Laboratory Rearing of The Peach Fruit Fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) on Semi-Artificial Diet Based on Soybean Protein

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ABSTRACT

Impact of three different larval diets on various growth parameters of fruit fly *Bactrocera zonata* was studied in the Faculty of Agriculture, Alexandria University, Egypt. Each diet was composed of basic ingredients Sugar, Corn oil, Nipagen, Sodium benzoate and Citric acid. Soybean flour (23.07% protein)was added to Diet (I) and (II) while Gelatin (90.28 % protein) was added to Diet (I) and (III). All ingredients were solved in water.Rearing *B. zonata* was carried out in small rearing cabinet under controlled conditions of 25 ± 2 ºC, 65-75 % RH, and photoperiod of 14:10 (L: D) The highest egg hatchability was 49.29% (diet III) following by diet (I) and (II); 44.83 and 21.14, respectively. Diet II produced, the highest development of larvae to pupal stage, adult emergence and fecundity of female.Different biological aspects i,e adult rearing, egg deposition oviposition stimulants, evaluation of larvae rearing diets and flight ability test of adult fly were assessed.

Key words: *Bactrocera zonata*, rearing diets, Soybean, Gelatin.

INTRODUCTION

Egypt have been suffering from pest problems with fruit flies ever since the beginning of the last century. *Bactrocera zonata* was recorded as a new pest in Egypt in 1997 at Agamy and Sabahia districts at the West Northern coast near Alexandria(El-Minshawy *et al*., 1999). Since then, it has been spreading over the Nile valley displacing the indigenous fruit fly *Ceratitis capitata* (Wiedemann). Egyptian farmers were forced to use large quantities of broad-spectrum insecticides to protect their crops from the aggressive attack of *B. zonata* and other fruit fly pests. Furthermore, the Egyptian Ministry of Agriculture has decided to target *B. zonata* through an emergency action plan adopted by FAO/AIEA funded project for insect eradication using bait annihilation technique (BAT) and/or male annihilation technique (MAT). Because of the wide spreading of infested host fruits, the strategy of area wide eradication of *B. zonata* by insect lures in Egypt did not intend to eradicate the target pest. Populations of *B. zonata* are considered as a threat to orchards till now and farmers still control this pest with the conventional intensive use of insecticides.

Suppression program using Sterile Insect Technique (SIT) is not feasible now because of the large fly populations supported by host plants available the whole year around. An incredible numbers of irradiated flies (billions per week) are needed for SIT costly eradication program that Egypt alone is not ready to support it. In addition, the fly populations must be suppressed first for an SIT program to be effective. Current surveys in Egypt show that the insect population are spreading throughout the whole Nile Delta and valley, the Oases, and further east to the Sinai Peninsula (Draz *et al*.,2016).No efficient control action has yet been undertaken and farmers still rely on costly chemical control measures to protect their crops. However, in some agricultural firms such as Nubaria and Delta valley the annihilation technique in addition to partial spray with poisonous bait for both *Ceratitis capitata* and *B. zonata* are applied. The technique is effective especially in farms cultivated with mixed trees of citrus and peach. **(**El-Minshawy, unpublished *work*).

A principal requirement for success of SIT of the peach fruit fly is mass production of the flies on a larval diet of high efficiency and low cost. Fruit flies may require several generations for the insects to adapt to the artificial diets (Economopoulos, 1992). In many cases, attempts to raise a colony from wild populations on artificial diets have not been successful (Rossler, 1975), but, if achieved, long-term rearing on artificial diet may improve insect performance e.g. through longer oviposition period and an elevated egg production (Vargas and Carey, 1989).

Available information on larval nutritional requirements for Tephritid flies has been focusing on improving survival, growth, and development by using dry plant materials and yeasts (Vargas *et al*.,1994). In general, nutrients from some diets apparently are sufficient to support larval development. However, for optimization of growth and development, a completely chemically defined

diet is required for better understanding of nutritional responses for *C. capitata* **(**Chang *et al*., 2001).Bulking and nutritive components in an insect diet is expensive and in some countries is difficult to import. The replacement of imported components by local products has been the concern of researchers in many countries that have fruit fly mass rearing facilities.

The objective of the present work was to develop suitable and economic diet based on soybean and gelatin proteins for laboratory mass rearing of the peach fruit fly, *B.zonata* in the future.

