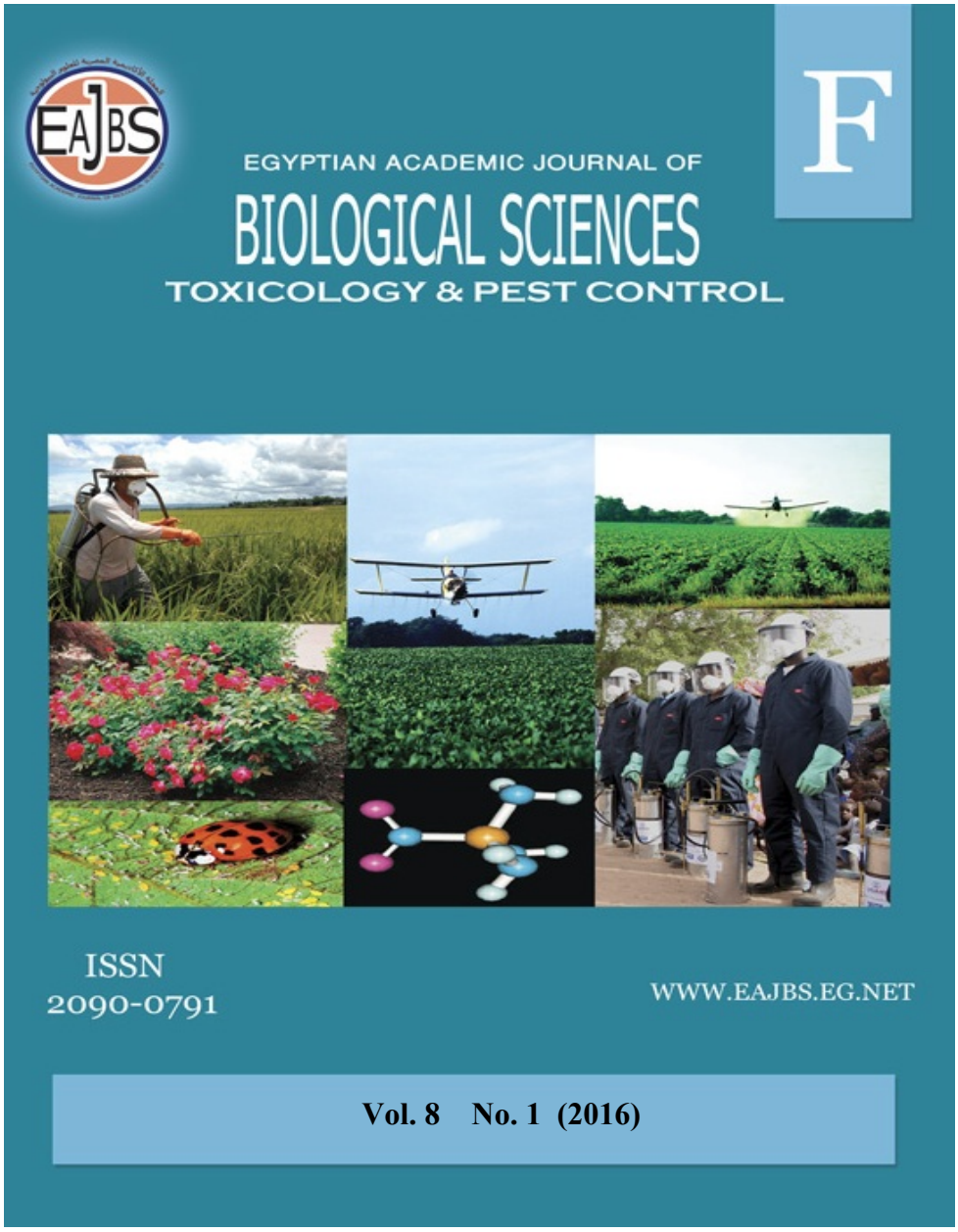

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Toxicological and Biochemical Effects of Malathion and Spinosad on the Peach Fruit Fly, *Bacterorcera zonata* (saunders)

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ABSTRACT

Toxicity of Malathion and Spinosad to pupal and adult (male and female) stages of the peach fruit fly, *Bacterorcera zonata* (Saunders) was investigated in laboratory. The adult stage was more susceptible than pupal stage and adult females were more tolerant than males to effect the two insecticides. Malathion and Spinosad were more effective by using residual thin film(LC_{50}=4.28 & 4.51 and 1.14 & 2.50 ppm for males & females, respectively) than feeding technique (LC_{50} = 6.40 & 6.49 and 2.83 & 4.13 ppm for males & females, respectively). The results revealed that the resistance ratio of Spinosad (10.0 &10.9 folds for female & male) in field population was higher than Malathion (5.4 & 4.8 folds for female & male) compared with lab insects. The treatment of lab insects with LC_{50} of Malathion and Spinosad for five generations produced 16 and 4 folds of resistance ratio. Malathion and Spinosad caused a significant depletion of total protein contents in the whole body tissues of treated and resistant insects. Significant increase in activity of Glutathione-S-Transferase and Acetylch-olinesterase enzymes was detected in treated male & female adults.

