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Effect of α-Cypermethrin on Esterases of 6th Instar Larvae and 10 Days Old Adults of Three Different Strains of *Tribolium castaneum*

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Abstract.-The 6th instar larvae and 10 days old adult beetles of three strains (Pak, FSS-II, CTC-12) of *Tribolium castaneum* were exposed to sublethal doses of a synthetic pyrethroid insecticide α-cypermethrin 5 EC and analyzed for estimation of different esterase activities. The 6th instar larvae of Pak and CTC-12 strain had lower LC_{50} (36 and 19%, respectively) as compared with that of FSS-II strain, whereas the 10 days old adults of Pak and CTC-12 strains had higher LC_{50} (459 and 1220%, respectively) when compared with the FSS-II strain. Generally the larvae had lower concentrations of acetylcholinesterase (AChE), arylesterase (AE), carboxylesterase (CE), cholinesterase (ChE) and total esterases (TE) as compared with those in adult beetles. Treatment with α-cypermethrin did not cause any significant change in the AChE and CE activity of 6th instar larvae in all the three strains. However, AE activity decreased 61% in Pak strain larvae, ChE activity increased 96% in FSS-II larvae, and TE decreased 13, 19 and 33%, respectively in Pak, FSS-II and CTC-12 strain larvae after α-cypermethrin treatment. In adult beetles after treatment with α-cypermethrin the AChE activity increased 132% in Pak strain adult, whereas FSS-II showed 836% decrease; ChE activity in Pak and FSS-II strains showed respectively 96 and 42% decrease; CE activity increased 24 and 38% respectively in adults of FSS-II and CTC-12, AE activity decreased 14% and 46% in Pak and CTC-12 adults, respectively, whereas it increased 39% in FSS-II adults. The TE increased 40% and 37% in Pak and FSS-II, respectively and decreased 23% in CTC-12 strain. The overall biochemical and electrophoretic analysis showed that esterase activities decreased slightly in larvae of all the three strains, and increased in adults of CTC-12 after treatment with α-cypermethrin. Thus we can conclude that α-cypermethrin is not a very effective insecticide against Pak strain.

Key words: Cholinesterase, carboxylesterase, stored grain pest, red flour beetle, enzyme induction.

INTRODUCTION

Red flour beetle, *Tribolium castaneum* can be controlled either through management practices or with the help of chemicals *i.e.* residual insecticides, grain protectants, fumigants etc. As *T. castaneum* has developed resistance against several of already in use insecticides, therefore, there is a need to look for more effective new insecticides which could eliminate this pest. Resistance among population of *T. castaneum* is widespread (Saleem and Shakoori, 1984; Speirs and Zettler, 1969; Champ and Campbell-Brown, 1970; Dyte and Blackman, 1970; Bhatia and Pradhan, 1972; FAO, 1973, Zettler and Jones, 1973; Shakoori and Saleem, 1989; Saleem and Shakoori, 1990; Irshad and Gillani, 1990).

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High total esterase activity has been implicated with resistance in aphids than in susceptible ones. Resistant aphids were associated with high esterase isoenzyme activity of complex bands II-V, while in susceptible aphids these bands were virtually missing. In many other species of insects, insecticides resistance has been shown to be correlated with levels of esterase activity (Oppenoorth and Welling, 1976; Pasteur and Georghiou, 1989). They have a wide range substrate specificity, they are able to cleave triester phosphate, halides, esters thioesters, amides and peptides.

Esterases exhibit enormous multiplicity in both vertebrates and invertebrates. Due to extreme sensitivity to inhibitors and substrate specificity, these are categorized into many forms (Holmes *et al*., 1968; Haites *et al*., 1972; Hart and Cook, 1976). Arylesterases (AE) hydrolyze aromatic esters and are sensitive to inhibition by para-chloromercuric benzoate (PCMB) or para-hydroxy mercuri-benzoate. Carboxylesterases (CE) hydrolyze aliphatic esters and are inhibited by OP compounds.

Cholinesterases (ChE) hydrolyze the cholinesterase and are inhibited by OP compounds; acetylcholinesterase (AChE) hydrolyze acetate esters (Holmes *et al*., 1968; Haites *et al*., 1972).

In saw-toothed grain beetle, *O. surinamensis* (L.) resistance appeared to involve both esterase and mono-oxygenase-mediated mechanisms (Kotze and Wallbank, 1996). A number of insecticides have been known to induce detoxication enzymes in insects, for example the glutathione transferases and carboxylesterase (Terriere and Yu, 1974; Hayaoka and Dauterman, 1982), have been correlated to resistance (Matsumura and Brown, 1963; Motoyama and Dauterman, 1980; Rathore and Wood, 1981; Shamaan *et al*., 1993).

AChE plays a crucial role in insect cholinergic synaptic transmission and is the target site of inhibition of OP and carbamate insecticides (Smallman and Mansingh, 1969). Alterations in the structure AChE can reduce the level of inhibition by these extensively used insecticides and confer resistance in insects and other arthropod species (Oppenoorth, 1985; Fournier and Mutero, 1994). Quantitative change of AChE has also been suggested to contribute to the resistance in *Drosophila* (Fournier *et al*., 1992). Recent molecular studies have demonstrated that decreased sensitivity of AChE is due to structural changes in the AChE gene (Fournier *et al*., 1992; Fournier and Mutero, 1994). Furthermore, different resistance patterns can originate from combinations of several point mutations in the AChE gene. High levels of AChE insensitivity could come from the combination of several point mutations (Zhu *et al*., 1996).

