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Glyphosate Use in Agriculture: Declining Africans Giants Snails Populations

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ABSTRACT

The study carried out on the snails *Achatina fulica* and *Ahatina achatina* at the Jean Lorougnon GUEDE University achatinicole farm aimed to evaluate the effect of glyphosate on the growth and survival of these animals. After a month of acclimatization where the snails were regularly watered and fed every other day with fresh lettuce leaves, the 210 juvenile snails of each species were divided into seven (7) batches of 30 snails each. The experiment consisted in modifying the diet of the snails by adding every 6 days, lettuce leaves soaked in glyphosate diluted at different concentrations: D_0, D_4, D_6, D_8, D_{10}, D_{12}, and D_{14},, for 60 days. Live weight and shell length were measured on each individual and the number of deaths from each batch was recorded every 15 days. The results reveal a negative effect of glyphosate on both weight growth and shell growth. Thus, a weight loss of the order of 0,2g (0,06%); 0,67g 2,23%); 1,65g (5,5%); 4,12g (13,73%); 6,43g (21,43%) and 8,04g (26,8%) is noted in *A. fulica*. In contrast, *in A. achatina*, it is 0,39 g (1,3%); 2,86 g (9,53%); 4,01 g (13,46%), 5,72 g (19,06%), 7,82 (20,06%) and 9,59 g (31,96%) respectively in subjects exposed to doses D_0, D_4, D_6, D_8, D_{10}, D_{12} and D_{14}. As for the shell length, that of *A. achatina* increased by 20.33%; 4.7%; 2.1%; 1,73%; 0,8%; 0,4%, and 0,66% respectively in snails subjected to doses D_0, D_4, D_6, D_8, D_{10}, D_{12} et D_{14}, versus 31% 2,4%; 1,96%; 1,03%, 0,83%; 0,76% and 0,53% in *Achatina fulica* for the same doses. The cumulative mortality rate is 27,14% in *A. fulica* and 33,8% in Aachatina. Plant protection products, namely glyphosate, could be one of the causes of the decline of snails in the wild. Therefore, these results may help the conservation of these animals by limiting the misuse of plant protection products.

INTRODUCTION

Yield losses of agricultural production due to diseases, pests, and weeds have reached significant proportions [1]. This is why several researchers as well as decision-makers and many international organizations such as the FAO have implemented modernization strategies since 1947 to increase agricultural production [2]. These strategies focus on encouraging farmers to use fertilizers, agricultural machinery and pesticides [3]. Therefore, the use of pesticides is considered the preferred route to the rapid increase in production [4]. Indeed, this method allowed the development of agriculture and contributed to the increase in yields and the regulation of agricultural production after the Second World War [5]. In terms of variants of these products, herbicides are the most widely used worldwide (46,9%) ahead of fungicides (25,9%), insecticides (24,1%), and other products [6]. Côte d'Ivoire has not remained on the sidelines of this practice based on the use of agricultural inputs. Thus, in 2016 alone, the quantity of pesticides imported by the country was estimated at 20,000 tons, two-thirds of which were herbicides [7]. This control method, very common in large areas, reduces weeding time and optimizes farm yields. It can reduce weeding time by 40-60% and increase production by 10-20% compared to manual weeding [8]. This method is cited by some authors as an economic practice in industrial oil palm plantations [9,10]. Today, labor is scarce or when it exists, it is very expensive. [11] and [12] have shown that manual weeding in palm groves is more expensive than chemical treatments. However, these pesticides are frequently implicated in the degradation of freshwaters, and coastal waters and the reduction of biodiversity observed in agricultural areas [13]. Neurotoxic carcinogenic or endocrine-disrupting effects of pesticides have been demonstrated in animals [14,15]. The active substances present in these products are able to enter directly into the food chain and concentrate in certain living organisms. However, in some developing countries, some naturally available food resources play a fairly substantial role in feeding populations [16]. These resources include African giant snails (or achatines) belonging to the Achatinidae family. According to [17] although the main targets of herbicides are plants, only 0,1% of the quantities applied reach their targets. Unlike earthworms, which are mainly exposed to soil and decaying organic matter, terrestrial snails can be exposed to 3 sources of contamination: air, soil and plants. The soil is therefore the matrix that receives all forms and types (physical, chemical and biological) of pollution. However, the soil is a source of food for snails in which they ingest the resources necessary for their growth, a habitat, and a support to