INTRODUCTION

Family Tephritidae is a group of polyphagous flies which contain some of the most damaging fruit pests in the world. The peach fruit fly (PFF), *Bactrocera zonata* (Saunders) is one of the most destructive tephritid pests which spread in several regions of the world (Drew, 1989). It attacks a large host range of fruit, such as mango, peach, fig, guava, citrus, and apple (White and Elson-Harris, 1994). *B. zonata* being established in Egypt was in Qalubia and Faiuom governorates in 1993 from guava (*Psidium guajava*) samples.

In 1995, the insect was found in further fruit-producing governorates and it was putted throughout Egypt including the Dakhla and Kharga oases and Sinai In 1997 (El-Minshawy *et al.,* 1999 and Hashem *et al.,* 2001).

Control of *B. zonata* is based primarily on applications of organophosphrous insecticides, especially malathion. The mode of action of Malathion is anticholinesterase enzyme (Sharma *et al.,* 2005). This insecticide is the most commonly used in aerial, ground treatments and mixed with protein baits. The intense and repeated applications of Malathion led to development of fly resistance and harmful effects on beneficial insects (Daane *et al.,* 1990 and Urbaneja *et al.,* 2004). Spinosad is a natural compound with insecticidal activity , it is a mixture of two macrocyclic lactones (spinosyns A and D) derived from metabolites of the actinomycete bacterium *Saccharopolyspora spinosa* (Mertz and Yao) (Sparks *et al.,*1998 and Thompson *et al.,* 2000). Spinosad was classified as an environmentally and toxicologically reduced-risk insecticide because it have limited impact on non target organisms that may be exposed to it (Cleveland *et al.,* 2001 and BCPC, 2006). Spinosad excite neurons in the central nervous system, causing involuntary muscle contractions that lead to paralysis from neuromuscular fatigue (Salgado 1998). It has high effect on a wide range of pest species. At present, bait treatment containing Spinosad and a mix of sugars, water and attractants, is successfully being used to control different tephritid pests' worldwide (Bruns *et al.,* 2001).

This study aim to evaluate the potency of Malathion and Spinosad against pupae and adults of peach fruit fly. Also, the total protein content and activity of Glutathion S Transderase and Acetcholinesterase enzymes of lab, field, treated and resistant insects were detected.

MATERIALS AND METHODS

Insect culture

Eggs of the Laboratory peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) were obtained from Plant Protection Research Institute and kept in the Central Agricultural Pesticides Laboratory, Agricultural Research Center for several generations without exposure to any insecticide under conditions of (25±2°C, 60±5% R.H and photoperiod of 14 L: 10 D). The eggs were scattered on surface of the artificial diet according to (Tanaka *et al.,* 1969) and was modified by (Shehata *et al.,* 2006) which was placed in plastic trays of (20 x 10 x 8cm) until larval pupation. Pupae were separated and kept in cages until emergence of flies. Adult Flies were fed on sugar and fortified protein hydrolyzate.

Field insects were collected from infested Mango fruits of Giza Governorate in July 2014 and kept under laboratory conditions for two generations to increase the number of insects.

Insecticides used

Two pesticides were used:

1-Malathion: (Organophosphorous insecticide), Trade name: malason. Formulation tested: 57% E.C, Produced by : Ficom Organics.

2-Spinosad: (Bio-insecticide), Trade name: Spintor, Formulation tested: 24% Produced by : Dow Agro Sciences.

Toxicological studies:

The insecticidal activity of two pesticides was tested against pupae and adults of *B. zonata*.

Pupicidal activity:

Dipping method was used to evaluate the toxicity of Malathion and Spinosad against lab *B.zonata* pupae. A piece of muslin containing 20 pupae (3-days old) was dipped in each concentration of Malathion (44.53, 89.06, 178.13, 356.26 and 712.52 ppm) and

Spinosad (60, 120, 240, 480 and 960 ppm) for 60 seconds. The untreated pupae were dipped in water only (five replicates for each pesticide concentration and control). After dryness, the pupae were incubated at laboratory constant conditions. Daily inspections for pupal mortality and adult emergence were carried out.

