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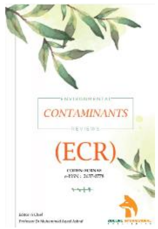

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RESEARCH ARTICLE

ECOTOXICOLOGICAL CONSIDERATION OF DICHLORVOS (SNIPER®) ON NON-TARGET ENVIRONMENTAL RECEPTORS

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ABSTRACT

The deleterious ecotoxicological influence of Sniper® a dichlorvos-based pesticide was evaluated on non-target environmental receptors – onions - (*Allium cepa L*) and snails – (*Archachatina marginata*). The mean effective concentration (EC_{50}) of Sniper® was 7.07 ± 0.08 mg/L for *Allium cepa L*, with a safe limit estimated at 0.707 ± 0.04 mg/L, while the mean EC_{50} for *Archachatina marginata* was 0.762 ± 0.04 mg/kg with a safe concentration of 0.0762 ± 0.02 mg/kg. The Ecotoxicological Risk Assessment Matrix (ERAM) was used to evaluate the deleterious ecological consequences resulting from exposure to the test pesticide. The risk level designated for Sniper® in the *Allium cepa L* and *Archachatina marginata* bioassay was E3 or 15 (P; E; C) and E4 or 20 (A; E; C) respectively, which was considered moderate to high risk to plants (P), animals (A), the environment (E) and community (C) based on the frequency of application / exposure. The results from the study implied that continuous exposure and misuse of Sniper® could pose significant risk and damage to non-target plants, soil dwelling species and humans.

KEYWORDS

Dichlorvos, onions, pesticide, risk assessment matrix, snail.

1. INTRODUCTION

Globally, anthropogenic operations have contributed more than 250 billion tonnes of synthesized chemicals, used yearly in a toxic avalanche that is deleterious to organisms and humans. Based on this, scientists have categorized the Earth as a 'toxic planet'. According to Cribb, "Earth, and all life on it, are being saturated with man-made chemicals in an event unlike anything in the planet's entire history," (Cribb, 2017). Thus, we all end up paying for the toxicity of chemicals either at the shop during purchase or at the hospice after exposure. Existing way back in the early 60s, a chemical recently re-introduced into the Nigerian market is Sniper®. It is an organophosphate insecticide dichlorvos (2,2-dichlorovinyl dimethyl phosphate - *DDVP*) used mainly to control household pests or to protect stored food crops / products from pests' destruction. It is effective against houseflies, mosquitoes, cockroaches, spider mites and a wide range insect infesting vegetable crops and animal products. The chemical was banned in the European Union (EU) in 1998. The toxicity of DDVP extends beyond insects and it is usually underestimated in its capability to eliminate organisms exposed to it, acting like a sniper and killing them within few second to minutes. Some species die without getting in contact with the chemical but rather from perceiving the vapour from it since it is almost odorless.

In another abusive way, Sniper® has been illegally applied above the specified / recommended concentration by farmers and vendors not on insects it is intended for but on grains, crops and stored products that are or may likely be infested by weevils and other pests that devour / destroy such stored food items / crops. These include grains (rice, corn, beans, millets) and dry meat products (stock fish, camel meat, donkey meat) amongst others. Recently, Sniper® was used at an alarming rate by humans to commit suicide or annihilate the lives of higher living species, rodents, birds, reptiles etc., other than the insects it was intended to control or kill. With the increasing number of deaths and suicide cases, the government of Nigeria placed a ban on the product in July 2019 but with effect from 1st September 2019. The long duration between the date of pronouncement and when the banning was to be effected made a lot of shop owners to stockpile / hoard the pesticide in large quantities. However, after September 2019 and till date, Sniper® was still very much available, now more in open retail shops with vendors and online ordering than supermarkets or malls, who secretly sell the product off counter to buyers at an exorbitant price or under disguise (Guardian Newspaper, 2019).

In Nigeria, due to poor control of chemicals, food and drugs by the regulatory authority - National Agency for Food and Drug Administration and Control (NAFDAC), most end users obtain, use and dispose these pesticides indiscriminately. Humans are surrounded by various chemicals, they are exposed to daily at home, work, or the environment. The pesticide - dichlorvos may reach non-target species / environment directly through spraying or as vapours dispensed from aerosol cans. Indirect contact could be via spray drift, runoffs during rains / irrigation or on treated stored products etc. (Michaelidou et al., 2000; Ezeji et al., 2015). We tend to think of some chemicals as toxic and others as nontoxic, but the fact is that any chemical is toxic at a high enough concentration. In this perspective, we can say that all substances are potential poisons since all of them can cause injury or death following excessive exposure and as Paracelsus, noted that "all substances are poisons and there is none that is not a poison". Thus, what differentiates a poison and a remedy is the right dose / concentration (Hodgson, 2004).

