Effect of cypermethrin on biochemical aspect of land slug Laevicaulis alte.

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ABSTRACT: ***The toxic compounds act as one of the stresses to organism and organism responds to it by developing necessary potential to counter act that stress. The slugs Laevicaulis alte were exposed to Lc_{10} and LC_{50} values for 96 hours exposure to assess the impact of pesticides like cypermethrin during pre reproductive periods. The Protein,Glycogen and Lipid content from different body parts like mantle, hepatopancreas and foot were done from the sluge from control and Lc_{10} and Lc_{50} groups. The total protein,Glycogen and Lipid content was found to be depleted from all the body parts. So in this Study an attempt has been made to find out the impact of Cypermethrin on Biochemical Content like Protein, Glycogen and Lipid of Laevicaulis alte.***

KEYWORDS: Biochemical, Laevicaulis alte., Cypermethrin.

INTRODUCTION

Environmental pollution by pesticides is on a steady rise following their ever increasing utilization in modern science and technology. Different pesticides are likely to affect biological system in different ways according to their respective chemical properties.

A number of studies have been carried outon the pesticide toxicity in aquaticgastropods. (Ramana Rao and Ramamurthi, 1978; Muley and Mane,1989; Magare, 1991;Chaudhari and Lomte, 1992, Jadhav et. al.,1995; Lomte and Waykar, 2000; Ahirrao et al.,2004; Ahirrrao and Kulkarni, 2005; Ahirrao and Khedkar, 2012; Ahirrao andBorale, 2013And Borale and Ahirrao, 2013.The toxic compounds act as one of the stresses to organism and organism responds to it by developing necessary potential to counter act that stress.The biochemical changes occurring in the body indicates the stress (Mayes, 1977).A number of changes in biochemical parameters of aquatic organisms due to pesticide toxicity have been noted by several investigators (Subhadradevi, 1985; Muley and Mane, 1990).It has also been reported that acute and chronic toxicities due to pesticide, caused biochemical alterations in organs involved in detoxification metabolism (Dikshit *et al.*, 1975; and Sastry and Sharma, 1979).Carbohydrates are the major source of energy for vital activities of the organisms.Glycogen is the chief carbohydrate of the tissue just as glucose is the blood and other body fluids. Kalarani*et al.* (1984) reported the impact of endosulfan toxicity on certain aspects of carbohydrate metabolism of the fresh water snail, *Pila globosa.* Kulkarni *et al.* (2001) have worked on effect of sublethal concentration of Cypermethrin on organic constituents of snail *Macrochlamys indica.*

Proteins are the most abundant chemical compounds of the organisms and are the important class of biological macromolecules. Many workers reported the effects of pesticides on protein metabolism (Shakoori et al., 1976; De Bruin, 1976; Schmidt-Nielson, 1977; Ansari and Gupta, 1980; Suryaprakash *et al*., 1981; Saxena *et al.*, 1981; Krishnamurthy, 1981; Reddy, 1981.Lipids are responsible for a variety of functions in molluscs. Ramana Rao and Ramamurthi (1980) reported changes in total lipids under Sumithion stress in snails.

MATERIAL AND METHODS

Fresh specimens of *Laevicaulis alte* were collected from the cultivated fields and gardens in and around Kalwan area of Nashik district and were maintained under laboratory conditions in troughs with sufficient moist soil. They were fed once in a day on carrot, potato or calatropis leaves. They were kept in laboratory for 3-4 day under laboratory conditions for acclimatization. Healthy and mature animals were of same size, 8 to 10 cm in length and 2-3 cm in width were chosen for the experiments.

The slugs were exposed to LC_{10} and LC_{50} values for 24, 48, 72 and 96 hours exposure to cypermethrin and during pre-reproductive periods. The different body parts like mantle, hepatopancreas and foot are separated and dried to powder in a hot air oven at 65 to 80°C for 24 hours. The biochemical analysis from body parts like mantle, hepatopancreas and foot were done from the slug, *L. alte* belonging to the control, LC_{10} and LC_{50} groups. Every time samples from three different individuals belonging to the above groups were used to estimate total protein (Lowry *et al*., 1951), glycogen (Kemp *et al.*, 1954), lipid (Barnes and Blackstock, 1973).