MATERIALS AND METHODS

Insect collection:

Adults of *B.zonata* were obtained from infested guava *Psidium guajava* fruits collected from house backyards. Emerged adults were reared for three generations on guava fruits in the laboratory for adaptation. Also, *B. zonata* was reared for six generations on semi-artificial diet on small scale in insect rearing cabinet. Some biological aspects such as egg hatchability, larval duration, pupal weight, percentage of pupal recovery, percentage of adult emergence and adult longevity was calculated.

The insectary:

Rearing of *B.zonata* was carried out in small rearing cabinet (2 X 3 X 3 m) in the Department of applied Entomology. The cabinet walls were made from double metal layers provided with foam layer 5 cm in thickness to maintain constant temperature. The room was provided with heat and fluorescent light systems. Rearing conditions was adjusted to 25 ± 2 ºC, 65 – 75 % RH and a photoperiod of 14:10 (L: D).

Adult rearing:

Collected pupae from infested guava fruits were placed inside plastic cages (30x30x27 cm). Cages were covered with muslin cloth for ventilation (Fig.1). Emerged adult flies were provided with a 3:1(volume: volume) mixture of sugar and hydrolysate protein (Bominal) each week. Also, plastic cup provided with wet cotton wick was put in the cage as a source of water. A plastic container (400 cm^3), perforated with 40 punctures 0.5-mm-diameter on the wall, was used as an oviposition device (Fig. 2 and 3). The cup is placed upside down on a piece of sponge (3X4X2 cm) soaked in a 1:1 mixture of guava juice and water to stimulate flies to lay eggs in the punctures. A circular black plastic disc was put above the sponge for collecting deposited eggs. Oviposition device unit was placed inside the adult rearing cage. Deposited eggs were collected by using Camel hair brush. Eggs were collected until adults reach up to 30 days from the beginning of egg laying.

Rearing Larvae:

To prevent desiccation, collected eggs were put on the semi-artificial diets inside plastic trays (15X10X3 cm) covered with clear Parafilm lids until egg hatching to prevent desiccation. Containers lids were ventilated with 2 to 4 mm-diameter holes during larval duration period. Larval trays were stored on metal shelves. Before larval maturation, larval trays were put in ventilated boxes provided with (half kg of wet sterilized sand) on the bottom. After one week the mature larvae begin to leave the diet and jump to the sand for pupation.

Handling pupae:

Collecting pupae was done for 4 days after pupation. Pupae were sieved from the sand and held in the adult rearing cages until emergence. Number of pupae recovered was determined and percentage of pupal recovery was calculated based in the initial numbers of eggs put on the diet.

