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Histopathological and physiological effects of selected agrochemicals on non-target *Archachtina marginata*

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ABSTRACT: The chronic and acute toxicity of the foliar fertilizer, Boost Xtra®and the pesticide, Cypercot® were investigated against the non-target agro ecosystem fauna *Archachatina marginata*. The results of 96 hours acute toxicity test indicates differential toxicity of both chemicals (LC_{50}:Cypercot=74.286, Boost Xtra =250.935) to *A. marginata*. Based on the calculated 96hrs LC_{50}, Cypercot was found to be approximately three times more toxic than Boost Xtra. Sub-lethal exposure to $1/10^{th}$,$1/100^{th}$ and $1/100^{th}$ of the LC_{50} of Cypercot and Boost Xtra for 28days did not significantly impair growth because weight changes were not significant ($p>0.05$) although unexposed control snails recorded higher average weight gain. Histological assessment of the gut and reproductive tract of the mollusc exposed to different sub-lethal concentrations of the agrochemicals indicates that Boost Xtra is much more persistent than Cypercot, causing severe necrosis and sloughing of the gut epithelium at $1/10^{th}$ of the LC_{50} (55.3ml/L). Cypercot induced no visible lesion in the gonadal epithelium while the sub-lethal exposure to Boost Xtra initiated massive necrosis in the gonadal duct. Sublethal concentrations of both chemicals induced aestivation but this was not always concentration dependent. The importance of these findings are discussed.

Keywords: Pesticides, fertilizer, non target fauna, agro-ecosystem, *Archachatina marginata.*

Introduction

The rapid annual increase in global human population and the consequent increased demand for crops produced mostly on perennially cultivated plots demands that huge investments and emphasis is placed on the application of agrochemicals to increase yield.

Agro-chemical production began as a relatively simple process, based primarily on the combination of a few chemical substances such as copper, mercury salts, elemental sulphur, arsenic and cyanide (Robbins, 1991). The increasing use of these chemicals among other agricultural activities in many parts of the world has posed a potential danger to both non-target wild life and humans.

Pesticides are often broad spectrum, impacting negatively on non-target species such as arthropods, annelids and molluscs in agro-ecosystems (Don-Pedro,2010). They may immobilize terrestrial gastropods (Gordan, 1983) and interfere with neural control of feeding behaviour (Barley et al., 1989). Afomezie et al.,(2011) in their study on the effects of different soil on the giant African land snail, *Archachatina marginata* found that relatively less toxic inputs such as NPK fertilizers also impairs the growth.

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Molluscs are common, highly visible, ecologically and commercially important on a global scale as food and as non food resources (Rittschof and McClellan-Green, 2005) and a number of them are classified as non-native and invasive species (Cowie et al., 2009). The extent of the spread of *A. marginata* and *A. fulica* have been shown to be confounded by humans (Raut and Baeker,2002). Simpson et al.,(1994)have earlier reported that application of nitrogen fertilizer and pesticide management of rice fields in the Philippines were associated with reduced molluscan population densities.

A. marginata Swainson, 1821 is an essential source of protein in many West African countries (Yoloye, 1994) and is a common fauna in most farmlands in Nigeria, hence its selection as a suitable non-target species for this study.

Materials and Methods

Bioassay

All bioassay was carried out at the ectoxicology laboratory of the Zoology Department, University of Lagos, Nigeria at room temperature.

Bioassay Animals

The West African pulmonate gastropod, *Archachatina marginata* was the farm animal used for this study. All snails were obtained from the same stock in a snail farm within the Lagos metropolis. A total of 80 snails weighing between 50g and 99g were used.

Bioassay Chemical

Two agrochemicals, a pesticide (Cypercot®) and a foliage fertilizer (Boost Xtra®) NPK 20:20:20 were selected for use in this study. Boost Xtra a foliar fertilizer complex is a highly concentrated fully soluble emulsion fertilizer designed for application to the foliar parts of crops. Cypercot is an agricultural pesticide with the active ingredient cypermethrin. It is a synthetic pyrethirin formulated for crop protection.

