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EFFECT OF MALATHION ON THE TOTAL BODY GLUCOSE AND PROTEINS IN THE MANGO MEALY BUG, *DROSICHA STEBBINGI* (COCCIDAE: HOMOPTERA)

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Abstract.- The effect of starvation and malathion (50 PPM and 100 PPM) has been described on the total body proteins and glucose contents of the adult female of *Drosicha stebbingi* (Green). The insects were starved for 4, 8, 12 and 16 hours. The protein contents increased upto 8 hours and then started decreasing as a result of starvation and the minimum protein level was observed at 16th hour of their laboratory life. The glucose contents, on the other hand, showed an increase which reached its peak at the 12th hour, after that it started decreasing. Malathion treatment showed the highest protein contents at the 6th hour of their laboratory life and the minimum contents at the 16th hour in case of 50 PPM, but at the 12th hour in case of 100 PPM. The lowest glucose level was observed at 12th hours and the highest at the 16th hour of exposure to the insecticide.

INTRODUCTION

The control of insect pests is one of the major problems especially as insects, apart from being a very serious competitor of man for food are also vectors for many serious diseases. Starting from the use of DDT after the 2nd World War various other types of insecticides have been synthesized and used to suppress their populations. The development of resistance in insect populations against various insecticides has forced the workers to use different biologically active chemicals against them. Organophosphates introduced by Scharder (1952) are widely used now. They have the ability to inhibit cholinesterase activity in cholinesgic nerve transmission. Apart from that they also inhibit non-specific esterases and during this proces they themselves are inactivated. (Metcalf, 1955; Patton, 1961; Plopp and Bigley, 1961). This involves deactivation through phosphorylation of the active enzyme centres.

Malathion also seems to interfere with energy substrate metabolism. According to O'Brian (1957) malathion may have its toxic effect against insects through interferring with carbohydrate metabolism. Depletion of glucose has been reported in *Blattella germanica* as a result of malathion poisoning by Mansingh (1965) also. Recently studies about the effect of different insecticides on a variety of insects have been carried out by various workers like Agosin *et al.*, 1965; Hadaway, 1972; Moore, 1980; Saleem and Shakoori, 1986 and many others.

The study of mode of action of various biologically toxic substances is of primary importance if we want to control the hazards caused by insect pests. The present work was undertaken to study the effect of malathion exposure for different durations on some metabolites of the mango mealy bug, *Drosicha stebbingi*.

MATERIALS ANE METHODS

Collection and maintenance of the mango mealy bugs

The mango mealy bugs, *Drosicha stebingi* were collected from the trunks and branches of the mulberry trees in the vicinity of New Campus, Punjab University, Lahore. They were found abundantly during the months of March, April and May. Only females were collected directly in jam jars using a small soft camel brush and foreceps. They were sorted out in the laboratory according to their size and kept separately in glass containers covered with muslin. These bugs were maintained on small mulberry shoots and leaves at 30°C, 15 hours photoperiod and relative humidity ranging from 60 to 65 percent. Adult females with the length and width range of 1.5 cm to 1.8 cm and 0.8 cm to 1.0 cm, respectively, and with a mean weight of 0.50 gms were selected for these experiments. These criteria were chosen for the experimental insects as it was difficult to determine the age of the females thus collected.

Toxicant used

Malathion used in the present study was obtained from M/s Jaffer Brothers, (Pakistan) Limited, The Mall, Lahore. For the present experiments its 50 ppm and 100 ppm solution were prepared by dissolving it in acetone.

Exposure to insecticide

Pure acetone in equal amount was used for control experiments. For the application of the insecticide and acetone treatment a group of 3 mealy bugs was transferred in petridishes with a 4" diameter. 1.3 ml of the respective insecticide concentration and pure acetone was given to each such group separately. The total amount of insecticide in the case of 100 ppm was 5.143 and in the case of 50 ppm was 2.571 mg. The effect of insecticide was analysed for different exposure times. The female bugs were exposed to each dose for 4, 8, 12 and 16 hours, respectively. Three replicates were used for each test dose and three for the controls. They were provided with mulberry shoots of equal size (in area) and weight.

