Effects of Endosulfan, an organochlorine pesticide on growth, reproduction and avoidance behavior of earthworm Eisenia foetida.

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INTRODUCTION

Earthworms are the common prey of many invertebrates such as birds and shrews, and therefore, they play a key role in the biomagnifications process of several soil pollutants and in the occurrence of indirect effects on terrestrial vertebrates from soil pollution (Dell'Omo et al., 1999). Considering their leading role in the monitoring of soil pollution changes in earthworm abundance or species richness have been positively correlated to point sources to the level of soil degradation by agricultural activity (Spurgeon et al., 1999). In addition these organisms are suitable indicators for monitoring the effectiveness of polluted soil remediation procedures (Lock et al., 2003). The presence of earthworms in wide range of soils and their high contribution to soil biomass makes them suitable to determine the effects of soil pollutants such as pesticides (De Silva et al., 2009).

Earthworm biomarkers of xenobiotics exposure have experienced a significant progress in the last decade as they are common organisms in agro ecosystems with direct beneficial effects on plant growth and soil functioning, but unfortunately very few studies have assured the impact of agricultural pesticide applications on earthworm populations through the use of biomarkers. Laboratory experiments are therefore needed to examine to what extent abiotic variables influence the normal fluctuations of earthworm biomarkers. (Ali et al., 2010).

Venter and Reinecke (1988) suggested that acute toxicity is insufficient to predict environmentally acceptable concentrations of chemicals as they do not reveal sub lethal effects of low concentrations on growth, behavior, and reproduction. The parameters such as growth and fecundity must be investigated to observe the sub lethal effects of pollutants. (Phillips and Bolger 1998; Yasmin and D’Souza 2007). The reduction of population size due to mortality or reduced reproduction is an ecological consequence of exposure to chemicals in soil. Worms are highly mobile in soils and hence have the ability to detect and avoid areas of contamination, which would have significant ecological implications (Yeardley et al., 1996). Thus behavior seems to be a promising biomarker in earthworm studies since it can give different end points, that could linked, at least theoretically, to soil functioning (Annette, 2009).

Endosulfan is a pesticide belonging to the organochlorine group of pesticides. It is used against a broad spectrum of insects and mites in agriculture and allied sectors. It acts as contact and stomach poison and has a slight fumigant action. It is used in vegetables, fruits, paddy, cotton, cashew, tea, coffee and timber crops. It is one of the most extensively used pesticides in the agricultural fields of M.P., Central India. But its toxicological effects on nontarget soil organisms like earthworms have been totally ignored and therefore present studies have been undertaken to evaluate its effects on growth reproduction and avoidance behavior of earthworm, Eisenia foetida.

MATERIAL AND METHODS:

E. foetida has been selected as test species because it has been suggested as a sensitive and standard species for ecotoxicological studies by OECD (2007). These were brought from M.P Council of Science and Technology (MPCST) nursery Obedullahganj (District Raisen, M.P.). Prior to exposure all worms were acclimatized for one day.
month in the uncontaminated soil medium in the laboratory which was the mixture of cow dung manure and virgin black soil, as per the method of Rao and Kavitha, (2004).

After calculation of LC50 according to the OECD guidelines No.207 (1984), artificial soil test substrate was prepared (using an evenly blended dry weight mixture of 68%, mesh silica sand, 20% kaolin clay and 10% sphagnum peat. Different concentrations of the pesticide that is endosulfan (organochlorine) formulated as (endosulfan 35% EC, Hyderabad Chemicals Limited, India) were prepared in 1000 ml of distilled water and mixed with 1 kg of the soil (dry weight) described above and placed into earthen pots. The pH was maintained at 6.9±0.5 and moisture content was maintained at 35%.

Batches of 40 adult earthworms of approximately (9.52±0.25cm) length and weight (845.6±18.27) were divided into four replicates of 10 earthworms, each batch was exposed to each concentration of Endosulfan i.e. (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4.5 and 5mg/kg dry weight of soil). The control media was the same quantity of water without any additive agent. Testing was done in continuous light at 20±2°C. The mortality was recorded after 14 days of exposure. The concentration verses percentage mortality was subjected to Probit analysis using Stat plus (2009) portable software for calculating the median lethal concentration (LC50) of the test substance during the exposure tenure.