which they are strongly subject. Thus, because of their sedentary lifestyle, these species are good indicators of environmental pollution. Today, in snail-producing regions whose main activity is based on agriculture, there is a strong and record proliferation of garden snails (*A. fulica*) [18,19] which are not very popular because of their muddy taste to the detriment of *A. achaina*, the most prized species on the markets by the Ivorian populations [20]. It seems important to us to assess the resistance of these two species to the uncontrolled use of pesticides. The objective of this study is to compare the impact of glyphosate on giant snails. More specifically, it is a question of evaluating the impact of glyphosate on the shellfish and weight growth of *A. achaina and A.* fulica and determining the related mortality rates.

Study environment

The city of Daloa is located in the center-west of Côte d'Ivoire, the capital of the region of Haut Sassandra between 6°52′38" north latitude and 6°27′00" of the western nozzle. The site of the Jean Lorougnon Guédé University Purchase Farm served as a framework for evaluating the effect of pesticides on the growth of African giant snails.

Biological material

This study focused on two species of snail of the same genus Achatina belonging to the family Achatinidae. These are *Achatina achatina and Achatina fulica* (Figure 1 and 2*).*

1.5 cm **Figure 1: *Achatina achatina***

2,5 cm

Figure 2: ***Achatina fulica***

Chemical material

It is essentially composed of the herbicide (glyphosate) in the liquid form called on the market Kalach 360 SL (Figure 3) widely used by growers for plowing plots before sowing or weeding in plantations of perennial crops.

METHODOLOGY

Impacts of plant Protection products on Snail Growth

A total of 210 specimens of *Achatina achatina* and 210 specimens of *Achatina fulica* with an average weight of 19,49±0,80 g and 19,88±4,33 g for an average shell length of 52,66±2,1 mm and 50,64±2,97 mm were acclimatized for four (4) weeks in breeding tanks of 1 m 2 of surface built of bricks and used in this study which lasted sixty (60) days. The snails come from a breeding farm, all from the first generation aged about 6 months. Seven (07) lots of thirty (30) snails each were formed and arranged in these snails. Lettuce leaves that were consumed preferably during the period of acclimatization of the snails served as food for the experiment. The animals were watered every day, except in case of rain. The animals were subjected to lettuce leaves soaked in glyphosate solution at different doses every six (6) days. According to the results of the survey of lettuce growers, no herbicides were used during plowing. Watering and frequency of exposure lasted eight (8) weeks. Seven (7) dilution doses D_0, D_4, D_6, D_8, D_{10},

D_{12} et D_{14}, were used in accordance with Table 1. Every 15 days, the live weight and shell length of the snails were determined.

Table 1: Different dilutions of glyphosate

Glyphosate g/L(water)	Dilutions
D0	Control snails 0 g_{gly}/L_{water}
D4	Snail treated with 4 g_{gly}/L_{water}
D6	Snail treated with 6 g_{gly}/L_{water}
D8	Snail treated with 8 g_{gly}/L_{water}
D10	Snail treated with 10 g_{gly}/L_{water}
D12	Snail treated with 12 g_{gly}/L_{water}
D14	Snail treated with 14 g_{gly}/L_{water}

Data processing

Descriptive statistics were mainly used for data analysis. It consisted of calculations of averages and percentages. The calculations and figures were performed with Excel 2019 software.

RESULTS

Effect of Glyphosate on the feeding behavior of *A. achatina* and *A. fulicas snails*

Snails exposed to glyphosate showed several symptoms, the first of which is the loss of chemoreceptors. Indeed, snails were no longer attracted to food in a dose-dependent way compared to control snails. Very often, they remain fixed on the walls of the bins or on the closure of the bins without showing locomotor and nutritional activity and others become embedded in the ground. This way of life also concerns the control snails with the difference that they go out from time to time to feed. Snails exposed to concentrations 4 and 6 of glyphosate are significantly active compared to snails exposed to higher doses of glyphosate during the eight (08) weeks of experiments.

