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Evaluation of the effect of herbicides on African giant snails: Case of glyphosate on *Achatina achatina*

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Abstract

The study carried out on the *snail Achatina achatina* at the achatinicole farm of the Université Jean Lorougnon guédé aimed to evaluate the effect of glyphosate on the weight and shell growth of this snail. After acclimatization in the breeding tanks for four (4) weeks, 210 snails were divided into seven (7) batches of 30 snails each. These snails are regularly watered and fed every other day with fresh lettuce leaves soaked or not in a diluted solution of glyphosate at different doses: D_0, D_4, D_6, D_8, D_{10}, D_{12} and D_{14}. The weight and shell measurement is done every two weeks. The results obtained show that the weight and linear growth of snails are inhibited according to the dose of glyphosate. All snails exposed to the product are directly impacted. The use of this product by farmers could reduce the availability of snails in orchards and consequently lead to a shortage of snail meat on the markets. In order to anticipate this ecological and environmental problem, this study must be extended to all giant snails consumed in Côte d'Ivoire.

Keywords: Giant snails; Glyphosate; *Achatina achatina;* Herbicides

1. Introduction

The forest is a privileged habitat for all animal and plant species. Its area was estimated in Côte d'Ivoire at more than fifteen million hectares before the country's independence. But today, the forest covers less than three million hectares of area [6]. This significant drop in Ivorian forest cover is justified by the fact that Côte d'Ivoire's economy is based on agriculture. Thus, several hectares of forest have been decimated in favor of perennial crops. Animal species including snails, which reside there, are doomed to extinction in the face of this disappearance. The snails are forced to find refuge in the fields (cocoa, rubber, coffee fields...) which serve as shelters. However, losses in agricultural yields related to diseases, pests but also and especially weeds [19] will lead to the increased use of phytosanitary products (PPS). Indeed, this method of control, very common in field crops, makes it possible to reduce weeding time and optimize farm yields. In addition, chemical control can reduce weeding time by 40 to 60% and increase production by 10 to 20% compared to manual weeding [18]. This method is cited [12, 13] as an economic practice in industrial oil palm plantations. Today, labor is becoming increasingly scarce and when it exists, is very expensive [10] Pesticides have thus allowed the development of agriculture and contributed to the increase in yields and the regulation of agricultural production in the world. Based on product targets, herbicides are the most used worldwide (46.9%) ahead of fungicides (25.9%) insecticides (24.1%) and others (rondenticides, molluscides, etc.). [21]. In developing countries, even highly toxic products banned in developed countries are still widely used [2]. About 30% of pesticides marketed in developing countries do not comply with international quality standards, as they contain a lot of very toxic impurities [1]." The use of plant protection products has also limited or eradicated a number of highly lethal parasitic diseases. However, today these products are suspected of causing a risk to human health and his environment. They are frequently implicated in the degradation of fresh and coastal waters and in the reduction of terrestrial biodiversity observed in agricultural

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areas. Neurotoxic carcinogenic effects or endocrine disruption of pesticides have been demonstrated in humans [1]. The question of the risks for the snails that reside there therefore remains raised both at the level of their growth and at the level of their reproduction. In Côte d'Ivoire, 1 snail is a highly valued commodity [16]. Its tender, tasty flesh, very rich in proteins, minerals and especially iron, represents an alternative source of animal protein in both rural and urban areas [5]. On the other hand, the use of glyphosate-based herbicides continues to grow especially in the agricultural field where large quantities of active ingredients are used in the treatment of orchards and / or weeding areas. According to [17] glyphosate commonly known as "all burned" in peasant environments is the most used active ingredient in the Nawa region (south-west of Côte d'Ivoire). On the one hand, the purpose of that increased use of that herbicide is justified by the fact that it contains agricultural fertilisers. On the other hand, it contributes to a better agricultural yield by limiting the human or animal motive power. However, snails that now have as their gite only the fields following the destruction of their natural environment are exposed to this same herbicide. Also, no study to our knowledge has been devoted in Côte d'Ivoire to the evaluation of the contamination of the snails so prized under our orchards. This is why the overall objective of this study is to assess the impact of a glyphosate-based herbicide with a broad spectrum of use on African giant snails. More specifically, it is a question of evaluating the effect of this phytosanitary product on weight and shell growth in the *snail Achatina achatina*.

