Toxicological and Biochemical Effects of Some Insecticides on Peach Fly, *Bactrocera zonata* (Diptera: Tephritidae)

Yahia Youssef MOSLEH[1], Lamiaa Housein YOUSRY[2] and Adel ABO-EL-ELAA[3]

[1]Plant Protection Department, Faculty of Agriculture and [2]Chemistry Department, Faculty of Science, Suez Canal University, Ismailia, Egypt; [3]Zoology Department, Faculty of Science, Fayoum University, Al Fayyum, Egypt

Abstract

Mosleh Y.Y., Yousry L.H., Abo-El-Elaa A. (2011): **Toxicological and biochemical effects of some insecticides on peach fly**, *Bactrocera zonata* (Diptera: Tephritidae). Plant Protect. Sci., **47**: 121–130.

The peach fruit fly, *Bactrocera zonata* (Saunders, Diptera: Tephritidae), has been a serious pest in the last decade attacking a wide range of fruits in Egypt. The toxicity of Malathion, Diazinon, Methoxyfenozide and Lufenuron to adult males and females of *B. zonata* was studied under laboratory conditions. The results showed that Diazinon was the most toxic among the tested compounds followed by Malathion, Lufenuron and Methoxyfenozide. LC_{50} values for adult males and females were 0.20 ppm, 0.09 ppm and 0.02 ppm (for males), 0.91 ppm, 0.14 ppm and 0,01 ppm (for females), respectively. The results showed that the level of glutamic oxaloacetic transaminase (GOT) of treated adult males and females in 24 h, 48 h, and 72 h post treatment increased compared to untreated adults. The highest activities of GOT in treated adult males in 24 h, 48 h, and 72 h were 92.11µM, 101.99µM and 112.21µM pyruvate released × 10^3/min/g FW (fresh weight), respectively, for Methoxyfenozide LC_{10}, and in treated adult females after 24 h, 48 h, and 72 h they were 84.24µM, 94.33µM, and 111.12µM pyruvate released × 10^3/min/g FW, respectively, for Diazinon LC_{25}. The activities of acetylcholine esterase of treated adults decreased compared to untreated adults. The highest activities of acid phosphatase in adult males after 24 h and 48 h were 249.43 µg and 270.52 µg AchI hydrolysed/min/g FW, respectively, for Methoxyfenozide LC_{25}. The highest activities of alkaline phosphatase in adult males were 139.04 µg, 175.67 µg, and 199.29 µg phenol × 10^3/min/g FW for Malathion LC_{10} and in adult females they were 123.31 µg, 162.10 µg and 199.59 µg phenol.10^3/min/g FW, respectively, for Lufenuron LC_{25} in 24 h, 48 h, and 72 h post treatment.

Keywords: *Bactrocera zonata;* insecticides; glutamic oxaloacetic transaminase – GOT; glutamic pyruvic transaminase – GPT; acid phosphatase; alkaline phosphatase; peach fruit fly

The fruit flies belong to the most important insect pest groups of horticulture production and export throughout the world. Over 4500 species occur worldwide, about 50 species are regarded as major pest species; at least another 30 species are of minor economic importance. Twenty four of the major pest species occur within the Pacific region. Four hundred species belonging to the genus *Bactrocera* are widely distributed in tropical Asia, South Pacific and Australia regions, but very few species of this genus were recorded in Africa (Drew & Hancock 1994). Recently, in 1993 the peach fruit fly *Bactrocera zonata* (Saunders) was recognised in Egypt causing most of fruit damage. *B. zonata* was found in Egypt to attack a variety of fruits including mango, guava, apricot, peach, apple and fig, although this insect species was recorded in Egypt as early as in 1924 (Efflatoun 1924).

In India, *B. zonata* is active throughout the year except the cold winter months of January and February (Grewal 1981). The aim of the present study is to evaluate the efficiency of some insec-

ticides belonging to different groups which differ in their mode of action against *B. zonata* and to study the effect of sublethal concentrations of the tested insecticides on some biochemical components of adult males and females of *B. zonata* such as total soluble protein, transaminases (glutamic oxaloacetic – GOT and glutamic pyruvic – GPT), phosphatases (acid and alkaline) and acetylcholine esterase enzyme activities.

MATERIAL AND METHODS

***Mass rearing technique of** B. zonata*. The present study was carried out in the laboratory of Ismailia Agricultural Research Station, Ismailia Governorate, under laboratory conditions (24 ± 3°C and 70 ± 3% RH). The insects were obtained from infested mango fruits from the farm of El-Qasasin Agricultural Research Station. The insects were reared in the laboratory according to the rearing method described by Youssef (2004). According to this method, the infested fruits were kept under laboratory conditions (24 ± 3°C and 50:70% RH). Plastic containers were filled with sterilised sand and the infested fruits were placed into the containers where eggs hatching to 1st instar, then 2nd and 3rd instar of larvae until pupation took place. Pupae were collected daily and transferred to adult rearing cages (30 × 30 × 30 m^2). The sides of the adults cages were coated with wire screen except one side which had a sieve opening (for daily examination) and the cage floor was made of wooden sheet. The newly emerged flies were provided with a source of drinking water and food made of sugar mixed with buminal (3:1), the adults cages were supplied with artificial plastic fruit having many small pores (as ovipositor sites) except 3 cm at the bottom and one wide pore at the top which was covered with suitable lid; this plastic fruit was filled with water to a height of the above-mentioned 3 cm at the bottom to receive the eggs. The deposited eggs were collected every 24 h and placed on an artificial diet containing wheat bran (1000 g), brewer's yeast (250 g), sugar (300 g), sodium benzoate (2 g), HCl (10%, 2 ml), and water (500 ml). The diet was kept in plastic containers and stored in a refrigerator until use. The plastic containers which contained collected eggs in the artificial diet were covered with plastic lids during the 1st three days to provide maximum moisture for egg hatching. The plastic lid was replaced by the muslin fabric (from 4 to 7 days), on the 8th day the muslin fabric was removed to allow the full grown larvae to jump out of the trays and pupate in fine sand. The pupae were collected daily and transferred to the rearing cages. The adults emerged after 1–2 weeks to start a new generation in the same way.