Adulticidal activity

The toxic effect of Malathion and Spinosad on adult flies of *B. zonata* tested by using two methods of treatment (residual thin film and feeding).

The method of residual thin film (Plapp *et al*., 1987) was applied on lab male and female flies(5-days old) by dipping 100 ml (9Dx15H) glass scintillation vial in different concentrations of Malathion (1.43, 2.86 , 5.72, 7.13 and 11.44 ppm) and Spinosad (0.6, 1.2 , 2.4, 4.8 and 6 ppm), but the control vials were dipped in water. All vials were left to dry in the air, then 5 male or female flies) were placed in each vial (five replicates for each treated and control vials) for 24 hours.

Toxicity experiments by feeding method on lab and field flies (5-days old) were conducted in small and clean glass jars, each jar received ten male or female flies that were confined separately without food for 6 hours. The top of each jar were covered with muslin, held in place by means of rubber bands. Flies were fed on treated sugar solution which prepared by mixing 2 ml of sugar solution (5%) with 2 ml of each concentration of Malathion (3.57, 7.13, 14.25, 28.5, and 57 ppm for lab and 35.62, 71.25, 142.5, 285 and 570 ppm for field) and Spinosad (2.4, 4.8, 6, 12 and 24 ppm for lab and 30, 60, 120, 240 and 480 ppm for field). The treated sugar solution was added to a piece of cotton placed on a small feeding try and inserted in each experimental cage for 24hours. Control male or female flies were fed on sugar solution only (five replicates for each pesticide concentration and control).

Mortality percentages were recorded and corrected using Abbott's formula (Abbott, 1925). The sub-lethal concentration (LC_{50}) and slope value of each insecticide toxicity line were determined according to SAS (1997). The resistance ratio of field *B. zonata* flies to Malathion and Spinosad was determined as:

R.R = LC_{50} of the field flies / LC_{50} of the laboratory flies (Fold).

Development of resistance in lab *B. zonata* strain:

The development of resistance in lab strain of the peach fruit fly against Malathion and Spinosad carried out by using the residual thin film method. The adult flies were selected for five generations with LC_{50} of Malathion and Spinosad and resistance ratio of the fifth generation was determined as:

R.R = LC_{50} of the fifth generation flies / LC_{50} of the parent flies (Fold)

Biochemical studies

Preparation of samples:

One gram from the untreated (lab), field, LC_{50} treatment and resistant to Malathion and Spinosad peach fruit fly, *B. zonata* adults (female and male) were homogenized in 3ml of sodium phosphate buffer (pH=7) by using a manual Teflon glass homogenizer surrounded by jacket of crushed ice. Centrifugation was carried out by a cooler centrifuge (Mikro 22 R Hettich Zentrifugen- Germany) for 15 min at 6000 rpm / min at 4^{o}C then supernatant was transferred to new tubes and frozen at (-20^{o}C) for biochemical analyses. The total protein content and activity of Glutathione-S-Transferase (GST) and Acetylcholinesterase (AChE) enzymes was determined by Unico UV 2100 Spectrophotometer (U.S.A.).

The total protein content:

The total protein content of all insect samples was determined based on Biuret test (Henry, 1964), using Kit purchase from dp international laboratory. The reaction mixture consists

of 1.0 ml of the total protein reagent [Sodium hydroxide 0.2 N, Sodium, Potassium tartrate (18 mM), Potassium iodide (12 mM) and Cupric sulfate (6 mM)] and 20 µl of sample or deionized water (for blank) or standard protein (for standard). After 5 minutes of incubation at 25°C, the absorbance (A) of samples and standard were recorded at 546 nm compared with blank (five replicates for each sample). The total protein content of samples expressed as mg protein /gm body weight.

Glutathione-S-Transferase activity

The activity of Glutathione-S-Transferase (GST) in all insect samples was determined according to the method of (Habig *et al*., 1974). The procedure depend on incubation of 50mM from the substrate; CDNB (1-chloro-2,4-dinitrobenzen) with 50mM GSH (reduced glutathione) and 50µl of sample in 0.1M phosphate buffer (pH7) for 5min. at 27°C (five replicates for each sample). The activity monitored at 340 nm and expressed as nmoles of CDNB conjugated /mg protein^{-1} /min^{-1}

Acetylcholinesterase activity

Acetylcholinesterase (AChE) activity of all insect samples was determined according to Simpson *et al*., (1964). A mixture of 200µl of sample, 500µl phosphate buffer and 500µl of acetylcholine bromide (substrate) was incubated for 30 min at 37°C. After incubation; 1.0 ml alkaline hydroxylamine solution was added (shaking and leaved for 2 min) , 500µl of HCl solution were added (shaking and leaved for 2 min) and 500µl of ferric chloride solution were added to the mixture (mixed well and filtrated). The activity of AChE was measured at 515 nm and expressed as µg of acetylcholine bromide (AChBr) hydrolyzed / mg protein^{-1} /min^{-1}.

