Pakistan J. Zool., vol. 39(3), pp. 171-178, 2007.

In vivo Effect of ß-Cyfluthrin on Proteolytic Enzyme Activities in Insecticide-Resistant and -Susceptible Strains of *Musca domestica*

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Abstract.- To further elucidate the possible involvement of intracellular protein catabolism in the development of insecticide resistance, we determined *in vivo* effect of the potent synthetic pyrethroid, ß-cyfluthrin on the activities of cytoplasmic and lysosomal proteolytic enzymes in insecticide-resistant and susceptible strain of housefly, *Musca domestica.* ß-Cyfluthrin at LC_{50} dose after 48 hour treatment decreased all cytoplasmic and lysosomal proteases in living (ranging from 15 to 87%) and dead flies (ranging from 17 to 88%) of susceptible strain except proline endopeptidase, which increased. ß-cyfluthrin also considerably inhibited all proteases of both live (ranging from 20 to 70 %) and dead flies (ranging from 53 to 77%) of resistant strain. Higher level of inhibition of all proteases of resistant strain by ß-cyfluthrin revealed efficacy of the insecticide to control resistant strain effectively, as well as possible involvement of proteases in the development of insect resistance to insecticides.

Key words: ß-cyfluthrin, resistant strain of *Musca domestica*, cytoplasmic proteases, lysosomal proteases, aminopeptidases, endopeptidases, cathepsins.

INTRODUCTION

Proteases are involved in protein digestion outside of the cells and also in the expression and regulation of cell proteases. In a strict sense, these intracellular proteases degrade exclusively resident cellular proteins and peptides although protein that are transported in to the cell are also degraded (Wilkins and Ahmed, 1998). Nevertheless, cellular proteases function to (a) create biologically active molecules, or (b) destroy biologically active proteins and peptides (Bond and Butler, 1987; Rivett, 1990).

The key role played by intracellular proteins (particularly enzymes) in the biochemical compensatory mechanisms associated with the development of pesticide resistance in insects subjected to toxic stress has become increasingly recognized. Resistant strains of insects may be endowed genetically such that the activity of key metabolic enzymes normally present within insect tissues may be quantitatively or qualitatively modified (in terms of overall activity levels and / or modification of the enzymatic site), or may be more efficiently induced following insecticide exposure (Wilkins *et al.,* 1999).

It is now well established that insecticide detoxication enzymes such as mixed function oxidases, carboxylesterases and glutathione-S-transferases are mainly induced in insects resistant to insecticides. These enzymes are important in the degradation of almost all conventional insecticides (Terriere, 1984). Less attention has been paid to study the effect of xenobiotics on a wide range of cytoplasmic and lysosomal proteolytic enzymes in insecticide-resistant and susceptible strains of insects as well as possible role of proteases in development of resistance in insects to insecticides. Proteolytic enzymes have also been reported to be important in the general process of intracellular protein catabolism essential for normal functioning of cells in tissues (Pennington, 1977; Turner, 1986; Anonymous, 1990) and may have additional specialized functions. Most of the previous studies have been based on the use of nonspecific protease assay procedures and focused primarily on proteases associated with gastrointestinal tract such as the identification of cysteine type and serine type

0030-9923/2007/0003-0171 $ 8.00/0

proteases as the major digestive enzymes in the gut of many coleopteran insects (Murdock *et al.,* 1987) and lepidopteran pests (Johnson *et al.,* 1990; Christetter *et al.,* 1992). In this study very sensitive assay procedures were adopted. Moreover, studies were conducted on various aminopeptidases, endopeptidases and cathepsins instead of crude analysis of total proteases.

We have already reported increased proteolytic activities in insecticides-resistant strain of *Musca domestica* (Saleem *et al.,* 1994a,b) and *Tribolium castaneum* (Saleem *et al.,* 2000) compared to the corresponding susceptible strains. In an attempt to further elucidate the biochemical mechanism by which increased proteolytic capacity may confer pesticide resistance, in this study we aimed to compare changes in the activity of a range of proteolytic enzyme types in insecticide-resistant and susceptible strains of *M. domestica* following exposure to LC_{50} dose level of a synthetic pyrethroid, ß-cyfluthrin.

