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Effect of Furadan on Superoxide Dismutase And Catalase Activity of *Eudrilus Eugeniae*

KEYWORDS: Furadan, catalase, superoxide dismutase, *Eudrilus eugeniae*

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ABSTRACT *Eudrilus eugeniae was treated with furadan spiked soil for different time interval (0 h, 24 h, 48 h and 72 h) and the change of antioxidant enzyme (superoxide dismutase and catalase) between treated and untreated were measured spectrophotometrically. It is observed that the superoxide dismutase decreases at 24 h and gradually increases in case of 48 h and 72 h respectively, but a reverse case is observed in case of catalase i.e. it increases at 24h and then gradually decreases at 48 h and 72 h respectively in comparison to control.*

INTRODUCTION

Pesticides have major impact in decreasing the biodiversity of soil inhibiting organisms (Edwards and Thomson, 1973). Pesticides also decrease the biodiversity of aquatic insects and fishes in aquatic systems contaminated with agricultural pesticides and have very similar effects to those on terrestrial system (Edwards, 1977). Biochemical changes induced by pesticidal stress lead to metabolic disturbances, inhibition of important enzymes, retardation of growth and reduction in the fecundity and longevity of the organism (Murty, 1986; Khan and Law, 2005). Carbofuran or furadan (2, 3-dihydro -2, 2-dimethyl-7-benzofuranyl-N-methylcarbamate) is a widely used systemic and contact insecticide, acaricide and nematicide which has broad spectrum of activity against many agricultural pests.

This study was designed to see the toxic effects of carbofuran on *Eudrilus eugeniae* by measuring superoxide dismutase and catalase activity at different time intervals of (control) 0hr and experimental (24hr,48hr and 72hr) .

MATERIALS AND METHODS

Eudrilus eugeniae were purchased from Soil Chemist Office, Takatpur, Baripada, Mayurbhanj. A culture of *Eudrilus eugeniae* was maintained in the laboratory. They were reared in buckets with perforated base, lined with gravel, coconut husk, farmyard manure on which worms are released. Bucket was covered with nylon net and gunny cloth and kept moist by sprinkling water. Care was taken that bedding material and feed for vermiculture were free from any type of pesticide contamination.

Soil testing methods of the vermiculture bed soil

The soil sample was tested by using KO54 Soil Testing Kit of Himedia Laboratories Pvt. Limited.

Treatment process

Thirty numbers of earthworms were transferred to C_F as control where as ten number of earthworms were transferred to other three trays (E_{F1}, E_{F2}, E_{F3}) as experimental. The soil in E_{F1}, E_{F2}, E_{F3} was spiked with 15 mg of furadan dissolved in 1ml of acetone

Table-1: Experimental Set-Up

Tray No.	No. of earthworm	Furadan dose/ kg soil	Time interval (Hours)
C_F	30	Nil	0 hr, 24 hr, 48 hr, 72 hr
E_{F1}	10	15 mg	24 hr
E_{F2}	10	15 mg	48 hr
E_{F3}	10	15 mg	72 hr

Toxicity of furadan on the morphology of the earthworm *Eudrilus eugeniae*

Thirty numbers of earthworms were transferred to C_F as control where as ten number of earthworms were transferred to other three trays (E_{F1}, E_{F2}, E_{F3}) as experimental. The soil in E_{F1}, E_{F2}, E_{F3} was spiked with furadan (15 mg kg^{-1}) The test containers were covered with moist gunny bag and plastic net to avoid the moisture content in the medium. The control medium was the same quantity of water without any additive agent. Testing was done in continuous light at 22 ± 2° C. The coiling behavior, morphological abnormalities and the percentage mortalities were recorded and photographed after 24 hr, 48 hr, and 72 hr after exposure to Furadan (Figs 3, 4, 5, 6).

Preparation of supernatant

Eudrilus eugeniae, 5-6 in number were picked up from each tray and their pooled weight was measured in digital monopan balance (Shimadzu; ELB 300). A 20% homogenate was prepared in ice-cold 50mM phosphate buffer (pH 7.4) using pre-chilled porcelain mortar and pestle by up and down strokes at 4°C. The homogenate was centrifuged at 4500 rpm (1000 Xg) for 10 minutes at 4°C in Cooling Centrifuge (Remi). The supernatant was taken for biochemical assay.

