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Impact of Certain Compounds on Toxicity and Latent Biochemical Effects of Some Soil Born Stages of the Peach Fruit Fly, *Bactrocera zonata* (Saunders)

[1]Saneya R.M. Farag, [1]Ghada M.A. Morsi and [2]Gamila Sh. Selem

[1]Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt
[2]Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt

Abstract: The toxicity effect of the bio-insecticide, Biomectin 5%EC and the insect growth regulator, Novo 10% DC on some soil born stages of the peach fruit fly *Bactrocera zonata* (Saunders) was evaluated as well as, their latent effects on some biochemical constituents of newly emerged adults resulting from treated full-grown larvae. The results indicated that, the two compounds showed toxic effects to full- grown larvae of *B. zonata*. Recorded values of LC_{50} and LC_{90} for Biomectin were lower than those recorded with Novo. When 1-day and 5-day old pupae treated in sand with the two tested compounds, recorded values of LC_{25} and LC_{50} for Novo were lower than those obtained with Biomectin. 1-day old pupae were more susceptible to the two tested compounds than 5-day old pupae. The results clearly showed that, full-grown larvae were more susceptible to the two compounds than 1-day and 5-day old pupae. Biochemical analysis revealed that, the amount of total protein and total carbohydrates were markedly decreased in newly emerged adults resulting from full- grown larvae treated with the two compounds compared with untreated control. The activity of glutamic oxaloacetic transaminase (GOT) decreased with high significance and also that of glutamic pyruvic transaminase (GPT) was significantly decreased for flies resulting from larvae treated with Biomectin. On the contrary, values of GOT and GPT were significantly increased for flies resulting after larval treatment with Novo compared with untreated control. Value of alkaline phosphatase was highly significantly decreased with the two compounds while, acid phosphatase was significantly decreased with Biomectin and no significant increment found with Novo. The amount of alpha-esterases and beta –esterase were highly significantly decreased with Biomectin. No significant decrement in the activity of phenol oxidase enzyme was recorded when either Biomectin or Novo were used. The present study proved that, both Biomectin and Novo cause death of different soil- born stages of *B. zonata* and alteration in biochemical contents of newly emerged adults resulting from treated full-grown larvae, so they can be used in the control of this insect.

Key words: IGR -bioinsecticide · Toxicity · *Bactrocera zonata* · Biochemical effects

INTRODUCTION

The peach fruit fly, *Bacterocera zonata* (Saunders) (Diptera: Tephritidae) is considered one of most serious and destructive insect pests of fruits over the world. It is a polyphagous pest that infests many horticultural fruits and some vegetables [1]. It was recorded for the first time in Egypt in 1998 [2]. Recently, it became a serious pest that cause a great loss of fruit production annually [3]. Soil treatment with insecticides beneath host trees may play an important role in killing fruit fly larvae or pupae to suppression of fruit fly population as an item in eradication programme [4]. Because soil treatment has resulted in less or no residues on fruits, so the purpose of this work attempted to study: The toxicological effects of soil treated with the bio-insecticide, Biomectin 5%EC and the insect growth regulator Novo 10 % DC on some soil born stages (full-grown larvae, 1-day and 5-day old pupae) of *B. zonata.* Latent biochemical effects on newly emerged adults resulting from full -grown larvae exposed to soil treated with the tested compounds.

MATERIALS AND METHODS

Insects Rearing: The peach fruit fly *B. zonata* was obtained from the rearing laboratory of Horticultural

Corresponding Author: Saneya R.M. Farag, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt

Insect Research Department, Plant Protection Research Institute, Agricultural Research Center (ARC), Giza, Egypt. The insect larvae were reared according to Afia [5] using artificial diet consisted of 3 g sodium benzoate, dissolved in 500water and 3 g citric acid, 84.50 g sugar, 84.50 g brewer's yeast and 330 g wheat bran, were carefully mixed in a large plastic container. Then eggs were scattered on the surface of the diet which was placed in small plastic plates that were tightly covered with muslin cloth After that the plates were placed in a large cage containing sand to allow the popping larvae to pupate. Adult flies were provided with food (sugar and enzymatic yeast hydrolysate in a ratio 3:1, respectively) [6]. In the present investigation full-grown larvae, were collected immediately after leaving diet to pupate.

