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Effect of Malathion on Antioxidant Parameters (Lipid Peroxidation and Reduced Glutathione) in Different Regions (Pre-Clitellar, Clitellar and Post Clitellar) of *Eudrilus Eugeniae*

KEYWORDS: Malathion, Eudrilus eugeniae, clitellar, reduced glutathione, lipid peroxidation

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ABSTRACT *Protein content, lipid peroxidation (LPX) and reduced glutathione (GSH) level in pre-clitellar, clitellar and post-clitellar region of Eudrilus eugeniae exposed to the soil, spiked with malathion @ of 20mg/kg (at different time interval of 24 h, 48 h, 72h) were measured and compared with that of the control (0 h).*

INTRODUCTION

Malathion is a broad-spectrum insecticide used to control a variety of outdoor insects in both agricultural and residential settings. Malathion is registered for use on food, feed, and ornamental crops and in mosquito, boll weevil and fruit fly eradication programs (Ware, 2000). Malathion is toxic through skin contact, ingestion, and inhalation exposure (Tomlin, 2006). Malathion and other organophosphate insecticides bind to the enzyme Acetylcholinesterase (AChE) at nerve endings throughout the bodies of insects and other organisms. Under normal circumstances, AChE binds to the neurotransmitter acetylcholine (ACh) at the nerve junction, effectively ending the stimulation of the next neuron. When AChE is bound by malathion's metabolite malaoxon, ACh accumulates at the nerve junction and results in overstimulation of the nervous system (Reigart and Roberts, 1999). Malaoxon is considered to be 22 times more toxic than the parent malathion from acute dietary exposure and 33 times more toxic by all routes of exposure from short-term and medium-term exposures (RED, EPA, 2009).

Malathion is also harmful to earthworms. According to Panda and Sahu (2000) malathion treatment reduced the number of earthworms in a rice field, and decreased the rate at which they reproduced. Omar and Edurado (2004) reported that sub lethal doses of malathion alter male reproductive parameters of Eisenia fetida. Worms make direct contact with the ground and absorb pesticides both from the skin and digestive system, thus the pollutants are absorbed 5 to 10 times (Sorous and Larink, 2001). Hence, ranges of toxicity tests have been proposed to assess the potential hazards of pollutants to earthworms (Van Gestel and Van Straalen, 1994). In the present work generation of ROS in response to Malathion were estimated by measuring reduced glutathione (GSH) and lipid peroxidation level (LPX) in different region (pre cliteller, clitellar, post clitellar) of earthworm, Eudrilus eugeniae at different time intervals (24h, 48h, 72h) and compared with the control earthworm.

MATERIALS AND METHODS

Animal

Eudrilus eugeniae were purchased from soil conservation office, Baripada, Mayurbhanj and kept in plastic tray (30cm x 25cm x 6.5cm) in the laboratory. Each tray contains soil covered with net and moist gunny cloth (maintain temperature and darkness). Eudrilus were acclimatized for seven days in the laboratory condition prior to the experiment.

Treatment process

Four trays were taken and labeled as C (for control or untreated) and E24, E48 and E72 (for experimental or treated). Each tray containing 20 matured earthworm per 1.5 Kg soil and. The Furadan (dose @ of 20mg/kg soil) was added to the soil of E24, E48 and E72 numbered tray.

Preparation of tissue samples

For preparation of sample a pool of 2 numbers of Eudrilus eugeniae were taken from each experimental tray (E24, E48 and E72) at different time intervals and also from the control (C). The Body of the earthworms was cut into 3 parts with the help of a sharp blade i.e. pre-clitellar, clitellar, and post-clitellar. The tissues (pre-clitellar, clitellar and post-clitellar) were kept at 4^0C in ice box.

The weights of the different parts were taken by the help of monopan digital weight machine (Shimandzu). Homogenate of tissues (different parts of the earthworm) were prepared with phosphate buffer (pH 7.4) and then centrifuged at 4°C in 4000 rpm for 10 minutes with high speed cold centrifuge (Remi).

Measurement of protein content

Protein estimation of samples was made according to the method of Lowry et al. (1961). The data were expressed in mg/g tissue.

Measurement of Lipid Peroxidation

Lipid peroxidation of the sample was estimated as thiobarbituric acid reacting substance (TBARS) by thiobarbituric acid (TBA) according to the method of Ohkawa et. al. (1979). The data were expressed as nmoles of TBARS/mg protein.

