

Laboratory Evaluation of Fipronil on Biological, Parameters, Gut Microflora and Physiology of *Eudrilus Eugeniae*

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Abstract- Earthworms are economically important organisms and play key role in environmental management. Vermiculture is entirely natural process which maintains the environmental balance. In the present studies an attempt was made to observe the effect of soil mixed insecticide, viz. Fipronil on biological parameters, reproductive physiology of and total count of gut microflora of *Eudrilus eugeniae* and the ability of these microflora to produce enzymes. It was observed that in Fipronil treated soil the earthworm population was reduced and its growth and reproductive potential was adversely affected. The number of cocoons and juveniles formed was decreased in Fipronil treated soil than in control. In addition to that carbon and phosphorus content of the treated soil is significantly reduced as compared to the control. In case of earthworms from treated soil there was reduction in total viable count of gut microflora as well as enzyme production ability of microflora as compared to that in control.

Index Terms- Fipronil, gut microflora of earthworm, soil fertility

I. INTRODUCTION

In order to get maximum crop yield there is a need to protect the crop from insect pests. Farmers are applying several kinds of insecticides to get rid of pests. Tons of synthetic pesticides are applied annually to crops worldwide. Whereas pesticides are used for precise purposes, to eliminate weeds, kill fungus, and eradicate insects all of which can damage crops chemicals can move off site and create non-target impacts. A small share of pesticides applied on a given field or in a given area actually reaches the intended target. It is estimated that less than 0.1 per cent of the pesticides applied to crops reach the target pests^[1]. Thus, more than 99 per cent of applied pesticides have the potential to impact non-target organisms and become widely dispersed in the environment as potential contaminants. These uncontrolled and continuous malpractices result in the accumulation of insecticides in the soil. Consequently the quality and fertility of the soil is reduced due to reduction in the essential microflora and fauna and soil dwelling animals like earthworms. The later are animals which are the most essential to build the soil to support the vegetation. They benefit soil quality by shading residues and improving soil fertility. Earthworms are part of a host of organisms that decompose organic matter in the soil. As earthworms digest the microorganisms in soil and

organic matter, the form of nutrients is changed as materials pass through the earthworm's gut. Thus, worm casts are richer in nutrients than the surrounding soil and containing nutrients changed into forms that are more available to plants. It was found that in a sample of soil with 4% organic matter worm casts contained 246 pounds of N per 1000 square feet while the surrounding soil contained 161 pounds of N per 1000 square feet^[2]

Insecticides are proved to be toxic to earthworms by various workers^[3,4]. They have been used to assess impact of soil pollution^[5,6,7,8]. The laboratory toxicity of insecticides on various species of earthworms have been investigated^[9,10,11,12]

Fipronil is a broad spectrum phenyl pyrazol insecticide toxic by both contact and ingestion in insects. It is neurotoxic, contact poison. It is used as pesticide to control horticultural and pests on agricultural products. Chemically the Fipronil is 5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4- (trifluoromethylsulfonyl)-1H-pyrazole-3-carbonitrile. Fipronil was 1st registered for use by the U. S. Environmental Protection Agency in May 1996. Currently, 49 registered products contain Fipronil as an active ingredient. It is used to control ants, cockroaches, termites, weevils, beetles, flea, ticks and other insects^[13]. It is used in granular turf products, seed treatments, topical pet care products, gel baits, liquid termiticides and in agriculture.

Earthworms effectively harness the beneficial soil microflora, destroy soil pathogens and convert organic wastes into enzymes, antibiotics, growth hormone and protein rich casts. Earthworm bioreactors have an in-house supply of enzymes such as amylase, cellulose, nitrate reductase and alkaline phosphatases. The digestive enzymes of earthworm are responsible for the decomposition and humidification of organic matter. The compost thus formed has high economic value as soil conditioner for plant growth.

In the present work an attempt was made to study effect of soil mixed insecticide, Fipronil on exotic species of earthworm viz. *Eudrilus eugeniae* and on the soil properties with respect to pH, carbon, phosphorus, potash content under laboratory conditions. Also observations on gut microflora and assay of gut enzymes of *E. eugeniae* in Fipronil treated and control samples were made.