Protein estimation

Protein estimation of the samples was made according to the method of Lowry *et al.*, (1951). Protein content was expressed as mg/g wet weight of the tissue and aqueous BSA (Bovine Serum Albumin) was taken as standard protein.

Estimation of superoxide dismutase (SOD) activity

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined ccording to the method of Das *et al.*, (2000).

Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

SOD activity was expressed as units/mg protein (Fig 8).

Estimation of catalase (CAT)

Catalase (CAT; EC 1.11.1.6) activity was estimated according to Beers and Sizer (1952). The activity of catalase was expressed as nkat/mg protein (1nkat=1mole of substrate converted to product per sec, 1U=16.67 nkat) (Fig 9).

Statistical methods

One-way ANOVA and Post Hoc analysis was carried out to find out the level of significance between *Eudrilus eugeniae* treated with lindane over a period of 24 hr, 48 hr, 72 hr and in control. A difference was taken as significant when P was less than 0.05. Statistical analysis was done with the help of software SPSS package 16.0.

RESULTS AND DISCUSSIONS

Biomass of *Eudrilus eugeniae* at 0 hour (control) was 0.791667 ± 0.182612 g. At 60^0 , the biomass were 0.225 ± 0.083217 g after 5 hour and 0.191667 ± 0.045977g after 10 hour (Fig 1). One way ANOVA revealed that the biomass is significant [F (2, 17) = 40.239, (P=0.00)] It was found that, the biomass in *E. eugeniae* in control and in those after 5 hours and 10 hours showed significant differences. Post Hoc analysis revealed that the biomass at 5 hr and 10 hr is significant 05; LSD) with respect to control.

Body weight of *Eudrilus eugeniae* were 1.094 ± 0.279328 at 0 hour (control), 0.98 ± 0.22163 after 24 hour, 0.922 ± 0.183891 after 48 hour, 0.894 ± 0.183913 after 72 hour and 0.74 ± 0.161617 after 30 days (Fig. 2). In other words, body weight of *Eudrilus eugeniae* was highest at 0 hour (control) and gradually the body weight decreases after 24, 48 72 hour and after 30 days.

One way ANOVA revealed that the body weight is significant [F (4, 24) = 1.511, (P=0.237)]. It was found that, the body weight in *E. eugeniae* at different time intervals showed significant differences. Post Hoc analysis revealed that the body weight was significant only at 720 hour (P≤0.05; LSD) with respect to control.

Table1: Comparison of protein content (mg/g tissue), Superoxide dismutase activity (SOD) in Unit/mg protein and Catalase (CAT) level in nkat/mg protein of *E. eugeniae* after treatment of furadan at different time interval. The value are expressed in Mean± S.D.

Duration after treatment with furadan	Protein content	SOD activity (Unit/mg protein)	CAT level (nkat/mg protein)
C_F (0h)	20.83±5.07	102.21±5.42	0.01 ± 0.01
E_{F1} (24h)	16.25±2.52	4.61 ± 1.15	0.03 ± 0.01
E_{F2} (48h)	10.73±0.47	21.78 ± 3.72	0.02 ± 0.01
E_{F3} (72h)	17.72±3.55	25.6 ± 5.31	0.02 ± 0.00

Fig. 1: Comparison of biomass at 60° C temperature in *Eudrilus eugeniae* at different time intervals.

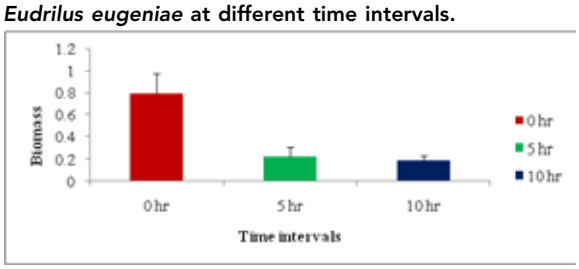


Fig. 2: Comparison of body weight in *Eudrilus eugeniae* at different time intervals.

