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Earthworm Avoidance Behaviour Test As A Screening Tool To Assess Soil Conservation

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INTRODUCTION

Earthworms contribute to soil structure formation and organic matter activation through nutrient cycling and decomposition of residues. Charles Darwin was the first person to recognize the significance of earthworms, since then, they are labeled as 'keystone species' as they affect soil properties.
Soil avoidance by earthworms has been considered a valid and sensitive test for analyzing soil contamination by xenobiotics. The result of avoidance behaviour response test can increase sensitivity in the evaluation of a quick analysis of an ecological endpoint (Yeardley et al., 1996). Hence, avoidance behaviour is used as a parameter to evaluate adverse and stressed conditions.
Vermicomposting is a solution to large amounts of organic agro wastes being burned. This method can promote our agricultural development in a more effective, economic and eco friendly manner. The soil enriched with vermicompost provides additional substances that are not found in chemical fertilizers.
Vermicomposting is marked as an answer to the effective waste management system. This process allows the composting of degradable materials as well as the utilization of products obtained after composting to increase and improve crop production by the effective elimination of chemical fertilizers. It has also been headlined as the remedial method to a soil surrounding treated with chemicals (Edwards and Bohlen, 1996).
The study is acting as a guide to local farmers and agricultural workers as it provides the details on optimum amount of pesticides, soil contamination test and domestic vermicomposting.

Avoidance behaviour:
Avoidance (escape) behaviour is a type of activity seen in animals exposed to adverse stimuli, in which the tendency to act defensively is stronger than the tendency to attack. Most animals capable of locomotion show a rapid locomotor reflex to stimuli. Animals show avoidance to stressful conditions such as chemical exposure, high temperature etc. The motor function of earthworms is limited to locomotion and rapid writhing escape movements.
Soil avoidance by earthworms has been considered a valid and sensitive test for analyzing soil contamination by xenobiotics. The result of avoidance behaviour response test can increase sensitivity in the evaluation of a quick analysis of an ecological endpoint (Yeardley *et al.*, 1996). Hence, avoidance behaviour is used as a parameter to evaluate adverse and stressed conditions. Toxicity test to earthworms is enlisted in commission regulation (EC) No.440/2008 of 30 May 2008.

Vermicomposting
Vermicomposting is the method of biological degradation and decomposition leading to stabilized organic waste by earthworms and microbes to form vermicompost. Organic farming is maintained effortlessly with the help of vermicomposting which is easily prepared and harmless to the environment.
Epigeic earthworms like Eudrilus eugeniae are used for vermicomposting and the resultant compost is called as vermicompost, which is a fine granular material with excellent properties of structure, porosity, aeration, drainage and moisture holding capacity (Edwards, 1982, 1988). Vermicompost enhances the physical, chemical and biological properties of soil (Kale, 1998). It acts as a material enhancing growth of crops like wheat, paddy

and sugarcane (Ismail, 2005). Vermiculture is the culture of earthworms and vermicast is the faecal matter released by the earthworms (Ismail, 2005).
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The objectives of the study are
1. To study the avoidance behaviour of *Eisenia fetida* in soil treated with different doses of the insecticide- Chlorpyrifos + Cypermethrin and pesticide- Quinalphos.
2. To assess vermicomposting of vegetable wastes with the help of Eudrilus eugeniae using cow dung and thereby obtain awareness on eco friendly organic waste management.
3To enhance the awareness of local people, especially agricultural workers about soil contamination test and effective cum nutritional compost, hence understanding the relationship between earthworm and ecosystem functioning.

METHODOLOGY

Avoidance behaviour of *Eisenia fetida* to commonly used agro chemicals

The study comprising avoidance behaviour of the earthworms *Eisenia fetida* to some commonly used agro chemicals was undertaken during the month August 2019.

A. Collection of earthworms:
The earthworms of the species *Eisenia fetida* were collected from the vermiculture pits created in the house backyard (Aripra) and from Agricultural Research Station (Anakkayam) with assistance. Sixty healthy juvenile clitellated worms were randomly sampled from the population for inoculation.