II. MATERIALS AND METHODS

1. Preparation of mother culture:

A container with an outlet at the bottom to drain excess of water is used to prepare mother culture. A polythene paper was spread at the base of the box on which 3 inch layer of soil was spread. Above the soil layer 1 inch layer of vermiculture bed was prepared by mixing 1/3rd of soil with 2/3rd of organic matter such as kitchen wastes. Above vermiculture bed 1 inch layer of soil was made. This was followed by 1 inch layer of cow dung. Top most 3 inch layer was of dried leaves, roots, stems and grass. Water was sprinkled to maintain the moisture. Top of the box was covered by wet Jute cloth.

2. Experimental sets:

a) To study the effect of Fipronil on biological parameters of earthworm, *Eudrilus eugeniae*: Earthworm cocoons were isolated, maintained in separate containers and observed for hatchings. The juveniles of the same age were collected for experimental purpose. Soil is mixed with Fipronil to make different concentrations. Vermiculture beds were prepared using Fipronil mixed soil. Three replicates of each concentration were prepared. Ten earthworms were released in each replicate. Untreated soil was used to make controls. Observations were made on mortality of juveniles after 24hr. of treatment. Dose response was calculated by regression analysis.

A dose of LC₂₀ (20.7mg Fipronil/Kg. of soil) was used to determine the effect of Fipronil on biological parameters such as growth, development and reproductive potential of *E. eugeniae*.

Effect on soil properties: Analysis of control and treated soil for pH, Phosphorus and potash content was done.

b) **To study effect of Fipronil on gut microflora of *E. eugeniae*:** Earthworms from control and Fipronil treated soils were dissected under aseptic conditions. Guts were collected in the sterile homogenizer and homogenized them in the saline. Serial dilutions were made and used to streak the plate on the sterile nutrient agar plate.

c) **To study effect of Fipronil on gut enzymes of *E. eugeniae*:**

Cellulase assay: Plates of Czapek dox agar plates were prepared with 1% cellulose and streaked with the culture suspension. They were incubated at room temperature for 24 hours. Plates with growth of microorganisms were taken for assay. They were inoculated with one colony in different flask with 100ml sterile cellulose broth and incubated on rotary shaker for 4days at room temperature at 100rpm. After 4days of incubation broth was taken in microfuge tubes and spin for 5min at room temperature. Supernatant was collected as a test sample and cellulase content

was estimated using DNSA reagent at 530nm absorbance using colorimeter.

Same procedure as that for cellulase assay was repeated except the media used that is 1% starch agar plates were streaked for amylase assay, 1% sucrose agar plates were streaked for invertase assay and 1% pectin was used for pectinase assay.

III. RESULTS

LC values calculated by regression analysis were LC₂₀, 20.7mg Fipronil/Kg. of soil and LC₄₀ 33 mg Fipronil/Kg. of soil (Fig. 1)

Effect of Fipronil on biological parameters of *Eudrilus eugeniae*:

In *Eudrilus eugeniae*, it was observed that the cocoon formation was reduced to 7.6±0.3 in Fipronil treated soil as compared to that in control (12±0.3) on 90th day (Table 1). Further it was found that the number of juveniles developed was less (4±1.3) in experimental sets as compared to that in control sets (10±0) on 90th day (Table 1).

Effect of Fipronil mixed vermiculture bed with *Eudrilus eugeniae* on Soil properties: It was observed that in Fipronil treated vermiculture bed there was reduction in pH, carbon, phosphorus and potash content of the soil than that of control. Also, under laboratory conditions pH, phosphorus and potash content of the soil in control is more than the normal poeta soil (Table 2)

Effect of Fipronil on Gut Microflora of *E. eugeniae* :

