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BIOCHEMICAL ABNORMALITIES INDUCED BY ABAMECTIN IN SIXTH INSTAR LARVAE OF THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST)

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ABSTRACT:- The sub lethal effects of abamectin (Sure 1.8 EC) were studied on malathion-resistant (PAK) and organophosphate susceptible (FSS-II) strains of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) larvae in the laboratory. The objective was to examine changes in production or activities of carboxylesterase (CE), total esterases (TE), alpha-amylase, glucoamylase, alkaline phosphatase (AkP), acidic phosphatase (AcP), total protein, soluble protein and free amino acids (FAA). The sixth instar larvae of *T. castaneum* were released and exposed for 48h without food on abamectin treated glass petri dishes. The surviving ones were then homogenized in saline and centrifuged prior to biochemical analyses. Results showed differences in the activities of enzymes and quantities of total protein, soluble protein and FAA between strains and among concentrations. Abamectin, at LC_{10} and LC_{20}, changed the activities /levels of TE, CE, AcP, total protein and FAA in the larvae of both the strains. The activities of alpha-amylase, glucoamylase and AkP remained non-significant at both doses in the two strains. In PAK strain larvae, the TE activity was inhibited with depletion of total protein contents and elevation of FAA contents. In FSS-II larvae, the effect of abamectin on levels of α-amylase, glucoamylase, AkP, total protein and soluble protein remained non-significant. The activities of TE and AcP were reduced at both doses, while activities/levels of CE reduced at LC_{10} and FAA increased at LC_{20}. It is concluded that abamectin affected the overall body functioning of PAK strain more as compared to FSS-II strain considering disturbances caused in the levels/activities of biochemical components.

Key Words: Tribolium castaneum; Larvae; Abnormalities; Abamectin; Enzymes; Biochemical Components; Proteins; Pakistan.

INTRODUCTION

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is one of the major stored grain pests, specially pulses, millets and cereals in Pakistan (Hamed and Khattak, 1985) and other parts of the world (Obeng-Ofori, 2008). The decade-long use of organophosphates and pyrethroids has developed resistance in some stored-product insects (Subramanyam and Hagstrum, 1996; Kljajić and Perić, 2005; Boyer et al., 2012).

The insecticides with novel

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modes of action are usually highly toxic to insects but low to mammals. Therefore, they are putting back the conventional organochlorine, organophosphate, carbamate, and pyrethroid insecticides in insect pest management programmes. Abamectin is a biopesticide, isolated from fermentation of *Streptomyces avermitilis* Kim and Goodfellow. It is effective against insect pests including Coleopterans due to its cuticular and stomach actions (Ishaaya and Degheele, 1998). It was found as effective as some contact insecticides used against stored-product insects (Kavallieratos et al., 2009). It acts by stimulating the release of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter. It is rapidly eliminated mainly via feces and urinary excretion in animals (Tomlin, 2000). Iqbal and Wright (1997) reported that there might be the involvement of microsomal monooxygenases and/or esterases in resistance to abamectin with a limited evidence of involvement of glutathione-S-transferases.

The insecticide usage against stored grain insect pests causes extensive damage to insect hemolymph besides affecting the other systems (Mehmood and Yousuf, 1985). There are numerous reports available on damages to enzymes and other biochemical constituents of *T. castaneum* caused by conventional insecticides (Saleem and Shakoori, 1985; 2000 a & b; Saleem et al., 2001). Hussain et al. (2012) studied the effects of LC_{10} and LC_{20} of abamectin on 10-day old adults of insecticide-resistant and susceptible strains of *T. castaneum*. Some other workers also reported remarkable biochemical changes in fourth instar larvae of *Spodoptera littoralis* (Boisd.) treated with emamectin benzoate, abamectin and spinosad (Megahed et al., 2013). Ghoneim et al. (2013) reported effects of feeding on different host plants, viz., *Vicia faba* L. *Trifolium alexandrinum* L. and *Lactuca sativa* L. on the general body metabolism of adult females of the black blister beetle, *Meloe proscarabaeus* L.

The present study was conducted to understand the biochemical basis of effects of abamectin in larvae of *T. castaneum* exposed to sub-lethal dose levels.

MATERIALS AND METHOD

Rearing of Beetles

Two strains of *T. castaneum*, malathion-resistant (PAK) and organophosphates-susceptible (FSS-II), were brought from the Department of Zoology, University of the Punjab, Lahore, Pakistan to the Eco-toxicology Lab. Department of Entomology, University of Agriculture, Faisalabad, Pakistan. The latter strain belonged to the Ecotoxicology Centre, School of Biology, Faculty of Sciences, Agriculture and Engineering, University of Newcastle UPON Tyne, UK.