Glycogen:

The glycogen content in the tissues was estimated by the method of Kemp *et al.* (1954). A known amount of tissue powder was taken and homogenized in 5 ml of TCA solution (5% Trichloroacetic acid + 0.1 % silver sulphate solution) and kept in boiling water bath for 15 minutes and allowed to cool. The original volume of TCA solution was restored and centrifuged for 10 minutes at 3000 rpm. 1 ml of supernatant solution was taken and 6 ml of concentrated H_2SO_4 was added to it. It was kept in a boiling water bath for 6.5 minutes and cooled to bring it at room temperature. The optical density was recorded using 520 nm wavelength on the spectrophotometer. The amount of glycogen was calculated by multiplying the glucose value by the factor 0.927. The results were expressed in percentage of glycogen by using regression equation. Pure D-glucose (AR grade) was used as a standard. Simultaneously, blank was also run with distilled water, and the amount of glycogen was calculated from the standard graph regression equation and represented as mg of glycogen / gram dry weight.

Proteins:

Total protein content was determined by the method of Lowry *et al.* (1951). For the estimation of protein, 1 % tissue homogenate was prepared in 10% TCA (10%Trichloroacetic acid) and centrifuged at 3000 rpm for 15 minutes. To the residue, known volume of 1 N sodium hydroxide was added. To 1.0 ml of aliquot 5 ml of reagent containing copper sulphate, sodium carbonate, sodium potassium tartarate and NaOH were added. The mixture was kept at room temperature for 10 min. Then 0.5 ml of diluted (1 N) Folin phenol reagent was added and colour developed was read at 620 nm in spectrophotometer after half an hour. Simultaneously a blank was also run with distilled water. The amount of protein was calculated from the standard graph and represented as mg protein/gram dry weight of the tissue. Here pure BSA (Bovine serum albumin) is used as standard.

Total Lipids:

The total lipids content in the tissues was estimated by the method of Barnes and Blackstock (1973).

The known amount of tissue powder was taken, homogenized in 5 ml of Folch's mixture (2:1 chloroform : methanol) and the tubes were kept in boiling waterbath with constant shaking till the solvent begins to boil. The tubes were removed with constant shaking for 5 minutes, cool to room temperature and centrifuged for 10 minutes at 3000 rpm. The supernatant was taken into another tube and evaporated to dryness in a boiling waterbath. 1.0 ml of concentrated H_2SO_4 was added and kept in the boiling water bath for 15 minutes and cooled to bring it at room temperature. 5.0 ml vaniline reagent was added and after 15 minutes the optical density was recorded using 660 nm wavelength in the spectrophotometer. The results were expressed as mg/gm dry weight of the tissue. Here AR grade cholesterol was used as a standard.

RESULTS

This study was done by analyzing the glycogen protein, free amino acid, lipid, ascorbic acid and cholesterol contents from the body parts like Mantle, hepatopancreas and foot of land slug *Laevicaulis alte*. The content expressed in terms of mg/100mg body parts on dry weight basis from respective control LC_{10} and LC_{50} groups in pre-reproductive (March to Jun)showed significant changes due to pesticides. The results are expressed in fig(1-3).

1) Glycogen (Fig. 1): During pre-reproductive period, the glycogen content in control group was high from mantle (14.7661 ± 0.0971) followed by hepatopancreas (16.7527 ± 0.145) and foot (4.2926 ± 0.0162). During reproductive period the content in control group was high in mantle (27.9539 ± 0.5138) followed by hepatopancreas (15.0199 ± 0.0421) and foot (4.2769 ± 0.0377). In cypermethrin (Table 3; Fig. 3) in LC_{10} compared to control the content in pre-reproductive period decreased from all the body parts. This decrease was more from hepatopancreas (34.0494%; $P < 0.005$) followed by mantel (17.1142; $P < 0.001$) and foot (1.6484%; $P < 0.05$). In LC_{50} group also the content decreased from all the body parts. This decrease was more form hepatopancreas (53.0571%; $P < 0.001$) followed by mantle (32.6019%; $P < 0.001$) and foot (15.6928%; $P < 0.01$). The contents when compared between LC_{10} and LC_{50} in LC_{50} group it was significantly decreased from all the body parts.

