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Avoidance behavior of *Eisenia fetida* and *Metaphire posthuma* towards two different pesticides, acephate and atrazine

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HIGHLIGHTS

- The avoidance test provides the first hand information to check the sensitivity of earthworms towards the pesticides.
- The avoidance response of earthworms towards pesticides depends on earthworm species and soil medium type.
- The avoidance test provides a screening method for evaluation of the habitat function of the soil.
- Tropical species, *M. posthuma* was found to be less sensitive than standard species *E. fetida.*

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ABSTRACT

The avoidance behavior is regarded as the method that provides first hand information about the behavior of an organism in the presence of contaminants in the soil. Very little data is found in literature regarding the effect of pesticides on tropical earthworms. Two pesticides, acephate and atrazine which are widely used in Indian tropical area were investigated for their avoidance behavior on standard species, *E. fetida* (ISO 2007) and on a tropical species, *M. posthuma*. The avoidance tests are rarely replicated on tropical species, *M. posthuma* in comparison to standard species, *E. fetida or E. andrei*. The standard avoidance test (ISO 2007) was taken into consideration for two different species of earthworm. Significant difference in the distribution of earthworms in the control and test soils was found depicting that soil composition plays a vital role in affecting the distribution of worms. The results also show higher sensitivity of *E. fetida* in comparison to *M. posthuma* in terms of avoidance response for both the pesticides. For risk assessment, the soil types and indigenous soil species of earthworms must be taken into consideration for evaluation of soil contamination. Avoidance tests forms the basis to study the molecular mechanisms underlying the receptor proteins responsible for the process of chemesthesis in annelids.

1. Introduction

In tropical nations, the climatic condition of high temperature and humidity being favorable for rapid increase in pest population which leads to substantial crop losses (Kannan et al., 1992; Lakshmi 1993; Das et al., 2020). There is a considerable increase in pesticide market in tropical areas especially in the last decade (De Silva et al., 2009; Goulson 2020; Andersson and Isgren 2021). The resultant is increased pesticide residues in the soil. No doubt, pesticides including insecticides play an important role in increasing the crop yield, but at the same time they are also responsible for causing a steep fall in the diversity and richness of biota associated with cropland ecosystem (Stockdale and Watson 2012; Beketov et al., 2013; Wietzke et al., 2020; Almond et al., 2020). Accelerating functional biodiversity in agricultural ecosystems is a principal ecological approach improving sustainability of agricultural

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production (Altieri 1999).

The organization for Economic Co-operation and Development (OECD) has provided the guidelines used by professionals in academia, industry and government that are involved in testing and assessment of various chemicals and contaminants. These guidelines are updated time to time to ensure that they reflect the state-of-the-art science and techniques to meet the regulatory needs of the member countries. Pesticides sprayed in agricultural fields affect non target organisms such as earthworms and also damage the ecosystem (Datta et al., 2016). Several toxicity tests have been devised to evaluate the effects of chemicals on soil organisms. These includes acute tests with earthworms, (OECD 1984; ISO 1993; ISO 1998; OECD 2004), springtails (ISO 1999), and pot worms (Enchytraeus sp.) (ISO 2004), and reproduction tests with earthworms (ISO 1998; OECD 2004). The standardized acute and reproduction toxicity tests detects the toxicity incurred on the test species by the contaminants in soils through mortality, reproduction and other sublethal endpoints (Lokke and van Gestel, 1998). These studies deliver the information about toxic effects of contaminants on exposed organisms but they fail to provide first hand information about the reaction and behavior of organisms when exposed to contaminants in soil. Earthworms are known to react to natural and anthropogenic stress quickly which makes them an ideal early warning system (McGuirk et al., 2020). Many engineered nanomaterials like silver nanomaterials (Mariyadas et al., 2018); nano-carbon black surface (Xu et al., 2019) and other chemicals like neonicotinoids (Ge et al., 2018) gold mining tails (McGuirk et al., 2020); metal contaminated soil (Delgadillo et al., 2017) have been studied for avoidance by earthworms using avoidance test.

