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Effect of an Organophosphate, Pirimiphos-Methyl, on Esterases of Different Developmental Stages of Stored Grain Pest Red Flour Beetle, *Tribolium castaneum* (Herbst.) – Spectrophotometric Analysis

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Abstract.- The effect of sub-lethal doses of an organophosphorus insecticide, Pirimiphos-methyl, has been described on esterases of *Tribolium castaneum* collected from stored grain godown and compared with those of insecticide resistant (CTC-12) and susceptible (FSS-II) strains to establish insecticide resistance of the pest and hence suggested alternate control measures. The multiple forms of esterases activities were determined biochemically, using spectrophotometer. After treatment with pirimiphos-methyl the soluble proteins of 4th instar larvae of Pak strain decreased (30%), while that of other strains increased. The other stages of the three strains did not show any significant change after insecticide treatment. The pirimiphos-methyl treatment significantly decreased the carboxylesterase (CE) activity in all the developmental stages (4th and 6th instar larvae, newly emerged and 15 days old beetles) of Pak strain (56, 29, 76 and 66%), FSS-II strain (81, 75, 64 and 27%) and CTC-12 strain (61, 58, 51 and 48%). The acetylcholinesterase (AChE) activity decreased significantly with the treatment in all stages of the three strains. The cholinesterase (ChE) activity increased (65, 59 and 37%) only in the 4th and 6th instar larvae and 15 days old adult beetles of Pak strain, while it decreased (31%) in the newly emerged adults. The various developmental stages in FSS-II and CTC-12 strain, however showed opposite response. ChE activity of 15 days old beetles increase (21 and 80%), while that other stages decrease after treatment with pirimiphos-methyl. The arylesterase (AE) activity in all the three strains decreased from the 4th instar larvae to 15 days old adults after pirimiphos-methyl treatment. The total esterase (TE) activity was significantly inhibited in the four stages of the three strains (Pak 54, 48, 69 and 59%; FSS-II 80, 69, 62 and 6%: CTC-12 73, 62, 67 and 71%).

Key words: Insecticide resistance, esterases activity, red flour beetles.

INTRODUCTION

Pakistan, having different climatic zones and complex cropping patterns and practices, suffers severe economic losses due to pests, where the product is grown, picked, or harvested, in the storage bins or granaries. If proper and timely preventive measures are not adopted, heavy losses occurs. There are several pests which infest some specific food products but *Tribolium castaneum* and *T. confusum* are very important because they infest large variety of food products. *T. castaneum* can be controlled either through management practices or with the help of toxic chemicals *i.e.* residual insecticides, grain protectants and fumigants etc. Organophosphates (Pirimiphos-methyl) has been most popular to control the stored grain pests.

There are numerous reports from various countries on the widespread resistance in several stored-product insect to the OP grain protectants and fumigants (Subramanyam and Hagstrum, 1995). Malathion resistance as detected in all species, with a large portion of the *T. castaneum* strain testing positive for malathion resistance followed by *T. confusum* and *Rhyzopertha dominica.* Bansoda and Campbell (1979) found 44 fold malathion resistance in a composite field strain of *T. castaneum* from North Carolina that was collected from storage bins. The malathion resistance was triphenyl phosphate (TPP) suppressible, and this strain was not cross resistant to pirimiphos-methyl, fenitrothion, or bromophos. This TPP suppressible malathion resistance, and lack of cross resistance to related OP, has been independently reported by researchers in other countries (Dyte and Rowlands, 1968; Dyte and Blackman, 1972; Pasalu and Bhatia, 1974).

Resistance to chlorpyriphos-methyl, pirimiphos-methyl, and malathion was detected in

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lesser grain borer, *R. dominica* (F), collected from 8 sites in the sites of Minas Gerais and Sao Paulo in Brazil and from 7 sites of northeast Kansas. Six insect species collected during 1979 and 1980 from stored grain in 34 of 46 counties of South Carolina (USA) were tested for resistance to malathion and pirimiphos-methyl (Horton, 1984). Only malathion specific resistance was detected in *T. castaneum* and these *T. castaneum* strain were susceptible to pirimiphos-methyl. A previous survey showed that *T. castaneum* strain were susceptible to dichlorovos. None of the strains showed resistance to chlorpyrifos-methyl, two strains showed resistance to synergized pyrethrins and one to pirimiphos-methyl (Halliday *et al.*, 1988). Six insect species (*T. castaneum, T. confusum, R. dominica, Sitophilus oryzae, S. granarius* and *Oryzaephilus surinamensis)* collected during 1986 from 63 farms in South Wales, Australia were tested for resistance to malathion, fenitrothion, carbaryl, bioresmethrin, pirimiphos-methyl, chlorpyrifos-methyl and phosphine using the filter paper method (Herron, 1990). Malathion resistance was detected in all species. About 70 % of the *O. surinamensis* strain were resistant to pirimiphos-methyl, 50% to fenitrothion, and 39% to chlorpyrifos-methyl (Halliday *et al.,* 1988). Pimentel *et al*. (2007) also reported resistance to fumigants in ten populations of *T. castaneum*, nine populations of *R. dominica* and seven populations of *O. surinamensis*.

