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Effect of endosulfan on the bacterial and fungal populations in the gut of the Indian earthworm *Lampito mauritii* (Kinberg)

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Abstract

Sublethal lower concentration (1/10th of 96 h LC_{50} value 0.005 ppm kg^{-1}) and sublethal higher concentration (1/3rd of 96 h LC_{50} value 0.016 ppm kg^{-1}) (T_2) of endosulfan reduced the gut microbial proliferation of *Lampito mauritii* upto 15 days of exposure. However on the 30th day of exposure the microbial populations recuperated to near control values. Further, out of the eight bacterial and five fungal species that inhabit the guts of *L. mauritii* only four bacterial and three fungal species were able to survive in the endosulfan exposure.

Keywords : endosulfan, *Lampito mauritii*, microbial population, microbial species, soil substrate

INTRODUCTION

Earthworms are commonly present in a wide range of soils and may even represent 60-80% of the total soil biomass. They are well known for their proximate effects on soil respiration, organic matter decomposition and water infiltration and for their contribution to large-scale soil process such as soil fertility and pedogenesis (Wolters, 2000; Dominguez *et al*., 2004). In developing countries like India where the economy depends largely on agricultural products, 15-20% of the total harvest is destroyed by pests which has resulted in uncontrolled use of pesticides by the Indian farmers causing soil contamination by pesticide residues. Endosulfan (6, 7, 8, 9, 10, 10-hexachlora-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 3, 4-benzodioxa thiepin-3-oxide) is an organochlorine pesticide used throughout the world for higher agricultural production. Earthworms may take up pesticides such as endosulfan through contact with contaminated soil or through ingesting of contaminated soil or plant materials, and are thus most likely to be affected by pesticides used on agricultural lands (Brunninger *et al*., 1994). The earthworm *Lampito mauritii* is widespread occurrence in aerable and pasture lands and is consequently vulnerable to surface applied pesticides such as endosulfan making it an ideal candidate for assessment of agroecosystem contamination.

Earthworms are also believed to have a mutualistic relationship with soil microorganisms passing through their digestive tract, but the nature and function of the microbiota inhabiting their gut are largely unknown. It is hypothesized that mucus and water secretion from the earthworm gut wall into the digestive tract causes dormant ingested microbiota to be activated, increasing microbial activity and enzyme production and hence facilitating digestion (Lavelle and Spain, 2001). Thus earthworm gut microbiota have been attributed to have a central role in the digestion of organic substrates (Horn *et al*., 2003).

This paper reports the effect of endosulfan on the gut bacterial and fungal population of Indian earthworm *L. mauritii*.

MATERIALS AND METHODS

The earthworm *L. mauritii,* clay loam soil and fresh cowdung were collected from agricultural farms and dairy yards of Annamalai University, Taminadu, South India. Matured and healthy earthworms were acclimatized under laboratory conditions in cowdung substrate for one week. The sun dried and powdered cowdung (earthworm's nitrogen rich natural food) was mixed with soil (low in nitrogen) in the ratio of 1:3 (vol/vol) and used as soil substrate throughout the study. The organochlorine pesticide, endosulfan (35% E.C.) was procured from Corromandal Fertilizers Ltd., Secunderabad, South India.

Our earlier acute toxicity evaluation of endosulfan on *L. mauritii* indicated that the 96 h LC_{50} value of endosulfan was 0.047 ppm kg^{-1} (Kavitha and Ramalingam, 2008). From 96 h $LC_{50,}$ two sublethal concentrations of endosulfan such as one lower concentration 0.005 ppm kg^{-1} (1/10th of 96 h LC_{50} value) (T_1) and one higher concentration 0.016 ppm kg^{-1} (1/3rd of 96 h LC_{50} value) (T_2) have been selected. Three plastic troughs (diameter 25 cm x height 6 cm) each filled with 1 kg of soil substrate were designated as C (control), T_1 and T_2. The control was mixed only with water. The required quantity of endosulfan for T_1 and T_2 was mixed with soil substrate using 300 ml of water to ensure homogenous mixture and required moisture. Three replicates were maintained for C, T_1 and T_2. Four adult

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L. mauritii were introduced into C, T_1 and T_2. The troughs were covered by nylon net, and maintained at room temperature (28 ± 2º C) with 50 - 60 % moisture.

