

ANTIAPAPETANT AND REPELLENT EFFECT OF ~~THE~~ BIOPESTICIDES ASTOUN 50 EC AND NECO 50 EC ON ~~ELDANA SACCHARINA~~ELDANA SACCHARINA Walker (LEPIDOPTERA: PYRALIDAE) UNDER ~~invitro~~CONTROLLED CONDITIONS

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Abstract

This study is part of the search for means and methods of sustainable control of *Eldana saccharina*, the main pest of sugarcane cultivation in Côte d'Ivoire. Despite efforts to control the pest through the selection of resistant varieties, the cultivation of healthy cuttings and the elimination of alternative host plants, the damage, which is already economically significant, continues to increase in the sugarcane-growing areas of Côte d'Ivoire. The aim of this study, ~~was therefore~~ to evaluate the effect of two proven biopesticides, ASTOUN 50 EC and NECO 50 EC, on the palatability of ~~E. saecharina~~*E. saccharina* larvae and adults. Firstly, different ages of larvae were reared on ~~two~~ nutrient media ~~such as artificial medium and natural medium~~ treated with increasing concentrations of the two biopesticides. ~~Two nutrient media were used: an artificial medium and a natural medium.~~ ~~The larvae were~~ 14, 21 and 28 days old ~~larvae were incubated and~~ ~~After incubation,~~ changes in larval palatability were assessed over a 5-day period. The concentrations of biopesticide tested were 0.5, 1, 2, 4, 8 and 10 ml/l. In addition, the repellent effect of the biopesticides on the adults was assessed using the preferential zone method. The results showed that the biopesticide ASTOUN 50 EC is highly anti-apertaining for larvae and repellent for adults of *E. saccharina*, unlike NECO 50 EC. It also greatly reduced the rate of oviposition in the treated compartments. This biopesticide can ~~therefore~~ be tested *in vivo* for use against *Eldana saccharina*.

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Keywords: *Eldana saccharina*, biopesticide, sustainable control, sugar cane, Ivory Coast

1. Introduction

Eldana saccharina is the main constraint to sugarcane cultivation in Côte d'Ivoire [1]. The larva of this pest, which constitutes the harmful phase, digs galleries in the cane stalks and feeds on the cane pulp, which is supposed to accumulate sugar at maturity. The galleries left in the stalks make the plant more vulnerable to disease and other pests such as termites and rodents, as well as to bad weather, which causes more lodging than usual. As a result, ~~Eldana saecharina~~*Eldana saccharina* causes huge losses in terms of both quantity and quality in sugar cane cultivation [2, 3]. Statistics show losses of up to 0.1% for every 1% of cane attacked, and up to 70% of the sugar content in attacked internodes. Since the 2015-2016 sugar campaigns, when attack rates exceeded the critical threshold of 6% [1], control trials have been carried out in sugarcane production areas in order to get to grips with this pest, whose damage is increasing year on year. These control methods, which

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are mainly based on eliminating alternative host plants, destroying crop residues and growing resistant varieties, have shown their limitations. Hence the need to develop other control methods that can be used on their own or as part of an integrated pest management system. ~~It is in this context that this study was initiated.~~ Its aim is to demonstrate the efficacy of biopesticides such as ASTOUN 50 EC and Neco 50 EC, which ~~have been~~ shown to have insect-repellent and insecticidal potential against a wide range of pests [4, 5, 6]. It was carried out in vitro on ~~Eldana saecharina~~*Eldana saccharina* larvae and adults.

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1. Materials and methods

The plant material was sugar cane (*Saccharum officinarum*) and the variety was R570 ~~used for the study.~~ Cane stalks of this variety with a diameter of between 0.9 and 1.5 cm were cut into pieces 3.5 to 4.3 cm long. These pieces of cane stalk were used to feed the ~~Eldana~~ *saccharina* larvae and formed the natural nutrient medium. In addition to this nutrient medium, an artificial medium was prepared following the method used by [7]. The composition of this medium is given in Table 1.

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The animal material consisted of ~~Eldana saecharina~~*Eldana saccharina* larvae and adults ~~and 14, 21 and 28 day old larvae.~~ Different ages of larvae were used. ~~These were 14, 21 and 28 day old larvae.~~ For adults, only imagos were used.

The technical equipment consisted mainly of rearing dishes for ~~Eldana saecharina~~*Eldana saccharina* larvae and adults, alcohol and sodium hypochlorite.

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The biological control materials tested consisted of the biopesticides ASTOUN 50 EC and NECO 50 EC. The biopesticide ASTOUN 50 EC comprises neral (39.33%), α -citral (31.89%), sulcatone (2.49%), oxygenated monoterpenes (73.71%) and hydrocarbon monoterpenes represented by β -myrcene (26.29%) [8]. As for NECO 50 EC, the major ~~itaires~~ ~~components~~ ~~antes~~ are: Thymol, Gamma-Terpinene and Eugenol [9]. These two biopesticides, obtained ~~from at the~~ Industrial Research Unit of the Université Félix Houphouët Boigny, are registered in Côte d'Ivoire.