The pest has developed resistance against chlorinated hydrocarbons (*e.g*. BHC/Lindane), OP (malathion, fenitrothion, pirimiphos-methyl), carbamates and certain fumigants (Speirs and Zettler, 1969; Champ and Campbell-Brown, 1970; Dyte and Blackman, 1970; Bhatia and Pradhan, 1972; FAO, 1973, 1974; Zettler and Jones, 1977). The activity of detoxifying enzymes was evaluated in codling moth (Lepidoptera: Tortricidae), such as glutathione S-transferases (GSTs), cytochrome P450 polysubstrate monooxygenases (PSMOs), and esterases, which are likely to be involved in resistance to insecticides (Fuentes-Contreras *et al*., 2007).

Esterases exhiblit enormous multiplicity in both vertebrates and invertebrates. Arylesterases (AE) hydrolyze the aromatic esters and are sensitive to inhibition by parachloromercuribenzoate (*p*-CMB) or parahydroxymercuribenzoate (*p*-HMB). Carboxylesterases (CE) hydrolyze aliphatic esters and are sensitive to inhibition by OP compounds. Cholinesterase (ChE) hydrolyze the choline esters and are inhibited by OP compounds. Acetylcholinesterases (AChE) hydrolyze acetate esters (Holmes *et al*., 1968; Haites *et al*., 1972).

Esterase activities toward α and β-naphthylacetate, p-nitrophenylacetate, methylthiobutyrate, and phenylacetate were correlated with resistance to chlorpyrifos-methyl, and pirimiphos-methyl. Esterases of insects are known to be inhibited both in *vitro* and in *vivo* by OP and carbamate insecticides (Chadwick, 1963; O'Brien, 1966; Casida, 1973). These enzymes have also been reported to bring about detoxification of these insecticides in the resistant strains of insects (Oppenoorth and Van Asperen, 1960; Casida, 1973).

The aims and objective of present study was to correlate insecticide resistance with levels of different categories of esterases, and to assess the effects of an OP (pirimiphos-methyl) on three different strains of *T. castaneum* (Pak, FSS-II, CTC-12) with specific reference to esterase activities. This work is expected to help in understanding the chemical control mechanism of stored grain pests and biochemical basis of insecticide resistance in *T. castaneum.* This will also provide some information about improving the future pest control programme.

MATERIALS AND METHODS

Three strains of *T. castaneum* (Herbst) *viz*. Pak, FSS-II and CTC-12 were used in this study. The master culture of Pak strain was obtained from Food Storage Division of Pakistan Agricultural Research Council, Malir Halt, Karachi. The susceptible (FSS-II) and resistant (CTC-12) strains were obtained from University of New Castle upon Tyne, England. FSS-II is susceptible to malathion (Lloyd and Ruczkowski, 1980), whereas CTC-12 is a multi-OP resistant strain of the above pest, reported first by Champ and Campbell-Brown (1970).

Insect cultures were set up in sterilized jam jars at 30±1°C in a laboratory at relative humidity 65±5%. The culture medium used was whole meal

flour sterilized at 60°C for 60-90 minutes (Saleem and Shakoori, 1984). Insects were left in culture media for 2-3 days for egg laying and then were removed with the help of separating sieves and fine camel brush. Flour containing eggs was placed back in the same jars, in which the 4th instar larvae, 6th instar larvae, the adults and 15 days old beetles emerged after 20±1, 28±1, 38±1 and 53±1 days. These larvae and adults were then used for toxicological studies.

Toxicants used

The insecticide for the present study, pirimiphos-methyl (0-2-diethylamino-6-methylpyrimidine-4-yl 0 0-dimethyl phosphorothioate) is an OP insecticide for industrial, amenity, domestic and public health uses. It has a broad-spectrum of activity, against flies, mosquitoes (larvae and adults), cockroaches, wood-boring beetles, carpet beetles, clothes moth, termites, fleas, crickets, wasps, body lice, bugs and various veterinary pests.

Determination of LC_{50}

For determination of LC_{50}, Residual Film method was used for which the serial dilutions of pirimiphos-methyl (50 EC) were prepared in acetone. LC_{50} of each insecticide was determined against each developmental stage separately. For this purpose three sets, each of four Petri plates, for different doses were used. These doses were applied on the center of glass Petri plates (size 130 cm) and rotated manually to make a thin film. The 1.3 ml of insecticide solution was sufficient to spread as a thin film on entire surface of Petri plates. In the control Petri plates, only acetone was applied. After the dishes were air dried and acetone evaporated, ten healthy insects (4th instar larvae, or 6th instar larvae, or newly emerged adult or 15 days old beetles) were introduced in different Petri plate and then covered. After 48 hours, mortality was recorded. Larvae/ beetles showing no movement after pressing with brush were considered dead. The criterion of mortality used in this study was the one described by Lloyd (1969). The mortality data were, thereafter, subjected to probit analysis by Finney (1971). LC_{50} values were derived from these analyses and expressed in ppm of insecticide for each developmental stage of *T. castaneum*.