Effect of glyphosate on mortality of *A. achatina* and *A. fulicas* snails subjected to different doses

During the experiment, the mortality was a function of the species and distributed as follows. In *A. achatina snails*, it is 0; 03;05; 09; 12; 16 and 25 dead at doses D_0, D_4, D_6, D_8, D_{10}, D_{12} and D_{14}, respectively. As for *A. fulica*, it is of the order of 0; 3; 4; 8; 11; 14 and 17 respectively in snails exposed to doses D_0, D_4, D_6, D_8, D_{10}, D_{12} and D_{14}. Mortality is relatively lower in snails of *A. fulica* species compared to that of *A. achatina* (Table 2) with respectively 57 individuals out of 210 or 27,14% and 71 individuals out of 210 or 33,81%.

Table 2: Mortality rate of A. achatina and A. fulica during sixty days of glyphosate treatment

Dose (g/l)	**Number of deaths per fortnight in *A. achatina***					**Sixty-day cumulative mortality (%)**
	T_0	T_{15}	T_{30}	T_{45}	T_{60}	
D_0	0	0	0	0	0	0,00
D_4	0	1	1	1	0	10
D_6	0	2	2	1	0	16,66
D_8	0	6	2	1	10	30
D_{10}	0	7	4	1	0	40
D_{12}	0	11	3	2	0	53,33
D_{14}	0	16	6	3	0	83,33
Number of deaths per fortnight in *A. fulica*						
D_0	0	0	0	0	0	0,00
D_4	0	1	1	1	0	10
D_6	0	2	1	1	1	13,33
D_8	0	5	2	1	1	26,66
D_{10}	0	6	4	1	0	36,66
D_{12}	0	8	3	2	1	46,66
D_{14}	0	12	4	1	0	56,66

Effect of the herbicide glyphosate on the evolution of the shell of A. *achatina* and *A. fulica snails*

The shellfish growth of snails *Achatina achatina* and *Achatina fulica* not exposed to glyphosate shows similarities between the two snail species during the eight (8) weeks of exposure (Figure 4). In the control medium, the growth of *Achatina achatina* (D0a*)* and *Achatina fulica* (D0f) *is* substantially identical with a slight advance of *Achatina fulica* until the end of the experiment. Thus, the size of *Achatina achatina snails* increased by 20,33%; 4,7%; 2,1%; 1,73%; 0,8%; 0,4%, and 0,66% respectively in snails subjected to doses D_0, D_4, D_6, D_8, D_{10}, D_{12} and D_{14} against 31% 2,4%; 1,96%; 1,03%, 0,83%; 0,76% and 0,53% in *Achatina fulica* corresponding to D_0, D_4, D_6, D_8, D_{10}, D_{12} and D_{14}. Glyphosate therefore has an inhibitory effect on the shellfish growth of *Achatina achatina* and *Achatina fulica.* On the other hand, doses D_{10}, D_{12}, D_{14} seem more toxic. Indeed, the curves (reflecting the shellfish growth of the two snails (*Achatina achatina* and *Achatina fulica))* remained substantially static from the beginning to the end of the experiment thus expressing an almost total absence of shell growth.

