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Effect of Fury, a Synthetic Pyrethroid, 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 Synthetic Pyrethroids insecticide Fury has been checked 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 recognized insecticide resistance of the pest and hence suggested alternate control measures. The different types of esterases and total soluble protein activities were determined biochemically, using spectrophotometer. The total soluble protein contents significantly decrease (30%), after Fury treatment in 4th instar larvae whereas newly emerged adults and 15 days old beetles showed increase (28 and 12%). The various developmental stages of FSS-II and CTC-12 strains showed increase of total soluble contents after treatment with Fury. The Fury treatment however did not show any significant carboxylesterase (CE) activity in all the developmental stages of Pak strain. The 4th and 6th instar larvae of FSS-II and 4th instar larvae of CTC-12 strain showed decrease CE activity, whereas all other developmental stages remained unaffected. After the treatment with Fury the AChE activity decreased significantly in all stages of the three strains except 15 days old beetles of Pak and CTC-12 strains. The Fury treatment resulted in increase of ChE activity in the larval stages of Pak and FSS-II, while the adult stages were not affected. In CTC-12 strain the ChE activity decreased (46 and 67%) significantly in the 4th instar larvae and newly emerged adult after Fury treatment whereas in 15 days old adult the ChE activity increased (40%) significantly. The arylesterase (AE) activity decreased (33%) in 4th instar larvae and increased (38%) in 6th instar larvae whereas the other stages remained unaffected of Pak strain after Fury treatment. The 15 days old beetles of FSS-II and 4th and 6th instar larvae of CTC-12 strain showed significantly decreased AE activity, while the other stages showed non-significant changes after treatment with Fury. The Fury treatment, caused decrease (15%) of total esterases (TE) in the 4th instar larvae of Pak strain, whereas 4th instar larvae, 6th instar larvae, newly emerged and 15 days old beetles of FSS-II strain showed decrease (17, 26, 20 and 36%) respectively. In CTC-12 strain the TE activity of 15 days old beetles increased (24%) after Fury treatment, while the remaining stages showed no significant change.

Key words: Synthetic pyrethroids, carboxylesterase, acetylcholesterase, insecticide resistance, red flour beetle.

INTRODUCTION

Pyrethroid insecticides have been widely used since the late 1970s and now they are the major insecticides used in agriculture and household pest control. Pyrethroids, are of two types, natural pyrethroids and synthetic pyrethroids. Natural pyrethroids are obtained from flower of a plant, *Pyrethrum cinerarifolium* (genus *Chrysanthemum*, family Compositae). The flower contains about 90 % of pyrethrins. Pyrethrins have been used for many years to control insect attacking food products including *T. castaneum* (Carte *at al.*, 1975; Halliday *et al.*, 1988).

The synthetic pyrethroids are highly potent neurotoxic insecticides which greatly affect the behavioural and neuro-endocrine functions of mammals. They alter the normal function of sodium (Na^+) channel. DDT and many of its structural analogues have similar mode of neuro-toxic action (Beeman, 1982). The introduction of ∝-cyano group and halogenation of esters further enhanced the activity and stability of pyrethroid. Pyrethroid encompass an increasingly diverse range of lipophilic and insecticidal compounds capable of assuming similar configuration on binding at the relevant neuroreceptor (Elliott, 1977). Metabolic studies in insects, mammals, plants and environmental system are needed to evaluate pyrethroid mode of action, resistance mechanism,

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interaction with synergists, selective toxicity relationship, residual properties and safety in use (Casida and Ruzo, 1980).

Pyrethroid insecticides are characterised by high knockdown and lethal activity, a wide spectrum, good residual activity together with repellent and antifeeding activity (Hirano, 1989). Pyrethroids possess high toxicity to insects, low toxicity to mammals, photostability, high degradability and effective application at minimum dose (Barlow *et al.,* 1971; Hadaway, 1972). With these characteristics, pyrethroid insecticides have become widely used for plant protection. Their major use has been for the control of bollworm and leafworms in cotton but they have also achieved wide spread use for controlling various species of Lepidopterous pests of fruits and vegetables, aphids in cereals, and many other minor outlet. Although the early synthetic pyrethroid were ineffective against mites and soil pests, later additions such as fenpropathrin, have combined high acaricidal activity with insecticidal activity, and further pyrethroid are being introduced for use in soil. The extent of pyrethroid use has increased progressively since the first photostable pyrethroid was registered.

