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Assessing Cocoon Production by Earthworm in Response to Soil Pesticide Contamination

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Abstract

The global rise in human population has escalated food demand, leading the agricultural sector to heavily rely on pesticides, which over time contaminate soil. To preserve the environment, it is imperative to adopt sustainable agricultural practices. Pesticides adversely affect earthworm survival and cocoon production, rendering them unsuitable for agricultural purposes. Bioremediation combined with bioaugmentation offers a solution to improve pesticide-contaminated agricultural soil. Although earthworms are effective in soil bioremediation, even sublethal doses of pesticides can diminish their lifespan and reproductive capabilities. This study specifically highlights the impact of sublethal toxicity from AlP (Aluminium phosphide) and DDVP (dichlorvos) on *Eisenia fetida.* Initial findings indicate a substantial reduction in cocoon production in the early stages following pesticide exposure, yet subsequent bioaugmentation with cow dung marginally increased cocoon production. Thus, incorporating bioaugmentation with cow dung is essential when employing earthworms for bioremediation in pesticide-contaminated soil. Furthermore, this study underscores the significant harm that pesticide residues and sublethal doses inflict on soil fauna and the microorganisms residing within macrofauna.

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Introduction

Rapid urbanization and industrialization have led to enormous solid waste generation. This includes domestic, industrial, and agricultural waste, which is escalating at an alarming rate. Failure to address this issue seriously could lead to ecological imbalance.[1,2] Municipal solid waste generated annually in the world is around 1.3 billion metric tons and it is expected to rise by 2.2 billion metric tons by 2025.[3] One of the well-known reasons for environmental degradation is the pollution of land, water, and air through different toxic chemical substances.[4,5]

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Pollutants in the soil cause both quantitative (decrease in biomass, affects reproduction) and qualitative (depletes natural fertile capacity of soil) changes in fauna. Soil microfauna is a good indicator of toxicity in soil. Protozoans, Nematodes and mesofauna (oligochaete annelids) are used for ecotoxicological studies. The soil macrofauna (snails and terrestrial gastropod molluscs) is utilized for bioaccumulation studies.[6]

Technologies such as vermiremediation are economical and undemanding to maintain and apply. Earthworms contribute positively to soil productivity and fertility without disturbing the top layer of the soil, thereby causing minimal environmental impact. The coelomic fluid of earthworms has antibacterial properties hence, minimises the amount of pathogens in the soil. Earthworms are good organisms for bioremediation but only in low or moderately-polluted soils as earthworms' growth, reproduction and physiology will be affected in contaminated soil.[7] Assessing the response of earthworms is crucial in determining the soil ecological toxicity in contaminated soil.[8]

Eisenia fetida, an epigeic earthworm can be used for soil bioremediation. They have been shown to survive in soil contaminated with oil (Petroleum and Diesel) (3500mg/kg). Moreover, the survival rate depends on exposure and metabolism. It was also found that even polycyclic aromatic hydrocarbon concentration decreased upon bioremediation with *Eisenia fetida and Lumbricus rubellus.*[9]

Toxic pesticides such as aluminium phosphide and organophosphates like dichlorvos (esters of phosphoric acid) and their residue have contaminated the air, water, and soil and thus they are a threat to the co-existence of different plant and animal species.[10] A plentiful amount of applied pesticides (15%- 40%) is dispersed in the air through volatilization.[11] Pesticides can be harmful even after many years of application because of their characteristics such as bioaccumulation, lipophilicity, and long half-life.[10]

The first step towards sustainable management is soil protection. Invertebrates like earthworms help modify the soil environment by accumulating cast, pellets, and galleries (biogenic structures). Earthworms also play a major role in the carbon and nitrogen cycle. Urine, mucus and fresh earthworm casts contain more N, P, K and Ca than dry casts. When casts dry, the breakdown of solid organic matter ceases for several months.[12,13] However, all these services earthworms provide are at risk due to the excessive use of pesticides. The most lethal toxic class of pesticides is insecticides, which also pose a risk to the non-target soil fauna.[14]