Experimental procedure

The sublethal doses were used to determine the effects of insecticides against different developmental stages of three different strains of *T. castaneum.* Four sets, each of three Petri plates, both for control as well as experiment, were used. Another Petri dish with acetone alone served as a control. After the acetone evaporated, fifty 4th instar larvae, 6th instar larvae, newly emerged and 15 days old beetles were introduced in different Petri plates in the absence of food. The larvae and beetles were exposed to insecticide for a period of 48 hours. Live insects from each Petri dish were then weighed and used for the estimation of acetylcholinesterase (AChE; acetylcholine acetyl hydrolase; 3.1.1.7) according to Devonshire (1975a), arylesterases (AE; arylester hydrolase; 3.1.1.2) activity was estimated according to Junge and Klees (1981), carboxylesterase (CE) and total esterases (TE) (carboxylic ester hydrolase; 3.1.1.1) activity was estimated according to Devonshire (1975b), cholinesterases (ChE; acetylcholine acyl hydrolase; 3.1.1.8) activity was estimated according to Rappaport *et al.* (1959), The soluble proteins were estimated according to Lowry *et al*. (1951).

Statistical analysis

The data was subjected to statistical analysis. Mean, standard deviation of the mean, standard error and least significant difference (LSD) with two way analysis of variance was done according to Steel and Torrie (1981). The LSD was calculated among the different age groups (4th instar larvae, 6th instar larvae, newly emerged adults and 15-days old beetles), control *vs* treatment and among the different strains at P= 0.05 level.

RESULTS

Lethal concentration (LC_{50})

The relative toxicity (LC_{50}) of pirimiphos-methyl against the 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old beetles of Pak strain were 681 ppm, 1753 ppm 4508 ppm and 3.22 ppm, respectively. The LC_{50} against the 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old beetles of FSS-II strain was 88 ppm, 743 ppm, 2674 ppm and 1.08,

respectively, whereas CTC-12 strain showed 662 ppm, 5446 ppm, 5821 ppm and 81 ppm, respectively, in the above mentioned stages.

Total esterase (TE) activity

The insecticide, pirimiphos-methyl drastically inhibits the TE activity of all the three strains of *T. castaneum*. TE of 4th instar larvae, 6th instar larvae, newly emerged adult beetles and 15 days old beetles of Pak strain decreased 54, 48, 69 and 59% after insecticide treatment. In FSS-II strain this decrease was 80, 69, 62 and 6%, respectively, in the 4th instar larvae, 6th instar larvae, newly emerged adult beetles and 15 days old beetles after treatment with insecticide. The total esterases of different developmental stages of CTC-12 strain were inhibited 73, 62, 67 and 71% after the administration of pirimiphos-methyl (Table I).

The specific activity of Pak strain significantly decreased 30, 51, 76 and 70% in the 4th instar larvae, 6th instar larvae, newly emerged and 15 days old beetles after the treatment of insecticide. The FSS-II strain showed 85, 68 and 58% significant reduction in 4th instar larvae, 6th instar larvae, newly emerged, whereas the 15 days old stages showed only 5% decrease of specific activity. In CTC-12 strain the insecticide treatment resulted in 77, 64, 68 and 72% inhibition of TE in the 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old beetles.

Soluble protein contents

After the treatment of different developmental stages of Pak strain with pirimiphos-methyl, the soluble protein contents of 4th instar larvae showed 30% decrease, whereas the other stages do not show any significant change. In FSS-II strain the 4th instar larvae showed 38% increase after insecticide treatment, whereas the 6th instar larvae, newly emerged adult beetles and 15 days old beetles showed 9, 9 and 13% decrease, respectively. The CTC-12 strain, likewise, showed 24% increase in the 4th instar larvae while the remaining stages showed non significant increase of 5, 1.5 and 7% in the 6th instar larvae, newly emerged adult beetles and 15 days old beetles (Table II).

Carboxylesterase (CE) activity

Table III shows the total and specific activities of CE in four different developmental stages after the treatment with sub-lethal dose of pirimiphos-methyl. In Pak strain the total CE activity decreased significantly in the 4th instar larvae (56%), 6th instar larvae (29%), newly emerged adults (76%) and 15 days old adult beetles (66%). In FSS-II strain this decrease was, respectively, 81, 75, 64 and 27%, whereas in the CTC-12 strain the total CE activity was inhibited, respectively, 61, 58, 51 and 48% in the 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old adult beetles of *T. castaneum*.

