International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Acute Toxicity of Cyantraniliprole 7.3% W/W + Diafenthiuron 36.4% W/W SC on the Non – target species, Earthworm Eisenia Fetida

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Abstract

Earthworms play a crucial role in sustaining the soil ecosystems, particularly agroecosystems, where they are both abundant and indispensable. Their activities enhance soil health by boosting nutrient availability, improving drainage, and fostering a more resilient soil structure all of which contribute to greater agricultural productivity. Often referred to as 'ecosystem engineers,' earthworms profoundly influence the soil's physical, chemical, and biological properties, shaping its overall profile. These changes can impact the habitat and behaviours of other organisms within the soil ecosystem. Earthworms also serve as bioindicators, playing a vital role in monitoring and assessing the health of natural ecosystems in their environment (Wang et al., 2015a, Ye et al., 2016). Soil pollution has become a growing environmental issue in agricultural regions worldwide, largely due to the extensive use of pesticides. Cyantraniliprole 7.3% w/w combined with Diafenthiuron 36.4% w/w SC is a recently introduced systemic insecticide, extensively employed to manage chewing and sucking insect pests. When sprayed on plants, these compounds are absorbed and transported within the plant, rendering parts or the entire plant toxic to insects that consume its tissue. To fully understand the ecological effects, it is crucial to conduct comprehensive studies on the application of this combined product. To assess the toxic impact of Cyantraniliprole 7.3% w/w + Diafenthiuron 36.4% w/w SC on earthworms, acute study was conducted with different concentrations viz.,10, 50,100, 250, 500, 750 and 1000 mg/ kg dry artificial soil. Mortality and sublethal observation were done on Day 7 and Day14. No mortality was found up to 1000 mg/ kg dry artificial soil on Day 7 and Day14 but there was a reduction in body weight observed. The data was analysed using IBM SPSS Statistics Version 29. The data was normally distributed and homogeneous. Hence, for Biomass analysis parametric One-way ANOVA Dunnett t (2-sided) test was performed. Further research will be carried out to assess the toxic impact of test item on earthworms, the levels of reactive oxygen species (ROS) (Mittler, 2002); and malondialdehyde (MDA), activities of superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST), Acetylcholinesterase (AChE) enzyme activity as well as DNA damage will be measured.

Keywords: Earhworms, Eisenia fetida, Cyantraniliprole 7.3% w/w + Diafenthiuron 36.4% w/w SC, Mortality, Sublethal, IBM SPSS Statistics

INTRODUCTION

Cyantraniliprole and Diafenthiuron are widely used systemic and contact insecticides to control a range of chewing and sucking pests in agriculture (Ishaaya et al. 1993; Lingappa et al. 2004) and used in various crops to control a variety of pests (Schwarz et al., 2011, Zhang et al., 2012). While effective on target pests, it is crucial to assess their potential impact on non-target soil organisms, particularly earthworms, which are vital to soil health and fertility.

There is limited publicly available data on the combined effects of Cyantraniliprole 7.3% w/w and Diafenthiuron 36.4% w/w SC on earthworms. Previous studies have shown that sub-chronic exposure to Cyantraniliprole can cause several adverse effects in earthworms (Eisenia fetida) (Awaknar and Karabhantanal, 2004). Cyantraniliprole can alter the intestinal enzyme activity, can reduce cocoon production and the number of juveniles, changes in the relative expression of functional genes and can affect carbohydrate, protein, lipid digestion and absorption of earthworms (Zhihua Qiao et al., 2020). Although individual studies on Cyantraniliprole highlight its significant toxicity, information on the impact of Diafenthiuron remains sparse. Given this, it is reasonable to assume that the combination of these two pesticides may pose a greater risk to earthworms, potentially intensifying the effects observed with Cyantraniliprole alone.

Therefore, to better understand the ecological implications of such pesticide combinations on non-target soil organisms, further research is essential. As part of this effort, an acute toxicity study of Cyantraniliprole 7.3% w/w + Diafenthiuron 36.4% w/w SC was conducted on the non-target species, earthworms, specifically Eisenia fetida.

MATERIALS AND METHODS

Test System Details

Earthworms Eisenia fetida which were used for the study were bred under standardized conditions (OECD 207) by IIBAT. Earthworms from the same source were used for test item treatments and 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 the study according to the OECD and ISO (OECD Test Guideline No.: 207 and ISO 11268-1) test guidelines. The artificial soil was prepared by mixing the ingredients 10% Sphagnum-peat air-dried and finely ground (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 Hardware, Padappai, 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 Test Guideline No.: 222: 6.0 ± 0.5).

Test Conditions

The minimum and maximum temperature (18.3 ºC – 20.2ºC) was maintained throughout the test. The containers used for the test were uniquely identified with, treatment and replicate number. The experimental containers were kept under photoperiod of 544 to 754 LUX continuous light intensity as per OECD guideline (OECD Test Guideline No.: 207 - 1984).

