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MALATHION INDUCED CHANGES IN TOTAL PROTEIN AND PROTEIN FRACTIONS IN DIFFERENT TISSUES OF A FRESHWATER LEECH, *HIRUDO BIRMANICA* [BLANCHARD].

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ABSTRACT

Leeches are medicinally important, and are non-target organisms as far as the pestcontrol operations are concerned. Leeches become the victims of toxicity of various agrochemicals, pollutants etc. in the freshwater ecosystem, due to which their very existence is unnecessarily threatened. Changes in the protein fractions such as sarcoplasmic , contractile and stromal of skin (botryoidal tissue), anterior sucker and posterior sucker of the leech. Hirudo birmanica were studied after treatment with sublethal concentration of malathion (0.01ppm), for 24, 48 and 96 h. The values of the fractions are presented as mg% on the dry weight basis. Amongst the protein fractions in control leeches the contractile proteins were quantitatively more than sarcoplasmic and stromal proteins in all tissues. The protein fractions decreased progressively and significantly ($p<0.05$) in all tissues of leeches as the period of exposure to sublethal concentration of malathion increased. The results are discussed in the light of physiological response of leech to pesticidal stress.

Key words : *Protein, Leech, Skin, Anterior and posterior suckers, Pesticides.*

INTRODUCTION

The use of pesticides to control hazardous and nuisance causing organisms dates back, but the effect such pesticides is stress on the non-target organisms inducing undesirable changes in them. Many reports are available on changes due to pesticidal stress (Patole et al., 2016 and Verma,2017). In protein in various tissues of different aquatic animals like molluscs, prawns, fishes, beetles, bugs, crabs.

The leeches have ancient medicinal importance. They are being used even today in therapeutic and post-surgical treatments. Owing to indiscriminate use of agrochemicals and their reach to the aquatic ecosystem by many ways has been threatening the existence of this precious treasure (Banerjee et al., 2015). Literature indicate no work is done on changes in protein fractions (sarcoplasmic, contratile and stromal) in different body tissue of leech under pesticidal stress. Therefore, in the present study the effect of sublethal concentration of pesticide malathion, on changes protein fractions of different tissues of the leech, *Hirudo birmanica* has been studied.

MATERIALS AND METHODS

The leeches, *Hirudo birmanica* were collected from the freshwater ponds around Partur town. They were acclimatized 5 days to the prevailing laboratory conditions under normal day/night illumination at 27 ± 0.5°C. The leeches having approximately equal size and weight (9.0 + 0.5 g) were selected for experimentation. Leeches were divided into four groups of 10 each and exposed to sublethal concentration (0.01mg/l) of malathion for 24, 48 and 96 h along with concurrent control in tap water. After the respective exposure period, the anterior sucker, skin (botryoidal tissues) and posterior sucker were dissected out separately in watch glasses and were dried in oven at 70 ± 0.5° C for 72 h. The dried tissues were finely powdered and used for the analysis protein fractions.

Total protein was estimated by the method of (Lowry et al., 1951).While protein fractions were estimated by the method of Helander(1957).The experiments were repeated thrice and the results averaged. The data are statistically analysed using student 't' test and presented as mg% on the dry weight basis.

RESULTS

The results presented in Table-1 show that in control leeches, skin contained more protein (10.5%) as compared to anterior sucker (6.8%) and posterior sucker (5.8%). The data in Table show changes in the sarcoplasmic, contractile and stromal proteins in skin, anterior sucker and posterior sucker of leeches exposed to sublethal concentration of malathion. Among the protein fractions, the contractile was found quantitatively more than sarcoplasmic and stromal protein in all tissues.

Total protein and protein fractions decreased progressively and significantly ($p<0.05$) in all tissues as the period of exposure to malathion increased from 24 through 96 h.

