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Full Length Research Article

EFFECT OF PHYTOPESTICIDE, ON BIOCHEMICAL CHANGES IN CERTAIN SELECTED TISSUES OF THE ADULT MALE INSECT, *MYLABRIS INDICA* (THUN.) (COLEOPTERA: MELOIDAE)

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The blister beetle *Mylabris indica* (Thenberg) a harmful crop pest, voracious feeding to flowers and to cause the Sevier damage of different agricultural plants, due to the problem of pesticide resistance, alternative techniques for chemical control, such as plant derived natural biopesticides, have been object of this research. The present study was aimed to understand the biochemical changes in fat body and reproductive organs such as testis, vas deferens seminal vesicle, MARGs (accessory glands)and ejaculatory duct of *M.indica* when treated sublethal concentration of phytopesticide, vijay neem.

Key words: Phytopesticide, Biochemistry, Meloidae.

INTRODUCTION

Reproductive physiology of male insects is a complicated phenomenon that deals with the structure and function of various tissue components of the system. The insect reproductive system if the merits of pesticides include enhanced economic potential for their use in agriculture to contain pests, on the debit side are the serious health implications for man and animal life (Bhatnagar, 2001).It is usually formed by one pair of testes, two deferent ducts and one ejaculatory duct. Pesticides may induce oxidative stress leading to the generation of free radicals and alteration in antioxidant or oxygen free radical scavenging enzymes such as superoxide dismutase, catalase, glutothione peroxidase, glutathione reductase and glutothione transferase Many plant extracts could be an alternative source for insect-control agents because they constitute a rich source of bioactive chemicals and many of them are largely free from adverse effects to the environment. Much effort has, therefore, been focused on plant-derived material as potential sources of commercial insect-control agents (Konstantopoulou *et al.,* 1992; Shaaya *et al.,* 1997; Ngamo Tinkeu *et al.,* 2007; Thonte *et al.,* 2009). The increasing amount of research on insect–plant chemical interactions has unveiled the potential of utilizing botanical insecticides in the form of secondary plant metabolites, or allelochemicals (Kubo, 2006). Natural pesticidal products have low toxicity to humans and natural enemies (Rahman and Siddiqui, 2004). Botanicals are naturally occurring secondary metabolites extracted from plants.

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Botanical insecticides break down very quickly in the environment and do not accumulate in plant or animal tissue (Schmutterer, 1990; Copping and Menn, 2000). Energy metabolism in insects during various vital activities has been drawing increasing attention among the insect physiologists. An understanding of the metabolism of protein and amino acids are important. The carbohydrates, which are the principal source of immediate energy, are stored in the fat body as glycogen, which is rapidly used for the energy requirements of other larval tissues (Keeley, 1985; John and Muraleedharan, 1993). Lipids are the chief form in which energy is stored in insects. The ability to synthesize lipids for storage is widespread, but except for specific item as small amounts they are not usually essential constituents of their diet. Insects utilize lipids and can also synthesize from proteins and carbohydrates. The lipid content of insect fluctuates during the animal life history. The changes reflect variations in the metabolic balance between lipid synthesis and lipid utilization and result from direct or indirect action of hormones. The fat body tissue plays a key role in the insect lipid metabolism, this in the site of both lipid storage and mobilization. The major lipid component of the insect fat body is long chain fatty acid, triglyceride (Gilby, 1965). The fat body is a source of fat supply as an energy fuel to the muscle especially for sustained activity as during migratory flight (Gilby, 1965). In insects, lipid is converted into carbohydrate by glyoxylate path way. Accumulation of pesticides and their elimination mostly depends upon the amount of lipid content in the body of the animal. Male accessory glands of insects secrete proteins and hormones whose functions include stimulation of oviposition, inhibition of female receptivity, activation and nourishment of sperm and formation of spermatophore (Barker and Davey, 1981; Chen, 1984; Happ, 1984; Selvisabhanayakam *et.al*., 2002). In view of this, an attempt has been made in the present studies, of the biochemical changes in the in the fat body, testes, vas deferens, seminal vesicle, MARGs and ejaculatory duct of

adult male insect *M. indica* when treated with the Phytopesticide, Vijay neem.