2. Material and methods

2.1. Hardware

The site of the Jean Lorougnon Guédé University of Daloa served as a framework to evaluate the effect of pesticides on the growth of the giant African snail. This study focused on a single species of snail belonging to the Achatinidae family: *Achatina achatina*.

The herbicide Kalach 360 with glyphosate as the active ingredient, is the chemical material used during the experiment. According to [17] glyphosate is the most widely used herbicide in the Nawa region (Ivory Coast).

2.2. Methods

A total of 210 *Achatina achatina* specimens with an average weight of 19.48 g and an average shell length of 52.66 mm were acclimatized for 4 weeks in tanks of 1 m2 of area built of bricks and used in this study which lasted sixty (60) days. Seven (07) lots of thirty (30) snails each were formed and arranged in snail snails. Lettuce leaves were best consumed during the snail acclimatization period and served as food for experimentation. The animals were regularly watered and fed every two (2) days with fresh lettuce leaves. Seven (7) dilution doses (D_0, D_4, D_6, D_8 D_{10}, D_{12} and D_{14}) were used in accordance with Table I. Animals are subjected to lettuce leaves soaked in glyphosate solution in different doses only every 6 days. Every 15 days, the live weight and shell length of the snails were determined using a precision scale and an electronic caliper, respectively.

Table 1 Dilution of herbicide used

Glyphosate in mL/L	Dilutions
D_0	Control snails 0 mL/L glyphosate
D_4	Snail treated with 4 mL/L glyphosate
D_6	Snail treated with 6 mL/Lde glyphosate
D_8	Snail treated with 8 mL/L glyphosate
D_{10}	Snail treated with 10 mL/L glyphosate
D_{12}	Snail treated with 12 mL/L glyphosate
D_{14}	Snail treated with 14 mL/L glyphosate

2.3. Statistical analysis

The various measurements obtained were statistically analysed using statistica 7.1 software. They were first subjected to Shapiro-Wilk normality and Bartlett homogeneity tests to verify their distribution and the equality of variances. Then, analyses of variance to a classification criterion (ANOVA 1) followed by Fisher's LSD tests were applied to compare the different means. The differences were considered significant at the 5% threshold ($p < 0.05$).

3. Results

3.1. Effect of glyphosate on shell growth

The results of our study show that glyphosate has a negative effect on shellfish growth (Figure I). Analysis of histograms reveals an inhibitory effect of glyphosate on the shell growth of *Achatina achatina* snails. Thus, there is a low or no growth rate of the length of shells of snails subjected to different doses of glyphosate. After 60 days of experimentation, shell lengths increased from 54.58 to 55.99 mm, from 54.15 to 54.78 mm, from 56.96 to 57.48 mm, from 56.03 to 56.27 mm, from 52.66 to 52.77 and from 54.81 to 55.02 mm, respectively for doses D_2, D_4, D_6, D_8, D_{10}, D_{12} and D_{14}. In contrast, control snails experience a significant increase in shell length from 56.52 to 62.53 mm. Analysis of the data through Statistica 7.1 shows that there is a significant difference ($p<0.05$) between the shell length of control snails and that of snails subjected to different doses of glyphosate from the 60th day of experimentation (Table II). On the other hand, there is no statistical difference between the shell lengths of snails subjected to different doses of glyphosate.

