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Effect of Natural Biopesticides Neem and Commercial Chemical Pesticide Fipronil on Microbial Diversity of Earthworm Gut

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ABSTRACT

Study of gut microbial flora from Earthworm species *Eisenia foetida* was conducted. Three treatment feeds were applied for feeding the earthworms; T1 – neem leaves as organic raw material, T2 - Soil mixed with Fipronil + Neem Leaves Waste, T3 - Soil + Garden Waste. The effect of these three different feeds on gut microflora was studied. The microbial growth was enhanced in gut of earthworms fed with neem leaves followed by garden waste and Soil mixed with Fipronil + Neem Leaves Waste. This study explains the importance of naturally available biopesticides and the need of eliminating the chemical insecticides and pesticides.

Key words : *Eisenia foetida, Microbial, Gut, Neem, Fipronil*

Introduction

In the context of soil health, earthworms are the pioneer engineers by maintaining relationship with microbes, which are proliferated in the gut and vermicompost. Earthworms also help in the distribution of microbes and with their joint action the duo mineralize and humify organic matter and facilitate chelation of some metal ions.. Earthworms breakdown the organic matter, loosen it there by increasing surface area for colonization of microbes aiding the agroecosystem sustainability.

The increasing use of pesticides and chemicals in the agricultural fields is a concerning factor as it is pressurizing the global environment by disturbing the flora and faunal diversity. The interaction of earthworm in pesticide infested soil can be used as a bioindicator for pesticide pollution. Continuous use of chemical pesticides is not only making negative impact on living and non-living entities with a threat, but also the pests are becoming resistant to these pesticides by altering their genetic makeup for survival (Kaiser, 2005).

Biopesticides are produced from the source of animals, plants and microorganisms with the potential of neutralizing plant infested pests in an eco-friendly way replacing the chemical pesticides which are environmentally unsafe. This study also aims with special emphasis on need for use of biopesticide and replacing chemical pesticides by identifying the change in microbial gut flora of earthworm, the earthworm activity and effect on vermicompost production a value-added end product. Vermicompost is organic manure produced by activity of earthworms in collaboration with gut micro flora.

(Asst. Professor)

Many studies have been conducted on earthworm's role as bioindicator species for the ecotoxicological analysis of pesticide soil pollution (Schreck *et al*., 2008; Belanger, 2009). Some experiments also revealed that microorganisms in the soil help degrade the chemicals (Abdullah *et al*., 1997).

Microbes enrich the soil by supplying nutrients, enzymes it also enhances the plant growth by producing the plant growth promoters like auxins, gibberellins and antibiotic (Gyaneshwar *et al*. 2002; Jacoby *et al*., 2017). Inside the gut of earthworms, the digestion of soil organic matter takes place through a mutualistic relationship between ingested microflora and earthworm (Lemtiri *et al*. 2014) during which a direct contact occurs between soil, microorganisms and pollutants.

Fipronil is a most commonly used effective phenylpyrazole insecticide in farming to control the pest. According to Xianjiang *et al.* (2020), fipronil can be metabolized to many forms of toxic metabolites in living organisms. These insecticides can contaminate or enter the foodstuffs, posing a threat to human population. Earthworms named as the intestines of earth contribute a huge deal in maintaining the soil health, principally through their activities in consuming organic matters fragmenting it and mixing it intimately with mineral particles to form aggregates. During their feeding, earthworms promote microbial activity greatly which in turn also accelerates the breakdown of organic matter. However, very little information is available about the ecotoxicological effects of pesticides on soil fauna.

This study thus aims at evaluating the effect of biopesticide and chemical pesticide on microbial diversity of earthworm gut.

Materials and Methods

The earthworm species *Eisenia foetida* were collected from Krishi Vigyan Kendra, Aurangabad. Around thousand authenticated earthworms were cultured in a pot with natural day light condition. The feeding substrate for earthworms was partially decomposed neem leaves waste. Experimental pots were set-up, T1 included soil mixed with neem leaves powder in the proportion of 50:50. T2 - soil mixed with fipronil + neem leaves waste. Fipronil was diluted in water in the proportion of 1.5 ml/l. This water was later added in the soil (T2). T3 as Control consisted of soil mixed with garden waste.