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, vas deferens, seminal vesicle, MARGs and ejaculatory duct 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. 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 rate of mortality was observed at 24, 48, 72 and 96 hours. Individuals showing no movement and no response to tactile stimuli were considered as dead and were removed immediately. The concentration at which 100% mortality was observed within 24 hours were considered as lethal concentration (24 hours LD_{100}) and 100 percent survival were considered as sublethal concentration below 24 hours. 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.

Biochemical analysis: Adult male insects of both control and phytopesticide treated insects were collected from rearing cages and vivisected in insect Ringer solution (Ephrusassi and Beadle, 1936). The following biochemical estimations were made in the tissues of fat body, testes, vasdeferens, seminal vesicle and male accessory reproductive glands. The colorimetric micro-method of Kemp A. Kits van Heijninger *et al.,* (1954) was employed for the quantitative estimation of glucose and glycogen. The protein content in the fat body, testis, vasdeferens, seminal vesicle and male accessory reproductive glands (MARGs) were determined by adopting the procedure of Lowry *et al.* (1951). Colorimetric micro method of Moore and Stein (1954) was adopted for the quantitative estimation of total free amino acid in the fat body, testis, vasdeferens, seminal vesicle and male accessory reproductive glands of control and pesticide treated insects. Lipid content was estimated by the semi-micro determination method of Pande *et al.* (1963).

RESULT

Estimation of carbohydrate: The widespread use of insecticides has amounted the biochemical and physiological changes, which may be of adaptive significance to the life of an animal. The glycogen content of the fat body, testis, vas deferens, seminal vesicle and MARGs ($MARG_1$, $MARG_2$ and $MARG_3$) insects were found to be decreased than the control insects about 6.21 ± 0.32 to 4.42 ± 0.30; 4.42 ± 0.30 to 2.77 ± 0.18; 2.65 ± 0.20 to 1.91 ± 0.04; 3.45 ± 0.13 to 2.35 ± 0.34; 2.82 ± 0.18 to 1.05 ± 0.04; 2.76 ± 0.15 to 1.71 ± 0.11 and 2.52 ± 0.05 µg/mg to 2.04 ± 0.03 µg/mg, respectively (Table.1). From the table, it is clear that the t-values 10.01, 11.47, 8.69, 7.51, 22.82, 13.47 and 20.74 were significant at 0.05% level. Therefore, it may be concluded that the glycogen content of the fat body, testis, vas deferens, seminal vesicle and male accessory reproductive glands significantly differ in control and treated insects. Similarly, the quantity of glycogen has decreased significantly during the treatment with phytopesicide Vijay neem, indicating the utilization of these substances in all the reproductive tissues as an energy demand. Glucose content in the fat body, testis, vas deferens, seminal vesicle, $MARG_1$, $MARG_2$ and $MARG_3$ were found to be increased in the treated insects than the control insect of about 4.63 ± 0.13 to 6.38 ± 0.34; 4.13 ± 0.14 to 5.47 ± 0.29; 2.97 ± 0.17 to 3.94 ± 0.23; 3.28 ± 0.22 to 5.43 ± 0.32; 2.99 ± 0.09 to 3.40 ± 0.13; 2.87 ± 0.18 to 3.42 ± 0.14 and 3.05 ± 0.12 to 3.65 ± 0.25 µg/mg, respectively (Table 2).

