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Effect of Pirimiphos-Methyl on Esterases of Different Developmental Stages of Three Strains of *Tribolium castaneum* (Herbst.) – An Electrophoretic Study

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Abstract.- The effect of sub-lethal doses of Pirimiphos-methyl was studied on esterases of three different strains of *Tribolium castaneum viz.*, Pak, CTC-12 (resistant) and FSS-II (insecticide susceptible). The multiple forms of esterases were resolved by polyacrylamid gel electrophoresis (PAGE) and their activities analyzed by band patterns of different strains of *T. castaneum.* The control electrophoretic pattern of Pak and FSS-II strains showed seven distinct esterases bands. The 4th and 6th instar larvae of both the strains showed carboxylesterase (CE), cholinesterase (ChE), arylesterase (AE) and acetylcholinesterase (AChE) bands, whereas AChE (band No. 6) was only visible in the FSS-II strain. The newly emerged adults of FSS-II had all the esterases, while the newly emerged adults of Pak strain had low level of CE, ChE and AE activity, whereas AChE was absent. In 15 days old beetles of Pak and FSS-II strains all the seven esterases bands were prominent. After pirimiphos-methyl treatment the ChE, AE and AChE of all the developmental stages in Pak and FSS-II strains were completely inhibited. The higher molecular weight CE, however, was not much affected. The CTC-12 control pattern comprises eight esterase bands in newly emerged adults and 15 days old beetles. The 4th and 6th instar larvae had almost similar pattern except for the absence of AChE (band No. 8). The pirimiphos-methyl treatment generally reduced the intensity of all the esterases. The AE and AChE (bands 5, 7, 8) were completely inhibited in the newly emerged adults and 15 days old beetles after insecticide treatment. However, one band of AChE (band 6) remained unaffected. In CTC-12 strain esterase bands were decreased in intensity after treatment with pirimiphos-methyl, whereas in other strains these bands were completely inhibited in all the developmental stages. The variable thickness of the band in the gel indicated relative esterases induction that could be correlated with the development of resistance in *T. castaneum.*

Key words: Insecticide resistance, electrophoretic analysis of esterases, red flour beetles.

INTRODUCTION

Red flour beetles, *Tribolium castaneum* can be controlled either through management practices or with the help of chemicals *i.e.*, residual insecticides, grain protectants, fumigants etc. Application of insecticides for protection against infestation is a relatively new approach to the problem of preventing injury to crop seeds and keeping grains free from insects. As *T. castaneum* has developed resistance against several of already in use insecticides, therefore, dire need is being felt to look for more effective new insecticides which could eliminate this pest.

The pest has developed resistance against chlorinated hydrocarbons (*e.g.,* BHC/lindane), organophosphate (malathion, fenitrothione, pirimiphosmethyl), carbamates (carbaryl) and certain fumigants (Speirs and Zettler, 1969; Champ and Champbell-Brown, 1970; Dyte and Blackman, 1970; Bhatia and Pradhan, 1972; FAO, 1973, 1974; Zettler and Jones, 1977; Saleem and Shakoori 1984).

Esterases, in general, have been reported to play significant role in development of resistance to insecticides, particularly OP (Needam and Sawicki, 1971; Hama, 1976; Hughes and Devonshire, 1982; Oppenoorth, 1982; De Malkenson *et al.*, 1984). Most insecticides in use today are esters of substituted phosphoric, carbamic or cyclopropane-carboxylic acids, and are consequently subjected to degradation by esterases (Devonshire, 1991). Esterases are able to cleave halide esters, thioesters, peptides and amides. Level of esterase activities not constant throughout life cycle as larvae of some species exhibit higher tolerance to contact insecticides than adults (Parkin, 1956; Lloyd and

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Hewlett, 1958). Esterases exist in multiple forms due to extreme sensitivity to inhibitors and substrate specificity (Holmes *et al.*, 1968; Haites *et al*., 1972; Hart and Cook, 1976).

The electrophoretic analysis of homogenate of tissue or entire organism is the most faithful technique for obtaining biochemical and ecological information on insects on the fields (Maruyama *et al.*, 1984). The extensive esterase polymorphism in insects can be resolved by electrophoresis (Devonshire, 1989).