Metabolism of pyrethroids by esterases and oxidase action usually limits their toxicity to mammals more than to insect, thereby conferring useful selective toxicity properties (Casida and Ruzo, 1980; Ishaaya and Casida, 1980; Soderlund *et al.,* 1983). Hence they are expected to supplement and possibly replace some of the conventional OP, OC and carbamate insecticides. Appearance of resistance to OP insecticide in *T. castaneum* is the major reason for failure of control mechanisms against the pest (Hoppe, 1981). The two most important detoxification pathways for pyrethroid involve esterases and hydroxylation by MFOs enzyme which involve cytochrome P-450 (Shono *et al.,* 1979; Lee *et al.,* 1989). Zhao *et al.* (1996) suggested that both cytochrome P-450 and esterases play an important role in Tobacco budworm resistance and cross resistance between carbamates, OPs and pyrethroids. Resistance to pyrethroids is clearly a growing problem (Twine and Reynold, 1980; Plapp and Campanhola, 1986).

Resistance to the pyrethroid was first reported in Australian population of *Helicoverpa armigera* by Gunning *et al.* (1984). This resistance has been characterized as being due to gut site insensitivity, penetration resistance and increased metabolic detoxification (Gunning *et al.,* 1991). Resistance to pyrethroid was reported for *H. armigera* in Thailand, India, Indonesia, Egypt and Zimbabwe (Sawicki and Denholm 1987, 1989; Denholm and Rowland, 1992). Resistance to pyrethroid, like that to most insecticides, can be due to number of causes, including reduced sensitivity of target site, reduced cuticular penetration and increased rates of detoxification (Brattsten *et al.,* 1986). The pyrethroid resistance also observed in diamondback moth, *Plutella xylostella* most likely attributed to decreased target site sensitivity (Yu and Nguyen, 1996). *Rhyzopertha dominica* and *Sitotroga cerealella* are major insect pests of stored grain in Taiwan. Synthetic pyrethroids and carbamates synergised with piperonyl butoxide (PB) exerted siginificant improvement against Phoxim resistant strain of *R. dominica* and *S. cerealella* (Chi and Chen, 1997).

Marked characteristics of pyrethroids is their susceptibility to attack by metabolic system. All well known pyrethroids are esters. They are inactivated by cleavage with esterases. AChE is the most usual enzyme to be inhibited in sensitive organism (Carbett, 1974) but CE has been suggested as possible target in some case (Oppenoorth and Van Asperen, 1960).

Pyrethroid esterases are a major factor in the tolerance of adults of the whitefly, *Bemesia tabaci*, to cypermethrin and related pyrethroids (Ishaaya *et al.,* 1987). Collins (1990) reported failure of pyrethroids, such as deltamethrin, cypermethrin or cyfluthrin, used in the field to control *T. castaneum*, even if they are synergised with piperonyl butoxide. The resistance dose not jeopardise OP materials *e.g*. fenitrothion, chlorpyrifos methyl, pirimiphos-methyl, methacrifos or methoprene. Stuart *et al.* (1998) reported that the *T. castaneum* (Herbst.) strain QTC 279 is highly resistant to deltamethrin and other synthetic pyrethroids. This strain was shown to carry at least one resistance gene.

The development of resistance emphasizes the need for control strategies to prolong the useful life of insecticides. These might involve the use of integrated control in situations where this is

practicable, improved forecasting system to minimize insecticide use, and the development of novel control chemicals such as antifeedants or other behavior-controlling chemicals. It might be expected that the performance of such compounds lacking an ester group would be unaffected by the known resistance mechanism in aphids (Dawson *et al.*, 1983).

The present study aims to correlate insecticide resistance with levels of different categories of esterases, and to assess the effects of synthetic pyrethroid (Fury) on three different strains of *T. castaneum* (Pak, FSS-II, CTC-12). 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.*

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, Fury (S-Cyano (2-Phenoxyphenyl (±) cis/trans 3-(2,2-dichloroethenyl)-2,2 dimethylcyclopropane carboxylate) is a pyrethroid insecticide. Fury is designed to provide effective control of a wide range of insects on cotton and other crops. In t plant safety and minimal environmental impact. This pesticide is extremely toxic to fish and aquatic inverteaddition to outstanding insect control, Fury provides excellenbrates, and it is highly toxic to bees exposed to direct treatment or residues on blooming crops or weeds. It has a broad-spectrum of activity, including tobacco bud worm, cotton boll worm, cabbage looper, corn earworm, codling moth, soya bean looper, tomato fruit worm and peach borer.