Although several studies have been done to understand the mechanism of insecticide resistance in various insect pests such as houseflies and fruit flies, but little is known about stored grain pest *T. castaneum*. The present study aims at studying the phenomenon of insecticide resistance with reference to the variety of esterases present in the insect body. For this purpose the effect of synthetic pyrethroid (α-cypermethrin) has been studied on esterases of *T. castaneum*. This work is expected to help in better understanding of chemical control strategies of stored grain pests in the field.

MATERIALS AND METHODS

Insects

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

Maintenance of insect culture

The insect culture was maintained in sterilized jam jars at 30±1°C in the 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). Each jar was filled 1/4th with wheat flour, and about 200 beetles were added to each jar. The jars were then covered with muslin cloth and tied with rubber bands to avoid the escape of beetles and entry of ants, mites and other predators. Beetles were left in the culture medium for about 2-3 days for egg laying and then were removed with the help of sieves and fine camel brushes, and added to another set of sterilized jars filled 1/4th with sterilized wheat flour for continuation of culture. The flour containing eggs was placed back in the same jars, in which the 6th instar larvae and 10 days old beetles emerged after 28 days and 48 days, respectively. These 6th instar larvae and adult beetles were then used for biochemical analyses and for gel electrophoresis.

Toxicant used

A synthetic pyrethroid α-cypermethrin (5 EC) [(RS)- α-cyano-3-phenoxybenzyle (IRS, 3RS : 1RS, 3RS) -3- (2,2 dichloroninyl)-2,2= dimethlycyclopropanocarboxylate)] was used for the present study.

Determination of LC_{50}

The LC_{50} values were determined for sixth instar larvae and 10 day old adult beetles of three strains (Pak, CTC-12, FSS-II) of *T. castaneum*, individually. For determination of LC_{50}, serial

dilutions of α-cypermethrin were prepared in acetone and residual film method was used. Serial dilutions of α-cypermethrin (5 EC) were prepared in acetone as follows:

Pak	6^{th} instar larvae	$18x10^3 – 2.25x10^3$ ppm
	10 days old adult	$4x10^3 – 5x10^3$ ppm
FSS-II	6^{th} instar larvae	$20x10^3 – 2.5x10^3$ ppm
	10 days old adult	$1x10x^3 – 0.0125x10^3$ ppm
CTC-12	6^{th} instar larvae	$30x10^3 – 3.75x10^3$ ppm
	10 days old adult	$12x10^3 – 1.5x10^3$ ppm

These dilutions were applied on the center of glass Petri plates (size, 130 cm) which were rotated manually to make a thin film, 1.3 ml of insecticide solution was sufficient to spread as a thin film on entire surface of Petri plates. In control Petri plates only acetone was applied. After the plates were dried and acetone evaporated, ten healthy 6^{th} instar larvae and 10 days old beetles were introduced in three replicates of each dose as well as in control. The Petri plates were then covered. After 48 hours, mortality was recorded. Larvae and beetles showing no movement after pressing with needle of brush were considered dead.

The mortality data was then subjected to probit analysis as described by Finney (1971). The LC_{50} values were then derived from these analyses and expressed as ppm of insecticide for 6^{th} instar larvae and 10 days adult beetles of *T. castaneum,* separately.

Experimental procedure

Sublethal (LC_{20}) doses of α-cypermethrin against the 6^{th} instar larvae and 10 days old beetles of Pak, FSS-II and CTC-12 strains were used, as shown in the Table I. Each dose was applied on the three Petri plates by a method described above. Three Petri dishes with acetone alone served as control. After acetone evaporated, 6^{th} instar larvae and 10 days old adult beetles were introduced to each Petri plate.

The larvae/beetles were exposed to insecticide for 48 hours, after which live insects were collected from each plate, weighed and used for estimation of saline soluble proteins according to Lowry *et al.* (1951), acetylcholinesterase (AChE; EC 3.1.1.7) according to Devonshire (1975a), cholinesterase (ChE) according to Rappaport *et al.* (1959), carboxylesterase (CE; EC 3.1.1.1) according to Devonshire *et al.* (1975b), arylesterase (AE; EC 3.1.1.2) according to Lorentz *et al.* (1979) and total esterases (TE) according to Devonshire (1991). These parameters were also analysed electrophoretically by polyacrylamide gel electrophoresis (PAGE).

Polyacrylamide gel electrophoresis for esterases

Esterases were analyzed electrophoretically according to Devonshire (1979). Sixty larvae and beetles of *T. castaneum* were homogenized in ice cold esterase extraction buffer. The sample was kept cold during homogenization. The homogenate was centrifuged at 6000 rpm at 4°C for 30 minutes. The supernatant (200 µg protein) was loaded on 7.5% polyacrylamide gel and electrophoresed at 250 volts with continuous cooling. Bromophenol blue 1X was used as loading dye. The gel was stained for esterases at 37°C for 30 to 45 minutes and then destained. Staining solution was prepared by dissolving 40 mg α-naphthyl in 1 ml acetone, 1 ml distilled water, 98 ml 0.2 M phosphate buffer pH 6.0 and 76 mg fast blue R.R. salt, mixed thoroughly and filtered. The destainer was prepared by mixing 300 ml distilled water, 150 ml methanol, and 50 ml glacial acetic acid.