The role of esterases in dichlorvos resistance of the cotton aphid, *Aphis gossypii* Glover was studied by vertical slab polyacrylamid gel electrophoresis (PAGE) by using a susceptible and dichlorvos-resistant strain. The resistant strain, had additional bands which probably reflected changes in conformation. Although bands of both strains exhibited equal affinity, bands E-11 and E-12 in both strains were classified as eserine sulfate sensitive carboxylesterasae (Owusa *et al.*, 1996). The PAGE of general esterases from eastern subterranean termite, *Reticulitermes flavipes* (Kollar) workers revealed the presence of seven esterase bands, named E1-E7. Bands E1-E4 were determined to be ChEs based on inhibition by paraoxon (Davis *et al.*, 1995). Prabhakaran and Kamble (1993) reported that electrophoretic studies of German cockroach homogenate revealed 10 esterase isozymes that differ in isozyme composition between insecticide resistant and insecticide susceptible strains. Sujatha *et al.* (1993) reported the esterase pattern of two termite species (*Coptotermes ceylonicusare* and *Odontotermes wallonensis*) for which substrate specificity and inhibitor sensitivity of the individual esterase bands has been tested to classify them into different categories. Sivakumaran and Mayo (1991) investigated the esterases of greenbug, *Schizaphis graminum* (Rondani) by PAGE and identified ChE and CE. Parker *et al.* (1991) identified eight esterase bands, in a susceptible and an OP resistant strain of *Lucilia cuprina*.

In present study the inhibitor were used to identify different esterases and their activities have been correlated with insecticide resistance. Besides that effect of pirimiphos-methyl has been studied on esterases of three different strains of *T. castaneum* (Pak, FSS-II, CTC-12).

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).

The methodology adopted to maintain the culture, toxicant used and determination of LC_{50} has already been described in Mujeeb and Shakoori (2012).

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 and experimentals. Acetone alone was used for control plates, whereas insecticide. 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 homogenized for the extraction of esterases. The esterases like acetylcholinesterase (AChE; acetylcholine acetyl hydrolase; 3.1.1.7), arylesterases (AE; arylester hydrolase; 3.1.1.2), carboxylesterase; (CE carboxylic ester hydrolase; 3.1.1.1), cholinesterases and (ChE; acetylcholine acyl hydrolase; 3.1.1.8) were analyzed electrophoretically by polyacrylamide gel electrophoresis (PAGE), as described by (Devonshire and Moores, 1982).

The larvae and beetles of *T. castneum* were homogenized in ice cold specific esterase extraction buffer. The sample was kept cold during homogenation. The sample was then centrifuged at

2500 xg at 4°C for 30 minutes. Supernatant (100 µg Protein) after mixing with loading dye (1 X bromophenol blue) was loaded on 8.5 % polyacrylamide gel and electrophoresed at 250 volts with continuous cooling 4°C.

After electrophoresis the gel was sliced into two strips. One strip was soaked for 15 minutes in 0.2 M phosphate buffer pH 8.0 containing specific inhibitor for specific esterases. The other strip was soaked in phosphate buffer alone and considered as a control. Both the strips were then subsequently stained with staining solution for specific esterases at 37°C for 30-45 minutes. The level of inhibition was determined by visual comparison of the staining intensity of esterases in gels treated with inhibitor relative to controls.

Inhibitor sensitivity

Four inhibitors, diagnostic for particular classes of esterases, were tested for their ability to inhibit the activity of esterases under study. Parachloromercurobenzoic acid (*p*-CMB), $5x10^{-3}$ M, was used as inhibitor of AE. Eserine sulfate, $2x10^{-3}$ M, was used as inhibitor of AChE. paraoxon, 10^{-2} M, was used as inhibitor of ChE, and triphenyl phosphate (TPP), $2x\ 10^{-3}$ M, was used as inhibitor of CE. These chemicals permit the classification of esterases according to the criterion of Holmes and Masters (1967).

RESULTS

The saline extracts of 4th instar larvae, 6th instar larvae, newly emerged adult beetles and 15 days old beetles, with and without treatment of pirimiphos-methyl, were run on polyacrylamid gel and the number of esterases bands produced were studied. Figure 1 show electrophoretic pattern of Pak strain, while Figure 2 shows electrophoretic pattern of FSS-II strain. Figure 3 exhibit electrophoretic pattern of CTC-12 strain.

Pak strain

Arylesterase (AE)

After pirimiphos-methyl treatment the bands No.4-7 of all the developmental stages were completely inhibited (lanes 1-4). The higher molecular weight esterases (bands 1-3), however, were not affected much, except that these bands became more defined after pirimiphos-methyl treatment.

