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Effects of Sublethal Doses of Talstar on Biochemical Components of Malathion-Resistant and -Susceptible Adults of *Rhyzopertha dominica**

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Abstract.- To evaluate biochemical differences between Malathion-resistant and -susceptible adult populations of lesser grain borer, *Rhyzopertha dominica* a sublethal dose (LC_{20}) of Talstar, a pyrethroid insecticide, was administered at 1.4ppm to resistant and at 0.22ppm to susceptible population for a period of 48 hours. Effects on various biochemical components such as some enzymes *e.g.,* acid phosphatase (AcP), alkaline phosphatase (AkP), amylase, cholinesterase (ChE), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), and trehalase and among metabolites free amino acids (FAA), glucose, glycogen, soluble protein, total protein, total lipids, trehalose, DNA and RNA contents were studied. AcP, amylase, lactate dehydrogenase (LDH) and trehalase activities were increased, whereas, a decrease in AkP activity and glycogen and trehalose contents was observed in both populations. Inhibition of ChE activity in resistant but induction in susceptible population indicates non-involvement of this enzyme in resistance of this beetle. Among transaminases, both GOT and GPT activities decreased by 53% and 32% in resistant adults respectively, resulting in accumulation of FAA and depletion of total protein contents which can be correlated with increase (52%) in FAA level and the reduction (17%) in the total protein contents. The increase in GOT (23%) and GPT (15%) activities in susceptible adults is an indication of accelerated amino acid catabolism. High activities (43% and 117%) of LDH and isocitrate dehydrogenase (ICDH), respectively, in resistant insects indicates that both catabolic pathways (glycolysis and TCA cycle) are switched on to cope with the insecticidal stress, thus keeping up its ability to resist the insecticide which is obvious by significant decrease (25%) in glucose level. In susceptible population, LDH activity also elevated (38%) with no change in ICDH activity exhibiting its inability to defend the insecticidal stress by complete oxidation of carbohydrates. Huge accumulation of lipids (50% and 119%) in resistant and susceptible populations respectively, reveals enhancement of lipid biosynthesis. The higher enzyme activities induced by Talstar may be justified by 70% and 76% rise in RNA contents in both resistant and susceptible populations, respectively, through a probable increase in transcription rate. Conclusively, both populations of *R. dominica* defended greatly against Talstar stress by the induction of the major enzymes under the present experimental conditions.

Key words: Pyrethroid, *Rhyzopertha dominica*, pest control, LC_{50}, LC_{20}, enzymes, metabolites, insecticides.

INTRODUCTION

In Pakistan, besides low productivity large percentages of grains are destroyed in the stores by the ravages of certain noxious stored grain pests including lesser grain borer, *Rhyzopertha dominica* (F.) (Alam and Ahmad, 1989). The origin of *R. dominica* is tropical but it is well established in the temperate regions of the world (Schwardt, 1933; Potters, 1935; Irshad and Talpur, 1993; Bennett, 2003). This pest is of great economic significance in the United States of America, Southern Canada, Argentina, New South Wales, South East Australia and Indo-Pakistan sub-continent (Cuperus *et al*., 1986; Shakoori *et al*., 2000; Toews and Subramanyam, 2003; Flinn *et al*., 2004; Toews *et al*., 2005; Fields, 2006). It is the widespread and predominant pest collected from storage bins, which readily feeds on whole grain (Arthur, 1992). The first instar larvae have been observed to enter the grain through the intact kernel (Rees, 2007) and cannot be removed from the grain through normal cleaning procedures (Flinn, 1998).