The insecticide treatment showed the same pattern of change in the specific activity of CE, as for the total activity of this enzyme (Table III). The specific activity of Pak strain larvae and beetles decreased significantly after insecticide treatment *viz.*, 38%, 34%, 73% and 67%, respectively, in the 4th instar larvae, 6th instar larvae, newly emerged beetles and 15 days old beetles. In the susceptible strain the insecticidal inhibition of CE specific activity was 85%, 75%, 60% and 17%, respectively, for the 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old adults beetles, whereas in the resistant strain (CTC-12), this inhibition was 70%, 60% 52% and 52%, respectively. The Pak strain appears to be least sensitive.

Acetylcholinesterase (AChE) activity

After treatment with pirimiphos-methyl the total AChE activity significantly decreased in the 4th instar larvae (82%), 6th instar larvae (74%), newly emerged adults (81%) and 15 days old adult beetles (42%) of Pak strain, while in the FSS-II strain this decrease was 15, 6, 29 and 25%, respectively. The AChE activity of CTC-12 strain showed 72, 86, 80 and 83% decrease in the 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old beetles of *T. castaneum* (Table IV).

The Pak strain showed significant decrease in AChE specific activity in the 4th instar larvae (83%), 6th instar larvae (79%), newly emerged adults (86%) and 15 days old beetles (45%) after treatment with pirimiphos-methyl. In FSS-II strain the specific activity of AChE decreased 32, 22 and 15% in the 4th instar larvae, newly emerged adult

Table I.- Effect of sub lethal doses of an OP, pirimiphos-methyl, on the total esterases of 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old adult beetles of three different strains (Pak, FSS-II, CTC-12) of *T. castaneum.*

Developmental stages		Total enzymatic activity (IU/mg body weight)			Specific activity (mIU/mg soluble protein)		
		Pak (n=3)	FSS-II (n=3)	CTC-12 (n=3)	Pak (n=3)	FSS-II (n=3)	CTC-12 (n=3)
4th instar larvae	Control	5.33±0.16	4.95±0.07	8.95±0.33	36.19±1.07	53.93±1.35	72.27±1.73
	Treated	2.45±0.15	1.02±0.03	2.43±0.07	25.21±0.45	7.82±0.33	16.48±1.20
6th instar larvae	Control	3.64±0.05	3.61±0.01	6.89±0.17	35.45±2.23	37.53±3.01	60.25±1.24
	Treated	1.89±0.12	1.11±0.01	2.59±0.06	17.10±1.92	11.86±0.59	21.61±1.019
Newly emerged adults	Control	3.66±0.15	1.98±0.01	6.68±0.21	35.13±3.15	21.28±1.21	61.63±3.13
	Treated	1.214±0.14	0.75±0.03	2.16±0.17	8.35±1.07	8.86±0.47	19.63±1.50
15 days old adults	Control	3.01±0.07	2.02±0.01	6.17±0.10	31.20±1.86	21.69±0.15	69.48±3.30
	Treated	1.24±0.02	1.90±0.06	1.82±0.07	12.43±0.76	20.66±1.10	19.20±
LSD at P=0.05	**For age**	0.35	0.15	0.46	4.03	2.56	3.86
	Treatment	0.49	0.20	0.65	6.09	3.62	5.46
LSD among the strains at P=0.05			1.04			18.43	

*IU(International unit), transformation of one micromole substance in one minute under the condition of the test.

Table II.- Effect of sub lethal doses of an OP, pirimiphos-methyl, on the total protein of 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old adult beetles of three different strains (Pak, FSS-II, CTC-12) of *T. castaneum.*

Developmental stages		Soluble protein contents µg/mg		
		Pak (n=3)	FSS-II (n=3)	CTC-12 (n=3)
4th instar larvae	Control	147.5±4.46	91.65±4.33	125.65±7.42
	Treated	103.66±2.56	126.51±6.55	155.67±6.88
6th instar larvae	Control	103.0±2.08	103.67±3.28	114.32±0.49
	Treated	110.40±6.59	94.47±6.16	120.66±7.85
Newly emerged adults	Control	132.33±1.76	93.04±1.08	108.51±0.04
	Treated	136.64±1.13	85.12±5.84	110.03±3.31
15 days old adults	Control	96.45±5.83	78.79±2.79	88.88±2.43
	Treated	100.18±3.59	68.86±0.73	95.51±4.32
LSD at P=0.05	For age	9.83	10.51	17.59
	Treatment	13.9	14.87	12.44
LSD among the strains at P=0.05			25.7	

beetles and 15 days old beetles after insecticide treatment. The CTC-12 strain showed 84, 80, 80 and 84% inhibition of AChE specific activity in the 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old beetles of *T. castaneum*, respectively, after the treatment of insecticide.

Cholinesterase (ChE) Activity

Table V shows the effect of pirimiphos-methyl on the total and specific activity of ChE. In Pak strain the 4th instar larvae, 6th instar larvae and 15 days old beetles showed 65, 59 and 37% increase in the total activity of ChE, while the newly emerged adult beetles showed 31% decrease. The FSS-II strain showed 82, 47 and 33% decrease in 4th instar larvae, 6th instar larvae and newly emerged adult beetles, whereas in 15 days old beetles the total ChE increased 21% after insecticide treatment.

