Influence of Two Insect Growth Regulators on Chitinase Activity

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ABSTRACT

Toxicity of two insect growth regulators (IGRs) (lufenuron and hexaflumuron) against two larval instars c leafworm *Spodoptera littoralis*, laboratory and field strains were determined. Chitinase activity in the two strains investigated. Results revealed that, 2nd instar larvae were more sensitive than 4th instar larvae to both insecticic sensitivity of chitinase activity was measured by I_{50} values. The I_{50} values of lufenuron were 0.31, and 0.64 µM and field strains of *S. littoralis* 2nd larvae respectively, while I_{50} values were 0.44, and 0.75 µM for lab and field s *S. littoralis* 4th larvae respectively. The hexaflumuron were 0.57, and 0.76 µM for lab and field strains of *S. littc* larvae respectively, the I_{50} values were 0.65, and 0.81µM for lab and field strains of *S. littoralis* 4th larvae resp Also, chitinase enzyme kinetic parameters, as Michaelies-Menten Kinetics (K_m and V_{max}) values and the in constant (K_i) were determined. The obtained data proved that lufenuron and hexaflumuron compounds are con inhibitors of chitinase activity. Results indicated that, the IGRs have shown high potentiality against larvae of *S. l* so, these IGRs may be recommended for *S. littoralis* larvae control, it could be concluded that the use of IGRs in conventional hazardous insecticides; may avoid increasing selection pressure of *S. littoralis* populations to conv insecticides, hazard effects on human health, environmental components and natural enemies, IGRs may important role in future insect pest management programs.

Key words: Chitinase- *S. littoralis* – insect growth regulators (IGRs).

INTRODUCTION

The development of multiple insecticide resistance in field strain of the *Spodoptera littoralis* to several insecticides has been recorded by several investigators. Due to severe applications of insecticides for the control of *S. littoralis* larval instars, which are the most destructive stages of the insect on cotton and vegetable crops, the larval stages have become extremely tolerant to the action of pesticides (Ware 2000 and Temerak 2002). So the need to develop novel alternatives or functional combinations of pest control techniques is emphatically a product of this decade and many sources for alternative pesticides were found such as insect growth regulators (IGRs) compounds which are considered nowadays one of the mainly component of IPM program. Term IGRs describe a new class of bio-rational compounds, this group are active against larvae of many lepidopterous species (Fisk & Wright 1992; Schneider *et al*., 2003, and Sandeep & Bhamare 2006).

Therefor the present work was conducted to study the efficiency of two IGRs (lufenuron and hexaflumuron) upon the 2nd and 4th larval instar of *S. littoralis*, and describe the development of biochemical assay system for measuring the sensitivity of chitinase enzyme to two IGRs (lufenuron and hexaflumuron), in laboratory and field strains.

MATERIALS AND METHODS

1. Test insects:

Susceptible laboratory strain of leafworm, *Spodoptera littoralis* was provi central lab of pesticides, Agricultural R Center (ARC) Cairo, Egypt which was rea several years on artificial diet under s laboratory conditions of 27 ± 2 °C and 65-70

Field strain of cotton leafworm, *Spo littoralis* egg masses were collected from fields at Abeis area Alexandria, governorate The 2nd and 4th larval instars were cho bioassay and biochemical assessment.

2. Test insecticides:

Lufenuron (Match, 5% EC), and hexafl (consult, 5% EC), were supplied by Syngenta

3. Bioassay tests:

3.1. Toxicity of the tested IGRs aga *littoralis*:

Lufenuron and hexaflumuron were bio against the 2nd and 4th larvae of *S. littora* castor leaves were dipped in different concen of the tested IGRs. Lufenuron and hexafl concentrations were prepared in distilled Treated and control leaves plants were air-d 3 hrs, the treated leaves were placed in clea container at the laboratory conditions of (27 and 65-70 % RH, ten larvae (lab and field were used for each test with three replicate Number of alive and dead larvae per replic

counted 24, and 48 hr, after treatment. Concentrations-mortality percentage were calculated and corrected for natural death according to Abbott equation (Abbott, 1925). LC_{50} values were calculated and statisticaly and analysed by using the probit-analysis method of Finney (1971).