Protein: The protein content in the fat body, testis, vas deferens, seminal vesicle and MARGs of control and treated insects are presented in Table 3. The protein content in the fat body, testis, vas deferens, seminal vesicle and MARGs were found to be decreased in the treated insects than that of the control insects. The amount of protein content ranges in the fat body, from 61.57 ± 1.33 to 45.27 ±0.96 µg/mg; in the testis, from 48.52 ± 1.26 to 38.58 ± 0.78 µg/mg; in the vas deferens from 49.28 ± 0.62 to 39.8 ± 0.81 µg/mg; in the seminal vesicle, from 59.79 ± 1.35 to 46.54 ± 1.56 µg/mg; and in the MARGs ($MARG_1$, $MARG_2$ and $MARG_3$) from 79.52 ± 2.67 to 61.87 ± 3.84; 78.01 ± 3.69 to 57.69 ± 2.88 and 83.52 ± 3.24 to 65.41 ± 3.08, respectively. The amino acid contents of the treated insect tissues were found to be increased than that of control insects which were about 65.61 ± 3.07 to 108.87 ± 7.74; 59.65 ± 7.62 to 94.01 ± 4.56; 55.88 ± 3.07 to 72.86; 65.86 ± 3.05 to 100.00 ± 6.32; 72.55 ± 3.51 to 112.45 ± 4.17; 71.67 ± 3.44 to 110.17 ± 3.71 and 84.83 ± 2.48 to 122.50 ± 4.85 µg.mg, respectively (Table.4).

Lipid: The lipid contents of the experimental insects were found to be decreased than that of the control insects and were about 25.37 ± 1.46 to 16.42 ± 0.92; 18.82 ± 0.82 to 12.78 ± 0.60; 10.05 ± 1.18 to 7.36 ± 0.32; 15.90 ± 1.14 to11.26 ± 0.36; 12.01 ± 0.91 to 7.85 ± 0.48; 9.96 ± 0.87 to 6.50 ± 0.42 and 10.09 ± 0.91 to 6.89 ± 0.23 µg/mg, respectively (Table 5).Thus, it is evident that the quantity of the lipid of the fat body, testis, vas deferens, seminal vesicle and MARGs has decreased significantly during treatment with Vijay neem, suggesting that the utilization of these substances as an energy source to avoid stress by the insects.

DISCUSSION

One of the most fundamental requirements of any organism is energy which is needed for various metabolic activities (Boell, 1965). Glucose is an immediate source of energy, and it can quickly be mobilized from glycogen stores when sudden demands for energy are made (Lehninger, 1984). When there is a demand of energy, glucose is oxidized to carbon dioxide, water and energy in the form of ATP molecules. The hyperglycaemic hormones were involved in the regulation of carbohydrate metabolism have been reported in many insects (Steele, 1961; Witten *et al.,* 1984; Gaede and Rinehart, 1987; Gaede *et al.,* 1988). In the present study, it has been shown for *M. indica,* the quantity of glycogen content in the control insect tissues like fat body, testis, vas deferens, seminal

Table 1. Glycogen content of selected tissues in control and phytopesticide treated adult male insect, *M. indica*

Tissue	Control (µg/mg)	Treated (µg/mg)	Percentage over control	't' value
Fat body	6.21 ± 0.32	4.42 ± 0.30	-28.86	10.04*
Testes	4.42 ± 0.30	2.77 ± 0.18	-37.40	11.47*
Vas deferens	2.65 ± 0.20	1.91 ± 0.04	-27.84	8.69*
Seminal vesicle	3.45 ± 0.13	2.35 ± 0.34	-31.95	7.51*
$MARG_1$	2.82 ± 0.18	1.05 ± 0.04	-62.60	22.82*
$MARG_2$	2.76 ± 0.15	1.71 ± 0.11	-37.93	13.47*
$MARG_3$	2.52 ± 0.05	2.04 ± 0.03	-19.02	20.74*

Data represent values are mean ± S.D (n=6).
There is significant difference in the control and treated tissues.
*Significant at 0.05% level.

Table 2. Glucose content of selected tissues in control and phytopesticide treated adult male insect, *M. indica*

Tissue	Control (µg/mg)	Treated (µg/mg)	Percentage over control	't' value
Fat body	4.63 ± 0.13	6.38 ± 0.34	37.70	-11.89*
Testes	4.13 ± 0.14	5.47 ± 0.29	32.38	-10.37*
Vas deferens	2.97 ± 0.17	3.94 ± 0.23	32.53	-8.22*
Seminal vesicle	3.28 ± 0.22	5.43 ± 0.32	65.63	-13.68*
$MARG_1$	2.99 ± 0.09	3.40 ± 0.13	13.61	-6.26*
$MARG_2$	2.87 ± 0.18	3.42 ± 0.14	18.92	-5.84*
$MARG_3$	3.05 ± 0.12	3.65 ± 0.25	19.70	-5.20*

Data represent values are mean ± S.D (n=6).
There is significant difference in the control and treated tissues.
*Significant at 0.05% level.