Table 1: Composition of the artificial nutrient medium

Ingredient	Quantity	Role
Canne à sucre séchée et écrasée	22 g	Nutrients
Pois chiches en poudre	120 g	
Saccharose	10 g	
Caséine	12 g	
Levure de boulangerie	12 g	
Acide ascorbique	4 g	Vitamins
Acide sorbique	2 g	

Agar	10 g	Binding substance
Eau distillée stérile	1 000 mL	
Nipagine	1,6 g	
Formaldéhyde	1.2 mL	Anti-microbial
Méthanol	50 mL	

Source [10]

Multiplication of *E.dana saccharina* larvae and adults

A basic sample of larvae and pupae was collected from the sugarcane plots. From this sample, rearing was carried out in the laboratory to obtain the number of larvae and adults required for the biopesticide tests. ~~To do this,~~ the adults ~~that~~ had emerged from the larvae and pupae collected in the field were incubated in rearing boxes under conditions of temperature between 26 and 29°C and relative ~~air~~ humidity between 60 and 70%. The eggs were collected every 24 hours and incubated on blotting paper in Petri dishes. Once the eggs ~~had~~ hatched, the larvae were incubated to produce 14-, 21- and 28-day-old larvae and adults. The larvae were reared either on pieces of sugarcane stalk or on an artificial nutrient medium. A total of 520 larvae of each age and 96 adults were used in this study.

Preparation of concentrations and treatment of nutrient media

The two biopesticides were tested at concentrations of 0.5 ml/l, 1 ml/l, 2 ml/l, 4 ml/l, 8 ml/l and 10 ml/l. These concentrations were obtained by diluting distilled water with Tween 20. Pieces of cane stalks were treated by soaking in the various concentrations of ASTOUN 50 EC biopesticide or NECO 50 EC biopesticide for 5 minutes, then exposed to the open air for 5 minutes. As for the artificial medium, the biopesticides were dissolved directly in the nutrient medium. These treated nutrient media were ~~then~~ used to feed the larvae. ~~Two controls were created.~~ One consisted of untreated nutrient media and the other of nutrient media treated with Tween 20 ~~served as control~~.

Evaluation of ~~the effect of~~ biopesticides on the palatability of *E.dana saccharina* larvae

This test was carried out on 14-, 21- and 28-day-old larvae. These larvae were deposited on the treated nutrient media and then ~~their~~ feeding or not was observed at regular 24-hour intervals according to the method used by [11]. A larva was considered to be feeding on the medium when droppings were present in the Petri dish. The number of larvae having consumed the nutrient media was recorded at each observation and the rate of larvae having consumed the nutrient medium (TLC) was calculated according to the following formula

$$\text{TLC(\%)} = \frac{\text{Number of larvae that consumed the medium}}{\text{Total number of larvae}} \times 100$$

TLC : Rate of larvae having consumed the nutrient medium

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Evaluation of the repellent effect of biopesticides on *E. saccharina* imagos

The repellent effect of biopesticides was evaluated using the preferential zone method [12]. For this experiment, the rearing box was divided into two compartments connected by a central opening. On one side were sugarcane leaves treated with biopesticides and on the other, sugarcane leaves treated only with Tween 20. The leaves were soaked for 5 minutes in the different concentrations and then exposed to the air for 5 minutes. Four *E. saccharina* imagos (two females and two males) were then deposited in each compartment. Ten replicates were used for each concentration of biopesticide tested. After 24 hours, the number of adults in each compartment of the rearing box was counted and the percentage repulsion (PR) was calculated using the formula in [12]:

$$PR (\%) = \frac{NC - NT}{NC} \times 100$$

PR: Percentage of repellency; NC: Number of adults in the compartment treated with biopesticides; NT: Number of adults in the compartment treated only with Tween 20.

The average repellency percentage for each biopesticide concentration was calculated (PR) and assigned to one of the different repellency classes established by [12], ranging from 0 to V, recorded in Table 2.

Similarly, the number of eggs laid in each compartment of the rearing box was counted after 24 hours. The rate of eggs laid in the treated compartment (TOPCT) was then calculated using the following formula:

$$TOPCT (\%) = \frac{\text{Number of eggs laid in the treated compartment}}{\text{Total number of eggs laid in the box}} \times 100$$

TOPCT : rate of eggs laid in the treated compartment

Table 2: Biopesticide repellency classification scale

Class	Repulsion interval	Properties
0	$PR \leq 0,1\%$	Not repellent
I	$0,1 < PR \leq 20\%$	Very low repellency
II	$20,1 < PR \leq 40\%$	Weakly repellent

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III	$40,1 < PR \leq 60\%$	Moderately repellent
IV	$60,1 < PR \leq 80\%$	Repellent
V	$80,1 < PR \leq 100\%$	Highly repellent

Source : [12]

Data analysis

The data collected were recorded on the computer using Excel 2013 software, which was also used to draw up the graphs, curves and tables.