4. Biochemical studies:

4.1. Chitinase preparation and activity assay:

Chitinase was prepared from *Spodoptera littoralis* 2nd and 4th instars larvae (lab and field strains) according to the method of Deul *et al*., (1978). Larvae homogenate was prepared in 10^3 M Clelands' reagent (dithiotheritol, DTT) (v/w=2), centrifuged at 12.000 g for 15 min. Then an equal volume of saturated ammonium sulfate solution was slowly added to the supernatant. After stirring for 1 hr, the suspension was centrifuged at 10.000 g for 10 min. The precipitate was washed with half-saturated ammonium sulfate solution and recentrifuged. Then it was suspended in a small volume of water, followed by dialysis for 20 hr at 0-2 °C.

The chitinase activity measurements were done according to the method reported by Reissig *et al*., (1955), which modified by Andrew *et al*., (1982), using sodium acetate buffer instead of tris-HCl buffer and wave-leangth of 416 nm instead of 544 nm. 25 µl of chitin (20mg/ml), 100 µl of enzyme preparation were used and 225 µl of sodium acetate (pH 4.5) in total volume 350 µl. The enzyme substrate mixture was incubated at 35 °C for 60 min, then the reaction was stopped by adding 100 µl of 0.8 M borate buffer (pH 10.0) followed by determination of n-acetylglucoseamine by method of Reissig *et al*., (1955) by adding 1.5 ml of p-dimethyl amino benzaldhyde (DMAB, reagent). The samples were incubated in shaker water bath at 35 °C for 20 min and were measured spectrophotometrically at λ412 nm.

The protein content in prepared homogenates of *S. littoralis* was assayed by the method of Lowery *et al*. (1951) at λ750 nm using Bovine Serum Albumin (BSA) as a standard protein.

4.2. *In vivo* inhibition of chitinase activity

The inhibition percentage of chitinase activity was determined in the 2nd and 4th instars larvae previously feed on leaves treated with the concentration of LC_{50} values of each of the tested insecticides (lufenuron and hexaflumuron). 10 µl of the enzyme preparation was incubated with the substrate for 30 min, the enzyme-substrate was used to measure the remaining activi percent inhibition was calculated usi following formula:

$$\% \text{ Inhibition} = \frac{V-Vi}{V} \times 100$$

Where:-

(V) is the specific activity in larvae feed on castor leaves.

(Vi) is the specific activity in larvae feed treated castor leaves.

4.2. *In vitro* inhibition of chitinase activity

The inhibitor of chitinase activity was ev to determine enzyme kinetic parameters, the of Dixon and Webb (1964) was adopted to d Dixon-plots by plotting 1/V versus concentra the inhibitor (lufenuron and hexaflumuron) concentrations of the substrate, chitin (the s of chitinase) concentrations of 3.0 and 5. Estimation of I_{50} value was carried preincubating the enzyme with the inhibitor min, using the following concentrations 0. 10; 50, and 100 µM. K_i (the inhibition c values for each inhibitor were estimate Dixon-plot. Michaelies-Menten Kinetics (V_{max}) values were calculated by a linear re of 6 point on each Lineweaver and Bu (1934).

RESULTS AND DISCUSSION

Toxicity of IGRs against *S. littoralis* larvae

The toxicity of the lufenuron and hexafl in terms of LC_{50} are given in table (1) for 2nd larvae of *S. littoralis*. LC_{50} values were 0. 0.55 ppm for lufenuron and hexafl respectively against 2nd instar larvae of *S. l* after 24 hr for lab strain, while for field stra values were 0.54 and 0.76 ppm for the tw respectively. Also LC_{50} values were 0.052 an ppm after 48 hr for lab strain, while for fiel LC_{50} values were 0.068 and 0.095 ppm f IGRs, respectively. LC_{50} values were 0.44 a ppm for lufenuron and hexaflumuron resp against 4th instar larvae of *S. littoralis* after 2 lab strain, for field strain LC_{50} values were 0 0.97 ppm for the two IGRs respectively. LC_5 were 0.061 and 0.077ppm after 48 hr for la respectively, while for field strain LC_{50} valu 0.080 and 0.096 ppm for two IGRs respective

Table 1: Toxicity of IGRs on *S. littoralis* larvae.