Bioremediation is an inexpensive, effective and eco-friendly technology to clean up hazardous materials from the soil.[15] This technique includes the breakdown of organic materials by fungi, bacteria and earthworms (soil organisms).[16] In-situ bioremediation involves bioaugmentation (degrading unwanted compounds by adding pre-grown microbes) which enhances the degradation rate.[17,18] Earthworms are tolerant to heavy metals, pesticides and other organic pollutants. This tolerance capacity of earthworms makes them convert even unstable solid organic wastes such as sewage sludge, industrial waste and animal waste into compost (vermicompost) which has high NPK (nitrogen, phosphorous and potassium) content. An Increased amount of Ca (calcium), and Mg (magnesium) is also found in such composts.[19] This vermicompost can be used as organic fertilizer in crops, it increases the leaf number and leaf area, root length, and number. It also improves stem height and seed germination.[20,21] The study investigates how sub-lethal doses of pesticides AIP and DDVP affect the reproductive capacity of earthworms. In agriculture, even small amounts of pesticide residues can adversely affect the reproductive health of earthworms. Therefore, it is essential to prioritize techniques such as bioaugmentation using substances like cow dung and biochar to protect populations of soil fauna.

Material and Methods

Rearing of earthworms (*Eisenia fetida*)

Rearing of worms was done according to OECD guidelines no. 207 (1984), ISO (1993,1998), Tropical Artificial soil was utilized as a medium.

- Plastic trays, Size 50 cm X 25 cm X 22 cm for culture and rearing - For Tropical Artificial soil (adapted from Garcia, 2004 and De Silva and Van Gestel, 2009) – 10 % Non-decomposed Coconut coir dust/ coconut peat, decomposed Coconut coir dust/ coco peat was used as alternative for sphagnum peat along with rice husk dust and saw dust moistened to 50

% of its water holding capacity, 20 % kaolin clay, 70% fine industrial sand/silica. Calcium carbonate was added, and pH was adjusted to 6 ± 0.5. Nutritive medium - 30 days-old cattle manure was finely ground and sieved through a 500µm sieve. The nutritive medium was placed in a plastic tray and stabilized for 24 hours. Muslin cloth was used to cover the culture trays and to maintain proper aeration. The temperature of the medium was maintained at 25 ± 2º C and relative humidity at 80 %. Scraps of paper and cardboard were used as the bedding material.

- For testing acute toxicity, six concentrations in increasing geometric value were chosen. Aluminium phosphide powder is a dry test substance so it was mixed with artificial soil for exposure, but for Dichlorvos, the concentrations were mixed with distilled water and then evenly sprayed in artificial soil. Ventilation of test medium was done before use. Test was conducted in replicates of four with control. Adult clitellated worms were picked from culture medium, washed in distilled water, dried and introduced in the test medium. Any behavioural and physiological change observed was noted. Mortality was plotted versus Log concentration and LC_{50} was calculated by probit analysis. Moisture and pH were assessed at the start and end of the test period.

Experimental Tests

Earthen pots were used for exposure to sublethal doses of AlP and dichlorvos. The exposure to sub-lethal dose was done in artificial soil, finely ground, and sieved cattle manure was given weekly as food. Post-exposure to two sub-lethal dose (1/2 and 1/3 of LC50 for both pesticides) constant monitoring was done. To check the combined effects of both pesticides, combined exposure was also done, along with control. All the tests were performed in replicates of four. For analysis of reproduction in earthworms (cocoon production), the test period was 8 weeks. After the end of each week, the earthen pot was emptied to check cocoon production. Every week the test medium was augmented with cattle manure, finely sieved, and ground. Any physical and behavioural changes were also observed in earthworms.

All the data collected of mean weight of 4 replicates of control, AlP, DDVP exposed worms as well as combined exposure of both pesticides, was subjected to analysis by two way ANOVA. The standard deviation and standard errors were also calculated. After statistical analysis using ANOVA, Bonferroni correction (multi comparison correction) was also done. The results were subjected to Tukey-Kramer multiple comparison post hoc T- test.

Results

For analysis of cocoon production, the test media were emptied every week and the artificial soil medium was sieved and searched for any cocoons laid by the worms. The cocoon production was assessed for 8 weeks.

As compared to the control, there was a significant decline in the cocoon production in both AlP and DDVP exposed worms as well as combined exposure. In the control, average cocoon production per 10 worms per week reached close to 2 in week 5 and remained 1.3 to 1.8 throughout the test period of 8 weeks.

