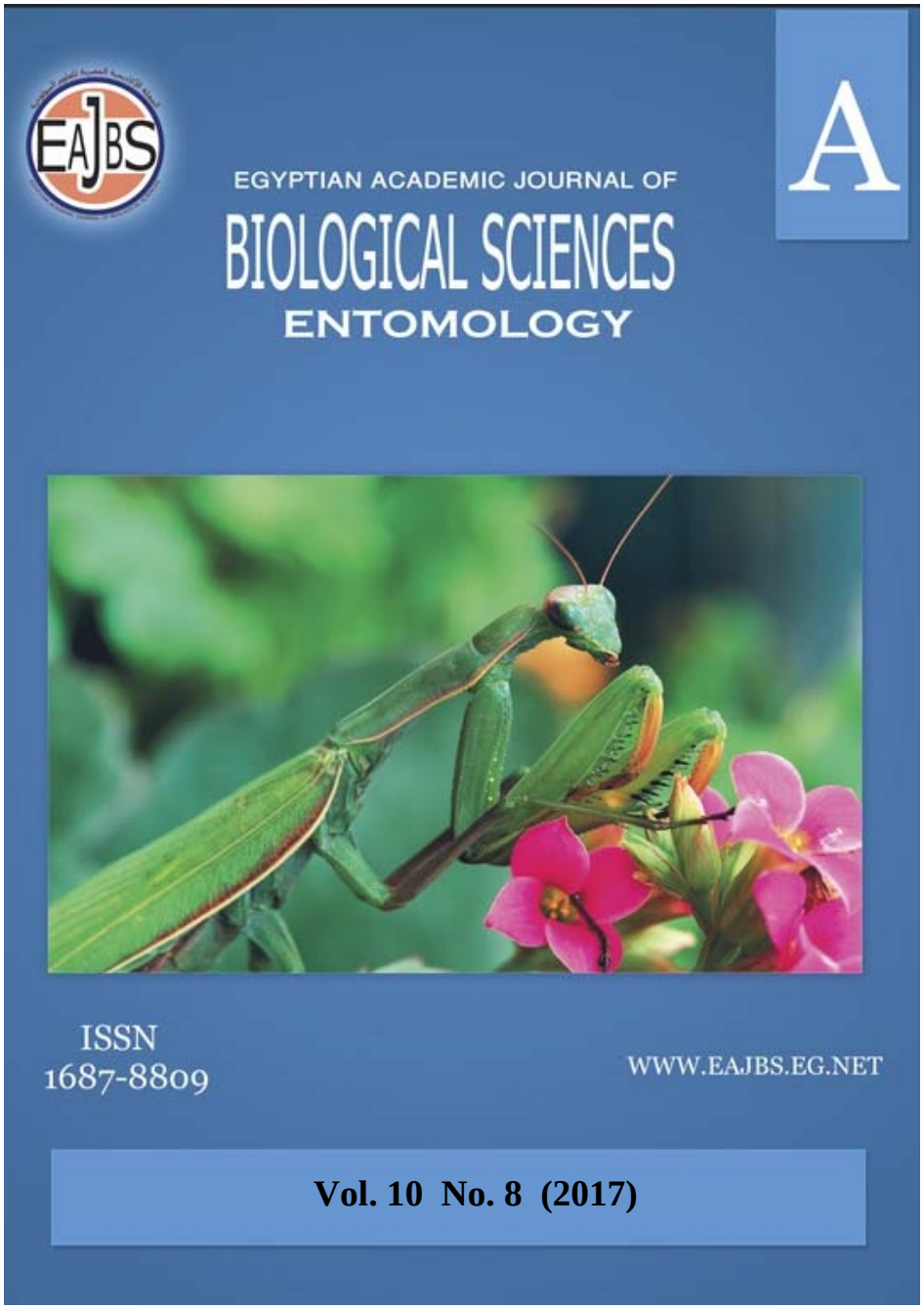

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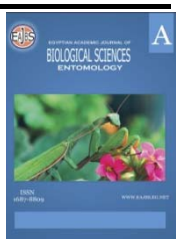

Insecticidal Activity and Biochemical Effects of Two Bioinsectidal on *Bactrocera zonata* (SAUNDERS) (Diptera: Tephritidae)

