

Effect of Abamectin on Body Protein Content and Activity of Selected Enzymes in Adults of Insecticide-Resistant and -Susceptible Strains of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract.- The effects of LC₁₀ and LC₂₀ of a biopesticide, abamectin, as a formulation (Sure 1.8EC), were studied, in the laboratory on 10 days old adults of insecticide-resistant and -susceptible strains of red flour beetle, *Tribolium castaneum*. The beetles were released on the biopesticide-treated glass Petri dishes and exposed for 48 h. The surviving beetles were then homogenized in saline and centrifuged to analyse the various soluble biochemical components of whole body. The biopesticide affected the activities and levels of carboxylesterase (CE), total esterases (TE), acid phosphatase (AcP) total protein, and free amino acids (FAA) in both the strains. However, in adults of resistant strain, the TE and CE activities were inhibited with depletion of total protein contents and elevation of FAA contents in both the doses. In susceptible strain, at LC₁₀ the CE activity was increased with increase of soluble protein and depletion of total protein and FAA contents, while at LC₂₀, the TE and CE activities were elevated and AcP was inhibited with increase of total proteins.

Key words: Abamectin, carboxylesterase, alkaline phosphatase, free amino acids.

INTRODUCTION

The red flour beetle, *Tribolium castaneum* (Herbst), is one of the major pests of stored grains, especially pulses, millets and cereals in Pakistan and other parts of the world (Saleem and Shakoori, 1989). The insecticide usage to save stored products from the ravages of this pest is likely to cause extensive damage to insect hemolymph besides 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 from our laboratories (Saleem and Shakoori, 1985, 2000a,b; Saleem *et al.*, 2001).

The insecticides with novel modes of action might well herald a new era in pest control. They possess many desirable properties including high toxicity to insects and low toxicity to mammals. Therefore, they are replacing the conventional organophosphate, carbamate and pyrethroid insecticides in controlling the resistant insect pests.

Abamectin is a biopesticide, isolated from fermentation of *Streptomyces avermitilis*. It has cuticular and stomach actions, and is effective against insect pests including Coleopteran (Ishaaya and Degheele, 1998). 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. Wang and Wu (2007) found that abamectin resistance in abamectin selected strain of *Bemisia tabaci* was due to elevated activity of P450 monooxygenase and glutathione S-transferase instead of esterase. Therefore, the present endeavor was made to explore the effects of abamectin on some enzymes and proteins of *Tribolium castaneum* adult beetles.

There are no reports on the effect of LC₁₀ and LC₂₀ of abamectin on activities / levels of enzymes and other macromolecules in adult beetles of *T. castaneum*. The objective of this research was to study the biochemical response by the insects exposed to sublethal dose applied in field.

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MATERIALS AND METHODS

Rearing of beetles

The malathion-resistant, PAK and organophosphate-susceptible, 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 insects were reared in the laboratory as described by Saleem and Shakoori (1984). The insect culture was maintained in sterilized glass jars (diameter 6 cm and height 14 cm) at $30\pm 1^{\circ}\text{C}$ in the laboratory at relative humidity $65\pm 5\%$. The culture medium was whole meal wheat flour sterilized at 60°C for 90 minutes.

Toxicant used

Commercially available formulation of abamectin (Sure 1.8EC) was obtained from M/S Pan Pacific (Pvt.) Ltd, Vehari, Pakistan. Its chemical abstract name: 5-*O*-demethylavermectin A1a (i) mixture with 5-*O*-demethyl-25-de (1-methylpropyl)-25-(1-methylethyl) avermectin A1a (ii), was used in the present study. Formulation of abamectin instead of its technical grade material was used in this study because the chemical was available in this form for use in the field in Pakistan. Acetone was used as solvent for the preparation of different concentrations of the insecticide.

Insecticide exposure

The insects were exposed to insecticide according to Saleem and Shakoori (1989, 1990). The LC_{10} and LC_{20} of abamectin were prepared for 10 days old adult beetles of resistant (0.57 and 2.02 mg L^{-1}) and susceptible (0.55 and 2.07 mg L^{-1}) strains. Each concentration (1.0 ml) was applied to the bottom of a glass Petri dish of 90mm diameter and 15mm height 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, without discrimination of sex, were released in each Petri dish in the absence of food for a period of 48 h. After the lapse of the prescribed period the surviving beetles were weighed and used for biochemical studies, *i.e.*, estimation of changes in activities of CE, TE, α -amylase, glucoamylase, AkP, AcP, and levels of some macromolecules such as soluble protein, total protein and FAA.