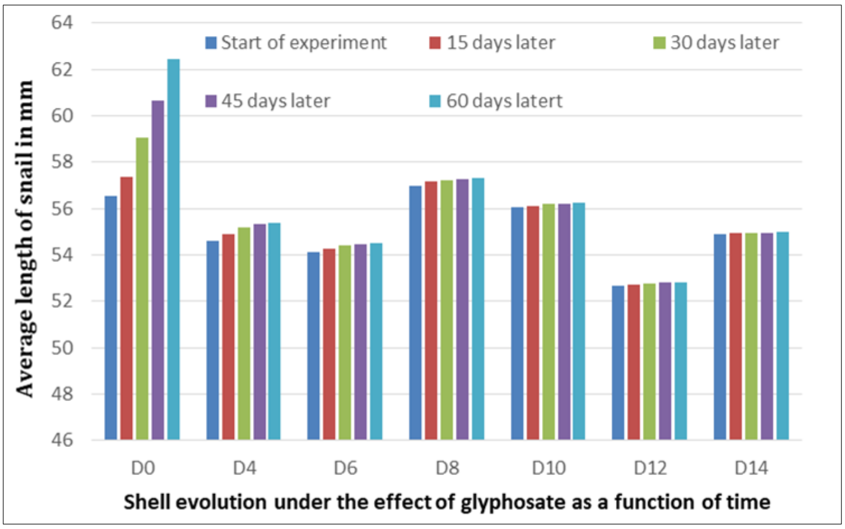

Figure 1 Effect of glyphosate on the shell growth of *Achatina achatina*

Table 2 Fisher's LSD test: analysis of differences between the sizes of *Achatina achatina* at T0, T15, T30, T45 and T60 subjected to glyphosate

Time	D_0	D_4	D_6	D_8	D_{10}	D_{12}	D_{14}
T_0	56.52±4.04^b	54.58±1.30^a	54.15±4.40^a	56.96±1.52^a	56.03±4.47^a	52.66±2.1a	54.81±0.86^a
T_{15}	57.59±3.52^b	54.92±1.75^a	55.14±3.40^a	57.31±3.09^a	56.05±1.96^a	52.72±2.14^a	54.93±4.72^a
T_{30}	58.50±3.52^b	54.87±1.75^a	54.3±1.11^a	57.40±1.40^a	56.09±1.08^a	52.77±2.07^a	54.96±5.17a
T_{45}	60,66±2.12^a	55.83±2.04^a	54.78±1.25^a	57.46±1.17^a	56.21±1.66^a	52.78±0.86^a	54.96±6.08^a
T_{60}	62.53±1.44^a	55.99±1.85^a	54.78±1.20^a	57.48±0.72^a	56.27±1.4a	52.78±0.70^a	55.02±1.20^a
F	3,93	2,3	0,65	0,4	0,06	0,00	<0.01
P	<0.001	0,51	0,70	0,82	1	1	1

NB: On the same column, the indexed average values of the same letters are not statistically different ($p < 0.05$); The letters correspond to the ranks obtained at the end of the classification of the doses; Highly significant: $p \leq 0.001$; very significant: $p \leq 0.01$; p: % probability; F: Smallest Significant Difference test: Gly: glyphosate, D_0: control tank, D_4: 4g gly dose, D_6: 6g gly dose, D_8: 8g gly dose, D_{10}: 10g gly dose, D_{12}: 12g gly dose, D_{14}: 14g gly dose; The average values of the rows indexed by the same letters are not statistically different at the 5% threshold according to the Fisher LSD test

3.2. Effect of glyphosate on the weight growth of snails

The results obtained show that glyphosate negatively impacts the weight growth of treated snails, as shown in Figure 2. Analysis of weight growth histograms shows that all snails subjected to glyphosate lost weight. Snails subjected to doses D_4, D_6 and D_8 mL/L record less weight loss unlike those subjected to doses 10, 12 and 14 mL/L which record significant weight losses from 19.65 to 13.98 g, from 19.80 to 11.84 g and from 19.59 to 10g respectively. In addition, only control snails experience a remarkable increase in weight, from 19.48 to 25.26 g. Thus, Fisher's LSD test performed on all live weight data of snail batches grouped according to different doses of glyphosate shows that there is a significant difference ($p<0.05$) between the live weight of controls and that of snails treated over time.