Table III.- Effect of sub lethal doses of an OP, pirimiphos- methyl, on the carboxylesterase of 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old adult beetles of three different strains (Pak, FSS-II, CTC-12) of *T. castaneum*.

Developmental stages		Total enzymatic activity (IU/mg body weight)			Specific activity (mIU/mg soluble protein)		
		Pak (n=3)	FSS-II (n=3)	CTC-12 (n=3)	Pak (n=3)	FSS-II (n=3)	CTC-12 (n=3)
4th instar larvae	Control	3.89±0.08	4.42±0.22	7.2±0.27	26.48±0.86	42.73±1.26	60.44±0.96
	Treated	1.71±0.06	0.82±0.04	2.79±0.04	16.49±0.48	6.227±0.19	17.97±0.66
6th instar larvae	Control	2.67±0.08	3.08±0.05	5.86±0.16	25.98±0.67	33.28±1.26	51.26±1.53
	Treated	1.90±0.14	0.77±0.09	2.43±0.05	17.23±0.77	8.28±1.16	20.34±1.86
Newly emerged adults	Control	2.88±0.18	1.55±0.06	4.84±0.15	21.79±0.78	16.72±0.57	44.6±1.52
	Treated	0.69±0.01	0.56±0.03	2,35±0.07	5.59±0.44	6.63±0.43	21.38±0.57
15 days old adults	Control	2.89±0.25	1.65±0.07	5.73±0.24	29.94±1.32	20.92±0.19	64.87±4.78
	Treated	0.98±0.04	1.20±0.07	2.96±0.13	9.81±0.24	17.41±0.92	31.06±0.79
LSD at P=0.05	**For age**	0.32	0.25	0.73	2.97	1.77	3.89
	Treatment	0.45	0.35	1.04	4.19	2.49	5.51
LSD among the strains at P=0.05			1.09			14.66	

*IU(International unit), transformation of one micromole substance in one minute under the condition of the test.

Table IV.- Effect of sub lethal doses of an OP, pirimiphos-methyl, on the acetylcholinesterase of 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old adult beetles of three different strains (Pak, FSS-II, CTC-12) of *T. castaneum*.

Developmental stages		Total enzymatic activity (IU/mg body weight)			Specific activity (mIU/mg soluble protein)		
		Pak (n=3)	FSS-II (n=3)	CTC-12 (n=3)	Pak (n=3)	FSS-II (n=3)	CTC-12 (n=3)
4th instar larvae	Control	11.32±0.29	8.25±50.16	9.58±0.89	92.27±3.37	93.82±0.80	77.40±0.80
	Treated	1.99±0.08	7.8±0.11	2.66±0.12	12.78±0.31	63.35±2.72	12.61±0.27
6th instar larvae	Control	13.56±0.14	8.11±0.15	13.498±0.35	125.60±3.10	78.48±2.34	115.88±22.4
	Treated	3.48±0.27	7.44±0.09	1.92±0.05	32.05±4.58	79.6±5.71	22.31±2.27
Newly emerged adults	Control	14.68±0.14	10.56±0.24	11.74±0.08	140.92±7.38	113.56±3.07	108.24±0.56
	Treated	2.72±0.24	7.47±0.23	2.31±0.09	19.89±1.59	88.74±7.84	21.04±0.30
15 days old adults	Control	5.84±0.53	7.66±0.05	6.00±0.09	60.71±4.84	97.49±3.52	67.50±1.54
	Treated	3.36±0.23	5.66±0.24	1.01±0.02	33.58±2.07	82.42±3.69	10.66±0.8
LSD at P=0.05	**For age**	**0.72**	0.52	0.72	9.52	8.17	4.37
	Treatment	**1.02**	0.73	1.01	13.46	11.56	6.18
LSD among the strains at P=0.05			4.08			42.92	

*IU(International unit), transformation of one micromole substance in one minute under the condition of the test.

In CTC-12 strain, however the 4th and 6th instar larvae had 29 and 15 low activity after insecticide treatment, while in the newly emerged and 15 days old adult beetles, an increase of 8 and 80%, respectively, was observed after insecticide treatment.

The above data was also interpreted in term of specificity of ChE. In Pak strain ChE specific activities increased 119, 43 and 86% in the 4th instar larvae, 6th instar larvae and 15 days old beetles after insecticide treatment, whereas in the newly emerged adult beetles this activity decreased 36%. In FSS-II strain, the ChE specific activity was inhibited 87, 41, and 26% in the 4th instar larvae,

Table V.- Effect of sub lethal doses of an OP, pirimiphos-methyl, on the cholinesterase of 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old adult beetles of three different strains (Pak, FSS-II, CTC-12) of *T. castaneum*.