The above electrophoretic pattern was also studied after treatment with *p*-CMB, which is a known inhibitor of AE (Fig. 1A, lanes 9 - 16). The control esterase pattern after inhibitor treatment (lanes 13 - 16) is fairly comparable with control pattern, which is without inhibitor treatment (lanes 5-8), except for the band No. 5 which disappeared after *p*-CMB treatment. Lanes 9 - 12 showed esterase pattern after double treatment *i.e.*, with pirimiphos-methyl and *p*-CMB. It is concluded that band No. 5 is AE, which is expressed during all developmental stages and is inhibited by *p*-CMB, pirimiphos-methyl and both *p*-CMB + pirimiphos-methyl.

Acetylcholinesterase (AChE)

After pirimiphos-methyl treatment, the bands 4-7 disappeared (Fig. 1B), whereas bands 1 - 3, though became dimmer, were not completely eliminated. The esterases of different developmental stages of Pak strain were treated with eserine sulfate, which is an established inhibitor of AChE. The bands 4–7 were partially or completely inhibited, whereas bands 5 and 6 were completely inhibited. The bands No. 4-7 were not completely inhibited, which was because of original higher concentration of these enzymes. It is concluded that bands No. 6 and 7, which are inhibited by pirimiphos-methyl, eserine sulfate, and pirimiphos-methyl + eserine sulfate, are AChE bands.

Cholinesterase (ChE)

After pirimiphos-methyl treatment, the bands 2, 4-7 disappear. The control esterases pattern was treated with ChE inhibitor, paraoxon. The bands 4 - 6 were completely inhibited by paraoxon, whereas bands No. 1-3 were not completely eliminated, although the intensity was slightly affected. The band No. 7 was also not inhibited. The Figure 1C also shows the effect of both insecticide as well as paraoxon (lanes No. 9-12). All esterase bands were completely inhibited by these two chemicals *i.e.* insecticide+paraoxon. It is concluded that bands No. 4, 5, 6, which are inhibited by paraoxon are bands of ChE.