DISCUSSION

Proteins are the important group of macromolecules which occupy a central place ... both structural and dynamic aspects of living matter. They act as biocatalysts, contractile elements, elastic and inelastic elements of connective tissues and from important constituents of body fluids.Organic pesticides are known to alter various aspects of protein metabolism in different animals and all events of protein metabolism offer compensatory mechanisms during altered environmental conditions. (Singh et al., 1998)Observed a decrease in protein content in aldrin treated fish and concluded that decrease in protein may be either through the inhibition of RNA synthesis at the transcriptional level or due to impaired incorporation of amino acids into polypeptide chain. Protein decrease observed in the present study could be attributed to the physiological strategy adopted by leeches when they are in need of more energy to adapt themselves to the new environment . Such adaptation could be through the stimulation of degradative processes like proteolysis and utilization of degraded products of increased energy demand. Kondekar(1998) has reported increased protease activity in different tissues of *Hirudo birmanica* treated with organic pesticides. Begum and Vijayaraghavan(1996) supported the above notion while tracking changes in muscle proteins of the fish *Clarias batrachus* exposed to dimethoate.

Further analyses of protein fractions gave the picture of protein profile with type of fractions in different vital tissues. The sarcoplasmic and contractile proteins are the important constituents of the muscle, whereas the stromal protein is a mixture of remaining heterogenous group of membranes. It is observed in the present study that malathion has induced changes in the structural and functional sites of muscular unit in *Hirudo birmanica*. This is in agreement with observations of protein depletion in the fish, *Labeo rohita* after metacid treatment,(Raja N, & Arivoli K et al.,) and the snail, *Bellamya dissimmilis* after the treatment of organophosphate and organochlorine pesticides (Singh NN, Das VK et al.,) . The sarcoplasmic proteins are important constituents of the muscles, since the muscles are the seat of glycolytic activity. Earlier, Kondekar(1998) reported changes in carbohydrate and respiratory metabolism in *Hirudo birmanica* after treatment with malathion. This alteration in glycolytic activity may be attributed to depletion in sarcoplasmic protein. The contractile proteins are important in contraction of muscle depending upon their concentration, composition and arrangement in the muscle. These proteins form part of structure and seat of enzymatic actions. Level of contractile protein depleted more in skin of *Hirudo birmanica* in all media as the period of exposure increased. Skin plays two very important roles (1) as a wrapper for protection, and (2) as a respiratory organ. A poor undulatory activity and loss of movement an holding capacity of suckers were observed which are the indicators of loss of contractile action.

TABLE 1. Per cent changes in the total protein level in various tissues of freshwater leech, *H.birmanica* Exposed to sublethal concentration of Malathion pesticide.

Treatment	Tissue	Exposure period in hours		
		24	48	96
Malathion	Skin	9.61 ± 0.21 9 [-8.47 %]	8.52 ± 0.14 [8.85%]	7.73 ± 0.16 [-26.38%]
	Anterior Sucker	5.28 ± 0.02 [-22.35%]	4.39 ± 0.01 [-35.44%]	3.69 ± 0.05 [-45.73%]
	Posterior Sucker	4.73 ± 0.12 [-18.44%]	3.76 ± 0.10 [-35.17%]	3.39 ± 0.03 [-41.55%]

Control values (mg protein/100 mg of dry weight):
Skin 10.5 ± 1.2; Anterior sucker 6.8 ± 0.8; Posterior sucker 5.8 ± 0.7

TABLE 2. Per cent change in the protein fractions in various tissues of freshwater leech, *H.birmanica* after exposure to sublethal concentration of malathion.

Tissue	Fraction	Exposure period in hours		
		24	46	96
Skin	Sarcoplasmic	2.38 ± 0.03 [-24.20 %]	2.22 ± 0.03 [-29.29 %]	2.21 ± 0.03 [-29.61 %]
	Contractile	3.73 ± 0.06 [-20.63 %]	3.17 ± 0.03 [-32.55 %]	2.78 ± 0.11 [-40.85 %]
	Stromal	2.18 ± 0.02 [-16.15%]	1.85 ± 0.09 [-28.84 %]	1.66 ± 0.07 [-36.15 %]
Anterior Sucker	Sarcoplasmic	1.99 ±0.06 [-5.23 %]	1.76 ± 0.11 [-16.19 %]	1.8 ± 0.15 [-14.28 %]
	Contractile	2.3 ± 0.06 [-25.80%]	2.12 ± 0.01 [-31.61%]	1.57 ±0.07 [-49.35 %]
	Stromal	1.48 ± 0.02 [-12.94 %]	1.45 ± 0.05 [-14.70%]	1.27 ± 0.03 [-25.29%]
Posterior Sucker	Sarcoplasmic	1.56 ± 0.07 [-13.33 %]	1.36 ± 0.08 [-24.44 %]	1.02 ± 0.02 [-43.33%]
	Contractile	2.12 ± 0.22 [-26.89%]	1.83 ± 0.13 [-36.89%]	1.31 ± 0.03 [-54.82%]
	Stromal	1.01 ± 0.01 [-15.83 %]	0.87 ± 0.08 [-27.5 %]	0.89 ± 0.03 [-25.83 %]

