Pakistan J. Zool., vol. 46(2), pp. 853-861, 2014.

Biochemical Response of Malathion-Resistant and -Susceptible Adults of *Rhyzopertha dominica* to the Sublethal Doses of Deltamethrin*

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Abstract. - *Rhyzopertha dominica,* lesser grain borer both in larval and adult forms is a primary pest of stored grains and mainly attack wheat, rice, millet and corn. Various recommended insecticides are routinely used to control its population which has led to the development of resistance. The aim of present study was to determine the biochemical differences in the malathion-resistant and -susceptible adult populations of *R. dominica.* Sublethal doses of deltamethrin were applied at 1.50 ppm for resistant and 0.966 ppm for susceptible populations for a period of 48 h. Highly significant rise in activities of acid phosphatase (AcP) (135%), amylase (55%), glutamate oxaloacetate transaminase (513%), trehalase (348%) and concentration of trehalose (110 %) was observed in susceptible population. In resistant population alkaline phosphatase (33%), amylase (40%), glutamate pyruvate transaminase (32%) and trehalase (62%) activities and concentrations of glucose (32%) decreased significantly, while free amino acids (23%), total lipids (39%), and RNA (29%) content increased significantly after deltamethrin treatment. Cholinesterase and AcP activities increased in both populations. Of respiratory enzymes, lactate dehydrogenase remained unaffected in both populations but isocitrate dehydrogenase (ICDH) activity showed significant increase (87%) in resistant and decrease (81%) in susceptible populations. Similarly, contrasting trend was found in amylase and trehalase activities with significant decrease in resistant and increase in susceptible population. Total lipids and RNA content showed rise in resistant and decline in susceptible beetles. All these biochemical changes, especially in amylase, ICDH, trehalase, glucose, total lipids and RNA in susceptible and resistant beetles are indicative of development of severe disturbances in respiration, carbohydrate, lipid and protein metabolism. It can be concluded that deltamethrin at above sublethal concentrations produced significant metabolic alterations by hitting at different secondary targets in resistant and susceptible populations that render the beetles unable to survive long.

Key words: Pyrethroid, K-Othrin, stored grain pests, pest control, *Rhyzopertha dominica*, deltamethrin.

INTRODUCTION

World food losses during storage are 200 million tons per year worth US$ twenty billion (Credland *et al*., 2003). In Pakistan, the losses of stored grains caused by insects, range from 5-10% of the world grain production (Ahmad and Mahmood, 1991). Lesser grain borer, *Rhyzopertha dominica*, is a primary pest of stored grain in many regions of the world (Edde, 2012), causing huge damages to stored grains. This beetle is a strong flyer and may rapidly migrate to begin new infestation elsewhere (Mahroof *et al*., 2010).

Different control measures *e.g.,* physical such as temperature (Watters *et al.,* 1983; Bennet, 2003), pressure, (Mbata *et al*., 2004) aeration (Arthur, 1995; Flinn *et al.,* 1997), relative humidity and starvation (Ahmad *et al.,* 1982; Ofuya and Reichmuth, 2002; Ali *et al*., 2006), use of plant extracts (Naqvi *et al.,* 1998; Fields, 2006), biological (Flinn *et al.,* 2004; Nayak *et al*., 2005) and the chemical control (Hooper *et al*., 2003) by the use of contact insecticides (Kljajic and Peric, 2006; Ali *et al*., 2003, 2007; Athanassiou *et al.,* 2007) and fumigants (Daglish, 2004) have been adopted.

The control of stored-grain insect pests by organophosphate (OP) insecticides and fumigants (methyl bromide, phosphine) had led to development of resistance (FAO, 1974, 1975; Collins, 1998; Emery, *et al.,* 2003; Daglish, 2004; Ali *et al*., 2013). Friedlander *et al*. (1981) and Collins *et al*. (1992) linked resistance to chlorpyriphos-methyl with general esterase levels in laboratory selected strains of *Oryzaephilus*

* Part of PhD thesis of first author.
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0030-9923/2014/0003-0853 $ 8.00/0

surinamensis. Resistance to OPs may result by altering the structure of the esterases that increase the ability to hydrolyze the insecticide (Hama and Hosoda, 1983; Oppenoorth, 1985; Pasteur *et al.,* 1986; Devonshire, 1987).

