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Toxicological, Biological and Biochemical Effects of Certain Insecticides on *Bactrocera zonata* (Saunders) (Diptera, Tephritidae)

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Abstract: The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is a serious pest attacking a wide range of fruits in Egypt. The toxicity, biology and biochemical effects of certain insecticides belonging to different chemical groups Beticol, Biosad, Elsan, Lufox, Mani, Match and Radiant against full grown larvae and pupae of *B. zonata* with different concentrations as contact poisons or in sandy soil treatments were assayed under laboratory conditions. The results showed that Radiant was the most contact poison on full grown larvae among the tested insecticides, followed by Elsan, Beticol, Lufox, Match, Biosad and Mani. The respective LC_{50} values were 4.65, 18.8, 20, 35.5, 122, 158 and 330ppm, respectively. On 1-day old pupae, Elsan was the most potent toxicant compound followed by Match, Lufox, Beticol, Radiant, Mani and Biosad. While, in sandy soil treatments, Lufox was the most potent toxicant against 1-day old pupae at ($LC_{50=}$ 200ppm). Considerable number of larvae, pupae and adults showed obvious malformations after treatments as surface contact or in sandy soil. Also, certain biological aspects of adults emerged from pupae treated with LC_{50} values of the tested insecticides as surface contact application was detected. The obtained results show that pre-oviposition, oviposition and post-oviposition periods were highly significantly different. The tested insecticides reduced the fecundity and hatchability percentage. Also, these insecticides caused high levels of sterility for adult females emerged from treated pupae. So, it can be used as chemosterilizing agents against *B. zonata.* Also, Biosad, Radiant, Beticol, Lufox and Mani decreased adults longevity and induced significant differences in enzymes and total proteins appeared after treatment with the tested insecticides compared with control.

Key words: AchE · *Bactrocera zonata* · Bioactivity · Fruit fly · Insecticide · Toxicity · Malformations

INTRODUCTION

The fruit flies belong to the most important insect pest groups of horticulture production and throughout the world. The peach fruit fly, *Bactrocera zonata* (Saunders) is one of the most destructive fruit pests which spread in several regions of the world [1, 2]. It is considered that *B. zonata* was recorded in Egypt in 1999, where it caused a severe damage to a wide range of fruits including guava, peach, apricot and mango [3,4]. Chemical control has been the most important measure used. Malathion is the most commonly used in both aerial and ground treatments. This insecticide was the first example of a wide spectrum Organophosphorus insecticide combined with a low mammalian toxicity and its cheap price. The intense and repeated applications of insecticides which are often from the same chemical group led to development of insects resistance to pesticides. To prevent the resistance phenomenon, there is a need for insertion of different compounds having different modes of action in control programs [5]. The aim of the present work is to evaluate the efficiency and biological effects of some insecticides belonging to different groups which differ in their mode of action against certain stages (full grown larvae and 1-day old pupae) of *B. zonata.* The effect of sublethal concentrations of the tested

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insecticides on some biochemical constitutes (acetylcholine esterase, α-esterase and total proteins) are carried out on 1-day old pupae of *B. zonata* under laboratory conditions.

MATERIALS AND METHODS

Insect Stock Culture: The laboratory susceptible colony of the peach fruit fly, *Bactrocera zonata* was established from a strain continuously reared in the laboratory of Horticultural Insects Department, Plant Protection Research Institute, Agricultural Research, Center, Dokki, Giza under conditions of (25±3°C, 60±5% R.H and photoperiod of 14 L: 10 D). Adults of *B. zonata* were reared in a cage (30 x 30 x 30 cm) with wooden frames and covered from each side with metal screen. Flies were fed with sugar and fortified protein hydrolysate at ratio of 3:1, respectively. Adults were provided with a small plastic bottle filled with water as drinking sites, until mating took place then females started oviposition. The cage was supplied with false plastic fruits that had many fine pores (as oviposition receptacles). These plastic fruits are filled with 3ml water to receive and prevent drying of the eggs. Also, at the top of these plastic fruits, small plastic vials containing cotton wicks saturated with guava juice were put to enhance egg lying within these false fruits. Larvae were reared on an artificial diet consisted of 500 ml distilled water, 3.00g sodium benzoate, 3.00g citric acid, 84.50g sugar, 84.50g brewer's yeast and 330gwheat bran. These ingredients were carefully mixed in large plastic container. The eggs were scattered on the surface of the diet which was placed in plastic trays of 20 x 10 x 8cm that were tightly covered with muslin clothes using rubber bands. The trays were placed in a wooden cage with sand at the bottom to allow the jumping larvae to pupate. All pupae were separated by sieving the sand [6].

