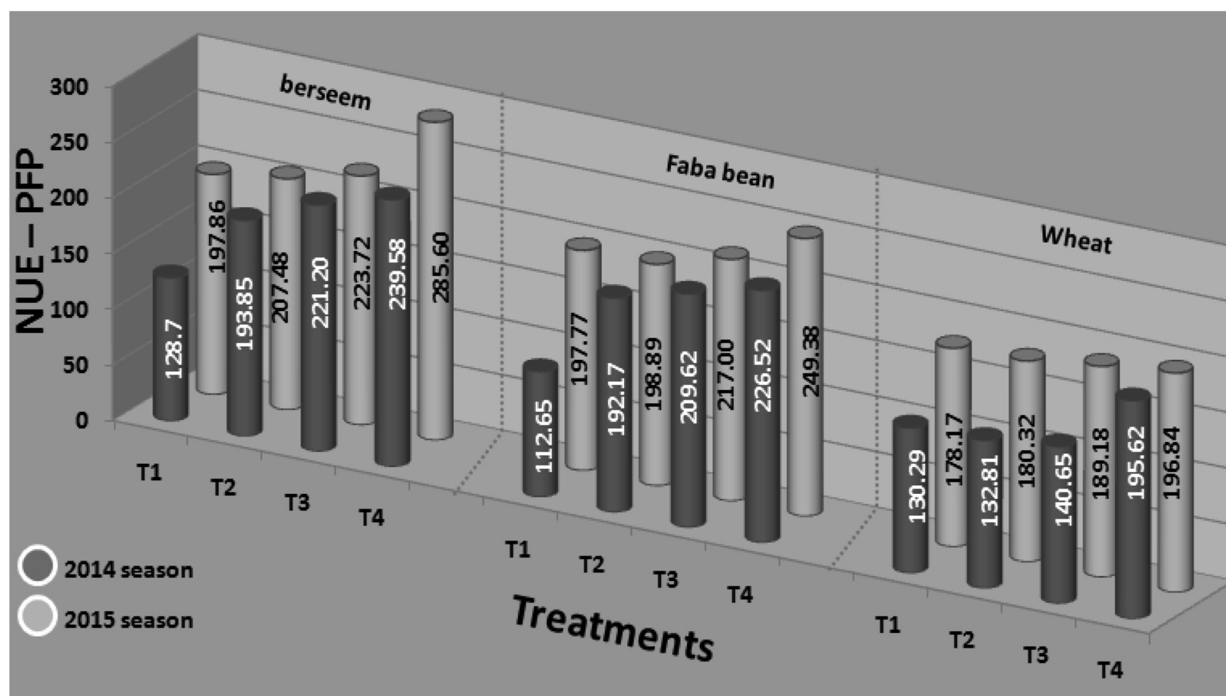

Figure (1). NUE as affected by the preceding winter field crops, tillage systems and N fertilizer rates in the two growing seasons (2013/2014 and 2014/2015).

Economic Return

The highest of total and net returns values of maize crop was obtained by growing maize crop after berseem cutting and using T_4 system with 83.3 % of the recommended mineral N fertilizer rate of maize compared to the other treatments (Table 4). Net returns of maize crop differed largely according to the preceding crop, tillage system and N fertilizer rate.

After berseem cutting, net return of maize crop ranged from L.E. 12816 / fed in the first season and L.E. 12522/ fed in the second season by using T_4 system with 100 kg N / fed to L.E. 7821/ fed in the first season and L.E. 7637/ fed in the second season by using T_1 system with 70 and 85 kg N / fed in the first and second respectively. After faba bean harvest, net return of maize crop was ranged from L.E. 5879/ fed in the first season and L.E. 5332/ fed in the second season by using T_4 system with 100 kg N/fed to L.E. 1530/ fed in the first season and L.E. 1083/ fed in the second season by using T_1 system with 70 kg N/ fed. After wheat harvest, net return of maize crop was ranged from L.E. 5665 / fed in the first season and L.E. 5190 / fed in the second season by using T_4 system , with 85 kg N /fed to L.E. 2710 / fed in the first season and L.E. 2279 / fed in the second season by using T_1 system with 70 kg N/ fed.

Also, differences between the highest and the lowest values of net return were L.E. 4995 and 4885/ fed for maize crop after berseem cutting in 2013/2014 and 2014/2015 seasons, respectively, L.E. 4349 and 4249/ fed for maize crop after faba bean harvest in 2013/2014 and 2014/2015 seasons, respectively, and L.E. 2959 and 2911/ fed for maize crop after wheat harvest in 2013/2014 and 2014/2015 seasons, respectively. On the other hand, there were gradual and consistent decreases in net return values with deceasing mineral N fertilizer rate from 100 to 70 kg N/ fed.

Obviously, using T_4 system after berseem cutting with application of 83.3% of the recommended N fertilizer for the successive maize crop resulted in high net return and could be recommended. These results are in parallel with those observed by Abdel-Wahab *et al.* (2016) who investigated that farmer's benefit was achieved by intercropping cowpea with maize that received 87.5% of the recommended mineral N fertilizer rate of maize after berseem cutting.

CONCLUSION

This study emphasizes there is a critical need for importance of the crop sequence with adequate nutrient management which did not lead to deterioration in soil health. This study can also be helpful in matching crops to the soil for which they are best suited. Using combined of moldboard plow + chisel plows tillage system after berseem cutting could be recommended to decrease 16.6% of the recommended mineral N fertilizer for the successive maize crop and achieve farmer's benefit.

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Table (4). Economic return as affected by the preceding winter field crops, tillage systems, N fertilizer rates and their interactions during the two growing seasons, 2013/2014 and 2014/2015

Treatments	Traits	Grain yield of maize (ardab/fed)								Yields of winter crops (forage yield of berseem 'ton/fed', seed yield of faba bean 'ardab/fed' and grain yield of wheat 'ardab/fed')							
		2013/2014 season				2014/2015 season				2013/2014 season				2014/2015 season			
		N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean
Berseem	T_1	13.52	13.79	21.2	16.17	12.86	13.24	20.46	15.52	29.56	29.56	29.56	29.56	27.81	27.81	27.81	27.81
	T_2	18.66	20.77	22.23	20.55	17.85	19.89	21.4	19.71	29.56	29.56	29.56	29.56	27.81	27.81	27.81	27.81
	T_3	17.69	23.7	23.97	21.78	17.03	22.9	23	20.97	29.56	29.56	29.56	29.56	27.81	27.81	27.81	27.81
	T_4	20.78	25.67	30.6	25.68	17.96	24.64	29.63	24.07	29.56	29.56	29.56	29.56	27.81	27.81	27.81	27.81
Mean		17.66	20.98	24.5	21.04	16.42	20.16	23.62	20.06	29.56	29.56	29.56	29.56	27.81	27.81	27.81	27.81
Faba bean	T_1	11.72	12.07	21.19	14.99	11.17	11.51	20.38	14.35	9.22	9.22	9.22	9.22	8.84	8.84	8.84	8.84
	T_2	13.09	20.59	21.31	18.33	12.5	19.72	20.57	17.59	9.22	9.22	9.22	9.22	8.84	8.84	8.84	8.84
	T_3	16.64	22.46	23.25	20.78	16.01	21.53	22.46	20	9.22	9.22	9.22	9.22	8.84	8.84	8.84	8.84
	T_4	17.34	24.27	26.72	22.77	16.64	23.38	25.84	21.95	9.22	9.22	9.22	9.22	8.84	8.84	8.84	8.84
Mean		14.69	19.84	23.11	19.21	14.08	19.03	22.31	18.47	9.22	9.22	9.22	9.22	8.84	8.84	8.84	8.84
Wheat	T_1	10.87	13.96	19.09	14.64	10.29	13.28	18.34	13.97	21.17	21.17	21.17	21.17	20.55	20.55	20.55	20.55
	T_2	11.42	14.23	19.32	14.99	10.84	13.61	18.63	14.36	21.17	21.17	21.17	21.17	20.55	20.55	20.55	20.55
	T_3	11.43	15.07	20.27	15.59	10.92	14.43	19.4	14.91	21.17	21.17	21.17	21.17	20.55	20.55	20.55	20.55
	T_4	15.2	20.96	21.09	19.08	14.49	20.22	20.29	18.33	21.17	21.17	21.17	21.17	20.55	20.55	20.55	20.55
Mean		12.23	16.05	19.94	16.07	11.63	15.38	19.16	15.39	21.17	21.17	21.17	21.17	20.55	20.55	20.55	20.55
Recommended culture of maize					29.81				28.94				29.56				27.81

T_1= no tillage T_2= chisel plow T_3= moldboard plow T_4= moldboard + chisel plow

Table (4). Continued.

Treatments	Traits	Total return/fad/year (L.E.)								Net return/fad/year (L.E.)							
		2013/2014 season				2014/2015 season				2013/2014 season				2014/2015 season			
		N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean
Berseem	T_1	15244	15326	17571	16047	15044	15159	17347	15850	7837	7821	9968	8542	7637	7654	9744	8345
	T_2	16801	17441	17883	17374	16556	17174	17632	17120	9394	9936	10280	9870	9149	9669	10029	9615
	T_3	16508	18329	18410	17747	16308	18086	18117	17501	9101	10824	10807	10244	8901	10581	10514	9998
	T_4	17444	18926	20419	18929	16589	18613	20125	18441	10037	11421	12816	11424	9182	11108	12522	10937
Mean		16499	17505	18572	17523	16123	17256	18305	17226	9091	9999	10968	10020	8716	9751	10701	9723
Faba bean	T_1	10373	10480	13243	11364	9926	10029	12716	10889	1530	1539	4204	2424	1083	1088	3677	1949
	T_2	10789	13061	13279	12376	10329	12516	12774	11871	1946	4120	4240	3435	1486	3575	3735	2932
	T_3	11864	13628	13867	13119	11392	13065	13346	12601	3021	4687	4828	4178	2549	4124	4307	3660
	T_4	12076	14176	14918	13722	11583	13625	14371	13192	3233	5235	5879	4782	2740	4684	5332	4252
Mean		11274	12834	13825	12643	10808	12308	13302	12138	2430	3893	4786	3704	1964	3366	4262	3198
Wheat	T_1	11994	12930	14485	13136	11563	12469	14003	12678	2710	3548	5005	3754	2279	3087	4523	3296
	T_2	12161	13012	14554	13242	11730	12569	14090	12797	2877	3630	5074	3860	2446	3187	4610	3414
	T_3	12164	13267	14842	13424	11754	12818	14324	12963	2880	3885	5362	4042	2470	3436	4844	3583
	T_4	13306	15051	15091	14482	12836	14572	14593	14000	4022	5669	5611	5100	3552	5190	5113	4618
Mean		12407	13564	14743	13570	11970	13106	14252	13109	3122	4182	5262	4189	2685	3724	4771	3727
Recommended culture of maize					20180				19916				11515				11251

Prices of main products are that of 2014: L.E. 400 for ardab of wheat, L.E. 3700 for one cut of berseem, L.E. 720 for ardab of faba bean and L.E. 290 for ardab of maize.

Prices of main products are that of 2015: L.E. 411 for ardab of wheat, L.E. 3716 for one cut of berseem, L.E. 740 for ardab of faba bean and L.E. 303 for ardab of maize.

T_1= no tillage T2= chisel plowT3= moldboard plowT4= moldboard + chisel plow N1 = 70 kg N/fed N2 = 85 kg N/fed N3 = 100 kg N/fed

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تأثير المحصول الشتوى السابق ونظم الخدمة ومعدلات السماد الأزوتى على محصول الذرة الشامية ومكوناتة

محمد خالد حمدنى عامر

قسم بحوث التكثيف المحصولى- معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية.

أجريت هذة التجربة بمحطة البحوث الزراعية بالجميزة، محافظة الغربية موسمى٢٠١٣ / ٢٠١٤ و ٢٠١٤ / ٢٠١٥ بهدف دراسة تأثير المحاصيل الشتوية السابقة و نظم الخدمة و معدلات السماد الازوتى على محصول الذرة الشامية (هجين ثلاثى أصفر ٣٥٣) ومكوناتة اشتملت التجربة على ٣٦ معاملة عبارة عن التوافيق بين ثلاث محاصيل سابقة هى البرسيم المسقاوى (صنف هلالى) و الفول البلدى (صنف جيزة ٧١٦) و القمح (صنف جميزة ١١) وأربع نظم حرث مختلفة و هى بدون حرث (T_1) الحرث بالمحراث الحفار(T_2) الحرث بالمحراث القلاب (T_3) الحرث بالمحراث الحفار متبوعا بالمحراث القلاب (T_4) و ثلاث معدلات سماد أزوتى هى (٧٠ و ٨٥ و١٠٠ كجم N/ فدان).

أستخدم تصميم القطع المنشقة مرتين في ثلاث مكررات حيث وزعت المحاصيل السابقة فى القطع الرئيسية و معاملات الحرث فى القطع الشقية و معاملات السماد الازوتى فى القطع تحت الشقية و كانت اهم النتائج المتحصل عليها فيما يلى :

- أظهرت المحاصيل الشتوية السابقة تأثير معنوى على محصول النبات الفردى – محصول الحبوب للفدان – ووزن المائة حبة فى كلا الموسمين حيث كانت أعلى قيم تحصل عليها عند زراعة الذرة الشامية بعد البرسيم المسقاوى يليها زراعة الذرة الشامية بعد الفول البلدى و أقلها عند زراعة الذرة الشامية بعد القمح.
- أثرت نظم الخدمة معنويا على ارتفاع النبات – ارتفاع الكوز – طول الكوز – قطر الكوز –محصول النبات الفردى – محصول الفدان من الذرة الشامية مقارنة بمعاملة عدم الخدمة.
- أثرت معدلات السماد الازوتى معنويا على ارتفاع النبات – ارتفاع الكوز – طول الكوز – قطر الكوز – محصول النبات الفردى – محصول الفدان من الذرة الشامية حيث ازدادت قيم هذه الصفات بزيادة معدلات السماد الازوتى من ٧٠ الى ١٠٠ كجم نيتروجين / فدان و قد تأثر محصول حبوب الذرة الشامية ومكوناته بمعظم التفاعلات بين عوامل الدراسة إلا أن وزن الـ ١٠٠ حبة كانت الصفة الوحيدة التى تأثرت بالتفاعل بين جميع عوامل الدراسة .
- تراوحت قيم كفاءة إستخدام النيتروجين من ٦٥,١١٢ إلى ٥٨,٢٣٩ كجم حبوب لكل كجم نيتروجين مضاف فى الموسم الأول ومن ١٧,١٧٨ إلى ٦٠,٢٨٥ كجم حبوب لكل كجم نيتروجين مضاف فى الموسم الثانى مما أدى إلى زيادات كبيرة في محصول حبوب الذرة ومكوناتها.
- أدى التفاعل بين المحاصيل الشتوية السابقة و نظم الخدمة و معدلات السماد الازوتى الى الحصول على أعلى انتاجية من الفدان عند زراعة الذرة الشامية بعد البرسيم المسقاوى أو بعد الفول البلدى باستخدام المحراث الحفار متبوعا بالمحراث القلاب إلى نقص التسميد النيتروجينى للذرة الشامية بنسبة ٦,١٦٪ من السماد النيتروجينى الموصى به للذرة الشامية مع زيادة العائد النقدى للمزارع.

الكلمات الدالة: محصول الذرة الشامية – المحاصيل الحقلية الشتوية – نظم الخدمة – كفاءة إستخدام النيتروجين – العائد النقدى.

Effect of Pre-harvest Treatments (Gibberellic Acid and Biofertilizers) and Postharvest Treatments on Fruit Characteristics of Egypt Lime (*Citrus aurantifolia*) During Cold Storage

Gihan M. Ali*, Hamdy M. Marzouk, Farouk M. Mostafa and Ahmed H. Mansour
Horticulture Research Institute-Faculty of Agriculture, Assiut University*

ABSTRACT

The study was conducted to investigate the effect of organic fertilization, biological fertilization and spraying gibberellic acid on the properties and behavior of the fruits of Egypt lime through some cold storage treatments during the 2003-2004 seasons. Trees were treated with one of the following treatments during growth season: A) organic fertilizer as control trees, B) Organic fertilizer with biofertilizer phosphorine, and C) Organic fertilizer with biofertilizer phosphorine after fruit set plus gibberellic acid (20 ppm)sprayed 2 weeks before harvest date. The fruits of each field treatments were treated with one of following treatments: 1) Packaging fruits in polyethylene bags perforated, 2) dipping fruits in GA_3 50 ppm,3) dipping fruits in Sida film 5%,4) dipping fruits in (GA_3 50 ppm, with Sida film 5%),5) wrapped in Silk paper,6) dipping the fruits in (GA_3 50 ppm) then wrapping ,7) dipping the fruits in a (SIDA film 5%) then wrapping and 8) dipping in (GA_3 50 ppm +Sida film 5%) then wrap.

The results showed that fruit treated with phosphorine +GA_3 during growth season induced the highest weight, volume, rag weight. Apply phosphorine plus spray GA_3 20 ppm during the growing season as well as dipping fruits in Sida film5% + GA_3 50 ppm were more effective in decreasing weight loss%, gave the lowest percentage of unmarketable fruits weight, resulted in the highest level of juice weight %, the highest level of TSS%, the lowest level of TA%, the highest value in vitamin C content in juice of stored fruit, keep good fruit quality and prolong cold storage period.

Key words:

INTRODUCTION

Citrus is the most important fruit crop in Egypt, as far as its acreage, production and exporting potential are concerned. The economic value put citrus fruit on the top of all other important fruit crops in Egypt. Egyptian area of lemons and lime is equal to 0.0211% of world area. On the other hand, Egyptian production of lemons and limes is equal to 1.20% of world production of lemons and limes as reported by the F A O (2004). Egyptian lime is considered as the favorite fruit crop in Egypt and it's considered as one of the important source of citric acid. They can grow under different environmental conditions and soil types. One of the important tools to increase its yield is the phosphorus fertilization. Egyptian soils having alkaline pH are low in their available P that approximately 90-95% of P occurring as unavailable form; it is thought that P biofertilizers are of the almost important for plant production and soil fertility as they improve the biological, physical and chemical properties of soils (Abd el- Rahman, 2002).

Growth regulators were used with film- forming or without wax emulsion to eliminate decay and to improve quality of citrus fruits during cold storage (Farag ,2001). El-Hammady *et al.*(2000)found that all preharvest treatments with GA_3 were effective in reducing weight loss% compared to control Balady mandarin fruits at 5 ^{0}C. Fruit wrapping decreased weight loss during storage period on Orablanco fruit (Citrus grandis L.xC. paradisimacr) (Rodov *et al.*, 2001).Therefore, the objectives of this study were comparing the effectiveness of some field treatments applied during growth seasons, i.e. farmyard manure, farmyard manure+phosphorine and farmyard manure+ phosphorine +GA_3. As well as some storage treatments wrapping, GA_3, Sida film with or without GA_3 in order to extend storage period and enhance physical and chemical properties of Egyptian Lime fruits at harvest and during cold storage.

MATERIALS AND METHODS

This study was carried out on Egyptian Lime grown at the Experimental Orchard, Faculty of Agriculture, Assuit University in 2003 and 2004 seasons. Trees were seedy and planted at 3.5x3.5 meters a part. Trees were treated with one of the following treatments during growth season: organic and bio-fertilizers were applied to the selected trees as follows:

1- Organic fertilizer (Farmyard manure) as control in the middle of December (50 kg /tree).

2- Organic fertilizer (Farmyard manure) + bio-fertilizer phosphorine (phosphate dissolving bacteria, *Bacillus megaterium*) (100 g/ tree) after fruit set.

3- Organic fertilizer (Farmyard manure)+ bio-fertilizer (phosphorine) after fruit set +GA_3 (20 ppm) sprayed 2 weeks before harvest date. The fruits were handpicked and carefully were brought soon to Postharvest Laboratory, Agriculture Research Station when color of fruit was yellowish green skin, and juice percentage fruit was approximately 37%-40% as an indicator of commercial fruit harvest standard. Mature fruit samples were taken for physical and chemical characteristics determination at harvest time (Weight, volume, rag weight %, juice weight, TSS %, TA %, TSS/TA ratio and Vitamin C). The other samples

were divided into 8 equal groups; each of these was treated with one of the following treatment:

a- Washing under tab water and then packing in perforated polyethylene bags

b- Dipping for 5 minutes in GA_3(50ppm) and then were packing.

c- Dipping for 5 minutes in Sida film 5% (wetting agent and surfactant compound, registered under No.(072) and then were packing

d- Dipping for 5 minutes in GA_3 (50ppm) with Sida film 5% and then were packed and stored

e- Washing and wrapping in silk paper then were packed perforated polyethylene bags

f- Dipping for 5 minutes in GA_3 (50ppm) and were wrapped and packed.

g- Dipping for 5 minutes in Sida film 5% and were wrapping and packing.

h- Dipping for 5 minutes in GA_3 (50ppm) with Sida film 5% and then wrapping and packing.

All the groups were stored at 7-10°C and 80-85% R.H and the following determination were carried out biweekly interval period through cold storage:

1- physical characteristics :
- 1-1- Fruit weight loss percentage
- 1-2- Fruit number unmarketable percentage
- 1-3- Juice weight percentage/fruit

2- Chemical fruits characteristics:
- 2-1 Total soluble solids percentage (TSS): was determined by a hand refractometer
- 2-2- Titratable acidity according to the A.O.A.C. (1985)
- 2-3- Total soluble solids/ acidity ratio (TSS/Acid ratio)
- 2-4- Ascobic acid (V.C.) content according to the A.O.A.C. (1985)

Statistical Analysis:

Means were compared by the L.S.D. value at 5% level (Snedecor and Cochran,1990).

RESULTS

1- Effect of pre-harvest treatments of organic fertilizer, phosphorine plus GA_3 on fruit physical characteristics at harvest time:

1-1- Weight and volume of mature limes:

As shown in Table (A) it is clear that both of phosphorine or phosphorine plus GA_3 (20 ppm) applied at 2 weeks pre-anticipated harvest date resulted in significant increase of fruit weight and volume in both seasons. This positive influence of phosphorine might be due to improving uptake of nutrients specially phosphate. The positive effect of GA_3 could be attributed to enhancement effects of GA_3 in cell division and enlargement at pre-mature stage. These results are in harmony with those found by Tawfiek and Gamal(2000) and Gamal and Ragab(2003) on Balady mandarin.

1-2- Percentage of rag weight and juice weight:

Appling phosphorine to organically fertilized lime trees resulted in significant decrease in rag weight %. On the other hand, spraying GA_3 (20ppm) 2 week at pre-anticipated harvest time induced significant increase in rag weight%. It could be observed that phosphorine caused an improve in juice weight %. While, spraying GA_3 induced reduction in juice weight % of mature fruits. These effects of GA_3 could be due to delaying maturity degree of lime and decreasing juice %. These results are in harmony with those found by Tawfiek and Gamal (2000) and Attia *et al.*(2002).

2- Effect of pre-harvest treatments of organic fertilizer, phosphorine plus GA3 on fruit chemical characteristics at harvest time:

2-1- Total soluble solids% (TSS %)

It could be noticed that treated fruits with phosphorine induced the highest level of TSS% in first season, while organic fertilizer gave the highest level of TSS% in second season (Table A). These results are in agreement with those reported by Tawfiek and Gamal (2000) and Gamal and Ragab(2003)on balady mandarin

2-2- Titratable Acidity% (TA %)

Data in Table (A) showed that fruits treated with phosphorine plus GA_3 during growth season induced the highest level of TA% in 2003 while organic fertilizer gave highest level in 2004 season, these results are in agreement with those found by Gamal and Ragab(2003).

2-3- TSS/TA ratio

As shown in Table (A) treated fruits with phosphorine induced the highest level of TSS/TS ratio in both season. the obtained results are in agreement with those of Ibrahim *et al.* (1994)they found that the application of GA_3 increased TSS/acid ratio on Washington Navel orange.

2-4- Vitamin C content in fruit juice

During the first season it was noticed that GA3 gave the highest level of vitamin C, while during the second season, it was found that organic fertilizer alone gave the highest level. These results are in agreement with those reported by Gamal and Ragab (2003) on Balady mandarin.

3- Effect of storage treatments on physical characteristics of Egyptian Lime fruits treated with phosphorine or phosphorine plus GA_3 applied during growth season:

3-1 Fruit weight loss percentage

Fruit weight loss % increased gradually as storage period advanced, data presented in Table (1) indicated that wrapping fruits gave the least percentage of loss in comparison with unwrapped fruits (control fruits) or unwrapped fruits treated with GA_3 or Sida film.

Table (A): Effect of pre-harvest treatments with organic fertilizer phosphorine and phosphorine plus GA_3 (20ppm) on physical and chemical characteristics of Egypt Lime fruits in 2003 and 2004 seasons

Season	Field treatments	Fruit weight (g)	Fruit volume (cm_3)	Reg weight%	Juice weight%	TSS%	Titrable acidity%	TSS/TA ratio	V.C in juice mg/100 ml
2003	Organic fertilizer	20.30^{C}	19.50^{C}	60.60^{B}	39.40^{B}	9.20^{B}	7.70^{B}	1.19^{B}	27.63^{B}
	Organic fertilizer + phosphorine	25.68^{B}	25.33^{B}	59.04^{C}	40.06^{A}	9.27^{A}	7.63^{C}	1.21^{A}	25.18^{C}
	Organic fertilizer + phosphorine + GA3 (20 ppm)	27.76^{A}	27.00^{A}	61.85^{A}	38.15^{C}	9.13^{C}	7.78^{A}	1.17^{B}	35.41^{A}
2004	Organic fertilizer	21.95^{C}	21.00^{C}	58.18^{B}	41.82^{B}	9.67^{A}	9.55^{A}	1.01^{B}	33.53^{A}
	Organic fertilizer + phosphorine	26.56^{B}	26.20^{B}	56.72^{C}	43.28^{A}	9.00^{C}	9.36^{B}	1.07^{A}	28.77^{C}
	Organic fertilizer + phosphorine + GA3 (20 ppm)	30.46^{A}	29.00^{A}	58.66^{A}	41.34^{C}	9.33^{B}	9.03^{C}	1.03^{B}	29.47^{B}

Mean separation by L.S.D at 0.0

Table (1): Average changes in weight loss% during cold storage of Egyptian Lime fruits treated with phosphorine and phosphorine+GA_3 (20ppm)applied through growth season as affected by storage treatments in 2003 and 2004 seasons

Storage treatment	Average of storage periods							
	2003				2004			
	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments
1- unwrapped fruits(control)	19.95	18.1	19.22	19.09 B	19.26	18.48	19	18.91 C
2-unwrapped+GA3	22.58	20.38	20.17	21.04 A	24.09	20.83	21.19	22.04 A
3-unwrapped+Sida film	18.53	18.83	18.6	18.65 C	19.23	19.8	19.24	19.42 B
4-unwrapped+GA3+Sida film	18.2	17.84	17.86	17.97 D	18.84	18.05	17.85	18.25 D
5-wrapped fruits	16.67	17.49	14.26	16.14 E	18.9	16.76	14.37	16.69 E
6-wrapped+GA3	14.27	12.93	14.26	13.82 F	14.67	12.8	14.22	13.86 F
7-wrapped+Sida film	14.01	14.59	10.46	13.02 G	15.84	13.92	10.67	13.48 G
8-wrapped+GA3+Sida film	13.67	10.13	11.57	11.79 H	13.85	10.82	11.77	12.15 H
Average of storage periods	17.24A	16.29 B	15.80 C	16.44	18.09 A	16.44 B	16.05 C	16.86

Mean separation by L.S.D at 0.05

It was found that wrapping fruits treated with GA_3 plus Sida film was more effective in decreasing weight loss % than treating with GA_3 or Sida film alone. Stored fruits treated with phosphorine plus GA_3 during growth season resulted in the lowest level of weight loss % (15.80, 16.05%) followed by phosphorine (16.29, 16.44%), while untreated control fruits gave the highest level of weight loss % (17.24, 18.09%). Furthermore, unwrapped fruits treated with GA_3(50ppm pre-storing) gave the highest level of weight loss% (20.04, 22.04%), while wrapped fruits treated with GA_3 (50ppm) plus Sida film (5%pre-storing)resulted in the lowest level of weight loss % (11.79,12.15%)under the condition of cold storage in two season, respectively.

These obtained results are in harmony with those found by Attia *et al.* (1996) on Banzahir and Houssini Lime, Ladaniya *et al.* (1997) on Nagpur Mandarin ,Subedi(1999)on Mandarin and Oranges, Sonkar and Ladaniya (1999)on Mandarin ,Tariq *et al.* (2002) on Banzahir Limes.

3-2- Unmarketable fruit number percentage:

It could be noticed that unmarketable fruit number increased gradually as storage period advanced. Unwrapped fruits and unwrapped fruits plus Sida film gave the highest percentage of unmarketable fruit number%. Table (2), wrapped fruits +Sida film 5%+GA3 (50ppm pre-storage) gave the lowest unmarketable fruit number (11.72, 14.86%). Fruits treated with phosphorine plus GA3 (20ppm) during growth season resulted in the lowest values of unmarketable fruit number (8.12, 8.60%), while treated fruits with phosphorine gave the highest level of unmarketable fruit number (26.85,26.20%) in two season, respectively. These results are in harmony with those found by El-Mughrabi(1999) on Baladi orange, El-Hammady *et al.* (2000) on Balady Mandarin, and El-Helaly (2002) on Banzahir Limes.

3-3- Fruit Juice weight%:

Fruit Juice weight percentage gradually decreased with prolonged storage duration. It could be noticed that fruit treated with phosphorine during growth season resulted in the highest level of juice weight %,(40.86, 42.99%), table (3). It was also found that wrapped fruit gave the highest value of juice weight %(40.71, 42.71%). Wrapped fruits treated with phosphorine +GA3 (50ppm pre-storing) plus Sida film resulted in the highest level of juice weight %(43.67, 46.33%) in the two studied seasons. These obtained results are coincided with those found by El-Mughrabi (1999) on Balady orange and Farag(2001) on Navel orange.

4- Effect of some storage treatments on chemical characteristics of Egyptian lime fruits treated with phosphorine or phosphorine plus GA_3 applied during growth season

4-1- Total Soluble Solids% (TSS %)

TSS% gradually increased with prolonging storage period. However, it was clear that storage treatments induced slight differences in TSS% in fruit juice under the conditions of cold storage Data presented in Table (4) indicated that wrapping fruits treated with GA_3 (50ppm pre-storing) plus Sida film resulted in the highest values of TSS% (10.49,10.41%) in two season, respectively. Moreover, fruits treated with phosphorine during growth season induced the highest level of TSS %(10.23%) in juice of pre-stored fruits during cold storage in 2003 season, while fruits treated with phosphorine plus GA_3 (20ppm) during growth season produced the highest level of TSS %(10.58%) in juice of stored fruits in 2004 season. These obtained findings are in harmony with those reported by Farag (2001) who reported that TSS% on Navel oranges treated with GA did not differ greatly than those of untreated ones. Meanwhile, El-Helaly (2002) on Banzahir Limes obtained that waxed fruits had lower values of TSS%.

4-2-Titratable acidity% (TA %) in fruit juice:

All storage treatments reduced TA% in Lime juice, and the reduction was associated with prolonging storage period duration. Table (5) observed that treated fruits with phosphorine plus GA_3 (20ppm) produced the lowest level of TA% (6.65, 8.20%) in juice of stored fruits during the two studies season. Moreover, it could be noticed that both of unwrapped control (6.90, 9.11%) or unwrapped fruits treated with Sida film resulted the highest level of TA %(7.36, 8.64%) in juice in two season respectively. While, unwrapped fruit treated with GA_3 (50ppm pre- storage) gave the lowest level in TA% (6.75%) in 2003. Also, wrapped fruits treated with GA_3 (50 ppm pre-storage) gave the lowest level of TA %(8.37%) in juice of stored fruit during season 2004. Those results are in partical agreement with those found by El-Mughrabi(1999) on Baladi orange and El-Helaly (2002) on Banzahir Limes fruits, they reported that waxed fruit treated with or without GA , generally had the least percentage of citric acid than those of the other applied treatments.

4-3- Total soluble solids/ titratable acidity ratio (TSS/TA ratio) in fruit juice:

TSS/TA ratio increased gradually as storage period advanced. As shown in Table (6) data indicated that fruits treated with phosphorine plus GA_3 (20ppm) gave the highest value of TSS/TA ratio (1.51, 1.29%) in juice. Wrapped fruits treated with or without GA_3 + Sida film induced the highest level of TSS/TA (1.49, 1.25%) ratio, meanwhile, unwrapped fruits (control) gave the least value (1.42, 1.19%) in both seasons, respectively. These obtained results are in harmony with those findings reported by Farag (2001)

4-4- Vitamin C content in fruit juice:

Data presented in Table (7) indicated that phosphorine plus GA_3 (20ppm) induced slight differences in response to effect of some storage treatment during cold storage in the two studied season. It could be noticed that

Table (2): Average changes in unmarketable fruits number% during cold storage of Egyptian Lime fruit treated with phosphorine and phosphorine + GA_3 (20ppm)applied through growth season as affected by storage treatments in 2003 and 2004 seasons

Storage treatment	Average of storage periods							
	2003				2004			
	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments
1- unwrapped fruits(control)	16.42	34.33	27.32	26.02^{A}	19.78	36.83	27.32	27.98^{B}
2-unwrapped+GA3	11.88	32.84	29.91	24.88^{C}	14.67	34.78	30.76	26.74^{C}
3-unwrapped+Sida film	14.71	31.08	30.39	25.39^{B}	17.18	34.33	33.48	28.33^{A}
4-unwrapped+GA3+Sida film	11.46	30.36	31.77	24.53^{C}	13.44	31.73	32.8	25.99^{D}
5-wrapped fruits	13.17	25.62	10.71	16.68^{E}	16.08	22.94	11.74	16.92^{F}
6-wrapped+GA3	15.48	2305	11.86	16.80^{E}	17.07	19.2	12.98	16.42^{G}
7-wrapped+Sida film	22.03	25.98	11.13	19.71^{D}	27.16	22.94	11.63	21.24^{E}
8-wrapped+GA3+Sida film	15.48	11.56	8.12	11.72^{F}	19.55	16.44	8.6	14.86^{H}
Average of storage periods	15.15^{C}	26.85^{A}	20.15^{B}	20.72	18.37^{C}	26.20^{A}	21.16^{B}	21.91

Mean separation by L.S.D at 0.05

Table (3): Average changes in juice weight % during cold storage of Egyptian Lime fruits treated with phosphorine and phosphorine+GA_3 (20ppm) applied through growth season as affected by storage treatments in 2003 and 2004 seasons

Storage treatment	Average of storage periods							
	2003				2004			
	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments
1- unwrapped fruits(control)	36.3	37.98	38.16	37.48 D	40.73	41	40.09	40.61 C
2-unwrapped+GA3	37.32	39.19	38.24	38.25 B	42.88	41.1	39.51	41.16 B
3-unwrapped+Sida film	33.29	38.79	36.58	36.22 F	339.54	41.15	37.19	39.29 E
4-unwrapped+GA3+Sida film	35.52	38.25	35.32	36.36 F	41.97	40.67	35.88	39.51 D
5-wrapped fruits	40.81	45.52	35.79	40.71 A	44.85	44.58	38.71	42.71 A
6-wrapped+GA3	37.63	40.97	34.96	37.85 C	41.5	43.42	36.69	40.63 C
7-wrapped+Sida film	34.8	42.47	33.6	36.96 E	39.19	45.69	33.32	39.40 D
8-wrapped+GA3+Sida film	38.63	43.67	30.67	37.66 C	42.08	46.33	33.03	40.48 C
Average of storage periods	36.79 B	40.86 A	35.42 C	37.69	41.59 B	42.99 A	36.84 C	40.47

Mean separation by L.S.D at 0.05

Table (4): Average changes in TSS% during cold storage of Egyptian Lime fruits treated with phosphorine and phosphorine+GA_3 (20ppm) applied through growth season as affected by storage treatments in 2003 and 2004 seasons

Storage treatment	Average of storage periods							
	2003				2004			
	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments
1- unwrapped fruits(control)	9.84	9.72	9.91	9.82^{D}	10.57	10.03	10.6	10.40^{A}
2-unwrapped+GA3	9.95	10.4	9.76	10.04^{C}	10.4	10.03	10.5	10.31^{B}
3-unwrapped+Sida film	9.81	10.63	10.6	10.35^{B}	10.47	10	10.8	10.42^{A}
4-unwrapped+GA3+Sida film	9.96	10	9.73	9.89^{D}	10.67	10.2	10.57	10.48^{A}
5-wrapped fruits	10.03	9.98	10	9.97^{D}	10.37	10.17	10.37	10.30^{B}
6-wrapped+GA3	10.24	10.11	10.12	10.16^{C}	10.53	9.93	10.2	10.22^{C}
7-wrapped+Sida film	10.27	10.2	10.11	10.19^{C}	10.4	9.83	10.73	10.32^{B}
8-wrapped+GA3+Sida film	10.12	10.85	10.52	10.49^{A}	10.63	9.77	10.83	10.41^{A}
Average of storage periods	10.03^{B}	10.23^{A}	10.09^{B}	10.11	10.51^{A}	9.99^{B}	10.58^{A}	10.36

Mean separation by L.S.D at 0.05

Table (5): Average changes in TA% during cold storage of Egyptian Lime fruits treated with phosphorine and phosphorine+GA_3 (20ppm) applied through growth season as affected by storage treatments in 2003 and 2004 seasons

Storage treatment	Average of storage periods							
	2003				2004			
	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments
1- unwrapped fruits(control)	6.9	7.72	6.18	6.86^{B}	9.11	8.64	8.61	8.78^{A}
2-unwrapped+GA3	6.63	7.23	6.39	6.75^{C}	8.59	8.3	8.51	8.47^{C}
3-unwrapped+Sida film	7.01	7.01	7.36	7.13^{A}	8.49	8.59	8.64	8.57^{B}
4-unwrapped+GA3+Sida film	6.47	7.14	6.94	6.85^{B}	8.72	8.14	8.34	8.40^{D}
5-wrapped fruits	6.76	7.46	6.23	6.82^{B}	8.65	8.68	8.24	8.52^{B}
6-wrapped+GA3	6.74	7.32	6.51	6.85^{B}	8.66	8.84	7.59	8.37^{E}
7-wrapped+Sida film	6.92	7.01	6.65	6.86^{B}	8.64	8.63	7.95	8.41^{D}
8-wrapped+GA3+Sida film	6.55	7.15	6.74	6.81^{B}	8.87	8.73	7.7	8.43^{C}
Average of storage periods	6.75^{B}	7.25^{A}	6.65^{C}	6.88	8.72^{A}	8.68^{B}	8.20^{C}	8.53

Mean separation by L.S.D at 0.05

Table (6): Average changes in TSS/TA% during cold storage of Egyptian Lime fruits treated with phosphorine and phosphorine+GA_3 (20ppm) applied through growth season as affected by storage treatments in 2003 and 2004 seasons

Storage treatment	Average of storage periods							
	2003				2004			
	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments
1- unwrapped fruits(control)	1.43	1.26	1.57	1.42^{D}	1.16	1.19	1.23	1.19^{D}
2-unwrapped+GA3	1.5	1.44	1.48	1.47^{B}	1.21	1.24	1.25	1.23^{B}
3-unwrapped+Sida film	1.4	1.52	1.46	1.46^{B}	1.23	1.14	1.2	1.19^{C}
4-unwrapped+GA3+Sida film	1.54	1.4	1.43	1.45^{C}	1.22	1.29	1.27	1.26^{A}
5-wrapped fruits	1.48	1.33	1.51	1.44^{C}	1.2	1.2	1.28	1.22^{C}
6-wrapped+GA3	1.52	1.39	1.52	1.47^{B}	1.21	1.17	1.25	1.21^{C}
7-wrapped+Sida film	1.48	1.47	1.54	1.49^{A}	1.2	1.18	1.37	1.25^{A}
8-wrapped+GA3+Sida film	1.44	1.47	1.53	1.49^{A}	1.2	1.15	1.42	1.25^{A}
Average of storage periods	1.47^{B}	1.41^{C}	1.51^{A}	1.47	1.20^{B}	1.20^{B}	1.29^{A}	1.22

Mean separation by L.S.D at 0.05

Table (7): Average changes in vitamin C (mg Ascorbic acid/100ml juice)during cold storage of Egyptian Lime of fruits treated with phosphorine and phosphorine+GA3(20ppm)applied through growth season as affected by storage treatments in 2003 and 2004 seasons

Storage treatment	Average of storage periods							
	2003				2004			
	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments	Control	Phosphorine	Phosphorine + GA_3	Average of storage treatments
1- unwrapped fruits(control)	30.89	31.34	28.98	30.43^{B}	36.17	29.41	32.15	32.58^{B}
2-unwrapped+GA3	27.69	29.11	28.86	28.55^{D}	33.88	26.48	28.13	29.49^{E}
3-unwrapped+Sida film	26.15	29.93	38.08	30.39^{B}	32.33	25.26	30.96	29.52^{E}
4-unwrapped+GA3+Sida film	32.76	31.41	32.38	32.18^{A}	33.83	24.78	33.44	30.68^{D}
5-wrapped fruits	28.13	31.85	31.12	30.37^{B}	37.28	33.71	28.8	33.26^{A}
6-wrapped+GA3	28.82	32.35	29.96	30.38^{B}	33.63	30.34	30.87	31.61^{C}
7-wrapped+Sida film	30.6	27.18	30.54	29.44^{C}	31.35	27.69	27.03	28.69^{F}
8-wrapped+GA3+Sida film	28.61	32.06	27.89	29.52^{C}	32.37	28.69	27.84	29.63^{E}
Average of storage periods	29.21^{B}	30.66^{A}	30.60^{A}	30.16	33.85^{A}	28.29^{C}	29.90^{B}	30.68

Mean separation by L.S.D at 0.05

unwrapped fruits treated with GA_3 (50ppm pre-storage) plus Sida film gave the highest value in vitamin C content in juice of stored fruit (32.76 mg/ 100ml), followed by unwrapped content fruits (30.89 mg/ 100ml), while unwrapped fruits treated with Sida film produced the lowest value of V.C in season 2003. On the other hand, wrapped fruits gave the highest level of V.C (37.28 mg/ 100ml), followed by unwrapped content fruits, while wrapped fruits treated with Sida film induced the least value of V.C content (31.35 mg/100ml) in fruit juice stored during the second season.

These obtained findings of this study are in agreement with those reported by El- Maghrabi (1999) on Balady orange who found that wrapping had no effect on vitamin C. Moreover, Farag (2001) noticed that ,no significant differences was noticed in V.C content of navel oranges during storage .

CONCLUSION

Generally it could be concluded that Egyptian Lime fruits treated with phosphorine (after fruit set) plus GA_3 (20ppm 2 weeks before harvest date) during growth season resulted in the lowest values of weight loss%, the lowest values of unmarketable fruit number%, the lowest level of acidity %, the highest value of TSS/TA ratio in juice of stored fruits during the two studies seasons. Furthermore, wrapping treated fruits with GA_3 (50ppm) plus Sida film (5% wetting agent) was more effective in decreasing weight loss% than treating with GA_3 or Sida film alone. Also, wrapping fruits +Sida film + GA_3 (50ppm pre-storage) gave the lowest percentage of unmarketable fruits number, the highest value of juice weight percentage , the highest level of TSS/TA ratio in juice, keep good quality and prolong cold storage period .

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تأثير معاملات ما قبل الجمع (حمض الجبريليك والمخصبات الحيويه) وما بعد الحصاد على خصائص الليمون المصرى المالح خلال التخزين المبرد

*جيهان محمد على , حمدى محمد مرزوق , فاروق محمد مصطفى , احمد حسن منصور

*معهد بحوث البساتين - كليه الزراعه جامعه اسيوط

اجريت الدراسه لبحث تأثير التسميد العضوى والتسميد الحيوى والرش بحمض الجبريليك على خصائص وسلوك ثمار الليمون المالح المصرى خلال بعض معاملات التخزين خلال موسمى ٢٠٠٣-٢٠٠٤ فى مزرعه كليه الزراعة جامعة اسيوط حيث تم معامله الاشجار بثلاثه معاملات : تسميد عضوى. تسميد عضوى مع تسميد بالفوسفورين بعد العقد. تسميد عضوى مع تسميد بالفوسفورين والرش بحمض الجبريليك (٢٠ جزء فى المليون) قبل الميعاد المتوقع للجمع ٢-٣ اسابيع ثم معامله الثمار باحدى المعاملات:

تعبئه الثمار فى اكياس بولى اثيلين مثقب ,غمر الثمار فى محلول GA_3 تركيز ٥٠ جزء فى المليون ,غمر الثمار فى محلول Sida film 5% ,غمر الثمار فى محلول (GA_3 ٥٠ جزء، Sida film 5%)، لف الثمار فى ورق حرير، غمر الثمار فى محلول (GA_3 ٥٠ جزء) ثم لف الثمار، غمر الثمار فى محلول (Sida film 5%) ثم لف الثمار، غمر الثمار فى محلول (GA_3 ٥٠ جزء +Sida film 5%) ثم لف الثمار.

أوضحت النتائج انه للحصول على ثمار ذات جوده عاليه عند الجمع ولاطول فتره ممكنه (٦٠ يوم) مع صفات ثمريه جيدة تحت ظروف التخزين يفضل رش الاشجار بحمض الجبريليك ٢٠ جزء فى المليون مع التسميد بالسماد الحيوى الفوسفورين اثناء موسم النمو مع لف الثمار ومعاملتها Sida film 5 %+ GA_3 ٥٠ جزء فى المليون قبل التخزين على درجه ٧-١٠°م.

Effect of Water Deficit on Fruit Quality and Storability of Valencia Orange Fruits Under Cold Storage Conditions

Hamdy A. E.[1*] , S. M. Khalifa[1] and W. F. R. Abobatta[2]

(1) Department of Horticulture, Faculty of Agriculture, Al-Azhar University, Nasr city, Cairo, Egypt.

(2) Citrus Dept., Horticulture Research Institute, A.R.C., Giza, Egypt.

ABSTRACT

The current study was carried out during the two successive seasons of 2014/2015 and 2015/2016 to investigate the effect of different water regimes (100%, 80% and 60%) of crop evapotranspiration (Etc) on fruit quality and storability of Valencia orange fruits under cold storage conditions. Fruit samples were collected at maturity stage and stored at 5 °C and RH at 85% ± 2 for 105 days. Results indicated that fruit weight loss (%), decay (%), TSS % and TSS/Acid ratio of fruits were increased with extending the period of cold storage. Whereas, firmness of fruit peel and pulp, juice content (cm^3), vitamin C as well as total acidity (%) decreased by increasing the period of cold storage. Irrigation tree with 80% of (Etc) was the best treatment for improving fruit quality under cold storage conditions in comparison to other treatments. The 80% irrigation treatment proportionate a water saving of 20%, which consists in lower irrigation costs that might compensate the slight yield losses in fruit production.

Key words*: irrigation regime, Valencia orange, water saving, fruit size, weight loss, fruit firmness.*

INTRODUCTION

Citrus is one of the greatest important fruit crops all over the world and Egypt as well. Citrus fruit measured as the best exportation fruit promising, moreover it is the popular fruit in Egypt, because of its taste, excellent flavor and high content of vitamin C. Citrus fruits are one of the major sources of human diet due to its high nutritive value, particularly vitamin C which is considerable essential for human fitness because of its unique functioning including increased resistance versus influenza and decreased accumulation of calcium oxalate in the kidneys (Haleblian *et al.*, 2008). Valencia orange [*Citrus sinensis* L. (Osbek)] is the most export cultivar among other citrus species in Egypt. Citrus in terms of fruit yield and also juice of fruit products are highly consumed and demanded worldwide (FAO, 2013). The total cultivated area of Valencia orange in Egypt reached 111807 feddan(**) in the season 2014, produced about 1182667 tons /year. Irrigation and fertilization practices can also have significant effects on quality of fruit and shelf life during harvest, packinghouse operations, storage, and distribution. These include impacts on fruit color, smoothness, juice content, and the development of physiological disorders (Ritenour *et al.*, 2002). Deficit irrigation has been widely demonstrated to be effective and sustainable under limiting water conditions by increasing water efficiency while stabilizing yield (García-Tejero *et al.* 2011). Kallsen *et al.* (2011) reported that irrigation seems to influence fruit volume, quality, yield, and harvest earliness, but little scientific information is available on how it affects fruit size, quality, and harvest date of early maturing navel oranges. Water stress can affect yield and fruit quality. Summer, fall, and season-long water deficits have increased the concentration of juice TSS, especially sugars, through short-term fruit dehydration or longer-term osmotic adjustment, although the ratio of TSS to percent Total acidity changed little (Hutton *et al.*, 2007 and Pérez-Pérez *et al.*, 2009). Although water deficit irrigation has been used to increase grower financial returns by increasing fruit grade and value through a reduction in rind creasing in 'Frost Nucellar' navel orange (Goldhamer and Salinas, 2000) and by reducing fruit granulation and moderating fruit size in 'Lane Late' navel oranges (Goldhamer, 2007). Under cooled storage conditions (about 4–6ºC at 90 percent RH), juice content, TSS, and total acidity contents declined progressively in Kinnow and Nagpur mandarins (Ladaniya and Sonkar, 1996). Losses in total acidity, TSS and ascorbic acid contents of Hayashi Satsuma were very slower at 5ºC than at 15ºC up to 3 months. Johnson and Hofman (2009) reported that rapid cooling of fruits is needed to minimize the respiration rate of fruits and to provide the best possible eating quality. Therefore, the main aim of the present study is to determine the effect of water deficit as a pre-harvest treatments on fruit quality and storability of Valencia fruits under cold storage conditions.

MATERIALS AND METHODS

Plant Materials: The present study was carried out in the two successive seasons of 2014/2015 and 2015/2016 on trees of Valencia orange [*Citrus sinensis*, L. (Osbeck)], (eight years old) budded on Volkamer lemon (*C. volkameriana* Ten. and Pasq.) rootstock, grown in sandy soil irrigated through drip irrigation system and planted 4 × 6 meters apart in a private orchard located at Wadi Almollak, Ismailia Governorate to elucidate the effect of different irrigation regimes on yield, fruit quality and storability of Valencia orange fruits under cold storage conditions. The water irrigation regimes were equivalent to 100%, 80% and 60% of crop evapotranspiration (Etc), which determined by using the Penman-Monteith according to Allen *et al.* (1998) equation:

Corresponding author: ashrafezat@azhar.edu.eg

(**) According to the last census, issued by Ministry of Agric. - Egypt (2014).

Crop evapotranspiration (Etc) mm/day= $Et_o \times Kc$

Twenty-seven trees nearly similar in growth, health and subjected to the same cultural practices were selected and then divided to three treatments each has 3 replicates, each replicate has 3 trees. A complete randomized block design was followed in analyzing the samples. Ninety fruits from each treatment were harvested at maturity stage according to Kader (1985) then divided into two groups, each has 45 fruits. Fruits of the first group was devoted to measure the fruit decay and loss weight while the second one was devoted for measuring fruit firmness and the chemical analysis. The harvested fruits were transferred to the post-harvest lab where they washed with tap water and then dipped in 2% boric acid solution. Thereafter, air dried under room temperature. All fruits were sound selected without any decay and then put in carton boxes and stored at 5°C and 95% relative humidity (RH) as well as analyzed biweekly.

Measurements:

Weight loss was determined as follows:

Weight loss (%) = $[(W_0 – W_1)/W_0]$ x100

Where w_0 is the initial weight and w_1 is the weight measured at start of each storage period.

Decay % was determined by calculating the number of decayed fruits at harvesting date expressed as percentage of initial fruit number according to (El-Anany *et al.*, 2009).

Fruit firmness (lb. /inch^2) was measured in peel and pulp using pressure tester (Digital force-Gouge Model FGV-0.5A to FGV-100A. shimpo instruments).

Juice content (cm^3): Juice content of the fruit was determined at 15 days intervals from zero storage to 105 days of storage.

The total acidity: (TA) was determined by titration and expressed as citric acid according to A.O.A.C. (2005).

The percentage of total soluble solids (TSS %) was determined by using a digital refractometer.

Total soluble solids/ total acidity ratio: was recorded by dividing TSS value by total acidity value.

Vitamin C was estimated by titrating juice sample with 2, 6 dichlorophenol indophenol dye according to A.O.A.C. (2005).

Statistical Analysis:

A complete randomized block design was followed and the results were analyzed using CO-STAT software program and the means compared according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Effect of water stress on physical properties and chemical constituents

Physical properties of fruit under cold storage conditions

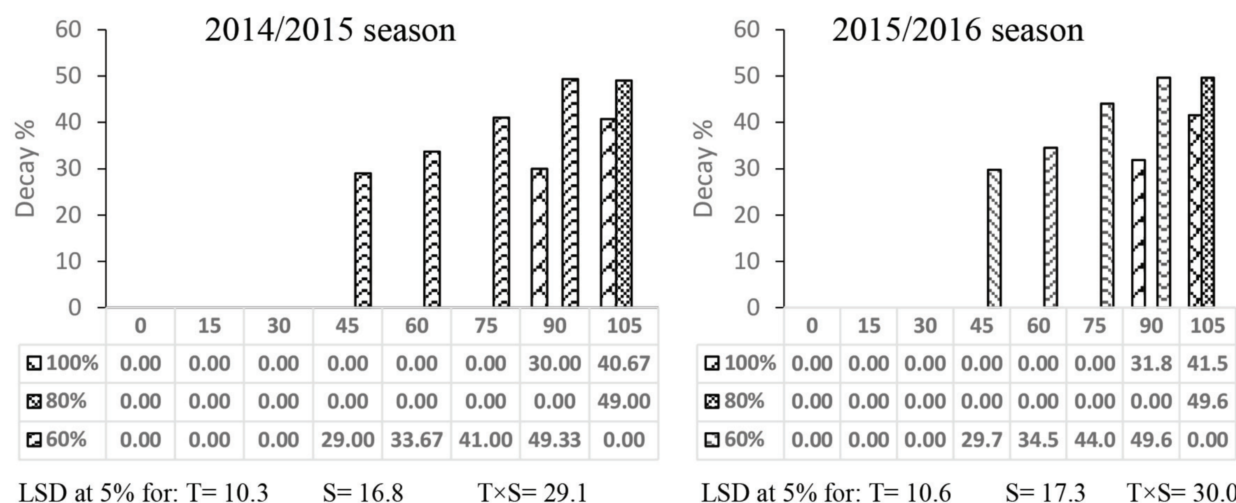
Fruit weight loss %:

Data in Table. (1) did not show a real significant difference between the effects of three studied irrigation treatments on fruit weight loss % under cold storage conditions. Hifny *et al.* (2012) on Valencia orange cv., stated that weight loss of fruits increased by increasing the storage period. They also reported that the increase in fruit weight loss under cold storage may be due to respiration and transpiration of water through peel tissue and perishable fruit is a serious concern in its storage because loss of moisture decreases visual quality;

Table 1: Effect of water regime on weight loss % of Valencia orange fruits under cold storage conditions

Storage periods (days) under 5°C									
2014/2015 season									
Treatments	**0.0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**Mean**
100%	0.0	4.25	3.18	3.26	3.31	4.72	7.90	7.78	3.75
80%	0.0	1.61	2.12	2.64	2.44	3.83	3.50	7.16	3.19
60%	0.0	3.18	3.58	4.19	4.16	5.41	4.25	0.00	3.32
Mean	0.0	3.09	2.88	3.34	3.37	4.51	5.33	4.98	--
LSD at 5% for:	T= 3.48			S= 1.51			T×S= 3.47		
2015/2016 season									
100%	0.0	3.95	3.43	3.84	4.03	5.47	4.17	7.05	3.16
80%	0.0	1.77	2.36	2.99	2.74	3.22	2.66	8.27	3.09
60%	0.0	2.66	2.84	3.60	3.52	4.56	3.27	0.00	2.97
Mean	0.0	2.56	2.95	3.37	3.52	4.28	3.46	4.43	--
LSD at 5% for:	T= 0.83			S=1.52			T×S= 2.63		

T: treatments; S: storage period; T×S: treatments × storage period.



2014/2015	0	15	30	45	60	75	90	105
100%	0.00	0.00	0.00	0.00	0.00	0.00	30.00	40.67
80%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	49.00
60%	0.00	0.00	0.00	29.00	33.67	41.00	49.33	0.00

2015/2016	0	15	30	45	60	75	90	105
100%	0.00	0.00	0.00	0.00	0.00	0.00	31.8	41.5
80%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	49.6
60%	0.00	0.00	0.00	29.7	34.5	44.0	49.6	0.00

LSD at 5% for: T= 10.3 S= 16.8 T×S= 29.1

LSD at 5% for: T= 10.6 S= 17.3 T×S= 30.0

T: treatments; S: storage period; T×S: treatments × storage period.

Fig. 1 Effect of irrigation regime on decay (%) of Valencia orange fruits under cold storage conditions during 2014/2015 and 2015/2016 seasons

salable weight and may result in physiological disfunction. A decrease in fruit weight loss percentages was noticed when the trees received 60 % of water compared with 100%, while the loss in weight percentages were increased in general by increasing number of days being under cold storage. Fruits of Valencia orange cv. at 80% irrigation showed fewer loss in weight up to 4 weeks being under cold storage than that of 100% or 60% irrigation treatments. The present results are in agreement with those of Abdel-Razik (2012) who studied that fruits of mango trees treated with 70% of Etc gained lower weight loss as compared with that of other treatments.

Decay Percentage.

The results in Fig. (2) clearly showed that decayed fruits could not be detected for thirty days being under cold storage in both studied seasons. Data indicated that the reduction in irrigation amount from 100% to 80% of Etc decreased the decayed fruit under cold storage. Regarding, the effect of the interaction between storage period and irrigation treatments, data in Fig. 1 showed that the highest decay (%) was gained by the interaction between remaining 90 days under cold storage and fruits due to using 60% in comparison to 80% or 100% irrigation treatments. These results are in agreement with those reported by Abdel-Razik (2012) on mango, who

Table 2: Effect of irrigation regime on peel firmness (lb. /inch²) of Valencia orange fruits under cold storage conditions during 2014/2015 and 2015/2016 seasons.

	Storage periods (days) under 5°C								
	2014/2015 season								
Treatments	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**Mean**
100%	4.22	4.25	4.01	3.76	3.62	3.37	3.20	3.00	3.78
80%	3.72	3.59	3.72	3.41	3.41	3.10	2.91	2.68	3.32
60%	5.48	4.86	4.79	4.28	4.16	3.90	3.82	0.00	4.39
Mean	4.47	4.23	4.17	3.82	3.73	3.46	3.31	2.84	--
LSD at 5% for:	T= 0.24			S= 0.39			T×S= 0.68		
	2015/2016 season								
100%	3.97	3.93	3.78	3.71	3.53	3.18	2.99	2.91	3.50
80%	4.27	4.22	4.18	4.13	3.72	3.59	3.35	3.12	3.92
60%	5.48	4.84	4.40	4.40	4.26	4.18	3.82	0.00	4.36
Mean	4.57	4.33	4.12	4.08	3.84	3.65	3.39	3.02	--
LSD at 5% for:	T=0.08			S=0.13			T×S= 0.23		

T: treatments; S: storage period; T×S: treatments × storage period.

Table 3: Effect of water regime on pulp firmness (lb. /inch²) of Valencia orange fruits under cold storage conditions

Storage periods (days) under 5°C									
2014/2015 season									
Treatments	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**Mean**
100%	5.64	5.32	5.00	4.83	4.24	4.03	3.91	2.16	4.39
80%	6.28	5.59	5.37	5.06	5.01	4.67	3.04	2.68	4.71
60%	6.52	6.04	5.40	5.26	4.27	4.15	2.36	0.00	4.86
Mean	6.15	5.65	5.26	5.05	4.51	4.28	3.10	2.42	
LSD at 5% for:	T= 0.22			S= 0.36			T×S =0.64		
2015/2016 season									
100%	5.51	5.37	5.18	4.83	4.27	4.15	2.36	2.42	4.47
80%	5.63	5.31	5.14	5.01	4.83	4.24	3.91	2.16	4.52
60%	7.30	6.52	5.30	5.13	4.61	4.30	3.04	0.00	4.98
Mean	6.14	5.73	5.20	4.99	4.57	4.23	3.10		
LSD at 5% for:	T= 0.13			S= 0.21			T×S = 0.37		

T: treatments; S: storage period; T×S: treatments × storage period.

reported that irrigation trees with 70% increased decay % when compared with those irrigated with 100% Etc under cold storage, Abdrabboh (2012) on Canino apricot as he found that decay (%) of fruits were increased with prolonging the period of cold storage and Hifny *et al.* (2012) on Washington navel orange who indicated that decay % of fruits was increased by prolonging cold storage period.

Fruit firmness.

Data presented in Tables (2 and 3) indicated that peel and pulp firmness gradually decreased with increasing number of days being under cold storage in all irrigation treatments. Yashoda *et al.* (2006) observed that softening in fruit texture from unripening to ripening stage of mango was a result of a reduction in starch content, pectin, cellulose and hemicellulose. Data also in Tables 2 and 3 showed a sharp decrease in fruit firmness after being under cold storage condition for the first month. Fruit firmness could also be an index for determining the optimum stage of fruit maturity as stated by Proietti and Antognozzi (1996) who reported that fruits of trees that received 60% of (Etc) possessed the best values of pulp firmness under cold storage conditions compared with irrigated with 100%. This is may be due to the thinner peel and the lower peel moisture content of ‘Valencia’ orange variety compared to ‘Marsh’ grapefruit fruits (Oberbacher, 1965).

Chemical characteristics of fruit:

Total Soluble Solids %

The results in Fig. (2) showed that juice total soluble solids % of Valencia orange fruit gradually increased with the prolonging of cold storage period in the two studied seasons. Thus, the highest values of TSS% were gained at the end of storage period in both seasons. The results are in agreement with the finding of Rub *et al.* (2010) on sweet orange and Hifny *et al.* (2012) on Washington navel orange and Abobatta (2015) on Valencia orange who found that TSS% were increased by increasing storage period. They added that TSS% is a function of total dissolved solids and moisture content of fruit and the increase in TSS% may be due to loss of fruit moisture content during cold storage period. The present results indicated that TSS% was increased by decreasing of irrigation water given to the orchard whereas maximum increase was recorded at 60% of Etc. These results are in harmony with those found by Abdel-Razik (2012) on mango trees, who reported that irrigation trees with 70% of Etc increased TSS% under cold storage conditions as compared with other treatments.

Total acidity %:

Data in Fig. (3) showed that total acidity % of Valencia orange juice was significantly decreased with prolonging cold storage period, hence the minimum fruit acidity percentage was recorded at the end of storage period during the two studied seasons. Similar results were reported by Kamel (2014) on Valencia orange fruits as he noticed decreasing trend in total acidity during 15 days of storage period. Rathore *et al.* (2007) reported that the decrease in acidity may be attributed to the increase in activity of citric acid gyloxylase during ripening. The reduction in acidity also may be due to their conversion into sugars and their further utilization in metabolic process in the fruit. The highest value of acidity was found at 80% of irrigation treatment while the lowest one was at 60% of Etc in the two studied seasons. The possible causes of TSS increase and total acidity decrease were the conversion of organic acids to sugars through gluconeogenesis (Echeverria and Ismail, 1987). Moreover, the degradation of cellulose, hemicellulose and pectin from cell walls within fruit segments might

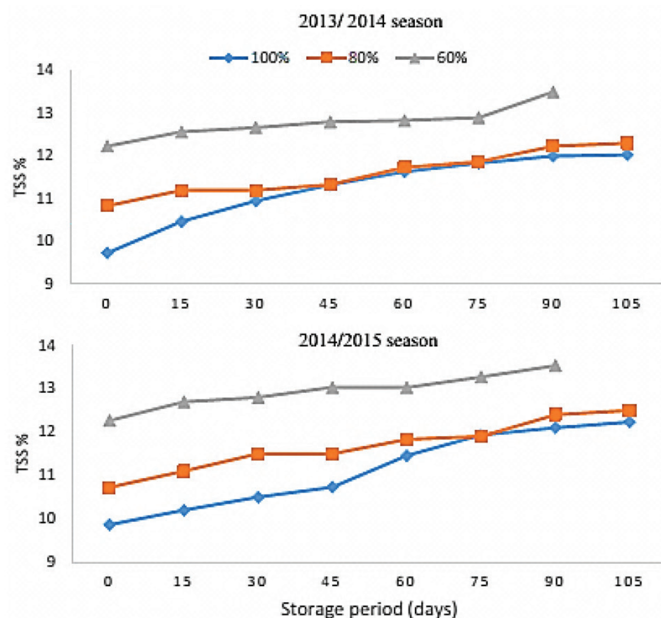


Fig. 2 Effect of water regime on TSS % of Valencia orange fruits under cold storage conditions

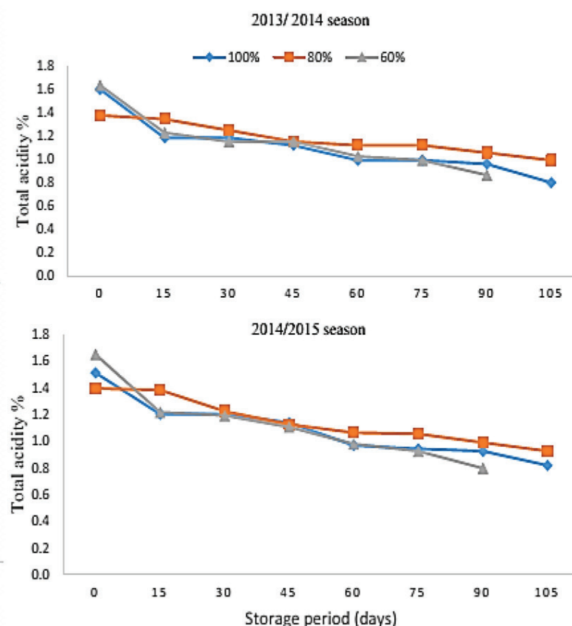


Fig. 3 Effect of water regime on total acidity % of Valencia orange fruits under cold storage conditions

release soluble components which could have a direct effect on TSS (Echeverria *et al.*, 1988). It was found that solubilization of cell water constituents by galactosidases and glucosidases present in citrus fruit, might have contributed to increase TSS levels (Echeverria, 1990).

Data in Fig. (4) indicated that both cold storage period and irrigation treatments affected the TSS/Acid ratio. TSS /Acid ratio was significantly increased by increasing the storage period, as TSS% was increased by increasing the storage period, total acidity was decreased. TSS/Acid ratio was increased as a result of increasing TSS content in the fruit. Regarding the effect of irrigation treatments on fruit TSS/acid ratio under cold storage conditions, the results showed that fruits of trees treated with 60% of Etc led to an increase in ratio of TSS/acid compared with those treated with 100% or 80%. The results are in agreement with those obtained by Nasir and Haq Main (1993) who reported that excessive moisture has depressing effect on TSS/acid ratio of mango. On the other hand, Abdraboh and Abdel-Razik (2009) found that TSS/acid ratio was increased by increasing the period of cold storage.

Vitamin C (Ascorbic acid):

Fruit V.C content was affected by irrigation treatments as shown in Table (4). Significant differences were noticed between fruits in relation to water regime as fruits of trees received 60% of Etc possessed the highest vitamin C values under cold storage conditions followed descendingly by those receiving 80% and 100%. Data in Table (4). also revealed that vitamin C content in fruit juice was gradually decreased by increasing the period of cold storage. Various reports had shown that vitamin C decreased under ambient and refrigerated

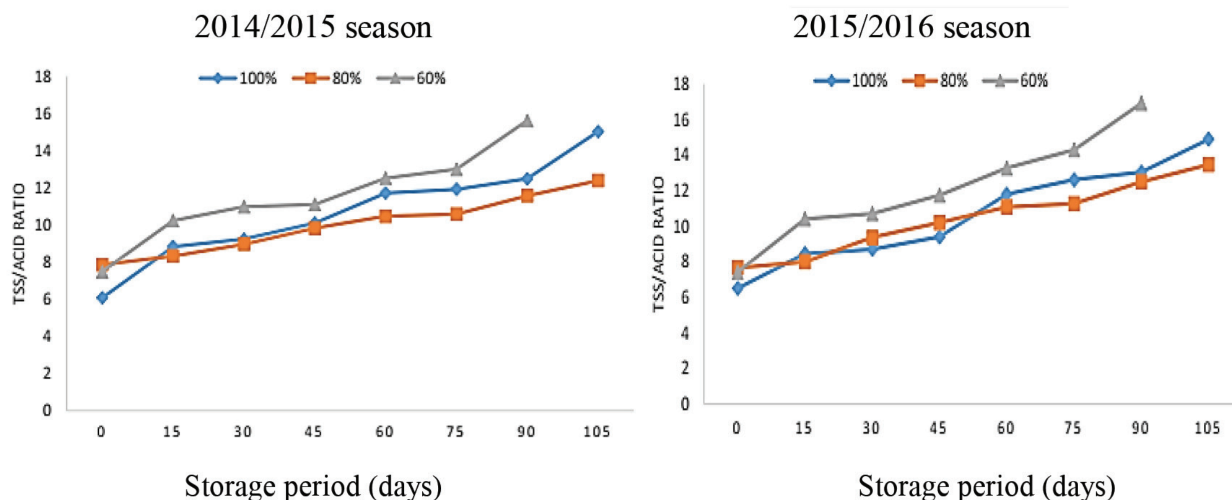


Fig. 4 Effect of water regime on TSS/acid ratio of Valencia orange fruits under cold storage conditions

Table 4. Effect of water regime on V. C (mg/100ml juice) content of Valencia orange fruits under cold storage conditions

Storage periods (days) under 5°C									
2014/2015 season									
Treatments	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**Mean**
100%	42.00	33.00	33.00	31.90	31.90	30.40	27.50	27.50	32.81
80%	48.30	38.40	36.30	34.10	30.80	30.80	29.70	29.70	34.72
60%	52.50	48.40	42.90	38.50	36.80	31.90	29.70	00.0	40.10
Mean	47.60	39.93	37.40	34.83	33.17	31.03	28.97	28.60	--
LSD at 5% for:	T= 1.04			S= 1.68			T×S = 2.93		
2015/2016 season									
100%	42.00	33.00	31.63	31.40	31.21	29.84	28.19	25.44	32.47
80%	46.99	38.40	34.24	32.04	30.80	30.39	30.39	29.43	34.08
60%	51.84	51.19	48.40	39.80	36.44	31.21	29.01	0.0	35.99
Mean	46.94	40.86	38.09	34.41	32.82	30.48	29.20	27.43	--
LSD at 5% for:	T= 0.78			S= 1.27			T×S =2.84		

T: treatments; S: storage period; T×S: treatments × storage period.

conditions during storage of citrus fruit such as orange and mandarin (Rapisarda *et al.*, 2001). Luis and Andrea (2010) stated that prolonging storage period reduced vitamin C in guava. Our result was confirmed also by EI-Kassas (1983) and Khattab *et al.* (2011) on Manfalouty pomegranate as they reported that ascorbic acid decreased by increasing soil moisture.

3.1.2.5. Juice content:

Data in Fig. (5) showed that fruit juice was gradually decreased with the prolonging of cold storage period in the two studied seasons. The fruits of trees treated with 100% irrigation showed an increase in fruit juice content than those treated with 80 % or 60% of Etc. The losses in fruit weight (g) and moisture content (%) of the peel were mainly caused by transpiration of fruit in which water moved out and resulted in wilted rind and a shriveled appearance (Wills *et al.*, 2007 and Roongruangsri *et al.*, 2013).

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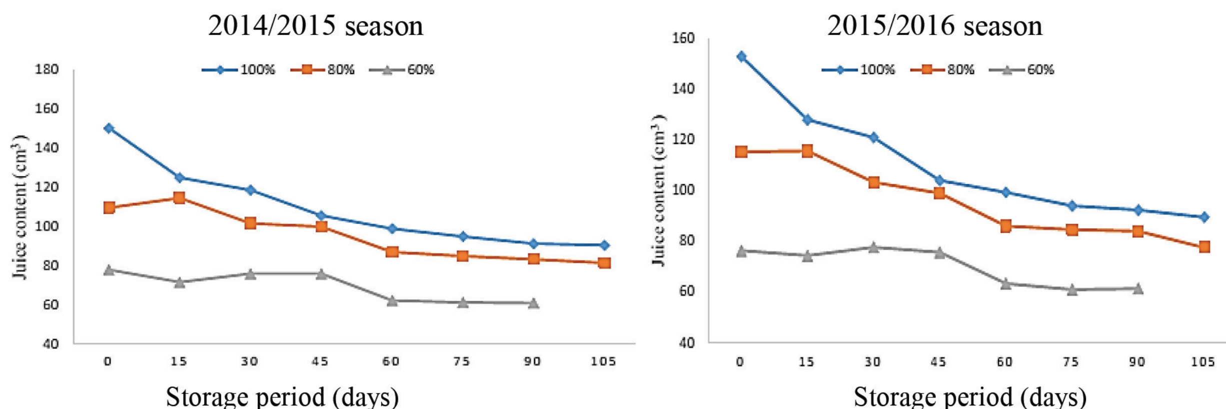

Fig. 5 Effect of water regime on juice content (cm³) of Valencia orange fruits under cold storage conditions

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الملخص العربي

تأثير نقص الماء على جودة الثمار والقدرة التخزينية لثمار البرتقال الفالنسيا تحت ظروف التخزين المبرد

أشرف عزت حمدى[١]، صبحى محمد خليفة[١]، وليد فؤاد ابوبطة[٢]

[١] قسم البساتين – كلية الزراعة بالقاهرة – جامعة الازهر

[٢] قسم بحوث الموالح – معهد بحوث البساتين

أجريت هذه الدراسة خلال موسمين متتاليين ٢٠١٤/٢٠١٥ و ٢٠١٥/٢٠١٦ لدراسة التأثير من مستويات مختلفة من الرى (١٠٠٪ و ٨٠٪ و٦٠٪ من البخر نتح المرجعى (Etc) على جودة الثمار والقدرة التخزينية للبرتقال الفالنسيا تحت ظروف التخزين المبرد . هذا وقد جمعت عينات الثمار فى مرحلة اكتمال النمو (Maturity) وتم تخزين الثمار على درجة حرارة (٥ درجة مئوية) ورطوبة نسبية ٨٥٪ ± ٢ لمدة ١٠٥ يوم. هذا وقد اوضحت النتائج حدوث زيادة فى كلا من الصفات التالية النسبة المئوية للفقد فى الوزن والنسبة المئوية للثمار التالفة و النسبة المئوية للمواد الصلبة الذائبة الكلية و النسبة المئوية للمواد الصلبة الذائبة الكلية: النسبة المئوية للحموضة الكلية بازدياد فترة التخزين المبرد للثمار ، بينما أدت النتائج إلى حدوث نقص معنوى فى صلابة الثمرة (قشرة ولب) ومحتوى الثمرة من العصير (سم٣) وفيتامين ج وكذلك النسبة المئوية للحموضة الكلية بطول مدة التخزين المبرد للثمار. كذلك أظهرت النتائج أن الرى بمستوى (٨٠٪) كانت ملائمة لتوفير ٢٠٪ من كمية مياه الرى والتى أدت إلى تقليل تكلفة الرى وهذا ربما يعوض نوعا ما بعض النقص الحادث فى محصول الثمار.

الكلمات الدالة : الاحتياج المائي، البرتقال الفالنسيا ، توفير الماء، حجم الثمرة ، الفقد فى الوزن، صلابة الثمرة

Study on Storage of Barhey Fruits (Consumer Package)

Gihan Mohamed Ali
Horticulture Research Institute

ABSTRACT

This research has been carried out over two seasons (2005- 2006). Fruits of date palm cv. Barhey were obtained from private orchard at South Tahrir area in order to determine fruit quality, storage life and marketability and the effect of consumer polyethylene and mesh package on keeping quality of fruits during storage at 0°C and 20°C. Results revealed that there was an increase in fruit weight, length, diameter, TSS% and 'a value' of peel color with age advanced during maturation period. Storage life was 60 days at 0°C plus 7 days at ambient temperature (18-20°C), 28 days at (20-25°C). Fruits that stored in polyethylene bags showed lower values of weight loss and rutab percentage, higher texture than fruits in mesh bags, besides, delayed the development of peel and pulp color values. Therefore, shelf life was 28 days at 20°C without any symptoms of decay. However, no noticeable chilling injury was found in fruits of present study.

INTRODUCTION

Date palm (*Phoenix dactylifera*) is the major fruit tree in most Arabian countries and it is widely grown in Middle Eastern countries. North Africa and the Arab-Muslim world are the main production areas, according to a 2006 study for the years 2003 and 2004. Thus, 11 countries in these regions carry out 94% of world production; the 1st place was occupied by Egypt followed successively by Iran and Saudi Arabia, Abd Elkader, (2016). The date fruit takes about six months to ripen completely. Barhi dates can be eaten at Khalal stage, when they are free of astringency, sweet and crunchy. Fresh dates, such as Barhi cultivar, are popular and widely consumed at the Khalal stage of maturity (first edible stage, crunchy and sweet) during the date production season. In this respect Mortazavi *et al*. (2010) reported that 'Barhee' is mainly harvested at the Khalal stage. Growth of date fruits follows a sigmoid curve and is usually divided into five development stages known by the Arabic terms: 'hababouk', 'kimri', 'khalal', 'rutab' and 'tamr' (Yahia, 2004).

One of the primary technical challenges in marketing fresh Barhi fruits at the Khalal stage of maturity is the preservation of quality for the longest possible period after harvesting and during the marketing process. Abdullah M. Alhamdan *et al*. (2015).

Alhamdan & Al-Helal (2008) reported that there was no commercial method available to preserve fruit at the khalal stage of maturity beyond the few days provided by traditional refrigeration methods. Other postharvest preservation techniques that may be useful in commercially extending fruit shelf life include controlled atmosphere (CA), modified atmosphere packaging (MAP) (Sidhu, 2006; Omar, 2008 and Al-Redhaiman, 2005).

Storage at modified atmosphere (MA) conditions delays fruit senescence, and extends storage life of horticultural crops through reducing postharvest loss (Kader 2002).

Somboonkaew & Terry (2010a) reported that perforated polyethylene bags were used to extend the storability of litchi fruit cv. Mauritius through reducing abrasion damage, minimizing weight loss and delaying of ripening and senescence processes. They are also maintaining the organoleptic properties of the fruit (Ramin & Khashbakhat 2008).The improved method of high density polyethylene (HDPE) and low density polyethylene(LDPE) reduced respiration rate, weight loss, soluble solids content and titratable acidity; improved fruit firmness and ascorbic acid content; and extent fruit storability (Adamicki 2001; Aharoni et al., 2008).

It is necessary to find out a cheap and effective storage method to increase fruit shelf-life by means of retarding the natural physiological deterioration and preventing the activity of decay organisms. Different types of packaging can be used depending on requirements of the produce and the target market. These are polymer film bags, trays with ridged or sealed film lids, overwrapped trays, and clamshells, Rajkumar and Mitalid (2009) and (Forney, 2007).

The aim of this study is to determine the maturity stage of Barhey fruit, studying effect of pre-storage packaging with polyethylene and mesh bags on fruit characters during cold storage and evaluate fruit quality during shelf-life.

MATERIALS AND METHODS

This study was carried out during two successive season, 2005 and 2006 on Barhey date palm fruit trees grown at a private orchard at South Tahrir district, Behera Governorate. The study comprises the following:

1- Fruit characters at maturity stage:

Samples of Barhey fruits at maturity stage (color khalal) were taken at 220, 230, 240 days after full bloom to determine fruit properties (flesh 5 fruit weight "g", fruit texture "g/cm^2, peel and pulp color, TSS%, Acidity%, TSS/ acid%, Tannin%) according to (A.O.A.C. 1991).

2-Fruit characters during cold storage:

Fruit were picked at maturity, transferred immediately to laboratory, washed, divided into two groups, one

was packed in polyethylene bags (20 micron in thickness) and the other one was packed in mesh bags. Both of them stored at 0°C (85-90% RH) 60days and at ambient temperature (20-25° C) 28 days. Fruits were examined every 10 days to detect weight loss %, Rutab %, peel and pulp color, texture, TSS%, Acidity%, and TSS/acid ratio.

3- Fruit characters during shelf- life:

Fruits were weighted and put at ambient temperature (18-20°C) for 7 days to stimulate the marketing period, at the beginning 15 days, middle 40 days and the end 60 days of cold storage period at 0°C. Changes in fruit characters were recorded. Weight loss was measured using a digital balance according to A.O.A.C. (1991). Soluble solids content (SSC) was determined using a hand-held refractometer (Model K-0032, Cosmo, Japan) at room temperature according to A.O.A.C. (1991). Titratable acidity was calculated with the titrated volume of standard NaOH to pH 8.1 and expressed as mg of malic acid according to A.O.A.C. (1991).texture was estimated by measuring resistance of fruit flesh (at a middle position) for a penetrating needle of a texture analyzer instrument (Lfra texture analyzer) for a fixed distance of 2 millimeters inside fruit flesh and firmness is expressed in gram unit. Color measurements for the fruit were obtained using Hunter colormeter (DP9000), (a,value:green,red) (b,value:blue,yellow), (L,value: lightness), McGure, (1992).

Statistical analysis:

Data obtained were statistically analyzed using the L.S.D. value at 5% level (Scedecor and Cohran, 1972).

RESULTS

1- Fruit characters at maturity stage:

Data presented in Table (1) showed that, in Barhey fruits which picked at the color Khalal stage, the fruit weight; length and diameter were significantly increased till the age of 240 days. No statistical differences were detected regarding fruit shape index during maturation time Fruit firmness was decreased gradually toward the maturity stage. TSS percentage increased significantly and gradually during maturation period, meanwhile acid percentage and tannin content in date fruit, decreased significantly, these results indicate that tannins synthesis is reduced in late stages of fruit development and at harvest, Nihad *et al*., (2013) came to the same findings.

Regarding fruit color, it is clear from Table (1) that Fruit became more yellowish, where "a" values were increased till the end of maturity stage while "b" values were decreased during maturity stage. These results are in agreement with the finding of Salah *et al*. (2012)

2- Fruit characters during cold storage:

2-1. Weight loss:

It can be concluded that weight loss % was increased with prolonged storage period in both seasons of the study. (Table 2 & 3)

Control treatments (mesh bags) lost 7.4%, 14. 2% and 20% of their total weight over the 20, 40 and 60 days of storage period in the first season, while polyethylene-treated dates lost 2.8%, 5.8% and 9.4%. At second season, packaging in polyethylene bags showed the least significant percentage of weight loss. The same trend was observed by Salah *et al*. (2012).

2-2. Rutab fruits percentage:

Rutab fruits (they are undesirable character), It means that the fruits begin to soften and acquiring a darker and less attractive color as reported by Saied *et al*. (2014). Rutab fruit% increased as the cold storage period increased, reached 24%, 22.0%in polyethylene treatment, while control gave the highest significant percentage 55%, 51% in 2005, 2006 respectively. (Table 2 & 3).

Even though consumers may find these softer fruit desirable at later date ripening stages such as in the rutab and tamr stages, they would not be considered 'crunchy' as expected from a khalal stage fruit and therefore would not be a suitable fruit quality, Salah *et al*. (2012).

2-3. Peel and pulp color

Both external color of peel and pulp were increased during storage period, generally, polyethylene bags de-

Table (1): Fruit characters at maturity

season	Days from full bloom	Weight 5 fruit	Length	Diameter	shape index	firmness	Texture%	TSS%	Acid%	TSS/acid	Tannins%	Peel color a	Peel color b	Pulp color a	Pulp color b
2005	220	83.2^{C}	3.6^{C}	2.5^{C}	1.44^{A}	26.0^{A}	116^{A}	30.1^{B}	0.5^{A}	60.2^{C}	0.44^{A}	0.60^{C}	22.9^{A}	-2.4^{A}	17.5^{A}
	230	95.4^{B}	4.0^{B}	2.7^{B}	1.48^{A}	24.0^{B}	110^{B}	32.9^{A}	0.5^{A}	65.8^{B}	0.43^{A}	0.71^{B}	21.5^{C}	-2.1^{B}	17.3^{A}
	240	106.1^{A}	4.4^{A}	3.0^{A}	1.46^{A}	22.0^{C}	112^{B}	33.2^{A}	0.4^{B}	83.0^{A}	0.29^{C}	0.75^{A}	22.0^{B}	-1.7^{C}	16.5^{B}
2006	220	87.1^{C}	3.6^{C}	2.5^{C}	1.44^{A}	24.0^{A}	120^{A}	31.0^{C}	0.5^{A}	62.0^{C}	0.45^{A}	0.66^{C}	21.4^{B}	-1.8^{B}	19.5^{A}
	230	93.9^{B}	4.2^{B}	3.0^{B}	1.40^{A}	20.0^{B}	116^{B}	33.2^{B}	0.4^{B}	83.0^{A}	0.40^{B}	0.72^{B}	21.3^{B}	-5.1^{A}	17.4^{B}
	240	105.6^{A}	4.5^{A}	3.1^{A}	1.45^{A}	20.0^{B}	110^{B}	33.7^{A}	0.4^{B}	84.2^{A}	0.27^{C}	0.75^{A}	21.7^{A}	-2.1^{B}	16.7^{C}

Mean separation by L.S.D at 0.05.
Means followed by the same letters are not significantly different.

Table (2): Fruit physical characters at cold storage at (0°C) first season

Days of storage	Weight loss			Rutab %			Peel color a			Peel color b			Pulp color a			Pulp color b		
	mesh	P.E*	mean	mesh	P.E	mean	mesh	P.E	mean	mesh	P.E	mean	mesh	P.E	mean	mesh	P.E	mean
Start	0	0	0	0	0	0	0.6	0.72	0.66^{G}	22.9	22.2	22.5^{B}	-3.8	-2.5	-3.15^{G}	16.2	16.3	16.25^{A}
10	2.2	1.6	1.9^{F}	5.5	0	2.75^{F}	1.9	0.76	1.33^{F}	21.6	26.4	24.0^{A}	-2.5	-1.2	-1.85^{F}	16.5	15.8	16.15^{A}
20	7.4	2.8	5.1^{E}	9	2	5.5^{E}	3.52	3.01	3.26^{E}	22.3	22.7	22.5^{B}	-0.5	-0.2	-0.35^{E}	14.4	14.9	14.65^{C}
30	10	4.2	7.1^{D}	15	5.1	10.05^{D}	4.48	3.77	4.12^{D}	19.3	24.4	21.8^{C}	-0.4	-0.14	-0.27^{D}	16.2	15.3	15.75^{B}
40	14.2	5.8	10.0^{C}	30	9.9	19.95^{C}	7.37	6.74	7.05^{C}	19.5	23.7	21.6^{C}	-0.4	0.63	0.11^{C}	14.6	14.3	14.45^{C}
50	17.3	7.2	12.2^{B}	40	20	30.0^{B}	7.64	8.05	7.84^{B}	20.4	23.1	21.7^{C}	0.3	0.92	0.61^{B}	17.1	14.9	16.00^{A}
60	20	9.4	14.7^{A}	55	24.4	39.7^{A}	9.99	10.84	10.41^{A}	20.1	22.4	21.2^{C}	0.5	0.9	0.70^{A}	16.8	15.2	16.00^{A}
M	10.1^{A}	4.4^{B}		22.0^{A}	8.7^{B}		5.1^{A}	4.8^{B}		20.8^{B}	23.5^{A}		-0.9^{B}	-0.22^{A}		15.9^{A}	15.2^{B}	

Mean separation by L.S.D at 0.05. P.E*: Polyethylene.
Means followed by the same letters are not significantly different.

Table (3): Fruit physical characters at cold storage at (0°C) second season

Days of storage	Weight loss			Rutab %			Peel color a			Peel color b			Pulp color a			Pulp color b		
	mesh	P.E*	mean	mesh	P.E	mean	mesh	P.E	mean	mesh	P.E	mean	mesh	P.E	mean	mesh	P.E	mean
Start	0	0	0	0	0	0	0.66	0.9	0.78	21.1	23.4	22.25^{A}	-1.22	-2.1	-1.66^{G}	19.1	19.1	19.1^{A}
10	3	2	2.5^{F}	4.2	0	2.1^{F}	0.95	1.2	1.07^{F}	21.2	22.3	21.75^{B}	-2.11	-1.7	-1.90^{F}	17.5	17.3	17.4^{C}
20	5.8	3.1	4.45^{E}	8.3	2.3	5.3^{E}	2.2	3.4	2.8^{E}	19.5	21.1	20.3^{D}	-0.68	-0.3	-0.49^{E}	17.8	18.2	18.0^{B}
30	8.4	4.6	6.5^{D}	12	4.8	8.4^{D}	5.35	5.2	5.27^{D}	20.3	21.5	20.9^{C}	-0.33	-0.22	-0.27^{D}	14.4	16.6	15.5^{E}
40	11.5	6	8.75^{C}	28	8.6	18.3^{C}	6.45	7.6	7.02^{C}	20.6	21.4	21.0^{B}	1.03	0.32	0.67^{C}	16.2	13	14.6^{F}
50	15.4	8	11.7^{B}	37	17	27.0^{B}	8.33	6.8	7.56^{B}	19.9	20.9	20.4^{D}	1.62	0.63	1.12^{A}	17.1	15.5	16.3^{D}
60	17.7	9.3	13.5^{A}	51	22	36.5^{A}	8.14	9.8	8.97^{A}	20.1	21.1	20.6^{C}	0.96	0.55	0.75^{B}	14.3	14.7	14.5^{F}
M	8.8^{A}	4.7^{B}		20.0^{A}	7.81^{B}		4.58^{B}	4.9^{A}		20.3^{B}	21.6^{A}		-0.1^{A}	-0.4^{B}		16.6^{A}	16.3^{B}	

Mean separation by L.S.D at 0.05 P.E*: Polyethylene
Means followed by the same letters are not significantly different

layed the development of peel and pulp color in comparison with mesh bags, it is evident from Table (2) that treated dates had significantly higher "b" values (23.5) of peel color, higher "a" values (-0.22) of pulp color than control dates in the first season, while in the second season, fruits treated with polyethylene bags had higher «a» and "b" values of peel color, suggesting that by this time MAP dates were notably more yellow in appearance than control dates.

The results of two seasons were confirmed with Salah *et al*. (2012) who indicate that the use of MAP had some potential to reduce rate of color change of Khalas dates packed at the khalal stage of maturity and stored at 0°C.

