Effect of Endosulfan on Glycogen and Protein Content of Gastropod Laevicaulis Alte

D.B. Goswami

P.G. Department of Zoology, K. V. N. Naik Arts, Commerce and Science College Nasik. Maharashtra, India
Corresponding Author Email ID: dbgoswami.1967@gmail.com

Abstract

The effect of endosulfan pesticides was carried out in batch laboratory experiment for the study of its interactions with glycogen and protein of terrestrial pulmonate gastropod. The *Laevicaulis alte* was exposed for LC_{10} and LC_{50} concentration for 24 to 96 hours. Bioassay test was carried out with the body part such as mantle. hepatopancreas and foot.

Proteins are most abundant chemical compounds of organisms and are the important class of biological macromolecules. They not only serve as fuel to yield energy but also play the vital role in every aspect of structural and functional characteristics of the organism. In this study, the attempt was made to investigate the effect of endosulfan on protein and glycogen content of land slug gastropod *Laevicaulis alte*

KEYWORDS: Endosulfan, Gastropods, LC_{10}, LC_{50}, Glycogen, Protein

---

INTRODUCTION

The toxic compounds act as one of the stresses to organism and organism responds to it by developing the necessary potential to counteract that stress. The biochemical changes occurring in the body indicates the stress (Barton *et al.,* 1991). 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 a pesticide, caused biochemical alterations in organs involved in detoxification metabolism (Dikshit et al., 1975; and Sastry and Sharma, 1979; Jayanthi P,Prabhu S (2015) Yekeen TA (2009). Yekeen, Taofeek A. and Fawole, Olatunde O (2011.); Chaudhari, T.R. and Lomte, V.S. (1992). . In recent times, investigations on the physiological and biochemical responses of the mollusks to environmental agents have been expanded significantly(Chinoy, N.J. et.al(1977).. Studies on the tissue-specific on time-dependent alterations in the metabolites on enzymes have provided clues to mechanisms of toxicity.(Ahmed Kabeer et.al, 1978) Analysis of biochemical profiles in the toxicant-treated animals has provided indices of chemical toxicosis. Amongst the several molecules available in the cells, carbohydrates play an important role in the cellular processes. Metabolic pathways of carbohydrates provide

energy and essential structural and functional components of cells. Bicknell, F. and Prescoot, F. (1953); Bragmann, C.G. and Brown, G.W. (1974). . Quantitative measurements of metabolites and enzymes related to carbohydrate metabolism in the tissue of animals exposed to toxicants indicate the nature of functional impairment (K.suneetha (2012). 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. Glycogen as reserve or storage carbohydrate is reversible _' converted to blood glucose and normally serves to maintain blood sugar level, when a supply of carbohydrates from intestinal ‘absorption is inadequate. Glycogen synthesis and breakdown appear to occupy a central position being controlled by extrinsic and intrinsic factors thereby altering the physiological state of the organism. (Chaudhari, T.R(1999); Chetty, A.N. (1995);

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 the laboratory for 3-4 day under laboratory conditions for acclimatization. Healthy and mature animals were of the 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 endosulfan during pre-reproductive, reproductive and post-reproductive periods. The different body parts like the mantle. hepatopancreas and foot are separated and dried to the 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 LC50 groups. Every time samples from' three different individuals belonging to the above groups were used to estimate total protein (Lowry et al., 1951) and glycogen (Kemp et al., 1954).

The values of the estimates from the replicates of three different estimates from each sample were subjected to the calculation. For each biochemical composition of the given body part, all the mean values of estimates were subjected to statistical analysis of variance. Multi range tests for comparison of the means (Campbell, 1975; Casida, J.E., 1983; Chaudhari R.D. (1994).) to find out the significant differences among the control, LC_{10}, and LC_{50} groups. The analysis is performed as above on the animals belonging to pre-reproductive, reproductive and post-reproductive periods as above.

Glycogen

The glycogen content in the tissues was estimated by the method of Kemp *et al.* (1954). Also proceed with the Zootecus sp by Chaudhari R.D. (1993). 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, the 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 l N sodium hydroxide was added. To 1.0 ml of aliquot 5 m1 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 (1N) 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 1 gram dry weight of the tissue. Here pure BSA (Boyine serum albumin) used as the standard.