B. Preparation of bed:
Six plastic boxes (17X18X16 cm) with tiny holes for aeration were procured and the inner sides of the boxes were wrapped with paper to prevent direct contact of the worms with the plastic walls of the boxes. A hard cardboard sheet was placed in each box to compartmentalize it into two chambers, one control and the other treated. Soil weighing 1kg was fed into each chamber. A lid covering the boxes was also set ensuring no escape of earthworms.

C. Soil:
For the study, the soil was collected from the backyard of the house where vegetables and banana plantations are maintained. The pH value of soil was 6.8 as determined by soil analysis report. It was treated with organic cow dung manure in the ratio 50:50.

D. Test chemicals:
1.Ekalux: it is an organophosphate pesticide. The chemical is manufactured by Biostadt India Ltd.
Chemical composition: Quinalphos 25% EC
Quinalphos technical 35.70% w/w
Solvent xylene (dimethyl benzene) 54.30% w/w
Emulsifier (calcium alkyl)
Sulfonate and polyoxyethylene ether 10.00% w/w

Three different concentrations were taken:
0.1ml in 100ml water (Low)
0.2ml in 100ml water (Optimal and recommended dose field application)
0.3ml in 100ml water (High)

2. Koranda 505: It is a combination of organophosphate and pyrethroid insecticide. The chemical is manufactured by Rallis India Ltd.
Chemical composition: Chlorpyrifos 50% w/w & Cypermethrin 5% w/w
Chlorpyrifos 50.00% w/w
Cypermethrin 5.0% w/w

Emulsifier A (anionic oxide base) 5.6% w/w
Emulsifier B (nonionic oxide base) 2.4% w/w
Three dosage of the chemical was prepared:
0.1ml in 100ml water (Low)
0.2ml in 100ml water (Optimal and recommended dose field application)
0.3ml in 100ml water (High)

E. Procedure:
(i) Acclimatization of worms: The collected earthworms were inoculated into soil kept in prepared chamber termed as T (treated) for acclimatization before each treatment and placed in such chambers undistributed for 2 hours.
(ii) Experimental setup: Three different set ups for treatment were taken carrying low (T1), optimal (T2) and high (T3) concentration of the test chemicals. Earthworm acclimatized chambers (treated chambers) are served with soil treated with different concentrations of the test chemicals. Then the untreated soil (control) is filled in the other chamber named as control (C) chamber. The cardboard sheet was removed, lids were placed on boxes and were allowed to stay undisturbed for 48 hours. For each concentration, the procedure was carried out 3 times to facilitate average observation.
(iii) Evaluation: After 48 hours, lids were removed and cardboard sheet was reinserted to avoid further migration of earthworms. Then the number of worms were counted in both the chambers and recorded . The percent avoidance was calculated.
Also to record normal percent migration, a control box with untreated soil and manure was experimented with the same number of earthworms.

Vermicomposting using *Eudrilus eugeniae* with dry leaves, vegetable waste and cow dung manure.
Phase 1: Field experiment
- Construction of the vermicompost station

A vermicompost station of three buckets (height of 25cm and diameter, 17cm) are used as containers for culturing the earthworms. These units have tiny holes for effective water drainage.
- Preparation of culture bed:

The culture bed was prepared as described by Ismail (2005).
1st layer: A basal layer of vermibed comprising broken bricks, then a layer of sand of appropriate thickness was set up to ensure proper drainage.
2nd layer: Soil collected from the backyard of the house is moistened and layered. The earthworms, *Eudrilus eugeniae, were* inoculated into this layer.
3rd layer: Lumps of fresh or dry cattle dung were scattered over the soil, mixed with vegetable waste.
4th layer: The soil was then covered with dry grass and leaves.
The entire unit was covered with banana leaves to protect the earthworms from sunlight and birds. It was kept moist by sprinkling water weekly and was turned over once a week up to the harvest of the vermicompost.

