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

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Abstract. - The effects of sublethal doses of an organophosphate insecticide, Malathion, administered at 20ppm and 3.10ppm to adults of Malathion-resistant and -susceptible populations of lesser grain borer, *Rhyzopertha dominica*, respectively, for a period of 48 hours, were studied on various biochemical components. Malathion produced significant decrease (32%) in acid phosphatase (AcP) activity in resistant though remained unchanged in susceptible strain. Alkaline phosphatase (AkP) activity decreased (21%) significantly in susceptible population. Among transaminases, significant change (53% increase) occurred in glutamate pyruvate transaminase (GPT) activity in susceptible population only. Lactate dehydrogenase (LDH) activity showed 40% increase in resistant while 43% decrease in susceptible population. Free amino acids (FAA), glycogen and DNA contents also showed similar behavior with 38%, 34% and 22% rise in resistant, and 24%, 41% and 25% decline in susceptible population, respectively. Other activities like amylase, cholinesterase, trehalase and glucose, total lipids and trehalose contents did not undergo any significant change. Soluble proteins and RNA contents were depleted 16 and 20%, respectively, in resistant population. It can be concluded that AcP, AkP, GPT and LDH activities and FAA, glycogen, soluble protein, DNA and RNA contents which were changed in one population and remained unchanged in other population and parameters with contrasting values, may have some role in the development of resistant and susceptible populations. These metabolic and macromolecular derangements induced by Malathion treatment provide a baseline biochemical data to adopt better control strategy by regulating the pesticides and their concentration for both populations of this stored grain pest.

Key words: Organophosphate, lesser grain borer, $LC_{50,}$ $LC_{20,}$ stored grains pest, insect pest, enzymes, biochemical components, metabolic and biochemical effects.

INTRODUCTION

Stored grains and milled cereal foods can be infested and damaged by a number of stored grain pests. *Rhyzopertha dominica*, the lesser grain borer, is the predominant stored grain pest in the United States (Cuperus *et al.*, 1986; Toews *et al.*, 2005; Flinn *et al.*, 2004), Southern Canada (Fields, 2006), New South Wales and South East Australia (Collins *et al.*, 1993), South Asia including India and Pakistan (Shakoori *et al.*, 2000). It is one of the most important insect pests infesting whole, dried, and sound grain of many cereals and legumes throughout the world (Hagstrum and Flinn, 1984, Hagstrum *et al.*, 1994). First instar larvae have been observed to enter the grain through the intact kernel (Arthur, 1999) and cannot be removed from the grain through normal cleaning procedures (Flinn, 1998). Several adult lesser grain borers may occupy one whole grain. Stored products losses resulting from insects have been controlled through the use of insecticides. Number of organophosphate (OP) insecticides have been and are being used against stored grains pests. Important among these are Malathion, Sundaphos, pirimiphos methyl (Actellic), chlorpyrifos-methyl (Reldan) and coumaphos (acaricide). *etc*., which resulted in the development of severe resistance in many insect species including lesser grain borer (Champ and Dyte, 1977; Haliscak and Beeman, 1983; Zettler and Cuperus, 1990; Ali *et al*., 2003; Ma *et al*., 2004; Ali *et al*., 2007; Cao *et al*., 2008)

Malathion has low mammalian toxicity and brief to moderate in persistence. Its use was spread to several countries in the 1960's, which resulted in the development of severe resistance in many insect species including lesser grain borer (Ali *et al*., 2007;

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Ali *et al*., 2003; Badmin, 1990; Zettler and Cuperus, 1990; Haliscak and Beeman, 1983).

Resistance to Malathion along with pirimiphos-methyl, lindane, methyl bromide and phosphine has been reported to develop in *Tribolium castaneum*, *Oryzaephilus surinamensis, Sitophilus oryzae, R. dominica,* and *T. confusum* (Champ and Campbell, 1970; Saleem and Wilkins, 1983; Champ and Dyte, 1977). Likewise, resistance to Malathion in *T. castaneum* adults and larvae has also been reported by earlier workers (Saleem and Shakoori, 1989; Shakoori and Saleem, 1989). In China, it has been used since 1990 to control the grasshopper, *Oxya chinensis* (Sun and Peng, 1991).

