

Effect of Sub Lethal Concentration of Coragen and 2,4-D Ethyl Ester on Biomass and Reproductive Parameters of Earthworm Species, *Eudrilus eugeniae* Kinberg, 1867.

Shaikh. H. M¹, More, B. C², Patel, N. G³.

Department of Zoology, N. N. C. College, Kusumba Dist- Dhule. (M.S.) India¹

Professor, Department of Zoology Karm, A. M. Patil Arts, Com. and Kai N. K. Patil Science College, Pimpalner, Sakri, Dhule (M.S.) India².

P.G. and Research Centre, , Department of Zoology, Pratap College, Amalner, Dist-Jalgaon, (M.S.) India³

ABSTRACT: Present study revealed with chronic effect of sub lethal concentrations of two commonly used pesticides like Coragen (insecticide) and 2,4-D Ethyl ester (herbicide) on biomass and reproductive parameters on earthworm species, *Eudrilus eugeniae*. The results showed there was significantly decrease in biomass of treated worm than control group. Similar results find in cocoon production as well as juvenile count in treated worms. Maximum reduction in all parameters were found to be occur in worms treated with higher (3/4th) sub lethal dose concentration than the lower (1/4th) dose concentration i.e. toxicity is dose dependent. The results of present study can be used to plan and implement remediation strategies for the pesticide polluted soils where risk of soil pesticide contamination is associated with population of soil invertebrates.

KEY WORDS: Chronic, Coragen, herbicide, juvenile, cocoon, biomass, toxicity.

I. INTRODUCTION

Today's agriculture practices are pesticidal based. To get maximum yield, tons of synthetic pesticides and chemical fertilizers are applied annually to crops worldwide to eliminate unwanted weeds, fungus and insect pests etc. The use of pesticides has increased with the increasing awareness of their utility in agriculture, animal husbandry and post harvest technology as well as public health [1]. In our country, agriculture is major backbone of economy but unfortunately it is depends on chemical fertilizers and pesticides. India is the largest manufacturer of pesticides; around 128 pesticides are registered in India [2]. Pesticides which includes insecticides, molluscides, bacteriocides, fungicides, weedicides and nematicides [3]

A great proportion of biomass of terrestrial invertebrates is represented by earthworms, as it plays an important role in increasing the nutrient content of the soil and plays the vital role in the maintenance of soil structure, functions and fertility [4]. Earthworm activity not only modifies soil aeration and drainage but also provides essential nutrients for plant growth. They are the key components of soil biota and contribute to the overall productivity of agricultural soil through their feeding, casting and burrowing activities [4, 5]. The population of earthworms is greatly affected by excess usage of inorganic fertilizers and pesticides especially they affect reproductive potential of earthworms and causes decline the population of the worms. Pesticides are also known to produce morphological, anatomical and physiological changes in the reproductive aspects of different non-target animals including earthworm [6].

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The earthworm species, *Eudrilus eugeniae* is commonly called African night crawler, which is used extensively for composting in tropics especially in Africa and India. This species is more productive in terms of rates of growth than other earthworm species. Effect of pesticides on growth of reproductive parameters of earthworms exposed to agro pesticides loaded soils seems to be useful indicator of soil pollution [7].

Coragen (Chlorantraniliprole) is a novel substituted anthranilamide insecticide. The commercially available Coragen has 18.5% EC concentration. It is recommended for controlling pests of rice, cabbage, cotton and sugarcane. Similarly, 2, 4-D ethyl ester is a good wide spectrum weedicide. In market it is available in the concentration of 38% EC and uses for the control of broad leaf weeds.

The present study is undertaken to determine the effect of Coragen (insecticide) and 2, 4-D Ethyl ester (herbicides) on biomass and reproductive parameters of earthworm species *Eudrilus eugeniae*.

II. MATERIALS AND METHODS

Biological Material: Earthworm species, *Eudrilus eugeniae* were obtained from State Government agricultural nursery, Sakri (Dhule) M. S. They were maintained in the laboratory as per OECD guideline [8]. Only healthy adult worms having well developed clitella were used for the experiment.

Chemical Material: Two chemicals i.e. commercially available Coragen (18.5 % EC) an insecticide and wide spectrum herbicide, 2, 4-D ethyl ester (38.5 % EC) commonly were purchased from local pesticide shop. The quality soil and a month old cow dung were collected from agriculture field and cow shed respectively.

Experimental Set-Up: The experiment was performed in plastic tough having five kg capacities. A dried quality soil was ground and sieved. In a tough 800 g of fine soil thoroughly mixed with 200 g Cow dung (CD) and appropriate amount of water was added to moisten the mixture.

Treatment: For sub lethal dose, the LC₅₀ values of the both the pesticides in earthworm were already estimate in our previous study, it was 17.9 ml/ kg for Coragen and 0.350 ml/ kg for 2, 4 - D ethyl ester. The lower (1/4th) and higher (3/4th) sub lethal dose concentration are 4.48 and 13.42 ml/ kg respectively for Coragen and 0.088 and 0.260 ml/ kg respectively for 2,4-D ethyl ester was added separately in 100 ml of distilled water and that was mixed thoroughly in experimental groups only. On next day 20 mature worms were added to each tough. The tough was covered with perforated lid and kept for 60 day. The experiment set up was prepared in triplicate for each treatment.

At the end of experiment earthworms were separated by hand sorting washed with water and kept on filter paper. Weigh the worms to calculate the growth of biomass. Similarly the number of cocoons and juveniles are also hand sorted, counted and tabulated, the data was statistically analyzed.