Texture:

It is evident from Tables (4 & 5) that fruit texture was decreased gradually during storage period; moreover, polyethylene bags kept fruit texture higher than mesh bags (102.8, 10.4.4%) in the two studies seasons, respectively. Storage control-packaged dates continued to record low penetration measurements, but this was most likely due to that the fruit being firmer through dehydration and weight loss.

Total Soluble Solids (TSS):

Soluble solids content(SSC)was gradually increased till 60 days in the two seasons (Tables 4 & 5), polyethylene bags significantly reduced the degradation of TSS% the highest total soluble solids was obtained from fruits in mesh bags (34.2%) in the two seasons.

Slow increases in control date fruit TSS may be due to that the fresh dates being stored at their optimum storage temperature of 0°C (Kader & Hussein, 2009).

Acidity

It is evident from tables (4 & 5) that fruit acidity percentage decreased with the advancing of storage period. The highest acidity was obtained from fruits in polyethylene bags.

TSS: acid ratio

Significantly increased till the end of storage pe-

Table (4): Fruit characters at cold storage at (0°C) first season

Days of storage	Texture%		mean	TSS%		mean	Acid%		mean	TSS :acid%		mean
	mesh	P.E*		mesh	P.E*		mesh	P.E*		mesh	P.E*	
Start	110	120	115^{A}	33.1	33	33.05^{C}	0.4	0.4	0.4^{A}	82.75	85.5	
10	106	116	111^{A}	32.4	33.4	32.9^{D}	0.4	0.4	0.4^{A}	81	83.5	85.6^{D}
20	100	114	107^{B}	33	34	33.5^{C}	0.3	0.4	0.35^{B}	110	85	82.25^{E}
30	88	95	91.5^{C}	34.1	34.7	34.4^{B}	0.3	0.3	0.3^{C}	113.6	115.6	97.5^{C}
40	79	90	84.5^{D}	35.2	34.5	34.8^{B}	0.3	0.3	0.3^{C}	117.3	115	114.6^{B}
50	77	88	82.5^{D}	35.6	34.3	34.9^{B}	0.3	0.3	0.3^{C}	118.6	114.3	116.1^{B}
60	56	79	67.5^{E}	36	34.8	35.4^{A}	0.2	0.3	0.25^{D}	180	116	116.4^{B}
M	88^{B}	102.8^{A}		34.2^{A}	34.1^{B}		0.3^{A}	0.3^{A}		114.7^{A}	102.1^{B}	148.0^{A}

Mean separation by L.S.D at 0.05 P.E*: Polyethylene

Table (5): Fruit characters at cold storage at (0°C) second season

Days of storage	Texture%		mean	TSS%		mean	Acid%		mean	TSS :acid%		mean
	mesh	P.E*		mesh	P.E*		mesh	P.E*		mesh	P.E*	
Start	112	122	122^{A}	34	33.5	33.7^{D}	0.4	0.4	0.4^{A}	85	83.75	84.35^{D}
10	100	120	110^{B}	33.4	33.4	33.4^{E}	0.4	0.4	0.4^{A}	83.5	83.5	83.5^{D}
20	98	115	106.5^{C}	33.4	33.6	33.5^{DE}	0.3	0.4	0.35^{B}	111.3	84	97.65^{C}
30	94	107	100.5^{D}	33.6	34	33.8^{D}	0.3	0.4	0.35^{B}	112	85	98.5^{C}
40	80	99	89.5^{E}	34.4	34.1	34.2^{C}	0.2	0.3	0.25^{C}	172	113.6	142.8^{B}
50	69	90	79.5^{F}	35	34.4	34.7^{B}	0.2	0.3	0.25^{C}	175	114.6	144.8^{B}
60	54	88	71^{G}	35.6	35	35.0^{A}	0.2	0.2	0.2^{C}	178	175	176.5^{A}
M	86.7^{B}	104.4^{A}		34.2^{A}	33.9^{B}		0.2^{B}	0.3^{A}		130.9^{A}	105.6^{B}	

Mean separation by L.S.D at 0.05 P.E*: Polyethylene

riod, polyethylene bags treatment had scored the lowest values (102.1, 105.6%) in both seasons, respectively (Tables 4 & 5).

3- Fruit characters during shelf- life:

Data from table (6) showed that weight loss %, TSS%, TSS: acidity ratio and Rutab % were increased during shelf-life at ambient temperature (18-20^{0}C) at the beginning, middle and the end of storage period, while texture % and acidity % were decreased with prolonged storage. The lowest weight loss, TSS % and rutab % were found of fruits in polyethylene bags, Moreover, there was no incidence of physiological disorder during storage and shelf-life.

It is clear from table (7) that fruits were stored for a period of 28 days at 20-25^{0}C with good appearance, whereas, fruits treated with polyethylene had the lowest weight loss(7.5,7.4%),the lowest Rutab% (7.1,9.2%) , the highest texture% (77.6,80.0%) compared with control treatment (mesh bags) in the two season respectively.

Table (6): Fruit characters at shelf-life ambient temperature (18- 20o C)

Season	Period/ treatment	Weight loss		Texture		TSS%		Acidity		TSS/acid ratio		Rutab %	
		M	P.E*	M	P.E*	M	P.E*	M	P.E*	M	P.E*	M	P.E*
2005	After 15 days	12.4	3.9	87	116	36	35	0.4	0.4	90	87.5	20	9
	After 40 days	15.1	6.2	75	92	36.4	36	0.3	0.3	121.3	120	25	12
	After 60 days	22	9.8	55	75	37	36.5	0.2	0.3	185	121.6	30	20
2006	After 15 days	9.9	3.4	93	111	37	35.6	0.3	0.4	123.3	89	15	5
	After 40 days	13.8	5.8	71	90	38	36.2	0.3	0.3	126.6	120.6	20	10
	After 60 days	20.3	9.5	56	77	37.5	36.5	0.2	0.3	187.5	121.6	25	15

Mean separation by L.S.D at 0.05 P.E*: Polyethylene

Table (7): Fruit characters at shelf life 28 days at (20-25°C)

Season	Treatment	Weight Loss%	Texture%	TSS%	Acid%	TSS:Acid%	Rutab%
2005	Mesh (control)	11.6A	61.6B	37.9A	0.23B	164.7A	16.3A
	polyethylene	7.5B	77.6A	36.1B	0.26A	138.8B	7.1B
2006	Mesh (control)	11.2A	61.2B	37.8A	0.20B	189.0A	22.1A
	polyethylene	7.4B	80.0A	36.9B	0.30A	123.0B	9.2B

Mean separation by L.S.D at 0.05.

CONCLUSIONS

Perforated polyethylene bags are useful in the storability of Barhey date palm fruit, as they delay fruit ripening, maintain quality and extend cold storage period. Fruit could be stored for 60 days at 0°C and 28 days at 20°C in high density polyethylene bags (HDPE 20) with the optimal quality parameters than perforated with mesh bags treatments as control. However, no noticeable chilling injury or decay was found in fruits of present study.

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دراسه عن تخزين ثمار البلح البارحى (عبوات مستهلك)

جيهان محمد على

معهد بحوث البساتين

أجرى البحث على ثمار البلح صنف البارحى المأخوذه من مزرعه خاصه بمنطقه جنوب التحرير – محافظه البحيرة خلال موسمى ٢٠٠٥– ٢٠٠٦ وهو من الاصناف العالية الجودة المرتفعة الثمن حيث تم دراسه التغيرات فى الصفات الطبيعية والكيمائيه وصولا لمرحله اكتمال النمو وجمع الثمار وكذلك دراسه تأثير التعبئة فى مواد مغلفه (البولى ايثيلين والشباك) على جوده الثمار خلال التخزين على درجه الصفر المئوى ودرجه حراره الغرفة.

وأظهرت نتائج البحث ان وزن الثمار وابعادها ونسبه المواد الصلبة الذائبة ولون القشرة يزداد مع تطور عمر الثمار بينما تقل نسبه الحموضة والصلابة والتانينات. تغليف ثمار البلح البارحى بالبولى ايثيلين كان اكثر ثاثيرا فى تقليل كل من نسبه الفقد فى الوزن ونسبه الترطيب الى جانب تأخر تطور لون قشره الثمار ولون اللب الداخلى والمحافظه على صفات الجودة التخزينية والتسويقية لاكثر من ٦٠ يوما على درجه الصفر المئوى ورطوبة نسبيه ٨٥–٩٠٪ بالاضافة الى اسبوع على درجه حراره ١٨ – ٢٠ درجه مئوى للتسويق .كما امكن تخزين الثمار٢٨ يوم بجوده عاليه على درجه ٢٠ درجه مئوى .

Maturity and marketability of new cultivars of Jujube (*Ziziphus jujube* Mill) fruit grown under south Tahrir region

Gihan Mohamed Ali and Hesham Allam
Horticulture Research Institute

ABSTRACT

In this study an attempt was carried out to collect information about the fruits of new cultivars (Lee, Lang, Balahy, and Seedy) and to determine fruit quality and marketability. The fruits were obtained from Southern Tahrir region, Behera governorate during two successive seasons 2005-2006. Fruits were picked at maturity stage and put at ambient temperature (18-20°C) to prolong the marketing period.

Considering all the physical and chemical characters matching with subjective parameters, results revealed that fruits of all cultivars were found commercially mature after 90-100 days of fruit set when the fruit color is converted to light greenish yellow to greenish yellow color.

Among the studied cultivars, Lee produced the greatest fruit weight, flesh thickness, texture%, TSS % and also the highest vitamin C content. The fruit weight of the other cultivars varied from 9.60 to 85.09g/5 fruits.

Mature fruits in perforated polyethylene bags were held at 18-20°C for 15 days turned to ripe. The lowest value of fruit weight loss and ripe % and the highest texture % were noticed for Lee cv. Ripe fruits can be held for another 15 days without significant quality deterioration or decay.

INTRODUCTION

Jujube, (Chinese date or ber), is a deciduous fruit of tropical and subtropical origin, the jujube trees can withstand a wide range of temperatures and tolerant marginal land, the fruits are a drupe (Reddy *et al.*, 1998).

Ber is one of the most nutritious fruits with medicinal value. It is one of the richest sources of vitamin C, next to guava but better than citrus fruits and apple (Islam *et al.*, 2015, and Bal and Uppal, 1992).

Aulakh *et al.* (2005) studied the performance of 8 jujube (*Zizyphus mauritiana Lamk*) cultivars, they found fruit set of all cultivars was observed during the second week of October.

Lal and Dhaka (2003) found that the greatest fruit weight and fruit size were recorded for Umran, Sanaur No. 2 produced fruits with higher total soluble solids and vitamin C content.

Islam (2007) observed what the fruits after 4 to 5 days of storage life for different cultivars of ber grown in Rajshahi region. In fact, the short storage life of BARI Kul-2 was considered in this study due its rapid browning tendency of fruit skin, i.e. pericarp browning within 2 to 5 days of storage at ambient condition. The pericarp of ber was found to become brown and shrivel with storage time. The skin of the fruits got blemish through browning, shriveling and undesirable.

Fruits harvested at the mature green stage were stored at 10°C or room temperature (27-30°C) for up to 22 and 6 days, respectively. Data concerning fruit TSS, ascorbic acid and total carotenoid content, on the percentage of titratable acidity, and on fruit physical indices such as colour, flavour, taste and texture throughout the storage period were tabulated. The overall evaluation indicated that fruits held at room temperature for 6 days and at 10° for 22 days were in good and very good condition, respectively. (Abbas *et al.*, 1990).

The storage life of ber was found to be short likewise too many other tropical fruits (Abbas, 1997), depending on cultivar and storage conditions, it may vary from 4 to 15 days (Pareek, 2001)

The aim of this study was determining the maturity stage of four cultivars of Jujube fruit, studying fruit characters and evaluating fruit quality during shelf-life.

MATERIALS AND METHODS

The experiment was conducted at Southern Tahrir region, Behera governorate during two successive seasons 2005-2006. Fruits were picked at maturity stage and put at ambient temperature (18-20°C) to improve characteristics of the fruits through the marketing period.

Three plants of similar age from each cultivar were selected for this study, fruits were tagged at fruit setting stage and harvested at 90, 110, 130 days after fruit set .50 Uniform mature fruits were collected from each tree to determine the physical and chemical characteristics.

The physicochemical characters such as fruit weight, shape index, flesh thickness, TSS (%), acidity (%), TSS-acid ratio and pH% were determined according to A.O.A.C. (1991), texture was estimated by measuring resistance of fruit flesh (at a middle position) for a penetrating needle of a texture analyzer instrument (L fra texture analyzer) for a fixed distance of 2 millimeters inside fruit flesh and firmness is expressed in gram unit. Color fruit measurements were obtained using Hunter colormeter (DP9000), (a, value: green, red) (b, value: blue, yellow), (L, value:lightness), McGure (1992).

Storage life: Fruits were packaged with polyethylene, the shelf- life of fruits , physiological weight loss (%), ripening %, texture %, color (a, b, L., values), TSS% and nonmarketability parameters like decay at ambient condition were determined.

Statistical analysis:

The recorded data for the studied parameters of the fruits were analyzed statistically using the L.S.D. value at 5% level (Snedcor and Cochran (1990).

RESULTS AND DISCUSSION

Fruit character at maturity stage

Fruits of all cultivars were found commercially mature after 90-100 days of fruit set when the fruits turned into light greenish yellow to greenish yellow color.

The physical fruits characteristics are presented in Tables (1 & 2), significant differences were observed in the study among the cultivars in most of the characteristics.

The fruits are a drupe, fruit shape varied from round to elongate and at size of plum. The flesh was white, crispy, juicy, acid, or sub acid to sweet. Fully ripe fruits were wrinkled, the flesh buff-colored. The fruit contained one central brown stone.

Data presented showed that the averages of fruit weight (125.7,108.5 g /5 fruits), flesh thickness (1.7, 1.8), texture % (160, 180), peel color "a values" (1.2, 0.11), TSS% (20.8, 20.9), vitamin C (591.7, 588.3), pH% (5.0, 5.1) of Lee cultivars were higher than other cultivars in both seasons, respectively. The longest fruit (1.29, 1.27 cm) was observed in Balahy.

Among the varieties the seed weight of the fruit was maximum (7.2, 7.1g) in Lee cultivars, during the two seasons. These results were in agreement with those of Obeed *et al.* (2008), Ezz *et al.*, (2011).

The data revealed the significant difference among these cultivars in respect of total soluble solids. The highest total soluble solids were found in Lee (20.8-20.9 %) which was followed by Lang and Balahy (20.13-20.73, 20.47%). Lowest total soluble solids were recorded (19.5-19.0%) in Seedy cultivar.

Concerning total acidity, it was noticed that there were no significant differences between cultivars in the first season, while in the second year; seedy fruits contained the lowest values.

TSS/ acidity ratio of Balahy fruit was the highest in comparison with the rest of varieties (100.65) in the first season, while in the second year; Seedy cultivar had the highest ratio (146.6).

The pH value varied from 4.86 to 5.9 during first season and from 4.82 to 5.10 during the second in different varieties. Among the cultivars, the acid content was maximum in Lee and Lang which was determined as citric acid followed by Balahy and Seedy. Such results are in accordance with those of Ibrahim *et al.* (2009).

Ascorbic acid content was in the range of 463.3-591.7mg/100g with the lowest and highest value in seedy and Lee, respectively, these results are in harmony with those findings of Ezz *et al.* (2011) who reported that the average of ascorbic acid in Tofahy were higher than in Balahy variety by 16.16 % and 17.62% , respectively.

Shelf life of mature fruits

Mature fruits in perforated polyethylene bags held at 18-20 ^{0}C for 15 days turned to ripe, the lowest values of weight loss, ripe % and the highest texture % were noticed for fruits of Lee cv.

Table (1): Fruit characteristics at maturity stage in the first season 2005

Cultivar	Weight of 5 fruits g.	Shape index	Seed weight g.	Flesh thickness cm.	Peel color L	Peel color a	Peel color b	Texture%	TSS%	Acidity%	TSS/acid ratio	pH	Vitamin C mg/ 100gm
Lee	125.7^{A}	0.95 ^{D}	7.20^{A}	1.73^{A}	40.1^{A}	1.2^{A}	40.0^{A}	160^{A}	20.80^{A}	0.23^{A}	90.4^{C}	5.09^{A}	591.7^{A}
Lang	76.54^{C}	1.23^{B}	5.03^{C}	1.23^{B}	38.8^{B}	-2.0^{B}	36.8^{C}	144^{B}	20.13^{AB}	0.26^{A}	77.4^{D}	4.96^{A}	549.7^{AB}
Balahy	85.09^{B}	1.29^{A}	6.56^{B}	1.23^{B}	38.4^{B}	-2.2^{C}	39.1^{B}	135^{C}	20.13^{AB}	0.20^{A}	100.65^{A}	4.77^{AB}	543.3^{B}
Seedy	9.00^{D}	1.07^{C}	1.90^{D}	0.43^{C}	37.0^{C}	-5.8^{D}	32.2^{D}	110^{C}	19.53^{B}	0.20^{A}	97.65^{B}	4.86^{AB}	463.3 ^{C}

Mean separation by L.S.D at 0.05

Table (2): Fruit characteristics at maturity stage in the second season, 2006

Cultivar	Weight of 5 fruits g.	Shape index	Seed weight g.	Flesh thickness cm.	Peel color L	Peel color a	Peel color b	Texture%	TSS%	Acidity%	TSS/acid ratio	pH	Vitamin C mg/ 100gm
Lee	108.5^{A}	0.95^{D}	7.16^{A}	1.80^{A}	44.2^{B}	0.11^{A}	32.8^{C}	180^{A}	20.9^{A}	0.26^{AB}	80.3^{C}	5.10^{A}	588.3^{A}
Lang	75.70^{B}	1.20^{B}	4.96^{C}	1.20^{C}	43.1^{C}	-3.4^{D}	34.4^{B}	155^{B}	20.73^{B}	0.30^{A}	69.0^{D}	4.99^{A}	545.0^{B}
Balahy	84.17^{B}	1.27^{A}	6.33^{B}	1.26^{B}	50.1^{A}	-1.7^{C}	36.0^{A}	150^{C}	20.47^{C}	0.23^{B}	88.6^{B}	4.88^{AB}	546.7^{B}
Seedy	11.07^{C}	1.05^{C}	1.80^{D}	0.50^{D}	40.4^{D}	-1.4^{B}	30.9^{D}	145^{D}	19.07^{D}	0.13^{C}	146.6^{A}	4.82^{AB}	505.7^{C}

Mean separation by L.S.D at 0.05

After 5, 10 and 15 days of storage at room temperature18-20°C, the data of the four cultivars showed that shelf life of cultivars was ranged from 15 days to mature fruits and another 15 days to ripe fruits at room temperature 18-20°C during two seasons (Table 3). The weight loss percentage increased significantly during shelf life and the highest weight loss was observed in Seedy cultivar after 15 days (13.3, 12, 7) during the two seasons.

Ripe% of mature fruits

Data presented in Table (4) showed that the percentage of ripe fruits was increased significantly at ambient temperature, fruits of Lee cultivar was recorded the lowest percentage. Such result in accordance with those of Adel *et al.*(1982). They concluded that after 15 days at 20^{0}C ,75% of the fruits were more than 75% brown , 15% were 50- 75% brown, 7% were 25-50% brown ,and 3% were less than 25% brown ,thus ,fruits picked whitish-green can continue their maturation and ripening after harvest.

Texture % of mature fruits

The present results in Table (5) showed a significant decrease in texture percentage for fruits all cultivars during two seasons, the highest texture of fruit was recorded for fruits Lee; the lowest texture was found in fruits of seedy cultivar in both seasons.

Color of peel fruit

It is clear from Table (6) that there was significant increase in “a values” at 15 days from shelf-life during the two seasons of the study, the increase in “a values” due to loss of green color, while the decrease in “b values” reflects brown color development with ripening. These results were in agreement with those reported by Abbas *et al.* (1990).

TSS% of mature fruit

Data in Table (7) indicated that TSS content increased significantly during shelf-life, it increased from

Table (3): Weight loss% of mature fruit at shelf-life at 18-20^{0}C during two seasons 2005&2006

	Days / Cultivars	5 days	10 days	15 days	Means
2005	Lee	5.06^F	9.06^D	10.87^C	8.33^C
	Lang	6.50^E	9.26^D	11.23^C	9.00^B
	Balahy	5.50^F	9.53^D	12.20^B	9.07^B
	Seedy	6.66^E	10.60^C	13.33^A	10.20^A
	Means	5.93^C	9.61^B	11.91^A	
2006	Lee	5.267^E	8.333^C	10.93^B	8.178^C
	Lang	5.967^{DE}	9.033^C	12.50^A	9.167^B
	Balahy	5.367^E	9.067^C	12.40^A	8.944^B
	Seedy	6.567^D	10.73^B	12.73^A	10.01^A
	Means	5.792^C	9.292^B	12.14^A	

Mean separation by L.S.D at 0.05

Table (4):%Ripe of mature fruit at shelf-life at 18-20^{0}C during two seasons 2005&2006

	Days / Cultivars	5 days	10 days	15 days	Means
2005	Lee	13.33^H	41.00^F	71.67^{CD}	42.00^C
	Lang	18.67^{GH}	64.33^D	77.33^{BC}	53.44^B
	Balahy	22.33^G	52.67^E	75.00^{BC}	50.00^B
	Seedy	50.00^E	79.33^B	91.33^A	73.56^A
	Means	26.08^C	59.33^B	78.83^A	
2006	Lee	7.233^F	50.00^C	79.00^A	45.41^C
	Lang	15.00^{EF}	70.33^B	83.00^A	56.11^B
	Balahy	16.67^E	37.33^D	82.67^A	45.56^C
	Seedy	36.33^D	66.00^B	83.33^A	61.89^A
	Means	18.81^C	55.92^B	82.00^A	

Mean separation by L.S.D at 0.05

Table (5): Texture % of mature fruit at shelf-life at 18-20⁰C during two seasons 2005&2006

	Cultivars / Days	5 days	10 days	15 days	Means
2005	Lee	161.0^{A}	144.0^{B}	121.7^{D}	142.2^{A}
	Lang	140.0^{B}	121.0^{D}	97.00^{E}	119.3^{B}
	Balahy	131.7^{C}	122.3^{D}	86.67^{F}	113.6^{C}
	Seedy	130.0^{C}	98.00^{E}	82.67^{F}	103.6^{D}
	Means	140.7^{A}	121.3^{B}	97.00^{C}	
2006	Lee	158.3^{A}	145.0^{B}	130.3^{C}	144.5^{A}
	Lang	143.7^{B}	130.7^{C}	117.7^{D}	130.7^{B}
	Balahy	140.3^{B}	116.3^{D}	90.67^{E}	115.7^{C}
	Seedy	134.0^{C}	96.67^{E}	82.67^{F}	104.4^{D}
	Means	144^{A}	122.1^{B}	105.3^{C}	

Mean separation by L.S.D at 0.05

Table (6): Peel color (a ,b, L values) of mature fruit at shelf-life at 18-20⁰C during two seasons

		a				b				L			
	Cultivars / Days	5 days	10 days	15 days	Means	5 days	10 days	15 days	Means	5 days	10 days	15 days	Means
2005	Lee	-0.90	3.4	8.3	3.6^{A}	41.6	37.8	28.2	35.9^{A}	34.4	30.1	21.6	28.7^{B}
	Lang	-1.2	2.2	8.0	3.0^{B}	34.8	34.0	29.5	32.8^{C}	39.3	35.5	23.1	32.6^{A}
	Balahy	-2.1	3.0	7.8	2.9^{C}	40.1	38.1	27.6	35.3^{B}	32.5	28.8	22.1	27.8^{C}
	Seedy	-3.5	5.1	7.5	3.0^{B}	34.4	30.2	29.0	31.2^{D}	37.1	26.4	17.7	27.1^{D}
	Means	-1.9^{C}	3.4^{B}	7.9^{A}		37.7^{A}	35.0^{B}	28.6^{C}		35.8^{A}	30.2^{B}	21.1^{C}	
2006	Lee	0.8	4.0	8.7	4.5^{A}	37.2	32.8	26.6	32.2^{C}	35.0	28.1	25.7	29.6^{B}
	Lang	-3.4	4.4	7.9	3.0^{C}	37.4	35.1	29.2	33.9^{B}	38.4	36.0	22.0	32.1^{A}
	Balahy	-2.3	3.6	6.9	2.7^{D}	40.0	38.0	30.1	36.0^{A}	31.5	29.2	24.1	28.3^{C}
	Seedy	-1.8	4.7	7.8	3.6^{B}	36.6	30.1	23.9	30.2^{D}	38.0	26.1	16.6	26.9^{D}
	Means	-6.7^{C}	4.2^{B}	7.8^{A}		37.8^{A}	34.0^{B}	27.5^{C}		35.7^{A}	29.9^{B}	22.1^{C}	

Mean separation by L.S.D at 0.05

Table (7): TSS %of mature fruit at shelf-life at 18-20⁰C during two seasons

		a				b				L			
	Cultivars / Days	5 days	10 days	15 days	Means	5 days	10 days	15 days	Means	5 days	10 days	15 days	Means
2005	Lee	19.67^{EF}	23.10^{D}	25.73^{B}	22.83^{B}	19.67^{EF}	23.10^{D}	25.73^{B}	22.83^{B}	19.67^{EF}	23.10^{D}	25.73^{B}	22.83^{B}
	Lang	19.37^{F}	22.77^{D}	27.73^{A}	23.29^{B}	19.37^{F}	22.77^{D}	27.73^{A}	23.29^{B}	19.37^{F}	22.77^{D}	27.73^{A}	23.29^{B}
	Balahy	22.00^{D}	24.33^{C}	28.10^{A}	24.81^{A}	22.00^{D}	24.33^{C}	28.10^{A}	24.81^{A}	22.00^{D}	24.33^{C}	28.10^{A}	24.81^{A}
	Seedy	18.73^{F}	20.80^{E}	25.17^{BC}	21.57^{C}	18.73^{F}	20.80^{E}	25.17^{BC}	21.57^{C}	18.73^{F}	20.80^{E}	25.17^{BC}	21.57^{C}
	Means	19.94^{C}	22.75^{B}	26.68^{A}		19.94^{C}	22.75^{B}	26.68^{A}		19.94^{C}	22.75^{B}	26.68^{A}	
2006	Lee	18.90^{H}	22.00^{E}	25.07^{B}	21.99^{C}	18.90^{H}	22.00^{E}	25.07^{B}	21.99^{C}	18.90^{H}	22.00^{E}	25.07^{B}	21.99^{C}
	Lang	20.90^{F}	23.03^{DE}	25.20^{B}	23.04^{B}	20.90^{F}	23.03^{DE}	25.20^{B}	23.04^{B}	20.90^{F}	23.03^{DE}	25.20^{B}	23.04^{B}
	Balahy	20.00^{FG}	24.33^{BC}	28.67^{A}	24.33^{A}	20.00^{FG}	24.33^{BC}	28.67^{A}	24.33^{A}	20.00^{FG}	24.33^{BC}	28.67^{A}	24.33^{A}
	Seedy	18.33^{H}	19.00^{GH}	23.33^{CD}	20.22^{D}	18.33^{H}	19.00^{GH}	23.33^{CD}	20.22^{D}	18.33^{H}	19.00^{GH}	23.33^{CD}	20.22^{D}
	Means	19.53^{C}	22.09^{B}	25.57^{A}		19.53^{C}	22.09^{B}	25.57^{A}		19.53^{C}	22.09^{B}	25.57^{A}	

Mean separation by L.S.D at 0.05

(19.9, 19.5%) to (26.6, 25.5%) at the end of shelf-life in both seasons, respectively.

Fruits of Balahy cv. contain the highest values (24.8, 23.3%), while the lowest values were recorded for fruits seedy cv. (21.5, 20.2%). These results are in line with those of Abbas *et al.* (1990).

Ripe fruit at ambient temperature

Ripe fruits can be held for another 15 days without significant quality deterioration or decay.

Weight loss% of ripe fruits: It is evident from Table (8) that weight loss increased with advanced shelf-life ,there were significant differences between cultivars, the lowest weight loss was observed in fruits Lee cv. (9.9, 8.8 %) during the two seasons of the study.

Texture% of ripe fruit

The data introduced in Table (9) showed that the percentage of texture were decreased gradually as the shelf-life advanced, the differences among all cultivars were significant, fruits Balahy cv. recorded the highest percentage of texture, while those of seedy cv. had the lowest texture percentage during the two seasons, this may be due to the spongy nature of the flesh and its relatively low water content.

Peel color of ripe fruit

The rate of color development increases with temperature and it is optimum at 20^{0}C, brown spots develop on the surface of fruits and increase in size until the entire skin becomes reddish brown. Data recorded in Table

Table (8): Weight loss % of ripe fruit at shelf-life during the two seasons 2005 & 2006

		a				b				L			
	Days / Cultivars	5 days	10 days	15 days	Means	5 days	10 days	15 days	Means	5 days	10 days	15 days	Means
2005	Lee	6.767^{H}	8.000^{FG}	15.07^{C}	9.944^{D}	6.767^{H}	8.000^{FG}	15.07^{C}	9.944^{D}	6.767^{H}	8.000^{FG}	15.07^{C}	9.944^{D}
	Lang	7.267^{GH}	8.467^{F}	16.67^{B}	10.80^{C}	7.267^{GH}	8.467^{F}	16.67^{B}	10.80^{C}	7.267^{GH}	8.467^{F}	16.67^{B}	10.80^{C}
	Balahy	7.833^{FG}	10.10^{E}	17.33^{B}	11.76^{B}	7.833^{FG}	10.10^{E}	17.33^{B}	11.76^{B}	7.833^{FG}	10.10^{E}	17.33^{B}	11.76^{B}
	Seedy	8.300^{F}	14.17^{D}	20.03^{A}	14.17^{A}	8.300^{F}	14.17^{D}	20.03^{A}	14.17^{A}	8.300^{F}	14.17^{D}	20.03^{A}	14.17^{A}
	Means	7.542^{C}	10.18^{B}	17.27^{A}		7.542^{C}	10.18^{B}	17.27^{A}		7.542^{C}	10.18^{B}	17.27^{A}	
2006	Lee	6.500^{J}	7.967^{GH}	11.93^{D}	8.800^{D}	6.500^{J}	7.967^{GH}	11.93^{D}	8.800^{D}	6.500^{J}	7.967^{GH}	11.93^{D}	8.800^{D}
	Lang	6.967^{IJ}	8.333^{G}	14.47^{C}	9.922^{C}	6.967^{IJ}	8.333^{G}	14.47^{C}	9.922^{C}	6.967^{IJ}	8.333^{G}	14.47^{C}	9.922^{C}
	Balahy	7.133^{HIJ}	9.833^{F}	15.70^{B}	10.89^{B}	7.133^{HIJ}	9.833^{F}	15.70^{B}	10.89^{B}	7.133^{HIJ}	9.833^{F}	15.70^{B}	10.89^{B}
	Seedy	7.867^{GHI}	10.87^{E}	17.77^{A}	12.17^{A}	7.867^{GHI}	10.87^{E}	17.77^{A}	12.17^{A}	7.867^{GHI}	10.87^{E}	17.77^{A}	12.17^{A}
	Means	7.117^{C}	9.250^{B}	14.97^{A}		7.117^{C}	9.250^{B}	14.97^{A}		7.117^{C}	9.250^{B}	14.97^{A}	

Mean separation by L.S.D at 0.05

Table (9): Texture % of ripe fruit at shelf-life during the two seasons 2005 & 2006

		a				b				L			
	Days / Cultivars	5 days	10 days	15 days	Means	5 days	10 days	15 days	Means	5 days	10 days	15 days	Means
2005	Lee	112.0^{B}	85.00^{D}	67.67^{F}	88.22^{B}	112.0^{B}	85.00^{D}	67.67^{F}	88.22^{B}	112.0^{B}	85.00^{D}	67.67^{F}	88.22^{B}
	Lang	104.3^{BC}	86.00^{D}	70.33^{EF}	86.89^{B}	104.3^{BC}	86.00^{D}	70.33^{EF}	86.89^{B}	104.3^{BC}	86.00^{D}	70.33^{EF}	86.89^{B}
	Balahy	120.7^{A}	99.67^{C}	66.67^{F}	95.67^{A}	120.7^{A}	99.67^{C}	66.67^{F}	95.67^{A}	120.7^{A}	99.67^{C}	66.67^{F}	95.67^{A}
	Seedy	107.3^{BC}	78.33^{DE}	52.00^{G}	79.22^{C}	107.3^{BC}	78.33^{DE}	52.00^{G}	79.22^{C}	107.3^{BC}	78.33^{DE}	52.00^{G}	79.22^{C}
	Means	111.1^{A}	87.25^{B}	64.17^{C}		111.1^{A}	87.25^{B}	64.17^{C}		111.1^{A}	87.25^{B}	64.17^{C}	
2006	Lee	130.0^{A}	94.00^{C}	75.00^{E}	99.67^{A}	130.0^{A}	94.00^{C}	75.00^{E}	99.67^{A}	130.0^{A}	94.00^{C}	75.00^{E}	99.67^{A}
	Lang	115.7^{B}	94.00^{C}	64.67^{F}	91.44^{B}	115.7^{B}	94.00^{C}	64.67^{F}	91.44^{B}	115.7^{B}	94.00^{C}	64.67^{F}	91.44^{B}
	Balahy	125.7^{A}	98.33^{C}	81.67^{D}	101.9^{A}	125.7^{A}	98.33^{C}	81.67^{D}	101.9^{A}	125.7^{A}	98.33^{C}	81.67^{D}	101.9^{A}
	Seedy	109.7^{B}	80.00^{DE}	60.67^{F}	83.44^{C}	109.7^{B}	80.00^{DE}	60.67^{F}	83.44^{C}	109.7^{B}	80.00^{DE}	60.67^{F}	83.44^{C}
	Means	120.3^{A}	91.58^{B}	70.50^{C}		120.3^{A}	91.58^{B}	70.50^{C}		120.3^{A}	91.58^{B}	70.50^{C}	

Mean separation by L.S.D at 0.05

(10) showed that “a, values” showed significant increases till the end of shelf-life, the highest value (22.7) on fruits of Balahy in the first season and (22.5) on fruits of seedy cv. in the second one “b, values” showed significant decreases till the end of shelf-life, the highest value (21.1) on Lee in the first season and (15.6) on Lang cv. in the second one. “L , values”” showed significant decreases till the end of shelf-life ,the highest values (13.7 ,15.2) on Lee and the lowest values (11.8 ,11.5) on seedy cv. in the two seasons. These results are in agreement with those of Adel *et al.*, (1982) and Islam (2007).

TSS% of ripe fruit

The data presented in Table (11) showed a significant increase in TSS5 of ripe fruits at shelf-life during the two seasons, seedy cv.had highest content of TSS% during the two seasons. These results were in agreement with Obeed et. al. (2008). They reported that the fruit of Um-suleam cv. had high content of juice acidity percentage compared with the other cultivars. Also, it had the highest TSS percentage.

CONCLUSION

Considering physical and chemical characters, it might be concluded that jujube fruit can achieve horticultural maturation within 110 to 120 days after fruit set. Lee, Lang and Balahy have good characteristics under south Tahrir region. Mature fruits in perforated polyethylene bags held at 18-20°C for 15 days whereas it turned to ripe. Ripe fruits can be held for another 15 days without significant quality deterioration or decay.

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Table (10): peel color (a, b, L) of ripe fruit at shelf-life at 18-20°C during two seasons

		a				b				L			
	Days / Cultivars	5 days	10 days	15 days	Means	5 days	10 days	15 days	Means	5 days	10 days	15 days	Means
2005	Lee	13.4	16.0	19.5	16.3^{D}	28.9	24.4	10.2	21.1^{A}	17.8	13.5	9.8	13.7^{A}
	Lang	15.1	19.9	23.7	19.6^{C}	24.1	18.8	15.4	19.4^{B}	18.8	12.4	10.3	13.8^{A}
	Balahy	19.5	21.2	27.3	22.7^{A}	25.9	19.9	8.7	18.1^{C}	18.1	12.7	6.1	12.3^{B}
	Seedy	17.0	21.7	23.4	20.7^{B}	24.1	17.3	13.1	18.1^{C}	18.4	9.7	7.4	11.8^{C}
	Means	16.3^{C}	19.7^{B}	23.5^{A}		25.75^{A}	20.1^{B}	11.8^{C}		18.2^{A}	12.0^{B}	8.4^{C}	
2006	Lee	16.5	17.9	18.7	17.7^{C}	16.3	11.9	9.0	12.4^{D}	22.2	15.4	8.1	15.2^{A}
	Lang	15.6	16.4	21.2	17.7^{C}	18.2	15.4	13.2	15.6^{A}	16.4	10.8	7.1	11.4^{C}
	Balahy	17.1	23.3	23.1	21.1^{B}	17.1	13.2	11.8	14.0^{B}	19.2	10.1	7.8	12.3^{B}
	Seedy	19.6	23.4	24.5	22.5^{A}	15.6	13.9	11.9	13.8^{C}	17.4	9.8	7.4	11.5^{C}
	Means	17.2^{C}	20.3^{B}	21.9^{A}		16.8^{A}	13.6^{B}	11.4^{C}		18.8^{A}	11.5^{B}	7.6^{C}	

Mean separation by L.S.D at 0.05

Table (11): TSS % of ripe fruit at shelf-life during the two seasons 2005 & 2006

		a				b				L			
	Days / Cultivars	5 days	10 days	15 days	Means	5 days	10 days	15 days	Means	5 days	10 days	15 days	Means
2005	Lee	24.00^{C}	25.83^{C}	33.83^{A}	27.89^{B}	24.00^{C}	25.83^{C}	33.83^{A}	27.89^{B}	24.00^{C}	25.83^{C}	33.83^{A}	27.89^{B}
	Lang	23.50^{C}	25.00^{C}	32.47^{AB}	26.99^{B}	23.50^{C}	25.00^{C}	32.47^{AB}	26.99^{B}	23.50^{C}	25.00^{C}	32.47^{AB}	26.99^{B}
	Balahy	24.67^{C}	26.00^{C}	31.90^{AB}	27.52^{B}	24.67^{C}	26.00^{C}	31.90^{AB}	27.52^{B}	24.67^{C}	26.00^{C}	31.90^{AB}	27.52^{B}
	Seedy	25.67^{C}	30.07^{B}	33.63^{A}	29.79^{A}	25.67^{C}	30.07^{B}	33.63^{A}	29.79^{A}	25.67^{C}	30.07^{B}	33.63^{A}	29.79^{A}
	Means	24.46^{C}	26.73^{B}	32.96^{A}		24.46^{C}	26.73^{B}	32.96^{A}		24.46^{C}	26.73^{B}	32.96^{A}	
2006	Lee	21.90^{G}	24.40^{F}	29.30^{C}	25.20^{C}	21.90^{G}	24.40^{F}	29.30^{C}	25.20^{C}	21.90^{G}	24.40^{F}	29.30^{C}	25.20^{C}
	Lang	21.23^{G}	25.17^{EF}	31.77^{B}	26.06^{C}	21.23^{G}	25.17^{EF}	31.77^{B}	26.06^{C}	21.23^{G}	25.17^{EF}	31.77^{B}	26.06^{C}
	Balahy	24.00^{F}	27.30^{D}	32.13^{B}	27.81^{B}	24.00^{F}	27.30^{D}	32.13^{B}	27.81^{B}	24.00^{F}	27.30^{D}	32.13^{B}	27.81^{B}
	Seedy	26.00^{DE}	30.97^{B}	34.50^{A}	30.49^{A}	26.00^{DE}	30.97^{B}	34.50^{A}	30.49^{A}	26.00^{DE}	30.97^{B}	34.50^{A}	30.49^{A}
	Means	23.28^{C}	26.96^{B}	31.93^{A}		23.28^{C}	26.96^{B}	31.93^{A}		23.28^{C}	26.96^{B}	31.93^{A}	

Mean separation by L.S.D at 0.05

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الملخص العربى

اكتمال النمو والقدره التسويقيه لاصناف جديده من العناب تحت ظروف منطقه جنوب التحرير

جيهان محمد على , هشام علام

معهد بحوث البساتين

اجريت هذه الدراسة لتحديد اكتمال النمو والقطف ودراسه القدره التسويقيه لثمار أصناف جديدة من العناب (Lee, Lang Balahy, seedy) وقد تم الحصول على ثمار من منطقة جنوب التحرير بمحافظة البحيرة خلال موسمين متتاليين ٢٠٠٥-٢٠٠٦. قطفت الثمار في مرحلة اكتمال النمو وضعت في درجة حرارة الغرفة (١٨-٢٠°م) لإطالة فترة التسويق.

اظهرت النتائج أنه يمكن جمع ثمار جميع الاصناف فى مرحله اكتمال النمو والتى تتراوح من ٩٠-١٠٠ يوما من العقد

أنتج الصنف لي أكبر قيمه لوزن الثمرة، سمك اللحم، والصلابه ٪، نسبه المواد الصلبه الكليه الذائبه ٪ وأيضا أعلى محتوى فيتامين C.

يمكن ان تخزن الثمار المعباه في أكياس البولي إثيلين مثقبة علي درجه حراره الغرفه ١٨- ٢٠ درجه مئوية لمدة ١٥ أيام إلى ان تتحول الى مرحله النضج .وقد لاحظت أقل قيمة من فقدان وزن الثمرة واقل نسبه ثمار ناضجه وأعلى نسبه صلابه لصنف Lee. ويمكن تخزين الثمار الناضجة لمدة ١٥ يوما أخرى على درجه حراره الغرفه دون تدهور لجودتها او ظهور اى اعراض للعفن.

Efficiency of Different Nitrogen Levels and Calcium Spraying Rates on Vegetative Growth, Yield, and Quality of Cucumber under Greenhouse conditions

Elsharkawy A.M, Sanaa M. El-Araby, Ibrahim M. Ghoneim and Shimaa M. Hassan
Department of Vegetable, Faculty of Agriculture, Alexandria, Egypt.

ABSTRACT

A field experiments were carried out during the winter seasons 2009/2010, 2010/2011 at the Experimental Station Farm of the Faculty of Agriculture, Alexandria University, at Abeis, Alex, governorate, A.R.E to study three levels of nitrogen, as main plots, were used in the present study; 80, 130 and 180 kg N fed^{-1}. Nitrogen fertilizer; in the form of ammonium nitrate (33.5% N) was added through drip irrigation system. Three concentrations of calcium chloride; 270, 390 and 510 ppm Ca, as sub-plots, were foliar sprayed. The results showed that plant height, number of leaves plant^{-1} and average leaf area plant^{-1} appeared to be positively correlated with the sampling dates. Hence, it was markedly increased with the progress of growth time. The highest efficient yield potentials were produced from the application of 130 kg N fed^{-1} combined with 390 or 510 ppm , in both seasons. The highest mean values of leaf N content were produced from the combined treatment of 180kg N fed^{-1} and 510 ppm Ca. Meanwhile, the application of 180kgN fed^{-1} combined with the lowest Ca concentration (270ppm) had the highest leaf P and K contents.

Keywords: ***Greenhouse, Cucumber, Nitrogen fertilization, Calcium.***

INTRODUCTION

Cucumber is a very sensitive plant to nutritional balance, particularly nitrogen fertilization, since cucumber plants are known to grow quickly and should never be allowed to suffer from lack of water or nutrients; beside, it produces a huge vegetative growth during a long growing season (Johnson and Hickman, 1984). Nitrogen is vitally an important nutrient and is frequently the most deficient of all nutrients.

There are many reports on nutritional trials with cucumber, but the majority of these are for field crop or pickling cucumbers. Little information, in Egypt, was available concerning nitrogen fertilization in greenhouse cucumber production. Application of optimum levels of nitrogen and calcium were always thought to have a key role on improvement of cucumber production and quality. Generally, nitrogen is more likely known to affect plant growth and yield potential; however its direct effect on fruit quality is not so clear. Reports are contradictory concerning the effects of nitrogen on some fruit quality such as firmness, calcium, organic acids, pH, soluble solid compounds and sugars (Cummings and Reeves, 1971).On the other hand, calcium is an important nutrient that seems to have a positive effect on fruit quality.

The object of this study was: to study the effects of different rates of fustigated nitrogen and different concentrations of calcium as a foliar spraying on growth, yield and quality of cucumber, grown in clay loam soil under polyethylene greenhouses conditions.

MATERIALS AND METHODS

This study was conducted during the two winter seasons of 2009/2010 and 2010/2011 at the Experimental Station Farm of the Faculty of Agriculture, Alexandria University, Abeis, Alex, Governorate, Egypt.

This study was concerned the effects of different nitrogen levels, calcium foliar application and their interactions on vegetative growth characters, yield, and quality of cucumber cv. Hesham F_1 parthenocarpic (Royal Sluis Company, origin Mexico) grown under greenhouse.

Soil samples at 30 cm depth were collected and analyzed for some soil's physical and chemical properties of the two experimental sites according to stander methods, described by Black (1965) and the results are listed in Table (1).

Treatments

1. Nitrogen fertilization

The levels of nitrogen used in the present study were 80, 130 and 180 kg N fed^{-1}. Nitrogen fertilizer; in the form of ammonium nitrate (33.5% N) was added one time weekly starting from one week after transplanting through drip irrigation system. The fertigation method in the two seasons is presented in Table (2).

2. Calcium fertilization

Three concentrations of calcium were used in the present study; (270,390 and 510 ppm Ca). Calcium fertilizer; in the form of calcium chloride ($CaCl_2 .6 H_2O$) was used as a foliar spraying. The foliar application of calcium treatments was applied one time weekly starting from one week after transplanting. Five dosages at the rate of 90, 130 and170 ppm Ca were practiced during the vegetative growth stage till the begging of flowering (40 days). The rest of Ca concentrations (180-260-340 ppm) was took place during the flowering and fruiting stages.

The experimental design was a split-plot system in a randomized complete blocks design (R.C.B.D) with sex replicates. Main plots consisted of the three rates of mineral nitrogen fertilization whereas; sub-plots were allocated to the three concentrations of foliar sprayed cal-

Table 1: Soil's physical and chemical properties of the experimental sites* in the two winter seasons of 2009/2010 and 2010/2011 before cultivation

Seasons / Properties	Winter 2009/2010	Winter 2010/2011
Physical properties		
Sand %	32.9	33.6
Silt %	25.8	25.3
Clay %	41.3	41.1
Soil texture	Clay loam	Clay loam
Chemical properties		
pH	7.96	8.00
E.C. (dS.m-1)	3.00	2.86
Soluble cations (m.eq/ l)		
Ca++	2.30	2.29
Mg++	1.90	1.95
Na+	2.63	2.56
K+	0.38	0.42
Soluble anions (m.eq/ l)		
Co3--	Trace	Trace
HCO3-	2.20	2.19
Cl-	1.90	1.93
SO--	3.11	3.10
Total N %	0.15	0.168
Available P (ppm)	0.48	0.55

* These analyses were carried out at the central laboratory, Faculty of Agriculture, Alexandria University.

cium .Each sub-plot consisted of two ridges,1,65cm long including 12 plants and occupying an area of 4 m^2. Each replicate contained nine treatments which represented the combinations among the three levels of nitrogen (80 kg.N-130 kg.N-180kg.N) fed^{-1} and the three concentrations of foliar calcium spraying (270-390-510) ppm.

Seeds of Hesham cultivar were sown in the nursery in foam trays filled with a mixture of peat moss and vermiculite (1:1 v/v) on October 25 in both seasons. Seedlings of 25 days old were transplanted in the greenhouses. Polyvinyl chloride (P.V.C) plastic cover was used only in this experiment. Greenhouse area was 270 m^2; 9m in width and 30m in length. During soil preparation, chicken manure as a source of organic fertilizer was uniformly added into the soil at the rate $1m^3$ 100 m^{-2}.Also, all experimental plots received calcium super phosphate at the rat of 10 kg100 m^{-2}, potassium sulphate at the rate of 10 kg100 m^{-2} and sulfur at the rate of 5 kg 100 m^{-2}as a basal soil dressing. Greenhouses were divided into six ridges 1 m wide, 40 cm space between ridges and 50 cm between plants. Cucumber transplants were grown in double rows. During the entire growing season, the rest of phosphorous and potassium fertilizers were added one time weekly through drip irrigation system starting one week after transplanting. Phosphoric acid (61.5% P_2O_5) was used as a source of phosphorus at the rate of (80 liter phosphoric acid fed^{-1} and potassium sulphate (48% K_2O) was applied as a source of potassium at the rate of 310 kg potassium sulphate fed^{-1} .Schedule of fertigation system applied for cucumber plants are given in Table (2).

Plants were drip irrigated once weekly, using drippers of 2 liter hr^{-1} capacity.

The first picking of cucumber fruits was started on 10th and 13th of January for 2010 and 2011 growing seasons, respectively .Picking was ended on April 25th and May 25 thin the first and second season, respectively.

Table 2: Schedule of fertigation system applied for cucumber plants grown in clay loam soil under greenhouse in the two winter seasons of 2009/2010 and 2010/2011

Percentage of total fertilization %	Week after transplanting	Ammonium nitrate(270 g m^{-2})			Phosphoric acid (270cm^3 m^{-2})	Potassium sulphate (270 g m^{-2})
		N_1 80 kg N fed^{-1}	N_2 130 kg N fed^{-1}	N_3 180 kg N fed^{-1}		
2%	2	360g	540g	720g	100 cm^3	420 g
4%	3	720g	1080g	1440g	200 cm^3	840 g
6%	4	1080g	1620g	2160g	300 cm^3	1260 g
8%	5	1440g	2160g	2880g	400 cm^3	1680 g
12%	6	2160g	3240g	4320g	600 cm^3	2520 g
12%	7	2160g	3240g	4320g	600 cm^3	2520 g
12%	8	2160g	3240g	4320g	600 cm^3	2520 g
12%	9	2160g	3240g	4320g	600 cm^3	2520 g
8%	10	1440g	2160g	2880g	400 cm^3	1680 g
8%	11	1440g	2160g	2880g	400 cm^3	1680 g
8%	12	1440g	2160g	2880g	400 cm^3	1680 g
8%	13	1440g	2160g	2880g	400 cm^3	1680 g

Table 3: Plant height (m) of cucumber plants grown under greenhouse conditions as affected by nitrogen levels, calcium spraying rates and their interactions, during the two winter seasons of 2009/2010 and 2010/2011 at 30,60,90 and 120 days after transplanting

Character / Season	Winter season of 2009/2010				Winter season of 2010/2011			
	Plant height (m)				Plant height (m)			
Treatments / Days	30	60	90	120	30	60	90	120
Nitrogen levels (kg N fed^{-1})								
80 kg	0.620^{B}	1.310^{B}	1.675^{C}	2.176^{C}	0.623^{C}	1.280^{C}	1.656^{C}	2.193^{C}
130 kg	0.690^{A}	1.347^{A}	1.818^{B}	2.270^{B}	0.666^{B}	1.320^{B}	1.790^{B}	2.253^{B}
180 kg	0.690^{A}	1.350^{A}	1.869^{A}	2.310^{A}	0.687^{A}	1.336^{A}	1.893^{A}	2.403^{A}
Calcium spraying rates (ppm)								
270 ppm	0.640^{B}	1.277^{B}	1.732^{B}	2.198^{C}	0.644^{C}	1.250^{C}	1.720^{C}	2.166^{C}
390 ppm	0.676^{A}	1.364^{A}	1.805^{A}	2.270^{B}	0.662^{B}	1.333^{B}	1.780^{B}	2.316^{B}
510 ppm	0.686^{A}	1.363^{A}	1.825^{A}	2.298^{A}	0.676^{A}	1.353^{A}	1.840^{A}	2.366^{A}
Nitrogen levels × Calcium rates								
80kg fed^{-1} ×270ppm	0.571^{f}	1.240^{e}	1.590^{f}	2.120^{e}	0.610^{g}	1.210^{f}	1.580^{g}	2.130^{f}
80kg fed^{-1} × 390ppm	0.641^{e}	1.358^{ab}	1.710^{e}	2.190^{d}	0.620^{f}	1.310^{c}	1.690^{f}	2.200^{e}
80kg fed^{-1} × 510ppm	0.650^{h}	1.333^{bc}	1.720^{e}	2.220^{d}	0.640^{e}	1.320^{c}	1.700^{c}	2.250^{d}
130kg fed^{-1} × 270ppm	0.676^{bc}	1.283^{de}	1.770^{d}	2.210^{d}	0.650^{d}	1.250^{e}	1.750^{e}	2.100^{f}
130kg fed^{1} × 390ppm	0.695^{abc}	1.366^{ab}	1.833^{c}	2.300^{b}	0.660^{c}	1.340^{b}	1.790^{d}	2.300^{c}
130kg fed^{-1} × 510ppm	0.700^{ab}	1.392^{a}	1.850^{bc}	2.320^{ab}	0.690^{b}	1.370^{a}	1.830^{c}	2.360^{b}
180kg fed^{-1} × 270ppm	0.671^{cd}	1.310^{cd}	1.830^{c}	2.260^{c}	0.673^{c}	1.290^{d}	1.830^{c}	2.270^{cd}
180kg fed^{-1}× 390ppm	0.693^{abc}	1.366^{ab}	1.875^{ab}	2.320^{ab}	0.690^{b}	1.350^{b}	1.860^{b}	2.450^{a}
180kg fed^{-1} × 510ppm	0.710^{a}	1.366^{ab}	1.900^{a}	2.350^{a}	0.700^{a}	1.370^{a}	1.990^{a}	2.490^{a}

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

All Agricultural practices such as cultivation, plant training and disease and pest control were carried out whenever it was necessary according to the recommendations of the commercial production of greenhouse cucumber as outlined by Ministry of Agriculture (1989).

All the data recorded in the experiment were determined for vegetative growth characters which were measured at 30, 60, 90 and 120 days after transplanting such as plant height, number of leaves plant^{-1} and average leaf area plant^{-1},leaf chlorophyll content (mg.100gm^{-1}f.w.) according to the method described by Yadava (1986), using a Minolta SPAD and fruit components such as early yield, total yield, number of fruits plant^{-1}and m^{-2}. Average fruit weight, total soluble solids (T.S.S %), fruit length (L), fruit diameter (D) fruit shape index (L/ D), dry matter content of leaves and fruits and chemical constituents of leaves and fruits according to A.O.A.C. (1990)

Statistical analysis

All obtained data were statistically analyzed, according to the analysis of variance as illustrated by Snedecor and Cochran (1980) and the revised L.S.D.test at 0.05 level was used to compare the differences among the means of the various treatments combinations, as illustrated by El- Rawy and Khalf-Allah (1980).

RESULTS AND DISCUSSIONS

Vegetative growth parameters:

The results showed that plant height, number of leaves plant^{-1} and average leaf area plant^{-1} appeared to be positively correlated with the sampling dates. Hence, it was significantly increased with the progress of growth time Tables (3, 4 and 5). The highest mean values of plant height, number of leaves plant^{-1} and average leaf area plant^{-1} seemed to be associated with 120 days after transplanting; whereas, the lowest ones was obtained at 30 days after transplanting. Such an increase in vegetative growth parameters with the progress of time might be expected due to the growth curve of cucumber plants as reported by Johnson and Hickman (1984), who clarified that greenhouse cucumbers grow very quickly and should never lack water or nutrients. De Kreij *et al.* (1992) pointed out that nitrogen is required in the production of chlorophyll (the green pigment in leaves),

Table 3: Plant height (m) of cucumber plants grown under greenhouse conditions as affected by nitrogen levels, calcium spraying rates and their interactions, during the two winter seasons of 2009/2010 and 2010/2011 at 30, 60, 90 and 120 days after transplanting

Character / Season	Winter season of 2009/2010				Winter season of 2010/2011			
	Plant height (m)				Plant height (m)			
Treatments / Days	30	60	90	120	30	60	90	120
Nitrogen levels(kg N fed^{-1})								
80 kg	0.620^{B}	1.310^{B}	1.675^{C}	2.176^{C}	0.623^{C}	1.280^{C}	1.656^{C}	2.193^{C}
130 kg	0.690^{A}	1.347^{A}	1.818^{B}	2.270^{B}	0.666^{B}	1.320^{B}	1.790^{B}	2.253^{B}
180 kg	0.690^{A}	1.350^{A}	1.869^{A}	2.310^{A}	0.687^{A}	1.336^{A}	1.893^{A}	2.403^{A}
Calcium spraying rates (ppm)								
270 ppm	0.640^{B}	1.277^{B}	1.732^{B}	2.198^{C}	0.644^{C}	1.250^{C}	1.720^{C}	2.166^{C}
390 ppm	0.676^{A}	1.364^{A}	1.805^{A}	2.270^{B}	0.662^{B}	1.333^{B}	1.780^{B}	2.316^{B}
510 ppm	0.686^{A}	1.363^{A}	1.825^{A}	2.298^{A}	0.676^{A}	1.353^{A}	1.840^{A}	2.366^{A}
Nitrogen levels × Calcium rates								
80kg fed^{-1} × 270ppm	0.571^{f}	1.240^{e}	1.590^{f}	2.120^{e}	0.610^{g}	1.210^{f}	1.580^{g}	2.130^{f}
80kg fed^{-1} × 390ppm	0.641^{e}	1.358^{ab}	1.710^{e}	2.190^{d}	0.620^{f}	1.310^{c}	1.690^{f}	2.200^{e}
80kg fed^{-1} × 510ppm	0.650^{h}	1.333^{bc}	1.720^{e}	2.220^{d}	0.640^{e}	1.320^{c}	1.700^{c}	2.250^{d}
130kg fed^{-1} × 270ppm	0.676^{bc}	1.283^{de}	1.770^{d}	2.210^{d}	0.650^{d}	1.250^{e}	1.750^{e}	2.100^{f}
130kg fed^{1} × 390ppm	0.695^{abc}	1.366^{ab}	1.833^{c}	2.300^{b}	0.660^{c}	1.340^{b}	1.790^{d}	2.300^{c}
130kg fed^{-1} × 510ppm	0.700^{ab}	1.392^{a}	1.850^{bc}	2.320^{ab}	0.690^{b}	1.370^{a}	1.830^{c}	2.360^{b}
180kg fed^{-1} × 270ppm	0.671^{cd}	1.310^{cd}	1.830^{c}	2.260^{c}	0.673^{c}	1.290^{d}	1.830^{c}	2.270^{cd}
180kg fed^{-1} × 390ppm	0.693^{abc}	1.366^{ab}	1.875^{ab}	2.320^{ab}	0.690^{b}	1.350^{b}	1.860^{b}	2.450^{a}
180kg fed^{-1} × 510ppm	0.710^{a}	1.366^{ab}	1.900^{a}	2.350^{a}	0.700^{a}	1.370^{a}	1.990^{a}	2.490^{a}

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

Table 4 : Number of leaves plant^{-1} of cucumber plants grown under greenhouse conditions as affected by nitrogen levels, calcium spraying rates, during the two winter seasons of 2009/2010and 2010/2011 at 30,60,90 and 120 days after transplanting

Character / Season	Winter season of 2009/2010				Winter season of 2010/2011			
	Number of leaves plant^{-1}				Number of leaves plant^{-1}			
Treatments / Days	30	60	90	120	30	60	90	120
Nitrogen levels(kg N fed^{-1})								
80 kg	6.77^{B}	13.22^{C}	25.00^{C}	31.72^{C}	6.22^{B}	12.72^{C}	24.66^{C}	31.77^{C}
130 kg	7.83^{A}	15.33^{B}	28.50^{B}	33.66^{B}	7.38^{A}	15.05^{B}	28.11^{B}	34.00^{B}
180 kg	7.66^{A}	15.94^{A}	29.11^{A}	35.50^{A}	7.27^{A}	16.05^{A}	29.05^{A}	35.88^{A}
Calcium spraying rates (ppm)								
270 ppm	7.27^{B}	14.44^{B}	27.22^{B}	33.16^{B}	6.66^{B}	14.11^{B}	26.88^{B}	33.33^{B}
390 ppm	7.38^{AB}	14.94^{A}	27.61^{B}	33.77^{A}	7.05^{AB}	14.66^{A}	27.38^{AB}	34.00^{A}
510 ppm	7.61^{A}	15.11^{A}	27.77^{A}	33.94^{A}	7.16^{A}	15.05^{A}	27.55^{A}	34.33^{A}
Nitrogen levels × Calcium rates								
80kg fed^{-1} × 270ppm	6.50^{i}	12.83^{f}	25.00^{c}	31.50^{e}	5.66^{e}	12.33^{e}	24.83^{c}	31.83^{c}
80kg fed^{-1} × 390ppm	7.00^{g}	13.16^{e}	25.00^{c}	31.50^{e}	6.66^{cd}	12.66^{de}	24.50^{c}	31.00^{d}
80kg fed^{-1} × 510ppm	6.83^{h}	13.66^{d}	25.00^{c}	32.16^{d}	6.33^{de}	13.16^{d}	24.66^{c}	32.50^{c}
130kg fed^{-1} × 270ppm	8.00^{b}	14.66^{c}	27.66^{b}	33.66^{c}	7.50^{ab}	14.33^{c}	27.16^{b}	34.00^{b}
130kg fed^{1} × 390ppm	7.66^{d}	15.66^{b}	28.83^{a}	33.83^{bc}	7.16^{abc}	15.16^{b}	28.50^{a}	34.33^{b}
130kg fed^{-1} × 510ppm	7.83^{c}	15.66^{b}	29.00^{a}	33.50^{c}	7.50^{ab}	15.66^{ab}	28.66^{a}	33.66^{b}
180kg fed^{-1} × 270ppm	7.33^{f}	15.83^{ab}	29.00^{a}	34.33^{b}	6.83^{bcd}	14.33^{c}	28.66^{a}	34.16^{b}
180kg fed^{-1} × 390ppm	7.50^{e}	16.00^{a}	29.00^{a}	36.00^{a}	7.33^{abc}	15.16^{b}	29.16^{a}	36.66^{a}
180kg fed^{-1} × 510ppm	8.16^{a}	16.00^{a}	29.33^{a}	36.16^{a}	7.66^{a}	15.66^{ab}	29.33^{a}	36.83^{a}

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

Table 5 :Leaf area (cm^2) plant^{-1} of cucumber plants grown under greenhouse conditions as affected by nitrogen levels, calcium spraying rates and their interactions during, the two winter seasons of 2009/2010and 2010/2011 at30,60,90 and 120 days after transplanting

Character / Season	Winter season of 2009/2010				Winter season of 2010/2011			
	Leaf area (cm^2)plant^{-1}				Leaf area (cm^2)plant^{-1}			
Treatments / Days	30	60	90	120	30	60	90	120
Nitrogen levels(kg N fed^{-1})								
80 kg	122.00^{C}	149.61^{C}	249.72^{C}	258.88^{C}	123.11^{C}	150..27^{C}	250.38^{C}	259.77^{C}
130 kg	128.77^{B}	159.94^{B}	270.33^{A}	280.11^{A}	131.33^{B}	160.83^{B}	271.33^{A}	281.00^{A}
180 kg	133.94^{A}	165.22^{A}	268.83^{B}	279.11^{B}	135.00^{A}	166.22^{A}	270.16^{B}	280.27^{B}
Calcium spraying rates (ppm)								
270 ppm	127.77^{B}	154.72^{C}	258.44^{C}	266.61^{C}	129.33^{A}	155.44^{C}	259.33^{C}	267.44^{C}
390 ppm	128.1^{AB}	158.11^{B}	262.44^{B}	273.27^{B}	129.94^{A}	159.11^{B}	263.50^{B}	274.38^{B}
510 ppm	128.77^{A}	161.94^{A}	268.00^{A}	278.22^{A}	130.16^{A}	162.77^{A}	169.05^{A}	179.11^{A}
Nitrogen levels X Calcium rates								
80kg fed^{-1} X270ppm	121.33^{e}	144.00^{e}	245.50^{h}	252.00^{h}	122.16^{d}	144.00^{e}	246.16^{h}	252.83^{f}
80kg fed^{-1}X 390ppm	121.33^{e}	149.16^{d}	249.00^{g}	260.16^{g}	122.33^{d}	150.50^{de}	249.66^{g}	261.00^{e}
80kg fed^{-1}X 510ppm	123.33^{d}	155.66^{c}	254.66^{f}	264.50^{f}	124.83^{c}	156.33^{cd}	255.33^{f}	265.50^{e}
130kg fed^{-1}X270ppm	129.00^{c}	156.33^{c}	266.83^{d}	275.16^{d}	130.66^{b}	157.33^{c}	267.66^{d}	275.83^{d}
130kg fed^{-1}X390ppm	129.00^{c}	161.16^{b}	270.83^{c}	282.16^{b}	132.33^{b}	162.00^{bc}	271.83^{c}	283.00^{b}
130kg fed^{-1}X510ppm	128.33^{c}	162.33^{b}	273.33^{b}	283.00^{b}	131.00^{b}	163.16^{b}	274.33^{b}	283.83^{b}
180kg fed^{-1}X270ppm	133.00^{b}	163.83^{b}	263.00^{e}	272.66^{e}	135.16^{a}	165.00^{ab}	264.16^{e}	273.66^{d}
180kg fed^{-1}X390ppm	134.16^{ab}	164.00^{b}	267.50^{d}	277.50^{c}	135.16^{a}	164.83^{ab}	269.00^{d}	279.16^{c}
180kg fed^{-1}X510ppm	134.66^{a}	167.83^{a}	276.00^{a}	287.16^{a}	134.66^{a}	168.83^{a}	277.33^{a}	288.00^{a}

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

which is responsible for converting sunlight to usable plant energy. Therefore, shortage of nitrogen reduces the plant capacity to trap energy through photosynthesis. They, also, reported that when cucumber plants reaching the flowering stage the plant will be well developed vegetatively; or it will have a low yielding potential.

The obtained results clarified that plant height; number of leaves plant^{-1} and leaf area plant^{-1} were significantly affected by the foliar application of Ca concentrations, at the four sampling dates as shown in Tables (3, 4 and 5). It was noticed that increasing the foliar application of calcium from 270 to390 and up to510 ppm resulted in corresponding increases in plant height; number of leaves plant^{-1} and leaf area plant^{-1}. Such results seemed to agree with those Liang *et al.* (2008), who studied the effects of calcium in different concentration on plant height, leaf area; photosynthetic rate and yield of cucumber in solar-greenhouse. Results showed that $CaCl_2$ can promote the plant growth, increase its photosynthetic rate, and improve the yield of cucumber remarkably. Calcium is essential for structure and function of cell walls and membranes. Recently, Al-Hamzawi (2010) found that the higher concentration of Ca were superior in their effect on plant vegetative characters of cucumber plants.

Fruit yield:

Results indicated that application of N up to130 kg N fed^{-1} was associated with progressive significant increases in early and total yields and number of fruits per plant and square meter in both seasons as shown in Tables (6 and 7). Increasing N application rate above 130 kg N fed^{-1}, however, significantly exerted negative remarkable influences on fruit yield and its components indicating that this medium N rate (130 kg N fed^{-1}) is sufficient to meet cucumber plants requirements. Reviewing to the leaf N-content as shown in Table (10), it was noticed that analysis of leaf samples, taken at flowering stage, showed that a leaf N-status of 3.03% on dry matter basis (average two seasons) was associated with the highest total yield per plant and per square meter. It can be concluded that leaf analysis for N content might be used as an indicator for prediction the yield of cucumber plants. These positive results could be related to the best utilization of N fertilizer in metabolism and meristimic activity which caused better growth which is able to intercept and trap more light energy through photosynthetic products accumulation required for fruit formation and development and subsequently yield components. On the other hand, the deteriorated effect of the highest N level (180 kgfed^{-1}) on fruit yield potentials might be explained on the basis that the excessive amount of nitrogen affect the carbohydrate/nitrogen ratio; so that, encouraged the vegetative growth to go more forward on the expense of flower initiation and finally decreased fruit yield and its components (Janick,1974). The obtained results are in according with those reported

Table 6 : Early and total yields (kg m^{-2}) of cucumber plants grown under greenhouse conditions as affected by nitrogen levels ,calcium spraying rates and their interactions, during the two winter seasons of 2009/2010 and 2010/2011

Characters	Early yield (kg m^{-2})		Total yield (kg m^{-2})	
Season / Treatments	Winter 2009/2010	Winter 2010/2011	Winter 2009/2010	Winter 2010/2011
Nitrogen levels (kg N fed^{-1})				
80 kg	1.99^{B}	1.78^{B}	8.97^{B}	10.42^{B}
130 kg	2.17^{A}	2.09^{A}	9.77^{A}	12.31^{A}
180 kg	1.59^{C}	1.55^{C}	7.94^{C}	9.34^{C}
Calcium spraying rates (ppm)				
270	1.74^{B}	1.70^{B}	8.61^{B}	10.32^{B}
390	1.94^{A}	1.86^{A}	8.95^{A}	11.00^{A}
510	2.07^{A}	1.85^{A}	9.13^{A}	10.75^{A}
Nitrogen levels × Calcium rates				
80kg fed^{-1} × 270 ppm	1.76^{d}	1.77^{e}	8.64^{cd}	10.23^{f}
80kg fed^{-1} × 390 ppm	2.08^{b}	1.79^{d}	8.84^{c}	10.58^{d}
80kg fed^{-1} × 510 ppm	2.14^{b}	1.77^{e}	9.43^{b}	10.45^{e}
130kg fed^{-1} × 270 ppm	1.89^{c}	2.01^{c}	9.54^{b}	12.20^{c}
130kg fed^{-1} × 390 ppm	2.18^{a}	2.20^{a}	9.70^{ab}	12.50^{a}
130kg fed^{-1} × 510 ppm	2.44^{a}	2.06^{b}	10.08^{a}	12.25^{b}
180kg fed^{-1} × 270 ppm	1.58^{e}	1.32^{h}	7.65^{e}	8.55^{i}
180kg fed^{-1} × 390ppm	1.55^{e}	1.59^{g}	8.30^{d}	9.92^{g}
180kg fed^{-1} × 510ppm	1.64^{e}	1.73^{f}	7.86^{e}	9.55^{h}

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

Table 7: Number of fruits per plant and square meter of cucumber plants grown under greenhouse conditions as affected by nitrogen levels, calcium spraying rates and their interactions, during the two winter seasons of 2009/2010 and 2010/2011

Characters	Number of fruits plant^{-1}		Number of fruits m^{-2}	
Season / Treatments	Winter 2009/2010	Winter 2010/2011	Winter 2009/2010	Winter 2010/2011
Nitrogen levels (kg Nfed^{-1})				
80 kg	23.56^{B}	41.01^{B}	106.22^{B}	123.03^{B}
130 kg	26.37^{A}	48.25^{A}	114.17^{A}	144.77^{A}
180 kg	19.28^{C}	36.44^{C}	92.99^{C}	109.34^{C}
Calcium spraying rates (ppm)				
2 270 ppm	33.79^{C}	40.29^{C}	101.38^{C}	120.87^{C}
3 390 ppm	34.90^{B}	42.89^{A}	104.72^{B}	128.68^{A}
5 510 ppm	35.79^{A}	42.51^{B}	107.38^{A}	127.55^{B}
Nitrogen levels × Calcium rates				
80kg fed^{-1} × 270 ppm	34.22^{f}	39.93^{f}	102.67^{f}	119.81^{f}
80kg fed^{-1} × 390 ppm	34.74^{e}	41.48^{e}	104.24^{e}	124.45^{e}
80kg fed^{-1} × 510 ppm	37.25^{d}	41.62^{d}	111.75^{d}	124.86^{d}
130kg fed^{-1} × 270 ppm	37.31^{c}	47.82^{c}	111.94^{c}	143.46^{c}
130kg fed^{-1} × 390 ppm	37.66^{b}	48.63^{a}	112.98^{b}	145.89^{a}
130kg fed^{-1} × 510 ppm	39.23 a	48.39 b	117.70 a	145.17 b
180kg fed^{-1} × 270ppm	29.87 i	33.24 i	89.60 i	99.73 i
180kg fed^{-1} × 390ppm	32.27 g	38.55 g	96.81 g	115.67 g
180kg fed^{-1} × 510ppm	30.80 h	37.54 h	92.42 h	112.63 h

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

by Adams *et al.* (1974), who noticed that the early yields were improved by the highest concentration of 300 mg l^{-1} N in the liquid feed but used at every watering; this subsequently became excessive and depressed the final yield by 10%. Similarly, Alan (1989) studied the effect of nitrogen nutrition on growth, and yield of cucumber (*Cucumissativus*, L.), and found that the nitrate significantly increased fruit yield, whereas; ammonium decreased it. Results, also, indicated that yield of cucumber plant was best with calcium nitrate application .These results agree with Choudhari and More (2002), who found that increase nutrient level improved plant growth of cucumber plants .They reported that the adequate nutrient availability in the nutrient solution lead to increase various physiological processes, better uptake of nutrients and higher rates of photosynthesis, which might be reflected on more number of fruits and highest fruit yields.

The obtained results reflected that the foliar spraying of calcium at the rates of 390 ppm or 510 ppm, significantly, increased early and total yields, number of fruits per plant and square meter as shown in Tables (6 and 7). Reviewing to the leaf Ca-content, it was noticed that analysis of leaf samples, taken at flowering stage, showed that a leaf Ca-status of 0.795 % on dry matter basis (average two seasons) was associated with the highest fruit yield and its components .Such a result were obtained by Mcfeeters *et al.* (1995), who showed that early and total yield of fancy grade peppers increased linearly as the rate of supplemental Ca (34-68 kg Ca ha^{-1}) increased. Hochmuth (1988) reported a tentative adequate range of 1.8 % of Ca in young mature leaf at fruit set stage was sufficient for cucumber plants to obtain its requirement. On the contrary, Yann *et al.* (2011) demonstrated that leaf analysis standards for greenhouse cucumbers (in dry matter of 3rd-5th leaves from the top) is<1.2 % for Ca -deficient plant and 2.2–2.4% Ca for the normal growth.

Chemical constituents of leaves:

The results showed that leaf chlorophyll content appeared to be significantly decreased with the progress of growth time as shown in Table (8). The highest mean values of leaf chlorophyll content seemed to be associated with 30

Table 8 : Leaf chlorophyll content (mg/ 100 g f.w) of cucumber plants grown under greenhouse conditions as affected by nitrogen levels ,calcium spraying rates and their interactions, during the two winter seasons of 2009/2010 and 2010/2011 at 30, 60, 90 and 120 days after transplanting

Character / Season	Winter season of 2009/2010				Winter season of 2010/2011			
	Leaf chlorophyll content (mg/ 100 g f.w)				Leaf chlorophyll content (mg/ 100 g f.w)			
Treatments / Days	30	60	90	120	30	60	90	120
Nitrogen levels(kg N fed^{-1})								
80 kg	44.25^{B}	42.77^{B}	41.07^{C}	40.07^{C}	42.30^{B}	41.35^{C}	40.15^{C}	39.31^{C}
130 kg	44.45^{A}	43.24^{A}	42.05^{B}	41.10^{B}	45.81^{A}	44.08^{B}	42.81^{B}	41.81^{B}
180 kg	44.48^{A}	43.17^{A}	42.23^{A}	41.39^{A}	46.25^{A}	45.13^{A}	43.78^{A}	42.95^{A}
Calcium spraying rates (ppm)								
2 270 ppm	42.00^{B}	40,.90^{C}	39.75^{C}	38.87^{C}	44.72^{A}	43.16^{A}	41.62^{B}	40.57^{B}
3 390 ppm	45.32^{A}	43.58^{B}	42.31^{B}	41.25^{B}	44.95^{A}	43.68^{A}	42.49^{A}	41.65^{A}
5 510 ppm	45.81^{A}	44.69^{A}	43.29^{A}	42.45^{A}	44.68^{A}	43.72^{A}	42.63^{A}	41.83^{A}
Nitrogen levels × Calcium rates								
80kg fed^{-1} × 270 ppm	42.13^{e}	41.06^{e}	39.10^{g}	38.08^{i}	42.63^{b}	41.40^{d}	39.60^{d}	38.41^{c}
80kg fed^{-1} × 390 ppm	44.95^{d}	42.88^{d}	41.26^{e}	40.05^{f}	42.65^{b}	41.81^{d}	40.55^{d}	39.66^{bc}
80kg fed^{-1} × 510 ppm	45.68^{bc}	44.36^{b}	42.58^{cd}	42.10^{c}	41.61^{b}	40.83^{d}	40.31^{d}	39.85^{b}
130kg fed^{-1} × 270 ppm	42.15^{e}	41.32^{e}	40.05^{f}	39.00^{h}	45.45^{a}	43.38^{c}	41.93^{c}	40.71^{b}
130kg fed^{-1} × 390 ppm	45.46^{c}	43.86^{c}	42.71^{d}	41.77^{e}	45.96^{a}	44.36^{bc}	43.04^{b}	42.26^{a}
130kg fed^{-1} × 510 ppm	45.75^{ab}	44.53^{b}	43.40^{b}	42.53^{b}	46.03^{a}	44.50^{b}	43.46^{ab}	42.45^{a}
180kg fed^{-1} × 270 ppm	41.91^{f}	40.33^{f}	40.12^{f}	39.52^{g}	46.08^{a}	44.70^{b}	43.35^{ab}	42.60^{a}
180kg fed^{-1} × 390 ppm	45.54^{bc}	44.00^{c}	42.96^{c}	41.95^{d}	46.25^{a}	44.86^{ab}	43.90^{ab}	43.03^{a}
180kg fed^{-1} × 510 ppm	46.00^{a}	45.18^{a}	43.62^{a}	42.72^{a}	46.41^{a}	45.85^{a}	44.11^{a}	43.21^{a}

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

days after transplanting; whereas, the lowest ones was obtained at 120 days after transplanting. Abd-Rabbo (2005) noticed that the highest nutrient concentrations were recorded in the fourth week from transplanting date (vegetative stage), but different nutrient concentration were progressively decreased after the second week after harvest. Similarly, Gharib (2001) found that using the full dose of nitrogen led to significant increment in leaf chlorophyll content cucumber plants

The obtained results indicated that increasing foliar application of calcium from 270 to 390 ppm and then to 510 ppm resulted in corresponding increases in leaf chlorophyll content at the four sampling stages, in the first season. However, in the second season, the differences between 390 and 510 ppm, at the four sampling dates, were not significant. Such results were previously documented by Kaya *et al.* (2002), who reported that supplementing irrigation water with calcium nitrate resulted in an increase in dry matter and chlorophyll concentrations over plants irrigated with saline water in both melon and cucumber.

The results showed that leaf dry matter content appeared to be significantly increased with the progress of growth time as shown in Table (9). The highest mean values of leaf dry matter content seemed to be associated with 120 days after transplanting; whereas, the lowest ones was obtained at 30 days after transplanting. Increasing N applied rate from 80 to 130 and raising to 180 kg N fed^{-1} was accompanied with significant increases in leaf dry matter content application of the highest N level, significantly, accumulated the highest leaf dry matter content relative to other N levels, at the four tested sampling dates, in both seasons. Similar findings were found by Choudhari and More (2002) who reported that dry matter accumulation in the cucumber plants during the crop period indicated that there was a linear increasing in dry matter, with increasing nitrogen and phosphorus fertilizer levels.

Results indicated that increasing foliar application of calcium from 270 to 390 ppm and then to 510 ppm, significantly, increased leaf dry matter content at the four tested sampling dates, in both seasons. Similar findings were previously documented by Kaya *et al.* (2002), who found that plants sprayed with 15 mM of $Ca(NO_3)_2$ accumulated more dry weight (16.30%) than the other treatments. The highest shoot dry weight was observed due to the spraying with $Ca(NO_3)_2$ at 15 mM .

The comparisons among the nine interactive treatments indicated that the combination treatment of 180Kg N fed^{-1} and 510ppm Ca concentration, significantly, produced the highest leaf dry matter content at the four tested sampling dates, in both seasons. The positive effects of N and Ca on leaf dry matter content may be due to that increasing the amount of N fertilizer encouraged the absorption of N, which enhanced the vegetative growth consequently; more dry weight can be formed.

Leaf mineral content except for the leaf Ca content appeared to be negatively correlated with the sam-

Table 9: Leaf dry matter content(%) of cucumber plants grown under greenhouse conditions as affected by nitrogen levels ,calcium spraying rates and their interactions, during the two winter seasons of 2009/2010 and 2010/2011 at 30, 60, 90 and 120 days after transplanting

Character / Season	Winter season of 2009/2010				Winter season of 2010/2011			
	Leaf dry matter content (%)				Leaf dry matter content (%)			
Treatments / Days	30	60	90	120	30	60	90	120
Nitrogen levels(kg N fed^{-1})								
80 kg	11.37^{C}	14.56^{C}	14.86^{C}	14.90^{C}	10.98^{C}	13.89^{C}	14.25^{C}	14.34^{C}
130 kg	12.80^{B}	15.80^{B}	16.05^{B}	16.24^{B}	12.38^{B}	15.25^{B}	15.44^{B}	15.52^{B}
180 kg	14.12^{A}	16.57^{A}	17.08^{A}	17.49^{A}	13.45^{A}	16.27^{A}	16.30^{A}	16.66^{A}
Calcium spraying rates (ppm)								
2 270 ppm	12.36^{C}	15.31^{C}	15.72^{C}	16.04^{C}	11.95^{B}	14.80^{B}	15.10^{B}	15.37^{B}
3 390 ppm	12.73^{B}	15.57^{B}	15.85^{B}	16.20^{B}	12.26^{AB}	14.98^{AB}	15.19^{B}	15.54^{A}
5 510 ppm	13.20^{A}	16.06^{A}	16.43^{A}	16.39^{A}	12.61^{A}	15.62^{A}	15.70^{A}	15.61^{A}
Nitrogen levels × Calcium rates								
80kg fed^{-1} × 270 ppm	11.03^{i}	14.00^{i}	14.30^{i}	14.80^{i}	10.53^{d}	13.16^{e}	13.80^{d}	14.30^{e}
80kg fed^{-1} × 390 ppm	11.27^{h}	14.38^{h}	14.50^{h}	14.89^{h}	10.94^{d}	13.88^{de}	14.00^{d}	14.39^{e}
80kg fed^{-1} × 510 ppm	11.82^{g}	15.30^{g}	15.78^{g}	15.01^{g}	11.49^{cd}	14.63^{cd}	14.95^{c}	14.34^{e}
130kg fed^{-1} × 270 ppm	12.58^{f}	15.62^{f}	15.87^{f}	16.00^{f}	12.25^{bc}	15.1^{bcd}	15.20^{c}	15.34^{d}
130kg fed^{-1} × 390 ppm	12.82^{e}	15.81^{e}	16.00^{e}	16.21^{e}	12.23^{bc}	4.98^{bcd}	15.33^{c}	15.54^{cd}
130kg fed^{-1} × 510 ppm	13.00^{d}	15.96^{d}	16.30^{d}	16.51^{d}	12.66^{ab}	5.64^{abc}	15.80^{b}	15.68^{c}
180kg fed^{-1} × 270 ppm	13.48^{c}	16.30^{c}	16.98^{c}	17.31^{c}	13.07^{ab}	16.13^{ab}	16.31^{a}	16.47^{b}
180kg fed^{-1} × 390 ppm	14.11^{b}	16.51^{b}	17.07^{b}	17.52^{b}	13.60^{a}	16.09^{ab}	16.23^{a}	16.69^{ab}
180kg fed^{-1} × 510 ppm	14.78^{a}	16.92^{a}	17.20^{a}	17.66^{a}	13.68^{a}	16.60^{a}	16.37^{a}	16.82^{a}

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

pling dates and markedly decreased with the progress of growth time. The highest mean values of leaf's N content were produced from the application of 180kg N fed^{-1}, or from the foliar spraying with 510 ppm Ca and, also ,from the combined effects of N and Ca (180kg N fed^{-1} and 510 ppm Ca). The previously reported results on the leaf's N- content may be due to that increasing the amount N fertilizer encouraged the absorption of N. These findings are, partially, in agreement with those found by Abd-Rabbo (2005), who noticed that the N percentages was declined during growth of cucumber. The highest nutrient concentrations were recorded in the fourth week from transplanting date (vegetative stage), but different nutrient concentration were progressively decreased after the second week after harvest. On the other hand, N percentage was increased with increasing nutrient level in nutrient solution. The presented results indicated that increasing nitrogen level, significantly and consistently raised the leaf contents of P and K at all growth periods (30, 60, 90 and 120 days), in both seasons. The highest leaf P and K contents were produced by applying the high level of nitrogen (180kgNfed^{-1}) at all sampling dates. These findings are, partially, in agreement with Gharib (2001)

Result showed the highest percentage of leaf P and K contents were obtained when the lowest Ca concentration was sprayed, in both seasons. However, increasing Ca concentration to the highest rate (510ppm) caused significant decreases in leaf P and K contents suggesting the presence of an antagonistic effect between the higher levels Ca and K uptake (Marschner ,1995).

Regarding the effect of N and Ca on leaf P and K contents, the obtained results indicated that the highest leaf P and K contents were produced when the combination between high N level (180kgN fed^{-1}) and the lowest Ca concentration (270 ppm) was applied, in both seasons. However, the lowest mean values of leaf P and K contents were obtained by the application of the combined treatment having the lowest N level (80 kg N fed^{-1}) plus the high Ca concentration (510 ppm), in both seasons. These results are in conformity with those reported by Song and Fujiyama (1996), who noticed that the maximum K application combined with high level of N diminished Ca uptake, due to the antagonistic relationship between these two nutrients. These findings are, partially, in agreement with those found by Gharib (2001), who showed that using the full dose of mineral nitrogen led to increasing fruit phosphorus content.

The reported results exhibited the presence of significant differences among the mean values of leaf Ca content due to the main effects or the interaction between nitrogen levels and calcium spraying rates, in both seasons. It was noticed that the highest leaf Ca content was obtained from the high nitrogen level (180 kg N fed^{-1}) or/ and the highest calcium concentration (510 ppm) was sprayed. On the other hand, the combination treatment having the first N level (80 kg N fed^{-1}) plus the lowest Ca concentration (270 ppm) recorded the lowest mean values of leaf Ca content, in both seasons. These findings are, partially, in agreement with those found by Cantliffe (1977), who reported that fertilizer influenced

cucumber tissue concentrations of P, K, and Ca in cucumber plants ,where increasing the nitrogen level led to increase the tissue concentration of K, Ca, Fe and Mn. This may have been due to more active root growth to slight changes in soil availability of these nutrients due to the addition of N fertilizer to the soil .In this concern, Hashem (2007) noticed the greatest content of Ca, K and Mg percentages in cucumber leaves and roots occurred when NO_3 consistent (100% NO_3 -0% NH_4) from the applied N fertilization in leaves and roots was applied.

Results indicated that fruit's dry matter content recorded the highest mean values due to 180Kg N fed^{-1} and / or 510ppm Ca concentration as shown in Table (9). The previously results on fruits dry matter content confirmed the results obtained by Altunlu and Gul (1999) found that nitrogen concentrations of 200 and 300 ppm increased dry matter content of cucumber plants as compared to 100 ppm. Similar, results were found by Choudhari and More (2002), who reported that dry matter accumulation in the cucumber plants during the crop period indicated that there was a linear increasing in dry matter, with increasing nitrogen and phosphorus fertilizer levels.

The obtained results indicated that the combined treatment that having the highest level of nitrogen and calcium (180 kg N fed^{-1} plus 510 ppm of Ca) exhibited the highest mean values of cucumber fruit's N, K, Ca contents, in both seasons as shown in Tables (10, 11, 12 and 13). However, the highest mean values for fruit's P content was obtained when the plants were fertilized with 180 kg N fed^{-1} and were sprayed with the low concentration Ca (270 ppm). Such results were obtained by Ruiz and Romero (1998), who found that increasing N fertilization, decreased the fruit Ca concentration. Gharib (2001) reported that using the full dose of mineral nitrogen led to increasing fruit phosphorus content. Similar findings were noticed by Kotsiras *et al.* (2002), who pointed out that the highest concentration of K, Ca, Mg and NO_3 in leaves was obtained when NO_3 constituted 75% or more of the total N in the nutrient medium but was reduced by increasing concentrations of NH_4. Similarly, Abd-Rabbo (2005) noticed that the Ca percentage in cucumber leaves and fruits was decreased with increasing potassium concentration of the nutrient solution with different nitrogen and phosphorus combination levels. Yann *et al.* (2011) indicated that fertilizer management practices need to assure that plant requirements are satisfied to achieve good yields of high-quality fruit .

CONCLUSION

Based on the obtained, it could be generally concluded that, the fertilization progaramme of cucumber plants grown in clay loam soil, under polyethylene plastic house might be included the application of nitrogen at the rate of 130kgNfed^{-1} in a fertigation system combined with the foliar spraying of calcium chloride at 390 ppm Ca concentration to insure balanced vegetative growth, produce higher yield potential with acceptable quality , under the prevailing environmental condition of Alexandria province and other similar regions .