Determination of LC_{50}

For determination of LC_{50}, Residual Film method was used for which the serial dilutions of Fury 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 *et al*. (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 Fury against the 4th instar larvae, 6th instar larvae, newly emerged adults and 15 days old beetles of Pak strain were 405 ppm, 648 ppm 560 ppm and 121 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 856 ppm, 1088 ppm, 555 ppm and 58, respectively, whereas CTC-12 strain showed 1442 ppm, 1285 ppm, 1420 ppm and 671 ppm, respectively in the above mentioned stages.

Carboxylesterase (CE) activity

The insecticide treatment did not produce any significant change in total CE activity of various developmental stages of Pak strain, whereas in the FSS-II strain the 4th and the 6th instar larvae showed, the 11 and 17% decrease, respectively, after the insecticide treatment. The other two stages did not show any significant change. In the CTC-12 strain the 4th instar larvae showed 15% decrease after the treatment with Fury, while the 6th instar larvae, newly emerged adults and 15 days old beetles showed no significant change (Table I).

The insecticide treatment had similar type of effect on the specific activity of CE in various developmental stages. In Pak strain the CE specific activity decreased 15, 22 and 17% in the 4th instar larvae, newly emerged adults and 15 days old beetles after insecticide treatment. In the FSS-II strain, the 4th instar larvae, 6th instar larvae, and 15 days old beetles had their CE specific activities reduced 36, 31, and 8%, after insecticide treatments, whereas the newly emerged adult have 3.5% increase. In CTC-12 strain this decrease was 20% in all the developmental stages after insecticide treatment (Table I).

Acetylcholinesterase (AChE) activity

After the administration of Fury total activity of AChE of 4th instar larvae, 6th instar larvae, newly emerged and 15 days old beetles of Pak strain decreased 34, 51, 36, and 8%, respectively. In FSS-II strain this decrease was 17, 22, 4 and 29%, respectively, in the 4th instar, 6th instar larvae, newly emerged beetles and 15 days old beetles after the treatment with insecticide. The AChE activity decreased 53, 49, 32% and 9% in 4^{th} instar larvae, 6th instar larvae, newly emerged beetles and the 15 days old beetles of CTC-12 strain, after the treatment of insecticide (Table II).

The specific activity of Pak strain decreased 13, 50, 47 and 9% in the 4th instar larvae, 6th instar larvae, newly emerged beetles and 15 days old beetles, respectively, after the insecticide treatment. The FSS-II strain exhibited 40, 31 and 32% decrease in the specific activity in the treated 4th instar larvae, 6th instar larvae and newly emerged adult beetles. The treated CTC-12 strain showed 61, 52 and 36% decreased in the specific activity of AChE in the 4th instar larvae, 6th instar larvae and newly emerged adults respectively. The 15 days old