Resistance to OP in the laboratory selected and field strains of the sheep blowfly *Lucilia cuprina* has been reported to be primarily caused by several carboxylesterase (CE) enzymes (Raftos, 1986; Heidari *et al.,* 2004). In *T. castaneum* adults, cholinesterase (ChE) and in its larvae lactate dehydrogenase (LDH) activities were drastically induced by malathion at sublethal doses (Saleem and Shakoori, 1989; Saleem and Shakoori, 1987a). The resistance in adults of OP resistant *T. castaneum* strain is correlated to significantly raised levels of CE, ChE, protease, and LDH activities and lipid contents and significantly reduced activities of acid phosphatase (AcP). Other enzymes like alkaline phosphatase (AkP), isocitrate dehydrogenase (ICDH), glutamate oxaloacetate transaminase/aspartate aminotransferase (GOT), glutamate pyruvate transaminase/alanine aminotransferase (GPT), trehalase and amylase were found elevated in larvae of OP resistant *T. castaneum* strains. Macromolecules like, total and soluble proteins, glycogen, nucleic acids and metabolites, *e.g.,* FAA, glucose, trehalose, *etc.,* also found to have some possible involvement in the development of resistance in the larvae and adult beetles of *T. castaneum* (Shakoori and Saleem, 1991). Other studies revealed that a mild resistance to malathion in the insecticide-resistant and –susceptible populations of the oriental migratory locust, *Locusta migratoria manilensis* was associated with reduced sensitivity and increased catalytic activity of AChE (Yang *et al.,* 2008). Decrease in LDH activity and disturbance of the enzymes of Krebs cycle was also reported after OP insecticide exposure (Nath, 2000). Increase in trehalase activity and significant depletion of fat body glycogen reserves, significant hyper and hypo-trehalosemia, hyper and hypo-glycemia in hemolymph and fat body induced by both lethal and sublethal doses of fenitrothion and ethion (OPs) in silkworm, *Bombyx mori* was also noted (Nath, 2002). Malathion did not develop any change in DNA and RNA contents of *T. castaneum* larvae with a significant decrease in total protein contents (Saleem and Shakoori, 1985a).

Very few workers have paid attention to determine the effects of organophosphates on the enzymes of the citric acid cycle, glycolytic pathway and other related biochemical components of *R. dominica* adults. None of the above mentioned reports from various authors provide any information about the biochemical changes in *R. dominica* after Malathion, treatment.

The objective of the present study was to determine the biochemical toxicity of sublethal doses of malathion in susceptible and resistant populations of adult *R. dominica* collected from the godowns/of different areas of Pakistan, against malathion.

MATERIALS AND METHODS

Beetle culture and its maintenance

The original culture of six populations of *R. dominica* was taken from godowns of different regions of Pakistan, like Chichawatni, Karachi, Wazirabad, Sialkot, Lahore and Multan. These populations were named as C, K, W, S, L and M, respectively. The sterilized jam bottles covered with muslin cloth were used to maintain these populations in a culture room at 30±2°C with 65±5% relative humidity. Whole wheat, 24 h after fumigation with phosphine was used as food of these beetles. Adult beetles collected 43±2 days after egg laying were used in the present study (FAO, 1974).

Insecticide

Formulation of Malathion [diethyl (dimethoxyphosphino-thioylthio) succinate] 57 EC was obtained from the Agricultural Chemical Group of FMC Corporation, Lahore, Pakistan.