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It is important to state that the essence of synthesizing the colourless, crystalline, tasteless, and almost odourless organochlorine pesticide - dichlorodiphenyltrichloroethane (DDT) by the Swiss chemist Paul Hermann Muller in 1939 was for the purpose of controlling malaria / typhus among civilians and troops during the second half of World War II. However, after the war, DDT was used as an agricultural insecticide and its production and use increased. Although, Muller was awarded the Nobel Prize in Physiology or Medicine "for his discovery of the high efficiency of DDT as a contact poison against several arthropods" in 1948, non-target species were the worse hit from exposure to the lethal insecticide DDT.

Similarly, when, Carson, noticed that birds that sing by her window during spring no longer do so, it was difficult to believe that an organochlorine chemical, DDT, meant to kill insects would have such lethal effects on higher non-target animal species (Carson, 1962). Hence Carson wrote about the dying of birds and other non-target species during spring and the revealing facts from her book ‘‘*Silent Spring*’’ lead to the ban of DDT in 1972 in the United States (Carson, 1962). This changed the existing view on pesticides and has stimulated public concerns on their impact on health and the environment since most producers / manufacturers of chemicals are usually concern or interested in achieving the purpose for which the products are synthesized without considering the impact these chemicals can cause upon release, or exposure / disposal (Mufflin, 2012).

Information contained in the safety data sheet (SDS) for dichlorvos with respect to the toxicity for most species is scanty except for fish and rodents, with an information stating that the toxicological effects of the product have not been thoroughly studied. In the same vein, most non-professionals handling the pesticide may not have information or access to the safety data sheet (SDS) and there is always a high tendency for people to misuse or abuse such hazardous chemicals due to lack of adequate knowledge. These indiscriminate anthropogenic actions could possibly lead to the decline / extinction of many ecological species to the detriment of humans who are major end consumers of a myriad of food crops and animals that may have been exposed to these pesticides. Thus, the aim of this study was to evaluate the deleterious ecotoxicological consequences of Sniper®, on non-target receptors - onions - *Allium cepa L* and snails (*Archachatina marginata*).

2. MATERIALS AND METHODS

2.1 Test chemical

Sniper®, a synthetic organophosphate pesticide was obtained locally from the vendors in Effurun, Delta State, Nigeria. The test chemical is a dense volatile pale-yellow clear liquid with a sweetish smell, which mixes readily with water and contains 1000 g/L dichlorvos (2,2-dichloroethenyl dimethyl phosphate - DDVP 1000 EC), as the major active ingredient. It has a molecular formula: $C_4H_7Cl_2O_4P$ or $CCl_2{=}CHOPO(OCH_3)_2$ with a molecular mass of 220.97 g/mol. As an insecticide, the test chemical is commonly used by farmers in Nigeria to control insects / pests in food storage areas, barns and livestock. It is also used by non-farmers for the same purpose in homes, workplaces, industries while veterinarians use it to control parasites on pets and domestic animals.

2.2 Test species

The test species *Allium cepa L* and *Archachatina marginata* were conditioned in the laboratory. *Allium cepa L* (Plate 1) is an important edible vegetable crops used worldwide for seasoning and as an antioxidant while *Archachatina marginata* (plate 2) is consumed by humans as a rich source of protein and also to indicate the health of soils since a major aspect of their cycle - metabolic activities and reproduction takes place in soils.

Plate 1: Onion (*Allium cepa Linn*) (Source: www.shutterstock.com)

Plate 2: The giant African snail (*Archachatina marginata*) (Source: www.shutterstock.com)

2.3 Allium cepa root tip assay

The Organization for Economic Co-operation and Development, (OECD) protocol #208 was applied for root growth inhibition bioassay (OECD, 2003a; Olorunfemi et al., 2015). *Allium cepa L.*, (2n = 16) of average weight and length of 110 ± 0.87 g and 8.58 ± 0.08 cm respectively previously air-dried was prepared by exposing the fresh meristematic tissues after removing the dead root ends. A preliminary test was carried out so as to determine the range of concentrations for the actual test. The acute toxicity bioassay was carried out in the dark for 96 hours. The roots of each onion bulb were removed with a forcep and the root growth measured and used to determine the effective concentration (EC_{50}) (concentration that inhibits 50% root growth on exposure to the toxicant) after subjecting the assessment to a Probit analysis (Finney, 1971).

