

Ecotoxicity analysis of a commercial tobacco based pesticide on zebra fish (*Danio rerio*)

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ABSTRACT:

Studies on the commercial products for gardening have been lesser studied for their conditions, in and outside South America are extremely rare. Brazil is not an exception that the commercial products based on natural ingredients of free commerce for pest control in organic agriculture has few scientific papers analyzing impacts from the point of view of environmental contamination and effects on non-target organisms. The commercial product based tobacco (unreported amount of nicotine in the package) was tested for its effect on the lethality of known Zebrafish (*Danio rerio*), since this organism is recommended by the International Organization for Standardization - ISO, as they are a bioindicator of environmental pollution. *D. rerio* were purchased and collected at random; it was then acclimated to the laboratory conditions and used for in two bioassays. These bioassays were conducted with the middle aged zebra fish viz., eleven months old and five months old with the spraying volume ranged from 0.25 mL.L⁻¹ to 10 mL.L⁻¹ and number of sprays number ranged from 2 to 25. Mortality was observed over four sprays, because it is a concern that four sprayings or more is the recommended dosage to control plague while gardening. The organism *D. rerio* was taken as the test animal because it has many characteristics similar to that of mammals.

Keywords:

Tobacco, Zebrafish, *Danio rerio*, vegetables gardens, pesticide.

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INTRODUCTION

Toxicology of plant products to non target organisms is a science still not established in developing countries. Brazil is not an exception. Commercial products based on plant ingredients, which can be freely sold to control plagues in organic agriculture in Brazil, are not very frequently studied on a scientific basis, to verify the impacts on the environment and on non target organisms. For this reason, one of the most widely used products to control caterpillars and plant louses in gardening are taken into consideration for our study. In this study a tobacco based commercial product "Dimy Premium - Pronto Uso (amount of nicotine not mentioned in the package) was tested to verify its toxicity and lethal effect on zebra fish (*Danio rerio*). This species is standard for toxicity tests and recommended by ISO and ABNT (2010), because it is a good bioindicator to be used in bioassays with chemical substances.

MATERIALS AND METHODS

Two tests were performed at the Toxicology Laboratory of the Center for Research and Environmental Protection Development of the Biological Institute in São Paulo, Brazil. For the bioassays, five month old specimens of *Danio rerio*, were acclimated and their mortality rate was checked according to ABNT (2010). In the first test, the fish were grown in the laboratory until 11 months. In May 2014, after 11 months, some of them were randomly collected using a net and weighed. A 48 h static assay was performed to analyse their external toxicity. Only the initial concentration of the test substance was taken into consideration as the final lethal concentration could not be determined, due to the lack of adequate equipment and methodology. The physical and chemical characteristics of the water used to prepare the solutions were: Temperature: 20.9°C, pH: 7.9 and dissolved oxygen: 11,1 mg/L. The test had was done with six

replicates (each fish was considered a replicate) for each treatment and one control, (ABNT 2010). There were seven treatments with a fixed volume of water (2 L) and a variable concentration of the commercial product (CP) from 0 mL (T_0) to 20 mL (T_6). The amount of the commercial product was calibrated and obtained with 0; 2; 5; 10; 15; 20 and 25 sprayings in each aquarium, respectively: viz., T_0 =control; $T_1 \cong 1.00 \text{ mL (CP) L}^{-1}$; $T_2 = 2.00 \text{ mL (CP) L}^{-1}$; $T_3 = 4.00 \text{ mL (CP) L}^{-1}$; $T_4 = 6.00 \text{ mL (CP) L}^{-1}$; $T_5 = 8.00 \text{ mL (CP) L}^{-1}$ and $T_6 = 10.00 \text{ mL (CP) L}^{-1}$ respectively.

In the second bioassay, after the acclimation, the toxicity to five month old fishes were evaluated, using nine treatments with a higher volume, in relation to the prior test with, 4L of water per aquarium. The increase of water volume was due to the high toxicity which was verified in the previous test, but the ratio of 1L of water per gram of *Danio rerio* and eight replicates per aquarium was maintained throughout the test period. The physical and chemical characteristics of the water used to prepare the solutions were: Temperature: 19.6°C; pH: 7.6 and dissolved oxygen: 6.3 mg/L. the concentrations of the commercial product (CP) were: T_0 Control $T_1 = 0.25 \text{ mL (CP) L}^{-1}$; $T_2 = 0.50 \text{ mL (CP) L}^{-1}$; $T_3 \cong 1.18 \text{ mL (CP) L}^{-1}$; $T_4 = 1.25 \text{ mL (CP) L}^{-1}$; $T_5 = 1.88 \text{ mL (CP) L}^{-1}$; $T_6 = 2.50 \text{ mL (CP) L}^{-1}$; $T_7 = 3.25 \text{ mL (CP) L}^{-1}$; $T_8 = 4.13 \text{ mL (CP) L}^{-1}$. These volumes were obtained respectively by 0; 2; 4; 6; 7; 10; 14; 18 and 22 sprayings in each aquarium, measured in a 50 mL graduated cylinder and a 5 mL graduated pipette.

RESULTS AND DISCUSSION

The behavior of the fish was observed during 30 min after the spraying of the test product and the readings were performed after 24 and 48 h for both assays.

In the first test, after 30 min of exposure the animals of all replicates were dead in the two treatments with the higher concentrations of the commercial



Figure 1: Fish showing internal bleeding above four sprays in the aquarium (second bioassay)

product, in the two assays. The LC_{100} was obtained with five sprayings, except for T_0 (control) and T_1 (1 mL CP/L), where the fish remained alive; in all other concentrations the fish showed stress in the first 15 min and after 24 h, all the fish were dead and showing internal bleeding in the ventral side mainly, near the head (Figure 1).

In the second assay where lower concentrations were used, the LC_{100} was 0.50 mL CP/L, or 4 sprayings, however during the first 30 min a small increase in opercula opening frequency and erratic movement of the animals, were noted in 0.50 mL CP/L, 1.18 mL CP/L, 1.25 mL CP/L, 1.88 mL CP/L and 2.50 mL CP/L concentrations and stress followed by death with 3.25 mL CP/L and 4.13 mL CP/L. All dead fishes showed internal bleeding in the ventral side near the head.

The experiments were conducted in the winter, and considering that *Danio rerio* is a tropical fish that also develops well in mild temperatures, it is possible to conclude that the laboratory room temperature (20 to 21°C) contributed to the lower assimilation of the product, due to the less intense metabolic activity, one can expect that in natural conditions in tropical countries, the effects are more harmful to fishes.

This is serious because five sprayings or more is the recommended dosage to control plague in gardening and *D. rerio* has many characteristics similar to mammals and recently a case of epilepsy in zebra fish.

It must be noted that toxicity of tobacco was not expected, since this substance is widely used in organic agriculture and there are no reports of fish mortality.

CONCLUSION

It is possible to conclude that formulated products can be more toxic than the active principle (BEVILACQUA *et al.*, 2008) and that toxicity tests with such products are essential for us to analyse. An adequate control is also necessary for products of free commercialization and may be an agronomic prescription is also inevitable for better usage of these products.

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