***Laboratory assay of some selected insecticides against the adult stage of** B. zonata*. The experiments were carried out to evaluate the toxicity of various insecticides belonging to different chemical groups: Malathion (Malathin® 57% EC), Diazinon (Bassudin® 60% EC), Methoxyfenozide (Runner® 24% SC), and Lufenuron (Match® 5% EC) to the adult stage of *B. zonata* under laboratory conditions (24 ± 3°C and 70 ± 3 % RH).

Toxicity experiments were conducted in small glass jars; each jar received ten adults of male and female flies of *B. zonata* that were confined separately without food. Pieces of cotton were immersed in a series of different concentrations (six concentrations) of each of the tested insecticides. The cotton pieces were placed in small plastic cups. Three replications of each concentration were used and three untreated replications were also set up as a check (control). All the experiments were performed three times on different dates.

The small jars were examined after 24 h, 48 h, and 72 h and dead flies were counted and recorded. The average percentage of adult mortality for each concentration was calculated and plotted against each test concentration on a logarithmic probit paper. The concentrations killing 10% (LC_{10}), 25% (LC_{25}), 50% (LC_{50}), and 90 %(LC_{90}) of tested adults were calculated according to Finney (1971).

***Effect of sublethal concentrations of the tested insecticides on some enzymes of** B. zonata*. This experiment was carried out to determine the effects of sublethal concentrations (LC_{10} and LC_{25}) of the above-mentioned insecticides after 24 h, 48 h, and 72 h post treatment on some enzyme activities, total soluble protein, GOT, GPT, acid phosphatase, alkaline phosphatase and acetylcholine esterase (AchE) of *B. zonata* adults.

Preparation of** B. zonata **adult homogenates. Samples of treated and untreated adults of both sexes (males and females) were taken in 24 h, 48 h, and 72 h post treatment and confirmed in Eppendorf tubes under freezing conditions and replicated four times. The adult samples were homogenised in 5 ml 0.1M phosphate buffer pH 7.4 for both sexes using a glass homogeniser which was surrounded

with ice. The total homogenates were centrifuged at 4000 rpm for 20 min at 4°C. The supernatants were used for biochemical tests.

Determination of the activity of total soluble protein. The activity of total soluble protein was determined colorimetrically according to the method of Bradford (1976).

Glutamic oxaloacetic transaminase (GOT). The activity of GOT, which is known as aspartate aminotransferase (AST), was determined colorimetrically according to the method of Reitman and Frankel (1957), where GOT transfers the amino group from aspartate to α-ketoglutarate, producing a new amino acid glutamate and oxaloacetate.

Glutamic pyruvic transaminase (GPT). The activity of GPT, which is known as alanine aminotransferase (ALT), was determined colorimetrically according to the method of Reitman and Frankel (1957), where GPT transfers the amino group from alanine to α-ketoglutarate, producing a new amino acid glutamate and pyruvate.

Acid phosphatase. The acid phosphatase (Ac-P) activity was determined according to the method described by Powell and Smith (1954). In this method, the liberated phenol from the enzymatic hydrolysis of phenyl phosphate was measured colorimetrically in the presence of 4-aminophenazone, and by the addition of potassium ferricyanide the characteristic brown colour was produced, according to the following equation:

$$\text{phenyl phosphate} \xrightarrow[\text{pH 4.9}]{\text{acid phosphatase}} \text{phenol + phosphate}$$

Alkaline phosphatase. The alkaline phosphatase (Alk-P) activity was determined according to the method described by Powell and Smith (1954). In this method, the liberated phenol from the enzymatic hydrolysis of phenyl phosphate was measured colorimetrically in the presence of 4-aminophenazone, and by the addition of potassium ferricyanide the characteristic brown colour was produced, according to the following equation:

$$\text{phenyl phosphate} \xrightarrow[\text{pH 10}]{\text{acid phosphatase}} \text{phenol + phosphate}$$

Determination of the activity of acetylcholine esterase (AchE). The acetylcholine esterase (AchE) activity was measured according to the method described by Ellman *et al.* (1961); it is based on the hydrolysis of acetylthiocholine iodide (ATchI) as a substrate of the tested enzyme to produce thiocholine and acetic acid. The reaction of thiocholine with DTNB (5,5-dithiobis-(2-nitrobenzoic acid) will produce the anion of 5-thio-2-nitrobenzoic acid. The rate of colour production as a function of the enzyme activity was measured spectrophotometrically at 405 nm according to the following equations:

$$\text{acetylthiocholine} + H_2O \xrightarrow{\text{cholinesterase}} \text{thiocholine} + \text{acetic acid}$$

$$\text{thiocholine} + \text{DTNB} \longrightarrow \text{5-thio-2-nitrobenzoic acid}$$

In all cases a minimum of three independent experiments was conducted and each sample was in triplicate. Data presented are means ± standard deviations. Data were analysed by two-way analysis of variance, and a pairwise multiple comparison procedure (Student-Newman-Keuls method) was used to compare insecticides at $P < 0.05$.

