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Assessment of the toxic effects of the organophosphate insecticide Malathion (Cythion) on non-target earthworm growth

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Abstract

Earthworms are recognized as important bioindicators of chemical toxicity within soil ecosystems. This is significant because they serve as common prey for many terrestrial vertebrates, including birds and small mammals, and therefore play a key role in the biomagnification of various soil pollutants. The present study examined the concentration-dependent effects of the pesticide Cythion on earthworm growth. The experiment was conducted over a 60-day period using different concentrations of Cythion, starting from 3 mg/kg soil and increasing to 9 mg/kg. These concentrations produced non-significant effects on earthworm growth throughout the 60-day toxication period when compared with the control. A further increase from 9 mg/kg to 12 mg/kg soil likewise showed no significant difference in growth relative to the control during the same period. The results suggest that growth can be considered a sensitive parameter for evaluating the toxicity of Cythion in earthworms. Cythion demonstrated an impact on earthworm growth, resulting in a decrease in earthworm size.
Keywords: Earthworm, Cythion, Concentration, Soil, Growth, Toxicity

Introduction

Earthworms are one of the most important organisms responsible for mechanical mixing of soil and play a major role in maintaining physical soil characteristics and processes such as aeration, water permeability, and mineral turnover. Earthworms are key components in natural food chains, providing a food source for many small mammals, birds, fish, and prawns. Pesticides are known to produce morphological, anatomical, and physiological changes in the vital organs such as reproductive, nervous, respiratory, and osmoregulatory of different non-target animals, such as earthworms and other beneficial organisms (Guo et al., 2019). Earthworms are, as a matter of fact, important components of soils because they fine-tune femininity of soils, their saturation, and pH (Ye et al., 2016). But these organisms are destroyed (Roubalová et al., 2014) by interaction of industrial pollutants, effluents, flues, at the boiling point wastes, municipal wastes, household wastes, hut wastes, artificial fertilizers, pesticides, insecticides, herbicides, particulates, corrosive gases, fog, and global warming. These harmful substances changed the physical, chemical, and biological properties of soils. These substances are regularly acidic and basic in bias, and they give a pink slip, constitute a microbiological corrosion prison (Elyamine et al.,2018) by the whole of earthworms, thus biological corrosion life starts these species mutually, and in this by the number, their morphology can be displaced which leads to the rack and ruin of these organisms in soils. Ponds, canals, rivers, and reinforce raw material for the irrigation of soils, and this polluted water creates a corrosive environment for earthworms (Hu et al., 2016). Earthworms represent a significant, if not a dominant part of the soil biomass, and are regarded as soil engineers regulating important soil processes, and are being broadly used to assess the environmental impact of heavy metal pollution (Cao et al., 2013). In recent years, there has been a growing interest in the development of sub-lethal earthworm biomarkers; one of them is the neutral red retention assay, which measures the membrane stability of lysosomes within the coelomocytes of earthworms in response to contaminants. Thus, it can be used for the evaluation of the toxicity of a range of toxicants under different exposure conditions. Recently, from this laboratory, a series of publications have come up, where the effects of metals like lead, copper, aluminium, and some pesticides have been studied on growth, reproduction and avoidance behavior of earthworms, *E. foetida* and *Lumbricus terrestris*, have reported that the hazardous pollutants significantly affect the sensitive parameters of these soil organisms (Elyamine et al., 2018). Earthworms play a critical part in the maintenance of soil structure, roles, and richness. Their activities modify soil aeration, drainage, and availability of nutrients for plants and generally integrate soil organic and mineral ingredients to form aggregates and improve soil structure. Earthworms are an indicator of soil quality because they respond to and contribute to healthy soil. Earthworms are abundant and meet several conditions that are associated with soil quality and agricultural sustainability: moderate pH, surface residue in food and protection, and soil that is not waterlogged, compacted, drought, or excessively sandy (www.earthworm biology.com). Red worms are common soil organisms in most environments and play an important role in the structure and fertility of soil ecosystems (Markad et al., 2015). Earthworms play a significant role in decomposing litter material and in structuring soils. By burrowing, they create holes and pores in the ground and stabilize these structures with their slime see for an overview, Parelho et al., 2018). As a sound indicator of land quality, earthworms were used as testing organisms by OECD in the early 1980s for the registration of industrial fertilizers and pesticides before implementing them into the ground. More than forty of them are currently registered, although all operate the risk of acute and sub-acute toxicity. Organophosphates are used in agriculture, on the home lawn, in gardens, and in veterinary practice etc. The availability of earthworms in soil plays a very important role in the enrichment of the fertility of soil. But this non-target animal is affected by fertilizers. Therefore, we had taken a step to estimate the effects of pesticides on earthworms. The capacity of macro-invertebrates for monitoring has contributed to the art and science of procedures for evaluating the severity of environmental folded from changes in crowd structure. Many writers did what one is told that the barest pretty monitoring environmental feature on crowd is to cash in on plenitude word for hit species, and by means of this, the whole system off the rack up to detect pollutants from their impacts on crowd gregarious halls of knowledge must explain temporal amendment in abundance

