



ORIGINAL ARTICLE

THE EFFECT OF PHYTOPESTICIDE, AZHADIRACHTIN ON THE ACTIVITY OF SOME
HYDROGENASE ENZYMES IN SELECTED TISSUES OF MALE BLISTER BEETLE
MYLABRIS INDICA (THUN) (COLEOPTERA: MELOIDAE).

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ABSTRACT

the present study investigated the toxicological effect on enzymological changes in fat body, testis vas deferens, seminal vesicle and male accessory glands (MARGs) of blister beetle, *Mylabris indica*. The results have been observed that the pattern of activity of respiratory enzymes MDH and GDH which is found increased all the test experimental tissues treated with phytopesticide than the control insects. Whereas the enzymes LDH and SDH in tested insect tissues have decreased significantly.

Keywords: Enzymological changes, *Mylabris indica*, MDH, GDH, LDH, SDH,

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1. INTRODUCTION

Enzymes are attractive as indicators because they are more easily quantified than the other indicators. Metabolic path way mainly depending on enzyme activities may be affected due to the destruction under stress, reflecting the changes for enzyme activities. Glycolysis is known to yield pyruvate and glycoliphosphate. Pyruvate apart from being formed by glucose metabolism is also formed from the carbon skeletons of a number of amino acids such as serine, alanine and cysteine. -ketoglutarate is found as a preferred energy substrate for the spermatozoa and as an accelerator for sperm respiration in insects. In addition, energy metabolism of reproduction of male insects has not been much studied. Investigations were made on the TCA enzymes, SDH, GDH, LDH and MDH in the fat body and the accessory reproductive gland of *Odontopus varicornis* before and after mating by Selvisabhanayakam (1995). A study of oxidative enzymes will reveal whether pyruvate is being utilized to supply energy or from other pathways.

The insecticides act as enzyme inhibitors, leading to hyperexcitability of the nervous system. It may also cause

various side effects, e.g. change in DNA structure (Griffin and Hill 1978), cause sperm malformations (Mathew *et al.*, 1992), generate reactive oxygen species (Bagchi *et al.*, 1995), and act as inducers of heat shock protein (Bagchi *et al.*, 1996). Pesticides and other xenobiotics may increase the level of free radicals (Freeman and Crapo, 1982) and influence (mobilise) an antioxidant defence system in tissues and cells. Antioxidants can so other neurotoxic effects of insecticides (Bagchi *et al.*, 1993, 1996; Minakata *et al.*, 1996). However, cytochrome p450 mediated detoxification of xenobiotics results in enhanced free radical content of cells. Insecticides may be broken down in different ways, which can lead to different products of a higher or lower toxicity than the mother compound (Brattsten *et al.*, 1986; Chambers *et al.*, 1994).

Lactate dehydrogenase (LDH) is an important glycolytic enzyme being present in virtually all tissues (Kaplan and Pesce, 1996), it is also involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Wu and Lam, 1997; Diamantino *et al.*, 2001), although, it is usually used as an index of anaerobic metabolism (Chamberlin and King, 1998). To show correlation between some enzyme activity and non-enzymatic compounds, the amount of glucose and protein was measured. Lactate dehydrogenase catalyzes the last step in glycolysis, as it reduces pyruvate to lactate. Succinic dehydrogenase is the active regulatory enzyme of the Tricarboxylic acid cycle. Lactic

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dehydrogenase (LDH) is a hydrogen transferring enzyme that catalyzes the oxidation of L- lactate to pyruvate with the mediation of NAD⁺ as hydrogen acceptor. LDH activity is present in all cells of the body and is invariably found only in the cytoplasm of the cells

