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In vivo Effect of Spinosad on Proteases of Insecticide-Resistant and Susceptible Strains of *Musca domestica*

Mushtaq A. Saleem*, Muhammad Ashfaq and A.R. Shakoori

Department of Entomology, University College of Agriculture, Bahauddin Zakariya University, Multan-60800, Pakistan (MAS), Department of Agricultural Entomology, University of Agriculture, Faisalabad (MA), and School of Biological Sciences, University of the Punjab, New Campus, Lahore, Pakistan (ARS)

Abstract.- To further elucidate the possible involvement of intracellular protein catabolism in the development of resistance to insecticides, we determined *in vivo* effect of one of the environment friendly naturalite insecticide available and marketed in Pakistan, spinosad, on the activities of cytoplasmic and lysosomal proteases in insecticide-resistant and -susceptible strains of adult houseflies, *Musca domestica* (Muscidae: Diptera) under laboratory conditions. Spinosad at LD_{50} dose level after 48 hour treatment decreased all cytoplasmic proteases in living (ranging from 21 to 93%) and dead flies (ranging from 20 to 97%) in susceptible strain except proline endopeptidase which was increased considerably. Likewise spinosad also depleted all proteases in live (in the range of 28-74%) and the dead flies (in the range of 41-82%) except proline endopeptidase which was elevated. The results also showed considerably higher levels of all cytoplasmic (54 to 95%) and lysosomal proteases (142 to 158%) in control groups of resistant compared with susceptible strains. This probably reveals the efficacy of spinosad to control the resistant strain of *M. domestica* effectively and suggests involvement of proteases in the development of insect resistance to this insecticide.

Key words.- Spinosad, resistant and susceptible strains of *Musca domestica*, cytoplasmic and lysosomal proteases, aminopeptidases, endopeptidases, cathepsins.

INTRODUCTION

Spinosad is derived from the actinomycete, *Saccharopolyspora spinosa* and following fermentation, it is obtained from a whole broth extraction. It was first identified in a soil sample by Eli Lilly & Co. in 1982 and commercially introduced by Dow Agro Sciences during 1997 (Tomlin, 2000). It is active against a range of insect species. Studies on relevant susceptible and resistant strains of different insect pests indicate that spinosad efficacy varies greatly between species (Scott, 1998; Sayyed *et al.,* 2004; Hussain, 2005). It exhibits both stomach and contact activities against insects and is commercially registered on about 250 crops (such as cotton, vegetables, and fruits) in over 50 countries of the world as well as for urban pest control, termites, chewing and sucking lice of livestock animals including *Musca domestica* (Tomlin, 2000). Fiang *et al.* (2002) reported that spinosad which is a bacterium fermentation product, has low mammalian toxicity.

M. domestica has an important bearing upon the welfare of man as carrier of the germs of summer diarrhoea, typhoid and other diseases (Scott *et al.*, 2000; McLeod *et al.,* 2002). Several prevention and remedial measures have been adopted against larvae at its breeding places and to control adult flies. Keeping in view the extensive use of insecticides, the pest has developed resistance to several insecticides such as DDT and malathion etc. It is now well-established that insecticide detoxification enzymes such as mixed function oxidases, DDT-dehydrochlorinases, carboxylesterases and glutathione-S-transferases are mainly involved in insects including house flies which are resistant to insecticides (Terriere, 1984).

Keeping in view the fast development of resistance in different insects including house flies, it is important to look for an alternate insecticide to control this house-hold pest and also to determine possible defense/resistance mechanism in order to adopt suitable strategies under Integrated Pest Management. The present experimentation was, therefore, planned (i) to determine efficacy of one of the recently developed naturalite insecticides spinosad, against susceptible and resistant strains of *M. domestica* and (ii) to determine activities of cytoplasmic in insecticide-resistant and -susceptible strains of *M. domestica* and (iii) elucidate possible

* Corresponding author: mushtaqasaleem@hotmail.com
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involvement of intracellular protein catabolism in the development of insecticide resistance.