Developmental stages		Total enzymatic activity (RU/mg body weight)			Specific activity (mRU/mg soluble protein)		
		Pak (n=3)	FSS-II (n=3)	CTC-12 (n=3)	Pak (n=3)	FSS-II (n=3)	CTC-12 (n=3)
4th instar larvae	Control	0.35±0.03	0.82±0.04	0.35±0.02	2.42±0.19	8.92±0.43	2.62±0.14
	Treated	0.55±0.02	0.15±0.02	0.25±0.01	5.32±0.04	1.12±0.04	1.63±0.10
6th instar larvae	Control	0.37±0.03	0.71±0.01	0.39±0.04	3.54±0.10	6.89±0.18	3.41±0.04
	Treated	0.58±0.01	0.38±0.08	0.33±0.02	5.08±0.30	4.06±0.33	2.72±0.23
Newly emerged adults	Control	0.14±0.02	0.57±.0.02	0.36±0.02	3.87±0.33	6.16±0.28	3.98±0.17
	Treated	0.34±0.02	0.39±0.01	0.39±0.08	2.49±0.12	4.57±0.31	3.60±0.18
15 days old adults	Control	0.26±0.01	0.59±0.04	0.42±0.01	2.68±0.08	7.51±0.48	4.69±0.15
	Treated	0.50±0.03	0.72±0.03	0.76±0.02	4.99±0.47	10.04±0.52	8.01±0.22
LSD at P=0.05	**For age**	0.05	0.07	0.14	0.65	1.18	0.52
	Treatment	0.07	0.10	0.19	0.92	1.31	0.73
LSD among the strains at P=0.05			0.16			2.25	

*RU(Rappaport unit), amount of enzyme that will liberated one micromole of acetic acid from acetylcholine in 30 minutes under the test conditions.

Table VI.- Effect of sub lethal doses of an OP, pirimiphos-methyl, on the arylesterase of 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old adult beetles of three different strains (Pak, FSS-II, CTC-12) of *T. castaneum*.

Developmental stages	00	Total enzymatic activity (IU/mg body weight)			Specific activity (mIU/mg soluble protein)		
		Pak (n=3)	FSS-II (n=3)	CTC-12 (n=3)	Pak (n=3)	FSS-II (n=3)	CTC-12 (n=3)
4th instar larvae	Control	18.74±0.26	32.74±1.24	34.27±2.62	127.49±5.65	357.26±11.47	276.37±9.29
	Treated	3.05±0.31	3.70±0.22	11.06±1.43	29.43±2.92	28.18±1.64	70.57±6.30
6th instar larvae	Control	12.70±0.58	27.01±1.17	30.83±0.97	123.29±4.27	261.71±13.69	269.64±7.36
	Treated	2.80±0.25	2.96±0.73	6.71±0.17	25.40±2.04	31.09±1.45	52.62±0.25
Newly emerged adults	Control	9.16±0.36	17.91±0.97	22.85±1.42	72.23±1.34	192.55±11.70	210.76±13.33
	Treated	3.24±0.27	3.62±0.11	9.13±0.32	25.97±2.5	43.27±5.14	83.14±4.75
15 days old adults	Control	6.72±0.10	14.56±0.32	14.99±0.48	70.62±3.96	184.94±5.33	170.99±13.15
	Treated	2.72±0.49	9.29±0.84	11.19±.051	27.15±0.71	135.18±13.72	117.39±7.72
LSD at P=0.05	**For age**	2.28	2.01	3.02	10.01	24.97	21.35
	Treatment	**3.23**	2.85	4.24	14.16	35.32	30.19
LSD among the strains at P=0.05			4.7			59.79	

*IU (International unit), transformation of one micromole substance in one minute under the condition of the test.

6th instar larvae and newly emerged adult beetles after pirimiphos-methyl treatment, whereas in 15 days old beetles this activity increased 38% after the insecticide treatment. The ChE was inhibited in the CTC-12 strain. The inhibition was 37, 20 and 9%, respectively, in the 4th instar larvae, 6th instar larvae and newly emerged adult beetles while it showed 71% increase in 15 days old beetles after pirimiphos-methyl treatment.

Arylesterase (AE) activity

The Table VI shows drastic inhibitory effect of pirimiphos-methyl on the AE activity. In Pak strain the total AE activity was inhibited 84, 78, 65

and 60%, respectively, in the 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old beetles. The FSS-II strain also showed significant decrease in the 4th instar larvae (89%), 6th instar larvae (89%), newly emerged adults (80%) and 15 days old beetles (36%) after insecticide treatment. A significant decrease of 67, 78, 60 and 25% was also observed in CTC-12 strain 4th instar larvae, 6th instar larvae, newly emerged and 15 days old beetles, with respect to control.

Similar trend was observed when the enzyme activity was interpreted as specific activity. After treatment with insecticide the specific activities of 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old adult beetles of Pak strain decreased 77, 79, 64 and 62%, respectively, while this decrease was 92, 88, 77 and 27% in FSS-II strain and 74, 80, 61 and 31% in all four stages of CTC-12 strain, respectively.