Estimation of LC_{50}

To determine LC_{50}, serial dilutions of Malathion *viz*., 256, 128, 64, 32, 16, 8, 4, 2 and 1ppm concentration in acetone, were prepared by using calculated amount of insecticide in acetone. These concentrations (1.3 ml) were applied in the centre of glass Petri plates (diameter, 9cm) by residual film method, in triplicate. To spread the insecticides uniformly the Petri plates were rotated manually. The acetone was allowed to evaporate after which ten healthy beetles were placed in each Petri plate and covered. Three control Petri plates containing acetone only, with ten healthy adult beetles were also prepared. The beetles were kept starved for about a period of 48 hours after which they were checked for mortality by using camel hair brush. They were considered dead if on touching with brush they did not show any movement. Lloyd method was used for counting the mortality (Lloyd, 1969). LC_{50} was calculated by computerized probit analysis (Finney, 1979).

On the basis of LC_{50} values, the most resistant population (having the highest LC_{50} values) and the most sensitive population (having the least LC_{50} values) were selected for further experimentation. Both populations were considered as malathion-resistant and malathion-sensitive respectively, throughout the study.

Estimation of sublethal (LC_{20}) doses

The sublethal doses equivalent to LC_{20} were used to ascertain toxic effects of insecticides as at these doses the mortality was low though physiological/biochemical responses were significant enough to understand the mode of action. LC_{20} values for resistant and susceptible populations were worked out separately from the same data which was used for LC_{50} determination.

Administration of insecticide

Healthy and newly hatched adult beetles (150) of both malathion-resistant and the malathion-susceptible population were exposed separately to the sublethal doses of malathion respectively, by the residual film method as described above along with their controls. Three replicates were used in each experiment. The beetles were kept unfed for about 48 h 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 h period were discarded and only alive beetles were used for biochemical analyses.

Biochemical analysis

Following exposure to sublethal doses of Malathion 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 4900g 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 (Andersch and Szcypinski, 1947); alkaline phosphatase (AkP; orthophosphoric monoester phosphohydrolase alkaline optimum EC: 3.1.3.1) activity (Bessey *et al.,* 1946); lactate dehydrogenase (LDH; L-lactate NAD: oxidoreductase; EC: 1.1.1.27) activity (Cabaud and Wroblewski, 1958); isocitrate dehydrogenase (ICDH; Threo-Ds-isocitrate: NADP:oxidoreductase, EC: 1.1.1.42) activity (Bell And Baron, 1960); aspartate aminotransferase (ASAT/GOT; L-aspartate: 2-oxoglutrate aminotransferase, EC: 2.6.1.1) and alanine aminotransferase (ALAT/GPT; L-Alanine: 2-oxoglutarate aminotransferase, EC: 2.6.1.2) activities both according to (Reitmann and Frankel, 1957); cholinesterase (ChE; acetylcholine acetylhydrolase, EC: 3.1.1.7) activity (Rappaport *et al.,* 1959); amylase (1,4-D glucan glucanhydrolase, EC: 3.2.1.1) activity (Wootton and Freeman, 1982) [52] and trehalase activity (Dahlqvist, 1966).

The supernatant was also analyzed for soluble and total proteins contents (Lowry *et al.*, 1951); free amino acids (FAA) content (Moore and Stein,1954); glucose content by the *o*-toluidine method (Hartel, *et al.,* 1969); glycogen content according to the procedure described by the anthrone method (Consolazio and Iacono,1963); trehalose content by the anthrone method (Carroll *et al.,* 1956) as modified by Roe and Dailey (1966) and Steele and Paul (1985); total lipids were estimated from ethanol extract of beetles following centrifugation at 1300g by the methods of Zollner

and Kirsch, (1962) and Liebermann-Burchardt reaction as mentioned by Henry And Henry (1974). Nucleic acid estimation (DNA and RNA) follow Schmidt and Thannhauser procedure (Schneider, 1957). For detailed methods see (Ali *et al.*, 2011).