Inhibitor sensitivity

Four inhibitors, diagnostic for particular classes of esterases were tested for their ability to inhibit the activity of the esterase under study. Parachloromercuribenzoic acid $5x10^{-3}$ M (p-CMB), eserine sulphate $2x10^{-3}$ M, Paraoxon 10^{-2} M and Triphenyl phosphate (TPP) 2 x 10^{-3} M permit the classification of esterases according to criteria of Holmes and Masters (1967) into arylesterase, acetylcholinesterase, cholinesterase and carboxyl-esterase.

After electrophoresis the gel was sliced into strips. The strips were soaked for 15 minutes in 0.2 M phosphate buffer pH 8.0 containing inhibitor. Control strip was soaked in phosphate buffer alone. The strips were then subsequently stained with α-naphthyl acetate as described above. The level of inhibition was determined by visual comparison of the staining intensity of esterases in gels treated with inhibitor relative to controls.

RESULTS

Lethal concentration (LC_{50})

Table I shows LC_{50} of α-cypermethrin against 6th instar larvae and 10 days old adults of three strains (Pak, FSS-II, CTC-12). In all these strains the 10 day adult beetle requires, respectively, 76%, 97% and 56% lesser insecticide for LC_{50} values, as compared with their respective 6th instar larvae.

Table I: LC_{50} and LC_{20} values (in ppm) of three strains of *T. castaneum*. LC_{20} values were used for treatment of different strains.

Strains	6th instar larvae		10 days old adults	
	LC_{50}	LC_{20}	LC_{50}	LC_{20}
Pak	10200	3607	2433	763
FSS-II	16172	2015	435	59
CTC-12	13045	1792	5744	552

The 6th instar larvae of Pak and CTC-12 strains have lesser LC_{50} (36% and 19%, respectively) than that of FSS-II strain, while 10 days adults of Pak and CTC-12 strain have higher LC_{50} (459%, 1220%, respectively) when compared with FSS-II strain.

Acetylcholinesterase

Table II shows the total and specific activities of AChE, ChE, CE and AE, with and without treatment of insecticide, of 6th instar larvae and 10 days old adults of three strains of *T. castaneum*. The 6th instar larvae of Pak strain had total AChE activity 16.6±0.70 IU/mg body weight, while the 10 days old adults had 62% lower activity, when compared with the larvae. In FSS-II strain this activity in the 6th instar larvae was 16.66±1.0 IU/mg body weight but in 10 days old adults, the activity increased significantly (363%). In the CTC-12 strain the total AChE activity in the 6th instar larvae was 13.5±0.95 IU/mg body weight, while it decreased (56%) in 10 days old adults.

Specific activity of AChE in Pak strain was 37.81±2.25 mIU/mg soluble protein which decreased significantly (77%) in 10 days old adults; while in FSS-II, the 6th instar larvae had specific activity of 97.31±2.82 mIU/mg soluble protein, which increased significantly (467%) in 10 days old adults. In CTC-12 strain, the 6th instar larvae had specific activity of 450.24±121.58 mIU/mg soluble protein, which decreased 61% in 10 days old adults.

The 6th instar larvae of Pak strain had the same AChE activity as that of FSS-II, whereas the 10 days old adults of Pak strain had 92% less activity than the FSS-II strain adults. Similar results were observed for CTC-12 and FSS-II strains. The 6th instar larvae of CTC-12 strain had 19% less activity than that of FSS-II strain, while 10 days old adults had 98% less activity with respect to the corresponding stage of FSS-II strain.

Considering specific activity, the 6th instar larvae and 10 days old adults of Pak strain had 61% and 98% less AChE activity, respectively, when compared with the respective stages of FSS-II strain. In CTC-12 strain the 6th instar larvae had 352% more activity than that of FSS-II strain.

The α-cypermethrin treatment did not affect the total AChE activity of 6th instar larvae of Pak strain, while in 10 days old adults, the AChE activity increased 132%. In FSS-II strain, the AChE activity of 6th instar larvae increased 11%, whereas, in 10 days old adults, the AChE activity decreased 836% after insecticide treatment. In CTC-12 strain, the AChE activity in both 6th instar larvae and 10 days old increased 4 and 10%, respectively (Fig. 1).

The specific activity of AChE also showed similar trend. In 6th instar larvae of Pak strain the specific activity decreased 13%, while in 10 days old adults, the specific activity increased 114% after α-cypermethrin treatment. In 6th instar larvae of FSS-II strain, the specific activity of AChE increased 23%, while in the 10 days old adults it decreased 63% in 6th instar larvae and increased 23% in the 10 old adults after insecticide treatment (Fig. 1).

Cholinesterase

Table II shows the total and specific activities of cholinesterase (ChE) before and after the insecticide treatment. The 6th instar larvae of Pak strain had total ChE activity 7.61±0.57 RU/mg body weight, which decreased 56% in 10 days old adults of Pak strain. The 6th instar larvae of FSS-II strain had total ChE activity of 0.552±0.36 RU/mg body weight, which increased 82% in 10 days old adults of the same strain. In CTC-12 strain, the 6th instar larvae had 0.424±0.24 RU/mg body weight, which increased 13% in 10 days old adults.

Table II.- Effect of α-cypermethrin on the activities of acetylcholinesterase (AChE), cholinesterase (ChE), carboxylesterase (CE) arylesterase (AE) and total protein (TE) of 6th instar larvae and 10 days old beetles of three different strains (Pak, FSS-II, CTC-12) of *Tribolium castaneum*.