The succinate dehydrogenase (SDH), also known as respiratory chain complex II, is a universal and key component of the mitochondrial respiratory chain. As part of the Krebs cycle, this enzyme oxidizes succinate into fumarate with the concomitant reduction of respiratory chain ubiquinone. SDH is one of the important key enzymes and exhibits an important function in energetics which catalyses the reversible oxidation of succinate to fumarate and serves as a link between electron transport system and oxidative phosphorylation. SDH is the only enzyme that participates in both the citric acid cycle and the electron transport chain and acts as indicator of aerobic respiration. Since the activity of SDH in mitochondria is greater than the other enzymes of TCA cycle, an insight into the alterations of this enzyme activity may be taken as index to assess the function of TCA cycle in different organs of the fresh water animals. SDH is unique among the Krebs cycle enzymes, in that it is tightly bound to the inner mitochondrial membrane. Any alterations in its activity indicate changes in the structure and function of mitochondria.

Glutamate dehydrogenase (GDH) is a regulatory enzyme known to check the deamination process to minimize the ammonia level and plays a significant role in the catabolism of amino acids. These enzymes function as a link between protein and carbohydrate metabolisms and the net outcome is the incorporation of keto acids into the TCA cycle. There is much evidence for the shifts in the activities of these enzymes to a variety of environmental and physiological conditions (Knox and Greengard, 1965). MDH is an enzyme which catalyzes the NAD/NADH-dependent interconversion of the substrates malate and oxaloacetate. This reaction plays a key role in the malate/aspartate shuttle across the mitochondrial membrane, and in the tricarboxylic acid cycle within the mitochondrial matrix. The TCA cycle is completed when the oxidation of L-Malic acid to oxaloacetic acid is accomplished by the enzyme MDH.

From the above cited literature, it is evident that information on the functional significance of male reproductive system in insects are inadequate, particularly in coleopteran beetles, in view of this, it has been programmed to study enzymological changes the male reproductive organs and fat body in Coleopteran meloid blister beetle *Mylabris indica*.

2. MATERIALS AND METHODS

The material used in the present investigation is *Mylabris indica*, coleopteran blister beetle of the family Meloidae. It can be easily maintained in the laboratory at normal temperature and humidity. It is very convenient for dissection as the size of the animal is somewhat larger. Fat body, testes, s, seminal vesicle and MARGs were removed from the alive

specimens subjected to either anesthesia or without chloroform for the investigation.

In the present investigation, the phytopesticide Vijay neem commercial product has been selected, and the commercial formulation of neem pesticide (Vijay neem), active ingredients Azadirachtin 0.03% was obtained from India (manufactures FORTUNE BIO-TECH LIMITED FBL – Hyderabad) for our experiments used it against the *M. indica* at concentrations of 50, 40, 30, 20, 10 and 5 ppm, respectively. The concentration at which 100% mortality was observed within 24 hours were considered as lethal concentration (24 hours LD₁₀₀) and 100 percent survival observed below 24 hours were considered as sublethal concentration. Detailed probit analysis of phytopesticide concentration and present mortality of *M. indica* for 48 hours of exposure were determined. The sublethal concentration values were calculated for 48 hours at 18.349 ppm. The enzyme LDH was assayed by the method of King (1965), SDH, Bernath and Singer (1962), GDH, Stretcker (1965) and MDH, Ochoa (1955) method.

3. RESULT

The LDH activity in the fat body of control and treated insect of about 0.90 ± 0.056 to 0.12 ± 0.01 $\mu\text{moles/mg/protein}$; The SDH activity in the fat body of control and treated insect of about 18.82 ± 0.82 to 4.25 ± 0.60 $\mu\text{moles/mg/protein}$; The MDH and GDH activity in the fat body of control and treated insect of about 17.04 ± 0.11 to 28.38 ± 0.34 ; 7.29 ± 0.23 to 14.42 ± 0.34 $\mu\text{moles/mg/protein}$, respectively (Table 1).