The insect culture was maintained in sterilized glass jars (300 ml) at 30±1 ^{0}C in laboratory at relative humidity of 65±5%. The culture medium used for beetle's rearing was whole meal flour sterilized at 60 ^{0}C for 90 min.

Toxicant Used

Abamectin as a formulation, Sure 1.8 EC, with chemical abstract name:5-O-demethylavermectin A_{1a} (i) mixture with 5-O-demethyl-=25-de (1-methylpropyl)-25-(1-methylethyl) avermectin A_{1a} (ii) marketed by M/S

Pan Pacific (Pvt.) Ltd. Pakistan, was used in the present study. Acetone was used as solvent for the preparation of different concentrations of the insecticide.

Determination of LC_{50} Values

The sixth instar larvae of *T. castaneum* of both strains were separated with the help of a camel hair brush. Each concentration (1.0 ml) of abamectin was applied to the bottom of a glass petri dish (130 cm^2), with a micropipette, and then spread uniformly by rotating the dish. Only acetone was applied to the control strains. Three replicates of each dose were used. After evaporation of acetone at room temperature, about 10-20 healthy sixth instar larvae of same size were introduced without food into each petri dish and covered with a lid. The mortality was counted after 48h.

Larvae showing no movement after squeezing with a brush were considered to be dead as per criterion given by Lloyd (1969). The percentage mortalities, in the treated batches, were corrected by Abbott's formula (Abbott, 1925), as follows:-

$$\text{Mortality (\%)} = \frac{\text{Test mortality -\% control mortality}}{\text{100 -\% control mortality}} \times 100$$

Concentration-mortality data, so obtained, were subjected to probit analysis as described by Finney (1974) and outlined by Busvine (1971). The LC_{50} values were derived from these analyses and expressed in mgl^{-1} of insecticide, for larvae of both strains.

Biochemical Analyses

The LC_{10} and LC_{20} doses of abamectin were made from regression equations for sixth instar larvae of PAK (1.87 and 5.85 mg l^{-1}) and FSS-II (2.93 and 9.0 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 control strains. After evaporation of acetone, about 100 larvae of sixth instar were released in each petri dish in the absence of food for 48 h. After the prescribed period; the surviving ones were weighed and used for biochemical studies, i.e., estimation of changes in production of 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).

About 100 larvae of each strain were weighed and homogenized in 0.89% saline solution with a motor driven Teflon-glass homogenizer. Four replicates were used throughout biochemical experimentation following a completely randomized design. The homogenates were centrifuged at 4900 rpm 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 (Devonshire, 1975); α-amylase (Bernfield, 1955); glucoamylase (Dubious et al., 1956); AkP (Bessey et al., 1946); AcP (Andersch and Szcyphinski, 1947); soluble and total protein (Lowry et al., 1951); and FAA contents (Moore and Stein, 1954) by using Hitachi U-1100 Spectrophotometer.

The activities of enzymes were measured as IU mg^{-1}, 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 Analysis of Variance Technique was used to analyze the data on Pentium-IV Computer, using MStatC. In significant variations, the differences between mean values were compared by the LSD test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Toxicity against Larvae Administered for 48h

The toxicity of abamectin against larvae of PAK strain for mortality, was 13.33%, 25.00%, 55.00%, 76.67%, and 90.00% at 50, 100, 200, 400 and 800 mgl^{-1} doses, respectively. The regression equation was Y = 0.45 + 2.02*X* (Figure 1). There was no heterogeneity in the test population (x^2 = 1.82). The LC_{50} value was obtained as 178 mgl^{-1} (Table 1).

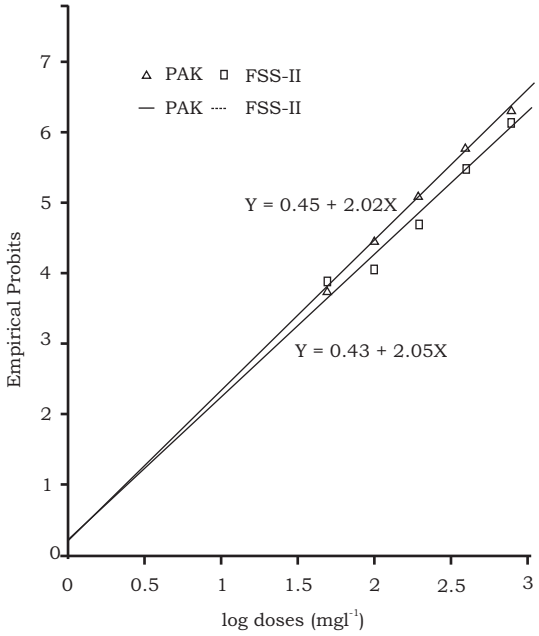

Figure 1. Regression lines of toxicity of abamectin against PAK and FSS-II larval strains of *T. castaneum*

Table 1. Toxicity of abamectin against sixth instar larvae of PAK and FSS-II strains of *T. castaneum*

Particular	PAK Strain	FSS-II Strain
LC_{50} (ppm)	178	261
95% fiducial limits	146-216	223-323
Slope	2.02 ± 0.05	2.05 ± 0.08
Chi-Square (X^2) at 2df	1.82	2.70

The toxicity of abamectin against FSS-II larvae for mortality, was 11.67%, 16.67%, 38.33%, 68.33%, and 86.67% at doses of 50, 100, 200, 400 and 800 mgl^{-1}, respectively. The regression equation was Y = 0.43 + 2.05X (Figure 1). There was homogeneity in the test population of insects (x^2=2.7). The LC_{50} value was 261 mgl^{-1} (Table 1).