The rates of repulsion and eggs laid in the treated compartment were analysed using Statistica version 7.1 software or an analysis of variance was performed. In the event of a significant difference, the averages obtained were classified into homogeneous groups using the Newmann-Keuls test with a threshold of 5 %.

RESULTS

Effect of the biopesticides ASTOUN 50 EC and NECO 50 EC on the palatability of *Edana saccharina* larvae

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Palatability of 14-day-old larvae on treated nutrient media

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❖ In the presence of the biopesticide ASTOUN 50 EC

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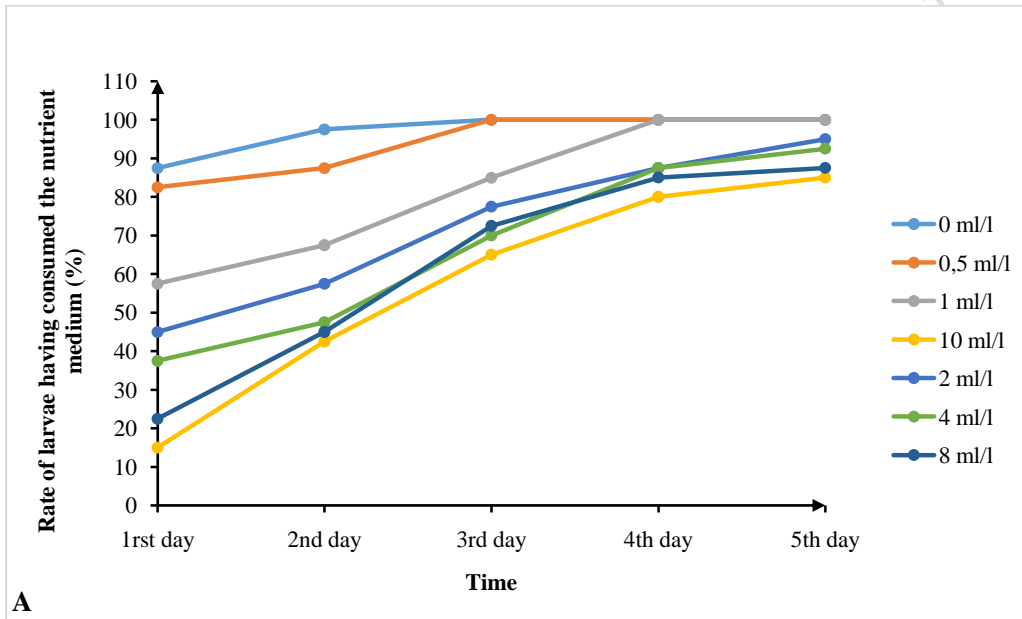
One day after culturing on nutrient media treated with the biopesticide ASTOUN 50 EC, all concentrations induced a lower palatability in 14-day-old larvae than in the control, regardless of the nutrient medium. Over 50% of the larvae did not consume the nutrient media treated at concentrations of 2 ml/l and above, whereas 87.5% of the larvae were already feeding on the control. Thereafter, larval palatability increased sharply over time. However, until the end of the observations, the palatability of 14-day-old larvae was significantly reduced by the biopesticide ASTOUN 50 EC from the concentration of 2 ml/l. The lowest rate of larvae consuming the treated nutrient media was obtained with concentrations of 8 ml/l and 10 ml/l (figure 1).