The Tested Compounds:

Biomectin 5% EC (active ingredient abamectin 5% wt/v). Chemical name: a mixture containing a minimum of 80 % avermectin B_{1a} (i): 5-O-demethylavermectin B_{1a} and a maximum of 20% avermectin B_{1b} (ii). 5-O-demethyl-25-de (1-methylpropyl)- 25- (1- methyl ethyl) avermectinB_{1b}. Produced by Heappy-Gowanlong-Agrochemical-Lemeted, China company.

Novo 10% DC (active ingredient fluophenoxyeron), chemical name:

1-(4-(2-chloro-a, a, a, trifluoro-p-tolyloxy)-2-fluorophenyl)-3-(2, 6-difluorobenzoyl)urea. Produced by Soltair company, Egypt.

Bioassay Method: In the present study the susceptibility of some immature stages (full-grown larvae, one day and five day old pupae) of *B. zonata* to the bioinsecticide, Biomectin 5% EC and the insect growth regulator, Novo 10% DC were tested in in plastic cups of 9.5 cm diameter and 8cm high each containing 100gm of fine sand sieved through 2 mm sieve. Different concentrations of each compound with water were prepared separately and 10 ml of each concentration were incorporated into the sand in each cup. Ten full-grown larvae of *B. zonata* were introduced on the surface of the sand which move down into the sand and buried themselves into it while in case of pupae, half of the treated sand was placed in the cup, then ten pupae were introduced to the sand and covered with the rest of sand. The cups were covered with muslin cloth and tightened by rubber bands. Three replicates were carried out for each concentration and three replicates with water only as untreated control. The experiment was conducted under laboratory conditions (25±2°C and 65-70% RH) and left until adult emergence. Number of emerged adults were recorded and mortality percentages were calculated. Probit analysis was used to calculate LC_{50} and LC_{25} of the tested compounds.

Latent Biochemical Effects: In this experiment the effects of Biomectin 5% EC and Novo10% DCon certain biochemical constituents (Total carbohydrates, Total protein) and some enzymatic activities (Alkaline phosphatase, Acid phosphatase, Alpha esterases, Beta esterasese and Phenoloxidases)of newly emerged adults of *B. zonata* that emerged from the full-grown larvae treated with $LC_{25}$of the two compounds were evaluated. About 30 individuals of the emerged peach fly for each compound and a control were collected and immediately kept in a deep freezer for biochemical analysis. All steps of samples preparation and analysis ran at Department of Physiology, Plant Protection, Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

Insect Preparation for Analysis: Preparation of the insects were carried out as described by Amin [7]. Then they were homogenized in distilled water (50 mg/ml)using a chilled glass Teflon tissue homogenizer(ST-2mechanic-preczyina, Poland). Homogenates were centrifuged at 8000 r.p.m. for 15 minutes at 2°C in a refrigerated centrifuge. The deposits were discarded and supernatants were kept in a deep freezer at -20°C till use for biochemical assays. the supernatants which is referred as enzyme extract can be stored at least one week without appreciable loss of activity when stored at less than °C.

Determination of Total Proteins and Carbohydrates: Total carbohydrates were estimated in acid extract of emerged flies by the phenol-sulphuric acid reaction of Dubios *et al.* [8]. Total carbohydrates were extracted and prepared for assay according to Crompton and Birt [9]. Total proteins were determined by the method of Bradford [10].

Determination of Some Enzymatic Activities

Phosphatases: Acid and alkaline phosphatases were determined according to the method described by Powell and Smith [11].

Transaminases: Glutamic pyruvic transaminase [GPT] and glutamic oxaloacetic transaminase (GOT) were determined colorimetrically according to the method of Reitman and Frankel [12].

Non Specific Esterase: Alpha esterases (α-esterases) and beta esterases (β-esterases) were determined according to Van Asperen [13] using α-naphthyl acetate or β- naphthyl acetate as substrates, respectively.

Statistical Analysis: Abbott' s formula, Abbott [14] was used to correct the mortality percentages. Obtained data was fitted to log- probit model according to Finney [15] using LDP line soft wear (Ehab soft). Toxicity index was calculated by Sun formula [16]. Differences between means in biochemical analysis were compared by using one way ANOVA, Duncan's multiple Rang test [17].