For worms exposed to sublethal dose of aluminium phosphide powder residue, cocoon production was significantly low throughout the test period, p-value was less than 0.05. There were fluctuations observed in the number of cocoons laid throughout the test period. The highest cocoon production was observed in the 7th week which was 0.7 ± 0.08 for 1/2 LC_{50} AlP and 0.75 ± 0.10 for 1/3 LC_{50} AlP (Table 1).

For worms exposed to a sublethal dose of dichlorvos, cocoon production was significantly lower, there was a significant difference observed between average cocoon production in control and DDVP-exposed worms, after ANOVA analysis. The p-value was << 0.05, pointing towards a statistically significant result. As compared to 1/3 LC_{50} DDVP, the cocoon production was lower in 1/2 LC_{50} DDVP, pointing towards the dose-dependent effect of DDVP on the cocoon production. Significant fluctuation was observed throughout the test period with maximum cocoon production in DDVP exposed worms observed in week 8 which was 0.6 ± 0.08 for 1/2 LC_{50} and 0.675 ± 0.09 for 1/3 LC_{50} (Table 2).

Table 1: Mean cocoon production over eight weeks in Control and AIP exposed worms, significant difference observed from control, p <<0.05. No Significant difference between AIP 1/2 LC_{50} and AIP 1/3 LC_{50}, p >0.05.

	CONTROL	AIP 1/2 LC_{50}	AIP 1/3 LC_{50}
WEEK 1	1.325 ± 0.12	0.575 ± 0.09	0.375 ± 0.17
WEEK 2	1.375 ± 0.15	0.5 ± 0.08	0.525 ± 0.05
WEEK 3	1.55 ± 0.30	0.45 ± 0.19	0.525 ± 0.05
WEEK 4	1.35 ± 0.19	0.5 ± 0.08	0.475 ± 0.05
WEEK 5	1.95 ± 0.23	0.575 ± 0.12	0.625 ± 0.05
WEEK 6	1.5 ± 0.24	0.65 ± 0.12	0.75 ± 0.05
WEEK 7	1.4 ± 0.18	0.7 ± 0.08	0.75 ± 0.10
WEEK 8	1.625 ± 0.15	0.6 ± 0.18	0.6 ± 0.14

Table 2: Mean cocoon production over eight weeks in Control and DDVP exposed worms, significant difference observed from control, p <<0.05. No Significant difference between DDVP 1/2 LC_{50} and DDVP 1/3 LC_{50}, p > 0.05

	CONTROL	DDVP1/2 LC_{50}	DDVP1/3 LC_{50}
WEEK 1	1.325 ± 0.12	0.375 ± 0.05	0.525 ± 0.12
WEEK 2	1.375 ± 0.15	0.175 ± 0.09	0.15 ± 0.05
WEEK 3	1.55 ± 0.30	0.175 ± 0.09	0.275 ± 0.09
WEEK 4	1.35 ± 0.19	0.3 ± 0.14	0.4 ± 0.11
WEEK 5	1.95 ± 0.23	0.35 ± 0.05	0.525 ± 0.05
WEEK 6	1.5 ± 0.24	0.525 ± 0.05	0.6 ± 0.08
WEEK 7	1.4 ± 0.18	0.55 ± 0.05	0.575 ± 0.09
WEEK 8	1.625 ± 0.15	0.6 ± 0.08	0.675 ± 0.09

In combined exposure to both AIP and DDVP, a significant decline from control was seen, after ANOVA analysis, p-value obtained was <<< 0.05 (significantly lower). There were fluctuations throughout the test period of 8 weeks, and the cocoon production in the combined exposure was also significantly lower than in the AIP and DDVP exposed worms (Table 3).