After calculation of LC50 which was found to be 3.57 mg/kg dry weight of soil the sub lethal doses of Endosulfan were selected as 0.5, 1 and 2 mg/kg dry weight of soil and were coded as E0 (control), E1(0.5mg/kg), E2(1mg/kg) and E3(2mg/kg). Statistical significance of all the values observed during the test was analyzed by comparing the values with their relevant controls at 95% confidence interval at significance of p < (0.05) and p < (0.001) using NCSS, (2007) version 7.1.14 statistical software.

RESULTS
Effects of Endosulfan on the growth of earthworm (Eisenia foetida)
In the present study the data of Fig No.1 clearly shows that endosulfan caused a gradual decrease in weight of all the groups of earthworms when they were exposed to different concentrations of endosulfan i.e. E1, E2 and E3 except the control group E0 in which the weight was found to increase over the period of 28 days. The initial fresh weight of sexually mature adult earthworm was found to be 852.35±18.17mg. Earthworms of group E1 i.e. 0.5mg/kg endosulfan exhibited the decline in weight from an initial fresh weight of 852.35mg to
Effects Of Endosulfan On Cocoon Production

Analysis of cocoon production rate as (no. of cocoons/worm/week) in treated worms (Eisenia foetida) indicated dose dependent effects. At the dose of 0.5 mg/kg there the rate of cocoon production was 0.30±0.14 which reaches up to 0.37±0.07 and found to be significantly low in comparison to its relevant controls a statistical differences were observed at (p<0.05) which is highly significant and clearly indicate that growth rate was hindered in treated worms while for control worms, increase in weight indicated a normal growth. (Fig No.1)

Cocoon Viability And Hatching Success Of Cocoons

The viability of cocoons produced during the test period is expressed as percentage (%) of viable cocoons at the dose of 0.5mg/kg the viability of cocoons found 14.67±1.62(%) at 1mg/kg it was found as 18.11±2.97 (%) while at the dose of 2mg/kg no viable cocoons were observed where as, for the control it was found to be 36±4.45 (%). (Table No.2)

The percentage of cocoons hatched at the dose of 0.5mg/kg dry wt. of soil was found as 16.11±2.47 and at 1mg/kg it was 7.44±0.75 (%) while that of control it was found to be 56.3±2.75. Statistically significant decrease was observed at (p<0.05) when the values obtained compared with that of control. (Table No.2) Effects of Endosulfan on avoidance behavior of earthworm (Eisenia foetida).

During the 48 hours of avoidance test the mean weight of earthworms from an initial value of 852.35±18.17mg to 427.05±14.08mg as observed by Mosleh et al., (2003) and they also detected the residues of endosulfan up to 40% after 2, 7, 14±4.00 (%) preferred the soil contaminated with 1mg/kg while there were no earthworms found in the soil containing 2mg/kg endosulfan and therefore 100% avoidance was observed at this dose.

DISCUSSION

In the present study the change in biomass of earthworm Eisenia foetida was found to be dose dependent during 28 days of exposure to endosulfan. A gradual decrease in biomass of earthworms was observed at all concentrations of pesticides i.e. 0.5, 1 and 2mg/kg dry weight of soil and the highest weight loss was observed at highest concentrations of 2mg/kg and found to be significantly different at (p>0.05) from that of control worms where there was gradual increase in weight over the entire period of experiment. The gradual decline in weight of endosulfan treated earthworms even after 3 weeks of exposure indicates the persistent toxicity of the pesticide in the soil as well as in the tissues of worms. Similar effects on growth rate of Lumbricus terrestris were observed by Mosleh et al., (2003) and they also detected the residues of endosulfan up to 40% after 2, 7, 15 days of exposure and concluded that concentration in the soil decreased faster at a higher initial concentration. Whereas, Antonious et al.,(1998) reported the differences in half lives of α and β isomers of endosulfan and therefore the weight loss may indicate a feeding inhibition situation, with the earthworm regulating the intake of the pesticides by reducing consumption rate. This strategy is commonly used to avoid poisoning with heavy metals and pesticides (Ribiero et al., 2001).

The findings of the present study show that the sublethal doses of organochlorine pesticide endosulfan altered the reproduction process of earthworm Eisenia foetida during exposure period of 28 days. The percentage of cocoons hatched at the dose of 0.5mg/kg dry wt. of soil was found as 16.11±2.47 and at 1mg/kg it was 7.44±0.75 (%) while that of control it was found to be 56.3±2.75. Statistically significant decrease was observed at (p<0.05) when the values obtained compared with that of control. (Table No.2) Effects of Endosulfan on avoidance behavior of earthworm (Eisenia foetida).

