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Toxicological and Biochemical Effects of Two Biopesticides on the Peach fruit fly, *Bactrocera zonata* Saunders (Diptera: Tephritidae)

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ABSTRACT

Different concentrations of the two biopesticides; spinosad and proclaim, were tested to evaluate their insecticidal effects against the adults of peach fruit fly, *Bactrocera zonata* Saunders. Moreover, their impact on some biochemical parameters of the adults of the fly. Recorded results showed that both spinosad and proclaim were effective on *B. zonata*. LC_{25s} was 0.156 and 0.233, LC_{50} was 0.42 and 0.620mg/l and LC_{90} was 2.909 and 3.978mg/L for spinosad and proclaim, respectively. Spinosad was more potent than proclaim on *B. zonata*. Toxicity results were correlated with some biochemical parameters such as electrophoratic patterns of protein and some isozymes. SDS-PAGE of protein showed a pattern of 7 normal bands in the control, while spinosad and proclaim treated flies showed 10 and 11 bands, respectively, with molecular weights ranged from 122.0 to 12.5 KDa. Treatment with spinosad and proclaim resulted in absence of two bands (9 and 10), with molecular weights 20.5 and 19.2 KDa. Proclaim treated flies were characterized by the presence of unique protein band number 4, with a molecular weight 51.7 KDa. Isozymes electrophoresis showed various changes as a result of utilization of spinosad than proclaim on *B. zonata*. α , β-esterases and Acid phosphatase showed 20, 17 and 20% polymorphism compared with control, respectively, while the most isozymes affected by biopesticides treatment were recorded in case of alcohol dehydrogenase, which showed changes 71 and 43% polymorphism in both of spinosad and proclaim, respectively. No effect appeared on aldehyde oxidase. This application may help to use biopesticides instead of conventional chemical pesticides to control *B. zonata* and to throw light on the role of some isozymes in the modes of action of these biopesticides.

Key words: Biopesticides, Spinosad, Proclaim, *Bactrocera zonata*, Toxicology, Biochemistry, Electrophoresis, Protein and Isozymes.

INTRODUCTION

The peach fruit fly, *Bactrocera zonata* Saunders is a newly recorded pest in Egypt (El – Minshawy *et al.*, 1999). Four hundred species belonging to genus *Bactrocera* are widely distributed in tropical Asia, South Pacific and Australia, but very few species of the same genus were recorded in Africa (White and Elson – Harris, 1994). *B. zonata* is one of the most serious polyphagous insect pests. It attacks a large host range of fruits and vegetables. According to FAO/IAEA report (2000) the economic loss of *B. zonata* infestation to horticultural plant fruits was estimated as 190 million EUR/year in Egypt. The natural products spinosad and proclaim with their insecticidal actions were recommended to control a variety of insects. The first record of toxic symptoms were death to involuntary muscle contractions caused by excitation of the central nervous system (Salgado, 1998). Spinosad is an insecticidal toxin of two macro cyclic lactones called spinosyns A and D, derived from metabolites of the actinomycete, *Saccharopolyspora spinosa*. This product is effective against many dipterous and lepidopterous pests (Sparks *et al.* 1998). Spinosad is effective against some fruit flies in family Tephritidae, especially the Med fly, *Ceratitis capitata* into protein baits at doses as low as 1 ppm active ingredient (Peek and Mcquate, 2000). Proclaim is a high potency insecticide, the second generation in the Novarties Avermectin family, its unique mode of action and physic-chemical properties are providing a superior performance against a broad range of lepidopteran at very low doses. Proclaim (Ememectin Benzoate) is a semi-synthetic Avermectin insecticide derived from the fermentation product Abamectin. Avermectins are gained from a naturally occurring soil bacterium, *Streptomyces avermitilis* (Jansson *et al.*, 1997).

The present work aimed to evaluate the toxicological and biochemical effect of some biopesticides (spinosad and proclaim) on the peach fruit fly, *B. zonata*.