S. littoralis strains	LC_{50} (ppm)							
	lufenuron				hexaflumuron			
	24hr		48hr		24hr		48hr	
	2nd	4th	2nd	4th	2nd	4th	2nd	4th
Lab	0.31	0.44	0.052	0.061	0.55	0.78	0.068	0.077
Field	0.54	0.63	0.071	0.080	0.76	0.97	0.095	0.096

According to LC_{50} values it is quite clear that the susceptibility of *S. littoralis* larvae to lufenuron and hexaflumuron decreased by increasing the posttreatment period. Also it was observed that the 2nd instar was more susceptible than the 4th instar. The present results are confirmed by the results of (Fisk & Wright 1992; Toscano *et al*., 2001, and Sandeep & Bhamare 2006).

The *in vivo* inhibition of *S. littoralis* chitinase activity:

The *in vivo* inhibitory effect of the LC_{50} values of tested IGRs against to the *S. littoralis* 2nd and 4th instars lab and field strains larval chitinase are shown in table (2). The data cleared that lufenuron and hexaflumuron concentration exhibited a high percentages of reduction of chitinase activity. The percentages of chitinase inhibition were 88.1, and 74.5 % for lab strain of *S. littoralis* 2nd instar larvae, respectively, while in field strain values were 74.3 and 62.8 % for the two IGRs respectively. Also the values were 73.6, and 63.1 % for lab strain of *S. littoralis* 4th instar larvae, and for field strain the values were 61.9, and 57.4 % for the two IGRs, respectively.

These results show that the tested IGRs act by reducing chitin incorporation in the cuticle of *S. littoralis*, similar results were obtained by Susan *et al*., 1990. Properties of the IGRs were originally recognized through their ability to initiate inappropriately timed and poorly coordinated moulting processes, the resulting perturbation of moulting and metamorphosis leads to death, usually because the insects cannot escape from the exuvie (Ascher & Nemny 1979; Aller & Ramsay, 1988, and Liburd *et al*., 2000). Therefore one may expect that these compounds will be very potent on cotton leafworm and other lepidopterous larvae.

Kinetic parameters of chitinase inhibition:

The kinetic studies were conducted to evaluate the effects of lufenuron and hexaflumuron on chitinase activity in both tested strains of *S. littoralis* 2nd and 4th larvae, table (3) shows the obtained Lineweaver-Burk (L-B) plots for chitinase in lab and field strains and the statistical analysis of the obtained values of K_m (Michaelis-Menten kinetics, constant) and V_{max} (maximum velocity) of the chitinase activity. The K_m values for chitinase were generally higher for field strain than lab strain, the change in K_m values of chitinase between the lab and field strains indicated changes in the affinities.

The present results show that the V_{max} v chitinase may reflect the physiological imp of the chitinase in the function of the mou the *S. littoralis* larvae. The V_{max} value generally higher in field strains than lab str indicated that the number of active sites chitinase of the larvae was increased in t strain, such change may be followed by dec the insect susceptibility which could be alt field application of the insecticides.

The *in vitro* inhibition of *S. littoralis* cl activity:

To characterize more details about the inhibition of chitinase by the inhibitors, the I of each inhibitor was estimated from the g method of Dixon and Webb (1964), table (sensitivity of chitinase activity to lufenur hexaflumuron were measured by I_{50} values case of lufenuron the I_{50} values were 0.31, a µM for lab and field strains of *S. littoralis* 2n respectively, while I_{50} values were 0.44, a µM for lab and field strains of *S. littoralis* 4t respectively. Similarly, in case of the hexafl the I_{50} values were 0.57, and 0.76 µM for field strains of *S. littoralis* 2nd larvae respe the I_{50} values were 0.65, and 0.81µM for field strains of *S. littoralis* 4th larvae respe The K_i values were 20, and 35 µM for lab a strains of *S. littoralis* 2nd larvae respectively, of lufenuron, while the values were 44, and for lab and field strains of *S. littoralis* 4t respectively. Also, in case of hexaflumu values were 34, and 51 µM for lab and field of *S. littoralis* 2nd larvae respectively, wh values were 52, and 63 µM for lab and field of *S. littoralis* 4th larvae, respectively.