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ABSTRACT

Two bio-insecticides, Biomctin and Tracer were evaluated for their toxicity as baits against peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), in the laboratory, also their effects on some biochemical constituts of the fly were investigated. The results showed that, both Biomectin and Tracer showed toxic effects to *B. zonata* flies with LC_{50} values of 2.39, 0.45 and 87.62, 5.61 ppm and LC_{90} to 849, 83.85 and 11359, 139.45 ppm at 24 hrs and 48 hrs, for Biomctin and Tracer respectively. Results of biochemical analysis showed that, the amount of protein level decreased in treated flies to 54.5 and 29.2 mg/g. b.wt of Biomectin and Tracer respectively compared to 61.1mg/g. b.wt in the control. The activity of acetylcholine esterase also decrease in treated individuals to 692 and 727 µg/g.b.wt respectively compared to untreated ones 738 mg/g.b.wt. Level of alpha-esterases increased to 730.7 and 573 µg/g.b.wt. compared to control flies to 563.3 µg.g.b.wt. while the amount of beta-esterase showed no significant difference 193 and 182 µg/g.b.wt respectively after treatment compared to 189 µg/g.b.wt in the untreated flies. The amount of glutamic oxaloacetic transaminase (GOT) decreased in treated flies 28.7 and 43.7 Ux 10^3/g.b.wt less than the untreated 113.3 Ux 10^3/g.b.wt, while the amount of glutamic pyruvic transaminase (GPT) increased after treatment with Biomectin and decreased after treatment with Tracer 2707 and 1582 Ux 10^3/g.b.wt, respectively compared to control ones 1937 Ux 10^3/g.b.wt. Level of phenoloxidas increased in treated individuals 19.1 and 17.6 O.D. units/min/g.b.wt, respectively, than control 12.9. Level of lactate dehydrogenase also more in treated flies 718 and574 Ux 10^3/g.b.wt more than control 496.3 Ux 10^3/g.b.wt. This study improves that the two bio-pesticides cause toxic effects and biochemical changes in *B. zonata* and this may help to use them in its control instead of chemical insecticides.

INTRODUCTION

Peach fruit fly, *Bactrocera zonata* (Saunders) is considered one of the most serious and destructive fruit pests in the world due to the losses caused by larvae as they feed and live in the fruit of host plants. It is a polyphagous insect; it attacks several kinds of fruit species including mango, peach, guava, apple and tomato (White & Elson-Harris, 1992 and Hashem *et al*., 2001). The two bio-insecticides, Biomectin and Tracer were recommended to control a variety of insects, both of them affect the insect's nervous system, shortly after exposure, the insects become immobile.

Biomectin (abamectin-avermectin) insecticide is a natural fermentation product produced by a soil microorganism (bacterium) *Sterptomycetes avermitilis* (Campbell, 1989). Tracer (active ingriedient spinosad) which is classified as biopesticide (Copping and Menn, 2000) is produced by the fermentation process from a soil bacterium *actinomycete, Saccharopolyspora spinosa.,* demonstrates lower mammalian and environmental toxicity with low risk to human and wild life than tradional insecticides (Dow Elanco, 1994) and rapid degradition in the environment (Thompson *et al*., 2000). The present study aimed to evaluate toxicity as baits and biochemical effects of two bio-insecticides (Biomectin and Tracer) against the peach fruit fly *B. zonata*.

MATERIALS AND METHODS

Insects:

The insects used in this study were obtained from the laboratory colony reared in the Horticulture Insects Department, Plant Protection Research Institute, Dokki, Giza, Egypt. The flies were provided with sugar and enzymatic yeast hydrolysate at ratio 3:1, respectively and water (El-Sayed, 1979).

Bioassay procedure:

In this assay the susceptiblity of *B. zonata* flies to two bio-insecticides was evaluated. Biomectin 5% and Tracer 24% were used in different concentrations with water. Three replicates of 10 pairs of flies (one week old) were used for each concentration. The flies were placed in plastic jars (9cm diameter and 23cm height) and were deprived from food and water for 2 hours. Ten ml of each concentration were added to a piece of cotton in a plastic vail and placed in each jar as a source of water, jars were supplied with adult's food and closed with muslin and rubber band.

The experiment was maintained at 25± 1C° and 70 ± 5 R.H. After 24 hrs. the cotton piece was replaced by other with water only. Number of dead flies were recorded after 24 and 48 hrs. Probit analysis was used to calculate LC_{50} of the two used insecticides.

