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# *In vivo* Effects of Lambda-cyhalothrin on Proteases of Various Body Compartments of *Periplaneta americana* Adults

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**Abstract.-** The overall activity and body comportment distribution of a wide range of cytoplasmic and lysosomal proteolytic enzymes was determined in the adults of American cockroach, *Periplaneta americana.* Individual proteolytic enzymes showed different overall and relative levels of activity in head, thorax, abdomen, leg, internal leg muscle and gut. The gut showed highest activities of alanyl aminopeptidase, dipeptidyl aminopeptidase (DAP) I, II and IV, and cathepsin B, L and H while head showed highest activities of arginyl, leucyl and tripeptidyl aminopeptidase. *In vivo* effects of lambda-cyhalothrin on proteases of various body compartments showed that almost all proteases in the gut, except cathepsin B, showed considerably elevated levels ranging from 19% in the case of proline endopeptidase and 429% in the case of DAP I. All DAPs manifested highest levels i.e. DAP I by 429%, DAP II by 355% and DAP IV by 178%, followed by cathepsin L (246%), cathepsin D (123%), tripeptidyl aminopeptidase (109%), arginyl aminopeptidase (78%), alanyl aminopeptidase (72%), leucyl aminopeptidase (49%), cathepsin H (37%) and proline endopeptidase (19%). Proteases of all other body compartments exhibited mixed responses, although in head and thorax, all cytoplasmic proteases were increased while almost all lysosomal proteases were decreased.

**Key words:** Lambda-cyhalothrin, *Periplaneta americana,* cytoplasmic proteases, lysosomal proteases, aminopeptidases, endopeptidases, cathepsins.

## INTRODUCTION

The importance of proteolytic enzymes in normal growth and development of various insects, particularly during digestion and moulting, has been well documented (Law *et al.,* 1977; Murdock *et al.,* 1987; Wolfson and Murdock, J987; Bai and Yi, 1991; Christetter *et al.,* 1992; Purcell *et al.,* 1992). Most of the previous studies have been based on the use of non-specific protease assay procedures and focused primarily on proteases associated with the gastrointestinal tract such as the identification of cysteine-type proteases and serine type proteases as the major digestive enzymes in the gut of many coleopteran insects (Murdock *et al., 1987;* Wolfson and Murdock, 1987; Oppert *el al.,* 1993) and lepidopteran pests (Johnson *et al.,* 1990; Christetter *et al.,* 1992; Turunen, 1993). Moreover proteolytic enzymes have also been reported to be important in the general process of intracellular protein catabolism essential for the normal functioning of cells in all tissues (Pennington, 1977; Turner, 1986; Anonymous, 1990), and may also have additional specialized functions such as their role in the metabolism of neurotransmitter oligopeptide within the nervous system (Turner, 1986), degeneration and regeneration in rat soleus muscle induced by venom from three subspecies of *Doboia russelli* (Faiz *et al.,* 1994) and their role in development of ressistance in insects to *Bacillus thuringiensis* (Johnson *et al.,* 1990) and to insecticides such as malathion (Saleem *et al.,* 1994 a, b).

As far as effects of some insecticides on proteolytic enzymes in various insects are concerned, we have already reported from our laboratories effects of insecticides such as deltamethrin, lambda-cyhalothrin, pirimiphos-methyl, malathion and gamma-hexachlocyclohexane and synengists such as piperonyl butoxide (PBO) *S, S, S*-tributyl phosphorotrithioate (DEF) and diethyl maleate (DEM) on resistant and susceptible starins of *Musca domestica* (Saleem *et al.,* 1994a, b; Ahmad *et al*., 2001); effects of deltamethrin, pirimiphos-methyl and gamma-hexachloro-cyclohexane on proteases of malathion-resistant and susceptible strains of red flour beetle, *Tribolium*

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*castaneum* (Saleem *et al*., 2000) and effects of starvation on proteases of *M. domestica* (Saleem *et al.,* 2003a) and *T. castaneum* (Saleem *et al.,* 2003b).