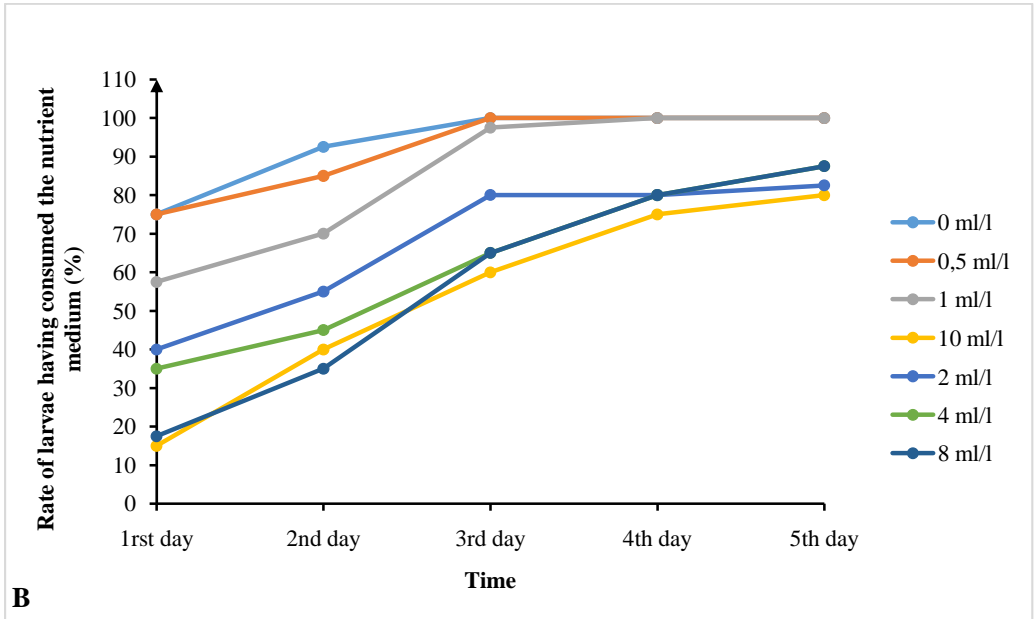
❖ In the presence of the biopesticide NECO 50EC

The palatability of 14-day-old larvae on nutrient media treated with the biopesticide NECO 50 EC was identical or very close to that of the control up to a concentration of 2 ml/l, throughout the observation period. Already on the first day after incubation, more than 70% of the 14-day-old larvae fed on the nutrient media treated, even at the highest concentrations. Larval palatability also increased sharply over time. At the end of the observations, only 15% of the 14-day-old larvae did not feed on the nutrient media treated with the 8 ml/l and 10 ml/l concentrations. All the other

concentrations induced palatability similar to that observed on the control, where 100% of the larvae fed.

Over the entire observation period, 14-day-old larvae fed less on nutrient media treated with the biopesticide ASTOUN 50 EC from 2 ml/l and ~~with~~ NECO 50 EC from 8 ml/l than ~~on~~ the control. After culture, the 14-day-old larvae fed less quickly on the nutrient media treated than ~~on~~ the control. Larval palatability dropped considerably as the concentration of biopesticides in the nutrient medium increased (figure 2).