Table 3: Mean cocoon production over eight weeks in Control, combined AIP and DDVP exposed worms, significant difference observed from control, p <<0.05. No Significant difference between combined 1/2 LC_{50} and combined 1/3 LC_{50}, p >0.05

	CONTROL	COMBINED 1/2 LC_{50}	COMBINED 1/3 LC_{50}
WEEK 1	1.325 ± 0.12	0.175 ± 0.09	0.15 ± 0.05
WEEK 2	1.375 ± 0.15	0.125 ± 0.05	0.15 ± 0.05
WEEK 3	1.55 ± 0.30	0.2 ± 0.08	0.15 ±0.05
WEEK 4	1.35 ± 0.19	0.375 ± 0.05	0.375 ± 0.09
WEEK 5	1.95 ± 0.23	0.4 ±0.08	0.375 ±0.09
WEEK 6	1.5 ± 0.24	0.375 ± 0.09	0.5 ± 0.08
WEEK 7	1.4 ± 0.18	0.525 ± 0.05	0.55 ± 0.12
WEEK 8	1.625 ± 0.15	0.6 ± 0.08	0.65 ± 0.10

The highest cocoon production in combined exposure was 0.65 ± 0.10 for combined 1/3 LC_{50} at the end of 8th week. The lowest cocoon production in all the tests conducted was in combined exposure of 1/2 LC_{50} which was 0.125 ± 0.05 at the end of 2nd week (Figure 1). It was during these initial two weeks that worms in the test media of combined exposure showed the most behavioural changes of coiling, jumping, excessive mucous secretion and extrusion of the coelomic fluid (Figure 2).

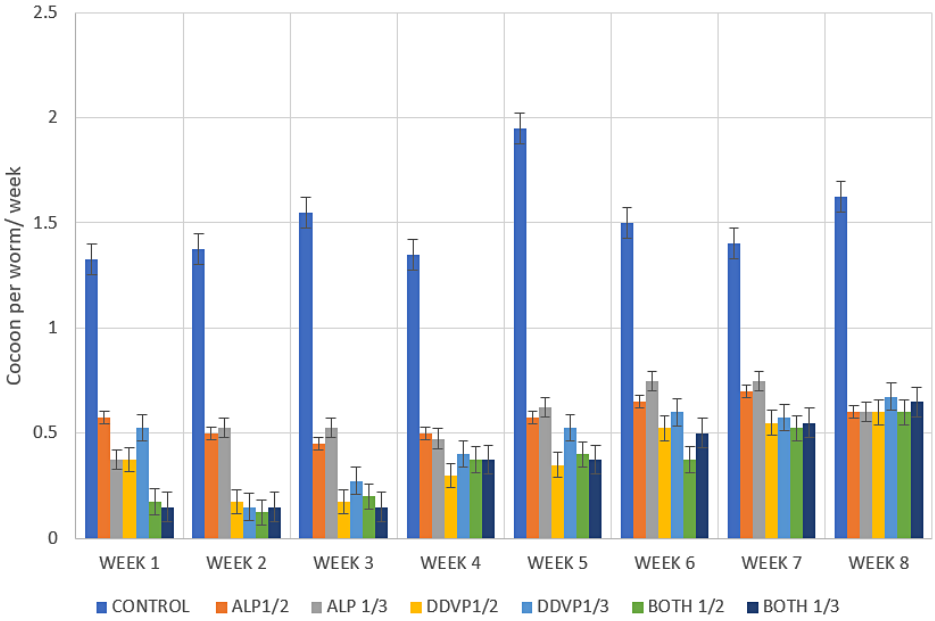

Fig. 1: Graphical representation of changes in cocoon production in control and AIP and DDVP exposed worms over 8 weeks