Enumeration of total bacteria and fungi was done from vermiproducts by serial dilution plate count technique using spread plate method (Aneja, 2008). For microbial analysis of vermicompost, one gram of sample was transferred into 10ml sterilized distilled water. This mixture was kept on shaker for about 30-40 minutes after which was processed for serial dilution of 10^3 and 10^4. Aliquots of 0.1ml was placed on sterile petri plates containing autoclaved media, spread with flamed glass spreader by spread plate method.

For culture of these microbes the media used were Nutrient agar for of bacteria, Sabouraud Dextrose agar for fungal culture. For fungal culture Sabouraud Dextrose agar was supplemented with 0.01% w/v Chloramphenicol for suppression of bacterial contaminants. The colonies were counted with the help of digital colony counter and given in terms of Colony Forming Units (CFU $gram^{-1}/ml^{-1}$). All the experiments were carried in triplicates. The standard incubation period for the growth of microbial cultures was 24 hours for bacterial growth, 48 hours for fungi at room temperature. Statistical Analysis of the result readings were made by using Graph Pad Prism 8.

Gut analysis of *Eisenia foetida* for microbial flora

The gut of earthworm was removed aseptically, placed on shaker to free the gut contents, 0.1ml of which was taken by serial dilution method for culture by spread plate method. Earthworm fed on T1 and T2 and Control were subjected for gut microbial analysis. Microscopic observations of bacteria were done with Gram's stain and fungi stained with Lactophenol Cotton Blue (LCB) was used (Wenyon, 1926).

From the Earthworms gut the bacterial and fungal isolates were selected and identified using various biochemical tests. Biochemical tests included bacterial identification through Triple Sugar Iron, Urease, Indole, H_2S strips, Glucose, Lactose, Citrate, Mannitol.

Results and Discussion

Earthworms from vermicompost of neem leaves waste showed enhanced gut microbial flora as compared to earthworms that fed on control (T3) and fipronil + neem leaves waste mixed soil (T2). It can be concluded that the use of chemicals containing synthetic compounds to control pests penetrates the soil and make harmful effect on animals dwelling

within the soil habitat. To prevent environmental pollution these chemicals can be replaced with eco-friendly naturally occurring products such as herbicides, vegetative waste etc.

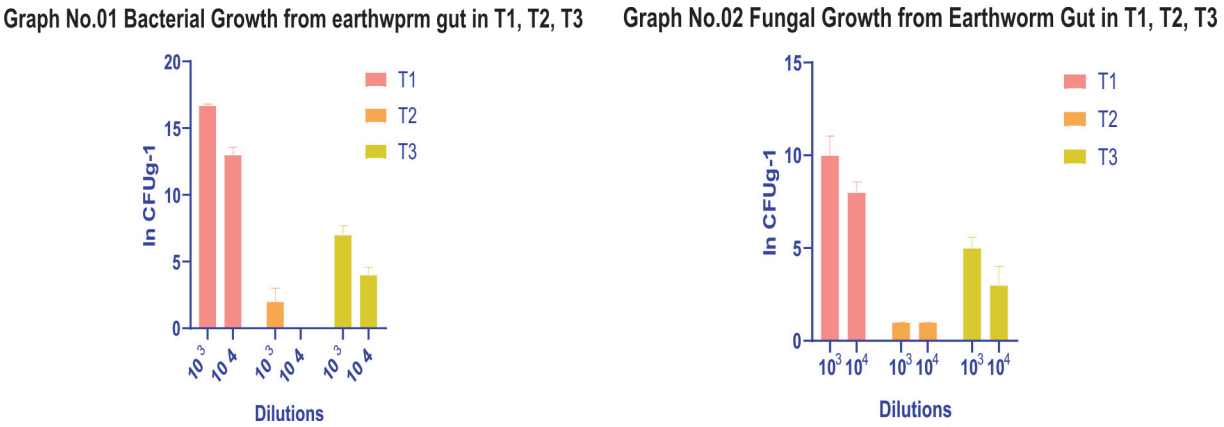
Bacterial count was increased maximum in the range of T1 (16.7 CFUml^{-1} x 10^3) > T3 (7 CFUml^{-1} x 10^3) > T2 (2 CFUml^{-1} x 10^3)/ 10^3 dilution, T1 (13 CFUml^{-1} x 10^4) > T3 (4 CFUml^{-1} x 10^4) > T2 (00 CFUml^{-1} x 10^4) / 10^4 dilutions.