Ugaki *et al*. (1985) found the major detoxification pathways in houseflies resistant to fenitrothion via glutathion-S-transferases (GST); mono-oxygenases played a minor role only. Contrarily, Hemmingway *et al*. (1991) concluded that resistance to fenitrothion in *Anopheles subpictus* (Grassi) was attributable to both mono-oxygenases and GST metabolism. Konno *et al*. (1989) demonstrated with a strain of tobacco budworm that a lower rate of activation of thion to oxon (via mono-oxygenases) coupled with more active hydrolases were responsible for resistance. Li *et al.* (2005) reported cytochrome P-450-based metabolic detoxification in a cattle tick.

Heidari *et al.* (2004) reported resistance to OP insecticides due to mutation in the gene encoding CE that enhances the enzyme's ability to hydrolyze insecticides. These enzymes protect the target site by catalyzing the hydrolysis of insecticides (Pasteur *et al*., 1986). Shakoori and Saleem (1991) correlated the resistance in OP resistant-*T. castaneum* adults to significantly raised levels of CE, ChE, protease and lactate dehydrogenase (LDH) activities and significantly reduced activities of acid phosphatase (AcP) and lipid contents.

The problems arising due to OP-resistance have directed the attention of researchers towards the use of pyrethroid insecticides (Elliot and Janes, 1973; Elliot *et al*., 1978; Mac Cuaig, 1980; Arthur, 1992, 1997; Ali *et al*., 2003, 2011) due to their significant insecticidal properties, low mammalian toxicity, and rapid breakdown in the environment (Pimentel *et al.,* 1992). Liu *et al.* (2006) reported the pyrethroids as the most widely used insecticides. Pyrethroids are found effective against a wide range of stored product pests (Carter *et al*., 1975; Watters *et al.* 1983). They exert their toxic effects primarily by altering properties of the sodium channel, which is essential for the generation and propagation of action potential in excitable cells (Narahashi, 1996; Yanola *et al.,* 2010).

Deltamethrin, a pyrethroid is a primary metabolite of tralomethrin and highly toxic than its parent compound (NRCC, 1986). Arthur (1997) have reported effectiveness of deltamethrin dust against three stored grain products including *R. dominica.* Its toxic effects have also been found against *Tribolium confusum* and *Sitophilus oryzae* on stored wheat (Athanassiou *et al.,* 2004; Athanassiou and Buchelos. 2004).

During stored grain spraying programs many target sites do not receive the desired concentrations of the applied materials, so stored grain pests are experienced with sublethal levels of insecticides. Sublethal effects result when insufficient molecules to cause death reach the sites of insecticidal action. Sublethal effects of pyrethroids on enzyme induction, glycaemia, lipemia, gut AcP, depletion of carbohydrate, protein and lipid reserves; decrease in growth rate, in adult fecundity, fertility and longevity and on reproductive rate have been discovered in a number of studies (Wongkobrat and Dahlman, 1976; Kumar and Chapman, 1984; Bounias *et al*., 1985; Jackson and Wilkins, 1985; Saleem *et al.,* 2000).

The main objective of the present study is to investigate the biochemical response of malathion-resistant and -susceptible adults of *R. dominica* to the sublethal doses of deltamethrin.

MATERIALS AND METHODS

Malathion-resistant and -susceptible populations of *R. dominica* were used for this study (see Ali *et al.,* 2007). The culture was developed in the sterilized jam jars covered with muslin cloth, in a culture room maintained at 30±2^{o}C with 65±5% relative humidity. Whole wheat grains following 24h phosphine fumigation were used as food of the beetles (FAO, 1974). The adults of the beetle collected at 43±2 days after egg laying were used in the present study.

Insecticide used

Technical grade deltamethrin [(S)-α-cyano-3-phenoxybenzyl ester; decis; K-Othrine] obtained from the Agricultural Chemical Group, FMC Corporation, Lahore, Pakistan was used for this study.

Determination of LC_{50} and LC_{20}

Serial dilutions of deltamethrin *i.e.,* 256, 128, 64, 32, 16, 8, 4, 2 and 1ppm concentrations were prepared in acetone. Each dilution (1.3ml) was applied in the center of a separate glass Petri plates. To spread the insecticide uniformly the Petri plates were rotated manually. The acetone was allowed to evaporate after which ten adult beetles of both malathion-resistant and -susceptible populations were placed in their respective Petri plates and covered. Three control Petri plates with solvent (acetone) and 10 adult beetles were also prepared for each population. The beetles were checked for mortality after 48 h. The beetles were considered dead if on touching with brush they did not show any movement. Lloyd (1969) was followed for mortality counts. LC_{50} and LC_{20} were calculated by computerized probit analysis (Finney, 1971).