	6th instar larvae		10 days old adults		LSD at
	Control (n=3)	Treated (n=3)	Control (n=3)	Treated (n=3)	P = 0.05
Acetylcholinesterase					
Total enzyme activity (IU/mg body wt.)					
Pak	16.6±0.70	16.9±0.32	6.17±2.24	14.37±1.23	4.79
FSS-II	16.66±1.20	14.83±0.78	77±5.51	12.6±1.05	11.54
CTC-12	13.5±0.95	14.16±1.02	5.83±0.44	11.73±1.15	2.97
Specific activity (µU/mg soluble protein)					
Pak	37.81±2.25	32.61±1.60	8.66±2.81	18.54±1.37	8.33
FSS-II	97.31±2.82	120.61±2.93	551.90±62.21	59.94±8.52	125.52
CTC-12	450.24±121.58	165.12±20.61	175.09±31.94	216.03±57.89	219.99
Cholinesterase					
Total enzyme activity (IU/mg body wt.)					
Pak	7.61±0.57	7.96±0.23	3.31±3.26	0.112±0.060	6.63
FSS-II	0.552±0.36	1.086±0.795	1.006±0.405	0.58±0.114	1.898
CTC-12	0.424±0.24	0.406±0.063	0.482±0.271	0.970±0.271	0.936
Specific activity (µU/mg soluble protein)					
Pak	17.63±2.87	15.29±0.404	4.95±4.88	0.149±0.085	11.34
FSS-II	3.32±2.22	9.62±7.44	7.81±3.89	17.63±2.87	17.35
CTC-12	13.15±6.91	4.62±0.369	11.52±3.81	17.15±5.14	18.81
Carboxylesterase					
Total enzyme activity (IU/mg body wt.)					
Pak	4.70±0.54	4.80±0.36	2.00±0.22	1.74±0.31	1.52
FSS-II	5.20±0.11	5.54±0.37	2.21±0.07	2.74±0.17	0.890
CTC-12	7.00±0.35	7.29±0.26	3.86±0.02	5.33±0.168	0.607
Specific activity (µU/mg soluble protein)					
Pak	10.58±0.45	9.29±1.02	2.85±0.19	2.18±0.36	2.38
FSS-II	30.52±1.20	45.87±6.29	15.85±1.55	11.11±1.77	13.61
CTC-12	226.43±41.00	85.23±9.44	118.87±27.08	94.61±13.89	103.59
Arylesterase					
Total enzyme activity (IU/mg body wt.)					
Pak	40.85±2.98	15.74±1.33	5.98±1.38	5.09±0.19	6.69
FSS-II	23.43±2.58	22.20±1.88	17.79±5.37	24.80±5.37	12.35
CTC-12	35.73±3.22	37.16±2.01	40.69±6.84	21.74±4.83	17.29
Specific activity (µU/mg soluble protein)					
Pak	93.33±8.85	30.46±3.50	8.71±2.22	6.42±0.07	19.49
FSS-II	137.53±16.57	180.08±10.07	120.51±27.57	99.05±9.74	70.15
CTC-12	1139.28±181.38	431.28±36.98	1264.88±301.56	370.97±1.73	77.608
Total esterases					
Total enzyme activity (IU/mg body wt.)					
Pak	11.01±0.90	9.48±0.57	5.34±0.25	7.54±0.09	3.79
FSS-II	16.31±1.57	13.14±1.06	1.68±0.21	2.31±0.30	3.86
CTC-12	19.26±1.17	12.90±0.89	22.53±1.50	17.28±1.30	4.96
Specific activity (µU/mg soluble protein)					
Pak	25.14±2.50	18.18±0.68	7.69±0.29	9.54±0.53	5.31
FSS-II	95.27±7.19	108.9±16.51	12.05±1.73	9.34±1.58	36.23
CTC-12	618.82±102.61	150.95±19.09	975.03±126.38	306.62±51.25	342.56

Fig. 1. Percent change in total (Left panel) and specific activity (Right panel) of 6^{th} instar larvae and 10 days old beetles of three strains of *Tribolium castaneum* after treatment with α-cypermethrin; Pak, Pakistan strain; FSS-II, multi-organophosphate insecticide susceptible; R (CTC-12), malathion resistance strain.

When considered in terms of specific activity, the 6th instar larvae of Pak strain had specific activity 17.63±2.87 mRU/mg soluble protein, which decreased significantly (71%) in 10 days old adults of Pak strain. The specific activity of 6th instar larvae of Pak strain was 3.32±2.2 mRU/mg soluble protein which increased 135% in 10 days old adults. In CTC-12 strain, the 6th instar larvae had specific activity of 13.15±6.19 mRU/mg, which decreased 12% in 10 days old adults.

On considering specific activities, the 6th instar larvae of Pak strain had 43% more activity as compared with FSS-II 6th instar larvae. The 10 days old adults of Pak strain had 36% less ChE activity, when compared with 10 days old adults of FSS-II strain. The 6th instar larvae and 10 days old adults of CTC-12 strain had 296 and 47%, more activity, respectively when compared with respective stages of FSS-II strain.

Table II shows the effect of α-cypermethrin on the activity of ChE. In Pak strain, the total ChE activity increased (4%) after treatment, while in 10 days old adults, the activity decreased 96%. In FSS-II strain, the ChE activity of 6th instar larvae increased 96%, while activity of 10 days old adults decreased 42% after treatment. In CTC-12 strain, the total of ChE activity of 6th instar larvae decreased 4% and 10 days old adults increased 10% after treatment with insecticide (Fig.1).