RESULTS AND DISCUSSION

Toxicity Assays: The toxicity of the two compounds, Biomectin and Novo against some soil associated developmental stages (full-grown larvae, 1 and 5day old pupae) of *B. zonata* are presented in Table (1) and illustrated in Figure (1). The results showed that, values of LC_{50} for full- grown larvae treated with the two compounds, were 0.927and 32.85 ppm while values of LC_{90} were 3.124 and 215.7 ppm for Biomectin and Novo, respectively, It could be concluded that, Biomectin was more toxic to full-grown larvae than Novo. As shown in Fig. (1) the slope value of LC-P line of Biomectin was 2.43 higher than that of Novo 1.57. Concerning the toxicity index, data in Table (1) clearly show that, efficacy of Novo 10% DC was much lower than Biomectin 5% EC (the standard) by 2.82% at LC_{50}

When 1-day and 5-day old pupae treated in the sand with the tested compounds the obtained data show that, values of LC_{25} and LC_{50} recorded for Novo with the two pupal ages were lower than those obtained with Biomectin. The slope values of LC-P lines of Novo were 0.539 and 0.559 lower than those of Biomectin 0.785 and 0.854 with the two pupal ages, respectively. Concerning the toxicity index results in Table (1) indicated that Novo was the standard recording 100 % efficiency while efficiency of Biomectin was lower than the standard by 15.62 and 62 with the two pupal ages respectively, so Novo10 % DC may possessed higher toxic effect to 1-day and 5-day old pupae than Biomectin 5%EC.

Comparing the susceptibility of the three developmental stages to the two tested compounds, the results cleared that full-grown larvae had the lowest values of LC_{50} and LC_{90}, so full-grown larvae were more susceptible to the bio-insecticide, Biomectin 5 %EC and the insect growth regulator, Novo10 % DC than1-day and 5-day old pupae, these results agree with Croft [18] who reported that, pupae are often less susceptible than larvae to the insecticides.

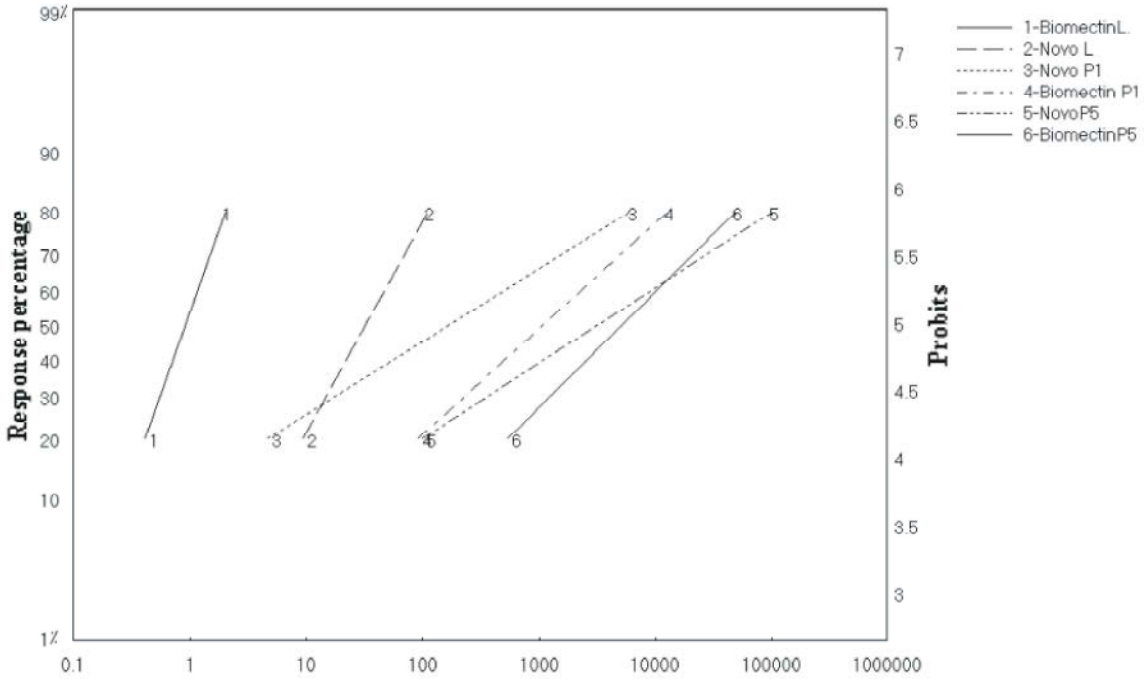
Comparing the susceptibility of the two pupal ages the results in Table (1) indicated that, 1-day old pupae recorded lower values of LC_{25}, LC_{50} and $LC_{90}$with the two compounds than 5-day old pupae, so the first stage was more susceptible than the later stage. The obtained results are in full agreement with Abdel Aziz [19] who showed that, tolerance of immature stages of *C. capitata* to the tested pesticides in sand decrease with increase of age. The obtained results agree also with Attia [20] who reported that, the three insecticides Runner, Movento and spincer exhibited toxicity rate against *B. zonata* full- grown larvae and one –day old pupae with dose dependent.