The difference in LC_{50} values of larvae of PAK and FSS-II strains was significant at 95 % Fiducial Limits (FLs) to LC_{50} (Table 1). It proved that abamectin was more effective against the larvae of PAK strain as compared to FSS-II strain of *T. castaneum*.

Biochemical Analyses

Biochemical changes were induced by the LC_{10} and LC_{20} doses of abamectin 1.87 and 5.85 mgl^{-1} as well as of 2.93 and 9.01 mgl^{-1} in the larvae of PAK and FSS-II strains of *T. castaneum*, respectively, after treatment by residual film method (Table 2).

The TE activity, in larvae, decreased up to 67.84% and 44.85%,

Table 2. Effects of abamectin (Sure 1.8EC) on some biochemical components of PAK and FSS-II strains of *Tribolium castaneum* larvae

Parameter	PAK					FSS-II					LSD value at 0.05
	Control (n=4)	LC_{10} (n=4)	%	LC_{20} (n=4)	%	Control (n=4)	LC_{10} (n=4)	%	LC_{20} (n=4)	%	
TE (IU mg^{-1})	2.55^{a}	0.82^{d}	-67.84	2.03^{b}	-20.39	1.94^{b}	1.07^{c}	-44.85	1.23^{c}	-36.60	0.163
CE (IU mg^{-1})	6.53^{b}	4.13^{c}	-36.75	25.07^{a}	283.92	3.63^{d}	2.57^{e}	-29.20	3.69^{d}	1.65	0.143
α-Amylase (IU ml^{-1} min^{-1})	0.03	0.03	21.43	0.04	42.86	0.07	0.07	4.35	0.06	-14.49	Non-significant
Glucoamylase (IU ml^{-1} min^{-1})	0.05	0.05	24.44	0.06	31.11	0.04	0.04	13.51	0.04	2.70	Non-significant
AcP (IU mg^{-1})	13.45^{b}	16.32^{a}	21.34	11.68^{d}	-13.16	12.64^{c}	9.26^{f}	-26.74	10.82^{e}	-14.40	0.432
AkP (IU mg^{-1})	3.06	4.18	36.60	4.83	57.84	2.60	4.08	56.92	4.60	76.92	Non-significant
Total Proteins (µg mg^{-1})	89.01^{a}	88.42^{a}	-0.66	70.64^{b}	-20.64	70.06^{b}	70.59^{b}	0.76	70.25^{b}	0.27	3.333
Soluble Proteins (µg mg^{-1})	30.05^{a}	17.59^{c}	-41.46	16.50^{c}	-45.09	28.02^{ab}	26.89^{b}	-4.03	28.94^{ab}	3.28	2.566
FAA (µg mg^{-1})	0.11^{e}	0.20^{b}	90.65	0.26^{a}	143.93	0.11^{de}	0.13^{d}	18.02	0.15^{c}	35.14	0.018

Means followed by same letter do not differ significantly at P<0.05

at lower and up to 20.39% and 36.60% at a higher dose of abamectin in PAK and FSS-II strains, respectively. The CE level decreased up to 36.75% at the lower and increased up to 283.92% at the higher dose in larvae of PAK strain, while, in FSS-II larvae, up to 29.20% decrease at lower and statistically no change at the higher dose, was observed (Table 2). Thus, abamectin exerted inhibitory effect on the CE activity in PAK strain larvae. The effect of lower and higher doses of abamectin remained non-significant on α-amylase, glucoamylase, AkP, total protein and soluble protein activities/ levels in the larvae of both the strains. An increase of up to 21.34% in the AcP activity at the lower dose and a decrease of up to 13.16% at the higher dose was observed in the larvae of PAK strain, while, in the larvae of FSS-II, a significant decrease of 26.74% at the higher and 14.40% at the lower dose was observed. In PAK strain larvae, the FAA contents increased by 90.65% and 143.93% at the lower and higher doses of abamectin, respectively, while, in FSS-II larvae, the only higher dose significantly caused an increase of 35.14%.