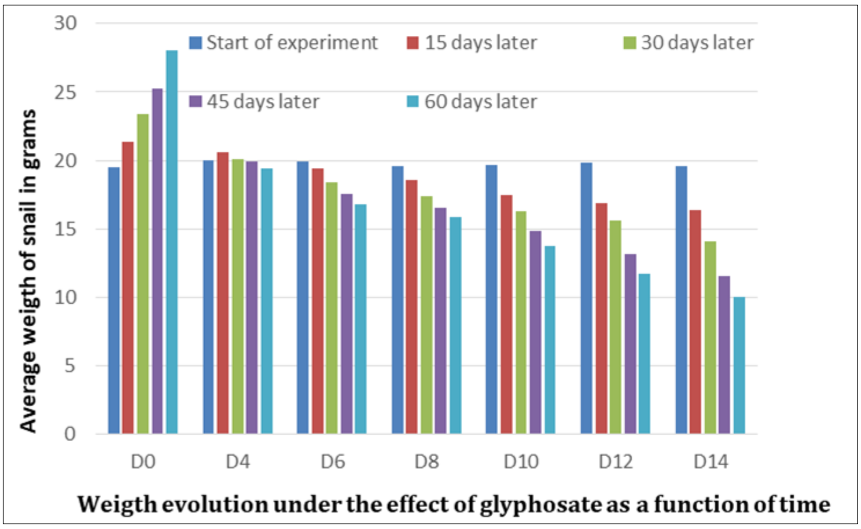

Figure 2 Effect of glyphosate on the average weight of *Achatina achatina* snails

Table 3 Fisher's LSD test: analysis of differences between the weights of *Achatina achatina* at T0, T15, T30, T45 and T60 subjected to glyphosate

TIME	D_0	D_4	D_6	D_8	D_{10}	D_{12}	D_{14}
T_0	$19.49±0.80^d$	$19.83±4.09^a$	$19.88±4.33^a$	$19.62±5.25^a$	$19.65±4.47^a$	$19.80±6.77^a$	$19.59±5.76^a$
T_{15}	$21.31±1.1^{cd}$	$20.65±1.39^a$	$19.45±2.64^a$	$18.5±1.7^{ab}$	$17.54±1.7^{ab}$	$16.9±2.75^b$	$16.33±2.62^b$
T_{30}	$23.27±1.9^{bc}$	$20.05±1.3^a$	$18.22±2.62^b$	$17.06±2.06^{bc}$	$16.41±2.57^b$	$15.57±1.6^{bc}$	$14.05±2.17^c$
T_{45}	$25.03±2.89^b$	$20.01±2.94^a$	$17.46±1.64^c$	$15.98±2.5^c$	$15.03±3.72^{bc}$	$13.35±2.57^{cd}$	$11.51±1.9^d$
T_{60}	$27.88±4.01^a$	$19.44±3.8^a$	$17.02±2.32^c$	$15.61±2.31^c$	$13.98±3.35^c$	$11.84±2.08^d$	$10.00±1.72^d$
F	52,31	0,53	4,22	7,3	11,18	16,04	31,94
P	<0.01	0,72	<0.01	<0.001	<0.001	<0.001	<0.01

NB: On the same column, the indexed average values of the same letters are not statistically different ($p < 0.05$); The letters correspond to the ranks obtained at the end of the classification of the doses; Highly significant: $p \leq 0.001$; very significant: $p \leq 0.01$; p: % probability; F: Smallest Significant Difference test: D_0: control tank, D_4: 4 g dose, D_6: 6g dose, D_8: 8g dose, D_{10}: 10g dose, D_{12}: 12g dose, D_{14}: 14g dose; The average values of the rows indexed by the same letters are not statistically different at the 5% threshold according to the Fisher LSD test