We have also described activities of proteases in various body compartments such as head, thorax, abdomen and gut of larval form of cotton leaf worm, *Spodoptera lilloralis* and cabbage butterfly, *Pieris brassicae* and head, thorax, abdomen, leg, internal leg muscle and gut of American cockroach adults, *Periplaneta, americana* (Saleem *et al.,* 1995). These studies have revealed possible role of protelytic enzymes in insect resistance to insecticides.

Keeping in view the current interest in the use of synthetic pyrethroids to control insect pests due to their potency against target pests and low mammalian toxicity, the present study was planned prior to determine their effects on resistant and susceptible strains of *P. americana.* Therefore objectives of the present research work described in this paper were (i) to determine the specific types of proteolytic enzymes in various body compartments of *P. americana* adults such as head, thorax, abdomen, leg, internal leg muscle and gut using specific fluorimetric assay procedures developed to identify proteases in tissues of higher animals (Blanchard *et al.,* 1993; Faiz *et al.,* 1994; Saleem *el al.,* 1995, 1998; Mantle *et al.,* 1997) and (ii) to determine effect of a synthetic pyrethorid, lambda-cyhalothrin on proteolytic enzymes in different body compartments of *P. americana* adults such as head, thorax, abdomen, leg, internal leg muscle and gut. It is expected that this study will provide an insight to exploring the distribution of the activities of a comprehensive range of cytoplasmic and lysosomal proteolytic enzymes in various body compartments of *P. americana* and their possible role in connection with the effect of a commonly used synthetic pyrethroid insecticide, lambda-cyhalothrin.

## MATERIALS AND METHODS

*Rearing of insects*

The colonies of cockroaches used in the present study were maintained in the laboratory with out exposure to insecticides for several generations and were reared according to the procedure described by Smith (1966) and modified by Hagenbuch *et al.* (1988).

*Insecticide and other chemicals*

Technical grade of a synthetic pyrethroid, lambda-cyhalothrin (81% w/w purity; a reaction product comprising equal quantities of (S)-α-cyano-3-phenonybenzyl (Z)-(1 R, 3R)-3-(2-chloro-3, 3, 3-trifluoroprop-l-enyl)-2, 2-dimethylcyclopropanecarboxylate and (R)-α-cyano-3-phenoxybenzyl (Z) (1S, 3S)-3-(2-chloro-3, 3, 3-trifluoroprop-l-enyl)-2, 2-dimethylcyclopropanecarboxylate) was obtained from Messers Zeneca Agrochemicals, Fernhurst, Haslemere, Surrey, U.K. Lambda-cyhalothrin is a non-systemic insecticide with contact and stomach action and repellent properties. It gives rapid knockdown and long residual activity. It is used to control a wide spectrum of insect pests e.g. aphids, Colorado beetle, thrips, lepidopteran larvae and coleopteran larvae and adults etc. in cotton, cereals, potatoes, vegetables, ornamentals and other corps. It is also used to control insect-borne plant viruses, and insect pests in public health. Its toxicity class according to WHO classification for a.i is II *i.e.* it is moderately toxic compound (Tomlin, 2000).

All other reagents including substrates were obtained from Messers Sigma Chemical Co., Poole, UK and were analytical grade, where available.

*Sample processing*

Six to seven adults of *P. americana* were dissected to remove different body parts such as head, thorax, abdomen, leg, internal leg muscle and entire gut according to the procedure described by Davenport and Wright (1985), Wadleigh and Yu (1988), Bai and Yu (1991) and Turunen (1993).