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animals whether vertebrates or invertebrates reaching sexual maturation can severely affect their fertility and lineage.

In the present investigation the avoidance responses of earthworms for the soil contaminated with endosulfan at all concentrations were observed. A 100% avoidance of earthworms was observed for the soil contaminated with the highest dose of 2mg/kg which clearly showed that the pesticide has detrimental effects on earthworm population. Although no mortality was observed at all concentrations during the avoidance test period of 48hrs, but 80% earthworms avoided even the lowest dose of 0.5mg/kg which indicates that *Eisenia foetida* is very much sensitive to this pesticide. Detection of a noxious chemical substance is likely to result in an avoidance reaction (Christensen and Mather, 2003) which may be followed by an escape response. Under natural conditions, if juvenile and mature individuals exhibit similar behavioral patterns with respect to migration from pesticide contaminated soil, it probably reflects a phase of dispersal in relation to the decrease in population at a living site (Stephenson et al., 1998). Similar responses were observed by Slimak et al., (1996) when earthworm *Lumbricus terrestris* were exposed to ten different pesticides including organochlorine pesticides and found that avoidance occurred for all pesticides studied and increased as concentration increased.

**Fig No. 1** showing the change in biomass during 7, 14, 21 and 28 days of exposure to different concentrations of endosulfan with well matched controls.

![Graph showing biomass change](image)

Table No. 1 Showing the effects on cocoon production of earthworms *Eisenia foetida* during 7, 14, 21 and 28 days of exposure to Endosulfan (No. of cocoons/worm/week, where n=9)(the values marked with asterisks are significantly different from control at p< 0.05)

<table>
<thead>
<tr>
<th>Dosage</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>E0(Control)</td>
<td>--</td>
<td>1.69±0.13</td>
<td>2.21±0.14</td>
<td>2.59±0.22</td>
</tr>
<tr>
<td>E1(0.5mg/kg)</td>
<td>0.30±0.14</td>
<td>0.26±0.10</td>
<td>0.43±0.15</td>
<td>0.37±0.07*</td>
</tr>
<tr>
<td>E2(1mg/kg)</td>
<td>0.09±0.04*</td>
<td>0.08±0.04*</td>
<td>0.12±0.07</td>
<td>0.13±0.06*</td>
</tr>
<tr>
<td>E3(2mg/kg)</td>
<td>0.32±0.06*</td>
<td>0.34±0.16</td>
<td>0*</td>
<td>0*</td>
</tr>
</tbody>
</table>

Table No. 2 showing the percentage of viable cocoons and percentage cocoons hatched in worms exposed to Endosulfan for 28 days as well as in control worms. (The values marked with asterisks are significantly different from control at p< 0.05)
<table>
<thead>
<tr>
<th>Dosage</th>
<th>% of viable cocoons</th>
<th>% of cocoons hatched</th>
</tr>
</thead>
<tbody>
<tr>
<td>E0(control)</td>
<td>35.89±3.19</td>
<td>55.11±2.64</td>
</tr>
<tr>
<td>E1(0.5mg/kg)</td>
<td>14.67±1.62</td>
<td>16.11±2.47</td>
</tr>
<tr>
<td>E2(1mg/kg)</td>
<td>18.11±2.97</td>
<td>7.44±0.75*</td>
</tr>
<tr>
<td>E3(2mg/kg)</td>
<td>0*</td>
<td>0*</td>
</tr>
</tbody>
</table>

Table No.3 Showing the Substrate preference, mean (% of total numbers n=40) ±S.E.

<table>
<thead>
<tr>
<th>Dosage</th>
<th>% of worms preference for substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>E0(control)</td>
<td>85±3.87</td>
</tr>
<tr>
<td>E1(0.5mg/kg)</td>
<td>20±3.16</td>
</tr>
<tr>
<td>E2(1mg/kg)</td>
<td>14±4.00</td>
</tr>
<tr>
<td>E3(2mg/kg)</td>
<td>0</td>
</tr>
</tbody>
</table>

REFERENCES:


ecotoxicological risk assessment.” Environmental Toxicology and Chemistry 18, 1591-1607.