Control values (mg protein/100 mg of dry tissue)
Skin : Sarcoplasmic 3.14 ± 0.6; Contratile 4.7 ± 0.4; Stromal 2.6 ± 0.2.
Anterior sucker: Sarcoplasmic 2.1 ± 0.2; Contractile 3.1 ± 0.3; Stromal 1.7 ± 0.07
Posterior sucker: Sarcoplasmic 1.8 ± 0.08; Contractile 2.9 ± 0.09; Stromal 1.2 ± 0.06.

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RESEARCH ARTICLE

EFFECTS OF MALATHION (50 % EC) ON THE GLYCOGEN CONTENT IN REPRODUCTIVE TISSUES OF A FRESHWATER LEECH, *HIRUDO BIRMANICA* (BLANCHARD)

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ABSTRACT

The present study was conducted to track changes in glycogen content of the reproductive tissues viz. testis, prostate gland, epididymis, vagina, albumen gland and ovary of the freshwater leech *Hirudo birmanica* exposed to sub lethal concentration of malathion. The glycogen in testis, prostate gland, epididymis, vagina, albumen gland and ovary tissues reduced significantly ($P<0.05$). The epididymis was affected more than other tissues.

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INTRODUCTION

The freshwater animals including medicinally important leeches are adversely affected by agricultural pesticides. Pesticide is defined by United Nations Environment Programme (UNEP, 2005) as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Chemical pesticides which are indiscriminately used in agricultural and household practices are generally designed for controlling pests and increase the economy of agriculture industry and to meet the world's need for abundant, safe and affordable food and fiber, at the same time such pesticides became highly toxic to the other organisms in the environment including human also (Pakhare, 2017). The use of such chemical pesticides in agriculture is a problem to ecosystem and resulting in the environmental pollution (Barbieri, 2008) and toxicity risk to the non-target organisms (Venkateswara Rao, 2006). Organophosphate compounds are widely used insecticides that account for about 70% of global insecticidal use (Ojha *et al.*, 2011). Malathion is an organophosphate pesticide which is extensively used in agriculture and household practices for pest eradication. It is hard insecticide having neurotoxic effects which cause persistent inhibition of acetylholinesterase (AChE). It is highly toxic to non-target organisms. Some organophosphate pesticides are highly soluble in water and can therefore easily contaminate aquatic ecosystem, thereby increasing the exposure risk of aquatic flora and fauna (Agdi *et al.*, 2000). Malathion (O,O-dimethyl phosphorodithioate of diethyl mercaptosuccinate) is a synthetic organophosphate, non-systemic, broad spectrum insecticide. Once malathion is introduced into the environment, it may cause serious intimidation to the aquatic organisms and is notorious to cause severe metabolic disturbances in non-target species (USEPA, 2005). Leeches are medicinally important animal which are adversely affected by pesticide pollution in freshwater ecosystem. Leeches are hermaphrodite animal which carries both the male and female reproductive organs. For the present study, *Hirudo birmanica* were selected as a test animal. Glycogen is the main biochemical component of the leech; it serves as a primary energy source for metabolic processes under the stressful condition. The stored glycogen may be utilized for their survival in the polluted environment and no further glycogen synthesis, so this could be the reason of glycogen depletion (Satyavardhan, 2013). The mode of action of malathion on treated animals are better understood by biochemical studies. Hence, the present work is aimed to assess the acute effect of malathion on glycogen content in the freshwater leeches *Hirudo birmanica*.