Table 10 : Leaf nitrogen content (%) of cucumber plants grown under greenhouse conditions as affected by nitrogen levels ,calcium spraying rates and their interactions, during the two winter seasons of 2009/2010 and 2010/2011 at 30,60,90 and 120 days after transplanting

Character / Season	Winter season of 2009/2010				Winter season of 2010/2011			
	Leaf N content (%)				Leaf N content (%)			
Treatments / Days	30	60	90	120	30	60	90	120
Nitrogen levels(kg N fed^{-1})								
80 kg	2.80^{C}	2.71^{C}	2.63^{C}	2.58^{C}	2.82^{C}	2.73^{C}	2.65^{C}	2.56^{C}
130 kg	3.02^{B}	2.87^{B}	2.73^{B}	2.65^{B}	3.05^{B}	2.88^{B}	2.75^{B}	2.63^{B}
180 kg	3.29^{A}	3.21^{A}	3.12^{A}	3.00^{A}	3.31^{A}	3.23^{A}	3.14^{A}	2.98^{A}
Calcium spraying rates (ppm)								
2 270 ppm	2.98^{C}	2.86^{C}	2.77^{C}	2.69^{C}	3.00^{C}	2.88^{C}	2.80^{C}	2.67^{C}
3 390 ppm	3.02^{B}	2.92^{B}	2.81^{B}	2.73^{B}	3.04^{B}	2.94^{B}	2.83^{B}	2.71^{B}
5 510 ppm	3.11^{A}	3.01^{A}	2.90^{A}	2.81^{A}	3.14^{A}	3.03^{A}	2.92^{A}	2.79^{A}
Nitrogen levels × Calcium rates								
80kg fed^{-1} × 270 ppm	2.74^{h}	2.64^{i}	2.59^{g}	2.53^{g}	2.76^{h}	2.66^{i}	2.61^{g}	2.51^{h}
80kg fed^{-1} × 390 ppm	2.77^{g}	2.70^{h}	2.60^{g}	2.55^{g}	2.79^{g}	2.71^{h}	2.62^{g}	2.53^{g}
80kg fed^{-1} × 510 ppm	2.90^{f}	2.78^{g}	2.71^{ef}	2.65^{e}	2.92^{f}	2.80^{g}	2.73^{ef}	2.63^{e}
130kg fed^{-1} × 270 ppm	3.00^{e}	2.80^{f}	2.69^{f}	2.61^{f}	3.02^{f}	2.81^{f}	2.71^{f}	2.58^{f}
130kg fed^{-1} × 390 ppm	3.01^{e}	2.86^{e}	2.71^{e}	2.64^{e}	3.03^{e}	2.88^{e}	2.73^{e}	2.62^{e}
130kg fed^{-1} × 510 ppm	3.06^{d}	2.96^{d}	2.80^{d}	2.71^{d}	3.09^{d}	2.98^{d}	2.82^{d}	2.68^{d}
180kg fed^{-1} × 270 ppm	3.20^{c}	3.14^{c}	3.04^{c}	2.94^{c}	3.22^{c}	3.16^{c}	3.07^{c}	2.92^{c}
180kg fed^{-1} × 390 ppm	3.28^{b}	3.20^{b}	3.13^{b}	3.00^{b}	3.30^{b}	3.22^{b}	3.14^{b}	2.97^{b}
180kg fed^{-1} × 510 ppm	3.38^{a}	3.30^{a}	3.20^{a}	3.07^{a}	3.40^{a}	3.32^{a}	3.22^{a}	3.05^{a}

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

Table 11: Leaf phosphorus content (%) of cucumber plants grown under greenhouse conditions as affected by nitrogen levels ,calcium spraying rates and their interactions, during the two winter seasons of 2009/2010 and 2010/2011 at 30, 60, 90 and 120 days after trans planting

Character / Season	Winter season of 2009/2010				Winter season of 2010/2011			
	Leaf P content (%)				Leaf P content (%)			
Treatments / Days	30	60	90	120	30	60	90	120
Nitrogen levels(kg N fed^{-1})								
80 kg	0.646^{C}	0.583^{C}	0.566^{C}	0.503^{C}	0.654^{C}	0.590^{C}	0.573^{C}	0.512^{C}
130 kg	0.680^{B}	0.637^{B}	0.606^{B}	0.553^{B}	0.688^{B}	0.644^{B}	0.615^{B}	0.560^{B}
180 kg	0.750^{A}	0.702^{A}	0.665^{A}	0.612^{A}	0.757^{A}	0.708^{A}	0.674^{A}	0.621^{A}
Calcium spraying rates (ppm)								
2 270 ppm	0.714^{A}	0.659^{A}	0.629^{A}	0.582^{A}	0.721^{A}	0.667^{A}	0.636^{A}	0.590^{A}
3 390 ppm	0.692^{B}	0.643^{B}	0.612^{B}	0.556^{B}	0.701^{B}	0.650^{B}	0.622^{B}	0.565^{B}
5 510 ppm	0.670^{C}	0.619^{C}	0.596^{C}	0.530^{C}	0.678^{C}	0.626^{C}	0.605^{C}	0.538^{C}
Nitrogen levels × Calcium rates								
80kg fed^{-1} × 270 ppm	0.661^{f}	0.601^{g}	0.581^{f}	0.528^{f}	0.666^{f}	0.613^{g}	0.586^{e}	0.536^{f}
80kg fed^{-1} × 390 ppm	0.648^{f}	0.580^{h}	0.558^{g}	0.501^{h}	0.656^{g}	0.585^{h}	0.565^{f}	0.510^{h}
80kg fed^{-1} × 510 ppm	0.630^{g}	0.568^{i}	0.560^{g}	0.481^{i}	0.640^{h}	0.573^{i}	0.570^{f}	0.490^{i}
130kg fed^{-1} × 270 ppm	0.701^{d}	0.668^{d}	0.628^{d}	0.581^{d}	0.710^{d}	0.673^{d}	0.638^{c}	0.586^{d}
130kg fed^{-1} × 390 ppm	0.678^{e}	0.631^{e}	0.610^{e}	0.558^{e}	0.686^{e}	0.638^{e}	0.621^{d}	0.565^{e}
130kg fed^{-1} × 510 ppm	0.660^{f}	0.611^{f}	0.581^{f}	0.520^{g}	0.670^{f}	0.621^{f}	0.586^{e}	0.530^{g}
180kg fed^{-1} × 270 ppm	0.778^{a}	0.708^{b}	0.678^{a}	0.638^{a}	0.786^{a}	0.715^{b}	0.683^{a}	0.646^{a}
180kg fed^{-1} × 390 ppm	0.751^{b}	0.720^{a}	0.670^{b}	0.610^{b}	0.760^{b}	0.726^{a}	0.680^{a}	0.620^{b}
180kg fed^{-1} × 510 ppm	0.720^{c}	0.678^{c}	0.648^{c}	0.588^{c}	0.725^{c}	0.683^{c}	0.660^{b}	0.596^{c}

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

Table 12: Leaf potassium content (%) of cucumber plants grown under greenhouse conditions as affected by nitrogen levels ,calcium spraying rates and their interactions, during the two winter seasons of 2009/2010 and 2010/2011 at 30, 60, 90 and 120 days after transplanting

Character / Season	Winter season of 2009/2010				Winter season of 2010/2011			
	Leaf K content (%)				Leaf K content (%)			
Treatments / Days	30	60	90	120	30	60	90	120
Nitrogen levels(kg N fed^{-1})								
80 kg	2.97^{C}	2.91^{C}	2.85^{C}	2.76^{C}	2.99^{C}	2.92^{B}	2.84^{C}	2.77^{B}
130 kg	3.04^{B}	3.00^{B}	2.88^{B}	2.85^{B}	3.06^{B}	2.97^{B}	2.88^{B}	2.77^{B}
180 kg	3.20^{A}	3.08^{A}	2.97^{A}	2.93^{A}	3.21^{A}	3.08^{A}	2.95^{A}	2.94^{A}
Calcium spraying rates (ppm)								
2 270 ppm	3.12^{A}	3.03^{A}	2.95^{A}	2.87^{A}	3.14^{A}	3.03^{A}	2.94^{A}	2.85^{A}
3 390 ppm	3.07^{B}	3.02^{A}	2.90^{B}	2.85^{B}	3.09^{B}	3.03^{A}	2.91^{B}	2.83^{AB}
5 510 ppm	3.04^{C}	2.94^{B}	2.85^{C}	2.82^{C}	3.04^{C}	2.91^{B}	2.83^{C}	2.80^{B}
Nitrogen levels × Calcium rates								
80kg fed^{-1} × 270 ppm	3.01^{d}	2.97^{e}	2.90^{c}	2.77^{e}	3.03^{d}	2.97^{bcd}	2.86^{e}	2.74^{b}
80kg fed^{-1} × 390 ppm	2.97^{de}	2.92^{f}	2.86^{c}	2.76^{e}	3.01^{d}	2.93^{cde}	2.88^{de}	2.76^{b}
80kg fed^{-1} × 510 ppm	2.92^{e}	2.87^{g}	2.79^{d}	2.74^{f}	2.93^{e}	2.86^{e}	2.78^{f}	2.79^{b}
130kg fed^{-1} × 270 ppm	3.11^{c}	3.01^{c}	2.94^{b}	2.88^{c}	3.13^{c}	3.01^{b}	2.95^{b}	2.78^{b}
130kg fed^{-1} × 390 ppm	3.01^{d}	3.00^{d}	2.89^{c}	2.85^{d}	3.03^{d}	2.99^{bc}	2.89^{cd}	2.78^{b}
130kg fed^{-1} × 510 ppm	3.00^{d}	3.00^{d}	2.81^{d}	2.77^{e}	3.00^{d}	2.91^{de}	2.80^{f}	2.74^{b}
180kg fed^{-1} × 270 ppm	3.24^{a}	3.11^{b}	3.02^{a}	2.98^{a}	3.25^{a}	3.11^{a}	3.00^{a}	2.98^{a}
180kg fed^{-1} × 390 ppm	3.21^{ab}	3.16^{a}	2.94^{b}	2.93^{b}	3.22^{ab}	3.17^{a}	2.95^{b}	2.94^{a}
180kg fed^{-1} × 510 ppm	3.17^{bc}	2.97^{e}	2.96^{b}	2.90^{c}	3.17^{bc}	2.97^{bcd}	2.91^{bc}	2.91^{a}

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

Table 13 : Leaf calcium content (mg/ 100 g D.w) of cucumber plants grown under greenhouse conditions as affected by nitrogen levels, calcium spraying rates and their interactions, during the two winter seasons of 2009/2010 and 2010/2011 at 30, 60, 90 and 120 days after transplanting

Season / Character	Winter season of 2009/2010 Leaf Ca content (mg/ 100 g D.w)				Winter season of 2010/2011 Leaf Ca content (mg/ 100 g D.w)			
Days / Treatments	**30**	**60**	**90**	**120**	**30**	**60**	**90**	**120**
Nitrogen levels(kg N fed^{-1})								
80 kg	0.765^{C}	0.767^{C}	0.788^{C}	0.832^{C}	0.768^{C}	0.772^{C}	0.788^{C}	0.841^{C}
130 kg	0.788^{B}	0.796^{B}	0.805^{B}	0.864^{B}	0.789^{B}	0.799^{B}	0.806^{B}	0.869^{B}
180 kg	0.814^{A}	0.828^{A}	0.879^{A}	0.991^{A}	0.815^{A}	0.830^{A}	0.878^{A}	0.996^{A}
Calcium spraying rates (ppm)								
2 270 ppm	0.784^{C}	0.790^{C}	0.818^{C}	0.884^{C}	0.786^{C}	0.794^{B}	0.819^{C}	0.888^{B}
3 390 ppm	0.789^{B}	0.795^{B}	0.823^{B}	0.893^{B}	0.790^{B}	0.797^{B}	0.822^{B}	0.898^{B}
5 510 ppm	0.794^{A}	0.805^{A}	0.832^{A}	0.910^{A}	0.796^{A}	0.810^{A}	0.832^{A}	0.920^{A}
Nitrogen levels × Calcium rates								
80kg fed^{-1} × 270 ppm	0.760^{i}	0.761^{h}	0.782^{i}	0.821^{i}	0.764^{g}	0.768^{f}	0.782^{h}	0.831^{f}
80kg fed^{-1} × 390 ppm	0.765^{h}	0.761^{h}	0.788^{h}	0.833^{h}	0.766^{g}	0.767^{f}	0.788^{g}	0.843^{ef}
80kg fed^{-1} × 510 ppm	0.770^{g}	0.780^{g}	0.795^{g}	0.841^{g}	0.775^{f}	0.783^{e}	0.795^{f}	0.850^{e}
130kg fed^{-1} × 270 ppm	0.781^{f}	0.790^{f}	0.800^{f}	0.856^{f}	0.782^{e}	0.791^{de}	0.805^{e}	0.857^{de}
130kg fed^{-1} × 390 ppm	0.789^{e}	0.797^{e}	0.803^{e}	0.865^{e}	0.790^{d}	0.797^{cd}	0.801^{e}	0.868^{cd}
130kg fed^{-1} × 510 ppm	0.795^{d}	0.801^{d}	0.813^{d}	0.873^{d}	0.795^{c}	0.810^{c}	0.813^{d}	0.881^{c}
180kg fed^{-1} × 270 ppm	0.811^{c}	0.822^{c}	0.871^{c}	0.975^{c}	0.812^{b}	0.823^{b}	0.870^{c}	0.976^{b}
180kg fed^{-1} × 390 ppm	0.813^{b}	0.827^{b}	0.878^{b}	0.982^{b}	0.814^{b}	0.828^{ab}	0.876^{b}	0.983^{b}
180kg fed^{-1} × 510 ppm	0.818^{a}	0.836^{a}	0.889^{a}	1.01^{a}	0.819^{a}	0.837^{a}	0.887^{a}	1.03^{a}

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

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تأثير مستويات مختلفة من النيتروجين ورش الكالسيوم على النمو الخضري والجودة في الخيار تحت ظروف الصوب الزراعية

عبد العزيز الشرقاوى – سناء مرسى العربى – ابراهيم غنيم –شيماء محمد رجب

قسم الخضر – كلية الزراعة – جامعة الإسكندرية

أجريت التجربة خلال موسمى الزراعة فى شتاء (٢٠٠٩/٢٠١٠)، (٢٠١٠/٢٠١١) وقد اشتملت كل مكررة على جميع التوافيق الممكنة بين ٣ معدلات من التسميد النيتروجينى وهى:(٨٠-١٣٠-١٨٠ كيلو جرام نيتروجين/ الفدان)بإستخدام نترات الامونيوم (٪ ٥ ,٣٣) و تم توزيعها فى القطع الرئيسية بالإضافة إلى ثلاث مستويات من الرش الورقى بكلوريد الكالسيوم وهى (٢٧٠-٣٩٠ -٥١٠ جزء فى المليون) وتم توزيعها فى القطع تحت الرئيسية وتتلخص النتائج التي حصل عليها على النحو التالي:

بزيادة معدلات التسميد النيتروجينى تدريجيا من (٨٠إلى ١٣٠ إلى١٨٠ كيلو جرام نيتروجين/ الفدان قد أدى إلى زيادة إرتفاع النبات و عدد الأوراق النباتية ومتوسط مساحة الورقة فى كلا الموسمين. وأوضحت النتائج بزيادة التركيز الورقى من٢٧٠ إلى ٣٩٠ إلى٥١٠ جزء فى المليون) من كلوريد الكالسيوم قد أدى إلى زيادة معنوية فى إرتفاع النبات وعدد الأوراق النباتية ومتوسط مساحة الورقة النباتية خلال مراحل النمو لمختلفة فى كلا الموسمين.

أوضحت النتائج أنه باستخدام المعدل العالى من التسميد النيتروجينى ١٨٠ كيلو جرام نيتروجين/ فدان مع المعدل العالى(٥١٠ جزء فى المليون) من الرش بالكالسيوم قد أعطت أعلى زيادة معنوية لعدد الأوراق/ نبات و متوسط المساحة الورقية لكل نبات خلال موسمى الزراعة.

كما لوحظ أعلى كفاءة إنتاجية فى جميع الصفات الخاصة بالمحصول باستخدام المعدل المتوسط من التسميد النيتروجينى (١٣٠ كيلو جرام نيتروجين/ الفدان) بالتداخل مع الرش بكلوريد الكالسيوم لكل من المعدل المتوسط (٣٩٠ جزء فى المليون) و المعدل العالى (٥١٠ جزء فى المليون) فى كلا الموسمين. سجلت النتائج وجود زيادة معنوية فى محتوى الأوراق من الكلورفيل بالإضافة إلى محتوى الأوراق من المادة الجافة عند زيادة التركيز الورقى من كلوريد الكالسيوم ٢٧٠ إلى ٣٩٠ إلى٥١٠ جزء فى المليون)

أوضحت النتائج أن التداخل بين المعدل المرتفع من التسميد النيتروجينى (١٨٠ كيلو جرام نيتروجين/ الفدان) مع الرش بكلوريد الكالسيوم عند تركيز (٥١٠ جزء فى المليون) قد أعطت أعلى القيم لمحتوى الثمار منN وK وCa فى كلا الموسمين بينما أدى التسميد النيتروجينى المرتفع مع الرش بكلوريد الكالسيوم عند تركيز (٢٧٠ جزء فى المليون) إلى أعطاء أعلى القيم لمحتوى الثمار من الفوسفور فى كلا الموسمين. في ضوء النتائج التي قد تم الحصول عليها ومناقشتها، من التجربة الثانية، يمكن أن نوصى بتضمن برنامج تسميدى لنباتات الخيار في الأراضى الطمية الطينية، تحت الصوب البلاستيكية على إضافة النيتروجين بمعدل١٣٠ كيلو جرام نيتروجين للفدان في نظام الرى بالتنقيط جنبا إلى جنب مع الرش الورقى بكلوريد الكالسيوم ٣٩٠ جزء في المليون لضمان النمو الخضري المتوازن لنبات الخيار ،و إنتاج أعلى لمحصول مبكر وكلى للثمار بأفضل مواصفات جودة وذلك تحت الظروف البيئية السائدة في محافظة الإسكندرية ومناطق أخرى مشابهة.

Effect of N forms and rates on the growth and head quality of endive grown under sandy soil condition

Ali,R.A.M.[(1)] ,Abd-ElKader, D.Y.[(2)], Shimaa M. Hassan[(3)]
1- Vegetable Department, Agriculture Rsearch Center, El-Dokki, Egypt.
2- Vegetable Department, Alexandria Univerity, Egypt.

ABSTRACT

Endive plants (*cv*. 'Fransawy') were grown for 11 weeks during winter seasons of 2013/ 2014 and 2014/2015 under sandy soil field condition to investigate the possibility of giving the mineral nitrogen fertilization at suitable sources and rates in harmony with the requirements of different endive growth stages. Nitrogen forms, used, were ammonium sulfate [$(NH_4)2SO_4$), (21% N)],ammonium nitrate [(NH_4-NO_3), (33% N)] and urea [($CO(NH_2)_2$) (46% N)], whereas, nitrogen rates were; 60, 90 and 120 kg fed.$^{-1}$. The effect of the above mentioned sources and rates as well as theirs interactions on vegetative growth, head characters, chemical characters of endive plants were recorded. Generally, the results showed that endive plants that treated by ammonium sulfate gave the highest mean values of all most characters in these study. Also,endive plants receiving N rate up to 120 kg fed.$^{-1}$ were achieved the highest vegetative, head and quality characters (e.g. plant height, number of leaves plant^{-1} leaf area plant^{-1}, dry weight of leaves, head weight and diameter V.C and phosphorous).Furthermore, the total phenol, and potassium were improved with N rate at 90 kg fed.$^{-1}$ whereas, the nitrate content of leaves was correlated with increasing nitrogen applied rate, as urea form, up to 120 kg fed.$^{-1}$.

Keywords: Nitrogen source, nitrogen levels, head quality, endive.

INTRODUCTION

Endive, (*Cichorium endivia* L.) is an annual and cool season leafy vegetable crop belongs to family (Compositae). It is a plant enjoying great popularity in thecountries of Western Europe. Where, it is one of the best-selling salad vegetable crops.It is a moisturizer for the human body and gives a sense of satiety. In addition, it is characterized by considerable nutritional value and a distinctive, slightly bitter taste. Therefore, it often used in fast food, quick serve restaurants and preparation the low-energy meals. In Egypt, it is a little-known leaf vegetable and considered as untraditional vegetable crop (Maraey *et al*., 2016; Abd-ElKader and Shimaa, 2016). As a leaf vegetable of a high growth rate, the endive has high nutritional demands in terms of nitrogen. Under favorable conditions, it takes up and accumulates this element in large quantities in the form of nitrates. Endive, like the lettuce, is a slow-growing plant that often accumulates 70:80 % of its head biomass and N uptake just during the last three to four weeks before the harvest (Gardener and Pew, 1979; Thomas and Thomas, 1995; Santos *et al*., 2013 and Mirdad, 2016) .

Therefore, the doses of N fertilizer preferably added in harmony with the different endive growth stages. Whereas, small doses of the N fertilizer must add during the early growth stage, while the great doses added during the last month before the harvest of endive plants.On the other hand, in sandy soil, the most important problems facing the production of leafy vegetable crops which have shallow root plants like endive under drip irrigation system is restricting the roots spread of crops and that concentrated only in the soil wetness zone, which requires to frequent supply of irrigation water and fertilizers (fertigation) for the plant root zone soil (Feleafel *et al*., 2014). Moreover, most N fertilizers used through the drip irrigation system are a high solubility and the crop uses about 50% of the amount applied and the rest lost by lixiviation in the soil, or volatilization into the atmosphere.Nitrogen is the major mineral nutrient that promotes sufficient leaf vegetables growth and consequently yield. It is absorbed by roots either as ammonium NH_4^+ and nitrate NO_3^- (Tsouvaltzis *et al*., 2014) .

Leafy vegetables required considerable high amount of N fertilizer.Unfortunately, it enhances the accumulation of nitrates in their edible parts (Wang and Li, 2004; Nurzynska-Wierdak, 2009 and Kaymak, 2013). Also, accumulation of NO^-_3 in field grown depend on fertilizer form used and doses applied (Inal *et al*., 2001 and Porto *et al.,* 2008).The nitrogen treatment affects vegetative growth, head characters and nitrate concentration of lettuce plants (Boroujerdnia and Ansari, 2007 and M'hamdi *et al*., 2014). Nitrogen fertilization showed a highly linear relationship between nitrate accumulation and water content (Qiu *et al*., 2014).

Vegetable's growers use excessive levels of nitrogen for leafy vegetables to give the highest yield (Porto *et al.*, 2008),overlooking pollution in soil and water as well as the quality (Wang *et al*., 2008).The source and quantity of nitrogen available to plants can affect the quality and yield of leafy vegetables (Wang *et al*., 2008 and Hassan *et al*., 2012).Significant effect of the fertilization forms and nitrogen application rate on the biological value of the endive and lettuce cultivars was documented (Sowinska and Uklanska, 2010 and Zeka *et al*., 2014). In Egypt, research information is quite scarce on response of endive to application of nitrogen sources and quantity.

Therefore, the aim of this study is to investigate the possibility of giving suitable sources and rate of mineral nitrogen fertilization on field grown endive by using fertigation system under sandy soil conditions.

MATERIALS AND METHODS

Two field experiments of the present study were conducted during the growing seasons of 2013/2014 and 2014/2015 at South Tahrir Horticulture Research Station, (Ali Moubarak village), El-Bostan region, Behera Governorate , Egypt. Seeds for endive *(Cichorium endivia* L.) Fransawy variety which is the known curly leaves (Sowinska and Uklanska, 2010), were sown on first September and the seedlings were transplanted into the field 30 days after sowing , in two lines on each row. The row spacing was 20 cm between the transplants and 70 cm between the lines in a row.

Treatments

The trial consisted of 9 treatments comprising combinations of three N forms. The nitrogen forms were ammonium sulfate [$(NH_4)2SO_4$), (21% N)],ammonium nitrate [$(NH_4$-NO_3), (33% N)] and urea [$(CO(NH_2)_2)$ (46% N)]. Nitrogen fertigation rates were; 60, 90 and 120 kg fed.$^{-1}$ and fertigation were executed as three doses weekly during the various growth stages of endive plants. The 40% of each studied rate was added during first six week. However, the rest of each rate 60% was applied during the last four week. The N fertigation process started after a week of transplanting and continue up to the 11th week. Phosphorus and potassium fertilizers were fertigated at rates of 70 and 100 kg P_2O_5 and K_2O fed^{-1}., respectively. Phosphoric acid (58%) and potassium sulfate (48% K_2O) were the P_2O_5 and K_2O sources, receptively. They were injected directly into the irrigation water, using a venture injector, mixed with nitrogen fertilizers, at fixed doses (33 doses season).The drip irrigation system consisted of laterals GR of 16 mm in diameter with drippers at 0.3 m distance. The drippers had a discharge rate 4 L h^{-1}. Irrigation water was applied through the drip irrigation system.

All agricultural practices were done as commonly followed in the commercial production of endive in the drip irrigation system.

Prior to the initiation of the experiments, soil samples were collected and analyzed according to the methods described by (Page *et al*.,1982). The results of the analysis are shown in (Table 1).

The experimental layout was a split-plot system in randomized compete block design, with three replications. Nitrogen forms were arranged as the main plots, while the three nitrogen rates were considered as the sub-plots.

Data Recorded

(a) Vegetative growth characteristics

The samples were taken at harvest stage after at 80 days after transplanting, ten plants were taken from each sub plot , to measure plant height (cm), number of leaves plant^{-1} and leaf area plant^{-1} (cm^2). Dry matter of leaves (%) was determined after drying the leaves at 70° C for 48h.

(b) Head characters

The sample were taken at harvest stage ,in both seasons. Ten plants were taken from each sub plot, to measured head fresh weight (g) and head diameter (cm) .

(c) chemical constituents of leaves

Vitamin C was measured by titration with iodide potassium (A.O.A.C.1990).Total soluble phenols were determined according to (Scalbert *et al*., 1989) and expressed as (mg.g^{-1}fw).The nitrates were determined according to (Cataldo *et al*.,1975).

Total nitrogen, phosphorus and potassium content of the leaves were expressed as a percentage on the dry weight basis according to (Cottenie, 1980).

Statistical analysis

Treatment means were separated and compared using the Revised L.S.D test at 0.05 level of significance according to (Snedecor and Cochran,1980).The statistical analysis was performed using CoStat software package for Windows.

RESULTS AND DISCUSSION

Vegetative growth characters:

It is obvious from the results in (Table 2) that there were significant increases on plant height, leaf area plant^{-1} and dry matter of endive leaves as a result of applied ammonium sulphate as a source of nitrogen compared with the two other sources used (ammonium nitrate and urea), in both seasons. However, no significant differences were detected in the number of leaves plant^{-1}. Similar results were reported by (Bozkurt *et al*.,2009; Engelbrecht *et al*.,2010; Dursun *et al*., 2013 and Zeka *et al*., 2014) on lettuce, swiss chard, rocket and spinach plant, respectively.

Progressive, significant, increases in plant height, number of leaves plant^{-1}, leaf area plant^{-1}and dry matter

Table 1: Some soil physical and chemical properties of the experimental sites, during the two winter seasons of 2013/ 2014 and 2014/2015

Properties / Seasons	Physical				Chemical		
	pH	E.C (dsm-1)	Sand (%)	Texture	Total N (%)	Available P (ppm)	K (meq/100 g soil)
2013/2014	8.21	0.59	92.90	Sandy	0.06	4.14	14
2014/2015	8.10	0.55	92.75	Sandy	0.05	3.88	12

of leaves occurred as a result of increasing N applied rate from 60 kg fed^{-1} to 90 and further to 120 kg fed^{-1} (Table 2). The trend was identical in both seasons. The enhancing effect of N on vegetative characters of leafy vegetables may be attributed to the promoting influence of N on the meristamic activity for producing more tissues and organs (Marschner, 1994). These finding appeared to agree with the results reported by many researchers (Boroujerdnia and Ansari, 2007; Lui *et al*., 2014; M'hamdi *et al*., 2014 and Mirdad, 2016).

The interactions of N sources and rates had significant effect on the vegetative growth characters of endive, in both years (Table 2) generally, the highest mean magnitudes for plant height, leaf area plant^{-1} and dry matter of leaves were attained with the application of ammonium sulphate at the rate of 120 kg fed^{-1}, while, the best significant result for number of leaves plant^{-1} was attained due to the combined application of urea at 120 kg fed^{-1} and ammonium nitrate at 120 kg fed^{-1}, in the first and second seasons, respectively. The obtained results matched well with those reported by (Abu- Rayyan, 2004) who stated that dry mass of lettuce was influenced by nitrogen level and nitrogen source. The greatest dry mass for lettuce was obtained where ammonium sulphate was applied as nitrogen source.

Head characters:

Fertilization with different sources of nitrogen was significantly associated with higher averages of head weight and diameter of endive (Table 3) through the two growing seasons. The obtained results clarified that the highest mean value of the head weight and diameter were given by the application of ammonium sulphate, in both growing seasons. However, the differences among the application of the various nitrogen sources on the values of head diameter, in the first seasons were found too small to be significant.

According to the results shown in (Table 3) there were significant increases on head weight and diameter due to the successive increases in N rate that gave the highest value was 120 kg N fed.$^{-1}$, the obtained results confirmed the findings of (Türkmen *et al*., 2004 and Mirdad, 2016) who found that nitrogen application significantly affected the head weight of lettuce. Also,(Boroujerdnia and Ansari, 2007) showed that nitrogen status significantly influenced yield attributes of lettuce.

Concerning the interaction effects between the different nitrogen source and nitrogen rates on head weight and diameter the results generally, indicated that the endive plant which applied with ammonium sulphate combined with nitrogen rate of 120 kg fed.$^{-1}$ gave the highest mean value of head weight and diameter characters compared with those of the other treatments, in both growing seasons.

Chemical constituents of leaves

Concerning the effects of different nitrogen sources on the chemical constituents of endive leaves, the results in (Table 4) showed that nitrogen sources, significantly, affected the chemical contents of leaves. Ammonium sulphate tended to increase the content of V.C, total phenol, potassium and phosphorus contents compared to the two used sources (ammonium sulphate and urea), the differences did not reach the significance level, in most cases. Whereas, ammonium nitrate gave the highest values of nitrogen content in, the two seasons. The highest value of nitrate content was obtained by using urea as a source of nitrogen compared with ammonium nitrate and ammonium sulphate, respectively. Similar results, regarding the NO_3-N content in leafy vegetable, were reported by (Santamaria *et al*., 2001; Stefanelli *et al*., 2011) who found that the use of fertilizers based on ammonia $NH4^+$ or on a mixture of NO_3^- and $NH4^+$ can reduce NO_3^- content in plants.

Results presented in (Table 4), generally indicated that the application of nitrogen at the rate of 120 kg fed.$^{-1}$ gave the highest significant mean values of chemical constituents of endive leaves (V.C, nitrate content, total N and P content) than those of the two other rates used (90 and 60 kg N fed.$^{-1}$), in both seasons. On the other side, total phenol and K content reflected reverse results. The highest values of these constituents were obtained due to the application of 60 kg N fed.$^{-1}$, in both seasons. The exception for K content was found in the first season, as the differences between 60 and 90 kg N fed.$^{-1}$ were insignificant. Similar findings were obtained by (Stefanelli *et al*., 2011) who found that high nitrogen availability is known to inhibit phenolic production and subsequently antioxidant capacity, in a range of leafy vegetables. In lettuce, fertilization treatments that resulted in relatively high soil N content caused a reduction in phenolic content, specifically coumaric acid.

The enhancing effect of raising nitrogen rates added to the growing plants on leaf NO_3-N content complemented the results of the nitrate increasing by increase N rate these results might be accepted on the basis that the soil of the experimental site contained relatively low amounts of N ranged from (0.05 to 0.06%) which were not sufficient to supply enough for the plants, thus increasing nitrogen rate led to differential rates of NO_3 uptake and may be related to the difference in nitrate reductase activity as mentioned by Hageman and Flesher (1960), who demonstrated an inverse relationship between nitrate content of leaves and nitrate reductase activity.

With respect to the interactions between different applied N sources and rates (Table 4), clearly indicated that ammonium nitrate combined with 120 kg fed.$^{-1}$ gave the highest value of V.C and total nitrogen, in both seasons. Nitrate content of leaves affected by the combined treatment of urea plus 120 kg N fed.$^{-1}$, in both seasons.

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Table 2 : Vegetative growth characters of endive plants as affected by nitrogen source ,nitrogen levels and their interactions ,during the two winter seasons of 2013/2014 and 2014/2015

Characters	Plant height (cm)		Number of leaves plant^{-1}		Leaves area plant^{-1}		Dry matter of leaves (%)	
Treatments / Season	Winter 2013/2014	Winter 2014/2015	Winter 2013/2014	Winter 2014/2015	Winter 2013/2014	Winter 2014/2015	Winter 2013/2014	Winter 2014/2015
Nitrogen source								
Ammonium sulphate	25.03^{A}	25.73^{A}	21.66^{A}	21.55^{A}	223.48^{A}	203.14^{A}	15.27^{A}	15.27^{A}
Ammonium nitrate	23.65^{B}	24.15^{B}	21.33^{A}	22.22^{A}	203.85^{C}	183.65^{B}	14.45^{B}	14.45^{B}
Urea	23.31^{B}	23.94^{B}	21.22^{A}	22.22^{A}	213.75^{B}	198.51^{A}	15.03^{AB}	15.03^{AB}
Nitrogen levels(kg N fed^{-1})								
60 kg fed^{-1}	22.38^{C}	23.15^{C}	20.11^{B}	21.11^{B}	190.22^{C}	171.06^{C}	13.8^{C}	13.8^{C}
90 kg fed^{-1}	24.45^{B}	25.03^{B}	21.77^{A}	21.77^{B}	212.68^{B}	195.68^{B}	14.76^{B}	14.76^{B}
120 kg fed^{-1}	25.15^{A}	25.64^{A}	22.33^{A}	23.11^{A}	238.18^{A}	218.55^{A}	16.2^{A}	16.2^{A}
Nitrogen source X Nitrogen levels (kg fed^{-1})								
Ammonium sulphate × 60	23.13^{de}	24.73^{cd}	19.66^{c}	20.66^{e}	196.4^{ef}	180.80^{c}	13.96^{d}	13.26^{ef}
Ammonium sulphate × 90	25.53^{ab}	25.96^{ab}	22.00^{ab}	21.00^{de}	220.5^{c}	199.53^{b}	15.23^{b}	14.23^{bc}
Ammonium sulphate × 120	26.43^{a}	26.5^{a}	22.33^{ab}	23.00^{ab}	253.56^{a}	229.10^{a}	16.63^{a}	15.63^{a}
Ammonium nitrate × 60	21.76^{f}	22.53^{e}	21.00^{bc}	21.33^{cde}	185.9^{f}	163.43^{d}	13.70^{d}	13.10^{f}
Ammonium nitrate × 90	24.16^{cd}	24.76^{cd}	22.00^{ab}	22.00^{bcd}	205.33^{de}	185.83^{c}	14.23^{cd}	13.76^{cde}
Ammonium nitrate × 120	25.03^{bc}	25.16^{c}	22.00^{ab}	23.33^{a}	220.33^{c}	201.70^{b}	15.43^{b}	14.43^{b}
Urea × 60	22.26^{ef}	22.20^{e}	19.66^{c}	21.33^{cde}	188.36^{f}	168.96^{d}	13.73^{d}	13.50^{def}
Urea × 90	23.66^{d}	24.36^{d}	21.33^{ab}	22.33^{abc}	212.23^{cd}	201.70^{b}	14.83^{bc}	14.00^{bcd}
Urea × 120	24.00^{cd}	25.26^{bc}	22.66^{a}	23.00^{ab}	240.66^{b}	224.86^{a}	16.53^{a}	15.73^{a}

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

Table 3: Head weight and diameter of endive plants as affected by nitrogen source ,nitrogen levels and their interactions, during the two winter seasons of 2013/2014 and 2014/2015

Characters	Head weight (gm)		Head diameter (cm)	
Treatments / Season	Winter 2013/2014	Winter 2014/2015	Winter 2013/2014	Winter 2014/2015
Nitrogen source				
Ammonium sulphate	296.11A	294.44A	16.38A	16.00A
Ammonium nitrate	290.00B	280.00B	16.03A	15.51B
Urea	287.77B	277.77B	16.38A	15.66B
Nitrogen levels(kg N fed^{-1})				
60 kg fed^{-1}	273.33C	266.11C	15.36C	14.76C
90 kg fed^{-1}	291.66B	282.22B	16.07B	15.61B
120 kg fed^{-1}	308.88A	303.88A	17.36A	16.8A
Nitrogen source X Nitrogen levels (kg fed^{-1})				
Ammonium sulphate × 60	273.33e	278.33d	15.26de	15.00de
Ammonium sulphate × 90	298.33c	291.66c	16.36b	15.60c
Ammonium sulphate × 120	316.66a	313.33a	17.53a	17.40a
Ammonium nitrate × 60	275.00e	261.66e	15.10e	14.46e
Ammonium nitrate × 90	288.33d	280.00d	15.90bc	15.70c
Ammonium nitrate × 120	306.66b	298.33bc	17.10a	16.36b
Urea × 60	271.66e	258.33e	15.73cd	14.83e
Urea × 90	288.33d	275.00d	15.96bc	15.53cd
Urea × 120	303.33bc	300.00b	17.46a	16.63b

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

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Table 4: Chemical constituents of endive leaves as affected by nitrogen source ,nitrogen levels and their interactions ,during the two winter seasons of 2013/2014 and 2014/2015

Characters	V.C (mg.100 g -1f.w)		Total phenol (ppm)		Nitrate content (mg. kg^{-1} FW)		Nitrogen		Potassium		Phosphorus	
Treatments / Seasons	Winter 2013/2014	Winter 2014/2015	Winter 2013/2014	Winter 2014/2015	Winter 2013/2014	Winter 2014/2015	Winter 2013/2014	Winter 2014/2015	Winter 2013/2014	Winter 2014/2015	Winter 2013/2014	Winter 2014/2015
Nitrogen source												
Ammonium sulphate	11.65^{A}	11.95^{A}	12.10^{A}	12.41^{A}	333.88^{C}	323.33^{c}	1.46^{B}	1.42^{B}	2.50^{A}	2.48^{A}	0.055^{A}	0.056^{A}
Ammonium nitrate	11.52^{A}	11.86^{A}	11.74^{B}	12.02^{C}	341.66^{B}	332.77^{b}	1.54^{A}	1.50^{A}	2.34^{B}	2.35^{C}	0.052^{B}	0.052^{C}
Urea	10.93^{B}	11.30^{A}	12.03^{A}	12.21^{B}	352.77^{A}	344.44^{a}	1.45^{B}	1.43^{B}	2.50^{A}	2.42^{B}	0.051^{B}	0.054^{B}
Nitrogen levels(kg N fed^{-1})												
60 kg fed^{-1}	1.38^{C}	1.35^{C}	12.26^{A}	12.57^{A}	325.55^{C}	317.77^{c}	1.38^{C}	1.35^{C}	2.51^{A}	2.64^{A}	0.052^{B}	0.051^{C}
90 kg fed^{-1}	1.48^{B}	1.45^{B}	12.04^{B}	12.29^{B}	341.11^{B}	333.33^{b}	1.48^{B}	1.45^{B}	2.50^{A}	2.33^{B}	0.052^{B}	0.054^{B}
120 kg fed^{-1}	1.59^{A}	1.55^{A}	11.57^{C}	11.78^{C}	361.66^{A}	349.44^{a}	1.59^{A}	1.55^{A}	2.32^{B}	2.28^{C}	0.053^{A}	0.056^{A}
Nitrogen source × Nitrogen levels(kg fad.$^{-1}$)												
Ammonium sulphate × 60	1.37^{f}	1.32^{f}	12.46^{a}	12.81^{a}	320.00^{f}	311.66^{g}	1.37^{f}	1.32^{f}	2.33^{f}	2.70^{a}	0.054^{c}	0.052^{d}
Ammonium sulphate × 90	1.44^{d}	1.40^{e}	12.03^{bcd}	12.43^{b}	333.33^{e}	321.66^{e}	1.44^{d}	1.40^{e}	2.74^{a}	2.39^{c}	0.055^{b}	0.057^{b}
Ammonium sulphate × 120	1.57^{b}	1.54^{b}	11.82^{cd}	12.00^{cd}	348.33^{c}	336.66^{c}	1.57^{b}	1.54^{b}	2.44^{e}	2.35^{c}	0.057^{a}	0.058^{a}
Ammonium nitrate × 60	1.42^{e}	1.41^{e}	11.98^{cd}	12.41^{b}	323.33^{f}	316.66^{f}	1.42^{e}	1.41^{e}	2.55^{c}	2.60^{b}	0.053^{d}	0.050^{f}
Ammonium nitrate × 90	1.55^{c}	1.50^{c}	12.05^{bc}	12.08^{c}	340.00^{d}	331.66^{d}	1.55^{c}	1.50^{c}	2.27^{g}	2.25^{de}	0.052^{e}	0.052^{d}
Ammonium nitrate × 120	1.65^{a}	1.58^{a}	11.19^{e}	11.57^{e}	361.66^{b}	350.00^{b}	1.65^{a}	1.58^{a}	2.20^{h}	2.21^{e}	0.051^{f}	0.053^{c}
Urea × 60	1.36^{f}	1.33^{f}	12.34^{ab}	12.49^{b}	333.33^{e}	325.00^{e}	1.36^{f}	1.33^{f}	2.67^{b}	2.64^{b}	0.051^{f}	0.051^{e}
Urea × 90	1.45^{d}	1.44^{d}	12.04^{bcd}	12.35^{b}	350.00^{c}	246.66^{b}	1.45^{d}	1.44^{d}	2.50^{d}	2.35^{c}	0.051^{f}	0.053^{c}
Urea × 120	1.55^{c}	1.54^{b}	11.70^{d}	11.78^{de}	375.00^{a}	361.66^{a}	1.55^{c}	1.54^{b}	2.32^{f}	2.28^{d}	0.053^{d}	0.057^{b}

* Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Revised L.S.D test at 0.05 level of probability.

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الملخص العربي

تأثير مصادر ومعدلات التسميد النيتروجينى على نمو وصفات الجودة للرأس فى نبات الهندباء تحت ظروف الأراضى الرملية

رمضان عبد العاطى محمد[١] – دعاء يحيى الدين حماد[٢] – شيماء محمد رجب على حسن[٣]

١– قسم الخضر، مركز البحوث الزراعية، الدقي، جمهورية مصر العربية.
٢– قسم الخضر، كلية الزراعة (الشاطبي)، جامعة الإسكندرية، جمهورية مصر العربية.

نفذت هذه الدراسة على محصول الهندباء – الصنف الفرنساوي – تحت ظروف الأراضي الرملية بمزرعة على مبارك – منطقة النوبارية بالإسكندرية و ذلك خلال موسمي شتاء عامي ٢٠١٣–٢٠١٤ و ٢٠١٤–٢٠١٥ و ذلك بهدف اختيار المصدر المناسب من مصادر السماد النتروجين المعدني (سلفات النشادر و نترات الأمونيوم و اليوريا) و استخدام ثلاث معدلات من التسميد النتروجينى المعدني (٦٠و ٩٠ و١٢٠ كجم للفدان) و التداخل بينهم على صفات النمو الخضري و صفات الرأس و الصفات الكيميائية في أوراق نبات الهندباء. و أظهرت النتائج بصفة عامة أن المعاملة باستخدام المصدر النتروجينى سلفات الأمونيوم أعطى اعلي النتائج لصفات النمو الخضري و صفات الرأس والمحتوى الكيميائي في هذه الدراسة و أن المعدل النتروجينى ١٢٠ كجم للفدان أعطى أعلى زيادة معنوية في قيمة المتوسط لصفات النمو الخضري و صفات الرأس والمحتوى الكيماوي .أيضا أوضحت النتائج أن المعدل ٩٠ كجم للفدان أعطى متوسطات للمحتوى الكلى للفينولات والنسبة المئوية للبوتاسيوم في الأوراق, و أظهرت النتائج أن تراكم النترات في الأوراق أرتبط بزيادة معدلات التسميد النتروجينى و استخدام مصدر السماد النتروجينى اليوريا.

Effect of Chitosan and Irrigation Intervals on the Growth of Codiaeum Variegatum Plants

Magd El Din F. Rida*and Naglaa M. Mostafa*
* Ornamental Plants & Landscape Gardening Research Department, Horticulture Research Institute, ARC, Alexandria, Egypt.

ABSTRACT

This experiment was conducted during seasons of 2015 and 2016 at Antoniades Research Branch, Horticulture Research Institute, A.R.C. Alexandria to study the effects of foliar spray of chitosan and irrigation intervals on the growth of *(Codiaeum variegatum* var. Gold Star) plants. The chitosan treatments were 0.0, 125,250 or 500 ppm and the irrigation treatments were 4 , 5 or 6 days . The results cleared that using 250 ppm chitosan combined with 6 days irrigation interval gave the highest significant increases in the number of leaves , number of branches, leaf dry weight , branches dry weight and carotene leaf content. Also the results showed that all chitosan treatments caused increases in the most of the studied characteristics. Also, using 250 ppm of chitosan caused the least significant decrease in the intensity of transpiration and the highest increases in leaves area , main branches diameter , roots volume, roots dry weight and leaf content of chlorophyll a , chlorophyll b and total carbohydrate percentage .

Key wards : *Chitosan - irrigation intervals -* *Codiaeum variegatum*

INTRODUCTION

Crotons (*Codiaeum variegatum L.)* are very popular plants in tropical gardens. Its native habitats are Malaysia, India and some of the South Pacific islands. They have two types of growth habits shrubs or small trees. Their leaf colors range from reds, oranges and yellows to green with all combinations of variegated colors, leaf shapes vary from broad and elliptical to narrow and almost linear and leaf blades range from flat to corkscrew-shaped (Robert and Lance, 2014).

One of the important factors that limits the growth and the distribution of natural vegetation and the performance of cultivated plants is water deficit stress (Safaei *et al*., 2014). Occasional or episodic drought events can be counteracted through the use of anti-transpirants compounds applied to foliage to limit the water loss. Chitosan (CHT) is one of the effective anti-transpirant compounds (Bittelli *et al*., 2001).

Chitosan (β-(1-4)-D-glucosamine) is a natural polycationic polymer obtained from the N-deacetylation of chitin, found in the exoskeleton of crustaceans and insects (McKnight *et al*.,1988). Chitosan application regulates plant growth, development, nutrition, and tolerance to abiotic stresses (Russell, 2013). There was a significant growth improvement in soybean sprouts (Lee *et al* .,2005), sweet basil (Kim *et al.,* 2005), grapevine (Ait *et al.,* 2004), as well as ornamental crops, such as *Gerbera* (Wanichpongpan *et al.,* 2000) and *Dendrobium* spp. (Chandrkrachang., 2002) after the application of chitosan.

The goal of this study is to determine the effects of chitosan on the production quality of croton plants under irrigation intervals.

MATERIALS AND METHODS

The experiment was conducted during the two seasons of 2015 and 2016 at Antoniades Research Branch, Horticulture Research Institute, A.R.C. Alexandria.

One year rooted cuttings of croton (*Codiaeum variegatum L* var. Gold Star) plants were transplanted in plastic pots of 14 cm diameter using a mixture of sandy and clay soils at the ratio of 1:1 by volume on 18th of May in both seasons, after one month the treatments started.

Three irrigation intervals treatments i.e. every 4, 5 or 6 days were used alone or combined with four chitosan concentrations i.e. 0.0, 125,250 or 500 ppm (producing 3x4 =12 treatments) .

After one month from the transplanted date on the 18th of June 2015 and the 20th of June 2016 (in the first and second seasons, respectively) similar plants in shapes were arranged into the experiment and the irrigation treatment started .The plants were irrigated till 100 % of the filed capacity of the used soil every 4,5 or 6 days. On the 20th of June 2015 and the 22nd of June 2016 (in the first and second seasons, respectively) the chitosan treatments stared at the rate of 0.0, 125,250 or 500 ppm and they were repeated three times at two weeks intervals. On the 18th of September 2015 and 20th of September 2016 (in the first and second seasons, respectively) the experiment was terminated.

The following data were measured in both of the two growing seasons:

1- Vegetative growth: Plant height (cm), leaves number, main branches number, main branch diameter (cm), leaf area (cm^2), branches number, number of leaves, leaves and branches dry weight (g).
2- Root characteristics: root volume (cm^3) and root dry weight (g).
3- Intensity of transpiration.
It was determined according to the method of Nguyen *et al.* (2011) .Three leaves of the three plants of each plot were collected and kept in the plants conditions. After 30, 60, 90, 120 min, the leaves were weighed to measure the loss of their weight.

The intensity of transpiration was determined as following:

$I = (W0 - Wt)\ S^{-1}\ t^{-1}$

Where I is intensity of transpiration (unit: mg cm^{-2} min^{-1}); S is area of the leaves (cm^2); W0: the weight of the leaves after cutting ; Wt: the weight of the leaves after t min in the plants conditions..

The average of the intensity of transpiration was determined by calculating the mean of four times.

4) Leaves chemical analysis: Chlorophyll a and b content (mg/100 g fresh weight) was determined according to Moran (1982) carotene (mg /100 g fresh weight according to Wellburn (1994), proline content (µg/g) according to Bates *et al.* (1973) and total carbohydrate content (%) according to (Hedge and Hofreiter 1962).

Statistical Analysis

The experiment layout was designed to provide complete randomized block design in split plot design which contained three replicates, each replicate contained twelve treatments. Three pots were used as an experimental unit for each treatment in each replicate. The main plot was water intervals. whereas the plot was chitosan concentrations .The means of the individual factors and their interactions were compared by L.S.D. at 5% level of probability according to Snedecor and Cochran (1989).

RESULTS

Vegetative growth characteristics:

Data presented in Table (1) cleared that there was a significant increases in plant height, leaves number, branches number, leaves and branches dry weights as a result of using chitosan combined with different irrigation intervals compared to zero chitosan. The tallest plants (43 and 45.05 cm) in both seasons were obtained with using chitosan at 500 ppm combined with the five days irrigation intervals. While the foliar application of chitosan at 250 ppm combined with the irrigation intervals at six days resulted in the highest increase in leaves number (68.56 and 69.17), branches number (3 and 2.86), leaves dry

Table (1) Means of Plant height (cm) , leaves number , branches number, leaves and branches dry weights (g) of *Codiaeum variegatum L* as influenced by the interaction between foliar spray of different concentrations of chitosan (Ch) and different irrigation intervals (I) during the two successive seasons of 2015 and 2016

Treatments		Plant height (cm)		Leaves number/ plant		Branches number/plant		Leaves dry weight (g) /plant		Branches dry weight(g) /plant	
Irrigation intervals (days)	Chitosan con. (ppm)	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
4	0	30.00[f]	34.83[f]	44.44[e]	46.50[e]	2.00[c]	2.14[c]	1.89[e]	2.15[d]	1.74[f]	2.18[f]
	125	39.44[abcd]	40.22[cde]	57.44[bc]	60.00[c]	2.22[b]	2.25[bc]	3.40[abcd]	3.58[ab]	2.91[bcde]	3.32[bc]
	250	41.22[ab]	43.06[ab]	62.11[ab]	66.33[ab]	2.11[c]	2.31[bc]	3.45[abc]	3.70[a]	3.34[ab]	3.62[abc]
	500	42.56[a]	44.68[a]	62.56[ab]	65.00[b]	2.00[c]	2.26[bc]	3.51[abc]	3.73[a]	3.40[ab]	3.80[ab]
Mean		38.31	40.70	56.64	59.46	2.08	2.24	3.06	3.29	2.85	3.23
5	0	36.78[cde]	35.97[f]	53.06[cd]	51.58[d]	1.89[c]	1.97,[c]	3.10[cd]	3.09[b]	2.40[de]	2.58[ef]
	125	38.26[bcd]	38.36[e]	61.11[ab]	60.94[c]	2.11[c]	2.00[c]	3.54[abc]	3.64[a]	3.01[bcd]	3.39[bc]
	250	36.56[de]	39.33[de]	57.00[bc]	63.17[bc]	2.33[b]	2.36[bc]	3.22[bcd]	3.43[abc]	2.78[cde]	3.06[cde]
	500	43.00[a]	45.05[a]	59.00[bc]	60.50[c]	3.00[a]	2.64[ab]	3.39[abcd]	3.56[ab]	2.73[cde]	3.22[cd]
Mean		38.69	39.68	57.54	59.05	2.33	2.24	3.31	3.43	2.73	3.06
6	0	33.50[ef]	35.69[f]	47.28[de]	51.81[d]	2.11[c]	2.15[c]	2.82[d]	2.94[c]	2.38[e]	2.68[de]
	125	37.22[cde]	39.72[cde]	68.22[a]	63.25[bc]	2.78[ab]	2.61[ab]	3.73[ab]	3.78[a]	3.10[bc]	3.36[bc]
	250	40.44[abc]	41.32[bcd]	68.56[a]	69.17[a]	3.00[a]	2.86[a]	3.90[a]	3.91[a]	3.82[a]	4.08[a]
	500	41.89[ab]	42.00[bc]	67.83[a]	66.14[ab]	2.00[c]	2.14[c]	3.54[abc]	3.66[a]	3.50[ab]	3.54[abc]
Mean		38.26	39.68	62.97	62.59	2.47	2.44	3.49	3.57	3.20	3.42
L.S.D.at 0.05	Ch	2.15	1.37	4.30	2.24	0.29	0.21	0.35	0.29	0.31	0.32
	I	N.S	N.S	3.72	1.94	N.S	N.S	N.S	N.S	N.S	N.S
	Ch × I	3.722	2.366	7.45	3.88	0.58	0.41	0.61	0.49	0.61	0.56

Means of treatments in the column have the same letters, are not significantly different at 5% level.

weight (3.9 and 3.91 g) and branches dry weight (3.82 and 4.08 g) in the first and second season, respectively .

Data in Table (2) showed that all chitosan treatments caused significant increases in main branch diameter and leaves area compared to the control. The highest increase in main branch diameter (0.55 cm) in both seasons was obtained with foliar spray of chitosan at 500 ppm. Application of chitosan at 250 ppm caused the highest significant increase in leaves area (721.94 and 841.91 cm^2) in the two seasons, compared with the other treatments.

Table (2) Means of main branch diameter (cm) , leaves area (cm^2), roots volume (cm^3) and roots dry weight (g) of *Codiaeum variegatum L* as influenced by foliar spray of different concentrations of chitosan during the two successive seasons of 2015 and 2016.

Chitosan concentrations	Main branch diameter (cm)		Leaves area (cm^2)/ plant		Roots volume (cm^3)/ plant		Roots dry weight (g)/ plant	
(ppm)	2015	2016	2015	2016	2015	2016	2015	2016
0	0.47^{C}	0.46^{b}	499.54^{b}	618.84^{c}	10.37^{c}	9.86^{b}	2.14^{b}	1.98^{b}
125	0.51^{b}	0.52^{a}	682.69^{a}	789.34^{b}	12.85^{b}	11.85^{a}	2.72^{a}	2.47^{a}
250	0.53^{ab}	0.53^{a}	721.94^{a}	841.97^{a}	15.15^{a}	12.85^{a}	3.23^{a}	2.81^{a}
500	0.55^{a}	0.55^{a}	688.17^{a}	799.12^{ab}	13.15^{b}	12.31^{a}	3.16^{a}	2.72^{a}
L.S.D at 0.05	0.03	0.03	65.82	48.64	1.33	1.21	0.55	0.40

Means of treatments in the column have the same letters, are not significantly different at 5% level.

It is clear from data presented in Table (3) that there was a significant effect of different irrigation intervals control on leaves area and the highest significant increase of leaves area (749.41 and 808.93 cm^2) was obtained with the irrigation intervals at 6 days in both seasons, compared with the other treatments.

Table (3) Means of leaves area (cm^2) of *Codiaeum variegatum L* as influenced by different irrigation intervals during the two successive seasons of 2015 and 2016

Irrigation intervals	Leaves area (cm^2)/plant	
(days)	2015	2016
4	514.79 c	715.26 c
5	680.05 b	762.75 b
6	749.41 a	808.93 a
L.S.D at 0.05	39.56	42.22

Means of treatments in the column have the same letters, are not significantly different at 5% level.

Root growth characteristics:

Data in Table (2) cleared that all chitosan treatments resulted in the highest significant increases in roots volume and roots dry weight compared to the control. The highest roots volume (15.15 and 12.85 cm^3) and roots dry weight (3.23 and 2.81 g) in the first and second season, respectively were obtained with using chitosan at 250 ppm .

Intensity of transpiration

Results in Table (4) indicated that all treatments of foliar spray of chitosan caused a significant decrease in intensity of transpiration. The lowest significant intensity of transpiration (0.015 mg $cm^{-2}min^{-1}$) was recorded with application of chitosan at 250 ppm in both seasons, compared with the control plants.

Leaves chemical analysis:

Data presented in Table (4) showed that all chitosan treatments caused significant increases in Chlorophyll a, Chlorophyll b and total carbohydrate content compared to the control. Also, foliar spray of chitosan at 250 and 500 ppm caused the highest significant increases in chlorophyll a, chlorophyll b and carbohydrate content in the two seasons, compared with the control treatment.

It is clear from data in Table (5) that there were significant effects due to the interaction between application of chitosan and different irrigation intervals on the carotene and proline content compared to zero chitosan treatment. The highest carotene content (19.13 and 18.36 mg / 100 g leaves fresh weight) was found using chitosan at 500 ppm combined with 5 days irrigation interval which was statistically similar to foliar spray of chitosan at 250 ppm combined with six days irrigation intervals. While the lowest significant amount of proline was found with application of chitosan at 500 ppm combined with six days irrigation intervals.

DISCUSSION

The increase in the studied vegetative and root growth parameters after the foliar spray of chitosan may be due to the fact that chitosan application increase the activities of key enzyme of nitrogen metabolism (nitrate reductase glutamine synthetase and protease). This enzyme improves plant growth and development (Li *et al.*, 2001 on rice). Also, this increment may be due to the increase in chlorophyll a and chlorophyll b content which lead to increase in carbohydrate content and consequently increase the plant growth. These results are in agreement with Sathiyabama, *et al.* (2014) who mentioned

Table (4) Means of intensity transpiration (mg cm^{-2} min−1), chlorophyll a and b (mg/100 g fresh weight) and carbohydrate (%) of *Codiaeum variegatum* L as influenced by foliar spray of different concentrations of chitosan during the two successive seasons of 2015 and 2016

Chitosan concentrations (ppm)	Intensity transpiration (mg cm^{-2} min^{-1})		Chlorophyll a (mg/100 g leaves fresh weight)		Chlorophyll b (mg/100 g leaves fresh weight)		Carbohydrate (%)	
	2015	2016	2015	2016	2015	2016	2015	2016
0	0.020 a	0.019 a	43.84 c	41.90 b	12.65 b	12.12 b	11.61 b	12.16 c
125	0.017 c	0.017 c	60.35 b	77.28 a	18.98 a	23.50 a	12.04 b	12.60 b
250	0.015 d	0.015 d	69.29 a	76.46 a	21.32 a	24.68 a	14.44 a	14.07 a
500	0.018 b	0.018 b	67.42 a	80.39 a	20.58 a	25.95 a	13.86 a	13.84 a
L.S.D at 0.05	0.001	0.001	6.00	6.07	2.35	3.05	0.83	0.50

Means of treatments in the column have the same letters , are not significantly different at 5% level.

Table (5) Means of *Carotene* (mg/100 g leaves fresh weight) and proline (µg/g) concentration in the leaves of *Codiaeum variegatum* L as influenced by the interaction between foliar spray of different concentrations of chitosan (Ch) and different irrigation intervals (I) during the two successive seasons of 2015 and 2016

Treatments		Carotene conc. (mg/100 g leaves fresh weight)		Proline conc. (µg/g)	
Irrigation intervals (days)	Chitosan con. (ppm)	2015	2016	2015	2016
4	0	13.92[e]	15.27[cd]	155.50[ab]	136.58[abc]
	125	16.29[cd]	16.57[abc]	118.25[cde]	109.84[def]
	250	17.47[bc]	15.42[bc]	94.92[gh]	90.62[fg]
	500	16.77[cd]	16.51[abc]	97.88[fgh]	105.87[def]
Mean		16.11	15.94	116.64	116.64
5	0	16.24[cd]	11.81[e]	169.38[a]	155.43[a]
	125	15.84[d]	18.09[a]	117.27[cdef]	103.33[defg]
	250	16.98[bcd]	17.55[ab]	136.53[bc]	141.89[ab]
	500	19.13[a]	18.36[a]	112.34[defg]	114.96[cde]
Mean		17.05	16.45	133.88	133.88
6	0	15.62[d]	13.17[d]	125.91[cd]	119.79[bcd]
	125	16.41[cd]	16.95[abc]	115.08[defg]	110.29[def]
	250	18.33[ab]	16.77[abc]	105.07[efgh]	95.50[efg]
	500	16.98[bcd]	17.25[abc]	86.72[h]	82.82[g]
Mean		16.83	16.03	108.20	108.20
L.S.D.at 0.05	Ch	0.88	1.29	11.68	13.13
	I	N.S	N.S	7.38	15.00
	Ch × I	1.53	2.24	20.23	22.74

Means of treatments in the column have the same letters , are not significantly different at 5% level.

that foliar application of chitosan enhanced fruit weight and productivity in tomato, Mondal *et al.* (2012) who found that foliar spray of chitosan increased fruit yield, plant height, and leaf number of okra (*Hibiscus esculentus* L.) and Yin *et al.,*(2012) who found that application of chitosan stimulated plant growth in Greekoregano.

Besides, there was a decrease in the intensity of transpiration with application of 250 ppm chitosan compared to the other treatments, which can be explained by the fact that foliar spray of chitosan decrease the transpiration by partial or full closure of stomata (Marco *et al.*, 2001). These results are in agreement with those obtained by Bittelli *et al.* (2001) who found that the water use of pepper plants treated with chitosan reduced by 26%–43%.

As for the chemical analysis there were significant increases in the values of chlorophyll a and chlorophyll b content. This increment may be due to the greater availability of amino compounds released from chitosan (Chibu and Shiayama 2001). These results were in harmony with those obtained by Farouk *et al.* (2008) on cucumber and Farouk *et al.* (2011) on radish. Also there was an increase in the percentage of carbohydrate which may be due to the increase of photosynthesis pigments (chlorophyll a, chlorophyll b content and total carotene).

All chitosan treatments at any irrigation intervals caused a decrease in leaves proline content compared to zero chitosan treatment which may due to the decrease in intensity of transpiration after chitosan treatments resulted in decrease in plant stress.

In conclusion chitosan can be used as a foliar spray on croton plants three times at the dose of 250 ppm combined with six days irrigation intervals. This treatment decreases the water use by 33.3 % and increases most vegetative growth parameters .

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تاثير الشيتوزان و فترات الري على نمو نباتات الكروتون

مجد الدين فؤاد رضا *، نجلاء محمد مصطفى *

*** فرع بحوث نباتات الزينة بأنطونيادس – الإسكندرية – قسم بحوث الزينة وتنسيق الحدائق**

معهد بحوث البساتين – مركز البحوث الزراعية

أجريت هذه الدراسة خلال موسمي ٢٠١٥ ، ٢٠١٦ بفرع بحوث الزينة وتنسيق الحدائق بحديقة انطونيادس التابع لمعهد بحوث البساتين– مركز البحوث الزراعية مصر وذلك لدراسة تأثير الرش بالشيتوزان وفترات الري على نمو نباتات الكروتون. وكانت معاملات الشيتوزان (صفر، ١٢٥، ٢٥٠ أو ٥٠٠ جزء في المليون) بينما فترات الري (٤، ٥ أو٦ ايام). وقد أظهرت النتائج أن التفاعل بين الرش ب ٢٥٠ جزء في المليون وفترة الري ٦ ايام أدى إلى زيادة معنوية في عدد الأوراق ،عدد الفروع ،والوزن الجاف للأوراق والفروع ومحتوى الأوراق من الكاروتين. أيضا أظهرت النتائج أن كافة معاملات الشيتوزان أدت إلى زيادة في أغلب الصفات المدروسة وأن المعاملة ٢٥٠ جزء في المليون من الشيتوزان أدت إلى أكبر نقص معنوي في معدل النتح كما أدت إلى أعلى زيادة معنوية في المساحة الورقية ، قطر الساق، حجم الجذور، وزن الجذور الجاف، محتوى الأوراق من كلورفيل أ، كلورفيل ب والنسبة المئوية للكربوهيدرات .

الكلمات الكاشفة : شيتوزان– فترات الري – الكروتون

IGFBP-3 Gene Diversity Among Egyptian Sheep Breeds

A. A. Saleh[1], M.H. Hammoud[1], Nassra A.Dabour[2], E.E. Hafez[3], M.A. Sharaby[1]

(1) Animal and Fish Production Department -Faculty of Agriculture (Alshatby) - Alexandria University.

(2) Dairy Science and Technology Department- Faculty of Agriculture (Alshatby) - Alexandria University.

(3) Plant Protection and Biomolecular diagnosis Department-Arid Lands Cultivation Research Institute (ALCRI) -City of Scientific Research and Technology Applications.

ABSTRACT

Insulin like Growth Factor Binding Protein-3 (IGFBP-3) gene plays a key role in mammalian growth, strain development and reproduction activity as well. It is recommended as a marker for different body functions due to its association with economic traits. Exploring this gene in Egyptian sheep may shed the light on the genetic architecture of the local breeds production profiles. The objectives of this study were to identify the genetic diversity between and within some Egyptian sheep breeds (Rahmani, Barki, Ossimi, Awassi) and crosses (Awassi X Suffolk and Rahmani X Barki) by examining polymorphism of IGFBP-3 gene using Restriction Fragment Length Polymorphism (RFLP). Also, to predict of the relationship between IGFBP-3 diversity and general behavior of these breeds with respect to reproduction and growth characteristics. Amplification of IGFBP-3 from DNA of the pure-bred sheep was successful, but failed from that of most individuals of specific crosses. Digestion of 654 bp with Hae III restriction enzyme yielded single restriction pattern of five fragments of sizes 201, 201, 87, 67, 57 in all tested breeds and crosses revealing absence of polymorphism.

Key Words: Egyptian Sheep, IGFBP-3 and animal breeding.

INTRODUCTION

Characterization and determination of genetic differences between and within sheep breeds will help the rapid improvement of their economically important traits. IGF growth factor system was recently found to be involved in the regulation of ovarian follicular development in sheep (Hanrahan *et al.*, 2004). It consists of two ligands (IGF-I and IGF-II) and two cell-surface receptor types I and II, IGF-R (Froesch *et al.*, 1985), both receptors show affinity for either ligand and mediate their effects. Further components of the system include the IGF-binding proteins, at least six of them are now known in the mammalian system (IGFBP-1, -2, -3, -4, -5 and -6) (Hwa *et al.*, 1999). IGFBPs belong to a family of at least six homologous proteins that bind IGFs and modulate many of their biological actions. Therefore, IGFBP is recommended as a marker for different body functions such as growth, body weight, reproduction, immunity, metabolism and energy balance….etc. In several species, components of the IGF system have been associated with the control of ovarian folliculogenesis, and the ovary is a major site for the synthesis of these components in human, rat, porcine, bovine and ovine (Adashi, 1994 and 1998; Campbell *et al.*, 1995; Giudice, 1992; Hammond *et al.*, 1991 & Monniaux *et al.*, 1992). Granulosa and theca cells have demonstrated the paracrine/autocrine regulatory effects of IGF-I and/or IGF-II on cell proliferation and differentiation, either directly or by interacting/synergizing with gonadotrophins (Hammond *et al.*, 1988). It is still a matter of speculation as to which ligands, receptor types or binding proteins contribute to the regulation of specific aspects of folliculogenesis at various key stages of development (Shukla, 2001).

IGFBP-3 is a structural gene responsible for the multiple effects of IGF. It plays a key role in mammalian growth, development and reproduction (Hastie *et al.*, 2004). Single-nucleotide polymorphism (SNP) of IGFBP-3 gene has been described in the bovine and buffalo to be associated with production traits (Maciulla *et al.*, 1997). No polymorphism was detected in Indian sheep IGFBP3 gene (Kumar *et al.*, 2006).Associations have been made between a limited number of the IGFBPs and follicle status. Growing follicles seem to have increases in levels of IGFBP-3 concomitant with decreases in levels of IGFBP-2, -4 and -5. This contrasts with atretic follicles where levels of IGFBP-3 are low, while levels of IGFBP-2, -4 and -5 are high (Besnard *et al.*, 1996 & Khalid and Haresign, 1996).Due to the key role of IGFBP-3 in the animal growth and development, it is considered as a candidate gene to be used as a marker for growth and production traits in livestock species for its association with economic traits (Ali et al, 2009 & Shafey *et al.*, 2014). A fragment of IGFBP-3 gene, comprising a part of exon 2, complete intron 2, exon3, and a part of intron3 was amplified and found to size 654, 651and 655 bp in sheep, cattle and buffalo respectively (Kumar *et al.*, 2006).

The objective of this study was to predict the genetic polymorphism in IGFBP-3 gene by Restriction Fragment Length Polymorphism (RFLP) and relate that to the diversity between and within some sheep breeds.

MATERIAL AND METHODS

Animals

A total of 110 animals belonging to six sheep breeds

viz; 20 Rahmani, 20 Barki, 10 Awassi, 8 Suffolk, 5 Ossimi and 47 Rahmani × Barki crosses were sampled for blood collection.

DNA isolation and manipulation

Genomic DNA was extracted from blood samples with QIAGEN kit (QIAGEN GmbH, Hilden Germany) as instructed by the manufacturer. All DNA extraction buffers and reagents were prepared and used according to Sambrook *et al.*, (1989). The isolated DNA were separated by electrophoresis on 0.8% agarose (Bioshop, Germany) in 0.5 × TBE buffer prepared, and contained 0.5 µg/ml ethidium bromide (Sigma, Germany). The electrophoresis run was performed using electrophoresis apparatus with power supply (Biometra, USA) and visualized by UV trans-illuminator and Gel documentation system (Gel Doc.Alpha-chem.Imager, USA).

PCR amplification

The IGFBP-3gene was amplified from the genomic DNA of the tested animals using primer: 5'CCA AGC GTG AGA CAG AAT AC'3 and 5'AGG AGG GAT AGG AGC AAG AT'3 (Kumar *et al.*, 2006).The PCR reaction volume of 100 µl consisted of 77.6µl H_2O, 10µl of 10X Taq buffer (Biometra, USA), 4µl of dNTP (A, T, G, and C at 10mM) (Bioshop, Germany), 4µl of temnplate DNA (25 ng mL^{-1}), 2µl of each primer (10 mM mL^{-1}) and 0.4µl (1.25 U) of Taq polymerase (Biometra, USA). PCR amplification was performed with a DNA Thermocycler Gene Amp 6700 (Applied Bio-system, USA) using the following cycles: After one cycle at 94 °C for 5 min to denature template DNA, 30 cycles were carried out with the following conditions: denaturation at 94°C for 30s, primer annealing at 54°C for 30s, and DNA extension at 72°C for 30s, finishing with a final extension of 5 min at 72°C. After cycling, the samples were rapidly cooled to 4°C until analysis. The PCR amplifications were separated by electrophoresis on 0.8% agarose (Bioshop, Germany) gel in 0.5X TBE buffer contained 0.5 µg/ml ethidium bromide (Sigma, Germany) (Sambrook *et al.*, 1989). The electrophoresis run was performed using electrophoresis apparatus with power supply (Biometra, USA) and visualized by UV transilluminator and the Gel documentation system (Gel Doc.Alpha-chem.Imager, USA).

Restriction fragment length polymorphism (RFLP)

The RFLP was used to detect the differences of genotyping between tested animals using the PCR of target genes. The PCR amplicons of IGFBP-3 gene (652 bp) were digested with *HaeIII* (Jena Bioscience, Germany). The RFLP-PCR reaction volume of 25 µl consisted of 12µl H_2O, 2µl of 10X *HaeIII* buffer (Jena Bioscience, Germany), 1 µl (5 unit/ul) Restriction enzyme and 10µl amplified DNA. All reactions were incubated at 37°C for 16 hours. Twenty µl of each reaction were separated by electrophoresis on 2.5 % agarose gel and visualized by UV trans-illuminator and Gel documentation system (Gel Doc.Alpha-chem.Imager, USA). The RFLP pattern was inspected to detect the differences and similarities between the samples (Bastos *et al.*, 2001; Sanger *et al.*, 1977; Stonking, 2001 and Vignal *et al.*, 2002).

RESULTS AND DISCUSSION

The PCR fragments of IGFBP-3 gene (654 bp) (Fig.1) were successfully amplified from the genomic DNA of the pure breeds Rahmani, Barki, Ossimi, Awassi and Suffolk with positive amplicons observed, but amplication failed for most individuals of Rahmani X Barki and Awassi × Suffolk (Fig.2). The amplification failure may be due to different genomic DNA arrangements in the crossbred animals in the location specific for the used primers. Further experimentation is required utilizing other primers to explore the reason behind the

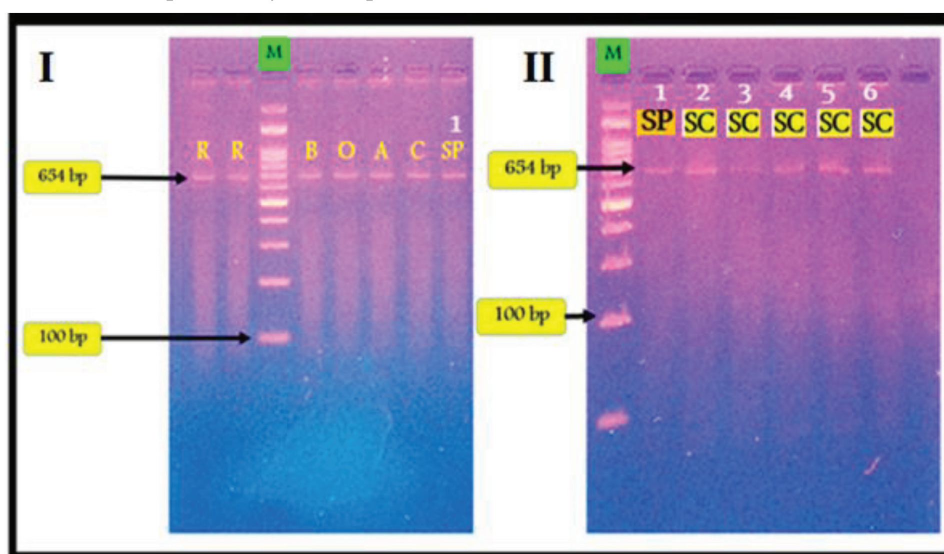

Figure 1: I-Purified PCR product of IGFBP-3 gene from Rahmani (R), Barki (B), Ossimi (O), Awassi (A), Rahmani X Barki cross (C) and pure Suffolk (SP) M, 100 bp DNA ladder and II- Purified IGFBP-3 gene (654bp) from gel in: Suffolk pure (1) Awassi X Suffolk Crosses (SC: from number 2 to 6) M= DNA ladder with 50 bp

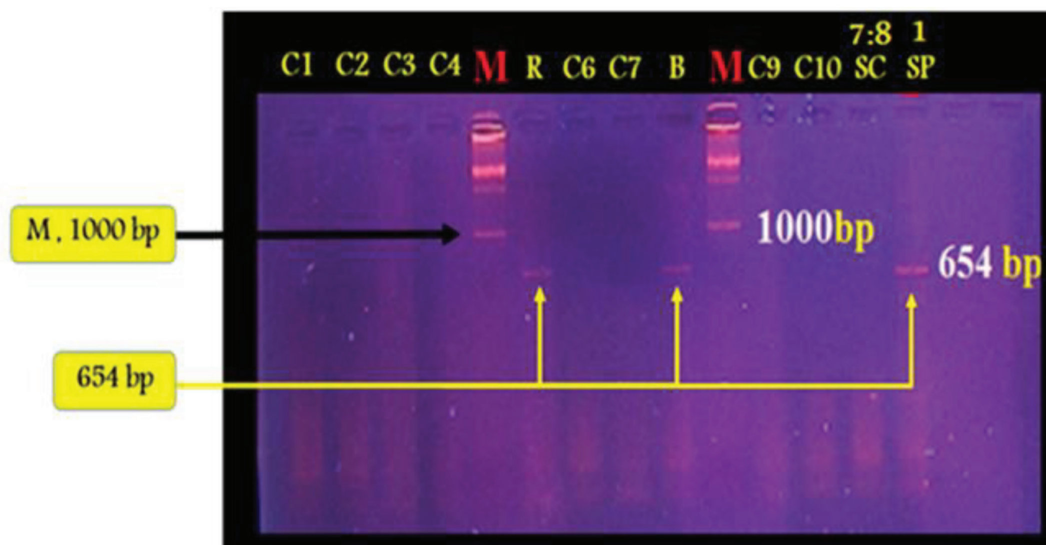

Figure 2: PCR amplification of IGFBP-3 gene (654bp) in: Rahmani × Barki crosses (unknown mixture ratio) (C1, C2, C3, C4, C6, C7, C9 and C10), Rahmani (R), Barki (B), Awassi × Sufollk crosses (SC: number 7 and 8) and pure Sufollk (SP: number 1)

disappearance of IGFBP-3 gene in some of the crosses. To confirm this result another PCR assay should be designed to amplify the whole gene with primers located out side of the target gene. Also, DNA hybridization could be used as potential tool to identify the absence or presence of gene.

Genotyping of IGFBP-3 gene by RFLP

The PCR products of IGFBP-3 gene obtained from the tested animals were digested with *HaeIII* (Fig.3). Digestion profile revealed only one pattern for five DNA fragments sized 201, 201, 87, 67 and 57 bp for all animals, which mean non detection of polymorphism within IGFBP-3 gene among the tested sheep. Ali *et al.*, (2009) reported that the RFLP pattern with *Hae III* of IGFBP-3 gene obtained from the four sheep breeds, Rahmani, Barki, Ossimi and Awassi yielded similarly one pattern of five fragments sized 201, 201, 87, 67, and 57 bp for all studied breeds revealing absence of polymorphism.

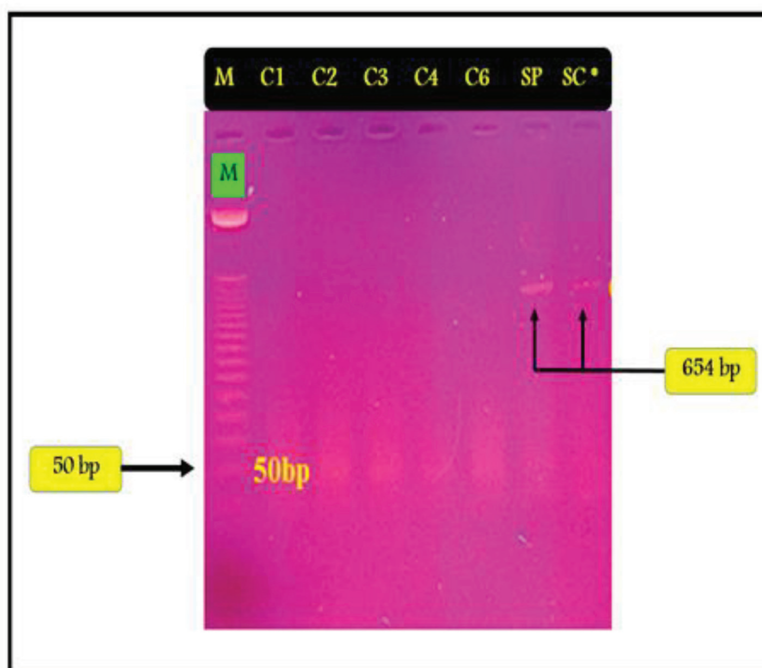

Figure 3: PCR amplification of IGFBP-3 gene from genomic DNA of Rahmani × Barki cross] C1, C2, C3, C4, C6: of unknown crossing ratio], Suffolk and Awassi × Suffolk Cross (SC: from number 4). M, 50 bp DNA ladder

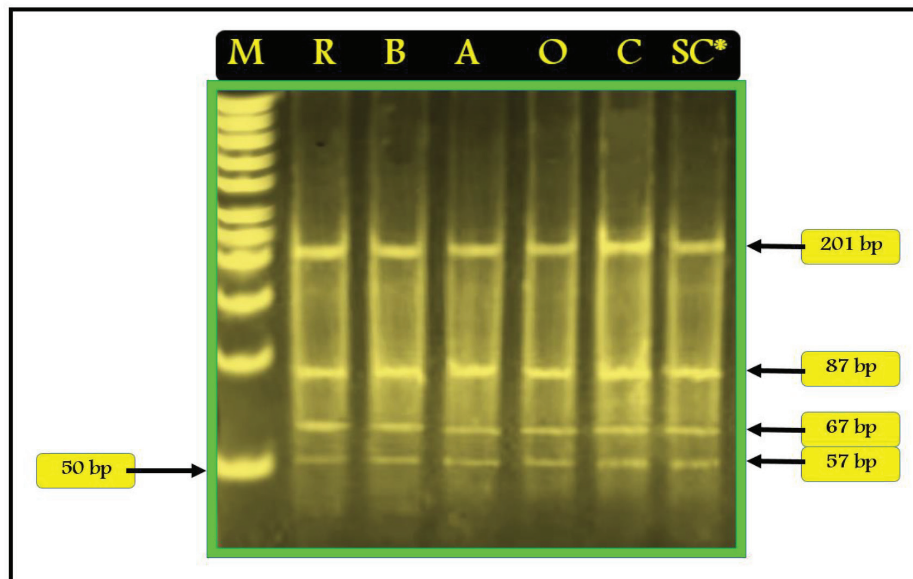

Figure 4: The PCR products of IGFBP-3 gene from genomic DNA of tested breeds digested by *HaeIII*. Rahmani (R), Barki (B), Awassi (A), Ossimi (O), Rahmani × Barki (C) and –SC*: Awassi × Suffolk crosses. M, 50 bp DNA ladder

The present results agree with those of Kumar *et al*., (2006) who studied the genetic diversity among Indian breeds of sheep; Marwari, Mandya, Madras, Red Muzaffarnagari and Banur based on sequencing and digestion profile by *HaeIII* of IGFBP-3 gene and reported that the digestion profile revealed only one pattern with eight DNA fragments sized 201, 201, 87, 67, 56, 19, 16 and 7 bp for tested animals and, consequently, detected no polymorphism. Choudhary (2004) reported that all sheep have intact *HaeIII* restriction site (GG CC) at the base number 300 of IGFBP-3 gene sequence also, indicating the absence of polymorphism at this site. The current results were in accordance with those reported on six breeds of buffalo (Choudhary, 2004), though, the sizes of restriction fragments were different (201, 165, 154, 56, 36, 19, 16 and 8 bp). That means no polymorphism has been detected among the six studied breeds of buffaloes with respect to IGFBP-3 gene.

In contrary, Choudhary (2004) identified three genotypes in exotic Holstein Friesian and Jersey cattle with restriction fragments of sizes 199, 164, 154, 56, 36, 18, 16 and 8 bp for AA genotype ; 215, 164, 154, 56, 36, 18 and 8 bp (BB genotype) and 215, 199, 164, 154, 56, 36, 18, 16 and 8 bp for AB genotype.

CONCLUSION

The PCR fragments of IGFBP-3 gene (654 bp) were successfully amplified from the genomic DNA of the pure breeds; Rahmani, Barki, Ossimi, Awassi and Suffolk with positive amplicons observed, but amplication failed for most individuals of Rahmani X Barki and Awassi X Suffolk. The amplification failure may be due to different genomic DNA arrangements in the crossbred animals in the location specific for the used primers. The RFLP pattern with *Hae III* of IGFBP-3 gene obtained from the tested sheep breeds, (Rahmani, Barki, Ossimi and Awassi (Awassi × Suffolk and Rahmani × Barki crosses) yielded similarly one pattern of five fragments sized 201, 201, 87, 67, and 57 bp for all studied breeds revealing absence of polymorphism.

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مورث IGFBP-3 يلعب دوراً هاماً، في نمو الثدييات وكذلك التطور والنشاط الجنسي، ويعتبر مميز للعديد من وظائف الجسم بسبب ارتباطه مع الصفات الاقتصادية واكتشاف الاختلافات المحتملة لهذا الجين في الأغنام المصرية قد يلقي الضوء على البنية الوراثية والصفات الإنتاجية للسلالات المحلية. هدف هذه الدراسة كان التعرف على التنوع الجيني بين وداخل بعض سلالات الأغنام المصرية، وهي الرحماني والبرقي والأوسيمي والعواسي وخلطان (العواسي والسفولك) و(الرحماني والبرقي)، وذلك باستخدام تقنية الـ RFLP–PCR، وكان تضخيم Amplificatioin مورث – IGFBP من الحمض النووي للأغنام النقية ناجحاً، لكنه فشل في معظم الأفراد الخليطة خاصة من خلطان غير خليط الجيل الأول، وتم الهضم باستخدام أحد إنزيمات الهضم وهو Hae–III لمورث السلالات النقية واتضح التشابه بين السلالات النقية لنفس المورث حيث أعطى الهضم Bands على ٥٧، ٦٧، ٨٧، ٢٠١، ٢٠١ في السلالات النقية وخليط الرحماني مع البرقي (للجيل الأول فقط)، هذا ويساعد اكتشاف الطفرات المفيدة في مثل هذه المورثات في تصميم وتنفيذ برامج عامة وشاملة للتحسين الوراثي في الأغنام سواء بالانتخاب لهذه العوامل الوراثية وطفراتها أو تنفيذ برامج عامة وشاملة للتحسين الوراثي في الأغنام سواء بالانتخاب لهذه العوامل الوراثية وطفراتها أو تنفيذ برامج محددة لخلط السلالات المحلية ببعضها البعض أو بسلالات أجنبية تحمل العوامل الوراثية المفيدة أو طفراتها والسماح لها بالانتقال عبر الأجيال لتصبح جزء من تكوين الجينوم الخاص بالأغنام المصرية.

A Note on the Potentials of Random Amplified Polymorphic DNA (RAPD) Technique to Depict Genetic Variability among Egyptian and Exotic Sheep Breeds Raised Locally

Ahmed A. Saleh, Sobhy M.A.Sallam, Mahmoud A. Sharaby

Animal and Fish Production Department -Faculty of Agriculture (Alshatby) - Alexandria University.

ABSTRACT

Genetic variability among Egyptian sheep breeds Rahmani, Barki and their cross and Ossim and exoit Awassi and British Suffolk were studied using random amplified polymorphic DNA (RAPD) technique. Three random primers were used to amplify DNA fragments and were formed to give recproducable pattern for the studies breeds. RAPD patterns with considerable level of polymorphism were detected among breeds. The genetic similarities among breeds were: Barki to Rahmani (96 %) , Rahmani to Awassi (92%), Barki to Awassi (91%), Barki to Ossimi (90%) while Suffolk to other breeds was below 85%. Genetic diversity exists among the Egyptian sheep breeds and Awassi and British Suffolk raised in locally. The RAPD profile generated for each breed can be effectively used as a supporting marker for taxonomic identification.

Key words: Sheep, RAPD, Molecoular Marker and Biodiversity.

INTRODUCTION

The common Egyptian sheep breeds are Rahmani, Ossimi and Barki**.** They have medium size and low rate of growth. Moreover, they breed all year round or possess extended breeding season and own small litter size ranging from 1.03 to 1.40. Fortunately, Egyptian sheep possess high favorable genetic diversity for most of production traits and conformation characteristics. The mature weight of Rahmani and Ossimi are higher than of Barki. While the fleece weight and meat quality of Barki are higher than those of Ossimi and Rahmani. Barki breed is well adapted to desert conditions, while Ossimi has a wider range of adaptability than Barki. Rahmani is believed to be more tolerant to internal parasites than other Egyptian breeds. The twinning rate is relatively high in Rahmani breed. The lactation period is longer in Barki, but total yield of milk is approximately the same in all three breeds. However, their inherent potential for growth and meat production have not been explored yet due to inadequate information about the genetic basis and breeding strategies (EL-Hanafy and Salem 2009).Attempts were made to increase meat production from local Egyptian breeds, primarily through changing production and reproductive management (EL-Hanafy and El-Saadani, 2009; Aboul-Naga and Afifi, 1982; Galal *et al*., 2005), and recently, by introducing highly prolific sheep genetics and detecting the changes in genes that affect fertility and growth traits (EL-Hanafy and El-Saadani, 2009).

Recent researches proved that some of such traits could be controlled with polymorphic major genes which can be traced along with their influence by the application of some molecular markers such as Restriction fragment length polymorphism RFLP, Amplified Fragment Length Polymorphism (AFLP), Random amplification of polymorphic DNA (RAPD) and Single-strand conformation polymorphism techniques SSCP (Allard *et al*., 1992; Dinesh *et al*., 1993; Appa Rao *et al*., 1996; Bahy, 2003 and William, 2014). Molecular markers are promising alternative for improvement that enables the animal breeders to select eligible animals with desirable traits such as longevity, fertility, litter size, and disease resistance at early ages. This will result in an increase in accuracy of selection response (Teneva, 2009). El Nahas *et al*. (2008), found polymorphic loci with significant deviation from Hardy-Weinberg equilibrium and with total number of alleles ranging from 6 to 14. Ossimi and Rahmani breeds clustered independently from Barki breed at 0.43 genetic distances.

The objective of present study was to utilize RAPD-PCR assay to depict the genetic variability and determine the degree of relatedness among four Egyptian sheep breeds and the exotic Middle Eastern Awassi and British Suffolk raised locally.

MATERIAL AND METHODS

Animals

A total of 110 sheep belong to six breeds viz; 20 Rahmani (ewes), 20 Barki (ewes), 10 Awassi (rams and ewes), 8 Suffolk (rams and ewes), 5 Ossimi (rams and ewes) and 47 Rahmani X Barki crosses (rams and ewes) were used in this study. Blood samples of 5 ml each were collected from the jugular vein, using venojects, treated with 0.5 ml of 2.7% EDTA (Pspark,UK), as anti-coagulant and transferred in an ice box.

Genomic DNA was extracted from blood samples with QIAGEN (QIAGEN GmbH, Hilden Germany) columns as instructed by the manufacturer. All the DNA extraction buffers and reagent were prepared and used according to Sambrook *et al,* (1989). The isolated DNAs were separated by electrophoresis on 0.8% agarose (Bioshop, Germany) in 0.5 X TBE containing 0.5 µg/ml ethidium bromide (Sigma, Germany). The electrophoresis run was performed using electrophoresis apparatus with power supply (Biometra, USA) and visualized by UV trans-illuminator and Gel documentation system (Gel Doc.Alpha-chem.Imager, USA).

The PCR reaction, Primers and cycling conditions are presented in Tables (1, 2 and 3). Thermal cycling (Gene Amp 6700 Applied Bio-system, USA) was carried out by initial denaturation at 94°C for 4 min, followed by 40-45 cycles each at 94°C for 30- 60s, annealing temperature at 28-58 for 30-60s , polymerization temperature at 72°C for 1 min and final extension at 72°C for 5 min., then the samples were held at 8°C. The amplified DNA fragments were separated on 2-3% agarose gel (Bioshop, Germany), stained with ethidium bromide (Sigma, Germany), visualized on a UV Transilluminator and photographed by Gel Documentation system (Gel Doc.Alpha-chem. Imager, USA).

Table 1: The PCR mixture carried out in a 25 µl

Serial addition	Content	Volume	Concentration
1	PCR buffer	2.5 µl	10 X
2	MgCl2	1 µl	1.5 µM
3	dNTP	0.5 µl	200 µM
4	Primer	1 µl	10 p.mol
5	dH2O	18.9 µl	----------
6	Taq DNA polymerase	0.1 µl	2 unit/µl
7	Templet DNA	1 µl	50 n.mol
Total volume		25 µl	

RESULTS AND DISCUSSION

To ensure that the amplified DNA bands originated from genomic DNA and are not primer artifacts, negative control was carried out for each primer/breed combination. No amplifications were detected in any of the control reactions. All amplification products were found to be reproducible when reactions were repeated using the same conditions (Figure 1). One of three primers was successfully amplified polymorphic bands among the six breeds (Table 4).