The LDH, SDH, MDH and GDH activities in the testes of control and treated insects were 2.91 ± 0.09 to 1.27 ± 0.11 , 12.31 ± 0.23 to 9.39 ± 0.27 , 8.59 ± 0.39 to 12.28 ± 0.49 and 15.41 ± 0.32 to 18.24 ± 0.46 $\mu\text{moles/mg/protein}$, respectively (Table 2).

The LDH and SDH activities in the vas deferens of phytopesticide treated insects were found to be comparatively less than that of control insect. In contrast, the MDH and GDH activities in the vas deferens of treated insects have increased significantly than the control insects. The LDH, SDH, MDH and GDH activities in the vas deferens of control and treated insects, of about 1.52 ± 0.12 to 0.73 ± 0.11 ; 9.19 ± 0.33 to 6.31 ± 0.36 ; 7.65 ± 0.28 to 11.44 ± 0.47 ; 13.09 ± 0.85 to 17.06 ± 0.58 $\mu\text{moles/mg/protein}$, respectively (Table.3).

The MDH and GDH activities in the seminal vesicle of phytopesticide treated insects were increased significantly than the control insects. The LDH and SDH activities in the seminal vesicle of treated insects were found to be decreased than that of the control insects. The LDH, SDH, MDH, and GDH activities in the seminal vesicle of control and treated insects of about, 1.61 ± 0.22 to 0.78 ± 0.11 ; 9.33 ± 0.30 to 6.66 ± 0.38 and 8.52 ± 0.36 to 12.40 ± 0.58 and 14.14 ± 0.27 to 18.04 ± 0.69 $\mu\text{moles/mg/protein}$, respectively (Table.4).

The LDH, SDH, MDH and GDH activities in the MARGs of control and the MARGs (MARG₁, MARG₂ and MARG₃) treated treated insects are presented in **Table 5-7**. The MDH and GDH activities in insects have increased significantly than that of control insects. The LDH and SDH activities in the MARG₁ of treated insects have decreased significantly than the control insects.

Table 1: Enzymes activity on fat body in control and phytopesticide treated adult male insect, *M. indica*

Fat body	Control ($\mu\text{moles/mg/protein}$)	Treated ($\mu\text{moles/mg/protein}$)	Percentage over control	't' value
LDH	0.90 \pm 0.05	0.12 \pm 0.01	-86.90	34.82*
SDH	18.82 \pm 0.82	4.25 \pm 0.60	-77.44	35.28*
MDH	17.04 \pm 0.11	28.38 \pm 0.34	66.52	-76.58*
GDH	7.29 \pm 0.23	14.42 \pm 0.34	97.78	-42.50*

Data represent values are mean \pm S.D (n=6). *Significant at 0.05% level.

LDH : Lactate dehydrogenase,SDH : Succinate dehydrogenase,MDH : Malate dehydrogenase,GDH : Glutamate dehydrogenase

Table 2: Enzymes activity on testes in control and phytopesticide treated adult male insect, *M. indica*

Testes	Control ($\mu\text{moles/mg/protein}$)	Treated ($\mu\text{moles/mg/protein}$)	Percentage over control	't' value
LDH	2.91 \pm 0.09	1.27 \pm 0.11	-56.47	27.97*
SDH	12.31 \pm 0.23	9.39 \pm 0.27	-23.71	20.16*
MDH	8.59 \pm 0.39	12.28 \pm 0.49	42.93	-14.38*
GDH	15.41 \pm 0.32	18.24 \pm 0.46	18.35	-12.35*

Data represent values are mean \pm S.D (n=6). *Significant at 0.05% level.

LDH : Lactate dehydrogenase,SDH : Succinate dehydrogenase,MDH : Malate dehydrogenase,GDH : Glutamate dehydrogenase

Table 3: Enzymes activity on vas deferens in control and phytopesticide treated adult male insect, *M. indica*

Vas deferens	Control ($\mu\text{moles/mg/protein}$)	Treated ($\mu\text{moles/mg/protein}$)	Percentage over control	't' value
LDH	1.52 \pm 0.12	0.73 \pm 0.11	-51.93	12.14*
SDH	9.19 \pm 0.33	6.31 \pm 0.36	-31.34	14.58*
MDH	7.65 \pm 0.28	11.44 \pm 0.47	49.51	-17.03*
GDH	13.09 \pm 0.85	17.06 \pm 0.58	30.34	-9.48*

Data represent values are mean \pm S.D (n=6). *Significant at 0.05% level.