Abamectin at both doses (LC_{10} and LC_{20}) inhibited the TE activities in larvae of both strains. CE activity was reduced at LC_{10} and increased at LC_{20} in both the strains. According to Lee and Clark (1996), CE, a polymorphic enzyme, plays an important role in the catabolism of juvenile hormones, during insect development. Its higher activity, in permethrin resistant strain, was primarily due to its overproduction in near isogenic strain of Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Abamectin inhibited the total esterase activity in larvae of both the strains. It was the indication of their susceptibility to the insecticide. Esterases were synthesized during the various development stages of embryo. The level of esterase activity was not constant, throughout the life cycle, as larvae of some species exhibited higher tolerance to contact insecticides than their adults (Nakakita and Winks, 1981; Price and Mills, 1988).

Abamectin, at LC_{10} and LC_{20}, did not affect the activities of α-amylase and glucoamylase, which suggested that the insecticide might not be interfering with the hydrolysis of starch and other related saccharides in the digestive tract of the insects (Horvathova et al., 2000). It also proved the non-utilization of carbohydrates, through the breakdown of available body starch through glycolysis and passed on to Krebs cycle, to provide extra energy.

Phosphatases played important role in the glucose phophorylation (Thibodeau and Patton, 1993) and digestion of phospholipids (Cook et al., 1969). According to Saleem and Shakoori (1985), the raised activities of phosphatases (AcP, AkP) supported the production of energy, through the breakdown of phosphate bonds of energy rich compounds such as ATP. The same had been reported by Nohel and Slama (1972), in a bug, *Pyrrhocoris apterus* (L). In the present investigations the LC_{10} and LC_{20} doses of abamectin affected the activities of AcP in sixth instar larvae of two strains of *T. castaneum*, but did not produce any pattern. On the other hand, the AkP activity remained unaffected. It means that abamectin

neither needed energy production by raising the AcP and AkP activities nor interfered with energy rich compound, ATP.

Total protein contents, decreased in the larvae of PAK strain and increased, non-significantly, in the FSS-II larvae, at both the doses. The decreased levels of total protein, in PAK strain, showed its utilization in energy production. In PAK larvae, soluble protein contents decreased significantly as compared to the FSS-II larvae. This again proved more utilization of the protein for energy production, in PAK strain, than in FSS-II strain. Etebari et al. (2007) reported significant decrease in protein contents in haemolymph of silkworm larvae due to pyriproxyfen residue; reduction in total head protein levels in *Spodoptera litura* F larvae treated with botanicals *Ageratum conyzoides* L. and *Artemisia vulgaris* (L.) (Renuga and Sahayaroj, 2009) and depletion of total protein contents in *T. castaneum* treated with spinosad (Hussain et al., 2009). In contrast, Rajender (1985) and Subba (1985) described the elevated protein contents in *Periplaneta americana* L. treated with quinalphos and monocrotophos. Proteins were the most abundant of the organic compounds in the insect body. Their functions were of first-rank importance, e.g., they provide structure to insect body and muscles, transported substances in haemo-lymph, provide energy, and catalyze chemical reactions in the form of enzymes (Mordue et al., 1980; Ross, 1982; Chapman, 1998). It may therefore, concluded that abamectin affected the overall body functioning of the malathion-resistant, PAK strain more as compared to organophosphates-susceptible, FSS-II strain.

In PAK strain larvae, FAA contents were raised after treatment with LC_{10} and LC_{20} of abamectin, but, protein contents decreased. According to Chapman (1998), FAA were used in production of tissues and enzymes. Abamectin, itself, stimulated the release of GABA, which was an inhibitory neurotransmitter (Tomlin, 2000). On the other hand, FAA contents were increased in larvae of FSS-II strain.

Thus the difference in LC_{50} values of larvae of PAK and FSS-II strains proves abamectin more effective against the larvae of malathion-resistant PAK strain as compared to organophosphate-susceptible FSS-II strain of *T. castaneum*. The LC_{10} and LC_{20} of abamectin changed the activities/ levels of TE, CE, AcP, total protein and FAA, but did not change the activities of alpha-amylase, glucoamylase and AkP, in larvae of both the strains. In larvae of PAK strain, the TE activity was inhibited with depletion of total protein contents and elevation of FAA contents. In FSS-II larvae, the effect of abamectin on levels of α-amylase, glucoamylase, AkP, total protein and soluble protein remained non-significant. The levels of TE, CE and AcP were reduced at both doses, while, levels of FAA increased at LC_{20}. It is suggested that effects of abamectin should also be studied against other insecticide resistant stored-product insects.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

S. No	Author Name	Contribution to the paper
1.	Dr. Riaz Hussain	Conceived the idea , Conducted research
2.	Dr. Muhammad Riaz	Conceived the idea, Technical input at every step
3.	Dr. Mushtaq A. Saleem	Supervised the research
4.	Mr. Muhammad Ishaque Mastoi	Conceived the idea, Technical input at every step

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