RESULTS

Toxicity of Malathion

The LC_{50} of Malathion for adults of *R. dominica* populations came out to be 115.50, 71.91, 90.39, 28.32, 47.18 and 12.40ppm for C, K, W, S, .L and M, respectively. The gradation followed by different populations was C > W > K > L > S > M. When LC_{50} of other populations of *Rhyzopertha* was compared with that of the most sensitive M populations, C, K, W, S, and L populations were found to have 9.31 fold, 5.80 fold, 7.29 fold, 2.28 fold and 3.80 fold higher $LC_{50.}$

Differentiation of the most resistant and the most sensitive populations

On the basis of $LC_{50,}$ the populations having maximum and minimum values of LC_{50} were differentiated. Therefore, C population (LC_{50} 115.50ppm) was considered as the most resistant and M population (LC_{50} 12.40ppm) as the most susceptible to Malathion. Both these populations were used throughout the experiment to find out the comparative effects of sublethal doses of Malathion on their biochemical components.

LC_{20}

LC_{20} of Malathion calculated in the study against Malathion-resistant and Malathion–susceptible populations of *R. dominica* came out to be 20ppm for C population (resistant) and 3.10ppm for M (susceptible) population.

Biochemical analysis

Table I shows the effects of Malathion on the various enzymatic activities and biochemical components of resistant and susceptible adults of *R. dominica*. Figures 1 and 2 shows % increase (+) or decrease (-) in the enzymatic activities as well as in macromolecular concentration of the resistant and sensitive populations of insects.

Effects of insecticide on resistant adult beetles

Enzyme activities

Amongst the enzymes, activities of ChE, GOT, ICDH and LDH increased 25%, 10%, 17% and 40%, respectively. This elevation of enzyme activities was highly significant for LDH. Of phosphatases, AcP and AkP activities decreased by 32% (highly significant) and 17%, respectively. Amongst carbohydrases, amylase activity decreased by 25%. No change was found in trehalase and GPT activities following Malathion treatment (Fig. 1).

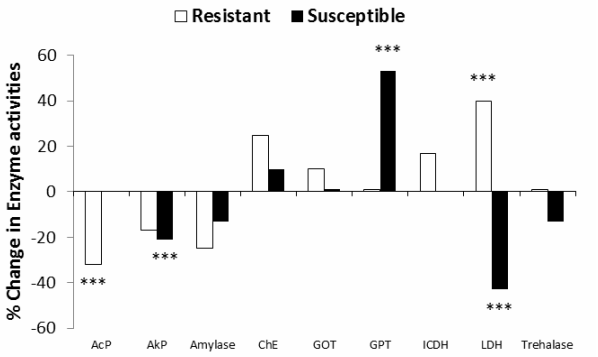

Fig. 1. Percent increase (+) and decrease (-) in various enzymatic activities of resistant and susceptible adults of *R. dominica* following Malathion treatment with reference to control.

Carbohydrates and total lipids

Sublethal doses of Malathion administered for 48 hours depleted glucose and trehalose level by15% and 12%, respectively, but glycogen and total lipids level was elevated 34% and 72%. The result indicated the utilization of glucose and trehalose accompanied by the gluconeogenesis and the mobilization of lipids from the hemolymph of the beetles under the influence of the Malathion treatment (Fig. 2).

Proteins, free amino acids and nucleic acids

Malathion exposure to adult beetles raised free amino acids (FAA) level significantly by 38%. Soluble protein contents decreased by 16% but total protein contents were elevated by 9%. There was 22% significant rise in DNA contents but RNA level was greatly depleted by 20% when compared with their respective controls (Fig. 2).

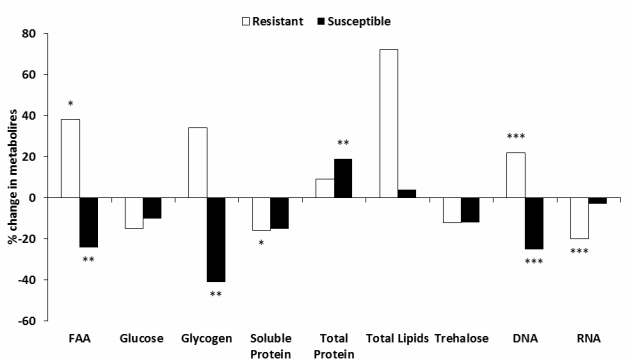

Fig. 2. Percent increase (+) and decrease (-) in various metabolites of resistant and susceptible adults of *R. dominica* following Malathion treatment with reference to control.