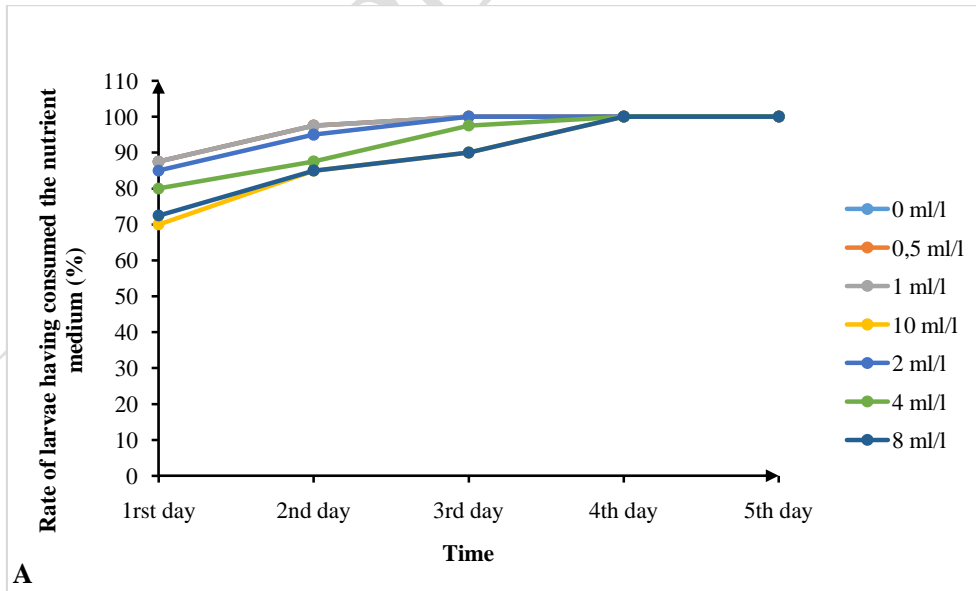


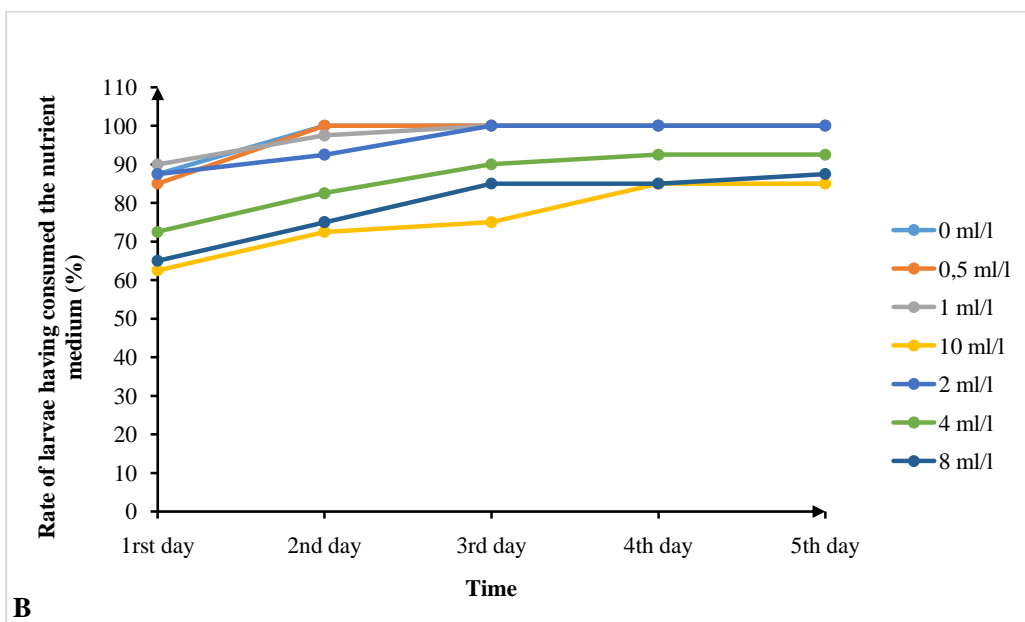


A : On pieces of cane stalk

B : On artificial nutrient medium

Figure 1: Changes in the rate of 14-day-old larvae that consumed nutrient media treated with the biopesticide ASTOUN 50 EC





A : On pieces of cane stalk

B : On artificial nutrient medium

Figure 2: Changes in the rate of 14-day-old larvae that consumed nutrient media treated with the biopesticide NECO 50 EC. Appétence des larves de 21 jours sur les milieux nutritifs traités

❖ **In the presence of the biopesticide ASTOUN 50 EC**

A concentration effect on the palatability of 21-day-old larvae was observed. On the first day after incubation, more than 50% of the larvae had not yet fed on the nutrient media treated with a concentration of 2 ml/l, whereas 85% had fed on the control. The feeding rate then increased sharply with time. At the end of the observations, the highest rate of larvae having consumed the nutrient media was observed on the control and the concentrations of 0.5 and 1 ml/l with values of 100%. The lowest rates were recorded with concentrations of 8 ml/l and 10 ml/l (figure 3).

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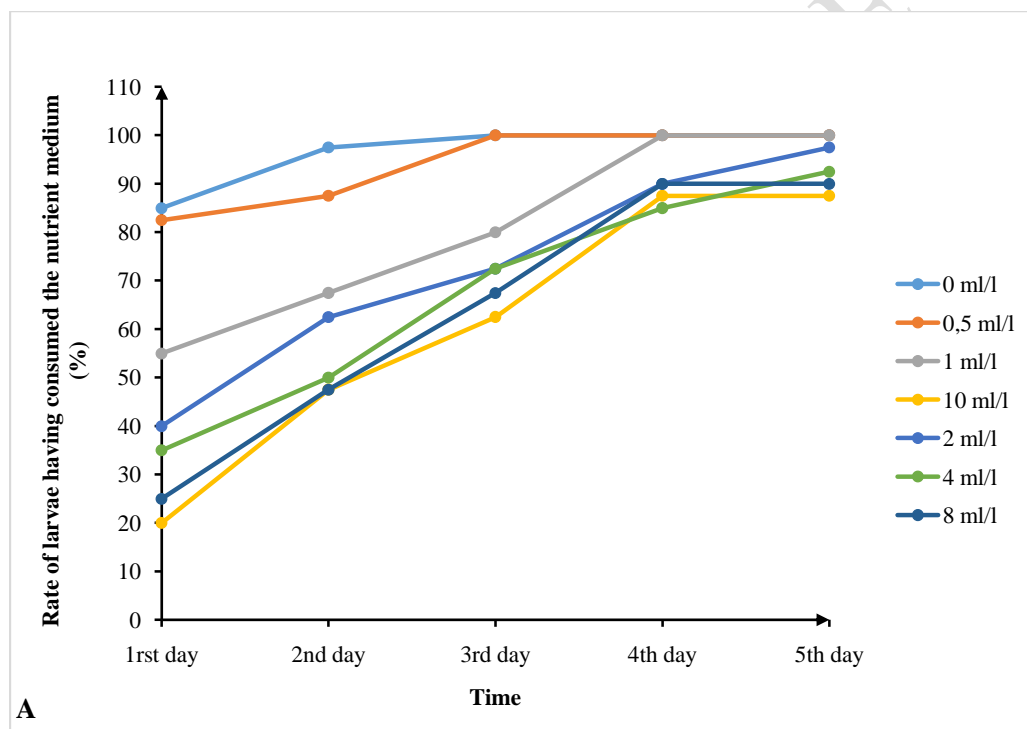
❖ **In the presence of the biopesticide NECO 50 EC**

