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Effect of Temperature on Acute Toxicity of Dimethoate 30%EC on Earthworm, *Eisenia fetida*

S. Geetha*[1], J. Kannadasan[1], and G. Chandrasehar[2]

[1]Department of Ecotoxicology, International Institute of Biotechnology and Toxicology (IIBAT), Padappai - 601 301, Tamil Nadu India; [2]Department of Plant Pathology, International Institute of Biotechnology and Toxicology (IIBAT), Padappai - 601 301, Tamil Nadu, India

Abstract: Earthworms are hermaphrodite, bilaterally symmetrical, segmented worms [2]. They are systematically classified under the phylum Annelida belonging to the class Oligochaeta [4]. The climate changes will have direct effects to all ecosystems, including the soil ecosystems. In ecotoxicological investigations, temperature is an important factor, since it can act as a stressor and induce the physiological status of organisms, besides affect the fate and transport of pollutants present in the environment. However, most of the ecological studies investigations neglected the possible effects of temperature and focused solely on the effects of toxicants on organisms. Considering the temperature can contribute to the toxicity of pollutants, it is of massive importance to scrutinize whether the change in the exposure temperature will impact the durability of the toxic effects present in soil ecosystems. Therefore, in the present study the toxicity effect and biomass change of Dimethoate 30% EC to earthworms was assessed under different exposure temperatures (20°C and 25°C). The results showed that changes in temperature led to variation in biomass and LC_{50}. Namely, exposure to the concentration at different temperatures lead to different toxicity responses. Increase in

temperature is caused increasing the toxicity, whereas decreasing the biomass change.

To assess the influence of temperature on the acute toxicity of Dimethoate 30% EC on Earthworm (*Eisenia fetida*), a laboratory study was conducted under two different temperature conditions *viz*., 20 ± 1 °C and 25 ± 1 °C. Earthworms were exposed to five different concentrations of 25, 50, 100, 150 and 200 mg Dimethoate 30% EC / kg dry artificial soil. Test concentrations were prepared using deionised water and applied to the artificial soil in which earthworms were exposed. This experiment set up was maintained for 14 days separately under two different temperature conditions. Observation on mortality and biomass change were made on Day 14 after treatment. Earthworms were assessed for mortality and body weights were assessed on day 0 and day 14. The results of the experiment showed that there was a temperature response toxic effect and biomass change noticed on the earthworm. The LC_{50} of Dimethoate 30% EC on day 14 was determined to be 83.165 mg/kg dry artificial soil incubated in 20 ± 1 °C and 64.80 mg/kg dry artificial soil incubated in 25 ± 1 °C. The 14-day No-Observed-Effect Concentration (NOEC) with respect to biomass was determined to be 50mg /kg dry artificial soil for both 20 ± 1 °C and 25 ± 1 °C. The LC_{50} values of Dimethoate 30% EC were significantly high at higher temperature range tested (25±1°C). It is concluded that the temperature has a pronounced effect on toxicity of Dimethoate 30% EC on earthworm.

Key words: Earhworms, *Eisenia fetida,* Dimethoate 30% EC, Temperature, Toxicity.

Introduction

International Institute of Biotechnology and Toxicology (IIBAT) is a Scientific and Industrial Research Organization (SIRO) which serves society through science. It provides scientific data by doing research work and publishing findings arrived through scientific research. At IIBAT, Effect of Temperature on Acute Toxicity of Dimethoate 30% EC on Earthworm, *Eisenia fetida* study has

been conducted in the month of April 2024 in the Department of Ecotoxicology (Earthworm Lab),) under laboratory conditions.