Insecticides Used: The commercial formulations of insecticides used in bioassays were (Beticol® 20% (SC) Neonicotinoid, Systemic insecticide (E)-N1-[(6-chloro-3-pyridyl) methyl]-N2-cyano-N1-methylacetamidine) have been obtained from (Nippon Soda, Co. LTD) (Elsan® 50% EC) Organophosphate, Phenthoate Non-Systemic, contact and stomach insecticides, *(S*- α-ethoxycarbonylbenzyl *O, O*-dimethyl phosphorodithioate) have been obtained from (Nissan Chemical Industries LTD). (Biosad®22.8% SC) Spinosad, Biochemical product of Actinomycetes. It was mixture of 50-95% (of two spinosoids, Spinosyn A, the major component and Spinosyn D the minor component), in an approximately 17:3 ratio. It was obtained from (Arabic Company for Pesticides and Veterinary Drugs (MIPCO) (Radiant® 12% SC) Spinotoram 12% SC (a mixture of 3'-O-ethyl-5,6-dihydro Spinosyn J and 3'-O-ethyl Spinosyn L) is the 2nd new generation of the spinosyn group with the same mode of action as spinosad. It is a mixture of two active spinosyns (spinosyn J and L), was obtained from (Dow Agro Sciences Co.) Lufox® is mixture of juvenile hormone mimic (Fenoxycarb 7.5% EC), ethyl [2-(4- phenoxyphenoxy) ethyl] carbamate and Chitin Synthesis inhibitor (Lufenuron), (Axor 3% EC). N-[[[2,5- dicholoro-4-(1,1,2,3,3,3-hexafluoropropxy) phenyl]amino] carbonyl] -2,6-difluorobenzamide, have been obtained from (Syngenta, AG.) (Mani ® 5% and Match® 5% SC) Lufenuron, Benzoylurea Inhibits chitin synthesis, (*RS*)-1-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy) phenyl]-3-(2,6-difluorobenzoyl) urea, was obtained from (Syngenta, AG).

Laboratory Bioassay: The bioassay tests were performed on full grown larvae and 1-days pupae of *B. zonata*. The contact toxicity was used. All concentrations of insecticides were prepared by dissolving in water. For control experiments, water was used.

Residual Film Method: Full grown larvae of *B. zonata* and pupae of 1-day old were treated as residual film method with acetone-solution of different concentrations of tested insecticides (5 concentrations). Group of 30 individuals larvae or pupae were used for each concentration and put in Petri dishes (9 cm in diameter) after insecticide treatment (1ml/dish). The control insects were put in Petri dishes treated with water only and they were left for 2 hrs at room temperature to dry. Three replicates were carried out for each insecticide concentration and for the control. The tested larvae or pupae were examined after 24, 48 and 72 hrs. All treatments were inspected for mortality and malformations. The treated larvae were kept under the aforementioned laboratory conditions till adult emergence. The observed mortality was corrected with Abbott's formula [7]. Toxicity index was obtained by comparing the efficiency of different insecticides at LC_{50} with that of the highly potent compound according to the equation of Sun [8] and plotted against concentrations as log/probit regression lines. LC_{50}, LC_{90} values and the toxicity index by well as the slope of the lines were calculated [9]. using LdPLine®" software. http://embakr.tripod.com/ldpline/ldpline.htm].

Sandy Soil Method: The efficacy of the tested insecticides on full grown larvae and 1-day old pupae of *B. zonata* were evaluated by sandy soil method. Sand was sieved and put in plastic cups (100g/each). Twenty or thirty full grown larvae or 1-day old pupae were confined and buried into sand in each cup. Fifteen milliliters (required amount for saturation) of each concentration were added in each cup. The cups were covered with muslin clothes which tightly secured with rubber bands and left under the above mentioned laboratory conditions till adult emergence. Five concentrations of each insecticide in tap water were prepared. For each concentration, three replicates were used. Control experiments using soil saturated with water only also carried out for comparison and correcting mortalities in treatments as previously mentioned in surface contact treatment.

Morphogenetic Action of Certain Insecticides Against Some Immature Stages of *Bacterocera zonata* under Laboratory Conditions: Also, the morphogenetic action was assessed as morphological deformities through larval-pupal or pupal-adult transformations. The obtained malformations were evaluated as graded scoring system. The degree of morphogenetic effect (score) was evaluated by multiplying the number of individuals by their numerical activity ratings and dividing the sum of total number of treated larvae or pupae. Unaffected stage was considered zero score, maximum observed responses and not the maximum theoretical response were the highest score with the intermediates arbitrarily fixed in between according to Redfern *et al*. [10].

Latent Effects on Some Biological Aspects of *B. zonata*: The effectiveness of the tested insecticides on some biological aspects of emerged adults was evaluated. Five hundreds 1-day old pupae were immersed for 5 seconds in LC_{50} of the tested insecticides. They were left for 30 minutes at room temperature to dry, the 1-day old pupae were placed in large glass jar (2 liters in capacity). The completely normal newly emerged adults were placed in two liters plastic cages and fed water, enzymatied yeast, protein hydrolyzed and sugar, until mating took place and the females were left for oviposition. In each cage ten pairs of adults were confined. Three replicates were used for each insecticide and for control as well. The cages were examined daily to record adult longevity and the females fecundity. The percentage of egg hatchability was recorded for the newly laid eggs (100 egg in 3 replicates) for each treatment. The sterilizing activity of the used pesticides was assessed according to Chamberlain formula [11] which modified by Toppozada *et al.* [12].