RESULTS

This study was done by analyzing the Glycogen, protein, contents from the body parts like Mantle, hepatopancreas and foot of land slug *Laevicaulis alte*. The content expressed in terms of mg/100 mg body parts on the dry weight basis from respective control LC_{10} and LC_{50} groups in pro-reproductive (March to Jun) reproductive (July to September) and Post-reproductive Period (October to February) showed significant changes due to pesticides. The results are expressed in table and figures.

In endosulfan (Table 1; Fig. 4) exposed groups the glycogen content in LC_{10} group compared to control in pre. reproductive period decreased form all the body parts. This decrease on the percent basis was more form hepatopancreas (44.3337%; $P < 0.001$) followed by mantle (31.0842%; P

<0.001) and foot (10.6900%; P < 0.05). In LC_{50} group, the content decreased in hepatopancreas (61.8103%; P < 0.001) and mantle (44.0013%; P < 0.001) but in foot (15.1457%; P < 0.01) it was increased significantly. The contents when compared between LC_{10}, and LC_{50} group, in LC_{50} group, it was also decreased from hepatopancreas (31.3952%; P < 0.001) and mantle (18.7462% ; P<0.001) but in foot it was significantly increased (28.928l%; P < 0.01). In reproductive period, the content in LC_{50}, group compared to control decreased in mantle (28.1627%; P < 0.001) and hepatopancreas (9. 3096% P < 0. 001) but increased significantly 1n foot (36. 8625% P < 0. 001). In LC_{50} group it was also decreased in mantle and hepatopancreas but in foot it increased significantly.

In LC_{50} group compared to LC_{10} the content increased from all the body parts. This increase was more from mantle followed by foot and hepatopancreas. In post-reproductive period in LC_{50} group compared to control, the content decreased in mantle (30.0189%; P < 0.001) and hepatopancreas (8. 1079%; P <0.00l) but in foot (28.6808%; P < 0.001) it increased significantly. In LC_{50}, group it also decreases from mantle and hepatopancreas but increased significantly from a foot, while in a LC_{50} group compared to LC_{10} it increased in mantle and foot but decreased in hepatopancreas.

The protein content from different body parts of *Laevicaulis alte* after exposed to LC_{10} and LC_{50} values of pesticide, Endosulphan at 96 h during pre-reproductive, reproductive and post reproductive period was shown in Table No. 2 and Figure No. 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 (12.5457:1: 0.1929) and mantle (l 1.3 184 1!: 0.1929) In reproductive period

The range of endosulfan (Table 2 Fig. 2) during a pre-reproductive period the protein content in LC_{10} group decreased from all the body parts. This decrease was more from foot (1 1.6561%; P < 0001) followed by hepatopancreas (9.757l%; P < 0.001) and mantle (8.593 1%; P < 0.01). In LC50 group the content decreased significantly from hepatopancreas (19.319%; P < 0.001) and mantle (20.6915%; P < 0.001) but increased in foot (2.8770%) nonsignificantly. The contents in LC50 group compared to LC_{10}, group increased from mantle (20.6915%;P < 0.001) and foot (16.4513%; P < 0.001) but decreased from hepatopancreas significantly. From the reproductive period in LC_{10} group, the content decreased significantly from all the body parts while in the LC_{50} group the same trend is observed. In LC_{50} group compared to LC_{10} group, the content increased significantly from all the body parts.