DISCUSSION

The development of resistance to chemical insecticides in arthropod pests constitute worldwide economic problem (Georghiou, 1986). *T. castaneum* has been subjected to considerable selection pressure with pesticide in the storage environment. As a consequence of the selection pressure, these beetles have developed resistance to many of the commonly used pesticides (Champ and Dyte, 1976).

Esterase activity is monitored in arthropod species because high level of these enzymes can be associated with insecticide resistance. These enzymes may be responsible for insecticide resistance through increased detoxification, or sequestration, or both (Devonshire and Moores, 1982).

In the present work, the level of insecticide resistance has been assessed and evaluated in Pak strain larvae and adult beetles, using established resistant (CTC 12) and susceptible (FSS-1I) strains of *T. castaneum* as positive and negative controls. The effect of sublethal doses of insecticides (pirimiphos-methyl) has also been studied on esterase activities.

Total esterases

The 4th instar larvae of the Pak, FSS-II and CTC-12 strains have higher TE activity as compared with other developmental stages. On comparison of three strains, the CTC-12 strains showed high level of TE as compared with FSS-II and Pak strains. Lewis and Madge (1984) reported that the TE activity was appreciably higher in resistant aphids than in susceptible ones. Resistant aphids were associated with, higher esterase isoenzyme activity of complex bands II-V. while in susceptible aphids these bands were virtually missing.

In the present study pirimiphos-methyl treated larvae and beetles have low level of TE at every stage under study with reference to control in Pak, FSS-II and CTC-12 strains. Moores *et al.* (1996) also reported inhibition of TE aetivity and AChE by insecticides (Carbarmate and OP) in three strains of *Aphis gossypii* Glover. Inhibition of insect permethrin esterases by OP compounds and carbamates occurs in several species (Jao and Casida. 1974; Holden, 1979; DeVries and Georghiou, 1981). Similarly Chang and Jordan (1982b) also noted significant inhibition of esterases by carbaryl and pirimiphos-methyl.

Carboxylesterase

Pirimiphos-methyl treatment significantly decreased the activities of various esterases in all the developmental stages of the three strains. It is well established that CE is responsible for OP resistance through an increased degradation or sequestering of insecticide molecules (Devonshire, 1991; Georghiou and Saito, 1983). This increased detoxication by CE is one of the most important factors in OP insecticide resistance, especially in homopterous, dipterous and acarine pest species (Devonshire 1991). Wanchun *et al.* (1999) reported toxicity of cytisine from *Sophora alapecuroides* (L) (Papilionaceae) against the mustard aphid *Lipaphis erymisi* (Kaltenbach) and its effect on esterase activity. Cytisine inhibited TE and CE activity in this species.

Haubruge *et al.* (2002) reported specific resistance to malathion in a strain of *T. castaneum* is due to a 44-fold increase in malathion carboxylesterase (MCE) activity relative to a susceptible strain. The kinetic analysis suggests that MCE of resistant insects hydrolyses malathion faster than the purified carboxylesterase from susceptible

beetles and that this enzyme has greater affinity for malathion. Malathion-specific resistance is due to the presence of a qualitatively different esterase in the resistant strain.

OP resistance in *T. castaneum appeare*s to have developed by at least two routes, malathion specific CE the predominant resistance mechanism in most strains of flour beetles and high resistance frequencies coupled with the high storage temperature that cause rapid residue breakdown have made malathion treatments extremely inefficient so that they are probably no longer cost effective (Haliscak and Beeman, 1983; Zettler and Cuperus, 1990).

Acetylcholinesterase

Degree of inhibition of AChE depends both on the concentration of inhibitor in its vicinity, and the duration of contact, so either or both must increase to maintain the same degree of inhibition, if the enzyme is modified to an insensitive form in resistant insects. This increased concentration of insecticide and extended survival of the insect permit other factors, especially metabolic activation and detoxication, in otherwise susceptible insects to influence the amount of poison reaching the target. Such factor either facilitate or obstruct the insecticide reaching its target (Devonshire, 1979). Yuan and Chambers (1998) reported both *in vivo* and *in vitro* studies that AE played an important role in non catalytic detoxication of OP insecticides in boll weevils. Acting as an alternative phosphorylation site, AE reduced the concentration of OP insecticides reaching AChE and thus, provides a protection to this target. The level of this detoxication depends on relative sensitivities or affinities of the axons for AE and AChE.

