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EFFECT OF MONOCROTOPHOS ON THE PROTEIN METABOLISM IN THE OF OVARY, FAT BODY AND HAEMOLYMPH OF LACCOTREPHES RUBER (LINN.) (HETEROPTERA: NEPIDAE)

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ABSTRACT

The present study is aimed to investigate the protein metabolism in the ovary, fat body and haemolymph of Laccotrephes ruber. The insects were exposed to monocrotophos of 96 hr LC_{50} In the present study, the total protein and total free amino acids were observed in ovary, fat body and haemolymph of Laccotrephes ruber. The present study showed the level of total protein was decreased and total free amino acids was increased. The results are discussed with available literature

Keywords: Workaholism, IT Sector, and Software engineers

1.INTRODUCTION

Monocrotophos[3 hydroxy-N-methyI-cis-crotonamide dimethylphosphate], an organophosphorous insecticide is widely used as an effective crop protectant. It has both systemic and contact properties and has been used against a wide range of insects including mites, Boll worms, sucking insects, leafeating beetles and other larvae on variety of Insect reproduction is an essential physiological process from the point of view of propagation and it has received relatively little direct attention perhaps due to the fact that its processes are so inteimately associated with other systems and are controlled by both intrinsic and extrinsic factors.

The problem of vitellogenesis, a complicated process that takes place during oogenesis, still remains controversial although extensive work has been done on this dynamic aspect of reproduction (review; Engelman, 1970; Mahowald, 1972; Adiyodi and Adiyodi, 1974).

In another study conducted to determine the effects of host density on development time, egg dispersion, fecundity, sex ratio, longevity, and glycogen, total sugar and lipid levels of *Bracon hebetor* Say, 1836 (Hymenoptera: Braconidae). As a result of research conducted that Host density had no significant effect on glycogen levels of female and male wasp, whereas sugar and lipid levels showed some variations in both sexes (Isitan *et al.*, 2011). The proteins are important organic constituents of the animal playing the vital role in the process of interactions between intra and extra cellular media being a part of cell membrane and protein participates the intricately balanced sub-cellular fractions [Ramalingam *et al*., 1995]. The amino acids are the building blocks of protein-there are number of amino acids present in the animal body and those vary in accordance with the number and sequence of amino acids [Linder, 1985].

Investigations on the effects of pesticides have revealed their interference with protein metabolism in several species of insects Ravichandran et al., 1993;Sing et al., 1993). Invariably pesticide treatment has led to an increase in the concentration of protein in ovarian tissues of. insects (Sridharan, 1984). Such an increase has been reported for the ovary of Periplanetaamericana exposed to malathion (Saxena,1989), DDT (Fell et al, 1984). Stomaxys calcitrans exposed to sodium sulphate (Housemann et al., 1986), Catacanthus incarnatus exposed to dimethoate (Vijayaprabha, 1990) and Chrysocoris stolli exposed to cadmium chloride (Islam and Roy, 1983).Contrary to these observations, treatment with

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certain pesticides has led to a decrease in the quantity of protein in certain tissues of insect body. Thus, the protein content in the tissues of testis, accessory reproductive gland and fat body has decreased following treatment with dimethoate in *Odontopus varicornis* (Jayakumar, 1988). In support of these findings treatment with endrin has also resulted a decrease in the content of protein in the liver of Salmo gairdneri (Grant and Mehrle, 1973). It is evident, therefore, that the action of pesticides seems to differ in different groups of insects, perhaps, due to differences in their power of toxicity, concentration and duration of exposure. This warrents further investigation on this aspect of study using monocrotophos in median lethal and sub lethal concentrations.

