

# *In Vivo* Evaluation On The Deleterious Effects of Fipronil on Earthworms

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## Abstract

Present study quantified the impact of the insecticide fipronil, on morphological and behavioral changes in earthworms. Study on fipronil treated earthworms showed significant changes in their behavior and morphology. Worms were adversely affected in its mobility, rate of amputation and regeneration. Mortality rate of earthworms were found to be increased with increased concentration of fipronil. Fragmentation tendency in fipronil treated earthworms were found to be high in lower concentrations. Fipronil treated live worm were seen on the surface of experimental soil and noted as restless and highly mobile. Histological study of fipronil treated worms exhibited histological anomalies. Intestinal villi were found to be shrunk and height of villi was reduced. Proximal part of villus covered by large misshapen cell of villus and finally collapses into small mound. From the study it was concluded that fipronil is highly toxic to earthworms

**Keywords:** Fipronil, Earthworms, Toxicity, Mortality

## 1. Introduction

Earthworms are vitally important members of the soil fauna, especially in agricultural soils where they can constitute up to 80 % of total soil animal biomass [Luo *et al.* 1999]. They play critical roles in the development and maintenance of soil physical, chemical and biological properties [Lee 1985]. Their activities improve soil structure by increasing porosity and aeration, facilitating the formation of aggregates and reducing compaction [Edwards and Bohlen 1996; Mostert *et al.* 2000]. Soil fertility is enhanced by earthworm effects on biogeochemical cycling [Coleman *et al.* 1988; Bartlett *et al.* 2010], the modification of microbial biomass and activity [Sheehan *et al.* 2008], breakdown of plant

litter [Knollengberg *et al.* 1985] and the mixing of litter with soil [Wang *et al.* 2012a]. The earthworm's borrowing creates a multitude of channels through the soil and is of great value in maintaining the soil structure, enabling process of aeration and drainage [Boyer and Wratten, 2010]. Use of specific herbicides, fungicides and insecticides in the agricultural field can be highly toxic to earthworms and they will suppress or nearly eliminate earthworm population [Williamson 2000]. Fipronil is highly toxic for crustaceans, insects and zooplankton, as well as bees, termites, rabbits, fringe-toed lizard and certain groups of gallinaceous birds. It is highly toxic to many fish, though its toxicity varies with species conversely, the substance is relatively innocuous to passerines, and wild fowl and earthworms [Jacob *et al.*, 2013]. Present study evaluated in vivo effect of Fipronil on earthworms.

## 2. Methods

Earthworms were collected from various sites of Assumption college campus. They were introduced into a vermicomposting tank of size 45cm length and 35cm breadth. A uniform layer of brick and pebbles were covered on the bottom portion of tank. The bottom was again topped with coarse sand in 6-7.5cm thickness. A layer of loamy soil was moistened and was set over the layer of coarse sand. Small lumps of cow dung were spreaded over the loamy soil. Water was regularly sprinkled in order to ensure water content in the tank. The unit was covered with broad leaves of coconut palm and papaya. Finally the setup was covered with a layer of hay and net. Entire unit was regularly moistened with water. The unit was kept undisturbed and wet for 25 days and it was opened on the 26th day. All earthworms of similar length were collected for the experiment procedures. Present experiment study constitutes two groups. Group 1 and Group 3. Each group consists of four

boxes. 8 plastic boxes with similar length and breadth (22x15cm) were bought from local market. The boxes were serrated with good air passage. Humus rich soil was prepared by mixing normal soil with cow dung in the ratio 4:1. Each serrated box were filled with 1kg of soil which was prepared as above. Fipronil (HPM Chemical and fertilizers Ltd.) were bought for market experimental purpose. For the experiment, Group 1 was assigned to study the toxicity of pesticide fipronil. Group 1 boxes were named as A, B, C, and D. In box A 0.05g fipronil was weighed and mixed with soil. In box B 0.3g fipronil was weighed and mixed with soil. Similarly boxes C and D were mixed with 0.9g and 1g of pesticide fipronil. Group II boxes were kept as control which contains humus rich soil. The harvested earthworms from vermin compost tank were washed in water. Then six worms were introduced into each box of the two groups. Moisture content was constantly maintained by spraying water frequently. The boxes were closed and kept for 7 days. Every day the morphological behavioral changes of worms from each box were studied. Number of worms, their morphological characters, and behavioral changes such as mortality, mobility, activity, fragmentation, regeneration, color were studied in respect to worms of controlled group and recorded. Studied worms were reintroduced into their respective boxes. The percentage of mortality was calculated by using formula.

$$\frac{\text{No: of earthworms dead}}{\text{Total number of earthworms}} \times 100$$

After 7 days of study, live worms from boxes of each were selected. Then a small longitudinal section was dissected out using a sharp end blade from the posterior end of each of the worm and the sections were fixed in 40% formaldehyde. Then the sections were cut using microtome and stained with haematoxylin and eosin mounted in DPX. The whole experiment was done in triplicates. Intestinal segments were studied with haematoxylin-eosin and examined by light microscopy.