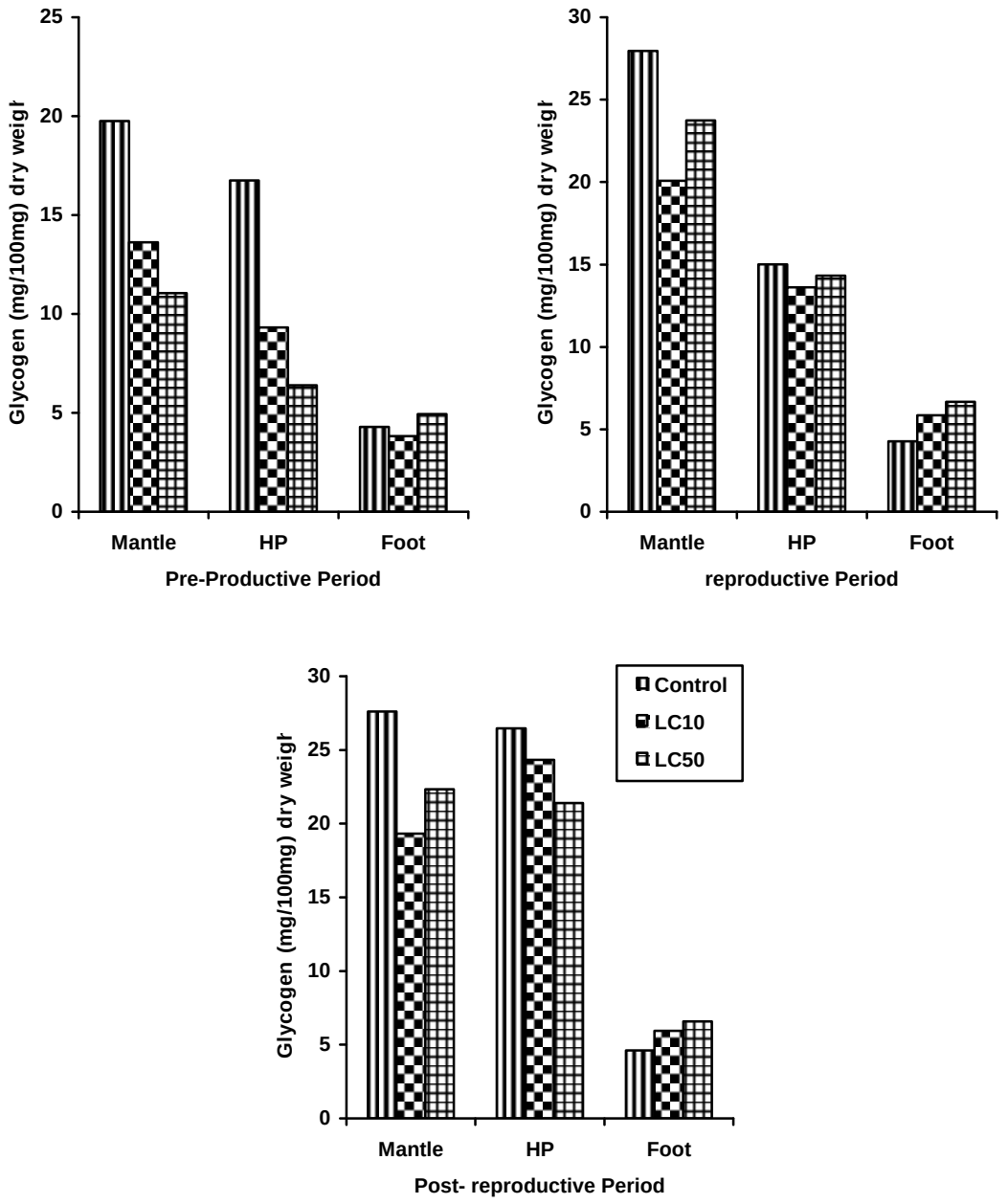

Fig: 1 Changes in the Glycogen content from different body parts of Laevicaulis alte after exposed to LC_{10} and LC_{50} values of pesticide, Endosulphan at 96 h during pre-reproductive, reproductive and post reproductive period

Table 1. Changes in the Glycogen content from different body parts of *Laevicaulis alte* after exposed to LC_{10} and LC_{50} values of pesticide, Endosulphan at 96 h during pre-reproductive, reproductive and post reproductive period.