Dimethoate is one of the insecticides referred to as organophosphates. Among different classes of pesticides, organophosphates are more frequently used because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in environment. It is a systemic insecticide used for control of a wide variety of insect pests of fruit, vegetables and crop plants. Therefore, the present study was done to evaluate its toxic effects on weight and mortality rate of *E.foetida*. Berrya and Jordanb (2000) studied temperature and soil moisture content effects on the growth of *Lumbrics terrestris*. Desilva [10] studied influence of temperature and soil type on toxicity of pesticide to *E.andrei*. Edwards [2] stated that the ideal growth of E. fetida in different animal and vegetable wastes occurred at 25-30°C. When species are exposed to above-ideal temperatures, it can cause alterations in their homeostasis, which can result in metabolic disorders [6]. Temperature increases may also change the environmental fate of pesticides in the terrestrial parts [7]. For instance, pesticide solubility may increase under high temperatures and their degradation and volatilization may also increase on these conditions. These events are also conditioned by soil properties as these factors hinder in the availability of chemicals to soil-dwelling organisms [8]. Furthermore, the metabolic activity of soil invertebrates is expected to rise with temperature increase, thereby the intake of the chemicals, and probably its toxicity, to these organisms may also be increased [9]. Nagavallemma [5] reported that earthworms can endure temperatures ranging from 0 to 40°C but regeneration efficiency is more at 25 to 30°C. The shortest growth period was 52 days at 25 degrees C, and the fastest growth rate was 0.0138 g per day as per J. Hou, Y.Qian [1].

MATERIALS AND METHODS

Test System Details

Earthworms *Eisenia fetida* which were used for the study were bred under standardized conditions (OECD 207) by IIBAT. Identification was done by **The New College, Chennai, Tamil Nadu, India**. Earthworms from the same source were used for the test item treatments and the control. Earthworms with 5 – 6 months of age with well-developed clitellum @ 300 - 600 mg body weight /worm were used for the study.

Artificial Soil Preparation

Artificial soil was prepared prior to study according to the OECD and ISO (OECD 207 and ISO 11268-1) test guideline. The artificial soil was prepared by mixing the ingredients 10% Sphagnum-peat air-dried and finely ground (≤ 2 mm) (*M/s. Pioneer Agro Industry, Coimbatore, Tamil Nadu, India*), 20% Kaolin clay, (Kaolinite content >30%) (*M/s. Uthaya chemicals, Tirunelveli, Tamil Nadu, India*), 70 % fine sand (grain size with more than 50 % by mass of particle size 0.05 -0.2 mm) (*M/s. Bhuvaneswari Hardwares, Chennai*) and thoroughly mixed using homogenizer for about 20 minutes. The pH of the soil was checked and was found to be 5.89 (OECD No 222: 6.0 ± 0.5).

Test Conditions

The minimum and maximum temperature (19.5 ºC – 20.3ºC & 24.6ºC – 25.3 ºC) was maintained throughout the test. The containers used for the test were uniquely identified with, treatment and replicate number. The experimental containers were kept in the photoperiod of continuous light as per OECD 207 [11].

Test Conduct

40 earthworms were used at each concentration level *viz.*, 25, 50, 100, 150 and 200 mg Dimethoate 30% EC/kg dry artificial soil along with control group for both the temperature and were observed 14 days after exposure.

Test Units

Glass beakers (1.5 L capacity with a cross sectional area of 130 cm^2) covered with perforated transparent lids for the gaseous exchange between the medium and the atmosphere and to enable the required access of light, to enable exchange of air and to minimize evaporation of the artificial soil. The containers were filled with approximately 570 g dry artificial soil plus 200 ml of deionised water (water content calculated based on 35 ml/100g dry artificial soil as per OECD 207 and ISO [11 & 12]). The wet weight of artificial soil in each replicate was 770.19 to 770.38 at beginning of the test.

Pre-moistening of Artificial Soil

One day before the test item application, dry artificial soil was moistened by adding 100 mL deionized water and mixed to avoid the dust emission during test item application day and covered with perforated plastic lid to avoid moisture loss. The pre moistening was done for two replicates together and it was distributed equally during test item application.

Preparation and application of Test Item

On the day of the experiment, the stock solution was prepared by weighing 4508.02 mg Dimethoate 30% EC and made up to 10 mL using deionised water and stirred continuously for 30 minutes to get homogenized solution. The required quantity of test solution per batch was applied to the artificial soil.