Biochemical Assays: Three hundred individuals of 1-day old pupae were immersed for 5 seconds in LC_{50} of the tested insecticides. Fifty individuals of pupae of 2 and 6 days as well as newly emerged adults were collected and immediately kept in a deep freezer for biochemical analysis. Biochemical assay has been carried out to determine the effect of sublethal concentrations LC_{50} of the tested insecticides on acetylcholine esterase, α-esterase and total proteins) of, *B. zonata* pupae. Insects were homogenized in distilled water (50mg/1ml) using a chilled glass Teflon tissue homogenizer (ST-2mechnic-Preczyina, Poland). Homogenates were centrifuged at 8000 rpm for 15 min at 5°C in a refrigerator. The deposits were discarded and the supernatants were kept in a deep freezer at -20°C till use. Double beam ultraviolet/visible spectrophotometer (spectronic 1201, Milton Roy Co., USA) was used to measure absorbance of coloured substances or metabolic compounds. Acetylcholine esterase (AchE) activity was measured according to the method described by Simpson *et al.* [13] using acetylcholine bromide (AchBr) as substrate. Alpha-esterase (α-esterase) was determined according to Van Asperen [14] using α-naphthyl acetate as substrates. Total proteins were determined by the method of Bradford [15]. Data of latent effects and biochemical analysis were statistically analyzed according to completely randomized design [9, 16].

RESULTS AND DISCUSSION

Toxicity of Certain Insecticides Against *B. zonata*

Residual Film Treatment

Full Grown Larvae: The results in Table 1 show that Radiant was the most effective toxicant to full grown larvae of *B. zonata* followed by Elsan, Beticol, Lufox, Match, Biosad and Mani recording LC_{50} values of 4.60, 17.13, 18.65, 34.97, 108.24, 143.03 and 286.81ppm, respectively. The toxicity line of the tested insecticides data indicate that Lufox showed the highest slope value of 2.50, while the line of Radiant showed the lowest slope value (1.03).The toxicity index values showed the superior efficiency of Radiant at LC_{50} (100%), followed by Elsan 26.82%, Beticol 24.65 and Lufox 13.14, respectively. While, the lowest percentage of the index toxicity were recorded with Match, Biosad and Mani was (4.25, 3.21 and 1.6%), respectively. Hertlein *et al*. [17] mentioned that Radiant 12% SC based on spinosad have been extensively used as successful agents for control of insect pest species in the Diptera, Lepidoptera, Coleoptera and Hymenoptera orders. The results of the present investigation clearly reveal that the Radiant was effective against the full

Table 1: Toxicity of certain insecticides as surface contact poisons against full grown larvae and 1-day old pupae of *B. zonata* under laboratory conditions

Insecticide	LC_{50} (95% FL)	LC_{90} (95% FL)	Slope ± SE	Toxicity index at LC_{50}
	Full grown larvae			
Beticol	18.65** (13.43-24.12)	213.58 (147.36-365.02)	1.21±0.13	24.65
Biosad	143.03** (76.92-214.20)	756.22 (558.73-2286.44)	1.77±0.16	3.21
Elsan	17.13** (13.63-20.57)	83.37 (64.84-119.24)	1.87±0.20	26.82
Lufox	34.97* (27.57-41.26)	114.03 (92.29-160.00)	2.50±0.36	13.14
Mani	286.81* (224.84-349.09)	1454.09 (1450.52-2862.03)	1.55±0.16	1.60
Match	108.24** (89.65-127.67)	520.83 (415.77-698.32)	1.88±0.16	4.25
Radiant	4.60* (3.31-6.11)	79.82(52.27-141.45)	1.03±0.09	100
	1-day old pupae			
Beticol	307.64** (252.64-365.72)	1855.90 (1402.09-5060.76)	1.64±0.16	23.53
Biosad	4107.04* (1971.34-8145.40)	29565.41	1.50±0.18	1.76
Elsan	72.39** (53.84-95.15)	1453.95 (842.55-3263.00)	0.98±0.11	100
Lufox	214.23	899.32	2.06±0.2	33.79
Mani	612.51	1593.97	3.09±0.28	11.82
Match	196.43** (93.97-295.68)	488.17	3.24±0.27	36.85
Radiant	492.33* (405.58-597.04)	3898.77 (2644.86-6857.66)	1.43±0.15	14.70

Table 1: continue

LC: lethal concentration; FL: fiducial limits

Table 2: Toxicity of certain insecticides applied in sandy soil against full grown larvae and 1-day old pupae of *B. zonata* under laboratory conditions