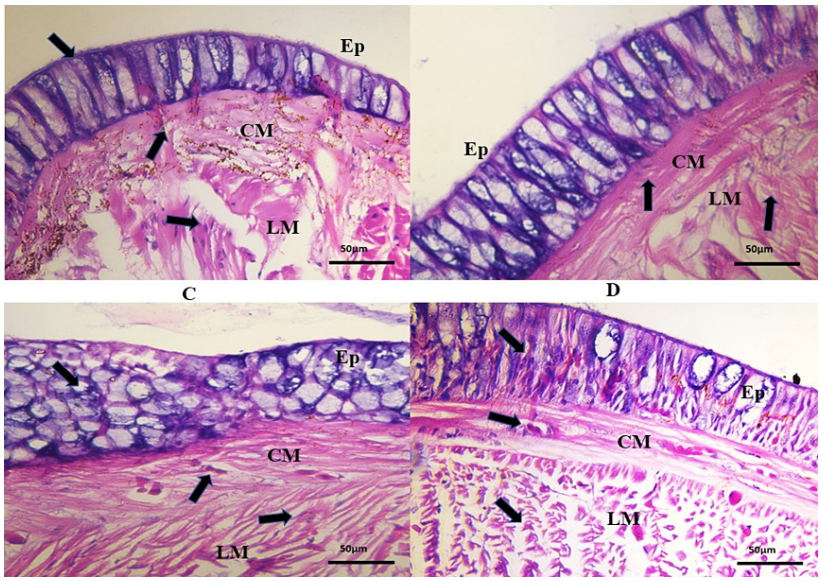

Fig. 2: Sections through the body wall of pesticide exposed worms A, B, C & D- the disintegration of the muscle layers and increased number of mucous gland cells in the epidermis can be seen.

The combined exposure had a significant decline in cocoon production. There was no significant difference in the dose of combined exposure. In fact, at the end of week 1, week 3 and week 5, the cocoon production was lower in 1/3 combined LC_{50} as compared to 1/2 combined LC_{50} (Table 3).

Discussion

When earthworms were exposed to sub-lethal doses of AlP, cocoon production reduced in all the weeks from 1 to 8 compared to control. But in both AlP ½ LC_{50} and AlP 1/3 LC_{50} in week 8th cocoon production was slightly increased compared to week 1. Almost similar results were observed when earthworms were exposed to dichlorvos. In the case of a combination of ALP and dichlorvos, the cocoon production by earthworms was significantly reduced in the first four weeks. Exposure to various pesticides causes considerable stress and reduces the capacity for cocoon laying in earthworms due to irregularities in mucus production and changes in subepidermal musculature. Over time, a slight increase was observed, after augmentation of the culture media with cow dung. The cocoons in earthworms are produced by the epidermis of the clitellar region; those exposed to pesticides exhibit an abundance of mucous glands and a modified muscle arrangement that disrupts cocoon formation.

In earthworms, cocoon development, shape, and size greatly vary among different species. In a study more weight and larger size cocoons were found in anecic earthworms whereas low-weight and smaller size cocoons were found in epigeic earthworms.[22] The incubation period of a cocoon differs among the species and also depends on the environment. The incubation period is short for epigeic and anecic species whereas for endogeic species it's long.[23] Further, the rate of cocoon production also depends on the substrate materials. Different species show different growth and reproduction patterns on the same substrate material.[24]

The use of earthworms in bioremediation, along with techniques like bioaugmentation and biostimulation, improves soil health and restores soil fertility.[25,26] In addition to bioremediation, phytoremediation (known for its high metal biosorption capacities) is essential for addressing heavily contaminated organic waste from industrial and agricultural sectors. The most commonly studied species of bacteria for bioremediation are *Mycobacterium, Pseudomonas* and *Rhodococcus*.[27] In addition to bioremediation, adequate supplies of water, oxygen, nitrogen, and phosphorus are essential to enhance degradation rates.[18] In this study, bioaugmentation was performed using cow dung. Cocoon production significantly decreased initially with sublethal doses of AlP and DDVP but showed improvement following bioaugmentation with cow dung.

Conclusion

Maintaining the health of agricultural ecosystems hinges on the preservation of optimum soil fauna populations. Pesticide exposure poses a significant threat to these crucial organisms, necessitating proactive measures to safeguard their survival. Earthworms, integral to soil health, play a pivotal role in bioremediating pesticide-contaminated soils. However, ensuring their vitality requires strategic interventions such as bioaugmentation techniques involving materials like cow dung and biochar. These practices not only support the well-being of earthworms but also contribute to the overall resilience and sustainability of agricultural ecosystems by fostering a balanced soil ecology. By prioritizing the health of soil fauna through thoughtful management practices, we can mitigate the adverse impacts of pesticides and promote sustainable agricultural practices for the future.

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Conflict of Interest

The authors have no competing interests to declare that are relevant to the content of this article.

Author's Contribution

All authors contributed to the study's conception and design. Material preparation, data collection and

analysis were performed by Himanshu Sharma and Yasha Yadav. The first draft of the manuscript was written by all authors. All authors read and approved the final manuscript.

Data Availability Statement

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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