Biochemical analysis:

One gm of *B. zonata* adults were used after treatment with LC_{50} (at 24 hrs) of the two bio-insecticides.

Preparation of insects for analysis:

The insects were prepared as described by Amin (1998). Then they were homogenized in distilled water (50 mg/ml). Homogenates were centrifuged at 8000≥ rpm. p.m for 15 minutes at 2°C in a refrigerated centrifuge. The deposits were discarded and the supernatants which is referred as protein and enzyme extract can be stored at least one week without appreciable loss of activity when stored at 5°C.

Determination of total protein

Total proteins were determined according to the method of Bradford (1976). Protein reagent was prepared by dissolving 100 mg of Goomassie Brilliant blue G-250 in 50 ml 95% ethanol, then 100 ml of phosphoric acid (85% w/v) were added. The resulting solution was diluted to a final volume of 1 liter. Sample solution (50 ml) or for preparation of standard curve 50 ml of serial concentrations containing 10 to 100 Mg bovine serum albumin were pipetted into test tubes. The volume in the test tube was adjusted to 1 ml with phosphate buffer (0.1M, PH6.6). Five millimeters of protein reagent were added to test tube and the contents were mixed either by inversion or vortexing. The absorbance 595 nm was measured after 2 min and before 1 hrs against blank prepared from 1 ml of phosphate buffer and 5 ml protein reagent.

Determination of enzyme :

Acetylchoinesterase (Ach E):

Ach E activity was measured according to the method described by Simpson *et al*. (1964), using acetyl choline bromide (A ch Br) as substrate.

Non specific esterases:

Alpha esterases (α- esterases) and beta esterases (β- esterases) were determined according to Van Asperen (1962) using α- naphthyl acetate or β- naphthyl acetate as substrate, respectively.

Transaminases:

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were colorimetrically determined according to the method of Reitman and Frankle (1957).

Phenoloxidase:

Phenoloxidase activity was determined according to a modification of Ishaaya (1971).

Lactate dehydrogenase (LDH):

The method preformed here is derived from the formulation recommended by the German Society for clinical chemistry (DGKC, 1972).

Statistical analysis:

The mortality percentages were corrected according to Abbott's formula (Abbott, 1925). Probit analysis was used to calculate Lc_{50}, Lc_{90} values and the slope of regression lines (Finney, 1971). Sun formula was used to calculate the toxicity index (Sun, 1950).Analysis of variance (ANOVA) (SAS Institute, 1988) was also used to determine differences between means of biochemical results.

RESULTS AND DISCUSSIONS

Toxicity of bio-insecticides:

Results in Table (1) and Figure (1) showed that, the toxicity of Biomectin and Tracer against *Bactrocera zonzta* adults after 24 and 48 hours of exposure. Data in the table indicated that values of Lc_{50} and Lc_{90} of Biomectin were 2.39, 849 ppm and 0.45, 83.85 ppm at 24 hrs. and 48 hrs.of treatment, respectively while, Tracer recorded, values of LC_{50} and LC_{90} of 87.62, 11359 ppm and 5.61,139.45ppm at 24 and 48 hrs, respectively. Based on LC_{50} and LC_{90} values of the tested bio-insecticides, Biomectin had the lowest values which lower than that obtained for Tracer, so Biomectin was more toxic to *B. zonata* adults as batis than Tracer. The results also indicated that both tested bio-insecticides, Biomectin and Tracer showed toxic effects to *B. zonzta* flies as baits. Abamectin was effective for controlling the apple maggot fly, *Rhagoletis pomonella* (Diptera: Tephritidae) compared to the two organophosphate insecticides (Diazinon and Dimethoate) under laboratory conditions (Hu *et al*., 2000). Also spinosad was used successfully under field condition for controlling fruit fly, *Ceratitis capitata* and named as environmentally friendly malathion replacement (Peck and Mcquate, 2000). Also Fetoh *et al*. (2009) reported that both bio-insecticides, spinosad and proclaim exhibited an ability to kill *B. zonata* adults as baits but spinosad was more effective than proclaim. Manrakhan *et al*., (2013) reported that, spinosad was found to induce the highest mortality on female Mediterranean fruit flies *C. capitata* with mortality reaching above 80% after 24hrs. when combined with a solution of either 2% Hymlure (a protein based attractant) or 10% Hymlure. Farag (2014) mentioned that different concentrations ranged from $2.3x10^{7}$ to $2.3x10^{11}$ of entomopathogenic fungi *Metrahizium anisopliae* and *Beauveria bassiana* caused mortality to *B. zonata* and *C. capitata* adults.