Data analysis

Enzyme activities were expressed as mean ± standard error (S.E.) and statistically analyzed by using SPSS program V.13. Differences were considered significant at $P < 0.05$ level.

RESULTS

Toxicological studies:

Pupicidal activity

Susceptibility of 3-days old pupae of the peach fruit fly, *B. zonata* to Malathion and Spinosad was shown in Table 1. Data indicated that the two pesticides had moderate insecticidal activity on pupae and less harmful effect on adult emergence. Also, the data revealed that Spinosad was more potent than Malathion against pupae of the peach fruit fly. The LC_{50} values were 258.30 and 397.80 ppm for Spinosad and Malathion. The slope value (≤ 1-2) revealed the homogenous response of *lab B. zonata* population to the two tested pesticides.

Table 1:Susceptibility of the peach fruit fly *B. zonata* pupae (3-days old) to Malathion and Spinosad

Insecticide	LC_{50} (ppm)	CI (95%)	(Slope ± SE)
Malathion	397.80	218.80-361.80	1.07 ± 0.33
Spinosad	258.30	148.55-897.05	1.03 ± 0.22

CI = Confidence interval LC_{50} = Lethal concentration.

Adulticidal activity

The toxic effect of Malathion and Spinosad on lab peach fruit fly, *B. zonata* adults (5-days old) by using residual thin film and feeding methods was shown in Table 2. The residual thin film treatment caused high percentages of fly's mortality. The LC_{50} values were 1.14 and 4.28 (for males) and 2.50 and 4.51 ppm (for females) of Spinosad and Malathion, respectively. These values were increased to 2.83 & 4.13 and 6.40 & 6.49 ppm from Spinosad and Malathion to male and female adults, respectively, by feeding method. These results revealed that the residual thin film technique was

more effective than feeding method. In addition male flies were more susceptible to the effect of Malathion and Spinosad than female flies and Spinosad proved more effective against lab flies than Malathion.

Table 2: Susceptibility of lab peach fruit fly *B. zonata* adults to Malathion and Spinosad with residual thin film and feeding methods

Adult fly	Insecticide	Residual thin film method			feeding method		
		LC_{50} (ppm)	CI (95%)	(Slope ± SE)	LC_{50} (ppm)	CI (95%)	(Slope±SE)
Female	Malathion	4.51	3.6-6.02	1.28±0.24	6.49	5.33-7.64	1.55 ± 0.15
	Spinosad	2.50	1.85-3.71	0.96±0.18	4.13	3.47-5.04	1.43 ± 0.12
Male	Malathion	4.28	3.31-5.91	1.13±0.24	6.40	5.25-7.96	1.32 ± 0.15
	Spinosad	1.14	0.62-1.66	0.76±0.17	2.83	2.00-4.24	1.49 ± 0.11

CI = Confidence interval LC_{50} = Lethal concentration.

Data in Table 3 show the effect of Malathion and Spinosad on field *B. zonata* male and female flies by feeding method. These data indicated that the LC_{50} values were 30.80 & 41.21 and 30.87 & 35.29 ppm from Spinosad and Malathion to male and female flies, respectively. The male flies were more susceptible to the effect of two pesticides than females and Malathion was more effective than Spinosad against field flies. The aforementioned results revealed that the values of LC_{50} of two pesticides against field insects were very high in comparing with those of lab flies by feeding method. This comparison produced the resistance ratio of field female and male flies (5.4 & 4.8 and 10.0 & 10.9 fold) to Malathion and Spinosad, respectively. The results revealed that the resistance ratio of Spinosad in field flies was higher than that of Malathion.

Table 3: Insecticidal effect of Malathion and Spinosad against field peach fruit fly *B. zonata* adult flies

Adult fly	Insecticide	LC_{50} (ppm)	CI (95%)	(Slope ± SE)	Resistance ratio (RR)
Female	Malathion	35.29	21.61-47.53	1.34 ± 0.21	5.4
	Spinosad	41.21	28.23-55.82	0.86 ± 0.14	10.0
Male	Malathion	30.87	21.37-39.27	1.94 ± 0.26	4.8
	Spinosad	30.80	18.99-42.96	0.82 ± 0.14	10.9

CI = Confidence interval LC_{50} = Lethal concentration

Development of resistance in lab *B. zonata*

The level of Malathion and Spinosad resistance in lab peach fruit fly, *B. zonata* adult flies through residual thin film was examined by comparing LC_{50} values of fifth generation with those of parent (Lab strain). The resistance ratio reached to 16 and 4-folds for Malathion and Spinosad, respectively.