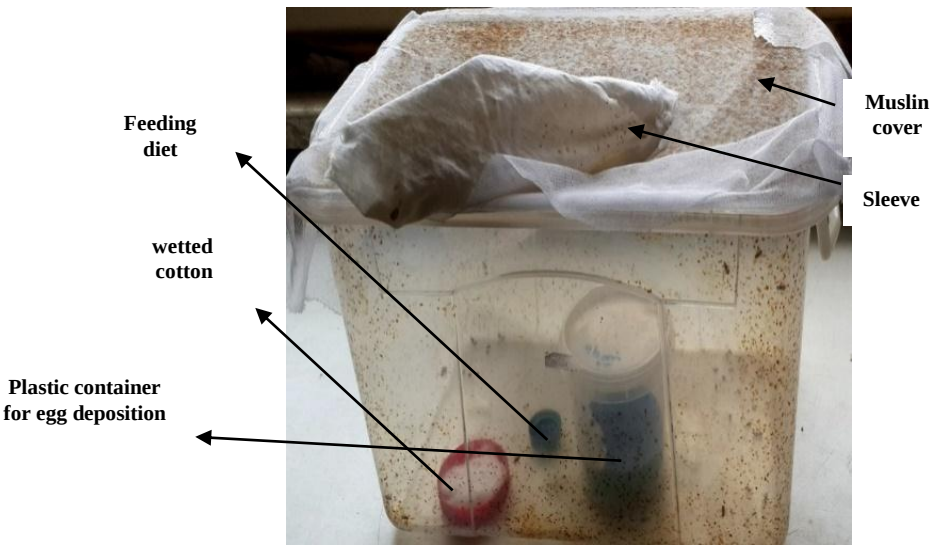

Fig. 1: Adult rearing cage of *B. zonata*

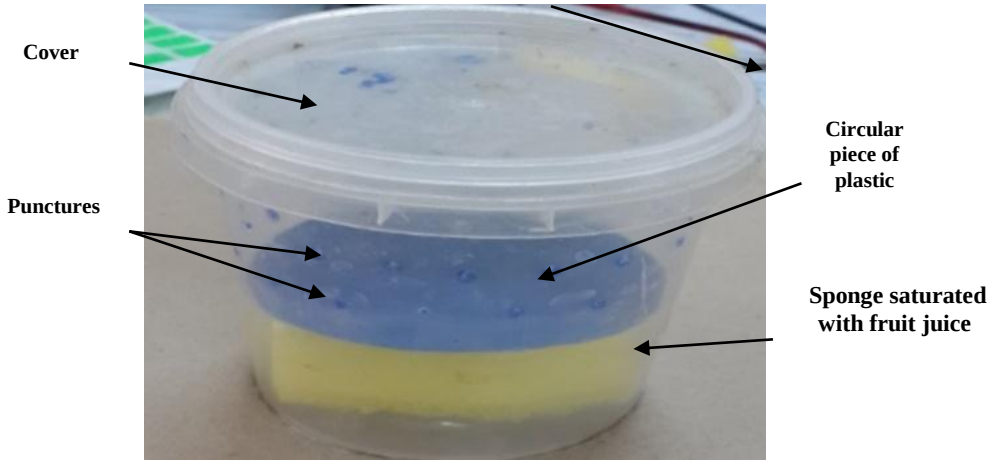

Fig.2: *B. zonata* container of egg deposition

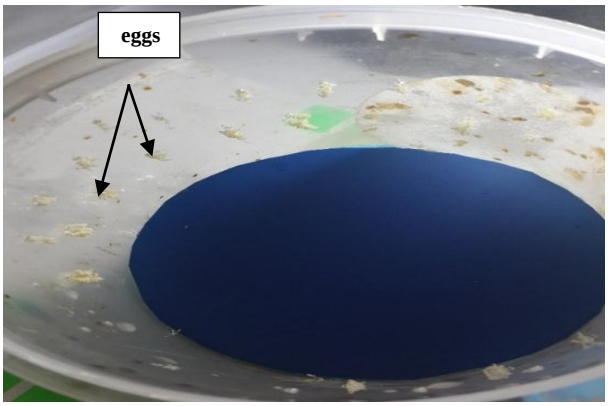

Fig. 3: Deposited eggs of *B. zonata* on the inner side of the container wall.

Tested Larval Diets:

Three different diets for rearing larvae of *B. zonata* listed in Table (1) were evaluated. Diet I, containing soybean and gelatin; diet II, without gelatin, and diet III, without soybean. Estimation of protein percent in soybean flour and gelatin was done in the Central Laboratory, Fac. of Agric. Alex. Uni. Egg hatchability, larval duration, pupal weight, percentage of pupal recovery, percentage of adult emergence and adult duration were calculated.

Egg depositing devices:

Three egg collecting devices were evaluated to select the most suitable one for egg laying. They were Plastic cup (150 cm^3), cartoon cup (250 cm^3) and plastic container (400 cm^3) (Fig2) The tested devices were prepared as mentioned before. Egg collection devices were put in adult rearing cage containing 50 pairs of 7 day-old flies of *B.zonata* and provided with a 3:1 (volume: volume) mixture of sugar and hydrolysate protein and wet cotton wick.

Table 1: Ingredients of different diets based on soybean-gelatin used for feeding larvae

Ingredient	Diet I		Diet II		Diet III	
	Weight (g)	%	Weight (g)	%	Weight (g)	%
Soybean flour *	150	15	150	17.16	-	-
Gelatin **	126.1	12.61	-	-	126.1	14.83
Sugar	89.9	8.99	89.9	10.29	89.9	10.58
Corn oil	14	1.4	14	1.6	14	1.65
Nipagen	1.5	0.015	1.5	0.017	1.5	0.018
Sodium benzoate	1.5	0.015	1.5	0.017	1.5	0.018
Citric acid	17	1.7	17	19.45	17	2.0
Water	600 ml	60	600 ml	68.66	600 ml	70.59

* Soybean flour (23.07 % protein) ** Gelatin (90.28 % protein).

