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Effect of Monocrotophos on Urea in Earthworm, *Eudrillus Eugeniae* (Kinberg).

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Abstract

Earthworm is one of the most important biotic component in the soil. Earthworm are ecologically important in field; hence there is an economic interest in testing side effect of environmental factors like temperature, salinity, pollutants etc. on biological aspects.

The study was carried out in plastic culture pots under laboratory conditions following the protocol of Panda and Sahu (2002). The test were conducted for 6hr, 12hr, 18hr and 24 hr to lethal concentration of monocrotophos (0.1ppm) applied to the soil. The urea content in the whole body tissue and nephridia was measured by using Urease Nesslerisation method as described by Varley (1976). Urea in the whole body of control earthworm *Eudrilus eugeniae* was found to be 0.06µg/mg. Urea in whole body of earthworm exposed to monocrotophos (0.1ppm) for 2h, 4h, 6h and12h it was found be 0.06 µg/mg, 0.07 µg/mg (16.7% increase), 0.07 µg/mg (16.7% increase), and 0.08 µg/mg (33.3% increase) respectively. Urea in the nephridia of control earthworm *Eudrilus eugeniae* it was found to be 0.05µg/mg. Urea in nephridia of earthworm exposed to monocrotophos (0.1ppm) for 2h, 4h, 6h and 12h was found be 0.05 µg/mg, 0.06 µg/mg (20% increase), 0.06 µg/mg (20% increase) and 0.07 µg/mg (40% increase) respectively.

Effect of monocrotophos on *Eudrilus eugeniae* for different time period produce a significant urea in whole body and in nephridia as compared to control. It is observed that concentration of urea is more in nephridia as compared to whole body.

Keywords: Earthworm, monocrotophos, nephridia, urea.

INTRODUCTION:

Earthworms are common soil organisms in most environments and play an important role in improving structure and fertility of soil ecosystems (Bartlett *et al.,* 2010).Soil fertility depends on physical, chemical and biological attributes (Huerta *et. al*., 2007). Worm casting play a significant role in improving the soil structure and fertility (Nijhawan and Kanwar, 1952; Edwards and Lofty, 1977; Edwards and Bohlen, 1996: Edwards, 1998). They act as miniature factories (Kitturmath *et. al*., 2007). Earthworm are not only gardener's best friend; they are also the recycler's as well. The Chinese characterized earthworm as the 'Angle of earth' Aristotle aptly referred to them as the intelligences of soil 'and Cleopatra decreed them sacred (Blakemore 2003).

It has been indicated that earthworms may represent upto 60–80% of the total animal biomass in soil (Ouellet *et al.,* 2008). The bioaccumulation of pesticides in earthworms but may produce serious damages (van Gestel *et. al.,* 2011). Therefore, earthworms are suitable bio-indicators of soil contamination, and can be used to provide safety thresholds for pesticides applications (Suthar *et al.,* 2008; Lourenc-o *et al*., 2011).To increase the yield of crop pesticides are used. Pesticides when used in agriculture, horticulture and forestry some part of them reach to soil by absorption and affect the soil inhabiting organisms (Corbett, 1974). According to Dash and Dash (2008) the earthworms tend to overcome the stress of pesticide and heavy metal by increasing mucous secretion, reducing burrowing activity and increasing reproduction.

The objective of this study was to evaluate the effect of monocrotophos on nephridial and whole body urea in the earth worm, *Eudrilus eugeniae* (Kinberg).

MATERIAL AND METHOD:

A large number of adult and healthy earthworm Eudrilus eugeniae were collected from fields around the Aurangabad city. They were maintained under 13:11day:night illumination at 26 ^{0} C ± 0.5 ^{0}C in plastic troughs containing the wet soil for three days before subjecting for experimentation. Earthworm looking health and having approximately equal size (10 ± 0.05) and weight (0.2 ± 0.05) were selected.