2.MATERIALS AND METHODS

2.MATERIALS AND METHODS

The insects used in the present investigation is *Laccotrephes ruber*.. 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, Ovary and haemolymph were collected from the alive specimens subjected to either anesthesia or without chloroform for the investigation

Toxicity studies

Acute toxicity tests were conducted to measure the impact of toxicant on aquatic animals within a short period of 2 days. The renewal technique of acute static test was adopted in which animals (insects) were periodically exposed to the test concentration of the same composition. usually once in every 24 hours, by transferring the animals from one test chamber to another (Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975).

Range finding test

The solutions were prepared over a wide range of (1-5 ppm) concentrations. The tests were conducted in adult aquatic insect of *L. ruber* with fully mature ovary. The experimental insects were maintained in different concentrations in plastic troughts (40 x 30 x13 cms) each containing 2 litres of water as follows:The results obtained from theses experiments were used to find out the range of the concentration of the insecticide to be used in the present investigation.

Full scale tests for toxicity evaluations

Different concentrations (1-5 ppm) of organophosphorus pesticide monocrotophos were prepared. Experimental and control insects (without insecticide) were maintained in plastic troughs each containing 2 litres of water for bioassay. The observations on mortality were made at 1, 3, 6, 12, 24 and 48 hours. Insects without movements and responses to a tactile stimulus were recorded as dead and were removed immediately. The recorded values were used for calculating LC_{50} values.

Calculation of LC_{50} values

Toxicity data are analysed following the method of Litchfield and Wilcoxon (1949) (abbreviated method) to determine the LC_{50} values. The LC_{50} values of 1, 3, 6, 12, 24 and 48 hours are derived by plotting the observed experimental data on the log probability sheet taking test concentration on the log scale and mortality rate on the probability scale. Then a straight line is drawn between 40 and 60 per cent of the mortality percentage Vs concentration.

By using the LC_{50} values, the test concentration for the experiment was selected.

Definitive tests

To ensure the acceptability of the test, to provide additional data for various length of exposures and to calculate the LC_{50} value with reasonable accuracy, elaborate treatments and desirable concentrations of the pesticide such as 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ppm were prepared (Committee on Methods for Toxicity Test with Aquatic Organisms, 1975).

Ten insects were introduced into the test trough containing 2 litres of water with a specific concentration of the pesticide. The trough was covered with a nylon net to prevent the escape of insects. The mortality rate for each concentration was observed and the data giving details of the survival percentage of insects, duration of exposure and the concentration of the pesticide were given below:

BIOCHEMICAL ESTIMATIONS

The tissues were used for the estimation of protein by the methods of Lowry et al., (1951) and estimation of amino acids is carried out by Moore and Stein (1954)

OBSERVATIONS

Treatment with monocrotophos results in marked changes in the quality of proteins and amino acids in the tissues of ovary, fat body and haemolymph of *Lacotrephes ruber*. The quantitative changes of protein in the tissues due to treatment with median lethal and sublethal concentrations are presented in Tables The tissues of ovaries, fat bodies and haemolymph of insects treated with median lethal concentration of monocrotophos for durations of 24 and 48 hours contain the following quantities of protein.

The tissues of ovaries, fat bodies and haemolymph of insects treated with sublethal concentration of monocrotophos for durations of 24 and 48 hours contain the following quantities

of protein:Treatment with monocrotophos results in makred changes in the quantity of amino acids in the tissues of ovary, fat body and haemolymph of *L. ruber*.

The quantitative changes of amino acids in the tissues due to treatment with median lethal and sublethal concentrations are presented in Tables 2.The amino acid contents of these tissues of control groups having completed durations of 24 and 48 hours of survival in a container with normal water without pesticide are given below and also in Table 2. The tissues of ovaries, fat bodies and haemolymph of insects treated with median lethal concentration of monocrotophos for durations of 24 and 48 hours contain the following quantities of amino acids:

Table 1 the level of protein content in the ovary, fat body and haemolymph of the Laccotrephes ruber exposed with Monocrotophos

