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ORIGINAL ARTICLE

EFFECT OF MONOCROTOPHOS ON THE CARBOHYDRATE 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 carbohydrate metabolism in the ovary, fat body and haemolymph of *Laccotrephes ruber*. The insects were exposed to median lethal and sublethal concentration 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: Monocrotophos, Carbohydrate metabolism.

1.INDRODUCTION

The carbohydrate metabolic changes caused by reatment with pesticide have been elucidated for few species of insects (Jabakumar and Jayaraman, 1988). These studies have shown that treatment with pesticides has led to a decrease in the content of carbohydrate in different tissues of the animals investigated. Such a decrease has been reported for Malacosoma pluviale exposed to Farnesyl methyl ether Ajai Mansingh,1972), *Dysdercus cingulatus* exposed to oxytetracycline and sulphanilamide (Venugopal and Subramaniam, 1979), Poecilocerus pictus exposed to endosulfan (Prakash et al., 1990) and Mylabris balteata exposed to cadmium chloride Shunmugavelu, 1993). Contrary to these observations carbohydrate content has been found to have increased in animal tissues when exposed to certain pesticides such as endrin and furadon (Grant and Mehrle,1973).Studies on quantitative changes of lactic acid content of the tissues treated with pesticides have been undertaken by few authors in different species of insects (Grant and Mehrle, 1973;Jayakumar, 1988).

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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 glycogen and glucose by the methods of Kemp and Kits van Heijningen (1954)

RESULTS

Treatment with monocrotophos results in marked changes in the quantity of carbohydrates in the tissues of ovary and fat body, glucose in the haemolymph and lactic acid in the ovary, fat body and haemoloymph of *Laccotrephes ruber* (Tables 1). The carbohydrate contents of these tissues of control groups having completed durations of 24 and 48 hours of survival in a container with normal water without pesticides are given below and also in Tables 1

The tissues of ovaries and fat bodies of insects treated with m edian lethal concentration of monocrotophos for durations of 24 and 48 hours contain the following quantities of carbohydrates.The tissues of ovaries and fat bodies of insects treated with sublethal concentration of monocrotophos for duration of 24 and 48 hours contain the following quantities of carbohydrates:The quantitative changes of glucose in the haemolymph due to treatemtnt with median lethal and sublethal concentrations are presented in Table 1.The glucose contents of the haemolymph of control groups have 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 haemolymph of insects treated with median lethal concentration of monocrotophos for durations of 24 and 48 hours contain the following quantities of glucose: The haemolymph of insects treated with sublethal concentration of monocrotophos for duration of 24 and 48 hours contain the following quantities of glucose.

The quantitative changes of lactic acid in the tissues of ovary, fat body and haemolymph due to treatement with median lethal and sublethal concentrations are presented in Table 1

The lactic acid contents of the tissues of ovary, fat body and haemolymph of control groups havaing completed durations of 24 and 48 hours of survival in a container with normal water without pesticide ware given below and also in 1 Te 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 lactice acid:The tissues of ovaries, fat bodies and haemolymph of insect treated with sublethal concentration of monocrotophos for duration of 24 and 48 hours contain the following quantities of lacatic acid content:

In the present study it has been shown that significant changes in the content of carbohydrate of the ovary and fat body have been observed in insects treated with median lethal and sublethal concentrations of monocrotophos. Treatment with median lethal concentration of monocrotophos has resulted in the decrese of total

carbohydrate content in tissues of ovary and fat body exposed to 24 and 48 hours of durations. Similarly treatment with sublethal concentration of monocrotophos has caused a decrese in the quantity of total carbohydrate content in these tissues when exposed to 24 and 48 hours of duration . From the t-values given in Table 51, it has been shown that carbohydrate contents of the ovary of experimental group treated with median lethal and sublthal concentrations for 24 and 48 hours of durations differ significantly only during sublethal concentration of pesticide suggesting that the action of monocrotophos on carbohydrate content of ovary appears to be dose dependent. From the t-values given in Table 2, it has been shown that carbohydrate contents of the fat body of experimental group treated with median lethal and sublethal concentrations for 24 and 48 hours of durations differ significantly during the treatment with pesticide, suggesting that the action of pesticide appears to be related to this duration of treatment with pesticide.