RESULTS AND DISCUSSION

Toxicity of selected insecticides to adult stage of *B. zonata*

Diazinon was the most toxic insecticide among the tested insecticides, followed by Malathion, Lufenuron and Methoxyfenozide, to males of *B. zonata* after 24 h, 48 h, and 72 h (Table 1). The respective values of LC_{50} of these compounds after 24 h were 0.20, 0.48, 8.97, and 9.73 ppm. The toxicity index values showed the superior efficiency of Diazinon at LC_{50} (100%) followed by Malathion (41.67%), Lufenuron (2.23%) and Methoxyfenozide (2.06%) after 24 hours. As for the slope values, the steep toxicity line of Lufenuron shows the highest slope value 1.73, while the line of Malathion shows the lowest slope value 1.21, whereas the rest toxicants lie between the values of the two above-mentioned compounds (Methoxyfenozide 1.57 and Diazinon 1.51).

The results also indicated that Diazinon was the most toxic insecticide among the tested compounds, followed by Malathion, Lufenuron, and Methoxyfenozide, to females of *B. zonata* after 24 h, 48 h, and 72 h (Table 1). The toxicity index values showed the superior efficiency of Diazinon at LC_{50} (100%), followed by Malathion (31.81%), Methoxyfenozide (8.33%), and Lufenuron (6.45%) after 48 hours. As for the slope values, the toxicity line of Diazinon shows the highest slope value 1.21, while the line of Malathion shows the lowest slope value 0.82, whereas the rest toxicants lie between

Table 1. Toxicity of some insecticides to adult males and females of *B. zonata* (Saunders) after 24 h, 48 h, and 72 h under laboratory conditions s

Insecticide	Time (h)	Lethal concentrations and their limits (ppm)				Slope ± SE	Toxicity index (%)			
		LC_{10}	LC_{25}	LC_{50}	LC_{90}		LC_{10}	LC_{25}	LC_{50}	LC_{90}
Males										
	24	0.04 ± (0.02–0.06)	0.13 ± (0.08–0.18)	0.48 ± (0.36–0.60)	5.50 ± (3.80–9.10)	1.21 ± 0.11	50.00	53.85	41.67	26.00
Malathion	48	0.03 ± (0.01–0.05)	0.10 ± (0.06–0.14)	0.34 ± (0.26–0.44)	3.46 ± (2.50–5.33)	1.28 ± 0.11	30.00	20.00	26.47	26.01
	72	0.004 ± (0.0006–0.014)	0.02 ± (0.005–0.05)	0.13 ± (0.06–0.20)	3.73 ± (2.12–9.73)	0.88 ± 0.13	50.00	35.00	15.38	9.92
	24	0.02 ± (0.01–0.04)	0.07 ± (0.04–0.10)	0.20 ± (0.15–0.24)	1.43 ± (1–2.48)	1.51 ± 0.18	100	100	100	100
Diazinon	48	0.009 ± (0.002–0.021	0.02 ± (0.009–0.04)	0.09 ± (0.05–0.12)	0.90 ± (0.63–1.64)	1.28 ± 0.19	100	100	100	100
	72	0.002 ± (0.00009–0.008)	0.007 ± (0.0007–0.02)	0.02 ± (0.006–0.05)	0.37 ± (0.26–0.61)	1.14 ± 0.23	100	100	100	100
	24	1.50 ± (0.95–2.09)	3.64 ± (2.71–4.56)	9.73 ± (8.12–11.63)	63.20 ± (46.08–97.25)	1.57 ± 0.14	1.33	1.92	2.06	2.26
Methoxyfenozide	48	0.21 ± (0.06–0.44)	0.55 ± (0.23–0.94)	1.60 ± (0.93–2.26)	12.00 ± (9.31–17.05)	1.46 ± 0.19	4.29	3.64	5.63	7.50
	72	0.03 ± (0.0005–0.17)	0.12 ± (0.005–0.42)	0.51 ± (0.06–1.12)	7.21 ± (5.09–11.91)	1.11 ± 0.25	6.67	5.83	3.92	5.13
	24	1.63 ± (1.09–2.20)	3.66 ± (2.80–4.51)	8.97 ± (7.56–10.56)	49.19 ± (37.37–70.91)	1.73 ± 0.14	1. 23	1.91	2.23	2.91
Lufenuron	48	0.13 ± (0.04–0.26)	0.46 ± (0.22–0.75)	1.88 ± (1.30–2.47)	26.50 ± (17.42–50.14)	1.11 ± 0.13	6.92	4.35	4.79	3.40
	72	0.006 ± (0.000001–0.076)	0.03 ± (0.00004–0.22)	0.22 ± (0.003–0.77)	8.23 ± (5.25–17.71)	0.82 ± 0.23	33.33	23.33	9.09	4.50
Fameůs										
	24	0.04 ± (0.01–0.06)	0.16 ± (0.08–0.25)	0.91 ± (0.67–1.22)	23.74 ± (12.33–65.15)	0.90 ± 0.10	75.00	50.00	28.57	9.01
Malathion	48	0.03 ± (0.01–0.05	0.11 ± (0.06–0.16)	0.44 ± (0.32–0.56)	5.93 ± (3.99–10.27)	1.13 ± 0.11	33.33	36.36	31.81	24.11
	72	0.004 ± (0.0005–0.01)	0.02 ± (0.006–0.05)	0.16 ± (0.08–0.25)	6.00 ± (3.08–19.95)	0.82 ± 0.13	100	100	43.75	14.00
	24	0.03 ± (0.01–0.05)	0.08 ± (0.05–0.12)	0.26 ± (0.20–0.32)	2.14 ± (1.39–4.34)	1.39 ± 0.18	100	100	100	100
Diazinon	48	0.01 ± (0.003–0.02)	0.04 ± (0.01–0.06)	0.14 ± (0.09–0.18)	1.43 ± (0.95–2.89)	1.27 ± 0.18	100	100	100	100
	72	0.006 ± (0.001–0.01)	0.03 ± (0.006–0.04)	0.07 ± (0.03–0.11)	0.84 ± (0.58–1.58)	1.21 ± 0.20	66.67	66.67	100	100
	24	1.60 ± (0.94–2.32)	4.49 ± (3.26–5.73)	14.12 ± (11.55–17.54)	124.61 ± (81.04–231.50)	1.35 ± 0.13	1.88	1.78	1.84	1.72
Methoxyfenozide	48	0.14 ± (0.03–0.34)	0.46 ± (0.16–0.86)	1.68 ± (0.92–2.46)	19.13 ± (14.03–30.22)	1.21 ± 0.16	7.14	8.70	8.33	7.48
	72	0.03 ± (0.0003–0.15)	0.12 ± (0.005–0.42)	0.62 ± (0.09–1.34)	14.14 ± (9.40–32.08)	0.94 ± 0.22	13.33	16.67	11.30	5.94
	24	1.98 ± (1.34–2.64)	4.51 ± (3.51–5.52)	11.26 ± (9.52–13.34)	63.96 ± (47.56–95.18)	1.69 ± 0.14	1.52	1.77	2.31	3.35
Lufenuron	48	0.14 ± (0.03–0.35)	0.52 ± (0.18–0.97)	2.17 ± (1.23–3.13)	32.23 ± (22.14–57.75)	1.09 ± 0.14	7.14	7.69	6.45	4.44
	72	0.02 ± (0.000003–0.24)	0.1 ± (0.0015–2.23)	0.55 ± (0.01–2.88)	14.17 ± (9.52–32.71)	0.90 ± 0.16	20.0	20.0	12.73	5.93