MATERIALS AND METHODS

Rearing of flies

Cooper, a susceptible strain of housefly, *M. domestica* (Chapman and Morgan, 1992) was used. The resistant strain, NH-I0, which was highly resistant to malathion, was obtained from Roussel Uclaf Environmental Health Ltd., Berkhamsted, UK. The stock cultures were maintained at 25°C in a mesh cage (50 x 30 x 30 cm). The adults were provided with granulated sugar and cotton wool soaked in milk-sugar solution as food in Petri dishes. Adult female flies laid eggs in a food cup (250ml) with larval food (yeast, milk powder and bran, 1: 2: 10 meshed in 500 ml distilled water). After 24 hours, food cups containing eggs were removed from the oviposition cage and placed in another cage at the same temperature for hatching of larvae and emergence of flies. Five day old adult flies were used in the experiments.

Insecticide and other chemicals

Technical grade of the synthetic pyrethroid, ß-cyfluthrin (Buldock; (RS)-oc-cyano-4fluoro-3-phenoxybenzyl (IRS, 3RS; IRS, 3SR)-3-(2, 2-dichlorovinyl) - 2, 2 dimethylcyclopropanecarboxy-late) was obtained from Bayer AG. ß-cyfluthrin is a non systemic insecticide with contact and stomach action. It acts on the nervous system, with rapid knockdown and long residual activity. It is effective against Lepidoptera, Coleoptera, Hemiptera and Homoptera. Its acute oral LD_{50} for rats is 500 mg/kg and acute percutaneous LD_{50} for rats is >5000 mg/kg and toxicity class according to WHO for a.i. is 1b *i.e.* it highly toxic compound (Tomlin, 2000).

All other reagents including protease assay substrates were obtained from Sigma Chemical Co. Poole, UK.

Bioassay procedure

Calculated quantities of ß-cyfluthrin were dissolved in acetone, which were then serially diluted to prepare five different concentrations. Each concentration was applied in the middle of Petri dish and was then spread uniformly by rotating the dish. For a Petri dish with an area of 130 cm^2, 1.3 ml of insecticide solution was sufficient to spread as a thin film on the entire surface. After evaporation of acetone and drying of dishes at room temperature, forty-five healthy insects of similar size and development stage were released in these dishes. Three replicates were used for each dose. Mortality counts were made after 48 hours exposure to insecticide and percentage kill was corrected by Abbott's formula for any control mortality (Abbott, 1925). The criterion for death was the one described by Lloyd (1969). Results were subjected to probit analysis. The LC_{50} values for susceptible and resistant strains of *M. domestica* adult flies were calculated as outlined by Busvine (1971) and described by Finney (1971). Resistance ratio was calculated by dividing the LC_{50} for the resistant strain with LC_{50} for the susceptible strain according to the method described elsewhere (Saleem and Wilkins, 1983).

For *in vivo* determination of the effect of ß-cyfluthrin on proteases, sufficient numbers of adult flies were exposed to LC_{50} values of susceptible and resistant strains. Thereafter analyses of proteases were determined from both live and dead beetles of both resistant and susceptible strains and compared

with their respective controls.

Isolation of proteases from adult flies

Six adult flies were weighed and homogenized in extraction buffer using an Ultra-Turrax homogenizer (2xl0 sec at 15000 rpm). Whole insect homogenate (1:40, w/v) was prepared in 50 mM Tris-acetate buffer, pH 7.5 containing 1 mM dithiothreitol (DTT), 0.15 M NaCl and 3 mM $NaNO_3$ for estimation of cytoplasmic protease activities. For lysosomal proteases, the same extraction buffer as that above was used, except Tris-acetate buffer was replaced with 50 mM acetate buffer, pH 5.3. The homogenates were centrifuged at 3000 x g for 20 min at 6°C and the resultant supernatants were used for determination of proteolytic enzyme activities.