Table I.- Effect of sub lethal doses of synthetic pyrethroid, Fury, 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	3.80±0.07	4.74±0.06	8.41±0.17	25.74±0.01	52.89±2.18	62.26±4.45
	Treated	3.30±0.04	4.2±0.14	6.56±0.42	21.94±1.38	34.11±2.36	49.65±3.58
6th instar larvae	Control	2.07±0.06	3.64±0.29	6.15±0.13	23.28±0.50	32.21±2.32	61.14±4.52
	Treated	2.78±0.08	3.02±0.05	5.83±0.06	24.33±0.10	22.24±1.71	48.72±3.80
Newly emerged adults	Control	2.75±0.14	2.00±0.05	5.63±0.27	17.76±0.99	22.33±1.38	48.41±2.12
	Treated	2.63±0.08	2.09±0.03	2.87±0.17	13.82±0.25	23.06±0.45	38.58±0.85
15 days old adults	Control	2.81±0.02	2.21±0.06	5.61±0.05	24.26±0.99	24.46±1.02	59.61±1.42
	Treated	2.61±0.06	1.83±0.19	6.13±0.47	20.23±1.23	22.55±2.91	60.59±2.95
LSD at P=0.05	**For age**	0.80	0.36	1.08	3.44	5.13	6.94
	Treatment	1.11	0.51	0.76	4.87	7.25	9.81
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 synthetic pyrethroid, Fury, 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	13.31±0.34	8.50±0.48	9.15±0.22	96.98±.67	94.81±6.03	90.97±0.72
	Treated	8.74±0.24	7.01±0.27	4.29±0.14	84.40±1.465	56.53±1.09	35.85±3.68
6th instar larvae	Control	12.59±0.73	8.42±0.43	13.00±0.32	108.4±6.83	74.18±2.94	97.00±2.65
	Treated	6.20±0.62	6.54±0.56	6.65±0.14	54.26±4.95	51.49±3.51	46.85±2.98
Newly emerged adults	Control	15.14±1.02	10.5±0.46	13.56±0.46	97.66±6.69	93.06±5.33	118.8±2.40
	Treated	9.76±0.65	10.1±0.97	9.20±0.11	51.58±4.49	63.17±3.13	73.44±1.44
15 days old adults	Control	6.34±0.17	6.61±0.27	6.80±0.34	56.40±2.87	73.60±2.09	72.47±4.58
	Treated	5.84±0.38	4.68±0.33	6.16±0.19	51.22±6.56	69.22±6.00	59.16±5.52
LSD at P=0.05	**For age**	1.50	1.13	0.83	13.43	10.94	10.61
	Treatment	15.08	2.11	1.60	1.18	18.59	15.45
LSD among the strains at P=0.05			2.91			24.63	

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

adult beetles of all the three strains showed no significant change in the specific activity of AChE (Table II).

Cholinesterase (ChE) activity

The Fury treated Pak strain showed 107 and 35% increased ChE activity in the 4th and 6th instar

larvae, while the remaining stages showed non significant changes. The 24% increase in the ChE activity was noted in the 6th instar larvae of FSS-II strain whereas other stages showed non significant increase after insecticide treatment. In CTC-12 strain the ChE activity decreased 46, 13 and 67%, respectively, in the 4th instar larvae, 6th instar larvae and newly emerged beetles, while the 15 days old beetles showed 40% increase after insecticide treatment (Table III).

The specific activity of Fury treated Pak strain increased 194 and 46% in the 4th and 6th instar larvae, whereas in the newly emerged beetles and 15 days old beetles the specific activity of ChE decreased 22 and 20%, respectively. The FSS-II 4th instar larvae and newly emerged beetles showed 35 and 44% decrease in the specific activity, respectively, while 15 days old beetles showed 45% increase in specific activity after the insecticide treatment. In CTC-12 strain the specific activity decreased 46 and 70% in the 4th instar larvae and newly emerged beetles while the 15 days old beetles, had 14% increase after the treatment of Fury. The 6th instar larvae of FSS-II and CTC-12 strains showed non significant changes after the treatment with insecticide (Table III).

Arylesterase (AE) activity

The Fury treatment of 4th instar larvae and newly emerged adult beetles of Pak strain resulted in 33 and 10% decrease in AE total activity, whereas the 6th instar larvae and 15 days old beetles showed 38 and 2% increase over the control, respectively. In the 15 days old beetles of FSS-II strain AE activity decreased 49% when compared with their respective control values. In CTC-12 strain the significant decrease of 37 and 15% was observed in the 4th instar larvae and 6th instar larvae after treatment with insecticide, whereas the other two adult stages did not show any significant effect (Table IV).

In term of specific activities the 6th instar larvae of Pak strain showed 40% increase after the insecticide treatment, while the 4th instar larvae, newly emerged adults and 15 days old beetles showed non significant decrease. The AE specific activity of 4th instar larvae, 6th instar larvae, newly emerged beetles and 15 days old beetles of FSS-II strain decreased 27, 10, 43 and 34% after treatment with insecticide. In CTC-12 strain the AE activity decreased 38 and 30% respectively, in the 4th and 6th instar larvae, whereas the remaining stages showed non significant decrease after the treatment of Fury when compared with the control (Table IV).

Total esterase (TE) activity

The Fury treatment caused 15% decrease in the 4th instar larvae of Pak strain, while the remaining stages showed no significant decrease. The FSS-II strain exhibited 17, 26 20 and 36% decrease after the treatment with insecticide in 4th instar larvae, 6th instar larvae, newly emerged adult beetles and 15 days old beetles. The 24% increase was observed only in 15 days old beetles of CTC-12 strain, while the remaining stages showed no significant decrease (Table V).