MATERIALS AND METHODS

Rearing of flies

A susceptible strain, named as Cooper, of housefly, *M. domestica* (Chapman and Morgan, 1992) was used in this study. The resistant strain, called 517, which was resistant to fenitrothion was obtained from Roussel Uclaf Environmental Health Ltd. Berkhamsted, UK. The stock cultures were kept in a temperature controlled room maintained at 25±2°C and 50-55% relative humidity in a mesh cage (50 x 30 x 30 cm). The adults were provided with granulated sugar as well as cotton wool soaked in milk-sugar solution as food, in Petri dishes. Adult female flies laid eggs in a food cup (250 ml) having larval food (yeast, milk powder and bran, 1:2:10 meshed in 500 ml distilled water). After 24 hrs, 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 days old adult flies were used in the experiments.

Insecticide and other chemicals

Technical grade of spinosad was obtained from Messer's Dow Agro Sciences. All other reagents including protease assay substrates were obtained from Messer's Sigma Co. Poole, UK.

Bioassay procedure

Adult houseflies aged 5 days were used to determine toxicity of spinosad against susceptible and resistant strains of *M. domestica*. The bioassays were carried out by topical application of a 1µl droplet of insecticide in acetone to the notum of flies with a programmable Arnold Micro-applicator (Burkard) and a micro-syringe (1 ml) with canulae (G 36 x 3″).

Total of 50 flies were treated for each concentration of insecticide with five replicates for each treatment. The flies were fed with milk-sugar (1:1) solution 2 hrs before dosing to eliminate the chances of desiccation. The flies were anaesthetized with CO_2 (flow rate 4 ml per sec) before dosing and held with padded entomological forceps during treatment. All the dosing of flies with insecticide was done at room temperature and the treated flies were then transferred to the breeding room (25+2°C).

Response in terms of mortality was recorded after 48 hours. Percent kill was corrected by Abbott's formula for any control mortality (Abbott, 1925). The criterion for death was the one described by Lloyd (1969). Dose-mortality data were analysed by probit analysis using Polo (LeOra Software, 2003) to obtain LD_{50}, chi square value, slope, probits and heterogeneity factors.

The resistance level of resistant strain was determined by dividing LD_{50} of resistant strain with that of susceptible strain.

Isolation of intracellular proteases from flies

For *in vivo* determination of the effect of spinosad at LD_{50} dose level on some intracellular cytoplasmic and lysosomal proteases, sufficient numbers of flies were allowed to drink this chemical along with sugar solution for 48 hrs. The resistant and susceptible strain flies were exposed alike, while their respective controls were allowed to drink sugar solution only. Each treatment had four replicates. Thereafter, the concentration of intracellular proteases and total protein were determined from the live flies of both the resistant and the susceptible strains and compared with their corresponding controls.

Three houseflies were weighed and homogenised in extraction buffer using an Ultra-Turrax T25 homogeniser (2x10 sec at 15,000 rpm). Whole insect homogenate was prepared in final volume of 1 ml of 50 mM Tris-acetate buffer, pH 7.5 containing 1 mM dithiotheritol (DTT), 0.15 M NaCl and 3 mM NaN3 for estimation of cytoplasmic protease activities. For lososomal proteases, the same extraction buffer as above was used, except for Tris-acetate buffer, which was replaced with 50 mM acetate buffer, pH 5.3. The homogenates were centrifuged at 10,000 x g for 15 min at 4°C and the resultant supernatant were used for determination of proteolytic enzyme activities.

Intracellular proteases assays

The quantification of intracellular 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) and was modified for use in Fluoroskan Ascent (Thermo Electron Corporation) with 96 microplate wells. Thus using Fluoroskan Ascent, the enzyme (40 µl from 60 times above mentioned diluted supernatant) was shaken gently, incubated with the appropriate assay medium (total volume 270 µl) at 37°C for 10 to 60 min. The fluorescence of the liberated aminoacyl 7-amino-4-methylcoumarin (AMC) was measured against 370 nm excitation and 425 nm emission filters. Standard curve was prepared from series of dilutions of AMC. 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 mixture for each enzyme given below:

Cytoplasmic proteases

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; 1 mM DTT; 0.25 mM Arg-AMC.