Chitinase plays an essential role during This enzyme is vital to moult in insects, a also affect gut physiology through their invo in peritrophic membrane turnover. The exos of insect might constitute a useful target insecticidal chemicals. The obtained cha enzymes activity between lab and field strai due to the variation in the protein synthes response to the different treatment (Clarke & 1990; Smagghe *et al*., 1997; Wilson & Crya Dean *et al*., 1999; Merzendorfer & Zimoch and Kostyukovsky & Trostanetsky 2006).

Table 2: *In vivo* inhibition of *S. littoralis* larvae chitinase activity by two IGRs (LC_{50}).

S. littoralis Strains	% inhibition of chitinase activity			
	lufenuron		hexaflumuron	
	2nd	4th	2nd	4th
Lab	88.1	73.6	74.5	63.1
Field	74.3	61.9	62.8	57.4

Table 3: Michaelies-Menten Kinetics of the chitinase of larval of *S. littoralis*.

S. littoralis Strains	lufenuron				hexaflumuron			
	K_m mM		V_{max} mM		K_m mM		Vmax mM	
	2nd	4th	2nd	4th	2nd	4th	2nd	4th
Lab	0.33	0.46	6.8	5.2	0.52	0.60	4.7	3.6
Field	0.54	0.65	4.7	3.2	0.64	0.77	2.9	1.8

Table 4: I*n vitro* inhibition of *S. littoralis* larvae chitinase activity by two IGRs.

S. littoralis Strains	Lufenuron				Hexaflumuron			
	I_{50} µM/L/min		K_i µM		I_{50} µM/L/min		K_i µM	
	2nd	4th	2nd	4th	2nd	4th	2nd	4th
Lab	0.31	0.44	20	44	0.57	0.65	34	52
Field	0.64	0.75	35	50	0.76	0.81	51	63

Finally, according to the results presented, lufenuron and hexaflumuron are potentially potent insecticides for controlling *S. littoralis*. These compounds are effective suppressors for the development of the entire life cycle of insects. They act preferentially by interfering with chitin synthesis metabolism (chitin synthesis inhibitors) and with the deposition of chitin in the insect cuticle. Therefore, these compounds could be used in the integrated pest management (IPM) programs, in order to minimize the negative effects of conventional insecticides on the environments and to protect the natural enemies.

1- Yield/ vine:

Data in Table (1) clearly show that spraying clusters of Early sweet grapevines with GA_3 at 10 to 40 ppm or Sitofex at 2.5 to 10 ppm was significantly effective in improving the yield relative to the check treatment. The promotion on the yield was accompanied with increasing concentrations of each plant growth regulator. Using GA_3 at 10 to 40 was significantly preferable than using Sitofex at 2.5 to 10 ppm in improving the yield. A slight and unsignificant promotion on the yield was attributed to increasing concentrations of GA_3 from 20 to 40 ppm and Sitofex from 5 to 10 ppm. The maximum yield was produced on the vines that received one spray of GA_3 at 40 ppm but the best treatment from economical point of view was the application of GA_3 at 20 ppm (since no measurable promotion on the yield was recorded between 20 and 40 ppm of GA_3). Under such promised treatment, yield/ vine reached 13.6 and 14.0 kg during both seasons, respectively. The control vines produced 9.1 and 9.6 kg during 2013 and 2014 seasons, respectively. The percentage of increase on the yield due to application of GA_3 at 20 ppm over the check treatment reached 49.5 and 45.8 % during both seasons, respectively. The beneficial effects of GA_3 on the yield might be attributed to their positive action on increasing cluster weight. The promoting effects of GA_3 on the yield was supported by the results of Dimovska *et al*., (2011) and Abu- Zahra and Salameh (2012) on different grapevine cvs.

The results regarding the beneficial ef Sitofex on enhancing the yield are in harmo those obtained by Juan *et al*. (2009); Abdel *et al*., (2010) and Al- Obeed (2011).