The specific activity of Pak strain increased 9% in the 6th instar larvae after treatment with Fury. The 4th instar larvae, newly emerged beetles and 15 days old beetles had lost TE specific activity 7, 27 and 16%, respectively, after the treatment of Fury. The FSS-II 4th instar larvae, 6th instar larvae, newly emerged beetles and 15 days old beetles showed decrease of 45, 33, 55 and 17%, respectively after treatment with Fury. The CTC-12 strain showed 14, 7 and 16% decrease, respectively in the 4th instar larvae, 6th instar larvae and newly emerged adult beetles, while the 15 days old beetles showed 11% increase after the treatment of insecticide (Table V).

Soluble protein contents

After the treatment with Fury, the 4th instar larvae exhibited 30% decrease and newly emerged adult beetles showed 28% increase of soluble proteins, while the remaining developmental stages showed no significant change. In FSS-II strain, the 4th instar larvae and newly emerged beetles showed soluble protein contents increased 38 and 78%, respectively, and, the 15 days old beetles showed 22% decrease after treatment with Fury. The 6th instar larvae of CTC-12 strain showed 28% increase while the remaining developmental stages did not exhibit any significant change (Table VI).

Table III.- Effect of sub-lethal doses of synthetic pyrethroid, Fury, 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	0.28±0.01	0.67±0.05	0.27±0.02	1.86±0.1	8.13±0.82	2.03±0.23
	Treated	0.57±0.03	0.65±0.04	0.15±0.01	5.54±0.27	5.28±0.85	1.07±0.01
6th instar larvae	Control	0.30±0.01	0.65±0.07	0.20±0.03	2.54±0.08	5.71±0.51	1.93±0.18
	Treated	0.40±0.02	0.81±0.05	0.18±0.01	3.50±0.40	6.19±0.62	1.45±0.14
Newly emerged adults	Control	0.33±0.02	0.65±.0.03	0.30±0.01	2.09±0.16	7.42±1.03	2.67±0.15
	Treated	0.31±0.05	0.67±0.02	0.10±0.01	1.64±0.24	4.18±0.27	0.79±0.05
15 days old adults	Control	0.30±0.01	0.74±0.04	0.50±0.01	2.65±0.04	8.15±0.83	5.32±0.01
	Treated	0.27±0.01	0.81±0.02	0.70±0.07	1.94±0.15	11.75±0.34	6.09±0.60
LSD at P=0.05	**For age**	0.08	0.16	0.04	0.43	1.02	0.64
	Treatment	0.11	0.23	0.05	0.68	1.45	0.91
LSD among the strains at P=0.05			0.13			1.94	

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

Table IV.- Effect of sub-lethal doses of synthetic pyrethroid, Fury, 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		Total enzymatic activity (mIU/mg body weight)			Specific activity (µIU/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	23.31±0.30	30.9±0.74	38.02±0.79	149.1±17.61	345.0±17.76	281.6±21.12
	Treated	14.8±1.06	28.7±1.67	23.83±1.98	142.5±7.43	252.9±20.84	173.0±12.37
6th instar larvae	Control	18.78±0.38	29.9±1.36	28.15±1.15	161.8±13.02	256.6±2.39	275.2±11.48
	Treated	26.01±0.99	28.9±0.50	23.70±0.18	227.7±5.99	229.5±18.50	193.4±14.24
Newly emerged adults	Control	8.99±0.30	16.8±0.69	21.94±1.4	57.99±14.27	187.9±1.19	201.2±12.89
	Treated	8.09±0.47	16.9±0.39	20.65±1.11	51.15±0.8	105.9±7.2	163.4±10.11
15 days old adults	Control	5.64±0.10	13.5±0.41	16.38±0.82	50.11±7.7	149.8±5.4	145.0±2.78
	Treated	5.77±0.11	6.88±0.24	15.03±0.42	44.83±2.86	101.2±3.62	149.1±2.1
LSD at P=0.05	**For age**	1.21	2.02	3.33	14.03	28.97	35.70
	Treatment	1.71	2.89	4.67	9.92	40.97	50.49
LSD among the strains at P=0.05			4.06			52.0	