Control insects were of the following three categories:

1. Insects without any food or acetone

2. Normal feds

3. Normal feds + acetone treated

The glass lids of the petridishes were kept tilted a little so as to provide a small amount of ventilation to the insects. All these experimental insects were kept under the previously mentioned contions.

Total proteins were estimated by Biuret method (see Henry, 1964), while the total body glucose was estimated by O-toluidine method of Hartel *et al.* (1969).

RESULTS

The total body proteins and glucose were quantitatively estimated in the female mango mealy bugs after exposing them to malathion for 4, 8, 12 and 16 hours respectively. 100 ppm and 50 ppm solutions were used for this purpose. Effect of starvation and pure acetone was also studied so that it could not be confused with the changes produced due to the treatment with malathion. Normal-fed females without any sort of treatment were also studied to be compared with the above mentioned experimental insects.

Changes in the total protein contents (Table I)

The protein contents of the normal-fed adult females of the mango mealy bugs were 75-75 μg/mg after the 4th hour of their laboratory life. They had increased 125% by the end of 8 hours and had reached the level of 114.26 μg/mg. After that the contents declined and fell down to 67.70 μg/gm and 10.57 μg/mg at 12 and 16 hours respectively. Increase of protein contents was observed at its highest at 8 hours of their life, followed by a 40% decrease at 12 hours as compared to the 8th hour of their laboratory life. Protein contents were still 33%, high. At the 16th hour, they were 5 times less as compared to the 4th hour, 12 times as compared to the 8th hour and nearly 7 times as compared to the 12th hour of their laboratory life.

The effect of starvation in these bugs also showed the same pattern of increase and decrease during their laboratory life. These contents were 48.34 μg/mg after the 4th hours of their laboratory life. At the 8 hour they showed a

TABLE I.- CHANGES IN THE PROTEIN LEVEL IN STARVED AND MALATHION TREATED FEMALES OF *DROSICHA STEBBINGI* AT DIFFERENT TIME INTERVALS IN μg/mg.

Time (Hours)	Fed (n=3)	Straved (n=3)	Fed+acetone treated (n=3)	Fed+50 ppm Malathion (n=3)	Fed+100ppm Malathion (n=3)
04	50.75±7.02[a]	48.34±2.42	36.26±2.53	90.27±5.21***	88.56±5.38***
08	114.26±5.66	140.46±15.98	148.07±16.07	140.18±17.89	145.71±12.68
12	67.70±4.49	61.41±5.78	71.12±9.34	74.55±4.81*	63.59±3.16
16	10.57±0.28	5.30±0.60***	53.37±4.62	57.45±3.74	66.50±4.18*

[a] = Mean ± SEM
* = Student 't' test P < 0.05
*** = Student 't' test P < 0.001

190% increase and became 140.46 μg/mg. After that at 12 hours a 56% decrease took place and they fell down to 61.41 μg/mg while at 16 hours the decreased was 90% and the contents were 5.30 μg/mg.

The total protein contents of the starved females were compared with those of their comparable normal-fed controls. In the initial stage no significant quantitative change was observed in the two cases. During the 8th hour the starved insects showed a slightly high amount i.e., 8 hours old fed controls had 114.26 μg/mg while the comparable starved insects had 140.26 μg/mg protein contents. During the 12th hour of their laboratory existantce, they were almost at the same level, but at the 16th hour the fed controls had double the amount as compared to the starved insects. In the former it was 10.57 μg/mg while in the latter it was only 5.30 μg/mg.

In the nomral-fed acetone treated females the protein contents were minimum initially as compared to the above mentioned two categories of insects. They were 36.25 μg/mg but increased nearly 4 times at the 8 hour followed by a 50% decrease at the 12th hour and a further 25% depletion was observed at 16 hours. By the end of the 16th hour the total body protein contents were 5 times greater than in the normal-fed controls and 10 times as compared to the starved insects.