RAPD analysis was used for constructing Phylogenetic tree to depict relationships among the studied breeds and cross (Figure 2). Data presented in (Table 4) showed closer proximity of Barki and Rahmani (96 %) , Rahmani and Awassi (92%), Barki and Awassi (91%), Barki and Ossimi (90%) but not of Suffolk and any of these breeds. This may be due to fact that Barki ,Rahmani and Ossimi are pure Egyptian breeds and Awassi originated in the Middle East but Suffolk has British origin. Some genes descended from common ancestor may had caused the high proximity between the three Egyptian sheep breeds and Awassi but not with Suffolk.

Table 2: List of the random primers used, their nucleotide sequence, G+C content % and annealing temperatures

Primer	Primer Sequence 5` - 3`	G+C content %	Annealing temperature(°)/Time (S)
1	GGA CTG GAG TGG TGA CGC AG	65%	58°/ 30 s
2	ATG ACG TTG A	40%	45°/ 30 s
3	ACC GCC GAA G	70%	28°/ 30 s

Table 3: PCR conditions

Primer	Denaturation		Annealing		Extension		Final extension		Number of cycle
1	Sec	°C	Sec	°C	Sec	°C	Sec	°C	N
	300	94	30	58	30	72	300	72	45
2	Sec	°C	Sec	°C	Sec	°C	Sec	°C	N
	300	94	30	45	30	72	300	72	40
3	Sec	°C	Sec	°C	Sec	°C	Sec	°C	N
	300	94	30	28	30	72	600	72	40

Table 4. Jaccard's similarity coefficients between sheep breeds based on RAPD data

Breeds	Awassi	Barki	Rahmani	Ossimi	(B × R)	Saffolk
Awassi	----------	91	92	89	89	85
Barki	91	----------	96	90	90	86
Rahmani	92	96	----------	88	90	89
Ossimi	89	90	88	----------	89	80
(B × R)	89	90	90	89	----------	79
Saffolk	85	86	89	80	79	----------

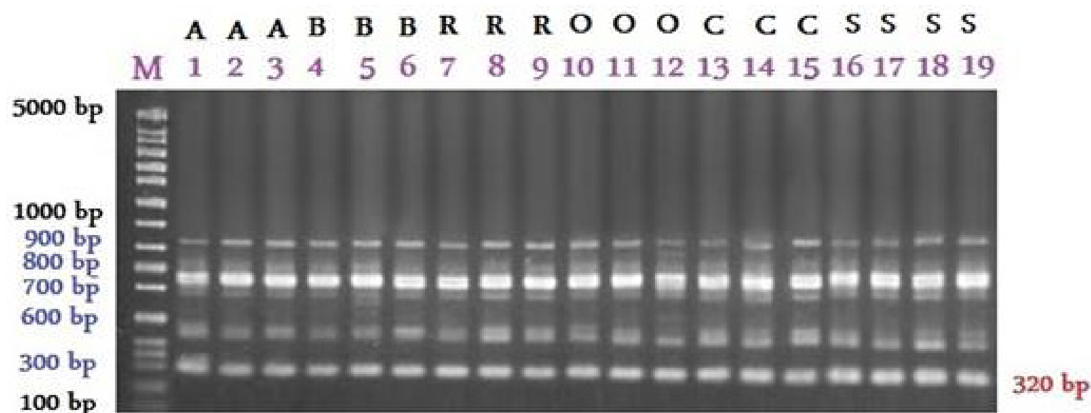

Figure 1. Example of RAPD patterns in six breeds of sheep obtained with random primer 1. Lane M: DNA marker (100:5000 bp). Lanes 1:3 Awassi , Lanes 4:6 Barki, Lanes 7:9 Rahmani, Lanes 10: 12 Ossimi, Lanes 13:15 (Barki X Rahmani crossbred) and Lanes 16:19 Suffolk.

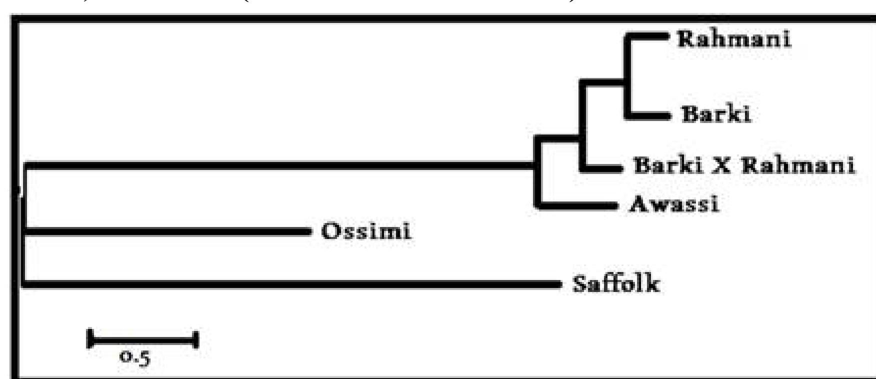

Figure 2. Dendrogram using Average Linkage (Between Groups) based on RAPD data analysis among the six breeds of sheep

The Phylogenetic tree showed three major classes for the studied breeds. Ossimi and Suffolk occupied classes 2 and 3 respectively with no partern Rahmani, Barki , Barki X Rahmani cross and Awassi were clustered together in class 1, which was divided into three sub-classes, the first accommodated, Rahmani and Barki while Barki X Rahmani cross was located in the second sub-class and Awassi existed in the third sub-class. These results are in agreement with those obtained by Mahfouz *et al*., (2008), who found that Rahmani and Barki breeds clustered together and Ossimi formed another class when used RAPD to verity the degree of association among several breeds of Egyptian sheep.

A genetic analysis by Ali (2003) was performed to detect genetic variation between Baladi, Barki, Rahmani and Suffolk sheep breeds in Egypt. RAPD patterns with a level of polymorphism were detected between breeds. Results showed close proximity of Barki to Rahmani and Baladi, while the Suffolk breed was the most different. Hassan *et al*., (2003) studied genetic diversity of Rahmani, Ossimi and Barki and reported that all loci under investigation were polymorphic and deviated from Hardy-Weinberg equilibrium. Similarly. El Nahas *et al*. (2008) studied the genetic diversity among; Rahmani, Ossimi and Barki sheep breeds and reported smaller genetic difference between Ossimi and Barki compared with between Rahmani and Ossimi. Mahfouz *et al***.** (2008) used biochemical and molecular genetic techniques to study diversity in some Egyptian sheep breeds. Ossimi breed showed higher homogeneity than Rahmani and Barki.

Mahfouz *et al.* (2008) studied the genetic variation of Rahmani, Ossimi, Barki, Saidi and Sohagi Egyptian sheep breeds and found specific bands that can be used as markers for each breed. Dendrogram analysis grouped the studied breeds into two clusters: the first included Ossimi and Rahmani while the second was divided into two sub-clusters: one included Barki and the other included Saidi and Sohagi.

CONCLUSION

The results of this study demonstrate the usefulness of RAPD approach for detecting DNA polymorphism in sheep and establishing the relationships among different breeds. The majority of random primers used gave distinctly reproducible patterns for the studied breeds though primers varied in the extent of information they generated with some producing highly polymorphic patterns and others producing less polymorphic products with some similar in size among the six breeds and others unique to a particular breed.

Thus, genetic diversity exists among the studied breeds. Further experimentations on RAPD profiles generated for each breed can be effectively used as a supporting marker for taxonomic identification.

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أجري هذا البحث بهدف تحديد التنوع الوراثي في سلالات الأغنام التالية : الرحماني، والبرقي، والعواسي، والأوسيمي وبعض الخلطان وكذلك سلالة السفولك البريطانية، تم عزل المادة الوراثية من الحيوانات، وتصميم عدد من البادئات غير المتخصصة Random Primers للكشف عن التنوع الوراثي، وذلك باستخدام تقنية (RAPD)، ومن خلال ذلك وجد أن التشابه بين البرقي والرحماني يصل إلى (٩٦٪)، بينما كان بين الرحماني والعواسي (٩٢٪)، في حين كان بين البرقي والأوسيمي (٩٠٪) في الوقت الذي تراوحت فيه نسب التشابه الوراثي بين السلالة البريطانية السفولك وبقية السلالات بين (٨٥٪)، و(٩٢٪) هذا ويساعد تحديد التنوع الوراثي في تصميم وتنفيذ برامج عامة وشاملة للتحسين الوراثي في الأغنام سواء بالانتخاب لهذه العوامل الوراثية أو تنفيذ برامج محددة لخلط السلالات المحلية بسلالات أجنبية تحمل العوامل الوراثية المفيدة والسماح لها بالانتقال عبر الأجيال لتصبح جزء من تكوين الجينوم الخاص بالأغنام المصرية وفي نفس الوقت تحقيق ميزة الاستفادة من وجود صفات الأقلمة للبيئة المصرية.

Induction of Systemic Resistance in Common Bean Against Root Rot Diseases

Wafaa T. Shahda *, Eid M. Abou-Taleb and Ragab G. Ismail
Department of Plant Pathology., Faculty of Agriculture, Alexandria University, El-Shtby 21545, Alexandria, Egypt.
Email: dr.wshahda@gmail.com

ABSTRACT

Four isolates of *Rhizoctonia solan*i (R1, R2, R3 and R4), two isolates of *Fusarium solani* (F1 and F2), one isolate of *Macrophomina phaseolina* (M), and one isolate of *Pythium* spp.(P) were isolated from common bean seedlings and plants showing damping-off and root-rot symptoms in El-Behera governorate. All tested isolates were pathogenic to all tested cultivars (Giza 6, Nebraska, Bronco and Paulista). Five antioxidants (benzoic acid, citric acid, hydroquinone, salicylic acid and sorbic acid) were tested for their effect on mycelial growth of the tested pathogens. The obtained data showed that all the tested compounds significantly inhibited most of the pathogens at 5 mM – 10 mM. The efficiency of seed treatment with the different antioxidant compounds in controlling common bean damping-off and root-rot caused by *R. solan*i (R1) and *M. phaseolina* (M)] was evaluated. All treatments were effective in reducing percentages of damping–off (from 14.3 – 56.6 %) and root-rot (from13.6 – 54.5 %) in Giza 6 and Bronco cultivars. The effect of seed treatment with the antioxidant compounds on the activity of some defence-related enzymes (peroxidase, polyphenol oxidase, chitinase and β-1,3-glucanase) and the total phenolic contents were estimated. The obtained results varied according to the different compounds, the pathogens, and the tested cultivars. In general, the activity of the enzymes were higher and faster (after one week) in the resistant cultivar (Giza 6), while the increase was less and slower (after two weeks) in the susceptible cultivar (Bronco).

Key words: ***Common bean, antioxidants, root-rot, defence-related enzymes.***

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of several crop species belonging to the Fabaceae family. It is one of the most widely cultivated legumes in the world (Gepts, 1998). Damping-off and root-rot diseases are widely distributed around the world and are economically important diseases in common bean, reducing both yield and quality (Abawi and Widmer, 2000). The major soil borne pathogens, which attack roots and causing damping-off and root-rot diseases, are *Fusarium solani* f. sp. *phaseoli*, *Fusarium oxysporum* f. sp. *phaseoli*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotium rolfsii*, *Pythium* spp., *Thielaviopsis basicola* and *Aphanomyce* spp. (Gonzalez *et al.,* 2006).

Commonly tested chemical elicitors are salicylic acid, methyl salicylate, benzothiadiazole, benzoic acid, chitosan, and so forth which affect production of phenolic compounds and activation of various defence-related enzymes in plants (Thakur and Sohal, 2013). Antioxidants, which save to human and environment, had been used successfully to control some plant diseases such as root- and pod-rot in peanut (Elwakil, 2003), Fusarium wilt in chickpea (Nighat-Sarwar *et al.*, 2005), faba bean chocolate spot (Hassan *et al.*, 2006), peanut root-rot (Mahmoud *et al.*, 2006), Fusarium wilt in tomato (El-Khallal, 2007).

Shahda (2000) showed that benzoic, salicylic and ascorbic acids significantly reduced linear growth of *F. solani, F. oxysporum* and *R. solani* (the causal organisms of tomato damping-off) at 20 mM. The three used antioxidants exhibited growth promoting effect where they increased shoot and root length and dry weight of tomato seedlings. Also, Shahda (2001) reported that salicylic acid was effective in inhibiting spore germination and hyphal growth of *Alternaria alternata* at 4.0 and 1.5 mM, respectively. Tomato seed soaking for one hour or soil drench with salicylic acid at 10 to 20 mM significantly decreased *A. alternata* damping-off. Hemeda (2009) recorded complete inhibition of linear growth of *Aleternaria aleternata, F. oxysporum* f.sp. *phaseoli, Macrophomina phaseolina* and *R. solani* the causal organisms of bean root-rot disease at 20 mM of benzoic, formice, malic, salicylic and sorbic acids. Sorbic, salicylic and benzoic acids increased fresh weight of total plant by 4-83%, 11-117% and 3-83%, respectively when used as seed soaking at 1 mM concentration for 2 hours. Abdel-monaim and Ismail (2010) reported that the use of antioxidants, e.g. coumaric acid, citric acid, propylgalate and salicylic acid each at 100 and 200 ppm as seed soaking, seedling soaking, and soil drench under greenhouse and field conditions reduced damping-off, root-rot and wilt of pepper plants caused by *Fusarium solani* isolate FP2 and *F. oxysporum* isolate FP4 compared with untreated plants. All chemicals significantly increased fresh and dry weight and plant growth parameters of seedling grown in soil drenching or seed treatment.

Zhang *et al*. (2016) reported that application of salicylic acid (SA) could improve resistance to Glomerella leaf spot (GLS) caused by *Glomerella cingulata* in a highly susceptible apple cultivar (*Malus domestica* Borkh. cv. 'Gala'. Concurrent with the enhanced disease resistance, salicylic acid treatment markedly increased the defence-related enzyme activities, including catalase (CAT), superoxide dismutase (SOD), peroxidase

(POD), phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO). As expected, SA treatment also induced the expression levels of five pathogenesis-related (*PR*) genes including *PR1*, *PR5*, *PR8, Chitinase* and *β-1,3-glucanase*.

This study aimed to control damping-off and root-rot of common bean using safe compounds as resistance inducers and explain the mode of action of these compounds on defence mechanisms.

MATERIALS AND METHODS

1- Isolation, Purification and Identification:

Common bean plants showing root-rot symptoms were collected from different fields of El-Behera governorate (Nubaria, Hosh-Essa and two locations of Abo-Elmatmer).

Roots and hypocotyls of the infected plants were thoroughly washed in running tap water for 30 min, cut into small pieces (5 mm length), surface sterilized with 1% sodium hypochlorite NaOCl for 3 min, rinsed several times in sterile distilled water and dried between sterilized filter paper.

The sterilized pieces were transferred to Petri plates containing potato dextrose agar (PDA) medium supplemented with streptomycin sulfate (50 mg/L.). Plates were incubated at 25±2°C and inspected daily.

The developed fungi were purified either by single spore isolation or hyphal tip techniques, then sub-cultured on PDA slants and kept at 4°C. Identification was carried out according to their morphological and microscopical characteristics as described by Booth (1985) and Barnett and Hunter (1986) in Plant Pathology Department, Faculty of Agriculture, Alexandria University.

2- Pathogenicity tests:

Seeds of four common bean cultivars namely; Bronco and Paulista (snap bean), Giza 6 and Nebraska (dry bean) were obtained from Horticulture Research Institute, Agricultural Research Centre (ARC) ,Giza,Egypt.

The purified cultures of identified *Rhizoctonia solani, Fusarium solani, Macrophomina phaseolina* and *Pythium* spp., were tested for their pathogenic capability to common bean cultivars under greenhouse conditions.

2.1. Damping-off test:

Clay pots, 25 cm diameter, filled with 1 kg autoclaved aerated sandy loam soil (1:1 v/v), were artificially infested with the inocula of each single species of isolated pathogens. Ten grams of infested barely grains were used / pot (kg soil), kept in the open air, watered daily for 7 days before sowing.

Surface sterilized seeds in 1% sodium hypochlorite for 3 min were sown at the rate of 10 seeds / pot after one week of soil infestation. Five replicates were used for each treatment, non-infested soil were served as control. Number of pre- and post-emergence damping-off were recorded after 15 and 30 days.

2.2. Root-rot and disease severity (DS) estimation:

To determine root-rot and disease severity on the emerged plants, a pot experiment was carried out. Sterilized seeds of different cultivars were used. Five common bean seeds were sown in each pot (25-cm-diam.) containing autoclaved sandy loamy soil (1:1 v/v), five replicates for each isolate were used. Common bean plants were inoculated, 4 weeks after sowing, with spores or hyphal suspension (50 ml / pot).

For assessment of root-rot and disease severity, visual examination of roots and hypocotyls was used after 60 days of inoculation. Number of plants showing root-rot were counted. Disease severity was estimated according to the C1AT 1-9 scale (Abawi and Pastor-Corrales, 1990). Disease severity index (DSI) as follows: 1 = no visible symptoms, 3 = light discolouration either without necrotic lesions or with approximately 10% of the hypocotyl and root tissue covered with lesions, 5 = approximately 25% of the hypocotyl and root tissue covered with lesions though the tissues remain firm, with some deterioration of the root system, 7 = approximately 50% of the hypocotyls and root tissues covered with lesions combined with considerable softening, rotting, and reduction of root system, 9 = approximately 75% or more of the hypocotyls and root tissues affected with advanced stages of rotting, combined with severe reduction in the root system.

DS % was calculated as follows:

$$DS = \frac{\Sigma d}{d\,max \times a} \times 100$$

Where: d is the disease rating possible, d max is the maximum disease rating and n is the total number of plants examined in each replicate.

3- *In vitro* Studies:

Five chemical inducers (benzoic acid, citric acid, hydroquinone, salicylic acid and sorbic acid) were tested for their effect on linear growth of the isolated pathogens.

Different concentrations *i.e.* 2.5, 5, 10, 20 and 30 mM of each compound were prepared. Hundred ml of aqueous solution of each compound was used as stock solution, a certain volume was added to warm PDA (100 ml in 250 conical flask) to obtain the required concentration, and then poured in 9 cm Petri plates. Plates contain PDA free of any compound served as control. Four replicates (plates) were used per each treatment. After solidification, plates were inoculated with 5mm disks of the tested fungi, incubated at 25±2 ºC. Percentage of growth inhibition of each pathogen was calculated using the formula below:

Inhibition (%) = (A − B)/A × 100

where, A = Colony diameter of growth in control,

B = Colony diameter of growth in the treated plates.

4. *In vivo* studies:

An experiment was carried out to evaluate the efficiency of different seed treatments by the most effective antioxidant compounds obtained from the *in vitro*

results (benzoic acid, salicylic acid and sorbic acid) and the fungicide Rizolex-T 50% in controlling common bean damping-off and root-rot caused by the most aggressive pathogenic fungi (*Rhizoctonia solani* isolate R1 and *Macrophomina phaseolina* isolate M) under artificially infested soil.

Disinfested common bean seeds (Giza 6 and Bronco cvs.) were individually soaked for 2 hrs. in each solution of either; benzoic acid, salicylic acid and sorbic acid at concentration of 5 Mm (which was completely inhibited the growth of *R. solani* isolate (R1) and *M. phaseolina* (M)), and then left to air drying before sowing.

Fungicide seed dressing: Common bean seeds (Giza 6 and Bronco cvs.) were dressed with Rizolex-T50 % at the recommended dose (3 g/kg seeds) then sown in infested soil and served as a comparison treatment.

Treated and untreated common bean seeds were sown in clay pots (25-cm-diam.) containing individually artificially infested sandy loam soil (1:1 v/v) with the most aggressive fungal isolates (*Rhizoctonia solani* isolate (R1) and *Macrophomina phaseolina* (M)) at the rate of 10g infested barely grains/pot (kg soil) . The inoculum of the pathogenic fungi was prepared as barley grains inoculum as previously described. Infested soil was irrigated for 1 week before sowing. Five replicates (pots) were used for each treatment (5 seeds / pot).

Percentages of damping-off at pre- and post-emergence stages were recorded after 15 and 30 days, respectively. Sixty days after sowing, root-rot and disease severity (DS) of root-rot was recorded by washing the survivors roots and hypocotyls under running tap water, and scoring the disease according to the C1AT 1-9 scale as previously described.

5. Effect of seed treatment by antioxidants on some defence-related enzymes activity:

An experiment was carried out to evaluate the efficiency of different seed treatments on the activity of peroxidase, polyphenol oxidase, chitinase and β-1, 3-glucanase, total phenolic contents.

Treated and untreated common bean seeds with the tested treatments were sown in clay pots containing autoclaved sandy loam soil (1:1 v/v). Five common bean seeds of any of the tested treatments were sown in each pot, and three pots were used as replicates for each treatment. Four weeks after sowing, common bean plants, in each treatment, were individually soil infested with mycelial suspension of each pathogen (*R. solani* (R1) and *M. phaseolina* (M)) at the rate of 50 ml / pot.

Roots of treated and untreated (control) plants with the tested treatments were collected at 0, 7 and 14 days after inoculation, then washed in running tap water and stored in a deep freezer (–20°C) until used for biochemical analysis.

5.1. Peroxidase (PO) assay:

PO activity was assayed spectrophotometrically (Spectro 22 colorimeter, Labomed, Inc, U.S.A.) at 470 nm using guaiacol as a phenolic substrate with hydrogen peroxide (Díaz *et al.*, 2001). The enzyme activity was expressed as changes in the absorbance at 470 nm min^{-1} g^{-1} fresh weight.

5.2. Polyphenol oxidase (PPO) Assay:

Polyphenol oxidase activity was determined as the procedure given by Mayer *et al.* (1965). The activity was expressed as changes in absorbance using (Spectro 22 colorimeter, Labomed, Inc, U.S.A.) at 495 nm min^{-1} g^{-1} fresh weight.

5.3. Chitinase assay:

The colorimetric assay of chitinase was carried out according to Monreal and Reese (1969). Colloidal chitin was prepared according to (Murthy and Bleakley, 2012). The enzyme activity was expressed as μmol N-acetyl-glugosamine 60 min^{-1} g^{-1} fresh weight.

5.4. β-1,3-glucanase assay:

β-1,3-glucanase activity was assayed by the laminarin-dinitrosalicylic acid method (Pan *et al.*, 1991). The enzyme activity was expressed as μg glucose released min^{-1} g^{-1} fresh weight.

5.5. Estimation of total phenolic contents:

The total phenolic contents were estimated as the procedure given by Zieslin and Ben–Zaken, 1993. Catechol was used as standard. The amount of phenolics was expressed as μg catechol g^{-1} fresh weight.

RESULTS AND DISCUSSION

1. Isolation and Identification:

Isolation was carried out from roots and hypocotyls of infected common bean seedlings and plants showing damping-off and root-rot symptoms, collected from different regions in El-Behera Governorate (Nubaria, Hosh-Essa and two locations of Abo-Elmatmer). The isolation revealed nine different isolates belonging to five genera were isolated, purified and identified according to their morphological and microscopical characters as described by Booth (1985) and Barnett and Hunter (1986). The isolated pathogens were four isolates of *Rhizoctonia solan*i (R1, R2, R3 and R4), two isolates of *Fusarium solani* (F1 and F2), and one isolate of each of *Macrophomina phaseolina* (M) and *Pythium* spp.(P).

Many investigators noted that *F. solani, R. solani*, *M. phaseolina*, *F. oxysporum* and *S. rolfsii* are considered among the main pathogens causing damping-off and root-rot diseases of common bean plants (Sallam *et al*., 2008; Abd-El-Khair, 2010; El-Mohamedy *et al*., 2013; Shehata, 2015).

2. Pathogenicity tests:

Pathogenic potentialities of the isolated pathogens were tested on four common bean cultivars (Bronco and Paulista[snap bean cvs.] and Giza 6 and Nebraska[dry bean cvs.]). Pre- and post-emergence damping-off were recorded after 15 and 30 days of sowing. Root-rot and disease severity index (DSI) were recorded after 60 days of sowing.

2.1. Estimation of damping-off disease:

All the isolated pathogens were pathogenic to the four tested cultivars and caused damping-off disease ranged from 4 - 96 %.

The obtained data proved that *Rhizoctonia solan*i (isolate R1) was the most pathogenic isolate to all the tested cultivars in causing pre- and post-emergence damping-off, where the total mortality was ranged from76 – 96 %, followed by *Rhizoctonia solan*i (isolate R4), where the percentages of the total mortality was ranged from 72 – 92%.

In case of the *Fusarium solani* (isolate F1) it showed a percentage of total mortality ranged from 52 – 80%. While, *Fusarium solani* (isolate F2) showed less damping-off percentages on the tested cultivars, which ranged from 4 – 48 %.

Percentage of total mortality was ranged from 4 – 76 % in Giza 6 cultivar, 12– 92 % in Nebraska cultivar, 48-96% in Bronco cultivar and 36-92% in case of Paulista cultivar. (Table 1).

2.2. Estimation of root-rot disease and disease severity:

Data presented in Table 2 showed the effect of the tested pathogenic isolates on root-rot and disease severity index (DSI) after 60 days of sowing. Tabulated data showed that:

Total mortality of root-rot disease was more significant on snap bean cultivars, which ranged from 32 – 72 %, than on dry bean cultivars, which ranged from 16 – 60 %.

The most aggressive pathogens in causing root-rot disease on Giza 6 cultivar were *R. solan*i (R1) followed by *Macrophomina phaseolina* (M) (36 and 28 %), respectively. Also, on Paulista cultivar, the highest root-rot disease percentages were (68 and 64 %) in *R. solan*i (R1) and *M. phaseolina* (M) respectively.

In case of Bronco cultivar, the most aggressive pathogens in causing root-rot disease were *R. solan*i (R1) and *M. phaseolina* (M) (72 % by both pathogens). In Nebraska cultivar, *M. phaseolina* (M) followed by *R. solan*i (R1) (60 – 56 %), respectively were the most pathogenic isolates.

Disease severity was recorded on the infected plants according to the C1AT 1-9 scale (Abawi and Pastor-Corrales, 1990) of root discoloration. Results showed that *M. phaseolina* (M) was the most aggressive pathogen in disease severity percentages (DS %) where it ranged from 84.4 – 93.3 %, followed by R. *solan*i (R1) where it ranged from 77.8 – 93.3 %.

Based on all of the aforementioned data, the obtained results showed that Giza 6 (dry bean) was the highest resistant cultivar, while Bronco (snap bean) was the highest susceptible one of the four tested cultivars.

Data also showed that the isolated pathogens were varied in their virulence, whereas R. *solan*i (R1) and R. *solan*i (R4) followed by *F. solani* (F1) were the most pathogenic isolates to all the tested cultivars in causing pre- and post-emergence damping-off. Where, *R. solan*i (R1) and *M. phaseolina* (M) were the most aggressive pathogens in causing root-rot disease.

3. *In vitro* studies:

The obtained results showed that:

1- The antioxidants used were very effective in inhibiting the growth of the tested pathogens on PDA medium compared with the control. The inhibition effect was different according to the pathogen and the compound.

2- Sorbic acid completely inhibited the growth of four pathogens at 5 mM and the fifth pathogen *M. phaseolina* (M) at 10 mM.

3- Benzoic acid was next to sorbic acid, where it completely inhibited the growth of three pathogens [*R. solan*i (R1), *M. phaseolina* (M) and *Pythium* spp. (P)] at 5 mM, and the rest pathogens [*R. solan*i (R4) and *F. solan*i (F1)] at 10 mM.

4- Salicylic acid followed the previous two in inhibiting the growth of one pathogen [*R. solan*i (R4)] at 5 mM and four pathogens at 10 mM *[R. solan*i (R1), *F. solan*i (F1), *M. phaseolina* (M) and *Pythium* spp. (P)].

5- Hydroquinone and citric acid could inhibit the growth of the tested pathogens at 10-30 mM.

Such results are in accordance with the work of Shahda (2000) who reported that benzoic acid completely inhibited the growth of *Rhizoctonia solani* at 25 mM, and salicylic acid against *Alternaria alternate* at 4.0 mM (Shahda, 2001). Salicylic acid was reported also against many pathogenic fungi (Ozganen *et. al.,* 2001; Shabana, *et al*.,2008; Abd El-Hai, *et al.,* 2009).

4. *In vivo* studies:

4.1. Control of damping-off disease:

Data in Table 3 showed that:

All the treatments were effective in reducing percentages of damping–off disease as compared with the control. The reduction ranged from 14.29–50.00%.

In case of Giza 6 cultivar, the most effective treatment against *R. solan*i (R1) was sorbic acid (44.44 % decrease). Also, sorbic acid was the most effective treatments against *M. phaseolina* (M), where the decrease reached 50.00 %. For Bronco cultivar, the most effective treatment against *R. solan*i (R1) was was sorbic acid (33.33 % decrease). Also, sorbic acid and salicylic acid were the most effective treatments against *M. phaseolina* (M) (37.50 % decrease). In all treatments the fungicide (Rizolex-T 50%) was the best in reducing damping-off incidence by the two tested pathogens in both cultivars.

4.2. Control of root-rot disease:

Data in Table 3 showed that:

Table 1. Effect of soil infestation with the isolated pathogens on pre– and post–emergence damping-off on common bean tested cultivars after 15 and 30 days of sowing

Cultivars		Pathogens Mean * no. of seedlings	Pre-emergence		Post-emergence		Total mortality %
			% of mortality	Mean * no. of seedlings	% of mortality	Mean * no. of seedlings	
Dry bean cultivars	Giza 6	Control	10.0 ^{a*}	00	10.0 ^{a}	00	00
		*Rhizoctonia solan*i (R1)	4.0 ^{d}	60	2.4 ^{f}	16	76
		*Rhizoctonia solan*i (R2)	8.8 ^{bc}	12	6.8 ^{d}	20	32
		*Rhizoctonia solan*i (R3)	8.4 ^{c}	16	8.0 ^{c}	04	20
		*Rhizoctonia solan*i (R4)	4.8 ^{d}	48	2.8 ^{f}	32	72
		Fusarium solani (F1)	8.4 ^{c}	16	4.8 ^{e}	36	52
		Fusarium solani (F2)	9.6 ^{ab}	04	9.6 ^{a}	00	04
		Macrophomina phaseolina (M)	8.4 ^{c}	16	8.4 ^{bc}	00	16
		Pythium spp.(P)	9.2 ^{abc}	08	8.8 ^{b}	04	12
	Nebraska	Control	9.2 ^{a}	08	9.2 ^{a}	00	08
		*Rhizoctonia solan*i (R1)	2.0 ^{f}	80	1.2 ^{e}	08	88
		*Rhizoctonia solan*i (R2)	2.8 ^{e}	72	1.2 ^{e}	16	88
		*Rhizoctonia solan*i (R3)	8.4 ^{bc}	16	6.4 ^{c}	20	36
		*Rhizoctonia solan*i (R4)	2.4 ^{ef}	76	0.8 ^{e}	16	92
		Fusarium solani (F1)	4.4 ^{d}	56	4.0 ^{d}	04	60
		Fusarium solani (F2)	8.0 ^{c}	20	8.0 ^{b}	00	20
		Macrophomina phaseolina (M)	8.0 ^{c}	20	6.8 ^{c}	12	32
		Pythium spp.(P)	8.8 ^{ab}	12	8.8 ^{a}	00	12
Snap bean cultivars	Bronco	Control	7.6 ^{a}	24	3.8 ^{a}	0	24
		*Rhizoctonia solan*i (R1)	0.8 ^{e}	92	0.2 ^{d}	4	96
		*Rhizoctonia solan*i (R2)	3.6 ^{c}	64	0.8 ^{c}	20	84
		*Rhizoctonia solan*i (R3)	3.6 ^{c}	64	1.4 ^{b}	08	72
		*Rhizoctonia solan*i (R4)	3.6 ^{c}	64	1.0 ^{c}	16	80
		Fusarium solani (F1)	2.0 ^{d}	80	1.0 ^{c}	00	80
		Fusarium solani (F2)	5.6 ^{b}	44	2.6 ^{b}	04	48
		Macrophomina phaseolina (M)	6.0 ^{b}	40	2.6 ^{b}	08	48
		Pythium spp.(P)	2.8 ^{cd}	52	2.2 ^{b}	04	56
	Paulista	Control	8.4 ^{a}	16	8.4 ^{a}	0	16
		*Rhizoctonia solan*i (R1)	0.8 ^{f}	92	0.8 ^{g}	0	92
		*Rhizoctonia solan*i (R2)	5.2 ^{c}	48	2.8 ^{e}	24	72
		*Rhizoctonia solan*i (R3)	5.6 ^{c}	44	2.8 ^{e}	26	72
		*Rhizoctonia solan*i (R4)	4.4 ^{d}	56	2.4 ^{ef}	20	76
		Fusarium solani (F1)	2.4 ^{e}	76	2.0 ^{f}	4	80
		Fusarium solani (F2)	6.4 ^{b}	36	5.6 ^{c}	8	44
		Macrophomina phaseolina (M)	6.4 ^{b}	36	6.4 ^{b}	0	36
		Pythium spp.(P)	4.8 ^{d}	52	4.0 ^{d}	8	60

* Mean of five replicates (pots), 10 seeds each.

Different letters indicate significant differences within the same column in each cultivar according to least significant difference test ($p \leq 0.05$).

Sorbic acid was the best tested antioxidant on both tested cultivars (Giza 6 and Bronco), which decreased root-rot percentages (40.0 and 31.8 %) against *R. solan*i (R1) in Giza 6 and Bronco cultivars respectively. In case of *M. phaseolina* (M) sorbic acid decreased root-rot percentages in both cultivars (54.5 and 47.4 % decrease) respectively.

5. Effect of seed treatment by different antioxidants on some defence-related enzymes activity:

The plant roots were collected at 0, 1 and 2 weeks after inoculation. The activity of peroxidase, polyphenol oxidase, chitinase, β-1, 3-glucanase and total phenolic contents were estimated.

Table 2. Effect of soil infestation with the isolated pathogens on root-rot and Disease Severity Index (DSI) after 60 days from sowing

Cultivars		Pathogens	Root-rot		DSI**	DS%***
			Mean* no. of survivors	% of mortality		
Dry bean cultivars	Giza 6	Control	5.0 a	0.00	2.00 d	22.22
		Rhizoctonia solani (R1)	3.2 c	36.00	7.00 b	77.78
		Rhizoctonia solani (R4)	4.0 b	20.00	6.20 c	68.89
		Fusarium solani (F1)	4.2 b	16.00	5.85 c	65.00
		Macrophomina phaseolina (M)	3.6 bc	28.00	8.05 a	89.45
		Pythium spp. (P)	4.2 b	16.00	6.40 c	71.11
	Nebraska	Control	4.8 a	4.00	2.50 c	27.78
		Rhizoctonia solani (R1)	2.2 d	56.00	8.00 a	88.89
		Rhizoctonia solani (R4)	3.2 c	36.00	6.50 b	72.22
		Fusarium solani (F1)	3.6 bc	28.00	6.75 b	75.00
		Macrophomina phaseolina (M)	2.0 d	60.00	8.25 a	91.67
		Pythium spp. (P)	3.6 ab	28.00	7.00 b	77.78
Snap bean cultivars	Bronco	Control	4.0 a	20.00	2.0 c	22.22
		Rhizoctonia solani (R1)	1.4 d	72.00	8.00 ab	88.89
		Rhizoctonia solani (R4)	2.8 c	44.00	7.25 b	80.56
		Fusarium solani (F1)	3.2 bc	36.00	7.70 ab	85.55
		Macrophomina phaseolina (M)	1.4 d	72.00	8.40 a	93.34
		Pythium spp. (P)	2.4 ab	52.00	7.85 ab	87.22
	Paulista	Control	4.2 a	16.00	2.75 e	30.55
		Rhizoctonia solani (R1)	1.6 e	68.00	8.40 a	93.33
		Rhizoctonia solani (R4)	3.0 bc	40.00	6.67 cd	71.11
		Fusarium solani (F1)	3.4 b	32.00	7.00 bc	77.78
		Macrophomina phaseolina (M)	1.8 de	64.00	7.60 ab	84.44
		Pythium spp. (P)	2.4 cd	52.00	5.67 d	62.95

* Mean of five replicates (pots), 5 seeds each.

** According to the C1AT 1-9 scale (Abawi and Pastor-Corrales,1990). In this scale; 1 = no visible symptoms 3 = approximately 10% of the hypocotyl and root tissue covered with lesions; 5 = approximately 25% of the hypocotyl and root tissue covered with lesions; 7 = approximately 50% of the hypocotyls and root tissues covered with lesions and 9 = approximately 75% or more of the hypocotyls and root tissues affected with advanced stages of rotting.

*** where d is the disease rating possible, d max is the maximum disease rating and n is the total number of plants examined in each replicate.

Different letters indicate significant differences within the same column in each cultivar according to least significant difference test ($p \leq 0.05$).

5.1. Peroxidase (PO) activity:

The obtained data showed that:

Peroxidase (PO) activity was increased in treated plants compared with control. The highest activity of the enzyme was recorded after one week of inoculation.

Soil infested with *R. solan*i (R1), salicylic acid (5 mM) showed the highest enzyme activity in Giza6 cultivar (533.5 U min^{-1} g^{-1} fresh weight). In case of Bronco cultivar, salicylic acid (5 mM) followed by sorbic acids (5 mM) were the highest treatments (5.3 and 4.6 U min^{-1} g^{-1} fresh weight) respectively.

Soil infested with *M. phaseolina* (M), sorbic acid (5 mM) recorded the most effective treatment followed by benzoic acid (5 mM) (251.5 and 233.5 U min^{-1} g^{-1} fresh weight) respectively, in Giza 6 cultivar. While, in Bronco cultivar sorbic acid (5 mM) followed by benzoic acid (5 mM) and then salicylic acid (5 mM) were recorded the most effective treatments (4.03, 4.00 and 3.95 U min^{-1} g^{-1} fresh weight) respectively.

5.2. Polyphenol oxidase (PPO) activity:

The obtained results showed that:

All treatments caused increase in polyphenol oxidase (PPO) activity after inoculation. The highest increase was recorded after 2 weeks of inoculation in Giza 6 cultivar, and after the third week in Bronco cultivar.

Table 3. Effect of seed treatment with antioxidants and the fungicide Rizolex-T 50% on the control of damping-off and root-rot diseases of common bean caused by the most pathogenic isolates on Giza 6 and Bronco cultivars

Cultivars	pathogens	Treatments	Conc.	Damping-off disease						Root-rot disease					
				Pre-emergence		Post-emergence		Total mortality %	% decrease	Root-rot			Disease severity		
				Mean* no. of seedlings	% of mortality	Mean no. of seedlings	% of mortality			Mean * no. of survivors	% Total mortality	% decrease	DSI	DS%**	% decrease
Giza 6	*R. solani* (R1)	Control	0	2.2 d	56	1.4 d	16	72	--	1.0 d	80	------	7.00	77.78	--
		Benzoic acid	5 mM	2.4 c	52	2.0 c	8	60	16.67	1.8 c	64	20.00	5.89	65.43	15.88
		Salicylic acid	5 mM	3.2 b	36	2.6 b	12	48	33.33	2.4 b	52	35.00	5.67	62.96	19.05
		Sorbic acid	5 mM	3.6 b	28	3.0 b	12	40	44.44	2.6 b	48	40.00	5.62	62.39	19.79
		Rizolex-T	3 g/kg seed	4.4 a	12	4.0 a	8	20	72.22	3.8 a	24	70.00	3.74	41.52	46.62
	M. phaseolina (M)	Control	0	4.0 b	20	3.8 b	8	24	**--**	2.8 b	44	------	8.00	88.89	--
		Benzoic acid	5 mM	4.6 ab	8	4.0 ab	4	20	**16.67**	3.4 a	32	27.27	6.53	72.55	18.38
		Salicylic acid	5 mM	4.2 ab	16	4.2 ab	4	16	33.33	3.8 a	24	45.45	6.47	71.93	19.08
		Sorbic acid	5 mM	4.6 ab	8	4.4 ab	8	12	**50.00**	4.0 a	20	54.54	6.10	67.78	23.75
		Rizolex-T	3 g/kg seed	5.0 a	0	4.8 a	4	4	**83.33**	4.4 a	12	72.72	5.27	58.58	34.10
Bronco	*R. solani* (R1)	Control	0	1.0 d	80	0.8 d	4	84	**--**	0.6 c	88	------	7.67	85.19	--
		Benzoic acid	5 mM	1.6 cd	68	1.4 cd	4	72	**14.29**	1.2 c	76	13.63	7.00	77.78	8.70
		Salicylic acid	5 mM	1.8 c	64	1.6 bc	4	68	**19.05**	1.2 c	76	13.63	6.67	74.07	13.05
		Sorbic acid	5 mM	2.6 b	48	2.2 b	8	56	**33.33**	2.0 b	60	31.81	5.90	65.56	23.04
		Rizolex-T	3 g/kg seed	3.6 a	28	3.4 a	4	32	**61.90**	3.0 a	40	54.55	5.20	57.78	32.18
	M. phaseolina (M)	Control	0	2.0 c	60	1.8 c	4	64	**--**	1.2 c	76	------	8.33	92.59	--
		Benzoic acid	5 mM	2.8 b	44	2.6 b	4	48	**25.00**	2.4 b	52	31.58	7.17	79.63	14.00
		Salicylic acid	5 mM	3.0 b	40	3.0 b	0	40	**37.50**	2.8 b	44	42.11	6.71	74.60	19.43
		Sorbic acid	5 mM	3.2 b	36	3.0 b	4	40	**37.50**	3.0 ab	40	47.37	6.60	73.33	20.80
		Rizolex-T	3 g/kg seed	4.2 a	16	3.8 a	8	24	**62.50**	3.6 a	28	63.16	5.00	55.56	39.99

* Mean no. of seedlings of five replicates (pots) / treatment, (5 seeds each).

** Mean no. of survivors plants of five replicates (pots) / treatment, (5 seeds each).

***, where d is the disease rating possible, d max is the maximum disease rating and n is the total number of plants examined in each replicate.

Different letters indicate significant differences within the same column according to least significant difference test ($p \leq 0.05$).

Sorbic acid (5 mM) was the best treatments, against *R. solan*i (R1), after the second week in Giza 6 cultivar (34.8 U min^{-1} g^{-1} fresh weight). In Bronco cultivar, the highest activity of enzyme was recorded with sorbic acid (5 mM) in the second week of inoculation (2.22 U min^{-1} g^{-1} fresh weight) against *R. solan*i (R1).

In case of the soil infested with *M. phaseolina* (M), salicylic acid (5 mM) followed by benzoic acid (5 mM) were the best treatments after the second week in Giza 6 cultivar (15.55 and 15.06 U min^{-1} g^{-1} fresh weight) respectively. In Bronco cultivar, the highest activity of enzyme was recorded with salicylic acid (5 mM) followed by sorbic acid (5 mM) in the second week of inoculation (1.79 and 1.14 U min^{-1} g^{-1} fresh weight) respectively.

5.3. Chitinase activity:

The obtained data showed that:

The highest activity of chitinase was after the second week of inoculation. Salicylic acid (5 mM) was the best treatment after the second week in Giza 6 cultivar (90.9 µmol N-acetylglugosamine 60 min^{-1} g^{-1} fresh weight) against *R. solan*i (R1). While, sorbic acid (5 mM) was better in case of Bronco cultivar, (51.81 µmol N-acetylglugosamine 60 min^{-1} g^{-1} fresh weight).

In case of soil infested with *M. phaseolina* (M), the highest activity of enzyme caused by sorbic acid (5 mM) at the first week (76.4 µmol N-acetylglugosamine 60 min^{-1} g^{-1} fresh weight), in Giza 6 cultivar. While, in case of Bronco cultivar sorbic acid (5 mM) was the best treatments at the second week (65.18 µmol N-acetylglugosamine 60 min^{-1} g^{-1} fresh weight).

5.4. β-1,3-glucanase activity:

The obtained results showed that:

The highest activity of β-1,3-glucanase enzyme was observed after 2 weeks of inoculation in both cultivars.

Sorpic acid (5 mM) was the best treatment against *M. phaseolina* (M) (460.30 µg glucose min^{-1} g^{-1} fresh weight) in Giza 6 cultivar. While, in Bronco cultivar salicylic acid (5 mM) was the best treatments after the second week (60.76 µg glucose min^{-1} g^{-1} fresh weight).

In case of *R. solan*i (R1), salicylic acid (5 mM) was the best treatment in Giza 6 cultivar (511.8 µg glucose min^{-1} g^{-1} fresh weight). While, in case of Bronco cultivar salicylic acid (5 mM) were the best treatment at the second week (52.96 µg glucose min^{-1} g^{-1} fresh weight).

5.5. Total phenolic contents:

The data showed that:

The highest quantity of total phenolic contents was recorded in the first week in case of Giza 6 cultivar, and in the second week in Bronco cultivar.

Salicylic acid (5 mM) was the best treatment against *R. solan*i (R1) in both cultivars (in Giza 6: 34.7, in Bronco: 18.4 μg catechol g^{-1} fresh weight).

Sorbic acid was the most effective treatment against *M. phaseolina* (M) in both cultivars (in Giza 6: 27.97, in Bronco: 13.05 μg catechol g^{-1} fresh weight).

The effect of seed treatment by antioxidant compounds on activity of peroxidase, polyphenol oxidase, chitinase and β-1, 3-glucanase revealed significant increase in all the enzymes activity. Also, total phenols significantly increased after seed treatment. Treatment with salicylic acid and its derivative induced expression of pathogenesis-related (PR) proteins. So, salicylic acid plays an important role in induction of plant defence against a variety of biotic and abiotic stresses through morphological, physiological and biochemical mechanisms. It regulates the activities of various enzymes such as, peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) etc., which are the major components of induced plant defence against biotic and abiotic stresses (Kumar, 2014).

The obtained results was in accordance with those obtained by Abdel Rahman, (2005) who recorded that cowpea root treatment with ascorbic and salicylic acids increased PPO and PO activity and phenolic contents against root- rot disease. This enhancement may lead to lignin formation and results an efficient physical barrier to restrict subsequent fungal penetration and infection. The role of oxidative enzymes such as peroxidase and polyphenol oxidase could be explained as an oxidation process of phenolic compounds to oxidized products (quinones) which may limit the fungal growth (Hassan, *et. al.,* 2007).

Tarred *et al*. (1993) reported that the increase in peroxidase activity enhanced lignification in response to chocolate spot infection which may restrict the fungal penetration. Peroxidase also produces free radicals and hydrogen peroxide which are toxic to many microorganisms.

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استحثاث المقاومة الجهازية في الفاصوليا ضد أمراض أعفان الجذور

أ.د. وفاء طاهر شهدة ، أ.د. عيد محمد أبوطالب ، رجب جمعه إسماعيل

قسم أمراض النبات – كلية الزراعة – جامعة الإسكندرية – الشاطبي (٢١٥٤٥) – الإسكندرية – مصر.

تم عزل أربع عزلات من الفطر *Rhizoctonia solani* (R1 ، R2 ، R3 و R4) و عزلتين من الفطر *Fusarium solani* (F1 و F2) و عزلة من الفطر *Macrophomina phaseolina* (M) وعزلة من المسبب المرضي *Pythium* spp. (P) من بادرات و نباتات الفاصوليا التي تعاني من الإصابة بمرض الذبول الطري للبادرات وكذلك عفن الجذور في محافظة البحيرة. وقد أظهرت كل العزلات مقدرة على إصابة الأربعة أصناف من الفاصوليا التي اختبرت في البحث وهي Giza ٦ و Nebraska (محصول جاف)، Bronco و Paulista (محصول أخضر).

أختبرت خمسة أنواع من المركبات المضادة للأكسدة وهي (حمض البنزويك ، حمض الستريك ، الهيدروكينون ، حمض السالسليك و حمض السوربيك) في قدرتها على تثبيط النمو في الأطباق وعلى بيئة البطاطس و الدكستروز للفطريات المعزولة و الممرضة. وقد أظهرت النتائج أن هذه المركبات أدت إلى تثبيط معنوي لنمو تلك الفطريات عند تركيز يتراوح بين ٥ مل مول – ١٠مل مول.

درس تأثير معاملة البذور للصنفين Giza ٦ (الأكثر مقاومة) و Bronco (الأكثر قابلية للإصابة) بالمركبات المختبرة ضد العدوى بالفطريات *R. solani* (R1) و *M. phaseolina* (M) (وهي الفطريات الأكثر إمرضية) على:

١) مقاومة المرضية وتقليل الإصابة بهما. وقد أظهرت المعاملات أنها كانت فعالة في تقليل مرض الذبول الطري من ٣,٤١ إلى ٦,٦٥ ٪ وتقليل مرض عفن الجذور من ٦,٣١ إلى ٥,٤٥ ٪.

٢) على نشاط الإنزيمات المتعلقة بالمقاومة وهي (بيروكسيديز ، بولي فينول أوكسيديز ، كيتينيز و بيتا ١-٣ جلوكانيز) و كذلك المحتوى من الفينولات الكلية . وقد أظهرت النتائج أن التأثير يختلف حسب المركب / المسبب المرضي / الصنف المختبر. وعموماً فإن نشاط الإنزيمات كان أسرع و أعلى في حالة الصنف المقاوم Giza 6 بينما كان أبطئ و أقل في الصنف القابل للإصابة Bronco.

Control of Fusarium Oxysporum Isolates Infecting Some Solanaceous Crops with Eco-Friendly Measures in Egypt

Wafaa T. Shahda[1],*, Ahmed F. El-Bebany[1] and Fayza H. Gomaa[2]

1 Department of Plant Pathology, Faculty of Agriculture, Alexandria University, El-Shatby 21545, Alexandria, Egypt.

2 Plant Pathology Institute, Agriculture Research Center, 12619, Giza, Egypt

(dr.wshahda@gmail.com)

ABSTRACT

Samples of diseased tomato, pepper and eggplant plants showing symptoms of wilt or root rot were collected from Alexandria, El-Behira, El-Fayoum, Qalyubia and Assiut at 2012. Two isolates were used; isolate 10 from Assiut University and isolate 11 was isolated from tomato seeds imported from China. Isolation trials revealed 19 different isolates in addition to Assiut isolate. The isolates were different in colour, mycelia growth pattern, growth rate, and microscopic characters. Pathogenicity tests showed that all the isolates were pathogenic to tomato cultivars (G.S9 and El-basha 1077) and pepper cultivars (Balady yellow and Balady green). Evaluation of neem leaves extract and dried orange peels on disease control of root rot and growth vigor was tested. The results showed that neem extract (10%) decreased the disease severity in tomato (54-86%) and pepper (60-80%). Same treatment increased root dry weight (24-88%) and shoot dry weight (53.5-93%) in both crops. Dried orange peels (100 g/Kg soil) significantly decreased root-rot disease by 46-80% in tomato and by 54-88% in pepper plants. The increase in root dry weight was 8.7-60% and shoot dry weight was 15.6-68.5% in both tested crops.

Key words: Fusarium oxysporum, tomato, pepper, neem extract, orange peel, control.

INTRODUCTION

Solanaceous plants tomato, pepper and eggplant are the most popular vegetable crops in Egypt. Many diseases and disorders can affect Solanaceous plants during the growing season. Vascular wilt disease caused by fungal pathogens is widespread and very destructive plant disease, causing enormous economic losses. The infection of tomato plants by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) was recorded for the first time in Egypt by Fahmi, 1930. Matuo and Ishigami, 1958 recorded *Fusarium oxysporum* f. sp. *melongenae* on eggplant, while *Fusarium oxysporum* f. sp. *capsici* was recorded by (Rivelli, 1989). Endo *etal.,* 1973, reported that all solanaceae plants infected by *Fusarium oxysporum* f. sp. *radicis- lycopersici* caused crown and root rot. Wokocha and Oparah 2004 isolated *Fusarium oxysporum f.sp. lycopersici* from wilted tomato plants in Nigeria.,while Nirmaladevi and Srinivas 2012 could isolate *F.oxysporum f.sp. lycopersici* strains from wilted tomato plants and soil samples collected from different tomato fields in and around Karnataka. Also, Altonk and Can, 2010 isolated *F.oxysporum f.sp. melongenae*, from diseased eggplants showing typical wilt symptoms.

The differentiation among the pathogen isolates involve cultural characters still most accurate too. *Fusarium oxysporum* f.sp. *ciceri* isolates from different plant species were identified on the basis of morphological characteristics (El-Kazzaz, *et. al.,* 2008 ; Mandhare, *et. al.,*2011; Nath 2011). Nirmaladevi and Srinivas 2012 differentiated between 114 isolates of *Fusarium oxysporum* f. sp. *lycopersici* based on cultural and morphological features, they found significant variations existed among the isolates with respect to rate and type of grown colonies. Benaouali *et al*., 2014 found that the isolates appearance of *Fusarium oxysporum f.sp radicis lycopersici* from different regions of western Algeria was differentiated into four morphotypes with different pigmentation. Chopada *et al*., 2014 studied the variability among *Fusarium oxysporum* f.sp. *lycopersici* collected from different tomato growing areas of south Gujarat. These studies based on cultural and morphological variation like mycelial colour, mycelial growth, dry mycelial weight, sporulation, conidial size and formation of clamydospores.

Many research efforts have been carried out to find alternatives and environmentally safe methods can be used to control plant diseases (Singh &Prithiviraj, 1997 and Paul and Sharma, 2002).Using some plant products in plant disease control seems to be an effective method to control many plant diseases. Aqueous extracts of several parts of neem (*Azadirachta indica*) plant showed inhibitory effects against fungal spore germination (Singh *et al*., 1980). Aqueous leaf extract of neem exhibited considerable control of *Fusarium oxysporum* disease development in banana (Singh *et al*., 1993). In Kenya, Kimaru *et al*., 2004 found that tomato plants grown in neem kernel cake powder (NKCP) amended and non amended soils were significantly different in wilt index of shoots and length of discoloration of vascular tissues. Agbenin and Marley 2006 reported that crude extracts of neem leaves or seeds (5% - 30%) inhibited mycelial growth of *Fusarium oxysporum* f. sp.

lycopersic. Dry neem seed extract gave 100% inhibition of mycelial growth. Fresh neem leaf extract reduced mycelial growth with increasing concentration. Hassanein *et al.,* 2010 found that aqueous neem extract (5, 10 and

20%) suppressed mycelial growth of *Fusarium oxysporum*, the causal pathogen of tomato wilt and high degree of diseases control (81%) and significant increase in growth parameters were achieved. Treatment with neem and willow (*Salix* sp.) aqueous extracts significantly exhibited a growth promotion of tomato shoot and root in infected or non infected seedling (Farag *et al.,* 2011). Hadian *et al.,*2011 compared neem extract with chemical control on *F. oxysporum* and *Meloidogyne incognita* complex of tomato. Results showed that neem seed powder significantly reduced the disease severity of *Fusarium* and root-knot nematode and all the treatments significantly improved the growth of the plants as compared to untreated inoculated plants. Neem not only controlled these diseases but also cause increase in growth characters such as plant weight and length. Singh *et al.,* 2015 used integrated approach for management of *Fusarium oxysporum f.sp. lycopersici* caused wilt of tomato plants including *Trichoderma harzianum,* soil application of neem cake powder and foliar spray of carbendazim, they reported reduction of disease and increase in fresh and dry shoot and root weight. Orange peels is one of the more promising methods of attaining biological control of soil-borne plant pathogens with the aid of plant residues as organic amendments added to the soil (Papavizas and Lumsden, 1980). Rold´an-Mart´ın *et al.,* 2006 demonstrated that mixing low amounts of orange peel (*Citrus reticulate)* with contaminated soil, under selected solid-culture conditions, is a good alternative for soil bioremediation. Umana *et al.,*2014 found that *Fusarium oxysporum f.sp lycopersici* was suppressed more effectively by orange peels ash with dry weight (DW) of 3.6g to 20.1g after 1-8weeks of growth. Maksoud, *et al.,* 2015 reported that organic fertilization with industrial orange wastes produced an improvement in nutritional status; fruit set and yield.

This study aimed to isolation and morphological differentiation between *Fusarium oxysporum* isolates infecting solanaceous plants, studying the use of some plant products for the diseases control as neem extracts and orange peels.

MATERIALS AND METHODS

1- Isolation of the pathogen:

Samples of diseased tomato, eggplant and potato plants showing symptoms of wilt and root-rot were collected from Alexandria, El Behira (El Nubaria and Elbanger village 5, 15) El Fayoum, El Dakahlia and Assiut Governorates in Egypt during 2012-2013 growing season. Samples of untreated seeds imported from China were used also for isolation. The collected seeds, seedlings and plant roots were thoroughly washed in running tap water for 15 minutes, then surface sterilized in 1% sodium hypochlorite for 1-2 min, rinsed in sterile water several times. The surface sterilized seeds and small segments of stem or roots were surface dried on sterilized filter paper, placed on potato dextrose agar medium PDA. The media were supplemented with streptopenicid at the rate of 2.5 mg/100ml of the medium. Plates were incubated at 28±2°C for 7days. The developed cultures were purified by single spore isolation technique, and sub cultured on PDA slants, kept at 4°C (Ainsworth, 1968).

2-Identification of the isolates:

The developed isolates were separately grown on PDA plates. The plates were inoculated centrally with 4 mm inoculums discs taken from periphery of 7 days old cultures and incubated at 28±2°C for 10 days. Examination of cultural characters (colony colour, growth pattern, growth rate) was recorded for the twenty isolates, microscopic examination was also used.

3-Pathogenicity test:

Pathogenicity of *F.oxysporum* isolates was tested on seedling of tomato *(Solanum lycopersicum)* cultivars G.S & Elbasha1077, pepper (*Capsicum annuum*) cultivars balady green& yellow and eggplant (*Solanum melongena*) cultivars balady black & arose white. Thirty five days old seedlings were inoculated separately with the isolates by immersing the roots in spore suspension of (5× 10^6spore/ml) for 30 min, then planted in clay pots 15 diameter, filled with autoclaved aerated sandy loam soil. Check treatment were kept un-infected. Four replicates (5 plants each) were used for each treatment. Disease index was recorded after 25 days of inoculation and planting according to Huertas-Gonzalez *et al.* 1999. The disease index was: 1- No symptoms.2-Beginning of wilt symptoms. 3-Leaves heavily wilted.4-All leaves completely wilted. 5- Dead plants.

4- Effect of neem leaves extract and orange peels on disease control and growth vigour:

The effect of neem extract, orange peels on disease control was carried out in greenhouse experiment. Clay pots (15 cm diameters), filled with sterilized aerated sandy-loam soil were inoculated with spore suspension (5× 10^6 spore/ml) of each of the tested isolate (20 ml/pot). Pots were daily watered for one week. In neem extract treatment, 35 days old seedlings were treated with 10% neem extract by suspending its roots in the extracts for one hour, and then transplanted after one week of soil infestation. In case of orange peels test, 35 days old seedlings were transplanted in the infested soil after one week of soil inoculation. Orange peels were added to the soil one week later at the rate of 100g/kg soil. Untreated plants or soil served as control. Data were recorded after 45 days of transplanting for disease control and growth vigour parameters (shoot and root fresh and dry weight).

RESULTS AND DISCUSSION

1- Isolation of the pathogen:

Isolation from samples of diseased tomato, pepper, eggplant and potato plants showing wilt and root rot symptoms revealed 18 isolates. In addition to one isolate (No-10) which obtained from Assiut University. All the isolates developed on PDA medium exhibited the taxonomic features of *Fusarium oxysporum*, and were identified according to Booth, 1971 (Table 1).

Table (1): Original hosts, collection sites, and time of isolation of 20 *Fusarium oxysporum* isolates used in this study

Isolates	Original hosts	Cultivar	Location	Year
1	Tomato root	Unknown	El-Behira	2012
2	Tomato root	Unknown	El-Behira	2012
3	Tomato root	Unknown	El-Behira	2012
4	Tomato root	Unknown	El-Fayoum	2012
5	Tomato root	Superhagin	Haron village El-nubaria El-Behira	2013
6	Tomato root	Unknown	El banger village 5 El-Behira	2013
7	Tomato root	Unknown	El banger village 15 El-Behira	2013
8	Tomato root	Unknown	El-Behira	2013
9	Tomato root	Unknown	Assiut	2013
10	Tomato root	Unknown	Assiut (AUMC)	2013
11	Tomato seeds	Alfarah F, Hybrid	China	2013
12	Pepper root	Unknown	Slah El-abd El noubaria El-Behira	2012
13	Pepper root	Kaha	Qalyubia	2012
14	Pepper root	Baldy	El banger village 4 El-Behira	2013
15	Pepper root	Unknown	El banger village 5 El-Behira	2013
16	Pepper root	Unknown	El banger village 15 El-Behira	2013
17	Eggplant root	Unknown	Alexandria	2013
18	Eggplant root	Aros	El banger village 4 El-Behira	2013
19	Eggplant root	Unknown	El banger village 5 El-Behira	2013
20	Potato root	Unknown	El-Behira	2012

2- Identification of the isolates:

All *Fusarium* isolates grown on PDA plates at 28±2°C were studied for their cultural and morphological characters. Observation on colony colour and pigmentation, mycelial growth pattern, radial growth and sporulation were recorded after 10 days of incubation (Table 2). The colour and pigmentation of the isolates on PDA medium varied between white, creamish white to cream, light pink to pink and light purple to violet. On the basis of the mycelial growth pattern, the isolates were categorized into two groups *i.e.* fluffy dense growth and sparse adherent smooth growth. Most of the isolates showed fluffy or dense growth while few isolates showed adherent sparse growth on the medium. Based on colony diameter, the isolates were divided into 3 groups. Fast growing (75-90 mm), moderate growing (60-75mm) and slow growing (less than 60 mm). (Table 3). Many investigators followed same method for differentiation between *Fusarium* isolates. (El-Kazzaz *et al.,* 2008; Nath, 2011; Deshwal and Kumari, 2012; Chopada *et al.,* 2014; Siddique *et al.,* 2014; Talaviya *et al*., 2014).

3-Pathogenicity assessment:

In tomato (Table 4) most of the isolates were pathogenic to G.S. cultivar (14 out of 20), while 10 isolates only were pathogenic to El Basha 1077 cultivar. The most pathogenic isolates to G.S. tomato cultivar were 5, 19 and 7 where the percentage of dead plants was 100%, 94% and 86%, respectively. In case of El Basha 1077 cultivar, isolates FO-5 (86%), FO-19 and FO-14 (80% for both) caused the highest value of dead percentage. For pepper plants (Table 5), isolates FO-19, FO-14, and FO-15 were the most pathogenic on balady yellow (94%, 86% and 80%, respectively), while isolate FO-16 (94%) FO-3 (86%) and FO-14 (80%) were more severe on balady green. The most pathogenic isolates on tomato or pepper plants showed that its severity not only to increase the dead plants percentage but also it exhibited severe symptoms on the survivals which ranged from beginning of wilt symptoms to death (2-5 on the scale) Table 4 & 5 and Figure1(A and B) . Eggplant was not susceptible to the twenty isolates, no symptoms were observed on it with any of the isolates. The infection of pepper plants with *F.oxysporum f.sp. lycopersici* in our results agree with Cafri *et al.,* 2005 who reported that *F.oxysporum f.sp.cucumerinum* infect cucumber (its host) and melon. For eggplant cultivars, no symptoms, or disease development were observed with all isolates. These results were compatible with the concept of *formae specilaes,* where the specific *formae specilaes* for eggplant is *F. oxysporum f.sp. melongenae* (Altonk and Can 2010).

Table (2): Colony characters of *Fusarium oxysporum* isolates after 10 days of incubation at 28±2°C.

Isolates	Growth pattern	Pigmentation	
		Upper surface	Reverse surface
FO-1	Dense	Creamy	Creamy
FO-2	Moderate dense	Light violet	Violet
FO-3	Fluffy dense	White	Dark pink
FO-4	Moderate dense	Light purple	Purple
FO-5	Dense	White	White to violet
FO-6	Moderate dense	White	Creamy
FO-7	Fluffy dense	White	Creamy
FO-8	Dense	Violet	Dark violet
FO-9	Moderate dense	White	Creamy
FO-10	Sparse	Dark purple	Purple
FO-11	Dense	White	Dark purple
FO-12	Dense	White	Creamy
FO-13	Dense	Light purple	Dark purple
FO-14	Dense	Creamy	Dark creamy
FO-15	Fluffy dense	White	Creamy
FO-16	Moderate dense	White to pink	Creamish
FO-17	Sparse	White to creamy	Violet to white
FO-18	Sparse	Dark creamy	Creamy
FO-19	Moderate dense	White to creamy	Creamy
FO-20	Dense	Light purple	Creamy

Table (3): Grouping of *F. oxysporum* isolates based on radial growth after 10 days of incubation at 28±2°C.

Radial growth of the twenty isolates after 10 days		
Fast growing (75- 90 mm)	Moderate growing (60- 75 mm)	Slow growing (less than 60mm)
FO-3	FO-1	FO-10
FO-4	FO-2	FO-12
FO-8	FO-5	FO-14
FO-9	FO-6	FO-17
FO-11	FO-7	FO-19
FO-13		
FO-15		
FO-16		
FO-18		
FO-20		
10 isolates	5 isolates	5 isolates

Table (4): Effect of soil infestation with the twenty isolates of *F. oxysporum* on tomato cultivars after 25 days of inoculation compared with uninfested soil (Cont.).

F. oxyrpertm isolate	Tomato cultivar C.S.			Tomato cultivar Elbasha 1077		
	*Mean no. of survivals	Dead %	DSI	*Mean no. of survivals	Dead %	DSI
Cont.	5.0a	0	1.0i	5.0a	0	1.0h
FO-1	2.7b	46	2.67f	5.0a	0	1.0h
FO-2	5.0a	0	1.0i	4.3a	14	2.0g
FO-3	1.0cde	80	4.0d	5.0a	0	1.0h
FO-4	1.0cde	80	4.0d	2.3b	54	2.3f
FO-5	0.0e	100	5.0a	0.7c	86	4.8a
FO-6	5.0a	0	1.0i	3.0b	40	3.67c
FO-7	0.7de	86	4.67c	5.0a	0	1.0h
FO-8	4.0a	20	2.0g	5.0a	0	1.0h
FO-9	4.3a	14	1.67h	5.0a	0	1.0h
FO-10	4.0a	20	2.0g	2.7b	46	2.6e
FO-11	1.3cd	74	4.0d	4.7a	6	2.0g
FO-12	1.3cd	74	4.0d	2.67b	46	3.3d
FO-13	1.3cd	74	4.0d	5.0a	0	1.0h
FO-14	2.0be	60	3.0e	1.0c	80	4.0b
FO-15	2.7b	46	2.67f	2.3b	54	3.3d
FO-16	5.0a	0	1.0i	5.0a	0	1.0h
FO-17	5.0a	0	1.0 i	5.0a	0	1.0h
FO-1S	5.0a	0	1.0i	5.0a	0	1.0h
FO-19	0.3de	94	4.8b	1.0c	80	4.0b
FO-20	5.0a	0	1.0i	5.0a	0	1.0h

Table (5): Effect of soil infestation with the twenty isolates of *F. oxysporum* on pepper cultivars after 25 days of inoculation compared with uninfested soil (Cont.)

F. oxyrpertm isolate	Tomato cultivar C.S.			Tomato cultivar Elbasha 1077		
	*Mean no. of survivals	Dead %	DSI	*Mean no. of survivals	Dead %	DSI
Cont.	5.0a	0	1.0e	5.0a	0	1.0h
FO-1	5.0a	0	1.0e	5.0a	0	1.0h
FO-2	5.0a	0	1.0e	5.0a	0	1.0h
FO-3	4.3b	14	2.0d	0.7de	86	4.4b
FO-4	5.0a	0	1.0e	3.0c	40	3.0e
FO-5	5.0a	0	1.0e	5.0a	0	1.0h
FO-6	5.0a	0	1.0e	3.7bc	26	2.3f
FO-7	5.0a	0	1.0e	5.0a	0	1.0h
FO-8	5.0a	0	1.0e	5.0a	0	1.0h
FO-9	4.7ab	6	2.0d	5.0a	0	1.0h
FO-10	5.0a	0	1.0e	5.0a	0	1.0h
FO-11	5.0a	0	1.0e	4.3ab	14	2.9g
FO-12	5.0a	0	1.0e	5.0a	0	1.0h
FO-13	4.3b	14	2.0d	5.0a	0	1.0h
FO-14	0.7cd	86	4.4b	1.0de	80	4.8a
FO-15	1.0c	80	4.0c	5.0a	0	1.0h
FO-16	5.0a	0	1.0e	0.3e	94	4.8a
FO-17	5.0a	0	1.0e	5.0a	0	1.0h
FO-1S	5.0a	0	1.0e	1.3d	74	4.0d
FO-19	0.3d	94	4.8a	5.0a	0	1.0h
FO-20	5.0a	0	1.0e	5.0a	0	1.0h

DSI: Disease severity index: was recorded after 25 days of inoculation, using a scale from 1 to 5 according to (Huertas-Gonzalez *et al.* 1999).

* Mean of four replicates (pots), 5 seedlings each.

Values within each column followed by same letter are not significantly different in each cultivar.

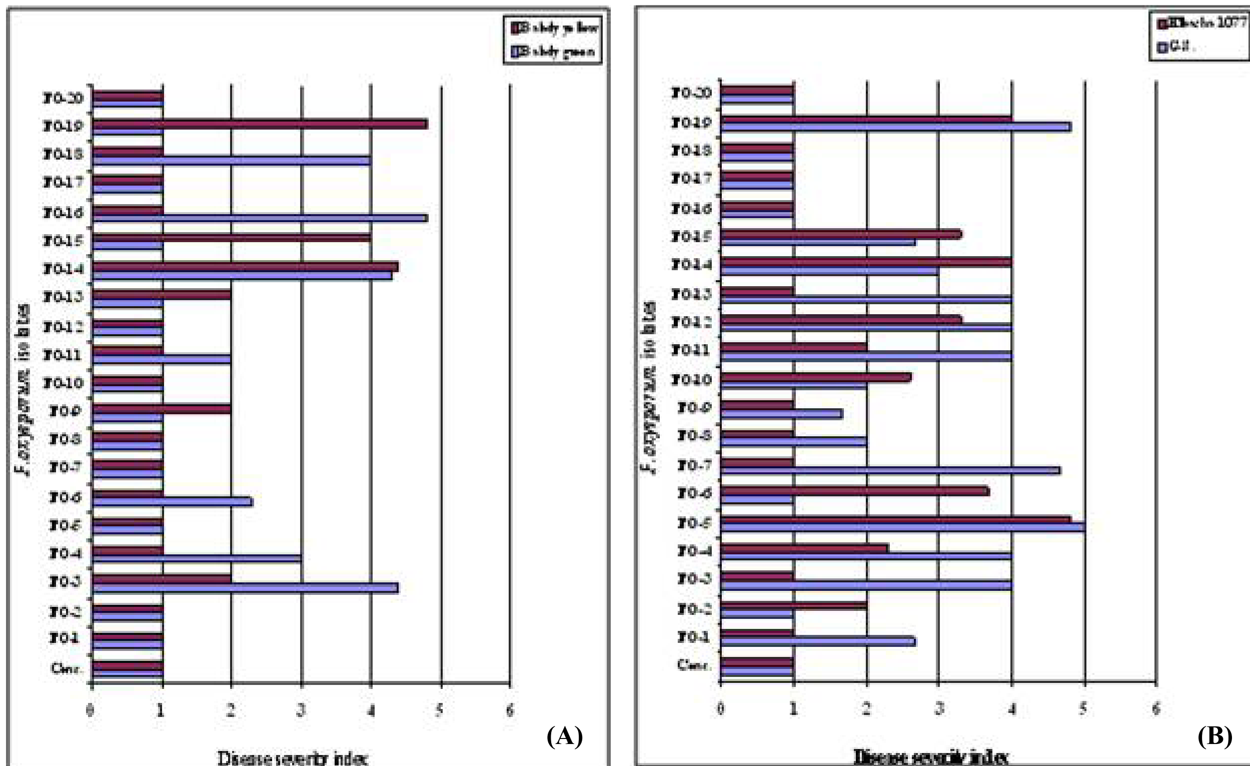

Figure (1): Mean of disease severity (DS) of the twenty isolates of *F. oxysporum* on tomato (A) and pepper (B) plants cultivars, using a scale from 1 to 5 after 25 days of inoculation and transplanting. Disease index: (1) No symptoms apparent. (2) Beginning of wilt symptoms on leaves. (3) Leaves heavily wilted. (4) All leaves completely wilted. (5) Dead plant.

4- Effect of neem leaves extract and orange peels on disease control and growth vigour:

Data presented in Tables (6, 7, 8 and 9) showed that the effect of neem leaves extract and orange peels on disease control was promising, where it recorded values nearby Rizolex-T in inhibiting the disease either on tomato or pepper cultivars. In case of tomato plants (Table 6 and 7), neem extract significantly decreased the disease from 54-86% in both cultivars. In pepper plants (Table 8, and 9), the same trend was observed with neem extract treatment, in which the decrease of the disease was significant than the untreated plants (60-88%) in both cultivars. In orange peels treatment, a significant decrease of the wilt disease was observed in tomato cultivars from 46-80% and in pepper cultivars from 54-88%.

Neem extract and orange peels recorded significant increase in shoot and root dry weight in each of tomato and pepper cultivars, compared with the fungicide in most cases (Tables 6, 7, 8 and 9). Neem extract significantly increased root dry weight from 30.7- 88.0% and shoot dry weight from 68.4-93.9% in tomato cultivars. While in pepper cultivars, the increase was from 24.8-64.3% in root dry weight and from 53.5-91.9% in shoot dry weight. Orange peels also increased significantly root dry weight from 8.7-60% and shoot dry weight from 28.1-68.5% in both tomato cultivars. In case of pepper cultivars, the increase in root dry weight was from 14.4-22.4% and in shoot dry weight was from 16.6-26.6%. Such results are in accordance with the findings of Kimaru *et al.,* 2004 (on *Fusarium* wilt in tomato), Agbenin and Marley 2006 and Hassanein *et al.,* 2010 (on *F.oxysporum f.sp. lycopersici*) and Obongoya *et al.,* 2010 (on *F. oxysporum f.sp. phaseoli)*. Hassanein *et al.,* 2010 and Farag *et al.,* 2011 recorded significant increase in growth parameters of tomato plants after neem extract treatment. The effectiveness of neem extract on disease control and enhancing growth vigour may be occur through a metabolic changes in plants including induction of phenol biosynthesis enzymes, antioxidants defensive enzymes and phenol accumulation (Paul and Sharma 2002; Guleria and Kumar, 2006; and Aboellil, 2007). Other investigators recorded that plant extracts can induce systemic resistance in many plants through accumulation of pathogenesis related proteins (PR proteins) (Sateesh *et al.,* 2004). Farag *et al.,* 2011 mentioned that application of neem aqueous extracts with *Fusarium oxysporum* significantly reduced the level of lipid peroxidation and induced high activities of antioxidants defensive enzymes after 3 and 7 days of infection. The use of orange peels in suppression plant pathogens was recorded by Rold´an-Mart´ın *et al.,* 2006; Umana *et al.,* 2014; and Mercy & Mubsira Banu, 2014. They explained that the effect of fruit peels is due to the increase of soil fertility and soil micro-organisms and its containment of potassium, vitamins, minerals and some essen-

Table (6): Effect of neem leaves extract and dried orange peels compared with Rizolex-T on disease control and growth vigour of tomato plants cv. G.S in infested soil with isolates 5, 14 and 19 of *F. oxysporum* after 45 days of inoculation

Isolate	Treatment	Disease control			Growth vigour					
		*Mean no. of survivals	% of mortality	Decrease %	Root system			Shoot system		
					F.W	D.W	% of D.W increase	F.W	D.W	% of D.W increase
FO-5	Infected only	0.0^{b}	100	-	3.5^{d}	1.17^{d}	-	15.7^{c}	2.S4^{d}	-
	Infected- Neem extract 10%	4.3^{a}	14	86	14.3^{a}	2.1^{a}	79.5	41.3^{a}	5.1^{a}	79.6
	Infected- oranee peel: 100g/Kg	4.0^{a}	20	80	9.4^{c}	1.63^{c}	39.3	37.3^{b}	4.6^{b}	62.0
	Infected- Rizolex-T 3g/L	4.0^{a}	20	80	13.6^{b}	2.0^{b}	71.0	37.6^{b}	4.5^{c}	58.5
FO-l4	Infected only	2.0^{b}	60	-	6.0^{d}	1.17^{c}	-	24.7^{c}	2.63^{d}	-
	Infected- Neem extract 10%	4.7^{a}	6	54	15.3^{a}	2.0^{a}	71.0	43.7^{a}	4.97^{a}	89.0
	Infected- oranee peel: 100g/Kg	4.3^{a}	14	46	9.43^{c}	1.3^{b}	11.1	37.2^{b}	3.^{Sc}	44.0
	Infected- Rizolex-T 3g/L	4.67^{a}	6	54	13.1^{b}	2.0^{a}	71.0	36.0^{b}	4.37^{b}	66.2
FO-19	Infected only	0.3^{b}	94	-	3.17^{d}	1.17^{d}	-	15.23^{d}	2.73^{d}	-
	Infected- Neem extract 10%	4.7^{a}	6	86	14.67^{a}	2.2^{a}	ss.o	42.0^{a}	5.1^{a}	S6.S
	Infected- oranee peel: 100g/Kg	4.3^{a}	14	80	19.63^{c}	1.67^{c}	42.7	36.7^{c}	4.6^{c}	6S.5
	Infected- Rizolex-T 3g/L	4.3^{a}	14	80	13.S3^{b}	2.0^{b}	71.0	3S.7^{b}	4.7^{b}	72.2

Table (7): Effect of neem leaves extract and dried orange peels compared with Rizolex-T on disease control and growth vigour of tomato plants cv. Elbasha 1077 in infested soil with isolates 5, 14 and 19 of *F. oxysporum* after 45 days of inoculation.

Isolate	Treatment	Disease control			Growth vigour					
		*Mean no. of survivals	% of mortality	Decrease %	Root system			Shoot system		
					F.W	D.W	% of D.W increase	F.W	D.W	% of D.W increase
FO-5	Infected only	0.7^{b}	S6	-	6.97^{c}	1.0^{d}	-	30.4^{d}	3.7^{d}	-
	Infected- Neem extract 10%	4.7^{a}	6	80	11.4^{a}	1.56^{a}	86	43.3^{b}	6.23^{a}	68.4
	Infected- oranee peel: 100g/Kg	4.3^{a}	14	72	8.8^{b}	1.6^{c}	60	35.67^{c}	4.74^{c}	28.12
	Infected- Rizolex-T 3g/L	4.0^{a}	20	66	8.4^{b}	1.S2^{b}	82	44.63^{a}	5.5^{b}	48.6
FO-l4	Infected only	1.0^{b}	80	-	8.4^{c}	2.37^{b}	--	31.25^{d}	3.3^{d}	-
	Infected- Neem extract 10%	4.7^{a}	6	74	13.3^{a}	2.1^{a}	53.3	46.25^{d}	6.4^{a}	93.94
	Infected- oranee peel: 100g/Kg	4.3^{a}	14	66	9.1^{b}	1.57^{ab}	14.6	34.3^{c}	4.7^{c}	42.4
	Infected- Rizolex-T 3g/L	4.3^{a}	14	66	9.27^{b}	1.78^{ab}	29.93	44.0^{b}	5.3^{b}	60.6
FO-19	Infected only	1.0^{b}	80	-	3.2^{c}	1.5^{b}	-	17.32^{b}	3.3^{d}	-
	Infected- Neem extract 10%	4.7^{a}	6	74	12.1^{a}	1.96^{a}	30.7	43.7^{a}	6.3^{a}	90.91
	Infected- oranee peel: 100g/Kg	4.3^{a}	14	66	8.7^{b}	1.63^{b}	8.7	34.6^{b}	4.6^{c}	39.4
	Infected- Rizolex-T 3g/L	4.7^{a}	6	74	8.5^{b}	1.76^{ab}	17.3	37.7^{a}	5.1^{b}	54.55

*Mean of 4 replicates (pots), 3 seedlings each. (FW) fresh weight and (DW) dry weight.
Values within each column followed by same letter are not significantly different in each isolate.

Table (8): Effect neem leaves extract and dried orange peels compared with Rizolex-T on disease control and growth vigour of pepper plants cv. Balady green in infested soil with isolates 14, 15, 16 and 19 of *F. oxysporum* after 45 days of inoculation

Isolate	Treatment	Disease control			Growth vigour					
		*Mean no. of survivals	% of mortality	Decrease %	Root system			Shoot system		
					F.W	D.W	% of D.W increase	F.W	D.W	% of D.W increase
FO-14	Infected only	1.0^{b}	80	-	8.5^{c}	1.47^{c}	.	32.4^{c}	3.7^{c}	-
	Infected- Neem extract 10%	4.3^{a}	14	66	12.7^{a}	2.4^{a}	63.3	45.7^{a}	7.1^{a}	91.9
	Infected- oranee peel: 100g/Kg	4.0^{a}	20	60	9.13^{b}	^{1}.8^{bc}	22.45	38.7^{b}	4.5^{be}	21.6
	Infected- Rizolex-T 3g/L	4.7^{a}	6	74	8.83^{be}	2.16^{ab}	46.9	45.7^{a}	5.5^{b}	48.6
FO-15	Infected only	5.0^{a}	-	-	8.4^{c}	1.57^{b}	-	31.17^{c}	3.9^{d}	-
	Infected- Neem extract 10%	5.0^{a}	-	-	12.6"^{a}	1.96^{a}	24.8	45.0^{a}	6.5^{a}	66.7
	Infected- oranee peel: 100g/Kg	5.0^{a}	-	-	9.03^{b}	1.9^{ab}	18.75	39.67^{b}	4.9^{c}	25.64
	Infected- Rizolex-T 3g/L	5.0^{a}	-	-	8.9^{bc}	1.8^{ab}	14.7	44.3^{a}	5.5^{b}	41.03
FO-16	Infected only	0.3^{b}	94	-	8.5^{c}	1.8^{c}	-	33.0^{c}	4.2^{c}	-
	Infected- Neem extract 10%	4.7^{a}	6	88	13.3^{a}	2.5^{a}	38.9	45.3^{a}	7.23^{a}	72.1
	Infected- oranee peel: 100g/Kg	4.7^{a}	6	88	9.4^{be}	2.1^{be}	16.7	39.0^{b}	4.9^{be}	16.7
	Infected- Rizolex-T 3g/L	4.7^{a}	6	88	9.4^{be}	2.3^{ab}	27.8	46.3^{a}	5.8^{b}	38.1
FO-19	Infected only	5.0^{a}	-	-	8.4^{b}	1.57^{b}	-	31.3^{e}	3.77^{d}	-
	Infected- Neem extract 10%	5.0^{a}	-	-	13.3^{a}	2.0^{a}	27.4	45.3^{b}	6.6^{a}	75.1
	Infected- oranee peel: 100g/Kg	5.0^{a}	-	-	9.07^{b}	1.93^{ab}	21.0	39.67^{c}	4.7^{c}	24.7
	Infected- Rizolex-T 3g/L	5.0^{a}	-	-	8.9^{b}	1.77^{ab}	12.7	46.0^{a}	5.4^{b}	43.24

*Mean of 4 replicates (pots), 3 seedlings each. (FW) fresh weight and (DW) dry weight.
Values within each column followed by same letter are not significantly different in each isolate.
FO-15 and FO-19 were not pathogenic to this cultivar.

Table (9): Effect of neem leaves extract and dried orange peels compared with Rizolex-T on disease control and growth vigour of pepper plants cv. Balady yellow in infested soil with isolates 14, 15, 16 and 19 of *F. oxysporum* after 45 days of inoculation

Isolate	Treatment	Disease control			Growth vigour					
		*Mean no. of survivals	% of mortality	Decrease %	Root system			Shoot system		
					F.W	D.W	% of D.W increase	F.W	D.W	% of D.W increase
FO-14	Infected only	1.0^{b}	80	-	6.4^{c}	1.6^{c}	-	25.97^{c}	3.9^{d}	-
	Infected- Neem extract 10%	4.0^{a}	20	60	11.8^{a}	2.3^{a}	43.73	46.3^{a}	6.7^{a}	71.8
	Infected- oranee peel: 100g/Kg	3.7^{a}	26	34	9.1^{b}	1.83^{be}	14.4	38.7^{b}	4.8^{c}	23.1
	Infected- Rizolex-T 3g/L	4^{Ja}	14	66	8.9^{b}	1.93^{b}	21.9	44.3^{a}	5.6^{b}	45.13
FO-15	Infected only	1.0^{b}	80	.	9.07^{a}	1.3^{b}	-	29.6^{e}	3.9^{d}	.
	Infected- Neem extract 10%	4.7^{a}	6	74	12.6^{a}	2.3^{a}	53.3	46.7^{a}	6.6^{a}	69.23
	Infected- oranee peel: 100g/Kg	4^{Ja}	14	66	9.2^{b}	^{1}.8^{ab}	20	38.7^{b}	4.8^{c}	23.1
	Infected- Rizolex-T 3g/L	4.7^{a}	6	74	9.8^{b}	1.83^{ab}	22	45.0^{a}	5.5^{b}	41.03
FO-16	Infected only	3.0^{a}	-	-	8.4^{c}	1.63^{b}	-	31.5^{c}	3.87^{d}	-
	Infected- Neem extract 10%	3.0^{a}	-	-	12.67^{a}	2.0^{a}	25	45.3^{a}	6.51^{a}	68.2
	Infected- oranee peel: 100g/Kg	3.0^{a}	-	-	9.06^{b}	1.9^{ab}	18.75	39.67^{b}	4.9^{c}	26.6
	Infected- Rizolex-T 3g/L	3.0^{a}	-	-	8.8'^{bc}	^{1}.88^{ab}	17.5	45.3^{a}	5.57^{b}	43.93
FO-19	Infected only	0.3^{b}	94	-	9.2^{b}	1.6^{b}	-	36.7^{b}	4.3^{d}	-
	Infected- Neem extract 10%	4.7^{a}	6	88	13.3^{a}	2.63^{a}	64.37	46.3^{a}	6.6^{a}	53.5
	Infected- oranee peel: 100g/Kg	4.7^{a}	6	88	9.6^{b}	1.6^{ab}	18.75	39.7^{b}	4.9"^{c}	15.6
	Infected- Rizolex-T 3g/L	4.7^{a}	6	88	9.5^{b}	2.1^{a}	31.25	45.7^{a}	5.7^{b}	32.56

*Mean of 4 replicates (pots), 3 seedlings each. (FW) fresh weight and (DW) dry weight.
Values within each column followed by same letter are not significantly different in each isolate. FO-16 was not pathogenic to this cultivar.

tial elements which enhance plant growth. They also added that peels of citrus varieties may used to kill the nematodes. They suggested that the chemical fertilizers can be replaced by the fruit peels powder and extract to protect the soil from the infertility. Maksoud *et al.,* 2015 mentioned that using orange wastes as organic fertilizers could represent a sustainable approach to recycling nutrients, solve environmental problems related to the citrus processing industry and reduced costs related to disposable.

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مقاومة عزلات الفطر *Fusarium oxysporum* الذى يصيب بعض محاصيل العائلة الباذنجانية فى مصر بواسطة طرق صديقة للبيئة

وفاء طاهر شهدة[١]، أحمد البيبانى[١] ، فايزة حسن جمعة [٢]

[١] قسم أمراض النباتات– كلية الزراعة (الشاطبى) جامعة الإسكندرية – مصر

[٢]معهد بحوث أمراض النباتات– مركز البحوث الزراعية– الجيزة– مصر

تم جمع عينات من نباتات الطماطم، الفلفل، الباذنجان المصابة بالذبول و عفن الجذور من محافظات الإسكندرية ، البحيرة، الفيوم، القليوبية و أسيوط فى موسم الزراعة ٢٠١٢–٢٠١٣ و قد أعطت نتائج العزل ١٨ عزلة من فطر الفيوزاريوم أكسوسبوريوم بالأضافة إإلى عزلة من بذور طماطم مستوردة من الصين (١١) و عزلة معرفة تم الحصول عليها من جامعة أسيوط. أظهرت عزلات الفطر فيوزاريوم أكسوسبوريوم إختلافا فى لون المزرعة، طبيعة نمو الميسليوم و معدل النمو الفطرى و الصفات الميكروسكوبية.

أظهرت نتائج أختبار القدرة الإمراضية أن كل العزلات المتحصل عليها كانت ممرضة لصنفى الطماطم(G.S و الباشا ١٠٧٧) و كذلك الفلفل (صنفى بلدى أصفر و بلدى أخضر).

أظهرت نتائج البحث أن لكل من المستخلص المائى لأوراق النيم و قشور البرتقال المجففة أثر فعال فى مقاومة مرض عفن الجذور الفيوزاريومى و كذلك تحسين قوة النمو و زيادة الوزن الجاف لكل من الجذور و السيقان.

أظهرت النتائج أن مستخلص أوراق النيم (١٠٪) أدت إلى تقليل حدوث مرض الذبول الفيوزاريومى فى نباتات الطماطم بنسبة تتراوح من ٥٤–٨٦٪ و الفلفل من ٦٠–٨٠ ٪ و كذلك زيادة الوزن الجاف للجذور (٢٤–٨٨٪) و للسيقان (٥٣,٥–٩٣٪) فى كلا المحصولين.

Genetic Variations of *Rhizoctonia solani* Isolates from Potato

Ahmed F. El-Bebany[1,*], Nader A. Ashmawy[1], Sayed S. Aboshosha[1], Fouad Daayf[2] and Sobhy S. H. Abdelsalam[1]

[1]Department of Plant Pathology, Faculty of Agriculture, Alexandria University, El-Shatby 21545, Alexandria, Egypt

[2]Department of Plant Science, 222 Agriculture Building, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada

*Email. aelbebany@yahoo.com.