Latent Biochemical Effects

Total Protein and Total Carbohydrates: Effects of the bioinsecticide, Biomectin 5%EC and the insect growth. regulator, Novo10%DC on the level of total protein content of *B. zonata* adults newly emerged from the full-grown larvae treated with LC_{25} of the two compounds are shown in Table (2), the data showed significant decrease in total protein content in newly emerged adults of *B. zonata* (24.33 ± 0.34) with Biomectin and (26.1±0.55) with Novo compared to (28.7±0.65) with untreated control. The difference between the two treatments was insignificant. The decrement in the amount of total protein may be due to action of the used insecticides, these results agree with Halawa [21] who recorded reduction in protein content of *B. zonata* larvae after treatment with Radiant (Spinosad based insecticide). The results are also in full agreement with Farag [22] who recorded a reduction in the amount of protein level of *B. zonata* flies treated as baits with Biomectin 5%EC and Tracer24%SC. Recently Morsi [23] showed a reduction in the level of total proteins of 1-day old pupae of *B. zonata* treated with *Moringa oleifera* oil, lemon peel oil and *M. oleifera* leaf extract. Data also clear that, the two compounds highly significantly decreased total carbohydrates content (52.8 ± 1.5, 67.8± 1.44 mg /g b.wt) for Biomectin and Novo, respectively compared to(87.8 ± 2.5 mg /g b.wt) for control, this reduction may be due to an increase in consumption of carbohydrates under toxicant stress as an important source of energy. This agree with Piri [24] who treated *Glyphodespyloalis* (Lepidoptera : Pyralidae) larvae with sub - lethal doses of Spinosad and recorded reduction in carbohydrates suggesting the possibility of active glycogenolsis and glycoltic pathway to provide excess energy in stress condition.

Table 1: Toxicity of the two compounds Biomectin 5%ECand Novo10%DC against *Bacterocera zonata* some soil born stages

Insect stage	Tested compounds	LC25 (ppm)	LC50 (ppm)	LC90 (ppm)	Slope ± S.E	Toxicity Index at Lc50
Larvae	Biomectin 5%EC	0.489	0.927	3.124	2.43 ± 0.215	100
	NOVO 10% DC	12.2	32.85	215.7	1.57 ± 0.204	2.82
(1-day) old pupae	Biomectin 5%EC	152.33	1101.4	47252.52	0.785 ± 0.159	15.62
	NOVO 10% DC	9.67	172.02	40823.4	0.539 ± 0.144	100
(5-day) old pupae	Biomectin 5%	855.98	5269.9	166573.6	0.181± 0.854	62.03
	NOVO 10% DC	203.73	3268.8	63766 2.8	0.559 ± 0.1144	100



1-Biomectin with full- grown larvae, 2-Novo with full- grown larvae, 3-Novo with 1-d old pupae
4-Biomectin with 1-d old pupae, 5- Novo with 5-d old pupae, 6- Biomectin with 5-d old pupae

Fig. 1: LC-P lines of Biomectin 5%ECand Novo10%DC against *Bacterocera zonata* some soil born stages

Table 2: Latent effect on total protein and total carbohydrate content in newly emerged adults of *Bacterocera zonata* resulted from full- grown larvae treated with sub- lethal dose (LC_{25}) of Biomectin 5%ECand Novo10% DC

	Mean ± S.E (mg/g body weight)	
Treatment	Total Protein	Total Carbohydrates
Biomectin 5%EC	24.33 ± 0.34 b	52.8± 1.5 c
Novo 10% DC	26.1±0.55 b	67.8± 1.44 b
Control	28.7 ± 0.65 a	87.8 ± 2.5 a
F	17.2	89.1
P	0.0033	< 0. 01

Within the column data with different letters are significant different ($p < 0.05$).

Some Enzymatic Activities: Results in Table (3) revealed that, the amount of glutamic oxaloacetic transaminase (GOT) decreased with high significance in newly emerged adults of *B. zonata* resulting from the full-grown larvae treated with $LC_{25}$of the two tested compounds (1510 ± 245.1) for Biomectin and (2903.3 ± 66.4) for Novo compared to(8136.7±238.1) for untreated control, while the activity of glutamic pyruvic transaminase (GPT) enzyme significantly decreased in flies resulting from larvae treated with Biomectin and significantly increased with those resulting from the full-grown larvae treated with Novo. Mosleh [25] showed that, in 24, 48 and 72hrs., post treatment of adult males and females of *B. zonata* with Malathion, Diazinon, Methoxyfenozoid and Lufenuron, the amount of GOT increased compared to control. The obtained results agree with Farag [22] who reported that, GOT amount high significantly decreased in *B. zonata* flies treated as baits with Biomectin and Tracer compared to control while the amount of GPT varied, high significantly increased in case of Biomectin and high significantly decreased in case of Tracer.