the values of the two above-mentioned compounds (Methoxyfenozide 0.94 and Lufenuron 0.90).

From the obtained results it is clear that the LC_{50} and LC_{25} values for treated adult females increased compared to treated adult males in 24 h, 48 h, and 72 h after treatment (Table 1). It means that adult males were more susceptible to the tested insecticides than adult females. However, the present findings confirm the results of Stark *et al*. (2004) that adult males of *C. capitata* were significantly more susceptible than adult females. It is also observed from the previous results that the slopes differed between both sexes of *B. zonata* and between 24 h, 48 h, and 72 h after treatment within each sex. Furthermore, the slope values also differed from one tested insecticide to another.

Generally, it could be concluded that Diazinon was the most effective insecticide against adult males and females of *B. zonata,* followed by Malathion, Methoxyfenozide and Lufenuron. The females of *B. zonata* are less sensitive to Malathion, Methoxyfenozide, and Lufenuron insecticide than the males. Diazinon is considered as organophosphorus (OP's) insecticide, its mode of action is cholinesterase (ChE) inhibitor. Synaptic transmission occurs through the synapses in the insect nervous system by neurotransmitters called cholinesterase (ChE), it is hydrolysed by acetylcholinesterase (AchE) that contains two active sites (esteratic site which contains OH group and anionic site which contains negative charge). At the beginning of hydrolysis of ChE by AchE it was binding with the two active sites giving acetic acid and choline. When Diazinon attacked, it was binding with AchE in the esteratic site only, forming a complex called phosphorylated enzyme, then the spontaneous recovery for this phosphorylated enzyme stopped. The inhibition of Diazinon for AchE leads to the accumulation of ChE in the nerve ends that leads to the continuous nerve conduction causing paralysis and then death of the insects.

Effect of sublethal concentrations of tested insecticides on the activity of total soluble protein

The activities of total soluble protein in untreated adult males were 0.27, 0.28, and 0.26 mg/g FW after 24 h, 48 h, and 72 h post treatment, respectively (Table 2). While the concentrations of total soluble protein in untreated adults females were 0.26, 0.32, and 0.20 mg/g FW in 24 h, 48 h, and 72 h post treatment, respectively.

Moreover, in treated adult males a decrease in the total soluble protein level was observed with the increasing exposure period when the highest rate of decrease was after 24 h with the values 51.85%, 44.44%, and 40.74%, for Methoxyfenozide at LC_{10}, Lufenuron at LC_{25} and LC_{10}, respectively.

The highest rate of decrease in the total soluble protein level in adult females was after 24 h with the values 50% and 46.15% for Lufenuron LC_{10} and LC_{25}, respectively, and with the value 46.15 for Methoxyfenozide at LC_{10} and LC_{25}. The obtained results agree with those obtained by Abd El-Hafez *et al.* (1988) and Ahmed & Mostafa (1989), who reported that the IGRs compounds caused a significant reduction in the level of total

Table 2. Effects of sublethal concentrations (LC_{10} and LC_{25}) of tested insecticides on total soluble protein (mg/g FW) of adult males and females of *B. zonata* (Saunders) under laboratory conditions