The per cent change in biomass, number of cocoon and juvenile were determined by applying following formula i.e.

$$\% \text{ change} = \frac{\text{Control} - \text{Experimental}}{\text{Control}} \times 100$$

III. RESULTS AND DISCUSSION

The result on biomass and reproductive parameters of earthworm treated with pesticides is presented in table-1. From this table we find following results;

On growth of biomass:- At the end of experiment significant increase in biomass is observed in all groups. Maximum (46.1 %) increase was finding in control group, while dose dependent but significant increases were recorded in Coragen (i.e. 32.5 % and 28.95 %) and in 2-4,D ethyl ester (i.e. 39.0 % and 34.30 %) in the worms treated with 1/4th and 3/4th sub lethal concentration respectively.

Our result are corroborated with the results of earlier workers like Spurgeon et al (2004) in earthworm exposed to pesticides and heavy metal like Copper and Cadmium may experience physiological disturbances such as reduction in biomass, population size and reproduction as well as lysosome deficiencies. Capowiez [9] and Olvera-Velora [10] reported that there are high losses in body mass of earthworm treated with insecticides like imidaclopride and ethyl parathion may lead to negative effects on reproduction and their survival. Ahmed [11] showed that the loss of body

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weight along with behavioral changes like coiling, swollen body, sluggish movement and discharge of coelomic fluid was observed in earthworm species, *Lumbricus terrestris* when exposed to pesticides viz., Cyren, Ridomil and Triplen for 4 weeks. Hundal [7] revealed that the higher dose of insecticide like Chloropyrifos were found to be detrimental to the growth and reproductive performance in the earthworm compare to low and recommended doses over the control group.

On cocoon and juvenile Production:- There was significant decrease in cocoon production and number of juveniles count in worm treated with pesticides over the control. Maximum reduction was find in the worms treated with higher sub lethal (3/4th) concentration i.e. for cocoon count it was 21.4 % and 28.6 % respectively in 2,4-D ethyl ester and Coragen treated and for juvenile count, it was 33.3 % and 48.4 % respectively as compare to control. Our finding is similar with earlier workers like [12] stated that contaminated dose samples have delayed cocoon production and it has been suggested that cocoon production rates were particularly sensitive during early period of reproduction. Gupta and Sexena [13], find higher sub lethal concentration of pesticides influence the reproductive parameters i. e. cocoon production and maximum number of hatching per cocoons and longer incubation periods of worms in a close dependent manner. Espinoza and Bustos [14] studied numerous reproductive parameters in earthworms exposed to various insecticides and chemicals like cocoon production, cocoon hatching and sperm production, ability of the worm produced, sexual maturation and genotoxicity etc. Yasmin and D'Sauza [15], reported that the cocoon production was found to be most sensitive parameter for pesticide treatment on worm. Rai and Bansiwala [16] reported exposure of worms to higher sub lethal dose of malathion produced lesser number of cocoons and hatching than the worms exposed to lower sublethal concentration. Zarea [17] revealed earthworms affected by herbicide in general show low toxicity towards earthworms than insecticide and Hundal [7] revealed when *Eudrilus eugeniae* exposed to chloropyrifos insecticide which impairs the cocoon production and number of hatchling

Amorium [18] tested earthworms with herbicides and found reproduction is the more sensitive end point than portality in *Enchytraeus albidus* and *Euchytraeus luxuriosus*. Frampton [19] stated that acute mortality test would not provide the most sensitive risk estimate for earthworms in majority of cases. Similarly, Rombke [20] suggested that sub lethal effect is more sensitive and more realistic approach for the prediction of environmental effects, because applied concentration of pesticides in the field are usually quite low.

IV. CONCLUSION

The earthworm is the major soil fauna to be exposed to soil contaminants and their presence is an bio indicator of soil health. In present investigation, potential negative impact of insecticide and herbicide on non target organism or beneficial species. Herbicide in general show low toxicity towards earthworms' species, *Eudrilus eugeniae*. Results of the present study provide evidence that sub lethal doses of pesticide affect on growth, cocoon production and numbers of juveniles. Result of the present study can be used to plan and implement remediation strategies for the pesticide polluted soils where risk of soil pesticide contamination is associated with population of soil invertebrates.

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Pesticides	Vermibed Groups	Weight of worms (g)		% increase	Number of	
		Initial	Final		Cocoon	Juveniles
	Control	14 ± 1.0	26 ± 1.5	46.10	14 ± 1.0	62 ± 3
2,4-D Ethyl ester	Expt. I (1/4 th)	12.5 ± 1.0	20.5 ± 1.2	39.0	13 ± 1.0 (7.1) NS	50 ± 2.0 (19.3) *
	Expt. II (3/4 th)	13.8 ± 1.2	21 ± 1.0	34.30	11 ± 1.0 (21.4) *	41 ± 2.0 (33.3) **
Coragen	Expt. I (1/4 th)	13.5 ± 1.0	20.0 ± 1.0	32.5	12 ± 1.0 (14.3) NS	39 ± 1.0 (37.1) **
	Expt. II (3/4 th)	13.5 ± 1.2	19 ± 0.4	28.95	10 ± 1.0 (28.6) *	32 ± 2.0 (48.4) **

Table -1
Biomass and reproductive parameter (cocoon and juvenile) count of earthworm.

Per cent values are % change over control. CD (Mean ± SEM, n = 3)