Animal behavior is known to get affected by the presence of chemicals in the environment. The organisms possess chemoreceptor's which are highly sensitive to chemicals in environment and this forms the basis of avoidance tests (Edwards and Bohlen 1996; Rombke and Schmidt 1999). Animal chemosensation like smell and taste relies on sensory circuits that involve recognition of wide range of chemical molecules by specialized transduction pathways transforming them into precise patterns or signals of neuronal activity to evoke the appropriate response (Ache and Young 2005; Hildebrand and Shepherd 1997). Aversive chemical signaling involves the gustatory system, olfactory system and chemesthesis through taste receptors, olfactory receptors and ion channels on nociceptive neurons respectively (Li and Liberles 2015; Mickle et al., 2015).

The earthworms influence the soil properties and significantly contribute to ecosystem by their activity and movement (Capowiez et al., 2003). It is therefore necessary to understand how these species are influenced by chemical stimuli. The study of avoidance behavior can form the basis for elucidating the molecular mechanisms of chemoreception and details of receptor proteins in earthworms. The avoidance test can be considered to investigate toxicity and mechanism of chemoreception of soil organisms like earthworms, enchytraeids and collembolans (Hund-Rinke and Wiechering, 2001; Natal-da-Luz et al., 2004; Loureiro et al., 2005; Diogo et al., 2007). The avoidance behavior is directly related with the energy budget of the individual worms and indirectly (i.e. via the moving and burrowing activity) with the soil structure and thus forms to be an important ecological endpoint (Amorim et al., 2005). Thus the soil properties and the earthworm activity correspond to the avoidance behavior of earthworm.

The International Standardization Organization (ISO 2007) and Environment Canada (2004) through an earthworm avoidance test, drafted guidelines for the determination of habitat function of the soil. Few authors have revealed that behavior of soil organisms like earthworms and springtails are altered greatly with respect to soil properties (Natal-da-Luz et al., 2008a). Earthworms avoid soils contaminated with fungicides (Garcia et al., 2008; Natal-da-Luz et al., 2008b), pesticides (Reinecke and Reinecke 2007; Dittbrenner et al., 2010; Rico et al., 2016), herbicides (Marques et al., 2009) and nanoparticles (Shoults-Wilson et al., 2011). This avoidance behavior caused by soil pollutants can affect soil animal communities as the worms may penetrate into deeper layers of soil or outside the area of contamination to avoid toxicants (Aldaya et al., 2006). Avoidance test is most suitable as a short-term screening test in comparison to other tests as it is useful in quick screening of either large number of soil samples or large areas with potential contaminants as total time duration for this test is 48 h (Hund-Rinke and Wiechering, 2001; Rombke, 2003).

The ISO (2007) recommends the use of *Eisenia fetida* or *Eisenia andrei* as standard species for avoidance tests. But *Eisenia* species being a manure or compost worm is not easily found in natural ecosystems and therefore have limited ecological relevance. Many alternative species with better ecological relevance such as *Lumbricus terrestris* (Schaefer 2004; Dittbrenner et al., 2010), *Aporrectodea caliginosa* (Hodge et al., 2000; Dittbrenner et al., 2010), *Lumbricus rubellus* (Lukkari and Haimi 2005), have been shown to be suitable for avoidance test. But *Lumbricus terrestris* and *Aporrectodea caliginosa* are not commercially available and thus have a limited usefulness for testing outside academic research labs. There is a dearth of studies on the impact of pesticides on tropical species of earthworms when compared to standard species, *E. fetida* and *E. andrei* (De Silva and Gestel, 2009b). Therefore an extrapolation of data pertaining to standard species to tropical species of earthworms can lead to invalid results (Garcia et al., 2008). Garcia et al. (2008) used a tropical variant of *E. fetida* as the test species; De Silva and Gestel (2009b) used *E. andrei* and *P. excavatus* as the test species. We hypothesize that (a) effect of pesticides on avoidance behavior of standard species *Eisenia fetida* (epigeic) and tropical species *Metaphire posthuma* (endogeic) is different. We further hypothesize (b) soil type plays a crucial role in earthworm behavior. Finally, we hypothesize that c) at higher concentration of pesticide atrazine and acephate, both the earthworm species show reduced soil habitat function.

2. Materials and methods

2.1. Test organisms

The tropical variant standard species *Eisenia fetida* and tropical indigenous species *Metaphire posthuma* were considered as test organisms. The culture of *E. fetida* were maintained on pre-composted cow dung and obtained from Department of Zoology, Khalsa College Amritsar. *M. posthuma* were collected from the moist soil from the garden of Lovely Professional University, Phagwara, Punjab, India. Adult earthworms with well-developed clitella were randomly chosen for the avoidance test. Three hours prior to the experiment, the worms were rinsed with water and placed on a moistened filter paper in a petridish and kept for 4 h to remove their gut contents.