2.4 Archachatina marginata bioassay

Archachatina marginata of length 1.23 ± 0.5 cm and weight 0.81 ± 0.05 g were collected from a cultured in Delta State at a coordinate of $N05^{o}\ 51^{1}\ 0.29^{11}$ and $E005^{o}\ 44^{1}\ 36.7^{11}$. The assay was carried out using the protocol of the International Organization for Standardization (ISO), # 15952 (ISO, 2006). Juvenile *Archachatina marginata* were acclimated for seven days in their habitat and were fed with cellulose to prevent starvation. Prior to the actual assay, a preliminary test was performed, which was used as a basis for estimating the concentrations for the actual bioassay. In each test vessel, 1000 g (1 kg) of native soil from the organism’s habitat was placed and homogenized with five (5 g) of cellulose as food for the organisms with 100 mL of each test concentrations of the pesticide. After attenuation of the soils, cellulose and the test chemical, ten (10) juvenile *Archachatina marginata* were placed in each test vessel in triplicate. A control vessel containing water in place of the test pesticide was also set up along with the other treatment groups. Dead count was taken on day 7 and 14 and the exposed species were regarded dead when organisms do not respond to a gentle prodding at the foot (pedal) region with a pointed metal or if after 5 minutes there was no activity of the organisms when placed on moist filter paper.

2.5 Growth rate evaluation

The growth rate, percentage growth rate relative to control and the percentage growth inhibition efficiency were calculated using the equations (1) – (3) (Owodeinde et al., 2017).

$$\text{Growth rate (cm/hr)} = \frac{mean\ length}{time} \quad (1)$$

$$\text{Percent growth rate (\%)} = \left(\frac{GRs}{GRc}\right) \times 100 \quad (2)$$

$$\text{Percent growth rate relative to control (\%)} = \frac{GRc - GRs}{GRc} \times 100 \quad (3)$$

Where:
GRs = growth rate for sample
GRc = growth rate for control

2.6 Ecotoxicological risk assessment (ERA) of pesticides

Risk levels can be classified as low, medium, or high on the Ecotoxicological Risk Assessment Matrix (ERAM). If an environment is polluted, then animals (A), plants (P), environment (E) and community (C) may be harmfully affected (Table 1). Some factors that can be used for risk classification include: exposure concentration, duration and potency of the chemical or toxicant. The risk levels are categorized in a numbered format (USEPA, 2015; Ogeleka et al., 2017). Each hazard is given a rating, and this was multiplied by the probability that these hazards would occur using the relationship:

Risk level = Hazard severity x likelihood of exposure

Hazard severity are rated as 1 (slight effect), 2 (minor effect), 3 (localized effect or damage). 4 [major effect (deaths)] and 5 [extensive effect (death of population)]. Similarly, the likelihood of occurrence or exposure are rated as 1 (seldom – A - yearly), 2 (frequent – B - quarterly), 3 (very likely – C - monthly). 4 (near certain – D - weekly) and 5 (certain – E – daily) (USEPA, 2015).

Table 1: Ecotoxicological Risk Assessment Matrix (ERAM)

SEVERITY	CONSEQUENCE		P	A	E	C	INCREASING PROBABILITY A	B	C	D	E
							Never experience the chemical in the area	Had been exposed / used in the area	Had been exposed/ used in the area and other locations	Had been exposed / used several times in the area	Had been exposed / used several times in the area and other locations
0	Practically non-toxic	>1000	No injury	No effect	No effect	No impact	Area 1				
1	Practically non-toxic	>1000	Slight injury	Slight effect	Slight effect	Slight impact					
2	Slightly toxic	100-1000	Minor injury	Minor effect	Minor effect	Limited impact					
3	Very toxic	10-100	Major injury	Localized effect	Localized effect	Considerable impact					
4	Extremely toxic	1.0-10	Single fatality	Major effect (deaths)	Major effect	National impact		Area 2		Area 3	
5	Super toxic	<1.0	Multiple fatality	Extensive effect (kills)	Massive effect	International impact					

Abbreviations: LC_{50} median lethal concentration in ppm (Ogeleka et al., 2017; OECD, 2003b).