Phase 2: Import of *Eudrilus eugeniae*:
In the 2nd phase, seventy five composting earthworms, *Eudrilus eugeniae* (epigeic species) were used for the production of vermicompost from vegetable waste and cow dung. The following parameters of the vermicompost were analyzed:
(a) The total amount of the vermicompost produced (weight in kg)
(b) Chemical analysis using the methods applied in the District Soil Testing Laboratory, Malappuram.
Phase 3: Experimental design for vermicomposting:
The vermicomposting experiment was conducted in plastic buckets. The vermibed of each combination of the cow manure was combined with dry leaves and vegetable waste in the ratio 2:1. In each unit, 25 earthworms were inoculated. The entire units were moistened and covered with banana leaves. It was moistened twice a week and turned once a week. To the inoculated soil,1kg of cow manure and 0.5kg of dry leaves (Treatment 1), 1kg of cow manure and 0.5 kg of vegetable waste (Treatment 2) and 1kg of cow manure and combination of 0.25kg of dry leaves and vegetable waste (Treatment 3) is applied.
After 120 days, the total production of vermicompost was determined and chemical analysis of the vermicompost was conducted. The vermicompost produced was collected in plastic trays and weighed in kilograms.
Productivity of vermicompost was calculated in percentage using the formula:
Productivity of vermicompost (%) = harvested vermicompost (kg) / Total mass of feed (kg) *100

RESULT AND DISCUSSION

Analysis of avoidance test using *Eisenia fetida*

All the results mentioned clearly depict that the percent migration of *Eisenia fetida* increases with the increase in the concentration levels of herbicides and insecticides. The tables and figures drawn can instantly indicate the told fact.

It is clear and evident that *Eisenia fetida* is most sensitive to treatment of soil with Cypermethrin + Chlorpyrifos at high concentration when compared to Quinalphos reactions. Avoidance behaviour fundamentally shows sublethal stress of earthworms in a short period of time.Avoidance in many cases has proved to be a sensitive indicator of chemical contamination of soil (Yeardley et al., 1996).

Table1: Percent migration of *Eisenia fetida* in response to treatment of different doses of Quinalphos in soil.

Type of Dose	C1	C2	C3	MEAN	Percentage of migration
Low(0.1ml in 100ml water)	0	1	0	1.0	10
Optimal(0.2ml in 100ml water)	2	2	2	2.0	20
High(0.3ml in 100ml water)	5	7	7	7.0	70

Table 2: Percent migration of Eisenia fetida in response to treatment of different doses of Cypermethrin and Chlorpyrifos in soil.

Type of Dose	C1	**C2**	**C3**	**MEAN**	**Percentage of migration**
Low(0.1ml in 100ml water)	3	2	3	3.0	30
Optimal(0.2ml in 100ml water)	2	3	3	3.0	30
High(0.3ml in 100ml water)	9	9	8	9.0	90

Table 3: Percent migration of earthworms in untreated soil

	C1	C2	C3	MEAN	Percentage of migration
Control soil	1	2	1	1.0	10

Table 4: Percent avoidance of *Eisenia fetida* to the soil treated with different concentrations of the test chemicals used in the study.

Type of doses	Quinalphos	Chlorpyrifos Cypermethrin
Low	10%	30%
Optimal	20%	30%
High	70%	30%

Due to the increasing population and constant requirement for food and other cultivated products, the agriculture industry faces a lot of pressure in order to expand its field to satisfy upcoming and present conditions. Hence, farmers are compelled to broaden their research to find out and experiment with various chemicals (like insecticides and pesticides) which can be harmful at some instances yet could provide a bug free and large yield in crops. For the selection and dosage of these chemicals to be used on certain plants and crops, farmers and agricultural workers utilize the type of study reports and experiments conducted. An earthworm based study can provide information regarding the soil fertility and soil types. Also the accurate pesticide to be used in what all dosages according to the avoidance behaviour of earthworms and isopods can be grasped with the help of this minimal yet beneficial study. Since earthworms are an affirmative indicator of a fertile soil, studies based on them are of economic importance worldwide. The stable soil structure provided by earthworms with increased nutrient availability and proper drainage, earthworms are encouragingly labelled as farmer's friends.

Analysis of vermicompost (after 120 days of composting period)

Table 5: Chemical properties of raw feedstock and vermicompost.