Biochemical studies

Effect of Malathion and Spinosad on the total protein content and activity of Glutathione-S-Ttransferase (GST) and Acetylcholinesterase (AChE) enzymes of body tissues of the peach fruit fly, *B. zonata* adults were detected.

Total protein content:

Data in Table 4 show the total protein content in the whole body tissues of untreated, treated, resistant and field peach fruit fly, *B. zonata* flies (male and female). This content reached to 183.63 and 200.15 mg/gm body weight of untreated female and male insects. It is clear that the male flies had high protein content (8.25%) than females. This concentration reached to 102.9 & 118.5 and 86.55 & 56.45 mg/gm body weight for treated female and male with LC_{50} of Malathion and Spinosad, respectively. In

resistant insects to Malathion and Spinosad, the protein content reaches to 59.55 & 64.6 and 144.2 & 192.95 mg/gm body weight for female and male, respectively. While, this concentration reached to 99.45 and 83.7 mg/gm body weight for field female and male.

The results indicated to presence of reduction in the protein content of all tested insects compared with control ones. The high significant decrease in total protein content (67.57 and 64.82%) of female was detected in resistant insects to Malathion and Spinosad. Significant decrease in protein contents was detected in field and treated females with Malathion and Spinosad (45.85, 43.97 and 35.48%), respectively. On the other hand, the high significant decrease in protein content of male was recorded in Spinosad and Malathion treated and field insects (71.79, 56.76 and 58.18%), respectively. Moderate decrease (27.95 %) in protein content presented in Malathion resistant males and a slight reduction (3.59%) in proteins observed in Spinosad resistant males.

Table 4: The total protein content of the peach fruit fly, *B. zonata* adul Conc. of protein Mean ± S.E. (mg//gm body weight)

Insect sample	Female		Male	
	Mean ± S.E	**% of change**	**Mean ± S.E**	**% of change**
Untreated (C)	183.65±21.00	0.0	200.15±44.34	0.0
Malathion (M)	102.9±00.00	(-) 43.97	86.55±10.68	(-) 56.76
Spinosad (SP)	118.5±12.02	(-) 35.48	56.45±4.03	(-) 71.79
Malathion resistance(MR)	59.55±9.40	(-) 67.57	144.20±29.13	(-) 27.95
Spinosad resistance (SPR)	64.60 ±1.56	(-) 64.82	192.95±3.46	(-) 3.59
Field (F)	99.45±8.98	(-) 45.85	83.70±1.41	(-) 58.18
	LSD = 27.486		LSD =54.336	

(+) increase (-) decrease LSD = Least Significant Difference

Glutathione-S-Transferase activity (GST):

The activity of Glutathione-S-Transferase in the whole body tissues of untreated, treated, resistant and field peach fruit fly, *B. zonata* flies (male and female) was shown in Table 5. The GST enzyme activity reached to 0.014 and 0.008 µmol conjugate CDNB /mg protein/min in untreated lab female and male, respectively. This result revealed that the female flies had a highly enzyme activity (42.86%) than males of lab strain. The enzyme activity reached to 0.006, 0.032, 0.009, 0.019 and 0.014 µmol conjugate CDNB /mg protein/min in field and treated & resistant female flies to Malathion and Spinosad, respectively. This value reached to 0.014, 0.045, 0.027, 0.009 and 0.008 µmol conjugate CDNB /mg protein/min in field and treated & resistant male flies to Malathion and Spinosad, respectively.

The activity of GST enzyme was varied in all insect samples compared with untreated one except Spinosad resistant male and female flies. The results revealed that the very high significant increase in enzyme activity (462.50 and 237.50%) was detected in Malathion and Spinosad treated males and Malathion treated females (128.60%). However, a high significant increase (75%) in enzyme activity was presented in field males and Moderate increase (35.71%) observed in Malathion resistant females. The lower increase (12.5%) in this activity was detected in Malathion resistant males. The moderate reduction in GST activity observed in field and Spinosad treated females (57.14 and 35.71%), respectively.