Tissue	Pre-reproductive period			Reproductive period			Post-reproductive period		
	Control	LC_{10}	LC_{50}	Control	LC_{10}	LC_{50}	Control	LC_{10}	LC_{50}
Mantle	19.766 ± 0.0971	13.6219 ± 0.3236 31.0842%***	11.0683 ± 0.1816 44.0013%*** 18.7462%•••	27.9529 ± 0.5138	20.0806 ± 0.6319 28.1627%***	23.7472 ± 0.4661 15.0457%*** 18.2514%••	27.6102 ± 0.3532	19.3219 ± 0.7243 30.0189%***	22.3438 ± 0.4219 19.0741%*** 15.6398%••
Hepatopancreas	16.7527 ± 0.1452	9.3256 ± 0.1662 44.3337%***	6.3978 ± 0.2317 61.8103%*** 31.3952%•••	15.0199 ± 0.0421	13.6216 ± 0.2316 9.3096%***	14.3219 ± 0.1942 4.6472%** 5.1411%^{NS}	26.4677 ± 0.1942	24.3217 ± 0.3338 8.1079%***	21.4017 ± 0.1762 19.1403%*** 12.0056%•••
Foot	4.2956 ± 0.0162	3.8364 ± 0.1819 10.6900%*	4.9462 ± 0.1634 15.1457%** 28.9281%••	4.2767 ± 0.0377	5.8532 ± 0.2661 36.8625%***	6.6629 ± 0.1318 55.9753%*** 13.8334%••	4.6233 ± 0.0299	5.9493 ± 0.2132 28.6808%***	6.5846 ± 0.2423 42.4220%*** 10.6786%•