Insecticide	LC_{50} (95% FL)	LC_{90} (95% FL)	Slope ± SE	Toxicity index at LC_{50}
	Full grown larvae			
Beticol	94.45 (76.44-115.71)**	848.39 (573.13-1488.11)	1.34±0.15	39.56
Biosad	597.16 (493.87-701.79)*	2711.59 (2222.35-3718.69)	1.92±0.18	6.258
Elsan	123.85(52.88-212.43)**	868.51 (633.187-4066.22)	1.52±0.14	30.16
Lufox	37.36 (28.58-47.52)*	525.65 (325.33-1079.82)	1.12±0.13	100
Mani	286.02 (241.19-337.16)**	1863.14 (1410.17-2676.55)	1.58±0.12	13.06
Match	157.74 (124.98-192.42)**	1362.85 (1015.36-2019.22)	1.37±0.12	23.68
Radiant	337.82 (264.62-429.82)**	6042.31 (3534.92-13363.46)	1.02±0.11	11.06
	1-day old pupae			
Beticol	655.90 (511.31-834.27)**	11751.09 (7081.18-24374.23)	1.02±0.01	23.78
Biosad	2914.93 (2189.34-3983.04)**	72029.06 (32352.37-30245)	0.92±0.14	5.35
Elsan	257.26 (192.10-325.55)**	2668.99 (1869.16-4441.06)	1.26±0.13	60.64
Lufox	156.02 (118.92-197.51)**	1496.51 (945.46-2423.48)	1.31±0.15	100
Mani	256.49 (207.88-304.19)**	1199.85 (926.57-1751.68)	1.91±0.22	60.83
Match	498.92 (386.81-615.08)**	3977.55 (2905.37-6186.14)	1.42±0.15	31.27
Radiant	2665.78 (2116.72-3378.95)**	33467.63 (1951.09-78402.15)	1.17±0.14	5.85

Table 2: continue

LC: lethal concentration; FL: fiducial limits

grown larvae of *B. zonata* a fact indicating the possibility of using these biocides as replacement to conventional insecticides or in alternation in IPM programs. Fetoh *et al.* [18] found that both spinosad and proclaim were effective on *B. zonata* and Spinosad was more potent than proclaim.

One-Day Old Pupae: Elsan was the most effective insecticide used against 1-day old pupae as toxicant as shown in Table 1 followed by Match, Lufox, Beticol, Radiant, Mani and Biosad, respectively. The LC_{50} values ranged between 72.39- 4107.04 ppm. LC_{50} values indicate that Match was the most effective compound. The descending order of activity against 1-day old pupae at LC_{90} was Match, Lufox, Mani, Elsan, Beticol, Radiant and Biosad, respectively The toxicity index values showed superior efficiency of Elsan at LC_{50} (100%) followed by Match (36.85%), Lufox (33.79%), Beticol (23.53%), Radiant (14.70%), Mani (11.82%) and Biosad (1.76%), respectively. As for the slope values, Match has highest slope value

(3.24). These results are in agreement with those obtained by Mosallam [19] and Md. Abdur Rashid *et al*. [20], who tested three different insecticides (pyrethroid, organophosphate and biopesticides) against the 3rd instar larvae, of melon fly, *B. cucurbitae* and found that malathion was found to be the most toxic insecticides used.

Sandy Soil Treatment

Full Grown Larvae: The efficacy of the tested insecticides on full grown larvae of *B. zonata* was evaluated by sandy soil method (Table 2) Lufox was the most toxic and Radiant was the least toxic one among the tested insecticides. The LC_{50} value of Lufox and Radiant were 37.36 and 337.82 ppm, respectively. While, LC_{90} values ranged between 525.65 to 6042.31 ppm. As for the slope values, the steep toxicity line of Biosad shows the highest slope value (1.92), while the line of Radiant shows the lowest slope value (1.02). The respective values of LC_{50} and LC_{90} Lufox were the most toxic insecticide among the tested insecticides was 37.36 and 525.65 ppm, respectively. These results are in agreement with those obtained by Mosallam [19], who found that Pyriproxyfen was the most potent compound against full grown larvae of *Ceratitis capitata* in sandy, salty and clay soils. Lufox is mixture of juvenile hormone mimic and chitin synthesis inhibitor. It is ovicidal and larvicidal to several species and effects on adult Diptera and Orthoptera [21-24].

One-Day Old Pupae: The efficacies of the tested insecticides on 1-day old pupae of *B. zonata* were evaluated in Table 2. Lufox with LC_{50} value of 156.02 ppm was the most toxic insecticide among the tested insecticides used. On the other hand, the LC_{50} values for Elsan and Mani were nearly similar (257.26 and 256.49 ppm). On contrast there were more variations with LC_{50} values of the other insecticides (Table 2). The LC_{90} of Lufox 1496.51 ppm was the most potent toxicant against 1-day old pupae of *B. zonata* in sandy soil.. Slope values of the examined insecticides ranged between 0.92 to 1.91. The results of our study confirmed with that of Mosallam [19], who evaluated certain pesticides as soil treatment (sand, silt and clay) under laboratory conditions against pupae of the Mediterranean fruit fly *C. capitata*. Pyriproxyfen was the most potent against the pupal stage of treatment.