2.2. Test substrate

Two different test substrates were used for determining avoidance test of pesticide on *E. fetida* and *M. posthuma*.

(1) Artificial soil-OECD was the common test medium used for both the earthworm species. OECD soil is basically composed of 70% fine sand, 20% kaolin, 10% sphagnum peat with a small amount of $CaCO_3$ for the adjustment of the pH. But in tropical countries like India, sphagnum peat is not readily available thus instead of that coco peat was used (De Silva and Gestel

2009a). All the components were thoroughly mixed and water was added to maintain 50% of maximum water holding capacity (WHC).

(2) The second test medium was different for both the earthworm species. It was basically the natural medium (NM) in which both the respective species generally inhabit. Garden soil (GS) is the natural medium for *M. posthuma* and pre-composted cow dung (CD) is the natural medium for *E. fetida*. The garden soil was collected from the garden of Lovely Professional University, Phagwara, Punjab, India. The soil was air dried and sieved (2 mm) to obtain a homogenous soil mixture. The cow dung was collected from a dairy farm and subjected to pre-composting for 10 days.

2.3. Test chemicals

Two pesticides acephate or atrazine were tested for earthworm avoidance behavior in various test mediums. Both these pesticides are widely used in tropical nations like India.

(a) Acephate (N-[Methoxy(methylsulfanyl)phosphoryl]acetamide) is an organophosphate foliar insecticide. It is primarily used for control of aphids in cultivation of potatoes, carrots, greenhouse tomatoes and lettuce. It is also applied on horticultural crops like rose and other greenhouse ornamentals. It keeps a check on leaf miners, sawflies, caterpillars, thrips, fireants etc. The commercial formulation of acephate used was Asataf SP 75% (Tata Rallis India Ltd.). Acephate is water soluble and thus was easily mixed with the test medium.

(b) Atrazine (6-chloro-N-ethyl-N′-isopropyl-1,3,5-triazine-2,4-diamine) is a herbicide of the triazine class. It is used for prevention of pre- and post-emergence broadleaf and grassy weeds in crops like sorghum, maize, sugarcane, lupins, pine, eucalyptus. It is also used to maintain turfs of golf courses and residential lawns. The commercial formulation of atrazine used was Atrataf WP 50% (Tata Rallis India Ltd.). Atrazine is moderately soluble in water and therefore for preparation of artificial soil, it was spiked with 10g of quartz sand and thereafter mixed thoroughly with the remaining medium. For preparation of both types of natural medium, GS and CD, emulsion of atrazine was prepared and mixed thoroughly. After this, water was added up to 50% of maximum water holding capacity (WHC).

2.4. Experimental procedure

The experimental procedure of the avoidance tests was based on ISO 17512-1 (2007). Five replicates for acephate and atrazine were used in the concentration of 3.90, 7.80, 15.62, 31.25, 62.50, 125, 250 mg/kg of dry soil. Control soils were similar to the test soil used but without pesticide. The test vessels were circular plastic trays of area 398.35 sq cm. The vessels were divided into two equal sections by a vertically introduced plexiglass divider. One half of the vessel was filled with test soil (Section A) and other half with control soil (Section B) (see Fig. 1). The separator was removed and thereafter 10 adult worms were placed one by one onto the line of separation of each vessel. The vessels were covered with jute mats permeable to air. The vessels were kept in dark to avoid lateral effects of light. They were kept at 26 ± 2 °C for 48 h after which the number of worms on both sides of the vessel was determined. The avoidance tests were also supported by dual control test to check if the worms in the absence of a contaminant, do not congregate but distributes themselves randomly in both sides of vessel and do not display behavior that might be mistaken for avoidance (Yeardley 1996). For this, control soil was used in both the sections of the vessel and earthworm avoidance behavior was analyzed.

2.5. Data assessment

For each replicate the avoidance response was calculated using

$$NR = [\frac{C - T}{N}]*100$$

where, NR = net avoidance response (%); C = number of worms in control soil; T = number of worms in pesticide-amended (treated) soil; N = total number of worms exposed.