2.7 Statistical analysis

Analysis of variance (ANOVA) in Statistical Package for Social Science (SPSS) statistical software in Version 22.0 was used to test the significant difference between the two variables – controls and test groups at significance level of $P = .05$.

Also, the effective concentration EC_{50} for growth inhibition for *Allium cepa L* and mortality for *Archachatina marginata* determined the susceptibility of the test species to the pesticide Sniper®.

3. Results

The results from this evaluation are presented in Tables 2 - 6 and Figures 1 - 3.

3.1 Toxicological effects of Snipper® to exposed Allium cepa L

There was no root inhibition in the control groups as 100% was recorded for the root growth inhibition efficiency (RGIE). However, there was 68% RGIE in the highest exposure concentration of 20 mg/L, which had a percentage growth of 32%. At 20 mg/L the specific growth rate was 0.0190 with a mean root growth of 1.82 ± 0.12 cm while the control had a mean root growth 5.66 ± 0.50. The values for the other concentrations are depicted in Table 2, Figure 1 and 2.

Some observed symptoms include: discolouration of the exposed bulbs, melting or very soft / weaken tissues of the bulbs at the higher concentrations especially 10 and 20 mg/L, fading, twisting and bending of the root tips, which were also very brittle.

For the mean root length, significant difference between the treatment groups and the control at a probability level of 5% had a value of f > Fcrit = 0.5. The f calculated (1) is > F Critical (0.15) for the one tail test. Implying that there was statistical significance between the control and the treatment groups.

Table 2: Average results of *Allium cepa L* exposed to Snipper®

Conc., mg/L	Mean root length (cm)	SGR	% Root Growth	%RGIE
Control (0)	5.66 ± 0.50	0.0590	100	0
1.25	4.54 ± 0.25	0.0473	80	20
2.5	4.00 ± 0.10	0.0417	71	29
5	3.42 ± 0.38	0.0356	60	40
10	2.22 ± 0.21	0.0231	39	61
20	1.82 ± 0.12	0.0190	32	68

Data were processed based on three replicates: SGR = specific growth rate; RGIE = root growth inhibition efficiency

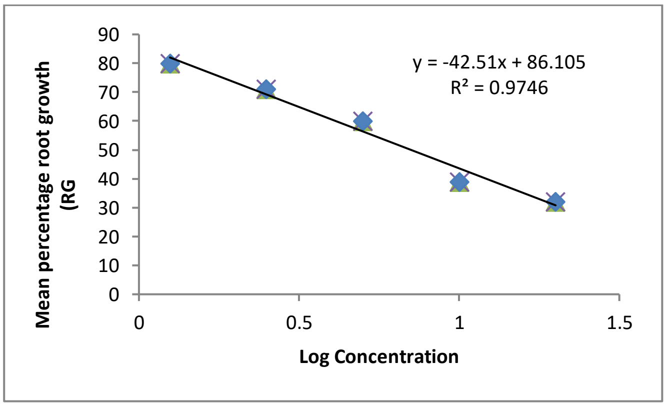

Figure 1: Average percentage root growth of *Allium cepa L* exposed to Snipper®

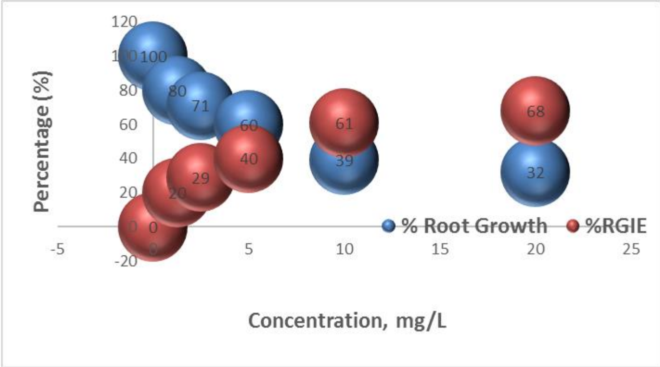

Figure 2: Average percentage root growth (RG) in comparison with root growth inhibition efficiency (RGIE)

3.2 Toxicological effects of Snipper® to exposed Archachatina marginata

The test chemical had adverse effect on the test organisms for the exposure period, however, no death or morphological changes was observed in the control groups. The exposure concentration was 0.3125, 0.625, 1.25, 2.5 and 5 mg/kg and at the end of the 14 day experimental assay, mean average % mortality of 15, 50, 70, 90 and 100% were recorded (Figure 3). As the concentration and exposure duration increased, the species became weaker and moved or burrowed more slowly when compared to their movement before the exposure (SETAC, 1997).