Morphogenetic Action of Certain Insecticides Against Some Immature Stages of *Bactrocera zonata* under Laboratory Conditions: Application of all concentrations of the tested insecticides (Beticol, Biosad, Elsan, Lufox, Mani, Match and Radiant) against full grown larvae or 1-day old pupae induced different morphological abnormalities with different scores and illustrated in Fig. 1. Considerable number of larvae, pupae and adults showed obvious malformations after its treatment as surface contact or in sandy soil. Many adults could not emerge completely include adults with wings slightly curled, adults with wings severely curled and malformed legs, adults seemed to be normal with abdomen or leg attached to puparium, partial emergence of adults with head and thorax and partial emergence of adults with head only. Also, intact dead pupae or larval-pupal intermediates and dead larvae.

Surface Contact Treatment

Full Grown Larvae: According to EC_{50} values, Elsan was the most potent toxicant (33.00ppm) followed by Radiant (36.5ppm) and Beticol (52.0ppm) (Table 3). While, the Mani was the least toxic one (LC_{50} 622.0 ppm). These results are in agreement with those obtained by Mosallam [19] and Burgos and Muniz [25]. Also, the teratogenic effects of lead acetate on external morphology of fruit flies (*Bactrocera dorsalis* and *B. zonata*) were observed by Rizwanual *et al.* [26] who stated that the effects of lead abnormalities and malformation were developed in the larvae of flies. Teratomorphic changes were observed as elongated wings, de-shaped wings, elongated and folded legs, change in melanization of larvae, pupae and adults as well as some other structural abnormalities of larval and pupal shape.

One-Day Old Pupae: The scoring system of pupal-adult transformations of *B. zonata* of the tested compounds to 1-day old pupae is shown in Table 3 revealed that Elsan was the most effective compound recording the lowest value of EC_{50} of (58 ppm).

Sandy Soil Treatment

Full Grown Larvae: Data in Table 3 showed that the efficiency of the tested compounds can be categorized in 4 groups, firstly the most efficient compounds (Lufox which recorded EC_{50} of 83ppm). The second group includes Beticol, Elsan and Match that showed moderate EC_{50} values of 265, 238 and 260ppm, respectively.

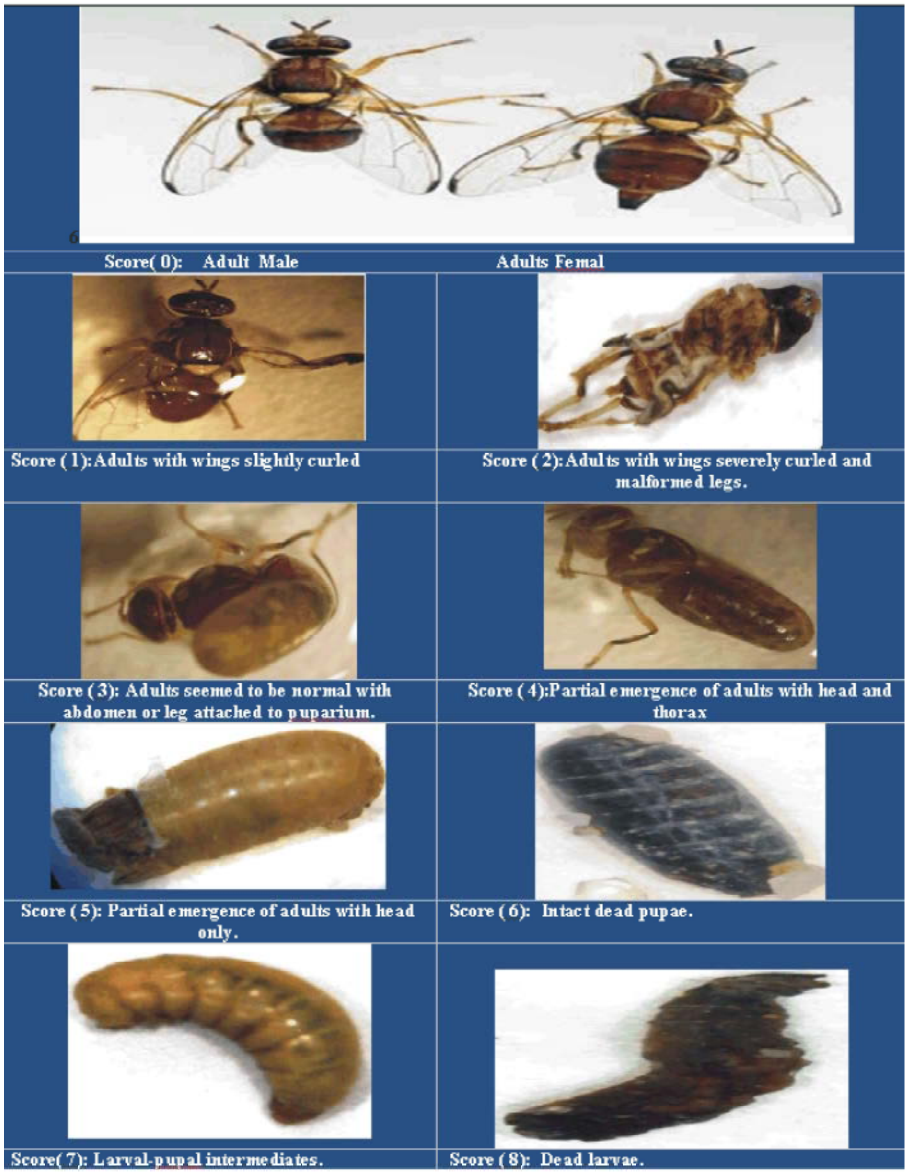

Fig. 1: Illustration of malformations resulted in certain pesticides applied as contact poisons for full grown larvae or pupae of *B. zonata.*