Table 3. Protein content of selected tissues in control and phytopesticide treated adult male insect, *M. indica*

Tissue	Control (µg/mg)	Treated (µg/mg)	Percentage over control	't' value
Fat body	61.57 ± 1.33	45.27 ± 0.96	-26.48	24.35*
Testes	48.52 ± 1.26	38.58 ± 0.78	-20.47	16.42*
Vas deferens	49.28 ± 0.62	39.8 ± 0.81	-19.17	22.69*
Seminal vesicle	59.79 ± 1.35	46.54 ± 1.56	-22.16	15.73*
$MARG_1$	79.52 ± 0.67	61.87 ± 3.84	-22.19	11.09*
$MARG_2$	78.01 ± 3.69	57.69 ± 2.88	-26.05	10.64*
$MARG_3$	83.52 ± 3.24	65.41 ± 3.08	-21.68	9.93*

Data represent values are mean ± S.D (n=6).
There is significant difference in the control and treated tissues.
*Significant at 0.05% level.

Table 4. Amino acid content of selected tissues in control and phytopesticide treated adult male insect, *M. indica*

Tissue	Control (µg/mg)	Treated (µg/mg)	Percentage over control	't' value
Fat body	65.61 ± 3.07	108.87 ± 7.74	65.93	-12.73*
Testes	59.65 ± 7.62	94.01 ± 4.56	57.60	-9.48*
Vas deferens	55.88 ± 3.07	72.86 ± 3.05	30.39	-9.62*
Seminal vesicle	65.86 ± 3.05	100.00 ± 6.32	51.84	-11.92*
$MARG_1$	72.55 ± 3.51	112.45 ± 4.17	55.00	-17.94*
$MARG_2$	71.67 ± 3.44	110.17 ± 3.71	53.72	-18.63*
$MARG_3$	84.83 ± 2.48	122.50 ± 4.85	44.41	-16.94*

Data represent values are mean ± S.D (n=6).
There is significant difference in the control and treated tissues.
*Significant at 0.05% level.

Table 5: Lipid content of selected tissues in control and phytopesticide treated adult male insect, *M. indica*

Tissue	Control (µg/mg)	Treated (µg/mg)	Percentage over control	't' value
Fat body	25.37 ± 1.46	16.42 ± 0.92	-35.29	12.74*
Testes	18.82 ± 0.82	12.78 ± 0.60	-32.10	14.65*
Vas deferens	10.05 ± 1.18	7.36 ± 0.32	-26.76	5.38*
Seminal vesicle	15.90 ± 1.14	11.26 ± 0.36	-29.18	9.47*
$MARG_1$	12.01 ± 0.91	7.85 ± 0.48	-34.68	9.94*
$MARG_2$	9.96 ± 0.87	6.50 ± 0.42	-34.78	8.76*
$MARG_3$	10.09 ± 0.91	6.89 ± 0.23	-31.75	8.36*

Data represent values are mean ± S.D (n=6).
There is significant difference in the control and treated tissues.
*Significant at 0.05% level.

vesicle and MARGs have increased significantly. In contrast, the amounts of glycogen in the phytopesticide treated insect have decreased significantly. The quantity of glucose of the treated fat body has revealed that reduction in the quantity of glycogen and increased in the quantity of glucose, indicating the possibility of breakdown of glycogen to glucose in order to meet the requisite energy demand during pesticide intoxication. Such an increase in the quantity of glucose in the reproductive tissues than the control appears to be due to transpiration of glucose from the storage organ namely, the fat body. These changes may be due to the toxic effect of the phytopesticide for *M. indica.* In the present study, it has been shown for *M. indica* that the quantity of protein content in the tissues like fat body, testis, vas deferens, seminal vesicle and MARGs have recorded more value. But in the amount of protein content in the phytopesticide treated tissues decreased significantly. Wigglesworth, (1977) has stated that the fat body in insects is the main site for protein synthesis as well as an intermediary metabolism of amino acids.