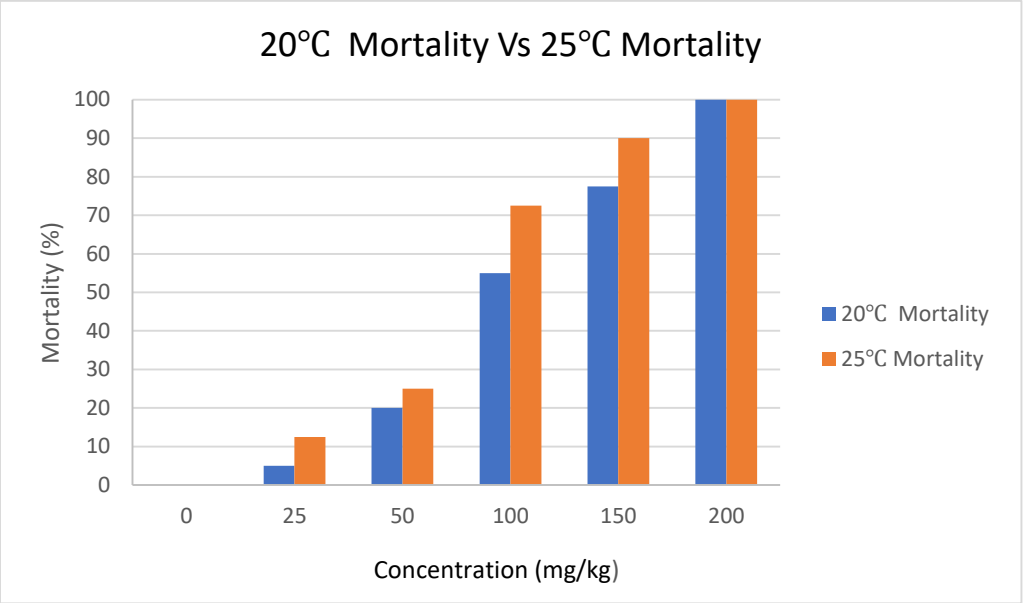
Test concentrations *viz.*, 25, 50, 100, 150 and 200 mg Dimethoate 30% EC/ kg dry artificial soil were attained from the stock solution. Application of test item was carried out in two batches, consisting of 2 replicates per batch (1.14 kg dry artificial soil/batch) for two different temperature study. The water content of the artificial soil in each container was checked by weight on day 7 and the difference in weight on day 0 and day 7 was matched by adding water. Therefore, artificial soil moisture loss was kept within 10% of the initial artificial soil moisture content.

Parameters Observed

Mortality

The artificial soil was emptied from the glass beakers and searched for earthworms on day 14 after application. The number of live and dead earthworms in each replicate was assessed..

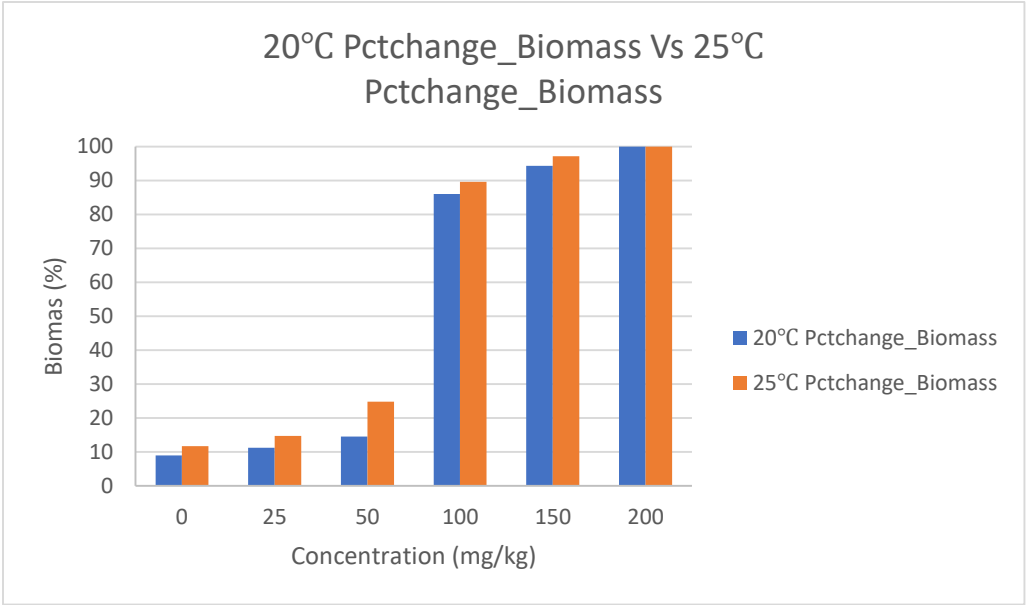
Figure 1 : Graphical representation on mortality data



Mean Biomass Change

The total and the mean body weights of all live earthworms in each test container were determined at the test start (day 0) and end (day 14).