Biochemical analyses

The procedure with regards to biochemical analyses have been already by our laboratories (Saleem and Shakoori, 1985, 1989, 1990) 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 minutes at 4°C , in a refrigerated centrifuge Kokusan H-200 nR. The supernatant obtained after centrifugation 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 Szcypinski (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 μmole of substrate per minute and IU/ml/min *i.e.*, the amount of enzyme, present in 1.0 ml of original enzyme solution, releases 1.0 μmole of glucose/maltose in 1.0 minute.

Data were analyzed by the Analysis of Variance Technique. In case of significant variations, the difference between mean values, were compared by the Least Significant Difference (LSD) test (Steel and Torrie, 1980).

The objectives were achieved through spectrophotometric methods by using Hitachi U-1100 Spectrophotometer.

RESULTS

Table I shows the effects of LC₁₀ and LC₂₀ of abamectin on activities / levels of some biochemical components of adults of PAK and FSS-II strains of *T. castaneum* in terms of unit body weight.

Effect on enzymes

The TE level was decreased up to 33.58 and 22.63%, at LC₁₀ and LC₂₀ doses of abamectin, respectively, in the adults of resistant strain. On the other hand, statistically no change in the TE activity, at LC₁₀ and 68.93% increase, at LC₂₀ dose, was observed in the adults of susceptible strain. In resistant adults, the CE activity decreased up to 14.18% at LC₁₀ dose and decreased up to 24.47%, at LC₁₀ dose of abamectin, while in susceptible adults, both the doses, showed a similar, but significant effect on the CE activity, i.e., by 19.31% increase at lower and 16.99% increase at higher dose. Thus, abamectin exerted inhibitory effect on CE activity in the case of resistant strain. The impact of lower and higher doses of abamectin remained non-significant on α -amylase activity, in the adults of resistant and susceptible strains. The effect of lower and higher doses of abamectin remained non-significant on glucoamylase activity, in the adults of resistant and susceptible strains. Abamectin decreased the AcP activity up to 29.68%, at lower dose and increased it up to 19.44%, at higher dose, in the adults of resistant strain, while in the adults of susceptible strain, no change in AcP activity at lower dose and a significant decrease of up to 19.39%, at higher dose, was observed. Both the doses caused non-significant effects on the AkP activity, in adults of resistant and susceptible strains.

Effect on proteins and free amino acids

In resistant adults, the total protein contents decreased up to 37.59 and 14.04%, at lower and higher sublethal doses of abamectin, respectively, while in the susceptible adults, 11.61% decrease, at lower dose and 81.07% increase, at higher dose was observed. The soluble protein contents increased up to 63.2%, at lower dose and did not affect at the higher sublethal dose, in adults of resistant strain. In adults of susceptible, these contents increased up to 27.87%, at a lower dose and remained unaffected at

a higher dose of abamectin. FAA contents in adults of resistant strain were increased up to 45.16%, at lower and up to 27.74%, at higher dose of abamectin, while in adults of susceptible, FAA contents, significantly decreased up to 13.66%, at the lower dose only and remained unaffected at the higher dose.

DISCUSSION

Abamectin (LC₁₀ and LC₂₀) inhibited the CE and TE activities in the adult beetles of resistant strain and elevated their activities in adult beetles of susceptible strain of *T. castaneum*. Abamectin resistance might be due to increased activities of microsomal oxidases in 5th instar larvae of *Spodoptera littoralis* (Christie and Wright, 1991), microsomal monooxygenases and / or esterases with a limited evidence of involvement of glutathione-S-transferases in *Plutella xylostella* (Iqbal and Wright, 1997) and P450 monooxygenase and glutathione S-transferase instead of esterase in *Bemisia tabaci* (Wang and Wu, 2007). The malathion-resistance in *T. castaneum* had been reported to be caused by higher levels of GSH transferase (Wool *et al.*, 1982) as well as by the esterases (Mackness *et al.*, 1983; Navarro *et al.*, 1986; Subramanyam and Harein, 1990; Haubruge *et al.*, 2002). According to Lee and Clark (1996), the higher activity of CE in permethrin-resistant strain of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), was primarily due to overproduction of the enzyme. The inhibition of the production of TE and CE by abamectin proves that it may be effective against malathion- and permethrin- resistant beetles.