The above body parts were separately weighed and homogenised in extraction buffer using an Ultra-Turrax homogeniser (2 x 10 sec at 15000 rev./ min). Tissue: buffer homogenate (1:60, w/v was prepared in 50mM Tris/acetate buffer, pH 7.5 at 6ºC containing / 1 mM dithiotherital (DTT), 0.15 M NaCI and 3 mM $NaN_3$ (for subsequent determination of cytoplasmic protease activities). For assay of lysosomal proteases, 50 µM acetate buffer pH 5.3 was incorporated in the above extraction buffer. The homogenates were centrifuged at 3000 x g for 20 min at 6°C and the resultant supernatants were used to determine 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 the addition of 0.6 ml of ethanol. The fluorescence of the liberated aminoacyl 7-amino-4-methylcoumarin (AMC) was measured with reference to a tetraphenylbutadiene fluorescence standard block (lambda$_{ex}$ 380 nm, lambda$_{em}$ 440 nm). Assay blanks were 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 the extent of 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 mixtures for each enzyme given below:

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

Arginyl aminopeptidase: 50 mM phosphate buffer pH 6.5, 0.15 M NaCl, I mM DTT, 0.25 mM Arg-AMC.

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

Dipeptidyl aminopeptidase-1: 50 mM Tris-acetate buffer pH 5.5, 2 mM DTT, 0.25 mM Gly-Arg-AMC.

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

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

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

Proline endopeptidase: 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 procedure of Pennington (1977) and Pluskal *et al.* (1978). The reaction mixture comprised of 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 10% PCA. The samples were centrifuged at 2000 x g 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

*Distribution of proteases in* Periplaneta americana *adults*

Table I shows distribution of various cytoplasmic and lysosomal proteolytic enzyme activities in various body compartments such as head, thorax, abdomen, leg, internal leg muscle and gut of *P. americana* adults. Of cytoplasmic proteases, activites of alanyl aminopeptidase (84 nmol/h/mg protein) and dipeptidyl aminopeptidase IV (29 nmol/h/mg protein) were highest in the gut followed by head (68 and 23 nmol/h/mg protein, respectively) than all other body compartments of *P. americana* adults.

On the other hand, head contained the highest activities of arginyl aminopeptidase (58 nmol/h/mg protein), leucyl aminopeptidase (49 nmol/h/mg protein), tripeptidyl aminopeptidase (51 nmol/h/mg protein) and proline endopeptidase (111 nmol/h/mg protein) than all the remaining body compartments. Next highest concentration of the first three types of cytoplasmic proteases were found in the gut while next highest concentration of fourth type of protease was detected in the leg, and it was found in the lowest concentration in the gut. The remaining body compartments have almost equal concentrations of proteases.

**Table I.- *In vivo* effects of lambda-cyhalothrin on proteolytic enzyme activities in various body compartments of *Periplaneta americana* adults (activity in nmol/h/mg protein)**