Table 5: Activity of Glutathione-S-Transferase in the Peach fruit fly, *B. zonata* adults

Activity of GST Mean ± S.E. (µmol conjugate CDNB / mg protein^{-1} /min^{-1})				
Insect sample	**Female**		**Male**	
	Mean ± S.E	**% of change**	**Mean ± S.E**	**% of change**
Untreated (C)	0.014± 0.001	0.0	0.008± 0.000	0.0
Malathion (M)	0.032± 0.000	(+) 128.6	0.045± 0.001	(+) 462.50
Spinosad (SP)	0.009± 0.010	(-) 35.71	0.027± 0.000	(+) 237.50
Malathion resistance(RM)	0.019± 0.000	(+) 35.71	0.009± 0.001	(+) 12.50
Spinosad resistance (RSP)	0.014± 0.001	0.0	0.008± 0.000	0.0
Field (F)	0.006± 0.001	(-) 57.14	0.014± 0.000	(+) 75.0
	LSD= 0.0087		LSD= 0.001	

(+) increase (-) decrease. LSD = Least Significant Difference

Acetylcholinesterase activity (AChE):

Table 6 illustrated Acetylcholinesterase (AChE) activity in the whole body tissues of untreated, treated, resistant and field peach fruit fly, *B. zonata* flies (male and female). The activity of AChE of untreated adult females and males were 0.042 and 0.112 µmol AChBr hydrolyzed/mg protein/min. Also, the female flies had a high activity (62.5%) of enzyme than males. The enzyme activity reached to 0.423, 0.245, 0.172, 0.570 and 0.298 µmol ACh Br. hydrolyzed /mg protein/min in field and treated & resistant female flies to Malathion and Spinosad, respectively. This value reached to 0.446, 0.251, 0.233, 0.108 and 0.047 µmol AChBr hydrolyzed /mg protein/min in Spinosad treated, Malathion resistant and treated & to Malathion, Spinosad resistant and field male flies, respectively.

Table 6:Activity of acetyl cholinesterase (AChE) in the Peach fruit fly, *B. zonata* adults

Activity of Acetylcholinesterase Mean ± S.E. (µg ACh Br/ mg protein^{-1} /min^{-1})				
Insect sample	**Female**		**Male**	
	Mean ± S.E	**% of change**	**Mean ± S.E**	**% of change**
Untreated (C)	0.042 ± 0.018	0.0	0.112± 0.121	0.0
Malathion (M)	0.245 ± 0.030	(+) 483.33	0.233 ± 0.002	(+) 108.04
Spinosad (SP)	0.172 ±0.002	(+) 309.52	0.446 ± 0.030	(+) 298.21
Malathion resistance (RM)	0.570 ± 0.050	(+) 1257.14	0.251 ± 0.030	(+) 124.11
Spinosad resistance (RSP)	0.298 ± 0.013	(+) 609.52	0.108 ± 0.060	(-) 3.57
Field (F)	0.423 ± 0.220	(+) 907.14	0.047 ±0.030	(-) 58.04
	LSD = 0.2312		LSD = 0.1433	

(+) increase (-) decrease. LSD = Least Significant Difference

The observed data revealed that the activity of this enzyme was highly significant increased in female flies of Malathion resistant, field, Spinosad resistant and Malathion & Spinosad treated (1257.14, 907.14, 609.52 and 483.33 & 309.52%, resp.) than the untreated insects. This increase in enzyme activity was detected also, in Spinosad treated (298.21%), Malathion resistant (124.11%) and Malathion treated males (108.04%). The highly significant reduction in enzyme was detected in field males (58.04), while a slight decrease was presented in Spinosad resistant male (3.57%).

DISCUSSION

Toxicological studies:

The presented data proved a low insecticidal activity of Malathion and Spinosad against 3-days old pupae of the peach fruit fly, *B. zonata*. These results are in agreement with those obtained by Mosallam, (1993) who reported that pupae of *C. capitata* were more tolerant to certain pesticides than the third larval instar placed in treated sandy, silty and

clay soils. The scoring system of pupal-adult transformations of *B. zonata* of the tested compounds to 3-day old pupae, revealed a satisfactory pupicidal activity of Spinosad than Malathion. Halawa *et al.,* (2013) studied the effect of certain insectidies belonging to different chemical groups on 1-day old pupae of *B. zonata* with different concentrations as contact poisons or in sandy soil treatments under laboratory conditions. The result showed considerable number of pupae and adults with obvious malformations after trearments as surface contact or in sandy soil. In general, the tolerance to toxicants increased with the development and transformation from stage to another.