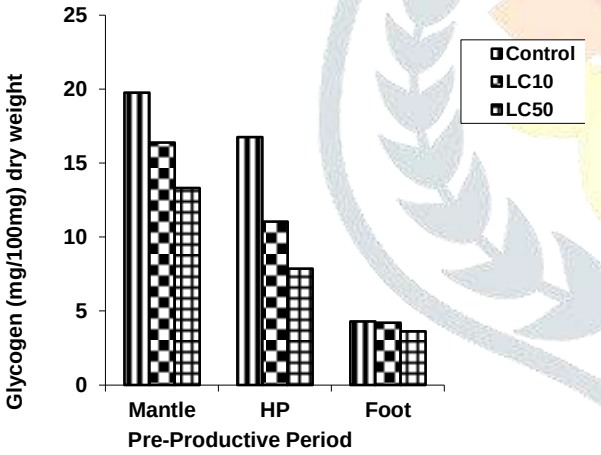

Fig. 1: Changes in *Glycogen* content from different body parts of *Laevicaulis alte* after exposure to LC_{10} and LC_{50} values of pesticide, *Cypermethrin* at 96 hours during pre-reproductive period. (*-P<0.05; **- P<0.01, ****- P<0.001 values compared to control; □- P<0.05, □□- P<0.01, □□□- P<0.001 values compared to LC10 group)

2) Total Proteins (fig 2): The total protein contents from control group in pre-reproductive period of Land slug *Laevicaulis alte* was high from foot (12.6003 ± 0.0889), followed by hepatopancreas (12.5457 ± 0.1929) and mantle (11.3184 ± 0.1929) In reproductive period it was higher in foot (17.9868 ± 0.051) followed by hepatopancreas (16.7731 ± 0.0668) and mantle (12.9957 ± 0.1350). On the other hand in post-reproductive period it was again high in foot (17.2503 ± 0.1265) and low in hepatopancreas (4.6365 ± 0.0841), while in mantle it was 8.2911 ± 0.0695 mg/100mg of protein content.

In cypermethrin (Table 7; Fig. 7), during pre-productive period the content in LC_{10} compared to control decreased from all the body parts. This decrease was more from mantle (18.1660%; $P < 0.001$) followed by hepatopancreas (2.4712%; N.S.) and foot (1.5793%; N.S). In LC_{50} group, it was decreased in hepatopancreas (19.1819%; $P < 0.001$) and mantle (5.9832%; $P < 0.01$) but increased in foot (9.8830%; $P < 0.01$). The contents when compared between LC_{10} and LC_{50} groups, in LC_{50} groups it increased significantly in mantle (14.8872%; $P < 001$) and foot (11.6463%; $P < 0.01$) but decreased significantly in hepatopancreas (17.1336%; $P < 0.001$).

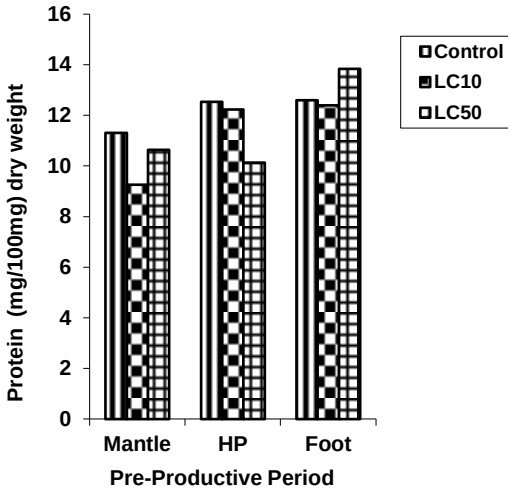

Fig. 2: Changes in *Protein* content from different body parts of *Laevicaulis alte* after exposure to LC_{10} and LC_{50} values of pesticide, *Cypermethrin* at 96 hours during pre-reproductive period. (*-P<0.05; **- P<0.01, ****- P<0.001 values compared to control; □- P<0.05, □□- P<0.01, □□□- P<0.001 values compared to LC10 group)