LDH : Lactate dehydrogenase,SDH : Succinate dehydrogenase,MDH : Malate dehydrogenase,GDH : Glutamate dehydrogenase

Table 4: Enzymes activity on seminal vesicle in control and phytopesticide treated adult male insect, *M. indica*

Seminal vesicle	Control ($\mu\text{moles/mg/protein}$)	Treated ($\mu\text{moles/mg/protein}$)	Percentage over control	't' value
LDH	1.61 \pm 0.22	0.78 \pm 0.11	-51.61	8.35*
SDH	9.33 \pm 0.30	6.66 \pm 0.38	-28.68	13.57*
MDH	8.52 \pm 0.36	12.40 \pm 0.58	45.56	-14.04*
GDH	14.14 \pm 0.27	18.04 \pm 0.69	27.59	-12.93*

Data represent values are mean \pm S.D (n=6). *Significant at 0.05% level.

LDH : Lactate dehydrogenase,SDH : Succinate dehydrogenase,MDH : Malate dehydrogenase,GDH : Glutamate dehydrogenase

Table 5: Enzymes activity on MARG₁ in control and phytopesticide treated adult male insect, *M. indica*

MARG ₁	Control ($\mu\text{moles/mg/protein}$)	Treated ($\mu\text{moles/mg/protein}$)	Percentage over control	't' value
LDH	1.91 \pm 0.05	1.28 \pm 0.12	-32.87	11.96*
SDH	11.07 \pm 0.31	7.64 \pm 0.38	-30.97	17.14*
MDH	0.90 \pm 0.02	1.49 \pm 0.04	64.76	-35.35*
GDH	0.84 \pm 0.02	1.52 \pm 0.04	80.04	-36.94*

Data represent values are mean \pm S.D (n=6). *Significant at 0.05% level.

LDH : Lactate dehydrogenase,SDH : Succinate dehydrogenase,MDH : Malate dehydrogenase,GDH : Glutamate dehydrogenase;GDH : Glutamate dehydrogenase

Table 6: Enzymes activity on MARG₂ in control and phytopesticide treated adult male insect, *M. indica*

MARG ₂	Control ($\mu\text{moles/mg/protein}$)	Treated ($\mu\text{moles/mg/protein}$)	Percentage over control	't' value
LDH	1.68 \pm 0.05	0.95 \pm 0.01	-43.54	33.95*
SDH	9.97 \pm 0.21	4.80 \pm 0.39	-51.88	28.47*
MDH	0.74 \pm 0.02	1.19 \pm 0.07	61.63	-15.57*
GDH	0.75 \pm 0.02	1.32 \pm 0.07	74.94	-17.92*

Data represent values are mean \pm S.D (n=6). *Significant at 0.05% level.

LDH : Lactate dehydrogenase, SDH : Succinate dehydrogenase, MDH : Malate dehydrogenase, GDH : Glutamate dehydrogenase

Table 7: Enzymes activity on MARG₃ in control and phytopesticide treated adult male insect, *M. indica*

MARG ₃	Control ($\mu\text{moles/mg/protein}$)	Treated ($\mu\text{moles/mg/protein}$)	Percentage over control	't' value
LDH	2.05 \pm 0.12	1.12 \pm 0.06	-45.62	17.04*
SDH	8.42 \pm 0.13	4.86 \pm 0.44	-42.30	18.94*
MDH	0.74 \pm 0.02	1.19 \pm 0.07	61.63	-15.57*
GDH	0.75 \pm 0.02	1.32 \pm 0.07	74.94	-17.92*

Data represent values are mean \pm S.D (n=6). *Significant at 0.05% level.