Feeding and Acclimatization

Pawpaw fruit, *Carica papaya* was selected for the experiment because it is a preferred food by the snail (Yoloye,1994). Snails were fed with the fruit for one week in laboratory condition during the acclimatization period. The pawpaw was selected because it is a common plant found growing in farm lands and gardens in Nigeria. It is also relatively easy to quantify for experimental purposes.

The snails were acclimatized in the laboratory at room temperature (37.6 ^{o}) in large plastic holding tanks (length=30.0cm,wdith=53.5,height=34.0cm). The tanks were floored with leaves and the top was covered with wire gauze to allow proper ventilation and prevent escape. Acclimatization was done for one week before the start of the experiment to ensure that only sturdy snails which survived this period were used for the study.

Bioassay container

The containers used were made of plastic (bottom diameter= 14.5cm, top diameter =21cm and height=9cm). Garden soil obtained from the biological garden complex within the campus free of any fertilizer or herbicide application was used to cover the floor of the plastic bowls to simulate a natural environment for the snails. Four snails were kept in duplicates per container for all concentrations.

Preparation of Bioassay fruit

Twelve sets of 200g fairly ripe paw-paw fruits were cut and weighed out using a Binatone® sensitive weighing balance. The first set were dipped in bowls containing 100ml/L, 400ml/L, 500ml and 600ml/L of Boost Xtra while the second set were immersed in separate bowls containing 50ml/L, 100mh/L, 150ml/L, 200ml/L and 300ml/L of Cypercot respectively. Each pawpaw was left in their respective solutions for 30 minutes to allow proper absorption of the test chemicals. The pawpaw fruits were later removed and introduced into bioassay bowls labelled with the corresponding concentrations, each housing four snails (73-95g).

Acute toxicity assay

The bioassay was allowed to stand for four days and dead snails were removed at 24hrs intervals. A snail was considered dead if the entire foot was completely out of the shell, limp and did not respond to gentle prodding with forceps or if it retracted completely into the shell and did not come out after repeated attempts to make it do so.

Chronic toxicity assay

Sub-lethal concentration (i.e $\frac{1}{10}th$, $\frac{1}{100}th$ and $\frac{1}{1000}th$) of the LC_{50} of both chemicals against *A. margninata* were selected for chronic toxicity studies to reflect happenings in real farm settings. These include 0.53ml/l5.53ml/l 55.3m/l and 0.19ml/l,1.9ml/l and 19.0ml/l of the herbicide and pesticide respectively.
The set up was allowed to last for 28 days within which weight changes and histopathological effects were recorded. The gut and gonads were taken from sacrificed molluscs for histological sections so as to determine possible tissue lesions and malformations resulting from such exposure.

Statistical analysis

The dose response of mortality were analyzed by probit analysis using SPSS 15® to derive the LC_{50}, $LC_{5,}$ LC_{95} and subsequently, toxicity factor (T.F). Graphs were plotted using Microsoft excel and GrapPad prism 5®.

Results

Acute toxicity

Acute toxicity test of both agrochemicals showed that *A. marginata* exhibits differential response upon single administration of the respective compounds (Table 1). The calculated toxicity factor indicates that Cypercot is about 3 times (2.92) more toxic than Boost Xtra. And this is significant because there was no overlap of confidence intervals of calculated LC_{50} values of both compounds (Table 1, Fig. 1).

Weight changes

Overall, weight continued to change during the course of the experiment, although this was not always proportional to the concentration of the test chemical (Table 2a and b, Fig. 2a and b)). Statistical analysis of the weight changes between the control populations and exposed snails over the 28 days period did not indicate any significant change ($P>0.05$).

