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Biochemical Abnormalities Produced by Spinosad in *Tribolium castaneum* Adult Beetles

RIAZ HUSSAIN[1], MUHAMMAD ASHFAQ AND MUSHTAQ A. SALEEM†
Ecotoxicology Lab., Department of Agricultural Entomology, University of Agriculture, Faisalabad, Pakistan
†*University College of Agriculture, Bahauddin Zakariyya University, Multan, Pakistan*
[1]Corresponding author's e-mail: riazhussain37@hotmail.com

ABSTRACT

The effects of LC_{10} and LC_{20} of a commercial formulation biopesticide, spinosad (Tracer 240 SC) in Pakistan, was tested in laboratory, on 10 d old adults of malathion-resistant, PAK and susceptible, FSS-II strains of the red flour beetle, *Tribolium castaneum* (Herbst). The objective was to examine the changes in production of carboxylesterase, total esterase, α-amylase, glucoamylase, alkaline phosphatase, acidic phosphatase, total protein, soluble protein and free amino acids. Beetles were released on to spinosad treated glass Petri dishes and exposed for 48 h without food. The surviving beetles were then homogenized in saline and centrifuged prior to biochemical analyses. The results showed differences in the production of enzymes and macromolecules between strains and among concentrations. Spinosad caused inhibition of CE activity at increased dose (LC_{20}) in PAK strain, while caused elevation of CE activity in FSS-II strain with depletion of total protein contents. Glucoamylase activity was inhibited in adults of both the strains. Involvement of these enzymes and macromolecules following exposure to the sublethal doses of spinosad is discussed.

Key Words: Spinosad; Esterases; Amylases; Phosphatases; Proteins; Free amino acids, *Tribolium castaneum*

INTRODUCTION

The development of environmentally friendly insecticides, having specificity to insects along with low toxicity to vertebrates, has captured worldwide attention of scientists (Ishaaya & Degheele, 1998). Spinosad is a new type of insecticide with natural origin. Spinosad is produced by one or more chemical mutants of the naturally occurring actinomycetes soil bacterium, *Saccharopolyspora spinosa* Mertz and Yao (Mertz & Yao, 1990). It has been used for the control of insect pests in the orders Lepidoptera, Diptera and Thysanoptera and some species of Coleoptera and Orthoptera (Sparks *et al.,* 1995; Cloyd & Sadof, 2000, Peck & McQuate, 2000; Thompson *et al*., 2000). It is a stomach poison with some contact action and shows some control on small beetle larvae (Thompson *et al.,* 2000; Tjosvold & Chaney, 2001). It kills insects through the activation of acetylcholine nervous system through nicotinic receptors. There may be some effects on the GABA and other nervous systems (Salgado, 1997 & 98). Within 48 h, 60-80% of spinosad or its metabolites are excreted through urine or feces, in rats (EPA, 1997; Dow, 1997). It exerts haematoxic, however comparatively less mutagenic and reproductive effects in albino rats (Mansour *et al.,* 2007; 2008). Many scientists have studied the toxic effects of spinosad against stored grain insect pests (Fang *et al.,* 2002a, b; Toews *et al.,* 2003, Toews & Subramanyam, 2003; Huang & Subramayam, 2004; Hussain *et al.,* 2005; Daglish & Nayak, 2006; Huang & Subramanyam, 2007; Daglish *et al.,* 2008). But there are no reports of the effect of spinosad on activities/levels of enzymes and other macromolecules in insects. Therefore, the present study was planned with an object to understand the biochemical basis of effects of spinosad in the resistant and susceptible strain adult beetles of the red flour beetle, *Tribolium castaneum* (Herbst) when exposed to sublethal dose levels, which usually occur after field applications.