Leucyl aminopeptidase. 50 mM glycine-NaOH buffer, pH 9.5; 5 mM $MgCl_2$; 1 mM DTT; 0.25 mM Leu-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.

Lysosomal proteases

Dipeptidyl aminopeptidase I. 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.

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.

Cathepsin D. Assay of cathepsin D 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 the addition of 0.5 ml 10% PCA. The samples were centrifuged at 2000xg for 10 minutes and the absorbance of acid soluble peptides determined at 280 nm. Assay blanks were run as above but without sample.

Total protein assay

Total protein assay was performed according to Undenfriend *et al.* (1972) and modified for microplates as described by Lorenzen and Kennedy (1993) and Kennedy and Jones (1994). A series of dilutions of Bovine Serum Albumin (BSA) ranging from 0 to 1000 µg/ml were used for preparing standard curve.

For statistical analysis, Student's 't' test was used to compare the data of the resistant strain with that of the susceptible strain and for other similar comparisons.

RESULTS

Resistance ratio against fenitrothion and spinosad

LD_{50} of fenitrothion against 5 days old adult flies of Cooper and 571 strains were 0.488 and 34.564 µg/fly, respectively. Thus resistance ratio was calculated as 70.83 fold to fenitrothion. The LD_{50} of spinosad was likewise 0.052 and 0.068 µg/fly for the two strains, respectively. According to the data, therefore, no resistance was found in the fenitrothion-resistant adult flies of *M. domestica* against spinosad.

Effect on total body weight

Table I shows the mortality and total body weight loss of 5 days old adult flies of susceptible and resistant strains after 48 hours exposure to

spinosad. Weight of four groups containing ten flies in each groups were weighed immediately after their removal from culture medium and exactly after 48 hours of treatment at LD_{50} dose level, and then their average weight was calculated.

LD_{50} dose level of spinosad caused 42 and 48% mortality in the susceptible and resistant strain adult flies, respectively, while it decreased weight by 56 and 58%, respectively.

Table I.- Mortality and total body weight loss of susceptible and resistant strains of *Musca domestica* adults following treatment with LD_{50} dose level of spinosad after 48 hours .

Parameters	Strains	
	Susceptible	Resistant
1. Weight of untreated fly in mg (n=40)	10.8	11.2
2. Weight of treated fly in mg (n=40)	4.5	4.9
3. % decrease in weight in treated compared with untreated	56	58
4. % mortality in treated compared with that of untreated	42	48

Proteases of resistant versus susceptible strain

Table II shows activities of cytoplasmic and lysosomal proteases in surviving susceptible and resistant strain adult flies. All activities were substantially higher in soluble extracts from the resistant strain compared with that of susceptible strain. Thus for the cytoplasmic proteases, alanyl aminopeptidase was increased by 21%, arginyl aminopeptidase by 28%, leucyl aminopeptidase by 13%, dipeptidyl aminopeptidase IV by 48%, tripeptidyl aminopeptidase by 38%, and proline endopeptidase by 32%. Likewise for the lysosomal proteases, dipeptidyl aminopeptidase I was elevated by 37%, dipeptidyl aminopeptidase II by 46%, cathepsin B by 22%, cathepsin L by 13%, cathepsin H by 8%, and cathepsin D by 26%. In contrast, the total protein concentration in cytoplasmic proteases extract was depleted by 24% whereas in lysosomal proteases extract was decreased by 36%.

Effect of spinosad on proteases of susceptible strain

Table III shows *in vivo* effect of spinosad at LD_{50} after 48 hours treatment on cytoplasmic and lysosomal proteases in surviving and dead adult flies of susceptible strain of *M. domestica*. Their percent increase (+) or decrease (-) following spinosad exposure when compared with the control groups is shown in Table IV.