The soils were considered to be toxic (habitat function reduced or limited) if > 80% of the worms stayed in the control soil (Hund-Rinke and Wiechering 2001).

2.6. Statistical analysis

JMP 13 software was used for the statistical analysis. Student's t-test was used to calculate the level of significance of avoidance. Avoidance response at various concentrations was used to determine the median effective concentration (EC_{50}) for avoidance by earthworms using a probit analysis model. Student t-test was also used to validate dual control test.

3. Results

3.1. Non-avoidance effect on earthworm

In all the tests conducted for *M. posthuma* and *E. fetida*, the concentration which showed greater than 10% mortality was eliminated from the statistical analysis of the avoidance behavior.

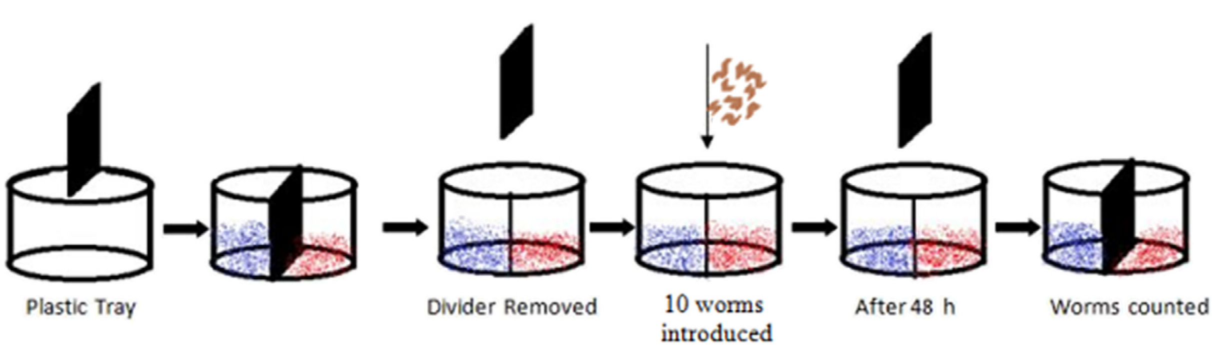

Fig. 1. Schematic representation of the procedure followed in earthworm avoidance test (modified from De Silva and Amarasinghe 2008).

Table 1
The calculated median effect concentration (EC_{50} mg/Kg) for the effect of acephate and atrazine on the avoidance behavior of *E. fetida* and *M. posthuma* in different exposure media.

Species	Exposure medium	Acephate (EC_{50})	Atrazine ((EC_{50})
E. fetida	OECD	26.37 (24.71–28.02)[a]	49.78 (41.70–57.85)
	CD	52.55 (48.50–56.10)	74.61 (70.83–78.39)
M. posthuma	OECD	49.88 (43.97–55.78)	112.09 (109.06–15.13)
	GS	85.03 (74.94–95.12)	110.71 (110.68–10.74)

[a] Parenthesis represent 95% confidence intervals.

The proportion of earthworms in the dual control tests, with control soils on both sides were found non-significant (student t-test, $p > 0.05$) depicting no significant preference or aggregation of earthworms to one side.

In the lower doses of atrazine and acephate, the number of earthworms in the test soil was found to be more than the control soil. Thus, non-avoidance or negative avoidance i.e. attraction (hormesis) of earthworms towards lower dose of pesticides was observed in both the species for both the pesticides in both types of soil media.

3.2. Effect of acephate on avoidance behavior of earthworm

The negative avoidance behavior (attraction) with respect to acephate was observed in the lowest concentration (3.90 mg/kg) for both soil types in *M. posthuma. E. fetida* also showed negative avoidance (attraction) in the lowest concentration (3.90 mg/kg) in OECD artificial soil but in case of CD two lower most concentrations (3.90, 7.80 mg/kg) depicted negative avoidance. The effective concentration (EC_{50}) of avoidance for acephate by *E. fetida* was found to be 26.37 mg/kg and 52.55 mg/kg in OECD and CD respectively (Table 1). While in case of avoidance by *M. posthuma*, EC_{50} was found to be 49.88 mg/kg and 85.03 mg/kg in OECD and GS respectively. We found that *E. fetida* was 1.9 times more sensitive than *M. posthuma* with respect to acephate in OECD artificial soil. In respective natural mediums (CD for *E. fetida* and GS for *M. posthuma*), sensitivity of *E. fetida* was similarly found to be more sensitive than *M. posthuma*. Habitat function of soil (i.e. 80% of the worms in the control soil or ≥60% avoidance response) was reduced at acephate concentration of ≥62.5 mg/kg in CD whereas in OECD it was reduced at 125 mg/kg dry soil *for E. fetida*. For *M. posthuma,* habitat function of soil was reduced at 62.5 mg/kg dry soil in OECD and ≥125 mg/kg dry soil in GS (Table 2).