Several methods (*i.e.* temperature, aeration, pressure, relative humidity, starvation, biological, natural and chemical control) have been used against *R. dominica* (Flinn *et al.,* 2004; Mbata *et al.*, 2004; Athanassiou and Kavallieratos, 2005; Ferizli and Beris, 2005; Ali *et al.,* 2006; Athanassiou *et al.*, 2005, 2006, 2007, 2008a,b 2010; Kavallieratos *et*

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Stored grain pests have developed resistance to organochlorine (OC) insecticides *e.g.*, BHC, γ-HCH/lindane (Bhatia and Pradhan, 1972), DDT, dieldrin, *etc.,* (Lloyd, 1969; Kulkarni and Mehrotra, 1973) and certain fumigants *e.g.*, methyl bromide, phosphine (FAO, 1975; Daglish, 2004). Collins *et al.* (2002) revealed the presence of dominant gene in phosphine resistant *R. dominica.* Depending on their type and application rate, insecticides can ensure long-term protection from noxious insects (Arthur, 1994; Desmarchelier, 1994). Similarly excessive use of organophosphate (OP) insecticides has also been reported to have developed resistance in *Rhyzopertha* in USA and south Asia (Beeman and Wright, 1990; Zettler and Cuperus, 1990; Ali *et al.*, 2003; Syed *et al.*, 2005). An acetylcholinesterase gene involved in malathion resistance has been studied and cloned by Zhou and Xia (2009).

Bifenthrin is a safe synthetic pyrethroid against non-target animals such as birds, reptiles and mammals and it lasts much longer than Ops (FMC, 1986). Bifenthrin controls insects by contact and stomach poison activity. It acts by paralyzing the nervous systems of insects (Miller and Salgado, 1985).

The objective of the present study is to evaluate the biochemical alterations induced as a result of sublethal exposure of Talstar (bifenthrin) in malathion-resistant and -susceptible *R. dominica,* which may be used as marker of insecticide exposure. The data may be helpful in formulating some control strategy for this pest.

MATERIALS AND METHODS

Malathion-resistant and -susceptible populations of *R. dominica* were used for this study (Ali *et al.,* 2003). The culture was maintained at 30±2°C with relative humidity of 65±5% in the sterilized jam jars, covered with muslin cloth. The adults of the beetle collected at 43±2 days after egg laying were fed on whole wheat grains 24 hours after phosphine fumigation (FAO, 1974).

Insecticide used

Technical grade of Talstar, 10 EC (bifenthrin (1α, 3α (z)-(±)-(2-methyl (1, 1'-biphenyl]-3-yl) methyl 3-(2-chloro-3, 3, 3-trifluoro -1- propenyl)-2, 2-dimethylcyclo-propane-carboxylate) was obtained from the Agricultural Chemical Group of FMC Corporation, Lahore, Pakistan.

Estimation of LC_{50} and LC_{20}

The detailed method to determine the range of toxicity (LC_{50}) of Talstar against six populations of adult beetles has been described elsewhere (Ali *et al.,* 2003). Lloyd method (1969) was used for counting the mortality. From the mortality data LC_{50} was calculated by computerized probit analysis (Finney, 1971) for each population separately. By using the same data and procedure, as for $LC_{50,}$ the sublethal doses **(**LC_{20}) of Talstar for both resistant and sensitive population were calculated, separately.

Exposure to insecticide

Adult beetles (150) of both malathion-resistant and -susceptible populations were exposed separately to the sublethal doses of Talstar by residual film method along with their controls. The residual film method is to coat the surface of the Petri plate with the very thin layer of the test substance such as any insecticide (Gupta, 1968). Three replicates were used in each experiment. The beetles were kept unfed in the Talstar treated triplicates of Petri plates for 48 hours at 30±2^{o}C and 65±5% relative humidity. Adult beetles which died due to natural causes or due to toxicity of insecticide during this 48 hours period were discarded and only alive beetles were used for biochemical analyses.