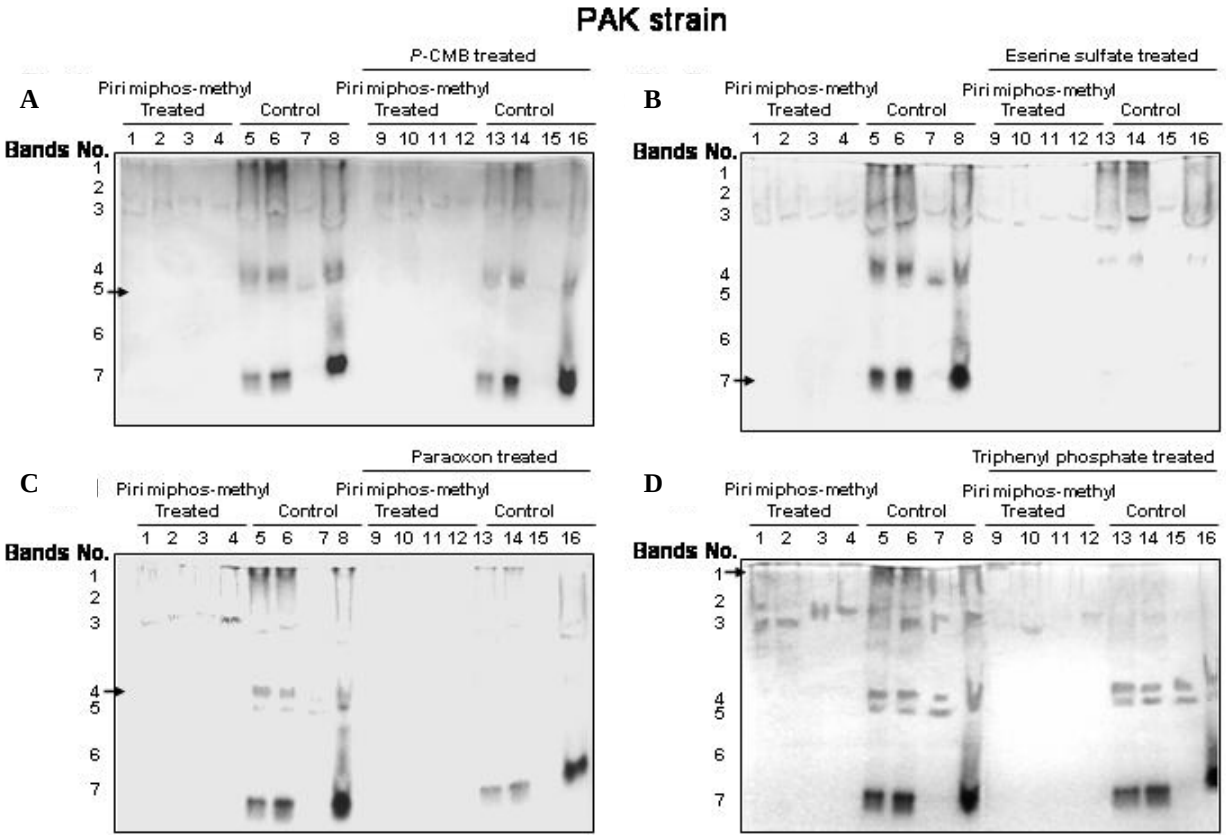

Fig. 1. Polyacrylamide gel electrophoretic pattern of esterases of different developmental stages of Pak strain of *T. castaneum* after the treatment with Pirimiphos-methyl insecticide, *p*-CMB (Arylesteras inhibitor) (Fig. 1A), Eserine Sulfate (AChE inhibitor) (Fig. 1B), Paraoxon, (Cholinesterase inhibitor) (Fig. 1C) and Triphenyl phosphate treated (Carboxylesterase inhibitor) (Fig. 1D). The first eight lanes (1 to 8) show the esterases of control (lanes 5 to 8) and Pirimiphos-methyl-treated (lanes 1 to 4) 4th instar larvae (lane 1,5), 6th instar larvae (lanes 2,6), newly emerged beetles (lanes 3,7) and 15-days old beetles (lanes 4,8). Lanes 9 to 16 shows the same sample arrangement but with the treatment of eserine sulfate, an inhibitor of acetylcholinesterase. Control (lane 13) and Pirimiphos-methyl treated (lane 9) 4th instar larvae, control (lane 14) and Pirimiphos-methyl treated (lane 10) 6th instar larvae, control (lane 15) and Pirimiphos-methyl treated (lane 11) newly emerged adults, and control (lane 16) and Pirimiphos-methyl treated (lane 12) 15 days old beetles are shown on the right side of the figure.

Carboxylesterase

After pirimiphos-methyl treatment, the esterase bands 4-7 disappeared, whereas, the bands 1-3 were distinctly visible. In the presence of triphenyl phosohate, the bands No. 4-7 remained unaltered, whereas bands 1–3 were distinctly decreased in intensity. The bands 1–3 were considerably inhibited, whereas bands No. 4–7 were completely obliterate from the pattern. It is concluded that esterase bands, which are inhibited by triphenyl phosphate, but not by pirimiphos-methyl, belong to CE.

FSS-II strain

Arylesterase (AE)

After pirimiphos-methyl treatment, the bands No. 4–7 were inhibited, whereas bands 1 – 3 did not completely fade. In fact, in some developmental stages, these bands are very distinct in spite of insecticide treatment. *p*-CMB treatment did not cause any significant effect on the control esterases except for band No. 5, which was less dark and less distinct than in the untreated pattern. Pirimiphos-methyl treated developmental stages when treated with *p*-CMB, had band 4-7 completely inhibited,