Exposure of beetles to sublethal doses of deltamethrin

Healthy adult beetles (150) of both populations were exposed separately to the sublethal concentrations (LC_{20}) of deltamethrin by the residual film method along with their respective controls in triplicate. The beetles were kept unfed for 48 h at 30±2°C and 65±5% relative humidity.

Biochemical analyses

A motor-driven glass homogenizer was used to homogenize 100 treated and 100 control beetles separately in 3ml of 0.89% cold (4°C) saline in triplicate for all biochemical analyses. All these homogenates were centrifuged at 4°C in refrigerated centrifuge to obtain clear supernatants which were later on used for the estimation of various enzyme activities like acid phosphatase (AcP) according to Andersch and Szcypinski (1947), alkaline phosphatase (AkP) according to Bessey *et al.* (1946), lactate dehydrogenase (LDH) according to Cabaud and Wroblewski (1958), isocitrate dehydrogenase (ICDH) according to Bell and Baron (1960), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) according to Reitmann and Frankel (1957), cholinesterase (ChE) according to Rappaport *et al.* (1959); amylase as given in Wootton and Freeman (1982) and trehalase by the procedure of Dahlqvist (1966). The supernatant was also analyzed for the soluble and total protein contents (Lowry *et al.*, 1951), glucose content by the *o*-toluidine method of Hartel *et al.* (1969) and trehalose content by the anthrone method of Carroll *et al.* (1956) as modified by Roe and Dailey (1966) and Steel and Paul (1985). Total lipids, nucleic acids and FAA were estimated from ethanol extract of treated and control beetles. For total lipids, nucleic acid and FAA estimation the method of Zöllner and Kirsch (1962), Schneider (1957) and Moore and Stein (1954) was adopted, respectively. Glycogen contents were extracted by crushing the whole beetles in KOH and estimated by the anthrone method of Consolazio and Lacono (1963). Total proteins were extracted in 2.5M hot NaOH.

Statistical analysis

Student's t test was used to compare the control and insecticide treated data. The "t" values < 0.05 were considered significantly different from control.

RESULTS

The effects of sublethal doses of deltamethrin on some enzyme activities and metabolites of malathion-resistant and –susceptible populations of *R. dominica* after an exposure of 48 h are shown in Table I. Changes (% increase or decrease) in the enzymatic activities as well as the metabolites are shown in Figure 1.

Effects of deltamethrin on enzyme activities of susceptible beetles

Most of the tested enzyme activities in susceptible beetles showed elevated values following deltamethrin exposed for 48 h. The increase in activities was135% for AcP, 55% for amylase, 97% for ChE, 513% for GOT and 348% for trehalase. On the other hand, ICDH activity was reduced by 81% after application of deltamethrin for 48 h with reference to control. The activities of AkP, GPT and LDH underwent nonsignificant changes (Fig. 1).

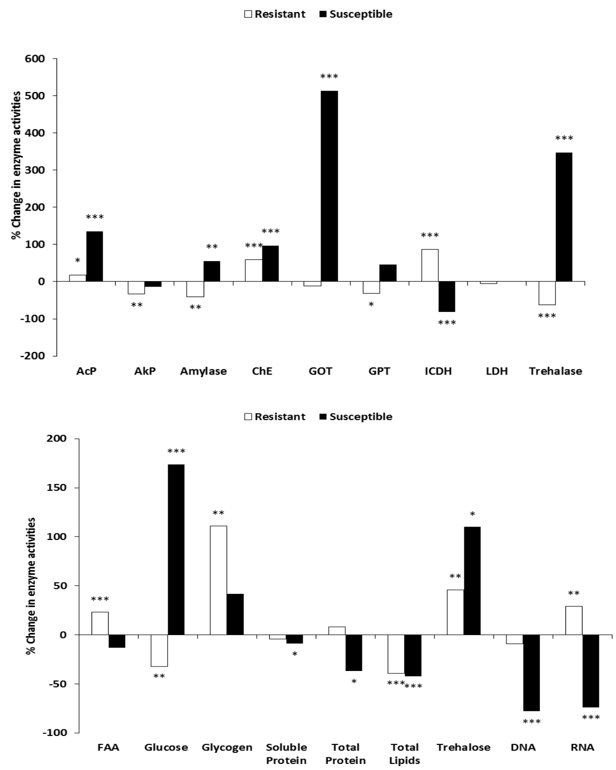

Fig. 1. Percent increase or decrease in enzymatic activities and metabolites concentration of malthion-resistant and –susceptible adult populations of *R. dominica* following deltamethrin treatment with reference to contol.