At 1, 5, 15 and 30 days one earthworm from each replicate of C, T_1 and T_2 were removed and sacrificed for the microbial study. The population of bacteria and fungi in the gut of *L. mauritii* were determined by dilution plate technique (Walksman, 1917). The gut contents (3-4 cm of gut ranging from 20-30 segments) were dissected out using sterile scissors and placed in sterile test tubes containing 1 ml of sterile saline (1 g $NaCl_2$ in 100 ml distilled H_2O). The tubes containing the gut contents were shaken thoroughly and used as inoculum. Using a micropipette, 0.01 ml of the inoculum was inoculated into Macconkey Agar (MA) (Anonymous, 1977) plates and spread over using a standard platinum loop for bacterial growth and Sabouraud's Dextrose Agar (SDA) (Emmon *et al*., 1970) plates for fungal growth. They were incubated for 18-24 h at 37º C for bacteria and for 4-7 days at 28º C for fungi. The different Colony Forming Units (CFU) developing on the media were estimated by using colony counter and expressed as CFU×10^6 cm^{-1} for bacteria and CFU×10^4 cm^{-1} for fungi according to the method of Baron *et al*. (1994).

On the 30th day of endosulfan exposure, the bacterial colonies were identified using Gram's strain and biochemical reactions according to the method described by Mahon and Manuselis (1995). The fungal colonies were identified by light microscopic examinations using Lactophenol cotton blue strain and also by cultural characteristics.

RESULTS AND DISCUSSION

The gut bacterial and fungal populations of *L. mauritii* were lower in T_1 and T_2 upto 15 days when compared to control (Table 1), Eventhough, thereafter they got increased, they were lesser than that of control on the 30th day of exposure. Comparatively the bacterial and fungal populations were less in higher concentration (T_2) than in lower concentration (T_1) of endosulfan. Earlier Kalam and Mukherjee (2001) have also reported that the total microbial count to get adversely affect at higher concentrations of hexaconazole and persisted in soil upto 21 days. So it can be concluded that the suppressive effect of endosulfan on microbial proliferation is dose dependent.

Nawab *et al*. (2003) reported that certain pesticides have inhibitory effects on bacterial growth. A reduction of fungi had been documented after organophosphorus pesticide treatment by Ambrogioni *et al.* (1987). Vig *et al*. (2008) reported about 75 % reduction in fungal population due to the application of six insecticides in cotton field. Vig *et al*. (2008) stated that the levels of bacteria in the pesticides treated field returned to levels of control after 20 days. Tu (1978) also reported decreased bacterial numbers after triazophos and lindane treatments with subsequent recovery. Similarly, in the present study also the bacterial and fungal populations recovered (increased) and reached near control values on the 30th day of exposure (Table 1). This could be due to biodegradation of endosulfan in the experimental media T_1 and T_2 during prolonged exposure (30 days) by the bacterial species *Klebsiella pneumonia, Enterobacter aerogens, Enterobacter cloacae* and *Bacillus subtilis* and fungal species such as *Aspergillus fumigatus, A. niger* and *A. flavus.* Awasthi *et al*. (2003) also found two *Bacillus* strains from soil that degrade endosulfan. Bhalerao and Puranik (2007) found initially the growth of *A. niger* to be suppressed in the presence of endosulfan, but after adaptation to endosulfan the culture could grow rapidly exhibiting high growth rate as compared to the growth in the absence of endosulfan medium. This could be due to availability of additional carbon and sulfur upon degradation of endosulfan in the medium. Kwon *et al*. (2002) identified a soil strain *K. pneumoniae* which degraded endosulfan without forming toxic metabolite endosulfan sulfate. Verma *et al*. (2006) using Gas Chromatography (GC) studied the degradation of endosulfan by *Rhodococcus* bacterial strain and reported that the endosulfan was degraded upto 92.58 % in 15 days. So it may be inferred that endosulfan either in lower or in higher concentration severely affected the *L. mauritii's* gut microbial proliferation upto 15 days, whereas on 30th day, perhaps due to biodegradation of endosulfan by specific bacterial and fungal species, the microbial populations could have got increased.