MATERIALS AND METHODS

A culture of adult flies of *B. zonata* was collected from infested Guava fruits at Ismailia governorate in October, 2008, maintained in the laboratory at 25±1°C and 65±5 % R. H., fed on a mixture consisted of 25% yeast hydrolysis enzymatic powder and 75% sugar in tap water. Spinosad and proclaim were used in different concentrations 0.10, 0.25, 0.50, 1.00 and 2.00 mg/L. Three teaspoonful of sugar (nearly 30g) were added to each concentration and stirred till sugar dissolved.

Three replicates of 50 flies each (two weeks old) were used for each concentration. Flies were removed from the stock cage with the aid of a vacuum hose and place in glass jars (750 ml) covered with muslin cloth. Flies were deprived from food and water for 24 hours. Each concentration of the spinosad and proclaim sugary solution was applied to each jar in a small bottle (3 x 2.5 x5 cm) contained cotton wick piece used as a source of food and water. For biochemical experiments LC_{50} of spinosad (S) and proclaim (P) were used.

Determination of median lethal concentration (LC_{50}):

Percentage mortality of the treated flies after 24 hours was calculated, corrected by using Abbott's formula (1925) and statistically computed according to Finney (1971). Computed percentages mortality were plotted with the corresponding concentrations probity (Ldp Lines).The lethal concentrations of 50% were determined for drawing regression lines. Also, toxicity index was calculated according to the equation of Sun (1950).

Bio-chemical experiments:

Protein electrophoresis:

SDS-polyacrylamide gel of denautrated protein electrophoresis was performed in 10% acrylamide slab gel following the system of (Laemmli 1970); protein extraction was conducted by homogenizing 0.2 gm samples with 0.5 ml saline solution in a mortar and pestle. The mixture was transferred to clean eppendorf tube then centrifuged at 10.000 rpm for 10 min. The supernatant of each sample (contained protein extract) was kept at -20°C until use for protein and isozymes electrophoic analysis, then a volume of 20 µl of protein extract was added to equal volume of buffer, then 25 ml of each sample of proteins was loaded in the gel.

Isozymes:

Effects of spinosad and proclaim on some biochemical parameters *i.e.*, α-and β-esterases (est.), Acid phosphatase (Acph.), alcohol dehydrogenase (Adh.) and of aldehyde oxidase (AO.) were determined. Isozymes were separated in 10% Native–polyarylamide gel electrophoresis as described by Stegemann *et al.*, (1985). 50 ml of each extract was mixed with 25 ml of buffer and 50 ml of this mixture was applied to the well. For gel staining, Scandalios protocols (1964) was used for α and β – est., Wendel and Weeden (1989) for Ao and Acph; Weeden and Wendel (1990) for Adh. Gels were washed two or three times with tap water and destained in ethanol 20% and glacial acetic acid (9: 11 v/v) for 24 hour and photographed. Changes occurred by biopesticides (polymorphisms %) in the isozymes were calculated according to the equation:

$$\text{Polymorphism \%} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

RESULTS AND DISCUSSION

Toxicological effects:

Results in Table (1) showed that the different concentrations of spinosad and proclaim exhibited an ability to kill *B. zonata* adults at low concentrations ranged from 0.10 to 2.00 mg/L. Percentages morality increased ascendingly with heightened of the concentration till reached the maximum of 91.3 and 87.3% for both of spinosad and proclaim (at the concentration of 2.00 mg/L). Both biopesticides were potent to *B. zonata* but spinosad was more effective than proclaim.

Results in Table (2) included the relative toxicity of spinosad and proclaim against *B. zonata*. Twenty five percent (LC_{25}) of the peach fruit fly were killed by 0.156 and 0.233 mg/L, LC_{50s} values were 0.428 and 0.620 mg/L, LC_{90s} values were 2.909 and 3.978 mg/L for spinosad and proclaim, respectively. Regarding the toxicity index referring to spinosad than proclaim was 100% after 24 hours, while in case of proclaim, it was 69.032. Figure (1) shows the probit lines of predicted percentage of death of *B. zonata*. Obtained data indicated that spinosad and proclaim were potent for controlling *B. zonata* as baits and can be successfully applied at a concentration not less than 0.4 mg/L for spinosad and 0.6 mg/L for proclaim.