Carbohydrates and lipids

Susceptible beetles showed highly significant elevation in glucose (P< 0,001) and trehalose (174% and 110%, respectively) and depletion (42%) in total lipid contents with nonsignificant effect on glycogen as compared to control (Fig. 1).

Proteins, free amino acids and nucleic acids

Prominent reduction in total protein, DNA and RNA contents (37%, 78% and 74%, respectively) was found, with reference to control with negligible effect on soluble protein and FAA contents (Fig. 1).

Effects of deltamethrin on enzyme activities of resistant beetles

After 48 h insecticide treatment majority of enzymes activities decreased significantly. The decrease in AkP was 33%, amylase 40%, GPT 32% and trehalase 62% but for other enzymes *e.g.* GOT (12%) LDH (6%), this decrease was minor with reference to the control. On the other hand, significant increase was noticed in the activities of AcP (18%), ChE (59%) and ICDH (87%) as shown in Figure 1. The decrease in activities of GOT and LDH was non-significant.

Carbohydrates and lipids

Among the fuel molecules, glycogen and trehalose contents were elevated by 111% and 46%, respectively, with reference to control, while glucose showed significant depletion by 32%. The total lipids content showed significant depletion of 39% (Fig. 1).

Proteins, free amino acids and nucleic acids

Following insecticide treatment, FAA and RNA contents were increased significantly by 23% and 29%, respectively, whereas, soluble protein, total protein and DNA remain unaffected (Fig. 1).

DISCUSSION

Exposure of *R. dominica* to sublethal concentrations of deltamethrin under laboratory conditions revealed the sensitivity of most of the enzymes in adults of malathion-resistant and –susceptible populations. In susceptible population most of the enzymes activities were raised significantly which is supported by increased synthesis of enzymes. The induction of detoxification enzymes after pyrethroid exposure were also revealed by other workers (Saleem and Shakoori 1987a,b, 1985; Kacew and Singhal, 1973). Ali *et al.* (2011) reported more or less similar findings after bifenthrin treatment to malathion-resistant and –susceptible populations. Highly reduced ICDH activity was an indication of probable low functioning of Krebs's cycle in the susceptible beetles, limiting their ability to generate energy to cope with the insecticidal stress. Likewise, Shakoori *et al.* (1994) and Saleem (1990) have found depletion of ICDH in Sumicidin super, Cymbush and Ripcord treated larvae and beetles of *T. castaneum.* Elevation of GOT activity seemed to induce the secondary process of respiration through transamination which is evident by depletion in the

Table I.- Effects of deltamethrin on the various enzyme activities and biochemical components of malathion-resistant and –susceptible populations of *R. dominica*.

Parameters[a]	Resistant population		Susceptible population	
	Control	Deltamethrin (n=3)	Control	Deltamethrin (n=3)
AcP (IU/mg)[c]	1.74±0.05[b]	2.05±0.14*	1.88±0.05[b]	4.41±0.63***
AkP (IU/mg)	1.21±0.12	0.81±0.06**	1.24±0.03	1.06±0.19
Amylase (mSU/mg)	12.81±0.12	7.72±1.20**	14.51±0.57	22.50±2.05**
ChE (IU/mg)	0.55±0.06	0.87±0.02***	0.25±0.03	0.49±0.04***
GOT (IU/mg)	1.37±0.06	1.20±0.16	1.18±0.05	7.24±0.71***
GPT (IU/mg)	0.09±0.06	0.06±0.01*	0.26±0.02	0.14±0.06
ICDH (IU/mg)	4.52±0.30	8.46±0.43***	4.68±0.17	0.88±0.13***
LDH (IU/mg)	7.66±0.18	7.21±0.87	10.82±0.56	11.13±0.83
Trehalase (IU/mg)	0.54±0.04	0.20±0.02***	0.39±0.03	1.74±0.20***
FAA (µg/mg)	97.16±3.97	119.65±3.26***	192.38±15.56	166.68±6.53
Glucose (µg/mg)	9.50±0.42	6.46±0.69**	98.72±0.32	23.91±2.79***
Glycogen (µg/mg)	7.78±0.32	16.49±2.80**	6.95±0.58	9.92±1.65
Soluble Protein (µg/mg)	85.85±2.64	82.20±9.17	87.40±1.14	79.14±3.32*
Total Protein (µg/mg)	182.63±4.60	198.74±13.80	161.43±3.20	101.52±26.77*
Total Lipids (µg/mg)	41.70±1.73	25.09±2.72***	39.96±2.20	23.11±2.41***
Trehalose (µg/mg)	19.34±0.80	28.27±2.24**	21.38±1.03	44.96±10.70*
DNA (µg/mg)	6.12±0.14	5.56±0.34	7.33±0.34	1.55±0.13***
RNA (µg/mg	11.87±0.38	15.36±0.86**	16.44±0.48	4.24±0.49***