Biochemical analyses

After exposure to sublethal doses, 100 adult insects were homogenized in 3 ml of 0.89% saline with the help of a motor-driven glass homogenizer under cold conditions (4^{o}C). Three replicates of each treatment were used throughout biochemical experimentation. The homogenate was centrifuged at 4900 x g for 45 minutes. The supernatant thus obtained was used for the estimation of various enzyme activities like acid phosphatase (AcP; orthophosphoric- monoester phosphohydrolase, acid optimum, EC: 3.1.3.2) activity according to Andersch and Szcypinski (1947), alkaline phosphatase (AkP; orthophosphoric monoester phosphohydrolase alkaline optimum EC: 3.1.3.1) activity as mentioned in Bessey *et al.* (1946), lactate dehydrogenase (LDH; L-lactate NAD: oxidoreductase; EC: 1.1.1.27) activity by a method based on Cabaud and Wroblewski (1958), isocitrate dehydrogenase (ICDH; Threo-Ds-isocitrate: NADP:oxidoreductase, EC: 1.1.1.42) activity by a procedure described by Bell and Baron (1960), glutamate oxaloacetate transaminase (GOT; EC 2.6.1.1) and glutamate pyruvate transaminase (GPT; EC 2.6.1.2) activities according to Reitmann and Frankel (1957), cholinesterase (ChE; acetylcholine aceylhydrolase, EC: 3.1.1.7) activity according to Rappaport *et al*. (1959), amylase (1,4- glucan, glucanhydrolase, EC: 3.2.1.1) activity according to the procedure described in Wootton and Freeman (1982) and trehalase activity by the procedure described by Dahlqvist (1966).

The supernatant was also analyzed for soluble protein contents according to 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 (RNA, DNA) and FAA contents were estimated from ethanol extract of treated and control adult beetles following centrifugation at 2500 rmp. Total lipids were estimated by the methods of Zöllner and Kirsch (1962); nucleic acid estimation followed Schmidt and Thannhauser procedure described by Schneider (1957) and free amino acids (FAA) content according to Moore and Stein (1954).

Glycogen contents were extracted by crushing the whole adult beetles in KOH according to the anthrone method described by the Consolazio and Lacono (1963). Total protein contents were also estimated by making the extract of treated and control homogenzing beetles in NaOH and analyzed according to Lowry *et al*. (1951).

Statistical analysis

Data were analyzed by student's `t' test at $P<0.05$.

RESULTS

Toxicity of Talstar

Treatment with Talstar revealed LC_{50} 4.37ppm for malathion-resistant population and 1.11ppm for –susceptible population respectively, whereas, their LC_{20} was 1.40ppm and 0.22ppm, respectively.

Table I shows the effects of sublethal doses of Talstar administered to resistant and susceptible adults for 48 hours on different enzymes such as AcP, AkP, amylase, ChE, trehalase, GOT, GPT, ICDH, LDH and metabolites *viz.,* glucose, glycogen, free amino acids, soluble protein, total protein, total lipids, DNA and RNA. Figure 1 show % increase (+) or decrease (-) in the enzymatic activities as well as concentrations of various metabolites in the two populations.

Effect on resistant beetles

Enzyme activities

Sublethal doses of Talstar administered for 48 hours to adult resistant beetles significantly raised (with respect to their controls) the activities of AcP, ICDH, LDH and trehalase by 33%, 117%, 43% and 103%, respectively, however 17% increase in amylase activity was insignificant as compared to respective controls. Other The enzyme activities which showed significant decrease were ChE (69%), GOT (53%) AkP (37%), and GPT (32%) (Fig. 1).

Carbohydrates and total lipids

Talstar treatment on adult beetles caused depletion of all carbohydrates tested. Glucose contents decreased 25%, glycogen and trehalose decreased 8% and 12%, respectively. Total lipids increased by 50% after exposing the adult beetles to the insecticide (Fig. 1).

Proteins, FAA and nucleic acids

After exposure of adult beetles to the Talstar for 48 hours, FAA and soluble proteins increased 52% and 33%, respectively, but total protein contents decreased by 17%. Amongst nucleic acid RNA contents showed 70% increase (with reference to control), whereas, the change in DNA was negligible (Fig. 1).

