

Effect of Sub Lethal Doses of Coragen and 2, 4-D ethyl Ester on Digestive Enzymes of Earthworm Species *Eudrilus eugeniae*, Kienberg 1867

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ABSTRACT : Present piece of research work deals with studies on effect of sub lethal concentration of pesticides like Coragen (insecticide) and 2, 4-D ethyl ester (herbicide) on digestive enzymes of earthworm, *Eudrilus eugeniae*. The digestive enzymes of worm are responsible for quick decomposition and humification of organic matter. Our results revealed that the activity of enzymes is significantly declined over the control. The activities of protease, amylase and phosphatase were found to be – 53.86 and - 43.33; - 30.55 and - 61.97, and – 39.40 and -32.00 per cent in the worms exposed to Coragen and 2, 4-D ethyl ester respectively. They also delayed in the process of decomposition and digestion.

KEYWORDS: Pesticides, insecticides, herbicides, Coragen, decomposition, humification.

I. INTRODUCTION

To support alarming population growth throughout the globe there is necessity of more food result in rapid growth of pesticides [1,2]. In modern agricultural practices to enhance productivity there is a need to protect the crop from attack of insect pest. Farmers are applying different kinds of insecticides to get rid of pests. They use pesticides for precise purpose to eliminate weeds, kill harmful fungi and eradicate insect pest; all of which can damage crops. In spite of their benefits, increasing trends of pesticides application has deleterious effect on human and ecosystem especially on non target organisms and these chemicals persist several years in the environment [3].

Earthworm effectively harness the beneficial soil microflora, destroy soil pathogens and convert organic waste into enzymes, antibiotics, growth hormones and protein rich cast [4,5] but most of pesticides especially herbicides adversely affect on beneficial organism like birds, mammal etc [6,7]. It is able to alter community dynamics [8]. Earthworm itself acts as a house which supply of enzymes such as amylase, cellulase, nitrate reductase and alkaline phosphatase. The digestive enzymes of earthworm are responsible for the decomposition and humification of organic matter. The compost thus forms as high as economic value of soil condition for plant growth [9,10].

Coragen (Chlorantraniliprole), a recently introduced diamide insecticide. It is recommended particularly for controlling of pest of rice, cabbage, cotton and sugarcane etc which have developed resistance to other chemical classes of insecticides. It has high biological activity, very low mammalian toxicity and selectivity to non- target arthropods [11]. Similarly a commercially available herbicide (2,4-D ethyl ester) is a good wide spectrum weedicide, recommended for the control of broad leaf weeds.

These two pesticide effects have not been reported so far in earthworm. Therefore the attempt has made to study the effects of sub lethal dose of Coragen (insecticide) and 2,4 D ethyl ester (herbicide) on digestive enzymes of earthworm *Eudrilus eugeniae*.

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II. MATERIAL AND METHOD

BIOLOGICAL MATERIAL: Earthworm species *Eudrilus eugeniae* were obtained from government of Maharashtra agricultural nursery, Sakri, Dist – Dhule. They were maintained in the laboratory [12]. Healthy adult clitellar worms were selected for the present experiment.

CHEMICAL MATERIAL: A commercially available Coragen, 18.5% EC is a novel substituted anthranilamide insecticide. Another pesticide, 2,4-D ethyl ester, 38% EC is a good wide spectrum weedicide. Both are purchased from local market.

The quality soil and a month old cow dung were collected from agriculture field and cow shed respectively.

EXPERIMENTAL SET-UP : In plastic tough of about 5 kg capacity, 800 g fine dried soil along with 200 g dried cow dung was used for this experiment. The mixture was thoroughly mixed with water and kept ready for experiment. The LC₅₀ value of an insecticide, Coragen and 2, 4 D ethyl ester was previously find out in our study. For Coragen it was 18 and 17 ml/kg for 7 and 14 days of exposure respectively. While 2, 4-D ethyl ester was 0.43 and 0.34 ml/kg for 7 and 14 days respectively. A higher sub lethal (3/4th of LC₅₀) dose i.e. 13.42 ml/kg of Coragen and 0.255 ml/kg of 2,4-D was selected for this study. A higher sub lethal dose of both the pesticides was added in 100 ml of water and it was mixed in the respective treatment groups only. 20 mature clitellar worms were released in each tough; they were covered with perforated lid. To maintain moisture water was sprinkled at the interval of 3 to 4 days. The entire tough was kept for 60 days at room temperature 26 ± 2 °C and moisture 70 ± 5 %. The experimental set-up was prepared in quadruplets for each treatment. A single bed was kept as a control without pesticides.