Mani and Radiant (the 3rd group) have the lowest efficiency exhibiting 720 and 765ppm. Finally, Biosad was the worst compound recording the highest values of EC_{50} of 1100ppm taking the fourth order of very low efficiencies scoring system of larval-pupal and pupal-adult transformations resulted in the tested compounds applied in sandy soil against 3rd larval instar of *B. zonata.*

One-Day Old Pupae: Scoring system of pupal-adult transformations of 1-day old pupae are shown in (Table 3). The effectiveness as EC_{50} values of the used insecticides' against 1-day old pupae in sand varied according to the compound and ranged between 113-1660ppm. In general, the tolerance to the tested toxicants in sandy soil increased with the development and transformation from stage to another. The 3rd larval instar was more sensitive than 1-day old pupae in treated sandy soil. These results are in agreement with those obtained by Mosallam [19] who reported that pupae of *C. capitata were* more tolerate to certain pesticides than the third larval instar placed in treated sandy, silty and clay soils.

Table 3: Morphogenetic activity of certain insecticides applied as contact poisons or in sandy soil for full grown larvae or 1-day old pupae of *B. zonata*

	Effective concentration (EC_{50}) ppm.			
	Surface contact		Sandy soil	
Insecticide	Full grown Larvae	1-day old pupae	Full grown Larvae	1-day old pupae
Beticol	52.0	4250	265	500
Biosad	345.0	6000	1100	940
Elsan	33.0	58	238	290
Lufox	457.0	208	83	113
Mani	622.0	770	720	230
Match	177.0	227	260	460
Radiant	36.5	625	765	1660

Table 4: Effect of certain insecticides LC_{50}'s as surface contact application on some biological aspects of *B. zonata* emerged from 1-day old pupae

	Oviposition periods (days)						Longevity (days)	
Insecticide	Pre.	Ovi.	Post.	No. of Egg/Female	% of Hatchability	Sterility %	Male	Female
Beticol	15.00^{BC}	2.67^{C}	0.33^{D}	28.94^{B}	62.72^{AB}	73.21	16.53^{C}	17.88^{CDE}
Biosad	19.33^{B}	2.00^{C}	1.67^{CD}	17.67^{C}	54.18^{B}	85.87	21.68^{BC}	22.67^{BCD}
Elsan	23.00^{A}	25.33^{A}	12.67^{A}	17.97^{C}	85.75^{A}	77.25	73.55^{A}	58.05^{A}
Lufox	13.67^{C}	1.67^{C}	0^{D}	33.81^{B}	64.70^{AB}	67.71	12.18^{C}	15.37^{DE}
Mani	8.67^{D}	2.00^{C}	0^{D}	15.75^{C}	65.20^{AB}	84.84	15.73^{C}	9.65^{E}
Match	22.67^{A}	2.67^{C}	6.33^{B}	17.08^{C}	49.99^{B}	87.40	31.29^{B}	30.96^{B}
Radiant	18.00^{B}	1.33^{C}	4.00B^{C}	18.50^{C}	44.51^{B}	87.85	28.06^{B}	22.54^{BCD}
Control	14.33^{C}	9.33^{B}	4.67^{B}	76.00^{A}	89.14^{A}	0	29.47^{B}	28.00^{BC}
"F" test	**	**	**	**	*	-	**	**

Within the column data with different alphabet superscripts are statistically significant (P<0.05 by LSD).

Table 5: Effect of certain insecticides applied as surface contact on 1-day old pupae of *B. zonata* on acetylcholine esterase, α- esterase and total proteins contents

	AchE (µg/g.b.w.)								
	Pupae			α-esterase (µg/g.b.w.)			Total proteins (mg/g.b.w.)		
Insecticide	2-day	6-day	Adult	2-day	6-day	Adult	2-day	6-day	Adult
Beticol	20.99^{F}	34.23^{D}	36.46^{CD}	429.33^{A}	586.00^{AB}	511.00^{F}	40.84^{C}	45.23^{C}	27.50^{CD}
Biosad	44.69^{A}	37.22B^{C}	38.67^{B}	183.67^{C}	526.67^{C}	823.67^{B}	34.20^{E}	54.04^{A}	30.82^{A}
Elsan	15.14^{H}	14.23^{E}	31.09^{E}	146.33^{D}	345.67^{E}	474.00^{G}	36.90^{D}	50.50^{B}	29.70^{AB}
Lufox	34.54^{D}	37.35^{BC}	26.78^{F}	143.67^{D}	527.67^{C}	665.00^{C}	43.37^{B}	43.44^{C}	25.33^{E}
Mani	18.01^{G}	39.56^{AB}	37.73^{BC}	131.67^{D}	419.33^{D}	623.67^{D}	42.94^{B}	45.03^{C}	26.23^{DE}
Match	27.78^{E}	35.83^{CD}	15.22^{G}	128.00^{D}	615.33^{A}	559.67^{E}	39.42^{C}	49.07^{B}	31.27^{A}
Radiant	38.66^{C}	40.23^{A}	34.99^{D}	220.33^{B}	570.67^{B}	890.33^{A}	49.16^{A}	51.07^{B}	28.64^{BC}
Control	41.90^{B}	38.15^{ABC}	40.55^{A}	195.67^{C}	502.67^{C}	820.33^{B}	48.61^{A}	51.27^{B}	29.80^{AB}
"F" test	**	**	**	**	**	**	**	**	**