Eight bacterial species and five fungal species were identified from the gut contents of *L. mauritii* kept as control (Table 2). In lower concentration (T_1) of endosulfan exposure, out of eight bacterial species *Escherichia coli* and *Morganella morganii* species were absent. In higher concentration, *Proteus vulgaris, P. mirabilis, E.Coli* and *M. morganii* were absent. Among the five species of fungi both in lower and higher concentrations (T_1 & T_2) *A. fumigatus*, *A. niger* and *A. flavus* were present, whereas *Mucor plumbeus* and *Rhizopus* sp. were absent. These results indicated that the growth of some of the microbial species *viz., P. vulgaris, P. mirabilis, E.coli, M. morganii, M. plumbeus* and *Rhizopus* sp. were suppressed due to endosulfan treatment. This could be probably due to the fact that these species were not able to degrade endosulfan and so suppressed by endosulfan. On the otherhand some of the bacterial such as *K. pneumoniae, E. aerogens, E.cloacae* and *B. subtilis* and the fungi such as *A. fumicatus*, *A. niger* and *A. flavus* were able to survive and degrade endosulfan during prolonged exposure (30th days). These results suggest the differences in the susceptibility and ability to degrade pesticids by different microbes.

Table 1. Total bacterial and fungal populations in the gut of *Lampito mauritii* exposed to control (C) (soil substrate alone), lower (T_1) and higher (T_2) sublethal concentrations of endosulfan mixed soil substrate for a period of 30 days

Exposure Period (days)	Bacteria CFU×10^6 / *Lampito mauritii* gut 3-4 cm^{-1}					Fungi CFU×10^4 / *Lampito mauritii* gut 3-4 cm^{-1}				
	C	T_1	%	T_2	%	C	T_1	%	T_2	%
1	467±12.13	445±10.11	-5^{NS}	382±8.95	-18*	185±6.64	124±3.18	-33*	116±2.91	-37*
5	451±11.27	320±8.37	-29*	248±5.78	-45*	180±5.49	115±2.60	-36*	109±2.60	-39*
15	473±10.69	266±6.07	-44*	185±3.46	-61*	182±5.20	107±2.60	-41*	100±2.60	-45*
30	465±10.39	308±7.80	-34*	241±5.78	-48*	180±4.91	132±3.48	-27*	125±2.91	-31*

Values are mean of 3 observations ± *S.E.*
% - indicates per cent decrease (-) over control (C)
* - significant at 1% level (P<0.01) for comparisons with control
NS - Not Significant (P>0.01) (based on t - test)
C - control (soil substrate alone)
T_1 - 1/10^{th} of 96 h LC_{50} value (0.005 ppmkg^{-1})
T_2 - 1/3^{rd} of 96 h LC_{50} value (0.016 ppm kg^{-1})

Table 2. Isolation of bacterial and fungal species (at the end of 30^{th} day) from the gut of *Lampito mauritii* exposed to control (C) (soil substrate alone), lower (T_1) and higher (T_2) sublethal concentrations of endosulfan mixed soil substrate

Sl. No.	Microbial species	Control	T_1	T_2
	Bacteria			
	Gram negative			
1.	*Enterobacter aerogenes*	+	+	+
2.	*Enterobacter cloacae*	+	+	+
3.	*Escherichia coli*	+	-	-
4.	*Klebsiella pneumoniae*	+	+	+
5.	*Morganella morganii*	+	-	-
6.	*Proteus mirabilis*	+	+	-
7.	*Proteus vulgaris*	+	+	-
	Gram positive			
8.	*Bacillus subtilis*	+	+	+
	Fungi			
1.	*Aspergillus flavus*	+	+	+
2.	*Aspergillus fumigatus*	+	+	+
3.	*Aspergillus niger*	+	+	+
4.	*Mucor plumbeus*	+	-	-
5.	*Rhizopus* sp.	+	-	-

C - Control (soil substrate alone)
T_1 - 1/10^{th} of 96 h LC_{50} value (0.005 ppm kg^{-1})
T_2 - 1/3^{rd} of 96 h LC_{50} value (0.016 ppm kg^{-1})
+denotes presence
- denotes absence

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