Tissues	Control		Experimental Group	
	24hrs	48hrs	4hrs	48hrs
Ovary(mg/g)	66.32±2.17	66.83±1.90	81.15±0.73	82.90±1.28
Fat body(mg/g)	13.86±0.54	13.40±0.29	62.15±1.42	89.96±1.97
Haemolymph(mg/g)	13.85±1.04	13.28±0.24	16.70±0.54	18.12±0.12

Values are Mean ±S.D

Table the level of pamino acidscontent in the ovary, fat body and haemolymph of the Laccotrephes ruber exposed with Monocrotophos

Tissues	Control		Experimental Group	
	24hrs	48hrs	4hrs	48hrs
Ovary(mg/g)	2.110.18	2.43±0.31	1.66±0.06	0.21±0.02
Fat body(mg/g)	1.35±0.07	1.36±0.06	1.19±0.11	0.25±0.03
Haemolymph(mg/g)	2.83±0.08	2.92±0.09	0.81±0.04	0.26±0.02

Values are Mean ±S.D

4.DISCUSSION

Effects of monocrotophos on protein contents

The median lethal concentration of monocrotophos causes an increase in the quantity of protein in the ovaries, fat bodies and haemolymph treated for 24 hours as well as 48 hours of ducation. However, the increase in the quantity of protein is relatively higher in the ovary, fat body and haemoloymph exposed to 48 hours of treatment with pesticide. The ovary, fat body and haemolymph when treated with sublethal concentration of this pesticide for a period of 24 and 48 hours also exhibits a similar trend of increase in the quantity of protein (Tables 1).

From the t-values given in Tables 1 has been shown that the protein contents of the ovary, fat body and haemoloymph of experimental groups treated with median lethal and sublethal concentrations for 24 and 48 hours of durations do not differ significantly during treatment with pesticide, suggesting that the action of monocrotophos on protein content of the ovary, fat body and haeolymph is not related to this period of treatment with pesticide. It has been shown that protein content of the ovary, fat body and haemoloymph of experimietnal groups treated with median lethal and sublethal concentrations for 24 and 48 hours of durations do not differ significantly during treatment with pesticide, suggesting that the action of monocrotophos on protein content of the ovary, fat body and haemoloymph is not related to this period of treatment with pesticide.

From the F-values given in Tables 42, 43 and 44, It has been shown that protein content of the ovary, fat body and haemoloymph of experimental groups treated with median lethal and sublethal concentrations differ significantly during treatment with different concentration of pesticide suggesting, that the action of monocrotophos on protein content of ovary, fat body and haemoloymph appears to be does dependent.

Further , monocrotophos seems to interfere with protein metabolism of this insect, perhaps, by inhibiting the process of its utilization as evidenced by an occurance of higher quality of protein and amino acids in ovarian tissues, fat bodies and haemoloymph treated for 48 hours of duration. This inference gains support from the observations indicating the presence of a lower quantity of protein and amino acids in ovarian tissues, fat body and haemolymph of control insects due to utilization of these substances during 48 hours of duration.

Insects in addition to sugars and lipids, use free amino acids as readily available source of respiratory fuels (Bursell, 1963;Candy and Kilby, 1975). Protein is stored after being synthesized from amino acids in the fat body where they are repaidly utilized to replace the loss of protein during physiological demands (Wigglesworth, 1979; Downer, 1982). Moreover, protein helps to synthesize microsomal detoxifying enzyme which assists to detoxify the toxicants entering into the animal body (Wilkinson, 1976).

Studies on the effects of insecticides have revealed their interference in protein metabolism in several insects. Invariably pesticide treatment has led to an increase in the concentration of proteins in ovarian tissues on insects. Such an increase has been reported for the ovary of Periplaneta Americana due to treatment with 2 per cent malathion (Saxena, 1989) and for the stable fly *Stomaxys calcitrans* exposed to sodium dodecyl sulphate (Houseman et al., 1986). Vijayaprabha (1990) has reported an increase in the quantity of protein content in the ovary of *Catacanthus incarnates* exposed to 24 hours of duration to the median lethal and sublethal concentrations of dimenthoate. Similar increase in protein content has also been reported for the ovary of *Odontopus varicornis* treated with sublethal concentration of monocrotophos (Ravichandran, 1990).