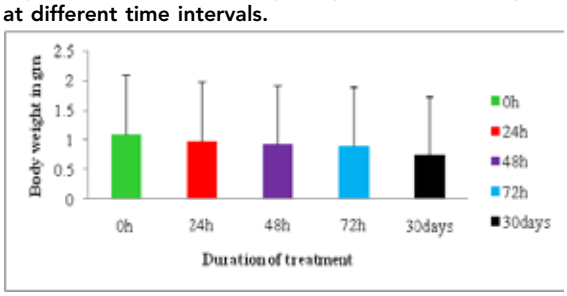


Fig.3 : Unexposed *Eudrilus eugineae*

Fig.4 : Coiling was seen after 24 hour of Furadan exposure

Fig. 5: Coiling was seen after 48 hour of Furadan exposure

Fig. 6: Coiling was seen after 72 hour of Furadan exposure

Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

Protein content (mg/g tissue) in *Eudrilus eugeniae* treated with furadan (15 mg/kg soil) were 20.83 ± 5.07 mg/g tissue, 16.25 ± 2.52 mg/g tissue, 10.73 ± 0.47 mg/g tissue, 17.72 ± 3.55 mg/g tissue after 0 hour, 24 hours, 48 hours and 72 hours respectively (Table 1 and Fig 7). The protein content (mg/g tissue) gradually decreased at 24 hour and 48 hours. It was lower in 48 hours in comparison to *Eudrilus eugeniae* exposed to furadan at different time intervals. The protein content was highest at 0 hours (Table 1 and Fig 7).

One way ANOVA was performed in order to analyse the effect of furadan (15 mg/kg soil) on the protein content at different time intervals in *Eudrilus eugeniae*. One way ANOVA revealed that the protein content at different time intervals in *Eudrilus eugeniae* is significant [F (3, 19) = 6.346, P=0.005]. Post Hoc analysis revealed that the protein content at different time intervals when treated with furadan in *Eudrilus eugeniae* was only significant at 48 hours (P<0.05; LSD). While, 24 hours and 72 hours are not significant with respect to control.

Superoxide dismutase activity (Unit/mg protein) in *Eudrilus eugeniae* treated with furadan (15 mg/kg soil) were 102.21 ± 5.42 Unit/mg protein at 0 hour (control), 4.61 ± 1.15 Unit/mg protein after 24 hours, 21.78± 3.72 Unit/mg protein after 48 hours, 25.6 ± 5.31 Unit/mg protein after 72 hours. The SOD level (Unit/mg protein) of *Eudrilus eugeniae* exposed to furadan was highest at 0 hour (control) and then decreased at 24 hour. The SOD level at 48 hour again increases and 72 hour then 24 hour (Table 1 and Fig 8.).

One way ANOVA revealed that the SOD activity (Unit/mg protein) in *Eudrilus eugeniae* exposed to furadan (15 mg/kg soil) at different time intervals is significant [F (3, 19) = 414.706, P=0.000]. Post Hoc analysis revealed that the SOD activity (Unit/mg protein) at different time intervals when treated with furadan in *Eudrilus eugeniae* was significant at 24 hours, 48 hours and 72 hours with respect to control (P<0.05; LSD)

Catalase activity (nkat/mg protein) in *Eudrilus eugeniae* treated with furadan (15 mg/kg soil) were 0.01 ± 0.01 nkat/mg protein, 0.03 ± 0.01 nkat/mg protein, 0.02 ± 0.01 nkat/mg protein, 0.02 ± 0.00 nkat/mg protein, after 0 hour, 24 hours, 48 hours and 72 hours respectively.

The CAT level (nkat/mg protein) differ greatly in *Eudrilus eugeniae* exposed to furadan at different time intervals. The CAT level (nkat/mg protein) was highest at 0 hour and 72 hours and was lowest at 24 hours (Table 1 and Fig 9).

One way ANOVA revealed that the CAT activity (nkat/mg protein) at different time intervals in *Eudrilus eugeniae* exposed to furadan (15 mg/kg soil) is significant [F (3, 19) =5.044, P=0.012]. Post Hoc analysis revealed that the CAT activity (nkat/mg protein) at different time intervals in *Eudrilus eugeniae* exposed to furadan (15 mg/kg soil) was only significant at 24 hours (P<0.05; LSD). While, 48 and 72 hours are not significant with respect to control.