While considering specific activity, the ChE activity of both 6th instar larvae and 10 days old adults of Pak strain decreased 13 and 96%, respectively after treatment with insecticide. The activity of both the 6th instar larvae and 10 days old adults of FSS-II strain increased 189 and 125%, respectively after treatment. In CTC-12 strain the specific activity of 6th instar larvae decreased 64%, while activity of 10 days old adults increased 48% (Fig.1).

Carboxylesterase

Table II shows the total and specific activities of carboxylesterase (CE) in 6th instar larvae and 10 days old adults before and after the treatment with α-cypermethrin. The 6th instar larvae of Pak strain had total CE activity 4.70±0.54 IU/mg body weight. This activity significantly decreased in 10 days old adults (57%). The 6th instar larvae of FSS-II strain had total CE activity of 5.20±0.11 IU/mg, whereas it decreased significantly in 10 days old adults (57%). In CTC-12 strain, the 6th instar larvae have total CE activity of 7.00±0.35 IU/mg body weight but it also decreased in 10 days old beetles significantly (45%).

When considered in terms of specific activity of 6th instar larvae of Pak strain the CE activity has specific activity of 10.59±0.45 mIU/mg soluble protein but this activity decreased significantly in 10 days old adults (73%), while in FSS-II, the specific activity of 6th instar larvae was 30.52±1.20 mIU/mg soluble protein but in 10 days old adults, this activity decreased significantly (48%). In CTC-12 strain, the 6th instar larvae had specific activity of 226.43±41.00 mIU/mg, which decreased 47% in 10 days old adults.

When compared with the respective development stages of susceptible FSS-II strain, the 6th instar larvae and 10 days old adults of Pak strain had 9 and 9% less CE activity, respectively. The 6th instar larvae and 10 days old adults of CTC-12 strain, however, had higher CE activity (*i.e.* 34% and 74%, respectively).

Considering specific activity, both 6th instar larvae and 10 days old adults of Pak strain had 65 and 82% less activity, respectively, when compared with the respective stages of FSS-II strain, while 6th instar larvae and 10 days old adults of CTC-12 strain both had 64% and 649% more specific activity as compared with that of FSS-II strain.

Table II also shows the effect of α-cypermethrin on CE activity of 6th instar larvae and 10 days old adults of three strains. In Pak strain, the total CE activity of 6th instar larvae remained unchanged, while in 10 days old adults the total CE activity decreased 13%. In FSS-II strain, the CE activity increased in both the 6th instar larvae and 10 days old adults (6, 24%, respectively). In CTC-12 strain this increase was 4% and 38%, respectively (Fig.1).

The specific activity of CE for Pak strain decreased in both 6th instar larvae and 10 days old adults (12, 23%, respectively) after treatment with insecticide. In FSS-II strain the specific activity in 6th instar larvae increased 50%, while in 10 days old adults this activity decreased 30%. In the CTC-12 strain, the specific activity for CE decreased in both 6th instar larvae and 10 days old adults (62 and 20%, respectively) (Fig.1).

Arylesterase

Table II shows the total and specific activities of arylesterase (AE) with and without treatment of insecticide. The 6th instar larvae of Pak strain had total AE activity 40.85±2.98 IU/mg body weight, which decreased 85% in the 10 days old adults. The 6th instar larvae of FSS-II strain had total activity 23.43±2.58 IU/mg body weight. The activity decreased 24% in 10 days old adults. In CTC-12 strain, the 6th instar larvae had total activity 35.73±3.22 IU/mg body weight which increased 13% in 10 days old adults.

When AE activity was considered in terms of specific activity, the 6th instar lavae of Pak strain had specific activity 93.33±8.85 mIU/mg soluble protein, which decreased significantly (90%) in the 10 days old adults. The 6th instar larvae of FSS-II strain had specific activity 137.53±16.57 mIU/mg soluble protein which decreased 12% in the 10 days old adults. In CTC-12 strain the 6th instar larvae had specific activity 1139.28±181.38 mIU/mg soluble protein which increased (11%) in 10 days old adults.

The 6th instar larvae of Pak strain had more AE activity (74%) than that of 6th instar larvae of FSS-II strain, while 10 days old adults of Pak strain had 66% lesser activity than that of the FSS-II strain. The 6th instar larvae and 10 days old adults of CTC-12 strain showed 52% and 128% more AE activity, respectively, with reference to that of the FSS-II strain.

Considering specific activity, the 6th instar larvae and 10 days old adults of Pak strain had lower specific activity (32%, 92%, respectively) when compared with the larvae and adults of FSS-II strain. The 6th instar larvae and 10 days old adults of CTC-12 strain showed higher (87%, 949%) specific activity when compared those of the FSS-II strain.

The total AE activity of 6th instar larvae and 10 days old adults of Pak strain showed 61% and 14% decrease, respectively after treatment with α-cypermethrin. In FSS-II strain the total AE activity of 6th instar larvae decreased only 5%, whereas that of 10 days old adults increased 39%. In CTC-12 strain, the AE activity of 6th instar larvae increased 40% while that of 10 days old adults decreased 46% (Fig.1).