2- Harvesting date:

It is clear from the data in Table (1) that and Sitofex treatments had significantly del the harvesting date of Early Sweet grapevine than the control treatment. The degree of de on harvesting date was correlated to the inc the concentrations of both GA_3 and Sitofex GA_3 significantly delayed harvesting comparing with using Sitofex. In concentrations of GA_3 from 20 to 40 p Sitofex form 5 to 10 ppm failed to show sig delay on harvesting date. A consi advancement on harvesting date was obse untreated vines the great delay on harvesti was observed on the vines that received GA ppm during both seasons. GA_3 and Sitofe shown by many authors to retard the rel ethylene and the disappearance of pigments chlorophylls and carotenoids and onest of r start. Also they were responsible for prolong maturity stages Nickell (1985). These regarding the delaying effect of GA_3 and Sit harvesting date were in harmony with obtained by Wassel *et al*., (2007), Kasser (2011), Abu- Zahra and Salameh (2012) and *et al*. (2012).

3- Cluster weight and dimensions:

It is evident from the data in Table (treating clusters with GA_3 at 10 to 40 Sitofex at 2.5 to 10 ppm was signi accompanied with enhancing weight, leng width of cluster relative to the control treatm

The promotion was significantly associate
increasing concentrations of GA_3 and Sitofe
GA_3 was significantly favourable than using
in this respect. The maximum values were r
on the vines that received one spray of GA
ppm. Meaningless promotion was detecte
increasing concentrations of GA_3 from 20 to

and Sitofex from 5 to 10 ppm. The untreated vines produced the minimum values during both seasons. The positive action of GA_3 on cluster weight and dimensions might be attributed to its essential role on stimulating cell division and enlargement of cells, the water absorption and the biosynthesis of proteins which will lead to increase berry weight. Dimovska *et al*., (2011); Abu Zahra and Salameh, (2012) and Dimovska *et al*., (2014).

The previous essential role of CPPU on cluster weight was attributed to its higher content of cytokinin when applied to plants (Nickell, 1985).

4- Shot berries %:

Data in Table (2) obviously reveal that percentage of shot berries in the clusters of Early Sweet grapevines was significantly controlled with spraying GA_3 at 10 to 40 ppm or Sitofex at 2.5 to 10 ppm relative to the check treatment. Using GA_3 was preferable than using Sitofex in reducing the percentages of shot berries. There was a gradual reduction on the percentage of shot berries with increasing concentrations of GA_3 and Sitofex. There was a slight reduction on such unfavourable phenomenon with increasing concentrations of GA_3 form 20 to 40 ppm and Sitofex from 5 to 10 ppm. The minimum values of shot berries (7.3 and 6.9 % during both seasons, respectively) were recorded on the clusters harvested from vines treated with GA_3 at 40 ppm. The maximum values of shot berries (12.0 & 12.5 %) during both seasons were recorded on the untreated vines during both seasons. The reducing effect of GA_3 on shot berries might be attributed to its important role on enhancing cell division and the biosynthesis of proteins Nickell, (1985). These results were supported by the results of wassel *et al*. (2007) and Abu Zahra and Salameh (2012).

5- Fruit quality:

Data in Tables (2, 3 & 4) clearly show that spraying clusters with GA3 at 10 to 40 ppm or Sitofex at 2.5 to 10 ppm significantly was accompanied with enhancing weight, longitudinal and equatorial of berry, total acidity%, proteins % and percentages of P, K and Mg and T.S.S. %, reducing sugars %, T.S.S. / acid and total carotenoids relative to the check treatment. The effect either increase or decrease was associated with increasing concentrations of each auxin. Using GA_3 significantly changed these parameters than using Sitofex. A slight effect was recorded on these quality parameters with increasing concentrations of GA_3 from 20 to 40 ppm and Sitofex from 5 to 10 ppm. From economical point of view, the best results with regard to fruit quality were observed due to treating clusters with GA_3 at 20 ppm. Untreated vines produced unfavourable effects on fruit quality. These results were true during both seasons. The effect of GA_3 on increasing berry weight and dimensions might be attributed to its effect in promoting cell division and enlarge cells, water uptake and the biosynthesis of Nickell (1985). These results were in cone with those obtained by Williams and Ayars and Dimovska *et al*., (2014).