Three replicates of each device were assembled. Cages were placed on the shelve closed to two rows of 60-w fluorescent lamps. Evaluation conditions is adjusted on 25±2 ºC,65-75 % RH and a photoperiod of 14:10 (L: D). Camel hair brush was used to collect laid eggs.

Oviposition stimulants for *B.zonata*:

Different juices of guava, mango, banana and grapefruit were tested as oviposition stimulants for *B.zonata*. Sponge pieces each was saturated with tested fruit juice was placed inside plastic egg laying device. Similar sponge piece saturated with water was used as a control. Each treatment was replicated three times. Egg laying devices were put in adult rearing cage contained 50 pairs of 7 days-old flies of *B.zonata* to estimate egg production. Adults were provided with water and 3:1 a volumetric mixture of sugar and hydrolysate protein(Bominal). Eggs laid were daily collected and counted for 7 days.

Flight ability test:

The method described by Collins *et al*.,(2008) was applied to assess flight ability of emerged adults. Three collections of 100 pupae each were used to assess percentage adult emergence, and percentage fliers. Pupae were placed in 55-mm Petri dish lids. The dish of pupae was then centered on 90 mm Petri dish lined with black paper. A 100 mm tall plastic tube (50 mm inner diameter), with a fine coat of talcum powder on the interior surface (to prevent flies walking out) was placed in the cage (Fig.1) and over set up on shelves near to the light in the rearing room. Flies that escaped from the tube were removed daily. When all emergence had ceased (4-5 days after the first flies emerged), the remainder pupae in the tubes were counted. The collected data were classified to three categories: i). not emerged, ii). Non-fliers (flies creeping on the tube wall and could not fly out of the tube), iii). Fliers (fly looked normal and had exit out of the tube).

Statistical analysis:

Experiments were laid out in a completely randomized design with 3 replicates. Results of oviposition devices and fruit juices tests were subjected to analysis of variance (ANOVA), and means were separated by LSD test at the $p \leq 0.05$ the statistical analysis was done using Mstat c. **(**Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Rearing on soybean – gelatin diet:

Mean duration of different stages of *B. zonata* reared on semi-artificial diet (diet I) under controlled conditions **(**25 ± 2 ºCand 65 - 75 % R.H.) was presented in Table (2). Data revealed that egg, larval and pupal duration was 3.0, 10.0 and 7.5 days, respectively. Pre-oviposition and oviposition periods were 10.0 and 41.67 days.

Biological parameters of *B. zonata* reared on semi-artificial diet (diet I) for six generations under controlled conditions of 25 ± 2 ºC and 65 - 75 % R.H. are shown in Table (3). Data revealed that a mean percentage of egg hatch was 34.0 % in generation 1 (G1), increased to 71.0 % G6 with 44.83 ± 16.28 % as mean. Weight of mature larva ranged between 6.55 mg and 6.98 with the mean weight of 6.67 ±0.38 mg. Also, weight of pupa ranged between 7.22 and 11.58 mg with the mean weight of 10.10 ± 1.52 mg. Percentage of pupal recovery recorded low value in G1 and G2 being 18.75 and15.0 % in the two generations, respectively. Then, it increased to 92.21 %in the G5. Percentage of adult emergence ranged between 85.0 and 97.0 % with mean of 91.0 ± 4.52.

Table 2: Duration (days) of different stages of *B. zonata* reared on semi-artificial diet (diet I) under controlled conditions (25 ± 2 °Cand 65 - 75% R.H.)

				Adult duration		
	Egg duration	**Larval duration**	**pupal duration**	**Pre-oviposition**	**Oviposition**	**Total**
Mean duration (days) ± SD	3± 0.9	10 ± 1.67	7.5± 1.05	10 ± 1.79	41.67 ± 12.69	52 ± 12.51

Data obtained from three replicates

Table 3: Biological parameters of *B. zonata* reared on semi-artificial diet (diet I) under controlled conditions (25 ± 2 °C and65 - 75% R.H.)

