

Original Research Article

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Comparative Assessment of Bacterial Population of Gut and Cast of *Pheretima posthuma* Exposed to Imidacloprid and Pendimethalin

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ABSTRACT

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The present study was carried out for comparative assessment of imidacloprid and pendimethalin on the bacterial population of gut and cast of *Pheretima posthuma*. The samples of gut and cast from earthworm treated for 90 days were cultured on five different culture media viz. King's B media, YEMA media, Malate media, Jensen's media and Nutrient agar. Dose dependent decrease in bacterial population was observed in the entire population of treated earthworm's gut and their cast in comparison to control. Maximum reduction in bacterial colonies of gut and cast were 53.13% and 58.53 % for *Rhizobium* (YEMA media) whereas minimum reduction 15.52% and 24.89% for total viable bacterial colonies (Nutrient agar media) in earthworm's gut and its cast respectively when the worms were exposed to combined dose of imidacloprid and pendimethalin @ 0.60 + 2.50 µl/kg of substrate. Results revealed that combined doses of these pesticides were more harmful than individual doses and among the two pesticides; imidacloprid was more harmful than pendimethalin.

Introduction

Earthworm is a vital soil biota and a very crucial indicator species in ecotoxicological and ecological risk assessment of soil pollution being caused by agrochemicals and other toxic substances (Zhang *et al.*, 2014; Wang *et al.*, 2015; Huang *et al.*, 2018). Earthworms influence the soil microbial communities directly and indirectly, mainly through feeding, burrowing and shedding casts. Latter have many beneficial microorganisms and buffering capacity which ultimately help in plant growth (Ali *et al.*,

2006; Kumar *et al.*, 2020). Earthworms increase bacterial population in soil and help in nitrification by improving soil aeration. Nitrogenase activities in earthworm's cast have been found higher than the surrounding soil, contributing to higher nitrogen fixation in worm castings and ultimately help in plant growth (Ranch, 2006; Kumar *et al.*, 2020; Blume-Werry *et al.*, 2020).

Earthworms depend on friendly microbes for rotting and decaying the wastes during the initial stages of vermicomposting, which make it suitable for ingestion. Then after

ingestion endosymbiotic gut microbial community help in digestion of organic waste (Owa *et al.*, 2013; Van Groenigen *et al.*, 2014). Earthworms function like biochemical reactors by converting labile plant compounds into microbial necromass in stabilised carbon pools without altering bulk measures, such as the total carbon content (Angst *et al.*, 2019). It was observed that efficacy of bacteria is better when they are present in earthworm's gut compared to their direct in vitro action (Owa *et al.*, 2013). Different type of bacteria are found in earthworm's gut like (Aira *et al.*, 2016) like *Aeromonas hydrophila* in *Eisenia fetida* (Toyota and Kimura, 2000), *pseudomonas* in *Lumbricus terrestris* (Devliegher and Verstraete, 1997) and *Actinobacteria* in *L. rubellus* (Kristufek *et al.*, 1993).

Insecticides and herbicides are being used in modern agriculture practices to control the unwanted insects and weeds etc. No doubts these chemicals kill or remove unwanted insects and weeds but also affect the beneficial organisms of soil like earthworms and soil bacteria, which play important role in maintaining soil fertility (Kumar *et al.*, 2020).

The change in qualitative and quantitative soil microbial populations by the herbicides was recorded by Saeki and Toyota, (2004). Insecticide, imidaclopridis a contact insecticide and act against a wide range of pest like aphids, whiteflies and leafhoppers; it is structurally similar to nicotine (Mahapatra *et al.*, 2017; Si *et al.*, 2018). Pendimethalin is also one of the extensively used dintroanaline herbicide used against *Phalaris minor*, *Cyperus sp.*, *Ludwigra parviflora* etc. (Singh and Singh 2009). So, the present study was carried out to investigate the impact of imidacloprid and pendimethalin on gut bacterial populations and vermicast of *Pheretimaposthuma*.

Materials and Methods

The earthworm species *Pheretima posthuma* was collected from the virgin soil of the CCS Haryana Agricultural University Hisar campus in the spring season of 2018. Before using soil as a substrate, the unwanted debris like wood pieces, stones etc. were removed from it. Appropriate moisture level was maintained in the tubs by sprinkling water for some days. Fully clitellated earthworms were exposed to different individual and combined doses of pesticides for 90 days to assess the impact of imidacloprid and pendimethalin.

Description of treatments given to earthworms along with control

Different doses were prepared according to the description given by Directorate of Plant Protection Quarantine & Storage and are given in table 1.

Preparation of media

Different media (King's B media, YEMA media, Malate media, Jensen's media and Nutrient agar of HIMEDIA) were prepared by using manufacturer's instructions in distilled water. These media were specific for *Pseudomonas*, *Rhizobium*, *Azospirillum*, and *Azotobacter* respectively whereas the nutrient agar was used for analysing total viable count in the cast and gut of *P. posthuma*.