The results indicated to a higher adulticidal activity of Spinosad compared with Malathion in lab and field strains in different methods of application. This result support the finding of Xin-Geng and Russell, (2006) who mentioned that the Spinosad-based fruit fly bait, has recently become a primary tool for area wide suppression or eradication of tephritid fruit flies pests. The obtained results concluded that female adults of *B. zonata* are less sensitive to Spinosad and Malathion than those of male ones. Such results are in agreement with that of El-Aw *et al.,* (2008) who reported that Spinosad was more efficient than Malathion in controlling *C. capitata* and *B. zonata* . They observed that LC_{50} values of all tested compounds were higher in the case of female than in male adults. The results indicated that LC_{50} values of Spinosad are 20.1 and 16.0 ppm for males and 27.0 and 19.1 for females of *B. zonata* at 24 and 48hrs posttreatment. Also, our results are consistent with the result of Stavridis *et al.,* (2013) that the adult females of *B. oleae* more tolerant to the tested insecticides than the adult males. This may be due to increased female resistance, since females generally live more than males in nature and are exposed for a longer period to pesticides. In such conditions resistant (mutant) alleles may be over expressed as those described by Hawkes *et al.,* (2005).

In addition, our results regarding that the flies when were forced to be in contact with the pesticide treated surface, there was still required lower dosages of Malathion and Spinosad to achieve LC_{50} values relative to the feeding application method. Maklakov *et al.,* (2001) reported that the forced contact experiments, showed low mortality comparin to oral administration of Malathion to *D. ciliatus* but in case of all tested pyrethroid the forced contact technique had higher mortality rate than feeding method. Hence, we concluded that the relatively low mortality rate of flies in the feeding experiments could not be explained by poor action of the pesticide but by the lack of good contact with the insecticides as a result of repellency.

The results of toxicological bioassay by feeding method on field peach fruit fly, *B. zonata* showed that these insects had higher level of resistance to Spinosad than Malathion when compared with lab insects This result confirm to do with Sato, (2005) who mentioned that Malathion-bait sprays, which was introduced for the control of fruit flies in 1960 and continue being used today, has been the most successful and widely used insecticide for the control of these pests throughout the world. Kakani *et al.,* (2010) mentioned that the widely use of Spinosad as an alternative to organophosphorus for control the tephritid species produced high levels of tolerance to it in field strain of peach fruit fly. This result was agree with Hsu *et al.,* (2012) who reported that LC_{50} of Malathion and Spinosad in field strain of melon fly more than LC_{50} of susceptible strain. Available evidence concluded that in insect resistance management (IRM) programs, the rotation of insecticides with different modes of action and incorporation of non

insecticide management practices is a desirable to avoid the development of further Spinosad resistance in the peach fruit fly.

The results showed development of resistance over relatively short periods of time. Laboratory selection of *B. zonata* with Malathion and Spinosad carried out by continuous exposure to LC_{50} for five generations by residual thin film. The results recorded that a sixteen-fold and four-fold increased level of resistance can develop in *B. zonata* after only five generations of selection. In the past studies have shown that many Ops insecticides including Malathion resulted in the development of resistance in many insect species (Li *et al.,* 2005). The results indicate that it is likely that peach fruit flies with resistance to Malathion also have higher potential to develop resistance to Spinosad. Resistance to insecticides in fruit flies has been attributed to selection pressure, fruit flies experience during life time. Furthermore, laboratory studies have shown that selection for Spinosad resistance can also be quite effective in species such as *B. dorsalis*, but it has been shown that up to 400-fold increased levels of resistance can develop after only eight generations of selection by topical application (Hsu and Feng, 2006).

Biochemical Studies

Colorimetric determination of the total protein contents in the body of *B. zonata* adults during all tested periods regarded significant reduction as compared to untreated, especially treated male with Spinosad followed by Malathion resistant female. The decrease in the protein content in treated adults might be due to inhibition of DNA and RNA synthesis and thus might affect the protein synthesis (Deloach *et al.*, 1981). Nath *et al.,* (1997) mentioned that protein depletion in tissues may constitute a physiological mechanism and might play a role in compensatory mechanisms under insecticidal stress to provide intermediates to the Krebs cycle by retaining free amino acid content in insect tissues. The results disagree with Zidan *et al.*, (2012) who mentioned that proteins are among most important compound of insects that bind with foreign compounds, the increase in the total protein of treated insects may reflect the increase in the activity of various enzymes related to insecticides.

The level of total proteins of adult female bodies tend to give the highest decrease at Malathion & Spinosad resistant and field flies, whereas, the moderate decrease showed in LC_{50} treatment of Malathion & Spinosad compared to untreated. The depletion of male fly body proteins was detected in Malathion & Spinosad and field but moderate decrease showed in Malathion & Spinosad resistant insects. The decreased levels of total protein in resistant strain revealed its possible utilization in energy production (Hussain *et al.,* 2009).