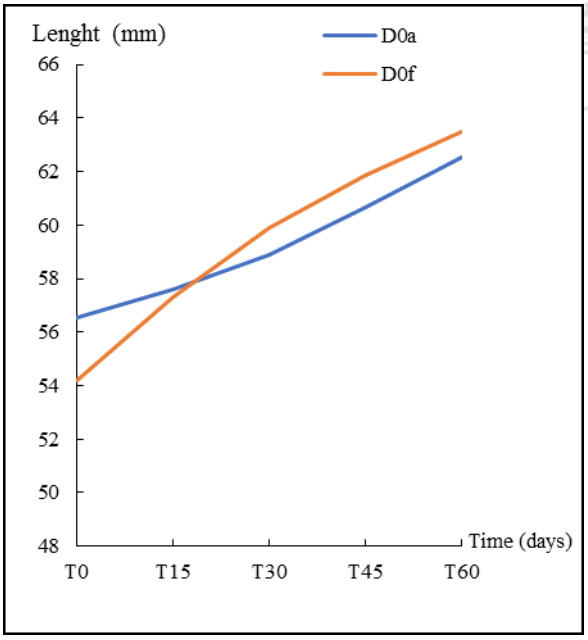

Figure 3 : Shell growth of A. Achaina and A. fulica controls

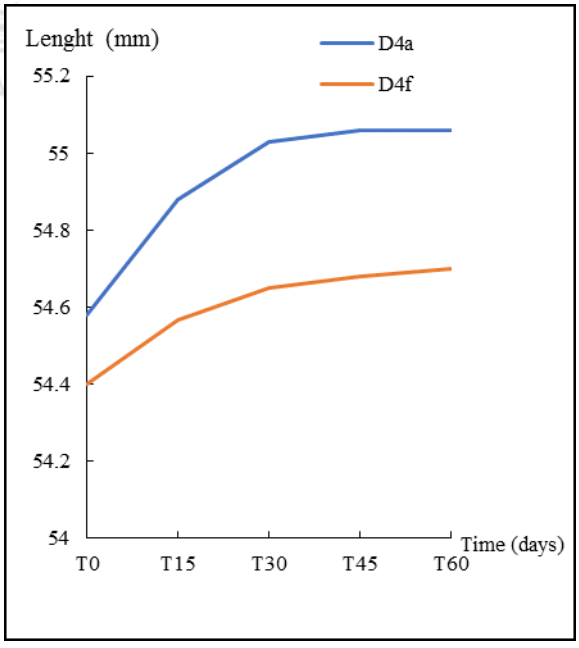

Figure 4: hell growth of A. Achaina and A. fulica treated with 4g/l

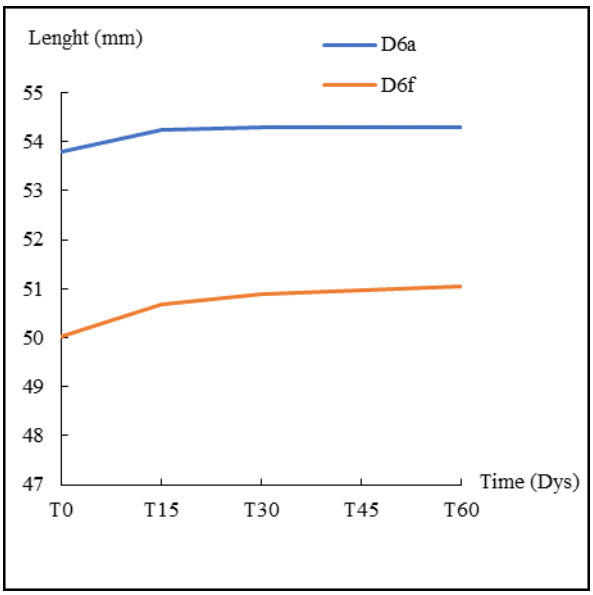

Figure 5 : Shell growth of A. Achaina and A. fulica treated with 6g/l

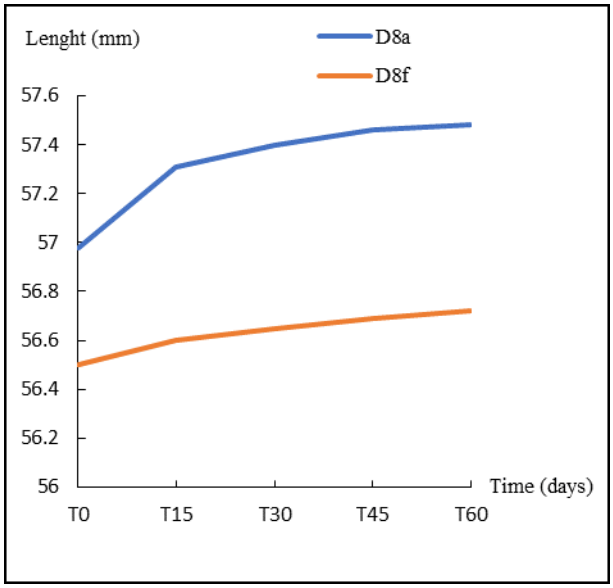

Figure 6 : Shell growth of A. Achaina and A. fulica treated with 8g