In surviving and dead adult flies of the susceptible strain, spinosad considerably decreased all cytoplasmic and lysosomal proteases except proline endopeptidase, which was increased. Thus spinosad decreased alanyl aminopeptidase by 28 and 35%, arginyl aminopeptidase by 41 and 26%, leucyl aminopeptidase by 49 and 43%, dipeptidyl aminopeptidase IV by 21 and 15%, tripeptidyl aminopeptidase by 27 and 20%, dipeptidyl aminopeptidase I by 77 and 85%, dipeptidyl aminopeptidase II by 86 and 94%, cathepsin B by 81 and 90%, cathepsin L by 93 and 97%, cathepsin H by 67 and 58% and cathepsin D by 36 and 43%, respectively. On the other hand, proline endopeptidase was increased by 48 and 22%, respectively. Likewise, spinosad increased soluble proteins in both *i.e.*, fraction used for extraction of cytoplasmic proteases by 54 and 95% and in fraction of lysosomal proteases by 142 and 158%, respectively.

Effect of spinosad on proteases of resistant strain

The effect of spinosad at LD_{50} dose level after 48 hours treatment on cytoplasmic and lysosomal proteases in surviving and dead resistant strain adult house flies is shown in Table III, while their corresponding percent increase (+) or decrease (-) compared with the controls are shown in Table IV.

Spinosad drastically depleted all cytoplasmic and lysosomal proteases both in the live and dead adult flies of resistant strain of *M. domestica* except for proline endopeptidase, which was elevated. Thus spinosad caused depletion both in surviving and dead flies of alanyl aminopeptidase, arginyl aminopeptidase, leucyl aminopeptidase, dipeptidyl aminopeptidase IV, tripeptidyl aminopeptidase, dipeptidyl aminopeptidase I, dipeptidyl aminopeptidase II, cathepsin B, cathepsin L, cathepsin H and cathepsin D by 57 and 69%, 65 and 72%, 61 and 80%, 28 and 41%, 52 and 74%, 70 and 82%, 74 and 79%, 61 and 72%, 58 and 52%, 57 and 51% and 49 and 57%, respectively. Conversely proline endopeptidase was elevated by 29 and 20%,

Table II.- Activities of proteases in susceptible and resistant strains of *Musca domestica*.

Protease types	Susceptible strain	Resistant strain	% increase (+) or decrease (-) [a]
Cytoplasmic proteases			
Alanyl aminopeptidase	624.65±10.47	758.45±10.82	+21.42
Arginyl aminopeptidase	538.84±8.15	691.17±8.10	+28.27
Leucyl aminopeptidase	44.37±2.13	50.26±2.74	+13.27
Dipeptidyl aminopeptidase IV	25.21±1.71	37.37±1.98	+48.23
Tripeptidyl aminopeptidase	126.32±5.32	174.89±6.47	+38.45
Proline endopeptidase	41.36±2.88	54.73±2.93	+32.33
Total protein	725.29±10.52	553.91±7.22	-23.63
Lysosomal proteases			
Dipeptidyl aminopeptidase I	94.22±3.47	128.74±4.45	+36.64
Dipeptidyl aminopeptidase II	80.35±3.12	117.25±4.21	+45.92
Cathepsin B	605.83±10.91	739.78±11.18	+22.11
Cathepsin L	351.30±7.55	397.92±7.96	+13.27
Cathepsin H	402.05±9.41	434.82±9.23	+8.15
Cathepsin D	212.61±6.67	267.40±6.18	+25.77
Total protein	685.45±10.12	439.65±6.16	-35.86

[a] Percent increase (+) or decrease (-) in enzyme activities of resistant strain has been calculated than those of susceptible strain.

Table III.- *In vivo* effect of spinosad on proteases of insecticide-resistant and -susceptible strains of *Musca domestica* adult flies after 48 hours treatment.