Measurement of reduced glutathione (GSH)

Glutathione content (GSH) was estimated by the method of Ellman (1959) and the amount of glutathione is expressed as mg/ g tissue.

All the solution was prepared by using Millipore distilled water. The above experiments were repeated for 10 times.

RESULTS AND DISCUSSION

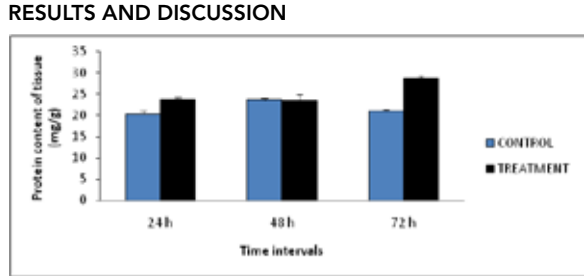

Fig-1: Comparison of protein content (mg/g) of pre-clitellar region of Eudrilus eugeniae at different time intervals.

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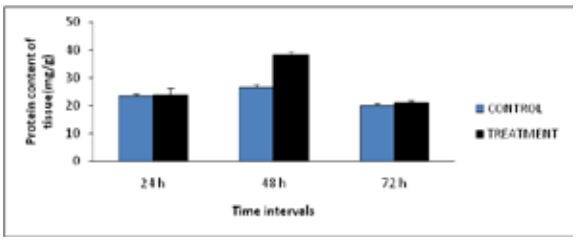

Fig-2: Comparison of protein content (mg/g) of clitellar region of Eudrilus eugeniae at different time intervals.

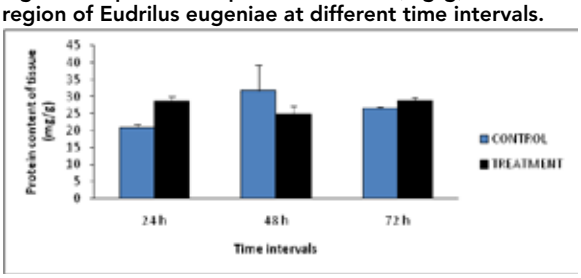

Fig-3: Comparison of protein content (mg/g) of post-clitellar region of Eudrilus eugeniae at different time intervals.

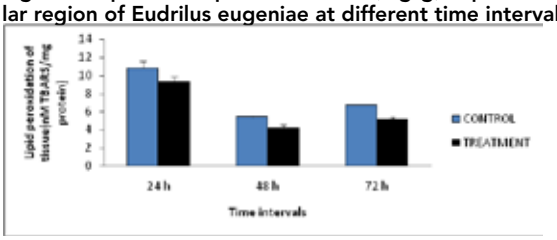

Fig-4: Comparison of lipid peroxidation (nM TBARS/mg protein) of pre-clitellar region of Eudrilus eugeniae at different time intervals.

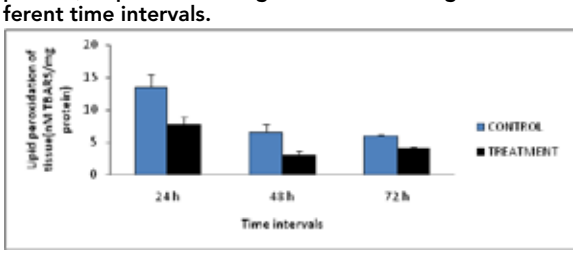

Fig-5: Comparison of lipid peroxidation (nM TBARS/mg protein) of clitellar region of Eudrilus eugeniae at different time intervals.

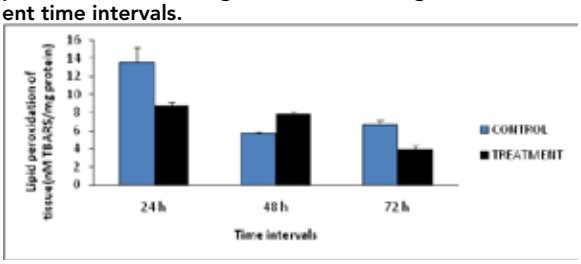

Fig-6: Comparison of lipid peroxidation (nM TBARS/mg protein) of post-clitellar region of Eudrilus eugeniae at different time intervals.