Material and methods

Experimental animal

Earthworm, *Eisenia foetida,* is a recommended earthworm test species by the Organization for Economic Co-operation and Development (OECD, 1984a) and European Economic Community (EEC, 1985).

Animal collection

Earthworm, *Eisenia foetida,* brought from commercial suppliers, Nursery Department of Forest, Wadali, Amravati, and adopted as the test species, recommended by OECD (1984) guideline for testing of chemicals, no. 207, earthworm, and acute toxicity tests.

Chronic test for growth

The long-term toxicity test was analyzed to show the impact of selected pesticides on the growth and reproduction of earthworms. This test was analyzed according to OECD guideline No. 207 (OECD, 1984) for growth and reproduction. The soil used for this test was the artificial soil. Soil water content was measured, and moisture content was maintained every week to reach the maximum holding capacity. In the present experiment, acute toxicity test set at different time periods 10, 20, 30, 40, 50 and 60 days and different concentrations of pesticides were added to the soil, cythion were 3mg, 6mg, 9mg, 12mg and 15mg/kg dry weight soil and dieldrine were 4ml, 8mg, 12mg, 16mg and 20mg/kg dry weight of soil. Fully clitellated mature worms were collected from the uncontaminated soil. At the beginning of the experiment, the worms were sorted, washed with deionized water, and blotted with filter paper. The weight was taken by an electric balance and added to the respective test jars containing soil. 3 replicates of each concentration were arranged, and 10 earthworms were kept in each experimental replicate and covered with a porous fabric plate to provide sufficient ventilation. At the end of the treatment period of the control group and intoxicated group, the worms were sorted by hand, and the result was assessed by comparing the mean final result of the treated group with the control as significant or non-significant.

Effects of cythion on the growth of earthworm

Table 1. Impact on the growth of earthworm exposed to cythion at different toxication periods

Toxicated periods (day) and Concentration(mg/kg)	10	20	30	40	50	60
Control	6.5±2.5	6.7±2.5	6.8±2.6	6.8±2.6	7.2±2.6	7.6±2.7
3mg	6.4±2.5	6.4±2.5	6.5±2.5	6.6±2.5	6.9±2.6*	7.1±2.6**

*Significant and **Highly Significant differences ($P < 0.05$) were found between treatment and control groups, with a difference found 0.0072 in concentration.