Proteolytic enzymes assays

The quantification of proteolytic enzyme activities in various tissues/species has been reported previously from our laboratories (Mantle *et al.,* 1992; Blanchard *et al.,* 1993; Faiz *et al.,* 1994; Saleem *et al.,* 1994a,b). In the present study, enzyme (0.05 ml supernatant) was incubated with the appropriate assay medium (total volume 0.3 ml) at 37°C for 10-120 min and the reaction terminated by addition of 0.6 ml of ethanol. The fluorescence of the liberated aminoacyl 7-amino-4-methy1-coumarin (AMC) was measured with reference to a tetraphenylbutadiene fluorescence standard block (λ_{ex} 380 nm, λ_{em} 440 nm). Assay blanks were also run in which the enzyme was added to the medium immediately before ethanol addition. Assay conditions were modified for samples with high enzyme activity such that substrate utilization never exceeded 15%. Stock substrate solutions (2.5 mM) were prepared in 10% ethanol. Assays were carried out for the following enzymes, with the corresponding reaction mixture for each enzyme given below:

Alanyl aminopeptidase (AlaAP): 50 mM Tris-acetate buffer pH 7.5, 5 mM $CaCl_2$, 1 mM DTT, 0.25 mM Ala-AMC.

Arginyl aminopeptidase (ArgAP): 50 mM phosphate buffer pH 6.5, 0.15 M NaCI, 1 mM DTT, 0.25 mM Arg-AMC.

Leucyl aminopeptidase (LeuAP): 50 mM glycine-NaOH buffer pH 9.5,5 mM $MgCl_2$, 1 mM DTT, 2 mM Leu-AMC.

Dipeptidyl aminopeptidase-I (DAP-I): 50 mM Tris-acetate buffer pH 5.5, 2 mM DTT, 0.25 mM Gly-Arg-AMC.

Dipeptidyl aminopeptidase-II (DAP-II): 50 mM acetate buffer pH 5.5, 2 mM DTT, 0.25 mM Lys Ala-AMC.

Dipeptidyl aminopeptidase-IV (DAP-IV): 50 mM Tris-acetate buffer pH 7.5, 2 mM DTT, 0.25 mM Gly-Pro-AMC.

Tripeptidyl aminopeptidase (TAP): 50 mM Tris-acetate buffer pH 7.5, 2 mM DTT, 0.25 mM Ala-Ala-Phe-AMC.

Proline endopeptidase (Pro EP): 50 mM Tris-acetate buffer pH 7.5, 2 mM DTT, 0.25 mM CBZ Gly-Pro-AMC.

Cathepsin B or cathepsin B + L: 50 mM acetate buffer pH 5.5, 2 mM DTT, 0.25 mM CBZ-Phe-AMC (cathepsin B + L) or 0.25 mM CBZ-Arg-Arg-AMC (cathepsin B only).

Cathepsin H: 50 mM phosphate buffer pH 6.0, 1 mM DTT, 0.5 mM puromycin, 0.25 mM Arg-AMC.

Assay of cathepsin D activity was based on the spectrophotometric procedures of Pennington (1977) and Pluskal *et al.* (1978). The reaction mixture comprised 50 mM acetate buffer pH 3.5, 1 mM DTT, and 3 mg/ml acid-denatured haemoglobin substrate (total assay volume 0.5 ml). The reaction was terminated by addition of 0.5 ml perchloric acid (10%). The samples were centrifuged at 2000 xg for 10 min, and the absorbance of acid soluble peptides determined at 280 nm. Assay blanks were run as above.

Supernatant protein levels were determined by the method of Lowry *et al.* (1951) with bovine serum albumin (BSA) as standard.

