ISSN: 0975-833X

Availableonlineathttp://www.journalcra.com

International Journal of Current Research
Vol. 11, Issue, 05, pp.3601-3603, May, 2019

DOI: https://doi.org/10.24941/ijcr.35407.05.2019

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

BUTTERFLIES AS AN INDICATOR SPECIES FOR THE TOXICITY ASSESSMENT OF A COMMERCIAL PESTICIDE FORMULATION CONTAINING D-TETRAMETHRIN AND CYPHENOTHRIN

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ARTICLEINFO

Article History:
Received 09th February, 2019
Received in revised form
14th March, 2019
Accepted 19th April, 2019
Published online 30th May, 2019

Key Words:

Pesticides, Larvae, Fogging, D-tetramethrin, Cyphenothrin.

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ABSTRACT

The use of pesticides has increased rapidly over the past few decades. Especially, in tropical regions pesticides are used extensively to control mosquitos which spread harmful diseases such as dengue and malaria. In Sri Lanka, mosquito borne diseases is a major concern. Infact, during the time period of January to July 2017, the Epidemiology Unit of the Ministry of Health has recorded 80,732 dengue victims and 215 deaths. A technique known as fogging/space spraying is used to control mosquito populations in urban areas. A chemical containing the active ingredients D-tetramethr in and cyphenothrin is mixed with diesel and fogged extensively to control mosquitos in Sri Lanka. This chemical can exert toxic effects on non-target species including butterflies which are a vital component in a balanced ecosystem. Therefore, this research was conducted to determine the toxic effects of the chemical using butterflies as indicator species. Butterflies were chosen because they are very sensitive to environment changes. The 5th instar larvae of *Tirumala limniace exoticus* (Blue Tiger) were used as the test species. The larvae used for the experiment were obtained by breeding the adult species in a butterfly cage. The larvae were exposed to 6 different formulations which included 'Aged Aerated Water' as Control, Diesel as Solvent Control and 4 increasing concentrations of the commercial pesticide (10, 100, 500 and 1000 ppm). A volume of 1µl was applied to the thorax region of the larvae (4 per each concentration). The chemical was allowed to air dry for a few seconds and observations were recorded. The larvae that were exposed to all the concentrations of the commercial pesticide including the solvent control did not manage to survive. This study concludes that the chemical has an effect on butterflies, thus on ecosystem. However, further studies including Acute and Chronic toxicity assessments must be conducted. The outcomes will be important in establishing conservation plans to protect butterflies as well as other insects that play a vital role in a balanced ecosystem.

***Citation: Karunananda, H.T.A.R. and Gunawardena, M.P.,* 2019.** "Butterflies as an Indicator species for the toxicity assessment of a commercial pesticide formulation containing d-tetramethrin and cyphenothrin.", *International Journal of Current Research*, 11, (05), 3601-3603.

INTRODUCTION

Environmental toxicology is a branch of toxicology that studies the impact of chemical, biological and physical agents on biological organisms. Ecotoxicology is a specialized form found in toxicology that focuses more specifically on the toxic substances and its effect on ecosystems (Klaassen, 2013). Over the past decade the human population has risen drastically. As a result, gradually harmful waste and chemicals are being discharged into the environment. Pesticides are chemicals used to control pests, including weeds. They are used mainly to protect agricultural farmlands from pests and to control diseases transmitted from vectors such as mosquitoes. However, the intensive and extensive use of pesticides is a major concern worldwide as well as in Sri Lanka. Several research studies have proved that these chemicals effect to humans in addition to non-target insect species including butterflies (Hoang, Pryor, Rand, and Frakes, 2011).Sri Lanka is home for 248 species of butterflies. The tropical climate and the lush vegetation provide a favorable habitat for animals, including insects such as mosquitoes. However, the increase of mosquito population, especially the disease spreading species has become a severe public health problem in Sri Lanka. There has been an increase in mosquito borne diseases such as dengue fever. Dengue fever and dengue hemorrhagic fever is a huge health problem to Sri Lanka. In fact, during the time period of January to July 2017, the Epidemiology Unit of the Ministry of Health has recorded 80,732 dengue victims and 215 deaths (Dengue fever - Sri Lanka, 2017). In order to protect the people from these diseases the Government of Sri Lanka has taken several measures which includes a technique known as space spraying. The chemical used in this technique contains the active ingredients d-tetramethrin and cyphenothrin.

The chemicals are mixed in diesel and sprayed extensively in urban areas in order to control the mosquito populations. This study was conducted to determine the effect of this chemical on non-target species using butterflies as indicators. This is the first research conducted in Sri Lanka using butterfly larvae as indicator species and only a handful of researches have been are conducted worldwide. The findings of this research will be important to implement conservation plans to protect non-target species including butterflies. Justification

the adult species that were bred in the cage. The test specimens were transferred to laboratory for chemical exposure.

Chemical Exposure: The specimens were then weighed in order to make sure they are of similar size and weight. Further, the specimens were closely examined for any parasitic attacks before exposing them to the chemical (4 larvae per each concentration) which were 'Aged Aerated Water'- Control, Diesel – Solvent Control and 4 increasing concentrations of chemical (10 ppm, 100 ppm, 500 ppm and 1000 ppm).