Table 1: Toxicity of two bio-insecticides Biomectin and Tracer as baits against *Bactrocera zonata* adults after 24 and 48 hours of treatment.

Tested bio-insecticides	After 24 hrs					After 48 hrs				
	LC_{50} (ppm)	LC_{90} (ppm)	Slope± S.E	Toxicity Index		LC_{50} (ppm)	LC_{90} (ppm)	Slope± S.E	Toxicity Index	
				Lc_{50}	Lc_{90}				LC_{50}	LC_{90}
Biomectin	2.39	849	0.5± 0.07	100	100	0.45	83.85	0.56± 0.083	100	100
Tracer	87.62	11359	0.61± 0.09	2.73	7.47	5.61	139.45	0.92± 0.14	8.02	60.12

1- Biomectin 48 hr 2- Biomectin 24 hr 3- Tracer 48 hr 4- Tracer 24 hr

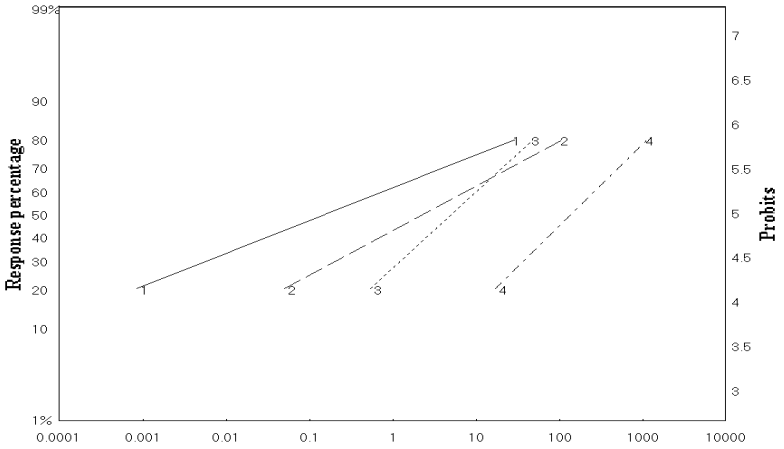

Fig. 1: LC-P lines of two bio-insecticides Biomectin and Tracer against *Bactrocera zonata* adults after 24 and 48 hours post treatment.

As shown in Table (1) and Fig. (1) after 24 hrs and 48 hrs of treatment, the slope values of the tested bio-insecticides were 0.5, 0.56 and 0.61, 0.92 for Biomectin and Tracer respectively. The two tested compound showed nearly paralled toxicity lines.

Respecting the toxicity index, results indicated that, Biomectin was the standard recording 100% efficiency; while efficacy of the other insecticide (Tracer) was lower than the standard by 2.73 and 8.02% at 24 hrs and48 hrs, respectively.

Biochemical analysis:

Effect on total protein:

Data in Table (2) indicated that, the two bio-insecticides treatment high significantly decreased the amount of total protein in *B. zonata* adults (54.5 ± 0.93) for Biomectin and (29.2 ± 1.05) for Tracer compared to control (61.1± 1.2). Fhamy (2000) reported that, bio-pesticides action reacts on different amino acids resulting in the production of subfractionation of the main protein types which may a ssume to be altering tissue function. Also, another researchers stated that total protein contents in treated individuals (2- days old pupae) of *B. zonata* and *C. capitata* by Dimectin and Neem force were less than that of untreated ones (El-sayed, 2016).

Effect on some enzymatic activities:

Obtained data in Table (2) showed that, the activity of acetylcholine esterase (Ach E) significantly decreased in Biomectin treated *B. zonata* adults (692± 5.04). But, respecting Tracer treated *B. zonata* (727± 6.11) the decrement was insignificant compared to control ones (738± 8.33). Ach E is a key enzyme that terminates never impulses by catalyzing the hydrolysis of neurotransmitter acetylcholine, in the nervous system of various organisms it is well known that, the altered Ach E activity, is one of the main resistance mechanisms in many insect species (Wang *et al*, 2004

and Nathan, 2013). Obtained results are in agreement with Mosleh *et al*., (2011) who reported that when *B. zonata* treated with Malathion, Diazinan, Methoxyfenozoide and Lufenuron, the activities of Ach E decreased compared to untreated adults.