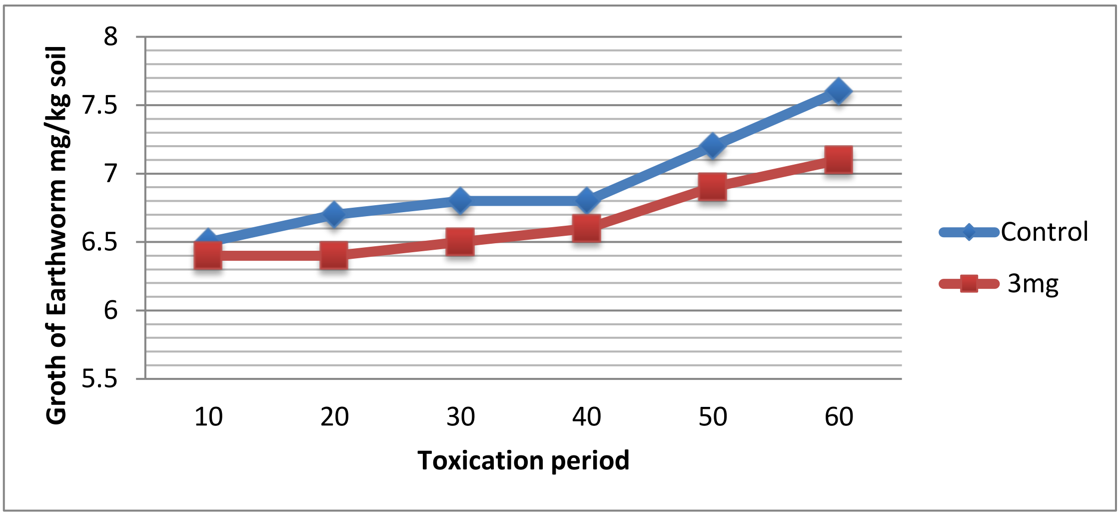

Figure 1. Impact on the growth of earthworms exposed to cythion at different toxication periods

Table and Figure 1 showed cythion toxicities of 3mg/kg soil at 10,20,30,40,50, and 60 days of experiment, showing a gradual decrease in growth of earthworm from 20 days to the end of the experiment, but the present data also showed a significant decrease in growth of earthworm, and it was dose and duration-dependent as compared to the relevant control.

Table 2. Impact on the Growth of earthworm exposed to cythion at different toxication periods.

Toxicity periods (days) and Concentration(mg/kg)	10	20	30	40	50	60
Control	6.5±2.5	6.7±2.5	6.8±2.6	6.8±2.6	7.2±2.6	7.6±2.7
6 mg	6.5±2.5	6.6±2.5	6.7±2.5	6.6±2.5	6.8±2.6	6.9±2.6

*Significant differences ($P < 0.05$) were not found between the treatment and control groups was found of 0.08 in concentration.

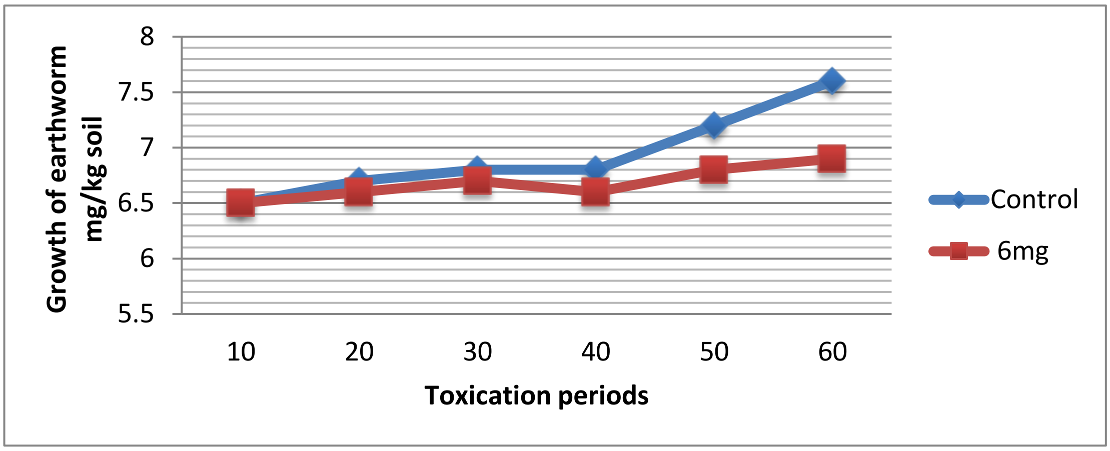

Figure 2. Impact on the growth of earthworms exposed to cythion at different toxication periods

The present study (Table and Figure 2) indicates that the 6mg/kg concentration of cythion showed a non-significant difference in the earthworm growth in all toxication durations. But the growth rate decreased from the initial to the end of the experiment as compared to the control.