The specific activity of AE of Pak strain 6th instar larvae and 10 days old adults, decreased 67% and 26% respectively after treatment with insecticide. In FSS-II strain this activity increased 30% in the 6th instar larvae, but decreased 17% in the 10 days old adults. In CTC-12 strain the AE specific activity of 6th instar larvae and 10 days old adults decreased 62% and 70%, respectively, after insecticide treatment (Fig.1).

Total esterases

Table II shows the total and specific activities of total esterases before and after the administration of α-cypermethrin. The 6th instar larvae of Pak strain has total esterase activity 11.0±0.90 IU/mg body weight. The 10 days old adults had less TE activity (51%) than that of 6th instar larvae. In FSS-II strain, the 6th instar larvae had TE activity 16.31±1.57 IU/mg body weight. This activity decreased significantly (89%) in 10 days old adults. In CTC-12 strain, the 6th instar larvae had 19.26±1.71 IU/mg body weight TE activity. During subsequent development this activity increased 16% in the 10 days old adults.

When considered in terms of specific activity, the 6th instar larvae of Pak strain had TE activity 25.14±2.50 mIU/mg soluble protein, while in 10 days old adults in decreased 69%. In FSS-II strain, the 6th instar larvae had TE activity 95.27±7.19 mIU/mg soluble protein which decreased significantly (87%) in 10 days old adults. The 6th instar larvae of CTC-12 strain showed TE specific activity of 618.82±102.61 mIU/mg soluble protein, which increased 9% in 10 days old adults.

The 6th instar larvae of Pak strain had 32% less total TE activity than that of FSS-II. The 10 days old adults of Pak strain had 218% more activity than that of FSS-II strain. Both 6th instar larvae and 10 days old adults of CTC-12 strain had 18% and 124% more total TE activity when compared with those of FSS-II strain.

The specific activity of TE in 6th instar larvae and 10 days old adults of Pak strain showed lesser activities (73%, 36%, respectively), when compared with those of FSS-II strain. The 6th instar larvae and 10 days old adults of CTC-12 strain showed more specific activity (549% and 5501%, respectively) than that of FSS-II strain.

After treatment with α-cypermethrin, the TE activity of 6th instar larvae of Pak strain decreased

13%, while 10 days old adults showed 40% increase. In the 6th instar larvae of FSS-II, the TE activity decreased 19%, while in the 10 days old adults it increased 37% after treatment. Both 6th instar larvae and 10 days old adults of CTC-12 strain showed 33 and 23% decrease, respectively, in the TE activity after insecticide treatment (Fig.1).

The specific TE activity of 6th instar larvae of Pak strain decreased 27%, while that of 10 days old adults increased 24% after insecticide treatment. In FSS-II strain, the specific TE activity of 6th instar larvae increased 14%, while that of 10 days old adults decreased 22% in CTC-12 strain. Specific TE activity decreased significantly (71% and 54%, respectively) in both the 6th instar larvae and 10 days old adults after insecticide treatment.

Soluble protein contents

The 6th instar larvae of Pak strain had 443.31±39.14 µg soluble protein / mg body weight. But as development proceeded, it increased significantly (56%) in 10 days old adults. The 6th instar larvae of FSS-II strain had 171.03±9.28 µg soluble protein/mg body weight, which decreased 17% in 10 days old adults. In CTC-12 strain, the 6th instar larvae had 33.45±7.0 µg soluble protein/mg body weight. The content increased (9%) in 10 days old adults.

The 6th instar larvae and 10 days old adults of Pak strain had more protein content (159% and 389%, respectively), when compared with those of FSS-II strain. In CTC-12 strain the 6th instar larvae and 10 days old adults had less protein content (80% and 74%, respectively) when compared with the respective developmental stages of FSS-II strain.

After insecticide treatment, the 6th instar larvae and 10 days old adults of Pak strain showed 17% and 14% increase, respectively in the soluble protein content, while in FSS-II, 6th instar larvae showed 27% decrease and 10 days old adults 78% increase in the soluble protein content. In CTC-12 strain, soluble protein content of 6th instar larvae and 10 days old adults increased 160% and 62%, respectively, after insecticide treatment (Fig.2).

Electrophoretic analysis of esterases

Pak strain

Figures 3 and 4 show the electrophoretic pattern of esterases of 6th instar larvae and 10 days old beetles of Pak strain of *T. castaneum* with and without the treatment of α-cypermethrin.

Fig. 2. Percent change in saline soluble protein content of 6th instar and 10 days old beetle of three strains of *Tribolium castaneum* after treatment with α-cypermethrin. For abbreviations, see Fig. 1.

Figure 3 shows that the bands number 5, 6 and 7 have been inhibited with insecticide as well as with the inhibitor of AChE, eserine sulfate. Band number 7 has not been completely inhibited with the insecticide though completely inhibited with eserine sulfate. Slight inhibition of band number 4 in both the larvae and adult beetles with insecticide, while this band is inhibited after paraoxon treatment. This band belongs to cholinesterase (ChE). Band number 5 was inhibited with the insecticide treatment as well as inhibitor of arylesterase (AE) *viz*. PCMB.

Figure 4 shows that the bands number 1 and 2 have been inhibited with the insecticide as well as triphenylphosphate, which is inhibitor of CE.