Table 2: Effects of two bio-insecticides Biomectin and Tracer on biochemical constitutes of *Bactrocera zonata* adults.

Tested insecticides	Mean ± S. E							
	Total protein (mg/g.b.wt)	Acetylcholine esterase (µg Ach Br/min/g.b. wt)	Alphae esterase (µ g α-naphthel /min b.wt)	Beta esterase (µ g B-naphthol /min /g.b.wt)	Glutamic Oxaloacetic Transaminase (GOT) ($Ux10^3$/g.b.wt)	Glutamic Pyruvic Transaminase (GPT) ($Ux10^3$/g.b.wt)	Phenoloxidases (PO) (O. D. units/ min/g. b. wt)	Lactate dehydrogenase (LDH) ($Ux10^3$/g. b. wt)
Biomectin	b 54.5± 0.93	b 692± 5.04	a 730.7± 13.86	a 193± 4.04	c 28.7± 1.86	a 2707± 72.8	a 19.1± 0.48	a 718± 14.2
Tracer	c 29.2± 1.05	a 727± 6.11	b 573± 4.36	a 182± 3.71	b 43.7± 2.73	c 1582± 34.4	b 17.6± 0.43	b 574± 8.76
Control	a 61.1± 1.2	a 738± 8.33	b 563.3± 6.69	a 189± 2.08	a 113.3± 6.74	b 1937± 84.8	c 12.9± 0.22	c 496.3± 8.57
F	31.36	12.9	103.47	2.88	108.7	129.06	66.75	108.02

Means with the same letter are not significantly different

The amount of α-esteras (Table 2) was significant increased in treated flies, for Biomectin (730.7± 13.86), but insignificant (573± 4.36 and 563.3±6.69) for Tracer and control respectively. On the other hand, no found significant difference between treated flies of the Biomectin (193±4.04), Tracer (182±3.71) and (189±2.08) for control. Increasing activity of enzymes such as esterases have been shown to protect insect from insecticide-poisoning as a part of defence mechanism or added stress on enzymes expression system to synthesize new and higher amount of detoxification enzymes where could be possible reasons for the arrested growth and mortality (Wheeler and Isman, 2000).

In Table (2) showed that, Glutamic oxaloacetic transaminase (GOT) amount high significantly decreased in treated flies, (28.7±1.86) and (43.7±2.73) for Biomectin and Tracer compared to untreated ones (113.3±6.74), respectively. While the amount of glutamic pyruvic transaminase (GPT) varied in treated flies, the activity of the enzyme high significantly increased in case of Biomectin (2707±72.8) and high significantly decreased in case of Tracer (1582±34.4) compared to the untreated flies (1937±84.8). The level of GOT of adult males and females of *B. zonata* in 24, 48 and 72 hrs., post treatment with Malathion, Diazion, Methoxyfenozoid and Lufenuron increased compared to untreated adults (Mosleh *et al*., 2011).

The present study also show high significantly increase in the activity of phenoloxidase (PO) enzyme in both bio-insecticides treatments, (19.1±0.48) and (17.6±0.43) for Biomectin and Tracer respectively, compared to control (12.9±0.22). These results are supported by Ratcliffe *et al*., (2004) who reported that insect PO is helpful in combating environmental stress from parasites, fungi and bacteria as one of their innate immune mechanisms. Also, among innate immune system factors, PO is critical in insect's defence (Ajamhassani *et al*., 2012 and Lavine & Strand 2002).

The activity of lactate dehydrogenase enzyme (LDH) high significantly increased in treated *B. zonata* adults, (718±14.2) and (574±8.76) for Biomectin and Tracer, compared to control ones (496.3±8.57) respectively and this may be due to utilization or censumption of biopesticides, LDH is an important enzymes present in

virtually all animals tissue (Kaplan and Pesce 1996), it is also involved in carbohydrate metabolism and has been used to indicate exposure to chemical stress (Diamantiro *et al*., 2001), and these reports supports the obtained results.

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