Insecticide	Concentrations (ppm)	Females			Males		
		72 h	48 h	24 h	72 h	48 h	24 h
Malathion	LC_{10}	0.16 ± 0.02	0.26 ± 0.02	0.22 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	0.23 ± 0.02
	LC_{25}	0.14 ± 0.02	0.21 ± 0.01	0.24 ± 0.01	0.14 ± 0.02	0.17 ± 0.01	0.18 ± 0.01
Diazinon	LC_{10}	0.14 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.14 ± 0.02	0.17 ± 0.02	0.19 ± 0.01
	LC_{25}	0.17 ± 0.01	0.20 ± 0.01	0.23 ± 0.05	0.15 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
Methoxy-fenozide	LC_{10}	0.10 ± 0.01	0.11 ± 0.01	0.14 ± 0.03	0.07 ± 0.02	0.10 ± 0.01	0.13 ± 0.04
	LC_{25}	0.11 ± 0.01	0.16 ± 0.01	0.17 ± 0.03	0.17 ± 0.01	0.20 ± 0.02	0.23 ± 0.06
Lufenuron	LC_{10}	0.08 ± 0.02	0.19 ± 0.18	0.13 ± 0.03	0.13 ± 0.01	0.15 ± 0.02	0.16 ± 0.05
	LC_{25}	0.08 ± 0.02	0.16 ± 0.19	0.14 ± 0.04	0.10 ± 0.02	0.12 ± 0.01	0.15 ± 0.04
Control	–	0.20 ± 0.02	0.32 ± 0.01	0.26 ± 0.04	0.26 ± 0.02	0.28 ± 0.01	0.27 ± 0.04

Values are mean ± standard deviations; $n = 3$

soluble protein of the 4th instar larvae of *Spodoptera littoralis*. Such a reduction in the level of total soluble protein may also be attributed to the observation of Deloach *et al.* (1981), who suggested that the effect of Diflubenzuron on insects was due to the inhibition of DNA and thus might affect the protein synthesis.

Effect of sublethal concentrations of tested insecticides on the activity of GOT and GPT

The activities of GOT in untreated adult males were 38.37 mg/g FW in 24 h post treatment, then they increased to 48.47 and 56.17μM pyruvate × 10^3/min/g FW in 48 h and 72 h post treatment, respectively (Table 3). While the activities of GOT in untreated adult females were 42.30, 44.09, and 43.91μM pyruvate ×10^3/min/g FW after 24 h, 48 h, and 72 h post treatment, respectively. It is noticeable that the level of GOT in treated adults in 24 h, 48 h, and 72 h post treatment was higher than in untreated adults.

The highest activities of GOT for Methoxyfenozide at LC_{10} after 24 h, 48 h, and 72 h were 92.11, 101.99, and 112.21μM pyruvate × 10^3/min/g FW, respectively, it means that the activity of GOT increased with the increasing exposure period. A positive correlation between the exposure period and the activity of GOT was observed in adult males of *B. zonata*.

The level of GOT in treated adult females in 24 h, 48 h, and 72 h post treatment was higher than in untreated females. The highest activities of GOT in 24 h, 48 h, and 72 h were 84.24, 94.33, and 111.12μM pyruvate × 10^3/min/g FW, respectively, for Diazinon at LC_{25} and the activity of GOT increased with the increasing exposure period.

GPT activities in untreated adult males were 4.83, 4.62, and 4.70μM pyruvate × 10^3 per min/g FW in 24 h, 48 h, and 72 h post treatment, respectively (Table 4). While the activities of GPT in untreated adult females were 5.69, 6.43, and 5.82μM pyruvate.10^3/min/g FW in 24 h, 48 h, and 72 h post treatment, respectively. The level of GPT of treated adult males in 24 h, 48 h, and 72 h post treatment increased compared to untreated adults. The highest activity of GPT in 24 h was 8.34μM pyruvate × 10^3/min/g FW for Malathion at LC_{25}, 8.16μM pyruvate × 10^3/min/g FW in 48 h for Lufenuron at LC_{10}, and 8.35μM pyruvate × 10^3 per min/g FW after 72 h for Lufenuron at LC_{25}.

The obtained results demonstrated that the level of GPT in treated adult females increased in 24 h post treatment compared to untreated females and the highest activity value was 10μM pyruvate × 10^3/min/g FW for Malathion at LC_{25}. After 48 h post treatment the activity of GPT in treated adults increased compared to untreated adults and the highest activity value was 9.73μM pyruvate × 10^3 per min/g FW for Malathion at LC_{25}, but there were some exceptions in Diazinon at LC_{10} and Lufenuron at LC_{25} when the activity value of GPT in treated adult females decreased compared to untreated females with the values 5.96 and 6.27μM pyruvate × 10^3/min/g FW, respectively. After 72 h post treatment the activity of GPT in treated adult females increased compared to untreated females and the

Table 3. Effects of sublethal concentrations (LC_{10} and LC_{25}) of tested insecticides on GOT activity (μM pyruvate released × 10^3/min/g FW) of adult males of *B. zonata* (Saunders) under laboratory conditions

