EVALUATION OF MONOCROTOPHOS AND THIOMEXOTHAM TOXICITY ON EARTHWORM AMYNTHAS ALEXANDRI USING CONTACT FILTER PAPER TEST METHOD

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Abstract: Toxicity of pesticides on earthworms has been categorized as acute (expressed as LC$_{50}$ or LD$_{50}$) and chronic, in field and laboratory conditions. An acute toxicity test of insecticides to earthworm Amynthas alexandri was performed using OECD (Organization for Economical and Cooperative Development) testing guideline no.207, to evaluate LC$_{50}$ for Monocrotophos and Thiomexotham the contact filter paper method. The worms were exposed to insecticides and kept uniform on filter paper for 48 h and the mortality was recorded. Based on the LC$_{50}$ value, the insecticides were classified as extremely toxic, very toxic and least toxic to earthworms. It was observed that the LC$_{50}$ mortality of Monocrotophos and Thiomexotham was found 0.15 μg/cm$^2$ and 0.12μg/cm$^2$ respectively in contact filter test method. The behavior of earthworms has been recorded and it was observed that earthworm turned slow and sluggish with the increase in dose. Earthworms treated with Monocrotophos also exhibit morphological changes such as coiling, heavy secretion of mucolytic fluid and loss of architecture whereas Thiomexotham revealed epidermal scars and degeneration of color.

Keywords: Amynthas alexandri, Monocrotophos, Thiomexotham, Filter paper Method, LC$_{50}$

I. Introduction

Earthworms living in soil are excellent bio-indicator of soil health specially for evaluating lethal and sub-lethal effects of toxic chemicals present in soil. Pesticides affect earthworms at each level like individual, population and community (Uwizeyimana et al., 2017). Number of pesticides are used in agriculture sector is to achieve high agricultural production, however the residues of pesticides increases soil contamination causing stressful environment for soil organisms (Choung et al., 2013). Exposure of earthworms to higher concentration of pesticides in soil ecosystem affects growth, sexual development, cocoon production, viability and density (Yasmin and D'Souza 2010; Gupta and Saxena 2003). The disproportionate use of agrochemicals threatened the fragile ecosystem causing exploitation of agricultural land (Ma WC et al., 1990; Miyazaki A et al., 2002). As per the OECD guideline earthworms have been used as test organisms before the registry of pesticides and industrial fertilizers prior to 1980’s (Pawar and Ahmed 2014). Many ecotoxicological studies have been carried out in recent years focusing on effect of single pesticides. Toxicity of pesticides is evaluated by performing filter paper test to enable a more realistic assessment of the toxic effect of pesticides on earthworms to provide risk evaluation data. Paper contact test method has been consecutively adopted by the scientists worldwide to determine the toxic effects of chemicals on earthworms (Karanjkar and Naik, 2010; Miyazaki et al., 2002; Robert and Dorough, 1984; Wang et al., 2012; and Velki et al., 2013). However, the chemicals used in the present study have been studied for the very first time with reference to Kumaun Region of Uttarakhand.

II. Materials and Methods

1) Experimental Animals- The earthworms were collected from grasslands free from agrochemicals and agri practices and cultured in large earthen pots using the grassland soil and fed with cow manure (Ismail 1997). Earthworms with well developed Clitellum were selected for testing and further experimentations (Iordache M and Borza I 2010).

2) Pesticides- The pesticides selected for the present study are of commercial grade (cg) organophosphate (OP), cyclodiene compound Monocrotophos and Thiomexotham belongs to
chemical class Neonicotinoids and subclass nitroguanidines and are highly toxic in nature. Insecticide Monocrotophos is extensively used in agricultural and animal husbandry and classified as ‘extremely toxic’ (Rao, 2004). In developing countries Monocrotophos has been withdrawn from use but in developing countries like India it is still in use due to lack of alternative replacements (Banaerjee et al., 2002). The concentration was prepared in mg/ml and the toxicity was measured as μg/cm².

3) Filter Paper Contact Test- The acute toxicity test was performed on earthworms using simple paper contact test method as described in OECD (2016). Adult earthworms those attained wet weight of 300-350mg, were selected for testing. Earthworms were kept on filter paper at 20±1°C for 48 hrs in dark to purge the gut content before being washed and dried for dose dependent test. A piece of Whatman filter paper (grade 1 quantitative) was placed in 9-cm petri-dish. After the solvent was evaporated, the piece of filter paper was remoistened with 2 ml of distilled water. Acetone was used as control. Five regular concentrations were prepared by dissolving test substance in 2mL of Acetone in a general geometric series. For each treatment 6 replicates of experimental series were used, each containing one earthworm per petri-dish. After exposure for 48 hrs the worms’ mortality was recorded. An Earthworm was considered dead if it failed to respond to a gentle mechanical touch on the front end.