Effects of insecticides on susceptible adult beetles

Enzyme activities

Except for GPT, AkP and LDH all other enzymes were affected only slightly. The GPT activity significantly increased by 53% but AkP and LDH activities decreased by 21% and 43%, respectively. Sublethal doses of Malathion were ineffective on AcP, GOT and ICDH. Slight increase in ChE (10%), and decrease in amylase (13%) and trehalase (13%) activities were also observed (Fig.1).

Carbohydrates and total lipids

Glycogen was depleted prominently (41%), while decrease in glucose (10%) and trehalose (12%) contents and rise in total lipids level (4%) was only negligible (Fig. 2).

Proteins, FAA and nucleic acids

The total protein contents increased by 19%, whereas there was an appreciable decrease in the total concentration of FAA, soluble protein, DNA and RNA by 24%, 15%, 25% and 3%, respectively, after exposure to Malathion (Fig. 2).

DISCUSSION

The adults of six populations of *R. dominica* behaved differently when exposed to malathion under laboratory conditions. These populations showed different levels of resistance to malathion. Multan (M) population was found to be the least resistant and Chichawatni population (C) the most resistant with 9.3 folds resistance than M population. Malathion-resistant and -susceptible strains of *T. castaneum* have also been reported by other researchers (Lloyd and Ruczkowski, 1980; Shakoori *et al.,* 1994a). Likewise, two populations of *Locusta migratoria manilensis* differentially resistant to Malathion and two populations of *Oryzaephilus surinamensis* resistant to another OP, fenitrothion were also revealed (Rossiter *et al*., 2001; Yang, *et al.*, 2008).

Studies in the past have shown that many OP insecticides including malathion resulted in the development of resistance in many insect species including lesser grain borer (Badmin, 1990; Rossiter *et al*., 2001; Li *et al.,* 2005). Freshly prepared malathion (2-8ppm) produced 100% mortality in adults of hairy fungus beetle, *Typhae stercorea* (Tigar and Pinniger, 1996). Failure of many other OP protectants *e.g.,* chlorpyrifos-methyl and pirimiphos-methyl to control the Brazilian and U.S. populations of *R. dominica* has also been reported (Guedes *et al*., 1996).

Sublethal doses of malathion greatly suppressed AcP activity in resistant beetles, which might be due to inhibition of this enzyme under insecticidal toxicity and impairing of the lysosomal activity to hydrolyze the macromolecules and in turn limiting the ability of the resistant beetles to use energy rich compounds to obtain energy. Phosphatases are sub-group of hydrolases that are distributed throughout the most cells and body fluids. They are characterized by their ability to hydrolyze a wide variety of monophosphate esters of phosphoric acid to alcohols and inorganic phosphates and are important in the absorption and metabolism of carbohydrates and nucleotides. The activity of these enzymes is related to the physiological state of the cell.

The application of sublethal doses of malathion to the susceptible adults of *R. dominica* for 48 hours resulted in depletion of AkP activity which is primarily concerned with energy production. It might be due to reduced synthesis of this enzyme under insecticidal toxicity limiting the ability of the beetles to use other energy rich compounds to obtain energy under stress thus

Table I.- The effects of Malathion administered at dose of 20 ppm and 3.10 ppm on the various enzyme activities and biochemical components of Malathion-resistant and -susceptible population of *R. dominica* respectively.