Insecticide	Concentrations (ppm)	Females			Males		
		72 h	48 h	24 h	72 h	48 h	24 h
Malathion	LC_{10}	82.05 ± 4.87	71.67 ± 6.34	65.82 ± 5.02	67.95 ± 6.85	58.41 ± 6.33	50.94 ± 5.21
	LC_{25}	90.87 ± 5.20	58.52 ± 5.38	54.16 ± 5.59	79.92 ± 5.02	71.13 ± 6.09	64.69 ± 4.98
Diazinon	LC_{10}	72.80 ± 4.97	58.46 ± 6.88	51.36 ± 5.99	63.82 ± 5.52	51.36 ± 6.94	46.84 ± 6.24
	LC_{25}	111.12 ± 5.15	94.33 ± 6.59	84.24 ± 6.55	103.90 ± 5.15	91.52 ± 6.99	83.70 ± 6.19
Methoxy-fenozide	LC_{10}	67.10 ± 6.15	58.11 ± 6.87	47.18 ± 4.57	112.21 ± 4.12	101.99 ± 6.36	92.11 ± 4.29
	LC_{25}	61.92 ± 6.12	53.64 ± 6.38	46.43 ± 6.31	83.78 ± 5.85	75.17 ± 6.87	66.19 ± 3.35
Lufenuron	LC_{10}	49.32 ± 5.41	55.05 ± 5.59	52.60 ± 2.12	60.07 ± 4.98	55.87 ± 6.03	57.77 ± 5.21
	LC_{25}	64.21 ± 6.01	54.85 ± 6.48	60.06 ± 5.00	65.69 ± 6.53	58.31 ± 6.84	47.76 ± 4.76
Control	–	43.91 ± 6.58	44.09 ± 5.65	42.30 ± 4.34	56.17 ± 5.78	48.47± 6.69	38.37 ± 4.92

Values are mean ± standard deviations; $n = 3$

Table 4. Effects of sublethal concentrations (LC_{10} and LC_{25}) of insecticides on GPT activity (μM pyruvate released × 10^3/min/g FW) of adult females of *B. zonata* (Saunders) under laboratory conditions

Insecticide	Concentrations (ppm)	Females			Males		
		72 h	48 h	24 h	72 h	48 h	24 h
Malathion	LC_{10}	7.38 ± 1.21	6.63 ± 0.51	7.41 ± 0.77	5.38 ± 0.83	5.85 ± 0.66	6.36 ± 0.96
	LC_{25}	8.40 ± 0.90	9.73 ± 0.96	10.00 ± 1.06	7.40 ± 0.90	7.85 ± 0.82	8.34 ± 1.15
Diazinon	LC_{10}	7.41 ± 0.86	5.96 ± 1.54	6.40 ± 1.58	5.39 ± 0.77	6.69 ± 0.87	7.15 ± 0.96
	LC_{25}	5.66 ± 1.38	7.90 ± 0.91	8.37 ± 0.96	6.34 ± 0.99	5.65 ± 0.90	6.14 ± 0.77
Methoxy-fenozide	LC_{10}	6.63 ± 0.60	7.72 ± 0.96	8.49 ± 0.66	6.40 ± 0.81	6.61 ± 0.72	7.42 ± 1.06
	LC_{25}	7.07 ± 0.56	7.90 ± 0.91	8.39 ± 0.94	7.26 ± 0.30	7.17 ± 1.06	7.40 ± 1.12
Lufenuron	LC_{10}	5.60 ± 0.88	6.88 ± 1.19	7.40 ± 1.27	7.16 ± 0.73	8.16 ± 1.29	8.25 ± 1.71
	LC_{25}	5.85 ± 0.57	6.27 ± 0.69	7.10 ± 1.06	8.35 ± 0.34	8.03 ± 0.65	5.53 ± 0.75
Control	–	5.82 ± 0.46	6.43 ± 0.76	5.69 ± 1.08	4.70 ± 0.07	4.62 ± 0.20	4.83 ± 0. 10

Values are mean ± standard deviations; $n = 3$

highest activity value was 8.40 μM pyruvate ×10^3 per min/g FW for Malathion at LC_{25}, but there were some exceptions in Diazinon at LC_{25} and Lufenuron at LC_{10} when the activity value of GPT in treated adults decreased compared to untreated adults with the values 5.66 and 5.60μM pyruvate × 10^3 per min/g FW, respectively.

From the previous results it is clear that these tested insecticides increased the activities of GPT and that there was a positive correlation between the concentration of tested insecticides and the increase of GPT activity.

These results show the same trend as in Ahmed *et al.* (1993), who postulated that on inducing the activities of transaminase enzymes GOT and GPT, this disturbance of *B. zonata* GOT and GPT can explain the reduction of its protein biosynthesis. It has been reported that disturbance of the levels of transaminases GOT and GPT was caused by some insecticides such as diflubenzeuron in earthworms.

The maintenance of the balanced amino acid pool in insects is the result of various biochemical reactions carried out by a group of enzymes called amino transferases (Meister 1957). Such reactions are mainly responsible for the degradation and biosynthesis of amino acids, linking the glucose and protein metabolism and the synthesis of some specific compounds.

Among the aminotransferases, especially alanine aminotransferase (GPT) is one of the components of oxidative metabolism of proline, which is utilided in some insects during the initial periods of flights (Bursell 1963), it also acts as a catalytic agent in the metabolism of carbohydrates (Katunuma *et al.* 1968). The effect of insecticides on enzymes catalysing the amino acid metabolism in insects might be important (Kamin & Handler 1957). A close relation was found in insects between protein synthesis and levels of transaminases, whereas these enzymes provide the building blocks for protein synthesis and are probably involved in the synthesis of amino acids during metamorphosis, thereby, GOT and GPT are often used as indicators of the metabolism of protein and amino acids (Wigglesworth 1972).

It can be concluded from the obtained results that the chronic effect of the tested insecticides on transaminase activities may lead to the disturbance of protein metabolism and synthesis of some specific compounds according to Bursell (1963).