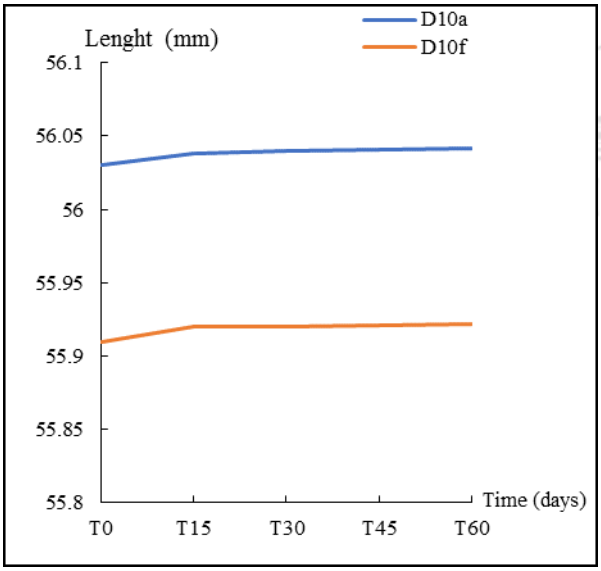

Figure 7 : Shell growth of A. Achaina and A. fulica treated with 10g/l

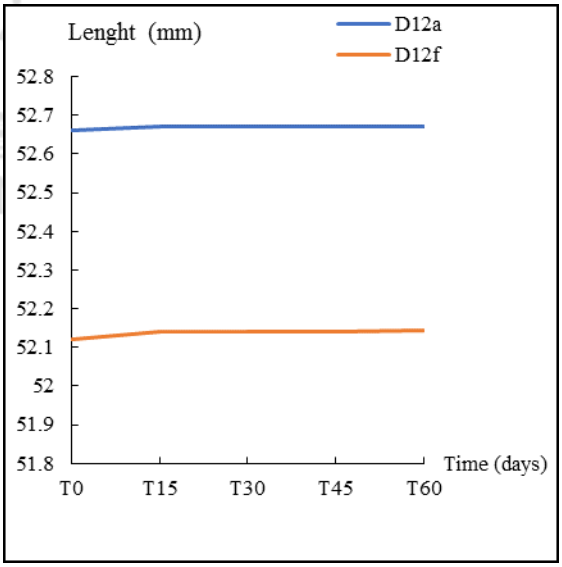

Figure 8 : Shell growth of A. Achaina and A. fulica treated with 12g/l

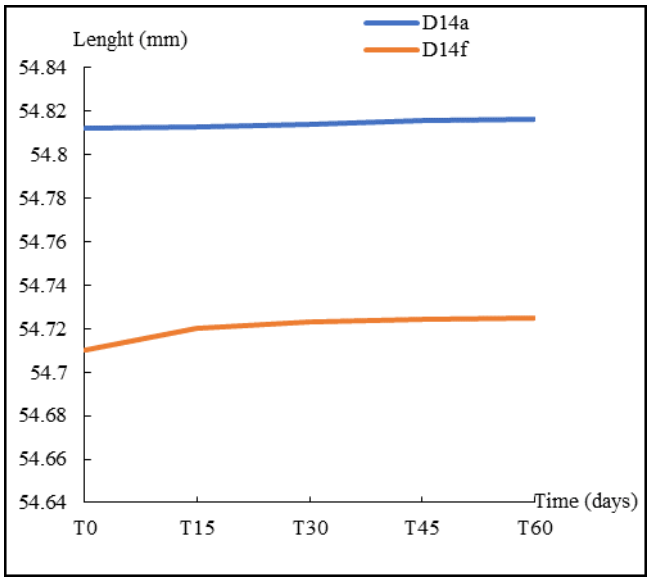

Figure 9 : Shell growth of A. Achaina and A. fulica treated with 14g/l

Effect of the herbicide glyphosate on the weight evolution of A. *achatina* and *A. fulica snails*