Fungal count from the end product was in the range of T1 (10 CFUml^{-1} x 10^3) > T3 (5 CFUml^{-1} x 10^3) > T2 (1 CFUml^{-1} x 10^3)/10^3 dilution, T1 (8 CFUml^{-1} x 10^4) > T3 (3 CFUml^{-1} x 10^4) > T2 (00 CFUml^{-1} x 10^4)/ 10^4 dilutions.

Bacteria isolated and identified by biochemical method were *Bacillus* spp, *E.coli, Klebsiella* spp, Pseudomonas spp, Streptococci etc, while Fungal species was commonly found in the vermicompost. There might be a positive correlation between the organic matter and the activity of earthworms which must have significantly increased these microbes. In an experiment conducted by Idowu *et al.*, 2006 isolated bacteria were identified as Bacillus spp., *Pseudomonas aeruginosa, Streptococcus mutans, Clostridium,* etc, molds were identified as, *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp and *Rhizopus* spp etc.

Tiwari *et al.*, (1989) stated that increased microbial populations might be due to favourable conditions for the growth of bacteria within the digestive tract of earthworm which is being facilitated by nutrient rich organic waste which provide energy and also act as a good substrate for microbial growth.

An experiment conducted to control the pod borer pest of rice crop by application of vermiwash in combination with botanical neem seed extract including neem extract proved to be an ensured method for pest control in an ecofriendly manner and an alternative for chemical pesticide (Haralu *et al.*, 2018).



Graph 1. Microbial Growth

Table 1. Microorganisms Isolated from Earthworm Gut

T1	T2	T3
Bacillus spp	*Bacillus* spp	*Bacillus* spp
Escherichia coli	*Escherichia coli*	*Escherichia coli*
Streptococcus Spp	——–	*Streptococcus* Spp
Pseudomonas spp	———	*Pseudomonas* spp
Klebsiella spp	———	*Klebsiella* spp
Rhizopus spp	———	*Rhizopus* spp
Fusarium spp	———	*Fusarium* spp
Penicillium spp	*Penicillium* spp	*Penicillium* spp
Mucor spp	———	*Mucor* spp
Trichoderma spp	———	*Trichoderma* spp
Aspergillus spp	———	*Aspergillus* spp
Cladosporium Spp	———	———

Pesticides and insecticides applied to the soil have deleterious effect on soil nutrients thereby affecting the soil fertility and soil health with an impact on the mobilisation and free availability of soil nutrients to plants and other organisms dwelling in the soil biota. Fipronil insecticides have been shown to pose a risk of harm to earthworms and other soil invertebrates (Pisa *et al*., 2014) and the potential to adversely affect soil ecosystem services (Chagnon *et al*. 2014). Therefore, an understanding of the fate and dynamics of insecticide residues in soils is necessary for an environmental risk assessment.

Conclusion

Earthworms are used as bioindicators against pollution. The use of ecotoxicological biomarkers such as earthworm behavioural responses such as avoidance, burrowing activity or cast production, decrease in the body weight, casts as significant hot spots of microbial proliferation and enzyme activities etc are widely practiced through many experiments. To study further symbiotic status of the microorganisms associated with the earthworm digestive tract.

Therefore, it is important to understand and predict how agrochemicals, especially insecticides, may affect soil fertility through their effects on microorganisms responsible for maintaining soil fertility and the populations of invertebrates responsible for the recycling and translocation of nutrients and maintain the ecological niches.

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