Table 3. Impact on the growth of earthworms exposed to cythion at different toxication periods.

Toxicated periods(days) and Concentration(mg/kg)	10	20	30	40	50	60
Control	**6.5±2.5**	**6.7±2.5**	**6.8±2.6**	**6.8±2.6**	**7.2±2.6**	**7.6±2.7**
9mg	**6.6±2.5**	**6.5±2.5**	**6.5±02.5**	**6.5±2.5**	**6.6±2.5**	**6.7±02.58***

*Significant differences (P < 0.05) were found between all treatment and control groups, with a difference found 0.059 in concentration.

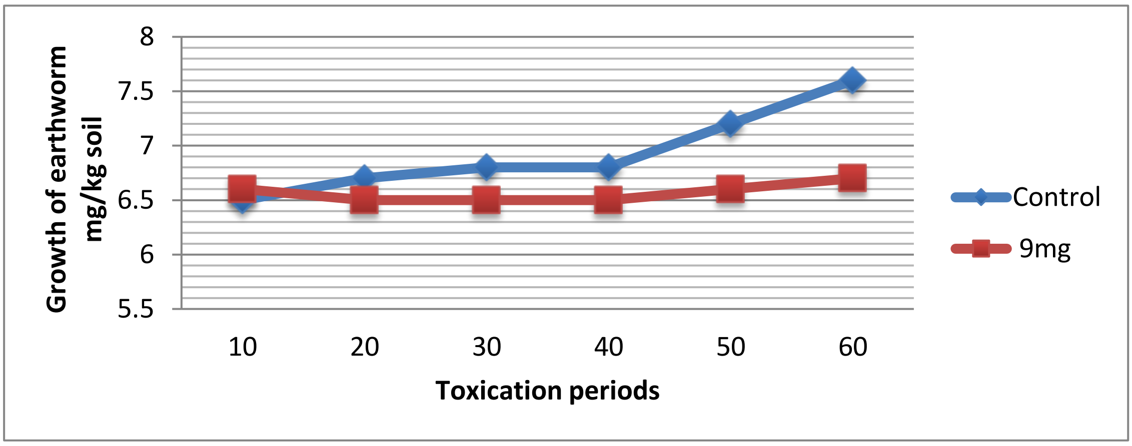

Figure 3. Impact on the growth of earthworms exposed to cythion at different toxication periods

The same toxicant was observed (Table and Figure 3) at 9mg/kg of soil, which was higher than the previous one in where concentration showed a non-significant result. But present concentration indicates a significant decrease in the growth of earthworm after 40 days of treatment, and a slight decrease in the growth of earthworm was observed from 10 to 20 days, then the constant growth rate was observed between 30 to 40 days of toxication as compared to the control.

Table 4. Impact on the growth of earthworms exposed to cythion at different toxication periods.

Toxicated periods (days) and Concentration(mg/kg)	10	20	30	40	50	60
Control	6.5±2.5	6.7±2.5	6.8±2.6	6.8±2.6	7.2±2.6	7.6±2.7
12mg	6.4±2.5	6.6±2.5	6.5±2.5	6.3±2.5*	6.5±2.54*	6.5±2.54*

* Significant differences (P < 0.05) found between treatment and control group was 0. 04.