ABSTRACT

Stem canker and black scurf caused by *Rhizoctonia solani* is a serious disease affects potato plants due to reduced marketability of potato tubers by forming hard black sclerotia on the surface of the tubers and stem cankers resulting in yield losses. Genetic variability among 20 isolates of *R. solani* was studied using Random amplified polymorphic DNA (RAPD) technique using 13 primers. Based on the banding patterns obtained by the amplification of genomic DNA, clear differences were observed among the tested isolates. Banding patterns were variable due to the primer and the tested isolate used. The primers used in this study generated 423 polymorphic bands which differ in their size that ranging from 200 bp to 2500 bp with primer USP among tested isolates. Banding patterns were screened by Totallab[M] software and incorporated into PAST 3.02 software program to construct the similarity matrix. Highly similarity (89%) was observed among two (RS 13-77 and RS 13-76) *R. solani* isolates recovered from one geographic region (Kafr EL-Sheikh), and minimum similarity level 5% were observed among two (RS 13-76 and RS 14-125) *R. solani* isolates obtained from different geographic regions (Kafr EL-Sheikh and Minufiya respectively). Investigating the genetic variation among *R. solani* isolates helps in understanding the mechanisms of host-pathogen interactions, resulting in development of new control strategies and improvement of breeding programs to control the black scurf and stem canker disease.

Key words: ***potato, black scurf,*** *Rhizoctonia solani*, ***RAPD, variations***

INTRODUCTION

In field, potato has several constraints such as diseases, climatic changes and new pathogenic strains, all of these may be leaded to reduce potato production (Day, 1959, Sharma, 2003, and Trigiano *et al*., 2006). *R. solani* is an important constraint to potato production (Balali-Dehkordi, 1996), due to quantitative and qualitative losses of the crop in many countries, including Egypt (Bounou *et al*., 1999, Metry *et al.,* 2001 and El-Bebany *et al*., 2014). *R. solani* considered as a collection of non-interbreeding population this recognized and manipulated by anastomosis groups (AG), which may by similar in their morphology but genetically isolated (Anderson, 1982). Potato plants affected with strains of *R. solani* belong to several AGs, thus, study genetics of *R. solani* is difficult (Warton *et al*., 2007).

Naturally, fungal pathogens vary in their physiological functions and virulence to overcome different environmental conditions and support their survival in various ecosystems (Narayanasamy, 2011). Pathogens with great evolutionary potential can break down the resistance genes of host. Also, pathogens may overcome control methods such as applications of fungicides or antibiotics depending on the genetic diversity of the isolates or strains (McDonald and Linde, 2002a).

Fungal plant pathogens are varying in life-history and strategies by which they interact with their hosts (McDonald *et al*., 1989). Understanding genetic diversity and the origin of fungal pathogens are necessary elements to provides information on the factors by which phytopathogenic fungi can breakdown the plant resistance.(McDonald *et al*., 2002b; Burdon and Silk, 1997), and also helps in developing sufficient and suitable methods for disease control (McDonald *et al*., 1989 and Narayanasamy 2011).

Limitations of biochemical and morphological criteria have been overcome easily by the recent development of the molecular biology. Several molecular techniques have been approved in molecular biology fields in order to discriminate and classify species and genera of pathogens depending on the significant variation that occurs naturally in the DNA (Xu, 2006).

Several DNA-based methods are used for study genetic variations in phytopathogenic organisms; biochemical and DNA-based markers are widely used. So instability of phenotypic criteria makes the DNA-based markers more effective for the detection of plant pathogens and characterization of variability among pathogens. (Sharma, 2003 and Xu, 2006). Recent development and applications of molecular methods have great growth in the field of analyses of fungal species and population. DNA sequencing and polymerase chain reaction (PCR) based methods became mainstream methods for study the population genetics (Xu, 2006).

Availability of genetic markers is very important to study population genetics, which is useful for investiga-

tion of the origin, genetic diversity and population structure of alleles (Liu *and* Muse, 2005). There are many genetic markers used in study genetic variation includes random amplified polymorphic DNA (RAPD) (Brown, 1996) that used as a powerful method for studying of genetic variation among fungal populations because of its simplicity and rapidity of detection, low reproducibility and highly standardized procedure of RAPD analysis.

RAPD-PCR was used in several studies for investigation of genetic variations among *R. solani* isolates. Bounou *et al*., (1999) used DNA from various *R. solani* isolates and forty short arbitrary primers of 10 mer primers in a RAPD experiment, they found that an amplicon of 2.6 kbp was detected in all tested isolates of *R. solani* belong to AG-3; a relation between RAPD pattern and origin of isolate was investigated and not be established. They also indicated that hybridization of amplicons generated by OPN-04-2.6 RAPD primer showed strong hybridization signal in dot-blot hybridization with all AG-3 isolates, and very faint signal with 3-1B1 isolate belonging to AG-8.

Toda *et al*., (1999) indicated that the use of RAPD-PCR is a useful tool for study genetic variations among *R. solani* isolates which collected from different geographical regions, whereas Isolates originated from the same geographical origin or host plants were not always genetically related, as well as some isolates obtained from one geographic region showed similar banding pattern, others vary in banding patterns with 13-33% similarity, also thy revealed that, RAPD-PCR showed considerable variation among the isolates belong to different AGs using arbitrary primers (P14, R28 and RC09) which generate 32, 25 and 23 fragments with a vary size ranged from 0.20 to 5.56 kb. among 41 tested isolates of *R. solani* and similarity was found to be ranged from 9% to 99%.

Random amplified polymorphic marker of 2.6 kb produced by one of 14 primers was assessed by southern blot analysis and partially sequenced, the result indicate that all AG-3 isolates obtained from potato were distinct from other *R. solani* isolates this finding was supported by restriction mapping of a specific band produced in tested isolates by specific primer yielded banding profile unique for AG-3 (Bounou *et al*., 1999).

Genetic variation among 30 isolates of *R. solani* have been studied by Duncan *et al.,* (1993) using RAPD-PCR by different primers (of which R2, R28, USP) and it was found that, no products were observed for primer R2 but USP primer gives fewer products than primers R1 and R28, higher level of variability was observed among isolates from the same AG but from different geographic location, so the relationship between banding patterns and geographic location do not established. Investigation of the genetic variation among 180 isolates of *R. solani* affected rice plant using RAPD markers reveled considerable variation within population, rather than among them (Wang *et al.,* 2013).

Random amplified polymorphic DNA (RAPD-PCR) have been used by Toda *et al*., (2004) for discrimination of *R. solani* isolates obtained from infected zoysia grass, the banding patterns generated by RAPD-PCR could be distinguished AG-2-2 into three cultural types (IIIB, IV and LP) and also could be separated into three clusters in a dendrogram constructed depending on the results of RAPD-PCR . Macnish *et al*., (2005) showed that four pectic isozyme groups belong to AG-8 of *R. solani* were confirmed by RAPD-PCR placed the studied isolates in four distinct groups.

Variations among 42 isolates of *R .solani* were investigated by Sharma *et al*., (2005) using RAPD-PCR with 11 random primers. They found that the RAPD-PCR patterns were varying in size (ranging from 0.3 to 3.5 kbp) in all isolates with RAPD, URP and ISSR, while isolates obtained from same hosts and same geographical regions showed similar results.

Yong *et al*., (2008) stated that most of *R. solani* strains obtained from Sichuan Province, China were genetically stable whereas 55 isolates were classified into 8 groups at 94 % similarity using 14 random primers which produced 638 polymorphic bands that have molecular length ranged from 0.2 kb to 2.3kb and they could differentiate between closely related banding patterns.

Compared to ISSR markers, RAPD markers were found to be able to detect more genetic variations (Guleria *et al*., 2007) whose used eight RAPD primers which produced 76 polymorphic (100) bands. Ten ISSR primers produced amplicons varied in molecular length whereas 79 were polymorphic and 9 were monomorphic. Based on data produced by RAPD and ISSR primers tested isolates were classified into five clusters with genetic similarity ranged from 49 to 89%. Also Guleria *et al*., (2007) found groping specific to the host variety. Genetic variations were assessed using RAPD and ISSR and showed that the polymorphism among *R. solani* isolates was 98.5% and 94% respectively with band size ranging from 0.1 to 5 kbp, whereas the use of SSR marker showed 93.6% polymorphism with the bands size ranging from 0.1 to 1.5 kbp. (Upadhyay *et al*., 2013).

Variation studies of 58 isolates of *R. solani* obtained from infected potato field in Iran were performed by Baitamar *et al.*, (2010) using morphological and molecular markers, tested isolates were classified into three groups (56 AG-3, 1 AG-4 and one isolate did not anastomose with any tester isolates available) according their ability to anastomose with tester isolates, AG-3 isolates were higher in Pathogenicity compared with AG- 4, also RAPD analysis supported and verified the morphological finds.

The objective of the current study was to assess the genetic diversity of R. solani isolates collected from potato fields in different geographic locations in Egypt.

MATERIALS AND METHODS

Collection of diseased samples and fungal isolation:

Infected samples were collected from several potato fields in Egypt, namely, Al-nubaria, Badr, Abou El-matamir, Hosh Eissa, Kafr El-Dawar, Itay Albaroud,

Wadi Alnetroun (El-Beheira), El-Khatatba (Menufia), Sakha (Kafr El-Sheikh) and El-Nahda (Alexandria). Fungal isolates were isolated from typical stem canker and black scurf symptoms which observed on the diseased potato plants. Small pieces from an advancing symptoms on diseased samples were cut (0.5-1.0 cm) long and surface sterilized with 1% sodium hypochlorite, rinsed in two changes of sterile water, placed on Potato Dextrose Agar (PDA) media and incubated at (27°C), after 48h; fungal growth from tissue pieces were examined with light microscope at 400X magnification. Fungal hyphae with characteristics of *Rhizoctonia solani* were transferred onto new PDA media and incubated at (27°C) for 3 days for purification. Pure cultures were then transferred onto PDA-slant and kept in refrigerator till used. Media preparation and isolation were performed according to Leslie and Summerell, (2006).

Fungal identification:

Morphological and molecular methods were used to identify the obtained isolates as it described in the following procedures:

Microscopic examination and culture characteristics:

Isolates were identified as *R. solani* using microscopic examination and culture characteristics (hyphal characteristics, colony color, growth pattern and location of sclerotia) according to Barnett and Hunter, (1998) and Trigiano *et al.,* (2004). Isolates which obtained from samples collected in 2013 and 2014 were assigned as RS 13 and RS 14 respectively followed by isolate number. Isolates were stained with lactophenol-aniline blue and examined using light microscope; dark hyphe right angle, septa and ramification, location of sclerotia, growth pattern were observed according to Lal and Kandhari, (2009) Growth rate was estimated by using different types of nutritional media Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Czapek-Dox Agar (Cz).

Random Amplified Polymorphic DNA (RAPD) analysis

Genomic DNA extraction:

A pure culture of each isolate was grown in potato dextrose broth (PDB) for 4 days, then mycelia were harvested and blotted by filter paper. DNA was extracted from *R. solani* isolates selected on the basis of cultural morphology and microscopic examination, following the procedure of Clapp, (1996). Briefly, 0.2-0.3 gm of fungal mycelium was grind with cetyltrimethylammonium bromide CTAB buffer (2% CTAB, 100 mM Tris-HCL PH 8.0, 20 mM ethylenediaminetetraacetic acid (EDTA), and 1.4 M NaCL) using mortar and pistil. Grind mycelium was transferred into 1.5 mL eppendorf tube; tubes were vortexed and incubated at 60°C for 30 min., then cold chloroform: isoamyl alcohol (24:1) solution was added, mixed and centrifuged at 5510 g for 15 min. Cold isopropanol was added and mixed with the supernatant in a new eppendorf tube and kept at -18°C for 30 min. in order to precipitate the DNA. Tubes were centrifuged at 13332 g for 15 min. Precipitated DNA was washed with 70% ethanol and left to dry in a laminar flow cabinet. DNA was re-suspended in 50 µL of TE buffer (10 mM Tris-HCL pH 8.0 and 1 mM EDTA).

RAPD-PCR amplification conditions

The 20 isolates of *R. solani* were used for RAPD analysis. Amplification reactions were performed using 50 ng of DNA for each isolate and 25 pmol of oligonnucleotide random primer; reactions were carried out in a total volume of 25 µl reaction mixture. Dream Taq Green PCR master mix (Thermo Scientific Company) was used according to the manufacturer instructions. The primers used in RAPD analysis are listed in (Table 1). PCR thermocycler(Techne- Progene) was programmed for one cycle of 3 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 35°c and 1 min at 72°C and one cycle of 7 min at 72°C for all tested primers except R1 and USP primers . For the primer R1, the amplification conditions contains two different stages after pre-denaturation step of 94°C for 1 min, the first stage contains 6 low stringency cycles of 94°C for 1min, 40°C for 1min and 72°C for 2min followed by the second stage of 26 cycles with high stringency of 94°C for 1min, 58°Cfor 1min and 72°C for 2min followed by the final extension cycle of 72°C for 10 min. For the USP, primer temperature profile was performed by 2 cycles of 94°C for 5 min, 48°C for 5 min and 72°C for 5 min, 40 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 2 min. The RAPD-PCR products were separated using 2% Agarose gel stained with ethidium bromide then visualized under UV transiluminator. Banding patterns of DNA were estimated using Totallab^{M} software (Nonlinear Dynamics Ltd, Durham, NC.) Reproducible products were incorporated into cluster analysis using the software PAST 3.02(Hammer *et al*. 2001).

Table 1. Sequence and annealing temperature of random primers used in RAPD analysis of *R. solani*

Primer no.	Sequence (from 5' to 3')	Annealing temperature (°C)
P14	CCACAGCACG	35°C
A01	TGCACTACAACA	"
A03	CGACGACGACGA	"
A04	ATCAGCGCACCA	"
A05	AGCAGCGCCTCA	"
A06	GCCAGCTGTACG	"
A07	TGCCTCGCACCA	"
A08	GCCCCGTTAGCA	"
A10	ACTGGCCGAGGG	"
A11	GATGGATTTGGG'	"
R28	ATGGATCCGC	"
USP	GTAAAACGACGGCCAGT	48 / 60
R1	GTCCATTCAGTCGGTGCT	40 / 58

RESULTS AND DISCUSSION

High variability among genetic material could be observed using several molecular markers, RAPD markers is one of the methods used in detection of genetic variability among fungal population because of its simplicity, does not require complicated preparations and rapidity of detection, also relationship between RAPD banding patterns and origin of tested isolates could be found (Bounou *et al.* 1999). The use of RAPD-PCR technique in this study revealed considerable variations among tested isolates (Figs. 1 to 9). Using 13 random primers, a total of 423 polymorphic bands were observed (Table 2), whereas no monomorphic bands observed between all tested isolate. The molecular length of the obtained banding patterns ranging from 200 bp to 2500 bp with Primer USP; these results support the findings of Moussa *et al*., (2014) which also showed that, the use of 14 RAPD primers with 20 isolates of *R. solani* produced 207 bands with 100% polymorphism, also banding patterns of tested isolates showed high variability depending on primer and isolates used. Using genomic DNA of 30 isolates of *R. solani* in RAPD PCR experiment showed that, primer R1 produced higher number of bands than USP (Duncan *et al.,* 1993). The obtained resultes showed that the highest number of bands (89) was observed using primer USP (Fig. 3), and the minimum number of bands (2) was observed with primer A01. Several types of bands have been observed among tested isolates, that have important role in specific detection of *R. solani* populations, several unique bands have been observed using primer tested which may be useful in specific detection of *R. solani* populations (Bounou *et al*., 1999). Several monomorphic bands could be detected in the current study with some primers which may be act as platform for specific detection of *R. solani*. Using primer P14 showed unique bands with some isolates, for instance a band of 1100 bp was found to be sheared between isolates RS 13-71, RS 13-56, RS 13-49, RS 13-19, RS 13-39, RS 13-82, RS 13-88, RS 13-91, RS 13-92, RS 13-99 and RS 14-129. In addition to that, fourteen isolates (RS13-82, RS 13-88, RS 13-91, RS 13-92, RS 13-99, RS 13-79, RS 13-32, RS 13-71, RS 13-49, RS 13-76, RS 13-77, RS 13-78, RS 13-79 and RS 13-39) have been distinguished from the other 6 isolates by a common band of 595 bp using USP primer. A clear small band of 250 bp was found only in eight isolates (RS13-82, RS13-88, RS13-92, RS13-99 "from Kafr El-Sheikh", RS 13-71, RS 13-49, RS 13-39 and RS 13-19 "from El-Behera"). A monomorphic band of 525 bp was found in nine isolates (RS13-82, RS 13-88, RS 13-91, RS 13-92, RS 13-99, RS 13-71, RS 13-19, RS 13-39 and RS 14-129) using primer A05, as well as eight isolates (RS13-82, RS 13-88, RS 13-91, RS 13-92, RS 13-99 , RS13-19, RS 13-32 and RS 13-71) found to be have a monomorphic band of 750 bp. Isolates of *R. solani* recovered from samples collected from the same geographic area and the same host showed similarity among banding patterns with some exception (Sharma *et al.*2005). Similar banding pattern was observed among *R. solani* isolates using RAPD analysis others

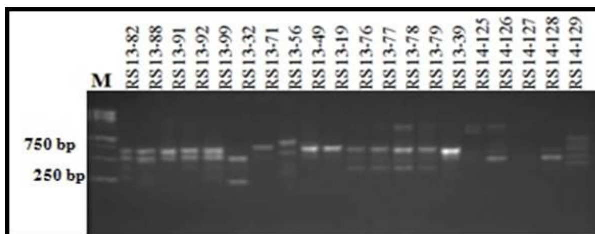

Fig. 1: RAPD-PCR pattern for 20 *R. solani* isolates with A07 RAPD primer. Isolates RS 13-82, 88, 91, 92 and 99 are those of El Nahda, (Alexandria); RS 13-32 from Abou El-matamir, (Elbehera); RS 13-71 from Wadi Alnetroun, (Elbehera); RS 13-56 from Itay Albaroud, (Elbehera); RS 13-49 from Kafr El-Dawar, (Elbehera); RS 13-19 from Badr, (Elbehera); RS 13-76, 77, 78 and 79 from Sakha, Kafr El-sheikh; RS 13-39 from Hosh Eissa, (Elbehera); RS 14-125, 126, 127, 128 and 129 from El Khatatba, (Menufia), M, molecular size marker.

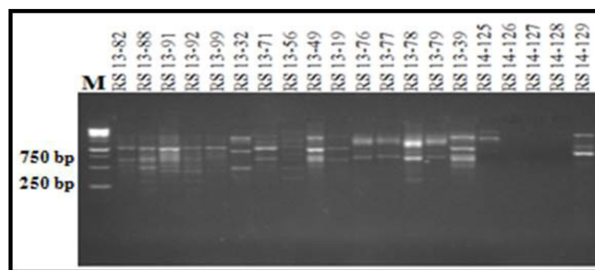

Fig. 2: RAPD-PCR pattern for 20 *R. solani* isolates with P 14 primer. Isolates RS 13-82, 88, 91, 92 and 99 are those of El Nahda, (Alexandria); RS 13-32 from Abou Elmatamir, (Elbehera); RS 13-71 from Wadi Alnetroun, (Elbehera); RS 13-56 from Itay Albaroud, (Elbehera); RS 13-49 from Kafr El-Dawar, (Elbehera); RS 13-19 from Badr, (Elbehera); RS 13-76, 77, 78 and 79 from Sakha, Kafr El-sheikh; RS 13-39 from Hosh Eissa, (Elbehera); RS 14-125, 126, 127, 128 and 129 from El Khatatba, (Menufia), M, molecular size marker.

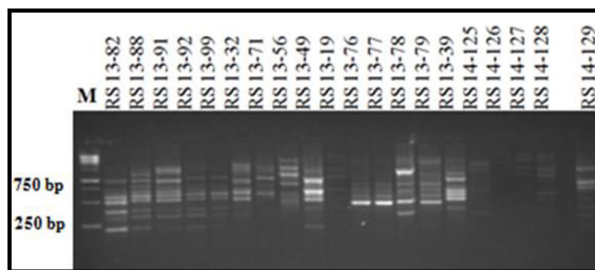

Fig. 3: RAPD-PCR pattern for 20 *R. solani* isolates with P USP primer. Isolates RS 13-82, 88, 91, 92 and 99 are those of El Nahda, (Alexandria); RS 13-32 from Abou El-matamir, (Elbehera); RS 13-71 from Wadi Alnetroun, (Elbehera); RS 13-56 from Itay Albaroud, (Elbehera); RS 13-49 from Kafr El-Dawar, (Elbehera); RS 13-19 from Badr, (Elbehera); RS 13-76, 77, 78 and 79 from Sakha, Kafr El-sheikh; RS 13-39 from Hosh Eissa, (Elbehera); RS 14-125, 126, 127, 128 and 129 from El Khatatba, (Menufia), M, molecular size marker.

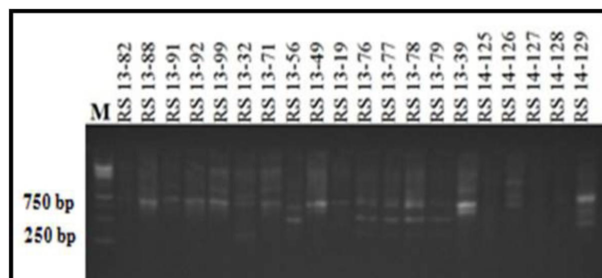

Fig. 4: RAPD-PCR pattern for 20 *R. solani* isolates with A03 primer. Isolates RS 13-82, 88, 91, 92 and 99 are those of El Nahda, (Alexandria); RS 13-32 from Abou Elmatamir, (Elbehera); RS 13-71 from Wadi Alnetroun, (Elbehera); RS 13-56 from Itay Albaroud, (Elbehera); RS 13-49 from Kafr El-Dawar, (Elbehera); RS 13-19 from Badr, (Elbehera); RS 13-76, 77, 78 and 79 from Sakha, Kafr El-sheikh; RS 13-39 from Hosh Eissa, (Elbehera); RS 14-125, 126, 127, 128 and 129 from El Khatatba, (Menufia), M, molecular size marker.

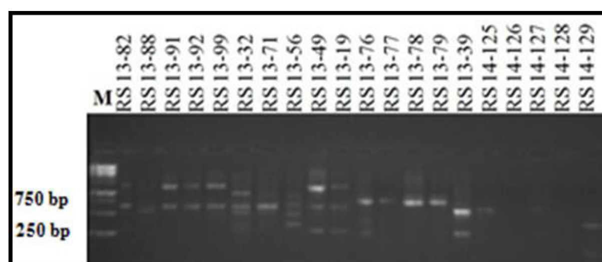

Fig. 5: RAPD-PCR pattern for 20 *R. solani* isolates with R28 primer. Isolates RS 13-82, 88, 91, 92 and 99 are those of El Nahda, (Alexandria); RS 13-32 from Abou Elmatamir, (Elbehera); RS 13-71 from Wadi Alnetroun, (Elbehera); RS 13-56 from Itay Albaroud, (Elbehera); RS 13-49 from Kafr El-Dawar, (Elbehera); RS 13-19 from Badr, (Elbehera); RS 13-76, 77, 78 and 79 from Sakha, Kafr El-sheikh; RS 13-39 from Hosh Eissa, (Elbehera); RS 14-125, 126, 127, 128 and 129 from El Khatatba, (Menufia), M, molecular size marker.

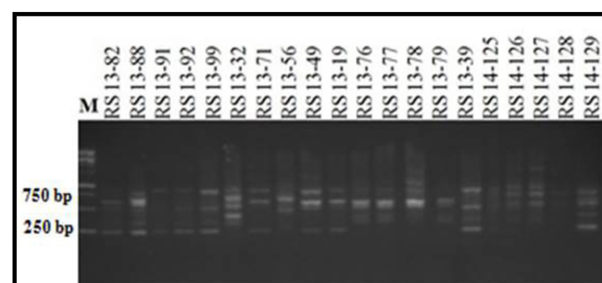

Fig. 6: RAPD-PCR pattern for 20 *R. solani* isolates with A04 primer. Isolates RS 13-82, 88, 91, 92 and 99 are those of El Nahda, (Alexandria); RS 13-32 from Abou Elmatamir, (Elbehera); RS 13-71 from Wadi Alnetroun, (Elbehera); RS 13-56 from Itay Albaroud, (Elbehera); RS 13-49 from Kafr El-Dawar, (Elbehera); RS 13-19 from Badr, (Elbehera); RS 13-76, 77, 78 and 79 from Sakha, Kafr El-sheikh; RS 13-39 from Hosh Eissa, (Elbehera); RS 14-125, 126, 127, 128 and 129 from El Khatatba, (Menufia), M, molecular size marker.

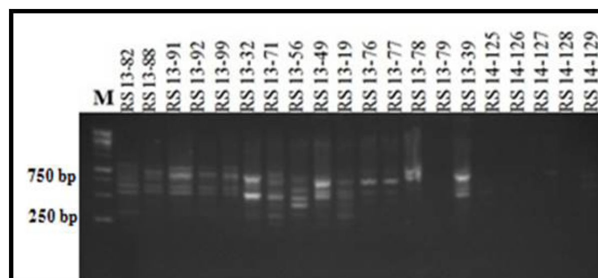

Fig. 7: RAPD-PCR pattern for 20 *R. solani* isolates with A10 primer. Isolates RS 13-82, 88, 91, 92 and 99 are those of El Nahda, (Alexandria); RS 13-32 from Abou Elmatamir, (Elbehera); RS 13-71 from Wadi Alnetroun, (Elbehera); RS 13-56 from Itay Albaroud, (Elbehera); RS 13-49 from Kafr El-Dawar, (Elbehera); RS 13-19 from Badr, (Elbehera); RS 13-76, 77, 78 and 79 from Sakha, Kafr El-sheikh; RS 13-39 from Hosh Eissa, (Elbehera); RS 14-125, 126, 127, 128 and 129 from El Khatatba, (Menufia), M, molecular size marker.

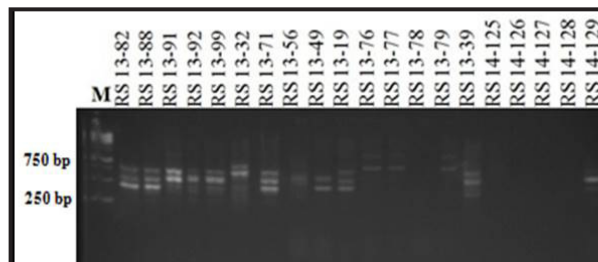

Fig. 8: RAPD-PCR pattern for 20 *R. solani* isolates with A05 primer. Isolates RS 13-82, 88, 91, 92 and 99 are those of El Nahda, (Alexandria); RS 13-32 from Abou Elmatamir, (Elbehera); RS 13-71 from Wadi Alnetroun, (Elbehera); RS 13-56 from Itay Albaroud, (Elbehera); RS 13-49 from Kafr El-Dawar, (Elbehera); RS 13-19 from Badr, (Elbehera); RS 13-76, 77, 78 and 79 from Sakha, Kafr El-sheikh; RS 13-39 from Hosh Eissa, (Elbehera); RS 14-125, 126, 127, 128 and 129 from El Khatatba, (Menufia), M, molecular size marker.

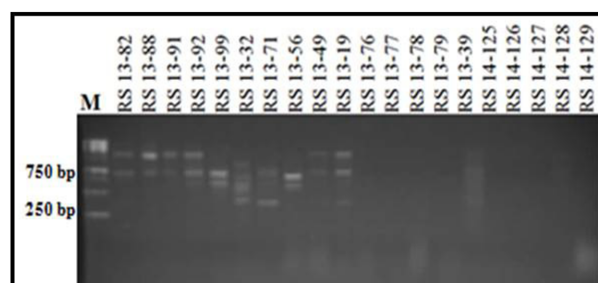

Fig. 9: RAPD-PCR pattern for 20 *R. solani* isolates with A06 primer. Isolates RS 13-82, 88, 91, 92 and 99 are those of El Nahda, (Alexandria); RS 13-32 from Abou Elmatamir, (Elbehera); RS 13-71 from Wadi Alnetroun, (Elbehera); RS 13-56 from Itay Albaroud, (Elbehera); RS 13-49 from Kafr El-Dawar, (Elbehera); RS 13-19 from Badr, (Elbehera); RS 13-76, 77, 78 and 79 from Sakha, Kafr El-sheikh; RS 13-39 from Hosh Eissa, (Elbehera); RS 14-125, 126, 127, 128 and 129 from El Khatatba, (Menufia), M, molecular size marker.

vary in banding patterns and shows similarity of 9% to 99% (Toda *et al.*, 1999). The dendrogram based on the banding patterns (Fig.10) obtained from the tested isolates using 13 different RAPD-PCR primers, grouped the tested isolates into two main clusters, that revealed considerable variation among tested isolates, whereas high similarity (89%.) was observed among two tested isolates of *R. solani* (RS 13-76 and RS 13-77, from the same location ,Kafr El-sheikh) based on the observed banding patterns and amplification products with each primer (Figs. 1: 9).While, low similarity (5%) was observed between isolates RS 13-76 and RS 14-128, from different locations, Kafr El-sheikh and Menufia respectively. Toda *et al.*, (2004) revealed that, *R. solani* isolates obtained from different geographical areas were classified into three clusters depending on the results of RAPD-PCR. High level of variability was observed among *R. solani* isolates from different geographical location using RAPD analysis on the other relationship between banding patterns and geographic location was established by Duncan *et al.,* (1993).

Table 2: Total number of bands and range of amplicons length (bp) for each primer

Primer name	Total number of bands	Range of amplicons length (bp)
P14	56	320-2500
A01	2	1000-1400
A03	28	500-1500
A04	42	250-1100
A05	30	400-1125
A06	22	475-2000
A07	33	250-1500
A08	35	290-941
A10	35	350-1050
A11	12	375-1500
USP	89	200-2500
R28	28	250-1450
R1	11	600-1500

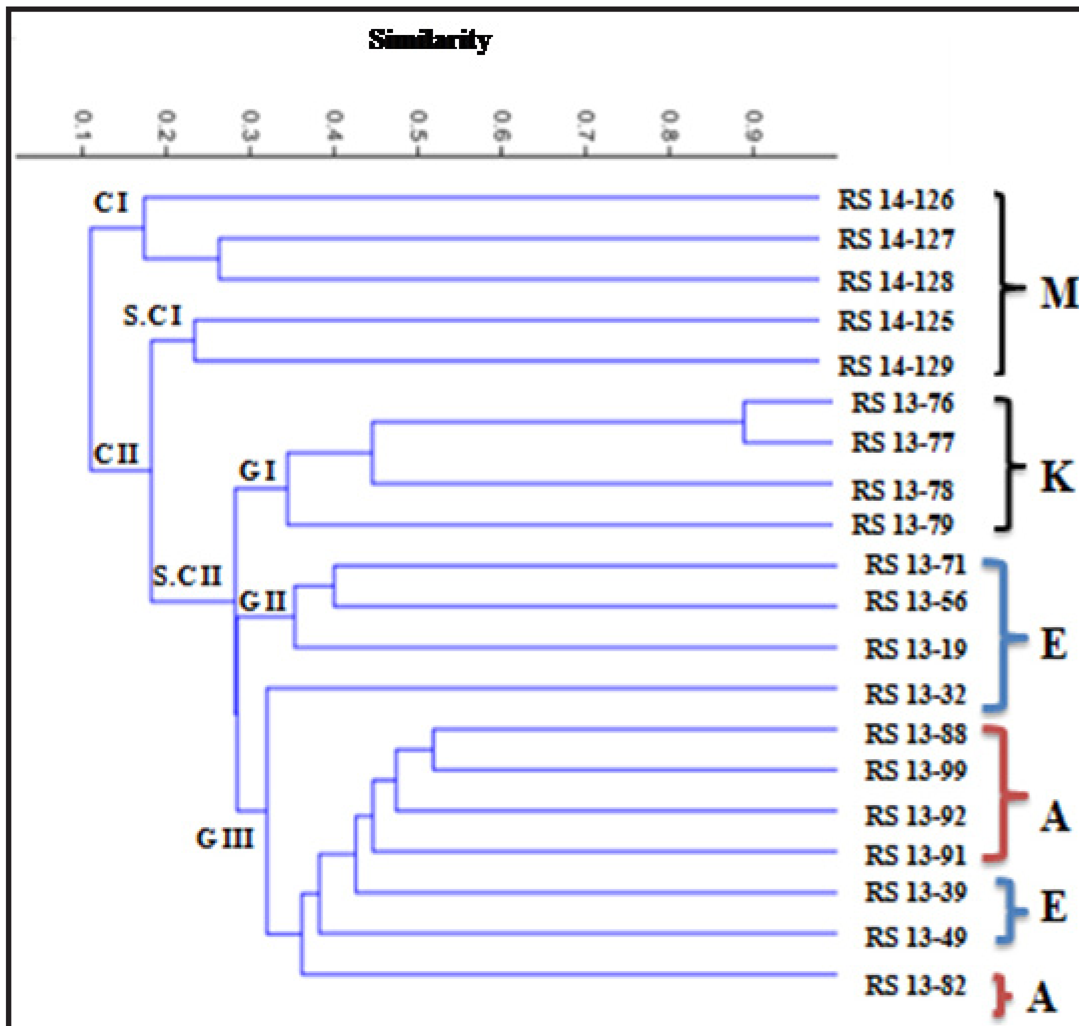

Fig. 10: Dendrogram obtained by clustering (UPGMA method) based on the banding pattern obtained by the RAPD-PCR analysis of twenty *Rhizoctonia solani* isolates from different locations according to Jaccard's similarity. Whereas, M (Minufiya), K (Kafr El-Sheikh), E (El-Beheira) and A (Alexandria).

Table 3: Similarity matrix (%) among *Rhizoctonia solani* isolates based on RAPD band pattern analysis and Jaccard's index

R. solani isolate	RS 13-82	RS 13-88	RS 13-91	RS 13-92	RS 13-99	RS 13-32	RS 13-71	RS 13-56	RS 13-49	RS 13-19	RS 13-76	RS 13-77	RS 13-78	RS 13-79	RS 13-39	RS 14-125	RS 14-126	RS 14-127	RS 14-128	RS 14-129
RS 13-82	100																			
RS 13-88	37	100																		
RS 13-91	43	43	100																	
RS 13-92	42	50	43	100																
RS 13-99	36	52	47	44	100															
RS 13-32	22	38	27	36	35	100														
RS 13-71	35	31	27	34	37	32	100													
RS 13-56	26	28	21	26	27	24	40	100												
RS 13-49	31	46	38	38	36	31	33	39	100											
RS 13-19	33	24	16	32	25	20	39	31	31	100										
RS 13-76	33	32	24	37	33	25	35	25	31	30	100									
RS 13-77	28	30	22	33	29	25	33	23	29	27	89*	100								
RS 13-78	19	28	25	24	37	31	34	27	27	14	46	43	100							
RS 13-79	28	24	19	37	28	20	30	25	13	26	40	34	28	100						
RS 13-39	28	47	39	41	42	34	30	26	33	24	39	33	28	33	100					
RS 14-125	15	11	17	14	15	10	20	12	13	14	16	13	21	19	20	100				
RS 14-126	9	13	11	11	12	16	15	11	12	13	12	12	18	8	15	7	100			
RS 14-127	7	8	9	11	6	9	7	11	13	6	6	6	15	9	15	21	21	100		
RS 14-128	14	10	17	13	14	8	7	11	7	9	5**	5	11	11	12	19	14	26	100	
RS 14-129	23	18	22	20	22	29	15	20	27	17	22	19	24	15	20	23	15	7	7	100

* High level of genetic similarity (89%) observed between tested isolates RS 13-76 and RS 13-77.
** Low level of genetic similarity (5%) observed between tested isolates RS 13-76 and RS 14-128.

The constructed dendrogram indicated that tested isolates were found in two major clusters cluster I contains 3 isolates (RS 14-126, RS 14-127 and RS 14-128) that obtained from the same geographical region (Menufia), cluster II was existed in two sub cluster, sub cluster I contains 2 isolates (RS 14-125 and RS 14-129) which obtained from the same geographical region Menufia, sub cluster II contains 15 isolates obtained from different geographical regions, which found in 3 different groups, group I en mpasses 4 isolates (RS 13-76, RS 13-77, RS 13-78, and RS 13-79) obtained from one geographic region (Sakha),. Group II includes 3 isolates (RS 13-71, RS 13-56, and RS 13-19) which were obtained from 3 different geographical regions (Wadi Alnetroun, Itay Albaroud, and Badr respectively). The third group contains 8 isolate obtained from different geographical regions, whereas 3 isolates (RS 13- 32, RS 13- 39 and RS 13- 49) were from Abou Elmatamir, Hosh Eissa and Kafr El-Dawar respectively and, the other five isolates (RS13- 88, RS 13- 99, RS 13- 92, RS 13- 91, and RS 13- 82) were from Alexandria. Random Polymorphic DNA markers are used for specific detection of phytopathogens populations; a RAPD marker of 2.6 kbp was detected in *R. solani* by Bounou *et al*., (1999) and used for specific detection of AG-3 from Canada and USA, in present study several RAPD markers have been observed using many arbitrary primers of which 1100 bp was detected using primer P14 and found to be sheared between ten isolates [RS 13-71, RS 13-49, RS 13-19, RS 13-39, (obtained from several areas in El-Behera governorate) RS 13-82, RS 13-88, RS 13-91, RS 13-92, RS 13-99 (collected from El-Nahda, Alexandria) and RS 14-129 (collected from El Khatatba, Minufiya]. It could be speculated that this is a part of genetic flow in these isolates that could be moved anyhow from El-Behera through its neighbor governorate, Alexandria, into El Khatatba, Minufiya. Toda et al., (1999) indicated that isolates originated from the same geographical origin or host plants were not always genetically related, as well as some isolates obtained from one geographic region showed similar banding pattern, others vary in banding patterns with 13-33% similarity. In this investigation, R. solani isolates revealed high genetic variability among tested isolates; each primer produced various numbers of bands which found to be polymorphic, distinguished and monomorphic bands. Reproducibility of the primers used in this study varied due to the isolate tested, poorly reproducibility was observed in several primers (A01, A11, and R1). Similarity between isolates form the same geographic region was varied according to primer used, some common bands were observed between some isolates which were obtained from the same and different geographic region. Similarity percentage was ranged from 20% to 40 % in case of isolates which originates from El-Behera governorate; no monomorphic bans were recorded with primers A01 whereas the remaining primers produces more than one common band between two isolates or more.

ACKNOWLEDGMENT

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الاختلافات الوراثية بين عزلات فطر الرايزوكتونيا سولاني التي تصيب البطاطس

أحمد فرج البيباني[1]، نادر عبد الوهاب عشماوي[1]، سيد أبوشوشة[1]، فؤاد ضعيف[2]، صبحي سلامه حسين[1]

١ قسم أمراض النبات– كلية الزراعة – جامعة الإسكندرية

٢ قسم علم النبات– جامعة مانيتوبا– وينيبج –كندا

تم دراسة وتعريف الاختلافات الوراثية بين عشرين عزلة من فطر الرايزوكتونيا سولاني *Rhizoctonia solani* علي المستوي المورفولوجي والجزيئي. درست هذه الاختلافات علي المستوي الجزيئي عن طريق التضاعف العشوائي لجينوم العزلات باستخدام طريقة RAPD–PCR. أظهر تكنيك (RAPD) نتائج إيجابية مع ثلاثة عشرة بادئا عشوائيا (١٣ ran-dom primers) تم استخدامها في هذة الدراسة وكان مجموع الحزم (bands) المتحصل عليها ٤٢٣ حزمة مختلفة في الطول , وكان طول أقل حزمة حصل عليها هي 200 bp وأعلي حزمة هي 2500 bp. وأظهرت دراسة الأنماط الوراثية fingerprinting patterns للعزلات المختلفة علي أساس اختبار RAPD أن هناك اختلافات واضحة في الأنماط الوراثية للعزلات المختلفة علي أساس ٣ أنواع من الحزم وهي الحزم المميزة distinguished (unique) bands والحزم المشتركة monomorphic bands وأخيرا الحزم المفرقة بين العزلات polymorphic bands. تم استخدام برنامج الـ Paleon-tological statistics software package (PAST) بهدف معرفة مستوي التشابه الوراثي بين العزلات المختلفة وذلك بإيجاد مصفوفة النسبة المئوية لمستوي التشابة الوراثي بين العزلات, أظهرت النتائج اختلافات وراثية معنوية بين العزلات المختلفة وكانت أعلي نسبة في مستوي التشابة الوراثي تساوي 89% وذلك بين العزلتين RS 13-76، RS 13-77 (كفرالشيخ) ,وأقل نسبة مستوي تشابة وراثي تساوي ٥٪ بين العزلتين RS 13-76، RS 14-125 (كفرالشيخ والمنوفية). تم عمل شجرة النسبPhylogenetic tree باستخدام برنامج PAST بين العزلات المختلفة علي أساس نتائج RAPD–PCR المتحصل عليها , أوضحت النتائج أن العزلات موضع الدراسة قسمت إلي عنقودين ٢ clusters ضم العنقود الأول علي ٣ عزلات من محافظة المنوفية (RS 14-126, 127 and 128) أما العنقود الثاني يحتوي علي تحت عنقودين sub cluster 2 ضم تحت العنقود الأول عزلتين فقط من محافظة المنوفية (RS 14-125 and 129) أما تحت العنقود الثاني فضم ٣ مجموعات ٣ groups المجموعة الأولي تضم عزلات كفر الشيخ (RS 13-76, RS 13-77, RS 13-78, and RS 13-79), وضمت المجموعة الثانية ٣ عزلات من محافظة البحيرة (RS 13-71, RS 13-56, and RS 13-١٩). ضمت المجموعة الثالثة ثمانية عزلات, منها ٣ عزلات من محافظة البحيرة (RS 13- 32, RS 13-39 and RS 13-49) وخمسة عزلات من محافظة الأسكندرية (RS13- 88, RS 13-99, RS 13-92, RS 13-91, and RS 13-82). دراسة الاختلافات الوراثية بين العزلات المسببة للمرض في مناطق مختلفة في مصر قد يساعد في معرفة شدة الإصابة المتوقعة وكذلك العزلات الأكثر أمراضية وأخذ ذلك في الاعتبار عند وضع برنامج مكافحة للمرض.

الكلمات المفتاحية :القشرة السوداء ، RAPD، *Rhizoctonia solani*، الاختلافات الوراثية

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Role of Fertilization on Development of Rhizoctonia Rot Disease of Faba Bean

Abd El-Magid Kamara[1], Sayed S. Aboshosha[1*], Ahmed F. El-Bebany[1], Mohsen Saleh[2] and Sherihan M. M. Bekheet[1, 2]

[1]Department of Plant Pathology, Faculty of Agriculture, Alexandria University, El-Shatby 21545, Alexandria, Egypt

[2]Plant Pathology Institute, Agriculture Research Center, 12619, Giza, Egypt*

ABSTRACT

Samples of diseased faba bean showed symptoms of root and stem cankers were collected from Alexandria and El-Behera governorates. Seven isolates of *R. solani* were recovered, purified and identified according to morphological microscopic and molecular characteristics of the fungus. Pathogenicity of obtained isolates was examined on faba bean seeds (Giza 3 cv.) .All tested isolates were pathogenic and differs significantly in their pathogenicity whereas isolate No5 was highly virulent, and isolateNo6 was weakly virulent, in case of pre-emergence. On parallel all isolates affected post-emergence damping off, the highest aggressive isolate was isolate No2. Effect of different types of biofertilizers HALEX®, Nitrobin®, Rhizobacterin® , Non commercial Biofertilizer (NC) and mineral fertilizer (NPK) on root rot was examined using isolate R5. It was found that the Biofertilizer (NC) leads to increase of fresh Weight of shoot and root up to 35 to 54% followed by Nitrobin (22%) and the mineral fertilizer (8%) compared to the check treatment. Treatments increased shoot length (17.1-21.4%) in compare to control. On parallel fresh weight of inoculated plant increased and the highest increase recorded with Biofertilizer (NC) (30.4%), on the other hand treatments did not significantly increase shoot length. The highest increase was related to Biofertilizer (NC) (35.3%) and Rhizobacterine (33.2%). Fresh weight increased by 77.1%with Biofertilizer (NC) and 60%with NPK. Dry weight increased by 85.4%and 79.2%with Nitrobine. Biofertilizer (NC) treatment significantly increased root length of both inoculated and uninoculated plants by 62.5and 54.2%, respectively, the highest increase of root fresh weight increased by 108.5% with Nitrobine in case of inoculated plants. Suppressive effect of some bioagents, *Bacillus subtilis, Pseudomonas fluorescens* and *Trichoderma harzianum* was examined on tested isolates *B. subtilis* was highly suppressive than *P. fluorescens*, tested isolates greatly affected by tested bioagents whereas R4 was highly suppressed by *B. subtilis* followed by moderately suppressed (R1, R2, R3, R6, R7) and weakly suppressed (R5). *P. fluorescens* has suppressive effect on isolate R7 and weakly suppressive effect on isolate R1, R2, R3, R4, R5 and R6. *T. harzianum* has highly suppressed isolate R5, moderately suppressed isolates R1, R2, R3, R6 and R7 and weakly suppressive effect on isolate R4.

Key words: *Rhizoctonia* ***, biofertilization, Faba bean, root rot***

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important crops in Egypt and worldwide. In Egypt, broad bean is grown in early spring or late summer to avoid high summer temperatures (Elwakil *et al.*, 2009). Broad bean is cultivated for both human and animal consumption, soil development, and medicinal uses. Broad bean is often used as either forage (leaves, plant material) or silage (fermented, high-moisture fodder) for animals (Munro, 1993).

Rhizoctonia solani can affect most annual plants including almost all vegetables, many field crops, perennial ornamentals, shrubs and trees (Ogoshi, 1987). The most frequently disease symptoms caused by *R. solani* are damping off seedlings, root rot and stem rot or stem canker of young and mature plants. *R. solani* overwinters as mycelium or sclerotia in the soil, in/on infected plants. In many plants for example tomato, rape, lettuce, Pea, red clover and broad bean, the fungus can be carried in the seeds (Papavizas and Davey 1960).

Many microorganisms have the ability to fix atmospheric nitrogen, thus they contribute immensely to soil fertility. Kapulnik *et al.* (1981) found that plant height significantly increased by inoculation with *Azospirillum.* Also, (Kapulnik *et al.* 1981) treated pea seeds (cv. Bonneville) for 10 min. with either bacterium (*Azotobacter* and *Rhizobium*) alone or in combination, and found that plant height was greater after seed treatment with both bacteria. Increasing the height of capsicum plants was recorded by (Paramaguru and Nataraian, 1993) Through the seed growing under semi-arid conditions treatment with *Azospirillium* combined with 56kg N/ha.

Moreover, (Ashour *et al.* 1997) studied the effect of biofertilizers (Nitrobien) as well as different levels of nitrogen on growth and yields of potato, they found that treating potato tubers with Nitrobien has significantly increased plants height. Similar trend was also demonstrated by Abd El- Fattah and Sorial (1998) through the inoculation of lettuce seedlings with single (*Azospirillum*) or mixed (HALEX®) biofertilizers, with respect to either the plant height or the leaves number per plant. On the other hand Merghany (1999) showed that the highest value of plant height of snap bean, was obtained under peat moss inoculation method compared with non-inoculated plants or seed inoculation method.

The effect of biofertilizer could be extended to the dry matter content of the plant organs as found by (Ni-

correspondence author: Dr. Sayed S. Aboshosh
Tel. 01227434663 - Email: sayed.aboshosha@yahoo.com

eto and Frankenberger1990) who studied the effect of a cytokinin producing bacterium, *Azotobacter chroococcum* on the morphological plant characteristic radish, and found that the inoculums enhanced the dry weight of shoot tissues. Rodelas *et al.* (1996) stated that strain sp7 (ATCC 29145) promoted substantial increases (> 30%) in shoot dry matter accumulation. Magnitude and direction of plant responses are strongly related to the cell density of *Azospirillum* applied as inoculum of faba bean. Ashour *et al.* (1997) found that treating potato tubers with Nitrobien has significantly increased foliage dry weight. On the other hand, Baltensperger *et al.* (1978) found that inoculation with *Azotobacter* or *Azospirillum* on turf give insignificant increase in total dry weight of all plant parts. Agwah and Shahaby (1993) stated that inoculation with *Azospirillum* significantly increased dry weight of lettuce in the first season regardless of N-fertility. However, in significant differences among treatments of the second season were recorded. El – Gizawy (1998) stated that leaves dry weight total nitrogen percent of molokhia plant was increased by inoculation with (*Azotobacter* a 104, *Azospirillium* AZ 307 and *klebsiella* K 201) from 0.24 to 0.58% under 0.0 kg (NH4)2 SO4/fed.

Baltensperger *et al.* (1978) found that leaf color differences were observed as a result of inoculation with *Azotobacter* or *Azospirillium* on turf-type. Nieto and Frakenbeerger (1990) studied the effect of a cytokinin producing bacterium, *Azotobacter chroococcum* on the morphological plant characteristics and, found that the inoculums enhanced chlorophyll a content of radish. Moreover, Abd El- Fattah and Sorial (1998) showed that inoculation of lettuce seedlings, with either single or mixed biofertilizer resulted in significant increments of leaf chlorophyll a and b.

One of the most commonly assumed relationships of N to disease is that high N rates lead to more disease. Adequate N uptake is essential to the formation of various structures, proteins and enzymes needed in both growth and disease resistance. A vigorously growing plant can often offset, or "outgrow" the most damaging effects of some diseases. Conversely, it has been observed that in some plants as the N content is increased beyond sufficient levels, the amount of antifungal compounds decreases. Feeding intensity and reproduction by sucking insects tend to be higher on plants with higher amino acid content. Potatoes deficient in either N or P are more susceptible to early blight (*Alternaria solani*) Randall *et al.*, (1993). The study was performed to investigate the role of biofertilizers in plant nutrition and suppression of *Rhizoctonia* rot disease of faba bean.

MATERIALS AND METHODS

Fungal isolation and identification:

Samples of faba bean plants showing typical symptoms of root rot were collected from several areas in Alexandria and Al-Behera governrates. Several isolation trilas were carried out from infected faba bean The infected roots were washed carefully with tap water, and then cut into small pieces, air dried and surface sterilized using 1% sodium hypochlorite solution , then rinsed several times in sterilized distilled water and dried using sterilized filter paper and placed on potato dextrose agar medium (PDA) supplemented with streptomycin sulphate at the rate of (50µg mℓ$^{-1}$) and incubated at 27ºc. Developing isolates were subcultured , successifuly , until fungal growth became homogenous. Further purifications was accertaned by hyphal tip technique (Lilly and Barnette 1951).

The purified fungi were identified as *Rhizoctonia solani* using cultural, morphological and microscopical characteristics that were discribed by **Gillman, (1957**), **Ellis, (1971), Barnett and Hunter (1972).**Seven isolates were confirmed as *R. solani* (*kühn*). Culture variations between the different isolates were recorded, i.e. rate of linear growth, fresh weight, dry weight, colour and zonation habit of mycelial growth ; four repilcates were prepared for each isolate.

A pure culture of each isolate was grown on potato dextrose broth (PDB) for 4 days; the mycelium was harvested and blotted by filter paper. DNA was extracted from *R. solani*, tested isolates according to the procedure of **Clapp (1996).**

The reaction was carried out in a technothermo cycler which programmed for an initial denaturation step of 94.0 °c for 1 min., followed by 35 cycle of 94.0°C for 1 min, 55.0°C for 2 min. and 72.0°C for 1 min. a final extension step at 72.0°C was added. PCR products were visualized under UV light. A 100bp ladder was.

Rhizoctonia solani isolates were mentaind on barley grain medium in order to enhance their mycelial growth. Soil infestation was carried out one week before planting broad bean seeds cultivar Giza 3. Ten grams of the barley grain media contains *R. solani* were mixed with one kg autoclaved sandy clay soil (1:1v/v) in plastic pots 16 cm in diameter. Seeds were planted in the infested soil by rate of 6 seeds/pot. For each isolate, four replicates were used, as well as the control replicates. Other pots without infestation were used as check.

The percentage of seed decay pre-emergence and post-emergence damping off was calculated after15 and 30 days of sawing, respectively, while the survival plants were assessed after 45 days. Disease assessments were calculated according to Yehia *et al.* (2007)

Effect of fertilization on faba bean resistace to R. solani :

The effect of three commercial biofrtilizers HALEX® Nirobin®, Rhizobacterin®, and NC (noni commercial) biofertilizers and one mineral fertilizer (NPK) were tested.

Halex®, Nitrobin and Rhizobacterin used as seed dressing at the rate of 10g/kg seed. Treatment repeated at the middle of growing seasons, (**Hassouna,** *et al.***, 1998**).

Halex® is a commerial biocontroler / biofertilizer / product contains the three nitrogen fixation bacterial

genera *Azospirillium brosilense, Azotobacter chrocco-cum* and *Klebsiella preumoniae*

(Nitrobein is the commercial name of nitrogen fixing bacteria containing (*Azotobacter chroococcum*) and (*Azopirillum lipoferum*) . (Rhizobacterin a commercial product that contains a specific strain of Rhizobiusm spp which fixes atmospheric N.) (**Hassouna, 1973**). NPK (31 / 5 / 0) use as mineral fertilizer at a rate of 20 grams per 1 liter of water with irrigation and repeated at the middle of growing seasons.Product of Abu Qir Fertilizers Company. Biofertilizers (noncommercial) used at the rate of 15 ml per 150 ml of water and that each was duplicated and repeated at the middle of growing seasons. (Expermintal (non commerial) biofertilizer contains three different genera of bacteria (Azotobacter, mychoriza and Rhizobium) were supplied , from soil departement, faculty of agriculture, Alexandria)

Control. (Untreated plants).

Evaluation of the impact of fertilization on broad bean:

Two weeks after sowing, the number of successful surviving seedlings of each hole were counted and the percentge was calculated for each replicate (row), for each treatment.

At the end of the experiment (45 day after sowing), ten plants were chosen randomly from each replicate, and examined for root rot symptoms.

Ten plants were chosen randomlyfrom each treatment, and the fresh weight of roots and shoots weight of each plant was weighed to the nearest gram.

Biological control

Two bacterial agents (*Pseudomonas fluorescens*- and *Bacillus subtilis)* were tested against *R.solani*.The bacterial strains were obtained as pure cultures from the bacteriology lab, Department of plant pathology, Alexandria University.

The suppressive effect of the bacteria on fungal growth in the streaking test was visually assessed by comparing the dishes of each treatment with their non-bacterial control. (Reddy and Patrrick, 1990and Wei and Tuzun 1990).

Trichoderma harzianum was tested against *R. solani. T.harzianum* strain was provided by Plant pathology lab, Agriculture Research Center Giza Eldokki. The fungal growth was examined for any change in growth shape or other antagonistic effects, (Papavizae, 1985).

RESULTS AND DISCUSSION

Isolation and identification of *Rhizoctonia solani*:

Faba bean is one of the most food commodities in Egypt, with cultivation area of 46200 Ha (FAO, 2013). Faba bean plants facing several plant pathogens that reduce productivity and quality of faba bean plants, root rot of faba bean caused by *Rhizoctonia solani* seems to be the most destructive disease attacks faba bean due to pre and post emergence damping off that cause losses in productivity (Abdel-Hafez 1988, Dubey and patel 2000, You*, et. al.,* 2008,).

Infected broad bean plants with typical Rhizoctonia canker and root rot symptoms were collected from Alexandria and EL-Behera governorates at the growing season (2009/2010). Seven isolates (R1, R2, R3, R4, R5, R6, and R7) of *Rhizoctonia solani* were recovered from these infected plants.

The tested isolates differed in rate of their linear growth and other characteristics. Data presented in Table (1) indicates that isolates 3, 5, 6 and 7 expressed the highest growth rate within the time, while isolate 1 is the lowest rates. Isolates 2, 5, and 6 expressed the highest weight, as they 3.037 g, 2.038 gm and 1.492 gm., respectively, while isolates 1 and 4 the lowest fresh and dry weight as 0.860 g and 0.626 g. Additionally the isolates differed noticeably in mycelia color, isolates No.3 and 4 were brown in color, while isolates No. 5 and 6 were light brown, and isolates No.1, 2 and 7were white in color. The isolates 1, 2, 3 and 4 were able to develop clear growth zonation but at different rates while the others (5, 6, 7) were not able to develop clear zonation, (Table 1)

Table1: Morphological characters of seven isolates of *Rhizoctonia solani*

Isolate NO	Linear growth (cm)			Fresh weight (gm)	Dry weight (gm)	Mycelim color	Zonation(1)	Arial mycelium(2)
	24 h.	48 h.	72 h.					
1	2.31^{c}	4.15^{c}	8.1^{b}	0.860^{f}	0.331^{b}	white	+ + + +	_
2	3.25^{b}	5.35^{b}	8.9^{a}	3.037^{a}	1.062^{a}	white	+ +	+
3	1.75^{d}	3.0^{e}	9.0^{a}	1.233^{d}	0.101^{b}	Dark brown	+ + +	_
4	0.83^{e}	1.55^{f}	8.9^{a}	0.626^{g}	0.190^{b}	brown	+ + +	_
5	3.42^{b}	6.85^{a}	9.0^{a}	2.038^{b}	0.352^{b}	light brown	_	+
6	3.41^{b}	5.75^{b}	9.0^{a}	1.492^{c}	0.131^{b}	brown	_	+
7	3.89^{a}	5.75^{b}	9.0^{a}	1.186^{e}	0.167^{b}	white	_	+

1– (+) = number of zones and (-) No zonation.
2– (+) = mycelium aerial and (-) No aerial mycelium.

Molecular identification of R. solani isolates using two universal primers (ITS1 and ITS2) showed that all of them have a specific DNA band of 700 bp (Fig. 1)

Pathogenicity test of *R. solani* isolates on broad bean plants:

The pathogenicity test of obtained *R. solani* isolates revealed that all isolates of *R. solani* were found to be pathogenic to faba bean plants and varied in pathogenicity whereas different types of symptoms were observed on faba been plants, infection was greater in the pre-emergence damping-off in case of plants that infected by isolate R5 followed by isolate R2 then isolate R1, R7, R4, and R3, while the lowest virulent one was R6.Also fresh and dry weight of infected faba bean plants greatly affected by tested isolates (Cubeta and Vilgalys, 2000). Our results indicated that the Rhizoctonia symptoms on the inoculated plants as they were observed previously on diseased samples diagnosed in fields.

Data presented in Table (2) revealed that all the tested isolates of *R. solani* were significantly varied in their pathogenicity to faba bean and produced typical symptoms of seed decay, root rot and stem canker diseases The most common symptom of Rhizoctonia was damping -off that characterized by non-germination of severally infected seed whereas infected seedlings were rotted either before or after they emerge from the soil.

Infected seedlings often had cankers as raddish-brown lesions on stems and roots. *R. solani* isolates affected both pre-emergence and post-emergence damping- off. According to Pre-emergence damping off, all isolates caused significant seed rot ranged 2.25 - 3.25, except isolate R6 (0.5) and R4 (1.25).

On the other hand, post emergence damping off was not significantly affected except with isolate R2 (1.0). It can be noticed that infection was greater in the pre-emergence damping-off for isolate R5 followed by isolates R1 and R3 then isolate R2, R7, and finally isolates R4, and R6, while the lowest aggressive one was R6 additionally data of post-emergence damping off showed that tested isolates could be arranged as follows: R2, then R1and R7 and finally R4, R3, R5 and R6 according to their pathogenic potential.

Regarding the root fresh weight, all tested isolates reduced the root fresh weight comparing to the control (Table 2).The isolate R5 was the most effective one that significantly decreased the fresh weight of shoot and root (4 g and 2.48 g), respectively comparing to the rest of tested isolates. On parallel the isolate R5 caused the most significant reduction to root dry weight and shoot dry weight 0.37g and 1.42g, respectively followed by R2 and R7.

Effect of fertilization on the growth of Faba bean plants inoculated with *R. solani*:

Five fertilizers were used, four biofertilizer and one mineral fertilizer as a check treatment.

The major sources of plant nutrient are mineral fertilizers, organic manure, recycled wastes and by product, biological nitrogen fixation, and natural minerals and to a lesser extent nutrient recycled through irrigation water and precipitation (Boddey *et al.*, 1995). These supplement major plant nutrients and the plant productivity for sustainable agriculture. They are important and cost effective inputs in agriculture, plantation and commer-

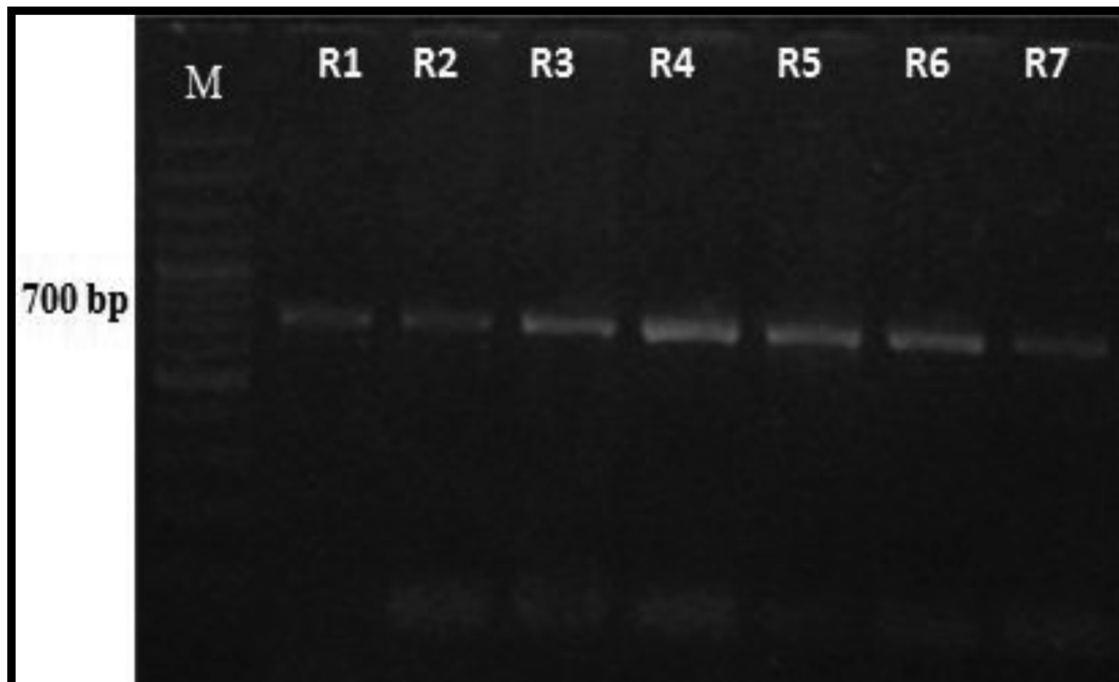

Fig. 1. PCR amplification of ITS1-5.8s-ITS2 region using ITS1 and ITS4 primers, R1 to R7 are *R. solani* isolates and M is a molecular marker

Table 2. Percent of pre, post-emergence and survival of faba bean plants inoculated with *R. solani* isolates

Isolates	Pre-emergence	post-emergence	Survival plants	Shoot system		Root system	
				FW	DW	FW	DW
CONTROL	0.25^{c}	0^{b}	4.75^{a}	7.15^{a}	0.77^{a}	5.93^{a}	2.95^{a}
R1	2.5^{ab}	0.75^{ab}	1.75^{c}	5.0^{bcd}	0.55^{abc}	3.75^{de}	2.16^{cd}
R2	2.25^{ab}	1^{a}	1.75^{c}	4.93^{cd}	0.47^{bc}	2.93^{ef}	1.69^{de}
R3	2.5^{ab}	0.25^{ab}	2.5^{bc}	6.08^{abc}	0.64^{ab}	5.35^{ab}	2.68^{abc}
R4	1.25^{bc}	0.5^{ab}	3.25^{abc}	5.83^{abc}	0.67^{ab}	4.88^{bc}	2.75^{ab}
R5	3.25^{a}	0.25^{ab}	1.5^{c}	4.0^{d}	0.37^{c}	2.48^{f}	1.42^{e}
R6	0.5^{c}	0.25^{ab}	4.25^{ab}	6.38^{ab}	0.66^{ab}	5.55^{ab}	2.49^{abc}
R7	2.25^{ab}	0.75^{ab}	2^{c}	5.5^{bc}	0.58^{abc}	4.18^{cd}	1.42^{e}

Values within column with the same letter are not significantly different according to the least significant difference test at $p<0.05$.

cial crops. Microbial inoculants/biofertilizers on their applications multiplies in soil rhizosphere and benefits the growing crops. If the soil conditions are favorable, the populations of the added microorganism are built up in the soil rhizosphere of plants and frequent application of microbial inoculants can be avoided. They are inexpensive and help reducing the consumption of chemical fertilizers. The cost of production of biofertilizer is low and so is the selling price (Fernando *et al.*, 2005)

Table (3) shows that, the shoot length of Faba bean plants significantly affected by treatments compared with the control "un treated plants" the increase ranged 17.1-25.7%. On parallel treatments significantly increased shoot length of inoculated plants by *R. solani,* and the highest was recorded with Biofertilizer (NC) (35%), Table (4).

Data in Table (3) indicate that shoot fresh weight of faba bean plants were increased with treatments of Biofertilizer (NC) and Rhizobacterin (30.4%) followed by Nitrobine (29.4%) and the lowest increase in HALEX® (1.5%). In case of the infection of *R. solani* the shoot fresh weight of bean plants were not affected with dif-

Table 3. Effect of fertilization on shoot system of non-inoculated faba bean plants

Treatment	Shoot system					
	Length (cm)	% of increase	Fresh weight (gm)	% of increase	Dry weight (gm)	% of increase
Control	29.25^{b}	0	4.83^{b}	0	0.55^{b}	0
HALEX®	35.5^{a}	21.4	4.9^{ab}	1.5	0.60^{ab}	9
Nitrobin	35.5^{a}	21.4	6.25^{a}	29.4	0.69^{a}	25.5
Rhizobacterin	36.0^{a}	23.1	6.3^{a}	30.4	0.64^{ab}	16.4
NPK	34.25^{a}	17.1	5.87^{ab}	21.5	0.59^{b}	7.3
Biofertilizer (NC)	36.75^{a}	25.7	6.3^{a}	30.4	0.64^{ab}	16.4

Values within column with the same letter are not significantly different at $p<0.05$.

Table 4. Effect of fertilization on growth of shoot system in inoculated faba bean plants with *R. solani* isolate (5)

Treatment	Shoot system of faba bean plants inoculated with *R. solani*					
	Length (cm)	% of increase	Fresh weight (gm)	% of increase	Dry weight (gm)	% of increase
Control	25.75^{ab}	0	3.67^{ab}	0	0.48^{ab}	0
HALEX®	31.5^{ab}	22.3	4.9^{ab}	33.5	0.60^{ab}	25
Nitrobin	32.25^{ab}	25.3	4.29^{ab}	16.9	0.89^{a}	85.4
Rhizobacterin	34.25^{a}	33	4.59^{ab}	25	0.54^{ab}	12.5
NPK	31.25^{ab}	21.4	5.87^{a}	60	0.72^{a}	50
Biofertilizer (NC)	34.75^{a}	35	6.3^{a}	71.7	0.86^{a}	79.2

Values within column with the same letter are not significantly different at $p<0.05$.

ferent treatments except with NPK and Biofertilizer (NC) which increased shoot fresh weight by (60%) and (71.7%), respectively compared with control (table 4).

Table (3) Showed that shoot dry weight of bean plants were not significantly affected with different treatments except with Nitrobine that increased shoot dry by 25.5%. In the case of the infection by *R. solani* the shoot dry weight of bean plants were affected significantly with treatments like Nitrobine 85.4%, Biofertilizer (NC) 79.2% and NPK 50% increase in root dry weight by compared with the control (Table 4).

Data in Table (5) Showed that root length of bean plants were not significantly affected with different treatments except with Biofertilizer (NC), which increased root length by 54.2% compared with the control and another treatment. On parallel in case of the infection of *R. solani* the root length of bean plants were not affected with different treatments except with Biofertilizer (NC) that increased the length by 62.5% compared with the control (Table 6).

Data in Table (5) illustrate that Biofertilizer (NC) increased the root fresh weight of faba bean plants by 40.5% while the increase with other treatments ranged 10.3-33.7%. In case of the infection of *R. solani* root fresh weight of bean plants significantly increased with treatments Nitrobine and Biofertilizer (NC), by 70% and 65.2%, respectively (Table 6).

According to the data in Table (5) the root dry weight of faba bean plants did not significantly affected by treatments and there were insignificant increase ranged from 1.3%-20.8% with HALEX® and Nitrobine, respectively. On the other hand, treatments resulted in increase in root dry weight of inoculated plants evaluated by (108.5%) with Nitrobine followed by Biofertilizer (NC) (88.3%) when the other treatments effect ranged (32.3%- 34.5%) (Table 6).

Obtained data revealed that treatments increased shoot and root length, fresh weight and dry weight while the increase ranged 17.1-25.7%, 1.5-30.4, 7.3-25.5% and 8.3-54.2, 10.3-40.5, 1.3-20.8%of both shoot and root system, respectively. Similarly, in case of inoculated plants the previously mentioned growth parameters were increased as result of treatment. The general improvements of lengths, fresh and dry weights of plants could be attributed to one or more of plant growth promoting mode of action caused by the biofertlizers such as N_2-fixation, which facilitates N_2 to the plant (Hassouna, 1962), hormonal effects which alter plant metabolism and growth and general improvement in nutrients and water (Glick, 1995).

Biological control treatments:

Management of faba bean diseases can be achieved through chemical, agricultural and biochemical control also fertilization has great effect on disease invasion,

Table 5. Effect of fertilization on growth of root system in non-inoculated faba bean plants

Treatment	Root system					
	Length (cm)	% of increase	Fresh weight (gm)	% of increase	Dry weight (gm)	% of increase
Control	18[bc]	0.0	7.27[b]	0.0	3.85[a]	0.0
HALEX®	21[b]	16.7	8.07[ab]	11.0	3.9[a]	1.3
Nitrobin	22[b]	22.2	9.72[ab]	33.7	4.65[a]	20.8
Rhizobacterin	20.5[b]	13.9	8.02[ab]	10.3	3.99[a]	3.6
NPK	19.5[b]	8.3	8.85[ab]	21.7	3.95[a]	2.6
Biofertilizer (NC)	27.75[a]	54.2	10.22[a]	40.5	4.20[a]	9.0

Values within column with the same letter are not significantly different at $p<0.05$.

Table (6) Effect of fertilization on growth of root system in inoculated faba bean plants with *R. solani* isolate (5)

Treatment	Root system inoculated with *R.solani*					
	Length (cm)	% of increase	Fresh weight (gm)	% of increase	Dry weight (gm)	% of increase
Control	14[b]	0.0	4.54[ab]	0.0	2.23[ab]	0.0
HALEX®	17[b]	21.4	5.12[a]	12.8	3.1[ab]	39
Nitrobin	15.25[b]	8.9	7.72[a]	70.0	4.65[a]	108.5
Rhizobacterin	14.5[b]	3.5	5.02[a]	10.6	3.0[ab]	34.5
NPK	16.75[b]	19.6	5.09[ab]	12.1	2.95[ab]	32.3
Biofertilizer (NC)	22.75[a]	62.5	7.5[a]	65.2	4.20[a]	88.3

Values within column with the same letter are not significantly different at $p<0.05$.

Rhizoctonia solani control is extremely difficult because it lives in the soil and combines high saprophytic competitiveness with a wide host range (Ogoshi, 1987; Cubeta and Vilgalys, 2000). To prevent the disease, the faba bean producers abandon the infected areas and migrate to uninfected fields, causing great economic losses because of the devaluation of the abandoned areas. Biological control is one of greatest methods that reduce inoculum level of plant pathogens (Papavizas and Lumasden 1980) which can be reached by introducing microorganisms that have antagonistic effect on plant pathogens. Instead of chemicals and fumigants, bioagents are widely used in plant disease management (Chet and Inbar, 1994 and Harman and Kubicek, 1998).

Suppressive effect of different types of bioagents (*Bacillus subtilis*, *Pseudomonas fluorescence* and *Trichoderma harzianum*) (Table 7 and Fig. 2) have been evaluated on the tested isolates of *R. solani.*The antagonistic effect of *B. subtilis* and *P. fluorescence* on tested isolates of *R. solani* (R1, R2, R3, R4, R5, R6, and R7) (Fig. 2) was recorded by measuring the radial growth of *R. solani* isolates treated with these bioagents. Data in Table (7) and Fig. (2) showed that *Bacillus subtilis* had the highest suppressive effect on radial growth of *R. solani* isolates (R3) as 1.4 cm, followed by its effect on *R. solani* (R1) and (R2) as 1.42 cm. while it caused little suppression effect (2.6 cm) to isolate R7. While *P. fluoresces* was the highest suppression effect on fungal growth of *R. solani* isolate (R4) as 1.27 cm followed by its effect on *R. solani* isolate (R3) as 1.6 cm and isolate R2as 1.72 cm while, it caused a little suppression effect 2.37 cm to isolate R5.

Interaction between *T. harzianum* and tested isolates of *R. solani* revealed meaningful suppression in growth rate of *R. solani* isolates. Data in Table (7) and fig. 3 Showed that *T. harzianum* isolate reduced the growth of all the tested isolates of *R. solani*. The highest suppressive effect of *T. harzianum* on growth of *R. solani* isolates R3 and R7 was 2.0 cm 2.27 cm, respectively, while little suppressive effect of 2.9 cm was caused to isolate R5.

Disease incidence could be reduced using *T. harzianum* as a biocontrol agent, so using commercial formula of *T. harzianum* reduced disease incidence in eggplants from 13% to 40% (Hadar *et al.*, 1979). Elad *et al.,* (1980) found that the use of *T. harzianum*in combination with lethal heat temperature reduced disease incidence in bean up to 99-100%. Data in this study showed that *T. harzianum* have great suppressive effect on growth of *R. solani* isolates whereas the radial growth of tested was significantly poor so it ranged between 2 cm (R3) to 2.9 cm (R5). *T. harzianum* actson plant pathogens through parasitism, lysis pathogens, competition and induction of plant defense mechanisms (Chet 1990).

It has been reported that *B. subtilis* has great effect on suppression or reduction of plant disease and also increase yield (Cambell *et al.*, 1989), through secretion of several types of antibiotics, antifungal peptides, lipopep-

Table7. Suppressive effect of *B. subtilis*, *P. fluorescens* and *Trichoderma* on tested isolates of *R. solani*

Treatment	Radial growth (cm) of R. solaniisolates						
	R1	R2	R3	R4	R5	R6	R7
Control	8.95a	9.0a	8.95a	8.95a	8.92a	8.95a	8.92a
B. subtilis	1.42d	1.42d	1.4d	1.45c	1.7d	1.9c	2.6b
P. fluorescens	2.12c	1.72c	1.6c	1.27d	2.37c	1.97c	2.0d
T.harzianum	2.4b	2.6b	2.0b	2.3b	2.9b	2.5b	2.27c

Values within column with the same letter are not significantly different at $p<0.05$.

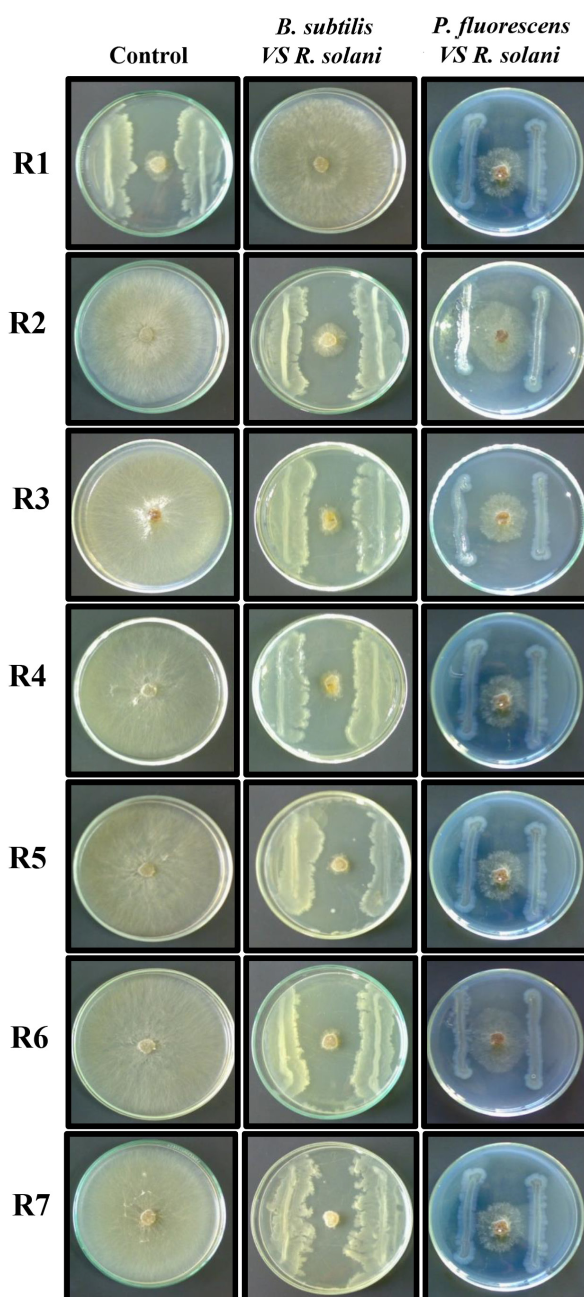

Fig. (2): Suppressive effect of *B. Subtilis* and *P. fluoresce*son *R. solani*isolates

tides and amino glycosides,*B. subtilis* can inhibit pathogenic fungi and bacteria as it was reported by Mcknight and Rossall, (1991) and Rossall and Mackinight, (1990) that strain MBI-600 of *B. subtilis* has antifungal activity against seedling disease of cotton (*R. solani*) and also increase nodulation in peanut. Furthermore *B. subtilis* had antifungal activity against *R. solani* on cotton seedlings and also increased nodulation in Pea nut. Our results showed that *B. Subtilis* caused great suppressive effect on tested isolates of *R. solani* whereas isolate R4 found to be greatly affected by *B. subtilis* followed by R1, R2, R3, R6 and R7 but isolates R5 weakly affected by *B. subtilis.* These finds were in agreement with results found by Jenesen *et al.,* (2002) who's indicated that root rot of bean has been reduced using *B. subtilis* and *T. harzianum* together or separately also yield has been increased up to 31 % compared to cheek. .

Pseudomonas fluorescence is an important growth promoting rhizobacteria (PGPR) that promote plant growth and it is used as a biocontrol agent and also used in bioremediation. *P. fluorescence* is effective bioagent against wide range of plant pathogens such as *R. solani* and *Pythium ultimum* via producing antagonistic compound (Schippers*et al.*,1987; Weller, 1988 and Paulitz, 1991).Weller, (1988) and leeman*et al*., (1996) and induction of callose formation in treated plants (M'Piga*et al*., 1997), in this study *P. fluorescence* has less effect on obtained isolates (R1, R2, R3, R4, R5 and R6 of *R. solani* than isolate R7, supporting data that were mentioned by Howell and Stipanovic, (1979) who reported that *P. florescence* had suppressive effects on *R. solani.*

Fig. (2) showed that *Bacillus subtilis* had the highest suppressive effect on radial growth of *R. solani* isolates (R3) as 1.4 cm, followed by its effect on *R. solani* (R1) and (R2) as 1.42 cm. while it caused little suppression effect (2.6 cm) to isolate R7. While *P. fluoresces* was the highest suppression effect on fungal growth of *R. solani* isolate (R4) as 1.27 cm followed by its effect on *R. solani* isolate (R3) as 1.6 cm and isolate R2as 1.72 cm while, it caused a little suppression effect 2.37 cm to isolate R5.

Fig. 3 showed that *T. harzianum* isolate reduced the growth of all the tested isolates of *R. solani*. The highest suppressive effect of *T. harzianum* on growth of *R. solani* isolates R3 and R7 was 2.0 cm 2.27 cm, respectively, while little suppressive effect of 2.9 cm was caused to isolate R5.

Generally the current study showed that biofertilizers play a vital role in crop productivity and they act as antagonists in addition to suppressing the incidence of soil borne plant pathogens (*Rhizoctonia sp.*) and thus help in the biocontrol of diseases. On the other hand mineral fertilizer support plant growth and the pathogenic fungi as well.

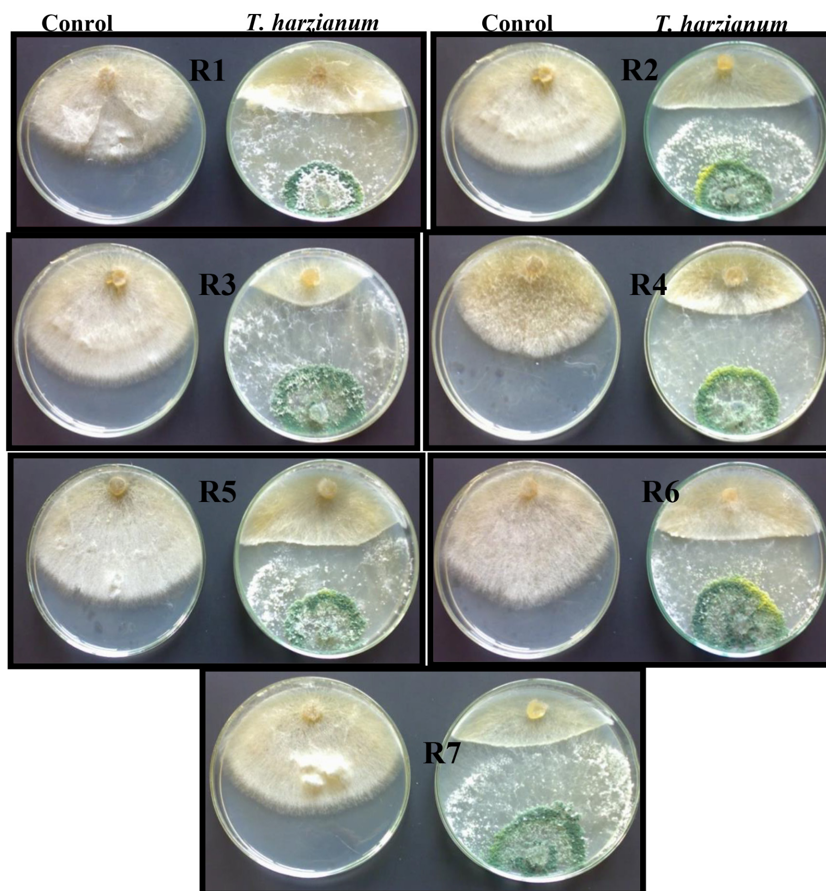

Fig.3: Suppressive effect of *T. harzianum* on growth of *R. solani* isolates

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اجريت هذه الدراسة على نبات الفول البلدى وفطر *Rhizoctoni solani* (Küh) بهدف التعرف على دور الأسمدة فى تغذية النبات والحد من المرض .تم إجراء عملية العزل من نباتات فول مصابة يظهر عليها أعراض تقرحات فى منطقة التاج والجذور على نباتات وبادرات الفول وذلك من عينات جمعت من محافظتى الاسكندرية والبحيرة. تم تعريف العزلات بناء على التعريف المظهرى والميكروسكوبى ثم التعرف عليها علي المستوي الجزيئي وذلك عن طريق عزل الحمض النووي واستخدم زوج من البوادئ المتخصصة لمناطق الـ internal transcribed spacer (ITS) والبوادئ هي (ITS1, ITS4) لمضاعفة مناطقة ITS1-ITS2 من جينات rRNA (rDNA) باستخدام تفاعل الـ Polymerase chain Reaction (PCR), أوضحت نتيجة التعريف أن العزلات تنتمي جميعها للفطر *R. solani*. اظهر اختبار القدرة الإمراضية للعزلات المختبرة وذلك باستخدام بذور الفول البلدى صنف جيزة ٣ اختلافات معنوية في قدرتها الإمراضية، بحيث كانت العزلة (R5) أشدهم قدرة امراضية. استخدم ٤ أنواع من الاسمدة الحيوية وهى كالتالى هاليكس والنيتروبين والريزوبكتيرين سماد حيوى مختبر وذلك عن طريق معاملة البذوروكذلك استخدام السماد المعدنى NPK (انتاج شركة ابو قير للاسمدة) فى صورة اضافة لماء الرى وذلك كمقارن مع الاسمدة الحيوية. وطبق ذلك على النباتات الملقحة والغير ملقحة بالفطر ريزوكتونيا سولانى العزلة رقم (٥) ذات القدرة الامراضية العالية حيث ان السماد الحيوى المختبر ادى الى الزيادة فى طول المجموع الخضرى والجذرى بمعدل زيادة ٣٥ و٥٤٪ على التوالى مقارنة بالكنترول يلية السماد الحيوى نيتروبين بنسبة زيادة ٢٢٪ مقارنة بالكنترول فى حين ان السماد المعدنى كان اقل تاثيرا على معدل طول المجموع الجذرى بنسبة زيادة ٨٪. اظهرت التجارب المعملية لدراسة مدى حساسية فطر الريزوكتونيا سولانى لبعض انواع مختلفة من الكائنات الحية الدقيقة.

بكتيريا *Bacillus Subtilis* و*Pseudomonas fluresencs*. اوضحت النتائج ان البكتيريا *B.Subtilis* أفضل من البكتيريا *P. fluresenc* حيث اظهرت الاولى تاثيرا قويا على العزلات رقم ٤ يليها العزلات ١ و٢ و٣ و٦ و٧ كانت اقل تأثيراً على العزلة رقم (٥) فى حين ان الثانية كانت اقل تاثيرا على العزلات رقم ١ و٢ و٣ و٤ و٥ و٦ وكانت قوية التأثير على العزلة رقم (٧).

كذلك استخدام الفطر *Trichoderma harzianum* للمقاومة الحيوية للفطر *R. solani* واوضحت النتائج ان له تاثيراً قويا على العزلة رقم (٥) وكان متوسط التأثير على كل من العزلات ١و٢و٣و٦و٧ وكانت اقلهم تأثرا العزلة رقم (٤). من ذلك اتضح أن السماد الحيوى ذو تأثير على زيادة معدل النمو للنبات كمقارنة بدور التسميد المعدنى الذى يمد كلا من النبات والفطر من العناصر الغذائية.

The Role of Plant Growth Promoting Rhizobacteria in Plant Defense Against Potato Stem Canker and Black Scurf Disease

Sayed S. Aboshosha*, Mohamed A. El-Saedy, Ahmed F. El-Bebany, Ahmed G. Gad and Mohamed G. Hassouna

Department of Plant Pathology, Faculty of Agriculture, Alexandria University, El-Shatby 21545, Alexandria, Egypt

ABSTRACT

Rhizoctonia solani is the causal agent of stem canker and black scurf disease of potato. *R. solani* was isolated from several areas in Alexandaria and El-Behera governrates. Pathogenicity of *R. solani* isolates were tested on two potato cultivars Spunta and Draga. The pathogenicity was evaluated on the whole seedlings and leaflets of potato plants. The pathogenic *R. solani* isolate SH gave 81.4% infection area on potato leaflets and 9.67 cm root lesion length on potato plants. The antagonistic effect of plant growth promoting rhizobacteria (PGPR) *Pseudomonas fluorescence* (isolates Pf1 and Pf2) was assessed against *R. solani* isolates. The ability of the two PGPR to produce hydrolytic enzymes was tested. Chitinase and β-1, 3- D glucanse increased gradually to the maximum at 36 hours after inoculation. Chitinase activity ranged between 26.4-73.2 and 28- 68.4 nM N-aceylglucose amine/ ml/ min with Pf2 and Pf1, respectively. β3-,١-D glucanase activity ranged between 5.6-20.3 and 9.6-16 µg glucose/min/ml with Pf2 and Pf1, respectively. Phenolic compounds were significantly accumulated in *R. solani* inoculated plants, 6 hr after inoculation. Treatment of the inoculated plants with Pf1, Pf2, chitosan and HALEX® accumulated phenolics at the same time as well. The increase was ranged from 24-52.2% as compare to check plants. The activity of peroxidase, polyphenoloxidase, chitinase, and β, 1-3, D- glucanase enzymes was determined in root system of potato cultivar Spunta inoculated with *R. solani* and treated with PGPR and chitosan. Peroxidase activity significantly increased by 26.9-51% after the 6th hr of application. Activity of polyphenoloxidase was increased by all treatments. The highest level of enzyme activity was determined at 12 hr after Pf1 treatment. Chitinase activity was increased 400-440% with Pf1 and Pf2, respectively at 6th hr after treatment as compared to non-treated plants. The activity of β, 1-3, D- glucanase was increased up to 980% in the Pf1- treated plants 6 hr after application in compare with the check treatment.

Key words: PGPR, potato, Rhizoctonia solani, and defense mechanism.

INTRODUCTION

One of the challenges of globalization to developing countries is increasing agricultural production and improving the quality of the products. Biofertilizer is defined as a substance that contains living microorganisms, when applied to seeds, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant parts and promotes growth by increasing the supply or availability of nutrients to plants. There are several benefits of using biofertilizer such as increasing germination percentages, yield, quality and reducing the application of inorganic fertilizers and pesticides (Vessey, 2003).

Plant growth promoting rhizobacteria (PGPR) are bacteria that colonize plant roots and in doing so, they promote plant growth and reduce diseases. PGPR are found in a very wide range of genera e.g. *Acinetobacter, Agrobacterium, Arthrobacter, Azospirillium, Bacillus, Bradyrhizobium, Burkholderia, Cellulomonas, Frankia, Pantoea, Pseudomonas, Rhizobium, Serratia, Streptomyces* and *Thiobacillus* (Somers *et al*., 2004). One of the most common ways of using these PGPR is improving plant nutrient uptake by altering plant hormone levels (Arshad and Frankenberger, 1993). In addition, promoting free-living nitrogen-fixing bacteria, which increasing supply of other nutrients such as phosphorus, sulphur, iron and copper, producing plant hormones and enhancing the growth of other beneficial bacteria or fungi (Vega, 2007). PGPR have attracted much attention in their role in controlling fungal or bacterial plant diseases.

The fungus *Rhizoctonia solani* is the causal agent of black scurf of potato disease. This disease causes severe economic impacts on potato production by reducing tuber yield and quality (Brewer and Larkin, 2005 and Hafeez *et al*., 2010). In Egypt, R. solani was recorded in several governorates, particulary Behera, Dakahlia, Gharbia, Menofia and Sharkia (Abd-Elsattar *et al*., 2009).

MATERIALS AND METHODS

Plant materials

Two commercial potato cultivars namely; Spunta and Draga were obtained from Sonac Company for Importing and Exporting Agriculture Crops, El-Behera governorate.