FSS-II strain

Figures 5 and 6 show the electrophoretic pattern of 6th instar larvae and 10 days old beetles with and without the treatment of insecticide of FSS-II strain of *T. castaneum*. The band number 6 was completely inhibited with eserine sulfate, inhibitor of AChE as well as α-cypermethrin. Band number 4 was not such much affected with insecticide, whereas it was completely inhibited with Paraoxon, inhibitor of ChE. Band number 1 was inhibited both with insecticide as well as TPP, inhibitor of CE in both adults and larvae, while, and number 2 was slightly inhibited in adult beetles

Fig. 3. Electrophoretic pattern of Pak strain of *Tribolium castaneum.* C is control, whereas T is the lane for insecticide treated insects.

though completely inhibited in larvae after treatment with insecticide as well as TPP.

Figure 6 shows that the band 5 was inhibited with p-CMB inhibitor of AE, while in adult beetles this band was slightly inhibited after treatment both with insecticide as well as p-CMB.

CTC-12 strain

Figure 7 shows the electrophoretic pattern of esterases of 6th instar larvae and 10 days old beetles of CTC-12 strain of *T. castaneum* with and without the treatment of insecticide. Band number 6 was inhibited very slightly in both adults and larvae, when treated with insecticide and also with eserine-sulfate, the inhibitor of AChE, while band number 7 was completely inhibited with both the insecticide as well as eserine sulfate. Band number 4 was slightly inhibited in adults and larvae when treated with insecticide, while it was completely inhibited with Paraoxon the inhibitor of ChE. Bands number 1 and 2 remained the same in larvae, whereas in adults it was slightly inhibited after treatment with insecticide, while they were completely inhibited with TPP, the inhibitor of CE.

Fig. 4. Electrophoretic pattern of Pak strain of *Tribolium castaneum.* C is control, whereas T is for insecticide-treated insects.

Fig. 5. Electrophoretic pattern of FSS-II strain of *Tribolium castaneum.* C is control, whereas T is for insecticide-treated insects.

DISCUSSION

Insecticide are used to control unwanted organisms. Almost all insecticide act by disrupting nerve function. The synthetic pyrethroids are in common use now a days. Pyrethroid insecticide are characterized y high knock down and lethal activity, a wide spectrum, good residual activity together with repellent and anti-feeding activity (Hirano, 1989). The 6^{th} instar larvae of susceptible FSS-II strain show the highest LC_{50} (16172 ppm) as compared with that of Pak and CTC-12 strains which have respectively LC_{50} of 10200 and 13045 ppm. However, the 10 days old beetles of CTC-12 strain showed highest LC_{50} (5744 ppm) when compared with Pak (2433 ppm) and FSS-II (435 ppm) strains.

Anspaugh *et al*. (1994) have reported 20-times greater KD_{50} for resistant roaches when compared with susceptible insects. Reduced penetration of (^{14}C) permethrin was observed in resistant insects during 24 hours after treatment along with increased *in vivo* metabolism as compared with susceptible controls.

Brattsten *et al*. (1986) have reported that resistance to pyrethroids, like that to most insecticides, can be due to a number of causes, including reduced sensitivity of the target-site, reduced cuticular penetration, and increased rates of detoxification. The two most important detoxification pathways for pyrethroids involve esterase cleavage and hydroxylation by mixed function oxidases enzyme (Shono *et al*., 1979; Bigley and Plapp, 1978; Lee *et al*., 1989) which involve cytochrome P_{450}s. The importance of cytochrome P_{450} is not unexpected. This family of enzyme has been well characterized in mammals (Wolf, 1986). Similarly, a range of different forms of the enzymes are also found in insects (Terriere and Yu, 1979; Reidy *et al*., 1987). These enzymes

Fig. 6. Electrophoretic pattern of FSS-II strain of *Tribolium castaneum.* C is control, whereas T is for insecticide-treated insects.

are thought to have evolved in animals as a general detoxification mechanisms to cope with broad array of toxic plant allelochemicals. In many strains of insects more than one of these mechanisms may be present and may interact, making it difficult to determine the relative importance of each in specific cases of resistance (Brattsten *et al.*, 1986). Prabhakaran and Kamble (1996) reported that insecticide resistance increase the risk of environmental contamination and human exposure to insecticide. Devonshire (1991) reported that most insecticides in use today are esters of substituted phosphoric, carbamicor cyclopropane-carboxylic acids and are consequently subject to degradation by esterases. The greatest diversity of chemical structures is seen in the organophosphorus (OP) insecticides. They are typically triesters involving two, usually identical O-methyl or O-ethyl substituents, with the third alcohol being a phenol, enol or mercaptan having electron withdrawing properties that enhance the electrophoretically and hence the phosphorylating ability of the phosphorus atom. It is the third "leaving group" that is lost when the insecticide inhibits its target enzyme AChE by forming an ester link with (*i.e.* acylating) the serine hydroxyl of the catalytic centre. The electrophilicity of the phosphorus also determines the reactivity of OP insecticides, to water (chemical hydrolysis) and other esterases which they inhibit.

In the present study, after treatment with α-cypermethrin the AChE activity in adults of FSS-II strain decreased significantly, while in all other strains, the AChE activity decreased very slightly. AChE in adults of Pak strain increased significantly, while it decreased in adults of resistant (CTC-12) strain. Saleem and Shakoori (1986) reported that the decrease in various enzyme activities are mainly due to enzyme inhibition and for rapid catabolic process to detoxify the insecticidal effect.

