Toxicity and Behavioural Studies on the Earthworm, *Lampito mauritii* (Kinberg) Exposed to Organophosphate Insecticide Monocrotophos

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Abstract: Earthworms have long been recognized as a 'friend of the farmer'. They plough, aerate and manure the soil ecosystem. Such a defense less friendly creature deserves attention and protection from the onslaught through the irrational use of pesticides. Indiscriminate use of pesticides is bound to disturb the ecological balance of the soil ecosystem. *Lampito mauritii*, a common earthworm in the West Godavari District has been chosen to study the influence of an organophosphate monocrotophos compound which is most commonly used in this area. On calculation the LC_{50} values for 24, 48, 72, 96 hrs were found to be 6.75, 6.25, 5.25 and 4.50 ppm respectively. During the present studies the changes in their morphological features and the patterns of behaviour were observed when exposed to monocrotophos pesticide. The changes were noticed to depend on the experimental periods and concentration levels of the pesticide compounds of the experimental media. The colour of the worms changed from brown to pale brown and mucous material was secreted by the worms. When exposed to monocrotophos the amount of mucous produced was comparatively high at higher concentration and low at low concentration. At higher concentration, *Lampito mauritii* developed swellings, sores and even rupture develop in the entire body when exposed to monocrotophos. Further, protrusion of internal visceral parts through ruptured regions on the body wall became visible in the monocrotophos exposed worms. The data obtained from LC_{50} calculation and the observed behavioural changes were discussed in the light of available literature.
Keywords: *Lampito mauritii,* monocrotophos, LC_{50}, behavioural changes.

INTRODUCTION

One of the major sources of pollution in the terrestrial environment is through indiscriminate and extensive use of biocidal chemicals which are collectively known as pesticides in agricultural operations. It has been now been increasingly realized that the pesticides play an important role in the soil ecosystem. The soil, therefore, acts as a reservoir of pesticides used in the eradication of pests. These pesticides, although primarily aimed at combating pests on crops reach soil system and slowly degrade, gradually spread and possibly get translocated to other environments as well as in the neighborhood through water or air. The rate of degradation of these toxic substances depends upon the constitution of the chemical used. In the above process of dissipation, persistence may result. The pesticides normally become transformed, modified or perhaps magnified through bio-concentrations at different tropic levels in soil ecosystems. Pesticides have been widely used all over the world to control insects, pests and disease vectors and they are one of the most potentially harmful chemicals introduced into the environment. Though they have contributed considerably to human welfare, their adverse effects on non-target organisms are significant.

The determination of LC_{50} values is useful in the evaluation of safe level of tolerance of pollutant and moreover it provides fundamental data to design more complex disposal modes of toxicity to the exposed animals. It is suggested that the chronic test, aiming at sublethal effects, is more sensitive and is a more realistic approach for the prediction of environmental effects because in the field, the exposure concentration of pesticides are usually quite low (Rombke *et al*., 2007). Toxicity test are basic tools for ecological risk assessment of toxic compounds. Earthworms are important biocomponents of ecosystem, although not numerically dominant in soil but their large size makes them major contributors to total biomass. They are extremely important in soil formation, principally through their activities in consuming organic matter, fragmenting and mixing it intimately with mineral particles to form aggregates. Pesticides are either directly applied to the soil to control soil borne pests or deposited on soil as runoff from foliar application. The pesticides residues will impair the physiological functions of earthworms leading to their mortality (Ahmed, 1991). Riepert, (2009) reported that the acute earthworm test is part of the basic test set, but the earthworm reproduction test is considered ecologically more relevant.

Several workers have also investigated the effects of organophosphate insecticides on earthworms' populations. Azinphosmethyl did not affect earthworm populations (Hopkins and Krik, 1957) but carbofuran did (Kring, 1969; Thompson, 1971) Chlorfenvinphos had slight effects (Edwards 1967). Parathion has been reported as moderately toxic to earthworms, particularly in large doses (Heungens, 1966). Senapati (1987) observed the impact of malathion on the population of the earthworms and reported its stressful effect on earthworm in agroecosystem. The earthworms when exposed to organophosphate pesticides showed increased ureotelic and ammonotellic activity (Patnaik and Madhab, 1991). In the same animal Kulkarni (1989) observed that fenvalerate produced pronounced changes in the behavior, and physiology bycausing hormonal and enzymatic imbalance. Goven (1993) have studied the cellular biomarkers for measuring toxicity of xenobiotics. Effect of polychlorinated biphenyls on the coelomocytes of earthworm *Lumbricus terrestris*. From the foregoing account it is clear that the work done on the effects of pesticides on earthworms is scanty. This is especially true in regard to earthworms in India. As mentioned earlier India in general and Andhra Pradesh, in particular, agriculture is the main occupation of a majority of the people.