The media were sterilized by autoclaving at 15 psi/121°C for 20 minutes, thereafter allowed to cool not so much that it solidified and after cooling media were poured in sterilized petri plates. After solidification these plates were kept inverted overnight. The enumeration of the different bacteria in the cast and gut of earthworm was carried out by following "Spread plate technique" after 90 days of treatment (Table 2).

Experimental set up

Surface sterilization of earthworm was done by using 70% ethanol, 0.1 gm. of the cast and gut contents of earthworm were transferred to 0.9 ml of distilled water and shaken properly. The dilutions up to 10^{-4} were prepared by following this step repeatedly. After shaking properly 0.1 ml aliquots of 10^{-4} dilution were spread on different media plates. These plates were incubated in B.O.D at $28 \pm 2^\circ\text{C}$ for 72 hours in inverted position and thereafter, the bacterial colonies were counted carefully, the number of bacterial colonies so obtained was used for assessing the total population of bacteria.

Formula for calculating total population of bacteria

$$\text{cfu/ml} = \frac{\text{(Number of colonies x dilution factor)}}{\text{Volume of culture plate (ml)}}$$

Statistical analysis

The experimental design for screen house and laboratory studies were completely randomized block (CRD) with three replicates. Critical difference (CD) was calculated between the various treatments by using Software ‘OPSTAT’, developed at the Computer Centre, College of Basic Sciences and Humanities, CCSHAU, Hisar, Haryana.

Results and Discussion

Numbers of bacterial colonies were reported significantly low in the gut of *P. posthuma* as

compared to its cast. Dose dependent decrease in bacterial population of gut and cast was reported in all the treatments and media. Maximum reduction in earthworm’s gut and cast bacterial colonies of *Rhizobium* were 53.13% and 58.53 % respectively when earthworms were treated with combined dose of imidacloprid and pendimethalin @0.60+2.50 $\mu\text{l/kg}$ of substrate in YEMA media (Table 2 and 3). Similar finding was reported by Parthasarathi *et al.*, (2007) they observed that the number of microorganism was higher in earthworms reared on the decomposed leaves whereas minimum in those earthworm which were fed on pesticides contaminated soil. Kadam and Pathade, (2017) observed that the lower count of bacterial population in gut as compared to vermicast may be due to digestive activities on ingested material in earthworm’s gut. Earthworms’ diet constitutes microorganisms as important nutritional component (Edwards and Bohlen, 1996; Kumar *et al.*, 2020) which is essential for their growth, development and reproduction (Parthasarathi and Ranganathan, 2000). Parthasarathi and Ranganathan, (1999) observed different kind of relationships between earthworms and microbes. They concluded from their studies that microbes are the part of worm’s diet or the earthworms provides suitable conditions in the gut for microbes to grow. Being a part of soil fauna, earthworms are easily exposed to soil pollutants either due to ingestion or due to contact with skin (Dallinger and Rainbow, 1993; Kumar *et al.*, 2020).

Table.1

Sr. No.	Treatments	Concentration ($\mu\text{l/kg}$ of substrate)
1.	Control	Without Pesticides
2.	Imidacloprid (48% FS)	0.6, 0.9 and 1.2
3.	Pendimethalin (30%EC)	2.50, 3.75 and 5.00
4.	Imidacloprid+Pendimethalin	0.30+1.25, 0.45+1.87, 0.60+2.50

Table.2 Effect of different concentration of pesticides on gut bacterial population of *P. posthuma*

Treatments ($\mu\text{l/kg}$ of substrate)	YEMA media	King's Media	Jenson's media	Azospirillum media	Nutrient Agar media
Control	42.67 \pm 1.76	54.00 \pm 1.16	44.33 \pm 2.03	74.67 \pm 1.45	141.67 \pm 0.88
Imidacloprid (0.6)	32.33 \pm 1.45	50.33 \pm 0.33	41.33 \pm 0.88	67.33 \pm 0.88	132.67 \pm 1.45
Imidacloprid (0.9)	28.00 \pm 2.31	46.33 \pm 0.88	38.67 \pm 0.88	65.67 \pm 1.20	130.67 \pm 1.76
Imidacloprid (1.2)	21.33 \pm 1.45	39.00 \pm 1.16	29.67 \pm 0.88	60.00 \pm 1.16	122.00 \pm 1.16
Pendimethalin (2.50)	27.00 \pm 1.53	47.00 \pm 1.16	39.67 \pm 1.45	67.67 \pm 0.88	128.00 \pm 1.16
Pendimethalin (3.75)	23.33 \pm 1.76	41.67 \pm 0.88	35.00 \pm 1.16	62.33 \pm 1.20	125.67 \pm 1.20
Pendimethalin (5.00)	23.00 \pm 1.16	40.33 \pm 1.20	33.33 \pm 1.45	61.00 \pm 1.16	124.00 \pm 1.16
Imidacloprid +Pendimethalin (0.30+ 1.25)	33.67 \pm 1.76	52.67 \pm 1.45	49.33 \pm 1.76	73.33 \pm 1.45	134.33 \pm 1.45
Imidacloprid + Pendimethalin (0.45+1.87)	26.00 \pm 1.73	45.00 \pm 2.08	34.33 \pm 1.20	59.33 \pm 1.45	127.33 \pm 1.45
Imidacloprid + Pendimethalin (0.60+ 2.50)	20.00 \pm 1.16	36.00 \pm 1.16	25.00 \pm 1.16	56.00 \pm 1.16	119.67 \pm 0.88
SE(m)	1.64	1.22	1.34	1.22	1.28
CD at 5%	4.87	3.63	3.97	3.61	3.81