3.3. Effect of atrazine on avoidance behavior of earthworm

The negative avoidance behavior or attraction with respect to atrazine was also observed in few lower doses. The effective concentration (EC_{50}) of avoidance for atrazine by *E. fetida* was found to be 49.78 mg/kg and 74.61 mg/kg in OECD and CD respectively (Table 1). While in case of avoidance by *M. posthuma*, the effective concentration (EC_{50}) was found to be 112.09 mg/kg and 110.71 mg/kg in OECD and GS respectively. The avoidance of *E. fetida* for atrazine in OECD artificial soil was 2.3 times more than *M. posthuma*. In natural soil, *E. fetida* was found to be 1.5 times more sensitive than *M. posthuma* for atrazine. Habitat function of soil (i.e. 80% of the worms in the control soil or ≥60% of avoidance response) was reduced at atrazine concentration of ≥62.5 mg/kg soil for *E. fetida* in OECD soil while in CD the habitat function of soil was reduced to ≥125 mg/kg dry soil. For both soil types in *M. posthuma*, the habitat function of soil was found to be reduced at ≥ 125 mg/kg dry soil (Table 3).

4. Discussion

According to ISO 17512-1 (2007), two criteria have to be fulfilled for the avoidance test to be considered as valid: (a) The experiment should be considered invalid if the number of dead or missing worms is >10% per treatment. (b) The avoidance behavior should be validated by a dual control test.

The first criterion was fulfilled in all the tests conducted for both earthworm species *M. posthuma* and *E. fetida.* The concentration at which greater than 10% mortality was recorded has been eliminated from the statistical analysis of the avoidance behavior. The proportion of earthworms on both sides in the dual control test with OECD soil, garden soil (GS) and cow dung (CD) were found to be non-significant (student t-test, $p > 0.05$) validating the second criterion.

A positive (+ve) net response indicates avoidance and a negative (-ve) net response indicates hormesis. In the lower doses of atrazine and acephate, hormesis effect was observed. Hormesis is a biphasic response phenomenon whereby a positive (beneficial) effect results from exposure to low doses of a chemical which otherwise is toxic or lethal at higher concentration. Within the hormetic zone, a favorable biological response to lower doses is observed. The effects of hormesis are more often observed below the No Observed Effect Levels (NOEL), in the zone of low dose (Calabrese 2005). Hormesis was also observed by De Silva and Gestel (2009b) while studying the sensitivity of *E. fetida* and *P. excavatus* towards chlorpyriphos and carbofuran by avoidance

Table 2
Mean net avoidance response (%) ± standard deviation of *E. fetida* and *M. posthuma* to acephate in artificial OECD soil and natural medium - cow dung and garden soil.

Concentration (mg	*E. fetida*		*M. posthuma*	
	OECD	CD	OECD	GS
3.90	−60 ± 18.70***	−60 ± 7.07***	−20 ± 7.07*	−40 ± 7.07**
7.80	30* ± 7.07	−20 ± 15.81*	0	0
15.62	40 ± 7.07**	20 ± 7.07*	10 ± 7.07	−20 ± 15.81***
31.25	6 ± 10***	0	40 ± 7.07**	10 ± 7.07
62.50	50 ± 14.14***	60 ± 12.24***#	60 ± 10 ***#	40 ± 15.81**
125	100***#	80 ± 14.14***#	70 ± 21.21***#	70 ± 21.21***#
250	100***#	100***#	100***#	100***#

Significant avoidance compared to control determined by Student's t-test: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.
# Indicates reduced habitat function (≥60% avoidance).

Table 3
Mean net avoidance response (%) ± standard deviation of *E. fetida* and *M. pos*thuma to atrazine in artificial OECD soil and natural medium - cow dung and garden soil.