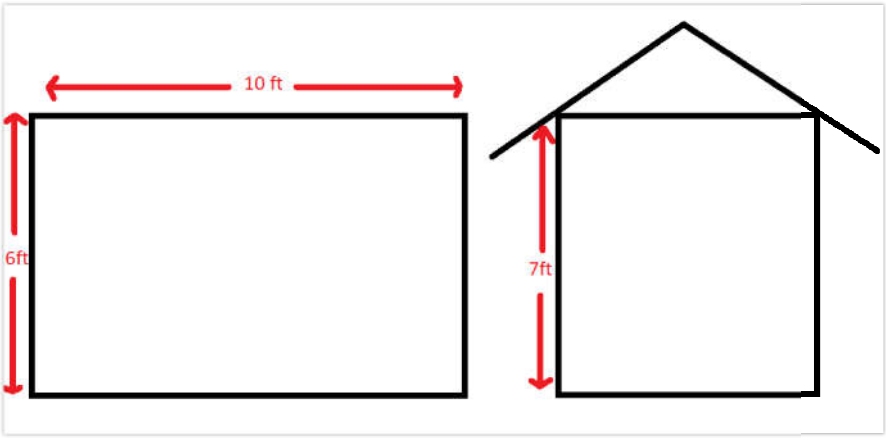

Figure. 2.1 - Structure of the Breeding Cage

Figure 2.2 **The Completed Butterfly Cage**

Figure 2.3. Thorax Application – 5th instar larvae of Blue Tiger (Indicated in red the region where the chemical is exposed)

Table 4.1. Results of toxicity exposure

Formulation	No. of Larvae	Concentration	Outcome
Aged Aerated Water'	4	--	Survived
Diesel	4	--	Dead
Exposure 1	4	10ppm	Dead/Took a long time 90 minutes and 45 seconds
Exposure 2	4	100ppm	Dead/65 minutes and 34 seconds.
Exposure 3	4	500ppm	Dead/50minutes and 20 seconds.
Exposure 4	4	1000ppm	Dead/ Took a period of 29 minutes and 50 seconds.

METHODOLOGY

The Blue Tiger is a butterfly species found commonly in urban areas. The high abundance of its larval food plant in urban areas makes it easier to follow its life cycle. Its IUCN status is Least Concern (LC). Considering the above facts, the larvae of this species (Figure 2.1) was selected as a good indicator species for the toxicity assessment. The butterfly species were bred in the home garden in a breeding cage (Figure 2.2). The required larvae for the toxicity assessment were obtained from

A volume of 1 µl was applied to the thorax region (Figure. 2.4) and left to air dry for 15 seconds, before moving on to the next specimen (Paramasivam and Selvi, 2017). The specimens were then closely observed for any changes and the time taken for the larvae to die was measured using a stopwatch.

RESULTS AND DISCUSSION

The larvae that were exposed to the all the concentrations of the commercial pesticide including the solvent control did not manage to survive.

Figure 2.4. Conducting the topical application technique to the test species

As the concentration of the chemical increased the time taken for the larvae to die reduced. The larvae at the highest concentration died after about 4305 minutes from the initial exposure. In the lowest concentration the larvae took a time of about 90 minutes. The larvae showed similarities in the way they died. After a couple of minutes upon exposure the larvae struggled, in time it caused paralysis and couldn't move before being killed. These results suggest that as the concentration increases the effect is high. However, further testing must be done. In Sri Lanka, this chemical is fogged, but in this study the topical application technique was carried out due to practical issues of simulating the open fogging conditions in a laboratory condition. The main reason to use this technique was due to the limited facilities and inability to suggest that the exact concentration to which the larvae died. The concentration in Sri Lanka that is used in fogging is 1000ppm. In this study, the larvae were exposed to this concentration as well as lower concentrations. The lowest concentration exposed was 10ppm which was $1/100^{th}$ of the concentration which is usually exposed. This suggests that the chemical is harmful to the larvae at even such low concentrations. Another study has been conducted in Kurunagala District of Sri Lanka to assess the toxic effects of this chemical on non-targeted species. In this research, the chemical has been fogged in two selected sites in the Kurunagala District.

The results from this study suggests that the chemical has affected species belonging to 12 orders. Diptera was the most affected insect order (36%) followed by Collembola (30%) and Thysanoptera (17%). More importantly, less than 1% of mosquitos had been killed, out of which only two belonged to the genus *Aedes* which causes dengue fever (Abeyasuriya, Nugapola, Perera, Karunaratne, and Karunaratne, 2017) Therefore, from the results of the present day study it proves that the chemical is harmful even with $1/100^{th}$ of the concentration which is usually exposed. The results from the previous studies shows that the chemical effects to a wide range of non-target species. Therefore, further assessments must be carried out in order to take measures to protect these non-target species.

Conclusion

In conclusion, from the results of the above-mentioned research and the present-day study it proves that the chemical is harmful to non-target species including butterflies. However, further studies of acute and chronic toxicity must be conducted for further analysis. The results from further analysis will help to reduce the killing of non-target species which plays a vital role in a balanced ecosystem.

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