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Effect of Carbofuran on the Survival Rate of *Eudrilles eugenia* and *Eisenia feotida*

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Abstract: ***Earthworms are an essential part of a healthy soil. They are the natural agents that convert complex organic agro - waste to compost. Use of chemical fertilizers, herbicides and pesticides have adverse effect on the earthworm population in a given locality. Though various chemicals are available in the market, carbofuran is used frequently. Carbofuran contaminated soil is a environmental hazard, due to the toxic effect it places on the natural ecosystem and thereby the organisms living in the soil. In this study the effect of carbofuran on the survival rate of two species of earthworms Eudrilles eugenia and Eisenia feotida was studied. The effect of carbofuran on the cocoon formation was also studied. The pesticide carbofuran was mixed with sterile soil and mature earthworms were introduced and the survival rate of the worms were observed. The cocoon formation was also analysed and the morphology of the hatchlings were also noted. The results clearly shows that the pesticide is lethal to the growth of the earthworms. Continuous spraying of the pesticide will reduce the earthworm population of the soil thereby reducing the fertility of the soil.***

Keywords: Carbofuran, *Eudrilles eugenia*, *Eisenia feotida*, survival rate, cocoon formation

1. Introduction

Earthworms are susceptible to pesticides causing immobility, rigidity, biomass reduction, reduced growth rate and reproduction by disrupting various physiological activities leading to loss of earthworm population and soil biodiversity. (Rashi Miglani and Satpal Singh Bisht.2019). Carbofuran is a carbamate pesticide, widely used around the world to control insects on a wide variety of field crops. It is a systemic insecticide, where the plant absorbs it through the roots and distributes it throughout its organs. Carbofuran is a pesticide widely used in Southern Africa to control nematodes on maize. Studies have already been made on the toxicity of this pesticide to the earthworm *E. fetida* (Bouwman and Reinecke.1987). Carbofuran also has contact activity against pests. It is one of the most toxic pesticides still in use. Carbofuran is used around the world for a wide variety of crops. It is widely used in Asia, Australia, and South America. It is commonly used in Malaysia for vegetables where it is a legally registered pesticide. Carbofuran acts through phloem sap against piercing - sucking pests such as green leafhoppers, brown plant hoppers, stem borers and whorl maggots (Sim *et al*.2019). Usage has increased in recent years because it is one of the few insecticides effective on soybean aphids.

Earthworms improve nutrient availability by ingesting organic residues of different C: N ratios (Patnaik and Dash, 1990). Activities of earthworms also help in enhancing beneficial soil microbes. The gut mucus secretion and excretion from earthworm are known to enhance the activity of microorganisms (Bhaduria and Saxena 2010). The incredible services provided by the earthworms to the ecosystem are at risk and recent research findings are now mainly focused on understanding earthworms and their responses to different pesticides. In the present study the effect of pesticide carbofuran on the survival rate of two species of earthworms namely *Eudrilles eugenia* and *Eisenia feotida* was studied.

2. Materials and Methods

The earthworms *Eudrilles eugenia* and *Eisenia feotida* were maintained in vermicompost pits. The worms were kept at a constant temperature of 25±2ºC and relative humidity of 80% in darkness (Reinecke and Kriel, 1980).

Garden soil along with leaf litter was sterilized by steam for 3 h to control all the possible organisms. Carbofuran was mixed with sterilized soil in the concentration of 10, 20, 30, 40 and 50 mg/Kg soil. Wide mouthed 10 L earthen pots with a hole in the lid, was used as containers. Soil treatments of 5 Kg was added to each container. Control was devoid of any pesticide. Triplicates were maintained for each treatment. Fully matured earthworms of 25 numbers were added to each treatment and control.

Cocoons were collected, counted and placed in separate containers. Hatchlings were removed every day and placed in soil containing carbofuran. The hatchlings were observed for any morphological changes.

3. Results and Discussion

Earthworms are important organisms in soil communities and are known for sustaining the life of the soil. They are used as a model organism in environmental risk assessment of chemicals and soil toxicology. Soil provides physical and nutritive support to agriculture system by regulating biogeochemical cycles, nutrient cycle, waste degradation, organic matter degradation *etc*. The biggest threat to soil health are pesticides and synthetic chemicals including fertilizers. Earthworms are most severely hit by these xenobiotic compounds leading to a sizeable reduction of their population and adversely affecting soil fertility. Earthworms play a crucial role in maintaining soil health. Pesticides used irrationally along with other soil toxicants, contribute to a quantum of damage to earthworms and other non - target organisms. (Rashi Miglani and Satpal Singh Bisht.2019).

In the present study, the effect of carbofuran on the survival rate of two species of earthworm namely *Eudrilles eugenia* and *Eisenia feotida* was presented in Table 1 and 2 respectively. At lower concentration 10 mg/Kg soil the worms were able to survive for longer periods whereas at higher concentration of 50 mg/Kg soil the worms perished within 15 d in *Eudrilles eugenia* and 18 d in *Eisenia feotida*. When compared to *Eudrilles eugenia, Eisenia feotida* was more sturdy and able to withstand carbofuran better.