MATERIALS AND METHODS

Rearing of beetles. The PAK and FSS-II strains of *T. castaneum* were obtained from the Department of Zoology, University of the Punjab, Lahore, Pakistan and the Ecotoxicology Centre, School of Biology, Faculty of Sciences, Agriculture and Engineering, University of Newcastle upon Tyne, UK, respectively. According to Saleem and Shakoori (1989), the PAK strain adults have developed 56-fold resistance against malathion as compared to FSS-II strain. The insect culture was maintained in sterilized glass jars (300 mL) kept in a temperature controlled room maintained at 30±1°C and 65±5% relative humidity. The culture medium was whole meal flour sterilized at 60°C for 90 min.

Toxicant used. Commercially available formulation of

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spinosad as Tracer 240 SC, contains about 85% spinosyn A and 15% spinosyn D with other spinosyns as minor impurities, was used in the present study. It was obtained from M/S SCL Agro-Sciences Pakistan (Pvt.) Ltd., Pakistan. Acetone was used as solvent for the preparation of different concentrations of the insecticide.

Insecticide exposure. The LC_{10} and LC_{20} of spinosad were made for 10 d old adult beetles of PAK (1.0 & 5 mg L^{-1}) and FSS-II (3 & 8 mg L^{-1}), strains. Each concentration (1.0 mL) was applied to the bottom of a glass Petri dish with a micropipette and then spread uniformly by rotating the dishes. Acetone alone was applied to the controls. After evaporation of acetone, about 100 adult beetles of same size and age were released in each Petri dish in the absence of food for a period of 48 h. Surviving beetles were weighed and used for biochemical studies i.e., estimation of changes in production of some enzyme activities such as carboxylesterase (CE), total esterase (TE), α-amylase, glucoamylase, alkaline phosphatase (AkP), acidic phosphatase (AcP) and some macromolecules such as soluble protein, total protein and free amino acids (FAA).

Biochemical analyses. About 100 adult beetles of each strain were weighed and homogenized in 0.89% saline solution with the help of a motor driven Teflon-glass homogenizer. Four replicates were used throughout biochemical experimentation following a completely randomized design. The homogenates were centrifuged at 4900 g for 45 min at 4°C, in a refrigerated centrifuge KOKUSAN H-200 nR. The supernatant was used for the estimation of activities of CE and TE according to Devonshire (1975); α-amylase according to Bernfield (1955); glucoamylase according to Dubious *et al.* (1956); AkP according to Bessey *et al.* (1946); AcP according to Andersch and Szcyphinski (1947); soluble and total protein according to the method of Lowry *et al.* (1951) and FAA contents according to Moore and Stein (1954). The activities of enzymes were measured as IU/mg i.e., International Units, the amount of enzyme, which under defined assay conditions will catalyze the conversion of 1.0 μ mole of substrate per minute and IU mL^{-1} min^{-1} i.e., the amount of enzyme, present in 1.0 mL of original enzyme solution, releases 1.0 μ mole of glucose/maltose in 1.0 min. The measurement of absorbance was undertaken by using Hitachi U-1100 Spectrophotometer.

RESULTS

Spinosad toxicity. The toxicity of spinosad, in terms of mortality, against adult beetles of PAK strain, was 13.33, 28.78, 32.75, 53.33 and 75% at 120, 240, 480, 960 and 1920 mg L^{-1} doses, respectively. The toxicity against adult beetles of FSS-II, was 11.42, 25.42, 28.96, 50.85 and 72.88% at same doses given for PAK strain, respectively. The overlapping fiducial limits show that spinosad is equally effective against adult beetles of both the strains (Table I).

The effects of the lower (LC_{10}) and higher (LC_{20}) sublethal doses of spinosad on the activities/levels of some biochemical components of the adults of PAK and FSS-II strains of *T. castaneum* are given in Table II.

Total esterase. There was 40.27% decrease at a lower dose and 21.16% increase at a higher dose of spinosad, in the TE activity in PAK adults, while in FSS-II adults, both the dose levels showed non-significant effect on the activity of the enzyme.

