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TOXIC EFFECTS OF TWO AGROCHEMICALS (CHLORPHYRIFOS AND GLYPHOSATE) ON THE NEUROSECRETORY CELLS IN THE BRAIN OF AN EARTHWORM SPECIES, *MEGASCOLEX SPECTABILIS*

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INTRODUCTION

Earthworms are used in recent investigation on neurons and neurone-glia relationships. Certain cells in the brain of earthworms release substances that function as hormones, which regulate physiological functions. There is evidence to show that chemicals can have various drastic effects on the nervous system of animals. Tests have been conducted to study the toxicity of various agrochemicals on organisms. Among the soil organisms, earthworms are particularly studied because of their role in soil fertility. Organophosphates and phosponoglycines are heavily used pesticides around the world. In Sri Lanka the weedicide, Glyphosate (Roundup) belonging to the phosponoglycine category and Chlorpyrifos an insecticide are heavily used.

OBJECTIVES

In this study, Glyphosate and Chlorpyrifos were used to find out the effects on the neurosecretory cells in the brain of *Megascolex spectabilis.*

METHODOLOGY

Megascolex spectabilis was selected based on the availability and the Culture was maintained in the laboratory using 50% sieved loamy soil and 50% crushed leaf litter. The experiment was carried out at room temperature and soil moisture of 65%.

The experiment was done by treating the soil with 3 levels of pesticide concentrations: (1) the recommended concentration (72ml Glyphosate /10 L water and 15ml Chlorpyrifos/10 L water), (2) a lower concentration than the recommended concentration (50ml Glyphosate /10 L water and 10ml Chlorpyrifos/10 L water) and (3) a higher concentration than the recommended concentration (100ml Glyphosate /10 L water and 20ml Chlorpyrifos/ 10L water. Control samples were treated with water. Two adult earthworms were exposed to pesticides for 12 days. There were two replicates from each sample.

The brains were dissected out, processed using required techniques and 5μm thick sections were stained with Heidenhain's haematoxylin and Pholoxin B, and observed for the types of neurosecretory cells. The diameter of five randomly selected neurosecretory cells and their nuclei were measured. Cellular and nuclear volume (N = 5) and the nucleocytoplasmic index was calculated using the formula,

Cellular Volume $(Vc) = \frac{4}{3} a^2 \frac{b}{8}$

b - Diameter of the cell along the longitudinal axis.

a - Perpendicular diameter to b.

Nuclear Volume $(Vn) = \frac{\pi}{6} d^3$

d - Maximum diameter of the nucleus.

Nucleo-cytoplasmic Index $(VP) = \frac{Vn}{Vc - Vn}$ (Siew, 1965)

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RESULTS AND DISCUSSION

Mainly three different types cells A, B, & C arranged in uni-layer at the peripheral region of the brain were identified based on the size and shape and staining of the nucleus. These cells were numerous in both extremes of the brain. Type A cells are somewhat oval in shape with a large central nucleus stained dark purple; Type B cells appeared pear shape and the nuclei appeared lighter with a darkly stained nucleus and type C cells appeared elongated with a central nucleus similar to type B cells.

Marked changes were observed in the histology of neurosecretory cells in the earthworm after 12 days exposure to treated soil. Among the three types of neurosecretory cells, type B cells were found to change in the histology of cytoplasm, size of cell and nuclei compared to other two types. Therefore, an effect on the secretory activity was expected in cell type B.

Different kinds of histological abnormalities were observed in all treatments. The most prominent abnormalities were seen as inter and intra cellular spaces in the brain tissues, cell necrosis, and cellular aggregations. The control did not show any of the abnormalities mentioned above.

Compared to the control, the cellular volume of the neurosecretory cells decreased in all Glyphosate and Chlorpyrifos exposed samples (Figure 1). Cellular volume of Chlorpyrifos exposed samples decreased more than in the Glyphosate treated samples. The nuclear volume also reduced in all the samples (Figure 2).The nucleocytoplasmic index also decreased, in all the Glyphosate and Clorpyrifos treated samples except the sample treated with low concentrations (Figure 3).

This study therefore indicates that volume of the neurosecretory cell and their nucleus decreased in all samples showing that agrochemicals affected badly on the neurosecretory cells by reducing the cell and nuclear sizes, leading to reducing the secretory activity.

In the literature, Chlorpyrifos is categorized as moderately hazardous and Glyphosate as an unlikely acute hazard in normal use.

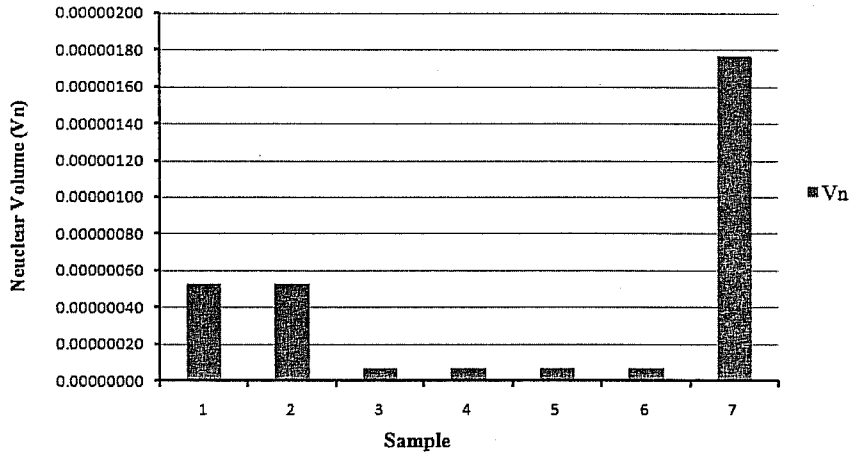

Figure 1. Effects of treatments on the nuclear volume of nucleus of neurosecretory cells.