Effects on susceptible beetles

Enzyme activities

All enzymes activities were enhanced after

Table I.- **Effects of Talstar on the various enzyme activities and biochemical components of resistant and susceptible population of *R. dominica.***

Parameters	Resistant population		Susceptible population	
	Control (n=21)	Talstar (n=3)	Control (n=21)	Talstar (n=3)
AcP (IU/mg)c	1.74 ± 0.057b	2.31 ± 0.19**	1.88 ± 0.05b	2.04 ± 0.17
AkP (IU/mg)	1.210 ± 0.12	0. 76 ± 0.44	1.24 ± 0.03	1.00 ± 0.06**
Amylase (mSU/mg)	12..81 ±0.12	15.06 ± 3.98	14.51 ± 0.57	24.29 ± 4.29*
ChE (IU/mg)	0.55 ± 0.06	0.17 ± 0.03***	0.25 ± 0.03	0.38 ± 0.16
GOT(IU/mg)	1.37 ± 0.06	0.64 ± 0.12***	1.18 ± 0.05	1.45 ± 0.09*
GPT(IU/mg)	0.09 ± 0.01	0.06 ± 0.02	0.26 ± 0.02	0.30 ± 0.10
ICDH(IU/mg)	4.52 ± 0.30	9.81 ± 1.49**	4.68 ± 0.17	4.70 ± 0.63
LDH(IU/mg)	7.66 ± 0.18	10.95 ± 0.88**	10.82 ± 0.56	15.03 ±1.18**
Trehalase (IU/mg)	0.54 ± 0.04	1.10 ± 0.03***	0.39 ± 0.03	0.55 ± 0.11
FAA (µg/mg)	97.16 ± 3.97	147.75 ± 25.88	192.38 ± 15.56	167.09 ± 12.75
Glucose (µg/mg)	9.50 ± 0.42	7.10 ± 0.72**	98.72 ± 0.32	9.10 ± 0.80
Glycogen (µg/mg)	7.78 ± 0.32	7.15 ± 0.15	6.95 ± 0.58	4.49 ± 2.49
Soluble protein (µg/mg)	85.85 ± 2.64	114.77 ± 14.32	87.40 ± 2.14	82.62 ± 16.99
Total protein (µg/mg)	182.63± 4.60	150.32 ± 26.45	161.43 ± 3.20	102.03 ± 6.59***
Total lipids (µg/mg)	41.70 ± 1.73	62.56 ± 11.15	39.96 ± 2.20	87.75 ± 7.44***
Trehalose (µg/mg)	19.34 ± 0.80	16.83 ± 4.06	21.38 ± 1.03	11.55 ± 1.86***
DNA (µg/mg)	6.12 ± 0.14	7.80 ± 0.85	7.33 ± 0.34	7.20 ± 1.00
RNA (µg/mg)	11.87 ± 0.38	20.21 ± 2.22**	16.44 ± 0.48	28.93 ± 5.54*

[a]Abbreviations used: AcP, acid phosphatase; AkP, alkaline phosphatase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; ICDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; IU, International unit; mSU, milli Somogyi unit; FAA, free amino acids; DNA, deoxyribo-nucleic acid; RNA, ribo-nucleic acid.
[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 5000 mg of starch in the experimental conditions used here.

treatment with Talstar except for AkP which was declined by 19% while ICDH activity remained unaltered. There was a significant increase of 67%, 23% and 38% in amylase, GOT, and LDH activities, respectively, whereas the non-significant rise in activities of AcP (8%), ChE (50%), GPT (15%) and trehalase (40%) was also noticed (Fig. 1).

Carbohydrates and total lipids

Talstar exposure to adult beetles produced significant increase (119%) (with reference to control) in total lipids and 45 decrease in trehalose. Similarly glucose content increased non-significantly by 4% while glycogen contents showed decrease (35%) as compared to control (Fig. 1).