Test Conduct

Forty earthworms were used at each concentration level viz., 10, 50, 100, 250, 500, 750 and 1000 mg / kg dry artificial soil along with control group and were observed for 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 (ISO 11268-2, (1998) and Soil Quality). The wet weight of artificial soil in each replicate ranged from 770.25 to 771.19g at beginning of the test.

Pre-moistening of Artificial Soil

One day prior to test item application, dry artificial soil was pre-moistened by adding 100 mL of deionised water. The soil was thoroughly mixed to prevent the dust generation during test item application and was then covered with a perforated plastic lid to minimise moisture loss. Pre-moistening was performed for two replicates together, and the moistened soil was evenly distributed between the replicates at the time of test item application.

Preparation and application of Test Item

On the day of the experiment, a stock solution was prepared by weighing 7.15 g of Cyantraniliprole 7.3% w/w + Diafenthiuron 36.4% w/w SC and diluting it to 25.0 mL with deionised water. The solution was homogenised for 30 minutes to ensure uniformity. Test concentrations of 10, 50, 100, 250, 500, 750, and 1000 mg/kg dry artificial soil were prepared from the stock solution. Application of the test item was conducted in two batches, each consisting of two replicates (1.14 kg dry artificial soil per batch).

Following application, the remaining portion of deionised water (post pre-moistening) was added to the soil and thoroughly mixed for approximately 5 minutes using a soil blender to ensure even distribution of the test substance. The prepared test medium was then aerated before introducing the test organisms.

Healthy adult earthworms, selected based on the presence of a well-developed clitellum, were collected from the breeding medium, gently washed with tap water, blotted dry using filter paper, and individually weighed (ranging from 302 mg to 490 mg) before being placed on the surface of each test container.

Moisture content of the artificial soil in each container was monitored by weight on Day 7. Any reduction in weight compared to Day 0 was corrected by adding deionised water, ensuring that soil moisture loss remained within 10% of the initial moisture level throughout the test period.

Parameters Observed

Mortality

On Day 7 and Day 14 following application, the artificial soil from each glass beaker was carefully emptied and examined for earthworms. The number of live and dead individuals in each replicate was recorded. No mortality and behavioural abnormalities were observed in adult earthworms across all tested concentrations by the end of the 14-day exposure period.

Table 1 Mortality and sub-lethal effect of the Earthworms after Exposure to Cyantraniliprole 7.3% w/w + Diafenthiuron 36.4% w/w SC

Concentration (mg/kg dry artificial Soil)	Mortality on Day 7 (%)	Mortality on Day 14 (%)	Sub-lethal effect on Day 7 & Day 14 (%)
Control (Deionised water)	0	0	0
10	0	0	0
50	0	0	0
100	0	0	0
250	0	0	0
500	0	0	0
750	0	0	0
1000	0	0	0

Mean Biomass Change

The total and mean body weights of all live earthworms in each test container were recorded at the beginning (Day 0) and at the end (Day 14) of the study. Biomass changes in earthworms exposed to Cyantraniliprole 7.3% w/w + Diafenthiuron 36.4% w/w SC ranged from -19.70% to -41.99%. Exposure to the test item negatively impacted the growth of earthworms, with weight loss frequently observed as a typical stress response (Su et al., 2013; Ye et al., 2016) and statistically significant reduction in biomass was observed starting from the 250 mg/kg treatment concentration, indicating adverse sublethal effects at and above this exposure level.

Table 2 Biomass Changes of the Earthworms after Exposure to Cyantraniliprole 7.3% w/w + Diafenthiuron 36.4% w/w SC

Concentration (mg/kg dry artificial Soil)	Average Pre-weight (mg)#	Average post weight (mg) #	Biomass loss (%)*	Significance
Control (Deionised water)	362.38	297.48	-17.91	-
10	361.50	290.28	-19.70	ns
50	360.60	286.75	-20.48	ns
100	354.63	274.75	-22.52	ns

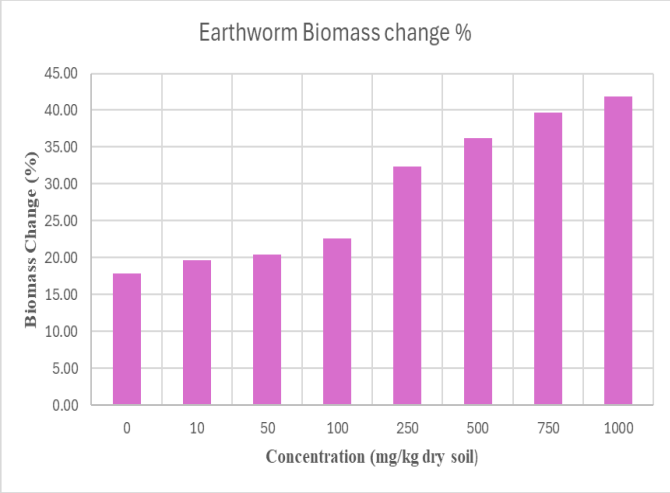
250	396.63	268.08	-32.41	s
500	374.60	238.73	-36.27	s
750	391.15	235.58	-39.77	s
1000	371.03	215.20	-41.99	s