Lampito mauritii, a common earthworm in the West Godavari District has been chosen to study the influence of one of the pesticide which is most commonly used in the area. The pesticide selected for the present study is organophosphate compound namely, monocrotophos (MCP), commonly known as Azodrin, is an extensively used, potent and highly toxic organophosphate insecticide with and acaricides belonging to the vinyl phosphate group and having a wide range of applications in agriculture (Kavitha and Rao, 2007). This is now in active use in agricultural practices in Lankalakoderu village, Palakol mandal, West Godavari District, Andhra Pradesh in India. *Lampito mauritii*is one of the most common terrestrial oligochaete inhabiting the upper horizon of soils in the southern parts of the Indian sub-continent. Since the use of insecticides has gone up by leaps and bounds now-a-days, it is thought that a study of toxicity of the commonly used organophosphate compounds to *Lampito mauritii* is worthwhile. It is reasonable to expect that the organophosphate compounds even at moderate doses exercise an immediate kill of the earthworm populations. Hence, the present investigations are designed to evaluate the dose-mortality levels of selected organophosphate insecticide namely monocrotophos.

MATERIALS AND METHODS

The earthworm specimens of *Lampito mauritii* were dug-out from the kitchen gardens of residential localities in Lankalakoderu village, Palakol mandal, West Godavari District. They were conveyed to laboratory in wide mouthed plastic jars along with some amount of damp soil collected from their habitat usually within an hour after their collections. After reaching the laboratory they were carefully isolated with a pair of brushes from the soil and gently washed in aerated freshwater. Only healthy, uninjured nearly equal sized worms weighing about 1.0 to 1.5g were selected and acclimated to the laboratory conditions (30 $\pm$ 1°C).Since overcrowding causes mortality of the worms, they were maintained in small numbers in batches in number of glass troughs containing fresh water. The media were periodically aerated. The fresh water used for maintenance analyzed to insure that the worms were in a medium of normal composition and therefore under normal physicochemical conditions. They were acclimated for 3 to 4 days to laboratory conditions. They were not fed either during acclimation or experimentation. Lest sudden and or large variations in temperatures should exercise deleterious effects on their survival, both acclimation and the experimentation on the worms were done at constant temperatures. Healthy, active and equal sized worms were chosen for toxicity studies. Standard renewal techniques recommended by (APHA *et al.*, 1998) have been adapted in the present exposure experiments. The renewal techniques followed here were simple and easy to follow in the laboratory.

The media, to which the specific toxicants were added, were freshly prepared. Accumulation excretory products which resulted in deoxygenation and other secondary effects were prevented by frequent renewal of experimental media. Simultaneously control experiments were also made by adding appropriate amounts distilled water to aqueous media. The distilled water added to the control experiments is identical to the quantities used at the maximum toxicant – concentration – exposures. Hence, it was thought fit simultaneously run controls at the same time. pH and dissolved oxygen content of water media are 7.1 to 7. 5 and 5.6 to 6.5 ml/litre respectively. Preliminary pilot experiments were conducted of exploratory nature to arrive at the broad concentration ranges of pesticides with in which the percentage mortality varied between 5 and 95percent. The concentrations resulting below 5 and above 95 percent mortalities were ignored for the final experiments. The pesticidal concentrations thus selected were used for the final experiments with *Lampito mauritii*. The glassware used was thoroughly cleaned and dried. Filtered and sufficiently aerated pond water was taken in uniform quantities into the various experimental troughs containing worms. Then, appropriate quantities of the stock solution were added to yield the desired concentrations at which the experiments were conducted. The concentration ranges used in the final experiments were in an increasing order at equal intervals of time. Exactly 200ml of water was provided per animal. The toxic media were repeatedly renewed in the experimental chambers as suggested by Environment Protect Agency (EPA, 1975). Periodically the oxygen levels in the experimental media were checked to insure that sufficient oxygen level were maintained.