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

Table V.- Effect of sub-lethal doses of a synthetic pyrethroid, Fury, on the total esterase 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	4.76±0.11	5.27±0.08	10.0±0.30	32.24±1.58	58.98±4.12	77.38±0.87
	Treated	4.05±0.17	4.35±0.17	9.16±0.43	30.02±1.27	35.32±2.96	66.35±1.53
6th instar larvae	Control	3.63±0.08	3.86±0.31	7.65±0.25	31.23±0.58	34.14±2.22	76.37±6.71
	Treated	3.89±0.07	2.67±0.07	7.09±0.12	34.15±1.73	22.95±2.61	70.74±4.98
Newly emerged adults	Control	2.75±0.17	2.67±0.08	7.48±0.22	17.76±1.26	29.86±2.11	65.49±0.79
	Treated	2.46±0.13	2.14±0.09	6.94±0.14	12.92±0.54	13.36±0.4	54.86±0.51
15 days old adults	Control	2.15±0.09	2.74±0.05	6.59±0.15	19.10±1.01	30.62±1.63	70.15±0.61
	Treated	2.07±0.16	1.76±0.09	8.14±0.012	16.08±1.53	25.52±0.73	77.96±6.5
LSD at P=0.05	**For age**	0.34	0.37	0.67	3.28	6.39	9.89
	Treatment	0.48	0.52	0.94	4.64	9.04	13.99
LSD among the strains at P=0.05			0.66			11.27	

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

Table VI.- Effect of sub lethal doses of synthetic pyrethroid, Fury, on the soluble protein of 4th instar larvae, 6th instar larvae, newly emerged adult and 15 days old adult beetle 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	148.25± 5.09	90.04± 4.61	136.74± 7.74
	Treated	103.79± 3.37	124.15± 10.15	137.92± 4.16
6th instar larvae	Control	116.21± 0.64	113.08± 1.99	94.88± 10.43
	Treated	114.25± 3.62	127.7± 10.47	121.24± 7.76
Newly emerged adults	Control	155.12± 1.06	90.07± 3.22	114.32± 4.79
	Treated	190.0± 6.42	160.68± 12.34	126.48± 3.56
15 days old adults	Control	116.22± 5.18	89.95± 3.47	94.01± 0.61
	Treated	129.81± 8.68	67.69± 1.18	105.46± 7.50
LSD at P=0.05 For age		11.19	14.53	21.11
Treatment		15.78	20.55	14.92
LSD among the strains at P=0.05			32.66	

DISCUSSION

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 Fury (synthetic pyrethroid) have been studied on esterase activities.

The use of synthetic pyrethroids is becoming common because of their certain desirable properties such as high toxicity against insects, low mammalian toxicity, photostability and sufficient persistence (Brattsten *et al.*, 1986). Pyrethrum is the only naturally occurring pyrethroid. Synthetic pvrethroids are also being developed. Pyrethroid insecticides are characterized by high knock down and lethal activity, together with repellant and antifeeding activity (Masachika, 1989). The conventional insecticides tested revealed a delayed and more or less gradual inhibition of oxygen uptake. A striking inhibitory effect was caused by the nereistoxin derivative bancol and by the pyrethroid deltamethrin. The carbamate carbaryl and the chlorinated hydrocarbons dieldrin DDT and lindane block oxygen uptake only weakly. No effect

was found with the OP trichlorfon. The degree of suppression of oxygen uptake can prove a criterion for the toxicity (Gundel *et al*., 1993).

In the present study low level of resistance was detected against the pyrethroid Fury. The above mentioned results also suggest that Fury may be of value of combating the growing threat of insecticide resistance. Lloyd (1973) and Carter *et al.* (1975) have reported almost similar results.

In three strains it was observed that the 15 days old beetles were the most susceptible stages of life cycle and they were more susceptible to pyrethroid treatment as compared with the larvae. The result of present study are in agreement with those given by Saleem and Shakoori (1990) who reported that the adult beetles were more susceptible to insecticide treatment as compared with the 6th instar larva. The result of their study also revealed that synthetic pyrethroid were more toxic against *T. castaneum* larvae and adult beetles than all the other groups of insecticides tested which is again in accordance with the presents results.

The organisms are becoming resistant to pyrethroids with the passage of time (Saleem and Shakoori, 1990; Pree *et al.*, 1989; Muller, 1989). The LD_{50} and knockdown effect of deltamethrin, permethrin and DDT to resistant and susceptible *Musca domestica* showed that susceptible strain had already developed very high level of resistance to knockdown by pyrethroid as well as DDT. This pyrethroid resistance is probably due to knockdown resistant gene mechanism. Stuart *et al.* (1998) reported that the *T. castaneum* (Herbst.) strain QTC 279 is highly resistant to deltamethrin and other synthetic pyrethroids.