[a]Abbreviations used: AcP, acid phosphatase; AkP, alkaline phosphatase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; ICDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; FAA, free amino acids; DNA, deoxyribose-nucleic acid; RNA, ribose-nucleic acid. IU, International unit; mSU, milli Somogyi unit.

[b]Mean ± SEM: Student's t test; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[c]Definitions of enzyme units: IU, international unit, the amount of enzyme, which under defined assay conditions, will catalyze the conversion of 1µ mol of substrate per minute; mSU, the amount of enzyme digesting 5 µg of starch in the experimental conditions used here.

protein and amino acid contents. Elevation of AcP suggests the activation of dephosphorylation mostly in the lysosomal fraction of the damaged cells enabling the organism to get energy through breakdown of energy rich compounds that could be supported by the reduction in DNA and RNA level. The breakdown of cellular components under the toxic stress and molecules thus released, may be used as source of energy by the organism. The inhibition of protein synthesis could also be related to the reduced levels of nucleic acids.

Increase in glucose and insect's specific disaccharide *i.e.* trehalose under insecticidal stress might be due to the inactivation of glycolysis and Krebs cycle which is supported by decrease in ICDH (a mitochondrial enzyme) activity. Increased glucose level may also be due to increased breakdown of trehalose which is evident from raised trehalase activity. This along with Increase in amylase without affecting the glycogen concentration points towards the development of severe abnormality in carbohydrate metabolism.

Deltamethrin treatment found to develop somewhat reverse situation in case of malathion-resistant population. The activities of AcP, ChE and ICDH increased significantly. Moreover, increase in ICDH activity indicated switching on of citric acid cycle in which metabolites were probably entering through dephosphorylation which can be supported by the 18% rise in AcP and inhibition in DNA contents. Other enzyme activities *i.e.,* AkP, carbohydrases (amylase and trehalase) and GPT were severely reduced which is obvious by the relevant rise in glycogen, trehalose and FAA content. RNA content was decreased in resistant while decrease in susceptible populations which might be attributed to increase in RNA biosynthesis by higher doses in resistant and decrease its synthesis by low doses of deltamethrin in susceptible populations. These results are similar to the reports of Naqvi *et al.* (1970), Ishaaya and Casida (1975) and Ishaaya *et al.* (1977).

Some parameters underwent same changes in both populations *e.g.,* enhancement in AcP and ChE activities, and glycogen and trehalose contents. Increase in ChE activity point towards the tendency of both populations to detoxify the insecticidal effects. Limoee *et al.* (2007) disclosed the same effects in the German cockroach *Blattella germanica* (L.) after permethrin treatment. Prominent reduction in the total lipids indicated these are being routed to the main metabolic pathway to utilize more and more energy production for the beetles struggling for existence, the finding which complies with the report of Saleem and Shakoori (1986). Moreover, it is suggested that these parameters might have some role in the defensive mechanism of the beetle.

These all biochemical modifications, especially contrasting trends in amylase, ICDH, trehalase, glucose and RNA contents and highly significant decrease in total lipids, in susceptible and resistant beetle populations are indicative of development of severe perturbations in respiration and carbohydrate, lipid and protein metabolism.

It can be concluded from this study that deltamethrin at above sublethal concentrations affect both populations severely but by targeting different biochemical and regulatory molecules in different metabolic pathways.

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(Received 11 September 2013, revised 27 May 2014)