The morphological and behavioural changes in the affected organisms could likely be due to the test chemical, which tends to weaken their locomotive cells since none was observed in the control. The species also formed protective layers at the lower concentrations of exposure, however, they were unable to do so at the higher concentrations. There was also discolouration at higher concentrations since most dead species became very pale to colourless at test termination.

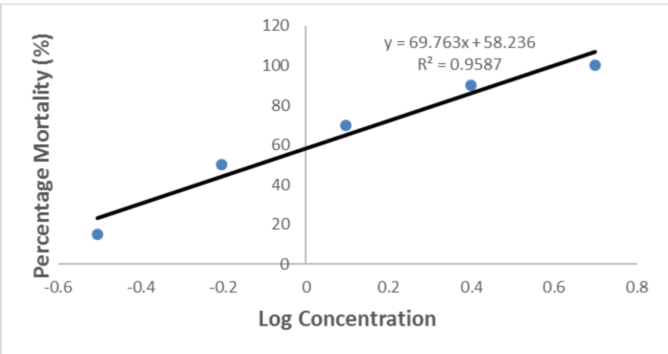

Figure 3: Average percentage mortality of *Archachatina marginata* exposed to Snipper®

3.3 Effective concentration (EC_{50}) of Sniper® exposed test organisms

According to the OECD rating for contaminants, the concentration of a toxicant / chemical in the environment should not exceed 10% of the EC_{50}, which is regarded as a safe limit. The results of this study revealed that Snipper® was highly toxic to *Archachatina marginata* and moderately toxic to *Allium cepa L* based on the OECD standard scale (Table 3 and 4) (OECD, 2003).

Table 3: Effective concentration (EC_{50}) values of Sniper® exposed to *Allium cepa L* and *Archachatina marginata*

Test Specie	Line equation (Y – value)	EC_{50}	Safe limit	OECD rating
Allium cepa L	y = - 42.51x + 86.105	7.07 ± 0.08 mg/L	0.707 ± 0.04	Moderately toxic
Archachatina marginata	y = 69.763x + 58.236	0.762 ± 0.04 mg/kg	0.0762 ± 0.02	Highly toxic

Data were processed and expressed as mean ± SD based on three replicates

Table 4: Toxicity rating

Ratings		EC50
5	Very highly toxic	< 0.1
4	Highly toxic	0.1-1
3	Moderately toxic	1-10
2	Slightly toxic	10-100
1	Practically non-toxic	100-1000
0	Non-hazardous	> 1000

Source: (OECD, 2003b; GESAMP, 2002)

3.4 Ecotoxicology Risk Assessment for the Sniper® bioassay

In Table 5, the ecotoxicological risk assessment was evaluated using the details indicated in Table 1.

Table 5: Ecotoxicology risk assessment of Sniper® exposed to non-target receptors

Test Specie	EC_{50} rating	Frequency of exposure (daily for 4 or 14 days) (a)	Hazard severity (b)	Risk level (a X b)	Hazard rating
Allium cepa L (4-day exposure)	Moderately toxic	E	3	E 3	E 3 or 15 (P,E,C)
Archachatina marginata (14-day exposure)	Highly toxic	E	4	E 4	E 4 or 20 (A,E,C)

Table 6 showed the likely consequences of the effect of the test pesticide - Sniper® on the two non-target environmental receptors. It can be seen that the effect / impact was more prominent on animals than plants.

Table 6: Consequences of the effects of Sniper® using ecological risk assessment matrix (ERAM)

	Consequences			Inference
Test Specie	Plant (P) / Animal (A)	Environment (E)	Community (C)	
Allium cepa L	Major injury	Localized effect	Considerable impact	Moderately toxic
Archachatina marginata	Major effect (deaths)	Major effect	National impact	Highly toxic

4. Discussion

More than two decades after its ban in Europe and eight months in Nigeria, the organophosphate insecticide, dichlorvos in the brand name of Sniper® is still very much available in the open market. The pesticide has lethal effects on organisms on exposure especially non-target biological receptors. The chemical is very volatile, meaning that it readily forms vapours which may be inhaled and inhalation is the most common way to be exposed to the chemical. High doses of test chemical may be very toxic, especially if inhalation exposure is continuous (Zhao et al., 2015). Exposure can occur by inhalation, ingestion, or contact and in human, acute (lethal) and chronic (sublethal) exposure may lead to death, genotoxic, neurological, carcinogenic, renal, respiratory, metabolic, dermal and other systemic effects. Death may be caused by respiratory failure or cardiac arrest. Cholinesterase inhibition may affect the nervous system and can cause abnormalities in the tissues of the lungs, heart, thyroid, liver and kidneys leading to death (Imam et al., 2018; Laudari et al., 2014).