Carboxylesterase. In adults of PAK strain, spinosad caused an increase of 8.30%, at lower and a decrease of 10.73%, at higher sublethal doses, while in FSS-II adults, increased activity of 31.01 and 9.30% was observed, at lower and higher doses, respectively.

α-amylase. In adults of PAK strain, both sublethal doses of spinosad, produced similar effects on the α-amylase activity, with a significant decrease of 10.44%, at lower dose only, while in FSS-II adults, both the doses, produced significant effect by showing 25.41% increase, at lower and 22.95% decrease, at the higher dose.

Glucoamylase. In adults of PAK strain, the enzyme activity decreased up to 18.87 and 9.43%, at lower and higher doses of spinosad, respectively, while in FSS-II, significant decrease (24.14%) occurred, at the higher dose only.

Acidic phosphatase. AcP activity, in the adults of PAK strain, increased up to 22.07 and 24.76%, at lower and higher doses of spinosad, respectively, while in the adults of FSS-II, the enzyme activity increased by 10.48%, at lower and decreased by 2.94%, at higher doses.

Alkaline phosphatase. The LC_{10} dose of spinosad decreased 35.53% and increased 46.98% the AkP activity, in the adults of PAK and FSS-II strains, respectively. The effect of LC_{20} remained insignificant in the adults of both the strains.

Total proteins. The lower and higher sublethal doses of spinosad decreased the total protein contents, in the adults of both the strains. In PAK adults, both doses showed similar effect with a decrease of 18.77%, at lower and 17.97%, at higher doses, while, a significant decrease of 8.34% was observed, only at a lower dose, in the adults of FSS-II strain.

Soluble proteins. The soluble protein contents, in the adults of PAK strain, were increased by 13.19%, at lower, but remained un-affected at the higher sublethal dose of spinosad. In case of FSS-II adults, there was an increase of 45.32%, at lower dose and a decrease of 22.51%, at the higher sublethal dose.

Free amino acids. The effects of LC_{10} and LC_{20} of spinosad on FAA levels, remained insignificant, except a significant increase of 149.01%, in FSS-II adults, at the higher sublethal dose, only.

DISCUSSION

In the present research endeavor, the effects of spinosad were observed on some hydrolases and body proteins of adult beetles of PAK and FSS-II strains of *T. castaneum*. In PAK strain adult beetles, spinosad at LC_{10},

Table I. Toxicity data of spinosad against PAK and FSS-II strains of *Tribolium castaneum*

Strain	LC50s (mg L^{-1})	95% Fiducial limits	Regression equation	χ^2 (2df)	Resistance Ratio
PAK	724	555-944	Y=0.996+1.40×	0.7	0.85
FSS-II	854	654-1116	Y=0.720+1.46×	0.65	-

Table II. Effects of spinosad on some biochemical components of PAK and FSS strains of *Tribolium castaneum*

Parameters	PAK			FSS-II			LSD at
	Control (n = 4)	LC_{10} (n = 4)	LC_{20} (n = 4)	Control (n = 4)	LC_{10} (n = 4)	LC_{20} (n = 4)	0.05
TE (IU/mg)	14.18 b	8.47 e	17.18 a	10.35 cd	11.29 c	9.83 d	1.127
CE (IU/mg)	2.89 c	3.13 b	2.58 d	2.58 d	3.38 a	2.82 c	0.101
α-Amylase (IU/ml/min)	0.182 a	0.163 b	0.171 ab	0.122 c	0.153 b	0.094 d	0.016
Glucoamylase (IU/ml/min)	0.106 a	0.086 c	0.096 b	0.058 d	0.064 d	0.044 e	0.009
AcP (IU/mg)	7.43 e	9.07 d	9.27 d	11.55 b	12.76 a	11.21 c	0.330
AkP (IU/mg)	3.04 a	1.96 b	2.95 a	2.15 b	3.16 a	1.90 b	0.190
Total Proteins (µg/mg)	55.20 a	44.84 b	45.28 b	43.26 bc	39.65 d	41.93 c	1.777
Soluble Proteins (µg/mg)	23.12 b	26.17 a	22.83 b	17.19 d	24.98 a	13.32 d	1.549
FAA (µg/mg)	0.171 b	0.146 b	0.181 b	0.151 b	0.141 b	149.01 a	0.064