ENZYMATIC MEASUREMENT: At the end of the experiment, to clear the digestive tract the earthworms were fasted for 24 h. Prior to biochemical analysis, the worms were cut into pieces and mixed with ice cold saline solution (0.86% Na Cl). The mixture was homogenized and centrifuged for 10 minute at 3000 rpm; the supernatant was used for determination of enzyme activities. The activities of enzyme like amylase, protease and acid phosphatase were determined by [13,14,15] respectively

III. RESULT AND DISCUSSION

Activity of the digestive enzymes protease, amylase and acid phosphatase of treated earthworm with 3/4th sub lethal dose of Coragen and 2, 4 D ethyl ester were presented in table-1. Our results revealed that the production of enzymes was significantly reduced over the control. The activity of protease (-53.86 %) and acid phosphatase (-39.40 %) was comparatively reduced in worm treated with insecticide than herbicide while the amylase activity (-61.96 %) was highly reduced in herbicide than insecticide.

Activity of amylase, cellulase, chitinase, lichenase, protease, lipase, urease, acid and alkaline phosphatase etc have been recorded in the gut of earthworm species like *Dichogaster bolawi*, *Drawida calebi*, *Drawida wilsii*, *Entyphoocus spp*, *Perionyx millardi* and *Pontoscolex corethrus* [16,17].

[18] found that feeding *Spodoptera littoralis* larvae with 5 ppm abamectin caused remarkable decrease in invertase, amylase and trehalase activities.

[19] reported ageing effect on enzyme activities in pressmud vermicasts of *Lampito mauritii* (Kinberg) and *Eudrilus eugeniae* (Kinberg).

[20] studied the level of various digestive enzymes like amylase, cellulose, xylanase, cellbiase, endonuclease and their activities in the gut of the two earthworms, *Eudirlus eugeniae* and *Eisenia fetida*. They reported overall enzymatic activity.

[21] reported reduction in the cellulose activity in the gut of earthworms treated with deltametrine pesticide and found inhibiting earthworm growth after 42 days of exposure.

[22] studied that the methanolic extract of *Artemisia annua* L. was investigated for its toxic effect on biochemical parameters of leaf beetle, *Xanthogaleruca luteola*, Mull. They reported within 24 h after treating 3rd instar larva with the extract, the level of alpha amylase was significantly changes.

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Treatment of rynaxyper and spinetoram (radiant) shown significant decrease in the activities of trehalose, invertase and amylase [23].

[24] studied effect of herbicides like Dhanutop and Racer on digestive enzymes of epigeic earthworm, *Perionyx excavatus*. They showed that decrease in digestive enzyme activity in sub lethal doses as increasing days of exposure. It is because of the herbicides is persisting in the food that prevents consumption of food by the worms. Our results are corroborated with earlier workers.

Table-1: Digestive enzymes activity of Earthworm exposed pesticides

Sr. No.	Pesticides	Sub lethal dose (mg/ kg of soil)	Enzymes activities					
			Protease ($\mu\text{g}/\text{min}$)		Amylase (Units/ ml)		Phosphotase (μ/L)	
			Control	Experimental	Control	Experimental	Control	Experimental
01	Coragen	13.42	5.80 \pm 0.6	2.40 \pm 0.4 (-53.86)**	720 \pm 8.0	500 \pm 10.0 (-30.55)*	0.40 \pm 0.02	0.24 \pm 0.02 (-39.40)**
02	2,4-D ethyl ester	0.255	6.0 \pm 0.6	3.4 \pm 0.4 (-43.33)**	710 \pm 10.0	270 \pm 8.0 (-61.96)***	0.40 \pm 0.04	0.32 \pm 0.02 (-32.00)*

* Significant value: $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Values in the parenthesis are per cent change over control. CD (Mean \pm SEM, n = 4).

IV. CONCLUSION

In nutshell we conclude that;

- Both insecticide i.e. Coragen and 2, 4-D ethyl ester were toxic to earthworm under chronic exposure.
- These results suggest that changes in enzyme activity during sub lethal exposure periods may be more sensitive parameter than LC_{50} . Since they provide early warning responses at sub lethal doses.
- Decrease in the enzyme activity represents the stress in organisms and disturbance in biochemical metabolisms.

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