Insects treated with 50 ppm and 100.0 ppm solutions of malathion at the end of 4 hours had 90.27 μg/mg and 88.56 μg/mg protein contents respectively. 8 hour exposure to these solutions resulted in no significant change when compared to the fed-acetone treated insects of comparable age. On the other hand, the treated insects body proteins showed a very marked increase as compared to the normal-fed and starved insects. It was 6 times more in the former when compared to the 100 ppm treated and 5 times to 50 ppm treated. In the latter this was 12 times when compared to 100 ppm treated and 10 times when compared to 50 ppm treated. The treated insects showed a significant rise when the exposure time was increased from 4 to 8 hours in the case of both the concentrations. But when this time was increased upto 12 hours, there was a significant decrease in the protein contents. Prolongation of the exposure time to 16 hours showed no significant change statistically, although in the case of 50 ppm it come down 22% but in 100 ppm treated there was almost no change.

Glucose contents (Table II)

The glucose content of the normal-fed adult females of the mango mealy bugs after the 4th hour of their laboratory life was 7.93 µg/mg, it increased to 44.60 µg/mg at 8 hours and to 84.20 µg/mg at 12 hours. After that a decline started and at 16 hours it had dropped to 23.41 µg/mg, but still it was nearly 3 times higher as compared to the initial amount.

TABLE II.- CHANGES IN THE GLUCOSE LEVEL IN STARVED AND MALATHION TREATED FEMALES OF *DROSICHA STEBBINGI* AT DIFFERENT TIME INTERVALS IN µg/mg.

Time (Hours)	Fed (n=3)	Straved (n=3)	Fed+acetone treated (n=3)	Fed+50 ppm Malathion (n=3)	Fed+100ppm Malathion (n=3)
04	7.93±0.74[a]	3.14±0.18**	10.84±0.55	29.61±1.50***	22.50±2.17**
08	44.60±3.48	46.88±3.66	40.63±2.41	51.01±3.47	52.63±2.48*
12	84.20±10.58	78.20±6.59	4.50±0.41	10.08±0.94**	15.40±0.20***
16	23.41±3.38	13.53±1.15	48.20±1.16	191.89±12.58***	186.38±25.29**

a = Mean ± SEM
* = Student 't' test $P < 0.05$
** = Student 't' test $P < 0.01$
*** = Student 't' test $P < 0.001$

The starved females showed the same pattern of increase and decrease during their laboratory life, but the glucose level was almost half as compared to the normal-fed after 4 hours of the laboratory life. The increased content after 8 hours come up to the same level as the fed bugs, but compared to the 4 hours starved bugs this increase was almost 15 times. At 12 hours the increase was again significant but at 16 hours of their laboratory life the glucose level had dropped considerably. This drop in the level was almost 85% when compared to the 12 hours starved bugs. But when compared to the normal-fed the level of glucose content was almost half at 16 hours of their laboratory life.

In the normal-fed acetone treated females the glucose level was highest initially as compared to the above mentioned two categories of insects. It was 10.84 μg/mg at the 4 hour stage and increased nearly 4 times at 8 hours, but then it had decreased 10 times at 12 hours, followed by a sudden rise which led to 11 times increase at 16 hours (from 4.50 to 48.21 μg/mg). At 8 hours all these three categories showed almost the same glucose level. At 12 hours the level was lowest in the normal-fed acetone treated insects, but at 16 hours these acetone treated insects showed the highest value.

Insects treated with 50 ppm concentration of malathion showed considerable increase in glucose contents when exposed for 4, 12 and 16 hours, but at the 8th hour of exposure almost no change was observed when compared to its corresponding normal-fed acetone treated controls. Exposure to 100 ppm solution of the insecticide also showed considerable increase of glucose content initially but at 12 hours a decrease in the content was noted when compared to the corresponding normal-fed controls and starved insects. On the other hand, the glucose content showed an increase in vlaue when compared to acetone treated fed-controls. As table 2 shows in the former it is 15.40 μg/mg while in the latter it is 4.50 μg/mg. 16 hours exposure to both the concentrations showed a comparable increase.

DISCUSSION

Malathion poisoning and starvation had variable effects on the total body proteins and glucose when compared to their normal-fed and acetone treated normal-fed controls. In both the controls the protein level increased up to the 8th hours and then it decreased significantly and was lowest in 16 hours old bugs. Towards the end of their laboratory life the normal fed controls had 5 times less proteins than acetone treated controls. The acetone must have somehow stimulated the utilization of proteins towards the end. The effect of aging on protein synthesis is variable and in many insects an initial rise follows a subsequent decrease as has been discussed by Rockstein and Miquel (1973).