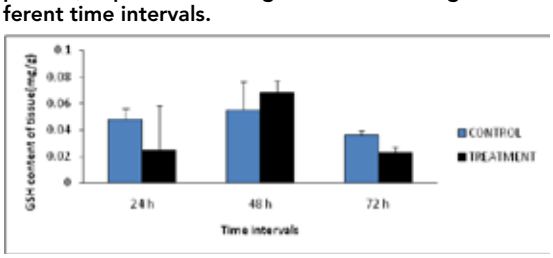

Fig-7: Comparison of reduced glutathione (mg/g) of pre-clitellar region of Eudrilus eugeniae at different time intervals.

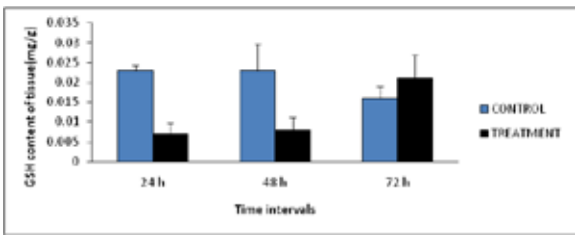

Fig-8: Comparison of reduced glutathione (mg/g) of clitellar region of Eudrilus eugeniae at different time intervals.

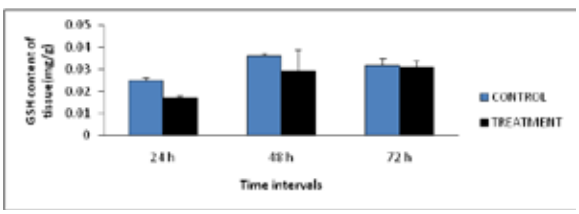

Fig-9: Comparison of reduced glutathione (mg/g) of post-clitellar region of Eudrilus eugeniae at different time interval.

The protein content (mg/ g tissue) of pre clitellar region of treated Eudrilus at 48 h and 72 h were slightly higher in comparison to the untreated Eudrilus (0 h), however the protein content of treated (24 h) is almost same to that of the untreated Eudrilus (Fig 1). In clitellar region, the protein content were always (after 24h, 48h and 72h of treatment) higher in comparison to untreated Eudrilus (Fig2). In post clitellar region the protein content increases at 24 h and decreases at 48 h and almost same at 72 h when compared with that of untreated Eudrilus (Fig 3).

In pre clitellar region lipid peroxidation of Eudrilus eugeniae decrease at 24 h and 72 h in experimental group and at 48 h it increases as compare to control test (Fig 4). Its decrease in different time intervals (24 h, 48 h and 72 h). In clitellar region LPX was decreased in experimental samples of Eudrilus eugeniae as compared to the control samples at 24 h, 48 h and 72 h (Fig 5). In post clitellar region LPX was increased at 48 h and 72 h and decreased at 24 h as compared to control(Fig 6).

GSH content of treated Eudrilus were found less than that of the untreated Eudrilus in all experimental tests in pre clitellar region of Eudrilus eugeniae. GSH content was almost same in 48 hour tests as compared to control, at 24 h it increased and 72 h GSH content was decreased in experimental group (fig 7). In clitellar region the concentration of GSH decreases more in 72 h as compared to 24 h and 48 h in experimental group (Fig 8). In post clitellar region the GSH content same in 24 h and at 48 h as compared to the GSH content in 72 h in experimental group (Fig 9).

STATISTICS

The statistical analysis was done with the help of statistical package SPSS 16.0. Correlation analysis test was carried out to find out the level of significance between Eudrilus eugeniae treated with Furadan at different time intervals of 24 h, 48 h, and 72 h along with that of untreated (0 h). According to Steven's guidelines if the correlation data is more than 0.72 then the data is significant. A difference was taken as significant when P was less than 0.05.

Correlation analysis of the data revealed that protein content of Eudrilus eugeniae of untreated and treated at 24h were varied significantly(-0.512). Protein content of clitellar and post-clitellar was also highly significant in experimental (treated) group with respect to control (untreated). At 48 h protein content are significant (0.835) in any region. At 72 h all regions are highly significant(0.910) with respect to control group at the level 0.05(2-tailed).

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Correlation analysis showed that GSH content of 24 h treated Eudrilus are not significant (0.469) in comparison to the corresponding untreated (24h) Eudrilus. At 48 h pre clitellar, clitellar and post clitellar are varied significantly (0.791). At 72 h experimental group and control group correlation is highly significant, so it not varied significantly(0.406) at the level 0.05(2-tailed).

LPX content in experimental group at 24 h is not varied significantly (0.525) but at 48 hour control and experimental group also not varied significantly (-0.373). A 72 h also both are highly significant (-0.756) and in all regions the correlation is significant at the 0.05 level (2-tailed).

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