| Protease type | Control | | | | | | Treated | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Head | Thorax | Abdomen | Leg | Internal leg muscle | Gut | Head | Thorax | Abdomen | Leg leg muscle | Internal | Gut |
| **Cytoplasmic proteases** | | | | | | | | | | | | |
| Alanyl aminopeptidase | 6838 | 49.57 | 50.66 | 42.35 | 29.30 | 84.12 | 111.57 | 55.55 | 43.43 | 46.88 | 22.42 | 144.45 |
| Arginyl aminopeptidase | 57.61 | 54.68 | 47.33 | 45.15 | 29.08 | 51.35 | 86.11 | 94.14 | 39.44 | 50.52 | 26.94 | 91.21 |
| Leucyl aminopeptidase | 49.22 | 37.32 | 30.05 | 30.61 | 26.54 | 37.62 | 107.38 | 64.54 | 28.47 | 44.71 | 30.46 | 56.11 |
| Dipeptidyl aminopeptidase IV | 23.14 | 12.51 | 9.72 | 14.40 | 9.19 | 28.81 | 74.73 | 20.78 | 8.92 | 17.79 | 11.50 | 80.03 |
| Tripeptidyl aminopeptidase | 50.55 | 45.05 | 19.77 | 12.75 | 10.86 | 48.92 | 110.29 | 147.81 | 26.57 | 14.73 | 10.52 | 102.09 |
| Proline endopeptidase | 110.61 | 81.27 | A.70 | 85.16 | 61.31 | 38.94 | 192.91 | 161.26 | 82.44 | 91.72 | 60.72 | 46.44 |
| Soluble protein (mg/ml) | 2.28 | 3.44 | 4.00 | 3.28 | 4.28 | 3.44 | 3.72 | 4.16 | 3.44 | 3.28 | 3.44 | 1.84 |
| **Lysosomal proteases** | | | | | | | | | | | | |
| Dipeptidyl aminopeptidase 1 | 11.18 | 7.52 | 36.25 | 6.29 | 8.41 | 52.13 | 4.23 | 3.57 | 18.35 | 2.48 | 2.96 | 275.90 |
| Dipeptidyl aminopeptidase 11 | 9.88 | 8.06 | 16.18 | 5.69 | 8A0 | 19.85 | 7.14 | 15.71 | 16.86 | 6.86 | 11.87 | 90.32 |
| Cathepsin B | 263.80 | 155.81 | 449.04 | 99.51 | 205.23 | 621.75 | 125.91 | 69.09 | 455.10 | 75.96 | 105.28 | 582.08 |
| Cathepsin L | 324.28 | 155.99 | 645.52 | 7L71 | 210.75 | 2409.25 | 97.74 | 46.56 | 324.37 | 27.82 | 77.96 | 8331.91 |
| Cathepsin H | 15.92 | 13.19 | 12.49 | 9.52 | 9.31 | 48.92 | 8.58 | 11.04 | 9.54 | 10.62 | 8.63 | 67.14 |
| Cathepsin D | 1000.12 | 8644.20 | 7991.75 | 1605.80 | 7883.65 | 4031.91 | 993.75 | 12383.68 | 6465.33 | 2222.86 | 8509.61 | 8989.55 |
| Soluble protein (mg/ml | 1.84 | 2.40 | 2.56 | 2.28 | 2.84 | 2.72 | 2.28 | 2.56 | 2.84 | 2.40 | 2.84 | 1 72 |

Of lysosomal proteases, the gut of adult *P. americana* has highest activities of dipeptidyl aminopeptidase I (52 nmol/h/mg protein), dipeptidyl aminoepeptidase II (20 nmol/h/mg protein), cathepsin B (622 nmol/h/mg protein), cathepsin L (2409 nmol/h/mg protein) and cathepsin H (49 nmol/h/mg protein) followed by abdomen in the case of first four types of proteases and by head in case of the last type of protease. Cathepsin D was highest in the thorax (8644 nmol/h/mg protein), followed by head (1000 nmol/h/mg protein) while its activity was the lowest in the gut. The remaining body compartments contained almost equal concentrations of proteases.

In vivo *effects of lambda-cyhalothrin*

Table I shows the effect of lambda-cyhalothrin at $LC_{50}$ dose level after 48 hours treatment on a comprehensive range of cytoplasmic and lysosomal proteolytic enzymes in various body compartments such as head, thorax, abdomen, leg, internal leg muscle and gut of *P. americana* adults whereas Table II shows percent increase or decrease in surviving adult cockroaches than their corresponding controls.

Of gut cytoplasmic proteases, all enzymes tested in this study exhibited increased activities than their controls. Thus alanyl aminopeptidase, arginyl aminopepetidase, leucyl aminopepetidase, dipepetidyl aminopeptidase IV, tripeptidyl aminopeptidase and proline endopeptidase were increased by 72%, 78%, 49%, 178%, 109% and 19%, respectively. Likewise all proteases in head, thorax and leg compartments also manifested elevated levels by 63, 12 and 11%; 49, 72 and 12%; 118, 73 and 46%; 223, 66 and 24%; 118, 228 and 16% and 74, 98 and 8%, respectively. Proteases of the remaining body compartments such as abdomen and internal leg muscle demonstrated mixed responses of either increase or decrease.