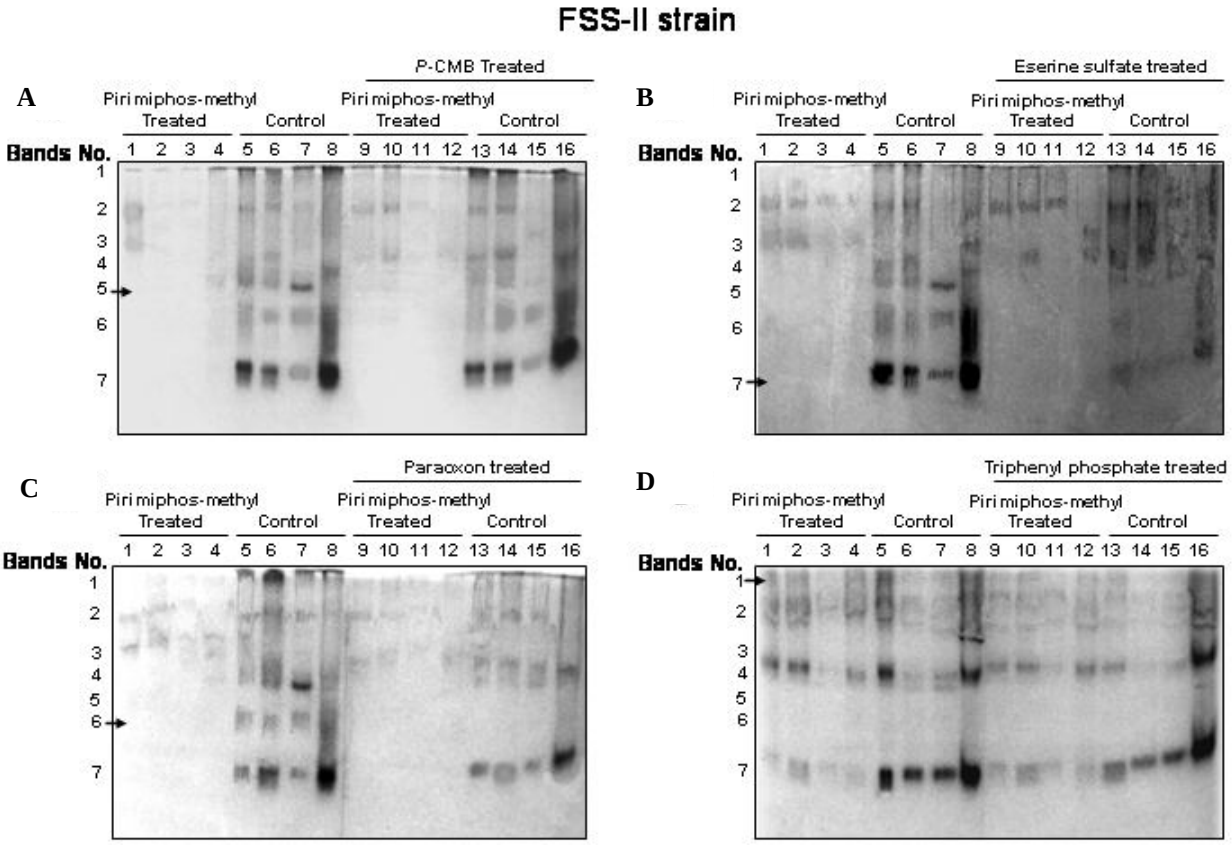

Fig. 2. Polyacrylamide gel electrophoretic pattern of esterases of different developmental stages of FSS-II strain of *T. castaneum* after the treatment with Pirimiphos-methyl insecticide, *p*-CMB (Arylesteras inhibitor) (Fig. 2A), Eserine Sulfate (AChE inhibitor) (Fig. 2B), Paraoxon, (Cholinesterase inhibitor) (Fig. 2C) and Triphenyl phosphate treated (Carboxylesterase inhibitor) (Fig. 2D). The first eight lanes (1 to 8) show the esterases of control (lanes 5 to 8) and Pirimiphos-methyl-treated (lanes 1 to 4) 4th instar larvae (lane 1,5), 6th instar larvae (lanes 2,6), newly emerged beetles (lanes 3,7) and 15-days old beetles (lanes 4,8). Lanes 9 to 16 shows the same sample arrangement but with the treatment of eserine sulfate, an inhibitor of acetylcholinesterase. Control (lane 13) and Pirimiphos-methyl treated (lane 9) 4th instar larvae, control (lane 14) and Pirimiphos-methyl treated (lane 10) 6th instar larvae, control (lane 15) and Pirimiphos-methyl treated (lane 11) newly emerged adults, and control (lane 16) and Pirimiphos-methyl treated (lane 12) 15 days old beetles are shown on the right side of the figure.

whereas band 1-3 were partially inhibited. Infect band 1-3 were denser in the larval stages than in adult stage. Apparently it implies that these esterases CE are more expressed in larval stages than in the adult of FSS-II strain (Fig. 2A).

Acetylcholinesterase (AChE)

After pirimiphos-methyl treatment, the bands No. 4–6 were considerably reduced in intensity, whereas band No. 7 was completely inhibited. Bands 1–3 remained unaffected, though these bands in larval stages were denser than in adult stages even after insecticide treatment. Eserine sulfate treatment did not affect the bands 1–3 (lanes 13–16), though other bands (No. 4–7) were considerably inhibited. Eserine sulfate treatment of pirimiphos-methyl treated developmental stages further inhibited the bands No. 4–7. The bands 1–3 also became less darker. It is concluded that bands No. 6 and 7, which are not completely inhibited by eserine sulfate, are AChE bands, but are completely inhibited, when bands of pirimiphos-methyl treated

insects are treated with eserine sulfate (Fig. 2B).