Parameter	**Cow dung manure**	**Raw material**	**Vermicompost**
pH-H2O	6	6.50	6.20
Total organic carbon (%)	21.02	39.84	22.53
Total-N (%)	0.84	0.80	1.42

C:N ratio	15:1	23:1	15:1
Total-P (%)	0.78	0.24	0.75
Total-K (%)	0.86	1.23	1.0
Total-Cu (ppm)	32.3	8.49	28.60
Total-Zn (ppm)	917	152	318

Table 6: pH and macronutrients of raw feedstock and vermicompost.

Treatments	pH-H2O	TOC	TN	C:N ratio	TP
Raw feedstock					
Dry leaves	6.8	41.87	39:1	0.96	0.28
Vegetable waste	6.5	40.95	21:1	0.63	0.19
Dry leaves+ Vegetable waste	7.3	36.70	23:1	0.82	0.26
Cow dung manure	6	21.02	15:1	0.84	0.78
After 8 weeks					
Dry leaves	6.5	20.60	13:1	0.52	0.86
Vegetable waste	6.2	16.76	15:1	0.84	0.78
Dry leaves on Vegetable waste	6.4	17.58	15:1	0.76	0.80
After 16 weeks					
Dry leaves	6.4	22.38	16:1	1.07	0.78
Vegetable waste	6	23.05	13:1	1.68	0.72
Dry leaves on Vegetable waste	6.2	22.17	15:1	1.53	0.76

Table 7: Harvest data of vermicompost.

Units (composition)	Dry leaves (T1)	Vegetable waste (T2)	Dry leaves+Vegetable waste (T3)
Total mass of feed (kg)	0.5	0.5	0.25+0.25
Harvested vermicompost (kg)	738	750	767
Productivity of vermicompost(%)	49.2	50	51.13

Vermicompost contains most nutrients in plant-available form such as phosphates, exchangeable calcium and soluble potassium (Suthar, 2007). In India, earthworms have been successfully utilized for vermicomposting of leaf litters, rice straw, municipal solid wastes, paper wastes, silkworm litter and beverage industry sludge (Singh et al.,1997).

Vermicompost is said to be an organic fertilizer and soil conditioner which can be processed and harvested domestically or via industrial procedures. It is used to enhance products and also used in small scale farming. For the agricultural industry which is expanding day by day, vermicompost from organic matters is helpful for better growth and nutrition filled products.

CONCLUSION

The avoidance behaviour test is termed as an easy and quick alternative to other eco-toxicological tests with many experiments, even though it cannot solely predict acute toxicity. The result obtained in the current study claims the idea of using avoidance test as a screening test for evaluating soil contamination by agro chemicals. However, studies involving other various chemicals and earthworm species are to be analysed before concluding soil quality evaluation by the avoidance test.

As per the vermicomposting procedure, it was a successful study using dry leaves, vegetable waste and cow dung manure with *Eudrilus eugeniae*. The produced vermicompost had a dark colour, earthy odour and was homogeneous. It possessed almost all the essential macro and micro-plant nutrients like N, P, K, Mn, Cu, Zn, indicating the study provided an environment friendly nutrient rich fertilizer for agriculture industry. The vermicompost product using other possible raw materials and manure should also be analyzed and explored when vermicomposting is carried out in the future.

REFERENCE

1. **Edwards, C.A., Humberto, J. and Dominguez, L. (2011).** Biology and ecology of earthworm species used for vermicomposting. *Vermiculture technology: earthworms, organic waste and environmental management*, 25-37.
2. **Farukh, S. and Ali, A.S. (2011).** Effects of Endosulfan, an organochlorine pesticide on growth, reproduction and avoidance behaviour of earthworm Eisenia fetida. *Iranian Journal of Toxicology,* 5(14), 495-501.
3. **Souza, A. and Andrea, M. (2011).** Earthworm (*Eisenia andrei*) Avoidance of soils treated with Cypermethrin. *Sensors (Basel, Switzerland),* 11. 11056-63.
4. **Suthar, S. (2009).** Vermicomposting of vegetable market solid waste using *Eisenia fetida*: Impact of bulking material on earthworm growth and decomposition ratio. *Ecological Engineering,* 35. 914-920.
5. **Yeardley, R.B., and Gast, L.C. (1996).** The potential of an earthworm avoidance test for evaluation of hazardous waste sites. *Environ Toxicol Chem 15,* 1532-1537.