Fig.7 : Comparison of protein content (mg/g tissue) in *Eudrilus eugeniae* treated with furadan (15 ml/kg soil) at different time intervals.

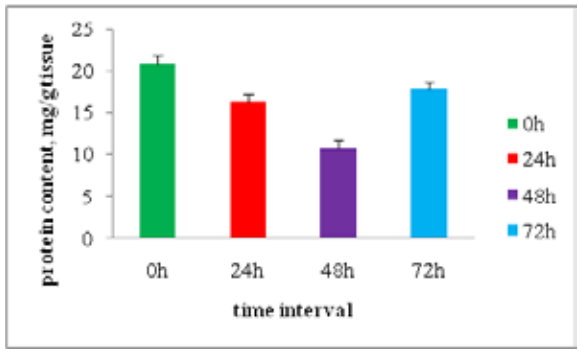


Fig.8 : Comparison of SOD activity (Unit/mg protein) in *Eudrilus eugeniae* treated with furadan (15 ml/kg soil) at different time intervals.

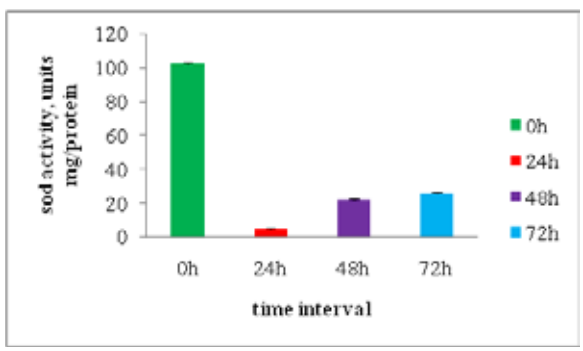
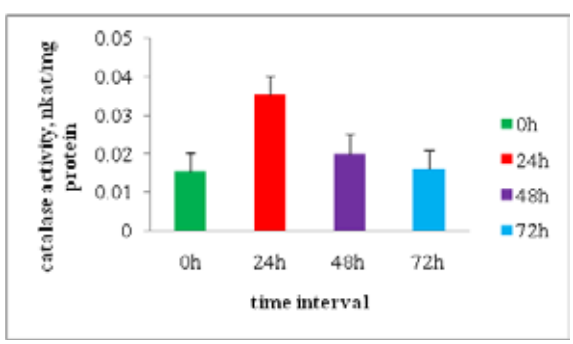


Fig.9 : Comparison of catalase activity (nkat. mg^{-1} protein) in *Eudrilus eugeniae* treated with furadan (15 ml/kg soil) at different time intervals.



CONCLUSION

Eudrilus eugeniae has two important antioxidative enzyme SOD and CAT to protect it from ROS produced due toxic insult of furadan. However, long exposure might lead to deleterious effect on earthworm and limit their survival rate

REFERENCE

Beers, R. F., Jr. and Sizer, I. W., (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem., 195: 133-140. | Das, K., Samanta, L. and Chainy, G. B. N., (2000) A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. Ind. J. Biochem. Biophys., 37: 201-204. | Edwards, C. A. and Thomson, A. R., (1973) Pesticides and soil fauna. Residue Reviews. 45:1-79. | Edwards, C.A., (1977) Nature and origins of pollution of aquatic systems by pesticides. In: Pesticide in Aquatic Environments, M.A.Q. Khan (Ed.), Plenum press, New York. Pp.11-37. | Khan, M. Z. and Law, F. C. P., (2005) Adverse effects of pesticides and related chemicals on enzyme and hormone systems of fish, amphibians and reptiles: a review. Proc. Pakistan Acad. Sci.,Vol. 42, pp. 315 - 323. | Lowry, O.H., Resbrough, N.J., Farr, A.L. and Randoll, R.J., (1951). Protein measurement with the Folin-phenol reagent. J.Biol. Chem. 19: 265-275. | Murty, A. S., (1986) Toxicity of Pesticides to fish. C.R.C Press Inc, I and II. pp. 483 - 355. |