Table 3: Latent effect on some enzymatic activities in newly emerged adults of *Bactrocera zonata* resulted from full –grownt larvae treated with sub -lethal dose LC_{25} of Biomectin 5%EC and Novo 10% DC

	Mean ± S.E						
Treatment	GOT (mU/g.bwt)	GPT (mU/g.b.wt)	Alkaline phosphatase (mU/g.b.wt)	Acid phosphatase (mU/g.bwt)	Phenoloxidases (O.D.units/min/g.b.wt)	Alpha esterases (ugánaphthol/min/g.b.wt)	Beta esterasese (ugânaphthol/min/g.bwt)
Biomectin 5%EC	1510 ± 245.1c	2904.3 ±29.2 b	2071.33±50.9 c	422.33±8.4 b	7.78±0.11 a	318±5.77 b	147.33±2.19 b
Novo10% DC	2903.3 ±66.4 b	3093.3 ±17.6 a	2316.33 ±49.4 b	557±13.5 a	7.4± 0.06 a	436.7±2.85 a	193.33±1.45 a
Control	8136.7 ±238.1 a	2996.7 ±43.3 ab	2663.7±48.3 a	522 ±21.9 a	11.13±0.09 a	426±4.6 a	188.67±3.18 a
F	302.19	7.65	36.07	23.7	0.947	203.9	113.1
P	***	*	***	**	Ns	***	***

Within the column data with different letters are significant different ($p < 0.05$)

As indicated in Table (3), the results also show high significant decrement in the activity of alkaline phosphatase in newly emerged adults, recording (2071.33± 50. 9) with Biomectin and 2316.33 ± 49.4) with Novo compared to (2663.7 ± 48.3) in the control, While the activity of acid phosphatase varied with the two tested compounds, it significantly decreased in case of Biomectin (422.33± 8.4) while non significant increase was noticed in case of Novo (557±13.5) in comparison with control (552±21.9). The obtained results disagree with Abdel Aziz [19] who reported that, the tested bio-insecticides (Emaskin1.9 % EC, Radian 12% SC, Proclaim 5% SG and Spintor 24%SC) significantly increased values of alkaline and acid phosphatase in newly emerged adults of the tested strains of *B. zonata* except in case of Proclaim 5% SG that decreased the activity of acid phosphatase in laboratory strain.

The activity of α-esterase (Table 3) was highly significantly decreased in newly emerged adults resulting from treatment with Biomectin (318± 5.77), while no significant difference was found in those resulting from treatment with the insect growth regulator, Novo (436. 7 ± 2.85) compared to the control (426± 4.6). The same trend was found also in the amount of beta-esterase, highly significantly decreased with Biomectin (147.33 ± 2.19) and no significant difference was found in case of Novo (193.33± 1.45) compared to the control (188.67± 3.18). The alterations occurred in the activity of the enzymes may be due to the defense system as a result of the action of the insecticides to protect the insect from insecticides poisoning and this agree with [26]. The results also agree with Farag [22] who reported that, level of alpha-esterase increased while the amount of beta- esterase showed no significant difference in *B. zonata* adults after treatment as baits with the bio-insecticides, Biomectin and Tracer compared to untreated adults. Data in Table (3) indicated also that, the amount of phenoloxidase (PO) activity decreased after both treatments but, there is no significant difference in case of Biomectin (7.78 ± 0.11) and Novo (7.4± 0.06) compared to (11.13± 0.09) in the control. PO enzyme is one of innate immune mechanisms in insects and is helpful in combating environmental stress [27]. The obtained results disagree with Farag [22] who illustrated a high significant increase in the activity of PO enzyme in *B. zonata* flies treated as baits with two bio-insecticides. The previous results indicated that both Biomectin and Novo cause changes in some biochemical constituents of *B. zonata* adults resulting from treated full- grown larvae.

CONCLUSION

This study was conducted in the laboratory; future study will be conducted in the field to show the efficacy of the two compounds in the field, the most effective dose and the suitable time of application.

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