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Full Length Research Paper

Impact of the insecticide endosulfan on growth of the African giant snail *Achatina achatina* (L.)

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The impact of the insecticide endosulfan was assessed on the growth of the African giant snails, litter-living animals found in cocoa fields throughout tropical Africa. Two doses of endosulfan, C1, 6.25 a.i g/l and C2, 12.50 a.i g/l were applied twice with one month of interval to the litter of the snails. After quarantine, snails were allowed to feed on this litter and the elongation of their shells as well as their growth was measured every nine days. The results, daily weight gain (g/j) of -0.028 ± 0.004 for C1 and -0.033 ± 0.007 for C2 showed that snails which received endosulfan in their feed had a very weak growth compared to the control one, which have a daily weight gain of 0.032 ± 0.006. The product seems to disrupt weight regulation. But the insecticide had little influence on the shell elongation. High dose of endosulfan reduced more the weight gain and shell elongation compared to the recommended dose for insects control in cocoa plantation. These results implied that the repeated application of endosulfan for pest control in cocoa plantations may impair the growth of African giant snails.

Key words: *Achatina*, endosulfan, pest control, insecticide, cocoa.

INTRODUCTION

The use of pesticides combined with irrigation, fertilisation and mechanisation helps to reduce damage to crops and maintain food production in order to feed African's growing population. But continuous use of pesticides may cause serious problems mainly due to their toxicity and environmental impact (Ngom, 1992; Gibsons et al., 1987; Niang, 1996). The African giant snail *Achatina achatina* (Linné) is found mainly in cocoa plantations from southern Côte d'Ivoire. They are vegetarian and live under the litter of fallen cocoa leaves. They are frequently gathered from cocoa plantations and sold on the markets for consumption. They are good source of proteins (13.42%), iron (49.07 mg/kg) and calcium (0.29 mg/kg). Their flesh is poor in fat (0.62%) compared to chicken (5.7%), making them a very good dietary item (Zongo et al., 1990). A large amount of pesticides are used in cocoa production for the pest control. Endosulfan is one of the insecticides used. It is a central nervous system poison (IPCS, 1988). Acute intoxication may lead to neurological disturbances such as irritability, restless-ness, muscular twitching, and convulsions that may end in death (NRC, 1975). The aim of the present study is to evaluate the effect of endosulfan on the growth of *A. achatina* exposed to litter-treated with different rates of endosulfan under field conditions. Growth was assessed by measuring the snail weight and the shell length.