The fungal isolates

Potato plants showing symptoms of stem canker or black spots as well as diseased tubers were collected from different commercial fields in major potato growing regions (Nubaria, Kafr eldawar and Elhadra Public Market). Pieces of plant tissues were taken from the margins of lesions appeared on stems or stolons or from sclerotia appeared on tuber were surface sterilized with 1% sodium hypochlorite for 1-3 min and plated on potato dextrose agar (PDA) containing streptomycin sulfate (50 mg/L). Isolation procedures and media preparation were done as

***correspondence author:** Dr. Sayed S. Aboshosh
Tel. 01227434663 - Email: sayed.aboshosha@yahoo.com

described by Tuite (1969). Hyphal tipped isolates tentatively identified as *Rhizoctonia solani* were transferred to potato dextrose agar slants and stored at 20ºC.

Chitosan extraction

Chitosan was extracted from crustacean shells specimens which were collected from the waste of a local sea food restaurant. Shrimp shells were boiled for 12 hr in order to remove soluble organics and binding protein, then dried at 80ºC for 24 hr. The dried shells were stored at room temperature (±25ºC). The next flow scheme (Fig. 1) shows the main steps of chitosan extraction according to No and Meyers (2000).

The biofertilizer

HALEX® is a commercial biofertilizer biocontroler product produced by Alssouna Company, El-Agamy, Alexandaria.

Pseudomonas fluorescence strains:

Two Egyptian strains of *P.fluorescence,* (Pf1 and Pf2) were kindly provided by Prof. Dr. Farag A. Saeed, Plant Pathology Department, Faculty of Agriculture, Assuit University, Egypt.

Pathogenicity tests

The pathogenic abilties of the *R. solani* isolates were investigated under greenhouse conditions as follow: A total of 40 plastic pots, 25 cm in diameter, filled with autoclaved sandy clay soil (1:1 v/v), and aerated for one week. Fungal inoculum was prepared by culturing fungal isolates on sterilized barely grains kept in 500 mLconical flasks, and incubated at room temperature (25 ˚C) for 2 weeks. The soil was infested with *R. solani* at the rate of 10 g infested seeds/kg soil and left one week for the inoculum establishment. Pots were watered daily for a week before planting. Two tuber pieces of each tested cultivar were planted in each pot. Four replicates were used for each treatment. Pots were irrigated after planting as needed. Four weeks after planting, the lesion length in cm was recorded.

Pathogenicity on detached leaflets

The four obtained isolates of *R. solani* were grown on tap water agar plates for three days at 22 ˚C. Potato leaflets were soaked in tap water for 6 hr, rinsed in sterile water and placed singly with the lower epidermis facing the culture of each isolate, then incubated for a further three days on the bench at about 16 ˚C in diffuse day

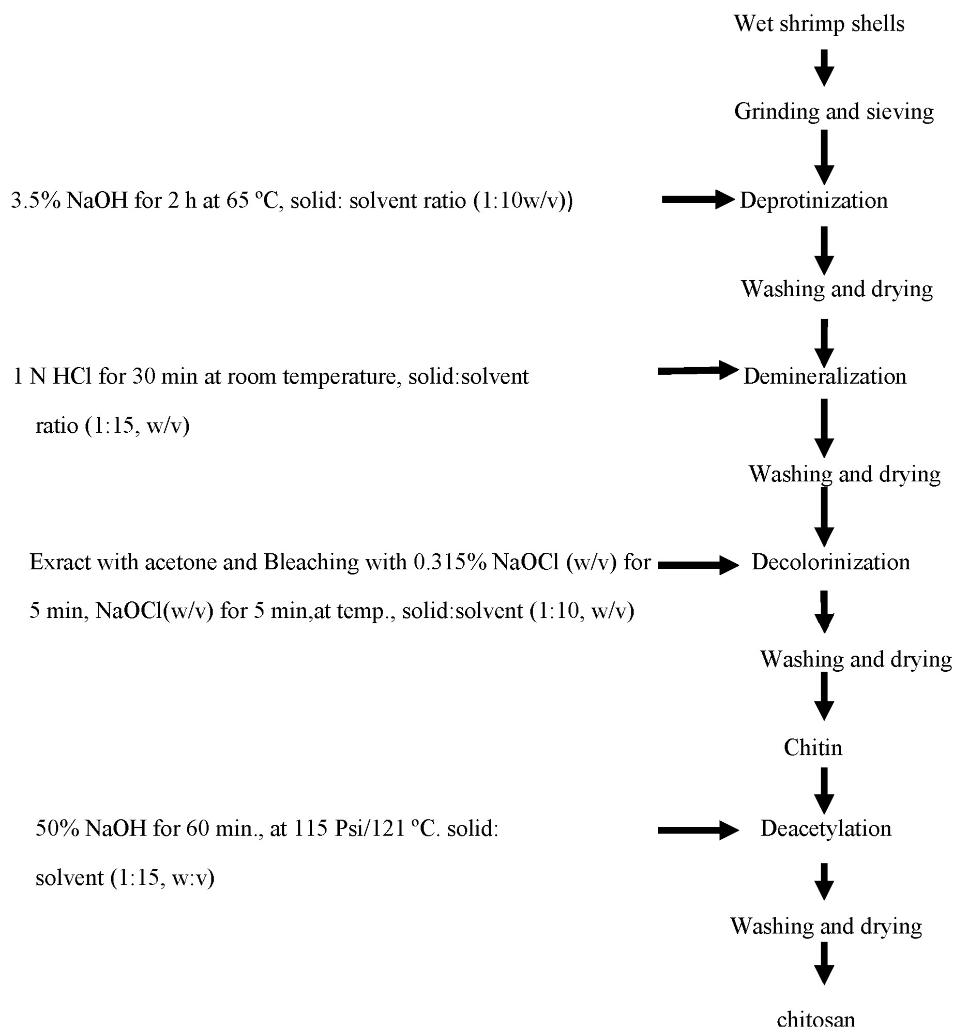

Fig. 1. Flow scheme of chitosan extraction

light. The virulence of each isolate was expressed as the percentage of discolored (invaded) leaflet tissue. The discoloration was measured using the computer software, "Leaf area measurement", version 1.3, England, Sheffield University (Spencer *et al*., 1978).

Antagonistic study

Potato dextrose agar (PDA) medium was poured into sterilized Petri dishes, 9 cm in diameter. The antagonistic effect of *Pseudomonas fluorescence* strains Pf1 and Pf2 was tested against the growth of *R. solani*. Two straight lines of 5 cm long and 3 cm apart of the investigated bacterial suspension were streaked with a loopfull needle. A disc of 0.5 cm in diameter of each tested isolate of *R. solani* was placed in a central position between the two lines, 1.5 cm apart from the streaks. Five plates were prepared for each treatment. The plates were incubated at 25 °C for 7 days. The examination of the fungal growth started after 24 hr and was followed up daily for 7 days. Inhibition zones of fungal growth were measured (Reddy and Patrick, 1990 and Wei and Tuzum, 1990).

Lytic enzymes production

Culture media

The two isolates of *P. fluorescence* were separately grown on the following medium described by Okon *et al* (1973) (g/L): $MgSO_4 .7H_2O$, 0.2 g/L; K_2HPO_4 , 0.9 g/L ; KCl , 0.2 g/L; NH_4NO_3 , 1.0 g/L; $FeSO_4.7H_2O$, 0.002 g/L; $MnSO_4$. H_2O, 0.002 g/L and $ZnSO_4.7H_2O$, 0.002 g/L (pH 6.5). The medium was supplemented with *R. solani* mycelium at the concentration of 1.0 % to induce production of enzymes and dispensed in 250 mL Erlenmeyer flasks. Each flask contained 50 ml of the medium. The flasks were autoclaved and each flask was inoculated with 1.0 mL of a precultured, *P. fluorescence*. The cultures were incubated on a shaker (180 rpm) at 28±2°C. Three flasks from each culture were analyzed daily for 5 days (Saad, 2006).

Chitinase activity assay in culture filtrate

One mL of 1 % colloidal chitin in 0.1 M citrate phosphate buffer (pH 6.5) was incubated with 1.0 mLof culture filtrate of Pf1 and Pf2 at 30 °C for 2 hr. Activity determined according to the methods of Moneral and Reese (1969) and was expressed as µM of N-acetylglucosamine units.

β -1, 3-glucanase activity assay in culture filtrate

β-1,3-glucanase activity was assayed by incubating 1.0 mL of 0.5% (w/v) laminarin in 0.1 M sodium phosphate buffer (pH 4.0) with 1.0 ml of culture filtrate of Pf1 or Pf2 at 55°C for 5 min . The reaction was stopped in an ice bath then 3 mL of 3,5 dinitrosalicylic acid (DNS) reagent was added and the mixture was heated in a boiling water-bath for 12 min. As a control, 1.0 mLof laminarin solution was incubated and cooled, then 1mLof enzyme solution together with 3 ml of DNS reagent were added to correct the reducing sugars in the substrate and the enzyme solution. Reducing sugar equivalents were measured in both the original and the control solutions by the colorimetric method (Miller, 1959) using glucose as standard at 450 nm (spectrophotometer 20).

Treatments

1- HALEX® used as seed dressing at the rate of 10 g/kg seed. Treatment repeated at the middle of growing seasons, 7 weeks later (Hassouna, *et al*., 1998).

2- *P. fluorescence* isolates were used as suspensions at a concentration of 2 ×10^8 cfu/mL at the rate of 10 mL/ kg soil.

3- Chitosan was used as a seed dressing for potato tuber piece pre-planting and as a foliar spray at the rate of 2 mL/l of water after emergence

4- Control, untreated plants.

Determination of plant defence reactions induced by treatments

Phenol content

Fresh root sample of 1 g was homogenized in 10 mL of 80% methanol and agitated for 15 min at 70 °C. One mL of the methanolic extract was added to 5 mL of distilled water and 250 μL of Folin-diens reagent (1N) and the solution was kept at 25 °C. The absorbance of the developed blue color was measured using a Spectrophotometer 20 at 725 nm. Catechol was used as a standard. The amount of phenolics was expressed as mg catechol/g sample (Zieslin and Ben–Zaken, 1993).

Assay of peroxidase

Fresh root sample of 1 g was homogenized in 2 mLof 0.1 M phosphate buffer, pH 7.0 at 4 °C. The homogenate was centrifuged at 16000 *g* at 4 °C for 15 min and the enzyme was measured in the supernatant. The reaction mixture consisted of 1.5 mL of 0.05 *m* pyrogallol, 0.5 mL of enzyme extract and 0.5 mL of 1% H_2O_2. The reaction mixture was incubated at room temperature (282°±C). The changes in absorbance at 420 nm were recorded at 30 s intervals for 3 min. The enzyme activity was expressed as changes in the absorbance unit/min (Hammerschmidt *et al*., 1982).

Assay of polyphenol oxidase

Polyphenoloxidase activity was determined as described by Mayer *et al*., (1965). Fresh root sample of 1 g was homogenized in 2 mLof 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 16 000 *g* for 15 min at 4 °C. The enzyme was measured in the supernatant. The reaction mixture consisted of 200 μL of the enzyme extract and 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction, 200 µL of 0.01M catechol was added and the activity was expressed as changes in absorbance at 495 nm.

Assay of β-1,3-D-glucanase

β-1,3-glucanase activity was assayed by the laminarin - dinitrosalicylic acid method (Pan *et al*., 1991). Root samples (1 g) were extracted with 2 mL of 0.05 M

sodium acetate buffer (pH 5.0) and centrifuged at 16000 *g* for 15 min at 4°C. The supernatant was used in the enzyme assay. The reaction mixture consisted of 62.5 µL of 4% laminarin and 62.5 µL of enzyme extract. The reaction was carried out at 40°C for 10 min. The reaction was then stopped by adding 375 µL of dinitrosalicylic acid and heated for 5 min on boiling water, vortexed and its absorbance was measured at 500 nm. The enzyme activity was expressed as µg glucose released/ mL/min.

Assay of chitinase:

Fresh root sample of 1 g was homogenized in 2 mL of 0.1 M sodium citrate buffer (pH5.0). The homogenate was centrifuged at 16 000 *g* for 15 min at 4°C the supernatant was used in the enzyme assay. The colorimetric assay of chitinase was carried out as the method described by Monreal and Reese (1969). One mL of 1% colloidal chitin in 0.1 M citrate phosphate buffer (pH 6.5) was incubated with 1.0 mL of enzyme extract at 37°C for 2 hr. Chitinase ctivity was expressed as µM of N-acetyl glucose amine /mL/min.

EXPERIMENTAL RESULTS

Fungal isolates

Cultural and microscopic characteristics of the collected isolates (hyphal characteristics, colony color, growth pattern and location of sclerotia) revealed clear diversity .

Table 1. *Rhizoctonia solani* isolates and locations

R. solani isolate	Location and Governrates
SH	El-Nagah region - Elbehera
R	Kafr Eldawar- Elbehera
M	Nubaria- Elbehera
S	Alexandria Market- Alexandria

Pathogenicity test

The soil-borne fungus *Rhizoctonia solani* causes Rhizoctonia disease of potato. This disease causes severe economic impacts on potato production due to reduction in both tuber yield and quality. *Rhizoctonia solani* has a wide host range, and is found everywhere potatoes are grown (Carling and Sumner, 1992). Symptoms of Rhizoctonia disease of potato include necrosis and girdling of young sprouts, stolon lesions, aerial tubers, sclerotia on the low tuber.

Isolates SH, R, M and S of *Rhizoctonia solani* were tested for their pathogenic potentialities on two potato cultivars (Spunta and Draga). The pathogenicity of *R. solani* was examined on the whole seedlings and leaflets of potato plants. Data in Table (2) showed that all tested *R. solani* isolates R, SH, and M were pathogenic. The highest infection area percentages (81.4 and 64.3%) on potato leaflets were recorded with isolate SH in case of Spunta and Draga cultivars, respectively. Also, *R. solani* isolate R caused 77.2% infection area on potato leaflets cultivar Spunta. While the moderate infection area

Table 2. Pathogenicity test of *Rhizoctonia solani* isolates on leaflets of Spunta and Draga potato cultivars

R. solani isolate code	Potat cultivar	
	Spunta	Draga
SH	81.40[*a]	64.29[a]
R	77.20[a]	57.66[a]
M	24.10[b]	24.10[b]
S	11.29[c]	9.95[c]

Data are means of 4 replicates.
Values, within each column, followed by the same letter(s) are not significantly different at P=0.05.
* % of infected areas in each leaflet.

percentage (57.66%) was recorded with isolate R of *R. solani* n Draga. Infection area percentage was 24.1% with *R. solani* isolate M in both potato cultivars (Table 2). On the other hand, *R. solani* isolate S had the lowest infection area percentage; (11.3 and 9.95%); on potato leaflets of both cultivars, Spunta and Draga, respectively. In the present study the four obtained isalates of *R. solani* were found pathogenic and produced the typical symptoms on the two tested cultivars. Spunta cultivar was more susceptible than Draga. The *R. solan*i isolate SH scored the highest lesions, lengths on the tested cultivars. The isolate SH was more severe than other tested isolates which followed by R, M and S isolates. Black scurf leads to a decrease in tuber quality and is serious problem for seed certification as the tuber-borne sclerotia could be a source of infection (Frank and Leach, 1980). Some studies have mentioned that Rhizoctonia potato disease may cause marketable yield losses up to 30% (Banville, 1989). Our results indicated that Rhizoctonia symptoms on the inoculated plants as they were observed previously on samples from fields and markets.Similar results were obtained with measuring to root lesion length. The isolate SH of *R. solani* had the highest root lesion length (9.67 and 6.17 cm) on both potato cultivars, Spunta and Draga, respectively then isolate R, which showed lesion length of 4.8 and 3.7 cm, respectively followed by the isolate M with root lesion length of 2.83 and 2.16 cm in Spunta and Draga, respectively. The lowest root lesion lengths (1.8 and 0.8 cm) were noticed with isolate S in Spunta and Draga, respectively (Table 3). These results are in harmony with those mentioned above and ensure the efficiency of these isolates to make infection.

Antagonistic effect of Plant Growth Promoting Rhizobacteria (PGPR) on *R. solani*

The use of biofertilizers and sometimes natural products to control diseases offer an alternative or supplement to pesticides for the management of plant diseases. Several bacterial genera with biocontrol activity have been identified, and meny of them proved efficiency in field application (Fernando *et al*., 2005). In the present work, the effect of *P. fluorescence* Pf1, Pf2, commercial product HALEX® and chitosan were evaluated against *R. solani* inoculated potato plants.

Table 3. Root lesions Lengths on the root of potato cultivars Spunta and Draga inoculated with *Rhizoctonia solani* isolates

R. solani isolate code	Potato cultivar	
	Spunta	Draga
SH	9.67*a	6.17 a
R	4.80 b	3.70 b
M	2.83bc	2.16 bc
S	1.80 c	0.80 c

Data are means of 4 replicates.
Values within each column followed by the same letter(s) are not significantly different at P=0.05.
*Root lesion length (cm).

The antagonistic effect of *P. fluorescence* strains Pf1 and Pf2 were investigated against the phytopathogenic fungus, *R. solani.* Data of Table (4) showed that plates streaked with Pf1 and Pf2 gave the highest suppressive effect (+++) on the fungal growth of *R. solani* isolates S, R, and M, which showed inhibition zone ranged 6-9 mm, except Pf1 with isolate M, which showed inhibition zone of 3 mm (moderate inhibition). Pf1 and Pf2 showed a moderate effect (++) on the fungus growth of *R. solani* isolate SH as they scored 4 mm. Data in Table (5) indicate the ability of Pf1 and Pf2 to produce hydrolytic enzymes chitinase and β-1, 3- D glucanse. The lytic enzymes production increased gradualy with time increase untill it was maximum on the 36th hr of inoculation. Chitinase activity ranged 26.4-73.2 and 28- 68.4 nM N-aceylglucose amine/ mL/min with Pf2 and Pf1, respectively. β3-,١-D glucanse activity ranged 5.6-20.3 and 9.6-16 µg glucose/min/mL with Pf2 and Pf1, respectively. The suppressions noticed by Pf1 and Pf2 could be a result of producing lytic enzyme like chitinase, which was determined in the culture filtrate of both Pf1and Pf2 bacterial strains as 68.4 and 73.2 nM N-aceylglucose amine/ mL/min after the 36th hr of inoculation, respectively or due to β-1,3-D glucanase activity which was 16 and 20.3 µg glucose/min/mL or protease and lipase that spread through the media (Saad, 2006).

Table 4. Antagonistic effect of plant growth promoting rhizobacteria (PGPR) on isolates of Rhizoctonia solani grown on PDA medium

Treatments	*R. solani* isolates			
	SH	R	M	S
P. fluorescence 1	++	+++	++	+++
(Pf1)	(4)	(7)	3)(	(9)
P. flourescence 2	++	+++	+++	+++
(Pf2)	(4)	(6)	(6)	(8)

- = No Suppression (0.0 mm)., + = Slight suppression (1-2 mm), ++ = Moderate suppression (3-4 mm), +++ = High suppression (5-10 mm).Values bettwen brackets are the inhibation zone in mm.

Table 5. Chitinase (nM N-aceylglucose amine/ mL/ min) and β,1-3 D- Glucanse (µg glucose/ min/mL(activity in the bacterial culture filtrate of P. fluorescence Pf1 and Pf2 at 12, 24 and 36 hr from inoculation

Bacterial strains	Chitinase activity			β,1-3 D- glucanse activity		
	12 hr	24 hr	36 hr	12 hr	24 hr	36 hr
Pf1	28.79	38.9	68.4	9.6	13.3	16
Pf2	26.4	31.19	73.2	5.6	8.2	20.3

Defense induction assay:

Phenolics:

Plants have endogenous defense mechanisms that can be induced in response to attack by insects and pathogens. It is well known that the defense genes are inducible by appropriate stimuli or signals (Van Loon, 1997). Using of fluorescent pseudomonads has been well documented (Weller and Cook, 1986; Paulitz and Loper, 1991). In the current study, *P. fluorescence* isolates Pf1, Pf2, HALEX® and chitosan were used as strong elicitors of plant defense reactions in potato under greenhouse conditions against black scurf disease. The present study reveald that *P. fluorescens* strains Pf1, Pf2, HALEX® and chitosan increased the activities of peroxidase, polyphenol oxidase, chitinase and β-1.3-D glucanase enzymes in potato root tissue in response to *R. solani* inoculation.

Data in Table (6a, b and c) indicated that phenolic contents were increased at the 3rdand the 6thhr from PGPR and chitosan applications. The highest increase (32.5 %) was recorded with chitosan at the 3rdhr, followed by that of Pf1 and Pf2 with 25.8 and 21.4 % increase, respectively. Whereas, the highest increase at the 6thhr (52.2%) was recorded with HALEX® application followed by that of Pf1 and Pf2 with 31.6 and 35.5% increases, respectively. On the other hand, phenolic contents were decreased at 12thhr and the 3rdday from Pf1 application with 6.4 and 21.9 % decrease, respectively. In the late determination (the 7thday), phenolic contents were increased again by

Table 6a. Phenolic contents (mg/g) in root tissues of potato plants cv. Spunta infected by isolate SH of Rhizoctonia solani and treated with Pseudomonas fluorescence Pf1, Pf2, HALEX® and chitosan

Treatment	Time after inoculation					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	1.51*b	1.92c	2.99ab	3.06a	3.22a	2.60c
Pf1	1.90a	2.50a	2.42c	2.39b	3.26a	3.22a
Pf2	1.83a	2.48a	3.13a	3.21a	3.25a	3.17a
HALEX®	1.38b	2.11b	2.78b	3.07a	3.25a	3.19a
Chitosan	2.00a	2.48a	2.98ab	3.06a	3.20a	2.98b

Control = plants inoculated only with R. solani.
*Values are means of three replicates. Values followed by the same letter(s) are not significantly different.

Table 6b. The increase percentage (%) in phenolics accumulation during the time

Treatment	Time after application					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	-	27.2	55.7	-	-	-19.3
Pf1	-	31.6	-	-	36.4	-
Pf2	-	35.5	26.2	-	-	-
HALEX®	-	52.2	31.8	10.4	-	-
Chitosan	-	24.0	20.2	-	-	-6.9

Table 6 c. The increase percentage (%) in phenolics accumulation in each time incompare to control

Treatment	Time after application					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	-	-	-	-	-	-
Pf1	25.8	30.2	-6.4	-21.9	-	23.8
Pf2	21.2	29.2	-	-	-	21.9
HALEX®	-	-	-2.3	-	-	22.7
Chitosan	32.4	29.2	-	-	-	14.6

(14.6-23.8 % increase) with PGPR and chitosan applications (Fig. 2). Treatment with PGPR and chitosan induced the accumulation of phenolics in potato root tissues. Phenolics accumulation started on early determinations (the 3rd and the 6th hr after application) except with HALEX®, followed by a decrease with Pf1 and HALEX® application at the 12th hr after application compared to the control. General increase was noticed on the 7th day after application. The results are agreement with several investigators. Accumulation of phenolics by prior application of PGPR in pea has been reported against *P. ultimum* and *F. oxysporum* f. sp. *pisi* (Benhamou *et al*., 1996). El Hassni *et al.* (2004) recorded an increase in the level of phenolics in date palm in response to *Fusarium oxysporum* f. sp. *albedinis* when treated with chitosan. The recent studies indicate that the higher and earlier accumulation of phenolics was observed in PGPR-treated root tomato tissue challenged with *F. oxysporum* f. sp. *lycopersici*. The increased accumulation of phenolic content and defense-related proteins has been noticed in interaction between bean roots and various rhizobacteria (Zdor and Anderson, 1992).The increased concentrations of phenolics may be toxic to fungal and bacterial pathogens. Phenolics may alter the membrane porosity of fungal cells and inhibit certain enzymes of pathogens. Phenolics may also inhibit production of toxins and pectic enzymes produced by pathogens (Vidhyasekaran, 1997).

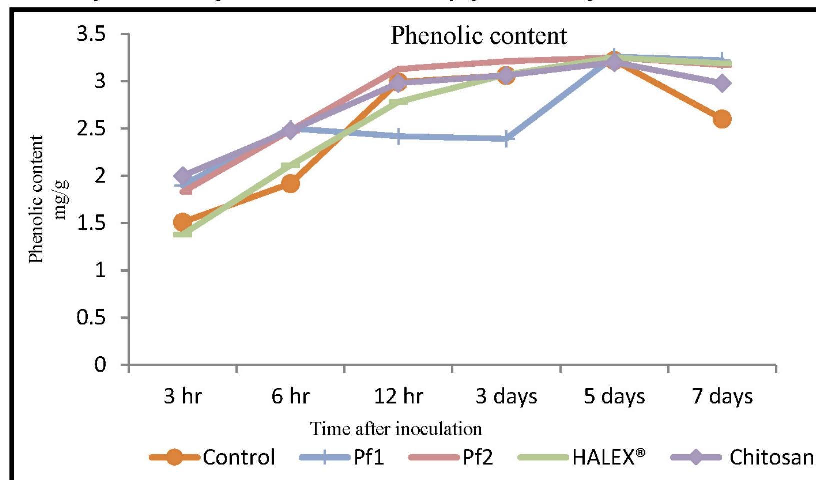

Fig. 2. Accumulation of phenolic contents induced by *Pseudomonas fluorescence* Pf1 and Pf2, HALEX® and chitosan in potato roots inoculated by *Rhizoctonia solani*

Peroxidase (PO) activity

Results in Table (7a, b and c) indicated that peroxidase (PO) activities were increased with all PGPR and chitosan applications at the 3rd, 6th and 12th hr from the applications. At the 3rd hr, the increase % ranged 45.6-49% whereas, the increase % were ranged 32.7-57.2% and 14-36.8% at the 6th hr and the 12th hr after the application, respectively. The highest increase % was obtained with chitosan treatment (57.2 and 36.8%) on the 6th and the 12th hr after application, respectively. On the late determinations, chitosan increased PO activity by 18.4% while decrease of 3.6% was recorded at the 3rd day after the application. At the 5th and 7th day determinations, showed increases in PO activity by 10.1-38.3% and 14-41.2% increase, respectively (Fig. 3). Increased activity of peroxidase due to the pathogen infection has been reported in different plants such as rice (Reimers *et al*., 1992), tobacco (Ahl Goy *et al*., 1992), tomato (Mohan *et al*., 1993), and cucumber (Chen *et al*., 2000) due to pathogen infection. Chen *et al*., (2000) reported that higher PO activity in cucumber roots treated with *Peudomonas corrugata* when challenged with *Pythium aphanidermatum*, was achieved. Peroxidases (POs) are key enzymes in the cell wall-building process, and it has been suggested that extracellular or wall-bound POs would enhance plant resistance to pathogen invasion and spread (Ride, 1983).

Polyphenol oxidase (PPO) activity

Data in Table (8a, b and c) showed that the polyphenoloxidase (PPO) activity was increased as a result of all PGPR and chitosan treatments. On the 3rd h from the application the highest increase (165.5 %) was noticed with HALEX® followed by that of Pf2 and chitosan with 139.8and 118.4% increase, respectively. The lowest increase (7 %) was recorded with Pf1. Similarly, the activity of PPO was increased at the 6th hr and the 12th hr from the application with arrange of 17.5-52.5% and 98-318% increase, respectively. On the other hand, the activity of PPO was decreased by 21% on the 6th hr after Pf1 application. In the late determinations, the 3rd, the 5th and the 7th day, PPO activity was increased with 63.4-125.6, 170-270.9

Table 7a. Peroxidase activity (unit/ min) in root system of potato plants cv. Spunta infected with *Rhizoctonia solani* isolate SH and treated with *Pseudomonas fluorescence* Pf1, Pf2, HALEX® and chitosan

Treatment	Time after inoculation					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	122.2*d	174.0c	206.8d	244.8b	220.2c	211.4d
Pf1	178.0c	235.7b	241.3b	245.6b	242.5b	241.0c
Pf2	182.0ab	231.0b	236.0c	236.0c	244.6b	244.8c
HALEX®	185.2a	235.0b	240.0bc	241.7b	246.0b	260.8b
Chitosan	181.4bc	274.0a	283.0a	289.8a	304.6a	298.6a

Control = plants inoculated only with *R. solani*.
*Values are means of three replicates. Values followed by the same letter(s) are not significantly different.

Table 7b. The increase percentage (%) in peroxidase activity with the time

Treatment	Time after application					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	-	42.4	18.9	18.4	-11.2	-04.0
Pf1	-	32.4	02.4	01.8	-	-
Pf2	-	26.9	02.2	-	03.6	-
HALEX®	-	26.9	02.1	-	01.8	06.0
Chitosan	-	51.0	03.3	02.4	05.1	-01.9

Table 7c. the increase percentage (%) in peroxidase activity in each time in compares to control

Treatment	Time after application					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	-	-	-	-	-	-
Pf1	45.6	35.5	16.7	-	10.1	14.0
Pf2	48.9	32.7	14.0	-	11.2	15.8
HALEX®	49.0	35.0	16.0	-	11.7	23.3
Chitosan	48.4	57.2	36.8	18.4	38.3	41.2

Table 8. Polyphenoloxidase activity (unit/min) in root of potato plants cv. Spunta inoculated with *Rhizoctonia solani* SH and treated with Pf1, Pf2, HALEX® and chitosan

Treatment	Time after inoculation					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	4.67*d	11.4d	15.3e	25.7d	22.0e	32.0e
Pf1	5.00d	09.0e	52.2b	54.0b	56.5c	54.0d
Pf2	11.2b	14.4c	64.0a	54.4b	60.0b	151.8a
HALEX®	12.4a	16.1b	30.3c	42.0c	59.4b	58.4c
Chitosan	10.2c	17.4a	27.6d	58.0a	81.6a	108.0b

Control = plants inoculated with the *R. solani*.
*Values are means of three replicates. Values followed by the same letter(s) are not significantly different.

Table 8b. the increase percentage (%) in polyphenol oxidase activity with the time

Treatment	Time after application					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	-	144	34	68	-14	-46
Pf1	-	80	480	3.4	5	-45
Pf2	-	29	344	-15	10	53
HALEX®	-	30	88	386	41	-
Chitosan	-	71	59	110	41	32

Table 8c. The increase percentage (%) in polyphenol oxidase activity in each time in compare to control

Treatment	Time after application					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	-	-	-	-	-	-
Pf1	7.0	-21	241	110	156.8	68.8
Pf2	139.8	17.5	318	112	172.7	374
HALEX®	165.5	41.2	98	63.4	170	82.5
Chitosan	118.4	52.6	80.4	125.6	270.9	273.5

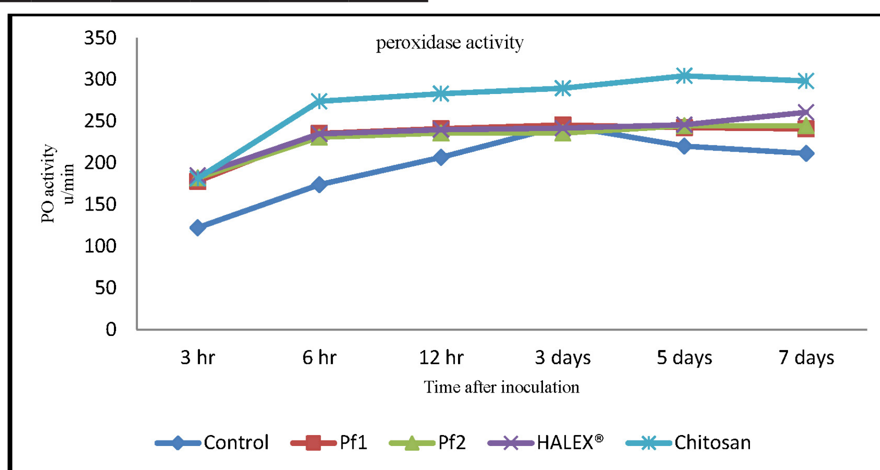

Fig. 3. Changes in peroxidase activity induced by inoculation *Pseudomonas fluorescence* (Pf1), (Pf2), HALEX® and chitosan in potato roots inoculated by *Rhizoctonia solani*

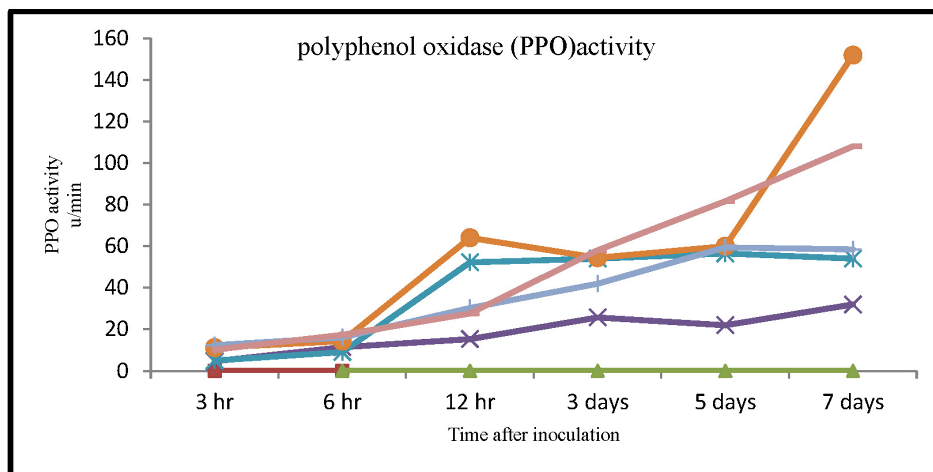

Fig. 4. Changes in polyphenoloxidase activity induced by *Pseudomonas fluorescence* (Pf1), (Pf2), HALEX® and chitosan in potato roots inoculated by *R. solani*

and 68.8-374% increase, respectively. High activity of PPO was recorded at the 7th day after Pf1 application with 374% increase followed by 270.9 % and 237.5% with the application of chitosan at the 5th and the 7th day after application, respectively (Fig. 4). The highest increase percentages was obtained from chitosan treatment. Chen *et al*., (2000) reported that various rhizobacteria and *P. aphanidermatum* induced the PPO activity in cucumber root tissues. Polyphenol oxidase activity increased in young leaves of tomato when matured leaflets were injured by insects and mechanical disorders (Thipyapong and Steffens, 1997). Recent studies provide evidence that PPO may participate in plant defense against pests and pathogens (Mayer and Harel 1979, Mayer 1987, Steffens *et al*., 1994, Constabel *et al*., 1995, Thipyapong *et al*., 1995 and Thipyapong and Steffens, 1997). Polyphenol oxidas are inducible enzymes in plants (Mayer and Harel 1979 and Mayer 1987). Polyphenol oxidase catalyze the oxygen-dependent oxidation of phenols to quinones. The quinonoid products of PPO are highly reactive molecules which can covalently modify and cross-link a variety of cellular nucleophiles, resulting in formation of melanin-like black or brown condensaed polymers. The active quinones produced by PPOs may possess direct antibiotic and cytotoxic activities to pathogens (Mayer and Harel 1979 and Peter 1989). In addition, systemic induction of PPO expression in response to wounding and pathogens may provide an additional line of defense to protect plants against further attack by pathogen and insects (Bashan *et al*., 1987; Constabel *et al*., 1995; Thipyapong *et al*., 1995 and Stout *et al*., 1999).

Chitinase activity

Data in Table (9a, b and c) showed that chitinase activity were increased by 22 % at the 3rd hr after the application with HALEX® while the application of Pf1, Pf2 and chitosan showed decreases in chitinase activity by 44, 44 and 78%, respectively. On the 6th hr from the application of Pf1 and Pf2, chitinase contents were increased by 38.9 and 50%, respectively and by 33 and 20% at the 12th hr after Pf1 and Pf2 application, respectively. On the other hand, chitinase contents were de-

Table 9. Chitinase activity (nM N-aceylglucose amine/ ml/min) in root of potato CV. Spunta inoculated with *Rhizoctonia solani* SH and treated with *Pseudomonas fluorescence* Pf1, Pf2, HALEX® and chitosan

Treatment	Time after inoculation					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	0.9*b	1.8c	3.0c	4.02c	3.84d	1.6e
Pf1	0.5c	2.5b	4.0a	6.77a	10.16a	6.3a
Pf2	0.5c	2.7a	3.6b	3.84d	6.55b	3.2d
HALEX®	1.1a	1.4d	2.5d	2.71e	3.84d	4.5c
Chitosan	0.2d	1.8c	1.6e	4.33b	4.84c	6.0b

Control = plants inoculated with the *R. solani*. *Values are means of three replicates. Values followed by the same letter(s) are not significantly different.

Table 9b. the increase percentage (%) in chitinase activity with the time

Treatment	Time after application					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	-	100	67	34	-	-58
Pf1	-	400	60	69	50	-38
Pf2	-	440	33	7	71	-51
HALEX®	-	27.3	77	-	42	17
Chitosan	-	805	-11	171	12	24

Table 9c. The increase percentage (%) in chitinase activity in each time in compare to control

Treatment	Time after application					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	-	-	-	-	-	-
Pf1	-44	38.9	33	69	165	293
Pf2	-44	50	20	-	71	100
HALEX®	22	-22	-17	-32	-	181
Chitosan	-78	-	-47	8.3	26	275

creased with HALEX® by 22 and 17% on the 6th hr and 12th hr from the application, respectively and decreased by 47% with chitosan at the 12th hr from the application. Late determination of chitinase activity at the 3rd day showed increase by 69 and 8.3% with Pf1 and chitosan applications, respectively and a decrease of 32% with HALEX® application. Chitinase activity at the 5th and 7th day showed increases with all treatments (except with HALEX® on the 5th) of 26-165 and 100-239%, respectively. The highest chitinase activity of 293, 275, 181 and 100% were noticed at the 7th day after the applications with Pf1, chitosan, HALEX® and Pf2, respectively (Fig. 5). Synthesis and accumulation of PR proteins have been reported to play an important role in plant defense (Maurhofer *et al*., 1994; Van Loon, 1997). Maurhofer *et al*., (1994) reported that induction of systemic resistance by *P. fluorescence* was correlated with the accumulation of β-1, 3-glucanase and chitinase. A combination of chitinase and β-1,3- D glucanase was demonstrated to be more effective than either enzyme alone against many fungi (Mauch *et al*., 1988). Resistance to leaf rust in wheat cultivars Karee and Lr35 was partially attributed to high constitutive levels of chitinase and induced β- 1,3- D glucanase activity, which resulted from a hypersensitive defense (Anguelova-Merhar *et al*., 2001).

β,1-3, D glucanase activity

Data in Table (10a, b and c) indicated that the application of Pf1 and HALEX® increased the activity of β, 1-3, D glucanase at the 6th and 12th hr with 1113 and 291% and 665 and 161% increase, respectively. On the other hand, β, 1-3, D glucanase activity were decreased by 87 and 46% at the 6th and 12th hr from chitosan application, respectively and by 65% with Pf2 at the 6th hr after application. The late determination of β,1-3, D glucanase showed significant increases ranged between 17-1082%, 122-1189% and 170-684% at the 3rd, 5th and 7th day of the applications, respectively. The highest increases of β,1-3, D glucanase 1082, 1189 and 684 % were recorded at the 3rd, the 5th and the 7th day after Pf1 application, respectively (Fig. 6).

β-1,3-D glucanase belongs to the PR-2 family which are widely distributed among plant species. Enough ex-

Table 10. Induction of β1-3, D Glucanase activity (μg glucose/min/ml) in root of potato cv. Spunta inoculated with Rhizoctonia solani SH and treated with Pseudomonas fluorescence Pf1, Pf2, HALEX® and chitosan

Treatment	Time after inoculation					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	0.85*a	2.3c	3.67c	4.8e	5.08e	5.00d
Pf1	0.28c	27.9a	28.1a	56.7a	65.46a	39.2a
Pf2	0.56b	0.8d	3.68c	5.6d	11.28d	15.8b
HALEX®	0.56b	9.0b	9.57b	10.7b	23.42b	15.8b
Chitosan	0.58b	0.3e	1.98d	8.8d	22.00c	13.5c

Control = plants inoculated with *R. solani* only.
*Values are means of three replicates. Values followed by the same letter(s) are not significantly different.

Table 10b. the increase percentage (%) in β1-3, D Glucanase activity with the time

Treatment	Time after application					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	-	165	63	31	-	-
Pf1	-	980	-	102	15	-67
Pf2	-	49	338	51	100	40
HALEX®	-	150	6	12	119	-32
Chitosan	-	-	633	342	151	-38

Table 10c. The increase percentage (%) in β,1-3, D Glucanase activity in each time in compare to control

Treatment	Time after application					
	3 hr	6 hr	12 hr	3 days	5 days	7 days
Control	-	-	-	-	-	-
Pf1	-	1142	665	1082	1189	684
Pf2	-	-63	-	18	122	216
HALEX®	-	301	161	123	361	216
Chitosan	-	-88	-46	82	334	170

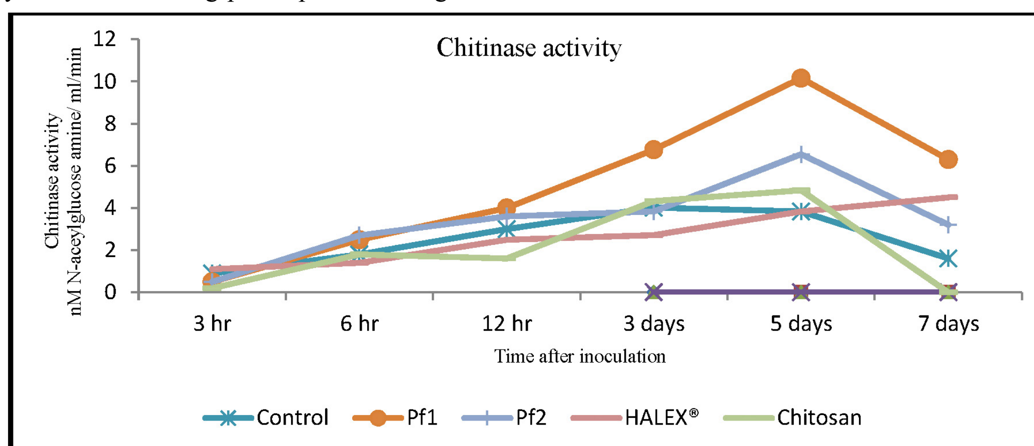

Fig.5. Induction of chitinase activity by Pseudomonas fluorescence (Pf1) and (Pf2), HALEX® and chitosan in potato roots inoculated by *Rhizoctonia solani*

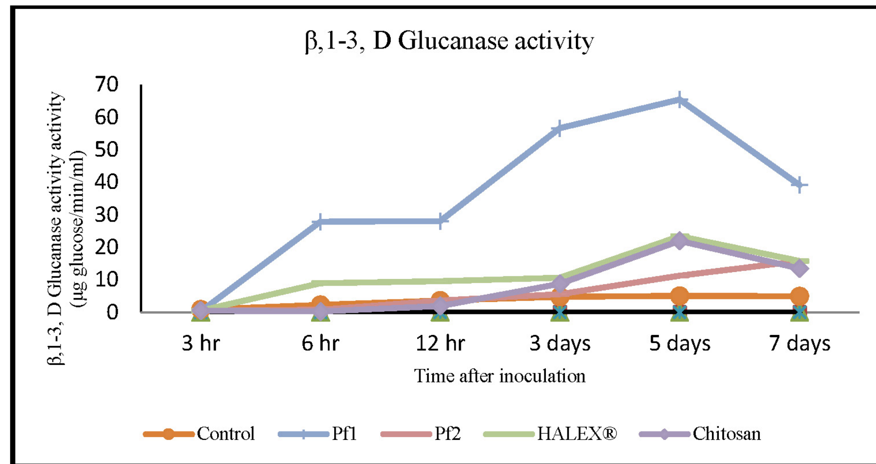

Fig. 6. Induction of β1-3, D glucanase activity by *Pseudomonas fluorescence* Pf1 and Pf2, HALEX® and chitosan in potato roots infected with *R. solani*

perimental evidence is available to support a defensive role for β-1,3- D glucanases against fungal pathogens, especially in a synergistic interaction with plant chitinases. These glucanohydrolases can exert their antifungal activity in at least two different ways: either directly by degrading the cell walls of the pathogen or indirectly by promoting the release of cell wall–degradation products that can act as elicitors to trigger a wide range of defense reactions (Leubner-Metzger and Meins, 1999). The fungal cell wall elicitors have been reported to elicit various defense reactions in greengram (Ramanathan *et al*., 2000). Several reports have confirmed the *in vitro* antifungal activity of β-1,3- D glucanases against various fungi (Velazhahan *et al*., 2003). The oligosaccharides released from the cell walls of the pathogen as a result of digestion by β-1,3- D glucanases acted as elicitors in soybean (*Glycine max* L.) plants. They induced the accumulation of a phytoalexins and glyceollin, which curtailed infection by *Phytophthora megasperma* f. sp. *glycinea* (Sharp *et al*., 1984).

In conclusion, the present study revealed that earlier and higher accumulation of peroxidase, polyphenol oxidase, β-1,3- D glucanases and chitinase enzymes have been found in potato tissues treated with PGPR and Chitosan in response to inoculation by *R. solani*. The plant-pathogen interactions have also included the activities of defense enzymes in the early phases of the interaction then drastically declined when the pathogen colonized the root tissues. Accumulation of phenolics, β-1,3-glucanase, PO, PPO and chitinasae by PGPR and chitosan in potato root tissues may have collectively contributed to induce resistance in potato plants against *R. solani.* Optimization of quantity and time of application of the studied PGPR and chitosan could be the next step to set up a successful management of black scurf disease on potato.

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Insecticidal and Antifungal Activities of *Helianthus annuus* Extracts

Amr A. M. Zoghroban, Shehata M.I. Kassem, Samir A. M. Abdelgaleil*
Department of Pesticide Chemistry and Technology, Faculty of Agriculture (Elshatby) Alexandria University, Alexandria 21545, Egypt
(samirabdelgaleil@gmail.com)

ABSTRACT

Plants may provide potential alternatives to currently used pest-control agents because they constitute a rich source of bioactive chemicals. The present study aimed to evaluate the insecticidal and antifungal activities of *Helianthus annuus* extracts against *Culex pipiens*, *Spodoptera littoralis* and five plant pathogenic fungi, namely *Fusarium oxysporum*, *Pythium debryanum Phytophthora infestans*, *Fusarium solani* and *Alternaria alternata*. *Helianthus annuus* air-dried leaves were extracted with n-hexane, methylene chloride, methanol and water sequentially. The resulting extracts were evaluated for their insecticidal activity against fourth instar larvae of *C. pipiens* by dipping assay. Hexane and methylene chloride extract showed strong toxic effect with complete mortality (100%) of the larvae at 500 mg/L. The LC_{50} values of hexane and methylene chloride extracts were 19.02 and 22.74 mg/L after 24 hours, and 9.02 and 9.28 mg/L after 48 hours, respectively. In addition, methanol extract showed moderate toxicity, while water extract was the less effective one. Residual film assay of methanol extract on the third instar larvae of *S. littoralis* revealed the high toxicity after 24 and 48 h of treatment with LC_{50} values of 15.99, 5.75 mg/L, respectively. Interestingly, methanol extract was more toxicant than the reference insecticide, chlorpyrifos, toward *S. littoralis* after two exposure times. Moreover, methylene chloride extract exhibited remarkable insecticidal activity, followed by water extract, while hexane extract was not toxic. On the other hand, water extract of *H. annuus* was the only effective extract among the tested extracts against the five plant pathogenic fungi with moderate antifungal activity.

Key words: *Helianthus annuus; Culex pipiens; Spodoptera littoralis;* ***Plant pathogenic fungi; Insecticidal activity; Antifungal activity.***

INTRODUCTION

Higher plants are a rich source of natural compounds that can be used effectively in pest control. Insecticidal, herbicidal and fungicidal activities of many plants against several pests have been demonstrated (Isman, 2006; Dyan *et al.*, 2009). Although botanicals are now a small part of the global pesticide market due to replacement by synthetics, the new environmental movement has provided a favorable environment for the renewal of botanical pesticides. Public concern over use of synthetic pesticides is growing. This has led to the great growth in organic agriculture in which botanicals play an important role in pest control. Controversy over genetically modified crops is another factor favoring the use of botanicals in pest control. Botanicals have certain advantages, such as rapid degradation, lack of persistence and bioaccumulation in the environment and low mammalian toxicity (Cantrell *et al.*, 2012).

Sunflower, *Helianthus annuus* L. (Asteraceae), is indigenous to North America and Mexico (Lentz *et al.,* 2008). Sunflower prefers a mild temperature regime and is grown in many semiarid regions of the world at 0–3,000 m altitude. It is suited to most dryland and irrigated farming systems but is not highly drought tolerant. It grows in a wide range of temperatures 17–33 °C conditions with an optimum of 21–26 °C. Sunflower has been an important resource of natural oil and lipid-rich nutrients for centuries. It is widely used in human foods because of its high protein concentrate in the seed flour. Additionally, it used as a preventive medicine against diuresis, diarrhea, and several inflammatory diseases. Several sesquiterpenes and sesquiterpenes lactones were isolated and identified from different plant parts of *Helianthus annuus* (Macias *et al.* 1998, 1999, 2008; Göpfert *et al.* 2005, 2009, 2010; Ghosh *et al.* 2007; Miyawaki *et al.* 2012). Extracts and isolated phytochemicals of *Helianthus annuus* have been described to possess antioxidant, immunomodulatory, antiinflammatory, antiviral, antimicrobial, antidiabetic, antihypertensive, antihyperchlolesteromic, skin moisturizing, phytotoxic and allelopathic activities (Lim, 2014).

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is the most destructive agricultural lepidopterous pests of cultivated crops primarily in tropical and subtropical regions (Bakr *et al.*, 2013). *Culex pipiens* are vectors of West Nile virus and an important pest to humans, causing allergic responses that include local skin reaction and systemic reactions such as angioedema, and urticaria (Cheng *et al.*, 2008). Plant pathogenic fungi cause significant preharvest and post-harvest loss in crop production. It is estimated that the world crop loss due to plant diseases may amount to 14%. Plant pathogenic fungi share 40-60% of total plant diseases loss (Mahy and van Regenmortel, 2009).

In our continuing efforts to find new natural products that can be used in pest control, the insecticidal activity of hexane, methylene chloride, methanol and water extracts of *H. annuus* was examined against *S. littoralis* and *C. pipiens*. In addition, the antifungal

activity of plant extracts was evaluated against five plant pathogenic fungi, *Fusarium oxysporum, Pythium debryanum, Phytophthora infestans, Fusarium solani* and *Alternaria alternata*.

MATERIALS AND METHODS

Plant material

Leaves of *Helianthus annuus* were collected from a farm located in El-Nubaria district, El-behera Governorate, Egypt in June, 2010.

Culex pipiens

Culex pipiens L. (Diptera: Culicidae) colony maintained in the laboratory of Mosquito Bioassay, Department of Economic Entomology, Faculty of Agriculture, Alexandria University, for more than 10 years was used. Mosquitoes were held at 262°±C, 70±5% RH, and a photoperiod regime of 14:10h. (light/dark). Adults were provided with a 10% sucrose solution as a food source. A pigeon was introduced twice per week for adult blood feeding. Larvae were reared in dechlorinated water under the same temperature and light conditions and were fed daily with baby fish food.

Spodoptera littoralis

A laboratory strain of *Spodoptera littoralis* (Boisd) was obtained from the Bioassay Laboratory, Department of Pesticide Chemistry and Technology, Faculty of Agriculture, Alexandria University. The colony was reared under laboratory conditions on castor bean leaves, *Ricinus communis* L. (Euphorbiaceae), at 26±2°C and 70±5% RH (El-Defrawi *et al*., 1964).

Fungi

Five phytopathogenic fungi species, *Fusarium oxysporum*, *Pythium debryanum Phytophthora infestans*, *F. solani* and *Alternaria alternata* were used in this study. The fungi were obtained from the Fungicide Bioassay Laboratory, Department of Pesticide Chemistry and Technology, Faculty of Agriculture, Alexandria University. The fungi were maintained during the course of the experiments on Potato Dextrose Agar Medium (PDA: Potato 200, dextrose 20 and agar 15 g/L in distilled water) at 25°C.

Extraction of *Helianthus annuus*

The dry leaves (1 kg) of sunflower (*H. annuus*) were extracted with n-hexane, methylene chloride, methanol and distilled water (2.5 l of each solvent) sequentially. The extraction was conducted at room temperature for two weeks. The solvents were evaporated under reduced pressure by using rotary evaporator to give 1.4 g of hexane extract, 8.0 g of methylene chloride extract, 8.0 g of methanol extract and 21.0 g of water extract. The crude extracts were kept in the refrigerator at 4°C until used for bioassay experiments.

Bioassay of crude extracts against *C. pipiens*

The WHO standard test method for mosquito larvae (Anonymous, 1996) was used to test the insecticidal activities of *H. annuus* extracts against the 4th instar larvae of *C. pipiens*. The concentrations of extracts were prepared in acetone. The extracts were tested at the concentrations ranged between 1 and 500 mg/L. Twenty larvae were used in each replicate and three replicates were considered as one treatment. Control was concurrently conducted under the same conditions with acetone only. Malathion 95% (Kafr El-zayat Pesticides and Chemicals Co., Egypt) was used as a reference insecticide. The mortality percentages were recorded after 24 and 48 hours of treatment. Mortality data were subjected to probit analysis to estimate the median lethal concentration values (LC_{50}) of extracts and the reference insecticide (Finney, 1971).

Bioassay of extracts against *S. littoralis*

Insecticidal activity of *H. annuus* extracts was tested by a residual film method (Ascher and Nissim, 1965) on the third instar larvae of *S. littoralis*. Leaf disks (1.8 cm diameter) of castor bean were immersed in acetone solutions of the crude extracts for 5 seconds. The crude extracts were tested at concentrations of ranged between 5 and 1000 mg/L. Three replicates were carried out for each concentration and control (leaf disks were immersed in acetone only). Ten larvae were placed with treated leaf disks in cups (200 ml) and kept at room temperature (25°C). The mortality percentages were recorded after 24 and 48 hours and LC_{50} values were calculated as previously described.

Bioassay of extracts against fungi

The antifungal activity of *H. annuus* extracts was tested by using radial growth inhibition technique (Zambonelli *et al*., 1996; Bajpai *et al*., 2007). Appropriate volumes of the stock solutions of the extracts in dimethyl sulfoxide (DMSO) were added to PDA medium immediately before it was poured into the Petri dishes (9.0 cm diameter) at 40-45ºC to obtain a series of concentrations (25, 50, 100, 200, 300, 400 and 500 mg/L). Each concentration was tested in triplicate. Parallel controls were maintained with DMSO mixed with PDA. The discs of mycelial felt (0.5 cm diameter) of the plant pathogenic fungi, taken from 8-day-old cultures on PDA plates, were transferred aseptically to the center of Petri dishes. The treatments were incubated at 27 ºC in the dark. Colony growth diameter was measured after the fungal growth in the control treatments had completely covered the Petri dishes. Percentage of mycelial growth inhibition was calculated from the formula: Mycelial growth inhibition = [(DC-DT) /DC] × 100 (Pandy *et al.*, 1982), where DC and DT are average diameters of fungal colony of control and treatment, respectively. The concentration of the extract inhibited the fungi mycelial growth by 50% (EC_{50}) was determined by a linear regression method (Finney, 1971).

Statistical analysis

The insect mortality of each concentration was calculated after 24 and 48 h of treatment as the mean of three replicates. The insect mortality and fungal growth

inhibition percentages were subjected to probit analysis (Finney 1971) to obtain the LC_{50} and EC_{50} values, using SPSS 12.0 (SPSS, Chicago, IL, USA). The values of LC_{50} and EC_{50} were considered significantly different if the 95% confidence limits did not overlap.

RESULTS AND DISCUSSION

Insecticidal activity of *Helianthus annuus* extracts against *Culex pipiens* larvae

The crude extracts of *H. annuus* were screened for their insecticidal activity against the 4th instar larvae of *C. pipines*. Hexane and methylene chloride extracts showed comparative strong toxic effect with complete mortality (100%) of the larvae at 500 mg/L. The LC_{50} values of hexane and methylene chloride extracts were 19.02 and 22.74 mg/L after 24 h, and 9.02 and 9.28 mg/L after 48 h, respectively. On the other hand, methanol extract showed relatively pronounced toxicity LC_{50} values of 75.58 and 21.77 mg/L after 24 and 48 h, respectively. In addition, water extract revealed moderate insecticidal activity. The LC_{50} values of this extract were 221.81 and 98.90 mg/L after 24 and 48 hours, respectively. Based on LC_{50} values of four extracts, hexane and methylene chloride extracts were the most potent toxicants to the 4th instar larvae of *C. pipines*, followed by methanol extract, while water extract was less effective one (Table 1). The results also showed that the toxicity of the tested extracts improved with increasing the exposure time. Although the tested extracts showed pronounced toxicity against *C. pipines* larvae they were less toxic than the reference insecticide, Malathion.

To best of our knowledge, there were no reported studies on the insecticidal activity of *H. annuus* extracts on the larvae of *C. pipines*. However, the extracts of *H. annuus* have been described to possess insecticidal activity against *Ploida interpunctella* and *Tetranychus urticae* (Lee *et al.*, 2001). In addition, Chou and Mullin (1993) found a positive correlation between the antifeedant activity and sesquiterpene contents of *H. annuus* on western corn rootworm. Moreover, the insecticidal activity of *H. annuus* essential oil has been reported against *Callosobruchus maculatus* in cowpea grains (Pérez *et al.*, 2010).

Insecticidal activity of *Helianthus annuus* extracts against *Spodoptera littoralis*

The insecticidal activity of *H. annuus* extracts of hexane, methylene chloride, methanol and water was evaluated by a residual film assay on the third instar larvae of *S. littoralis*. Table (2) presents LC_{50} values (mg/L), 95% confidence limits and other regression parameters generated from probit analysis of *H. annuus* extracts and the reference insecticide, chlorpyrifos. Methanol extract showed strong toxic effect after 24 and 48 h of treatment with LC_{50} values of 15.99, 5.75 mg/L, respectively. Based on LC_{50} values, methanol extract was more potent than chlorpyrifos after two exposure times. In addition, methylene chloride revealed remarkable insecticidal activity against the larvae of *S. littoralis*. On the other hand, the water extract exhibited moderate toxic effect, while hexane extract had the lowest toxicity among the tested extracts.

In the literature, there were not reported studies on the insecticidal activity of the *H. annuus* extracts against the third instar larvae of *S. littoralis*. Our results are supported by other studies in which the crude extracts of plants showed insecticidal, antifeedant and

Table 1. Comparative toxicity of *Helinthus annuus* extracts against the fourth instar larvae of *Culex pipiens* after 24 and 48 h of treatment

Treatment	Exposure time (h)	LC_{50}[a] (mg/L)	95% Confidence limits (mg/L)		Slope ±SE[b]	Intercept ±SE[c]	(x^2)[d]
			Lower	Upper			
Hexane extract	24	19.02	12.57	27.80	1.44±0.09	- 1.84±0.13	17.98
	48	9.02	4.70	15.10	1.22±0. 08	- 1.17±0.11	24.71
Methylene chloride extract	24	22.74	13.99	35.65	1.29±0.08	-1.75±0.13	21.78
	48	9.28	4.35	16.55	1.15±0.08	-1.11±0.11	28.93
Methanol extract	24	75.58	56.47	104.55	0.75±0.06	-1.41±0.11	2.36
	48	21.77	15.77	29.49	0.70±0.06	-0.94±0.10	6.92
Water extract	24	221.81	158.81	236.25	0.810.07±	-1.89±0.13	5.96
	48	98.90	71.90	143.30	0.71±0.06	-1.41±0.11	6.47
Malathion	24	2.2×10^{-3}	1.8×10^{-3}	2.7×10^{-3}	2.03±0.20	5.38±0.57	0.73
	48	1.6×10^{-3}	1.4×10^{-3}	1.9×10^{-3}	1.90±0.20	5.31±0.55	1.66

[a] The concentration causing 50% mortality.
[b] Slope of the log concentration- mortality regression line ± standard error.
[c] Intercept of the regression line ± standard error.
[d] Chi square value.

Table 2. Comparative residual toxicity of *Helianthus annuus* extracts against the third instar larvae of cotton leafworm, *Spodoptera littoralis*

Treatment	Exposure time (h)	LC_{50}[a] (mg/L)	95 % Confidence limits (mg/L)		Slope ±SE[b]	Intercept ±SE[c]	(x^2)[d]
			Lower	Upper			
Hexane extract	24	> 1000	-	-	-	-	-
	48	> 1000	-	-	-	-	-
Methylene chloride extract	24	50.26	24.72	96.66	0.96±0.08	- 1.63±0.15	10.95
	48	20.96	8.13	41.56	0.99±0.08	- 1.31±0.14	12.83
Methanol extract	24	15.99	7.80	27.07	0.50±0.07	- 0.60±0.13	3.528
	48	5.75	2.26	10.72	0.53±0.07	- 0.40±0.13	3.532
Water extract	24	367.83	218.6	750.6	0.52±0.07	- 1.34±0.14	0.774
	48	111.8	71.61	183.1	0.54±0.07	- 1.10±0.13	1.55
Chlorpyrifos	24	18.43	16.77	21.26	3.95±0.66	-5.00±0.79	1.00
	48	12.34	11.61	13.03	7.25±0.74	-7.92±0.85	0.89

[a] The concentration causing 50% mortality.
[b] Slope of the log concentration-mortality regression line ± standard error.
[c] Intercept of the regression line ± standard error.
[d] Chi square value.

growth inhibitory effects on *S. littoralis*. For example, Abd El-Rahman and Al-Mozini (2007) found that crude petroleum ether extracts of *Calotropis procera*, *Rhazya stricta* and *Solenostemma argel* exhibited significant antifeedant activity against the 4th larval instar *S. littoralis*. All plant extracts affected growth rate and consumption index of *S. littoralis* larvae. Hatem *et al.* (2009) reported that hexane, petroleum ether and ethanol extracts of three weed plants *Sonchus olearcues*, *Brassica niger* and *Raphanus sativa* had insecticidal and antifeedant activities against the 4th larval instar *S. littoralis.* Abdelgaleil *et al.* (2010) reported insecticidal activity of the extracts of three plants, *Zygophyllum coccineum*, *Majorana hortensis* and *Mentha microphylla*, against the fourth instar larvae of *S. littoralis*. Pavela (2011) screened the insecticidal activity of plant extracts obtained from 134 plant species of the Eurasian region for chronic toxicity and larval inhibition in *S. littoralis* larval growth. El-Kholy *et al.* (2014) studied the effects of three leaf plant extracts (*Ambrosia maritima, Datura stramonium, Eucalyptus globules*) on life parameters of *S. littoralis*. The extracts decreased the number of eggs laid per female and fecundity. Barakat (2011) described the insecticidal and antifeedant activities of acetone extract of *Casimiroa edulis* against 4th instar larvae of *S. littoralis*.

Antifungal activity of *Helianthus annuus* extracts against plant pathogenic fungi

The antifungal effect of the *H. annuus* extracts on *Fusarium oxysporum, Pythium debaryanum, Phythophthora infestans, Fusarium solani and Alternaria alternata* was determined by radial growth inhibition technique. The results of antifungal activity of the tested *H. annuus* extracts against fungi are shown in Table (3). In general the extracts showed moderate to weak inhibitory effect. At the highest tested concentration (500 mg/L) the radial growth inhibition was less than 50% for methylene chloride and methanol extracts, therefore EC_{50} values were not estimated. Water extract had the highest antifungal activity among the tested extracts with EC_{50} values of 164.9, 166.8, 242.2, 275.4 and 285.8 mg/L on *F. solani*, *P. infestans*, *F. oxysporum*, *A. alternata* and *P. debaryanum*, respectively.

The results of the present study showed that the water extract had antifungal activity against the tested fungi. These results are in accordance with those of Riaz *et al.* (2008) who stated that aqueous extracts of sunflower exhibited remarkable antifungal activity against *F. oxysporum.* Maximum inhibition in fungal biomass production (85%) was recorded in 2% extract treatment compared to control. Likewise, Bajwa *et al.* (2001) found that aqueous root extract of *H. annuus* suppressed the growth of *Aspergillus niger* and *A. fumigates* very effectively. In addition, the aqueous leaf extract of *H. annuus* significantly inhibited the growth of seed-borne fungi, *Aspergillus niger*, *A. fumigatus*, *Penicillium* sp. and *Rhizopus arrhizus*. Methanol and aqueous extracts of *H. annuus* seeds and ethanol extract of *H. annuus* stems were found to possess antifungal activity against *Aspergillus niger* and *Candida albicans* (Yagoub *et al.*, 2006; Adetunji *et al*., 2014).

In conclusion, the extracts of *H. annuus* leaves showed pronouncing insecticidal activity against the 4th instar larvae of *C. pipines* and the 3th instar larvae of *S. littoralis*. In addition, water extracts of the leaves revealed antifungal activity against five plant pathogenic fungi. These results suggest the possibility of using leaves and wastes of *H. annuus* in the management of these pests. This will be an excellent approach to dispose

Table 3. Comparative antifungal activity of *Helianthus annuus* extracts against plant pathogenic fungi

Fungi	Extract	EC_{50} (mg/L)	95 % Confidence limits (mg/L)		Slope ±SE	Intercept ±SE	(x^2)
			Lower	Upper			
Fusarium oxysporum	Hexane	n[a]	-	-	-	-	-
	Methylene chloride	> 500	-	-	-	-	-
	Methanol	> 500	-	-	-	-	-
	Water	242.2	130.2	706.3	0.71±0.08	- 1.68±0.15	8.57
Pythium debaryanum	Hexane	n	-	-	-	-	-
	Methylene chloride	> 500	-	-	-	-	-
	Methanol	> 500	-	-	-	-	-
	Water	285.8	149.2	945.2	0.85±0.09	-2.09±0.17	12.52
Phythophthora infestans	Hexane	n	-	-	-	-	-
	Methylene chloride	> 500	-	-	-	-	-
	Methanol	> 500	-	-	-	-	
	Water	166.8	127.3	230.8	0.95±0.09	- 2.12±0.17	1.37
Alternaria alternata	Hexane	n	-	-	-	-	-
	Methylene chloride	> 500	-	-	-	-	-
	Methanol	> 500	-	-	-	-	
	Water	275.40	206.9	394.3	1.05±0.09	- 2.56±0.19	5.16
Fusarium solani	Hexane	n	-	-	-	-	-
	Methylene chloride	> 500	-	-	-	-	-
	Methanol	> 500	-	-	-	-	-
	Water	164.9	126.5	226.7	0.97±0.09	-2.15±0.17	2.38

[a] Not tested.

of the plant wastes and at the same time will reduce the use of pesticides, which in turn reduces environmental pollution and positively affect human health.

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النشاط الإبادى الحشرى والنشاط التثبيطى الفطرى لمستخلصات نبات عباد الشمس

عمرو أحمد محمد زغربان وشحاته محمود ابراهيم قاسم وسمير عبد العظيم عبد الجليل
قسم كيمياء وتقنية المبيدات – كلية الزراعة – جامعة الأسكندرية

تعتبر النباتات بدائل جيدة للمركبات التى تستخدم فى مكافحة الأفات الآن وذلك لأنها مصدر غنى بالمركبات الفعالة بيولوجياً. تهدف هذه الدراسة إلى تقييم النشاط الإبادى الحشرى والنشاط المثبط للفطريات لمستخلصات أوراق نبات عباد الشمس على بعوضة الكيولكس ودودة ورق القطن وخمسة من الفطريات الممرضة للنبات وهى *Fusarium oxysporum* و *Pythium debryanum* و *Phytophthora infestans* و *Fusarium solani* و *Alternaria alternata*. حيث تم استخلاص اوراق نبات عباد الشمس بكل من الهكسان والميثلين كلوريد والميثانول والماء على التوالى. مستخلصات الهكسان والميثيلين كلوريد أظهرت سمية قوية ضد العمر اليرقى الرابع ليرقات بعوضة الكيولكس حيث سببا نسبة موت ١٠٠٪ على تركيز ٥٠٠ مجم/لتر. وكانت قيم LC_{50} لمستخلص الهكسان والميثيلين كلوريد ١٩,٠٢ و ٢٢,٧٤ مجم/لتر بعد ٢٤ ساعة و ٩,٠٢ و ٩,٢٨ مجم/لتر بعد ٤٨ ساعة على الترتيب. بالإضافة أن مستخلص الميثانول أظهر سمية متوسطة على يرقات بعوضة الكيولكس فى حين كان المستخلص المائى أقل المستخلصات سمية على الحشرة. وعند تقييم هذه المستخلصات على العمر اليرقى الثالث لدودة ورق القطن بطريقة التعرض للمتبقى أظهر مستخلص الميثانول سمية عالية بعد ٢٤ و٤٨ ساعة حيث كانت قيم LC_{50} ١٥,٩٩ و٥,٧٥ مجم/لتر على الترتيب. ومن الملفت للنظر أن مستخلص الميثانول كان أكثر سمية من المبيد الحشرى الكلوربيرفوس بعد ٢٤ و٤٨ ساعة كما أظهر مستخلص الميثيلين كلوريد سمية ملحوظة يليه مستخلص الماء فى حين مستخلص الهكسان كان غير فعال. على الجانب الأخر فإن المستخلص المائى لأوراق عباد الشمس هو الوحيد الذى أظهر تثبيطاً متوسطاً لنمو الفطريات الخمسة المختبرة.

Physico-Chemical Properties and Efficacy of Chlorpyrifos Commercial Formulations against *Spodoptera littoralis* under Laboratory and Field Conditions

Elham A. Sammour[1], Ahmed M. El-Bakry[1], Nahed F. Abdel-Aziz[1], Shehata M.I. Kassem[2], Samir A. M. Abdelgaleil[2,*]

[1]Department of Pests and Plant Protection, National Research Centre, Dokki, Cairo, Egypt

[2]Department of Pesticide Chemistry and Technology, Faculty of Agriculture, 21545-El-Shatby, Alexandria University, Alexandria, Egypt

(samirabdelgaleil@gmail.com)

ABSTRACT

The specifications of nine chlorpyrifos (48% Emulsifiable concentrate (EC)) commercial formulations, Chlorban, Chlorzane, Dursban, Ictafos, Pestban, Pyriban, Pyrifos El-Nasr, Tafaban and Terraguard were measured as described by Collaborative International Pesticides Analytical Council (CIPAC). These formulations were tested for their insecticidal activities contra the second and the fourth larval stages of *Spodoptera littoralis* under laboratory and field conditions. Chlorzane, Ictafos, Pyriban, Pyrifos El-Nasr, Tafaban and Terraguard passed the emulsion stability test. In the same trend, all chlorpyrifos formulations passed the foam test and gave full (100%) spontaneous emulsification. On the other hand, Chlorban was the only insecticide passed the acidity test, as its acidity was 0.05 calculated as percentage of H_2SO_4. Results of biological efficiency of chlorpyrifos formulations against 2nd and 4th instar larvae of *S. littoralis* revealed that, Dursban was the most potent under the laboratory conditions among all tested formulations with LC_{50} values of 0.2 and 1.11 mg/L on 2nd and 4th instar larvae, respectively, while Pyrifos El-Nasr was the least effective formulation with LC_{50} values of 1.4 and 3.78 mg/L on 2nd and 4th instar larvae, respectively. In the field experiments, Dursban and Pestban were the most efficient formulations under field conditions in controlling the 2nd instar larvae of *S. littoralis* as average of total mortality percentages were 78.1 and 75.6%, respectively, in cotton season 2010, and 80.6 and 78.1%, respectively, in cotton season 2011. Similarly, these two insecticides were also the most potent against 4th instar larvae. It is worth mentioning that, the efficiency of the most insecticides continued only for four days after application. Therefore, these formulations required adding suitable adjuvants to increase the persistence and enhance their insecticidal activities.

Keywords***: Chlorpyrifos; formulations; CIPAC specifications;*** *Spodoptera littoralis****; insecticidal activity.***

INTRODUCTION

Pesticide formulation is the process of transforming the active ingredient molecule to a product, which can be applied by practical methods to control pests in safe and at economical use (UNIDO, 1983). Pesticide formulations can undergo chemical and physical changes on storage. The rate at which these changes occur depends on the nature of the active constituent(s), the formulation type, the packaging and the storage conditions. The product remains fit for use as long as these changes have no adverse effects on application and biological performance. To ensure the quality of the formulations, the physico-chemical tests on formulations must be carried out.

Cotton is one of the major fibre crops of global significance. It is cultivated in tropical and subtropical regions of more than eighty countries of world occupying nearly 33 million hectare with an annual production of 19 to 20 million tones of bales. China, USA, India, Pakistan, Uzbekistan, Australia, Brazil, Greece, Argentina and Egypt are major cotton producing countries. These countries contribute nearly 85% of the global cotton production (Mayee *et al*., 2001).

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is a prolific and highly polyphagous insect. It is considered to be a major pest of great economic importance in many countries since it attacks a multitude of host plants. It is one of the most distractive insects infesting cotton plants. This insect causes a severe reduction in cotton yield and quality (Shonouda and Osmam, 2000; Magd El-Din and El-Gengaihi, 2000; El-Khawas and Abd El-Gawad, 2002). The use of insecticides is still having an important role in controlling such insect. Foliar applied insecticides are necessary when the economic threshold is exceeded. Therefore, insecticides are currently the principle method for controlling the Egyptian cotton leafworm in Egypt and will likely continue to be used until more biologically based management systems could be developed (Alotaibi, 2013).

Chlorpyrifos is a broad-spectrum organophosphorus insecticide, used for controlling cotton leafworm, aphids, whiteflies, roundworms and various crop pests (Fenske *et al.*, 2002). The objectives of the present study are to determine the physico-chemical properties of some chlorpyrifos (48% EC) commercial formulations and to evaluate the efficiency of these formulations under both laboratory and field conditions against 2nd and 4th instar larvae of *S. littoralis.*

MATERIALS AND METHODS

Insecticides

Nine commercial formulations of chlorpyrifos (48% EC) [O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate], namely Chlorban (CAM Agrochemicals Company. Nubariyah-Fertilizers and Diversified Chemicals Division, Alexandria), Chlorzane (Kafr El-Zayat Pesticides and Chemicals Company, Kafr El Zayat, Gharbia, Dursban H (Dow Chemical Company. Midland. USA), Ictafos (El-Nasr Company for Intermediate Chemicals. Cairo-Alexandria Desert Road km 28, Industrial Area, Abou-Rawash, Giza), Pestban (National for Agrochemicals & Investment. Elhorreya Road. Alexandria), Pyriban A (El-Helb Misr for Pesticides and Chemicals Company. Industrial Zone Area-Near Arab Contractors, New Damietta City), Pyrifos El-Nasr (El-Nasr Company for Intermediate Chemicals. Cairo-Alexandria Desert Road Kilo 28, Industrial Area, Abou-Rawash, Giza), Tafaban (El-Helb Misr for Pesticides and Chemicals Company. Industrial Zone Area-Near Arab Contractors, New Damietta City), Terraguard (Alexandria for Chemicals Company. Lewaa Al Eslam St., Kafr El Dawar, Al Beheira) were selected in the present study.

Physico-chemical properties of chlorpyrifos commercial formulations

Emulsion stability tests

The test was carried out according to CIPAC specifications (Anonymous, 1970). To prepare standard hard water, anhydrous calcium chloride (0.304 g) and magnesium chloride hexahydrate (0.139 g) were dissolved in distilled water and completed to 1 L. The resultant water has a hardness of 342 mg/L calculated as calcium carbonate.

a. Initial emulsification test

Measuring cylinders (100 ml) were filled to 95 ml mark with hard water at 30±1 ºC. The emulsifiable concentrate (EC) was poured gently (5 ml from a measuring cylinder) on to the surface of the water. The cylinder was stoppered and inverted once. After 30 sec., the formation of froth layer was noted.

b. Emulsion stability on standing

The cylinder, containing 95 ml hard water and 5 ml of EC formulation, was inverted 10 times and was allowed to stand undisturbed in the constant temperature bath at 30±1 ºC. The volume (ml) of free oil and/or cream layer, if any, formed either at the top or the bottom of the cylinder was recorded after standing for 30 min, 2 h and 24 h.

c. Re-emulsification after standing for 24 h

At the end of the 24 hours period, the cylinder was inverted 10 times and allowed to stand for 30 sec. The formation of free oil, cream or solid matter was observed.

d. Final emulsion stability test

The cylinder was allowed to remain undisturbed for a further period of 30 min. The volume of free oil, cream or solid matter, if any, was recorded. The volume of free oil, cream or solid matter, if any, should not exceed two ml at any period of the previous stages.

Foam formation

The method was carried out according to CIPAC specifications (Anonymous, 1970). The sample (5 g) was added to 95 ml of hard water in 100 ml measuring cylinder and made up to the mark. The cylinder was stoppered and inverted 30 times. The cylinder was stand on the bench and left undisturbed for one minute and examined for foam formation. The foam volume of chlorpyrifos should not exceed 20 ml.

Spontaneous emulsification test

The method was carried out as described by El-Akkawi (1970). Ninety five ml of hard water was placed in 100 ml graduated cylinder. Five ml of emulsifiable concentrate formulation were added at about 5 cm distance up the liquid surface in the cylinder. The amount of formed self-emulsion was recorded, directly, after addition of formulation.

Acidity determination

The test was carried out according to CIPAC specifications (Anonymous, 1970). The sample (10 g) was weighed and added to 100 ml (total volume) of distilled water and titrated with sodium hydroxide solution (0.02 M), by using methyl red as indicator. Blank determination was carried out, but without using the sample. The acidity values should not exceed 0.05% calculated as H_2SO_4.

Acidity = 0.0098 ($V_1 + V_2$);

Where: V_1 = volume (ml) of sodium hydroxide solution (0.02 M) used for the insecticide sample.

V_2 = volume (ml) of hydrochloric acid solution (0.02 M) used in the blank.

Insect

A laboratory strain of *Spodoptera littoralis* was reared on castor leaves according to El-Defrawi *et al.* (1964) under controlled conditions (25±2 ˚C and 65±5% R.H.) in laboratory of National Research Centre, Dokki, Cairo. The second and fourth instar larvae were used in the experiments.

Insecticidal activity against *Spodoptera littoralis*

Laboratory experiments

Dipping technique was carried out as described by Shepard (1958). Leaves of castor bean were soaked for 5 seconds in a series of concentrations of each tested formulation in water. The treated castor oil leaves were allowed to dry and the leaves were placed in Petri-dishes (15 cm diameter) with 10 larvae of either 2nd or 4th instar larvae. Four replicates were carried out for each treatment. Larvae in control treatment were fed on leaves treated only with water. The mortality percentages were recorded after 24 hours of treatment. The mortality data

were subjected to Probit analysis to obtain the LC_{50} values (Finney, 1971). The values of LC_{50} were considered significantly different, if the 95% confidence limits did not overlap.

Field experiments

Field experiments were carried out at Faculty of Agriculture Farm, Abees, Alexandria Governorate, during 2010 and 2011 seasons, cultivated with cotton, *Gossypium barbadens*, (Giza 86 variety). Seeds were planted in rows with width of 65 cm, the planting distance was 25 cm. The experimental design was randomized complete block design. Each treatment includes four replicates in experimental plots. The area of each plot was 42 m^2. The application of the formulations was carried out by using Knapsack sprayer (CP-3) equipped with one nozzle. Treatments were sprayed using the recommended rates (1 L/Feddan), as described in pest control program, Ministry of Agriculture, Egypt (2010). Each replicate was thoroughly sprayed using 6 liters of each insecticide dilution. Control plants were sprayed with an equal volume of water. Leaves of five plants of treated and control plots were collected randomly and transferred in perforated paper bags to the laboratory. Leaves were placed in cups (100 ml) with 10 larvae of each 2nd or 4th instar larvae. Leaves were collected at zero time and 1, 2, 4, 6 and 9 days after application. The mortality percentages of *S. littoralis* larvae were determined at each collection interval.

Statistical analysis

Statistical design was a randomized complete block design. Data were subjected to one-way analysis of variance followed by Student–Newman–Keuls test; Cohort software Inc. (Anonymous, 2004) to determine significant differences among mean values at the probability levels of 0.01. The concentration–mortality data were subjected to Probit analysis to obtain the LC_{50} values using the SPSS 21.0 software program (Anonymous, 2012).

RESULTS AND DISCUSSION

Physico-chemical properties of chlorpyrifos commercial formulations

Emulsion stability

The separation volumes (ml) after 30 sec (initial emulsification), 30 min, 2 and 24 h (emulsion stability on standing), re-emulsification after 24 h and final emulsion stability after 24.5 h were investigated. All chlorpyrifos formulations passed the test after 30 sec, 30 min and 2 h, except Dursban which failed in the test after 30 min and 2 h (creaming layers of Dursban were 3 and 5.1 ml after 30 min and 2 h, respectively) (Table 1). Chlorban, Dursban and Pestban failed in the test after 24 h, whereas the creaming layers were 3.6, 6.2 and 4.8 ml, respectively. All chlorpyrifos formulations passed the re-emulsification test after standing for 24 h. Similarly, the tested insecticides passed the final emulsion stability after 24.5 h, but Dursban failed in the test with creaming layer of 5.3 ml. The insecticides that passed the emulsion stability at all intervals were Chlorzane, Ictafos, Pyriban, Pyrifos El-Nasr, Tafaban and Terraguard.

Foam formation

The amount of foam was measured after one min. Results obtained showed that, all chlorpyrifos formulations passed the test (the limit of foam layer volume should not exceed 20 ml, Anonymous, 1970). This means that, the tested formulations can be sprayed in the field without any foam problems.

Spontaneous emulsification

Data revealed that, all chlorpyrifos formulations gave full (100%) spontaneous emulsification because all tested formulations had a specific gravity higher than that of water. The results are in good agreement with the results of El-Okda (1970), who reported that the emulsifiable concentrates which had a specific gravity more than one gave full spontaneous emulsification.

Table 1. Physico-chemical properties of chlorpyrifos commercial formulations

Product	Emulsion stability (separation ml)						Foam formation (ml)	Spontaneous emulsification (% emulsification)	Acidity (% H_2SO_4)
	Initial emulsification	Emulsion stability on standing			Re-emulsification after standing for 24 h	Final emulsion stability after 24.5 h			
	after 30 sec	after 30 min	after 2 h	after 24 h					
Chlorban	0	1.1	1.8	3.6	0	0.9	7	100	0.05
Chlorzane	0	0	0.5	1.9	0	0	11	100	0.24
Dursban	0	3	5.1	6.2	0.3	5.3	12	100	0.1
Ictafos	0	0.1	1.1	2	0	0.4	7.6	100	0.23
Pestban	0	0	0	4.8	0	0	10	100	0.32
Pyriban	0	0	0	0.8	0	0	7	100	0.12
Pyrifos El-Nasr	0	0.1	1	1.8	0	0.4	20	100	0.13
Tafaban	0	0	0.5	1.4	0	0.3	8	100	0.06
Terraguard	0	0	1.1	1.5	0	0	6	100	0.09

Acidity determination

Chlorban was the only formulation that passed the acidity test where its acidity was 0.05 calculated as % H_2SO_4 (the acidity values should not exceed 0.05% calculated as H_2SO_4, Anonymous, 1970).

Insecticidal efficiency of chlorpyrifos commercial formulations against *Spodoptera littoralis*

Under laboratory conditions

The LC_{50} (mg/L) values, 95% confidence limits and other regression analysis parameters of chlorpyrifos commercial formulations against the 2nd and the 4th instar larvae of *S. littoralis* are given in Table (2). Dursban was the most effective one among all the tested insecticides with LC_{50} value of 0.2 mg/L, followed by Pestban and Tafaban with LC_{50} value of 0.39 mg/L against the 2nd instar larvae of *S. littoralis*. Furthermore, Pyriban, Chlorzane and Ictafos showed high toxicity with LC_{50} values of 0.41, 0.54 and 0.55 mg/L, respectively, while Pyrifos El-Nasr was the least effective formulation with LC_{50} value of 1.4 mg/L against the previous instar larvae.

In the case of the 4th instar larvae, Dursban showed the highest insecticidal activity with LC_{50} value of 1.11 mg/L, followed by Pestban, Chlorzane, Pyriban and Tafaban with LC_{50} values of 1.23, 1.66, 1.68 and 1.7 mg/L, respectively. Pyrifos El-Nasr showed the least insecticidal activity with LC_{50} value of 3.78 mg/L. These results agreed with Ebeid and Gesraha (2012), who investigated the sensitivity of *S. littoralis* 6th larval instar toward Pyriban under laboratory conditions. El-Khayat *et al.* (2012) found that, chlorpyrifos was the most effective insecticide among six insecticides belonging to different groups; the LC_{50} values recorded 0.1 and 0.472 ppm for 2nd and 4th instars larvae, respectively.

Under field conditions

Mortality percentages of 2nd and 4th instar larvae of *S. littoralis* treated with chlorpyrifos (48% EC) commercial formulations at the recommended rate in cotton season 2010 are shown in Table (3). The recorded results revealed that, all chlorpyrifos formulations gave mortality percentages of 100 at zero time against 2nd instar larvae. Chlorzane, Dursban, Ictafos, Pestban, Pyriban, Tafaban and Terraguard gave the highest mortality percentages (there were no significant differences between

Table 2. Toxicity of chlorpyrifos commercial formulations against 2nd and 4th instar larvae of *Spodoptera littoralis* using dipping technique.

Product	Instar larvae	LC_{50}[a] (mg/L)	95% Confidence limits (mg/L)		Slope ±SE[b]	Intercept ±SE[c]	(χ^2)[d]
			Lower	Upper			
Chlorban	2nd	0.85	0.64	1.03	3.2 ± 0.28	0.22 ± 0.06	5.37
Chlorzane		0.54	0.47	0.62	2.28 ± 0.18	0.6 ± 0.07	3.63
Dursban		0.2	0.09	0.35	1.84 ± 0.14	1.27 ± 0.1	12.98
Ictafos		0.55	0.07	1.54	1.5 ± 0.14	0.38 ± 0.06	21.62
Pestban		0.39	0.22	0.64	1.96 ± 0.15	0.79 ± 0.08	10.76
Pyriban		0.41	0.14	0.77	1.74 ± 0.15	0.66 ± 0.07	15.15
Pyrifos El-Nasr		1.4	1.12	1.67	4.86 ± 0.38	− 0.72 ± 0.1	8.89
Tafaban		0.39	0.13	0.72	1.92 ± 0.16	0.77 ± 0.07	16.94
Terraguard		0.96	0.59	1.34	2.58 ± 0.24	0.03 ± 0.06	10.11
Chlorban	4th	3.31	3.07	3.53	10.5 ± 0.82	− 5.46 ± 0.44	6.01
Chlorzane		1.66	1.57	1.74	6.32 ± 0.47	− 1.39 ± 0.13	1.77
Dursban		1.11	0.79	1.44	4.01 ± 0.3	− 0.19 ± 0.07	12.36
Ictafos		2.23	2.13	2.33	7 ± 0.55	− 2.45 ± 0.21	1.54
Pestban		1.23	0.93	1.53	3.44 ± 0.3	− 0.31 ± 0.07	7.68
Pyriban		1.68	1.46	1.89	5.82 ± 0.44	− 1.31 ± 0.13	5.84
Pyrifos El-Nasr		3.78	3.68	3.87	11.95 ± 0.94	− 6.91 ± 0.55	4.5
Tafaban		1.7	1.42	1.96	5.63 ± 0.44	− 1.29 ± 0.13	8.35
Terraguard		3.25	3.15	3.34	10.31 ± 0.81	− 5.28 ± 0.43	4.76

[a] Concentration causing 50% mortality after 24 h. of treatment.
[b] Slope of concentration mortality regression line.
[c] Intercept of regression line.
[d] Chi square value.

Table 3. Mortality percentages of 2nd and 4th instar larvae of *Spodoptera littoralis* treated with chlorpyrifos (48% EC) commercial formulations under field conditions in season 2010[1].

Product	% Mortality after days of treatment									
	2nd instar larvae					4th instar larvae				
	0	1	2	4	Mean	0	1	2	4	Mean
Chlorban	100	77.5[bc3]	32.5[c]	17.5[de]	56.8[de]	100[a3]	60[cd]	20[de]	0[c]	45[d]
Chlorzane	100	90[a]	50[b]	35[abc]	68.7[b]	100[a]	87.5[a]	37.5[bc]	5[bc]	57.5[b]
Dursban	100	92.5[a]	75[a]	45[a]	78.1[a]	100[a]	90[a]	60[a]	37.5[a]	71.8[a]
Ictafos	100	85[ab]	35[c]	15[de]	58.7[cd]	100[a]	72.5[b]	30[bcd]	0[c]	50.6[c]
Pestban	100	92.5[a]	70[a]	40[ab]	75.6[a]	100[a]	87.5[a]	40[b]	7.5[b]	58.7[b]
Pyriban	100	90[a]	40[bc]	22.5[cde]	63.1[bcd]	100[a]	85[a]	30[bcd]	0[c]	53.7[c]
Pyrifos El-Nasr	100	72.5[c]	17.5[d]	12.5[ef]	50.6[e]	82.5[b]	52.5[d]	12.5[e]	0[c]	36.8[e]
Tafaban	100	90[a]	42.5[bc]	27.5[bcd]	65[bc]	100[a]	85[a]	32.5[bc]	0[c]	54.3[bc]
Terraguard	100	90[a]	37.5[bc]	2.5[f]	57.5[d]	87.5[b]	67.5[bc]	27.5[cd]	0[c]	45.6[d]
Control	0	2.5[d]	0[e]	0[f]	0.6[f]	2.5[c]	0[e]	0[f]	0[c]	0.6[f]
L.S.D $_{0.01}$[2]	–	11.84	14.2	13.14	6.73	7.51	11.23	11.43	5.96	4.6

[1] Data are means of 16 repetitions.

[2] L.S.D $_{0.01}$ least significant difference at 0.01 level of probability.