Generations	**% Egg hatching**	**Weight of mature larva (mg)**	**Pupal weight (mg)**	**% Pupal recovery**	**% Adult emergence**	**Sex ratio Female: Male**
G1	34%	6.55	11.06	18.75	85	2.30: 1.00
G2	55%	7.22	10.29	15.00	89	1.10: 1.00
G3	35%	6.68	10.29	35.27	92	0.83: 1.00
G4	47%	6.15	11.58	42.11	88	1.00: 1.00
G5	27%	6.44	10.17	92.21	95	2.30: 1.00
G6	71%	6.98	7.22	79	97	1.53 :1.00
Mean ± SD	44.83 ± 16.28	6.67 ± 0.38	10.10 ± 1.52	47.06 ± 31.79	91.0 ± 4.52	

Data obtained from three replicates

Mean numbers of eggs/female/day of *B. zonata* reared on semi - artificial diet of soy bean during six generations under controlled conditions of 25±2ºCand 70%RH are presented in Table (4). Data indicated that mean numbers of eggs/female/day fluctuated from 1.27 in G2 to 5.25 in G4. Generally, mean number of eggs (through the first 10 days of oviposition period) was 2.84 egg/female/day.

Mean egg production of *B. zonata* females and mean weight of both larva and pupa reared on guava fruit and semi-artificial diet (diet I) indicated in (Table 5).

Data revealed that mean number of eggs/day/ female reared on semi-artificial diet was significantly higher than that reared on guavafruit. Weight of larva and pupa reared on semi-artificial diet do not significantly differ from those reared on guava fruit. (Fig.4).

The presented data in table (6) of the flight ability test of *B. zonata* reared on natural fruit and semi-artificial diet indicated that the percentage of adult emergence were 49.33 % and 70.67 % of the pupae reared on guava fruit and semi-artificial diet, respectively. Flight ability of adult emerged from pupae reared on semi-artificial diet was significantly higher than those reared on guava fruit. In fact, no significant differences were observed between the two tested diets in percentage of creeping adults on the tube.

The gathered data indicated that, wild *B. zonata* collected from the field required many generations to be adapted for rearing on artificial diet. The present results are consistent with those of other investigators. Ekesi and Mohamed**,** (2012)reported that the laboratory adaptation of any insect species is a function of their inherited ability to adapt to the new rearing media. Souza*et al* (1988) observed that at least 10 generations were needed for the adaptation of *C. capitata* on artificial diet. In the olive fruit fly *Bactrocera oleae*, about three to four generations were required to adapt (Tsitsipis, 1983), while in *B. cucurbitae*, it took 14 generations to reach a permanent plateau (kamikado *et al*., 1987). It is therefore evident that several genetic, physiological and behavioral changes occur during colonization of wild populations of fruit flies and depending on the species could take several generations for the insect to fully adapt to the rearing medium (Economopoulos, 1992).

Data in Table (7) indicated that some biological parameters of *B. zonata* reared on three different diets (diet I, containing soybean and gelatin; diet II, decaffeinated gelatin, and diet III, decaffeinated soybean flour), under controlled conditions of 25±2ºC and 70 % R.H. Data indicated that % of hatching eggs were 44.83, 21.14 and 49.29 % of the three diets, respectively. Larval duration was reduced from 10.00 and 10.43 days of diet I and diet II to 3.71 days of diet III. Pupal weight was reduced from 10.10 mg of pupae resulted from rearing on diet I to 6.06 mg of diet II. In the meantime, larvae reared on diet III, decaffeinated soy bean did not complete their durations and no pupation occurred. Percentages of pupal recovery were 47.06 and 9.97 % of insects reared on diet I and diet IIfollowed with percentages of adult emergence, of 91.0 and 17.14 %. All adults emerged from pupae reared on diet III, (decaffeinated soybean) died.

Reduction in hatchability in diet II, decaffeinated gelatin, is probably due to the low humidity in the diet. Gelatin substance was used as bulking agent and source of protein. Gelatin gives the diet loose texture; hence it improves egg hatchability. Also, gelatin offers enough density and texture for larval mobility and jumping off the rearing tray easily. Decaffeinated of soy bean flour from the diet III resulted in reduction of larval duration and death of the hatched larvae. This indicates that soy bean contains some nutrients important for larval growth. Soy bean sale powder contains the essential requirements for development such as protein, sodium, potassium, calcium, phosphorus, iron, protein and oil. Also, Sobrinho *et al*.,(2006) reported that Brazilian soybean protein has chemical components that fulfill basic requirements for Medfly development, comparing with brewer yeast of the standard diet.