Immediately after the subjection of the worms to experimental media they were continuously observed hour after hour. Indication of immobility when a touch or mild prick by an entomological pin is given to the posterior part of the worm was considered as dead. The mortalities encountered in each experimental chamber were recorded periodically. In the dose mortality study the total kill observed at the end of each 24hrs period was taken into account for a period of 96hrs. Experiments were conducted in aqueous media to find out LC_{50} values for 24, 48, 72 and 96hrs exposure of the worms to the insecticide monocrotophos. All the experiments were repeated several times to register the constancy of the toxicity of each pesticide. After scoring the mortalities of the worms at various test concentrations, the data were processed employing unweighted regression method as suggested by Finney (1971) to evaluate the LC_{50} (Median Lethal concentrations). The data were recorded and calculated mortalities obtained in these investigations were further subject to heterogeneity test employing chi-square analysis (Finney, 1971). The dose-mortalities obtained in the present study were plotted on a probit graph paper against the calculated dose-mortalities at various concentrations.

RESULTS

The data obtained for *Lampito mauritii* on exposure to organophosphate Insecticide monocrotophos are presented in Table -1. The dose mortalities obtained for *Lampito mauritii* on exposure to 24 hrs to the concentration range of 2.5 to 11.0 ppm of monocrotophos showed 50 percent deaths at a concentration range of 5.90 to 7.59 ppm. On calculation the LC_{50} value for 24hrs obtained was found to be 6.75ppm for monocrotophos, similarly the LC_{50} value for 48hrs was also determined by exposing the worm to a concentration range of 2.0 to 10 ppm of at intervals of 1ppm and the LC_{50} pesticidal concentration for 48hrs was 6.25 ppm. The results indicate that the LC_{50}value for 72 hrs with the concentration range of 1 to 10 ppm was found to be 5.25 ppm. In 96hrs exposure study the pesticide concentration in the test media varied from 0.5 to 9.0ppm at a concentration of 4.50ppm, 50 percent of the test animals died in 96hrs. Hence the LC50 value for 96hrs exposure was found to be 4.50 ppm in Table 1& Fig -1.Concentrations below the LC_{50} concentration level are expressed as sub-lethal concentration. But in the present study a concentration which is 1/5th of the LC_{50} concentration has been considered. The sub- lethal concentration values were 1.35, 1.25, 1.05 and 0.90 ppm for 24, 48, 72 and 96 hrs respectively in Table1 and Fig.1. The results obtained on dose mortality studies of an organophosphate insecticide monocrotophos to *Lampito mauritii* reveal clearly that the worm reacted differently to pesticide concentrations in the present study.

Table 1: Toxicity of monocrotophos to the earth worm *Lampito mauritii*

Exp. Period (hrs)	^{LC}50 concentration in ppm	95% Fiducial Limit	1/5th Of ^{LC}50 Values	95% Fiducial Limit
24	6.75	5.90 -7.59	1.35	1.08-1.62
48	6.25	5.46 -7.03	1.25	1.0-1.50
72	5.25	4.59 -5.90	1.05	0.84-1.26
96	4.50	3.93 -5.06	0.90	0.72-1.08