Means having same letter are statistically insignificant

inhibited the TE activity, but at the increased dose (LC_{20}) elevated it and in FSS-II strain, both the doses affected the enzyme activity insignificantly. This shows detoxification of spinosad, at increased dose, in PAK strain adult beetles. As regards the CE activity, it was first increased, at the lower dose of spinosad and then, decreased at the higher dose, in the adult beetles of PAK strain, while it was found to be increased, at both the doses, in case of FSS-II strain. The CEs are polymorphic enzymes and have been reported to play an important role in the catabolism of juvenile hormone, during insect development. Their higher activity, in permethrin resistant strain, was primarily due to overproduction of CE, in the near isogenic strain of Colorado potato beetle (Lee & Clark, 1996).

Spinosad did not affect the α-amylase activity in PAK strain, while first increased and then decreased the enzyme activity in FSS-II strain adults. Glucoamylase was inhibited at LC_{20} in adults of both strains. The utilization of different carbohydrates depends on the ability to hydrolyze polysaccharides, the readiness with which different substances are absorbed and the possession of enzyme systems capable of introducing these substances, into the metabolic processes. Some insects can use a very wide range of carbohydrates. *Tribolium*, for instance, uses starch, the alcohol mannitol, the disaccharides and the monosaccharides (Dadd, 1960). So, inhibition of digestive enzymes, in the adult beetles of both the strains, because of spinosad, may result in ingestion of abnormal amounts of food and in consequence-more intensive or extensive craving for food. It also suggested non-utilization of carbohydrates, in glycolysis and in Krebs cycle, for provision of extra energy.

Spinosad caused changes in the activities of phosphatases (AcP, AkP), in adult beetles of both the strains, but did not produce any pattern. However, any change in the activities of these important enzymes, may affect the glucose phophorylation (Thibodeau & Patton, 1993), breakdown of ATP (Nohel & Slama, 1972; Saleem & Shakoori, 1985), the digestion of phospholipids (Cook *et al.,* 1969) and growth rate (Mosleh *et al.,* 2003). Spinosad decreased the total protein contents, in adult beetles of both the strain. It shows utilization of protein in energy production. In adult beetles of *T. castaneum,* gamma-HCH, at LC_{10} (10 mg L^{-1}) and LC_{20} (20 mg L^{-1}) also decreased the total protein contents (Saleem *et al.,* 2001); and bifenthrin, more prominently changed the total protein contents, in FSS-II strain, than, in PAK strain, after the treatment with a sublethal dose (200 mg L^{-1}) (Shakoori *et al.,* 1994).

The soluble protein contents increased significantly, at LC_{10} and decreased, insignificantly at LC_{20}, in adult beetles of both the strains. Subba (1985) studied the effects of organophosphates (quinalphos & monocrotophos) and a pyrethroid (Sumicidin) on the protein metabolism, in the haemolymph of *Periplaneta americana*. The author reported that the total protein increased for 2 h., following treatment and then declined. In another study on the effects of quinalphos and organophosphate, on the metabolism of the nerve tissues of *P. americana*, Rajender (1985) reported that after 24 h, there was a decrease in the total carbohydrates, glucose and glycogen contents *vis-à-vis* a simultaneous increase in proteins, free amino acids and total RNA. From the results, he suggested increased utilization of the carbohydrates and an increased protein formation.

From the results described above, it is concluded that spinosad reduced levels of CE activity at increased sublethal dose (LC_{20}) with depletion of total protein contents in PAK strain, while caused elevation of CE activity in FSS-II stain. Glucoamylase activity was decreased in the treated adults.

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