Parameters[a]	Resistant population		Susceptible population	
	Control (n =21)	Malathion treated (n =3)	Control (n =20)	Malathion treated (n =3)
[a]AcP (IU/mg)[m]	1.74 ± 0.057[l]	1.19 ± 0.05***	1.88 ± 0.05	1.88 ± 0.29
[b]AkP (IU/mg)	1.21 ± 0.12	1.00 ± 0.06	1.24 ± 0.03	0.97 ± 0.05***
Amylase (mSU/mg)[n]	12.81 ± 0.12	9.52 ±1.29	14.51 ± 0.57	12.50 ± 0.99
[c]ChE (RU/mg)[o]	0.55 ± 0.06	0.69 ± 0.07	0.25 ± 0.03	0.28 ± 0.06
[d]GOT(IU/mg)	1.37 ± 0.06	1.50 ± 0.07	1.18 ± 0.05	1.20 ± 0.14
[e]GPT(IU/mg)	0.09 ± 0.01	0.09 ± 0.02	0.26 ± 0.02	0.40 ± 0.02***
[f]ICDH(σ/mg)[p]	4.52 ± 0.30	5.31 ± 0.27	4.68 ± 0.17	4.69 ± 0.63
[g]LDH(BBU/mg)q	7.66 ± 0.18	10.71 ± 0.70[888]	10.82 ± 0.56	6.08 ± 1.12***
Trehalase(IU/mg)	0.54 ± 0.04	0.54 ± 0.02	0.39 ± 0.03	0.34 ± 0.02
[h]FAA	97.16 ± 3.97	134.26 ± 17.26	192.38 ± 15.56	144.51 ± 4.89**
Glucose	9.50 ± 0.42	8.05 ± 1.33	98.72 ± 0.32	7.82 ± 1.40
Glycogen	7.78 ± 0.32	10.44 ± 3.50	6.95 ± 0.58	4.08 ± 0.61**
Soluble protein	85.85 ± 2.64	71.82 ± 4.41*	87.40 ± 2.14	73.86 ± 9.72
Total protein	182.63 ± 4.60	200.47 ± 15.12	161.43 ± 3.20	192.12 ± 9.84**
Total lipids	41.70 ± 1.73	71.78 ± 20.06	39.96 ± 2.20	41.82 ± 6.420
Trehalose	19.34 ± 0.80	16.93 ± 2.69	21.38 ± 1.03	18.77 ± 1.49
[j]DNA	6.12 ± 0.14	7.50 ± 0.15***	7.33 ± 0.34	5.47 ± 0.20***
[k]RNA	11.87 ± 0.38	9.47 ± 0.44**	16.44 ± 0.48	15.81 ± 1.16

[a] Acid phosphatase; [b] Alkaline phosphatase; [c] Cholinesterase; [d] Glutamate oxaloacetate transaminase; [e] Glutamate pyruvate transaminase; [f] isocitrate dehydrogenase; [g] lactate dehydrogenase; [h] free amino acids; [J]Deoxyribonucleic acid; [k]Ribonucleic acid; [m] International unit, the amount of enzyme, which under defined assay conditions, will catalyze the conversion of 1 μ mol of substrate per minute; [n] milli Somogyi unit, the amount of enzyme digesting 5000 mg of starch in the experimental conditions used here; [o] Rappaport units; [p] Sigma units; [q] Berger-Broida units as written in sigma kit 0.48, factor for the conversion into units.
[r] ± SEM, Student's t test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

rendering it susceptible. The decreased AkP activity might also be due to binding of the insecticide at the active site of the enzyme. Decrease in AkP activity due to different stress and disease conditions has also been revealed from many studies (Miao, 2002; Etebari *et al.*, 2007).