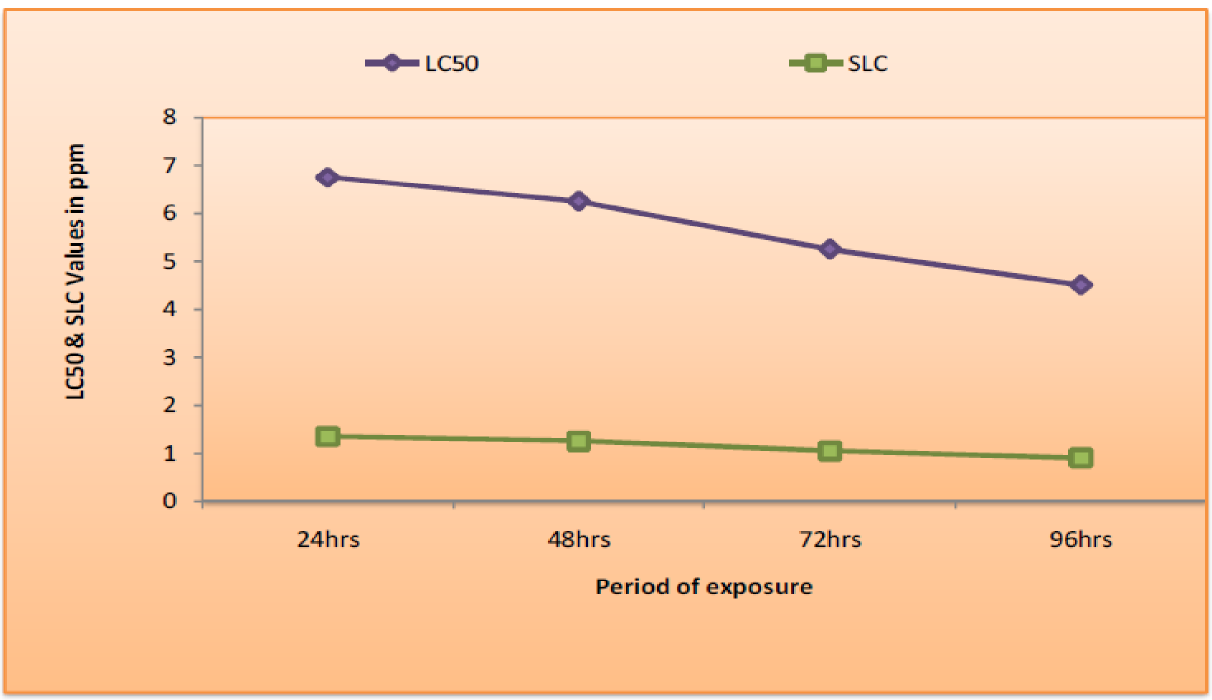

Fig -1: Regression lines showing dose mortality relationship of *L. mauritii* subjected to Monocrotophos for test periods in water medium.

BEHAVIOUR OF WORMS

The present studies revealed the changes in their morphological features and in the patterns of behaviour, when exposed to monocrotophos pesticide. The changes were noticed to depend on the experimental periods and concentration levels of the pesticide compounds of the experimental media. The colour of the worm's changed from brown to pale brown, mucous material was secreted by the worms. When exposed to monocrotophos the amount of mucous produced was comparatively high at higher concentration and low at low concentration. A gradual reduction was noticed in the quantity of mucous secreted with the increase in time of exposure to the pesticide. The intense coiling of the worm noticed after their exposure to toxic media monocrotophos is more effective, as noticed in the present studies, as evidence by the quick and haphazard moments of the worms. After two to three days of exposure the worms became lethargic and moribund and also become immobile and remained curled in a semicircular fashion.

At higher concentration, *Lampito mauritii* developed swellings; sores and even rupture develop in the entire body when exposed to monocrotophos. Further, protrusion of internal visceral parts through ruptured regions on the body wall became visible in the monocrotophos exposed worms (Plate 1-4). In general it was observed that the movement of the worm released in water, containing pesticide, the movements of the worms was invariably quick and erratic in behaviour obviously, as a reaction to 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 (Plate 1- 4). On certain occasions the worms showed swellings at the clitellar region. The swellings in the posterior segments were less frequently noticed in pesticide exposed worms. It may be mentioned, however, that although observation on the behaviour of the worms indicate the relative levels of toxic influence of the pesticide in the experimental media from the moment they are released into the above media, their movements and/ other changes in the worms noticed at short intervals of time could be made out as it was not possible to quantify the observed differences.

Plate-1: Coiling of the worm

Plate-2: Mucous secretion of the body

Plate-3: Reddening of the entire body

Plate-4: Swelling of the entire body

DISCUSSION

The present studies were principally aimed at investigating the 24, 48, 72 and 96 hrs LC_{50} values on exposure to monocrotophos. The differences between observed and calculated values were tested for significance using chi-square test which showed that the difference was not significant at P = 0.05 level at concentrations 6.75, 6.25, 5.25 and 4.50 ppm respectively. The experimental data clearly indicate the relative toxicity of the insecticide monocrotophos to *Lampito mauritii* under the laboratory conditions.

The results indicate that the worms showed higher mortality rate, even at lower concentrations of monocrotophos high mortality in water may be due to two reasons. The pesticide in medium of water diffuses into body easily through 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 variety of organophosphate, organochlorine and carbamate insecticides (Vijayalakshmi, 1980; Janardhanarao, 1984). 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 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). Stenersen et.al., (1973) is of the opinion that most of the carbamate insecticides like carbaryl, carbofuran are highly toxic to the earthworms like *Lumbricus terrestris* Cathey, (1973); Kring, (1969); Gilman & Vardanis (1974) on exposure. The pesticides were reported to cause characteristic sores and tumor-like swellings in earthworms. Like organophosphate insecticides and several carbamates were also found to be neurotoxic producing systemic changes in a number of aquatic and terrestrial organisms (Vijayalakshmi, 1980).At higher concentration, *Lampito mauritii* developed swelling in the entire body when exposed to dichlorvos. Protrusion of internal visceral parts through ruptured regions on the body wall became visible in the dichlorvos exposed worms.