Control snail populations recorded higher average weight gain compared to those exposed to the foliar fertilizer (Fig 2a and b). Although control *A. marginata* experienced steady increase in weight, those exposed to 1.9ml/L of Cypercot recorded the highest weight gain (Fig 2b).

Histopathological effects in the gut and gonads

No histological alteration or abnormality was observed in control tissues (i.e tissues of snails fed with the same food and kept under the same conditions, only separated by distance from the exposed snails) (Plates 1 and 2).

Cypercot did not induce significant histopathological effects in the digestive tissues and gonad of exposed snails examined . Exposure to $1/10^{th}$ of the LC_{50} (55.3ml/l) of the foliar fertilizer resulted in severe necrosis and sloughing of the gut epithelium (Plate 1 and 2). Various concentrations of the fertilizer induced histopathological effect on the gonads (Plate9 and 10) but this was not always proportional to concentration of exposure.

Aestivation

Aestivation of the snails were observed in groups exposed to the highest chronic concentrations of the herbicide (55.3ml/l) and two concentrations of the pesticide (0.19ml/l and 19.0ml/l). Some snails slowly emerged after repeated sprinkling of water.

Table1: Comparative Toxicity of Foliar fertilizer complex (Boost Xtra) and Pesticide (Cypercot) against *A. maginata*.

Time	LC_5	LC_{50}	LC_{95}	SE	DF	Equation of the line	TF
Boost Xtra	250.935 (68.949-352.743)	553.096 (428.143-722.582)	1219.103 (861.989-4565.230)	1.504	3	Y= -3.186 + X	2.92
Cypercot	74.286 (19.823-114.988)	189.528 (127.837-252.198)	483.547 (336.242-1356.199)	1.117	3	Y=-3.518 + X	1

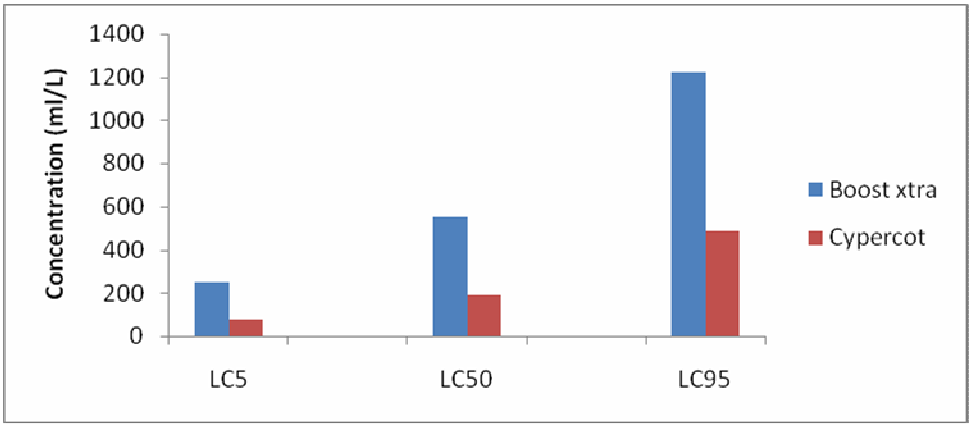


Fig 1: Relative acute toxicity of Boost Xtra and Cypercot

Table 2a: Mean weight± S.E of A. *marginata* exposed to Boost Xtra

Day	Control	55.3ml/L	5.53ml/L	0.553ml/L
Day 0	79.5 ±2.986079	80.75 ± 3.614208	80.75 ± 6.046693	79.75 ± 5.706356
Day 14	88.75±2.49583	79.75 ± 3.037954	88.5 ± 6.089609	80 ± 7.905694
Day 28	91± 2.160247	79.5 ± 3.840573	83.75 ± 4.366062	81.25 ± 7.284401