LDH : Lactate dehydrogenase, SDH : Succinate dehydrogenase, MDH : Malate dehydrogenase, GDH : Glutamate dehydrogenase

4. DISCUSSION

Enzyme is a biocatalyst which accelerates biological reactions. However, the concept of biocatalyst is a very wide source of enzymes used common in plant and animal cells. Energy is derived from the three major sources namely carbohydrates, proteins and fats, when they are oxidized (Gilmour, 1965 and Zubay, 1983). It is released from organic molecules, principally by oxidation. Biologically, such energy yielding oxidations are accomplished by the removal of hydrogen and electrons from the substrates and their transfer to other acceptors within the cell (Gilmour, 1965).

In the present study, the activity of, MDH and GDH in all the experimental phytopesticide treated insect tissues were increased than the control insects. In contrast, the activity of LDH and SDH in the test tissues of phytopesticide treated insects were decreased than the control insects. This observation is in conformity with Sumathi, (2002) for *Gryllotalpa africana* when exposed to endosulfan; Rajathi, (2004) for *Sphaerodema rusticum* exposed to heavy metal mercury. This is supported by the observations, indicating the formation of new protein in the gland during stress. Based on these findings, it is suggested that in this insects, energy may probably be supplemented through the oxidation of α -ketoglutarate as it has been shown for other insects such as *Plebiogryllus guttiventris* and *Chrysocoris purpureus* (Ranganathan, 1984); *Odontopus varicornis* (Selvisabhanayakam, 1995).

Lactate dehydrogenase (LDH) is an important glycolytic enzyme being present virtually in all tissues (Kaplan and Pesce, 1996), it is also involved in carbohydrate metabolism

and has been used as an indicative criterion of exposure to chemical stress (Diamantino *et al.*, 2001) and it is used as an index of anaerobic metabolism (Chamberlin and King, 1998). Activity level of Lactate dehydrogenase in *Culex* after treatment with DDT, malathion and cyfluthrin decreased 58.88%, 33.33% and 66.66%, respectively (Arshad *et al.*, 2002). Nathan *et al.*, (2005) have showed that feeding of *Spodoptera litura* on *Ricinus communis* L. treated with azadirachtin and nucleopolyhedrovirus decreases the amount of this enzyme in midgut that demonstrates low nutritional efficiency of the larvae. Similar results were also observed on effectiveness of *Melia azedarach* on rice leaf folder (Nathan, 2006). Hence, using chemicals may decrease activity level of LDH.

In the present study, the activity of LDH in the fat body, testis, seminal vesicle, vas deferens and MARGs of treated insects were lower than the control insects. From these findings, it may be suggested that the decreased LDH activity is probably for the conversion of lactate to pyruvate. In the present study, it has been observed that the SDH activity levels showed inhibition in all the reproductive tissues during treatment with the phytopesticide than the control insects, suggesting that the decreased amount of glycogen and increased level of glucose, signified their utilization for the energy requirement during the period of stress.

Inhibition of oxidation of succinate or succinate dehydrogenase by insecticides and heavy metals are well known. In the present investigation, the decreased level of SDH has been observed in all the tissues of phytopesticide intoxicated insects. As SDH is a key enzyme in the TCA cycle, it is logical to assume that the inhibition of SDH activity, the metabolic pathway might switch over from aerobic to anaerobic to meet the increased energy demand during the phytopesticide toxicity.