Effect of sublethal concentrations of tested insecticides on the activity of acid phosphatase and alkaline phosphatase

The activities of acid phosphatase in untreated males and females were 92.81, 126.58, 160.03 μg phenol × 10^3/min/g F and 160.60, 178.60, 180.30 μg phenol × 10^3/min/g FW after 24 h, 48 h and 72 h post treatment, respectively (Table 5). The level of acid phosphatase of treated adults was higher in 24 h, 48 h, and 72 h post treatment than in untreated adults. The highest activity of acid phosphatase in 24 h was 249.43 μg phenol × 10^3/min/g FW for Methoxyfenozide at LC_{25}. After 48 h the highest activity of acid phosphatase was 270.52 μg phenol × 10^3/min/g FW also for Methoxyfenozide at LC_{25}, while after 72 h the highest activity of acid phosphatase was 419.61 μg phenol × 10^3/min/g FW for Malathion LC_{10}.

Table 5. Effects of sublethal concentrations (LC_{10} and LC_{25}) of insecticides on acid phosphatase activity (μg phenol released × 10^3/min/g FW) of adult females of *B. zonata* (Saunders) under laboratory conditions

Insecticide	Concentrations (ppm)	Females			Males		
		72 h	48 h	24 h	72 h	48 h	24 h
Malathion	LC_{10}	301.03 ± 6.35	273.68 ± 5.85	260.02 ± 5.78	419.61 ± 5.91	197.58 ± 6.50	179.29 ± 4.82
	LC_{25}	317.53 ± 6.27	245.23 ± 5.00	178.35 ± 5.41	267.33 ± 4.33	228.57 ± 6.57	210.59 ± 6.41
Diazinon	LC_{10}	187.84 ± 5.70	244.39 ± 3.40	215.88 ± 4.86	255.81 ± 6.99	215.64 ± 4.79	187.12 ± 4.28
	LC_{25}	200.83 ± 6.30	182.13 ± 3.29	166.32 ± 5.58	231.94 ± 5.69	194.81 ± 5.49	154.75 ± 4.57
Methoxy-fenozide	LC_{10}	322.43 ± 4.54	295.91 ± 3.33	276.92 ± 4.58	342.62 ± 5.15	215.87 ± 5.74	183.75 ± 5.71
	LC_{25}	223.23 ± 5.61	189.98 ± 5.06	164.09 ± 4.49	206.22 ± 5.90	270.52 ± 5.99	249.43 ± 5.64
Lufenuron	LC_{10}	243.50 ± 5.54	214.03 ± 5.12	174.99 ± 4.58	274.62 ± 4.74	147.24 ± 5.03	197.02 ± 3.84
	LC_{25}	347.14 ± 6.52	326.07 ± 4.86	294.48 ± 4.76	213.85 ± 5.26	182.13 ± 5.28	245.10 ± 5.79
Control	–	180.30 ± 3.95	178.60 ± 4.93	160.60 ± 4.32	160.03 ± 3.99	126.58 ± 5.85	92.81 ± 6.69

Values are mean ± standard deviations; $n = 3$

The inhibitory effect of sublethal concentrations LC_{10} and LC_{25} of the tested compounds on alkaline phosphatase of the *B. zonata* adult females in 24 h, 48 h, and 72 h under laboratory conditions is shown in Table 6. The activities of alkaline phosphatase of untreated adult males and females were 41, 88.48, 136.58 μg phenol × 10^3 per min/g FW and 53.75, 55.32, 62.22 μg phenol × 10^3 per min/g FW in 24 h, 48 h, and 72 h post treatment, respectively. The level of alkaline phosphatase increased in treated adults after 24 h, 48 h, and 72 h post treatment compared to untreated adults. The highest activities of alkaline phosphatase were 139.04, 175.67 and 199.29 mg μg phenol × 10^3/min/g FW, respectively, for Malathion at LC_{10} after 24, 48, and 72 h post treatment.

These results concerning the effect of the tested insecticides on the alkaline phosphatase activity demonstrate that there is a positive correlation between the used concentrations and the induction effect on the enzyme activity.

These results agree with Mostafa (1998), who found out that the activity of alkaline and acid phosphatase increased in chlorfluazuron. Ahmed *et al.* (1993) reported that chlorfluazuron increased the activity of alkaline and acid phosphatase of *S. littoralis*.

Phosphatases are defined as enzymes that hydrolyse any phosphorus ester or anhydride bond, including P-O-C, P-F and others. One generalisation can be made safely; all the OP compounds can be hydrolysed in mammals, insects and plants by phosphatases, commonly the major metabolic route (O'Brien 1967).

Table 6. Effects of sublethal concentrations (LC_{10} and LC_{25}) of insecticides on alkaline phosphatase activity (μg phenol released × 10^3/min/g FW) of adult males of *B. zonata* (Saunders) under laboratory conditions

Insecticide	Concentrations (ppm)	Females			Males		
		72 h	48 h	24 h	72 h	48 h	24 h
Malathion	LC_{10}	156.24 ± 5.02	104.44 ± 4.98	64.24 ± 4.11	199.29 ± 4.13	175.67 ± 4.68	139.04 ± 6.36
	LC_{25}	116.97 ± 4.28	101.60 ± 3.15	61.70 ± 5.23	154.81 ± 3.35	104.97 ± 5.27	66.16 ± 5.13
Diazinon	LC_{10}	154.21 ± 4.78	102.23 ± 4.02	61.96 ± 4.55	139.52 ± 4.85	89.17 ± 4.61	48.42 ± 4.58
	LC_{25}	151.29 ± 3.50	102.52 ± 3.99	60.57 ± 5.86	153.33 ± 5.27	105.13 ± 3.94	63.06 ± 4.18
Methoxy-fenozide	LC_{10}	135.90 ± 3.20	85.57 ± 4.43	57.51 ± 5.05	140.50 ± 5.06	97.72 ± 5.56	55.29 ± 6.29
	LC_{25}	174.32 ± 4.80	122.90 ± 3.98	81.88 ± 4.70	176.65 ± 3.52	125.49 ± 4.90	88.05 ± 4.20
Lufenuron	LC_{10}	156.91 ± 4.19	105.53 ± 4.07	66.94 ± 5.25	149.43 ± 6.51	99.16 ± 5.00	49.65 ± 4.93
	LC_{25}	199.59 ± 3.78	162.10 ± 5.58	123.31 ± 2.94	144.15 ± 5.91	91.53 ± 5.07	45.27 ± 5.49
Control	–	62.22 ± 4.93	55.32 ± 4.47	53.75 ± 4.22	136.58 ± 4.39	88.48 ± 4.53	41.00 ± 5.29