Cholinesterase (ChE)

Bands number 4–7 were completely inhibited in all the developmental stages, except for the 15 days old adult beetles in which bands No. 4 and 5 were partially inhibited. Paraoxon treatment of control pattern completely eliminated bands No. 5 and 6 in all the developmental stages. On the other hand, paraoxon treatment of insecticide treated insects inhibited the bands No. 4–7 completely, whereas the bands No. 1-3 faded out, though not completely inhibited (Fig. 2C).

Carboxylesterase (CE)

CE bands were inhibited after pirimiphos-methyl treatment (see bands 4–7 in Fig. 2D). Out of three bands (bands No. 1–3) band No. 1 was eliminated after insecticide treatment in all the developmental stages. The bands No. 2 and 3 were slightly faded in larval stages, whereas these bands were almost completely inhibited in the adult stages (lanes 3 and 4). Triphenyl phosphate treatment of the control esterase pattern (lanes 13–16, Fig. 2D) resulted in decreased intensity of bands No. 1-3, whereas other bands remained unaltered. Triphenyl phosphate treatment of esterase bands of various developmental stages of FSS-II treated with pirimiphos-methyl resulted in elimination of esterases bands (lanes 9 – 12). The CE bands (bands No. 1, 2), however, were completely inhibited in adult insects, whereas some reduced activity was visible even after triphenyl phosphate treatment in the larval stages (lanes 9 and 10).

CTC-12 strain

Arylesterase

Pirimiphos-methyl treatment generally reduces the intensity of all esterase bands (lanes 1 – 4, Fig. 3A). Band No. 5 AE was completely inhibited by the insecticide. Bands No. 7 and 8 were also inhibited in the adult beetles with pirimiphos-methyl treatment. *p*-CMB treated control pattern is shown in lanes 13 – 16 in Figure 3. This pattern is similar to the one in lanes 5 – 8, except for the band No. 5, which is inhibited by *p*-CMB. On treatment of esterases of insecticide treated insects with *p*-CMB, several bands in the larvae as well as adults disappeared. In larvae band No. 6 became fairly reduced in intensity, when compared with the control bands and so did the bands No. 1 – 4 (lanes 9 – 12). In adults beetles, the bands No. 7 and 8 also disappeared after treatment of esterases of insecticide-treated insects with *p*-CMB.

Acetylcholinesterase (AChE)

After pirimiphos-methyl treatment, most of the esterase bands either disappeared or were significantly reduced in intensity. The bands No. 7 and 8 in adult beetles and bands No. 1, 4 and 5 both in larvae and adult beetles were eliminated. The band No. 6 was significantly reduced both in the larvae and adults. Bands No 2 and 3 were also partially inhibited. Eserine sulfate treatment did not affect the bands No. 1–3 of control insects, whereas bands No. 4, 5, 7 and 8 were also completely inhibited. The band No. 6 was partially inhibited (Fig. 2B, lanes 13–16). Eserine sulfate treatment of esterase bands of pirimiphos-methyl treated developmental stages completely eliminated almost all the bands (lanes 9–12). There was some indication however that band No. 3 was only partially inhibited.

Cholinesterase (ChE)

Pirimiphos-methyl treatment eliminated bands 4, 5, 7 and 8, whereas band No. 6 was significantly reduced in intensity. Paraoxon treatment of esterases of control insects inhibited all the bands from No. 4 to 8. The bands 1 – 3, however, remained uninhibited. The paraoxon treatment of esterases of pirimiphos-methyl treated insects showed complete inhibition of bands No. 4 – 8 (lanes 9 – 13, Fig. 3C).