Table the level of glucose 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)	14.03±0.98	14.00±0.72	12.55±0.78	13.52±1.34
Fat body(mg/g)	10.24±0.11	10.35±0.14	9.7±0.23	7.13±0.02
Haemolymph(mg/g)	2.31±0.05	2.39±0.08	3.68±0.14	3.05±0.04

Values are Mean ±S.D

Table the level of lactic acid 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)	240.93±5.75	240.85±85	416.83±20.90	445.56±7.51
Fat body(mg/g)	221.42±5.96	221.18±5.51	489.32±19.18	426.70±12.50
Haemolymph(mg/g)	2.07±0.06	2.09±0.08	4.96±0.37	4.87±0.57

Values are Mean ±S.D

DISCUSSION

The works of Bhatt and Krishna (1982) and Chaubey and Bhatt (1988) on rice moth, *C.cephalonia*, Premkumar et al. (1991) on *Laccotrephes gresius*, Sharma and Etheshamuddin (1992) on *Sphaerodema rusticum*, Peera lohr and Gerdgadi (1982) on *Carausiu morosus* and Rolf Ziegler (1981) on *Manduca sexta* contribute to our understanding of the importance of carbohydrate as an energy source.During the normal life of an insect, energy requiring processes such as moulting, development of gonad, vitellogenesis and muscular activity, demand an increase in metabolic flux. But, the metabolic activity of an insect subjected to a toxicant would be different due to the action of this substance on certain target organs.To understand that action of toxicants several works have been undertaken on carbohydrate metabolism in insects. Thus the effects of mercuric chloride on *Notopterus notopterus* (Verma and Rameshchand, 1988). Endosulfan on *Clarias batrachus* (Venkateswaralu et al., 1987) and dimethoate on *Odontopus varicornis* (Jayakuar, 1988) have been analysed in relation to carbohydrate metabolism.

In this regard, Grant and Schoettger (1972), McLeay and Brown (1975), Srivastava and Singh (1980) and Metelev et al., (1983) have shown that carbohydrate metabolism is impaired due to various pollutants. Similarly, treatments with dimethoate in *Odontopus varicornis* (Jayakumar, 1988). Endrin in *Salmo gairdneri* (Grant and Mehrle, 1973), lindane, disyston and furadon in *Anabas testudineus* Bakthavathsalam, 1980), sevin in *Channa punctatus* (Sastry and Siddique, 1982) have resulted in an increase in lactic acid content in these animals. It is evident from these studies that pesticides seem to interfere with the metabolic activities of the insect.

However, informations on the action of pesticides on these metabolic activities particularly with reference to carbohydrate appear to be fragmentary. Hence the present investigation has been undertaken to analyse the carbohydrate metabolism of the insect *Laccotrephes ruber* treated with the organophosphorus pesticide, monocrotophos.

Several other studies have revealed that treatment with other pesticides and certain chemicals has caused a significant reduction in the quantity of carbohydrates in different tissues of insects.

Chattoraj and Sharma (1988) have reported that R-20458 when administrated to the larvae of *Sopodoptera litura* has caused a decrease in the carbohydrate content. Farnesyl methyl ether has also caused a decrese in the content of glycogen in *Malacosma pluvial* (Ajai Mansingh, 1972). Shunmugavelu and Sekar (1992) have observed a reduction in the content of carbohydrate in the ovary, fat body and haemolyph of cadmium chloride treated *Mylabris balteata* and Mylabris pustulata Shunmugavelu, 1993). Prakash et al., (1990) have reported a decreased quantity of carbohydrate in the ovary and fat body of endosulfan treated *Poecilocerus pictus.*

In rice stem borer, *Chilo suppressalis*, treated with JH and ecdysone has resulted in a decline in the content of glycogen of fat body (Hasaaki Tsumuki and Katsuo Kanehisa, 1981). Babu et al (1988) have reported a decrease in total carbohydrate content of the liver and muscle of *Sarotherodon mossambicus* exposed to benthiocarb. Sastry and Aradhana (1991) have recorded a decrease in total carbohydrate level of liver of *Channa punctatus* when exposed to Nuvacron.

The results of the present investigation and those of the works referred to above indicate that the decreased carbohydrate content of the ovary and fat body of monocrotophos treted insects may be due to the production of high rate of energy to overcome the toxic stress caused by pesticides, as it has been suggested by Shunmugavelu (1988) and Shunmugavelu and Sekar (1992). In this regard Roan and Hopkins (1961) have shown that heavy metla toxicity causes an effect leading to an enhanced rate of catabolism and depletion of food reserves particularly carbohydrate in insects.

As pointed out by Peter (1973), carbohydrates from the key substreates of energy metabolism and they appear to h ave been utilized by the animal to meet the extra energy demand under conditions of acute stress.

The present study has revealed that the level of glucose has increased in the haemolymph of L. ruber treated with median lethal as well as sublethal concentration of monocrotophos for 24 and 48 hours of durations.