RESULTS

Proteases of susceptible strains

Table I and Figure 1 show the effect of ß-cyfluthrin at LC_{50} dose level after 48 hours treatment on a range of cytoplasmic and lysosomal

proteases in surviving and dead adult flies of

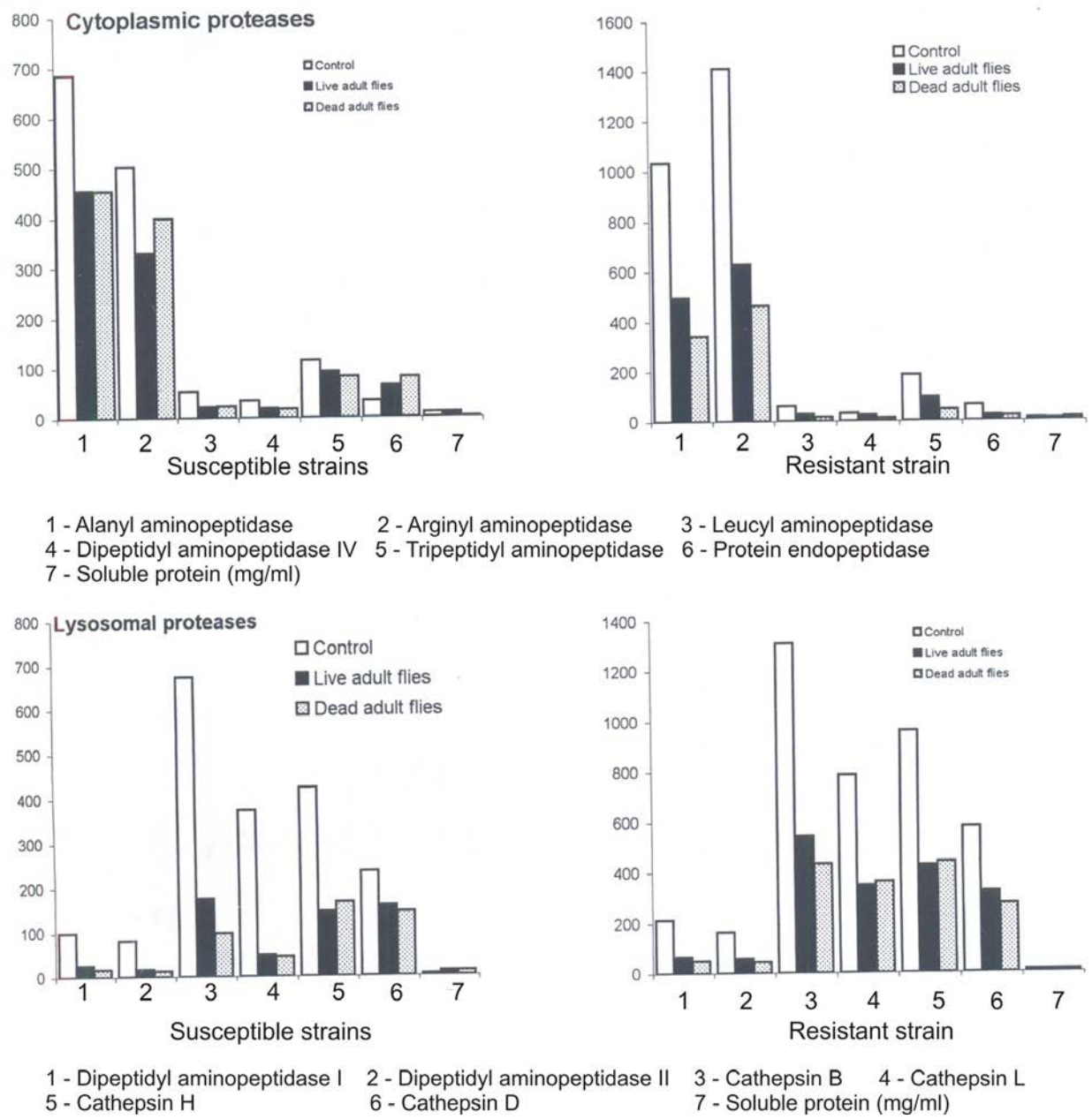

Fig. 1. Effect of ß-cyfluthrin on cytoplasmic and lysosomal proteases of susceptible and resistant strains of *Musca domestica*. The activities of various proteases are shown as nmol substrate hydrolysed/hour/mg soluble protein in control and insecticide treated live and dead insects.

susceptible strain of *M. domestica*. Their percent increase or decrease in living and dead flies of susceptible strain following ß-cyfluthrin treatment when compared with the controls is shown in Table II.