Concentration (mg)	*E. fetida*		*M. posthuma*	
	OECD	CD	OECD	GS
3.90	−20 ± 15.81*	−20 ± 15.81*	−10 ± 7.07	0
7.80	0	−60 ± 12.24***	0	−20 ± 7.07*
15.62	10 ± 7.07	−10 ± 7.07	20 ± 12.24*	−20 ± 15.81*
31.25	40 ± 15.81**	10 ± 7.07	30 ± 7.07	0
62.50	60 ± 12.24***#	20 ± 17.32*	40 ± 7.07**	20 ± 7.07*
125	70 ± 21.21***#	80 ± 12.24***#	60 ± 10***#	60 ± 10***#
250	100***#	100***#	100***#	100***#

Significant avoidance compared to control determined by Student's t-test: *$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$.
# Indicates reduced habitat function (≥60% avoidance).

test. De Silva and Amarasinghe (2008) also tested another organophosphate insecticide dimethoate depicting its high toxicity on earthworm by avoidance test. The EC_{50} value for dimethoate was found to be more in OECD than natural soil. In the present study, our results corroborated with the finding of De Silva and Gestel (2009b) that standard species, E. *fetida* was more sensitive than tropical species, *P. excavatus* towards pesticides chlorpyriphos and carbofuran. Other pesticides like benomyl and carbendazim were also found to be avoided by the earthworms (Garcia et al., 2008; Loureiro et al., 2005). Comparing the avoidance of *E. fetida* and *M. posthuma* towards acephate and atrazine in natural soil and OECD soil, it was found that acephate was more toxic to both the species in both the soil types with lower EC_{50} values in comparison to atrazine. The results of avoidance tests with respect to pesticides have been found to vary. This was also supported by Reinecke et al. (2002) and Garcia et al. (2008) who reported the avoidance behavior of earthworms towards mancozeb and lambda-cyalothrin respectively. However Hodge et al. (2000) reported a lack of avoidance response by *Apporectodea caliginaosa* towards diazinon and chlorpyriphos both in fields and laboratory analysis. According to Garcia et al. (2008) the avoidance test is clearly more sensitive than the acute toxicity tests and at least as sensitive as the chronic one.

Comparing both species of earthworms, it was found that standard species, *E. fetida* was much more sensitive than tropical species, *M. posthuma* as the EC_{50} for avoidance for both pesticides was higher in case of *M. posthuma* than *E. fetida*. Thus the first hypothesis stating that the effect of pesticide, atrazine and acephate on avoidance behavior of standard species, *E. fetida* (epigeic) and tropical species *M. posthuma* (endogeic) is different was confirmed. This was also supported by De Silva and Gestel (2009b) who also found *Eisenia* species being more sensitive with the local earthworms from Sri Lanka. *E. fetida* is an epigeic species and *M. posthuma* is an endogeic species. Epigeic species like *E. fetida* live on or near the soil surface, feeds on decaying leaf litter and plants, roots, dung etc. On the other hand, endogeic species like, *M. posthuma* form shallow semi-permanent burrows and are more resistant to unfavorable conditions (Dominguez 2018). The environment above the ground is a characteristic of high temporal and spatial heterogeneity. Thus exposure to contaminants of above group increases from endogeic to epigeic (Chatelain and Mathieu 2017). This is why epigeic earthworms face high fluctuating environment (temperature, humidity changes) and is directly exposed to soil inputs (pesticides, hydrocarbons, fertilizers, etc). This probably accounts for the high sensitivity of epigeic species. The endogeic nature of *M. posthuma* is a reason for their abundance (Jouquet et al., 2010; Ernst and Emmerling 2009; Hackenberg and Hackenberg 2014; Singh et al., 2016). The study of Singh et al. (2016; 2020) also concluded that *M. posthuma* has completely adapted to physical disturbance, intensive use of insecticide and pesticide and also human interventions. Epigeic and endogeic earthworms have relatively different ecological niches and therefore the difference in their sensitivities.