[3] Means followed by the same letter in a column are not significantly different at 1% level of probability (Duncan Test).

these formulations) ranged from 85 to 92.5 after one day from treatment, while Pyrifos El-Nasr was the least effective formulation with mortality percentage of 72.5. In addition, Dursban and Pestban gave the highest mortality percentages of 75 and 70, respectively after two days from treatment, followed by Chlorzane, Pyriban, Tafaban and Terraguard (there were no significant differences between these formulations), where mortality percentages ranged from 37.5-50; Pyrifos El-Nasr gave the least mortality percentage of 17.5. Moreover, Dursban, Pestban and Chlorzane were the most efficient formulations after four days from treatment, as mortality percentages were 45, 40 and 35, respectively, while there were no significant differences between Pyrifos El-Nasr, Terraguard and control treatment after four days. In general, Dursban and Pestban through 4 days were the most efficient formulations in controlling *S. littoralis* as average of total mortality percentages were 78.1 and 75.6, respectively, followed by Chlorzane, Tafaban and Pyriban as mortality percentages were 68.7, 65 and 63.1, respectively, while Chlorban and Pyrifos El-Nasr were the least efficient formulations as mortality percentages were 56.8 and 50.6, respectively.

Data of mortality percentages of 4th instar larvae of *S. littoralis* revealed that, all chlorpyrifos formulations gave mortality percentages of 100 at zero time, except Pyrifos El-Nasr and Terraguard as mortality percentages were 82.5 and 87.5, respectively. On the other hand, Chlorzane, Dursban, Pestban, Pyriban and Tafaban gave high mortality percentages (there were no significant differences between these formulations) ranged from 85 to 90 after one day from treatment, followed by Terraguard and Ictafos as mortality percentages were 67.5 and 72.5, respectively, while Pyrifos El-Nasr gave the least mortality percentage of 52.5. In addition, Dursban gave the highest mortality percentage of 60, followed by Chlorzane, Ictafos, Pestban, Pyriban and Tafaban, where mortality percentages ranged from 30-40, whereas Pyrifos El-Nasr gave the least mortality percentage of 12.5 after two days from treatment. In addition, Dursban gave the highest mortality percentage of 37.5 after four days, followed by Chlorzane and Pestban as mortality percentages were 5 and 7.5, respectively, while mortality percentages of Chlorban, Ictafos, Pyriban, Pyrifos El-Nasr, Tafaban and Terraguard were zero. In general, Dursban was the most efficient formulation in controlling *S. littoralis* as average of total mortality percentages was 71.8, followed by Tafaban, Chlorzane, and Pestban as mortality percentages were 54.3, 57.5 and 58.7, respectively, while Pyrifos El-Nasr showed the least efficiency as mortality percentage was 36.8.

Mortality percentages of 2nd and 4th instar larvae of *S. littoralis* treated with chlorpyrifos (48% EC) commercial formulations at the recommended rate in season 2011 are shown in Table (4). The obtained results were similar to those of season 2010, all chlorpyrifos formulations gave complete mortality at zero time against 2nd instar larvae. In addition, Chlorzane, Dursban, Ictafos, Pestban, Pyriban and Tafaban gave the highest mortality percentages (there were no significant differences between these formulations) ranged from 87.5-97.5 after one day of treatment, while Pyrifos El-Nasr was the least efficient as mortality percentage was 80. Moreover, Dursban and Pestban gave the highest mortality percentages of 77.5 and 75, respectively after two days of treatment, followed by Chlorzane, Ictafos, Pyriban and Tafaban, where mortality percentages ranged from 47.5 to 60, while Pyrifos El-Nasr gave the least mortality percentage of 40 at the same duration. In addition, Dursban, Pestban and Chlorzane were the most efficient formula-

Table 4. Mortality percentages of 2nd and 4th instar larvae of *Spodoptera littoralis* treated with chlorpyrifos (48% EC) commercial formulations under field conditions in season 2011[1].

Product	% Mortality after days of treatment									
	2nd instar larvae					4th instar larvae				
	0	1	2	4	Mean	0	1	2	4	Mean
Chlorban	100a3	82.5cd	42.5c	20ef	61.2e	100a3	65cd	25de	5cd	48.7ef
Chlorzane	100a	92.5abc	60b	37.5abc	72.5b	100a	85ab	42.5c	12.5bc	60bc
Dursban	100a	97.5a	77.5a	47.5a	80.6a	100a	90a	62.5a	40a	73.1a
Ictafos	100a	87.5abcd	47.5bc	25def	65cde	100a	75bc	30d	5cd	52.5de
Pestban	100a	95ab	75a	42.5ab	78.1a	100a	87.5a	50b	20b	64.3b
Pyriban	100a	90abcd	50bc	30cde	67.5bcd	100a	82.5ab	32.5d	7.5cd	55.6cd
Pyrifos El-Nasr	100a	80d	40c	17.5f	59.3e	90c	60d	20e	5cd	43.7f
Tafaban	100a	92.5abc	52.5bc	32.5bcd	69.3bc	100a	82.5ab	40c	7.5cd	57.5cd
Terraguard	100a	85bcd	45c	20ef	62.5de	95b	67.5cd	27.5d	5cd	48.7ef
Control	2.5b	2.5e	0d	5g	2.5f	0d	2.5e	0f	2.5d	1.2g
L.S.D $_{0.01}$[2]	3.07	10.35	12.8	11.08	5.61	3.55	10.5	7.1	9.39	4.88

[1] Data are means of 16 repetitions.

[2] L.S.D 0.01 least significant difference at 0.01 level of probability.

[3] Means followed by the same letter in a column are not significantly different at 1% level of probability (Duncan Test).

tions after four days of treatment, as mortality percentages were 47.5, 42.5 and 37.5, respectively. Chlorban, Ictafos, Pyrifos El-Nasr and Terraguard gave the least mortality percentages at the same duration.

All chlorpyrifos formulations gave complete mortality at zero time against 4th instar larvae, except Pyrifos El-Nasr and Terraguard as mortality percentages were 90 and 95, respectively. Chlorzane, Dursban, Pestban, Pyriban and Tafaban gave the highest mortality percentages (there were no significant differences between these formulations) ranged from 82.5 to 90 after one day of treatment, followed by Ictafos as mortality percentage was 75, while Pyrifos El-Nasr gave the least mortality percentage of 60. In addition, Dursban gave the highest mortality percentage of 62.5 after two days of treatment, followed by Pestban, where mortality percentage was 50. On the other hand, Pyrifos El-Nasr gave the least mortality percentage of 20 after two days. Similarly, Dursban gave the highest mortality percentage of 40 after four days of treatment, followed by Chlorzane and Pestban as mortality percentages were 12.5 and 20, respectively, while there were no significant differences between Chlorban, Ictafos, Pyriban, Pyrifos El-Nasr, Tafaban, Terraguard and control treatment. In general, Dursban was the most efficient formulation in controlling *S. littoralis* as average of total mortality percentages was 73.1, followed by, Pestban and Chlorzane as mortality percentages were 64.3 and 60.0, respectively, while Pyrifos El-Nasr was the least efficient formulation as mortality percentage was 43.7 through 4 days of treatment.

In general, Dursban and Pestban were the most efficient formulations for controlling *S. littoralis* followed by Chlorzane, Tafaban and Pyriban, while Pyrifos El-Nasr and Chlorban were the least efficient formulations. These results matched with the results obtained from laboratory experiments in which Dursban was the most effective formulation against *S. littoralis* and Pyrifos El-Nasr was the least effective one. These results are in agreement with Klein (1984) who mentioned that chlorpyrifos showed high activity to control young larvae of *S. littoralis* after one day of treatment and this efficiency was decreased with time. Moreover, Ahmed *et al*. (2004) mentioned that chlorpyriphos showed about 87% reduction in number of *S. littoralis* based on prespray data. In addition, Shivankar *et al*. (2008) indicated that chlorpyrifos 20 EC recorded high reduction (94.1%) of *S. litura* in sugar beet.

In conclusion, the results indicated that some of chlorpyriphos commercial formulations are not meeting CIPAC specifications which may decrease the efficiency and storage stability of these formulations. Moreover, chlorpyriphos commercial formulations revealed different insecticidal activity either in laboratory or in field indicating that the growers have to pay more attention to formulation manufactures. The results also showed that the formulations were not effective against *S. littoralis* after four days of spraying. Therefore, new strategies have to be taken to overcome the low persistence and enhance the efficiency of formulations in field. Among the suggested strategies are the uses of insecticides mixtures and tank-mix adjuvants.

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المواصفات الكيموفيزيائية والفعالية البيولوجية لتجهيزات مبيد الكلوربيريفوس التجارية ضد دودة ورق القطن تحت الظروف المعملية والحقلية

إلهام سمور[١]، أحمد البكري[١]، ناهد عبد العزيز[١]، شحاته قاسم[٢]، سمير عبد الجليل[٢]

[١] قسم آفات ووقاية النبات–المركز القومي للبحوث

[٢] قسم كيمياء وتكنولوجيا المبيدات–كلية الزراعة–الشاطبي

تم قياس المواصفات القياسية لتسعة تجهيزات تجارية من مبيد الكلوربيريفوس ٤٨٪ مركزات قابلة للاستحلاب (إي سي)، وهذه المبيدات هي كلوربان، كلورزان، دورسبان، اكتافوس، بستبان، بيريبان، بيريفوس النصر، تافابان، بالإضافة إلى تيراجارد، وذلك طبقا لمنظمة السيباك الدولية، هذا وقد تم اختبار الكفاءة الإبادية لهذه المبيدات معمليا وحقليا علي يرقات العمر الثاني والرابع لدودة ورق القطن. وقد أظهرت النتائج أن مبيدات كلورزان، اكتافوس، بيريبان، بيريفوس النصر، تافابان وتيراجارد قد اجتازت اختبار ثبات المستحلب، وفي نفس الاتجاه، فقد اجتازت جميع المبيدات اختبار تكوين الرغاوي، كما أن جميع المبيدات أعطت استحلاب ١٠٠٪، ومن ناحية أخرى، فإن مبيد كلوربان كان الوحيد الذي نجح في اختبار الحموضة، حيث أن درجة الحموضة لهذا المبيد كانت ٠,٠٥ محسوبة كنسبة مئوية لحامض الكبريتيك. وقد أظهرت النتائج المتعلقة بالكفاءة الإبادية لتجهيزات الكلوربيريفوس ضد يرقات العمر الثاني والرابع لدودة ورق القطن أن مبيد الدورسبان كان هو الأكثر فاعلية تحت الظروف المعلية، حيث سجلت قيم التركيزات المسببة لموت ٥٠٪ من الأفراد المعاملة قيم ٠,٢ و١,١١ مجم/لتر على يرقات العمر الثاني والرابع، على الترتيب، بينما كان مبيد بيريفوس النصر هو الأقل كفاءة، حيث كانت التركيزات المسببة لموت ٥٠٪ من الافراد المعاملة ١,٤ و٣,٧٨ مجم/لتر، على يرقات العمر الثاني والرابع، على التوالي. وقد كان مبيدي الدورسبان والبستبان هما الأكثر فاعلية تحت الظروف الحقلية، في مكافحة يرقات العمر الثاني لدودة ورق القطن، حيث كان متوسط نسب الموت الكلية ٧٨,١ و٧٥,٦٪ على التوالي، موسم ٢٠١٠، و٨٠,٦ و٧٨,١٪ على التوالي، موسم زراعة القطن ٢٠١١. وبطريقة مشابهة فقد كانا هذان المبيدان هما الأكثر كفاءة ضد يرقات العمر الرابع. من الجدير بالذكر أن الكفاءة الإبادية لمعظم المبيدات استمرت لمدة أربعة أيام فقط من وقت المعاملة، لذلك فإن هذه التجهيزات من المبيدات تحتاج لإضافة مواد محسنة مناسبة لزيادة فترة ثباتها، ومن ثم زيادة الكفاءة الإبادية تحت الظروف الحقلية.

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Persistence and Distribution for Three Alginate Controlled-Release and Granular Formulations of Aldicarb in the Soil-Tomato Plant System under Greenhouse Conditions

Hamdy R. Soltan, A. S. Marei and A.Mousa

Pesticide Chemistry Dept., Faculty of Agriculture, Alexandria University, Alex., Egypt

ABSTRACT

In pot experiment, the released aldicarb from three alginate controlled-released (FA_3, FA_6, and FA_9) formulations and the corresponding granule formulation (G15%) were evaluated, vis-à-vis leachability as well as its residue levels in sandy soil. Residues of aldicarb in soil could be taken up by the root system and translocated to all parts of tomato plants were determined under greenhouse conditions. The portion of the active ingredient still incorporated in different formulations was determined at the end of the experiment. Leaching potential of the alginate formulation (FA9) decreased more than 3.5 times (24.1%) compared with the G15% (82.49%) over 72 days after application. The concentration of aldicarb residues found in soil treated with the granular formulations, was lower than the concentration residues released from alginate formulations, after 18 days of application. Thereafter, the aldicarb residue was decreased rapidly with the time till the experiment ended for all the tested formulations. However, the total residues of aldicarb found were 23.06%, 16.7%, and 12.23% for FA_3, amount applied. Tomato plant grown in soil pot treated with alginate formulations; took up more amounts of these residues than G15%. Generally, aldicarb residues found in the root system were higher than that found in vegetation part treated with granular formulation. The opposite trend was found in case of tomato treated with the alginate formulations. No detectable amount of aldicarb was found in tomato fruits treated with G15%at the end of the experiment (72 days). Total concentration residues of aldicarb found in both vegetation and root systems were 5.45% and 4.40% for FA_3, 3.63% and 2.92% for FA_6, and 2.41% and 2.00% for FA_9, respectively. The amount of aldicarb still incorporated within the controlled release formulations ranged from 34.18% to 67.89%, after 18 days of application. The amount of aldicarb still incorporated in alginate formulations reached to the minimum (16.72% - 38.87%) whereas, for granule formulation was 0.0065%, after 72 days of application.

Key words: Alginate controlled release formulations, temik and environmental fate

INTRODUCTION

Meloidogyne incognita is one of the most harmful root-knot nematode species, which infects a wide range of vegetable crops in Egypt (Ibrahim *et al*., 2000). Root-knot nematodes infection of tomatoes limits fruit production by 30% (Anwar and McKenry, 2010). Among various control measures, use of chemicals has been considered as an effective strategy for control of plant parasitic nematodes when other methods like cultural practices, resistant varieties and bio-control agents are unable to protect crops from these pests (Randhawa *et al*., 2001; Sakhuja and Jain, 2001). Currently various fumigants and non-fumigants are available in the market against these pests (Rich *et al*., 2004). Granular non-fumigant nematicides are more easily applied and safer for farmers compared with fumigants (Lamberti *et al*., 2000). Among the most extensively used are granular form of aldicarb and cadusafos which are carbamate and organophosphate based nematicides. Although chemical nematicides are effective, easy to apply and show rapid effects, they have begun withdrawn from the market owing to concerns about public health and environmental safety (Rich *et al*., 2004). Controlled-release formulations technology offers the potential to reduce the environmental losses of pesticides and hence may be increased their efficacy, reduce their side effect to non-target organisms and reduce their harmful effect on growth and nutrient contents of crops (Marei, *et al*., 2000; Soltan 1996; Soltan, *et al*., 2001 and Soltan *et.al.,* 2015). Alginate gels are biodegradable and pesticides can be easily incorporated into the matrix using an aqueous system at ambient temperature. For all the reasons mentioned above, alginate gels were selected as matrices for preparation of new controlled-release formulations of aldicarb nematicide (Soltan *et.al.,* 2014). The objective of this study was to evaluate three developed alginate controlled-release formulations (FA_3, FA_6, and FA_9) compared to commercial formulation (15G) of aldicarb vis-à-vis leachability in the sandy soil under greenhouse conditions. In addition to, determination of aldicarb residue levels in soil, the amount uptake in tomato from soil, and unreleased portion of active ingredient still present in different formulations.

MATERIALS AND METHODS

Preparation on controlled – release formulations of aldicarb

Different types of alginate controlled-release formulations (CRF.s) for aldicarb were prepared in the present study according to the method described by Soltan *et.al.* 2014. Among the CRF·s prepared, FA_3, FA_6 and FA_9 were selected according to their appropriate release rate comparable (Table 1 and Fig.1). Commercial formulation (15G) for aldicarb was provided by Agrochem Company, Egypt.

Table (1): Average weight, number of beads/g and percentage of active ingredient of different controlled release alginate formulations of aldicarb

Pesticide	Formulation code No	Average weight/ bead (g)	Number of Beads/g	Active Ingredient %
	FA3*	0.989	1023	13.96
Aldicarb	FA6	3.355	298	17.24
	FA9	5.263	190	18.89

*FA= Aldicarb controlled release formulations.

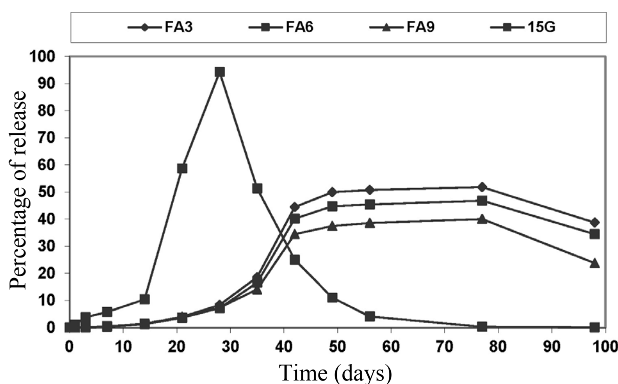

Fig. (1): Percentages of aldicarb released from different formulations in water in a closed bottle kept under static conditions

Experimental design:

Two separate experiments were carried out in this study. The first experiment, plastic pots (20 cm in diameter and 25 cm deep), with drainage tube in the bottom were prepared in order to collect leachate water. The pots were filled with 3 kg Sandy soil each. Two tomato seedlings 2 weeks old (*Lycopersicom esculentum* L. var. estrin B) were transplanted. Recommended agriculture practices for fertilization and irrigation were followed. The pots were left one week before pesticide application to acclimate the tomato seedlings under the greenhouse conditions. Aldicarb alginate controlled release formulations (FA_3, FA_6, FA_9,) and its granular formulation (15G), 3 days latter were applied on the surface layer of the soil at the recommended rate (1.5 mg a.i. /kg soil. All the different formulations were placed in plastic net in the form of tea packet, 2 cm depth from the surface. Untreated pots served as control. Treatments were arranged in a randomized complete block design. Temperature ranged between 24 to 39°C and the humidity ranged from 69% to 81% until the end of experiment. Water was added to each pot at the rate of 50 ml/pot, two times per weeks. Water leachate from the bottom of each pot was collected at intervals time (3,6,12,18,24,30,36,42,48,54,60,66, and 72 days).

The second experiment was carried out at the same condition under greenhouse, time and treatment as mentioned above. The residue levels of aldicarb in soil and tomato plant were evaluated at 18, 36, 54 and 72 days after treatment. In addition, the aldicarb formulations were collected to analyze the portion of aldicarb still incorporated in the different formulations at 18, 36, 54, 72 days. At the experiment ended, tomato fruits were collected and the residue levels of aldicarb were determined.

Determination of aldicarb in leachate water:

Water fractions (45±5ml) leached from the bottom of each pot were collected twice per week at the initial period of the experiment, and 6 day interval till the end of the experiment. One ml water from each fraction (4 replicates), without extraction, was transferred to a clean dry test tube. The procedure of determination of aldicarb pesticide was carried out as described by Soltan *et.al.,* 2014.

Extraction and determination of aldicarb in sandy soil:

Soil samples collected from each pot were air-dried for 24 hrs at room temperature. Aldicarb was extracted from soil according to the method described by Ambrus *et al.*, (1981). 50 gm homogenous dry soil sample was transferred to 250 ml conical flask and shaken for 2 hours with 200 ml mixture of 0.25 M HCl and acetone (7:3 V/V) to extract aldicarb residues. The Samples were filtered by using Buchner's funnel. The filtrate was transferred to 500 ml separatory funnel, and then sample was shaken three times with 3x50 ml methylene chloride and hexane (1:1 v/v). These extracts were combined, passed through anhydrous Na_2SO_4 and evaporated in a vacuum evaporator to dryness. Aldicarb residue was redissolved in total volume of 200 ml distill water, one ml quantitatively transferred to a clean dry test tube and then determined using new colorimetric methods (Soltan *et.al.,* 2014).

Extraction and determination methods of aldicab for shoot, root parts, and tomato fruit of tomato plant:

Aldicarb residues were extracted from shoot, root, and tomato fruit according to the method reported by Ambrus *et al.*, (1981). At 18,36,54, and 72 days after pesticide application, except for tomato fruit at 72 days only, the tomato plants were carefully removed from soil, washed with tap water to remove soil particles from roots and divided in two parts (shoots and roots) and the fresh weight was recorded. Plant parts were cut into small pieces with a single-edge razor blade, then 50 gm of fresh weight from each part was taken as sub-sample with three replicates and extracted by blending with 200ml distill water and acetone (2:1 v/v) and 0.2 gm of activated charcoal (to remove the chlorophyll dye) for 2 min., and filtered through Buchner's funnel. The residual tissues were washed with acetone one more time. Combined acetone extracts were quantitatively transferred to a 500 ml separatory funnel, then partitioned three times with 3x50 ml methylene chloride and hexane (1:1 v/v). The organic layer was combined, and passed through anhydrous sodium sulfate; then evaporated in a vacuum at 40 ^{O}C to dryness. Residues of aldicarb redissolved

in 150 ml distill water. One ml was taken to determine for aldicarb by colorimetric method (Soltan *et.al.,* 2014).

Extraction and determination of aldicarb incorporated in the different formulations:

At 18, 36, 54, and 72 days of formulations application, the plastic closed net was removed carefully from soil and cleaned up from soil particles. Different formulations (FA3, FA6, FA9, and 15G) were transferred to the laboratory. The beads were extracted by sonicting for 1hr with water 70% and acetone 30% mixture. The extract was decanted and the beads were extracted two more times. The combined extract was portioned three times with 3x50 ml ethylene chloride: hexane (1:1v/v). The procedure of extraction and determination of aldicarb was carried out as described by (Soltan *et.al.,* 2014).

RESULTS AND DISCUSSION

A- Residue levels of aldicarb in leachate water:

The aldicarb residue concentration in the leachate water of the sandy soil pots treated with commercial 15 G peaked to the maximum after 3 days of treatment (47.6%), and then declined rapidly, after 6 days of treatment Table (2). In contrast, the aldicarb residue levels in the leachate from alginate matrix slowly increased with time, mainly between 3 and 12 days. Thereafter, the amount of aldicarb which leached through the pots was almost constant up to 18 days after they were applied. The amount peaked to the maximum 24 days after applications, and then decreased slowly until the experiment ended. The FA_3, FA_6, and FA_9 as CRF leached at an end of the experiment (72 days) were 1.9%, 1.5% and 0.63%, respectively. Ttotal of 82.4997% of the applied aldicarb leached out from the commercial formulation in sandy soil over the 72 days period. As opposed to this, only 45.0544 %, 32.3444, and 24.108% of the aldicarb leached out from FA_3, FA_6, and FA_9 over the same period, respectively. This pattern is consistent with the rate of release of aldicarb from the three controlled release formulations and the granular formulation that was reported to be faster from the commercial formulation and slowest from FA_9. Generally, it should be noted that the alginate matrix incorporated aldicarb and filler materials such as kaolin slowed down the release rate of aldicarb that in turn decreased the environmental loss of aldicarb via leachability, and decreased the contamination of ground water and might be extended the pestcidal activity of aldicarb. To comparison between the CRFs and 15G, at the 54 days, the CRFs of aldicarb were constantly released until the end of experiment whereas, the 15G was non detectable at 48 days. These results are in agreement with Fatma *et al*., (1999). They found that carboxy methyl cellulose (CMC) as a matrix released 48-89% only aldicarb through eight weeks compared with the granular formulation which losses content immediately in water leachate. In addition, Kassem *et al*., (1985) they found that ethyl cellulose polymer as a matrix released 45.2% only aldicarb in water, while poly vinyl butyral released 83% of aldicarb after 16 weeks.

B- Persistent of aldicarb in sandy soil:

Aldicarb residue levels in sandy soil pots cultivated by tomato plants were evaluated at 18, 36, 54, and 72 days, under green house conditions, after treatment with different aldicarb formulations Table (3). The concentration of aldicarb residue in soil from granular formulation (15G) was lower than the concentration of aldicarb residue that released from the CR formulations (FA_3, FA_6, and FA_9) after 18 days of application. Thereafter, the aldicarb residue was rapidly decreased until the experiment ended for all tested formulations. The aldicarb residue levels found in the initial period (from 1-18 days) after aldicarb application were in the following order: FA_3 (14.32%), FA_6 (10.53%), FA_9 (8.18%) and 15 G (6.47%) However, after 72 days of application with the aldicarb formulations, the residue levels had decreased to 0.74-1.8% for alginate formulations, while the concentration from the commercial reached to 0.13%. Those results are consistent with that found in the release rate studies. However, the total amount of aldicarb found were 23%, 16.7%, 12.23% for FA_3, FA_6, and FA_9, respectively while, the granular aldicarb formulation was 9.18%. It could be noted the aldicarb was released quickly from the granular and had a short persist under temperature and humidity condition than that the aldicarb released from alginate formulations. This decreasing in aldicarb residue levels might be attributed to the aldicarb was rapidly oxidized to aldicarb oxide and sulfone ; and the aldicarb sulfoxide is approximately five-fold more soluble than aldicarb (James *et al*.,1974). Generally, the aldicarb released from CR formulations may be adsorbed on the soil particles more

Table (2): Residue levels of aldicarb in lachate water

Formulations	% Residue levels of aldicarb in leachate water (days)												
	3	6	12	18	24	30	36	42	48	54	60	66	72
FA3*	0.8	2.63	3.5	5	9.98	4.96	3.71	3.56	3.56	1.62	1.9	1.84	1.9
FA6	0.54	1.73	2.81	3.71	8.32	4.2	3.1	2.74	2.84	1.24	1.23	1.23	1.5
FA9	0.22	0.83	1.8	3.1	6.34	3.4	2.3	1.9	1.73	0.67	0.63	0.62	0.63
15G	47.6	23.8	5.85	4.31	0.92	0.068	0.0074	0.0008	*N.D	*N.D	*N.D	*N.D	*N.D

#N.D= non detected * FA = Alginate controlled release formulation

than that released from 15G. In addition, the aldicarb-released rate could be controlled by using alginate matrix and with Kaolin as filler material; and it may possible to change the release rate of it in sandy soil. These results also indicated that the aldicarb in FA_9, FA_6, and FA_3 formulations were persisted longer in soil than the 15G under experimental conditions and provided more protection to tomato plant against nematode at such a long period than the commercial formulation. These data are in agreement with those found by Kok *et al*., (1999) who mentioned that the ratio of lignin to carboxy methyl cellulose (CMC) and aldicarb to CMC may be delayed release effect of the controlled-release formulation compared to (a commercial preparation) aldicarb in soil, specially when lignin was incorporated.

Table (3): Percentage of aldicarb found in sandy soil cultivate with tomato seedlings under green house conditions.

Days after application	% of the total aldicarb recovered from sandy soil pots			
	FA3*	FA6	FA9	15G
18	14.3262	10.5339	8.1875	6.4776
36	5.1015	3.7910	2.5554	2.2184
54	1.7785	1.2543	0.7394	0.3556
72	1.8533	1.1232	0.7488	0.1310
Total	23.015	16.7024	12.2311	9.1826

* FA = Alginate controlled release formulation of aldicarb

C- Uptake and accumulation of aldicarb in vegetation part, root, and fruit of tomato.

Under green house conditions, aldicarb residue levels that up taken from soil treated with CR and granular formulations to tomato plant were determined (Table 4). The data showed that concentrations of aldicarb in vegetation part were accumulated; and the higher values recorded after 18 days treatment were 2.57%, 1.94%, 1.55%, and 2.1% for FA_3, FA_6, FA_9 and 15G, respectively. The residue levels found in the initial period were declined rapidly from the pots treated with the granular formulation and gradually for the pots treated with the CR formulations until the end of experiment. This might be referred to the amount released from the 15G formulation was converted to its metabolite products such as, aldicarb sulfoxide and aldicarb sulfone in soil that loss very rapidly via leachability (El-gendi *et al.,* 1978) and/or the dilution caused by the new growth in plant. At 72 days after treatments, the concentration of aldicarb from FA_3 (25.86 fold), FA_6 (15.61 fold), and FA_9 (4.8 fold) were equal approximately of the residue found in vegetation part that treated with 15G. It could be noted that the residue levels of aldicarb from CRFs may play an important role to prove an effective protection during the vegetation period against the insect attacking the leaves of tomato plant than the tomato plant treated with the 15G. This results, consistent with the release rate, may be related to the portion of aldicarb available in soil which in turn up taken and translocated by tomato plants.

Table (4): Uptake and accumulation of aldicarb in vegetation part of tomato treated with different formulations under green house conditions

Days after application	% of the total aldicarb residues recovered from vegetation part of tomato plant			
	FA3*	FA6	FA9	15G
18	2.5741	1.9413	1.55	2.1079
36	1.3703	0.7768	0.4908	0.5641
54	0.7544	0.453	0.2321	0.169
72	0.7656	0.4623	0.1422	0.0296
Total	5.4644	3.6334	2.4147	2.8706

* FA = Alginate controlled release formulation of aldicarb

Aldicarb residue levels in root system were determined and recorded as shown in Table (5). The data indicated that the aldicarb toxicant was up taken from the soil and the amount accumulated in to the root system was less than that found in the shoot system of tomato plant. This might be attributed to the highly water solubility of aldicarb. The concentration of aldicarb residues recorded was the values at the first 18 days after treatment, and then the concentration trend found after that period looks like the trend of residue levels found in shoot system. The highest concentration (2.29%) was recorded by FA_3 followed

Table (5): Percentage of aldicarb recovered form root system of tomato plant treated with different formulations under green house conditions.

Formulation treatments	% of the total aldicarb residues recovered in root system and fruit of tomato plant					
	Days after application				Total in root system	Total in fruit,72 days after application
	18	36	54	72		
FA3*	2.2952	1.1363	0.5223	0.3912	4.345	0.6308
FA6	1.6249	0.7263	0.3275	0.2414	2.9201	0.2826
FA9	1.1345	0.4979	0.2583	0.1141	2.0048	0.1235
15G	2.0967	0.8586	0.1753	0.0511	3.1817	ND**

* FA = Alginate controlled release formulation of aldicarb **ND= Non detectable

by 15G (2.1%), then FA_6 (1.62%) whereas, the lowest concentration (1.13%) was recorded by FA_9 at 18 days. At both times, 54 and 72 days, the amount of aldicarb that released from the CR formulations and absorbed into the root system were approximately FA_3 (2.98-7.65 fold), FA_6 (1.87-4.72 fold), and FA_9 (1.47-2.23 fold) compared to that of granular formulation (15G). These data indicated that the CR formulations may play an important role to reduce the losses of nematicide via degradation and/or leachability in soil (Marei, *et. al.* 2000).

This reduction in losing aldicarb, may be give a good distribution of toxic material in soil that help insure maximum benefits of nematode control and crop yield while, using the last possible amount of pesticides (El-gendi *et al.,* 1978). At the end of experiments the aldicarb residue levels in tomato-fruit was analyzed and tabulated in Table (5). The total amounts of aldicarb found were 0.6308%, 0.2826%, and 0.1235% of the initial amount of aldicarb formulations applied as FA3, FA6, and FA9, respectively. No detectable amount of aldicarb found in the tomato fruits treated with the commercial formulation, at the same time (72days). This data indicated that the pattern of aldicarb breakdown in plant was similar to that mentioned in soil. By other mean, the aldicarb that release quickly from the granular formulation was subjected to loss by converting to more soluble products such as sulfoxide and oxime, and this leads no detectable amount of aldicarb due to loss via leachability. This result was supported by Smelt *et al.,* (1995) who mentioned that the half-lives of aldicarb and its metabolites product, aldicarb sulfoxide, and aldicarb sulfone, ranged from 3.3 to 8.1 days. But the slower release rate from CRF.s, lead to more accumulated portion in soil which in turn lead to more residue translocated and accumulated in tomato fruit.

D- Determination of aldicarb unreleased from the different formulations:

Aldicarb unreleased from the different formulation types, CRFs and 15 G, were evaluated as observed in Table (6). The obtained data showed that the amount of aldicarb still incorporated within the controlled-release formulations were ranged from 44.184% to 67.89% compared to that treated with commercial formulation (1.25%) after 18 days of application, and then the CRFs released the aldicarb with a slow release rate till the second period (19-36 days), then the amount incorporated within the alginate beads started to decrease with the time to reach 16.72-38.87% of the initial amount applied at the end of the experiment. In other hand, the granular formulation released aldicarb quickly which lead to that the unreleased portion at initial period of estimation not more than 1.25% of the initial amount applied. This result supported our finding in the release rate studies that reported, there was a clear different between the three CR formulations in the release rate of aldicarb and the 15G.

Table (6): Percentage of aldicarb unreleased from the different formulations after application on soil cultivated with tomato under green house conditions

Formulation treatments	Days after application			
	18	36	64	72
	%Remaining aldicarb			
FA3*	44.184	41.062	25.274	16.724
FA6	57.475	55.727	38.878	28.269
FA9	67.896	65.587	50.797	38.878
15G	1.248	0.527	0.0561	0.0065

* FA = Alginate controlled release formulation of aldicarb

It can be noted that the CR formulations should be kept the aldicarb toxicant from loss via leachability and degradation be encapsulating the active ingredient within the alginate matrix.

Distribution of aldicarb residues as a parent compound in water, soil, tomato plant, and aldicarb that remaining within different formulations expressed as % of total residues aldicarb found in an end of the experiment are summarized in Table (7). The observed data showed that the aldicarb released the different formulations, transferred to soil pots, uptake to tomato plant, and leachout from the pots bottom was very different. Most of the residues aldicarb in the experiment which treated with CRFs were found in the water (45.05%, 32.34%, and 24.1%), followed by soil (23.06%, 16.7%, and 12.23%), plant (10.49%, 6.83%, and 4.54%), and then remaining portion into different CRFs (16.72%, 28.26%, and 38.87%) for FA_3, FA_6, and FA_9, respectively. It could be noted that the amount of aldicarb residue found in sandy soil was the half of that residue found

Table (7): Distribution of aldicarb released from different formulations in the sandy soil pots transplanted with tomato seedling under green house conditions

Formulation treatments	% aldicarb residues recovery				
	Leached water	Sandy soil	Tomato plant	aldicarb remaining into formulation	Unextractable aldicarb
FA3*	45.0544	23.0615	10.4996	16.7245	4.66
FA6	32.3444	16.7024	6.8361	28.2695	15.8476
FA9	24.1087	12.2311	4.5430	38.8783	20.2389
15G	82.4997	9.1826	6.023	0.0065	2.2589

* FA = Alginate controlled release formulation of aldicarb

in leachate. In addition, among of the plant parts, the aldicarb residues were accumulated more in vegetation parts than the root system, and fruit. On the other hand, the commercial formulation (15G) had a high concentration in water (82.5%), followed by soil (9.18%), total plant (6.05%), and then within a granules (0.0065%). It should be cited that the residue aldicarb found in soil was 11.13% comparable to the total amount of residue aldicarb found in leachate. In addition, the aldicarb residue levels were accumulated more in root system (3.18%) than the vegetation parts (2.87%) whereas, no residue was found in tomato fruit.

In comparison between developed CR and 15G formulations, the aldicarb residue leach out in water represented from (29.22% to 54.61%) of that leached out from the bottom of pots treated with 15G. In contrast, the amount of aldicarb residue found in soil treated with CRFs was higher than that aldicarb residue founding soil treated 15G (1.33-2.51 fold). This means that the persistence of aldicarb in soil were negatively correlated with the release rate of aldicarb released from the granule formulation and positive correlation in pots treated with CRFs. On opposite, the aldicarb adsorbed more by decreasing the aldicarb rate of release by CR Formulations and this might be explained that the unextractable portion was the highest from FA9. In conclusion, the data suggested that aldicarb in FA_9, FA_6, and FA_3 formulations persisted longer in the soil than the 15G under experimental conditions may reduce its losses via leachability and degradation. The CRFs were altered the release rate and consequently sustained the release period for such along time which might be extended the protection of plant against the nematode, and reduced the number of formulation applicable in soil and the environment al loss as well as the environmental hazards caused by aldicarb.

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مدى مثابرة وتوزيع مبيد الألديكارب المناسب من ثلاث تجهيزات لحبيبات الأليجينات المتحكم في تحرر المبيد والتجهيزات التجارية في نظام تربة – نبات الطماطم تحت ظروف الصوب الزراعية

حمدي سلطان، عبد السلام مرعي، أشرف موسى

قسم كيمياء المبيدات، كلية الزراعة، جامعة الإسكندرية، الإسكندرية، مصر

تم دراسة مدى امان استخدام المبيد النيماتودى الألديكارب المناسب من التجهزات في صورة حبيبات الاليجينات المتحكم في تحرر المبيد منها مقارنة بالنجهيزة التجارية فى صورة محببات بدراسة توزيع ومسار إختفاء المبيد في نظام يحتوي على نباتات طماطم مزروعة في أصص تحت ظروف الصوب الزراعية بتقدير متبقيات هذا المبيد في ماء الغسيل، التربة واجزاء النبات المختلفة مع تقدير الجزء الغير متحرر من المادة الفعالة لهذا المبيد في التجهيزات المختلفة. بالنسبة لتقدير متبقيات مبيد الألديكارب في ماء الغسيل فقد وجد أن النسبة المئوية لمبيد الألديكارب التي غسلت مع ماء الصرف كانت ٢٤,١٪ للـ FA_9 مقارنتها بالتجهيزات التجارية والتى فقدت خلال التجربة من ٨٢,٤٩٪ لمبيد الألديكارب. ومن تلك النتائج أتضح أن الاليجينات والكاؤلين المكونان للتجهيزات المتحكم في انسياب المبيد قد أظهرت قدر من التحكم في خروج المادة الفعالة لمبيد الألديكارب وقد أدى هذا إلى تقليل عملية الغسيل للمبيد. بالنسبة لبقاء مبيد الألديكارب في التربة وجد أن تركيز متبقيات المبيد المحررة من التجهيرات المتحكم في انسياب المبيد أعلى في التربة مقارنة بالتجهيزات التجارية وذلك خلال الـ١٨ يوم الأولى فقد كانت في الألديكارب ٢٣,٠٦٪ للـ FA_3، ١٦,٧٪ للـ FA_6 ، ١٢,٢٣٪ للـ FA9 على التوالي. بينما كانت في التجهيزة التجارية على نفس الزمن (١٨ يوم) ٩,٨١٪ للألديكارب. عموما بالنسبة لمتبقي المبيد في أجزاء نبات الطماطم فقد لوحظ ان اعلى تركيز تواجد للمبيد فى المجموع الجذرى مقارنة بالمجموع الخضرى كان من التجهيزة بينما تركيز المبيد المتواجد في المجموع الخضري للطماطم أعلى في النباتات المعاملة بالتجهيزات المتحكم في انسياب المبيد مقارنة بالتجهيزات التجارية. أيضا عند تقدير تركيز المتبقي داخل ثمار الطماطم في نهاية التجربة لوحظ ان في حالة التجهيزات التجارية لم يتم الكشف على أي آثار لمتبقيات المبيد عند تقدير المتبقي في ثمار الطماطم فى نهاية التجربة. كانت قيمة المتبقيات الكلية فى المجموع الخضرى و الجذرى لنبات الطماطم (٥,٤٥٪ و ٤,٤٠٪) لتجهيزة FA_3 و(٣,٦٣٪ و ٢,٩٢٪) للـ FA6 بينما كانت (٢,٠٠٪، ٢,٤١٪) للـFA_9. بالنسبة لكمية المبيد الألديكارب الغير متحرر من التجهيزات سواء المتحكم في انسيابها أو التجارية فقد وجد أن التجهيزات المتحكم في انسيابها يتراوح متبقي المبيد الغير متحرر بها ما بين ٣٤,١٨٪ الى ٦٧,٨٩٪ بعد الـ١٨ يوم الأولى من التطبيق وفي التقديرات التالية لذلك انخفضت النسبئة المئوية حتى وصلت الى قيمة تتراوح ما بين ١٦,٧٢٪ الى ٣٨,٨٧٪ في نهاية التجربة (٧٢) يوم. بينما في حالة المحبب التجاري للألديكارب كانت خلال الـ١٨ يوم ١,٢٤ ٪ وتصل الى ٠,٠٦٥٪ في نهاية التجربة (٧٢) يوم.

Persistence and Distribution of Cadusafos Released from Granular and Alginate Controlled-Release Formulations in The Soil-Tomato Plant System Under Greenhouse Conditions

Hamdy R. Soltan, A. S. Marei and A.Mousa
Pesticide Chemistry Dept., Faculty of Agriculture, Alexandria University, Alex., Egypt

ABSTRACT

The behavior of cadusafos released from three alginate controlled-release (CR) formulations (FC3, FC6 and FC9) and the corresponding granule formulation (10G) after application, vis-à-vis leachability as well as its residue levels in soil and tomato plant parts were discussed using data from green house trials as a basis. The accumulation pattern of cadusafos leached out from the bottom of the pots treated with FC3, FC6, FC9 and granular formulation during 72 days after application were 42.28%, 32.97%, 21.38% and 44.21%, respectively. The concentration of cadusafos residues found in soil treated with the CR formulations were higher than that found in pots treated with granular formulation, 18 days after application. Thereafter, the cadusafos residues were rapidly decreased with an increasing time for all the tested formulations. However, the total residues of cadusafos recovered from the pots treated with FC3, FC6, and FC9 were 24.45%, 21.24%, and 15.48%, respectively. On the other hand, the total residue was 6.38 % in soil treated with the granule formulation. Concentration of cadusafos in both vegetation part and root system were accumulated to some extended and the higher values recorded was 18 days after treatment. Thereafter, the residue levels were declined quickly in pots treated with the granular formulations (10G), and gradually for the pots treated with CR Formulations. Also, the total residues of cadusafos found in each vegetation part and root system were 0.33% and 0.77% for FC3, 0.24% and 0.54% for FC6 and 0.084% and 0.31% for FC9, respectively. On the other side, the total concentrations in the vegetation part and root system in the pots treated with the granular formulation were 0.34% and 0.74%, respectively. The portion of cadusafos still incorporated within the controlled release formulations were ranged from 15.911% to 21.680%, 72 days after application while for the granule formulation was 0.0994%.

Key words: Alginate controlled release formulations, cadusafos, and environmental fate

INTRODUCTION

Cadusafos (O-ethyl-S,S,-bis [1-methylpropyl] phosphorodithioate), is a new nematicide and it is introduced by the FMC Chemical Corporation and commonly marketed as Rugby. It has been used for control a wide range of soil insect pests and nematodes (Bourdoxhe, 1990) particularly on tea (Yao and Yu, 1993), banana (Queneherve *et al*, 1991), potato (Santo and Wilson, 1990), coffee (Vijayalakshmi *et al*, 1991) and citrus (Zou *et al*, 1992; McClure and Schmitt, 1996). It is classified by the World Health Organization (Anonymous, 1992) as "highly hazardous" (class Ib). Also, it was considered the most harmful compound against earthworm among the other tested nematicides such as phenamiphos and oxamyl (Beltagy, 2000). Moreover, the residues of cadusafos were highly mobile in sandy soil and could be leached into ground-water, so may be a potential for health hazard (Zheng *et al*, 1994). Soltan (2002) reported that the amount of cadusafos hydrolyzed was temperature dependant, and the rapid hydrolysis was achieved at 40 °C with short half live ($t_{0.05}$= 3.31 days) Controlled-release formulations technology offers the potential to reduce the environmental loss of pesticides and increase their efficacy. An approach to improving pesticide efficacy is the development of controlled-release formulations (Schacht and Vandichel, 1988). These could be useful in reducing losses of active ingredient by environmental factors (Marei, *et al*., 2000; Soltan, 1996; Soltan, *et al*., 2001). The objective of this research was to investigate if by using cadusafos in the form alginate controlled released formulations. Their release rate could be improved and its losses via leachability, volatility, degradation and etc… could be reduced under green house conditions.

MATERIALS AND METHODS

1- Pesticide formulations

Formulations (FC_3, FC_6, and FC_9) for cadusafos were prepared according to the method described by Soltan, 1996 and Soltan *et. al.,* 2014 and commercial granule formulation (10G) for cadusafose (Rugby) were provided by agrochem company, Egypt, and FMC Co., U.S.A, respectively.

2- Experimental design:

Two separate experiments were carried out in this study. The first experiment, plastic pots (20cm in diameter and 25 cm deep), with drainage tube in the bottom were prepared in order to collecting leachate water. Plastic pots were filled with 3 Kg Sandy soil each. Two tomato seedlings (*Lycopersicom esculentum* L. var. estrin B), two week old, were transplanted. Recommended agriculture practices for fertilization and irrigation were followed, and the pots were left one week before treatments were applied in order to acclimate the tomato seedlings under the greenhouse conditions. Treatments consisted of alginate controlled release formulations of cadusafos formulations (FC_3, FC_6, FC_9) and its granular formulation (10G). Each treatment replicated six times.

The formulations were applied at the rate 6 Kg a.i./ ha. All the different formulations were placed in plastic net in the form of tea packet, 2cm depth from the surface. Untreated pots served as control. Treatments were arranged in a randomized complete block design. Temperature ranged between 24 to 39°C and the humidity ranged between 69% to 81% until the end of experiment. Water was added to each pot at the rate of 50 ml/pot, two times per weeks. Water leachate from the bottom of each pot was collected at intervals time (3,6,12,18,24,30,36,42,48,54,60,66, and 72 days).

Another experiment was carried out at the same condition under greenhouse, time and treatment as mentioned above. The residue levels of cadusafos in soil and tomato plant were evaluated at 18, 36, 54 and 72 days after application. In addition, the cadusafos still incorporated in the different formulations were collected to analyze the un-releasing portion at 18, 36, 54, 72 days. At the experiment ended, tomato fruits were collected at analyzes the residue levels of cadusafos..

a- Determination of cadusafos in leachate water:

Threeten (45±5ml) water fractions leached from the bottom of each pot were collected twice per week at the initial period of the experiment, then water was collected at the rate of 6 days interval till the end of the experiment. One ml water from each fraction (4 replicates), without extraction, was transferred to a clean dry test tube. Cadusafos was determined according to the method described by Soltan (2002).

b- Extraction and determination of cadusafos in sandy soil:

Soil samples collecting from each pot were air-dried for 24 hrs at room temperature. Cadusafos was extracted from soil according to the method described by Ambrus *et al*., (1981). 50gm homogenous dry soil sample was transferred to 250 ml conical flask and shaken for 2 hours with 200 ml mixture of 0.25 M HCl and acetone (7:3 V/V) to extract cadusafos residues. The Samples were filtered by using Buchner's funnel. The filtrate was transferred to 500 ml separatory funnel, and then sample was shaken three times with 3x50 ml methylene chloride and hexane (1:1 v/v). These extracts were combined, passed through anhydrous Na_2SO_4 and evaporated in a vacuum evaporator to dryness. Cadusafos residues were redissolved in total volume of 200 ml distill water, one ml quantitatively transferred to a clean dry test tube and colorimetrical analyzed for cadusafos .

c- Extraction and determination methods of cadusafos for shoot, root parts, and tomato fruits of tomato plant:

Cadusafos residues were extracted from shoot, root, and tomato fruit according to the method reported by Ambrus *et al*., (1981). At 18,36,54, and 72 days after application of pesticides, except for tomato fruit at 72 days only, the tomato plants were carefully removed from soil, washed with tap water to remove soil particles from roots divided in two parts, shoots and roots and fresh weight recorded. Plant parts were cut into small pieces with a single-edge razor blade, then 50 gm of fresh weight from each part was taken as sub sample with 3 replicates and extracted by blending with 200ml distilled water and acetone (2:1 v/v) and 0.2 gm of activated charcoal (to remove the chlorophyll dye) for 2 min., and filtered through Buchner's funnel. The residual tissues were washed with acetone one more time. Combined acetone extracts were quantitatively transferred to a 500ml separatory funnel, then partitioned three times with 3x50ml methylene chloride and hexane (1:1 v/v). The organic layer was combined, and passed through anhydrous sodium sulfate; then evaporated in a vacuum at 40°C to dryness. Residues of cadusafos redissolved in 150 ml distill water. One ml was taken to determine cadusafos by colorimetric method..

d- Extraction and determination of cadusafos incorporated in the different formulations:

At 18, 36, 54, and 72 days after application of cadusafos, the plastic closed net was removed carefully from soil and cleaned up from soil particals. Different formulations (FC_3, FC_6, FC_9, and 10G for cadusafos) were transferred to the laboratory. In the extraction method, the beads were extracted by sonication for 1hr with solvent mixture of water: acetone (7:3 v/v). The extract was decanted and the beads were extracted two more times. The combined extract was portioned three times with 3x50 ml ethylene chloride: hexane (1:1v/v). One ml was taken to determine for cadusafos by colorimetric method..

RESULTS AND DISCUSSION

The objective of this study was to evaluate three controlled-release formulations (FC_3,FC_6, and FC_9) as well as the commercial formulation (10 G) for cadusafos vis-à-vis leachability of cadusafos released from these formulations in the sandy soil under green house conditions. In addition to, determination of residue levels in soil, the amount uptake in tomato from soil, and unreleased portion of active ingredient still present in different formulations.

A- Residue levels of cadusafos in leachate water:

Cadusafos leached out from the bottom of the sandy soil pots as leachate water is shown and illustrated in Table.(1). Data show that a maximum of 23% of the applied cadusafos-released leached out from the commercial formulation in soil over the 3 days period then declined rapidly. The low level was detected at 42 days after treatment and non-detectable amount after this time. As opposed to this, only 0.3%, 0.15%, and 0.11% of the applied cadusafos-released leached out 3 days after treatment from FC_3, FC_6, and FC_9 respectively. These concentrations were slowly increased with the time, and reached to the maximum between 18 and 24 days, then declined slowly. The accumulation pattern of cadusafos

Table (1): Residue Levels of Cadusafos in Lachate Water.

formulations	% Residue levels of causafos in leachate water (days)												
	3	6	12	18	24	30	36	42	48	54	60	66	72
FC_3*	0.3	0.91	1.98	5.19	8	5.64	4.68	3.8	3.72	1.81	1.87	1.87	2.24
FC_6	0.15	0.63	1.36	4.11	6.2	4.6	3.51	2.86	2.86	1.35	1.01	1.01	1.23
FC_9	0.11	0.39	0.93	3.11	4.7	2.55	2.4	1.94	1.93	0.28	0.51	0.52	0.53
10G	23	13.66	4.87	2.22	0.52	0.014	0.0019	0.012	#N.D	N.D	N.D	N.D	N.D

#N.D= non detected * FC = Alginate controlled release formulation (Soltan 2002)

leached out from the granular formulation was 44.2164% over the 72 days period whereas the accumulation pattern of cadusafos leached out from FC_3, FC_6, and FC_9 was 42.2881%, 32.4734%, and 21.3812%, respectively over the same period. This pattern is consistent with the rate of release of cadusafos from the three controlled release formulations and the granular formulation which was reported to be faster from the commercial formulation and slowest from FC_9.Generally, it could be noted that the alginate matrix incorporated cadusafos and Kaolin as filler material caused slow down in the release rate of which in turn decreased the portion of subject to loss by environmental factors such as temperature, pH, microorganisms, leachability…….*etc.*(Zheng, 1994 Soltan, 2002) This trend might be decreased the contamination of under ground water and may be extended the pesticidal activity of cadusafos. Also, we could concolouded that the CRF approach is one of the most applicable trend to reduce the leachability potential of pesticide in order to decrease the under ground contamination with this pesticide (Marei, *et al*., 2000).

B- Persistent of cadusafos in sandy soil:

Soil samples were collected at 18, 36, 54, and 72 days after treatment to find out the residue levels of cadusafos released from the different formulations in soil cultivated with tomato plants under greenhouse conditions. The results tabulated in Table (2) indicated that the greatest level of cadusafos residues was recorded at 18 days after treatment for all the tested formulations. But the residue level of cadusafos found in soil treated with the commercial formulation at 18 days (4.178%) decreased rapidly and the lowest concentration (0.00016%) was observed at the experiment ended (72 days after treatment). On the other hand, the residue levels of cadusafos in soil treated with the controlled release formulations (CRF) were varied from 2.395 (10.008%) to 3.666 (15.319%) folds of the granular formulation at 18 days after treatments. Thereafter the residue levels of cadusafos released in soil from the CRF decreased gradually with the time and reached to 0.0391, 0.022, 0.0071 and 0.00016% for CF_3, CF_6, CF_9 and 10G respectively. Also it should be noted that the difference between the commercial formulations and CRF in the amount recovered from the treated soil pots may be attributed to the difference in the release rate of cadusafos from the formulations which in turn related with leached rate which was higher for the commercial formulations and lower from CR formulations. Also, the total amount of cadusafos residues recovered from the soil were 24.455%, 21.246%, 15.486% and 6.389% for FC_3, FC_6, FC_9 and 10G, respectively. These mean from the point of environmental safety the amount and leached of cadusafos to the ground water from the soil treated with the CRF will be less and the protection period to tomato plant will be more than the commercial formulations. At the end of experiment,. FC_3 is highest persist in soil; while the 10G was lowest persist in soil.

Among of CRFs, the different residue level of cadusafos into the soil can be attributed to a difference in the adsorption capacity of the soil that might be referred to the amount of Kaolin different that contains into formulation. It appears that the CRFs will be more persist longer in the soil than the granular formulation. Generally it should be noted that the alginate matrix incorporated cadusafos and filler material such as Kaolin is not caused gradient down which may be referring to the another factors then presented in the environment such temperature, pH, water, microorganisms, soil enzymes, humidity……*etc*. These results are in agreement with Soltan, (2002).

Table (2): Percentage of cadusafos found in sandy soil cultivate with tomato seedlings under green house conditions.

Days after application	% of the total cadusafos recovered from sandy soil pots			
	FC3*	FC6	FC9	10G
18	15.3193	13.2115	10.0085	4.1784
36	6.4473	5.6514	4.2775	1.6738
54	2.6502	2.3609	1.1933	0.5372
72	0.0391	0.0226	0.0071	0.00016
Total	24.4559	21.2464	15.4864	6.3896

* FC = Alginate controlled release formulation (Soltan 2002)

They studied that the hydrolysis reaction rate of cadusafos under sterile condition at pH5, 6.5, and 8 as well as different temperatures of 5, 20, and 40°C. The data indicated that the effect of the tested temperature and different pH values on the persistence of cadusafos after differenttime intervals played an important role in this respect. Also Zheng *et al.,* (1994) studied the behav-

ior of cadusafos in different soil types in banana plant. They obtained indicating that the pluviometry and the soil characteristics in flounced the movement and persistence of cadusafos, and the time necessary to observe 50% dissipation of the applied dose in the 0-25 cm layer varied from 9-10 days in sandy loam soils to 18 days in Kaolinite clay soil, and 30 day in montmorillonite clay soil.

C- Uptake and accumulation of cadusafos in vegetation part, root, and fruit of tomato plant:

The tabulated data in table (3) indicated that concentrations of cadusafos in vegetation part were accumulated and the higher value was recorded at 18 days for all tested formulations, then the concentration decreased with the time till the experiment ended (72 days). This might be due to the degradation by tomato plant and/ or the dilution caused by new growth in tomato plant. Among the controlled-release formulations tested, the highest concentration (0.2127%) in vegetation part was recorded by FC_3 (approximately 63% of residue found in plant treated with 10G). Whereas, the lowest concentration (0.0619%) was recorded by FC_9. Approximately 18.38% of residue found in plant treated with 10G) at 18 days after application. At 72 days, the concentration of cadusafos was varied from 0.0391% to 0.0015% for FC_3, and 10G, respectively and the other formulation were in between. These data indicated that the residue levels of cadusafos in vegetation part might be provided an effective protection during the vegetation period against the sucking insects such as Aphid, gassed, trips, white fly … *etc*, than the tomato plant treated with the 10G formulation. The total cadusafos applied in vegetation part residue levels expressed as the percentage of the initial dose were 0.3363%, 0.2476%, 0.0843%, and 0.3409% for each FC_3, FC6, FC_9 and 10G, respectively. This consistent with the release rate and may be related to the portion of cadusafose available in soil which could be uptaken by plant.

Table (3): Uptake and accumulation of cadusafos in vegetation part of tomato plant treated with different formulations under green house conditions.

Days after application	% of the total cadusafos recovered from vegetation part of tomato plant			
	FC3*	FC6	FC9	10G
18	0.2127	0.1819	0.0619	0.3367
36	0.0567	0.0298	0.0123	0.0031
54	0.0278	0.0133	0.003	0.0010
72	0.0391	0.0226	0.0071	0.00016
Total	0.3363	0.2476	0.0843	0.34098

* FC = Alginate controlled release formulation (Soltan 2002)

The residue levels of cadusafos in root system were evaluated and recorded as shown in Table (4). The results showed that the concentration of cadusafos in root system was more accumulated than the shoot system.

The highest value was recorded at 18 days for all the formulations. The concentration of cadusafos residue found at the initial period was diminishaly decreased with time. The highest concentration (0.7439%) was recorded by 10G followed by FC_3 (0.4349%) and FC_6 (0.2954%) whereas, the lowest concentration (0.2251%) was recorded by FC_9 at 18 days after application. The roots of tomato plants treated with the CR formulation had the same amount of cadusafos of both 54 and 72 days after application, while the roots treated with 10G was non-detectable (N.D) at 72 days. This means that the accumulation of cadusafos in the root is due to non-systemic characteristic of cadusafos. In addition, it should be noted that the total amount of cadusafos taken up by the shoot and root from CR formulations were arranged from 0.0843-0.3363% (Table 3) and 0.3146-0.7755% (Table 4), respectively, while for 10 G was 0.3409 for the shoot and 0.7462% for the root. Generally, the CR formulations may play an important role to reduce the washout, evaporation, surface run-off and dispersion to unintended regions which also have undesirable economical consequences (Cohen *et al*., 1986), (Anonymous, 1992-1993). At the end of experiment, the residue cadusafos in tomato fruit was determined. The data tabulated in table (4) indicated that the residue cadusafos was reduced in tomato fruit treated with CRFs, (FC_3, FC_6, and FC_9). However, the total amounts of cadusafos found were 0.002%, 0.0061%, and 0.0144% of the total cadusafos applied as FC_9, FC_6, and FC_3, respectively. This result was consistent with release rate of the C.R. formulations. In contrast, no residue of cadusafos was found in tomato fruits at 72 days for plants treated with the granular formulation (10G). The cadusafos was released fast from the granules and had a short half live under warm and moist conditions. The major route of dissipations was volatilization (Anonymous, 1992-1993).

D- Determination of cadusafos unrleased from the different formulations:

Cadusafos unreleased from the different formulation were estimated four times during the experiment course as shown in Table (5). The amount of cadusafos still incorporated within the commercial formulation was represent 3.822% of the initial amount applied, while for CR formulations were ranged 38.6% to 50.2% at 18 days after application. Hence, it is obvious that the release of cadusafos from the three controlled-release formulations was not complete comparable with the granular formulation under the experimental conditions, and a large portion of the cadusafos was remained in the alginate beads. At the end of the study (72 days after application), only 0.0994%, 15.9%, 18.28%, and 21.68% were recovered from 10 G, FC_3, FC_6, and FC_9, respectively. It can be cited that the controlled-release formulations could be protected the cadusafos active ingredient from loss via leachability, volatility, and degradation by encapsulating the active agent within the alginate matrix. These formulations altered the release rate and consequently sustained the release period for

Table (4): Percentage of cadusafos recovered form root system of tomato plant treated with different formulations under green house conditions.

Formulation treatments	% of the total cadusafos recovered from root system and fruit of tomato plant					
	Days after application				Total in root system	Total in fruit, 72 days after application
	18	36	54	72		
FC3*	0.4349	0.19	0.0805	0.0701	0.7755	0.0144
FC6	0.2954	0.1301	0.0526	0.065	0.5431	0.0061
FC9	0.2251	0.0433	0.0257	0.0205	0.3148	0.0020
10G	0.7439	0.0017	0.0006	ND#	0.7462	ND#

* FC = Alginate controlled release formulation(Soltan 2002) #ND= Non detectable

such a long period, which might be, reduced the number of formulation application and increased the cadusafos efficiency against nematode.

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Table (5): Percentage of cadusafos unreleased from the different formulations after application on soil cultivated with tomato under green house conditions.

Formulation treatments	Days after application			
	18	36	64	72
	%Remaining cadusafos			
FC3*	38.606	36.679	24.212	15.911
FC6	47.528	45.772	30.308	18.2882
FC9	50.249	48.665	32.374	21.6807
10G	3.822	0.830	0.393	0.0994

* FC = Alginate controlled release formulation(Soltan 2002)

Distribution of cadusafos residues as parent compound in water, soil, tomato plant, and unreleased portion of cadusafos remaining into different formulations expressed as % the initial amount applied of each formulation, and different formulations are summarized in Table (6). The obtained results showed that the cadusafos release from the different formulations transferred to soil pots, translocated to plant and leach out from the pots bottom was very different. The cadusafos residues in the experiment treated with 10G were high in water (44.2164%), followed by soil (6.4823%), then tomato plant (1.0871%), and then in granular formulation (0.0994%). Most of the residues in the experiment

Table (6): Distribution of cadusafos released from different formulations in the sandy soil pots transplanted with tomato seedling under green house conditions.

Formulation treatments	% cadusafos recovery				
	Leached water	Sandy soil	Tomato plant	cadusafos remaining into formulation	unextractable cadusafos
FC3*	42.2881	27.1186	1.1262	15.9118	13.5553
FC6	32.4734	23.4865	0.7968	18.2882	24.9551
FC9	21.3812	17.1789	0.4009	21.6807	39.3583
10G	44.2164	6.4823	1.0871	0.0994	48.1148

* FC = Alginate controlled release formulation

that treated with controlled-release formulations (CRFs) were found in the water (42.2881%, 32.4734%, and 21.3812%), followed by soil (27.1186%, 23.4865%, and 17.1789%), tomato plant (1.1262%, 0.7968%, and 0.4009%), and then into different CRFs (15.9118%, 18.2882%, and 21.6807%) for FC_3, FC_6 and FC_9, respectively. Among the tomato plant parts, the cadusafos residue levels were concentrating in the root part more than the shoots and fruits for all the tested formulations. For the commercial formulation, the residue level of cadusafos was 0.7462% in the root system followed by shoot (0.3409%), and no residue found in tomato fruit whereas, the residue levels for pots treated with the CR Formulation were (0.7755%, 0.5431%, and 0.3146%) in the root system followed by shoots (0.3363%, 0.2476%, and 0.0843%) and then tomato fruit (0.0144 %, 0.0061%, and 0.0020%) for FC_3, FC_6, and FC_9, respectively. In comparing the three types of formulations, i.e., CRFs and 10G, there were differences in the behavior of the residues in the soil, leachate water and tomato plant. Residue in water of pots treated with 10G was little higher than in treated with the FC_3. In contrast, residue in soil treated with the CRFs were much higher than in the treated with the 10G; and the residue in tomato plant treated with FC_3 was nearly similar to that treated with the 10G. This means that the persistence of cadusafos in soil was negatively correlated with the release rate of cadusafos from the granular formulation . In other words, the cadusafos is adsorbed more by decreasing the cadusafos rate of release by CRFs. Generally, the results suggest that the release of cadusafos can be controlled by using alginate matrix with Kaolin as filler materials and by changing the properties of alginate and Kaolin; it is possible to alter the release rate into soil. These results also indicate that cadusafos in FC_9, FC_6, and FC_3 formulations persisted longer in the soil than the 10G under green house conditions which might reduce the losses via leachability, and provide adequate amount which may provide more protection to tomato plant against nematode and insect for long time than the 10G. The cadusafos unextractable from CR formulations were ranged from 13.5553 % to 39.3583% whereas; from 10G was 48.1148%. Therefore, when the C.R. formulations applied in a tomato plant field, the CR formulations are able to extend the persistence of cadusafos and at the same time reduce the environmental loss as well as the environmental risks caused by cadusafos.

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مدى مثابرة وتوزيع مبيد الكاديوسافوس المتحرر من ثلاث تجهيزات لحبيبات الأليجينات المتحكم في تحرر المبيد والتجهيزة المحببة التجارية في نظام تربة – نبات الطماطم تحت ظروف الصوب الزراعية

حمدي سلطان، عبد السلام مرعي، أشرف موسى

قسم كيمياء المبيدات، كلية الزراعة، جامعة الإسكندرية، الإسكندرية، مصر

نم دراسة سلوك مبيد الكاديوسافوس المنساب من ٣ تجهيزات الاليجينات المتحكم فى انسياب المبيد (FC9, FC6, FC3) مقارنة بتلك التجهيزة التجارية فى صورة محبب (10G) بعد التطبيق بتقدير متبقيات المبيد في ماء الغسيل والتربة والمجموع الخضري والجذري والثمار في الطماطم وتقدير المادة الفعالة الغير محررة من التجهيزات المختلفة سواء كانت المتحكم في انسياب المبيد أو التجارية تحت ظروف الصوب الزراعية. بالنسبة لتقدير متبقيات مبيد الكاديوسافوس في ماء الغسيل فقد وجد أن النسبة المئوية المتراكمة لمبيد الكاديوسافوس هي٤٢,٢٨ ٪ لـ FC3، ٣٢,٩٧ ٪ لـ FC6، ٢١,٣٨ ٪ لـ FC٩ على التوالى بينما التجهيزة التجارية والتى فقدت خلال التجربة ما يقرب من ٤٤,٢١ ٪ من مبيد الكاديوسافوس. بالنسبة لبقاء الكاديوسافوس في التربة وجد أن تركيز متبقيات المبيد المحررة من التجهيرات المتحكم في انسياب المبيد أعلى في التربة مقارنة بالتجهيزات التجارية وذلك خلال الـ١٨ يوم الأولى ثم بدأ التناقص سريعا في كل التركيزات حتى نهاية التجربة. كانت المتبقيات التى تم استعادتها من التربة للاصص المعاملة بتجهيزات الاليجينات هي٢٤,٤٥ ٪ لـ FC3، ٢١,٢٤ ٪ لـ FC٦، ١٥,٤٨ ٪ لـ FC9 على التوالى. على الجانب الاخر وجد ان تركيز المتبقيات لتربة الاصص المعاملة بالتجهيزة التجارية كان ٦,٣٨٪. بالنسبة لمتبقيات المبيد في كلا من المجموع الخضرى و الجذرى لنبات الطماطم فقد لوحظ ان اعلى تركيز تواجد للمبيد خلال فترة الـ١٨ يوم الأولى بعد التطبيق لكل التجهيزات ثم بدأت في الأنخفاض السريع حتى نهاية التجربة للتجهيزة التجارية وبصورة بطيئة لتجهيزات ا الاليجينات . أيضا وجد أن تركيزالكلى للمبيد في كلا من المجموع الخضري والمجموع الجذري كالتالى (٠,٣٣٪ و ٠,٧٧٪) لتجهيزة FC٣ و(٠,٢٤٪ و ٠,٥٤٪) للـFC٦ بينما كانت (٠,٠٨٤٪، ٠,٣١٪) للـFC9. هلى الجانب الاخر وجد ان تركيز متبقى الكاديسفوس في كلا من المجموع الخضري والمجموع الجذري للتجهيزة التجارية المبيد الكاديسفوس كانت ٠,٣٤٪ و ٠,٧٤٪ على التوالى. كانت التركيزات الغير متحرر من التجهيزات المتحكم في انسيابها تتروح مابين ١٥,٩١١٪ الى ٢١,٦٨٪ ٪ في نهاية التجربة (٧٢) يوم بهد التطبيق بينما في حالة المحبب التجاري كاديسفوس كانت ٠,٠٩٩٤٪ في نهاية التجربة .

Preparation of Silica Nanoparticles from Rice Husk and Examine It In Controlling Cotton Leafworm, *Spodoptera littoralis* (Boisd.) on Soybean Under Field Conditions

El-Samahy, M. F. M[1]. and R. E. Mashal[2]

1 Plant Protect. Res. Inst. (PPRI), Agric. Res. Station (ARS), Sakha, Kafr El-Sheikh, Egypt

2 Plant Protect. Dep., Fac. Agric., Tanta Univ., Egypt

ABSTRACT

The experiment was carried out at the Farm of Sakha Agric. Res. Station (SARS), and the laboratory of Soil, Water and Environment Research Institute, Kafr El-Sheikh, Egypt, during two successive seasons; 2015 and 2016. The aims of this study was to extract silica nanoparticles from rice and examined it in controlling the cotton leafworm, *Spodoptera littoralis* on soybean under field conditions. Results showed that the silica nanoparticles (SiNPs) extracted from rice husk ranged from 10 to 12 nm and the highest specific surface of the sample was about 320 $m^2 g^{-1}$. Data also showed that, the examined concentrations (0.1, 0.2 and 0.3 g/l) can be cause larval mortality reached to 97.55 and 94.44% reduction mortality at high concentration (0.3 g/l) of SiNPs during two seasons 2015 and 2016, respectively. This study showed efficacy of the SiNPs extracted from rice husk in controlling the cotton leafworm on soybean plants under field conditions.

Key words: Silica nanoparticles, rice husk, Spodoptera littoralis, soybean plants, field conditions, pest control

INTRODUCTION

Soybean (*Glycine max* (L.) Merr) was first originated in Southeast Asia around 1100 BC in China. In 1904, George Washington Carver from America discovered that soybeans are a rich source of protein and oil. In 1921, William Morse found out more than 10,000 different varieties of soybean. Earlier it was used for crop rotation and production of hay. During World War 2, soybeans were consumed as a high protein food and as edible oil (Sharma and Baluja, 2015).

Some of the natural medicinal plants are so common that we use them in daily life without knowing their medicinal importance. *G. max* is the best example of it. The plant is commonly known as soybean which is eatable. Bruised leaves applied to snake bites, Flowers used for blindness and corneal opacities. Green bean hulls chewed to a pulp are applied to smallpox ulcers, corneal ulcers and excoriations in children from urine, dried sprouts believed to be beneficial for hair growth and curative for ascites and rheumatism (Kanchana *et al.*, 2016). Tengnäs and Nilsson, 2002 showed that the dry soybean is the "king of beans." contains 38 percent protein—twice as much as pork, three times more than egg and twelve times more than milk. Furthermore, the protein in soybean has a more complete range of essential amino acids than most other foods. In addition, the dry seed contains 18.4 percent unsaturated fat. Many soybean products (e.g. miso, soy sauce, tempeh, and bean curd) originated in China. For example, bean curd (tofu) was invented in the Han dynasty (206 B.C. to 220 A.D.). The technology then spread to Japan around the year 700 A.D.

Cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is a highly polyphagous insect pest with numerous hosts causing economically important loses. In Egypt, the Cotton leafworm, *S. littoralis*, is considered one of the major pests attacking more than 112 host plants, such as cotton and soybean. Unfortunately, the rate of infestation may reach up to 119 048 egg-mass/ha, causing great damage to leaves, buds, flowers, and bolls (Temerak, 2002; El-Sheikh, 2012 and Ahmed *et al*., 2015a,b).

The control method of cotton leafworm is complicated due to its high resistance to most of the currently used pesticides classes. Their widely indiscriminate use moreover results in set up into environmental contamination, threat to wildlife populations, and serious public health concerns over food safety (Funderburk *et al*., 1993 and El-Geddawy *et al*., 2014). Recently, the global occurrence of cotton leafworm and its growing resistance problem have presented an area of great needs for more effective and acceptable control methods such as alternative safe pesticide with the advantage of its respect to the environment.

Great efforts have been made to control the insect pests chemically. Because of hazards of pesticides on public health and environmental balance in addition the harmful effects on beneficial insects such as natural enemies, relatively recent direction of using nanomaterials (NMs) in pest control management was introduced. Nanomaterials consist of one or more components present in various forms that possess at least one dimensional structure of diameters in the range of 1 to 100 nm (Warheit *et al.*, 2008). Among NMs, silica nanoparticles (SiNPs) have received considerable attention due to their unique properties. Silica NPs can be good alternative to the popular insecticides which are hazardous to human health and because of huge environmental concerns associated with them. The use of amorphous nanosilica as biopesticide has been reported (Barik *et al.*, 2008).

SiNPs obtained from commercial companies can be used in control some insect and animal pests in Egypt

such as; *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae), cotton leaf worm, *S. littoralis, Aphis craccivora* Koch. (Hemiptera: Aphididae), *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) and two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) (Derbalah *et al.*, 2012; Borei *et al.,* 2014; El-Samahy *et al.,* 2014; El-Samahy, 2015; El-Samahy *et al.,* 2015; Salem, 2015 and Alakhdar and El-Samahy, 2016).

Rice husks are formed from hard materials, including opaline silica and lignin. In addition to protecting rice during the growing season, rice hulls can be put to use as building material, fertilizer, insulation material, or fuel. In Egypt, about 3.5 million tons of rice straw and 0.5 million tons of rice hulls are produced every year from the rice fields and rice milling process respectively. There is no practical use for these by-products, up till now, except for fuel (Nour, 2003).

The aim of this study was to prepare silica nanoparticles from rice husks. Evaluate the efficacy of silica nanoparticles against the cotton leafworm, *S. littoralis* on soybean under field conditions.

MATERIALS AND METHODS

1. Preparation of Silica nanoparticles:

Silica nanoparticles (SiNPs) were prepared by extraction from rice husk according Hai Le *et al.* (2013) technique. While silica powder at nanoscale was obtained by heat treatment of rice husk following the sol–gel method (Figs. 1 and 2). The rice husk ash (RHA) is synthesized using rice husk which was thermally treated at optimal condition at 600°C for 4 h. The silica from RHA was extracted using sodium hydroxide solution to produce a sodium silicate solution and then precipitated by adding H_2SO_4 at pH = 4 in the mixture of water/butanol with cationic presence. In order to identify the optimal condition for producing the homogenous silica nanoparticles, the effects of surfactant surface coverage, aging temperature, and aging time were investigated.

2- Properties of silica nanoparticles from rice husk

The SiNPs size was examined by transmission electron microscopy (TEM). It was found that the obtained SiNPs size was ranged from 10 to 12 nm (Fig. 2). By using the Brunauer–Emmett–Teller (BET) method (Brunauer, 1945), the result showed that the highest specific surface of the sample was about 320 m^2 g^{-1}. The obtained results in the mentioned method was proved that the rice husk from agricultural wastes can be used for the production of silica nanoparticles.

3. Field experiment:

Soybean seeds of (*Glycine max* L.); Crawford variety, were obtained from Food Legumes Research Section, SARS, Kafr El-Sheikh, Egypt. This variety was found to be relatively susceptible to infestation with cotton leaf worm, *S. littoralis*.

The field experiment was carried out at the Farm of Sakha Agric. Res. Station (SARS), Kafr El-Sheikh, Egypt, during two successive seasons; 2015 and 2016. Seeds of soybean were planted in three replicates (each of 6×7 m) per treatment in Randomized Complete Block Design (RCBD). Four treatments were applied. The first treatment was the control which sprayed with water and the others were treated with the different concentrations of SiNPs. The normal cultural practices were applied as usual without using any insecticides.

Soybean seeds were planted in the first week of May during the two seasons; 2015 and 2016. Treatments of SiNPs were applied when about 25% of plant leaves were consumed by *S. littoralis* larvae. Randomized sample of ten plants per replicate were examined before treatment and after 1, 3, 5, 7, 10 and 15 days of spraying. Samples were examined by shake the plant in white bucket for counting live and dead larvae. The percentage of *S. littoralis* reduction for each treatment was calculated using Henderson Tilton's formula (Henderson and Tilton, 1955) as follow:

$$\text{Reduction \%} = 1- \frac{TA \times CB}{TA \times CB} \times 100$$

Where:

TA: No. of larvae after treatment, TB: No. of larvae before treatment

CB: No. of larvae in control before treatment, CA: No. of larvae in control after treatment

4. Statistical analysis:

Statistical analysis was conducted using analysis of variance (ANOVA) followed by Duncan's multiple

Fig. (1): Appearance of raw rice husk and extracted silica by different treatment schemes: a) raw rice husk, b) extracted silica by combustion of untreated rice husk at 600°C, and c) extracted silica from treated rice husk in H_2SO_4 followed by combustion at 600°C

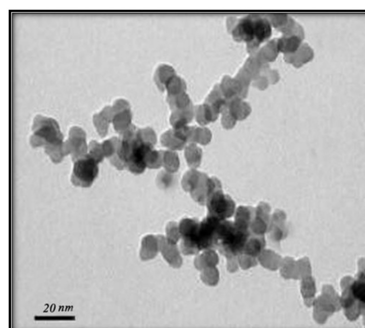

Fig. (2): TEM micrographs of silica nanoparticles

range test (1955) to compare the significance of differences between SiNPs concentrations and the results were considered significant at P < 0.05.

RESULTS AND DISCUSSION

The reduction percentages in the number of *S. littoralis* larvae under field conditions during 2015 and 2016 seasons were presented in Tables (1 and 2).

Table (1): Reduction percentages of *Spodoptera littoralis* larvae after SiNPs application under field conditions during 2015 seasons.

Conc. (g/l)	Day					
	1	3	5	7	10	15
0.1	22.12	41.20	60.11	87.23	89.11	94.55
0.2	28.51	53.12	65.28	88.22	95.33	96.10
0.3	35.31	52.61	70.10	90.35	96.21	97.55

Table (2): Reduction percentages of *Spodoptera littoralis* larvae after SiNPs application under field conditions during 2016 season.

Conc. (g/l)	Day					
	1	3	5	7	10	15
0.1	25.33	40.56	62.20	85.11	87.39	92.41
0.2	26.25	54.77	63.55	87.90	94.47	95.88
0.3	37.15	55.37	68.81	89.21	95.18	94.44

Data showed that the highest reduction percentages of larvae number; for the two seasons, were observed at all SiNPs concentrations after 7, 10 and 15 days. These reduction percentages after 15 days reached to 94.55, 96.10 and 97.55 % at 0.1, 0.2 and 0.3 g/l SiNPs, respectively during first season. In the second season the highest larvae reduction of cotton leafworm were observed after 15 days reached 92.41, 95.88 and 94.44% at 0.1, 0.2 and 0.3 g/l SiNPs, respectively.

In general, data presented in Figure (3) for 2015 season revealed that applications of 0.2 and 0.3 g/l of SiNPs; which did not differ significantly, occurred the highest reduction in the larvae number (71.09 and 73.69 %, respectively) while the concentration 0.1 g/l was recorded 65.72% reduction in the cotton leafworm larvae.

During 2016 season, all concentrations showed significant differences in the cotton leafworm larvae as reduction percentage. Data showed 65.50, 70.47 and 73.36% with 0.1, 0.2 and 0.3 g/l of SiNPs, respectively.

These results are in agreement with Debnath (2012), who revealed that SiNPs were effective against the leaf worm *S. litura* especially in its hydrophobic and lipophilic cases. Moreover, Debnath *et al.* (2012) found that silica based nanocide can be alternative to the commercial insecticides against this leaf worm. El-Bendary and El-Helaly (2013) reported that nano-silica application on the tomato plants can minimize the issues caused by *S. littoralis* providing resistance to the moderate level. Borei *et al.,* (2014) examined commercial SiNPs under laboratory conditions and their found that SiNPs effective in controlling cotton leafworm, *S. littoralis*. Recently El-Samahy and Mashal (2017) examined SiNPs extracted from rice husk on the cotton leafworm, *S. littoralis* under laboratory conditions. They found that SiNPs can reduce the ability of cotton leafworm larvae feeding and increase larval mortality.

Barik *et al*. (2008) revealed that SiNPs; when applied on leaves and stem surface, get absorbed into the culticular lipids by physisorption and caused death of insects purely by physical means.

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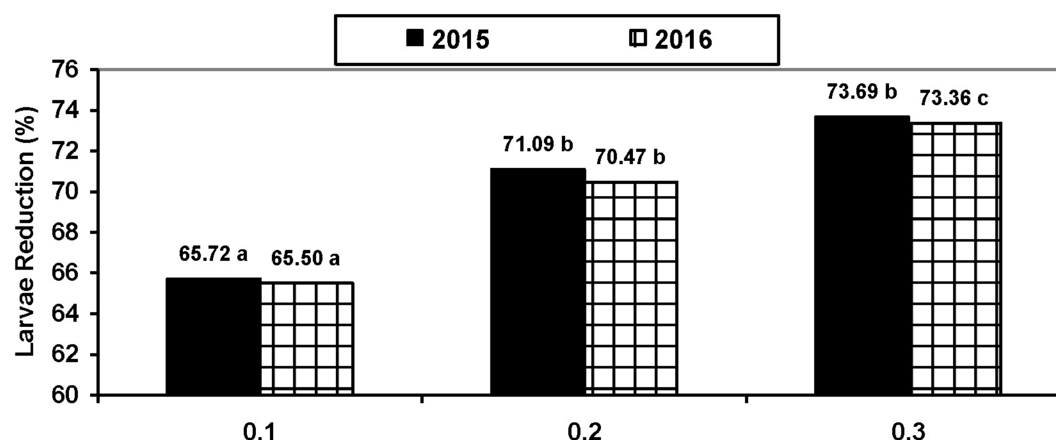

Fig. (3): General mean larvae reduction percent of *Spodoptera littoralis* (Boisd.) on soybean under field conditions according to Henderson and Tilton Formula (1955).

Means followed by the same letter are not significantly different at the 5% level by DMRT

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تحضير جزيئات السيليكا النانومترية من قشرة حبوب الأرز وأختبارها فى مكافحة دودة ورق القطن على محصول فول الصويا تحت الظروف الحقلية

مجدى فاروق محمد السماحى[١]، رانيا السيد مشعل[٢]

١. معهد بحوث وقاية النباتات، محطة البحوث الزراعية بسخا، كفر الشيخ، مركز البحوث الزراعية، مصر

٢. قسم وقاية النبات، كلية الزراعة، جامعة طنطا، مصر

أجريت هذه التجربة فى المزرعة البحثية ، ومعمل معهد بحوث الأراضى والمياه والبيئة بمحطة البحوث الزراعية بسخا، كفر الشيخ، مصر خلال موسمين متتاليين ٢٠١٥و٢٠١٦م. استهدفت هذه الدراسة استخلاص جزيئات السيليكا النانومترية من قشرة حبوب الأرز وأختبارها فى مكافحة دودة ورق القطن تحت الظروف الحقلية.

ولقد أظهرت النتائج أن جزيئات السيليكا النانومترية التى تم تحضيرها من قشرة حبوب الأرز تراوحت مابين ١٠ إلى ١٢ نانومتر، وكان أعلى سطح تغطيه لجزيئات السيليكا النانومترية ٣٢٠ م٢/جم. ولقد تم أختبار ثلاثة تركيزات من جزيئات السيليكا ٠,١، ٠,٢ و ٠,٣ جم/لتر على دودة ورق القطن فى محصول فول الصويا تحت الظروف الحقلية ووجد أن نسبة الخفض فى أعداد يرقات دودة ورق القطن وصلت إلى ٩٧,٥٥ و٩٤,٤٤٪ خلال موسمى الدارسة ٢٠١٥ و٢٠١٦م على التوالى عند استخدام التركيز العالى ٠,٣ جم/لتر. ومن نتائج هذا البحث يتضح إمكانية أستخراج السيليكا النانومترية من قشرة حبوب الأرز واستخدامها فى مكافحة دودة ورق القطن تحت الظروف الحقلية على محصول فول الصويا.

Compatibility Between the Entomopathogenic Fungus *Beauveria bassiana* and Insecticides Against Cotton Leafworm, *Spodoptera littoralis*.

Ibrahim, A.A.; M.F. Abd El-Aziz and El-Gabaly, A.R.
Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.

ABSTRACT

This study was to evaluate the efficacy of emamectin benzoate, methomyl, *Bacillus thuringiensis*, chlorantraniliprole, flufenoxuron and fipronil formulations as well as fungal isolate, *Beauveria bassiana* against cotton leaf worm, Spodoptera littoralis. Two concentrations (LC25 & LC50) of each insecticide were tested in the laboratory to study its effect on the mycelial growth of the entomopathogenic fungus Beauveria bassiana, then use of selective insecticides that associated with entomopathogens, increasing efficiency of control and which might reduce the use of required insecticides. Obtained results revealed that, flufenoxuron had the highest toxicity as it recorded the lowest LC50 against 2nd instar larvae (0.0225 ppm), followed by fipronil, emamectin benzoate, chlorantraniliprole, B. thuringiensis and methomyl (0.289, 0.326, 0.463, 1.143 and 10.283 ppm, respectively). Emamectin benzoate had the highest toxicity as it recorded the lowest LC50 against 4th instar larvae, followed by flufenoxuron, B. thuringiensis, fipronil, chlorantraniliprole, and methomyl (0.437, 2.235, 2.986, 3.253, 5.558 and 31.976 ppm, respectively). Emamectin benzoate, methomyl and *Bacillus thuringiensis* formulations at the two concentrations (LC50 & LC25) did not inhibit mycelial growth where percentage of reduction relative to the control less than 50%. On the other hand, chlorantraniliprole, flufenoxuron and fipronil formulations showed inhibition of mycelial growth at the LC50 concentration where percentage of reduction relative to the control were 100%, while at the LC25 concentration chlorantraniliprole, flufenoxuron and fipronil formulations were moderately toxic to *B. bassiana*. The Co-toxicity factor was > 20 indicating, only, additive effects against the 2nd instar *S. littoralis* larvae after treatments at rate LC25 + LC25 or LC50 + LC50. While the most of mixtures against the 4th instar gave antagonistic effect. This meant that the mixtures against the second larval instar was effective better compared with fourth larval instar.

***Key words:* .**

INTRODUCTION

A common feature of some micro-organisms, principally bacteria and fungi, is their natural ability to produce metabolic compounds that may be toxic against insect pests. The micro-organisms can be cultured in fermentation facilities and the resulting insecticidal compounds can be harvested, purified, formulated and used effectively against major arthropod pests. Two widely used commercial insecticides, spinosad and abamectin, were developed using this approach (Copping and Menn 2000; Godfrey *et al.* 2005).

Entomopathogenic fungi are important natural control agents of any insects, including several pests (Carruthers & Hural, 1990). Biological control, in particular when accomplished by entomopathogens, is a technique that should be considered as an important pest population density reduction factor in Integrated Pest Management (IPM) programs.

Therefore, the conservation of such entomopathogens, whether they occur naturally or when they are applied or introduced to control insects, is an interesting practice.

However, the use of incompatible insecticides may inhibit the development and reproduction of these pathogens, affecting IPM (Malo, 1993; Duarte *et al.*, 1992; Anderson & Roberts, 1983).

On the other hand, the utilization of selective insecticides in association with pathogens can increase the efficiency of control, allowing the reduction of the amount of applied insecticides, minimizing environmental contamination hazards and the expression of pest resistance (Quintela & McCoy, 1998 and Neves *et al.*, 2001).

The objective of this study was to evaluate the *in vitro* fungitoxic effect (selectivity/compatibility) of some important insecticides used to control cotton leafworm in relation to the entomopathogenic fungus *Beauveria bassiana*, an important natural control agent of cotton leafworm.

MATERIAL AND METHODS

Laboratory experiments:

The present study was conducted in the Bio-insecticides Production Unit and Cotton Leafwom Research Department at Plant Protection Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt.

Insect culture:

The stock colony of *Spodoptera littoralis* was obtained from Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza. This strain was reared under the technique described by El-Defrawy *et al.* 1964. Larvae were provided daily with fresh castor bean leaves *Ricinus communis*. The number of larvae per jar was gradually decreased as the larvae developed reaching twenty per jar for the 6th instar. The jars were incubated in the laboratory under constant laboratory conditions of 27±2°C and 65±5% RH. The resulting

moths were fed on 20 % sugar solution and allowed to lay their eggs on fresh *Nerium oleader* leaves as a physical surface for moths mating. Egg patches were collected daily and transferred into petri dishes until hatching.

Isolation of *Beauveria bassiana*:

Beauveria bassiana was isolated in pure culture on Czapekos Dox agar medium (CDA) from infected adults of *Rhynhophorus ferruginens* collected from Ismailia Governorate, Egypt (Ibrahim, 2006).

Mass production of the entomopathogenic fungus *Beauveria bassiana*:

Conidia were produced on agar Petri-dishes of semi-synthetic Czapek's Dox agar medium which consists of sucrose 20.0 g/L, K_2HPO_4 1.0 g/L, KNO_3 2.0 g/L, yeast extract 2.0 g/L, KCL 0.5 g/L, $MgSO_4$ $7H_2O$ 0.5 g/L, $FeSO_4$ $7H_2O$ 0.002 g/L, agar-agar 20.0 g/L, up to 1000 ml distilled H_2O. The medium was prepared and adjusted pH (5.5-6.5). Petri-dishes were inoculated with *Beauveria bassiana* and incubated for two weeks at 25 ± 1ºC & 50 – 60 % RH. At the end of incubation period the conidia were harvested from the surface of the culture directly by scraping with sterile solution of 0.01 % tween-80. The conidia were separated by filtration through sterilized glass-wool. The resulting suspension was counted using hemocytometer counts technique.

Insecticides:

The insecticide tested:

1- Jito 90% SP, distributed by Kafr El Zayat Pesticides & Chemicals Co. Egypt.
Common name: Methomyl
Recommended rate: 300g / feddan.

2- Coragen20% SC, distributed by DuPont Company, Egypt
Common name: Chlorantraniliprole
Recommended rate: 60ml / feddan.

3- Contrado 5% WP, distributed by Starchem Company, Egypt.
Common name: Fipronil
Recommended rate: 400g / feddan.

4- Novo 10% DC, distributed by Soltaire Company, Egypt.
Common name: Flufenoxuron
Recommended rate: 200ml / feddan.

5- Absolute 5% ME, distributed by Agrogroup Company, Egypt
Common name: Emamectin benzoate
Recommended rate: 75ml / feddan.

6- Dipel 2X 6.4% WP, distributed by My Trade Company, Egypt.
Common name: *Bacillus thuringiensis*
Dipel 2X; a selective bacterial insecticide containing 32000 IU of *Bacillus thuringiensis* subsp. *kurstaki* per mg of product.
Recommended rate: 200g / feddan.

These formulations were tested for their growth inhibition on *Beauveria bassiana* by poison food technique on Czapekos Dox agar medium (CDA) in three replications; the formulations were mixed at the different concentrations (LC_{25} & LC_{50}).

Each 100 ml portion of the medium was dispensed into a 250 Erlenmeyer conical flask and autoclaved at 121ºC for 20 minutes. It was then cooled to about 45ºC stock solutions of the insecticides were prepared in sterilized distilled water and incorporated into each flask to provide different levels of concentrations. Each flask was shaken well and poured into sterilized Petri-plates (90 mm). Medium without insecticides served as a control. Each plate was inoculated with 1 x 10^7 conidiospores from 12 days old culture of *Beauveria bassiana* The inoculated plates were incubated at 25±1ºC. After fifteenth-day of incubation, the growth of *Beauveria bassiana* colony in the Petri-plates that treated with different insecticides at different concentrates was recorded.