Gunning *et al.* (1999) also reported that one hundred percent mortality could he achieved when *H. armgera* were pretreated with some OP and then dosed with pyrethroid. Biochemical studies showed that pyrethroid associated resistance esterases in *H. armigera* were inhibited by OP compound, such as ethion, chloropyrifos and its oxon, profenofos and acephate. The OP binds to the active site of enzyme thus preventing pyrethroid detoxication.

Zettler *et al.* (1997) reported that in laboratory tests, carbonyl sulfide was toxic to 5 species of stored product insects. At the LC_{50} the most susceptible insects in order of decreasing susceptibility were larval navel orangeworm *Amyelois transitella* (Walker); adult sawtooth grain beetles, *Oryzaephilus surinamensis* (L); adult dried fruit beetles, *Carpophilus hemipterus* (L); adult cigarette beetles, *Lasioderma serricorne* (F.) and adult confused flour beetles, *T. confusum* (Jacquelin duVal). Post treatment end point mortality was immediate for *L. serricorne* adults but was delayed for *O. surinamensis* adults and *C. hemipterus* and *T. confusum* adults.

The resistant strain of *T. casteneum* has higher level of CE as compared with susceptible and Pak strains. The tissue distribution of CE associated with permethrin resistance in Colorado potato beetle *Leptinotarsa decemlineata* (Say) was investigated by Lee and Clark (1997). These CE from the permethrin resistance showed 1.7 to 6 times higher permethrin hydrolysis activities than those from the susceptible strain. Electrophoretic comparison and kinetic studies implied that higher activity in the permethrin resistance strain is primary due to the over production of the CE rather than any qualitative change this is in accordance with the results being reported here.

Fury treatment decreased CE of all the four developmental stages in three strains of *T. castaneum*. Shakoori *et al.* (1995) reported that CE activity of 6th instar larvae of Pak strain increased, whereas it decreased in adults. Fury inhibited the CE due to its high affinity binding with CE. Therefore, a sequestration of Fury by CE is the main reason of decrease of CE. The Colorado potato beetle *L. decemlineata* also showed that the hemolymph CE were inhibited by permethrin in a reversible manner, suggestive of high affinity binding of permethrin to the CE. Therefore, a sequestration of permethrin by hemolymnph CE through high affinity binding was proposed to be a major contributing factor of permethrin resistance in the permethrin resistant strain in addition to the low level of permethrin hydrolysis (Lee and Clark, 1997).

In this study the AChE activities decreased after pyrethroid treatment. According to Carbett (1974), AChE is the most usual enzyme to be inhibited in sensitive organism. Decrease in sensitivity of AChE to insecticide inhibition or target site insensitivity and metabolic detoxication

interact to confer overall resistance (Siegfried and Scott, 1992). This probably means that the insecticide inhibited the AChE activity resulting in the death of pest. Shakoori *et al.* (1995) reported that the 6th instar larvae and adults of three strains of *T. castaneum i.e.* Pak, FSS-II and CTC- 12 were exposed to newly introduced synthetic pyrethroid Ripcord 10 EC at LC_{10} and LC_{20} for 48 hours and then used for the estimation of AChE and CE and electrophoretic analysis of esterases. AChE activity decreased in adult beetles 54% and 61% for Pak, 57% and 84% for FSS-II, and 54% and 66% in CTC-12 stains, respectively for two doses. The decrease in larvae for these two doses, however, was 43% and 70% for Pak strain, 36% and 65% for FSS-II and 28% and 38% in CTC-12 strains, respectively Our results also show decrease of AChE and CE in the above mentioned stages.

Esterases (AChE and CE) are the detoxification enzymes, which are decreased with the administration of high dose of Fury. Degree of inhibition of AChE depends both on 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 insect. The interaction between AChE and insecticide esters is analogous to the hydrolysis of ACh by the enzyme except with the inhibitors the acetylated enzyme is stable whereas with substrate it is hydrolysed rapidly to active enzyme.