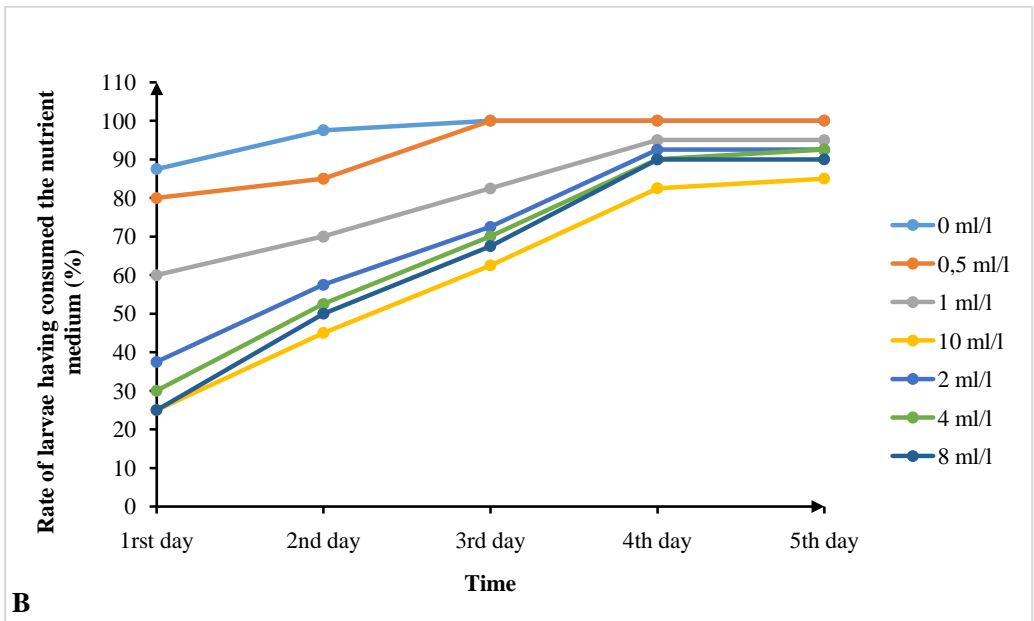
The results show a concentration effect of NECO 50 EC on the palatability of 21-day-old larvae. Also, the rate of larvae having consumed the treated nutrient media increased significantly with time. On the first day after incubation, more than 65% of the larvae had already fed on the media, whatever the concentration. The 8 ml/l and 10 ml/l concentrations stood out with the lowest rates of larvae having consumed the media, i.e. 62.5 and 72.5% respectively on pieces of cane stem and on artificial media. At the end of the observations, all the concentrations recorded a rate of larvae

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having consumed the treated media of over 97%. The control and all concentrations except 10 ml/l resulted in a 100% rate of larvae having fed. Only 2.5% of the larvae did not feed on the nutrient media treated with the 10 ml/l concentration (figure 4).

Thus, after incubation, the 21-day-old larvae fed less rapidly on the nutrient media treated than on the control. Over the entire observation period, 21-day-old larvae fed less on nutrient media treated with the biopesticide ASTOUN 50 EC than on the control from 8 ml/l onwards. Larval palatability dropped considerably when the concentration of ASTOUN 50 EC biopesticide or NECO 50 EC increased in the nutrient medium. The feeding rate of 21-day-old larvae was greater than 80% in the presence of ASTOUN 50 EC biopesticide and NECO 50 EC.