Rumpf *et al.* (1997) reported that the specific activities of head AChE and whole body glutathione-S-transferase (GST) were assayed as biomarkers of sublethal exposure to OP in larvae of 2 lacewing species. Inhibition of AChE in lacewings proved a useful tool to study the impact of different OPs used in integrated pest management. Oppenoorth (1985) has reported that AChE is the primary target for almost all OP and carbamate insecticides, reduced sensitivity of the enzyme to such inhibition is a common resistance mechanism in insects. Dary *et al.* (1990) have reported that esterase activity is monitored in mosquitoes and other arthropod species because high levels of these enzymes can be associated with pesticide resistance. A mosquito strain resistant to OP due to the presence of high levels of esterases showed about 200 times more esterase activity than a susceptible strain or a strain resistant due to insensitive AChE. Dary *et al.* (1990) also reported that in the absence of increased general esterases activity alone cannot indicate complete absence of resistance in a field population, because other unrelated mechanisms of resistance may be present. In the present study, there are no significant changes in ChE activity of 10 days old adults and 6th instar larvae of Pak, FSS-II and CTC-12 strains after treatment with α-cypermethrin. This means that there is no significant effect of insecticide on the ChE activity of the adults and larvae of *T. castaneum*. Ozturk *et al.* (1990) have reported ChE is considered in two forms as

Fig. 7. Electrophoretic pattern of CTC-12 strain of *Tribolium castaneum.* C is control, whereas T is for insecticide-treated insects.

ChE and pseudo-cholinesterase. The pseudo-cholinesterases are less well understood ChE, which hydrolyze acetylcholine and acetyl-beta-methylcholine. It is mainly located in the nervous system, skeletal muscle motor end plate and human erythrocytes. Insecticides which inhibit ChE include the OP and carbamates. OP insecticides strongly inhibit both ChE and pseudo-cholinesterase and therefore lead to an accumulation of AChE at the synapse (Namba *et al*., 1971; Dettbarn, 1984; Ferry, 1988; Kipling and Cruichshank, 1985). Excessive accumulation of ACh causes cholingeric overstimulation in the central and peripheral nervous system.

Shakoori and Saleem (1989) have reported the effect of α-cypermethrin on CE activity of three different strains of *T. castaneum*. The CE activity in 10 days old adults and 6th instar larvae of Pak, FSS-II and CTC-12 strain increased significantly.

Abdelaal *et al*. (1992) have reported that insecticide resistance in the tobacco aphid *Myzus nicotianae* Blackman, from different localities in the South Eastern United States was linked to high CE activity toward 1-naphthyl acetate. In general resistance to malathion appeared to be esterases mediated and some electro-focussing detectable esterase isozymes were associated with resistance.

Lee and Clark (1997) have reported that hemolymph CE were inhibited by permethrin in a reversible manner, suggestive of high affinity binding of permethrin to CEs. It is a well established mechanisms that CEs are responsible for OP resistance through an increased degradation or sequestering of insecticide molecules (Devonshire, 1991; Georghiou and Saito, 1983). Sakata and Miyata (1994) have reported that this increased detoxification by CEs is one of the most important factors in OP insecticide resistance, especially in

homopterous, dipterous and acarine pest species.

Lakshmipathi and Sujatha (1990) have reported that CE in *Barytelphusa guerini* were fairly specific to certain OP compounds. Paraoxon could reduce it more efficiently than oxydemetonmethyl (Zhu and Brindley, 1992). The physiological role of different classes of esterases is not clearly known. CEs have been reported to play an important role in the catabolism of juvenile hormones during insect development. In crustaceans they are thought to play a role in transporting lipids between sites of utilization. Quantitative and qualitative changes in the levels of activity of esterases have been studied in five stages of developing embryos of the fresh water crab *Barytelphusa guerini* (Lakshmipathi and Sujatha, 1990). Esterase activity levels increased enormously as development progresses.

The AE activity decreased significantly in Pak strain larvae, while slightly decreased in adult beetles after treatment. The AE activity decreased in larvae and increased in adults of FSS-II strain, while in CTC-12 strain the AE activity increased in larvae and decreased in adults of CTC-12 strain. Overall soluble proteins increased in amount after treatment of α-cypermethrin in all the three strains of *T. castaneum*, levels of esterases in larval and adult stages vary greatly (Parkin, 1965; Lloyd and Hewlett, 1958).

In the present study, we observed that amount of total esterases decreased almost in both adults and larvae of three strains of *T. castaneum* after the treatment with α-cypermethrin. Our analysis conclude that AChE and AE activity were significantly decreased in larvae as compared to adults of the three strains, whereas CE activity was found maximum in both stages of the three strain and there is no considerable change in ChE activity of both larvae and adults of all strains of *T. castaneum*.

Ono *et al*. (1994) have reported that results of *in vitro* metabolism studies in a susceptible and two parathion resistant green bug strains indicate that hydrolytic enzymes are active toward paraoxon but not parathion and are partially responsible for resistance. Native PAGE also supported this view. Since staining of enhanced bands associated with resistance was inhibited by incubation with paraoxon. The resistant strain apparently produced more of the isozymes, that are inhibited by paraoxon and are able to sequester a greater number of paraoxon molecules than susceptible green bugs. The band pattern of electrophoresis shows that all the four esterases were found in three strains (Pak, FSS-II and CTC-12) of *T. castaneum* in control as well as in treated larvae and adults.

In the present work, the biochemical and gel electrophoretic analysis showed that esterase activity decreased slightly in larvae of all the three strains, while only adults of CTC-12 strains showed some increase of esterases with α-cypermethrin treatment.

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