Table (1): Percentage mortality of different concentrations of spinosad and proclaim as baits against *B. zonata*, showing % mortality.

Concentrations mg/L	Spinosad	Proclaim
0.10	25.0±7.3 (16-34)	14.0±4.3 (8-18)
0.25	31.3±5.0 (26-38)	26.7±6.6 (18-34)
0.50	45.3±4.9 (40-52)	40.0±4.3 (36-46)
1.00	70.0±7.1 (64-80)	55.3±5.0 (50-62)
2.00	91.3±6.6 (84-100)	87.3±2.5 (84-90)

Number between the brackets refers to the range.

Table (2): LC_{25}, LC_{50} and LC_{90} for spinosad and proclaim as baits against *B. zonata*.

Toxicity parameters	Spinosad	Proclaim
LC_{25} (mg/L)	0156	0.233
95% FL	0.043-0.202	0.089-0.360
LC_{50} (mg/L)	0.428	0.620
95% FL	0.209-0.798	0.364-1.183
LC_{90} (mg/L)	2.909	3.978
95% FL	2.696-17.451	3.499-20.578
Index	100	69.032
Slope	1.54	1.59

Index compared with spinosad.

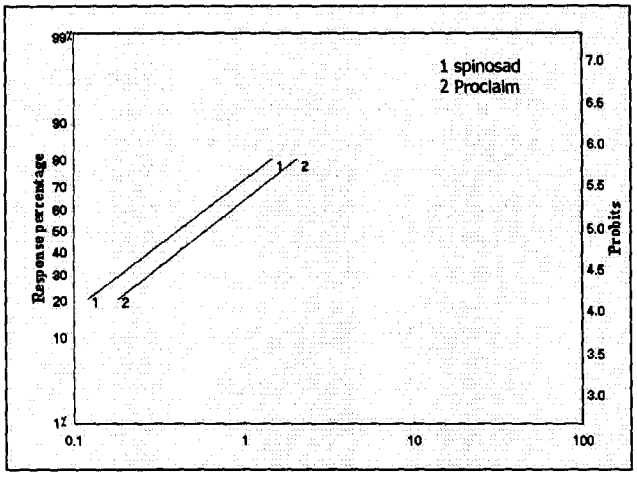


Fig. (1): Log- probit curve of predicted percentage of death of *B. zonata* treated with spinosad and proclaim.

In the same trend, Hu *et al.*, (2000) reported that abamectin was effective for controlling the apple maggot fly, *Rhagoletis pomonella* (Diptera: Tephritidae) compared with two organophosphate insecticides (Diazinon and Dimethoate) under laboratory conditions. Also, Peck and Mcquate (2000) used spinosad successfully for controlling the Med fly, *C. capitata* under field conditions and named spinosad as environmentally friendly malathion replacement. Also, Fahmy, (2005) stated that Spinosad had an adulticidal activity for either both sexes of *Spodoptera littoralis* adults.

Biochemical parameters:

i- Protein in SDS-PAGE:

Obtained data show that the protein of adult tissues of untreated samples of *B. zonata* had 7 normal bands in the control. Using spinosad and proclaim caused appearance and disappearance of some protein bands, treated flies with spinosad had 10 bands while treated flies with proclaim had 11 bands. Control had bands number 1, 2, 9, 10, 11, 12 and 13, with molecular weights 122.0, 102.0, 20.5, 19.2, 16.3, and 12.5 KDa, respectively. Treated flies with spinosad and proclaim confirmed absence bands number 9 and 10 with molecular weights 20.5 and 19.2 KDa, respectively.