The amount of protein in *Tribolium castaneum* decreased due to an application of sublethal concentrations of diazinon (Arash Zibaee *et al.,* 2008). Babu *et al.,* (1996) have showed that treatment of *Spodoptera littoralis* and *Agrotis ipsilon* with azadirachtin decreased protein of hemolymph. This could be due to the break down of protein into amino acids, so as to 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 hemolymph (Nath *et al.,* 1997). The depletion of protein content in treated insects may be due to a rapid utilization of proteins to meet the extra energy demand during phytopesticide intoxication. Another possible reason is that the protein synthesis is highly inhibited as a result of non availability of energy for protein synthesis (Saxena *et al.,* 1989; Singh *et al.,* 1992). Similar findings have been reported by Shanmugavelu, (1993) in *Mylabris pustulata;* Rameshkumar, (2004) for *Laccotrephus ruber;* Kowsalya, (2007) in *Odantopus varicornis.* In the light of these findings, it may be inferred that the decreased quantity of protein in all the tissue of *M. indica* treated with phytopesticide appears to be due to the inhibiting action of this pesticide on certain enzymes, affecting the process of synthesis of protein and utilization of protein for the metabolic activity. The present study has revealed that the amino acid contents in the fat body, testis, vas deferens, seminal vesicle and MARGs of treated insects were increased considerably than that of control insects. This inference gains support from the present observation, showing a reduction in the quantity of protein contents and an elevation of amino acid in all the tissues in *M. indica* due to the acceleration of protein break down into amino acid to meet an immediate energy demand. The present study clearly indicates that the high level of amino acids resulting from proteolysis in the tissues are transported into the haemolymph to the metabolic pathway by the pyruvate, which directly enter into TCA cycle in the form of keto acids to provide an additional energy during the stress period.

Insects and the life of most of the organisms are capable of utilizing digested lipid and converting non-lipoidal materials into lipid (Gillbert, 1967). Storey and Bailey, (1978) have proposed that the lipid may be converted into carbohydrate by glycolytic path way. The lipid is stored in the fat body in the form of triglycerides (Price, 1973). The lipid store is then utilized to provide energy for the metamorphosis (Rao and Agarwal 1969, 1971; Pol and Sawant, 1990). Fat body is the major site of fatty acid synthesis in insects (Gilbert, 1967). Lipid reserves are used as energy source for the processes such as flight, egg development etc. (Secktor, 1970 and Downer, 1978). Gilmour, (1965) has reported that benzene hexa chloride interfere with the metabolism of lipid in insects, which leads to the impairment of the functioning of the fat body (Prakash *et al.,* 1990) have found out that decreased level of lipid content in the fat body and haemolymph of *Poicelocerus pictus,* when exposed to endosulfan and these changes may be attributed due to environmental stress. Amasath *et al.,* (1993) have reported that the reduced level of lipid in the testis of *Sphaerodema molestum,* which might be due to lipid utilization of energy production during stress condition. Thiruvasagam (1994) has observed that the decreased level of lipid content in the testis, MARGs and haemolymph of *Aspongopus Janus* due to the toxic nature of nimbecilin. Similar results have also been observed by Ramanathan, (1995) for *Periplaneta americana* exposed to *Pongamia glabra* leaf extract; Ravichandran (1996) for *Laccotrephus ruber* when exposed to monocroptophos. Further, Palanisamy (1997) has also reported the level of lipid content in the testes, fat body and MARGs have decreased for *Laccotrephus ruber* when exposed to heavy metal mercury.

Generally, more energy is needed to mitigate any stress condition. This energy may be obtained from carbohydrate, protein and lipids. The decreased content of lipid in the tissues of the treated insect suggests that its utilization under stress condition as it is reported in the present study, when *M. indica* treated with phytopesticide. The other reason for the decline of all biochemical constituents might be due to the arrest of mobilization of nutrients from the fat body into all the reproductive tissues in the treated insects, *M. indica.*

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