[#] % mean of 4 replicates

- not relevant ns. - not significantly different compared to the control, s - significantly different compared to the control, Student t-test ($p > 0.05$)

* Average post weight - Average pre weight /Average pre weight × 100

Figure 1 : Graphical representation of Biomass Change



Statistical Analysis

Body weight change data of the earthworms were evaluated for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests, respectively. The results confirmed that the data were normally distributed and met the assumption of homogeneity. Consequently, a parametric one-way ANOVA followed by Dunnett's t-test (two-sided) was conducted to compare the biomass of worms in the control group with those exposed to treatment concentrations of 10, 50, 100, 250, 500, 750, and 1000 mg/kg dry artificial soil. Statistical analysis was performed using Statistics software (IBM SPSS Statistics, Version 29).

Results and Discussions

Although the combination of Cyantraniliprole 7.3% w/w and Diafenthiuron 36.4% w/w SC does not induce direct mortality in earthworms, it elicits notable sublethal effects, such as reduced biomass, indicating potential physiological and behavioral disturbances.

The control exhibited a biomass loss of -17.91%. Statistically, there was no significant change in biomass at concentrations of 10 (-19.70%), 50 (-20.48%), and 100 (-22.52%) mg/kg in dry artificial soil compared to the control. However, at higher concentrations of 250, 500, 750, and 1000 mg/kg, statistically significant

reduction in biomass was observed, with corresponding weight loss percentages of -32.41%, -36.27%, -39.77%, and -41.99%, respectively.

These effects might be attributed to factors such as feeding inhibition, muscle impairment, or disruption of energy metabolism. Moreover, the formulation might alter the activity of key enzymes like catalase (CAT) and acetylcholinesterase (AChE), which play essential roles in oxidative stress response and neural function, respectively (Zhihua Qiao et al., 2020). Such sublethal impacts underscore the importance of conducting chronic toxicity studies and ecological risk assessments, particularly in the context of integrated pest management (IPM), where the preservation of non-target organisms like earthworms is vital for soil health and ecosystem stability.

Conclusions

This study observed no acute lethal or sublethal effects on Eisenia fetida when exposed to Cyantraniliprole (7.3% w/w) and Diafenthiuron (36.4% w/w SC). However, significant reduction in biomass was recorded at the concentration viz., 250, 500, 750 and 1000 mg/kg in dry artificial soil. A decrease in biomass might lead to decline in cocoon production, reduced reproductive capacity, and a decrease in the number of juveniles (Zhihua Qiao et al., 2020). Future research has to be done to investigate potential chronic and sub-chronic effects of this test item by analysing DNA damage as it was the genetic material of all organisms and very sensitive to oxidative stress caused by ROS.

Acknowledgements

Authors wish to thank to the Management of IIBAT, for their support, encouragement and providing the facility to conduct the experiment. International Institute of Biotechnology and Toxicology (IIBAT), a Scientific and Industrial Research Organization (SIRO), contributes to society by conducting scientific research and publishing its findings.

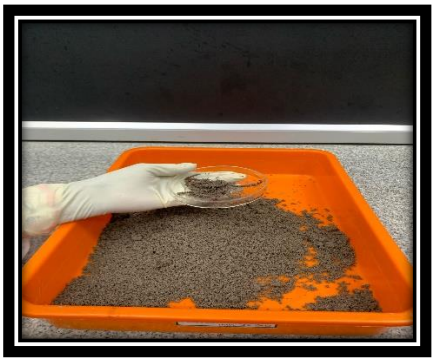
Representative Experimental Figure

Eisenia fetida

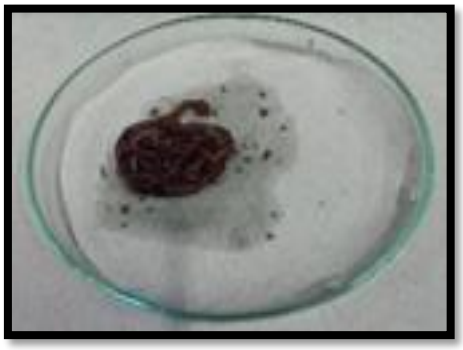
While weighing

While sorting

After sorting



Control worms



Treated worms

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