Samples treated with proclaim was characterized by the presence of unique protein band number 4, with a molecular weight 51.7 KDa. Treated samples with spinosad and proclaim had new bands not found in the untreated flies, these bands were 3, 5, 6, 7 and 8 with molecular weights 90.3, 25.0, 24.0, 22.4 and 21.5 KDa. Table (3) and Figs (1 & 2). These changes in the defractionated protein profile may reflect the insecticidal actions of the bioinsecticides in the treated adults being relatively higher with proclaim than those treated with spinosad. This variable protein profile was found as development of new protein bands of different molecular weights. This observation may reveal that the bioinsecticidal action reacts on different amino acids resulting in the production of subfractionation of the main protein types which may assume to be altering their tissue function Fahmy (2005).

Table (3): Quantative and qualitative protein patterns of *B. zonata* treated with spinosad and proclaim.

Band Number	Molecular Weight	C	S	P
1	122.0	+	+	+
2	102.0	+	+	+
3	90.3	-	+	+
4	51.7	-	-	+
5	25.0	-	+	+
6	24.0	-	+	+
7	22.4	-	+	+
8	21.5	-	+	+
9	20.5	+	-	-
10	19.2	+	-	-
11	16.3	+	+	+
12	14.0	+	+	+
13	12.5	+	+	+

C = Untreated samples, S = Treated samples by spinosad, P = Treated samples by proclaim, M.W. = Molecular weight in KDa, (+) = Band present and (-) = Band absent.

The development of new fractions on SDS PAGE of the extracted proteins of only the treated adults and not in the control ones may be considered as insecticide specific and this might be as a result newly synthesized proteins due to the effect of insecticidal action. This was more pronounced in case of proclaim than spinosad adult treatments. The presence or absence of such protein bands on fractionation was also reported by Eid *et al.*, (1979) and Abdel Fattah and Khaled (2008).

ii- Isozymes electrophoresis:

Obtained results in Tables (4 & 5) show that the spinosad was the most effective on different isozymes of *B. zonota*. Electrophoretic patterns of α-esterase revealed that 5 bands were present in the control and treated samples with proclaim. Samples treated with spinosad had 4 bands only. Percentage polymorphism induced in samples treated with spinosad was 20%. (Fig. 4). Electrophoretic patterns of β-esterase electrophoresis showed 6 bands for control and treated samples with proclaim. In treated samples with spinosad 5 bands only were present; the percentage of polymorphism was 17% (Fig. 5). El-Bokl (2003) reported that the presence of both quantitative and qualitative differences between the esterase patterns of treated and untreated larvae of *Culex pipiens* after treatment with different insecticidal agents indicated that this esterase may