The obtained results revealed that GST activity showed highly significant increase in males treated with Malathion and Spinosad and females of Malathion treatment, while, field males and Malathion resistant females showed moderate increase but Malathion resistant males had lower increase. Our finding in harmony with Yu (1996) who noticed that in insects GST have been induced and is becoming recognized for their importance in the metabolic detoxication of insecticides. Yaqoob *et al.,* (2013) investigate the effect of Malathion, Trichlorofon, and λ-cyhalothrin on GST activity in insecticide resistance in *B. zonata*. The result indicated significantly different in GST among different group of male flies. Also, results showed that GST activity was higher in Malathion and λ-cyhalothrin treated flies, while Trichlorofon treated flies did not differ significantly from control and Malathion exposed flies. The activities of GST activity among female flies also vary

significantly. Activities were higher in Malathion and λ-cyhalothrin exposed flies than control group, respectively, while Trichlorofon treated flies did not differ from control and λ-cyhalothrin treated flies.

GST showed moderate reduction in activity of Spinosad treated females and field females. Our findings, in harmony with Vontas *et al.,* (2001) who reported that the GST activity showed significantly lower in the field population of *B. oleae*. On the other hand, the results showed no effect on GST activity in Spinosad resistant females and males. This observation agree with Hsu *et al.,* (2004) who reported that there was none significant difference in GST activity between resistant and susceptible strains in *B. dorsalis*.

The results showed that activity of AChE was highly significant increased in Malathion resistant, field, Spinosad resistant, Malathion and Spinosad treatment females, moderate increase in Spinosad, Malathion resistant and Malathion males. Acetylcholinesterase is the primary target of organophophorus insecticides (Charpentier *et al.,* 2000). Yaqoob *et al.,* (2013) investigate the effect of Malathion, Trichlorofon, and λ-cyhalothrin on AChE activity in insecticide resistance *B. zonata*. The activity of esterases in insecticide treated male flies was significantly different from control group. Results showed that there was no difference in esterase level in male flies treated with Trichlorofon and Malathion, but both groups differ significantly from control group . It is also depicted that the level of esterases in λ-cyhalothrin treated flies was not only different from control group, but also from Trichlorofon and Malathion treated flies.

On the other hand, the results illustrated reduction in the AchE activity in Spinosad resistant and field males. Charpentier and Fournier (2001) showed that there was a correlation in natural populations of *D. melanogaster* between the amount of AChE in the central nervous system and their resistance to insecticides. Also Menozzi *et al.,* (2004) reported that the field populations are composed of mixture of different alleles with different sensitivities to each insecticide so the treatment with one pesticide would eliminate one allele but would select another one.

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ARABIC SUMMERY

التأثيرات السمية والبيوكيميائية لمبيدى ملاثيون وسبينوساد علي حشرة ذبابة ثمار الخوخ

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اختبرت سمية مبيدى ملاثيون وسبينوساد علي طور العذراء والطور البالغ (ذكروأنثي) لحشرة ذبابة ثمار الخوخ معمليا.

أوضحت النتائج أن الطور البالغ للحشرة كان أكثر حساسية لتاثير المبيدين من طور العذراء كما كانت الإناث أكثر تحملا من الذكور لتأثير المبيدين. وكان مبيدى ملاثيون وسبينوساد ذو كفاءة عند تطبيقهما بطريقة التعرض بالملامسة لسطح عليه طبقة رقيقة من متبقى المبيدين (التركيز القاتل ل 50% من العشيرة = 4.28 – 4.51 و 1.14- 2.50 جزء فى المليون للذكور - للإناث على التوالى)أكثر من طريقة التغذية (التركيزالقاتل ل 50% من العشيرة = 6.40 - 6.49 و 2.83 - 4.13جزء فى المليون للذكور - للإناث على التوالى) و تشير النتائج الى ان نسب مقاومة الحشرة البالغة لذبابة ثمار الخوخ الحقلية لمبيد سبينوساد (10 و 10.9 ضعف للإناث والذكور) أعلي منها لمبيد ملاثيون (5.4 و 4.8 ضعف للإناث والذكور) مقارنة بالحشرات المعملية. وقد نتج عن معاملة خمسة أجيال من الحشرات المعملية بالتركيز القاتل ل 50% من مبيدى ملاثيون وسبينوساد نسب مقاومة بلغت 16 و 4 أضعاف. وقد تسبب المبيدين فى حدوث إنخفاض معنوى فى المحتوي الكلى لبروتينات أنسجة جسم الحشرات المعاملة والمقاومة . كما لوحظ أيضا زيادة معنوية فى نشاط كل من أنزيمى جلوتاثيون إس ترانسفيريز وأسيتل كولين إستريزفى أنسجة الحشرات المعاملة.