Figure 2 : Graphical representation of Biomass Change



The biomass changes of the worms for dimethoate 30% EC exposed to 20°C ranged from -11.26 % to -100% and for 25°C the biomass changes ranged from -14.77 % to -100%. There was significant difference observed in biomass change from 50 mg dimethoate 30% EC for both the studies conducted in different temperatures *viz*., 20°C and 25°C.

Statistical Analysis

Data on body weight changes of the worms were tested for normality and homogeneity of variance using Shapiro-Wilk test and Levene's test. The biomass data was found to be normally distributed with equal variance and Student t-test ($p > 0.05$) between control group and treatment concentrations from 100 mg Dimethoate 30% EC/kg dry artificial soil for both 20°C & 25°C. Analysis was performed, using the statistical Software IBM SPSS Statistics Version 28 [14].

Results and Discussions

Earthworms which were exposed to two different temperature conditions at 20°C and 25°C, they exhibited increased toxicity level when exposing to higher temperature, where as there was not significance difference observed at biomass level when exposed to 20°C and 25°C. The test concentrations 25, 50, 100, 150 and 200 mg/ kg dry artificial soil recorded 5%, 20%, 55%,77.5%

and 100% and 12.5%, 25.0%, 72.5%, 90% and 100% when exposed to 20℃ and 25℃ temperature condition respectively.

The biomass changes of the worms for dimethoate 30% EC exposed to 20℃ dimethoate 30% EC ranged from -11.26 % to -100% and for 25℃ the biomass changes ranged from -14.77 % to -100%. There was significant difference observed in biomass change from 50 mg dimethoate 30% EC for both the studies conducted in different temperatures *viz*., 20℃ and 25℃.

According to the results of this study, 14-day LC_{50} of Dimethoate 30% EC was determined to be 83.165 mg/kg dry artificial soil tested at 20℃ and 64.800 mg/kg dry artificial soil tested at 25℃. The 14-day No-Observed-Effect Concentration (NOEC) with respect to biomass was 50 mg Dimethoate 30% EC / kg dry artificial soil for both the test conducted.

Table 2 Mortality Data of the Earthworms after Exposure to Dimethoate 30% EC

Test item concentration [mg/kg dry artificial soil][1]	**Body Weight Changes**	
	20℃	**25℃**
	Mortality (%) [1]	Mortality (%) [1]
Control (Deionised water)	0	0
25	5	12.5
50	20	25
100	55	72.5
150	77.5	90
200	100	100

[1] % mean of 4 replicates

Table 3 Body Weight Changes of the Earthworms after Exposure to Dimethoate 30% EC

Test item concentration [mg/kg dry artificial soil]	Body Weight Changes		Significance
	20°C	**25°C**	
	Weight change (%) [1]	Weight change (%) [1]	
Control (Deionised water)	-9.01	-11.75	-
25	-11.26	-14.77	n.s.
50	-14.59	-24.86	n.s.
100	-86.04	-89.61	s
150	-94.25	-97.16	s
200	-100	-100	s