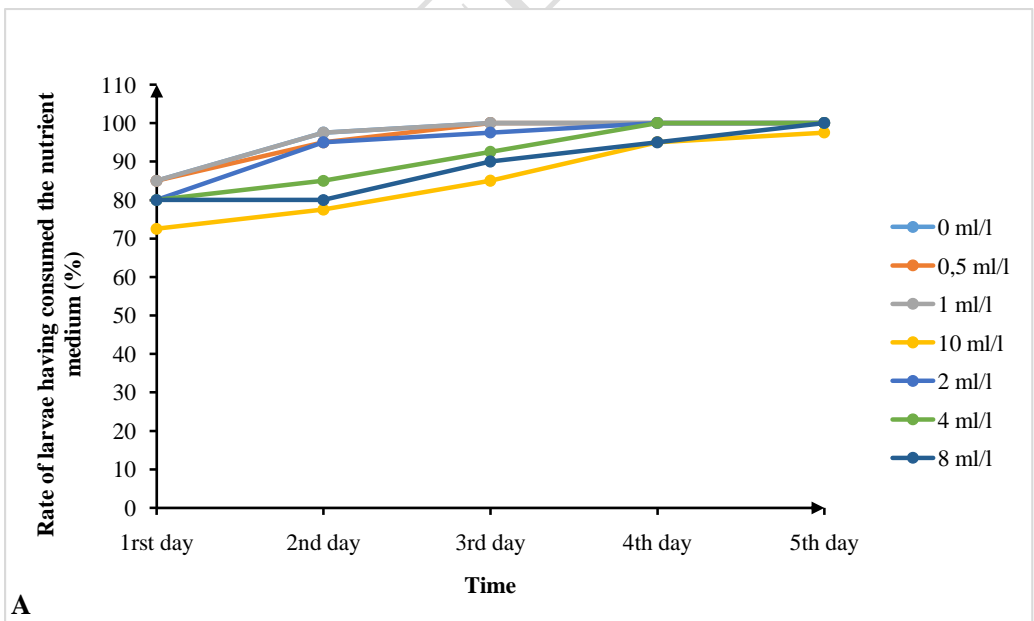


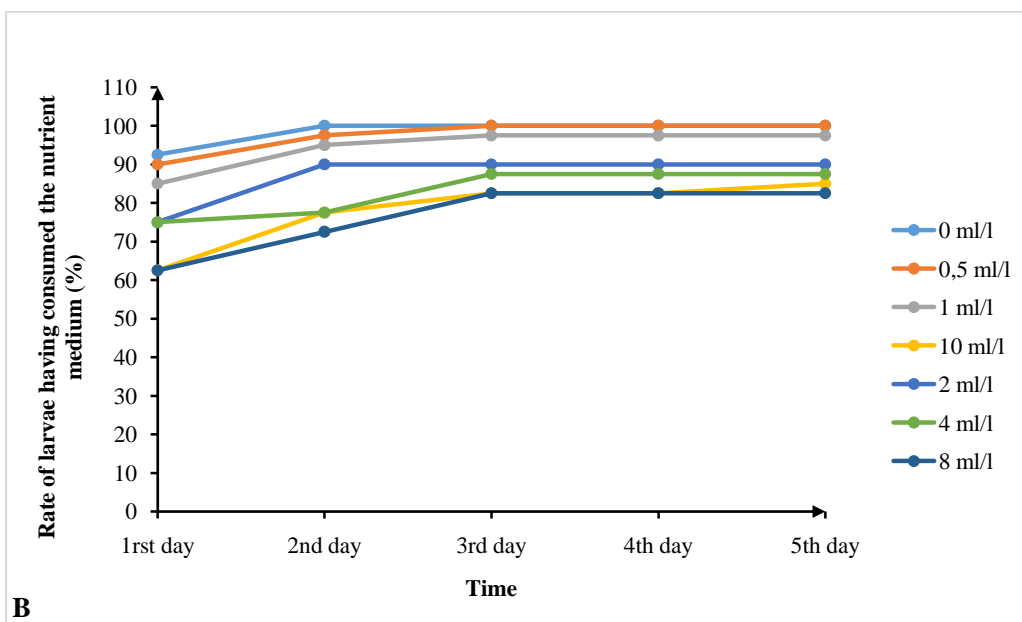


A : On pieces of cane stalk

B : On artificial nutrient medium

Figure 3: Changes in the rate of 21-day-old larvae that consumed nutrient media treated with the biopesticide ASTOUN 50 EC





A : On pieces of cane stalk

B : On artificial nutrient medium

Figure 4: Changes in the rate of 21-day-old larvae that consumed nutrient media treated with the biopesticide NECO 50 EC