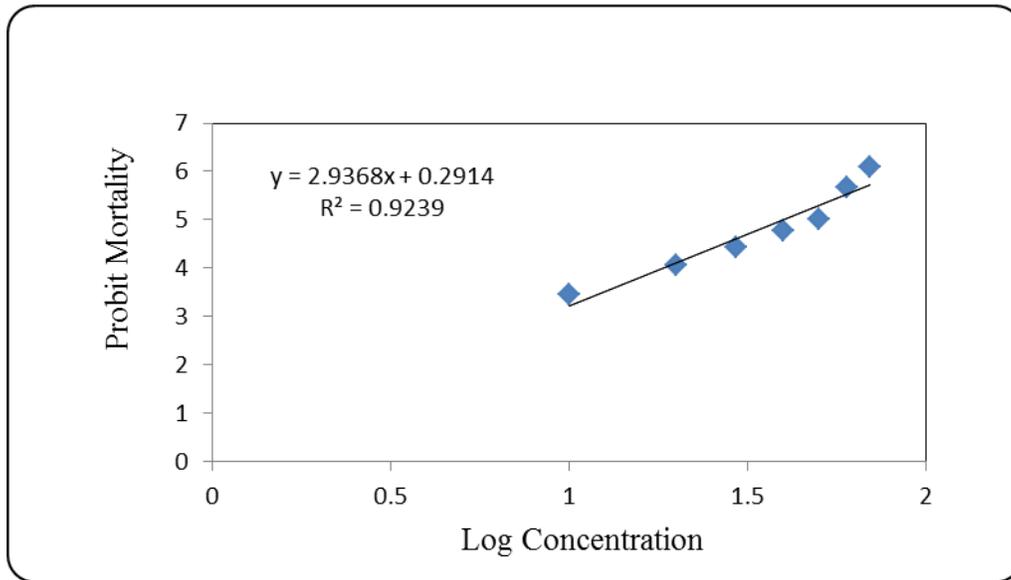
a) On the basis of morphological and biochemical characters colonies identified were:

- | | | |
|-------------|--------------------------------|-------------------|
| 1) Nocardia | 2) Cellulomonas | 3) <i>E. coli</i> |
| 4) Bacillus | 5) <i>Citrobacter freundii</i> | 6) Enterobacter |

b) Effect of Fipronil on total gut microflora of *E. eugeniae*: It was observed that the total count of the gut microflora of *E. eugeniae* was decreased with the days after treatment with Fipronil (Fig.2)

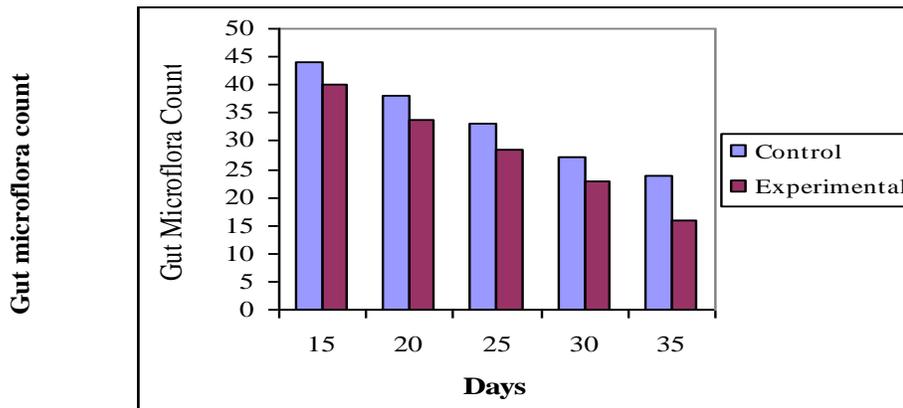
c) Assays of the enzymes amylase, cellulase, invertase and pectinase from the gut microflora of *E. eugeniae* revealed that their production was reduced in the Fipronil mixed with soil as compared to that of control (Fig. 3, 4, 5, 6).