The higher content of Sitofex from cy surly reflected on enhancing cell division elongation of berries Nickell (1985). These were in agreement with those obtained b Zahra (2013) and Retamales *et al*. (2015).

CONCLUSION

Treating Early Sweet grapevines once w average berries reached 6mm with GA_3 at was responsible for promoting yield an quality.

REFERENCES

Abbott, W. S. (**1925**). A method for compu effectiveness of an insecticide. J. Entomol. **18**: 265-267.

Aller, H. F., and Ramsay, J. R. (**1988**). Rh- novel insect growth regulator with a ne of action. Proceedings of the Brighto Protection Conference, Pests and Dise 511-518.

Andrew, C. C.; Mayer, R. T., and De-loac (**1982**). Purification and characteriza chitinase from the stable fly *St calciteans*. Arch. Biochem. Biophysic 314- 321.

Ascher, K. R. S., and Nemny, N. E. Toxcicity of chitin synthesis in diflubenzuron and its dichlorobenzoyl a to *Spodoptera littoralis* larvae, Pestic. S 9.

Clarke, B. S., and Jewess, P. J. (**199(** inhibition of chitin synthesis in *Spo littoralis* larvae by flufenoxuron, teflub and diflubenzuron. Pestic. Sci. **28**: 377-3

Dean, S. R.; Meola, R. W.; Meola, S. M., Bhatkar, H., and Schenker, R. (**1999**). M action of lufenuron in adult *Ctenocep felis* (Siphonaptera: Pulicidae). J. Entomol. **36**: 486-492.

Deul, D. H.; De-Jong, B. J., and Kortenbac M. (**1978**). Inhibition of chitin synth two 1-(2,6-disubstitut benzoyl)-3-phe insecticides. Pestic Biochem. and **8**: 98-105.

Dixon, M., and Weeb, E. C. (**1964**). E Academic press. Inc., New York. 2nd 328-330.

Finney, D. J. (**1971**). Probit analysis, 3 Cambridge Univ. Press, Cambridge, E pp: 318.

Fisk, T., and Wright, D. J. (**1992**). Speed o and toxicity of acylurea insect

regulators against *Spodoptera exempta* (Walk.) and *Spodoptera littoralis* (Boisd.) larvae: Effect of intomult age. Pestic. Since. **35**: 331-337.

Kostyukovsky, M., and Trostanetsky, A. (**2006**). The effect of a new chitin synthesis inhibitor, novaluron, on various developmental stages of *Tribolium castaneum* (Herbst). J. Stored Prod. Res. **42**: 136-148.

Liburd, E. O.; Funderburk, J. E., and Olson, S. M. (**2000**). Effect of biological and chemical insecticides on *Spodoptera* species (Lep. Noctuidae) and marketable yield of tomatoes. J. of Applied Entomol. **124**: 19-25.

Lineweaver, H., and Burk, D. (**1934**). The determination of enzyme dissociation constants. J. Amer. Chem. Soc. **56**: 658.

Lowery, O. H.; Rosebrough, N. J.; Farry, A. L., and Randall, R. J. (**1951**). Protein measurements with folin phenol reagent. J. Bio. Chem. **193**: 265-271.

Merzendorfer, H., and Zimoch, L. (**2003**). Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. The J. Exp. Biol. **206**: 4393-4412.

Reissig, J. L.; Strominger, J. L., and Leloir, L. F. (**1955**). A modified colorimetric method for the estimation of N-acetylamine sugers. J. Biol. Chem. **217**: 959-966.

Sandeep, D. Gaikwad, and Bhamare, K. V. (**2006**). Efficacy of newer insect growth regulators and insecticides against cotton leafworm. J. Plant Sci. **1**: 104-106.

Schneider, M.; Smagghe, G., and Vifiuela, E. (**2003**). Susceptibility of *Hyposoter didymator* (Hymenoptera: Ichenumonidae) adults to several IGRs pesticides and spinosad by different exposure methods. IBOC/wprs Bull. **26**: 111-122.

Smagghe, G.; Auda, M.; Laecke, K., and Van Degheele, D. (**1997**). Significance of penetration and metabolism on topical toxicity of diflubenzuron in *Spodoptera littoralis* and *Spodoptera exigua*. Entomol. Exp. Et Applicata. **82**: 255-260.