In surviving adult flies of the susceptible strain, ß-cyfluthrin considerably decreased all cytoplasmic proteases (except proline endopeptidase

which was increased by 42 and 29%, respectively).
Likewise all of the lysosomal protease activities

Table 1.- *In vivo* effect of ß-cyfluthrin on proteases in insecticide resistant and susceptible strains of *Musca domestica* adult flies.

Protease types	Susceptible strain			Resistant strain		
	Control	Live	Dead	Control	Live	Dead
Cytoplasmic proteases						
1. Alanyl aminopeptidase	684.28*	454.16	453.42	1031.93	496.33	341.25
2. Arginyl aminopeptidase	501.81	329.28	398.13	1409.46	627.71	464.48
3. Leucyl aminopeptidase	39.92	21.63	23.03	60.83	27.98	16.59
4. Dipeptidyl aminopeptidase IV	21.54	18.33	17.82	29.36	23.52	12.11
5. Tripeptidyl aminopeptidase	114.05	91.91	81.59	184.57	92.45	44.24
6. Proline endopeptidase	34.21	48.66	44.24	62.93	21.90	18.96
Lysosomal proteases						
1. Dipeptidyl aminopeptidase I	99.82	25.73	17.53	212.11	63.79	48.40
2. Dipeptidyl aminopeptidase II	81.10	17.55	13.15	165.32	57.86	44.47
3. Cathepsin B	673.75	175.45	98.63	1310.08	542.97	432.81
4. Cathepsin L	374.31	49.13	45.19	786.04	347.15	361.03
5. Cathepsin H	424.38	145.17	166.22	958.46	425.75	440.51
6. Cathepsin D	234.61	159.13	144.14	579.49	321.95	273.12

*The activities of various proteases expressed as nmol substrate hydrolysed/hour/mg soluble protein were estimated from 6-8 flies in each case.

Table II.- Percent increase (+) or decrease (-) in insecticide-resistant and susceptible strain of *Musca domestica* adult flies following ß-cyfluthrin treatment.

Protease types	Susceptible strain		Resistant strain	
	Live adult flies	Dead adult flies	Live adult flies	Dead adult flies
Cytoplasmic proteases				
1. Alanyl aminopeptidase	-33.63	-33.74	-51.90	-66.93
2. Arginyl aminopeptidase	-34.38	-20.66	-55.46	-67.05
3. Leueyl aminopeptidase	-45.81	-42.31	-54.00	-72.73
4. Dipeptidyl aminopeptidase IV	-14.90	-17.13	-19.89	-58.75
5. Tripeptidyl aminopeptidase	-19.41	-28.46	-49.91	-76.03
6. Proline endopeptidase	+42.24	+29.32	-65.20	-69.87
7. Soluble protein	+40.63	+ I 06.25	+72.41	+165.52
Lysosomal proteases				
1. Dipeptidyl aminopeptidase I	-74.22	-82.44	-69.93	-77.18
2. Dipeptidyl aminopeptidase II	-78.36	-83.79	-65.00	-73.10
3. Cathepsin B	-73.96	-85.36	-58.55	-67.65
4. Cathepsin L	-86.87	-87.93	-55.84	-54.07
5. Cathepsin H	-65.79	-60.83	-55.58	-54.04
6. Cathepsin D	-32.17	-38.56	-44.44	-52.87
7. Soluble protein	+166.67	+184.62	+110.26	+ 138.46

were also considerably decreased. AlaAP and ArgAP decreased by 34%, LeuAP by 46%, DAP IV by 15%, TAP by 19%, DAP I by 74%, DAP II by 78%, cathepsin B by 74%, cathepsin L by 87%, cathepsin H by 66% and cathepsin D by 32%. On the other hand, soluble protein contents were increased in the extract of cytoplasmic proteases by 41% and in the extract of lysosomal proteases by

167%.