LDH activity was immensely elevated in resistant adults after Malathion exposure. Raised LDH activity shows its induction due to toxic effect of the insecticide which finally leads to accumulation of lactic acid (Saleem and Shakoori, 1985b). Since this enzyme catalyzes reaction reversibly by the interconversion of pyruvate into lactate therefore, drastic increase in LDH activity suggests enhanced respiratory activities to neutralize the toxic effects of malathion. This may be due to activation of primary mechanism of energy production, so that insects could survive better under the insecticidal stress making this population resistant to malathion. The significant increase in LDH activity of larvae and adults of *T. castaneum* after treatment with sublethal doses of malathion has also been reported (Saleem and Shakoori, 1987a; Shakoori and Saleem, 1989). Application of both lethal and sublethal doses of other OPs (fenitrothion and ethion) also induced increase in LDH activity with elevated lactate levels in silkworm, *Bombyx mori* (Nath, 2000). Many other laboratories have reported elevated activities of enzymes after insecticidal applications (Kacew and Singhal, 1973; Shakoori *et al.*, 1994b; Terriere, 1984).

In resistant *R. dominica* adults the exposure of sublethal doses of malathion caused increase in DNA contents which may be due to elevated cells proliferation rate. It reflects the disturbances in basic metabolic activities. During this study the RNA contents decreased drastically which may result in decreased soluble protein and raised FAA level. While working on a plant bug, somewhat similar

findings have been reported (Nohel and Salama, 1972; Saleem and Shakoori, 1987a).

Transaminases are mitochondrial enzymes which transfer an amino group from amino acid to keto acid. They are found in most tissue cells. These enter the blood only when the cells to which they are confined are damaged or destroyed. The higher values of these enzymes indicate the probable site of tissue damage in higher animals. In the present study where whole beetle tissues are being used for tissue saline extract preparation, increased activity is indicative of increased synthesis of enzyme protein. During the study significant rise in GPT activity has been found. Somewhat accelerated transaminase activity supports the fact that the interconversion of amino acids to keto acids through transamination is also taking place. The raised transaminase activity might be required by insect to shift amino acids to TCA cycle to be used as fuel molecules to produce an additional energy or other biomolecules to resist the insecticidal stress. These interpretations also get support by the decrease in FAA contents. Decrease in FAA of cockroach haemolymph after OP and pyrethroid treatment has also been reported (Subba, 1985). The transaminase enzymes (GOT and GPT) serve as a strategic link between the carbohydrate and protein metabolism and are known to be altered during various physiological and pathological conditions (Etebari *et al.,* 2005; Ender *et al.,* 2005).

In susceptible population the LDH activity decreased drastically following malathion exposure, which is also an important factor contributing towards the susceptibility of adult population of *R. dominica*. The insect muscles depend almost entirely on anaerobic glycolysis (*i.e.,* conversion of glucose into lactic acid) for energy production during mechanical activity such as locomotion and flight (Crabtree and Newsholme, 1972). This report also complies with the study where glucose and glycogen reserves were enormously reduced without the synthesis of new ones. It is suggested that these macromolecules were assisting both aerobic and anaerobic glycolysis to provide the energy to the exhausted insects but insecticidal toxicity is not allowing their synthesis anymore due to which their quantity is reducing. Previously, the similar findings after OP, diazinon treatment have been reported (Zibaee *et al.* 2008).

Amongst the nucleic acids, the decrease in DNA content of susceptible population might be indicative of tissue damage induced by Malathion. The decrease in RNA was significant only in resistant population. Other investigators has also reported similar effects of Malathion on nucleic acid of *T. castaneum* adults (Saleem and Shakoori, 1987a).

Total protein content which normally contains both soluble and insoluble fractions was greatly increased in malathion treated susceptible population. This increase may be due to increase protein biosynthesis as a result of enzyme induction to counter the toxic effect of malathion. It can be inferred from the results that FAA were decreased due to their utilization for energy production and protein synthesis.

It can be concluded from this study that several populations of *R. dominica* have developed resistance against malathion and these cannot be controlled by this insecticide. Higher doses or other pesticides are required to control resistant population. Secondly, development of resistance and the susceptibility in insects is a biochemical phenomenon which is evident from number of macromolecular derangements in resistant and susceptible populations of *R. dominica.*

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