Other tissues such as fat boydy, haemoloymph and nervous tissues also exhibit a similar trend of increase in the concentration of protein and amino acids when treated with pesticides.

The quantity of protein has increased in the fat body, haemolymph and ovarian tissues of the stable fly, *Stomaxys calcitrans* after exposure to sodium dodecyle sulphate (Housemen et al 19086). Similarly the level of protein content has increased in the nervous tissues of *Periplaneta Americana* treate with DDT and DDE (Fell et al., 1984). Increased protein content has been reported for fat body of *Catacanthus incarnates* after 24 hours of exposures to median lethal and sublethal and sublethal concentrations of dimethoate Vijayaprabha, 1990). In *Preiplaneta americana* the protein content has increased in nervous tissues due to treatment with the insecticides bendiocarb and monocrotophos (Rajender, 1990).

The quantity of protein has increased in the haemoloymph of dimethoate treated *Odontopus varicornis* (Jayakumar, 1988) and in the haemoloymph of Farnesyl methyl ether treated *Periplaneta Americana* (Prabhu and Nayar, 1972b).

A similar increase in the quantity of protein has been reported for the haemoloymph of monocrotophos treated *Odontopus varicornis* (Ravichandran et al., 1993), cadmium chloride treated *Chrysocoris stolli* (Islam and Roy, 1983), danusban treated *Chrysocoris* sp. (Sridharan, 1984) and methyl parathion treated fish *Tilapia mossambica* (Rao and Rao, 1979).

Studies undertaken by Rajender (1986 a,b) have shown similar changes in the quantity of protein due to the effect of insecticides monocrotophos and bendicarb in the haemolymph and nervous tissue of the adult cockroach, *Periplaneta Americana*.

Contrary to these findings, Grant and Mehrle (1973) have reported that treatment with endrin has resulted in a decrease in the quantity of protein in the liver of *Salmo gairdneri*, when exposed to sublethal concentration of the pesticide. Later, in support of this findings, Jayakaumar (1988) was reported that in *Odontopus varicornis* the protein content in the tissues of testis, accessory reproductive gland and fat body has decresed following dimethoate treatment,

It is evident from these observations that chemicals such as monocrotophos, malathion, sodium dodecyle sulphate, dimethoate and endrin seem to interfere with protein metabolism resulting in significant changes in the quantity of protein and amino acids in tissues of ovary, fat body and haemolymph, perhaps, due to toxic stress. In this regard, Baumann (1971) has suggested that the increase in the level of protein in the haemolymph of *Periplaneta Americana* is due ot the effect of toxin (DDT) and hormones.

Further , it has been reported that organophosphorus compounds, including monocrotophos, apparently inhibit the action of several enzymes, including the enzyme acetyl cholinesterase (Ohkawa, 1982). Further, the mode of action insecticides has been shown to be related to their capacity to disturb lipid protein interactions (Doherty, 1979).

In the light of these findings it may be inferred that the increase quantity of protein in the tissues of ovary, fat body and haemoloymph of *L. ruber* treated with monocrotophos appears to be due to the inhibitory action of this pesticide on certain. Enzymes affecting the process of utilization of protein for metabolic activity.

Effects of monocrotophos on amino acid contents

In the present study, it has been shown that treatment with median lethal and sublethal concentrations of monocrotophos for a period of 24 hours and 48 hours has resulted in significant changes in the quantity of amino acids in the tissues of ovary, fat body and haemolymph. The amino acid content shows an inverse relationship with regard to protein concentration.