The study was carried out in plastic culture pots under laboratory conditions following the protocol of Panda and Sahu (2002) using gut evacuated earthworms. In brief, pesticide namely monocrotophos was obtained from Excel Crop Care Limited Mumbai and Devidayal (Sales)

Limited. The pesticide were chosen on the basis of their extensive use in this area.

The experiment was designed to study the histological effect and estimation of excretory product. The test were conducted for 6^{hr}, 12^{hr}, 18^{hr} and 24^{hr} to lethal concentration of monocrotophos (0.1ppm) applied to the soil. The concentrations of monocrotophos and sprayed on the soil surface, the treated soil was thoroughly mixed to distribute the monocrotophos evenly. Twenty healthy gut cleared earthworm were added to each pot. The experiment was maintained at 20% soil moisture at 25ºC ± 2ºC soil temperatures. The corresponding control worms were maintained in non-contaminated fresh moist soil for the experimental period. After completion of the experiment the nephridia of *Eudrilus eugeniae* were dissected out separately in watch glasses with the help of sharp scalpels, forceps and scissors. The estimation of excretory products were done using the methods as described below.

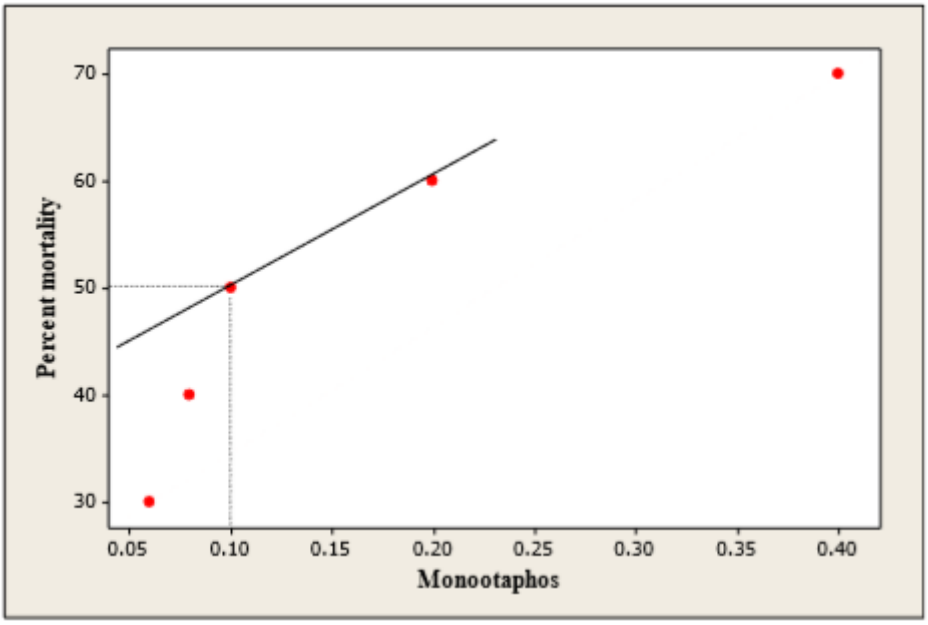
The urea content in the whole body tissue and nephridia was measured by using Urease Nesslerisation method as described by Varley (1976), $5^{\%}$ homogenate of nephridia was prepared in cold distilled water and centrifuged at 1500 rpm for 10 minutes. To 0.2 ml of supernatant 3.2 ml of distilled water and 20mg of soyabean meal was added. The mixture was incubated for 15 minutes at 40-500C. $10^{\%}$ sodium tunstate and 0.3ml 2/3N sulphuric acid were added to incubated mixture and mixed well to remove protein. After 10 minutes the mixture was centrifuged at 1500 rpm for 10 minutes again. The clear supernatant was used for the urea estimation. To 2 ml of supernatant 5ml of ammonia free water of Nesslers reagent was added. The optical density of the colour was read immediately at 480 nm in spectrophotometer against a reagent blank. The urea content was expressed as mg of urea per100mg of wet tissue (mg %).