Effect of starvations was also studied so that a compa· ison could be made among the two controls and malathion treated insects. The prolongation of starvation was very important, because both the protein and glucose contents increased initially. In the case of proteins the contents increased only up to 8th hour of their laboratory life, and then a rapid depletion occurred, after that until at 16th hours of their life the amount was nearly 28 times less than what was found at the 8th hour of their laboratory life. This indicated an initial synthesis but a later utilization without any simultaneous synthesis. In the case of glucose the contents reached their peak at 12 hours but at 16 hours a drastic decrease had occurred, thus showing an accumulation of glucose as a result of degradation and its utilization later on when the level of other energy substrates like proteins has fallen down.

During starvation the organisms have to depend upon their own reservoirs for differnt life activities which leads to drastic molecular readjustments (Parrilla, 1978a, 1978b; Shakoori and Haq, 1982) and changes in their energy substrates. This has been the case as found in the present study also. Mushtaq and Shakoori, (1986) working on the 6th instar larvae of *Tribolium castaneum* have reported the depletion of glucose contents starting immediately after starvation but in these mealy bugs an initial increase is followed by a decrease at the and, although Mwangi and Goldsworthy (1977) have reported an increase in haemolymph carbohydrates of the adult females of *Locusta migratoria* as a result of starvation. On the other hand, Hill and Goldsworthy (1965) have reported no change in haemolymph carbohydrates in 5th instar *Locusta* larvae under starved conditions. These differences in the relative concentration of glucose after starvation seem to depend upon the fact that what preferences an insect has concerning the utilization of its energy substrates.

In the female of these mealy bugs proteins underwent an initial increase and a later decrease but Mushtaq and Shakoori (1986) have reported no change in the protein level in the 6th instar larvae of *Tribolium castaneum*. This also gives support to the argument that different insects utilize different energy substrates under stress conditions like starvation and this can change with the developmental stage also.

Malathion was choosen as the test insecticide because it does not inhibit the Krebs' cycle (O'Brien, 1957). It also enhances the "*in vitro*" activity of transaminases in some insects like the American roach as discussed by Mansingh (1965). When insects were exposed to both the test dozes the protein contents increased upto the 8th hour and then a decrease was noted, and the lowest protein level was observed at the 16th hour in the case of 50 PPM treatment but in the 100 PPM treated insects almost the same amount of protein level was observed at the 12 as well as the 16th hour of their laboratory existence. The importance of the body proteins as one of the most important components of the biochemical milieu of the organism has been recognised universally and discussed by several authors. Ahmad *et al*. (1985) have also discussed its capacity to bind with foreign compounds including insecticides like dieldrin in *in vitro* and *in vivo* studies. This binding would reduce the amount of toxicant available for specific action, although may form a reservior (Just as when they are stored in fats) from which a supply of toxicant may be drawn over a prolonged period.

Depletion of several haemolymph amino acids like glycine, glutamate, proline and glutamine has been reported in the poisoned and paralysed roaches. The magnitude of depletion of these amino acids was directly dependent upon the degree of intoxication in this case. Corrigan and Kearns (1963) have also observed reduction of glutamine and proline of the blood of DDT poisoned American roaches. The correlation between the degree of intoxication and the reduction of amino acids indicates that the depletion of the amino acids was an indirect effect of poisoning. Malathion intoxication has been reported to increase the oxygen consumption (Harvey and Brown, 1951). These authors explain the depletion in these roaches as a result of their utilization to augment the usual substrate supply for the Krebs' TCA cycle, which is not inhibited by the insecticide (O'Brien, 1957). Thus the depletion of the amino acids was mainly a consequence of higher metabolic activity in the poisoned roaches in this case. In the present case the decrease of protein contents after 8 hours exposure showed that the binding of malathion with the protein contents of the body made them insoluble in saline, and in some unknown way it accelerated the synthesis of soluble proteins. This may be the reason for their depletion in the present study. The formation of stable complexes with the plasma lipoproteins has also been reported by Agostin *et al*. (1965).