### 3. Result

Fipronil treated earthworms showed significant changes in their behavior and morphology. Studies

revealed that live earthworms were confined to the surface of the soil. Excavating behavior was significantly reduced in all the fipronil treated groups. Worms in 0.05g(box b) and 0.3g(box c) of fipronil concentration, were found to be weak, sluggish and 50% mortality were observed within first four days. Worms in group treated with 0.9 g of fipronil were found to be pale and exhibited vigorous movement to escape from the container. Most of the worms seemed to be amputated in the posterior region and within two days, 100% of death were observed in boxes which were treated with 0.9% and 1 gm. of fipronil

Table:1. Morphological and Biological changes in experimental earthworms

Experimental groups	Mobility	Appearance	Mortality (%)
<b>Group I</b>	Less Active found above the soil	Fragmented, Sluggish and puffy Transparent	25
<b>Box A</b> (0.05g)			
<b>Box B</b> (0.3g)	Less Active found above the soil	Fragmented, Sluggish and puffy Transparent	50
<b>Box C</b> (0.9g)	Restless and found on the surface of the soil	Pale	100
<b>Box D</b> (1g)	Restless and found on the surface of the soil	Pale	100
<b>Group II</b> <b>Control</b>	Appeared to be burrowing and found inside of the soil	No change in appearance	0

Earthworms in normal control showed normal intestinal morphology. Histological examinations of untreated control group had no signs of

inflammation, degeneration or necrosis of intestinal mucosa. Ref Fig (1)



Figure I: Intestine OF Normal Control

Histological study of fipronil treated group exhibited histological anomalies. Intestinal villi were found to be shrunk and height of villi was reduced. Proximal part of villus covered by large misshapened cell of villus and finally collapsed Crypt architecture was completely lost. Degenerated nuclei, damaged epithelial lining of the villi, space formation and congestion of blood sinuses were found to be the remarkable necrotic changes in fipronil treated group Ref Fig (2)

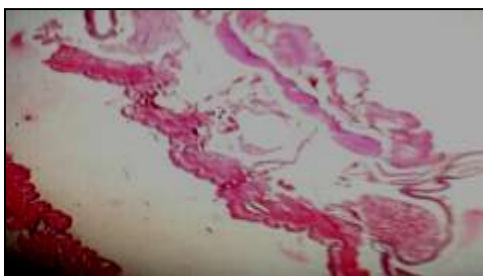


Figure2: Fipronil induced anomalies in Intestine

#### 4. Discussion

Ecotoxicological laboratory tests are considered as the basic tools for ascending pesticide toxicity in soil borne animals. Present study was done to find the toxic impact of fipronil in earthworms. Fipronil, an N-Phenylpyrazole, was introduced into the United States in 1996 for use in animal health, indoor pest control, and commercial turf and crop protection [Robert *et al* 2010]. Fipronil is used to control ants, beetles, fleas, cockroaches, ticks, termites, crickets, root worms and other insects. Fipronil were found to be stable without dehydration for longer periods and its half life is 125 days. [Jackson *et al* 2009] In our study fipronil treated worms were adversely affected in its mobility,

amputation and regeneration. Mortality rate of earthworms were found to be increased with increased concentration of fipronil. The toxic effect increased with increasing exposure, time, and concentration and could be lethal. This might be one of the possible cause of fipronil toxicity which caused the death of earthworms in a dose dependent manner. The worms were found to be excavated less into the soil in presence of fipronil. This means that they feed less and appeared to be thin and also reduced fragmentation. Fragmentation tendency in fipronil treated worms may be due to the deprivation of oxygen content in the cells and also due to the irritant chemical nature of fipronil. Fipronil treated worms were found to be seen on the surface of experimental soil and noted as restless and highly mobile. This may be due to the avoidance behavior of earthworms. Avoidance behavior of earthworm [Mo'nica. *et al* 2005] may be the reason for less excavating nature of earthworms. Avoidance behavior enable the worms to move away from fipronil source. Healthy intestinal mucosa plays an important role in gut function and it prevent pathogen and toxicogenic substance. Earthworms showed less excavation when exposed to fipronil that means that they feed less and have fewer intestinal contents. Necrosis is characterized by pyknotic nuclei, cytoplasmic swelling and mitochondrial damage which results from failure in osmotic regulation caused by loss of cellular energy supplies. [Bowen *et al* 1981] In fipronil treated earthworm intestinal villi was found to be shrunk and height of villi was reduced. Due to this dimorphology of villi gut barrier function was also get destroyed this may is leads to the lethality of fipronil treated earthworms. From the study it was concluded that fipronil is highly toxic to earthworms

#### 5. Conclusion

From the study it was concluded that insecticide fipronil will induce morphological, behavioral and histopathological changes in earthworms finally leading to death. Uncontrollable use of these insecticides will affect the basic ecological balance and soil texture.

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