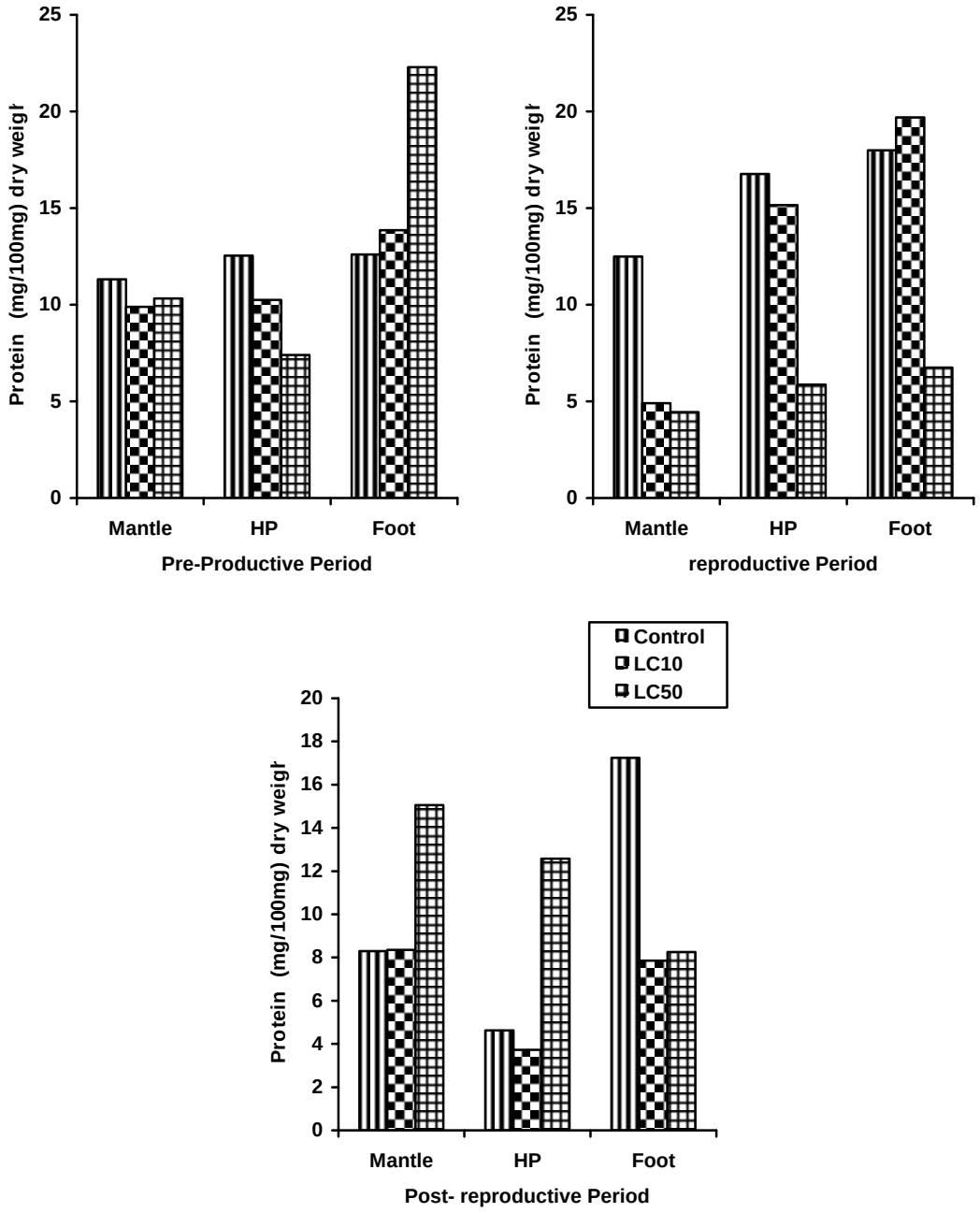

Fig. 2 Changes in the Protein content from different body parts of *Laevicaulis alte* after exposed to LC_{10} and LC_{50} values of pesticide, Endosulphan at 96 h during pre-reproductive, reproductive and post reproductive period.

Table 2. Changes in the Protein content from different body parts of *Laevicaulis alte* after exposed to LC_{10} and LC_{50} values of pesticide, Endosulphan at 96 h during pre-reproductive, reproductive and post reproductive period.

Tissue	Pre-reproductive period			Reproductive period			Post-reproductive period		
	Control	LC_{10}	LC_{50}	Control	LC_{10}	LC_{50}	Control	LC_{10}	LC_{50}
Mantle	11.3184 ± 0.1929	10.3458 ± 0.2619 8.5931%**	12.4865 ± 0.2316 10.3204%*** 20.6915%••	12.9957 ± 0.135	9.6483 ± 0.2629 25.7577%***	11.3219 ± 0.2162 12.8796%** 17.3460%•	8.9219 ± 0.3562	10.2826 ± 0.3429 15.2512%**	9.3216 ± 0.8023 4.4799%^{NS} 9.3459%^{NS}
Hepatopancreas	12.5457 ± 0.1929	11.3216 ± 0.0581 9.7571%***	10.1219 ± 0.0.2623 19.319%*** 99.9908%••	16.7731 ± 0.0668	14.2326 ± 0.3212 15.1462%***	15.0453 ± 0.0862 10.301%*** 5.7101%•	4.6365 ± 0.0841	5.9319 ± 0.2315 27.9391%***	7.5462 ± 0.4219 62.7564%*** 27.2139%••
Foot	12.6003 ± 0.0889	11.1316 ± 0.1926 11.6561%***	12.9629 ± 0.2816 2.8770%^{NS} 16.4513%•••	17.9868 ± 0.0511	15.3216 ± 0.1628 14.8175%***	17.0813 ± 0.3161 5.0342%* 11.4851%•••	17.2503 ± 0.1265	18.5465 ± 0.1265 7.5141%*	16.1942 ± 0.1239 6.1222%*** 12.6832%•••

The date regarding it also studied by Ali, S.M., Ilyas, R. and Bhusari, N.B. (1983) on fish, Channa gachua. In post-reproductive period the contents in LC_{10} group increased in mantle (15.2512%; P <0.01) and foot (7.5141%; P < 0.05) but decreased in hepatopancreas (27.9391%;-P3: 0.001).“

In LC_{50} group, it increased in hepatopancreas (62.756496; P < 0.001) and mantle (4.4799%; N. S.) but decreased in foot (6.1222%; P < 0.001). On the other hand in LCSO group compared to LClO it decreased significantly in the foot (12. 6832%; P < 0. 001) and mantle (9. 3459%; N. S) but in hepatopancreas it increased significantly (27 2139%; P < 0 01).

REFERENCES

Ahmed Kabeer, Ramana Rao, K.V. and Swami, K.S. (1978). Effect of malathion on enzyme activity in foot, mantle and hepatopancreas of snail, Pila globosa. Ind. J. Exptl. Biol. 6(2): 25-260.

Ali, S.M., Ilyas, R. and Bhusari, N.B. (1983). Effect of lethal and sublethal concentration of dimecron on ascorbic acid of fish, Channa gachua (Ham.). Marathwada University Journal of Science, XXII(15):69-74.

Bragmann, C.G. and Brown, G.W. (1974). In : Research in Fisheries, Annual Report of the College of Fisheries, Seattle Wash. University of Washington, Washington, pp. 56.

Bicknell, F. and Prescoot, F. (1953). The vitamins in medicine. Grunn and Stratton, New York.