Carboxylesterases (ChE)

The esterases bands were significantly reduced in intensity or they disappeared after treatment with pirimiphos-methyl. Bands 1, 2, 4, 5, 7 and 8 were completely inhibited, whereas bands 3 and 6 were partially inhibited (Fig. 3D, lanes 1–4). Triphenyl phosphate treatment did not seem to cause any appreciable change in the esterases pattern of CTC-12. Bands 1 and 2 were, however, not very distinctly visible. When esterases pattern of various developmental stages of pirimiphos-methyl

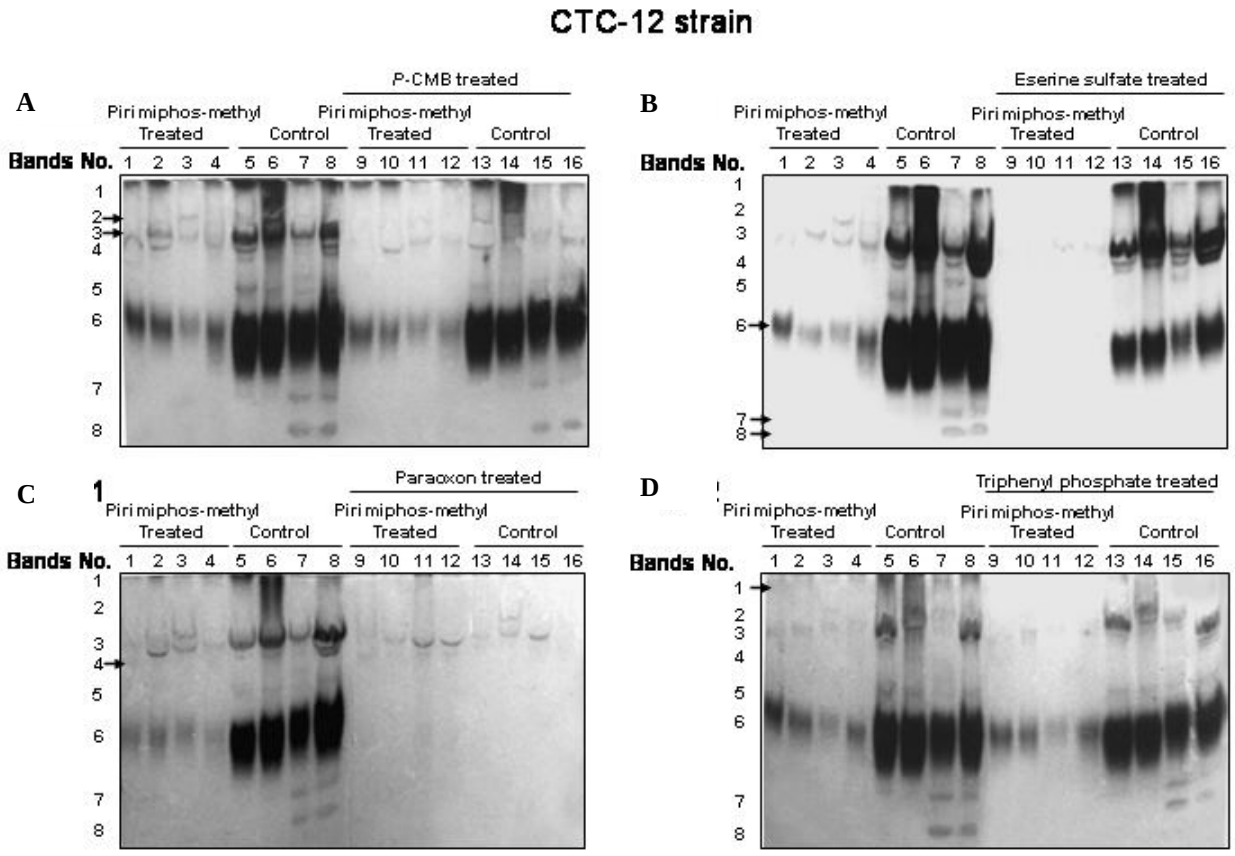

Fig. 3. Polyacrylamide gel electrophoretic pattern of esterases of different developmental stages of Pak strain of *T. castaneum* after the treatment with Pirimiphos-methyl insecticide, *p*-CMB (Arylesteras inhibitor) (Fig. 3A), Eserine Sulfate (AChE inhibitor) (Fig. 3B), Paraoxon, (Cholinesterase inhibitor) (Fig. 3C) and Triphenyl phosphate treated (Carboxylesterase inhibitor) (Fig. 3D). The first eight lanes (1 to 8) show the esterases of control (lanes 5 to 8) and Pirimiphos-methyl-treated (lanes 1 to 4) 4th instar larvae (lane 1,5), 6th instar larvae (lanes 2,6), newly emerged beetles (lanes 3,7) and 15-days old beetles (lanes 4,8). Lanes 9 to 16 shows the same sample arrangement but with the treatment of eserine sulfate, an inhibitor of acetylcholinesterase. Control (lane 13) and Pirimiphos-methyl treated (lane 9) 4th instar larvae, control (lane 14) and Pirimiphos-methyl treated (lane 10) 6th instar larvae, control (lane 15) and Pirimiphos-methyl treated (lane 11) newly emerged adults, and control (lane 16) and Pirimiphos-methyl treated (lane 12) 15 days old beetles are shown on the right side of the figure.

treated insects was treated with triphenyl phosphate, all bands except for No. 6 were completely inhibited (bands 9 – 12, Fig. 3D). The band No. 6, however, was significantly reduced in intensity.