The median lethal concentration of monocrotophos causes a decrease in the quantity of amino acids in the ovaries, fat bodies and haemolymph treated for 24 hours as well as 48 hours of duration. However, the decrease in the quantity of amino acids is relatively higher in the ovary, fat body and haemolymph exposed to 48 hours of treatment with pesticide. The ovary, fat body and haemolymph when treated with sublethal concentration of this pesticide for a period of o24 hours also exhibit a similar trend of decrease in the quantity of amino acids (Tables1).From the t-values given in Tables 1, it has been shown that amino acid contents of the ovary and haemolymph of experimental group treated with medium lethal and sublethal concentrations for 24 and 48 hours of durations differ significantly during the treatment with pesticide, suggesting that the action of monocrotophos on amino acid content of the overy and haemolymph appears to be related to this duration of treatment with pesticide. it has been shown that amino acid contents of the fat body of experimental group treated with median lethal and sublethal concentration for 24 and 48 hours of duratios differ significantly only during treatment with median lethal concentration of pesticide, suggesting that the action of monocrotophos on amino acid content of fat body appears to be concentration of the pesticide. It has been shown that amino acid content sof the ovary, fat body and haemolymph of experimental groups treated with median lethal and sublethal concentrations differ significantly during treatment with different concentrations of pesticide, suggesting that the action of monocrotophos on amino acaid contents of ovary, fat body and haemoloymph appears to be dose dependent.

According to Bursell (1963) and Candy and Kilby (1975) insects seem to use free amino acid as their readily available source of respiratory fuesl. In this regard, O'Brien (1957) has shown that insecticides affect the level of amino acids in the tissues and haemoloymph of insects.

The decreased quantity of amino acids in treated tissues of *L.ruber* thus, appears to be due to the utilization of these amino acids as fuels, perhaps, for respiratory activities during the period of strain and stress caused by pesticide.

Studies on the effects of insecticides have revealed their interference in protein and amino acid metabolism in several insects. The quantity of amino acid has decreased in ovary, fat body and haemolymph due to treatment with cadmium chloride in *Chrysocoris stolli* (Islam and Roy, 1983) and due to treatment with malathion in *Lamellidens marginalis* (Kabeer et al., 1978). Ravichandran *et al.,* (1993) have reported that the ovary of *Odontopus varicornis* treated with mediam lethal concentration of monocrotophos had a reduced quantity of amino acids.

A similar decreased quantity of amino acid has been reported for dimenthoate exposed ovarian tissues of *Catacanthus incarnates* (Vijayaprabha, 1990) and malathion exposed tissues of *Tilapia mossambica* (Kabeer et al., 1980).

Such a decrese in the quantity of amino acid has also been reported for other tissues such as fat body and haemolymph of *Chrysocoris stolli* due to treatment with cadmium chloride (Islam and Roy, 1983). Ravichandran (1990) has reported the presence of a decreased quantity of amino acids in the fat body of *Odontopus varicornis* treated with sublethal concentration of monocrotophos.

A reduction of amino acid content has also been reported for *Catacanthus incarnates* treated with median lethal concentration of dimethoate (Vijayaprabha, 1990), *Mylabris pustulata* treated with carbaryl (Bharathi and Govindappa, 1987), *Chrysocoris stolli* treated with cadmium chloride (Islam and Roy, 1983), *Dysdercus koenigii* treated with *Annona squamosa* extract (Damodar Reddy et al., 1993), *Dysdercus konigii* treated with malathion (Rambha Singh, 1982), *Odontopus varicornis* treated with monocrotophos (Ravichandran et al., 1993), *Lamellidens marginalis* treated with endosulfan, malathion and sevin (Khillare and Wagh, 1989) and *Tilapia mossambica* treated with malathion (Kabeer et al., 1980).

All the investigations indicate that treatment with pesticides results in a marked reduction in the quantity of amino acids in tissues of ovary, fat body and haemolymph possibly due to the utilization of amino acids as fuels to overcome the impending energy demands under toxic stress, as it has been reported for L.ruber.

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