Proteases type	Susceptible strain			Resistant strain		
	Control	Live flies	Dead flies	Control	Live flies	Dead flies
Cytoplasmic proteases						
Alanyl aminopeptidase	624.65±10.47	387.28±5.64	406.82±5.72	758.45±10.82	326.93±4.24	235.72+3.92
Arginyl aminopeptidase	538.84±8.15	317.92±3.73	398.04±3.61	691.17±8.10	241.11±2.65	193.13+2.72
Leucyl aminopeptidase	44.37±2.13	22.23±1.12	25.95±1.72	50.26±2.74	10.78±0.71	8.00+0.56
Dipeptidyl aminopeptidase IV	25.21±1.71	19.11±1.56	21.72±1.95	37.37±1.98	26.73±1.55	22.65+1.83
Tripeptidyl aminopeptidase	126.32±5.32	90.37±3.04	101.98±3.36	174.89±6.47	83.65±3.44	45.36+2.61
Proline endopeptidase	41.36±2.88	61.82±2.96	50.95±2.70	54.7±2.93	70.20±2.23	65.39+2.35
Soluble protein	725.29±10.52	1015.17±10.98	1214.31±11.43	553.91±7.22	1019.79±11.35	1210.61+12.33
Lysosomal proteases						
Dipeptidyl aminopeptidase I	94.22±3.47	21.67±1.43	14.13±1.84	128.74±4.45	38.12±1.95	23.17+1.54
Dipeptidyl aminopeptidase II	80.35±3.12	11.85±0.77	8.28±0.63	117.25±4.21	30.06±1.92	24.12+1.73
Cathepsin B	605.83±10.91	115.91±4.15	61.58±2.39	739.78±11.18	288.11±5.15	207.44+5.56
Cathepsin L	351.30±7.55	34.39±2.08	108.02±2.01	397.92±7.96	167.63±4.14	191.20+4.75
Cathepsin H	402.05±9.41	152.98±4.25	168.14±4.53	434.82±9.23	186.44±4.77	213.66+5.16
Cathepsin D	212.61±6.67	136.77±4.16	115.98±4.01	267.40±6.18	136.07±4.28	114.68+4.05
Soluble protein	685.45±10.12	1558.11±11.31	1758.33±13.26	439.65±6.16	857.32±7.72	931.89+8.43

respectively in surviving and dead resistant strain adult flies. Likewise spinosad elevated soluble protein in cytoplasmic protease extract by 84 and 151% and in lysosomal protease extract by 95 and 112% in living and dead flies, respectively.

DISCUSSION

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

Table IV.- Percent increase (+) or decrease (-) in proteases of insecticide-resistant and -susceptible strains of *Musca domestica* adult flies following spinosad treatment

Protease type	Susceptible strain		Resistant strain	
	Live flies	Dead flies	Live flies	Dead flies
Cytoplasmic proteases				
Alanyl aminopeptidase	-38.00	-34.87	-56.89	-68.92
Arginyl aminopeptidase	-40.99	-26.13	-65.12	-72.06
Leucyl aminopeptidase	-49.90	-41.51	-57.32	-68.13
Dipeptidyl aminopeptidase IV	-24.19	-13.84	-28.47	-39.39
Tripeptidyl aminopeptidase	-28.45	-19.27	-52.17	-74.06
Proline endopeptidase	+49.47	+23.19	+28.27	+19.48
Soluble protein	+39.97	+67.42	+84.11	+118.56
Lysosomal proteases				
Dipeptidyl aminopeptidase I	-77.00	-85.00	-70.39	-82.00
Dipeptidyl aminopeptidase II	-85.23	-89.68	-74.36	-79.43
Cathepsin B	-81.18	-90.00	-61.05	-71.96
Cathepsin L	-90.48	-70.10	-57.85	-51.95
Cathepsin H	-61.95	-58.18	-57.12	-50.86
Cathepsin D	-32.49	-42.76	-49.11	-57.11
Soluble protein	+127.31	+156.52	+95.00	+111.96