Table 2b: Mean weight± S.E of A. *marginata* exposed to Cypercot

Day	Control	19.0ml/L	1.9ml/L	0.19ml/L
Day 0	79.5 ± 2.986079	86 ± 3.188521	82.5 ± 6.062178	82.25 ± 3.614208
Day 14	88.75 ± 2.49583	86.75 ± 3.75	91.5 ± 5.722762	86 ± 2.081666
Day 28	91 ± 2.160247	88.5 ± 3.013857	96.75 ± 5.893146	88.25 ± 4.269563

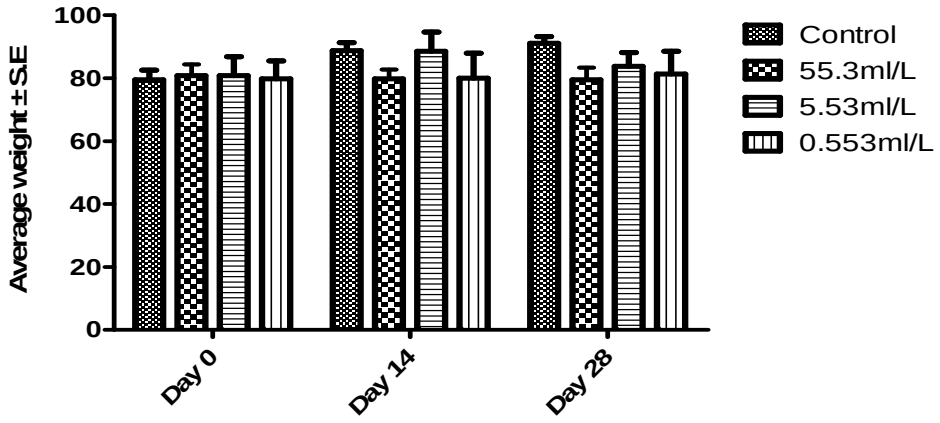


Fig 2a: Mean weight± S.E of A. *maginata* exposed to Boost Xtra (Foliar fertilizer)