From the t-values given in Table 52, it has been shown that glucose level in the haeolymph of experimental group treated with median lethal and sublethal concentrations for 24 and 48 hours of durations differs significantly during treatment with monocrotophos, suggesting that the action of this pesticide on glucose level appears to be related to this duration of treatment.

From the F-values given in Table 56, it has been shown that glucose content of the haemolymph of experimental groups treated with median lethal and sublethal concentrations differ significantly during treatment with pesticide suggesting that the action of monocrotophos appears to be dose dependent.
In sublethal treatment for 24 and 48 hours of duration the level of haemolymph sugar is found to have elevated, the elevation being higher for 24 hours of exposure.

Many toxicologists have reported an increase in the level of haemolymph sugar in insects treated with pesticides. Islam and Roy (1983) have observed an elevated level of sugar in the haemolymph of *Chrysocoris stolli* exposed to toxic medium. Jayakumar (1988) has reported an higher quantity of haemolymph sugar in *Odontopus varicornis* exposed to dimethoate. The total haemolymph sugar level has increased after treatment with formanidine in *Manduca sexta* (Ismail and Matsura, 1992). Treatment with mercury, copper and zinc has also caused an elevation in the level of sugar in the haemolymph of *Barytelphusa cunicularis* (George Verghese et al., 1992).

Regarding this trend, it has been suggested that an increased oxidation of glucose takes place through anaerobic glycolytic pathway to provide energy (Venkateswaralu etl al., 1987). Further, Mathews and Downer (1973) have shown that stress eeems to affect glycogenolysis in the fat body, leading to release of sugar into the haemolymph with the maximum hypertrehalosemic response in cockroach.

Thus, the present findings corroborate with those of Corbett (974) according to him the poisoned insects have shown an initial hyperactivity after administration of toxicide.

It was earlier reported for *L. ruber* that these insects when treated with median lethal and sublethal concentration of monocrotophos indicate significant changes in the content of lactic acid in the ovary, fat body and haemolymph.

The lactic acid content of the ovary, fat body and haemolymph of the insects treated with median lethal and sublethal concentrations of monocrotophos exhibit an increasing trend during 24 and 48 hours of exposure, the increase being higher during 48 hours of duration.

From the t-values given in table 57 and 59, it has been shown that lactic acid contents of ovary and haemolymph of experimental groups treated with median lethal and sublethal concentrations for 24 and 48 hours of durations do not differ significantly during the treatment with pesticide, suggesting that the action of monocrotophos on lactic acid content of ovary and haemolymph are not related to this period of treatment with pesticides.

From the t-values given in Table 58, it has been shown that lactic acid content of fat body of experimental group treated with median lethal and sulethal concentrations for 24 and 48 hours of durations differs significantly during the treatment with pesticide, suggesting that the action of monocrotophos appears to be related to this duration of treatment with pesticide.

From the F-values given in Tables 60, 61 and 62, it has been shown that lactic acid contents of the ovary, fat body and haemoloyymph of experimental groups treated with median lethal and sulethal concentrations differ significantly, suggesting that the action of monocrotophos on lactic acid content of ovary, fat body and haemolymph is dose dependent.

Thus, treatment with pesticide results in an increase in lactice acid content in tissues of L. ruber, as it has been reported for Odontopus varicornis exposed to rogor (Jayakumar, 1988) and Anabas testudineus exposed to furadon (Backthavathsalam, 1980).

Miny Samuel and Sastry (1989) have shown that lactate level has increased significantly in the blood and liver of the fish Channa punctatus treated with monocrotophos. Similarly, lactate content in the liver of *Clarias batrachus* has increased after exposure to endosulfan (Venkateswaralu et al., 1987).

These observations showing an increase in lactate content indicate that it could be due to an enhanced anaerobiosis as a result of toxic stress (Singh and Srivastava, 1981).In this regard, jayakumar (1988) has reported that an accumulation of lactic acid in tissues is probalpy pdue to anaerobic circumstances or hypoxia, as there is a concomitant inhibition in oxygen consumption and SDH activity in tissues. It is known that in anaerobic contraction, the decrease in glycogen is more since more glucose is oxidized to lctic acid yielding some energy.On the basis of the findings reported above it may be suggested that the accumulation of lactic acid in the haemolymph of *L. ruber* exposed to monocrotophos is due to its formation by anaerobic glycolysis in tissues.Thus, lactic acid may be utilized for the synthesis of glucose and glycogen through lactic acid cycle in invertebrats as suggested by Bodenskey (1947).

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