Egg collection:

Evaluation of different egg collection devices are indicated in Table (8). The mean number of collected eggs was significantly higher in the plastic container compared with the plastic cup and cartoon cup during 7-days test period. In this context, similar bottle for egg collection is adopted in Hawaii fruit fly mass rearing facility to protect eggs inside the saturated atmosphere of plastic containers with wet sponges (Tanaka, 1965; Tanaka*et al*., 1970).Vargas, (1984) demonstrated that oviposition by *C. capitata* was significantly higher in bottles than on the screen device. The oriental fruit fly *Bactrocera dorsalis*, which is a close relative of *B. ivadens* and *B. cucurbitae* are also mass reared in Hawaii by the use of bottle egg collection devices. Bottles have been found to be the best egg collection system and presently in use for mass rearing of the different fruit fly species. Increased female acceptance of this oviposition device has led to substantial reduction in labor and cost of mass rearing of the various fruit fly species. Meanwhile, Table (9) shows numbers of deposited eggs in oviposition device supplied with different fruit juices as oviposition stimulants through tested period of seven days. The highest numbers of eggs were collected from cups supplied with guava juice followed by mango, grape fruit then banana., No statistical differences occurred between deposited eggs from the tested fruit juices.

Table 5: Mean egg production of *B. zonata* females and mean weight of both larva and pupa reared on guava fruit and semi-artificial diet (diet I) under controlled conditions of 25 ± 2 °C and65 - 75% R.H.

	Guava fruit	Semi-artificial diet
Mean No. of eggs/day/female* ± SD	5.13 ± 1.23	9.38± 4.77
Mean larva weight (mg) ± SD	6.98±0.31	6.67±0.38
Mean pupal weight (mg) ± SD	9.74±0.38	10.10±1.52

*During oviposition period

Table 6: Flight ability test of *B. zonata* reared on guava fruits and artificial diet under controlled conditions of 25 ± 2 °Cand 65 - 75% R.H.

	%Adult flyers ± SD	%creeping adult ± SD	% Non-emerged adult ± SD
Fruit	49.33±6.11	31.33±9.02	19.33±9.45
Diet	70.67±8.33	29.33±8.33	0.00

Data obtained from three replicates

Table 7: Biological parameters of *B. zonata* reared on different diets (I, II, III) under controlled conditions (25 ± 2 °Cand 65 - 75% R.H.)

Diets	% Egg hatching	Larval duration (days)	%Pupal recovery	Pupal weight(mg)	%Adult emergence	Adult duration (days)
Diet I	44.83±16.28	10.00±1.67	47.06±31.79	10.10±1.52	91.00±4.52	52±12.51
Diet II	21.14±11.91	10.43±162	09.97±12.06	06.06±0.32	17.14±12.81	0
Diet III	49.29±15.01	03.71±0.76	0	0	0	0

Table 8: Mean number of eggs per day of *Bactrocera zonata* female in different oviposition devices containing fruit juice during seven days under controlled conditions of 25 ± 2 °C and65 - 75% R.H.

Oviposition Devices	No. of eggs/female/day							Mean No/day of Devices
	1st	2sd	3rd	4th	5th	6th	7th	
Plastic cup (250 cm³)	2.13	2.20	4.13	2.73	2.83	1.80	3.00	2.69 c
Plastic container (400 cm³)	6.73	6.50	4.87	5.07	4.10	5.03	6.67	5.57 a
Cartoon cup (250 cm³)	3.90	3.00	3.03	3.30	2.17	2.40	2.43	2.89 b
Means of days	4.26 a	3.90 b	4.01 b	3.70 b	3.03 c	3.08 c	4.03 b	

Means followed by the same letters do not significantly differ at the 5% level according to the LSD test.
L.S.D 0.05 days = 0.17 L.S.D 0.05 devices = 0.11 L.S.D 0.05 interaction = 0.29

Table 9: Mean number of eggs per day of *Bactrocera zonata* female offered to fruit juices as oviposition stimulants during seven days under controlled conditions of 25 ± 2 °C and65 - 75% R.H.