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MATERIALS AND METHODS

Test organisms and raising procedures

Young snails (sub-adults) (*A. Achatina*) with normal shell were

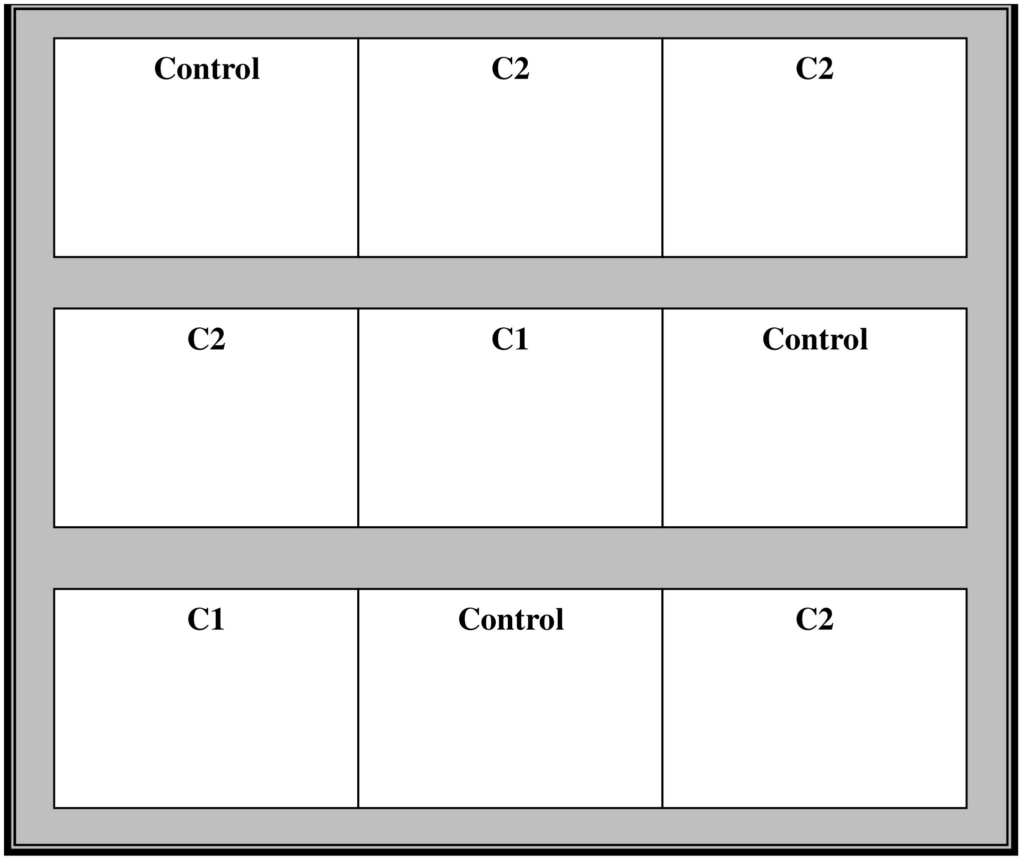

Figure 1. Illustration of the experimental setup and enclosure composed of three rectangular blocks (4.0 x 1.5 m) inside a fence of coconut palms around which passion fruit trees.

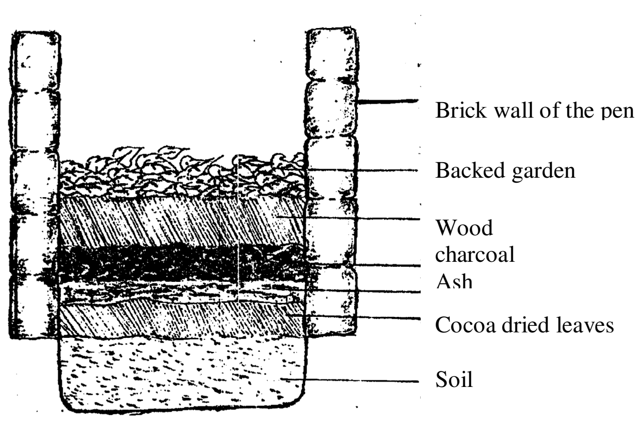

Figure 2. Longitudinal view of the litter.

bought from the market of Yamoussoukro in central Côte d'Ivoire and fed with cocoa (Cocoa theobroma Sterculiacées) leaves previously sun-dried. These leaves were chosen in order to simulate natural conditions. After 3 weeks, 162 snails in good condition were chosen for the study. Their mean weight was 29±0.8 g and the length of their shell was 58±0.5 mm. The snails were keptin an enclosure built on the experimental farm of the National Agricultural University (Yamoussoukro). The enclosure is composed of three rectangular blocks built with bricks (Figure 1). Shading was provided by a fence of coconut palms around which passion fruit trees (*Passiflora* sp., Passifloracea) were grown. Each box was covered with a nylon screen to confine snails and keep out birds and other predators. The litter (Figure 2) in each box was approximately 20 cm thick and was made at the bottom of loose uncompacted garden soil baked in order to kill all organisms (insects, nematodes, bacteria, etc.) likely to attack snails; followed by a layer of ash to disinfect the box and prevents ants to enter. The third layer was made of wood charcoal which allows water to gently infiltrate in the ground without destroying the pits. A second layer of baked garden soil was added. The last layer was made of dry cocoa tree leaves. In each box a container was installed to keep drinking water at the reach of snails.

Experimental setup

The experimental setup was three treatments with three replicates per treatment. For each treatment, 18 snails (29±0.8 g mean weight; 58±0.5 mm mean length) were confined in each box (4 x 1.5 m) covered with nylon. They were fed with contaminated cocoa leaves. During the experiment, water and calcium (chalk) were fed *ad libitum* to the snails (Ategbo and Zongo, 2000). The experiment was conducted at ambient temperature (24.5°C) with hygrometry reaching 86.4% which conform to the good raising condition (Stievenart, 1995; Zongo, 1994). pH and calcium in the litter were 6.8 and 38.6% respectively; these values were also in agreement with those recommended for snail raising (Stievenart, 1995).

Table 1. Effect of the treatment on the growth of *Achatina achatina*.