ß-cyfluthrin further intensified its effect in almost all proteolytic enzymes in dead flies. Thus ProEP was increased in dead flies by 29%, while AlaAP, ArgAP, LeuAP, DAP-IV, TAP, DAP-I, DAP-II, cathepsin B, cathepsin L, cathepsin H and catheprin D were decreased by 34%, 21%, 42%, 17%, 28%, 82%, 84%, 85%, 88%, 61% and 39%, respectively. In contrast soluble protein in cytoplasmic and lysosomal proteases extracts were increased by 106% and 185%, respectively.

Proteases of resistant strain

The effect of ß-cyfluthrin at LC_{50} dose after 48 hours treatment on cytoplasmic and lysosomal proteases in surviving and dead resistant strain adult house flies is shown in Table I and Figure 1, while their corresponding percent increase or decrease compared with their control groups are shown in Table II.

ß-cyfluthrin drastically inhibited all cytoplasmic and lysosomal proteases in both live and dead adult flies of resistant strain of *M. domesdica.* Of cytoplasmic proteases in live and dead flies, AlaAP was decreased 52 and 67%, ArgAP 55 and 67%, LeuAP 54 and 73%, DAP IV 20 and 59%, TAP 50 and 76% and ProEP 65 and 70%, respectively. Of lysosomal proteases in surviving and dead flies, DAP I was decreased 70 and 77%, DAP II 65 and 73%, cathepsin B 59 and 68%, cathepsin L 56 and 54%, cathepsin H 56 and 54% and cathepsin D by 44 and 53%, respectively. Soluble protein contents of cytoplasmic lysosomal proteases extracts were increased by 72 and 166%, and 110 and 138%, in living and dead flies, respectively.

DISCUSSION

Changes in the levels of specific intracellular proteins (generally enzymatic proteins such as xenobiotic detoxifying oxidases, esterases, and glutathione-S-transferases) induced in insect pests following insecticide exposure have been extensively reported previously (Terriere, 1984; Ishaaya, 1993; Wilson, 1993). More recently, pesticide induced alterations in the generalized processes of protein synthesis and degradation have been reported (Oppert *et al.*, 1993), and it has been suggested that intracellular protein mobilization resulting from increased proteolysis may represent an adaptive physiological response mechanism via reutilization of proteolytic products, following insecticide exposure (Ahmad *et al.*, 1998). In this regard, we have previously reported *in vivo* effects of various insecticides such as λ-cyhalothrin, malathion (Saleem, 1994a), deltamethrin, pirimiphos-methyl and γ-hexachlorocyclo-hexane (Saleem, 1994b) on proteolytic enzyme activities of malathion-resistant and susceptible strains of *M. domestica* In this study, we report *in vivo* effect of another potent synthetic pyrethroid, ß-cyfluthrin on a range of cytoplasmic and lysosomal proteases in malathion-resistant and susceptible strains of *M. domestica.*

ß-Cyfluthrin at LC_{50} dose level after 48 hours depleted all cytoplasmic and lysosomal proteolytic enzyme activities (except ProEP, which was increased) in living and adult flies of susceptible strain ranging from 15% to 87%. This *in vivo* effect was either more intensified in dead flies or was maintained at similar level and ranged from 17 to 88%. Likewise ß-cyfluthrin inhibited all proteases, without any exception, in both living and dead flies of resistant strain. The range of inhibition of cytoplasmic and lysosomal proteases in living flies was 20% to 70% and in dead flies was much more *i.e.* from 53% to 77%. Inhibition of proteolytic enzymes may be attributed to the higher potency and efficacy of ß-cyfluthrin than other insecticides to control resistant strain of *M. domestica* in particular and susceptible strain in general. The results, therefore, revealed that ß-cyfluthrin would be an effective and efficient alternative synthetic pyrethroid insecticide especially to control resistant strain as it possesses more potential to produce higher inhibition of cytoplasmic and lysosomal proteolytic enzymes in resistant than susceptible strain of *M. domestica.* According to Tomlin (2000) cyfluthrin was reported in 1981 and introduced by Bayer AG under trade names Baythroid® for agriculture use, Baygon® aerosol for public health use and Solfac® for animal health use, while more potent molecule of ß-cyfluthrin was reported in 1993 and introduced by Bayer A.G in the same year under trade names of Bulldock® for agriculture use