4)Lipids (fig 3): The Lipid contents from control group in land slug *Laevicaulis alte* during pre-reproductive period was higher in hepatopancreas (7.4322 ± .0.706) followed by foot (5.9092 ±.0.1412) and mantle (4.0652 ± 0.0281) during reproductive period it was higher in hepatopancreas (10.3162 ± 0.7060) and lower in mantle (1.8863 ± 0.1485). In foot it was 7.0549 ± 0.1623 mg/100mg. During post reproductive period the content was higher again in hepatopancreas (9.9787 ± 0.1749) followed by foot (8.5922 ± 0.353) and mantle (5.1686 ± 0.706).
In cypermethrin (Table 15; Fig. 15) during pre-reproductive period the lipid content in LC_{10} group decreased from mantle (10.8126%) and foot (8.1755; P < 005) but from hepatopancreas it increased non-significantly (2.5524%). In LC_{50} group compared to control and compared to LC_{10} group the content decreased significantly from all the body parts.

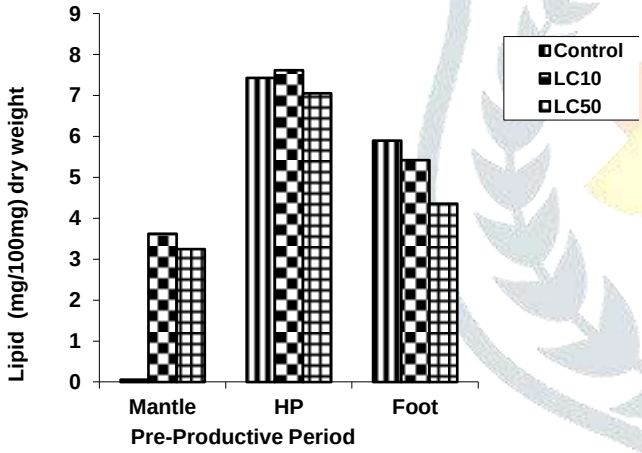

Fig. 3: Changes in *Lipid* content from different body parts of *Laevicaulis alte* after exposure to LC_{10} and LC_{50} values of pesticide, *Cypermethrin* at 96 hours during pre-reproductive , period. (*-P<0.05; **- P<0.01, ****- P<0.001 values compared to control; □- P<0.05, □□- P<0.01, □□□- P<0.001 values compared to LC10 group)

REFERANCES:

[1] ***Baldwin, E.and Needham** 1934. Biochem. J. **29**:1372-1392.
[2] ***Baldwin, E.** 1935. Biochem. J., **29,** 1538-1546.
[3] **Gilboe and Williams, J.N.** 1956. Proc. Soc. Exp. Biol. Med., 91:537.
[4] **Ghosh, R.C.** 1961. Observations on the digestive enzymes and cellulolytic bacteria of the giant land snail, Achatinafulica and their occurrence in the gastropoda. *Proc. Zool. Soc. London*, Pp137.
[5] **Birk, Y.K., Harpz, I., Ishaaya, I. and Bondi, A.** 1962. Studies on the proteolytic activity of the bettle, Tenebrio and Trilobium. J. Insect. Physiol. **8**:417-429.
[6] **Bailey, N.T.**1965. Statistical methods in biology. *ELBS* English University Press, London.
[7] **Brockerhoff, H., Stewart, J.E. and Tacreiter, W**. 1967. Digestion of triglycerides by lobster, *Homarusamericanus*. Can. J. Biochem. **45**:421-422.
[8] **Campbell, J.W., Speeg, K.V. and Vergl, Z.** Jr. 1968. Tissue distribution of enzymes of arginine biosynthesis in terrestrial snails. Physiol. **61**:164-175.
[9] **Biswas**and**Ghosh, K.C.** 1968. Elaboration of enzymes in the digestive gland Viviparusbengalensis (Mollusca: Gastropoda) during the first hour of digestion. J. Zool. Lond., **156**:325-332.
[10] **Campbell, J.W.** 1973. In comparative animal physiology. (Ed. Prosser, C.L. and W.B. Saunders Company, Philadelphia).
[11] **Coley, R.M. and Jansen, R.G**. 1973. Effects of parathion on liposis in isolated rate adipose tissue cells. Lipids, **8**:43-44.