Performances	Treatment		
	C0	C1	C2
Initial weight (g)	29.05a±1.28	29.75a ±1.32	29.9a±1.07
Final weight after 5 nine days (g)	31.07a±0.90	27.9b±1.25	27.85b±1.35
Daily weight gain (g/j)	0.032a±0.006	-0.028a±0.004	-0.033a±0.007
Initial shell length (mm)	5.91a±0.24	5.83a±0.35	5.75a±0.16
Final shell length after 5 nine days (mm)	6.47a±0.18	6.04b±0.21	5.97b±0.21
Daily shell elongation (mm/j)	0.09a±0.002	0.003b±0.001	0.003b±0.001

The mean values of the same line with the same superscript are not significantly different at $p<0.05$ according to Duncan test. Co, control: C1, 6.25 g a.i./l endosulfan; C2, 12.50 a.i. g/L of endosulfan.

The insecticide endosulfan, $C_9H_6Cl_6O_3S$ (Figure 5) is a chlorinated hydrocarbon insecticide and acaricide of the cyclodiene subgroup which acts as a poison to a wide variety of insects and mites on contact. It is used primarily on a wide variety of food crops including tea, coffee, fruits, and vegetables, as well as on rice, cereals, maize, sorghum, or other grains. The formulation Thiodan 50EC is applied at least once every two months for the control of insects in young cocoa plantations. The dose recommended by the CNRA, the National Center for Recherche in Agriculture is 0.50 L of Thiodan/ha or 125 ml for 10 L of water (Kebe et al., 2005).

Pesticide solutions were prepared as follows: 0.30 and 0.60 ml of the formulation (Thiodan 50EC) were added in a volumetric flask and 24 ml of distillate water was added to obtain a solution leading respectively to 6.25 and 12.50 a.i. g/L of endosulfan which corresponds respectively to the rate and twice the rate prescribed in cocoa plantation. All the solution was used to spray the litter of each box at the beginning of the experiment and one month later. The litter was thoroughly mixed to ensure even distribution of the pesticide solution. Distillate water was sprayed on the boxes which act as control.

Growth measurements

The length (shell length) and the weight of the snails in each treatment were measured. Before that, the snails were washed in order to eliminate soil and leaves caught by their pods. The length was measured with a laboratory slide calliper and the weight was obtained with a balance (Sartorius, precision 0.01g).

Results expression and data analysis

Weight growth and shell elongation were estimated from the daily mean growth every nine days and are expressed as mean weight growth (g/j) and mean shell elongation (mm/j).

$$GR - \frac{W_2-W_1}{T_2-T_1} \quad (1)$$

Mean growth rate:

P1: initial mean weight
P2: final mean weight
T1: initial time in days
T2: final time in days

$$MSE = \frac{L_2-L_1}{T_2-T_2} \quad (2)$$

Mean shell elongation:

L2: initial mean length
L1: final mean length
T1: initial time in days
T2: final time in days

SPSS was used to appreciate the factorial effects by ANOVA, the comparison of mean values according to the Duncan test. Results are presented as mean plus the standard deviation (Dagnelie, 1975).

RESULTS AND DISCUSSION

Mortality

The mortality observed with the control group is about the same as with the treated ones. The same rate of mortality (26%) was observed by Zongo (1994) in a normal raising condition. Thus, endosulfan seems not to induce acute toxicity in giant snails. Similar results were observed with the terrestrial isopod *Porcellio dilatatus* put in contact with endosulfan (Ribeiro et al., 2001) and bivalve molluscs (*Pisidium* spp.), after 24 h exposure to spray-drift contaminated water (Ernst et al., 2009).

Effect of Thiodan 50 EC on the weight growth and shell elongation of snails

Table 1 shows the growth characteristics of the snails on the three substratums. The results showed that for all the treatment, the weight gain of snails was very weak and was not stable after the application of the insecticide The daily weight gain was negative for the treatment (-0.033 ± 0.007 to -0.028 ± 0.004) compared to the control (0.032 ± 0.006 g/j). The comparison of mean growth rate by ANOVA using Duncan test ($p<0.05$) showed a significant difference between snails subjected to the insecticide and the control ones.

Temporal analysis of the growth (Figure 3) showed the rise of the weight of control animals during the first week to stabilize around 31.07±0.90 g. For the animals in contact with the insecticide, their weight was always