The increase of ChE activity with the treatment of Fury was observed in the 4th and 6th instar larvae of Pak strain, whereas the other two stages have minor decrease. High levels of detoxifying enzymes have been correlated to resistance (Matsumura and Brown, 1963; Motoyama and Dauterman, 1980; Rathor and Wood, 1981). The German cockroach *(Blattella germanica*) was tested in the laboratory for their susceptibility to 12 insecticides. They exhibited some resistance to all the pyrethroids. Pyrethroid resistance was partly attributed to an increase in esterases activity, but not oxidase activity. Nerves from individuals of this strain were also insensitive to aconitine, which blocks the inactivation of sodium channels, it was thus assumed that overall resistance was due to modifications to the sodium channels and an increase in esterase activity (Umeda *et al.,* 1988). The FSS-II and CTC-12 strain have non-significant change throughout the life cycle with the treatment of Fury. Saleem and Shakoori (1996) reported that talcord did not effect ChE activity of *T. castaneum.* In contrast to these results Sudderuddin and Lim (1978) reported inhibition of esterases of stored gain pest *viz., S. oryzae* and *Palembus dermestoides.* Likewise inhibition of esterases have also been described in the literature by synthetic pyrethroids and other insecticides in several other insects such as *Drosophila melanogaster* (Danford and Beardmore, 1978); *Trichoplusia ni* (Ishaaya and Casida, 1980); *Boophilus microplus* (Riddles *et al.*, 1983) etc. The pyrethroid insecticide as well as OP has also been reported to inhibit the activity of ChE (Chamberlain and Hoskins, 1951; Banyopadyay, 1982). It is reported that the organism becomes resistant to pyrethroids with the passage of time (Pree *et al*, 1989; Muller, 1989).

Resistance to pyrethroid involves esterases. The 4th instar larvae of Pak strain decreased, while the other stages have increased AE activity after treatment with Fury. The increase and decrease of esterases was also noted by Tang *et al.* (1993) who investigated the resistance of *Cavariella salicicola* to OP and pyrethroid insecticide. Aphid collected in the suburbs of Shanghai showed no apparent resistance to OP insecticides dimethoate, fenitrothion and methamidophos, but did show high resistance to the pyrethroids deltamethrin and fenvalerate. Studies with the synergists piperonyl butoxide and triphenyl phosphate indicated that resistance to pyrethroid involved esterases and to lesser extent MFOs.

The AE activity of the FSS-II strain was not affected until newly emerged stage while this enzyme decreased in the 15 days old beetles. Variation in esterases activity has been associated with insecticide resistance (Pasteure and Georghiou, 1989). These enzymes protect the target site by catalyzing the hydrolysis of insecticides, or by acting as alternative target or scavengers (Reiner *et al.* 1989). High levels of detoxifying enzymes have been correlated to resistance (Matsumura and Brown, 1963).

The 4th and 6th instar larvae of CTC-12 strain showed inhibition of AE activity, whereas the

other two stages have non-significant change. Chang and Jordan (1982b) reported that larvae of pyrethroid tolerant *Wiseana cervinata* larvae rapidly metabolize pyrethroids by hydrolytic and oxidative routes.

The LC_{50} and biochemical analysis results of this study revealed that synthetic pyrethroid Fury was more toxic against three strains of *T. castaneum* larvae and adult beetles than the OP. In a similar study Saleem and Shakoori (1986) concluded that Karate was more toxic, as compared with Decis, while polytrin-C and sumicidin were equally effective and were comparatively less toxic to sixth instar larvae of *T. castaneum.*

High mortality rate and less inhibition of esterases with Fury as compared with OP indicate that there is an alternate mechanism of inhibition of insecticides. Ishaaya *et al.* (1983) reported that in *T. castaneum* larvae oxidases seem to be more important than esterases for pyrethroid detoxication. Apparently the predominant pathway of pyrethrohd detoxication in insects, whether hydrolytic or oxidative, depends largely on the insect species and to some extent on the individual pyrethroid involved. Miota *et al*. (1998) investigated the mechanism of methyl parathion and ethyl parathion resistance in two populations (Phelps and York) of western corn rootworm adults *Diabrotica virgifera virgifera* (Le Conte). Results of these studies indicated that the resistance is due to combined effect of metabolic detoxification and target site insensitivity and that different processes (NADPH-dependont monoxygenases and general esterases) are involved in conferring resistance in the two resistant populations.

In the present study comparatively low level of resistance was detected against the pyrethroid insecticide. The above mentioned results suggest that synthetic pyrethroids may be of value in combating the growing threat of insecticide resistance.

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