The glucose contents showed considerable changes as compared to the protein contents after the treatment with malathion. Insects treated with 50 PPM showed 70% increase at the 16th hour as compared to the 4th hour of their laboratory existence. After 12 hours of exposure it decreased 5 times but then it again increased nearly 20 times at the 16th hour. Similarly treatment with 100 PPM of malathion showed 130% increase in the value of the glucose contents at the 8th hour followed by a decrease of 70% after 12 hous of exposure to the insecticide. After 16 hours exposure a great increase (160%) was noted. The present work showed that treatment with different concentrations of malathion resulted in increased protein and glucose contents towards the end of the experiment.

REFERENCES

AGOSIN, M., ARAVENA, L. AND NEGHIME, A., 1965. Enhanced protein synthesis in *Triatoma infestans* treated with DDT. *Exp. Parasitol.*, **16**: 318.

AHMED, M.S., ALI, F.A. AND SHAKOORI, A.R., 1985. Effect of Dieldrin on the whole body protein contents of *Pariplaneta americana*. *Pakistan J. Zool.*, **17**: 105-109.

CORRIGAN, J.J. AND KEARNS, C.W., 1965. Amino acid metabolism in DDT poisoned American roaches. *J. Insect. Physiol.*, **9**: 1-2.

HADAWAY, A.B., 1972. Toxicity of insecticides to tsetse flies. *Bull. WHO*, **46**: 353.

HARTEL, A., HELGER, R. AND LANG, H., 1969. A method for determination of glucose. *Z. Klin. : Chem. Kling. Biochem.*, **7**: 188.

HARVEY, G.T. AND BROWN, A.W.A., 1951. Effects of insecticides on the rate of oxygen consumption in *Blattella*. *Can. J. Zool.*, **29**: 42-53.

HENRY, R.J., 1964. *Clinical chemistry*, Principles and techniques. Harper and Row, New York.

HILL, L. AND GOLDSWORTHY, G.J., 1965. The utilization of reserves during starvation of larvae of migratory locust. *Comp. Biochem. Physiol.*, **36**: 61-70.

MANSINGH, A., 1965. The effect of malathion on the metabolism of amino acids in the German cockroach *Blattella germanica*. *J. Insect. Physiol.*, **11**: 1389-1400.

METCALF, R.L., 1955. *Organic insecticides*. Interscience, New York, pp. 77.

MOORE, R.F., 1980. Behavioral and biological effects of NRDC-161 as factors in control of the Boll weevil. *J. econ. Entomol.*, **73**: 265.

MWANGI, R.W. AND GOLDSWORTHY, G.J., 1977. Interrelationships between haemolymph lipid and carbohydrate during starvation in *Locusta*. *J. Insect. Physiol.*, **23**: 1275-1280.

O'BRIEN, R.J., 1957. The inhibition of Cholinesterase and Succinoxidase by malathion and its isomer. *J. econ. Ent.*, **49**: 484-490.

PARILLA, R., 1978. Flex of metabolic fuels during starvation in the rat. *Pfluegers Arch.*, **374**: 3-7.

PATTON, R.L., 1961. Haemocytes in the detoxication of Parathion. *Ann. Ent. Soc. Amer.*, **54**: 696-698.

PLAPPE, F.W. AND BIGLEY, W.S., 1961. Inhibition of fly esterase by Parathion and Malathion. *J. Econ. Ent.*, **54**: 103-108.

ROCKSTEIN, M. AND MIQUEL, J., 1973. Aging in insects. In: *Physiology* of Insects, 1: 371-501. (Ed. Rockstein, M.) Academic Press, New York, London.

SALEEM, M.A. AND SHAKOORI, A.R. 1986. Biochemical effects of sublethal dose of Cypermethrin on the 6th instar larvae of *Tribolium castaneum* (Herbst.) *Archives of Insect Biochemistry and Physiology*, **3**: 447-455.

SCHRADER, G., 1952. The development of new insecticides based on organic fluoride and phosphorous compounds. *Monograph*, **62**: *Angew. Chem*.

SHAKOORI, A.R. AND HAQ, R., 1982. Effect of starvation and refeeding on the blood and liver of domesticated rabbits. *Acta Physiol. Latinoam*, **32**: 131-145.