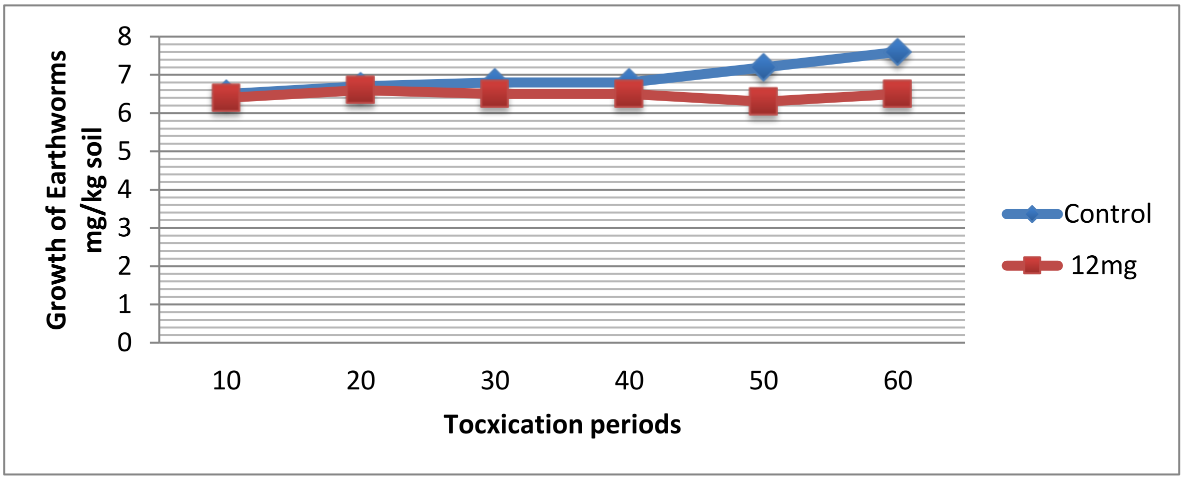

Figure 4. Impact on the growth of earthworms exposed to cythion at different toxication periods

Cythion administration found in the present (Table and Figure 4) depicted that the growth rate was found to be significantly decreased from 20 to 50 days and considerably increased from 50 to 60 days of toxication, but the concentration of toxicant was significantly affected as dose dependent in all days of toxication.

Table 5. Impact on the growth of earthworm exposed to cythion at different tocxcation periods.

Toxicated periods(days) and Concentration(mg/kg)	10	20	30	40	50	60
Control	6.5±2.5	6.7±2.5	6.8±2.6	6.8±2.6	7.2±2.6	7.6±2.7
15mg	6.5±2.5	6.4±2.5	6.4±2.4	6.2±2.4*	6.1±2.4*	6.4±2.5*

*Significant differences ($P < 0.05$) between treatment and control groups were found 0.02 in treatment.

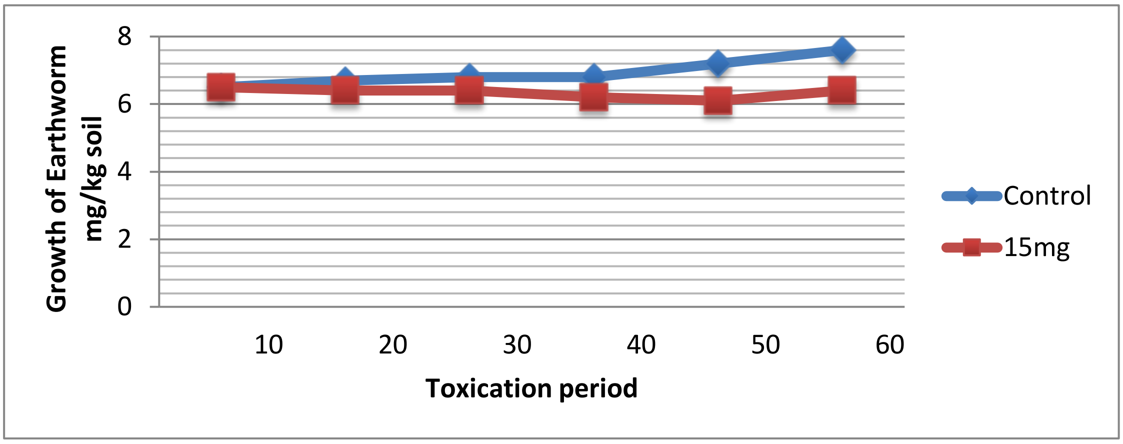

Figure 5. Impact on the growth of earthworm exposed to cythion at different toxication periods

The present study (Table and Figure 5) was conducted as a chronic test for a higher concentration of the toxicant 15mg/kg soil of cythion. It was observed that the growth rate gradually decreased with an increase in duration of toxication till 50 days. It slightly increased the growth of the earthworm from 50 days onwards. This happened due to adaptation and the decline in the stress of toxicants by long-duration treatment.