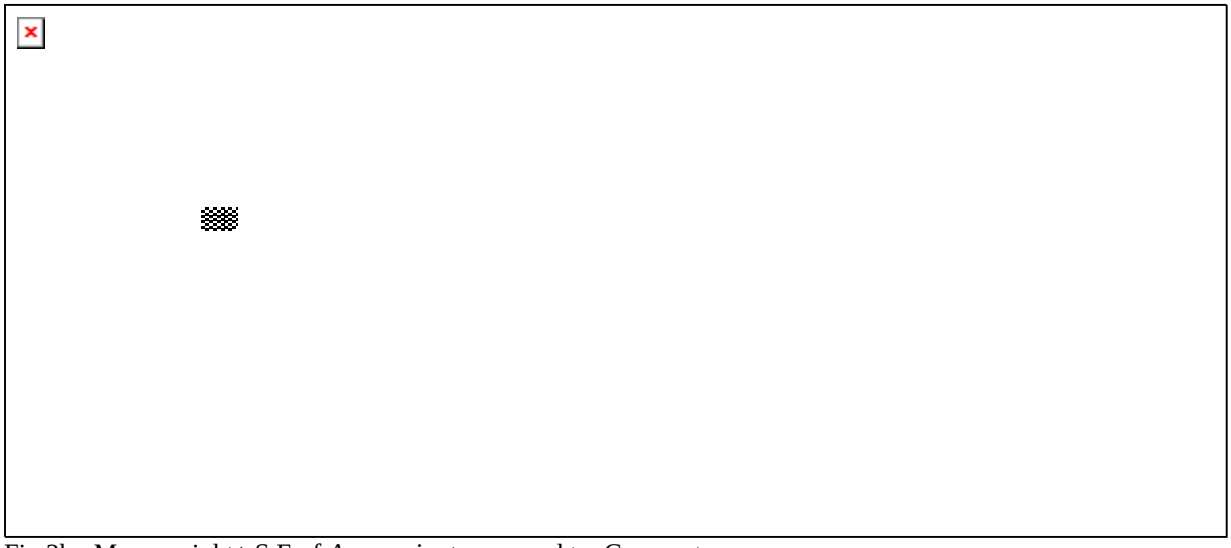


Fig 2b: Mean weight± S.E of *A. marginata* exposed to Cypercot

Table 3: Cumulative number of aestivating *A. marginata*

Concentration ml/l		Day 7	Day 14	Day 28
Cypercot	0.190	0	0	1
	1.900	0	0	0
	19.00	0	1	2
Boost Xtra	0.553	0	0	0
	5.530	0	0	0
	55.30	0	0	1