Table.3 Effect of different concentration of pesticides on vermicast bacterial population of *P. posthuma*

Treatments ($\mu\text{l/kg}$)	YEMA media	King's media	Jenson's media	Azospirillum media	Nutrient Agar media
Control	54.67 \pm 1.20	66.33 \pm 0.88	49.67 \pm 0.88	82.67 \pm 1.45	158.00 \pm 1.16
Imidacloprid (0.6)	45.33 \pm 0.88	60.67 \pm 1.20	43.00 \pm 1.16	78.67 \pm 1.20	141.33 \pm 1.20
Imidacloprid (0.9)	40.00 \pm 1.16	57.33 \pm 1.20	40.00 \pm 1.16	75.33 \pm 0.88	135.00 \pm 1.53
Imidacloprid (1.2)	37.33 \pm 1.45	50.33 \pm 0.88	34.00 \pm 1.16	57.67 \pm 1.45	127.67 \pm 1.45
Pendimethalin (2.50)	42.33 \pm 1.45	56.67 \pm 1.45	38.00 \pm 1.16	67.00 \pm 1.16	139.67 \pm 1.45
Pendimethalin (3.75)	38.33 \pm 1.45	55.00 \pm 1.16	36.00 \pm 1.16	65.33 \pm 1.20	134.00 \pm 1.53
Pendimethalin (5.00)	38.33 \pm 1.86	52.33 \pm 0.88	36.33 \pm 0.88	62.33 \pm 1.45	129.33 \pm 0.88
Imidacloprid +Pendimethalin (0.30+ 1.25)	32.33 \pm 1.20	51.00 \pm 1.16	38.33 \pm 1.45	63.33 \pm 1.45	135.33 \pm 1.20
Imidacloprid + Pendimethalin (0.45+1.87)	25.00 \pm 1.73	46.33 \pm 2.33	33.67 \pm 1.20	60.33 \pm 1.20	127.67 \pm 1.45
Imidacloprid + Pendimethalin (0.60+ 2.50)	22.67 \pm 1.45	44.67 \pm 1.45	29.67 \pm 0.88	55.00 \pm 0.58	118.67 \pm 0.88
SE (m)	1.41	1.33	1.12	1.23	1.30
CD at 5 %	4.19	3.94	3.33	3.67	3.85

The present studies revealed that minimum reduction in total viable bacterial colonies (Nutrient agar media) were 15.52% and 24.89% in earthworm's gut and its cast respectively after treating earthworms with combined doses of pesticides@ 0.60+ 2.50 $\mu\text{l/kg}$ of substrate and compared with control, which had maximum bacterial colonies in NA media. Our finding of dose dependent effect of insecticides (imidacloprid) and herbicides (pendimethalin) on bacterial population of *P. posthuma* earthworm's gut and its cast are similar to observations by Gundi *et al.*,

(2005), they found dose dependent effects of three insecticides like monochrotophos, quinalphos and cypermetharin on microbial population of earthworm, pendimethalin @0.75 Kg ha⁻¹ results in decrease of bacterial populations as compared to the non-treated plots (Singh and Singh 2009). Earthworm ingest the organic material and released the cast having beneficial bacteria, contaminated soil/organic material decreased these beneficial bacteria both in gut and cast. Present study revealed that both the pesticides imidacloprid and pendimethalin had

detrimental effects on gut and cast bacterial population of *P. Posthuma*, and their combined doses were more harmful than their individual application. It was also observed that among two pesticides imidacloprid was more lethal than pendimethalin.

In conclusion the bacterial counts were found more in *P. posthumacast* in comparison to their gut. Maximum number of bacterial colonies were reported in nutrient agar media whereas minimum in YAMA. The combined doses of insecticide and herbicide were found to be more harmful than their individual application. Individually, among two pesticides Imidacloprid was more harmful than Pendimethalin.

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