1-Glyphosate higher concentration 2- Glyphosate recommended concentration 3- Glyphosate lower concentration 4- Chlorpyrifos higher concentration 5- Chlorpyrifos recommended concentration 6 Chlorpyrifos lower concentration 7- Control.

This study also clearly shows that Chloropyrifos is more hazardous than Glyposate by showing smaller nuclear and cell volumes than Glyphosate treated samples, Glyphosate which is categorized as non -toxic in earlier studies, also showed bad effects on the neurosecretory cells.

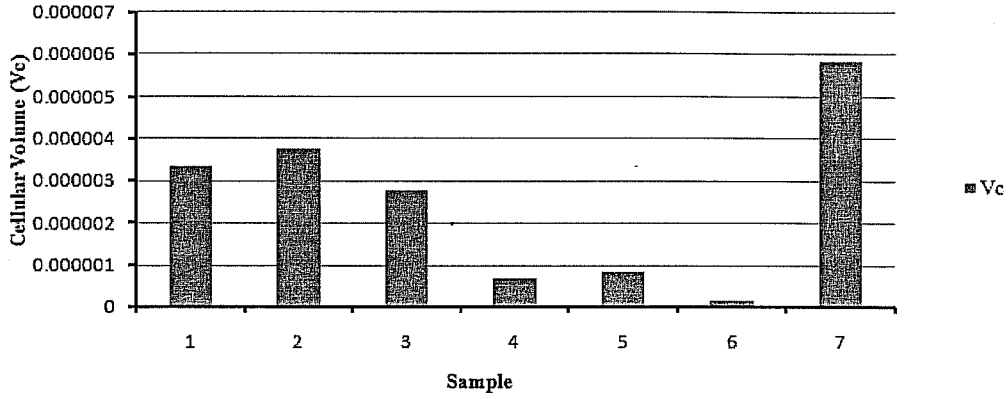

Figure 2. Effects of treatments on the volume of neurosecretory cells.

1-Glyphosate higher concentration 2- Glyphosate recommended concentration 3-Glyphosate lower concentration 4- Chlorpyrifos higher concentration 5- Chlorpyrifos recommended concentration 6- Chlorpyrifos lower concentration 7- Control

The nucleocytoplasmic index of the neurosecretory cells also decreased in all the treatments except the Glyphosate treated sample at the lower concentration. The reduction of the nucleocytoplasmic index indicated that the cell activity status and the functions have been reduced due to the stressed conditions. At the lower concentration of Glyphosate, the neucleocytoplasmic index has increased. This could be due to the enlargement of the nucleus under stress condition prior to the functionless state and the high secretion of neurosecretions

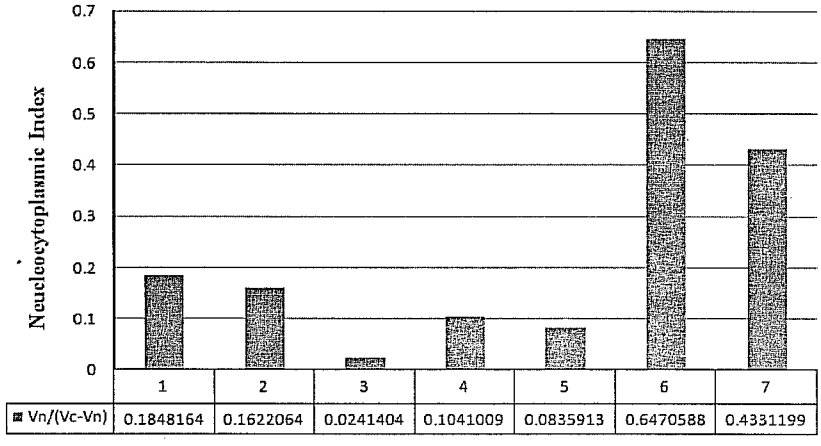

Figure 3. Effects of treatments on Nucleocytoplasmic Index of the neurosecretory cells.

1- Glyphosate higher concentration 2- Glyphosate recommended concentration 3- Glyphosate lower concentration 4- Chlorpyrifos higher concentration 5- Chlorpyrifos recommended concentration 6- Chlorpyrifos lower concentration 7- Control

CONCLUSIONS

The present study shows that Chlorpyrifos and Glyphosate affect the neurosecretory cells of *Megascolex spectabilis.*

The negative effects that can be induced by these pesticides are reduction of nuclear and cell size of the neurosecretory cells in the brain. Also these pesticides cause intercellular and intracellular vacuole formation, cell necrosis, and cellular malfunction.

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