Figure 7. Results showed the growth rate gradually decreased with the toxication treatment

(A) Normal Growth of the earthworm found before exposure to the herbicide cythion.

(B) Earthworm showing shrinking of body after 1 hour of exposure to herbicide, indicating unfavourable to normal functioning.

(C) Earthworm of smaller size found in the same environment after treatment of the soil with cythion.

Results

The Negative impact of pesticides on earthworm growth has been reported by various researchers. Xiao suggested that growth can be regarded as a sensitive parameter to evaluate the toxicity of acetochlor on earthworms (Cao et al., 2013). Respectively, the cythion showed an impact on the growth of the earthworm, due to which the size of the earthworm was decreased. From table and figure 1, it showed cythion toxication of 3mg/kg soil at 10, 20, 30, 40, 50, and 60 days of the experiment showed a gradual increase in growth of earthworm from 20 days to the end of the experiment. This result produced significant differences in dose and duration dependent as compared to the relevant control. Mosleh studied the effects of endosulfan and aldicarb on *Lumbricus terrestris* and has suggested growth rate as an important biomarker for contamination by endosulfan and aldicarb (Mosleh et al., 2003). The present study was conducted as a chronic test for a higher concentration of the toxicant 15mg/kg soil of cythion. It was observed that the growth rate gradually decreased with an increase in duration of toxication till 50 days. A slightly significant increase growth of earthworm was also observed from 50 days onwards. This happened due to adaptation and overcoming the stress of toxicants through long-duration treatment. Wang et al. (2020) assessed and found that chlorpyrifos had an adverse effect on growth in earthworms exposed to 5mg/kg chlorpyrifos after eight weeks. Similarly, the cythion showed an impact in the highest concentration on the earthworm growth. It was observed that the growth response rate gradually declined with an increase in the duration of days. A gradual decrease in the growth of earthworm was observed, which is significant and indicates that both responses were altered in the toxic earthworm than the control earthworm. Data from the present study showed a gradual decrease in the growth response of earthworm from 10 to 50 days, which later decreased significantly in response to long days of duration test, which was significantly different compared to the control. The growth rate was diminished on the initial day of experimentation, which later decreased significantly up to 60 days of the toxication period. At the end of the experiment highly significant result was observed, and we found that the effect was dose and duration-dependent. The present data of the growth response test showed that the growth was significantly decreased as compared to the control. The decrease was observed in all tested earthworms, meaning that dieldrin impacted the growth of earthworms. Some studies have shown that the growth of earthworms appeared to be more severely affected at the juvenile stage than at the adult stage (Ali & Naaz, 2013). Heavy metals have been shown to cause lysosomal membrane instability, changes in gene expression, and oxidative stress in earthworms (Jadhav & David, 2017), reducing growth. A gradual decrease in the growth of earthworm was observed, which is significant and indicates that both responses were altered in the toxic earthworm than the control earthworm. Depending upon the chemical nature of pesticides and soil properties, organs undergo a series of chemical pathways, transport, adsorption, and desorption processes (Thapar et al., 2015; Baishya, 2015). Rajashree et al. (2014) also found that Methyl parathion and phorate are very toxic to earthworms and showed progressive symptoms of toxicity, such as coiling, curling, and excessive mucous secretion with sluggish movements, swelling of the clitellum, degenerative changes in the nervous system, and loss of pigmentation, which is elicited by organophosphorus insecticides. In light of the present findings of a comprehensive assessment of the toxicity levels (LC50 values) of insecticides and their effects on the earthworms, is of great value. A perusal of literature dealing with the assessment of LC50 values of insecticides in respect of earthworms indicates that a quite a large number of publications appeared dealing with the toxicity levels of several agrochemicals based on field