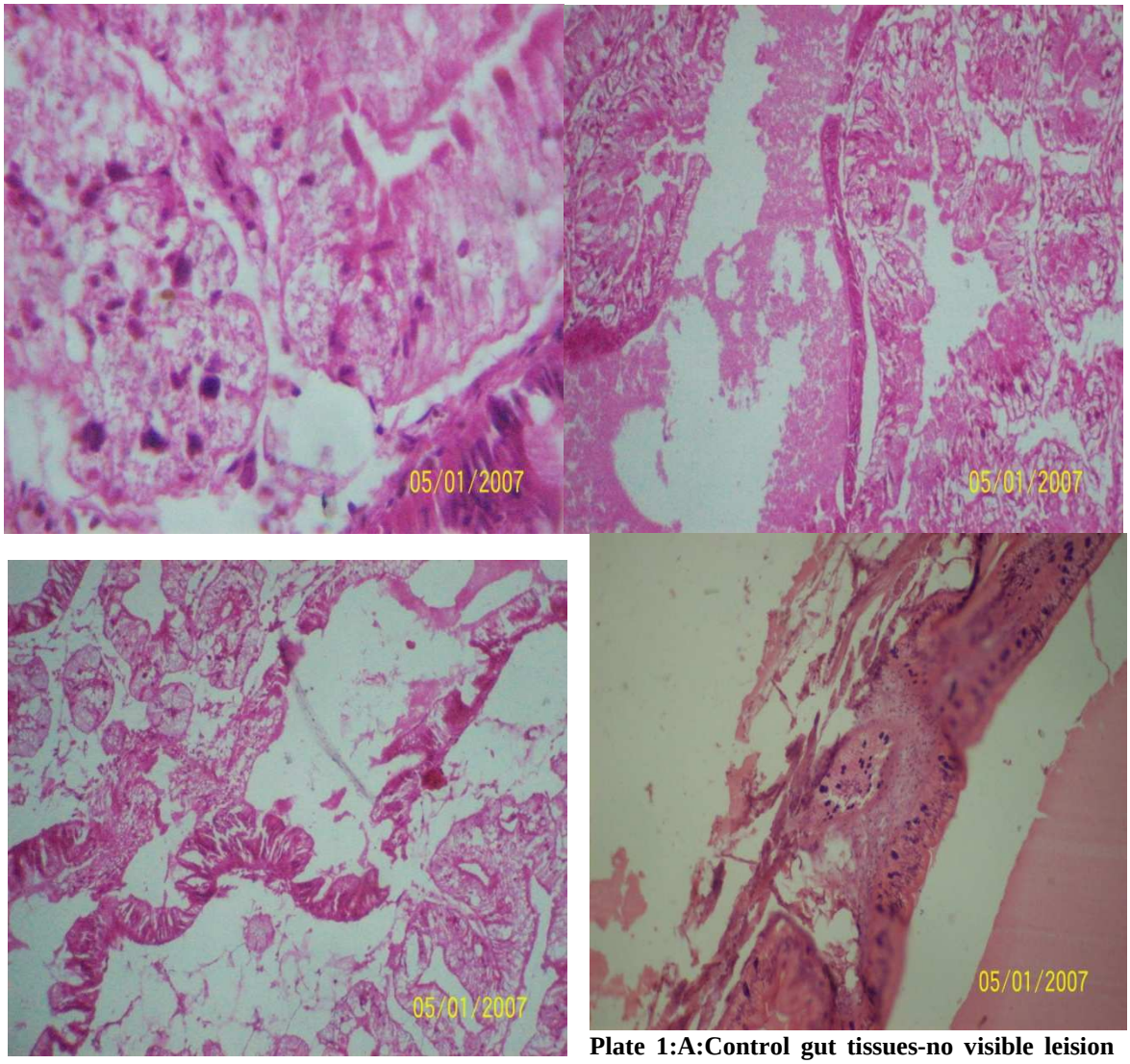
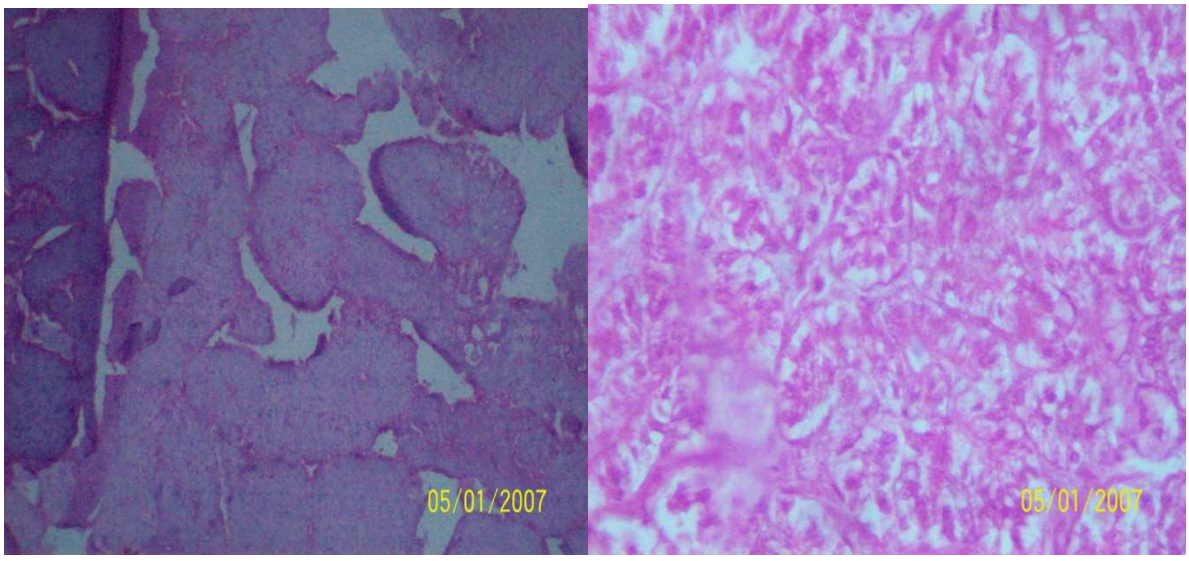
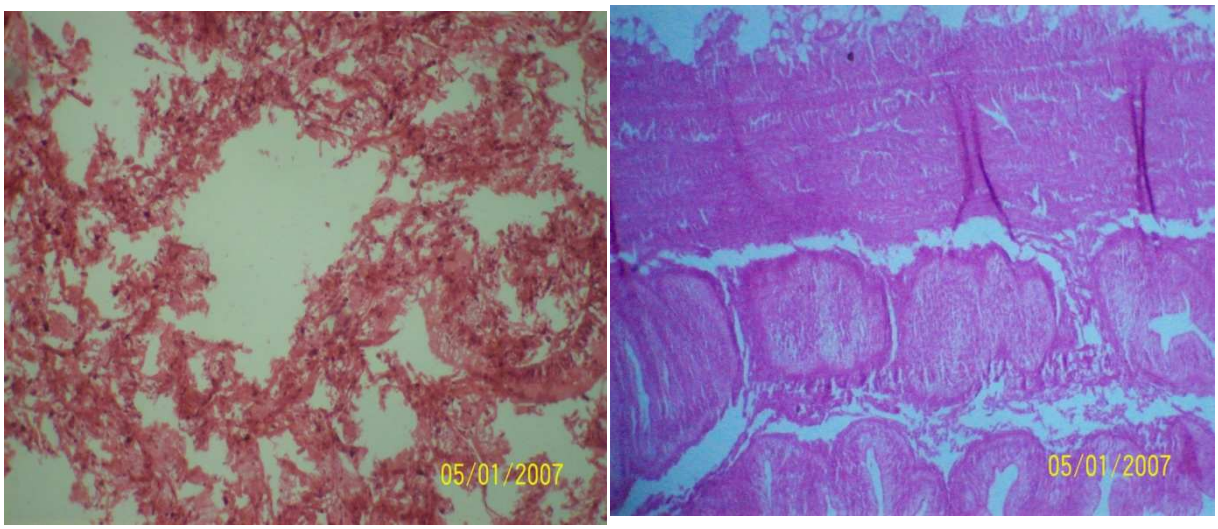