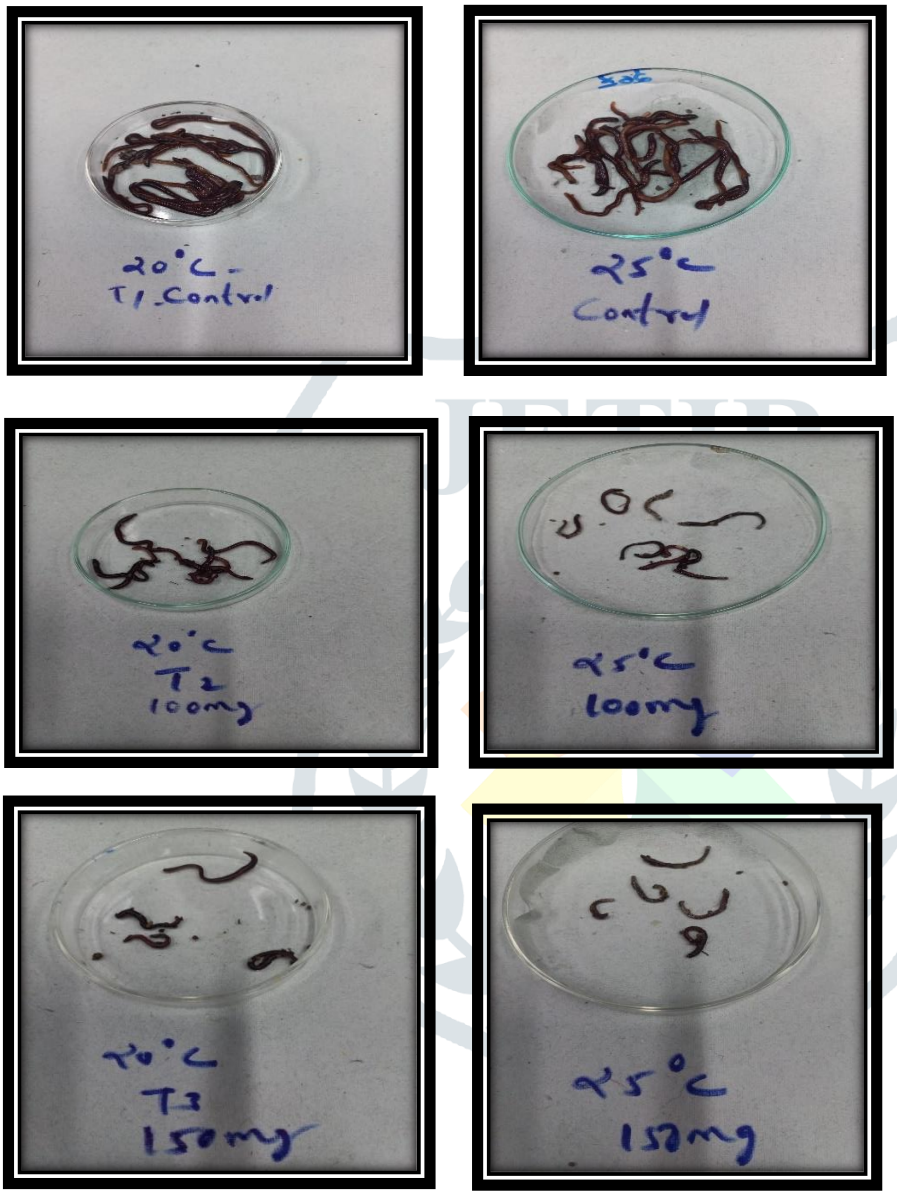
[1] % mean of 4 replicates
- = not relevant n.s. - not significantly different compared to the control, Student t-test ($p > 0.05$)
s - significantly different compared to the control, Student t-test ($p > 0.05$)

Conclusions

This study confirms that the temperature strongly influence the toxicity level of Dimethoate 30% EC to earthworms as Organophosphorus and carbamate pesticides mainly inhibit Acetylcholinesterase activity. Temperature is one of the most important factors for most of the biological activities. Most organisms have their specific temperature range which is ideal for their growth; this is because even small changes in the temperature can lead to significant changes in metabolism of organisms. The temperature mainly acts on neurosecretory cells and neurotransmitter inhibitor. The neurosecretory materials of earthworms have indirect and direct effect on regeneration. Earthworm showed sluggish movements and reduction in weight and increased toxicity effects due to alteration in temperature that affects the earthworm. Therefore, earthworms require an optimum and favorable conditions for its normal function.

Figure 3: Photographic representation of earthworms on 20℃ and 25℃ Condition

Earthworms at 20℃ condition **Earthworms at 25℃ condition**



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