Campbell, D. T. (1975). On the conflicts between biological and social evolution and between psychology and moral tradition. American psychologist, 30(12), 1103.

Casida, J.E., Gamon, D.W., Glickman, A.H. and Lawrence, L.J. (1983). Mechanisms of selective action of pyrethroid insecticides. Ann. Rev. Pharmacol. Toxicol., 23: 413-438.

Chaudhari R.D. (1994). Alterations in some physiological process in the terrestrial snail, Cerastus moussonianus due to monocrotophos intoxication. Ph.D. Thesis, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India.

Chaudhari R.D. and Kulkarni, A.B. (1993). LC50 and glycogen level of the terrestrial snail, Zootecus sp. exposed monocrotophos. J. Ecotoxicol. Environ. Monit., 3(1) : 77-79.

Chaudhari, T.R. and Lomte, V.S. (1992). Effect of pesticides on glycogen level and amylase activity in digestive gland of fresh water snail, Bellamya

(Viviparus) bengalensis. Environment and Ecology, 10(1): 188-190.

Chaudhari, T.R., Patil, P.N., Rao, K.R., Deshmukh, S.B. and Diwate, S.G. (1999). Effect of pesticide Rogor on some biochemical constituents in the freshwater snail, Thiara lineata. Environment and Ecology, 17(1): 146-148.

Chetty, A.N. and K. Indira (1995). Adaptive changes in the glucose metabolism of a bivalve to ambient ammonia stress. Bull. Environ. Contam. Toxicol. 54: 83-89.

Chinoy, N.J. (1969). Source of electron energy for animal metabolism. I. Role of ascorbic acid in the metabolism of avain muscle. Histochem., 19: 125-128.

Chinoy, N.J. and L. Seethalakshmi (1977). Biochemical evidence for the occurrence of a possible steroidal mechanism in the reproductive organs of slugs, Laevicaulis alte ferrussac. In: Advances in invertebrate reproduction, Vol. 1, eds. K.G. Adiyodi and R.G. Adiyodi, Peralamkenoth, Kerala, India, pp. 356-366.

Dixit, V. P., Lohiya, N. K., & Agawal, M. (1975). Effects of alpha-chlorohydrin on the testes and epididymides of dog: a preliminary study. Fertility and sterility, 26(8), 781-785.

Jayanthi P,Prabhu S (2015) Optimization Studies on Degradation of Endosulfan by Indigenous Soil Bacterial Isolates International Journal of Science and Research (IJSR) ISSN (Online): 2319-7064Index Copernicus Value (2013): 6.14 | Impact Factor (2013

K.suneetha (2012) effects of endosulfan and fenvalerate on carbohydrate metabolism of the freshwater fish, Labeo rohita (hamilton) International Journal of Pharmacy and Pharmaceutical Sciences.ISSN- 0975-1491Vol 4, Issue 1, 2012

Kemp, A., & Van Heijningen, A. J. K. (1954). A colorimetric micro-method for the determination of glycogen in tissues. Biochemical Journal, 56(4), 646.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. J biol Chem, 193(1), 265-275.

Muley, D. V., & Mane, U. H. (1990). Histopathological Changes in Body Parts of the Fresh-water Gastropod, Viviparus bengalensis (Lam.). J. Environ. Biol, 11(4), 413-425.

Sastry, K. V., & Sharma, S. K. (1979). Toxic effects of endrin on liver and kidney of Teleost fish. In Proc. Symp. Environ. Biol. Muzaffarnagar, India (pp. 337-342).

Subhadra Devi, G. (1985). Impact of endosulfan toxicity on whole animal oxygen consumption and biochemical composition of the haemolymph of a freshwater field crab Oziotelphusa senex senex (Fabricius). Tirupati, India Google Scholar.

Yekeen TA (2009). Studies on the toxic effects of some pyrethroid pesticides using catfish (Clariasgariepinus) and rat (Rattus novegicus) as test organisms. A Ph. D. Thesis submitted to Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Oyo State, Nig. p. 223.

Yekeen, Taofeek A.* and Fawole, Olatunde O (2011).Toxic effects of endosulfan on hematological and biochemical indices of Clarias gariepinus. African Journal of Biotechnology Vol. 10(64), pp. 14090-14096.