Plate 1:A:Control gut tissues-no visible leision B: Gut of *A. maginata* exposed to 55.3ml/l Boost Xtra showing severe necrosis and sloughing of glandula epithelium C: Gut exposed to 5.53ml/l Boost Xtra D: Gut exposed to 0.19ml/l of Cypercot showing tissue necrosis





A: Control gonad tissues-no visisble leisionm slightly autolysed B: Gonad exposed to 19ml/l Cypercot-.Presence of fat (glycogen) globules in the germinal tissue C: gonad of exposed to 55..3 ml/l foliar fertilizer showing tissue necrosis D: gonad exposed to 5.53 ml/l foliar fertilizer -No visible lesion

Discussion

Generally, pesticides are formulated to be acutely toxic to the target fauna and hence the preference in controlling pests of crops compared to other cultural methods. Non- target fauna such molluscs, earthworms, beneficial arthropods such as butterflies and other invertebrates are often on the receiving end of diverse assortment of chemicals introduced into farm lands.

The fact that the pesticide, Cypercot was found to be much more times toxic than the foliar fertilizer, Boost xtra resounds the results of past investigators who have consistently published scholarly articles on the toxicity of pyrethroids. The main environmental concerns of pyrethroids relate to their toxicity to fish and non-target invertebrates (Walker *et al.,* 2001).

The results obtained once again shows that physiological endpoints such as weight changes are somewhat unreliable in making pathological deductions because results of weight changes are often inconsistent. Hence there is need to use it in conjunction with other biomarkers. The unexposed snails (i.e negative control) recorded more consistent increase in growth throughout the study period but was not significantly different from exposed groups.

However, the fact that aestivation varies somewhat consistently, resounds this behaviour as a definite marker of exposure to stressors in gastropods. Despite advances in molecular biology, histopathological alterations remains key in understanding the effects of substances on the gross morphology an animal. Results from histological assessment of the gut and reproductive tissues reveals that the foliar fertilizer caused more severe tissue damage compared to the pesticide. This may be linked to the relatively low persistence of pyrethroids and other post-receptor mechanisms within the mollusc. Despite its low toxicity on the animal, boost xtra (foliar fertilizer) induced the greater damage and due to its low toxicity or some other protective mechanisms, the fertilizer is sequestered into tissues of the mollusc where they accumulate and exert tissue damage over time. Robert (2008) also reported that apart from its repelling effects, fertilizer causes disrupting in growth and reproduction in molluscs.

Overall, the observations from this study points to the ability of the agrochemicals to cause far reaching damage to snail tissues. However, a study that points to their bioaccumulation potentials and food chain transfer would provide a more vivid picture of their adverse effects.

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