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Table 1. Glycogen (mg/100g) content in reproductive tissues of *H. birmanica* exposed to acute exposure of Malathion.

Organs	Control	Experimental			
		24 hr (2.42 ppm)	48 hr (1.92 ppm)	72 hr (1.27 ppm)	96 hr (0.98 ppm)
Testis	21.32 ± 0.96	18.47 ± 0.34 (- 13.36)	15.14 ± 0.25 (- 28.98)	11.42 ± 0.26 (- 46.44)	8.92 ± 0.26 (- 58.16)
Prostate gland	28.34 ± 0.47	25.76 ± 0.18 (- 10.68)	22.75 ± 0.20 (- 21.12)	18.98 ± 0.19 (- 34.19)	14.97 ± 0.17 (- 48.11)
Epididymis	22.09 ± 0.22	19.64 ± 0.23 (- 14.24)	15.50 ± 0.29 (- 32.31)	11.64 ± 0.21 (- 49.17)	8.31 ± 0.23 (- 63.71)
Vagina	20.18 ± 0.81	18.18 ± 0.29 (- 9.91)	15.96 ± 0.15 (- 20.96)	13.60 ± 0.27 (- 32.60)	11.48 ± 0.28 (- 43.11)
Albumen gland	16.52 ± 0.36	15.18 ± 0.30 (- 8.11)	13.67 ± 0.31 (- 17.25)	12.11 ± 0.29 (- 26.69)	10.85 ± 0.35 (- 34.32)
Ovary	19.91 ± 0.19	17.95 ± 0.26 (- 9.84)	15.61 ± 0.28 (- 21.59)	13.47 ± 0.25 (- 32.34)	10.91 ± 0.18 (- 45.20)

Mg/g wet wt. of tissue. [Each value indicate the mean (X ± SD) of five estimations] [Values in the parenthesis indicate percent change over control] [Values are significant at $p<0.05$]

MATERIALS AND METHODS

Test Organism and Acclimatization: The freshwater leeches *Hirudo birmanica* (length 10 ± 1 cm and weight 8 ± 0.5 gm) were procured from freshwater ponds around Partur Dist. Jalna. These leeches were acclimatized to the laboratory conditions with wet mud and fed with for 10-15 days at a room temperature 27 ± 2°C prior to the experimental condition.

Toxicity assay: To the study of Malathion 50% EC toxicity, 10 leeches were exposed to different concentration of malathion viz. 2.42 ppm, 1.92 ppm, 1.27 ppm and 0.98 ppm for 24 hr, 48 hr , 72 hr and 96 hr exposure period respectively. Probit analysis was done for statistical analysis.

Glycogen estimation: To study the glycogen levels the leeches divided into two groups as control and experimental. After exposure, both control and experimental leeches were dissected and testis, prostate gland, epididymis, vagina, albumen gland and ovary tissues were processed for glycogen estimation, it was done by Anthrone reagent method (Dezwaan and Zandee, 1972) the optical density was measured at 620 µm. The data was subjected to one-way analysis of variance (ANOVA) and the significance difference was set up at $p < 0.05$.