Proteins, FAA and nucleic acids

Talstar treatment caused decrease in FAA (13%), soluble protein (5%) and total protein (36%). RNA contents increased (76%) with no change in DNA contents (Fig. 1).

DISCUSSION

The reduced levels of glucose, glycogen and trehalose contents recorded in the present study may suggest that energy production through glycolysis was switched on and accelerated to cope with the insecticidal stress (Tufail *et al.,* 1994). Vyjayanthi and Subramanyam (2002) also reported enhanced trehalase activity in the midgut of silkworms treated with insecticides. Saleem and Shakoori (1996) also reported elevation of LDH and ICDH when *Tribolium castaneum* larvae were treated with other pyrethroids such as cypermethrin and permethrin. Saleem and Shakoori (1985) related raised activity

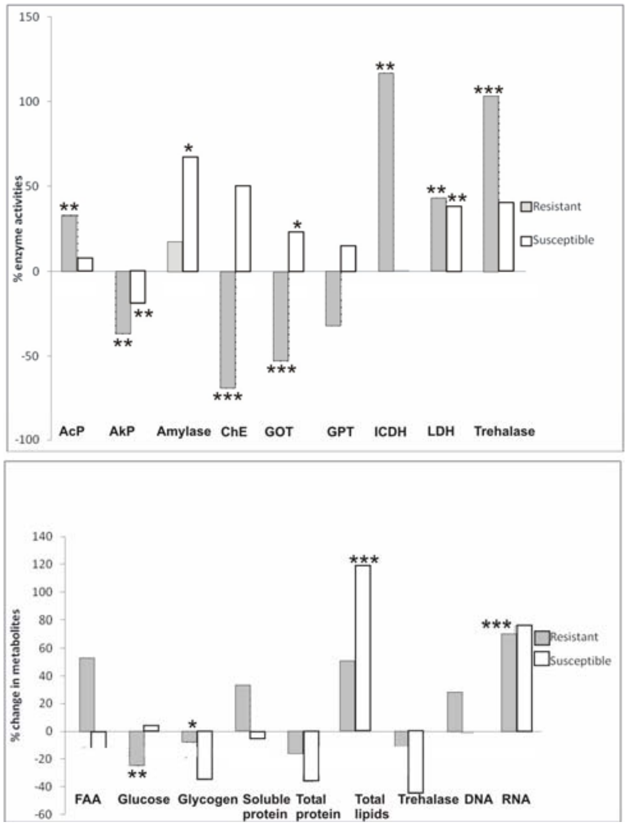

Fig. 1. Percent increase (+) and decrease (-) in various enzymatic activities and concentration of metabolites of resistant (■) and susceptible (□) adults of *R. dominica* following Talstar treatment with reference to control.

of LDH to its higher production and consequently accumulation of lactic acid from its substrate *i.e.,* pyruvic acid in the tissues. This might be the possible cause of resistance in the resistant population of *R. dominica*. Transaminases (GOT and GPT) were reduced after 48 hour of Talstar application. Reduction in GOT and GPT activities can be related to inhibition of transamination. Transaminase activities are based on the formation of oxaloacetate (GOT) or pyruvate (GPT) from aspartate and alanine, respectively, with 2, oxoglutarate. GOT shows a maximum activity at pH 8.0 and GPT has an optimum pH of 7.5. It can be suggested that Talstar treatment probably has disturbed these specific conditions for enzymatic activity resulting in their inhibition and thus blocking the additional energy production. Likewise, depleted level of transaminases is reported by Shakoori *et al.* (1994c) in *Tribolium* larvae after exposure to sublethal doses of Sumicidin Super (esfenvalerate). This reduced transamination can be related to increase in soluble protein and FAA. Reduction in the total protein may be due to its breakdown as a result of insecticidal stress. It can be related to the report of Etebari and Matindoost (2004) who has suggested that different stresses can decrease the amount of total protein in silkworm haemolymph. According to Nath *et al.* (1997) this could be due to the breakdown of protein into amino acids, so with the entrance of these amino acids to TCA cycle as a keto acid, they will help to supply energy for the insect. So, protein depletion in tissues may constitute a physiological mechanism and might play a role in compensatory mechanisms under insecticidal stress, to provide intermediates to the Krebs cycle, by retaining free amino acid content in haemolymph.