RESULT:

LC_{50} values for monocrotophos was found to be 0.1 ppm. Urea in the whole body of control earthworm Eudrilus eugeniae was found to be 0.06µg/mg. Urea in whole body of earthworm exposed to monocrotophps (0.1ppm) for 2h ,4h, 6h and12h it was found be 0.06 µg/mg, 0.07 µg/mg (16.7% increase), 0.07 µg/mg (16.7% increase), and 0.08 µg/mg (33.3% increase) respectively. Urea in the nephridia of control earthworm Eudrilus eugeniae it was found to be 0.05µg/mg. Urea in nephridia of earthworm exposed to monocrotophos (0.1ppm) for 2^h, 4^h, 6^h and 12^h was found be 0.05 µg/mg, 0.06 µg/mg (20% increase), 0.06 µg/mg (20% increase) and 0.07 µg/mg (40% increase) respectively.

I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
Conc. of Monocrotophos	No. of animal used	mortality	Corrected % of mortality	Log of conc.		Emperical probit	Expected probit	Working probit	Weighting coefficient	Weight						
			P	X				Y		W	WX	WY	WX2	WY2	WXY	Y'
0.06	10	3	30	0.025305865	1.06	4.4756	4.4	4.477	0.55788	5.5788	0.14118	24.97629	0.003573	111.819	0.63205	4.7084
0.08	10	4	40	0.033423755	1.08	4.7467	4.7	4.747	0.61609	6.1609	0.20592	29.24579	0.006883	138.83	0.9775	4.76939
0.1	10	5	50	0.041392685	1.1	5	5	5	0.63662	6.3662	0.26351	31.831	0.010908	159.155	1.31757	4.82926
0.2	10	6	60	0.079181246	1.2	5.2533	5.3	5.253	0.61609	6.1609	0.48783	32.36321	0.038627	170.004	2.56256	5.11317
0.4	10	7	70	0.146128036	1.4	5.5244	5.6	5.523	0.55788	5.5788	0.81522	30.81171	0.119126	170.173	4.50246	5.61614
				0.065086318						29.8456	1.91366	149.228	0.179116	749.981	9.99214	

Calculation 0f probit regression line for some experiment in which worms Eudrilus eugeniae were exposed to different concentration of monocrotophos (0.1 ppm) for a period of 24 hr.



Graph Showing LC50 value of monocrotophos for Eudrilus eugeniae using percent mortality

	Time period					
	Tissue	Control	2h	4h	6h	12h
Urea (µg/mg)	Whole body	0.06 ± 0.19	0.06 ± 0.19	0.07 ±0.23 (+16.7%)	0.07 ± 0.23 (+16.7%)	0.08 ± 0.27 (+33.3%)
	Nephridia	0.05 ± 0.17	0.05 ± 0.17	0.06 ± 0.18 (+20%)	0.06 ± 0.18 (+20%)	0.07 ± 0.24 (+40%)

Table: Effect of monocrotophos 24h LC_{50} (0.1ppm) on urea of earthworm Eudrilus eugeniae exposed for different time period. (Percent change is shown in parantheses).

DISCUSSION:

The earthworm *E.eugeneiae* (Kinberg) commonly know as the African Night crawler, is of West African origin end in plentiful in coastal shaded grasslands (Blakemore 2000). Treatment of worm with monocrotophos (0.1ppm) for different time period produced a significant increase in the urea in whole body and in nephridia. It is observed that concentration of urea is more in nephridia as compare to whole body. Such a observation we are also recorded earlier Kuikarni (1989) noted toxic effect of pyrethroids pesticides like cypermethrin and fen-fen on the nitrogenous excretory product of nephridia of worms *Lampito mauritii*. Patil (2002) reported a significant increase in urea and uric acid in earthworm *Perionyx excavatus* after exposing to fertilizers. Zeba (2006) reported decrease in urea and uric acid in *Pheretima* elongata exposed to organochlorines and organophosphate pesticides. Pesticides when used in agriculture, horticulture and forestry some part of them reach to soil by absorption and affect the soil inhabiting organisms (Corbett, 1974).

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