Susan, A. Lee; Barry, S. Clarke; Donald, W. Jenner, and Francis, A. Williamson. (**1990**). Cytochemical demonstration of the effects of the acylureas flufenoxuron and diflubenzuron on the incorporation of chitin into insect cuticle. Pestic. Sci. **28**: 367-375.

Temerak, S. A. (**2002**). History records of field cotton leafworm (*Spodoptera littoralis*) resistance to conventional insecticides as influenced by the resistance programs in Egypt. Resistant Pest Management Newsletter. **12**: 7-10.

Toscano, N.; Prabhaker, N.; Castle, S. Henneberrt, T. J. (**2001**). Inter differences in baseline toxicity of *argentifolii* (Homoptera: Aleyrodidae) two insect growth regulators, buprofe pyriproxyfen. J. Econ. Entomol. **94**: 153

Ware, G. W. (**2000**). The Pesticide Book, Thomson Publications, Fresno, Californi

Wilson, T. G., and Cryan, R. J. (**1997**). Lufe chitin synthesis inhibitor in development of *Drosphila melanoga* Exp. Zool. **278**: 37-44.

Abdel Fattah, M.E.; Amen, K.A.; Alaa, A Eman, A.A. (**2010**). Effect of berry t CPPU spraying and pinching on clus berry quality of two grapevine c Assiut J. of Agric. Sci., **40(4)**: 92-107.

Abu Zahra, T.R. (**2013**). Effect of plant h application methods on fruit qua Superior seedless grape. Bio Biotechnology Research Asia Vol. 527 531.

Abu Zahra, T.R. and Salameh, N. (**2012**). I of Gibebrellic acid and cane girdling size of Black Magic grape cultivar . East Journal of Scientific Research 718 722.

Al Obeed, R.S. (**2011**). Enhancing the shelf storage ability of Flame seedless gr by agrochemicals preharvest applications. Middle East Jour Scientific Research **8 (2)**: 319 327.

Association of Official Agricultural C (A.O.A.C.) (2000). Official Meth Analysis (A.O.A.C), 12th Ed., B Franklin Station, Washington D.C., pp. 490 510.

Dimovska, V.; Ivanova, V.; Ilieva, I Sofijanova, E. (**2011**). Influen bioregulator gibberellic acid on technological characteristics of clus berry from some seedless grape v Journal of Agric. Science and techno 1074 1058.

Dimovska, V.; Petropulos, V.I.; Salamovska, Ilieva, F. (**2014**). Flame seedless variety (*Vitis vinifera* L.) and c concentration of gibberellic acid Bulgarian Journal of Agric. Sci., **20** 137 142.

Dokoozlian, N.K. (**2001**). Gibberellic acid ap bloom reduces fruiit set and improves "Crimson seedless" Table Hort.science **36(4)**: 706 709.

Guiseppe; F.; Andream, M.; Guiseppe, N. C P. Angela, M.; Isabella, C. Pie

Mariangela, V. and Vito, G. (**2014**). Girdling, Gibberellic acid, and forchlorfenuron effect yield, quality and metabolic profile of table grape cv. Italia. Am. J. Enol. Vitic. 65.3.

Hiscox, A. and Isralstam B. (**1979**). Method for the extraction of chlorophylls from leaf tissue without maceration. Can. J. Bot. **57**: 1332-1334.

Juan, P.Z.; Bernardo, A.L. and Paulina, N. (**2009**). Preharvest applications of growth regulators and their effect on postharvest quality of table grapes during cold storage. Postharvest Biology and technology **51**. 183-192.

Kassem, H.A.; Al-Obeed, R.S. and Soliman, S. S. (**2011**). Improving yield, quality and profitability of Flame seedless grapevine grown under arid environmental by growth regulators preharvest applications. Middle East Journal of Scientific research **8 (1)**: 165-172.

Lane, J. H. and Eynon, L. (**1965**). Determination of reducing sugars by means of Fehlings solution with methylene blue as indicator A.O.AC. Washington D.C.U.S.A. pp. 490-510.