In the present study ChE greatly inhibited in the resistant population which are in contrast to the report of Saleem and Shakoori (1985, 1986, 1987a,b) that the detoxication enzymes are always induced after insecticide treatment, just as it happened in case of sensitive population. Other researchers, Sudderuddin and Lim (1978) have also reported the inhibition of esterases by the synthetic pyrethroids in stored grain pests.

In case of both populations of *Rhyzopertha* AkP activity was greatly decreased, while AcP was activated, a finding similar to Shakoori *et al.* (1994a). These are generalized enzymes involved in dephosphorylation and energy transfer. AkP inhibition according to Shakoori *et al.* (1994b) could be attributed to (i) the reduced enzyme synthesis and/or (ii) binding of insecticide at the active site of enzyme. RNA contents elevated enormously in resistant population suggests trend towards an increase in protein synthesis which also comply with the result of Shakoori *et al.* (1994c). Talstar treatment developed lipemia in both populations. Lipid turnover in insects is regulated by neuroendocrine-controlled feedback loops (Downer, 1985). Mulye and Gordon (1993) have shown that lipid synthesis and catabolism in the fat body was severely impaired in juvenile hormone analogue treated budworms. It also complies with the study of Shakoori *et al.* (1994b) and Saleem (1990) while looking into the toxicity of Talstar on susceptible and resistant strains of *T. castaneum*.

In contrast to resistant population, Talstar application caused induction of all other enzyme activities in susceptible population except ICDH which remained unaltered. Raised activity of LDH and unaltered ICDH activity was also reported by Tufail *et al.* (1994) while studying the biochemical changes in larvae of *Tribolium* after bifenthrin treatment. Shakoori *et al*. (1998) also reported similar results after treating the adults of *Tribolium* with Cymbush (cypermethrin 10 EC). Contrarily, Shakoori *et al*., (1994b) reported somewhat similar effects of the sublethal doses of bifenthrin in adults of OP-resistant *T. castaneum.* The elevated activities of various enzymes after insecticide poisoning have also been reported from other laboratories (Saleem and Shakoori, 1986). Shakoori *et al.* (1988) and Shakoori and Saleem (1989) also revealed that there is general tendency towards enhanced enzyme activities after insecticide application which is perhaps due to increased concentration of enzymes following induction at the gene level. The endogenous level of various enzymes increase to meet the condition of stress developed by the insecticide toxicity.

Induction of all enzymes in this susceptible population of *Rhyzopertha* could assist in defense mechanism under insecticidal stress which is accomplished through the utilization of body reserves. It is evident from decrease in glycogen, total and soluble protein and trehalose contents with an increase in the activity of related enzymes such as amylase, GOT, GPT, LDH and trehalase. It could also be inferred from this result that this beetle is utilizing all body reserves (glycogen and trehalose) in addition to glucose as primary source of energy production and the respiration has perhaps enhanced to cope with the environmental stress. Depleted levels of glycogen and trehalose contents and increase in lipids have also been mentioned by Shakoori *et al.* (1994c) and Orr and Downer (1982). Decrease in AkP and increase in GOT in susceptible population indicates malfunctioning of hepatic caeca caused by this insecticide. Elevation of RNA contents is also similar to the report of Shakoori *et al.* (1994a) while working out the effects of cyhalothrin on the larvae of *T. castaneum.* It can therefore, be summed up that Talstar caused major enzymatic and macromolecular abnormalities in both the populations of *Rhyzopertha* although their extent was more severe in the resistant population. Moreover, this insecticide can equally be used for the effective control of these populations of stored grain pest.

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