Within the column data with different alphabet superscripts are statistically significant (P<0.05 by LSD).

(µg/g.b.w.): µg of peptide per gram of wet weight of the pupae or adult.

(Mg/g.b.w.): mg of peptide per gram of wet weight of the pupae or adult.

Latent Effect of the Tested Insecticides on Certain Biological Aspects of *B. zonata*: Effect of the tested insecticides on certain biological aspects of *B. zonata* treated as 1-day old pupae was determined using the LC_{50}.

Oviposition Periods: Pre-oviposition, ovi-position and post-ovi-position periods, total number of deposited eggs (fecundity) and the percentage of hatchability of deposited eggs by females of *B. zonata* emerged from treated pupae with the tested insecticides in comparison to control were recorded (Table 4).The pre-oviposition period of *B. zonata* was significantly elongated in comparison with the control (P<0.05)by all tested insecticides with the exception of Mani 8.67 days when compared to control 14.33 days. The tested insecticides cause highly significant shortage of the ovi-position periods, except with Elsan which recorded the longest oviposition period 25.33 days of *B. zonata* females, while, the untreated individuals was 9.33 days. Statistical analysis of data in Table 4 shows highly significant differences in post-oviposition period between the tested insecticides. Elsan showed the longest period of 12.67 days, whereas Beticol recorded the shortest period of 0.33 days. Females of *B. zonata* emerged from pupae treated with both Lufox and Mani rapidly died during oviposition period showing 0.00 days of post-oviposition period.

Female Fecundity and Fertility: Data indicated that insecticides application caused significant reduction in the number of deposited eggs per female compared with control (Table 4). The maximum hatchability percentage was 85.75for eggs deposited by females resulted from Elsan and the lowest percentage hatchability for eggs deposited by females recorded with Radiant (44.51%), as compared with that in control 89.14% (Table 4).

Adult Longevity: As shown in Table 4 the females and males longevity were highly significantly affected by Elsan recording 73.55 and 58.05days, respectively. While, no significant difference in males longevity recorded with Biosad, Match and Radiant were 21.68, 31.29 and 28.06 days, respectively when compared with control 29.47days. Also it was shown that shortening in the longevity of females and males resulted from eggs treaded by Mani and Lufox. These periods were 15.73, 9.65 for females and 12.18, 15.37days for males, respectively.

Sterility Percentages: The tested insecticides exhibited high levels of sterility for females emerged from treated pupae (Table 4). The highest levels of sterility were 87.85, 87.40, 85.87 and 84.84%) resulted from females treated with Radiant, Match, Biosad and Mani, respectively. Whereas the lowest level of sterility percentages was 67.71% appeared with Lufox. The other tested insecticides Beticol and Elsan recorded intermediate values of 73.21 and 77.25% sterility. Generally, the conclusion of our results proved that the chitin synthesis inhibitors (Lufox, Mani and Match) which their active ingredient is Lufenuron could be used as insecticides of conventional agrochemicals for peach fruit fly, *B. zonata.* in orchards. The obtained results also, showed moderate effects to Lufenuron which was the most effective insecticide to *B. zonztza* followed by Spinosad (Radiant and Biosad). Similar conclusion was obtained by Mosallam [19], who studied the latent effect of sub-lethal doses of certain pesticides treated as topical application against 1-day old pupae of *Ceratitis capitata*. Results indicated that the pre-oviposition period was shortened due to treatment with all pesticides except fenamiphos which increased it comparing to control individuals, also increased the oviposition period of *C. capitata* except pirimiphos-methyl and found that the post-oviposition period was shortened with all tested pesticides. Budia and Vinuela [27] and Casana-Giner *et al.* [28] reported that the phenyl-benzoyl ureas with fluorinated alkoxy substituents in the phenyl group showed the highest activity as hatching inhibitors. Lufenuron at 1000 ppm concentration given to adult females (virgin or mated) for 3 h produced a total suppression of egg hatch. Also, Bachrouch *et al*. [29] reported that the Lufenuron bait, station technique could be involved as an appropriate strategy for the control of the Medfly in Tunisia. However, Lufenuron acts to stop eggs hatching and not to stop female flies from stinging fruits, it is possible that the larval population in the fruits decrease. Also, in Spain using the insect growth regulator (Lufenuron) under two application methods spraying and hanging traps showed a high reduction of Medfly population [30]. Casana-Giner *et al.* [28] tested the effect of certain insect growth regulators as chemosterilizing agents against *C. capitata*. The IGRs were administered to adult flies mixed with their food. The highest activity of sterilizing activity was observed in females that copulated with males treated with lufenuron in the food at 5000 ppm for 3h and Triflumuron caused a total suppression of egg hatch when administered to females at 10 000 ppm for 3 days. The degree of sterility differed according to the tested toxicant. Furthermore, Castillo *et al.* [30] showed that the Lufenuron used as chemosterilizing agent at a dose of 1,000 ppm against *C. capitata* lead to an important sterilizing activity as hatching inhibitors.