Of gut lysosomal proteases, all enzymes tested in this study except cathepsin B showed significantly increased levels than their corresponding controls. Thus dipeptidyl aminopeptidase I was increased by 429%, dipeptidyl aminopeptidase II by 355%, cathepsin L by 246%, cathepsin H by 37% and cathepsin D by 123%. On the other hand all these enzyme activities were

**Table II.- Percent increase (+) or decrease (-) of proteolytic enzyme activities of various body compartments of *Periplaneta americana* adults following lambda-eyhalothrin treatment *in vivo.***

| | Head | Thorax | Abdomen | Leg | Internal leg muscle | Gut |
|---|---|---|---|---|---|---|
| **Cytoplasmic** | | | | | | |
| Alanyl aminopeptidase | +63.16 | +12.06 | -14.27 | +10.70 | -23.48 | +71.72 |
| Arginyl aminopeptidase | +49.47 | +72.17 | -16.88 | +11.89 | -7.36 | +77.62 |
| Leucyl aminopeptidase | +118.16 | +72.94 | -5.26 | +46.06 | +14.77 | +49.15 |
| Dipeptidyl aminopeptidase IV | +222.95 | +66.11 | -8.23 | +23.54 | +25.14 | +177.79 |
| Tripeptidyl aminopeptidase | +118.18 | +228.10 | +34.40 | +15.53 | -3.13 | +108.69 |
| Proline endopeptidase | +74.41 | +98.43 | +20.00 | +7.70 | -0.96 | +19.26 |
| Soluble protein (mg/ml) | +63.16 | +20.93 | -14.00 | 0.0 | -19.63 | -46.51 |
| **Lysosomal** | | | | | | |
| Dipeptidyl aminopeptidase I | -62.16 | -52.53 | -49.38 | -60.57 | -64.80 | +429.25 |
| Dipeptidyl aminopeptidase II | -27.73 | -94.91 | +4.20 | +20.56 | +41.31 | +355.01 |
| Cathepsin B | -52.27 | -55.66 | +1.35 | -23.67 | -48.70 | -6.38 |
| Cathepsin L | -69.86 | -70.15 | -49.75 | -61.20 | -63.01 | +245.83 |
| Cathepsin H | -46.10 | -16.30 | -23.62 | +11.55 | -7.30 | +37.24 |
| Cathepsin D | -0.64 | +43.26 | -19.10 | +38.43 | +7.94 | +122.96 |
| Soluble protein (mg/ml) | +23.91 | +6.67 | +10.94 | +5.26 | 0.0 | -36.76 |

decreased by 62%, 28%, 52%, 70%, 46% and 0.64%, respectively. Proteases from all the remaining body compartments such as thorax, abdomen, leg and internal leg muscle showed mixed responses of either increase or decrease than controls.

## DISCUSSION

In order to further elucidate the biochemical mechanisms responsible for insecticide resistance in various resistant strains of insects such as housefly, *M domestics* (Saleem *et al.,* 1994a,b; Mantle *et al.,* 1997; Wilkins *et al.,* 1999; Ahmed *et al.,* 2001*;* Ahmed and Wilkins, 2002) and red flour beetle. *T. castaneum* (Saleem *et al.,* 2001), in this paper we have determined activity levels of a comprehensive range of proteolytic enzymes (cytoplasmic and lysosomal proteinases and peptidases, which play a key role in normal cell functioning) and *in vivo* effects of a synthetic pyrethroid, lambda-cyhalothrin on proteases.