The weight growth of the control *Achatina achatina* and *Achatina fulica* snails shows similarities between the two snail species during the sixty days of exposure (Figure 11). As for snails exposed to glyphosate at different doses, they reveal large differences in their growth. In the control medium (snails not exposed to glyphosate), the weight growth of the two snail species remains the same with a strictly linear growth for *Achatina fulica* throughout the experiment. In contrast, the weight growth of *Achatina achatina* is linear until the date T_{45}, from which date its growth undergoes a slight increase compared to that of *A. fulica.* At doses D4 *A. achatina*, first undergoes a slight increase in date T_0 to T_{30} compared to *A. fulica* before falling from T_{30} to T_{60}. At glyphosate concentrations D_6 and D_8, no weight gain is observed, there was even a slight weight loss from the beginning to the end of the experiment. Based on these doses of glyphosate, *Achatina achatina* is more sensitive to glyphosate as evidenced by increased weight loss. At concentrations D_{10}, $D_{12,}$ and D_{14}, the weight growth of both snail species appears to be consistently negative. However, the curves show a more pronounced weight loss in the species *Achatina achatina* than in *Achatina fulica.*

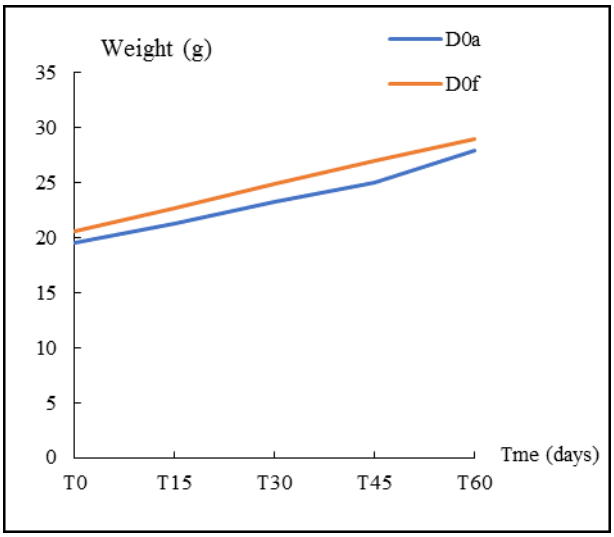

Figure 10 : Weight growth of A. Achaina and A. fulica controls

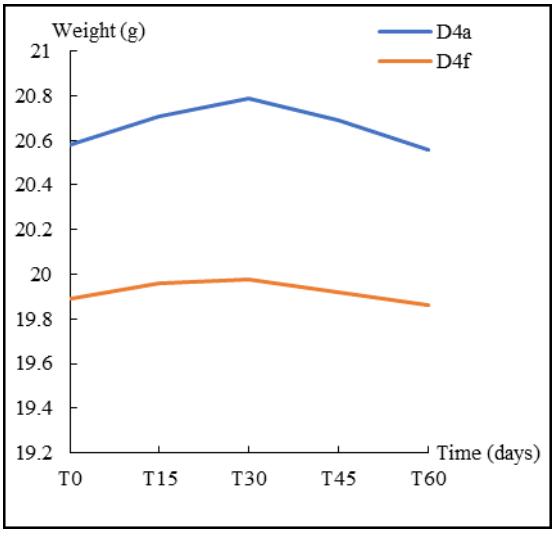

Figure 11 : Weight growth of A. Achaina and A. fulica treated with 4g/l

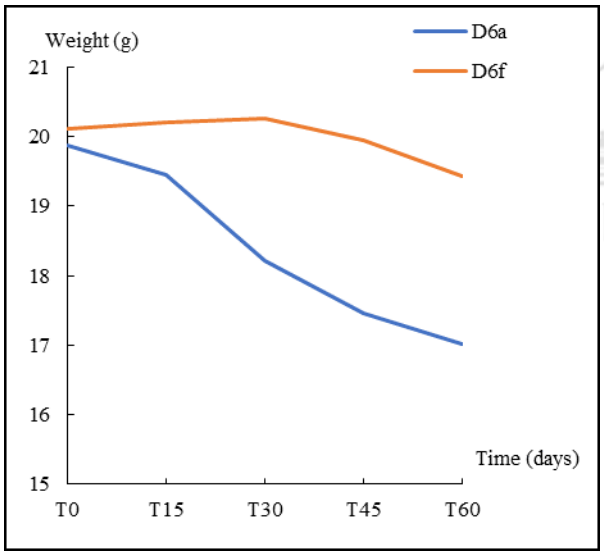

Figure 12 : Weight growth of A. Achaina and A. fulica treated with 6g/

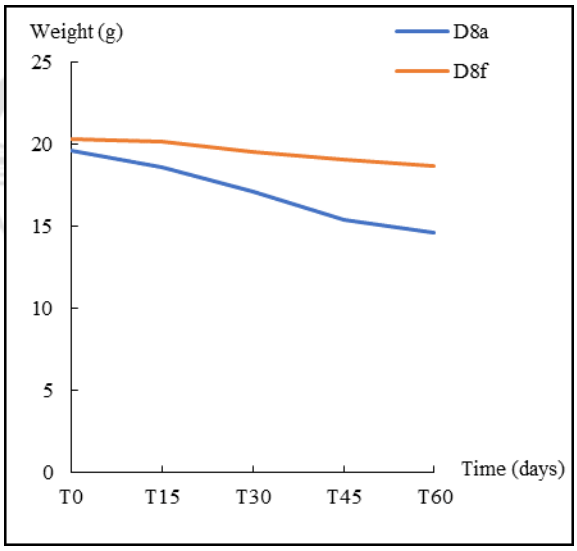

Figure 13 : Weight growth of A. Achaina and A. fulica treated with 8g/l

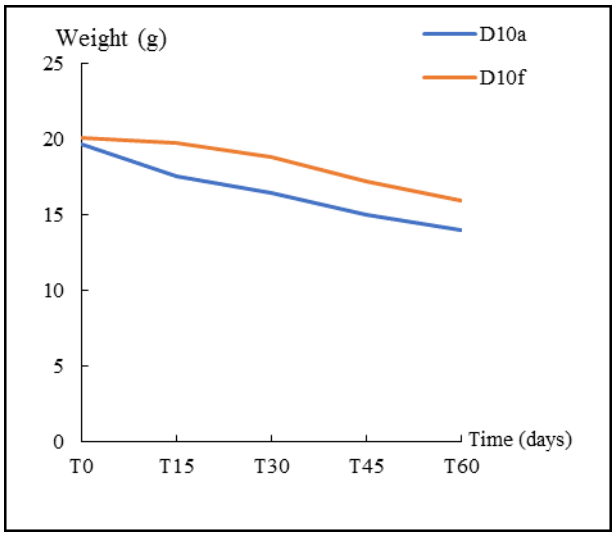

Figure 14: Weight growth of A. Achaina and A. fulica treated with 10g/l

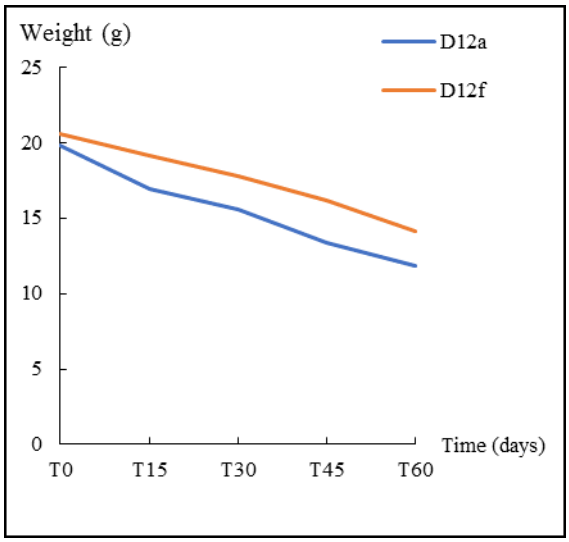

Figure 15: Weight growth of A. Achaina and A. fulica treated with 12g/l

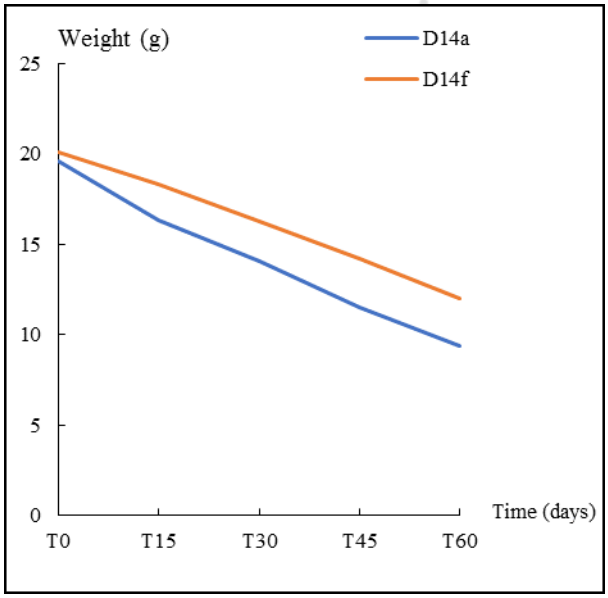

Figure 16: Weight growth of A. Achaina and A. fulica treated with 14g/l

DISCUSSION