Bioassay tests:

Treatments were applied by leaf-dipping bioassay technique (Tabashnik *et al.,* 1991 and Hafez *et al.* 2003) using castor bean leaves. The leaves were first washed with distilled water and were left to dry under room conditions, then dipped in solutions of different concentrations of the assayed insecticides. Each leaf was dipped for 5–10 seconds and left in the laboratory for about an hour until air dried. Each leaf was, then, placed in a 250ml glass jar. Freshly moulted 25 second or fourth instar larvae were, gently, placed in each jar with four replications including controls.

Five concentrations of methomyl (100, 50, 25, 12.5 and 6.25 ppm) and (20, 10, 5, 2.5 and 1.25 ppm) of chlorantraniliprole (6, 3, 1.5, 0.75 and 0.25 ppm) of fipronil & flufenoxuron, and (1, 0.5, 0.25, 0.125 and 0.0625 ppm) of emamectin benzoate and (4, 2, 1, 0.5 and 0.25 ppm) of B. thuringiensis and 109 , 108, 107, 106 and 105 conidia/ml of fungal isolate (*B. bassiana*) were prepared.

Larvae were allowed to feed for 48hrs on treated leaves with flufenoxuron, emamectin benzoate, B. thuringiensis and *B. bassiana* and for 24hrs in case of methomyl, fipronil and chlorantraniliprole.

After the exposure period, treated leaves were removed and replaced by fresh untreated ones. Larval mortality percentages were recorded and corrected by the formula of Abbott (1925). Probit analysis (Finney, 1971) was used to estimate the LC50 value.

Sequential treatments:

Twenty-five larvae in a Petri plate (9 cm diameter) lined with a filter paper were sprayed directly with 2ml conidial suspension of *B. bassiana* (LC_{25}) using a hand atomizer or hand sprayer. After air drying, the treated larvae were carefully transferred to sterile glass jar (250ml) containing castor leaves treated with LC_{25} of each insecticide for 48h. then mortality rate were recorded.

The combined action of the different mixtures was expressed as the Co-toxicity factor (CF) which was esti-

mated according to the equation given by Mansour *et al.,* 1966. CF was determined by calculating the observed mortality percentage minus expected mortality divided by expected mortality percentage.

$$\text{Co} - \text{toxicity factor} = \frac{\text{Observed mortality \%} - \text{Expected mortality \%}}{\text{Expected mortality \%}} \times 100$$

The Co-toxicity factors differentiate the results as follows:

A positive factor of 20 or more was considered as synergistic, a negative of 20 or less as antagonism, while intermediate values (–20 & +20) indicated additive effect.

RESULTS

Data presented in Tables (1 & 2), show the efficacies of the insecticides and fungal isolate, *Beauveria bassiana* represented as LC50's against the 2nd larval instars of *S. littoralis*. In general, data revealed, clearly, that flufenoxuron had the highest efficacy against 2nd larval instars, with LC50 of 0.0225 ppm, while emamectin benzoate had the highest efficacy against 4th larval instars, with LC50 of 0.4372 ppm. Concerning the toxicity of fungal isolate had efficacy against 2nd and 4th larval instars with LC50 of 22 x 104 and 22 x 109 conidia/ml, respectively. Data in Table (1) revealed that, flufenoxuron had the highest toxicity as it recorded the lowest LC50's against 2nd instar larvae (0.0225 ppm), followed by fipronil, emamectin benzoate, chlorantraniliprole, B. thuringiensis and methomyl (0.289, 0.326, 0.463, 1.143 and 10.283 ppm, respectively). Table (2) showed that, emamectin benzoate had the highest toxicity as it recorded the lowest LC50's against 4th instar larvae, followed by flufenoxuron, B. thuringiensis, fipronil, chlorantraniliprole, and methomyl (0.437, 2.235, 2.986, 3.253, 5.558 and 31.976 ppm, respectively).

Effect of the insecticides on *Beauveria bassiana* growth:

emamectin benzoate, methomyl and *Bacillus thuringiensis* formulations at the two concentrations (LC_{50} & LC_{25}) did not inhibit mycelial growth where percentage of reduction relative to the control less than 50% (Table 3).

Bacillus thuringiensis formulation more compatible especially at concentration LC_{25} where number of conidia was 175 x 10^9 conidia/ml with reduction 7.9%, followed by emamectin benzoate and methomyl were 167 x 10^9 conidia/ml (reduction was 12.1%) and 151 x 10^9 conidia/ml (reduction was 20.5%), respectively.

On the other hand, at the concentration LC_{25} of chlorantraniliprole, flufenoxuron and fipronil formulations were moderately toxic to *B. bassiana* where number of conidia were 84 x 10^9, 84 x 10^9 and 77 x 10^9 conidia/ml and percentage of reduction were 55.8, 55.8 and 59.5%, respectively (Table 3).

With regard to product incompatibility, the effect LC_{50} of chlorantraniliprole, flufenoxuron and fipronil formulations showed inhibition of mycelial growth, where percentage of reduction relative to the control were 100% this mean that LC_{50} of three insecticides previously mentioned very toxic.

Table (1): Toxicity of the tested insecicides on 2nd inster larvae of cotton leaf worm, *S. littoralis*

Treatments	LC_{50} (ppm)	Lower limit	Upper limit	Slope
Emamectin benzoate	0.326	0.2051	0.5942	0.5663±0.1342
Methomyl	10.283	5.5496	14.171	1.7572± 0.3078
Bacillus thuringiensis	1.143	0.8348	1.471	1.0397± 0.2322
Chlorantraniliprole	0.463	0.0916	0.9575	0.7212± 0.1508
Flufenoxuron	0.023	0.0108	0.1119	0.4204±0.1303
Fipronil	0.289	0.0556	0.5638	0.8414±0.1912
Fungal isolate	22×10^4 (conidia/ml)	2.26×10^4	4.39×10^5	2.29375±0.28135

Table (2): Toxicity of the tested insecicides on 4th inster larvae of cotton leaf worm, *S. littoralis*

Treatments	LC_{50} (ppm)	Lower limit	Upper limit	Slope
Emamectin benzoate	0.437	0.3081	0.7213	0.7521±0.1368
Methomyl	31.976	22.0766	42.5588	0.9418±0.2517
Bacillus thuringiensis	2.986	2.0536	8.2705	0.8721±0.2388
Chlorantraniliprole	5.558	4.2495	7.382	0.9895±0.1392
Flufenoxuron	2.235	1.5459	3.3111	0.8431±0.1908
Fipronil	3.253	2.5129	4.6613	1.012±0.1836
Fungal isolate	22×10^9 (conidia/ml)	2.1×10^8	3.4×10^{11}	2.44375±0.3115

Table (3): *In vitro* compatibility of fungal isolate (*B. bassiana*) with some insecticides at concentrations LC25 & LC50 of second instar larvae of cotton leaf worm, *S. littoralis*

Treatments	Rate	*B. bassiana*		
		Classification	**Number of conidia (conidia/ml)**	**(%) reduction**
Emamectin benzoate	LC_{25}	C	167×10^9	12.1
	LC_{50}	C	154×10^9	18.9
Methomyl	LC_{25}	C	151×10^9	20.5
	LC_{50}	C	145×10^9	23.7
Bacillus thuringiensis	LC_{25}	C	175×10^9	7.9
	LC_{50}	C	166×10^9	12.6
Chlorantraniliprole	LC_{25}	MoT	84×10^9	55.8
	LC_{50}	T	0	100.0
Flufenoxuron	LC_{25}	MoT	84×10^9	55.8
	LC_{50}	T	0	100.0
Fipronil	LC_{25}	MoT	77×10^9	59.5
	LC_{50}	T	0	100.0
Control		-	190×10^9	0

MoT= moderately toxic, C= compatible, T= very toxic.

Efficacy of combining *B. bassiana* with certain insecticides:

From data in Table (4), it could be deduced that, the *S. littoralis* 2nd instar larval treatments by tested compounds-*B. bassiana* mixtures, the observed mortality percentage after treatments at rate $LC_{25} + LC_{25}$ ranged from 51.3 – 57.5% and the Co-toxicity factor was always 20 < indicating, only, additive effects against the 2nd instar *S. littoralis* larvae.

As for the *S. littoralis* 4th instar larval treatments by tested insecticides plus *B. bassiana* mixtures, results showed that when treated larvae with Emamectin benzoate + *B. bassiana* and *B. thuringiensis* + *B. bassiana* at rate $LC_{25} + LC_{25}$ the observed mortality percentage were 48.8 and 47.5% and the Co-toxicity factor was 20 < indicating additive effect against the 4th instar *S. littoralis* larvae (Table 5). While treated larvae with methomyl + *B. bassiana* at rate $LC_{25} + LC_{25}$ the observed mortality was 41.3% and the Co-toxicity factor was-20 > this mean antagonistic effect (Table 5).

DISCUSSION

Variation in toxicity response of entomogenous fungi viz *B. bassiana* from synergistic, antagonistic or neutral to insecticides (Pevling and Weyrich, 1992; Mietkiewski and Gors- ki 1995) has been reported. In the present study, the different insecticides exhibited varying effects on toxicity against S. littralis when these were

Table (4): Effect of combining of *B. bassiana* and tested insecticides on 2nd instar larvae of cotton leaf worm, *S. littoralis*

Treatments	Application rate	% Expected mortality	% Observed mortality	Co-toxicity factor	Joint action category
Emamectin benzoate + *B. bassiana*	$LC_{25} + LC_{25}$	50	57.5	13.04	Additive
Methomyl + *B. bassiana*	$LC_{25} + LC_{25}$	50	52.5	4.76	Additive
B. thuringiensis + *B. bassiana*	$LC_{25} + LC_{25}$	50	51.3	2.53	Additive

Table (5): Effect of combining of *B. bassiana* and tested insecticides on 4th instar larvae of cotton leaf worm, *S. littoralis*

Treatments	Application rate	% Expected mortality	% Observed mortality	Co-toxicity factor	Joint action category
Emamectin benzoate + *B. bassiana*	$LC_{25} + LC_{25}$	50	48.8	-2.46	Additive
Methomyl + *B. bassiana*	$LC_{25} + LC_{25}$	50	41.3	-21.07	antagonistic
B. thuringiensis + *B. bassiana*	$LC_{25} + LC_{25}$	50	47.5	-5.26	Additive

mixed with *B. bassiana*. On the basis of these observations, it is apparent that the combinations of insecticides with *B. bassiana* were more toxic than when used alone except chlorantraniliprole, flufenoxuron and fipronil were found to cause the highest reduction levels and should never be used in mixtures with this entomopathogenic fungi or in pest control strategies where these fungi could be applied or could be an important natural pest population reduction factor. Which suggestion, the formula proposed by Alves *et al.* (1998) should consider the reduction in conidia germination an important factor in compatibility. Similar results were obtained by Prasad (1989) who found that endosulfan inhibited growth and conidial germination of *B. bassiana*.

Dayakar *et al.* (2000) found that the combination of insecticides with *B. bassiana* and *M. anisopliae* showed 1.05–1.24 and 1.19–1.42 fold increase in virulence over the sole treatment, respectively. In another study, Purwar and Sachan (2004) also found similar results with *Lipaphis erysimi*. Thus, the combination of insecticide and entomogenous fungi was more deleterious to the insect than application of insecticides or entomogenous fungi alone.

The sensitivity of different fungal isolates of the same species may differ greatly to a particular pesticide (Olmert and Kenneth, 1974). Generalization of the effect of insecticides on the fungus cannot be made since the interaction effects may vary from isolate to isolate and the chemical nature of the pesticides. Thus, it is apparent that the utilization of combination of *Bacillus thuringiensis* (Dipel 2X), emamectin benzoate (Absolute) and methomyl (Jito) with *B. bassiana* is feasible for the control of polyphagous insect, *S. littralis* especially the second instar. However, further studies on the mechanism of toxicity of these combinations in lepidopterans are needed.

Under field condition, compatibility germination should be considered as the most important factor (Malo 1993; Anderson and Roberts 1983) due to the fact that pathogens infect insects through conidia germination by ingestion or contact. Thus, when the treatment is compatible *in vitro*, there are strong evidences of its selectivity under field conditions. However, a high toxicity *in vitro* does not always mean that the same will happen in the field (Alves *et al.*1998).

Information about compatibility among entomopathogenic fungi and products used in organic agriculture, like fertilizers and insecticides, is scarce. Field studies with the application of products together with pathogens can provide extra information to that obtained by this study to help in the development of strategies for handling plagues in organic agriculture.

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التوافق بين فطر *Beauveria bassiana* وبعض المبيدات الحشرية ضد دودة ورق القطن

احمد عدلي ابراهيم و محمد فتحي عبد العزيز و علي ربيع محمد الجبلي

معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي – الجيزة

في هذه الدراسة تم تقييم ستة مبيدات حشرية ضد دودة ورق القطن معمليا وهي Emamectin benzoate, Methomyl, *Bacillus thuringiensis*, Chlorantraniliprole, Flufenoxuron and Fipronil وعزلة فطر *Beauveria bassiana*، ثم تم تحضير التركيزان LC_{25} & LC_{50} من المركبات السابقة ودراسة تأثيرهما على نمو الفطر وبعد ذلك اختيار المركبات والتركيز الامثل والذي يكون متوافقا مع الفطر محل الدراسة حتي يتم معاملة اليرقات بهم. اظهرت النتائج أن Flufenoxuron أكثر المركبات تأثيرا على يرقات العمر الثاني حيث كانت قيمة LC_{50} ٠,٠٢٢٥ جزء في المليون ثم يلية Fipronil, Emamectin benzoate, Chlorantraniliprole, *B. thuringiensis* and Methomyl حيث كانت قيم LC_{50} ٠,٢٨٩، ٠,٣٢٦، ٠,٤٦٣، ١,١٤٣ و١٠,٢٨٣ جزء في المليون على التوالي. اما بالنسبة للعمر اليرقي الرابع فكان المركب Emamectin benzoate هو الاكثر سمية ثم يليه المركبات الأخرى Flufenoxuron, *B. thuringiensis*, Fipronil, Chlorantraniliprole, and Methomyl حيث كانت قيم LC_{50} لهم ٠,٤٣٧، ٢,٢٣٥، ٢,٩٨٦، ٣,٢٥٣، ٥,٥٥٨ و ٣١,٩٧٦ جزء في المليون على التوالي. كما اوضحت النتائج ان المركبات Emamectin benzoate, Methyl, *Bacillus thuringiensis* عند التركيزان LC_{25} & LC_{50} ليس لها تأثير واضح علي نمو الفطر في المعمل. بينما المركبات الاخري و هي Chlorantraniliprole, Flufenoxuron, Fipronil كان لها تأثير مثبط لنمو الفطر تحت تركيز LC_{50}، و لكن تركيز LC_{25} كان له تأثير متوسط علي تثبيط نمو الفطر. وعند معاملة يرقات العمر الثاني لدودة ورق القطن بالفطر والمركبات Emamectin benzoate, Methomyl, *Bacillus thuringiensis* اعطت المخاليط تأثير اضافة عند التركيز LC_{25}. بينما معاملة يرقات العمر الرابع لدودة ورق القطن بالفطر والمركبات Emamectin benzoate, Methomyl , *Bacillus thuringiensis* اعطت تأثير تضاد في غالبية المعاملات ، و هذا يعني أن المعاملة بالخلط تكون افضل عند معاملة العمر اليرقي الثاني.

Characterization and Evaluation of Larvicidal Activity of some Bacillus Isolates Against *Agrotis ipsilon* Hfn. (Lepidoptera: Noctuidae)

Ibrahim[1], A. A., R. A. Arafa[2], N. N. Abed[2], S. K. M. Elshennawy[2]

[1] Bioinsecticides Production Unit, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

[2] Department of Botany and Microbiology, Faculty of Science, Al-Azhar University (Girls branch), Nasr city, Cairo, Egypt

ABSTRACT

A total of 10 *Bacillus* isolates were purified from 13 soil samples from different localities habitats in Egypt. They were characterized according to their phenotypic, physiological and biochemical parameters by conventional and routine techniques. In addition 16S rRNA gene sequencing was determined for the three most potent isolates which defined as *Bacillus thuringiensis Bt* 407(Be10), *Bacillus subtilis* IAM 12118(Ch2) and *Bacillus coagulans*(Da1) NBRC12583. The effective isolates exhibited strong insecticidal activity against the 2nd instar larvae of the black cutworm, *Agrotis ipsilon* (Hfn). Concentrations ranging from 2.7x10^{10}spores/ml to 2.7x10^{6}spores/ml were tested on the larvae for a period of 10 days. Accumulative mortality percentage of the 2nd instar larvae of *A. ipsilon* treated with the bacterial isolates were increased gradually with increasing the time elapsed after treatment. *Bacillus thuringiensis* displaying the highest mortality percentage on the second instar about 100%. *Bacillus subtilis* showed mortality percentage of 81.58%, while the least activity was recorded in *Bacillus coagulans* 64.07% mortality for the highest concentration 2.7x10^{10}spore/ml after 10 days of treatment. The SDS-PAGE protein profile analysis of the most potent bacterial isolate, *Bacillus thuringenisis* was showed one distinct protein band with molecular weight 64 kDa and was toxic to the 2nd instar larvae of *A. ipsilon*. The most common lepidopteran-specific toxin of *Bacillus thuringiensis* has been tested for this efficacy against larvae of *Agrotis ipsilon*. A single dose of 0.2mg were used and show mortality percentage about 100% after 3 days of treatment as compared to the control (0.0%). However, the mortality percentage of *B. thuringenisis* is higher than the percentage of other entomopathogenic isolates *B. bassiana*, *B. subtilis* and *B. coagulans* but their toxicity did not stop to this stage and was extended to the other stages of insect's life, including death and deformities until the end of the life cycle.

Key words: ***Entomopathogenic bacteria, Agrotis ipsilon (Hfn); 16S rRNA gene sequencing, Insecticidal activities.***

INTRODUCTION

The black cutworm, *Agrotis ipsilon* Hfn, (Lepidoptera: Noctuidae) is one of the most serious pests that possess an economic threat to many agricultural plant species in Egypt (Zethner, 1980). *A. ipsilon* is distributed in Europe, Africa, China, India, Canada and North America. It has a wide host range. Nearly all vegetables can be consumed, and this species also feeds on alfalfa, clover, cotton, rice, sorghum, strawberry, sugar beet, tobacco, and sometimes grains and grasses. Larvae can consume over 400 cm^2 of foliage during their development (Sahayaraj, 2015).

Extensive use of synthetic chemical pesticides resulting in environmental pollution, adverse effects on human health, other organisms and beneficial insects, which are natural enemies of target and non- target pest species (Ffrench-Constant *et al*., 2001). Recent concerns about the hazardous effects of chemical pesticides on the environment have encouraged scientists to consider finding more effective and safe control agents.

The Egyptian soil contains numerous types of living organisms like bacteria which are useful in discarded harmful organisms to plant like insects. Bacillus species have been used to control insects and the larvicidal Bacilli commonly used are *Bacillus thuringiensis, B. sphaericus, B. brevis, B. circulans* and *B. subtilis*. They have been demonstrated to produce larvicidal toxins (Darriet and Hougard, 2002; Das and Mukherjee, 2006 and Geetha *et al*., 2011).

The greatest successes in microbial pesticides have come from the uses of *B. thuringiensis.* The popularity of these microbes is due to their high insect toxicity, environmental safety and lack of toxicity to human and other vertebrates (Aronson and Yechiel, 2001 and Siegel, 2001).

The purpose of the present study is to explore the larvicidal activity of natural microbial isolates of *B. thuringiensis*-Be10*, B. subtilis*-Da1 and *B. coagulans*-Gh2 species which are isolated from different soil samples against the second instar larvae of *A. ipsilon* under laboratory condition.

MATERIALS AND METHODS

1. Insect Rearing:

The *Agrotis ipsilon* eggs were collected from the Insect Pathogen Unit of Plant Protection Research Institute, Dokki, Giza. Egg masses were used to maintain a colony in the laboratory adjusted under constant conditions of temperature and humidity (25 ± 2°C and 65±5% relative humidity) according to a constant technique described by Abdel-Hadi, M.A. (1968). Castor bean leaves, *Ricinus communis* was used for larval feeding under laboratory conditions and away from any intentional chemical pressure.

2. Isolation and identification of the bacterial isolates:

2.1. Sample Collection and isolation:

Thirteen soil samples were collected from several regions in Egypt (Beheira, Damietta, Dakahlyia, Gharbyia (clay), Gharbyia (sand), Giza, Kafer- Elsheikh (sand), Menoufia, Qalubyia and Sharkya) in clean plastic bags. Each 1g of the soil samples were suspended in 9ml of sterile distilled water and shaken vigorously for about 2min. The samples were heated at 80°C for 30 min in a water bath to destroy all vegetative microbial cells for the isolation of *Bacillus* spp. Isolation was done according to the method of Ohba and Aizawa (1986) and Travers *et al*. (1987).

2.2. Identification and purification of bacterial isolates:

- **Morphological and biochemical identification**

Isolated strains were identified based on its morphological and biochemical characteristics depending on phenotypic properties. Bacterial solates shared almost the same taxonomical characteristics according to classification of Bergye's Manual of systematic Bacteriology (Sneath, 1986; Holt *et al*., 1994 and Vos *et al*., 2009) at Al-Azhar University Center of Fermentation Biotechnology and Applied Microbiology.

- **Molecular identification**

In order to identify the most potent bacterial isolates, the genomic DNA was isolated and purified using purification kit (Wizard® Genomic DNA purification kit) provided by promega company. The DNA sample was amplified with the 16S rRNA specific primer and the PCR products were sent to Sigma Company for sequencing (at 23th Elesraa street Elmoalmean City Lebanon square Elmohandsean, Cairo, Egypt). The sequencing data was blasted on NCBI›s database (National Center for Biological Information) to compare the sequence data of unknown isolates with known sequence submitted before database. The sequence data was submitted to the gene bank database to get its accession number.

3. Preparation of bacterial concentrations:

All the microbial isolates were grown on Nutrient agar (NA) medium and the cultures were grown for 72h at 28ºC. The concentration of *Bacillus* spp. were 2.7×10^{10} spores/ml, detected by serial dilutions test for the suspension of the selected isolate in the laboratory of the Plant Protection Institute, Agriculture Research Center, Doki, Giza. The bacterial concentration which resulted from the previously technique was determined by plate count method, five concentrations of *Bacillus* spp. were prepared from (2.7×10^{6} to 2.7×10^{10} spores /ml).

4. Bioassay tests:

For studying the efficiency of the different concentrations of bacterial isolates. Five concentrations from$2.7\text{x}10^{6}$ to $2.7\text{x}10^{10}$ spores/ml were used for examined their toxicity against the second instar larvae of *Agrotis ipsilon* by leaf dipping technique according to Makkar and El Mandarawy, 1996. The larval mortality percentages were recorded after 48h to 10 days. The mortality percentages of treated larvae were corrected against the control by Abbott's formula (Abbott, 1925) as follows:

Abbott's corrected mortality =

$$\frac{\text{\% mortality in treatment - \% mortality in control}}{\text{100 - \% mortality in control}}\times 100$$

5. Statistical analysis:

All data obtained from the above experiments were analyzed statistically according to Finney (1971) using "Probit analysis LdP Line program"; software to calculate LC_{50} and as well as the slope and regression values.

6. Purification and estimation of crystal protein:

6.1. Preparation of spore-crystal mixture

Acetone powders of spore-crystal mixture were prepared as described by Ammoumeh *et al*. (2011).The dried residue was powdered and weighted then stored in airtight sterile glass vials at 4°C for further use . This powder was considered as an Acetone powder.

6.2. Solubilization of crystal proteins

Solubilization process was applied as described by Ammoumeh *et al.* (2010). Acetone powders of spore-crystal complex of *Bacillus* isolate was dissolved in solubilizing buffer and sonicated for 2 to 4 sec. The cell suspension was centrifuged at 43571.25 xg for 10 min after incubated them at 37°C for 4h. This protein estimation and characterization was determined on 10% SDS-PAGE. The solubilized crystal proteins were stored in clean autoclaved Eppendorf tubes at -20°C for further bioassays.

6.3. SDS-PAGE Analysis of parasporal inclusion protein

Parasporal crystal protein solution of the most potent Bacillus isolate was performed by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970) by using 10% separating and 4% stacking gels. After electrophoresis the gels were stained with 0.1% (w/v) Coomassie blue. The molecular weight of sample was calculated by comparing with the relative molecular weights of protein standard marker.

RESULTS AND DISCUSSION

Natural soil sample is an excellent residence for plentiful microbes which have specific activities against insect species of different orders. Basis of this, natural microbial isolates screened and isolate from the soil sample for larvae control program under laboratory condition.

1. Isolation and identification of the bacterial isolates:

In the present work, ten bacterial isolates were isolated from 13 soil samples, collected from different localities in Egypt. Only three clay soils from Damietta, Dakhliya, Giza governorates gave negative results (there is no bacterial growth), While soils of the other ten governorates gave a positive results.

The bacterial isolates were identified firstly by their morphology under light microscope and are suggestive to belonging to the genus Bacillus Gram-positive, rod shaped colony as shown in (Figure 1).

Then, physiological and biochemical studies were used according to Bergey's Manual of systematic Bacteriology, for further identification as shown in (Table1).

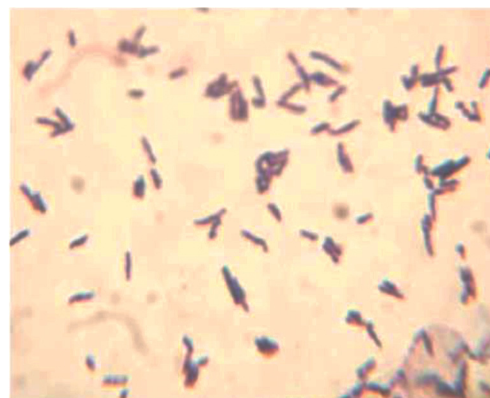

Figure (1): Gram stain test of *Bacillus* isolates showing vegetative cells with rod shaped and Gram positive

Table (1): Morphological and biochemical features

S. No.	Identification Tests	Bacterial isolates									
		Bacillus subtillis -Da1	*Bacillus coagulance* -Gh2	*Bacillus pseudomycoid* -Sh3	*Bacillus brevis* -Qa4	*Bacillus firmus* - KSh5	*Bacillus circulans* -Be6	*Bacillus licheniformis* -Gi7	*Bacillus macerans* -Me8	*Bacillus pantothenticus* - Gh9	*Bacillus thuringiensis* -Be10
	a. Preliminary tests										
1	Gram Staining	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
2	Spore staining	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3	Shape	Rod	Short rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
4	Motility	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
5	KOH	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	b. Biochemical tests										
1	Indole test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Catalase test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3	Utilization of Citrate	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
4	Urea test	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
5	Acetoine (AMC)	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve
	c. Carbohydrate fermentation tests (Acid production)										
1	D-Glucose	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^+
2	D-Xylose	A^+	A-	A^+	A^+	A-	A^+	A^+	A^+	A^-	A^-
3	D-manitol	A^+	A^+	A-	A^+	A-	A^+	A^+	A^+	A^-	A-
	d. Other testes										
1	Starch hydrolysis	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
2	Gelatin hydrolysis	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3	Anaerobic growth	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
4	Nitrate reduced to nitrite	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	–ve	+ve
5	Growth at pH 6.8	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	e. Growth at different temperature										
*	30°C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
*	40°C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
*	50°C	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve
*	60°C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	f. Tolerance to NaCl concentration										
*	2%	+ve	+ve	+ve	+ve	+ve	+ve	+ve	–ve	+ve	+ve
*	5%	+ve	+ve	+ve	+ve	+ve	+ve	+ve	–ve	+ve	+ve
*	7%	+ve	+ve	-ve	-ve	-ve	+ve	+ve	–ve	+ve	+ve
*	10%	–ve	–ve	–ve	–ve	–ve	–ve	+ve	–ve	+ve	+ve

(AMC): the production of acetyl – methyl carbinol

2. Bioassay tests

- **Bacterial susceptibility test:**

In screening assay, Different concentrations of the ten tested Bacillus strains were prepared to examine their insecticidal activity against the second instars larvae of *A. ipsilon,* where the microbial isolates have been effective using leaf dipping technique.

Results in Table (2) showed that after ten days of treatment, all the 10 bacterial isolates exert a percentage of mortality compared to the control (0.0%), the degree of toxicity varied greatly among them (from 13.4% to 100%). The lowest toxic strains were number Gh9, Me8 and Be6 which resulted in mortality % of 13.4, 15.6 and 22.3% respectively. Meanwhile, strains number Be10, Gh2 and Da1showed appreciable mortality of 100%, 81.5% and 64% respectively.

Table (2): Larvicidal effects of *Bacillus* isolates on the 2nd instars larvae of *Agrotis ipsilon* after ten days of treatment

Bacillus isolates code	Number of dead larvae	Mortality %
Control	0.0	0.0
Bacillus subtilis-Da1	32	81.5
B. coagulance-Gh2	25	64.0
B. pseudomycoid-Sh3	10	25.3
B. brevis-Qa4	22	52.3
B. firmus-KSh5	12	30.0
B. circulans-Be6	9	22.3
B. Licheniformis-Gi7	16	40.0
B. macerans-Me8	7	15.6
B. pantothenticus- Gh9	5	13.4
B. thuringiensis-Be10	40	100

- **Toxicity of the three most potent *Bacillus* isolates against 2nd larval instar of *A. ipsilon***

Accumulative mortality percentage of the 2nd instar larvae of *A. ipsilon* treated with the different concentrations of the most potent three tested bacterial isolates using leaf dipping technique was explained in Table (3). *B. thuringiensis*-Be10 *B. subtilis*-Da1 and *B. coagulans*-Gh2 caused 75.74 %, % 45.30% and 16.29% mortality at a concentration of $2.7x10^{10}$ spores/ml when feeding on castor leaves for two days and were 100%, 81.58% and 64.07% respectively for the concentration 2.7×10^{10} spores/ml after 10 days of infection, respectively. Mortality Percentage at the end of experiment caused by different concentrations of the bacterial isolate has been shown graphically (Figure 2).

The toxicity of this *Bacillus* spp. against 2nd larval instars of *A. ipsilon* clearly indicates that there is a positive relationship between the concentrations of the bacterial strains and their toxicity. These results may be comparable with these of other authors; for example, Salama *et al.* (1981) found that *B. thuringiensis* entomocidus caused 100, 98, 100, 88, 66, 58 and 32% mortality at concentrations of 500, 250, 200, 125, 100, 50 and 25 mg/ml, respectively, on 2nd instar larvae of *S. littoralis* that were fed on a treated artificial diet for two days.

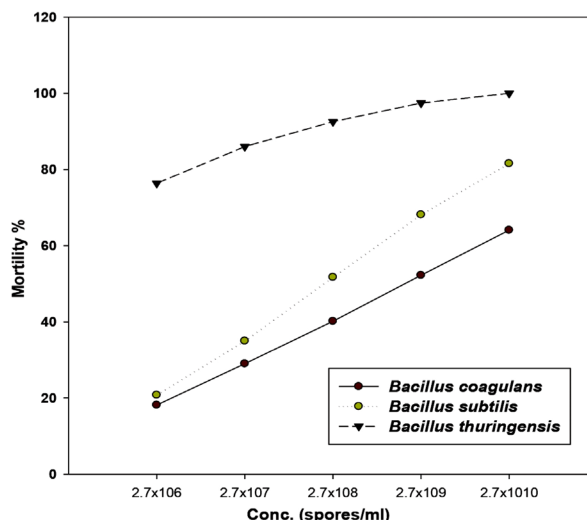

Figure (2): Effect of different concentrations of the three effective bacterial isolates on the 2nd instar larvae of *Agrotis ipsilon* after 10 days of infection.

However, Vandenberg and Shimanuki (1990) tested the pathogenicity of *Bacills coagulans* especially at doses higher than 2×10^6cfu/larva, they found about 62% mortality of larvae inoculated at 1 day of age was obtained at doses of 2.5×106and 3.5×10^6cfu/larva and 48% mortality occurred at a dose of 1×10^7cfu/larva.

Bacillus subtilis has also been shown to be capable of infecting and causing mortality of the 2nd instar larvae of the mosquito, *Anophelis culicifacies*, which is the primary insect vector of malaria in central India (Gupta and Vyas, 1989).

B. thuringiensis-Be10 was the most active strain, with an LC_{50} of 2.8 $x10^3$spores/ ml, *B. subtilis*-Da1 showed LC_{50} of $2.1x10^8$spores/ml while *B. coagulans*-Gh2 achieved LC_{50} of $1.7x10^9$ spores/ml (Table 3). The LC_{50} of *B. thuringeinsis* recorded agree with GuiLan *et al*. (2002) and Namvar *et al*. (2003).

The larvicidal activity of Bacillus isolates on the 2nd instar larvae induced as dead larvae with lysis of all component and dark color as shown in (Figure 3).There is no mortality was recorded within the control group and no bacterial growth was observed on dead control larvae.

3. Biological studies:

- **Latent effects of different entomopathogenic bacterial strains on *Agrotis ipsilon***

The data illustrated in Table (4) showed that the percentage of pupation and adult emergence decreased by increasing the bacteria concentration. The afore-

Table (3): Insecticidal effect of *B. thuringiensis*-Be10, *B. subtilis*-Da1 and *B. coagulans*-Gh2 against the 2nd instar larvae of *A. ipsilon* using leaf dipping technique

Isolates	Conc. (spores/ml)	% Accumulative mortality after indicated days					LC50 values in (Spores/ml)	Slope ± S.E.	N
		2	4	6	8	10			
Control		0.0	0.0	0.0	0.0	0.0			
Bacillus thuringiensis-Be10	2.7×1010	75.74	88.65	92.03	95.96	100	2.8 ×103	0.3627±0.1045	40
	2.7×109	67.17	80.78	86.61	92.09	97.43			
	2.7×108	55.58	71.73	78.56	85.93	92.55			
	2.7×107	49.82	60.85	68.42	77.13	86.02			
	2.7×106	37.62	49.95	65.96	69.31	76.38			
Bacillus subtilis-Da1	2.7×1010	45.30	53.70	64.87	75.21	81.58	2.1 ×108	0.4285±0.0702	40
	2.7×109	31.28	40.33	50.09	60.81	68.12			
	2.7×108	19.55	28.00	35.29	44.74	51.70			
	2.7×107	10.98	17.87	22.45	29.49	34.98			
	2.7×106	0.00	10.42	12.79	17.21	20.77			
Bacillus coagulans-Gh2	2.7×1010	16.29	25.33	36.45	48.38	64.07	1.7×109	0.2982±0.0671	40
	2.7×109	13.59	19.90	28.80	38.77	52.22			
	2.7×108	11.20	15.24	22.01	29.99	40.17			
	2.7×107	0.00	9.14	16.25	22.16	28.99			
	2.7×106	0.00	8.26	11.57	15.66	18.14			

N= number of tested larvae in each case

mentioned results in Table (3) showed that all larvae died at concentrations of (2.7×10^{10} and 2.7×10^{9} spores/ml), while at the concentrations (2.7×10^{8}, 2.7×10^{7} and 2.7×10^{6} spores/ml) all of the remaining larvae were died before pupation, so there are no latent effects observed of the tested larvae at different concentrations of *Bt*.

In agreement with the present results, Abu-Bakr (2015) found that two strains of *B. thuringiensis* gave high mortality against 1st instar larvae *spodoptera litoralis* and there are no any latent effects. However, *B. subtilis*-Da1 show 20% pupation and 37.5% adults emerged for the highest concentration, 2.7×10^{10}spores/ml compared to 100% pupation and adult emerged in the control. *B. coagulans*-Gh2 shows the less effective strain with 37.5% pupation and 66.6% adult emergency.

Figure (3): Normal and dead larvae cadavers of *Agrotis ipsilon*. (A) Normal larva of *A. ipsilon*. (B) Dark colored dead larva after treated with the three Bacillus isolates

In agreement with the present results, Atallah *et al.*, (2002) found that the percentage of pupation decreased by increasing the concentration; recording 46.0, 38.0, and 27.0% at concentrations of 0.625, 1.25 and 2.5 g/l, respectively; after 10 days, the percentage of adult emergence among the surviving individuals ranged from 20.0 to 41.0%. Moreover, Salama *et al.,* (1981) reported that the percentage of *S. littoralis* larvae that survived and succeeded to pupate increased with a decrease in toxin concentration and with a decrease in exposure time. Also, the percentage of moth emergence in *S. littoralis* was also shown to be affected since it was 83.0% after larval exposure to 100mg/ml for day, decreasing to 45.5% after exposure for two days.

Taking in consideration the percentages of Pupal Malformation, all tested bacterial strains revealed a good reduction in pupation percentage as compared to control larvae. Percentages of pupation failure were 0.0%, 80% and 62.5% for *B. thuringiensis*, *B. subtilis*-Da1 and *Bacillus coagulans*-Gh2 respectively at the concentration of $2.7\text{x}10^{10}$spores/ml.

Also, the percentages of adult Malformation decreased with a decrease in the concentration of 2nd instar larvae of *A. ipsilon*. The percentage of failure of adult emergence in 2nd instar larvae were 0.0%, 62.5% and 33.4% for the previous strains.

Table (4) Effect of *B. thuringiensis*-Be10, *B. subtilis*-Da1 and *Bacillus coagulans*-Gh2 on different biological parameters of the 2nd instar larvae of *Agrotis ipsilon*

Isolates	Concentrations (spores/ml)	% Pupation	% Pupal Malformation	% Adults emergence	% Adult Malformation
Control		0.0	0.0	0.0	0.0
Bacillus Thuringiensis-Be10					
2.7×10^{9}					
2.7×10^{8}	2.7×10^{10}	--------	--------	--------	--------
2.7×10^{7}					
2.7×10^{6}					
Control		100	0.0	100	0.0
Bacillus subtilis- Da1	2.7×10^{10}	20	80	37.5	62.5
2.7×10^{9}	32.5	67.5	53.8	46.2	
2.7×10^{8}	42.5	57.5	76.4	23.6	
2.7×10^{7}	67.5	32.5	81.5	18.5	
2.7×10^{6}	80	20	84.4	15.6	
Control		95	5	95	5
Bacillus coagulans-Gh2	2.7×10^{10}	37.5	62.5	66.6	33.4
2.7×10^{9}	45	55	72.2	27.8	
2.7×10^{8}	60	40	79.2	20.8	
2.7×10^{7}	72.5	27.5	86.2	13.8	
2.7×10^{6}	80	20	93.8	6.2	

The present work showed that, malformed individuals of *A. ipsilon* (pre-pupae) by *Bacillus coagulans*-Gh2 and *Bacillus subtilis*-Da1 were observed, in which high concentrations of this bacterial strains induced pre-pupae with dwarf and dark color of the whole body positively directed with dose,(Figure 4, 5).

Figure (4): (A) Normal pre-pupa of *A. ipsilon*, (B, C) Abnormal dwarf and dark color dead pre-pupae after 14 days of treatment by *B. coagulans*-Gh2

Figure (5): (A) Normal pre-pupa, abnormal dwarf and dark colored dead pre-pupa infected with *B. subtilis*-Da1 after 18 days of treatment

It is clear that the abnormalities extended to the resulted pupae, they were characterized by small size, dark color and appearance of larval pupal intermediate forms, Figure (6) in the case of *B. coagulans* and *B. subtilis* pupae were characterized by dwarf, dark color and appearance of larval pupal intermediate forms as shown in Figure (7).

Moreover, the deformities of adults *A. ipsilon* resulted from treated 2nd instar larvae were as a result of a side effect of bioinsecticide. *B. coagulans* show the deformities in Wings which were folded and crumbled (Figure 8).While, the deformities occurred by *B. subtilis* including severe shrinkage of appendages especially wings. Wings were folded and extremely reduced in size (Figure 9).

- **Molecular characterization of the three most potent entomopathogenic bacterial isolates:**

For a more detailed identification the three most potent insecticidal bacterial isolates Be10, Da1and Gh2 were sent to Sigma lab for partial 16S rRNA gene se-

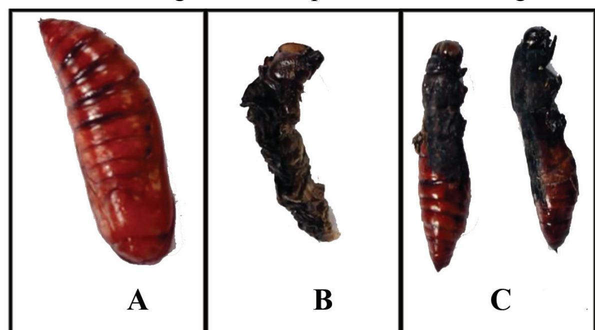

Figure (6): Photographs showing normal pupa and pupal cadavers after 20 days of infection by *B. coagulans*-Gh2, (A) Normal pupa of *A. ipsilon*, (B) Abnormal small and dark pupa, and (C) Abnormal larval pupal intermediate forms

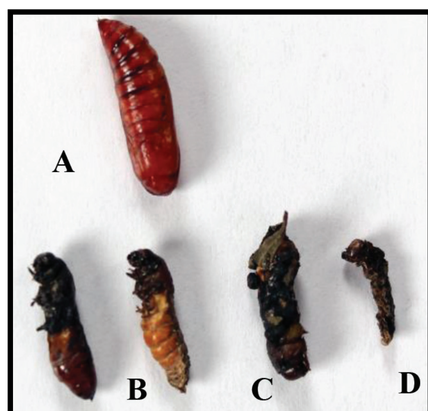

Figure (7): Photographs showing normal pupa and pupal cadavers at 22 days post-treatment by *B. subtilis*-Da1, (B) Abnormal dead larval pupal intermediate forms, (C, D) Abnormal dark and dwarf dead pupae

Figure (8): (A) Normal *A. ipsilon* adult, (B) Abnormal adult with folded and crumbled wings after 26 days post treatment by *B. coagulans*-Gh2

quence analysis at the species level.

Isolate *B. thuringiensis*-Be10 was confirmed by partial 16S rRNA sequence data and so it could be suggested of being *B. thuringiensis Bt*407 strain with Accession of (NR_102506.1). Dendrogram for the relation between *B. thuringiensis* and other NCBI Bacillus based on 16S rRNA gene is shown in Figure (10).

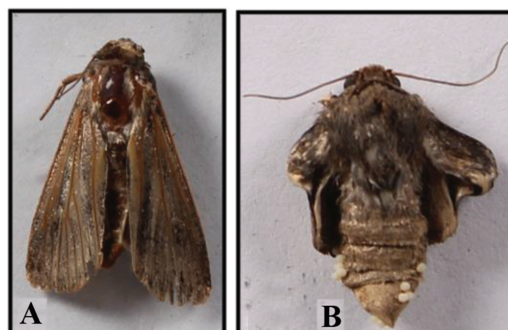

Figure (9): (A) Normal *A. ipsilon* adult, (B) Abnormal adult with sever small and shrinkage wings after 28 days of infection by *B. subtilis*-Da1

Also, *B. subtilis*-Da1 isolate suggested being belongs to the genus Bacillus according to Bergey's Manual of systematic Bacteriology. It could be given the tentative name *B. subtilis* IAM 12118 with accession number of (NR_112116.1) as shown in Figure (11).

Since the isolate *B. coagulans*-Gh2 was rod and Gram-positive bacteria. It was identified according to Bergey's Manual of systematic Bacteriology, and so it could be suggested as *B. coagulans* NBRC12583 with accession number of (NR_041523.1) as shown in (Figure12).

4. Analysis of crystals' protein profiles of *B. thuringiensis*-Be10:

The parasporal inclusion protein of bacterial strain that was the most toxic isolate to larvae of the black cutworm was analyzed by SDS-PAGE. Electrophoretic analysis revealed the presence of 7 distinct bands with molecular weights ranging from 20 to 225 kDa for standard proteins Marker and one distinct band with

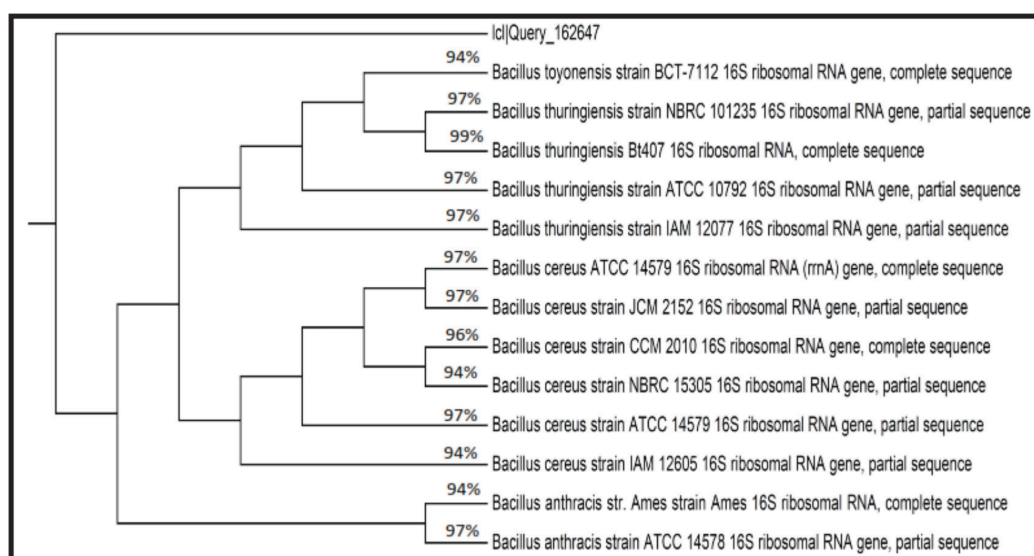

Figure (10): Dendrogram analysis showing the relationship between *Bacillus thuringiensis*-Be10 isolate and other NCBI Bacillus based on 16S rRNA gene

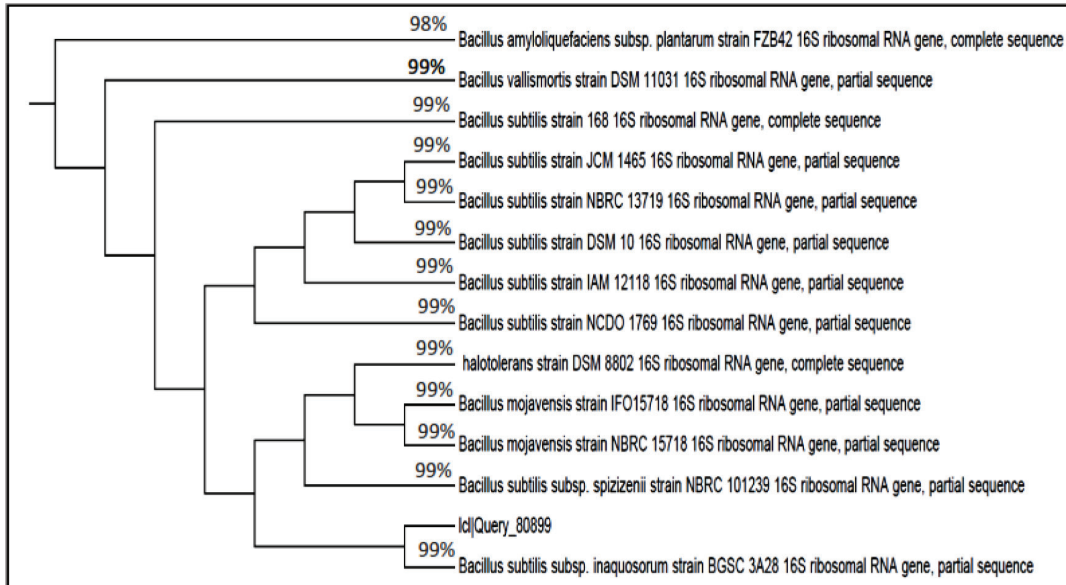

Figure (11): Dendrogram analysis showing the relationship between *B. subtilis*-Da1 strain and other NCBI Bacillus based on 16S rRNA gene

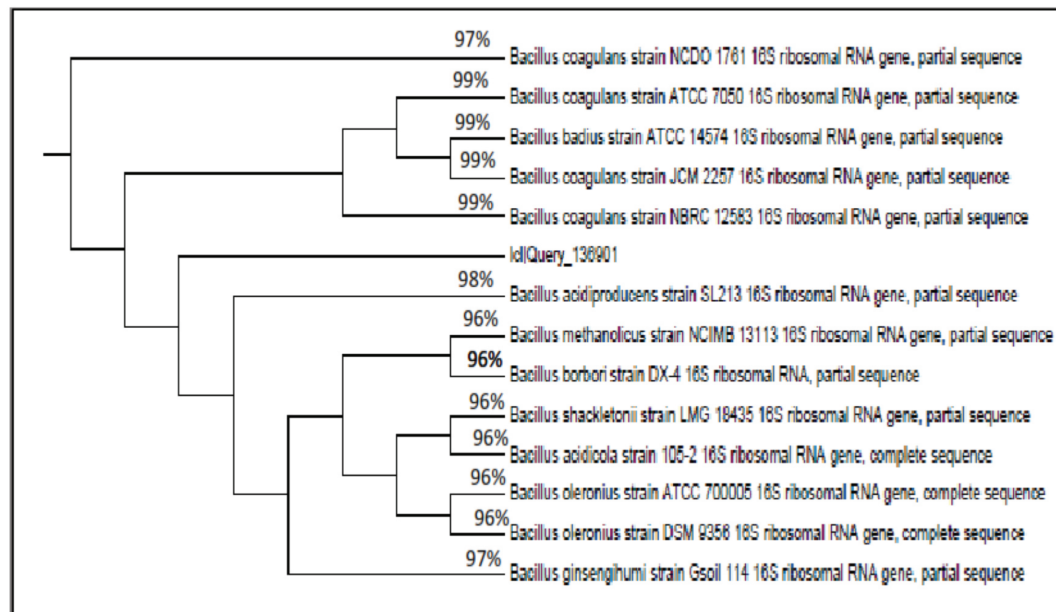

Figure (12): Dendrogram analysis showing the relationship between *B. coagulans*-Gh2 strain and other NCBI Bacillus based on 16S rRNA gene

molecular weight 64kDa for *Bacillus thuringensis*-Be10 isolate (Figure 13).

The results are in agreement with Obeidat *et al.*, (2004) who found that; by using SDS-PAGE, five strains of *Bacillus thuringiensis* (serotype: *kenyae, kurstaki, kurstaki*HD1 and *thuringiensis*) produced protein components, with molecular masses ranging from20 to 140 kDa and were toxic to the lepidoptera larvae of *Ephestiakuehniella* Zeller.

5. Effect of *B. thuringiensis*-Be10 toxin on the growth of the 2nd instar larvae of *A. ipsilon*

In screening assay, A single dose of 0.2mg of the activated toxin of *Bacillus thuringenisis*/g fresh castor

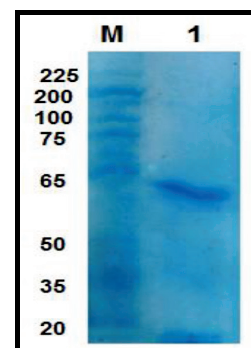

Figure (13): SDS – PAGE protein profile for bacterial isolate, Lane M: marker proteins, Lane 1: *B. thuringiensis*-Be10 strain

been leaves was tested against 2nd instar larvae of *A. ipsilon* under laboratory conditions 26 ± 2 °C, 60 ± 5 RH and photoperiod 16 lights: 8 dark. The mortality percentages of 2nd instar larvae of *A. ipsilon* by *B .thuringenisis*-Be10 were observed compared to the control (0.0%). No lethal effect was observed during the first 48 h. After3 days all the treated larvae died (100% mortality).An Injection of 0.2mg bacterial toxin resulted in an inhibitory effect on the feeding behavior of *A. ipsilon* larvae, Figure(14).

Figure (14): photograph showing the effect of *B. thuringenisis*-Be10 toxin on the 2nd instar larvae of A.ipsilon

In agreement with the present results, Abu-Bakr (2015) found that toxin of two *B. thuringiensis* strains isolated from Damietta and Sharkyia governorates gave mortality percentage (100% and 96.5%), respectively against 1st instar larvae of the cotton leaf worm, *spodoptera litoralis* post 7 days of treatment.

Also, Muñoz-Garay*et al.* (2010) suggested that the crystalline proteins (δ-endotoxins) produced by *Bacillus thuringiensis* are effective bioinsecticides against different insect pests. The primary threat to their long-term use is evolution of resistance by pests. Cry toxins are produced as protoxins activated in larval-gut lumen. The toxins bind to receptors that induce oligomerization and insertion into the membrane forming ionic pores that kill the cells and the larvae.

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الملخص العربي

تم تحديد وجود أنواع بكتيريا الباسيلس *Bacillus* الممرضة للحشرات في التربة بواسطة فحص ١٣ عينة تربة من مناطق مختلفة في مصرحيث تم الحصول على ١٠ عزلات بكتيرية من جينس الباسيلس وفقاً لشكلها الظاهري، والقياسات الفسيولوجية والبيوكيميائية عن طريق التقنيات التقليدية والروتينية. وبالإضافة إلى ذلك تم عمل التحليل الجيني لأقوى ثلاث عزلات على انها:

Bacillus thuringiensis Bt 407, *Bacillus subtilis* IAM 12118 and *Bacillus coagulans* NBRC12583

وقد أظهرت العزلات المؤثرة نشاط قوي ضد يرقات الطور الثاني للدودة القارضة السوداء، *Agrotis ipsilon*. حيث تم اختبار تركيزات تتراوح بين 2.7×10^{10} spores/ml إلى 2.7×10^{6} spores/ml على اليرقات لمدة ١٠ أيام ولوحظ زيادة النسبة المئوية التراكمية للموت على يرقات الطور الثاني للدودة القارضة السوداء بعد معاملتها بالعزلات البكتيرية تدريجياً بزيادة الوقت المنقضي.

وقد أظهرت بكتيريا *Bacillus thuringiensis* أعلى نسبة موت في الطور اليرقي الثاني حوالي ١٠٠ ٪ حيث كان التركيزاللازم لموت ٥٠٪ من اليرقات 8.2×10^{3} spores/ml وأظهرت بكتيريا *Bacillus subtilis* نسبة وفيات ٨١,٥٨ ٪ ، بينما سجلت Bacillus coagulans أقل نشاطا فكانت نسبة الوفيات ٦٤,٠٨ ٪ مع أعلى تركيز 2.7×10^{10} spore/ml بعد ١٠ أيام من المعاملة.

كما أظهر تحليل الفصل الكهربي للبروتينات SDS-PAGE للأقوى عزلة بكتيرية *Bacillus thuringiensis* شريط بروتيني واضح وزنه الجزيئي ٦٤ كيلو دالتون وكان سام ليرقات الدور الثاني للدودة القارضة السوداءحيث تم اختبار فعالية هذا السم باستخدام جرعة واحدة 0.2 mg على اليرقات وأظهرت النتائج نسبة وفيات حوالي ١٠٠ ٪ بعد ٣ أيام من المعاملة مقارنة بالكنترول (٠,٠ ٪) موت.

Design and Construction of a Pneumatic Harvesting and Cleaning Unit for Jojoba Seeds

Mahmoud A. El-Emam[1], Saad F. Ahmed[2], Mohamed A. Sabbah[2], Soliman Nasif[2]
Faculty of Agriculture, Department of Agriculture and Biosystms Engineering, Alexandria University, Alexandria, Egypt
(1): Assistant lecturer, Faculty of Agriculture, Alexandria University
(2): Professor emeritus, Faculty of Agriculture, Alexandria University

ABSTRACT

A pneumatic harvesting and cleaning unit of jojoba seeds was designed and constructed to harvest jojoba seeds from the soil surface. Some of physical and aerodynamic characteristics of jojoba seeds and material-other-than grain (MOG) were measured. The pneumatic harvesting unit was designed and constructed at the workshop of Agricultural and Biosystems Engineering Department, Faculty of Agriculture, Alexandria University, Egypt. To predict the performance of the harvesting unit, factors such as air suction velocity (m/s), unit forward speed (km/h), length of the suction hose (m), clearance of the suction hose from the soil surface (cm) and the percentage of materials other than jojoba seeds (MOG %) were tested. It was found that the optimum operating conditions was obtained at the collecting air velocity of 30 m/s, the hose length 2.5 m, the vacuum head clearance 5 cm from soil surface, the unit forward speed 1.2 km/h and the ratio of Grain/MOG 0.5.

Key words: **.**

INTRODUCTION

Jojoba (*Simmondsia Chinensis*) is being cultivated to provide a renewable source of unique high-quality oil. It is an ever green perennial desert shrub native to the Sonoran Desert of Arizona and Mexico and to Southern California. Jojoba cultivation was introduced in Egypt through the regional project for cultivating jojoba in some Middle East countries (FAO, from 1985 to 1990). Jojoba plant is an economic crop and had received a world attention for several reasons, the most significant being that the seeds contain 50 to 60 percent, by weight, liquid wax which is nearly identical in composition to sperm whale oil. Jojoba oil is odorless and colorless nonvolatile and free from rancidity. It has been utilized by the cosmetic and pharmaceutical industries for several years. Recently, its oil has received increased attention as a high quality lubricant and as additive for engine and transmission oils. Commercial production of jojoba began in the late 1970s with cultivated fields in the United States, now many hectares were cultivated in Egypt which the cultivated area is unspecific yet, with most of it planted in rows 4 m. Jojoba seeds don't mature at the same time, while the matured seeds fall down naturally to the soil surface, with harvesting commencing in July and normally being completed by late November. So, more than one of harvesting per season may be necessary, depending up on weather conditions and grower preferences. In Egypt, jojoba seeds are harvested from the soil surface by hand or primitive methods, which require intensive labor work force and low harvesting efficiency that makes it too expensive for most of their uses.

Universally, literature on pneumatic harvesting equipment of jojoba seeds is limited. Prior to 1986, jojoba growers and researchers concentrated their development efforts on harvesters which harvested seeds directly from the plants. The method which has received the most attention in recent years is soil surface harvesting, using pneumatic devices to collect the seeds from the soil surface. Anonymous (1984) describes a prototype vacuum harvester which was little more than a large vacuum cleaner. Four manually operated wands were used to vacuum seeds from underneath the plants. Pate (1987) described two growers' effort to construct pneumatic harvesters, both of which had limited success. Coates (1987) stated that all soil surface harvesting devices under development used vacuum to harvest seeds from the soil surface. In one case, a sweeper installed with a blower collected seeds into a windrow which was then vacuumed up from the soil surface by the harvester. The low capacities, relatively high power requirements and the large amount of soil, stones, rocks and trash that picked up with seeds were the main disadvantages of all developed units. These materials cause undue wear on many harvester parts, this increase the cost of harvesting material to a cleaning facility (in some cases 85% of the harvested material, by weight, is soil and rock), and require expensive cleaning operations before processing.

Coats and Lorenzen (1990) stated that two self-propelled vacuum harvesters were developed to harvest jojoba seed from the soil surface. One unit straddled the row and used mechanical agitation to dislodge the seeds, which then fell onto a collection surface located under the lower plant branches. Non-uniform seed ripening, low harvester field capacities, and inadequate harvesting efficiencies, combined with increasing plant size and consequent extensive shrub damage during harvesting, prompted producers and researchers to investigate alternative harvest methods. The other developed harvester passed between two rows and collected seeds from the soil surface from one side of each. After collection, the seed was interred to expansion chambers which separated seeds and heavier materials from the delivery airstream. The seed then was cleaned by mechanical and

pneumatic means by both units. The averaged harvesting speeds 0.85 km/h for the straddle unit, and 0.95 km/h for the other harvester. Field capacities were 0.61 ha/h and 0.35 ha/h for the two units, respectively, when operated in fields having 3.6 m rows.

Carnegie and Purcell (1988) reported that over 90% of seeds were harvested after four passes when using a sweeping and vacuuming system. However, 75% of the total volume of material removed from the field was rock. Coates and Yazici (1991) improved and developed a head for a vacuum harvesting jojoba seeds from the soil surface to reduce the amount of soil and stones collected with the seeds, thereby reducing harvester wear brought about through abrasion, reducing transport costs from the field to the processing facility, and lessening seed cleaning requirements. The head design permits the suction opening to be operated 50 to 75 mm above the soil surface, rather than skimming close to it as is commonly practiced this with head angle about 60º. The optimal head performance was found for travel velocities between 1.5 and 2.0 km/h.

Cyclone separators have been used for about 100 years ago since the middle of the 19th century. It is a type of centrifuge designed to separate solid particles or mist from gases or liquid streams within conical cylinder using centrifugal acceleration.

Knowlton (2003) reported that there are two types of cyclones: a) Uni-flow cyclone, the air-solid mixture enters the top of cyclone and spirals around the barrel to downward, then both of air and solids exit the cyclone at the bottom in the center, but solids exit along the wall of cyclone. b) The reverse-flow cyclone is by far the most common. The air-solid mixture enters the cyclone tangentially at its periphery, spirals around the barrel, and then the gas reverses flow and exits through a gas outlet tube at the top of the cyclone. The solids spiral down around the barrel of the cyclone and enter the cyclone cone attached to the bottom of the barrel and exit at the bottom of the cyclone cone. Shepherd and Lapple (1939), Parnell and Davis (1979) stated that 2D2D and 1D3D reverse-flow cyclone designs are the most commonly used. The D in the 2D2D designation refers to the barrel diameter of the cyclone. The numbers preceding each D relate to the length of the barrel and cone sections, respectively. A 2D2D cyclone has barrel and cone lengths two times the barrel diameter, whereas a 1D3D cyclone has a barrel length equal to the barrel diameter and a cone length of three times the barrel diameter. Simpson and Parnell (1996) introduced a new reverse-flow cyclone, called the 1D2D, for agriculture processing spectially in the cotton ginning industry to solve the cycling-lint problem. Wang (2000) cited that the 1D2D cyclones is a better design for high-lint content trash compared with 1D3D and 2D2D cyclones.

The main objectives of the research were to:

1- Study some physical and aerodynamic properties of jojoba seeds.
2- Design and construct a harvesting unit that suits cultivation conditions of jojoba plants in Egypt.
3- Carrying out laboratory tests to assess the harvesting unit performance.

MATERIALS AND METHODS

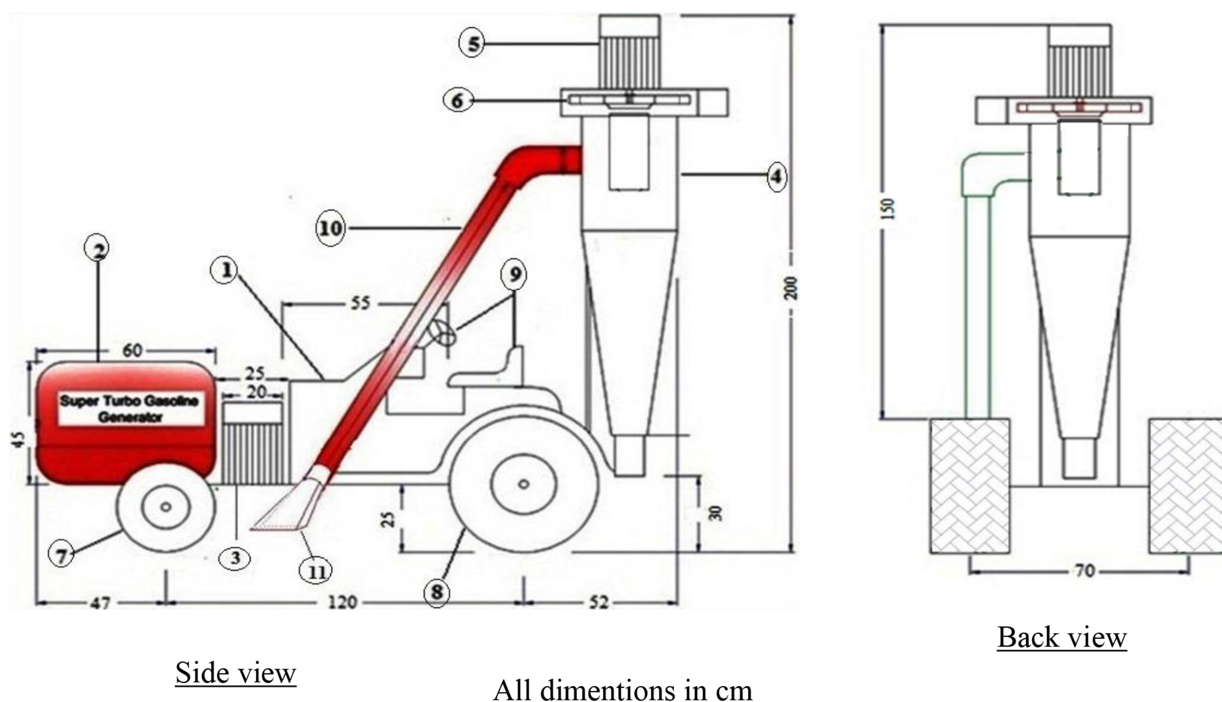
The laboratory method for harvesting operation was carried out by using collected samples of seeds and its associated matter, as it is, from under jojoba trees. The collected samples were put in windrow where seed density was distributed approximately as in natural conditions in the field under trees. While harvesting, the unit was moving at a constant forward speed.

The harvesting prototype unit (Figs. 1 and 2) was designed and constructed to harvest in these fields using modified lawn tractor. The overall frame dimensions are 1 m width, 2 m height and 2.2 m length, the clearance from the soil surface is 25 cm and the overall unit weight is 185.5 kg. The components used on the proposed harvester unit are categorized as:

1) **Power source:** the loan tractor engine was replaced by gasoline generator (45×45×60 cm in dimension, electric power 5kW- 220 V and weight 25 kg) that used for supplying electric power to two electric motors on the harvesting unit.

2) **Tran – Transmission unit:** it consists of four main parts, electric motor, pulleys and belts, differential crown gear, wheels and tires. It was designed to get a wide range of unit speeds from 1 km/h to 6 km/h by changing the reduction ratios between pulleys and belts manually in the range 1:1 to 1: 20. The power source of this unit is an electric motor (2 hp – 220V – 1400 rpm) that takes its power from the gasoline generator.

3) **Collecting and separating unit**: it was designed to collect seeds from the soil surface by pneumatic device to separate and clean seeds from the exotic materials. It consists of a) Centrifugal fan 42.5 cm in diameter with eight blades fixed at electric motor (1 hp - 2800 rpm) that takes the electric power from the gasoline generator to provide an air flow at 30 m/s. This air speed allows collecting and pick up jojoba seeds from the soil surface and deliver it to the cleaning unite (cyclone separator). This operation separates the materials other than grain from impurities by the air vortex. The speed of air stream is adjusted and controlled by a gate valve on the suction tube. b) Reverse-flow cyclone separator, air stream contains (seeds, leaves, stones, soil, small branches, etc.) enters tangentially at the top of the barrel portion and travel downward into the cone portion. The distribution of air velocity in the cyclone make an outer and inner vortex resulting increasing centrifugal force of seeds and separate them from the air stream. When the inner vortex that has exotic materials reaches the cone bottom, it reverses its direction to the cone center and leaves the cyclone with all impurities through the vortex portion, while the seeds fall toward the outlet that attached at the bottom of the cyclone. The cyclone was designed according to 1D2D as recommended by Simpson and Parnell (1996) and Wang (2000). Table (1) and Fig. (3) show the main dimensions of the cyclone separator and its components.



1) Frame and body.
2) Gasoline generator (Power source).
3) Transmission electric motor.
4) Cyclone separator.
5) Electric motor.
6) Centrifugal blower.
7) Front wheels.
8) Rear wheels.
9) Set and driving wheel.
10) Suction tube.
11) Vacuum head.

Fig. 1 Schematic line drawing showing main parts and dimensions for the prototype of jojoba seeds harvesting unit

a) Back view b) Side view c) Front view

Fig. 2 The prototype view of jojoba seeds harvesting unit

Table 1 The standard dimensions of the 1D2D cyclone separator

Component	Geometry	Dimension (mm)	Component	Geometry	Dimension (mm)
Barrel diameter	D_b	400	Inlet diameter	$D_i = D_b/4$	100
Barrel length	$L_b = D_b$	400	Outlet diameter	$D_o = D_b/4$	100
Cone length	$L_c = 2D_b$	800	Seeds outlet tube	$D_s = D_b/4$	100
Vortex diameter	$D_v = D_b/4$	100	Blower diameter	D	425
Vortex length	$L_v = D_b/8$	50	Case diameter	D_{bc}	460

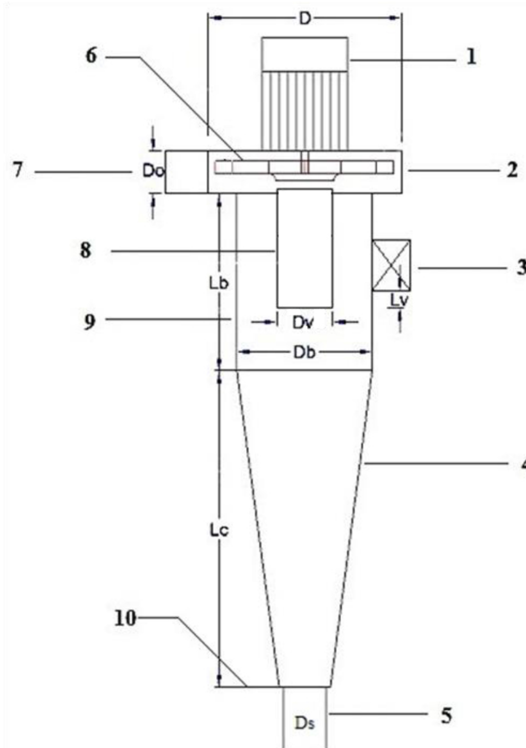
Factors affecting the performance of the prototype unit

Tests were carried out to determine the unit performance for harvesting seeds from soil surface and cleaning it from material-other-than grain (MOG). The collecting air velocity as 22, 24, 26, 28 and 30 m/s, unit forward speed was selected to be 0.8, 1.2 and 1.6 km/h, length of the suction tube was 3, 2.5, 2, and 1.5 m, clearance of the vacuum head from soil surface was 3, 5, 7 and 9 cm, and Grain/MOG ratio was 0.1, 0.2, 0.3, 0.4 and 0.5 were tested.

Evaluate the performance of the harvesting unit:

The following formulas were used to evaluate the performance of the harvesting unit:

1- Electric blower motor
2- Blower case
3- Suction inlet tube
4- Cyclone cone
5- Seed outlet
6- Centrifugal impeller
7- Outlet tube
8- Vortex tube
9- Cyclone barrel
10- Handle valve



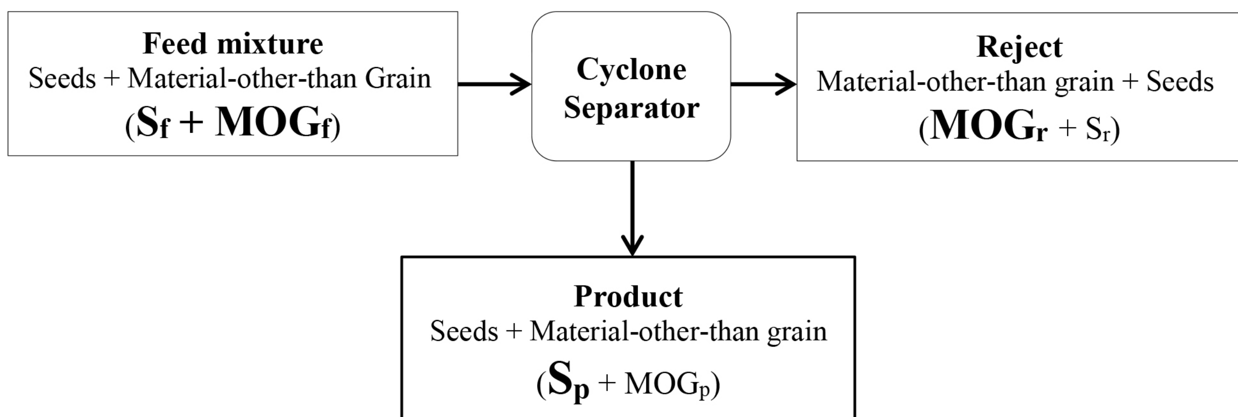


Fig. 3 The components and the operating theory of cyclone separator

1) Unit productivity, (kg/h): $M_p = \frac{S_p}{T}$

2) Cleaning efficiency, (%): $\eta_c = \frac{S_p}{(S_p + MOG_p)} \times 100$

3) Separating efficiency, (%): $\eta_s = \frac{S_p}{S_f} \times 100$

4) Seed losses, (%): $S_l = \frac{S_r}{S_f} \times 100$

5) MOGs separated, (%): $MOG_s = \frac{MOG_r}{MOG_f} \times 100$

6) Unit effectiveness, (%): $M_{eff} = \frac{\eta_s \cdot MOG_s}{100}$

7) Theoretical field capacity, (fed/h) : $FC_t = \frac{B \cdot V}{10}$

8) Specific energy consumption, (kW.h/ton): $E_c = \frac{P_t}{M_p}$

9) Seed damage, (%): $S_d = \frac{S_{pd}}{S_f} \times 100$

Where:

Symbol	Quantity	Formula
M_P	Unit productivity	kg/h
S_p	Seed weight in product	kg
T	Operating time	h
η_c	Cleaning efficiency	%
MOG_p	Material-other-than grain in product	kg
η_s	Separating efficiency	%
S_f	Seed weight in feed	kg
S_L	Seed loss	%
S_r	Seed weight in reject	kg
MOG_s	Material-other-than grain separated	%
MOG_r	Weight of material-other-than grain in reject	kg
MOG_f	Weight of material-other-than grain in feed	kg
M_{eff}	Unit effectiveness	%
FC_t	Theoretical field capacity	fed/h
B	Harvesting width	m
V	Unit forward speed	km/h
E_c	Specific energy consumption	kW.h/ton
P_t	Total electric power	kW
S_{pd}	Weight of Seed damage in product	kg

RESULTS AND DISCUSSION

All experiments were carried out under laboratory conditions at the Workshop of Agricultural and Biosystems Engineering Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Jojoba seeds were brought from Ismailia, Egypt and were used to run the tests in two seasons, 2012 and 2013. **Table (2)** shows some physical and aerodynamic properties of jojoba seeds that measured to design and construct a suitable harvesting unit which sample of 500 randomly selected seeds were used.

SD = Standard deviation, CV = Coefficient of variation, *Average of 500 seeds

The seed dimensions were measured using a digital Vernier caliber which having a resolution count of 0.05 mm. The projected area of seeds was determined by using camera (Kodak Corporation, japan, pentium II, ٢٣٣ MHz with software Impasse /32 Version 10). The sample was placed on white surface under the camera and the image was acquired and analyzed by using AutoCAD program 2010.

Fig. (4) shows the terminal velocity apparatus that used for measuring terminal velocity of seeds and MOG. It was designed and constructed in the workshop of Agricultural Engineering Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. It consists of two parts, anemometer (1) and blower fan powered by an electric motor which rotates at 2250 rpm and gives air flow rate of 0.05 m^3/s (2). The air pressure delivered from the fan blower passes through a horizontal tube 7 cm in diameter and 25 cm in length (4), connected with a vertical tube 25 cm in length by one elbow 90°. A transparent tube (3) 20 cm in length and 7cm in diameter was connected at the end of the tube that has a wire screen (6) fitted at the bottom of the transparent tube to put the sample on it. When the air passes though the tube the sample raises into a transparent tube and stop at certain level, whereupon the anemometer measure the terminal velocity of the air. The experimental measurements showed that the terminal velocity of jojoba seeds ranged from 14 to 20 m/s, however the terminal velocity of MOG ranged from 4 to 17 m/s; so it is so easy to separate the MOG from seeds as shown in Fig.(5).

Performance of the prototype harvesting unit

Effect of collecting air velocity

Five air velocities used were 22, 24, 26, 28 and 30 m/s. The experiments were carried out on 5kg of pure jojoba seeds without any foreign materials. The proposed fixed parameters were: hose length 2.5 m and vacuum head clearance 5cm where the forward speed of unit

Table (2) Physical and aerodynamic properties of jojoba seeds *(Egypt, 2012)

proprieties	Minimum Value	Maximum Value	Average Value	SD	CV, %
Length, mm	13.70	20	16.85	1.49	8.70
Width, mm	5.60	13.50	9.55	1.42	13.73
Thickness, mm	5.20	13.20	9.20	1.42	14.29
Projected area, mm^2	108.46	236.48	172.74	26.64	15.82
Thousand seed weight, g	1067.20	1132.80	1100	30.23	2.77
Bulk density, kg/m^3	674.22	702.13	688.17	12.36	1.80
Terminal velocity of seeds, m/s	14.60	19.50	17.05	0.72	3.87
Terminal velocity of MOG, m/s	4.25	16.75	10.5	—	—

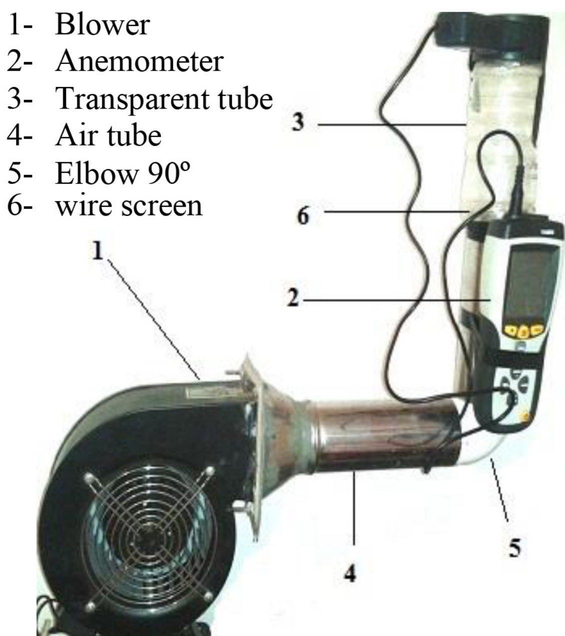


Fig. (4) Terminal velocity apparatus.

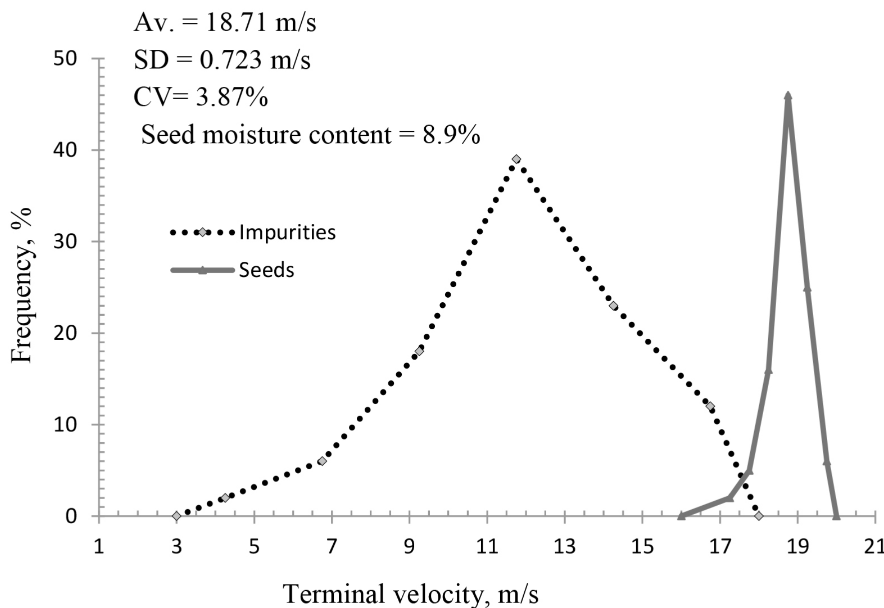


Fig. (5) Terminal velocity distribution of jojoba seeds and MOG.

equal zero. **Table (3)** shows the best collecting air velocity required was 30 m/s. This velocity permits to collect 100% of jojoba seeds from the soil surface and forces it into the cyclone body. At this level, the unit productivity was 166.7 kg/h without seeds damage; specific energy consumption decreased up to 4.47 kW.h/ton, and average operating time was less. On the other hand, at the air velocities less than 30 m/s, average operating time increased due to increasing the collecting time, this because of the time consumption to force the seeds into cyclone body increased. This means that the specific energy consumption increased and the unit productivity decreased.

Effect of unit forward speed.

Table (4) shows three levels of unit forward speed used: 0.8, 1.2 and 1.6 km/h and its effect on harvesting performance. The hose length 2.5 m, vacuum head clearance 5 cm, air collecting velocity of 30 m/s and Grain/MOG ratio of 0.5 were the proposed fixed parameters to pick up the sample was uniformly distributed in windrow. Using forward speeds of 0.8 and 1.2 km/h, all seeds on soil surface were sucked in and the unit performance remains the same. The second speed was better because the unit could pick up all seeds from the soil surface with very small amount of MOG. Under the previous conditions the separating efficiency, cleaning efficiency and unit effectiveness increased to reach 96.8%, 95.9% and 94.8% respectively while the specific energy consumption decreased to 13.5 kW.h/ton. Increasing forward speed to 1.6 km/h, about 8.27% seeds remained on the soil surface which resulted in decreasing the performance of harvesting unit.

Effect of Grain/MOG ratio

Five Grain/MOG ratios of 0.1, 0.2, .03, 0.4 and 0.5 were used by weight to evaluate its effect on the harvesting unit performance. The proposed fixed parameters to carry out these experiments were at hose length 2.5 m, vacuum head clearance of 5 cm and collecting air velocity of 30 m/s at unit forward speed of 1.2 km/h. Table (5) showed that at Grain/MOG ratio equal 0.1, the separating efficiency, cleaning efficiency and effectiveness reached to 87.86%, 69.91% and 84.5% respectively, while the specific energy consumption was 36 kW.h/ton. The average percentage of seed losses reached to 12.14 % which reduces the productivity to 20.67 kg/h. On the other hand, increasing the Grain/MOG ratio to 0.5, the separating and cleaning efficiencies increased to reach 98.74% and 96.7% respectively, while the specific energy consumption reached to 7.22 kW.h/ton and the effectiveness to 97.08%. The average percentage of seed loss decreased to 1.26 % this gave higher unit productivity as 103.3 kg/h.

Effect of suction hose length

Four suction hose lengths were: 1.5, 2, 2.5 and 3 m to determine which length is suitable for collecting seeds from soil surface and direct it to separation unit with minimum friction losses or pressure drop. The optimum parameters used under investigation were the vacuum head clearance from the soil surface 5 cm, collecting air velocity 30 m/s and 0.5 kg of pure seeds. Experiments were carried out while the unit was in idle

Table (3) Effect of collecting air velocity on the performance of harvesting unit

Unit performance	Air velocity, m/s				
	22	24	26	28	30
Average of collected seeds,%	40.1	51.3	75.2	99.4	100
Average of remained seeds,%	59.9	48.7	24.8	0.6	0
Average operating time, s*	16	15.8	15.3	11.2	10.8
Unit productivity, kg/h	45.13	58.44	88.47	159.75	166.67
Seed loss in reject, %	Nil	Nil	Nil	Nil	Nil
Specific energy consumption, kW.h/ton**	16.5	12.75	8.42	4.66	4.47
Seed damage in product, %	Nil	Nil	Nil	Nil	Nil

*Average operating time of collecting and separating.

Table (4) Effect of forward speed on the harvesting unit performance

Unit performance	Unit forward speed, km/h		
	0.8	1.2	1.6
Average of collected seeds, %	100	100	91.23
Average of remained seeds,%	0	0	8.77
Seed loss,%	3.39	3.16	12.22
Average of collected MOG, %	86.11	82.41	73.32
Average of remained MOG,%	13.89	17.59	26.68
MOG_s, %	98.12	97.92	97.43
MOG not separated,%	1.88	2.08	2.57
MOG in product,%	3.75	4.12	5.06
Average operating time, s*	42.62	42.13	41.63
Unit productivity, kg/h	163.2	165.5	152.3
Separating efficiency, %	96.61	96.84	96.55
Cleaning efficiency (Seeds in product), %	96.25	95.88	94.94
Unit effectiveness, %	94.79	94.82	94.06
Specific energy consumption, kW.h/ton**	13.71	13.52	14.7
Theoretical Field capacity, fed/h	0.38	0. 57	0.76

*Average operating time of collecting and separating seeds, ** Motors power = 2.25 kW

Table (5) Effect of Grain/MOG ratio on the performance of the harvesting unit

Unit performance	Grain/MOG ratio				
	0.1	0.2	0.3	0.4	0.5
Average of collected seeds,%	100	100	100	100	100
Seed loss, %	12.14	8.18	3.89	2.76	1.26
Seeds in product, %	69.91	85.20	91.75	95.29	96.70
MOG_s, %	96.22	96.81	97.41	98.08	98.32
MOG not separated,%	3.78	3.19	2.59	1.92	1.68
MOG in product,%	30.09	14.80	8.25	4.71	3.30
Average operating time, s*	15.3	15.9	16.1	16.7	17.2
Unit productivity, kg/h	20.67	41.58	64.47	83.84	103.33
Separating efficiency, %	87.86	91.82	96.11	97.24	98.74
Cleaning efficiency (Seeds in product), %	69.91	85.2	91.75	95.29	96.7
Unit effectiveness, %	84.53	88.89	93.62	95.37	97.08
Specific energy consumption, kW.h/ton**	36.09	17.94	11.57	8.9	7.22

*Average time of collecting and separating jojoba seeds.
** Motors power (Cyclone and transmission motors) = 2.25 kW

position. Table (6) shows that at hose length of 1.5 m all seeds on the soil surface were picked up, but only 20.45% were vacuumed into cyclone inlet while the other amount remained in the hose and clogged it. This is because the hose angle was approximately 90º which in turn increases pressure drop of air stream and prevents seeds to move into cyclone body. At this hose length the energy consumption increased to reach 19.35 kW.h/ and the unit productivity decreased to reach 38.54 kg/h. When using hose length 3 m the required air velocity on the vacuum head decreased to 27.2 m/s which made the amount of collected seeds and vacuumed to cyclone body decreased to 94.68%. As shown the most appropriate suction hose length is 2.5 m. This because the percentages of collected seeds 100% while the unit gave high productivity of 166.6 kg/h with minimum specific energy consumption 4.47 kW.h/ton.

Effect of vacuum head clearance from soil surface.

Vacuum head is a critical aspect of the pneumatic system design. The harvester was designed to use a vacuum head which passed along the soil surface and vacuumed seeds into the unit. The head clearance is very important in getting minimum amount of sand, dry soil, loose sand, small stones and gravels from the soil surface. Four vacuum head clearance of 3, 5, 7and 9 cm were used to find out the most suitable clearance required. The optimum parameters used under investigation were the hose length 2.5 m, collecting air velocity of 30 m/s and the Grain/MOG ratio equal 0.5 while the unit forward speed at 1.2 km/h. Table (7) showed that when vacuum head clearance was 3 cm, the sample was collected. Thus, the unit effectiveness, cleaning efficiency and separation efficiency were decreased to 95.08%, 97.14%, and 97.9% respectively. The separation time was increased because of a large amount of MOG vacuumed to cyclone body. This lead to increase the operation time and make the unit productivity decreased and the energy consumption increased. At vacuum head clearance 5 cm, the unit productivity 103.3 kg/h and energy consumption 21.6 kW.h/ton were preferable due to all seeds were sucked and 14.37 % of sand, small stones and gravels remained on the soil surface compared with other heights. The separating efficiency, cleaning efficiency and unit effectiveness reached to 98.74%, 98.32 and 97.08 respectively.

Table (6): Effect of suction hose length on the unit performance

Unit performance	Hose length (m)			
	1.5	2	2.5	3
Average of collected seeds ,%	20.45	65.23	100	94.68
Seed loss, %	79.55	34.77	0	5.32
Average operating time, s*	9.55	10.34	10.8	11.68
Unit productivity, kg/h	38.54	113.55	166.66	145.91
Separating efficiency, %	20.45	65.23	100	94.68
Specific energy consumption, kW.h/ton**	19.35	6.56	4.47	5.11

*Average operating time of collecting and separating. **Motor power = 0.75 kW.

Table (7): Effect of vacuum head clearance from the soil surface

Unit performance	Hose clearance (cm)			
	3	5	7	9
Average of collected seeds, %	100	100	95.68	87.23
Average of remained seeds,%	0	0	4.32	12.77
Seed loss,%	2.88	1.26	5.41	13.94
Average of collected MOG, %	100	85.63	70.82	50.53
MOG_s, %	97.12	98.32	98.94	99.02
Average operating time, s*	17.95	17.2	16.93	16.85
Unit productivity, kg/h	98.17	103.33	100.61	92.09
Separating efficiency, %	97.12	98.74	98.91	98.83
Cleaning efficiency (Seeds in product), %	97.14	98.32	98.94	99.02
Unit effectiveness, %	95.08	97.08	97.86	97.86
Specific energy consumption, kW.h/ton*	22.8	21.66	22.25	24.31

*Average operating time of collecting and separating. ** Motors power = 2.25 kW

CONCLUSIONS

The aim of this research is to design and construct a suitable pneumatic harvesting and cleaning unit of jojoba seeds from the soil surface in Egypt. It can be concluded that the best operation conditions for harvesting jojoba seeds using the recent prototype and obtain a higher unit performance and productivity at the air velocity 30 m/s, the hose length 2.5 m, vacuum head clearance 5 cm from the soil surface with unit forward speed 1.2 km/h and the ratio of Grain/MOG 0.5 %. The main recommendation for this study to double the unit productivity is to use two cyclone separators at the same time on the unit. This permit to harvest two windrows on the left and the right of unit.

In general, jojoba harvesting equipment is limited. The research is atrial to produce a prototype harvester, and then evaluated its performance under simulated field conditions. Although the performance of the harvester was considered satisfactory for a prototype, and it has been registered in Egypt as a patent with number (1991/2014); it requires additional modifications to make it suitable for commercial operation. The large amount of soil and rock picked up with the seed caused excessive wear on many of the components, leading to premature failure. To decrease the amount of these materials picked up, additional research is to be continued. Results of this work showed that significant improvements should be planned especially in designing additional cleaning device for use on the harvester. Reduction in the amount of impurities will help in reducing transport cost and would also reduce the cleaning load placed on the processing facility.

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