The results of the present study revealed that enzyme levels in different body parts varied widely for each protease type and there was no obvious generalized pattern of distribution of these enzymes (cytoplasmic or lysosomal, proteases or peptidases) in the different body compartments. Thus the head contained highest concentrations of arginyl aminopeptidase, leucyl aminopeptidase, tripeptidyl aminopeptidase, proline endopeptidase, and cathepsin D, while the gut contained highest levels of all lysosomal proteases tested in this study (except cathepsin D, which was highest in the thorax part) as well as alanyl aminopeptidase and dipeptidyl aminopeptidase IV out of cytoplasmic proteases. On the other hand the lowest levels of all cytoplasmic proteases (except proline endopeptidase) were observed in the internal leg muscle. Likewise the outer leg muscle contained the lowest concentration of all lysosomal proteases (except cathepsin H which was lowest in internal leg muscle). The remaining body parts contained almost similar levels of proteolytic enzymes. Similar results have already been reported from our laboratories (Saleem *et al*., 1995). Thus findings of the present study are in conformity with the previous findings from our laboratories.

The present study was also planned to determine the effects of a synthetic pyrethroid, lambda-cyhalothrin on proteases in various body parts of *P. americana* adults such as head, thorax, abdomen, leg, internal leg muscle and gut. For comparison, biochemical analyses were conducted in surviving adult cockroaches after 48 hours of treatment than their respective controls. The results

revealed that lambda-cyhalothrin significantly increased all cytoplasmic and lysosomal proteases and peptidases of gut except cathepsin B. Likewise all cytoplasmic proteases of gut, thorax and leg also exhibited elevated levels. Increased gut proteases in *P. americana* adults could be attributed to efficient degradation / detoxification of proteins or amino acids or related molecules. This could lead to the development of resistance in cockroach adults to insecticides. These results indicate the potential role of proteolytic enzymes in the development of resistance in insects to insecticides. However these results are in conformation with those already reported from our laboratories (Saleem *el al.,* 1994a,b, 2000)

On the other hand, the most obvious mechanism in context of the above, for synthetic pyrethroid, lambda-cyhalothrin action on proteases includes inhibition of almost all lysosomal proteases in head, thorax, abdomen, leg and internal leg muscle and almost all cytoplasmic proteases in abdomen and internal leg muscle. This reveals possible disruption of proteins and amino acid degradation cycle resulting in cell death. Similar results showing inhibition of proteases have been reported from our laboratories. Ahmed *et al.,* (2001) reported that *in vivo* treatment of piperonyl butoxide (PBO), *S, S, S,* tributyl phosphorotrithioate (DEF) and dietlyl maleate (DEM) caused 35-50% decrease in the activity levels of cytoplasmic and lysosomal intracellular proteases in an insecticide- resistant strain of *M. domestica.* A significant reduced hydrolytic activity of alanyl aminopeptidase, tripeptidyl aminopeptidase, proline endopeptidase, cathepsin B and H was found by the presence of DEF in the peptidase assay media. Likewise Ahmed and Wilkins (2002) reported that the application of fenitrothion alone and in combination with DEF and DEM at LD50 levels caused a significant decrease in activities of total esterase, acetylcholinesterase, (AChE) and glutathione-S-transferase (GST) in fenitrothion-resistant (232-fold) strain of *M. domestica.*

Above-mentioned findings of this study lead to accepting the hypothesis of involvements of cytoplasmic and lysosomal proteolytic enzyme activities in metabolic insecticide mechanism. It also leads to the conclusion that a wide range of cytoplasmic and lysosomal intracellular proteases are considerably affected either directly or indirectly.

The results of the present study are, however, in agreement to our previous findings already reported from our laboratories (Saleem *et al.,* 1994a, b; 2000; Ahmed *et al.,* 2001). Similar to the conclusion drawn by Wilkins *et al.* (1999), we therefore suggest that it must be the combination of intrinsically higher protease levels (prior to pesticide exposure) together with the capacity to further increase protease activities following insecticide exposure, which is important in the mechanism by which protease may confer survival advantages in insecticide resistant insects. We further speculate that this mechanism may involve increased supply to precursor amino acids from proteolytic degradation products to the intracellular pool, prior to *de novo* synthesis of detoxifying enzymes following insecticide exposure.