During the eight weeks of experimentation, high sensitivity in *A. achatina* and *A. fulica* exposed to glyphosate was found. It is observed as much on the shell length of snails as on the weight. Glyphosate acts negatively on the weight and shellfish growth of African giant snails (*Achatina*

achatina, Achatina fulica). Indeed, the live weight and shell length of control snails of these two species increase, while those of snails subjected to different doses of food contamination with glyphosate decrease significantly. This inhibition of the weight and shell growth of snails is a function of the dose of glyphosate which induces greater toxicity depending on its concentration. This explains the low weight loss observed in snails subjected to doses D_4 and D_6 g/l unlike the strong weight loss observed in those subjected to doses D_8, D_{10}, D_{12}, and D_{14} g/l. Our results are consistent with those of [21]. [22] and [23] who observed a significant reduction in the weight of snails treated with aminocarb, methyl parathion, paraquat, and glyphosate; this reduction is related to the nature and dose of pesticide administered. In addition, [24] observed a "dose-dependent" decrease in snail growth and survival by dimethoate. On the other hand, all our results go against those obtained by [25]. These authors showed that glyphosate did not have an inhibitory effect on the shell and weight growth of snails exposed to 2,8 $mg.kg^{-1}$ of active ingredients but rapid shell growth during the first 56 days of the experiment. However, a 43% decrease in albumin gland mass in exposed snails. Glyphosate has been described as an endocrine disruptor in some vertebrates [14,15]. Thus, this growth blockage could be due to a reduction in energy reserves, allocated to reproduction in snail tissue storage cells. In addition, it could be caused by the mobilization of resources for the initiation of detoxification processes. Studies conducted by [26] have found that the energy cost of environmental stress leads to a decrease in the amount of energy available for growth and reproduction and consequently inhibition.