DISCUSSION

In the present work, the level of insecticide resistance has been assessed and evaluated by electrophoresis analysis in Pak strain larvae and adult beetles, using established resistant (CTC-12) and susceptible (FSS-1I) strains of *T. castaneum* as positive and negative control.

In this investigation the electrophoretic pattern of esterases of different developmental stages of Pak strain show seven distinct bands, which were identified after treatment with specific inhibitors. Several esterases were shown to exhibit a mixed response to various inhibitors making it difficult to place them in any of the categories, such as in Pak strain the band No. 5 was inhibited with AE inhibitor (*p*-CMB) as well as eserine sulfate

which is AChE inhibitor (bands No. 6 and 7). Similarly bands No. 4-6 were inhibited by ChE inhibitor (paraoxone). Bands No. 1-3 were inhibited by triphenyl phosphate, hence representing CE.

The biochemical analysis and gel electrophoresis shows significant decrease of CE activity with the treatment of pirimiphos-methyl in all the developmental stages of three strains. Parker *et al.* (1991) identified eight esterases using native PAGE and characterized in a susceptible and an OP resistant strain of *Lucilia cuprina.* Developmental profile and tissue distribution were determined for all eight esterascs and substrate and inhibitor specificity was also determined for six of them. Esterase El and E2 were isozyme of AChE, with El present throughout the development and E2 largely confined to adults. Esterases E8, which was mainly confined to the neuromuscullar and digestive tissue of early larvae, and E7 which was only recovered from 2 to 3 days old adults, were not abundant enough for biochemical analysis. E4 and E13, classified as CE were only moderately sensitive to inhibition by OP and found mainly in the hemolymph. E3 and E9 were also identified as CE but were highly sensitive to inhibition by OP.

Prabhakaran and Kamble (1996) reported that three strains of German cockroach, *Blattella germanica* (L) showed varying levels of resistance to chlorpyrifos, methyl parathion propoxur, bendiocarb, and cypemethrin. The general esterase activity was at least two fold higher than susceptible strain. The present investigation depicts the same. Using non denaturing gel electrophoresis ten isozymes were identified in German cockroaches. These isozymes were isolated individually from the gels and analyzed for difference in activity. The isozyme E5, E6 and E7 of resistant strains had significantly higher specific activities when compared with the susceptible strain. About double the amount of E6 was recovered from the resistant strains when compared with the susceptible strain which is in accordance with the results being reported. The results suggest that the increased production of E6 esterase contribute to insecticide resistance in German cockroaches. In CTC-12 strain' also show the enhanced E6 band. Bush *et al.* (1993) also reported that elevated nonspecific esterase activities in Tufted apple bud moth (Lepidoptera: Tortricidae) were found in resistant laboratory strains and a field population with a high level of resistance. Among the non specific esterases, the presence of one esterase (E6) was closely associated with azinophosmethyl resistance in the field populations.

The esterases in pirimiphos-methvl treatment Pak strain are completely inhibited in all the developmental stages, this confirms the biochemical results. Insecticide is a potent inhibitor of esterases, and the resistance mechanism apparently involves rapid and tight binding of enzyme and insecticide that prevent the insecticide from reaching its target site and in turn decrease of esterase was observed. PAGE also supported this view since staining of enhanced bands associated with resistance was inhibited by incubation with inhibitor.

In FSS-II strain typical seven esterases bands can easily be identified in control lanes. After pirimiphos-methyl treatment all the developmental stages showed inhibition of esterases except CE bands. The esterase bands of CTC-12 strain was thick throughout the life cycle when compared with FSS-II and Pak strains. The CTC-12 strain has one additional band of esterase with reference to Pak and FSS-II strains. The pirimiphos-methyl treated larvae and beetles of CTC-12 strain, had inhibition of esterases in all the developmental stages. So it was concluded that in CTC-12 strain the esterases are not as drastically affected after insecticide treatment as they are in the other two strains.

The electrophoretic 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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