Leopold, A. C. (**1964**). Plant growth and development. pp. 133-143.TATA McGraw – Hill publishing Comp. LTD. Bombay New Delhi.

Marzouk, H.A. and Kassem, H.A (**2011**). Improving yield, quality and shelfe life of Thompson seedless grapevine by preharvest foliar application. Scientia Horticulruea **130**: 425-430.

Mead, R.; Currnow, R. N. and Harted, A. M. (**1993**). Statistical Biology. 2"d Ed. Meth Agriculture and Experimental ane London pp. 10-20.

Nickell, L.G. (**1985**). New plant growth re increase grape size. Proc. Plant grov Soc. of Am. 12. 1-7.

Refaat, S.S.E.; Ghada, Sh.Sh. and Ola, A.A Effect of foliar spraying with gibberl and/ or sitofex on bud behaviour, ve growth, yield and cluster qual Thompson seedless grapevines. Jou American Science, **8 (5)**: 99: 21-34.

Retamales, J.; Bangerth, F. Cooper, T. and C R. (**2015**). Effect of CPPU and GA3 quality of Sultanina table grape. Isl Hoerticulturae 394: plant Bioregula Horticulture.

Wassel, A.H.; Abdel-Hameed, M.; Gobara, attia, M. (**2007**). Effect of micronuitrients, gibberellic acid and a acid on growth, yield and quality o Banaty seedless grapevines. Africa Science Conference Proceeding Vo 547-553.

Weaver, R. J.(**1976**). Grape Growing . A Interscience Puplication John Wiley & New York. London. Sydney. Tronto. 175.

Wilde, S. A.; Corey, R. B.; Lyer, I. G. and V K. (**1985**). Soil and Plant Analysis f Culture. 3rd Oxford & IBH publishi New Delhi, pp. 1 – 218.

Williams, L.E. and Ayars, J.E. (**2005**). Wate Thompson seedless grapevines as affe the application of Gibberellic acid (G trunk girdling practices to increasin size. Agriculture and Forest Meterolo 85-94

الملخص العربى

تأثير أثنين من منظمات النمو الحشرية على نشاط أنزيم الكيتينيز.

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~~رش حامض الجبريليك والسيتوفكس فى تحسين المحصول وجودة حبات العنب الإيرلى سويت فى منطقة المنيا– مصر~~

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تقدير سمية أثنين من منظمات النمو الحشرية اليفيرون والهيكسافلوميرون ل لعمر اليرقى الثانى والرابع لسلالتين ورق القطن أحداهما حقلية والآخرى معملية وتم دراسة تأثيرالمركبين على نشاط أنزيم الكيتينيز للعمريين الحقلية والمعملية تحت الدراسة. وأضحت النتائج أن يرقات العمر الثانى أكثر حساسية للمركبين مقارنة بيرقات ابع. وقد تم دراسة المقدرة التثبيطية للمركبين على نشاط أنزيم الكيتينيز وبعض الثوابت الخاصة بأنزيم الكيتينيز ، ميخائيل (K_m) وأقصى نشاط نوعى (V_{max}) وقيم الـ I_{50} للمعاملة *in vitro* كانت 0.31 و0.64 ميكرومولر يرقات العمر الثانى للسلالة المعملية والحقلية بعد 24 ساعة من المعاملة باليفيرون على التوالى، أما ليرقات ابع فكانت 0.44 و 0.75 ميكرومولر على التوالى. أما بعد 24 ساعة من المعاملة بالهيكسافلوميرون ليرقات انى كانت 0.57 و 0.76 ميكرومولر للسلالة المعملية والحقلية على التوالى، بينما ليرقات العمر الرابع كانت 0.81 ميكرومولر على التوالى، وأيضاً تقدير قيم ثابت التثبيط K_i وقد أظهر المركبين تثبيط تنافسى على يم الكيتنيز. ومن هذه النتائج نجد أن عند أستخدام منظمات النمو الحشرية فى مكافحة دودة ورق القطن فإنه يل التأثيرات السلبية على صحة الأنسان والبيئة والأعداء الطبيعية وتفادى زيادة مقاومة هذه الآفة لفعل المبيدات

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