compensatory mechanisms associated with the development of pesticide resistance in insects subjected to toxic stress has become increasingly recognized. Resistant strains may be endowed genetically such that the activity of key metabolic enzymes normally present within insect tissue may be quantitatively or qualitatively modified (in terms of overall activity levels and /or modification of enzyme sites) or may be more effectively induced following insecticide exposure (Wilkins *et al.,* 1999). Likewise considerable induction of proline endopeptidase and soluble proteins in both surviving and dead adult flies following spinosad treatment manifests their possible involvement in creation of biologically active molecules followed by the destruction of biologically active proteins and peptides. An increase in protease activities and soluble proteins following insecticide treatment in the present study appears patterned after the induction of detoxification enzymes such as mixed function oxidases, carboxylesterases and glutathione-S-transferases (Terriere, 1984), although proteases hold little prospects of target of the insecticides and synergists (Saleem *et al.,* 1994a; Saleem *et al.,* 1994b). Wilkins *et al.* (1999) and Ahmad *et al.* (1998) have previously reported the dynamics of intracellular protease activities at different post-treatment intervals following DDT and fenitrothion treatments and the dynamics was related to increased supplies of necessary amino acids for the synthesis of insecticide metabolizing enzymes.

Spinosad at LD_{50} dose level after 48 hours depleted considerably all the remaining range of cytoplasmic and lysosomal proteases tested in this study in living and dead adult flies of the susceptible strain ranging from 14 to 90% as well as in the resistant strain in the range of 28 to 82%. Inhibition of almost all proteolytic enzymes (except proline endopeptidase) may be attributed to the higher potency and efficacy of spinosad than other insecticides to control resistant as well as susceptible populations of *M. domestica*. The results, therefore, revealed that spinosad would be effective and efficient alternate insecticide bearing novel mode of action than those conventional nerve poisons, especially to control resistant strains as it possesses more potential to produce higher depletion of cytoplasmic proteases in resistant than susceptible strain of *M. domestica*. According to Tomlin (2000), spinosad causes activation of the nicotinic acetylcholine receptor, but at a different site from nicotine or imidacloprid. Comparing efficacy of spinosad with other insecticides for

controlling *Helicoverpa armigera*, Saleem (2009) reported that recommended dose of spinosad is 80 ml/acre while it is 200 ml/acre for deltamethrin (synthetic pyrethroid) and 1000 ml/acre for chlorpyrifos (organophosphate). Thus spinosad is highly potent insecticide to control resistant and susceptible populations of some insect pests. Likewise results of the present study also revealed that spinosad produced higher depleted levels of most of the proteases in the resistant than that of the susceptible strain and therefore spinosad is possibly more potent and better alternate insecticide to achieve effective control of resistant strains of adult house flies, *M. domestica* especially. Likewise Moulton *et al.* (1999, 2000) reported resistance of beet armyworm, *Spodoptera exigua* to spinosad while Zhao *et al.* (2001) described monitoring and characterization of diamondback moth resistance to spinosad. The results of the present study, however, confirmed our hypothesis of possible involvement of a range of cytoplasmic and lysosomal proteases in the development of resistance in insects to insecticides. In this regard, we have previously reported *in vivo* effects of various insecticides (Saleem *et al.*, 1994a) such as lambda-cyhalothrin and malathion in *M. domestica* (Saleem *et al.,* 1994b) and effect of deltamethrin, pirimiphos-methyl and gamma-hexachlorocyclohexane in *Tribolium castaneum* (Saleem *et al.*, 2000) and cyfluthrin on the range of cytoplasmic and lysosomal proteases in malathion-resistant and -susceptible strains of *T. castaneum* (Saleem *et al.,* 2004). Sarath *et al.* (2001) reported inhibition of proteolytic enzymes. Likewise, 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, 2001).

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