Values are mean ± standard deviations; $n = 3$

Table 7. Effects of sublethal concentrations (LC_{10} and LC_{25}) of insecticides on acetylcholin esterase activity (µg AchI hydrolysed/min/g FW) of adult males of *B. zonata* (Saunders) under laboratory conditions

Insecticide	Concentrations (ppm)	Femals			Males		
		72 h	48 h	24 h	72 h	48 h	24 h
Malathion	LC_{10}	21.10 ± 5.00	14.80 ± 4.84	8.03 ± 3.93	33.31 ± 3.75	27.15 ± 6.14	21.43 ± 5.14
	LC_{25}	17.06 ± 5.06	15.40 ± 5.44	8.85 ± 5.76	27.85 ± 4.49	21.94 ± 5.00	14.58 ± 4.33
Diazinon	LC_{10}	25.83 ± 4.38	19.95 ± 6.08	13.23 ± 3.95	22.34 ± 4.16	16.88 ± 5.66	9.63 ± 3.87
	LC_{25}	18.24 ± 5.04	13.06 ± 4.75	5.21 ± 4.39	23.93 ± 5.08	17.94 ± 4.71	12.30 ± 2.67
Methoxy-fenozide	LC_{10}	27.91 ± 5.50	24.17 ± 4.84	16.53 ± 3.23	26.67 ± 4.56	21.19 ± 5.17	13.10 ± 4.05
	LC_{25}	24.97 ± 5.14	19.56 ± 6.75	11.70 ± 3.08	27.26 ± 3.87	22.77 ± 5.05	15.31 ± 4.21
Lufenuron	LC_{10}	31.36 ± 4.69	25.68 ± 5.03	18.06 ± 3.83	30.00 ± 4.87	23.76 ± 5.09	16.16 ± 4.29
	LC_{25}	39.34 ± 5.63	31.01 ± 7.76	24.91 ± 4.55	21.85 ± 3.41	17.78 ± 4.02	12.47 ± 4.13
Control	–	45.38 ± 3.96	39.08 ± 3.37	32.22 ± 4.98	37.63 ± 3.58	31.08 ± 3.50	25.38 ± 3.83

Values are mean ± standard deviations; $n = 3$

Effect of sublethal concentrations of tested insecticides on the activity of acetylcholine esterase (AchE)

The activities of acetylcholine esterase of untreated adult males were 25.38, 31.08, and 37.63 µg AchI hydrolysed/min/g FW in 24 h, 48 h, and 72 h post treatment, respectively (Table 7). The percentage values of acetylcholine esterase of treated males decreased after 24 h, 48 h, and 72 h compared to untreated males. While the activities of acetylcholine esterase of untreated adult females were 32.22, 39.08, 45.38 µg AchI hydrolysed/min per mg FW in 24 h, 48 h, and 72 h post treatment, respectively. The percentage values of acetylcholine esterase of treated females decreased after 24 h, 48 h, and 72 h compared to untreated females.

From these results it is clear that the used insecticides had a highly inhibiting effect on AchE of adult males and females of *B. zonata*, the inhibition occurred in 24 h, 48 h, and 72 h post treatment. Organophosphorus (OP's) insecticides had a higher inhibiting effect than IGR insecticides because the mode of their action is cholinesterase (ChE) inhibitor when AchE contains two active sites (esteratic site which contains OH group and anionic site which contains negative charge). At the beginning of hydrolysis of ChE by AchE it was binding with the two active sites giving acetic acid and choline, when the OP insecticides attacked it was binding with AchE in the esteratic site only, forming a complex called phosphorylated enzyme, then the spontaneous recovery for this phosphorylated enzyme stopped. The inhibition of OP insecticides for AchE leads to the accumulation of ChE in the nerve ends that leads to the continuous nerve conduction causing paralysis and death of the insects. This agrees with O'Brien (1967), who found out that the organophosphorus insecticide (Malathion) is a contact neurotoxin that inhibits esterases, particularly AchE in the insect nervous system, and Charpentier *et al.* (2000) reported that AchE is the primary target of organophosphorus (OP) insecticides. Acetylcholine esterase is one of the most important enzymes belonging to specific esterases, the great majority of traditional insecticides are more poisonous and the main target for most of them is the acetylcholine esterase. From the above results it is clear that the inhibition effect of tested insecticides on *B. zonata* considers another mode of action of these insecticides on such pest.

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Received for publication December 5, 2009
Accepted after corrections February 20, 2011

Corresponding address:

Dr. Yahia Youssef Mosleh, Suez Canal University, Faculty of Agriculture, Plant Protection Department, Ismailia 41522, Egypt
tel.: + 20 643 271 25, e-mail: yahia.mosleh@voila.fr