From the assessment of this study, it was found that the test chemical had impact on the species under investigation, although the effects was more prominent in the soil dwelling receptor - *Archachatina marginata* than on *Allium cepa L*. The test chemical has the capability to inhibit or deactivate acetyl cholinesterase (an enzyme required for proper nerve functioning) at the cholinergic junction of the nervous system, which could lead to damage / destruction the deoxyribonucleic acid (DNA) of the species (Okoroiwu and Iwara, 2018). In nature, soil constituted the support in which terrestrial species take around 40% of their nutrients and dwell. Research have shown that the longer the exposure of snail to chemicals, the greater the effects in the digestive gland, foot and mantle of the snails (Oliveira-Filho et al., 2005). Further studies by a group researcher revealed that dichlorvos can cause quantitative and qualitative changes in snail's activities especially reproduction ability (Rivadeneira et al., 2013).

Some plants tolerate dichlorvos very well and certain concentrations used for control of glasshouse pests may not be completely phytotoxic as reported long ago, who noted that, the only adverse effect recorded was slight discoloration, fading and severe leaf burning of chrysanthemum blooms after exposure for 15 hours at 20°C to 204 mg/L dichlorvos vapours to 42 plants and 20 flowering plants (Pass and Thurston, 1964). These observed symptoms were also corroborated in this study (discolouration, melting / weaken bulbs tissues, fading, twisting and bending of the root tips at the higher concentrations. The lethal concentration LC_{50} for some species have been evaluated by some researchers, although not much studies are available on the toxicity of dichlorvos to terrestrial plants and animals especially *Allium cepa L* and *Archachatina marginata*. However, a group researchers in their research on the impact of the insecticide endosulfan on growth of the African giant snail *Achatina achatina (L.)*, concluded that due to growth impairment on exposure to the insecticide, the repeated application of endosulfan for pest control may impair the growth of African giant snails (Wandan et al., 2010).

Similarly, some researchers in their study revealed that the three pesticides (endosulfan, dichlorvos, and carbendazim) assessed produce genotoxic effects in *Allium cepa* which could result in health risks to human populations (Kuchy et al., 2015). A concentration of 15 mg/L is expected to kill a rat in 4 hours while the 96-hour LC_{50} for dichlorvos in lake trout is 0.2 mg/L, and 12 mg/L for fathead minnows as reported (Ezeji et al., 2015; USEPA, 1999; Das, 2013). Some researchers reported that dichlorovos was significantly toxic to exposed earthworms at lethal and sublethal concentrations, which affected their weight and behavioural changes (pigment discoloration, decreased burrowing capability and increased propensity to coil) (Ogunwole et al., 2018). Ariole and Ezevununwo found the 96-hour LC_{50} acute toxicity of dichlorvos to freshwater snail (*Pila ovata*) to be 0.54 ppm, which can be considered very lethal according to the OECD rating (Ariole and Ezevununwo, 2013; OECD, 2003b; Laudari et al., 2014; Okoroiwu and Iwara, 2018; Ariole and Ezevununwo, 2013; Prosser et al., 2017).

5. Conclusion

This appraisal concluded that the test chemical Sniper® cause severe lethal ecotoxicological effects on exposed non-target environmental receptors - *Allium cepa L* and *Archachatina marginata* with effects more prominent on the exposed animals than the plants. The study established data that would be useful to determine the safe levels of the test pesticide so as to prevent / minimize unintended harm to non-target organisms. Similarly, the appraisal gave data that would assist users in safeguarding plants and animals in the environment and the lives of humans. For further evaluation, the authors recommend that biochemical markers such as the oxidant activity – malondialdehyde (MDA) and anti-defensive mechanisms (superoxide dismutase - SOD and catalase – CAT) should be used to evaluate the deleterious consequences of the test pesticide in addition to the risk associated with such exposure. Consequently, since humans are usually the end users and consumers of crops and animals, there is the need to ensure that these vulnerable species and man are protected from the deleterious impacts of this lethal test pesticide that could exterminate a community of exposed species in a short time.

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