studies (Sattibabu, 2013; Rakesh, 2014). The results indicate that the worms showed a higher mortality rate, even at lower concentrations of monocrotophos. High mortality in water may be due to two reasons. The pesticide in the medium of water diffuses into the body easily through the body wall. As there is no food supply, naturally, the animal will starve. The test animal being terrestrial animal and soft skinned, possible a quicker exchange of the toxicant is possible and there by neurotoxicity effects may ensure since the quantity of toxicant in water medium may directly enter more readily through the body openings resulting in reddening and in the appearance of swellings in the anterior segments in the worms within two days after their exposure to insecticide (Plates 1 & 2). Similar effects were reported in the case of Lumbricus terrestris, Lumbricus rubellus, Eisenia foetida, Aporrectodea caligilosa, Allobophora chlorotica, Lampito mauritii, and Parvularcula bermudensis when exposed to a variety of organophosphate, organochlorine, and carbamate insecticides (Rakesh 2014). In general, it was the movements of the worm released in water containing pesticide, the movements of the worms were invariably quick and erratic in behaviour, obviously as a reaction to the toxic action of the chemical. However, this apparent activity of the worms gradually became dissipated with increasing exposure periods of time and concentration. Moderately large swellings appeared in the anterior parts of the body in the first 3 to 4 segments, covering the esophageal region. On certain occasions, the worms showed swellings at the clitellar region. The swellings in the posterior segments were less frequently noticed in the toxic media-exposed worms (Sattibabu, 2013; Rakesh, 2014). (Pelosi et al., 2014) documented that the soil was artificially contaminated by different concentrations of cypermethrin, which were 5, 10, 20, 40, and 60 mg/kg based on the results of pilot experiments. He had found that carbendazim and dimethoate were greater toxic than glyphosate. There was a significant reduction in the number of young worms due to being exposed to carbendazim and to a mixture of three pesticides (Wang et al., 2019). They had reported a marked negative impact on the growth and reproduction of *Eisenia fetida*. A decrease in the reproductive rates of *Aporretodea longa* and *Aporretodea rosea* was also observed after the application of benomyl in the grassland ecosystem by Sun et al. (2020). The results of the present study also depicted the decrease in the growth of earthworms by the pesticide cythion when exposed at different concentrations and exposure periods. All of the above-mentioned studies indicate a negative impact of pesticides on earthworm growth and reproduction. Some studies also indicate that microorganisms in the soil help degrade the chemicals (Pelosi et al., 2013). The earthworms are a very important tool. We made out a test of (Organochlorin) dieldrin on earthworm growth. This research has investigated the organophosphates are impacting non-target animal earthworm growth. We increased the concentrations of cythion on earthworms, which affected the growth of the earthworms. The red worm is using very large amounts of dirt and organic waste, and cow dung as a nutrient. The cythion is mixed in the soil in which the earthworms survive. Malathion (cythion) is one of the most widely used organophosphate pesticides (Pelosi et al., 2013) in agriculture, therefore posing high potential risks to environmental contamination and human health and other living organisms (Pass et al., 2015). Similarly, the cythion has a very harmful impact on the earthworm. The metabolism of Malathion in vertebrates and invertebrates is complex, and its metabolites present varied toxicity. Several of them can inhibit acetyl cholinesterase (AchE), contributing to an aggregation of acetylcholine (ACH) (Kavitha et al., 2019). Similarly, the selected pesticide cythion varied in toxicity on the annelid, like an earthworm.

Conclusion

The present study was conducted as a chronic test for a higher concentration of toxicant cythion in soil. Earthworms are generally called the friend of farmers, so we should take a serious notice regarding the use of fertilisers. From the study, it appeared that we should use eco-friendly pesticides that bear a very low threat to the non-target organisms.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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