and Responsar® for animal health use. For agriculture use, cyfluthrin is recommended® 15-40 g/ha while dose of ß-cyfluthrin is 7.5 to 20 g/ha. Likewise the results of the present study revealed that ß-cyfluthrin is more potent and better alternate insecticide to achieve effective control of resistant strains of housefly, *M. domestica* especially. The results of the present study have also confirmed our hypothesis of possible involvement of a range of cytoplasmic and lysosomal proteases in the development of resistance in insects to insecticides.

REFERENCES

ABBOTT, W.S., 1925. A method for computing effectiveness of insecticides. *J. econ. Ent.*, **18**: 265-269.

AHMED, S., WILKINS, R.M. AND MANTLE, D., 1998. Comparison of proteolytic enzyme activities in adults of insecticide resistant and susceptible strains of the housefly, *Musca domestica.* L. *Insect Biochem. Mol. Biol.*, **28**: 629-639.

ANONYMOUS, 1990. *Protein degradation in health and disease.* Ciba Foundation Symposium Excerpta Medica, Amsterdam.

BLANCHARD, P., ELLIS, M., MALTIN, C., FALKOUS, G., HARRIS, J.B. AND MANTLE, D., 1993. Effect of growth promoters on pig muscle structural protein and proteolytic enzyme levels *in vivo* and *in vitro. Biochemistry*, **75**: 839-847.

BOND, J.S. AND BUTLER, P.E., 1987. Intracellular proteases. *Annu. Rev. Biochem.*, **56**: 333-364.

BUSVINE, J.R., 1971. *A critical review of the techniques for testing insecticides.* The Commonwealth Institute of Entomology, London.

CHAPMAN, P.A. AND MORGAN, C.P., 1992. Insecticide resistance in *Musca domestica* L. from Eastern England. *Pestic. Sci.*, **36**: 35-45.

CHRISTETTER, J.T., LAING, W.A., MARKWICK, N.P. AND BURGESS, E.P.J., 1992. Midgut protease activities in 12 phytophagous lepidopteran larvae; Dietary and protease inhibitors interactions. *Insect Biochem. Mol. Biol.*, **22:** 785-796.

FAIZ, M.A., HARRIS, J.B. AND MANTLE, D., 1994. Biochemical analysis of degeneration and regeneration in rat soleus muscle induced by venom from three sub-species of *Doboia russeli. Comp. Biochem. Physiol.*, **107B:** 231-240.

FINNEY, D.J., 1971. *Probit Analysis.* England.

ISHAAYA, I., 1993. Insect detoxifying enzymes: their importance in pesticide synergism and resistance. *Arch. Insect Biochem. Physiol.*, **22**: 263-276.

JOHNSON, D.E., BROOKHART, G. L., KRAMER, K.J., BARRETT, B.D. AND MCGAUGHEY, W.A., 1990. Resistance to *Bacillus thruringiensis* by the Indian meal moth, *Plodia interpunctella:* Comparison of midgut proteinases from susceptible and resistant larvae. *J. Insect Path.*, **55:** 235-244.

LLOYD, C.J., 1969. Studies on the cross-tolerance to DDT-related compounds of a pyrethrin-resistant strain of *Sitophilus granarius* L. (Coleoptera: Curculionidae). *J. stored Prod. Res.*, **5:** 337-356.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. AND RANDAL, RJ., 1951. Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193:** 265-275.