Effect of Tested Insecticides on Some Biochemical Responses of *Bactrocera zonata*

α-Esterase Enzyme: Data in Table 5 show that in all treatments of the 1-day old pupae of *B. zonata* with LC_{50} of tested insecticides caused a reduction in the level of α-esterase activity from 15.14 to 38.66 µg/g.b.w than that obtained with Biosad and control in case of 2-day pupae, Regard to the respecting pupae of 6-day, α- esterase level show significant difference between control and both of Beticol and Elsan. The level of α-esterase activity was 38.15, 34.23 and 14.23 µg/g.b.w, respectively. Also, data presented in Table 5 indicate that the activity of α-esterase enzyme in the adult of *B. zonata* was generally increased in the control compared to tested insecticides; it was 40.55 µg/g.b.w. The statistical analysis shows that a significant difference between control and all tested insecticides. Fetoh *et al*. [18] found that the toxicity results of the peach fruit fly were correlated with some biochemical parameters such as electrophoretic patterns of some isozymes. Isozymes electrophoresis showed various changes as a result of utilization of spinosad than proclaim on *B. zonata*. Alpha-, beta-esterase and acid phosphatase showed 20, 17 and 20% polymorphism compared to control, respectively.

AChE Enzyme: Data tabulated in Table 5 show that the activity of AChE enzyme in the 2-day pupae of *B. zonata* was generally increased in Beticol and Radiant compared to other insecticides and control. It was 429.33 and 220.33 µg/g.b.w than that obtained with control 195.67 µg/g.b.w, respectively. The activity of AChE enzyme tended to give the highest increase in 6-day pupae of *B. zonata* as affected by all insecticides with the exception of Elsan and Mani that give lowest AChE activity were (345.67 and 419.33µg/g.b.w). The statistical analysis shows that a significant difference between Match and other insecticides in 6-day pupae, while no significant difference between control and Biosad or Lufox. Also, results in Table 5 show a remarkable significant increase in the AChE enzyme activity in *B. zonata* adult using Radiant (890.33 µg/g.b.w) compared to other insecticides. Magana *et al.* [31, 32] reported that acetylcholine esterase activity was less inhibited *in vivo* by Malathion *in vitro* by malaxon in adult flies of *Ceratities captata*. Mosleh *et al.* [33] reported that the activities of acetylcholine esterase of treated peach fruit fly *B. zonata* with Malathion, Diazinon, Methoxyfenozoide and Lufenuron decreased compared to untreated adults.

Total Proteins: Regarding to the level of total proteins, there were decrease in the activity in *B. zonata* pupae resulted from all insecticides during all tested periods as compared to control (Table 5). The level of total proteins tend to give the highest decrease at 2-day pupae as affected by all insecticides with the exception of both Radiant and control were (49.16 and 48.61) respectively. Whereas, the reduction in the level of total proteins in 6-day pupae ranged between a minimum value of 43.44mg/g.b.w. for Lufox to maximum value of 54.04 mg/g.b.w. for Biosad. The level of total proteins in newly emerged adults of *B. zonata* shows that a highly significant difference according to treatments and ranged between 25.33 to 31.27 mg/g.b.w.. Generally, total proteins amounts differently increased with developing of pupae, but they sharply or drastically reduced in case of newly emerged adults. Abdel-Hafeez [34] revealed that gamma irradiation has unobvious effects on both total proteins and total carbohydrates in *B. zonata*, i.e. there were no clear relation between dose and the total content of carbohydrates or total proteins contents this is due to the differences in both proteins and carbohydrates uptake by different individuals.

CONCLUSIONS

Radiant was the most contact poison on full grown larvae, among the tested insecticides.While, Elsan was the most potent toxicant insecticide in sandy soil treatments (pre-oviposition, oviposition and post-oviposition periods) were highly significantly different. The tested insecticides reduced fecundity (amounts of eggs/female) and hatchability percentage. Also, these insecticides caused high levels of sterility for adult females emerged from treated pupae. So, it can be used as chemo sterilizing agents against *B. zonata.* Results indicated that Biosad, Radiant, Beticol, Lufox and Mani decreased adults longevity and induced significant differences in enzymes and total protein content appeared after treatment with the tested insecticides compared with control.

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