[12] Ghosh, A. and Konar, S.K. 1973. Effect of phosphamidon on the activity of the digestive enzyme invertase in the cat fish Clariasbatrachus (Linn.). *J. Inland Fish. Soc. India*, **5**:129-130.
[13] Coppage, D.V., Mathews, E., Cook, G.H. and Knight, J.C. 1975. *Pestic Biochemistry. Physiology*, **5**:536.
[14] Hinton, D.E. and Koehing, J.C. Jr. 1975. Acid phosphatase activity in subcellular fractions of fish liver exposed to methyl mercuric chloride. *Comp. Biochem. Physiol.*,**50**(B): 621-628.
[15] Harper, H.A., Rodwell, V.W. and Mayees, P.A. 1978**.** A review of physiological chemistry, 17th Edition, Lange Medical Publications, California, U.S.A.
[16] Banerjee, S.K., **DastidarS.G**., **Mukhopadhyay, P.K**. and **Dehadra, P.V**.1978. Toxicity of cd: A comparative study in the air breathing fish Clariasbatrachus and in the non-breathing one, Tilapia mossambica. *Indian J. Exp. Biol*., **16**:1275-1277**.**
[17] Barhanpurkar, S.N. 1979. *Some physiological aspects of armyworm Pseudaletiaseparata* (H.). Ph.D. Thesis, Marathwada University, Aurangabad, M.S., India.
[18] Bhagyalakshmi, A. 1981. *Physiological studies on the fresh water crab Oziotelphusasenexsenex (Fab) in relation to pesticidal impact*. Ph.D. Thesis, ShriVenketeshwara University, Tirupati, A.P.
[19] Bodhke, M.K. 1983. *Effect of some pesticidal pollutants on the physiology of Barytelphusacunicularis.* Ph.D. Thesis, Marathwada University, Aurangabad (M.S., India).
[20] Chaudhari, T.R. 1988. *Pesticidal impact on some physiological aspects of Bellamya (Viviparus) bengalensis.* Ph.D. Thesis, Marathwada University, Aurangabad (M.S., India).
[21] Geethanjali, K. 1988**.***Alterations in nitrogen metabolism during BHC intoxication in fresh waterfish, Channapunctatus.* Ph.D. Thesis, Osmania University, Hyderabad, A.P. India.
[22] Bhamre, P.R. 1993. Impact of pollutants on some physiological aspect of the fresh water bivalve, Parreysiafavidens. Ph.D. Thesis, Marathwada University, Aurangabad (M.S., India).
[23] Chaudhari, T.R. and Lomte, V.S. 1992. Effect of pesticides on glycogen level and amylase activity in digestive gland of freshwater snail, Bellamya (Viviparus) bengalensis. *Environ. And Ecology*, **10**(1): 188-190.
[24] DeshmukhMeenakshi, B. 1995. *Some physiological studies of Parreysiacorrugata in relation to pollutant stress*. Ph.D. Thesis, Dr. B.A. Marathwada University, Aurangabad (M.S., India).
[25] Gastron, S. and Campbell, J.W. 1996. Distribution of arginase activity in molluscs. *Comp. Biochem. Physiol.*,**17**:259-270.
[26] AhirraoK.D.2014.Body Indices of the Fresh Water Snail. ThiaraLineata.*SRJIS*,**2**/11.
[27] GhanbahadurAG.,GhanbahadurG.R.GaneshwadeRM.Wagh S.P.2015.Histopathological effect of organochlorideendosulfan on gills of larvivorous fish *Rarboradaniconius.Bioscience Discover**6***(2):121-124**.**
[28] AhirraoVD,Phand DL 2017. Impact of fenvalerateOn Whole Body of Freshwater Snail *BellamyaBengalensis*. *IJAR***3(**1): 334-336.