At times the worms showed swellings at the clitellar region (Bharathi and Subba Rao, 1987). Immediately after exposure to pentachlorophenol *Lampito mauritii* have become highly agitated and slowly curled-up into horse shoe and circular shapes followed by arresting their movements. In course of time the anterior part of the worms turned pale and characteristic wounds and sores appeared on different regions of the body. In higher concentration the worms became rigid similar to that reported in *Lumbricus terrestris* exposed to carbofuran and carbaryl (Stenersen, 1973). The present investigation on the worm involving organophosphate compound showed (Plate 1 & 2) similar changes with slight variations in the characteristic sores, swellings, cuts etc., in the pesticide exposed worms. Although the toxicity levels of the insecticide are different to *Lampito mauritii* the characteristic abnormalities found were nearly similar. The reddening, swellings, sores, cuts, blister, etc., in worms noticed in exposed in test media.

In the light of the present findings of comprehensive assessment of the toxicity levels (LC_{50} values) of insecticide and the effects on the earthworms is of a great value. A perusal of literature dealing with assessment of LC_{50} values of insecticide in respect of earthworms indicate that a quite a large number of publications appeared dealing with the toxicity levels of several agro chemicals based on field studies (Doane, 1962; Edwards *et al*., 1968; Thompson, 1973; Vijayalakshmi, 1980; Bharathi,1983; Sattibabu, 2013; Rakesh, 2014). These reports indicate the toxic nature of a number of organochlorine, organophosphate and carbamate compounds to a variety of non-target organisms including earth worms. Very few investigations are available dealing with the toxicity of pesticides to terrestrial oligochaete species under laboratory conditions (Martin and Wiggans, 1959; Stenersen *et al*., 1973, Chio and Sanborn, 1978; Stenersen, 1979a; Stenersen and Oien, 1980, Vijayalakshmi,1980; Bharathi,1983; Sattibabu, 2013; Rakesh, 2014).

In the case of monocrotophos, concentration levels above 1.0 ppm were noticed to be toxic to the aquatic oligochaete *Tubifex tubifex*. In the present instance *Lampito mauritii* being longer in size tolerated a high concentration of monocrotophos shows LC_{50} value as 0.85 ppm for 96 hrs exposure. Kale and Krishnamoorthy (1979) reported 30 day LC_{50} value for carbaryl compound Sevin as 375 ppm in *Pontoscolex corethrurus*. They reported higher concentrations of the carbamate compounds inhibiting release of castings, resulting loss in weight, survivability and retarded growth. They further felt that higher concentration of these pesticides might as well reduce populations in the ecosystem. Cathey (1973) made a valuable contribution in the field of oligochaete toxicology in his study on the toxic effects of carbamate pesticides to *Lumbricus terrestris*, she reported that a 21 day LC_{50} was 20 μg of carbaryl to *Lumbricus terrestris* in addition a good number of behavioral changes such as withdrawal response i.e., extensive coiling, bodily constrictions, swellings, blisters and reddening of anterior parts consequent to carbaryl treatment were also notice in *Lumbricus terrestris.*

A critical examination of the effects of the chemicals chosen reveals that *Lampito mauritii* exhibited initial hyperactivity at low pesticidal concentrations and developed lethargy at higher concentration of the organophosphate compound. The insecticide presently used is systemic in nature and neurotoxic like any other organophosphates or carbamates as reported by Cathey (1973), Stenersen *et al*., (1973), Stenersen (1979b), Kale and Krishnamurthy (1979), Vijayalakshmi (1980), Bharathi (1983), Sattibabu (2013), Rakesh (2014). Based on laboratory studies, Richards and Cutkomp (1946) and Martin and Wiggans (1959) showed that the DDT in water could survive amounts of at least 1: 10,000. The present findings also showed that at lower concentration the pesticide was stimulatory to the worm rather than inhibitory because the pesticide does act on central nervous system. The hyperactivity at low pesticide concentration and the lethargy developed by the worm at high concentration positively affect changes in population of the worms. Capacities for bioaccumulation and transformation by earthworms on repeated applications of the pesticides have been amply demonstrated (Stenersen *et al*., 1973). In the present study *Lampito mauritii* showed that the toxicity increased with exposure period. This suggests that the toxicity is associated with accumulation of monocrotophos in excess amounts that may be metabolized and prove injurious to the earthworms.

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