MANTLE, D., DELAY, M.L AND MAL TIN, C.A., 1992. Effect of clenbuterol on protease activities and protein levels in rat muscle. *Muscle and Nerve*, **15:** 471478.

MURDOCK, L.L., BROOKHART, G., DUNN, P.E., FORREST, D.E., KELLEY, S., KITCH, L., SLADE RE., SCHUKLE, R.H. AND WOLFSAN, J.L., 1987. Cysteine digestive proteases in Coleoptera. *Comp. Biochem. Physiol.*, **87B:** 783787.

OPPERT, B., MORGAN, T.D., CULBERTSON, C. AND KRAMER, KJ., 1993. Dietary mixtures of cystein and serine proteinase inhibitors exhibit synergistic toxicity towards the red flour beetle, *Tribolium castaneum. Comp. Biochem. Physiol.*, **105C**: 379-398.

PENNINGTON, RJ.T., 1977. Proteinases of muscle. In: *Proteinases in mammalian cells and tissues* (edited by Burnelt A. J.), pp. 515-545. North Holland, Amsterdam.

PLUSKAL, M.G., HARRIS, J.B., PENNINGTON, RJ.T. AND EAKER, D., 1978. Some biochemical responses of rat skeletal muscle to a single subcutaneous injection of a toxin isolated from the venom of the Australian tiger snake, *Notechis scutatus scutatus. Clin. exp. Pharmacol. Physiol.*, **5:** 131-141.

RIVETT, A. J., 1990. Intracellular protein degradation. *Essays Biochem.*, **25:** 39-81.

SALEEM, M.A. AND WILKINS, RM., 1983. Toxicity of pirimiphos-methyl against a malathion-resistant and a susceptible strain of saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.). *Pakistan J. Zool.*, **15:** 89-93.

SALEEM, M.A., SHAKOORI, A.R., WILKINS, R.M. AND MANTLE, D., 1994a. *In vitro* effects of insecticides on proteolytic enzyme activities in insecticide resistant and susceptible strains of *Musca domestica. Proc. Pakistan Congr. Zool.*, **14:** 341-350.

SALEEM, M.A., SHAKOORI, A.R., WILKINS, RM. AND MANTLE, D., 1994b. *In vivo* effect of lambda-cyhalothrin and malathion on the proteolytic enzymes of malathion-resistant and susceptible strains of *Musca domestica. Pakistan J. Zool.*, **26:** 327-333.

SALEEM, M.A., SHAKOORI, A.R, WILKINS, R.M. AND MANTLE, D., 2000. *In vitro* effects of deltamethrin, pirimiphos-methyl and gamma-HCH on proteases in insecticide-resistant and susceptible strains of *Tribolium*

castaneum. Pakistan J. Zool., **32:** 53-59.

TERRIERE, L.C., 1984. Induction of detoxication enzymes in insects. *Ann. Rev. Ent.*, **29:** 71-88.

TOMLIN, C.D.S., 2000. *A world compendium: The pesticide manual.* British Crop Protection Council, Farnham, Surrey, UK.

TURNER, A.J., 1986. Processing and Metabolism of Neuropeptidase. In: *Essays in biochemistry* (eds. R.D. Marshall and K.P. Tipton), vol. 22, pp. 69-119. Academic Press, London.

WILKINS, R.M. AND AHMED, S., 1998. Intracellular preteases: their role, insecticide toxicity and resistance mechanisms. *Brighton Conf. Pests Dis.*, 511-516.

WILKINS, RM., AHMED, S. AND MANTLE, D., 1999. Comparative effect of fenitrothion treatment on intracellular protease activities in insecticide-resistant and susceptible strain of *Musca domestica* L. *Comp. Biochem. Physiol.*, **3**: 337-343.

WILSON, M., 1993. Malathion specific resistance in the flat grain beetle. *Resist. Pest Managem.*, **5**: 8-9.

(Received 3 August 2006, revised 11 October 2006)