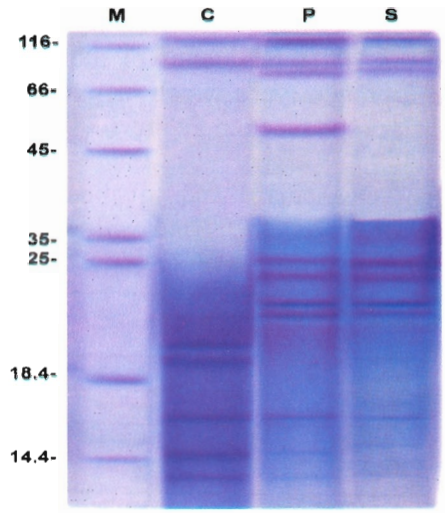


Fig. (2): SDS polyacrylamde gel of denaturated proteins patterns of *B. zonata.*

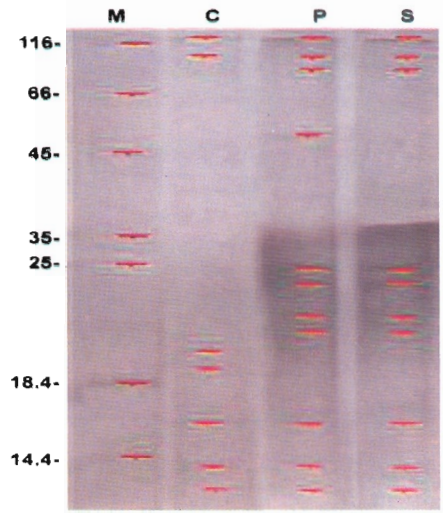


Fig. (3): Protein patterns of *B. Zonata.*

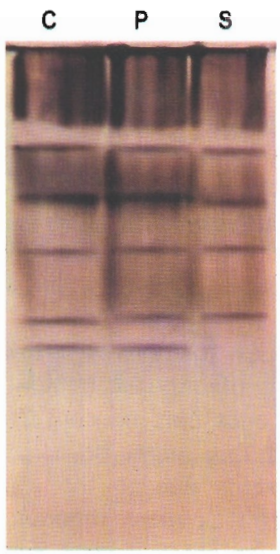


Fig. (4): α–Est Electrophoretic patterns of *B. zcnata.*

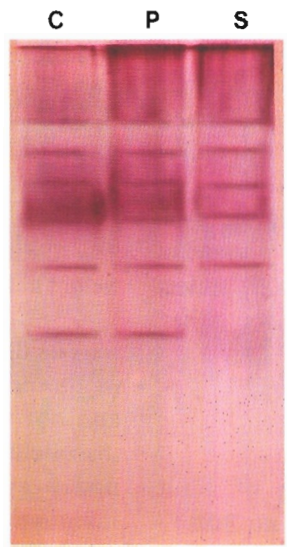


Fig. (5): β-Est Electrophoretic patterns of *B. zanata.*

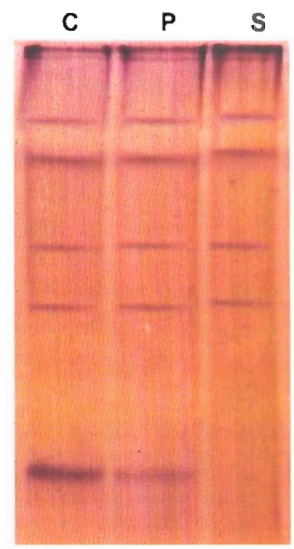
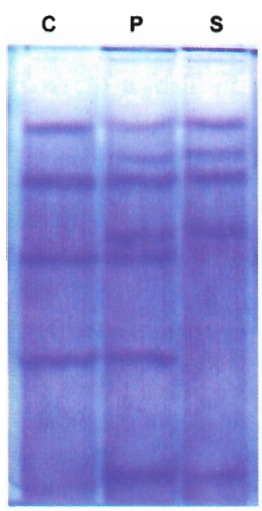
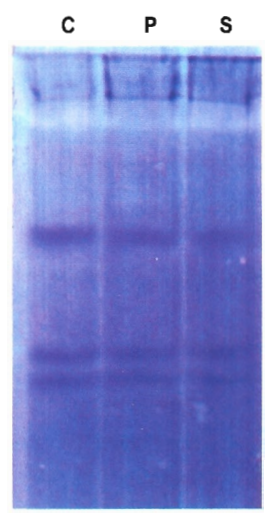


Fig. (6): Acph Elecirophoretc patterns of *B. zonata.*



Fg (7): Adh Eectrophoretc patterns of *B. zonata.*



Fig, (8): Ao. Eeotrophorehc patterns of *B. zonata.*

M= Molecular size marker C=Control P=Proclaim treated insects S=Spinosad treated insect

Table (4): Qualitative patterns of different isozymes for *B. zonata* treated by spinosad and proclaim.

Isozymes	Band Number	Sample C	P	S
α- est	1	+	+	+
	2	+	+	+
	3	+	+	+
	4	+	+	+
	5	+	+	-
β-est	1	+	+	+
	2	+	+	+
	3	+	+	+
	4	+	+	+
	5	+	+	+
	6	+	+	-
Acph	1	+	+	+
	2	+	+	+
	3	+	+	+
	4	+	+	+
	5	+	+	-
Adh	1	+	+	+
	2	-	+	+
	3	+	+	+
	4	-	+	+
	5	+	+	-
	6	+	+	-
	7	-	+	+
Ao	1	+	+	+
	2	+	+	+
	3	+	+	+

C= Untreated samples, S= Treated samples by spinosad, P= Treated samples by proclaim, (+) = Band present and (-) = Band absent.