Edward and Bohlen (1992) reported that earthworms are highly susceptible to pesticides and insecticides, therefore they are considered as a model organism to evaluate the effects of insecticides. There are certain pesticide families that are considered as harmful to earthworms i. e. neonicotinoids, strobilurins, sulfonylureas, triazoles, carbamates and organophosphates (Pelosi *et al.,* 2014). The pesticides affect mortality of earthworms by directly distressing them or by altering their physiology (Sabra and Mehana, 2015).

The effect of carbofuran on the reproduction rate was prominent as seen from the table 3 and 4. The cocoon production reduced in both *Eudrilles eugenia* and *Eisenia feotida* at higher concentration of 50 mg/Kg soil. *Eudrilles eugenia* was most affected when compared to *Eisenia feotida* as observed from the results. Pesticides have a negative effect on the survival and reproduction of earthworms especially at higher concentration. Possible effects of pesticides and insecticides on earthworms in the soil are also depended on earthworm species, type of contaminant and its concentration, soil characteristics etc. (Rodriguez - Campos *et al.,* 2014).

Various reproductive parameters such as maturation, cocoon production, viability, hatching and sperm production were studied by several workers (Espinoza - Navaroo and Bustos, 2004; Govindarajan and Prabaharan, 2014) with reference to the genotoxicity when exposed to different types of insecticides and other chemical classes. Pawar and Ahmad (2013) reported that the effect of Chlorpyriphos which is an organophosphate insecticide with the exposure period of 35 days, the dose concentration of 0.1 and 0.2 mg/Kg showed less effect on growth for the exposure period of 14 days, but effected the earthworms growth when exposed for more than 14 days.

Booth and O'Halloran (2001) found significant reduction in growth of *Aporrectodea caliginosa* by exposure to two organophosphates, diazinon and chlorpyrifos, at 60 and 28 kg/ha dose. Rajshree *et al.* (2014) also found that Methyl parathion and phorate are very toxic to earthworms and showed progressive symptoms of toxicity such as coiling, curling and excessive mucous secretion with sluggish movements, swelling of the clitellum, degenerative changes in nervous system and loss of pigmentation which is elicited by organophosphorus insecticide.

In the present study the cocoons were collected and incubated separately in Petri dishes with sterilized soil and carbofuran. The cocoons were monitored for number of hatchings and number of hatchlings. This was done by placing each cocoon in a Petridish containing sterile soil. Few hatched and those that did hatch had few hatchlings. The biological availability of the pesticide might differ according to the biological nature of the earthworms and the type of the soil. Adsorption of the pesticide molecules by the particles or organic matter could have reduced the amount of pesticide available for biological action. Because of biological activity, this might subsequently be released again as reported by Fuhremann and Lichtenstein (1978). Adsorption is usually correlated with the organic matter content (Lofs - Holmin (1981). The microbial activity should also be considered as an important factor in the removal of the chemicals. The previous exposure of the worms to pesticides may have had a direct influence on their susceptibility to carbofuran. The worms used in this study had no history of exposure to pesticides, since the worms were maintained in garden soil devoid of chemicals for atleast 10 generations. Physiological differences between different geographic populations of *E. fetida* exist and could account for differences in susceptibility. The most obvious sub - lethal response was the loss of weight by the worms that survived the exposure. The duration of the experiment was not long enough to determine whether the surviving worms would have reached maturity or not. Since optimal conditions were maintained, it is unlikely that these worms would have survived had harsher conditions prevailed. All the worms exposed to 50 mg/kg soil of carbofuran, showed a distinct coiling of the last few segments. After 24 hours on filter paper the coiling disappeared and normal behaviour was observed. This recovery may be due to excretion of the pesticide or metabolites from the body.

Carbofuran has one of the highest acute toxicities to humans of any insecticide widely used on field crops, 1 ml can be fatal to humans. In most developed countries carbofuran is applied by commercial applicators using closed systems with engineered controls so there is no exposure to it in preparation. However, in developing countries, occupational exposure to carbofuran and resultant carbofuran - serum protein labelling has been reported to impact human health and well - being (Rehman *et al*., 2016). Since its toxic effects are due to its activity as a cholinesterase inhibitor it is considered a neurotoxic pesticide. *Eisenia fetida* generally enhanced the physicochemical properties of contaminated soil. pH and electrical conductivity were significantly improved by earthworm activity in bioremdiation process. The presence of *Eisenia fetida* significantly improved nitrogen, phosphorous, and potassium concentration in soil.