Fig. 1



Dose response for Fipronil on mortality of *E. eugeniae*

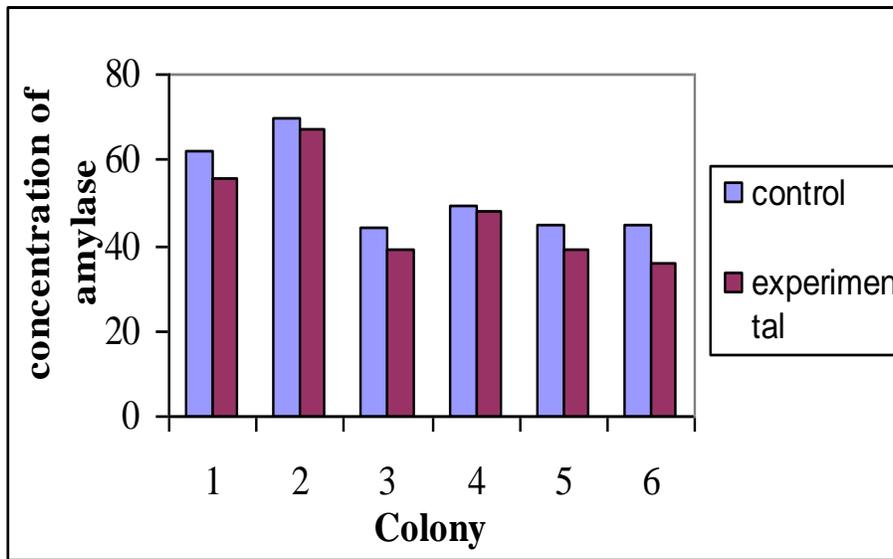
Fig. 2



Effect of Fipronil on total gut microflora of *E. eugeniae*

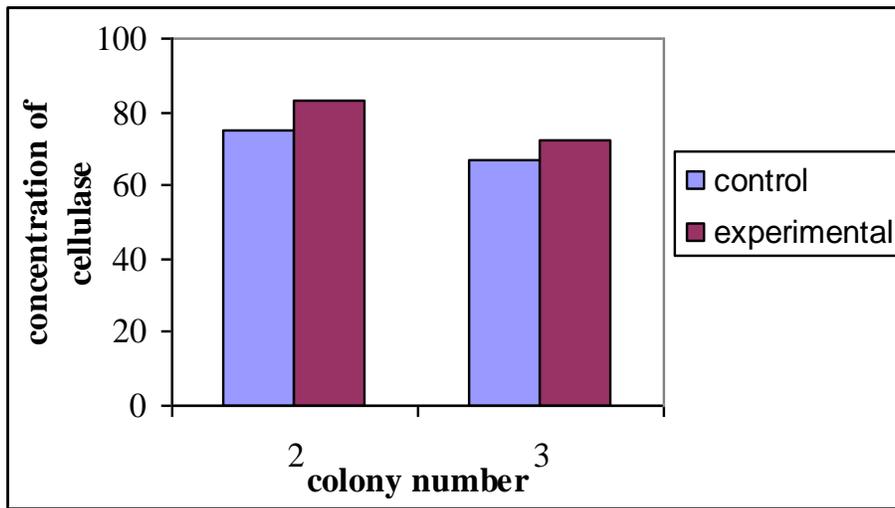
Enzymes assay

Fig. 3



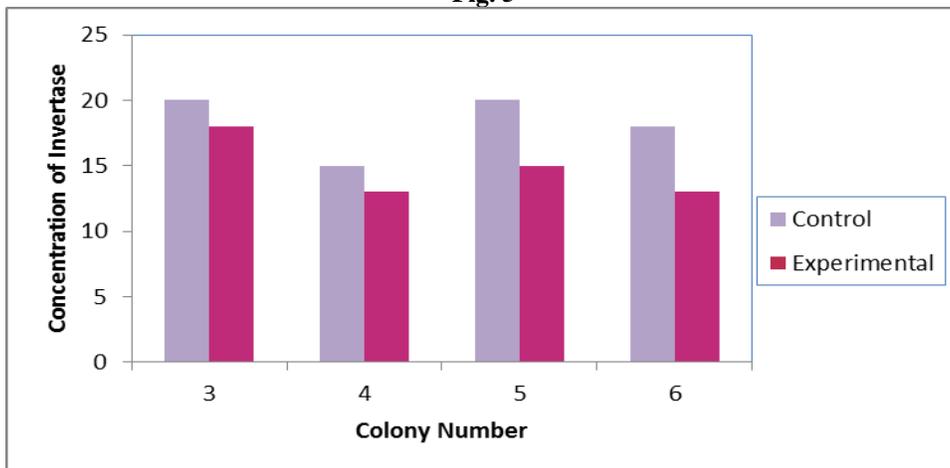
Amylase assay

Fig. 4



Cellulase assay

Fig. 5



Invertase assay

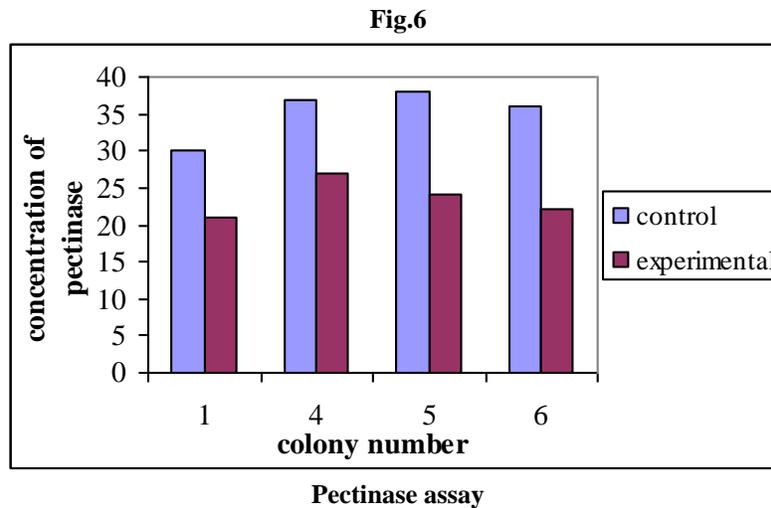


Table 1

Effect of Fipronil mixed vermiculture bed on biological parameters of *E. eugeniae*

Stages	21 st day		35 th day		90 th day	
	Control	Treated	Control	Treated	Control	Treated
Cocoons (Number)	8±0	4±1	9 ±0.2	7.6±1.02	12±0.3	7.6±0.3
Juveniles (Number)	5±1	3.3±1	8±0.5	4.6±1.5	10±0	4±1.3
Adults (Number)	10±.1	6.3±1	10±0.2	6.6±1	10±0	6±0.5

Table 2

Effect of Fipronil mixed vermiculture bed with *Eudrilus eugeniae* on Soil properties

Properties	Initial before treatment	On 35 th day	
		Control	Treated
pH	6.69	6.8	6.7
Carbon	0.453%	0.253%	0.134%
Potassium	14.745 kg/h	16.971kg/h	15.766kg/h
Phosphorus	359.09kg/h	389.09/kg/h	327.41kg/h

IV. DISCUSSION AND CONCLUSION

Vermiculture is entirely natural process that maintains the environmental balance. In the present studies an attempt is made to observe the effect of insecticide Fipronil on *Eudrilus eugeniae*. It was observed that the earthworm population is reduced in Fipronil treated soil and growth and reproductive potential of *Eudrilus eugeniae* was adversely affected as revealed by

reduction in number of cocoons and juveniles as compared to that of the control. Similar observations were made in parathion treated *Eiseina fetida*^[14]. A characteristic coiling of the body of insecticide treated earthworms was observed. It impairs with burrowing, copulation and feeding activities of earthworms. Our results thus, in agreement with those observed in different species of earthworm due to pesticides incorporated in the food material I (mixture of soil, dried leavs and farmyard

manure)^[15].

Studies were made to analyze properties of Fipronil treated and control soil of vermiculture bed with respect to pH, carbon, phosphorus and potash content to monitor the effect on soil fertility. It was observed that there was reduction in pH, carbon, phosphorus and potash content in Fipronil treated soil than in control. Also under laboratory conditions pH, carbon, phosphorus and potash content of the soil in control is more than the normal poeta soil, but the rate at which these components increases was lower in treated soil than in control.

Studies on different microorganisms isolated from the gut of *Eudrilus eugeniae* from treated and control soil revealed that total viable count of gut microflora as well as their enzymes production ability was reduced as compared to that in the control. In conclusion, the data suggests that insecticide Fipronil is not only toxic to earthworms but also reduces the fertility of soil to a greater extent.

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