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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 ± 2^0C 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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