The persistent nature of pesticides has impacted our ecosystem too, that have entered into various food chains and into the higher trophic levels such as that of humans and other large mammals. In order to reduce the effect of pesticides there should be input of sufficient organic manures instead of chemical fertilizers with minimal disturbances in soil and can be adapted for optimum activity of earthworms in the soil for healthy and fertile soil. Organic fertilizers are favourable in the long run, and the earthworm friendly agricultural practices would have a favourable impact on the environment and human health. Therefore, farmers must be educated regarding the beneficial role of earthworms because of its importance and to reduce or minimize the use of pesticide for safety and sustainability of fertile soil.

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Table 1: Effect of carbandazim on the growth of *Eudrilles eugenia*

S. No.	No. of days	Control	10 mg/g	20 mg/g	30 mg/g	40 mg/g	50 mg/g
1	2	25	24 ± 0.001	23 ± 0.001	23 ± 0.001	18 ± 0.005	16 ± 0.001
2	4	25	24 ± 0.001	23 ± 0.011	23 ± 0.011	23 ± 0.011	14 ± 0.011
3	6	25	23 ±0.003	23 ± 0.001	22 ± 0.001	21 ± 0.001	13 ± 0.005
4	8	25	23 ± 0.011	23 ± 0.005	22 ± 0.001	19 ± 0.005	13 ± 0.005
5	10	25	21 ± 0.003	20 ± 0.003	20 ± 0.005	19 ± 0.001	9 ± 0.003
6	12	25	21 ± 0.001	20 ± 0.005	19 ± 0.003	18 ± 0.003	7 ± 0.005
7	14	25	19 ± 0.005	18 ± 0.0 01	18 ± 0.005	17 ± 0.001	3 ± 0.011
8	16	25	19 ± 0.005	18 ± 0.005	17 ± 0.001	13 ± 0.005	-
9	18	25	17 ± 0.005	15 ± 0.011	14 ± 0.011	13 ± 0.011	-
10	20	25	16 ± 0.005	15 ± 0.005	13 ± 0.011	11 ± 0.005	-

Table 2: Effect of carbandazim on the growth of *Eisenia feotida*

S. No.	No. of days	Control	10 mg/g	20 mg/g	30 mg/g	40 mg/g	50 mg/g
1	2	25	24 ± 0.001	23 ± 0.001	22 ± 0.003	22 ± 0.003	18 ± 0.003
2	4	25	23 ± 0.001	23 ± 0.003	21 ± 0.001	21 ± 0.005	14 ± 0.005
3	6	25	23 ± 0.003	23 ± 0.005	21 ± 0.005	21 ± 0.001	14 ± 0.003
4	8	25	23 ± 0.001	23 ± 0.001	19 ± 0.001	19 ± 0.001	13 ± 0.005
5	10	25	21 ± 0.003	20 ± 0.005	19 ± 0.005	19 ± 0.003	11 ± 0.003
6	12	25	21 ± 0.005	19 ± 0.003	18 ± 0.003	16 ± 0.005	11 ± 0.005
7	14	25	18 ± 0.001	18 ± 0.001	18 ± 0.001	16 ± 0.001	8 ± 0.001
8	16	25	17 ± 0.005	16 ± 0.012	18 ± 0.005	14 ± 0.005	4 ± 0.005
9	18	25	17 ± 0.001	14 ± 0.001	14 ± 0.001	14 ± 0.001	-
10	20	25	15 ± 0.005	14 ± 0.011	14 ± 0.005	12 ± 0.005	-

Table 3: Effect of carbandazim on the cocoon formation of *Eudrilles eugenia*

S. No.	No. of days	Control	10 mg/g	20 mg/g	30 mg/g	40 mg/g	50 mg/g
1	10	13 ± 0.003	8 ± 0.005	9 ± 0.005	7 ± 0.005	7 ± 0.003	4 ± 0.003
2	20	25 ± 0.001	13 ± 0.003	14 ± 0.005	13 ± 0.005	11 ± 0.005	-
3	30	85 ± 0.001	57 ± 0.005	69 ± 0.001	73 ± 0.005	66 ± 0.0010.003	-
4	40	112 ± 0.001	78 ± 0.005	75 ± 0.003	54 ± 0.003	44 ± 0.005	-
5	50	349 ± 0.001	116 ±	117 ± 0.005	112 ± 0.005	112 ± 0.005	-

Table 4: Effect of carbandazim on the cocoon formation of *Eisenia feotida*

S. No.	No. of days	Control	10 mg/g	20 mg/g	30 mg/g	40 mg/g	50 mg/g
1	10	36 ± 0.003	19 ± 0.003	18 ± 0.003	21 ± 0.003	11 ± 0.005	8 ± 0.012
2	20	56 ± 0.005	19 ± 0.001	26 ± 0.001	25 ± 0.001	21 ± 0.003	14 ± 0.013
3	30	78 ± 0.001	75 ± 0.005	67 ± 0.003	86 ± 0.003	54 ± 0.012	-
4	40	132 ± 0.003	87 ± 0.003	87 ± 0.005	87 ± 0.003	66 ± 0.003	-
5	50	163 ± 0.005	113± 0.005	98 ± 0.003	94 ± 0.012	76 ± 0.011	-