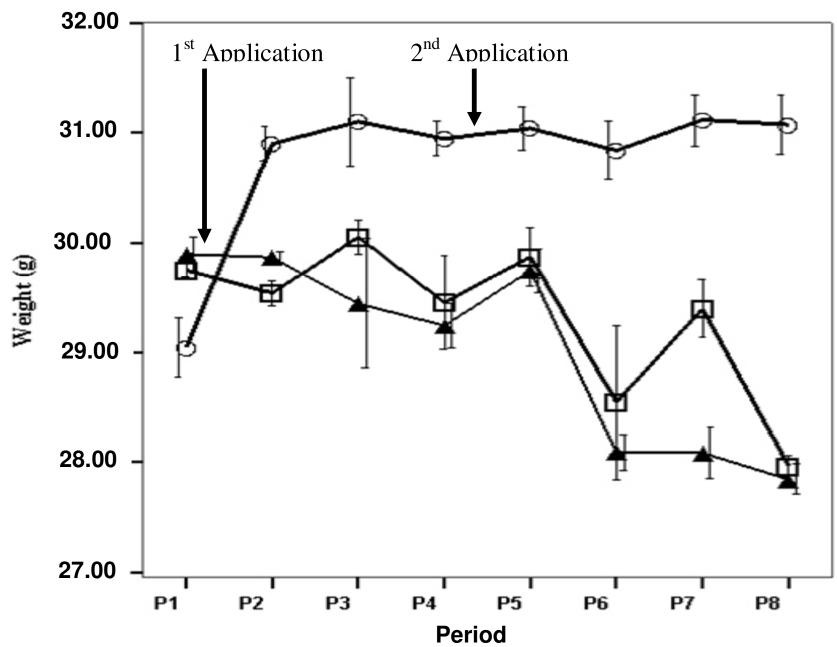

Figure 3. Evolution of weight gain of the snails; (○) control. (□) treatment C1. (▲) treatment C2. These various points represent the averages of each treatment at each period of measurement

below the weight of control animals and they follow two patterns. From the beginning of the assay to the 45th days (P5) during which the weight remain almost stable at 29.87±1,51 g. After this period, subsequent to the second application of the insecticide, the weight declined to around 6/0 g. The difference observed, mostly after the second application of the insecticide, in the weight gain between the snails in contact with the insecticide and those of the control indicated an effect of the insecticide on the weight gain of snails. It seems that endosulfan by contact or ingestion may have a negative retro-control on the regulation of the growth. Indeed, it was shown that external stimuli like odour could have an effect on the regulation of the growth of snails (Gomot and Vaufleury 2000).

The weakness of the growth of the snails observed in all the treatment may be attributed in part to the influence of the growth substratum. In nature, soil constituted the support in which snails take around 40% of their nutriments (Kouassi et al., 2007). On the other hand, studies have shown the higher susceptibility of freshwater snails to endosulfan, particularly to longer exposures (Oliveira-Filho et al., 2005). Sublethal concentrations caused adverse effects in the digestive gland, foot and mantle of the great ramshom snail in small ponds and streams (Otludil et al., 2004). But as the results show, these effects are less in terrestrial environment due to the low persistence of endosulfan in tropical soil. The daily mean shell elongation of the control snail (0.09 ± 0.002) was significantly different ($p < 0.05$) from the mean elongation of the snails subjected to the insecticide whereas no significant difference was found between snails subjected to the insecticide at the two different rates ($p < 0.05$). Mean shell length measured on control snails varied from 5.91 ± 0.24 to 6.47± 0.18 mm, which represents a daily length gain of 0.09 ± 0.002 mm/day. These results are somehow under those obtained by Waitkuwait (1987) which are around 0.16 mm/day. These results can be explained by growth troubles observed on snails even those fed correctly and raised in good conditions (Ministère de l'Agriculture, 2003). The observed stress may be due to frequent measures done on these snails with the slide calliper which can cause rupture on neo-formed parts leading to reduction of their shell growth. As shown by Figure 4, there were two patterns in shell elongation. During the first period from the beginning of the assay to the 27th day (P4), no significant difference ($p < 0.005$) was observed between the control snails and those subjected to the insecticide at the level C1 whereas there was a significant difference ($p > 0.05$). The second period started from the 27th day to the 63rd day of the experiment; during this period, no significant difference ($p < 0.05$). During the third period starting from day 63 to the end, we observe large increase of shell length of snails from the control compared to those subjected to the insecticide.