The high mortality rates (27,4% in *A. fulica and* 33,8% in *5*) of our results could be explained by the high dilution doses. These results are opposite to those obtained by some authors. For example, [25] showed that after 60 days of exposure to glyphosate and glufosinate at concentrations up to 30 mg kg^{-1} recorded respectively 20% versus 4% mortality in subjects. Also, he pointed out that no effects on mortality or growth were observed, either through exposure to soil or from food. In addition, [5] recorded a mortality rate of 9,61% in *Helix aspersa* exposed to thiamethoxam, tefluthrin, and their mixtures. As for [21], it obtained 13% mortality after exposure of *Helix aspersa* snails to 5mg/kg carbaryl for 14 days. The large difference in mortality between our results and those of the aforementioned authors could be explained by the difference between dilution doses, the lowest of which is 4000 mg/kg. This study showed that the *A. achatina* snail is more sensitive to glyphosate. The low resistance

capacity of *A. fulica* to glyphosate is explained by the large amount of slime that characterizes this species which would contain substances capable of digesting toxic substances over a period of time; which is not the case of species *A. achatina*. The mucus secreted by snails enhances microbial activity [27,28] which is itself known to increase the degradation, for example, of glyphosate [29]. This major impact of glyphosate on snails could indirectly impact other zoological species of animals such as birds [30]. The decrease or extinction of snails in the wild would result in a significant decrease in bird density and abundance. According to these authors, snails are essential to the diet of birds because they provide them with a source of calcium necessary for the construction of eggshells.

CONCLUSION

At the end of this work, we retain that glyphosate is an inhibitor to the growth of snails *A. achatina* and *A. fulica.* Weight loss and lack of shell growth are proportional to dilution doses. *A. achatina* is the most sensitive species to this active substance. Glyphosate is thought to be one of the main causes of snail extinction in the wild. The rarity of these snails could act on other zoological species. Appropriate measures should be taken to limit the uncontrolled use of this herbicide.

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