The SDH activity showed a decrease in the fat body which indicates a disturb in enzyme synthesis. The phytopesticide perhaps disturb the mitochondrial membrane of the fat body and all the reproductive tissues. This rupture leads to a decrease in the activity of membrane bound SDH. These results are in concomitant with the works of Sumathi, (2002) who has reported for inhibition of the activity of the dehydrogenase which may be due to the activity of changes in the mitochondrial membrane function in *Gryllotalpa africana* treated with the endosulfan. The inhibition of SDH activity in *M. indica* may be followed due to the reduction in O₂ consumption level, suggesting that the phytopesticide affects the TCA cycle, which leads to disturbances in the respiratory metabolism of these insects.

MDH is oxidized into oxaloacetate by the action of cytosolic MDH, to yield malate. The malate enters into the mitochondria via a carrier and is oxidized there into oxaloacetate by the action of mitochondrial MDH. It is evident that the gland is assured to supply energy by the TCA cycle during energy demand when insects are intoxicated with the phytopesticide. This suggested that more of the substrate either malate to oxaloacetate is combusted to sustain the TCA cycle.

In the present study, it has been observed that the pattern of activity of the respiratory enzyme, MDH is similar to the other respiratory enzyme GDH, which is found increased in all the reproductive tissues treated with the phytopesticide than the control insects. These changes might be due to the supply of energy by the TCA cycle for the treated insect which requires energy during phytopesticide intoxication.

Similar results have been observed by Sumathi, (2002) for *Gryllotalpa africana* when exposed to endosulfan; Rajathi (2004) for *Sphaerodema rustium* exposed to heavy metal mercury. Vijay Joseph and Jayantha Rao, (1991) have stated that SDH, MDH and LDH inhibited consequently on exposure to sublethal concentration of aldrin enhanced activities of LDH. Glutamate is the only amino acid for which specific and highly active dehydrogenase exists. This occurs principally through the amino transferases completed with the action of GDH (Smith *et al.*, 1985). In the present study, it has been shown that the dynamic nature of an increase in GDH activity in all the tissues of treated insects suggesting that the glutamate may be utilized for the conversion of α -ketoglutarate to meet out an extra energy demand by *M. indica* during phytopesticide intoxication. Similar results have been reported for *Melanoplus sanguinipes* (Chesseman *et al.*, 1990). On the basis of the observations made in the present study, it is evident that the inhibition of SDH and LDH activities and stimulation of MDH and GDH activities in all the target tissues of the reproductive system of *M. indica* when intoxicated with phytopesticide. The metabolic pathway of this insect has shifted towards anaerobic side rather than aerobic side to meet the increase in energy demands during phytopesticide treatment.

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activity in selected tissues of fresh water mussel, *Lamellidens marginalis* exposed to copper sulphate.21. Decreased SDH activity of muscle and heart tissues in the present study clearly indicates depletion in the oxidative metabolism at the level of mitochondria, leading to depression of TCA cycle on exposure to cypermethrin. Lactate dehydrogenase (LDH) is the key enzyme of anaerobic glycolysis, and catalyses the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. In the present study, the activity levels of MDH showed inhibition. Table 1 Alterations in dehydrogenase enzyme activities in muscle tissues of control and cypermethrin-treated albino rats.

Oxidative Enzymes	Control	Single Dose	Double Dose	Multiple Dose
(micromoles of Antioxidant enzymes: toward an active oxygen. balance. Small deviations from the physiological activity of antioxidant enzymes may have a dramatic effect on the resistance of cells to oxidant-induced damage to lipoprotein. Biochem J 1996; 320: 373-81. Moreover, the antimicrobial activity of these isolates was further assayed against some selected phytopathogens. The bacterial isolates demonstrated a wide-spectrum antimicrobial activity against the various phytopathogens as shown in Figure (1). Phytopathogens growth was suppressed by the endophytic isolates at different levels. Maltophilia was ascribed to the impact of alkaline serine proteolytic enzyme in addition to the induction of host systemic acquired resistance. Conclusion. Endophytic bacteria can release a wide array of extracellular bioactive metabolites with high capability to inhibit the growth of various bacterial and fungal species thus they can be used to manage different plant diseases. The present study.				