Abamectin at LC₁₀ and LC₂₀ did not affect the activities of α -amylase and glucoamylase, which suggested non-utilization of carbohydrates in glycolysis and Krebs' cycle to provide extra energy.

Phosphatases play an important role in the glucose phosphorylation (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 contention of the production of energy, through the breakdown of phosphate bonds of energy rich compound such as ATP. The same has been reported by Nohel and Slama (1972), in

Table I.- Effect of abamectin (Sure 1.8EC) on some biochemical components of malathion-resistant (PAK) and organophosphate-susceptible (FSS-II) strains of *Tribolium castaneum*, adults.

Parameters	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)	1.37 B	0.91 D	1.06 CD	1.03 CD	1.16 C	1.74 A	0.1635
CE (IU/mg)	2.82 B	2.42 D	2.13 E	2.59 C	3.09 A	3.03 A	0.1434
α-Amylase (IU/ml/min)	0.140	0.154	0.146	0.139	0.158	0.143	Non-significant
Glucoamylase (IU/ml/min)	0.148	0.151	0.158	0.129	0.139	0.137	Non-significant
AcP (IU/mg)	9.57 C	6.73 D	11.43 B	11.81 AB	12.15 A	9.52 C	0.4326
AkP (IU/mg)	1.98	1.51	2.66	1.82	2.39	1.58	Non-significant
Total proteins (μg/mg)	53.84 B	33.60 E	46.28 C	44.26 C	39.12 D	80.14 A	3.333
Soluble proteins (μg/mg)	13.75 C	22.44 A	13.69 C	12.81 C	16.38 B	11.44 C	2.566
FAA (μg/mg)	0.155 CD	0.225 A	0.198 B	0.161 C	0.139 D	0.147 CD	0.0178

AcP, acid phosphatase; AkP, alkaline phosphatase; CE, carboxylesterase; FAA, free amino acids; TE, total erythrocytes.

case of a bug, *Pyrhocoris apterus*. In the present investigations, the LC₁₀ and LC₂₀ doses of abamectin affected the activities of AcP in adult beetles of the two strains of *T. castaneum*, whereas AkP activity remained unaffected. It could be attributed to the reason that abamectin neither switched over this mechanism of energy production by raising the AcP and AkP activities nor interfered with energy rich compound, ATP.

Total protein contents decreased in the adult beetles of resistant strain at both the doses, while manifested decrease at LC₁₀ and increase at LC₂₀ in susceptible strain. The decreased levels of total protein in resistant strain revealed its possible utilization in energy production. The soluble protein contents increased significantly at LC₁₀ but remained unaffected at LC₂₀ in adult beetles of both the strains. This exhibited possible non-utilization of soluble protein for energy production. In contrast, Rajender (1985) and Subba (1985) described the elevated protein contents, in the American cockroach, *Periplaneta americana*, treated with quinalphos and monocrotophos.

In resistant strain adult beetles, the FAA contents were found elevated after treatment with abamectin but protein contents were found to be decreased. Abamectin, itself, stimulates the release of GABA, an inhibitory neurotransmitter (Tomlin, 2000). On the other hand in susceptible adult beetles, a decrease in FAA contents was observed because of abamectin treatments.

It was, therefore, concluded from the results

of the present studies that abamectin, at LC₁₀ and LC₂₀, affected the activities/levels of TE, CE, AcP, total protein and FAA in adults of resistant and susceptible strains. In adults of resistant strain, the TE and CE activities were inhibited with depletion of total protein contents and elevation of FAA contents. In susceptible adults, at LC₁₀, the CE activity was increased with increase of soluble protein and depletion of total protein and FAA contents, while at LC₂₀, the TE and CE activities were elevated and AcP was inhibited with increase of total protein. So abamectin may prove effective against malathion-resistant beetles.

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