There have been several other studies on the avoidance behavior of earthworms. According to Mather and Christen (1998), field application of pesticides leads to surface migration of earthworms which can be viewed as a consequence of avoidance. Capowiez and Berard (2006) reported endogeic species, *Allolobophora icterica* being more sensitive than anecic *Apporrectodea nocturna* in terms of avoidance behavior to imidacloprid. This difference in sensitivity could again be attributed towards the difference in niches of endogeic (*Allolobophora icterica*) and anecic (*Apporrectodea nocturna)* earthworms. It is very well known that anecic earthworms live deeper than endogeic earthworms. Similarly, Piearce and Piearce (1979) also found *E. fetida* (epigeic) to be more sensitive than *A. caliginosa* (endogeic) in avoidance test taking sand as substrate. In contrast, Owojori and Reinecke (2009) reported that irrespective of soil properties and ionic constitution, *A. caliginosa* (endogeic) was found to be more sensitive than *E. fetida* (epigeic). This suggests that the sensitivity of earthworms depends on the species (Edwards and Coulson, 1992) and on their ecological type (Tomlin, 1992; Lukkari and Haimi, 2005). In addition to ecological differences, the characteristic difference in chemoreceptors (Stephenson et al., 1998), physiological and morphological differences (Edwards and Bohlen, 1996) also account for the variation in the sensitivity of various species.

In the present study we found out that different exposure medium exerts different behaviors in earthworms. The differences in substrate medium could also be ascertained for varied response as soil properties on their own have the capability to affect behavioral response (Amorim et al., 2008). Ellis et al. (2007) compared the avoidance behavior of *E. fetida* in carbendazim in two artificial soils; one containing kaolin clay and other comprised of bentonite clay. Both the soil types showed significant differences in mortality indicating that clay type also play a role in influencing toxicity. Therefore, confirming our hypothesis that (b) soil type plays a crucial role in earthworm behavior. However, De Silva et al. (2009) found that temperature plays a bigger role in avoidance behavior than soil type. The avoidance tests can therefore have clear advantages as the first and foremost screening tool for risk assessment and secondly in soil quality criteria studies warranting quantitative assessment of the contaminants bioavailability and toxicity. Higher concentration of pesticides also leads to reduced habitat functions of the soil. De Silva and Amarasinghe (2008) reported reduced habitat function of soil at dimethoate concentration of 30 mg/kg soil. In our study also habitat function of soil for both earthworm species with respect to acephate and atrazine was found to be limited in higher concentrations of atrazine and acephate. Thus we hypothesize that (c) at higher concentration of pesticide; both the standard species and tropical earthworm species depict reduced soil habitat function.

The laboratory test conditions also account for some limitations in the avoidance test assessments. According to Ellis (2007), laboratory tests are unable to account for the influence of soil conditions such as moisture, other physical factors and subsequent exposure of other pollutants on the behavior of the earthworm. The avoidance behavior of earthworms are influenced by organic matter content and the texture of soils (acting singly or cumulative) (Natal-da-Luz et al., 2008a). Field studies or terrestrial model ecosystem experiments (Burrows and Edwards 2004) are required to estimate the modification of earthworm behavior with respect to soil conditions.

5. Conclusion

The tropical species, *M. posthuma* was found to be less sensitive than standard species *E. fetida* in avoidance tests for herbicide, atrazine and an insecticide, acephate. This study also depicted that the soil medium plays a crucial role in the avoidance behavior of earthworms. Moreover, for assessment of avoidance behavior of earthworms, *M. posthuma* as a tropical variant forms to be a suitable species. It thrives well in both OECD artificial soil and garden soil. Predictive models for influence of pedological properties of soil on the avoidance response needs to be validated further by testing more natural and manipulated soils. These cumulative present and future aspects of research could then be correctly utilized for analysis of real site specific avoidance.

Avoidance tests are undoubtedly useful screening tools for examination of potential contaminants in the soil. Magnanimous amount of literature depict the correlation of avoidance and mortality and therefore the avoidance test is an easy, time saving alternative to long term tests like survival and reproduction tests. The results in this study possibly try to make a contribution to overcome a lack of data on the effect of pesticides on tropical earthworm. The mechanism of action of chemoreceptor proteins is unknown for the earthworms. The avoidance test forms to be a first hand test to determine any kind of chemical sensitivity.

Credit author statement

Study concept and design: Jaswinder Singh and Joginder Singh; Obtained funding: Shivika Datta; Sampling and Experiment: Shivika Datta; Statistical Analysis and interpretation of data: Shivika Datta, Sharanpreet Singh and Simranjeet Singh ; Drafting of the manuscript: Shivika Datta; Critical revision of the manuscript: Jaswinder Singh and Joginder Singh; Study supervision: Jaswinder Singh and Joginder Singh. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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