4. Discussion

The weight loss and consistency observed in the shell lengths of snails subjected to different doses of incorporation of the feed to glyphosate; highlight the influence of glyphosate on the weight and shell growth of snails (*Achatina achatina*). The live weight of control snails is growing in contrast to the live weights of snails subjected to different doses of food contamination with glyphosate, which are regressing. This regression of the live weight of snails is a function of the dose of glyphosate which induces a greater toxicity depending on its concentration. This explains the low weight loss observed in snails subjected to doses D_4, D_6 and D_8 mL/L unlike the high weight loss observed in snails subjected to doses D_{10}, D_{12} and D_{14} mL/L. These results corroborate those obtained by authors who show that the evolution of the fresh weight of snails under the effect of pesticides is regressing compared to that of the control [1]. Also, others have reported that the significant reduction ($p<0.05$) in weight (total, shell and soft tissue) of snails treated with aminocarb, methyl parathion and paraquat is related to the nature and dose of pesticide administered [20]. For their part Coeurdassier *et al.* [3] observed a dose-dependent decrease in snail growth and survival induced by dimethoate. Therefore, exposure of snails through the epithelial route induces greater toxicity. This explains the growth in shell length of control snails, not exposed to glyphosate compared to the inhibition of shell growth of snails exposed to glyphosate.

On the other hand, all these results obtained on *Achatina achatina*, go against those obtained by Druart *et al.* , [4]. Indeed, during his work, this author showed that glyphosate had no effect on the growth (total mass and shell diameter) of snails exposed to 2.8 $^{mg.kg-1}$. This resulted in shell maturation and rapid growth during the first 56 days of experience. This difference between the results obtained could be explained by the large difference in the concentration rate of the active ingredient used. Indeed, in our study, the lowest glyphosate solution is 4 ml / l or 4g. Kg-1 much higher than the rate of 2.8 $^{mg.kg-1}$ used by this author.

The weight-dependent dose decrease and the absence of glyphosate-related shell growth in our results could also be explained by a possible interference of glyphosate in calcium (Ca) metabolism. Indeed, calcium plays an essential role in the development of the entire body of the snail and in particular that of the shell. This has been shown in *Helix aspersa* [9] and *Achatina fulicas* [14]. These authors explain this effect by an increase in thickness and shell mass. The shell consists mainly of calcium carbonate and an organic matrix called chonchiolin [11]. Thus, it has been shown a 30% decrease in the mass of the shell of snails exposed to artificial soil contaminated with calcium [7]. However, Jordan et al. [15] did not find a significant relationship between soil pollution by ETMs (Cd, Cr, Pb and Zn) and the strength of shells of *Cepea nemoralis* snails collected in situ.

In fact, the mechanism involved in inhibiting the growth of snails fed contaminated food is difficult to identify [8]. It could be a synthetic inhibition of a growth hormone essential for the growth of snails. In addition, this blockage could also be explained by the repellent nature of glyphosate. Indeed, we found that the snails refused to feed, after the first intake of food which forced them to dieback.

5. Conclusion

The present study, which evaluated the effect of glyphosate on the growth of the *Achatina achatina* snail, concludes that glyphosate has a definite effect on shell length and live weight of snails. This active substance causes weight loss of snails and limits the shell growth of them. In addition, it could be a source of toxicity for snails exposed to the herbicide both in the laboratory and in farmers. The inhibition of the weight and linear growth of snails is a function of the concentration of glyphosate in the solution. One of the main limitations of our study is that it was only circumscribed around *Achatina achatina.* However, the species *Achatina fulica* and *archachatina ventricosa* are also marketed in the different markets of the country. In the interest of food self-sufficiency, the impact of this herbicide on their growth could reduce their availability and consequently lead to a shortage of snail meat on the markets. Thus, in order to anticipate this problem, this study must be extended to all giant snails consumed in Côte d'Ivoire.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest

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