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**Table I.-** ***In vivo*** **effects of lambda-cyhalothrin on proteolytic enzyme activities in various body compartments of** ***Periplaneta americana*** **adults (activity in nmol/h/mg protein)**

| Protease type | Control | | | | | | Treated | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Head | Thorax | Abdomen | Leg | Internal leg muscle | Gut | Head | Thorax | Abdomen | Leg leg muscle | Internal | Gut |
| **Cytoplasmic proteases** | | | | | | | | | | | | |
| Alanyl aminopeptidase | 6838 | 49.57 | 50.66 | 42.35 | 29.30 | 84.12 | 111.57 | 55.55 | 43.43 | 46.88 | 22.42 | 144.45 |
| Arginyl aminopeptidase | 57.61 | 54.68 | 47.33 | 45.15 | 29.08 | 51.35 | 86.11 | 94.14 | 39.44 | 50.52 | 26.94 | 91.21 |
| Leucyl aminopeptidase | 49.22 | 37.32 | 30.05 | 30.61 | 26.54 | 37.62 | 107.38 | 64.54 | 28.47 | 44.71 | 30.46 | 56.11 |
| Dipeptidyl aminopeptidase IV | 23.14 | 12.51 | 9.72 | 14.40 | 9.19 | 28.81 | 74.73 | 20.78 | 8.92 | 17.79 | 11.50 | 80.03 |
| Tripeptidyl aminopeptidase | 50.55 | 45.05 | 19.77 | 12.75 | 10.86 | 48.92 | 110.29 | 147.81 | 26.57 | 14.73 | 10.52 | 102.09 |
| Proline endopeptidase | 110.61 | 81.27 | A.70 | 85.16 | 61.31 | 38.94 | 192.91 | 161.26 | 82.44 | 91.72 | 60.72 | 46.44 |
| Soluble protein (mg/ml) | 2.28 | 3.44 | 4.00 | 3.28 | 4.28 | 3.44 | 3.72 | 4.16 | 3.44 | 3.28 | 3.44 | 1.84 |
| **Lysosomal proteases** | | | | | | | | | | | | |
| Dipeptidyl aminopeptidase 1 | 11.18 | 7.52 | 36.25 | 6.29 | 8.41 | 52.13 | 4.23 | 3.57 | 18.35 | 2.48 | 2.96 | 275.90 |
| Dipeptidyl aminopeptidase 11 | 9.88 | 8.06 | 16.18 | 5.69 | 8A0 | 19.85 | 7.14 | 15.71 | 16.86 | 6.86 | 11.87 | 90.32 |
| Cathepsin B | 263.80 | 155.81 | 449.04 | 99.51 | 205.23 | 621.75 | 125.91 | 69.09 | 455.10 | 75.96 | 105.28 | 582.08 |
| Cathepsin L | 324.28 | 155.99 | 645.52 | 7L71 | 210.75 | 2409.25 | 97.74 | 46.56 | 324.37 | 27.82 | 77.96 | 8331.91 |
| Cathepsin H | 15.92 | 13.19 | 12.49 | 9.52 | 9.31 | 48.92 | 8.58 | 11.04 | 9.54 | 10.62 | 8.63 | 67.14 |
| Cathepsin D | 1000.12 | 8644.20 | 7991.75 | 1605.80 | 7883.65 | 4031.91 | 993.75 | 12383.68 | 6465.33 | 2222.86 | 8509.61 | 8989.55 |
| Soluble protein (mg/ml | 1.84 | 2.40 | 2.56 | 2.28 | 2.84 | 2.72 | 2.28 | 2.56 | 2.84 | 2.40 | 2.84 | 1 72 |