These results indicated an effect of the insecticide on shell elongation at the level two during the first period of the exposition to the insecticide. This effect was observed for the first level of application only after the second application of the pesticide. Since it was advised by the CNRA (Kebe et al., 2005) to apply the insecticide once

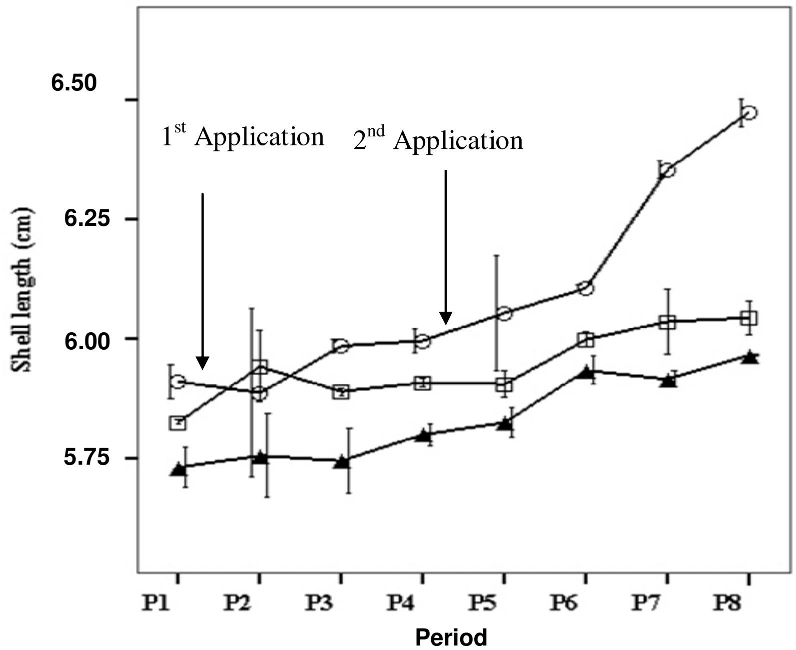

Figure 4. Evolution of the elongation of the snails; (O) control. (□) treatment C1. (▲) treatment C2. These various points represent the averages of each treatment at each period of measurement. Each point represents the mean of three replicate assays

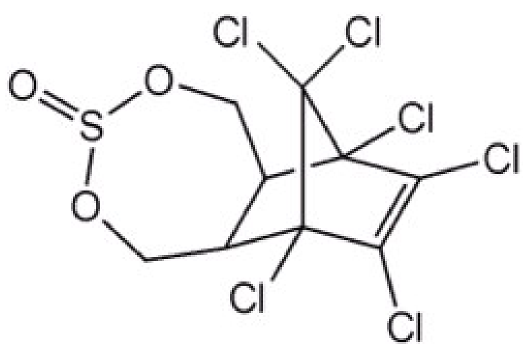

Figure 5. Chemical structure of endosulfan.

every two months that is 6 applications per year, we may conclude a serious negative impact of this insecticide to the African giant snail *A. achatina*. These results seemed to indicate a possible interaction between the rate of application of the pesticide and time; this means that continuous exposure of the snails to endosulfan even at the recommended rate of application may cause a stress to them if the application is repeated during the year. The observed reduction in the elongation due to the exposure to the insecticide may be explained by the fact that the insecticide could prevent the ingestion of calcium which is necessary to the formation of the shell. It was found that the snails subjected to stressing conditions of breeding present a slow shell production and of bad quality (Ministère de la coopération française, 2003).

Conclusion

The mortalities obtained in this test in the control snails (23,3%) and with snails which were in contact with the insecticide (25%) indicated that the product presents no acute toxicity hazard to the snails, even at twice the rate of application recommended in cocoa culture. Endosulfan seems to have a chronic toxic effect on the snails but this effect is very light even doubling the application rate of the insecticide. This toxic effect would result in reduction of the growth and shell elongation. The insecticide may have a deterrent effect on food ingestion by these animals. Long term negative impact of the insecticide was demonstrated by the study on both growth and shell elongation. But these effects may be attenuated by the fact that in plants, endosulfan is rapidly broken down to the corresponding sulphate. But monitoring data on incident reports confirm that endosulfan is moving through aquatic and terrestrial food chains and that its use has resulted in adverse effects on the environment adjacent to and distant from its registered use sites (US EPA, 2007). Besides that, one can attribute the weakness of the growth parameters obtained in this study by the lack of calcium on the substratum because the development of snails required a complementation of the diet with calcium from grocery or from shell powder.

Further work should evaluate the rates of consumption and assimilation in order to verify the effect of the product on the ratio of food ingestion/growth rate. Since the snails spent their energy reserve to avoid the product or to detoxify it, it would be desirable to measure the level of glucose, lipid and protein in exposed animals. The next studies should also evaluate herbicides used for weed control in cocoa plantation since these chemicals are directly applied to grasses. They can remain for a long period in the litter where snails live. If possible the level of these products on the flesh of snails should be investigated because these products can be toxic to consumers.

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