In present study the pirimiphos-methyl treated Pak FSS-II and CTC-l2 strains showed decrease in AChE activities. Among the three strains, Pak and CTC-12 strains holds a maximum decrease (82, 74, 83 and 42% in Pak strain, respectively and 72, 85, 80 and 83% in CTC-12, respectively) while the FSS-II strain showed comparatively less decrease (5, 6, 29 and 25%, respectively) of AChE in all the developmental stages. Reyes *et al*. (2008) reported the activity of detoxifying enzymes (glutathione-S-transferases (GST), mixed-function oxidases (MFO), and esterases (acetylcholinesterase) in codling moths. Activities of the three enzymatic systems across all populations were positively correlated. The co-occurrence of moths expressing significant elevated GST, MFO and low EST activities was also reported.

The inhibition of AChE activity by OP insecticides is a very well understood phenomenon. In houseflies, AChE. inhibition is the primary cause of death (Casida, 1956*;* O' Brein, 1961). Brown and Bryson (1992) reported that high concentration of methyl parathion against *Heliothis virescens* inhibit the AChE activity. The specificity of the insensitivity for one insecticide is unusual, and Silver (1984) suggested that inhibition by pirimicarb of AChE from *Aphis gossypii* might involve its binding to a site (allosteric) distinct from substrate binding site. A recent report of reciprocal cross-resistance when *Aphis gossypii* was selected with either pirimiphos-methyl or permethrin (Zil'bermints and Zhuravleva, 1984) clearly implicated some other biochemical mechanism in this particular case.

According to general classification of esterases, both AChE and AE are B-esterase which are characterized by inhibition by OPs. It has been widely accepted that inhibition of AChE in cholinergic synapses of the nervous system is the primary mechanism of acute toxicity of OP insecticides. Based on the studies on esterases in houseflies (Van Asperene, 1960, 1964), LC_{50} of the four phosphorothionate insecticides (parathion, methyl parathion, chlorpyrifos and chlorpyrifos-methyl) yield similar inhibition of AChE (44-60%) of boll weevils at their LC_{50} concentration (Yuan and Chambers, 1998). In lacewings species, the Chrysopid *Chrysoperla cameo* Stephens larvae, decline in AChE responses were significantly correlated with increasing OP concentrations. The degree of AChE inhibition observed at the LC_{50} concentration appeared to be specific for the respective toxin *i.e.*, methyl parathion reduced the AChE activity to 20% and azinophos-methyl to 51% of the control value alter 24 hours (Rumpf *et al.*, 1997). Zhu and Brindley (1992) used four OP compounds (paraoxon, dichlorvos, naled and oxydemeton-methyl) to study the inhibition of

AChE purified from five field population of *Lygus hesperus* (Knight). The sensitivity of AChE to the four OP compounds in different insects location was Logan> Roswell> Caldwell> Kuna> Star and the insensitivity spectrum of AChE to different OP compounds was rather broad.

Cholinesterase

In the present study ChE activity in FSS-II stain was higher than in the other two strains of *T. castaneum.* The AChE activity of the FSS-II was however, lower throughout the life cycle when compared with that of Pak and CTC-12 strain. The ChE: activity in the 4th and 6th instar larvae of Pak and CTC-12 strains and 15 days old adults of the three strains increased after treatment with pirimiphos-methyl . Biochemical studies suggested that insecticides produce inhibition because it is similar to ACh and react with esterase in the same way as the normal substrate. This effectiveness of the inhibitors results from the relatively long life of phosphorylated or carbamylated enzyme compared with the acetylated enzyme of normal reaction. So, the resistant organisms produce enzyme insecticide complex having short life, in this way amount of enzyme increase in the organism (Perry and Agosin, 1974).

The 4th and 6th instar larvae of CTC-12 and 4th and 6th instar larvae and newly emerged beetles of FSS-II strain have decreased ChE after the treatment with pirimiphos- methyl. Abiola *et al.* (1991) reported that OP compounds are widely employed in the control of crop and animal insects. The toxicity of these compounds result from their irreversible inhibition of ChE, resulting in accumulation of ACh at cholinergic sites.

Arylesterase

The maximum AE activity observed in the 4th instar larvae of the three strains, which decreased during the subsequent development. The comparison of three strains revealed that the CTC-12 strain has the higher AE activity than that of FSS-II, while the Pak strain has lower activity than FSS-II strain. Mackness *et al.* (1983) reported that α-naphthylacetate, p-nitrophenylacetate and phenylacetate were hydrolysed by homogenate of all the three strain, although there were inter strain differences. Kano C strain of *T. castaneum* showed only some 50% of the specific activity towards these three substrates shown by CTC-12 and FSS-II indicating that it has less AE per unit protein and or a less active esterase than CTC-12 or FSS-II.

In the present study AE activity of the three strains was inhibited in the larvae as well as adult beetles after treatment with pirimiphos-methyl. According to Mackness *et al.* (1983) the AE activity is inhibited by paraoxon more strongly in the insect homogenate in sheep serum.

The biochemical analysis depicts that after the treatment with pirimiphos-methyl the decrease of esterases was considerably high in three strains. From the results, it could be concluded that Pak strain possesses lowest level of esterases at the 4th instar larval stage and hence could best be controlled at this stage with pirimiphos-methyl.

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