RESULTS AND DISCUSSION

The observed values of glycogen content are tabulated in table 1. The depletion in glycogen content in reproductive tissues of *Hirudo birmanica* suggest that it is contributing to a greater extent to the general energy needs of leeches under to the stress. The glycogen content depletion may be due to the inhibition of enzymes which contribute to glycogen synthesis (Shobha *et al.*, 2007). Hence, it is assumed that glycogen content depletion may be due to an inactivation of the cellular enzyme involved in the biosynthetic process of these metabolites and more utilization of glycogen owing to the effects of pesticides (Dezwaan and Zandee, 1972). The depletion in glycogen synthesis is also attributed to the inhibition of the enzyme glucose-6-phosphatase or glycogen synthetase which mediates glycogen synthesis (Pakhare, 2017). The results also show glycogen content in epididymis was highly affected than the other tissue after 24 h of exposure (19.64 ± 0.23) mg/100mg, for 48 h exposure period (15.50 ± 0.29) mg/100mg, for 72 h exposure period (11.64 ± 0.21) mg/100mg and for 96 h exposure period (8.31 ± 0.23) mg/100mg in compare to control (22.09 ± 0.22) mg/100mg. The percent change over shows -14.24 %, -32.31 %, -49.17 % and -63.71 % for 24 h, 48 h, 72 h and 96 h respectively. This indicates that, the effect of malathion 50 % EC on glycogen content of epididymis tissue significantly affected than other tissues. In 1989 Rajender Sagar, reported similar significant reduction in glycogen content found in testis, prostate gland, epididymis, vagina, albumen gland and ovary tissues *Poecilobdella granulosa* after exposed to endosulfan, malathion and sevin. Ingle, (2014), observed significant decrease in the glycogen content in ovary of control leech *Poecilobdella viridis* when treated with Deltamethrin and Felvalerate. Similar glycogen content depletion was observed when freshwater fish *Ctenopharyngodon idella* was exposed to fenvalarate and malathion (Satyavardhan, 2013) and in *Channa gachua* exposed to quinalphos (Pakhare *et al.*, 2016) organophosphate pesticides. Glycogen is the main biochemical component of the leech; it serves as a primary energy source for metabolic processes under the stressful condition. The stored glycogen may be utilized for their survival in the polluted environment and no further glycogen synthesis, so this could be the reason of glycogen depletion (Satyavardhan, 2013). The mode of action of malathion on treated animals are better understood by biochemical studies.

Conclusion

The freshwater ecosystem was contaminated by the organophosphate pesticide which caused serious threat to the non target organism. The present study reveals that the exposure of malathion on the leech *H. birmanica* caused changes in the total glycogen content, when compare with controls which may be attributed to toxic stress, resulting in the disruption of enzyme associated with carbohydrate metabolism.

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Sr. No.	Academic year	Committee name	Designation
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		Dicipline	
		Time Table	
		Faculty Day celebration	
		Tree Plantation	
02	2017-2018	Tree Plantation	Member
		Faculty Day celebration	
		Discipline	
		Time Table	
		Student Seminar	
03	2018-2019	Time Table	Member
		Student Seminar	
		Discipline	
		Tree Plantation	
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AQUATIC ZOO FAUNA FROM HARSOOL LAKE DIST: AURANGABAD

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ABSTRACT
The study of Zooplankton is important to find out productivity of lake for pisciculture. There are large numbers of animals which are economically important for nature as well as human being. The investigation carried out for seasonal changes and their impact on zooplankton. The result showed that Rotifers were dominant in all seasons. The detail of results and analysis discuss in text.

Keywords: *Seasonal impact, Harsool Lake*

INTRODUCTION
The fish population of our aquatic system plays an important role in the economy. There are large numbers of living aquatic animals, which are significant for nature as well as human being for their using as food. Harsool Lake is present in Harsool area of Aurangabad which is important for fisheries and irrigation purpose. Since plankton data of this reservoir helps in fishery research and also beneficial for the fisherman for economically important aquatic animals and also for the water quality improvement.

MATERIAL AND METHODS
The zooplanktons are collected in the morning using plankton net. The size of plankton net is 30mm. Zooplankton were preserved in 4% formalin. Zooplanktons are identified as per guidelines given by Ward and Whiple (1958)

VALUES OF ZOOPLANKTONS STUDY

Zooplanktons	Rainy season	Winter season	Summer season	Total
Rotifers	35	44	65	144
Ostrocoderms	39	28	11	82
Copepods	25	34	30	89
Cladocera	23	30	25	78

RESULTS AND DISCUSSION
The population diversity shows the Rotifers were dominant all seasons. The data indicates that light intensity play important role in population of Rotifers. In summer seasons water is clear and helps in increase the population.

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U = O. D. of the unknown test solution

S = O. D. of the known test solution

1.11 = Conversion factor of glucose to glycogen

$$\text{Percentage of glycogen} = \frac{100 \times 0.45}{1.11 \times 2}$$

= 20.27 mg/100ml of solution.

The glycogen contain in host tissue was 20.27 mg/100ml of solution.

CONCLUSION

The results revealed that the percentage of lipid is high in the parasite than their host and also high as compared to glycogen and protein. Cestodes are depends upon the host for the lipid source. Results indicate that distinctiveness host parasite relationship.

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