Table (5): Polymorphism changes caused by spinosad and proclaim in the isozymes of *B. zonata*.

Isozyme name	Number of appearing bands	Monomorphic bands	Polymorphic bands	Total bands	Polymorphism %
α- est C	5	5	-	5	0
P	5	5	-	5	0
S	4	4	1	5	20
β- est C	6	6	-	6	0
P	6	6	-	6	0
S	5	5	1	6	17
Acph C	5	5	-	5	0
P	5	5	-	5	0
S	4	4	1	5	20
Adh C	4	4	-	4	0
P	7	4	3	7	43
S	5	2	5	7	71
Ao C	3	3	-	3	0
S	3	3	-	3	0
P	3	3	-	3	0

Monomorphic bands = Common bands, Polymorphic band = Bands appeared or disappeared as a result of treatments and % Polymorphism = Changes occurred after treatments.

have a major role in defensive functions. Certain quantitative differences were observed also in esterase bands induced by many insecticidal treatments by Eid *et al.*, (1979) and Fahmy (2005). Increases in enzyme activities were showed by Terriere (1984) who described the induction of several detoxication enzymes such as esterase in insects. Such increase in enzyme activities has been shown to protect insects from insecticidal poisoning as part of defense mechanism. Saleem *et al.*, (1998) reported that increased activities of esterase enzymes of the adult beetles of stored grains, *Tribolium castaneum* after cypermethrin treatment may be due to decreased body weight defend against insecticidal stress conditions and/or increase the energy production.

Aldehyde oxidase (Ao) electrophoresis revealed 3 bands in control, proclaim and spinosad treated samples of *B. zonata* as shown in (Fig. 6). No effect or change occurred on Ao after treatment of samples with proclaim and spinosad. Aldehyde oxidase isozyme patterns were studied in several insects; Garcin *et al.*, (1983) demonstrated the capacity of Aldehyde oxidase in *Drosophila melanogaster* to detoxify acetaldehyde and use it for energy production. Bakr *et al.*, (2006) reported that any unusual change of Ao patterns of *S. littoralis* larvae after treatment with IGRs and plant extracts might be on molecular levels and referred to depression or mutations of the regulating genes responsible for biosynthesis of polypeptide chain building these enzymes. Acid phosphates (Acph.) electrophoratic patterns showed 5 bands in control and proclaim treated samples. In flies treated with spinosad 4 bands were detected only, and the percentage of polymorphism was 20% (Fig.7). Saleem and Shakoori (1987) reported that phosphomono esterases such as Acph showed significant inhibition with insecticide doses. These decreases of activities of phosphates may be attributed to (i) reduced enzyme synthesis and/or (ii) binding of the insecticides at certain active sites of enzymes, thereby causing their inhibition. Alcohol dehydrogenase (Adh) electrophoretic patterns showed 4 bands in the control samples, treated samples with proclaim had 7 bands and samples treated with spinosad had 5 bands. The percentage polymorphism was 43 and 71% in treated samples with proclaim and spinosad, respectively, Fig. (8). The high percentage polymorphism in this isozyme revealed the great effect of these two biopesticides in the treated insects. Barkash and Shamina, (1994) reported that the alcohols are produced inside the living organism through the hydrogenation of aldehyde and ketones or through metabolism of fats (production of glycerol). The high concentration of alcohols inside the living organism causes high

disturbance in different metabolic path ways so the living organism must regulate these alcohols through alcohols metabolizing enzyme such as alcoholic dehydrogenase or Aldehyde oxidase. The previous data indicated that the biopesticides (spinosad and proclaim) were effective on *B. zonata* proteins and isozymes, especially denaturated protein, α-est., β-est, Acph and Adh. Furthermore no effects for both spinosad and proclaim on Ao were found.

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