4) Statistical analysis

A probit analysis was conducted to assess the acute toxicity of insecticides (Chi H 1997). Based on the results after 48 hrs LC₅₀ values, Monocrotophos and Thiomexotham were classified as super toxic at <1 μg cm⁻², extremely toxic 1-10 μgcm⁻², very toxic 10-100 μg cm⁻², moderately toxic 100-1000 μg cm⁻², or relatively nontoxic >1000 μg cm⁻² (Roberts and Dorough, 1984).

III. Result and Discussion

Mortality (LC₅₀) of Monocrotophos

The Monocrotophos exhibited very harmful effects on earthworms; different parameters were analyzed to determine the toxic effect of monocrotophos. The insecticide showed dose and duration dependent impact on earthworms. In this study, it was observed that Monocrotophos is slightly toxic in comparison to thiomexotham. Mortality was calculated by plotting probit analysis curve and it was observed that the LC₅₀ of Monocrotophos in Filter paper contact method was 0.15μg/cm².

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Earthworms Exposed</th>
<th>Concentrations (ppm)</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
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<td>0.1</td>
<td>33%</td>
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<tr>
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<td>50%</td>
</tr>
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<td>0.5</td>
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<tr>
<td>6.</td>
<td>6</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 1- Mortality (%) of earthworm (Amynthas alexandri) treated with Monocrotophos
Fig 1- Probit analysis curve showing LC₅₀ of Monocrotophos

Mortality (LC₅₀) of Thiomexotham

LC₅₀ of Thiomexotham in filter paper contact test method was 0.12μg/cm². The concentration of 0.14μg/cm² show high impact on earthworms showing curling, body lesions and bleeding. After 24 hrs of observation the defoliation started and finally the worm died.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Earthworms Exposed</th>
<th>Concentrations (ppm)</th>
<th>% Mortality</th>
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<td>6.</td>
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<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 2- Mortality (%) of earthworm (*Amynthas alexandri*) treated with Thiomexotham

Fig 2- Probit analysis curve showing LC₅₀ of Thiomexotham

Discussion

To assess the toxic effect of Monocrotophos (Organophosphate) and Thiomexotham on earthworm *Amynthas alexandri* the contact filter paper test was performed. It is an original screening technique used to evaluate the comparative toxicity of chemicals showing effect on earthworms. The chemicals are mainly absorbed through skin when earthworms move around filter paper. It was observed that the LC₅₀ of Monocrotophos and Thiomexotham was found 0.15μg/cm² and 0.12μg/cm² respectively in contact filter test
method. Both the insecticides observed as very toxic to earthworms because they were easily absorbed by the skin than by the gut. As per the previous investigations (Zohu et al., 2006) the weight of earthworms was more sensitive index compared to the mortality in indicating toxic effect of acetochlor and methamidphos. In case of dimethoate, pirimiphos-methyl and deltamethrin to L. rubellus through filter paper bioassay for 48 hrs the LC$_{50}$ was found to be 2.24, 0.41 and 0.11 μg/cm$^2$ (Velki and Hackenberger 2013). Respectively abamectin, emamectin and ivermectin exhibit LC$_{50}$ values of 23.08, 30.20 and 4.40 μg/cm$^2$ (Wang et al. 2012a) findings of these investigations substantiate the present results that Monocrotophos and thiomexotham are much more toxic insecticides in comparison to other routinely used chemicals. The highest dose of selected two insecticides showed a detrimental impact on earthworm and the mortality was observed even at the minimal concentration during the intoxicated period of 48 hrs. The 100% mortality was observed at 0.4 and 0.5mg/cm$^2$ concentration of Monocrotophos. During the present investigation exposure of earthworms to Monocrotophos and thiomexotham also showed morphological changes such as coiling, heavy secretion of mucolytic fluid and loss of architecture whereas thiomexotham revealed Epidermal Scars and degeneration of color. Though Monocrotophos is considered as most lethal insecticide as per the literature available and falls in the category of extremely toxic compounds but during the present investigation under laboratory condition the toxicity was restricted to very toxic category, whereas thiomexotham which is considered as slight toxic to earthworms caused heavy toxicity in the laboratory conditions on earthworms. The reports are scanty on earthworms killing by the insecticides but in case of the present study thiomexotham was also found a potent toxic chemical.

IV. Conclusion

The insecticide Monocrotophos manifests an extremely toxic action on earthworms, determining 100% mortality after 24 and 48 hrs tests. The only exception where earthworms survived for more than 48 hrs was the control test. The results obtained from filter paper test on this earthworm species confirmed the fact that organisms living in soil are highly sensitive to pesticides present in the soil. Therefore the use of chemical substances especially the chemicals used in agriculture like insecticides, herbicides, fungicides etc must be done with maximal responsibility and precautions special reference to dose and method of application. The present can serve as base line data for further investigations on effect of pesticides on earthworms.

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References