Oviposition Stimulants	No. of eggs/female/day							Mean of stimulants
	1st	2sd	3rd	4th	5th	6th	7th	
Guava	3.73	2.30	3.47	2.87	2.53	2.17	3.37	2.91 a
Mango	2.53	3.00	4.97	2.20	3.80	1.33	1.80	2.81 a
Grapefruit	1.37	2.37	4.13	3.10	2.53	1.27	3.57	2.62 b
Banana	0.87	1.07	4.00	2.67	2.37	2.33	3.17	2.35 c
Mean of days	2.13 d	2.18 d	4.14 a	2.71 c	2.81 c	1.77 e	2.98 b	

Means followed by the same letters, within a column, do not significantly differ at the 5% level according to the LSD test.
L.S.D 0.05 days = 0.14 L.S.D 0.05 Oviposition stimulants = 0.19 L.S.D 0.05 interaction = 02.29

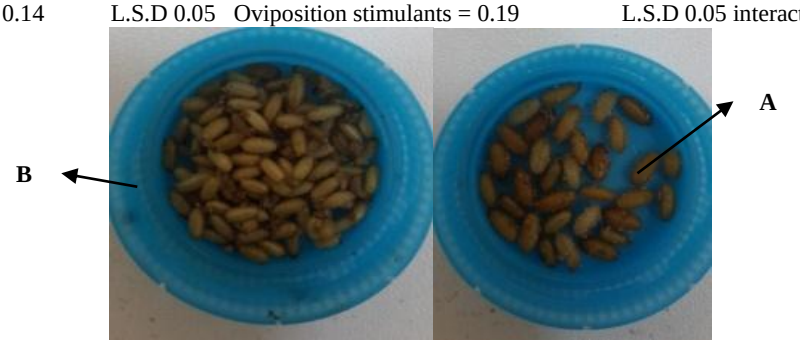

Fig. 4: Pupae of *B. zonata* reared on semi-artificial diet
A. Pupae reared on diet containing soybean flour and gelatin.
B. Pupae reared on diet containing soybean flour.

According to Tanaka,(1965), who recommended using a preferred host juiceor extract in an oviposition bottle to obtain a consistent egg harvest from tephritid species. Vargas and Chang, (1991) reported that artificial oviposition bottles treated with fresh frozen juice of *Citrus sinensis* (L.) Osbeck increased the egg harvest of *Bactrocera dorsalis* (Hendel) by 55.2% when compared with those treated with canned guava juice with sugar and/or corn sweetener, citric acid and ascorbic acid. Al-Eryan *et al*.,(2006) collected the highest numbers of *B. zonata* eggs from cups supplied with mandarin juices followed by banana, orange, guava and mango. Although, the gained results of the evaluated diet seem to be suitable for rearing *B. zonata* in small scale under laboratory conditions, further investigation would be required to improve the quality parameters of the modified diet for satisfactory mass production of *B. zonata*.

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الملخص العربى

التربية المعملية لذبابة ثمار الخوخ بكتروسيرا زوناتا (سوندرس)(رتبة ثنائية الاجنحة: عائلة تيفريتيدي)على بيئة فول الصويا النصف صناعية

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تم دراسة تأثير التربية المعملية على بيئات غذائية مختلفة على نمو ذبابة الخوخ بكتروسيرا زوناتا بكلية الزراعة جامعة الاسكندرية بمصر. تكونت البيئات الغذائية من المواد الأساسية وهى السكر، زيت الذرة، النيباجين، بنزوات الصوديوم، حامض الستريك. تم اضافة دقيق فول الصويا (٢٣,٠٧% بروتين) الى بيئتى(١) و(٢) بينما تم اضافة الجيلاتين(٩٠,٢٨% بروتين) الى بيئتى (١) و(٣). أذيبت المواد المكونة للبيئة فى الماء. ربيت ذبابةالخوخ في غرفة تربية محكمة(٢×٣×٣م) (تحت ظروف حرارة ٢٥ ± ٢م° و٦٥– ٧٥% رطوبة نسبية وفترة ضوئية(١٤ ساعة اضاءة: ١٠ ساعات اظلام). بدراسة الثلاث بيئات تبين أن أعلى نسبة فقس للبيض كانت ٤٩,٢٩% فى بيئة(٣) متبوعا ببيئتى(١) و(٢) حيث كانت نسبة فقس البيض ٤٤,٨٣ و ٢١,٤١ للبيئتين على التوالى. وكان أعلى معدل لنمو اليرقات والعذارى والحشرات الكاملة وخصوبة الاناث للحشرات المرباة على البيئة(١). كان لتأثير البيئات الغذائية نتائج مختلفة على بعض الحقائق البيولوجية لذبابة الخوخ مثل تربية اليرقات ووضع البيض والقدرة على الطيران.

الكلمات الدليلية: بكتروسيرا زوناتا، بيئة التربية، فول الصويا، جيلاتين.