Palatability of 28-day-old larvae on treated nutrient media

❖ In the presence of the biopesticide ASTOUN 50 EC

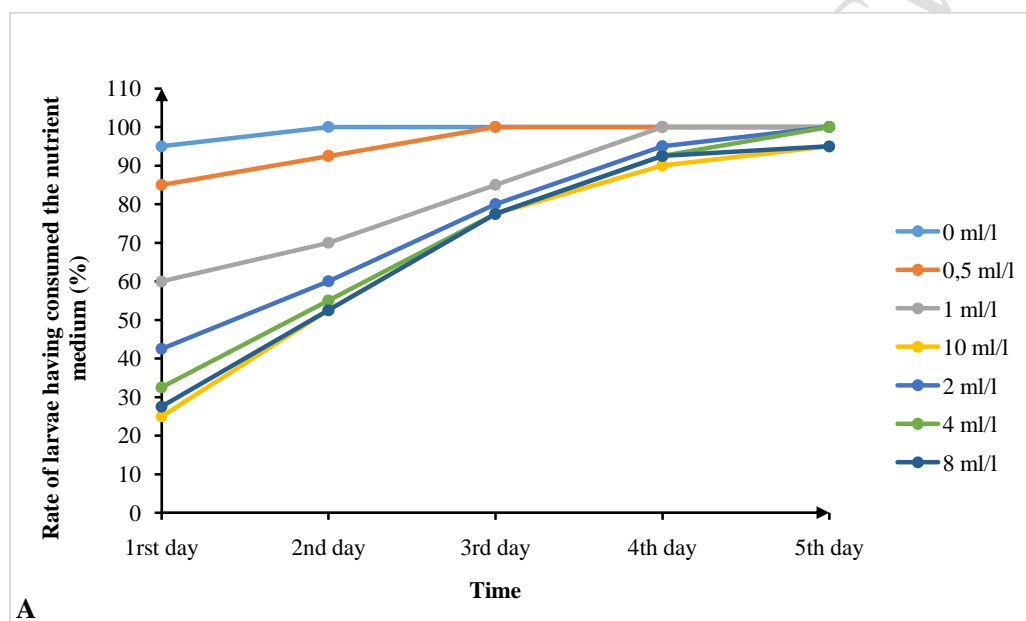
The results show that the rate of larvae having consumed the treated media increased considerably over time and decreased with increasing biopesticide concentration. On the first day after incubation, more than 50% of the 28-day-old larvae had not yet fed on the nutrient media treated at a concentration of 4 ml/l, whereas the control recorded a rate of more than 80%. At the end of the observations, 100% of the larvae had fed on the two artificial media up to a concentration of 4 ml/l. On the nutrient media treated with concentrations of 8 ml/l and 10 ml/l, only 5% of the larvae did not feed (figure 5).

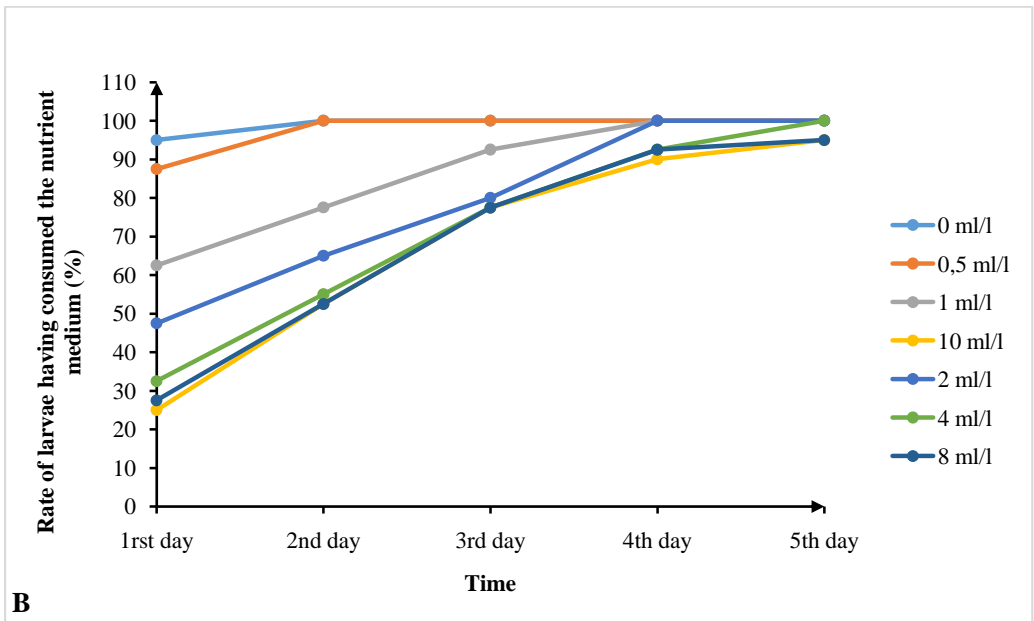
❖ In the presence of the biopesticide NECO 50 EC

The results show that the rate of larvae that consumed the treated nutrient media increased sharply over time for all the concentrations tested. A concentration effect was observed during the first few days of incubation. On the first day after incubation, more than 75% of the larvae fed on the treated nutrient media, whatever the biopesticide concentration. By the third day after culture, only 5% of the larvae were not feeding on the highest concentrations. At the end of the observations, all the

larvae fed on the pieces of cane stalk whatever the concentration and only 5% did not feed on the artificial medium for the concentrations of 8 and 10 ml/l (figure 6).

In short, over the entire observation period, 28-day-old larvae fed less on nutrient media treated with the biopesticides ASTOUN 50 EC and NECO 50 EC than on the control at 8 ml/l or more. After culture, the 28-day-old larvae fed less rapidly on the nutrient media treated than on the control. Larval palatability dropped considerably when the concentration of biopesticides in the nutrient medium increased. The rate of 28-day-old larvae consuming treated nutrient media was greater than 90% in the presence of the biopesticide ASTOUN 50 EC and NECO 50 EC.

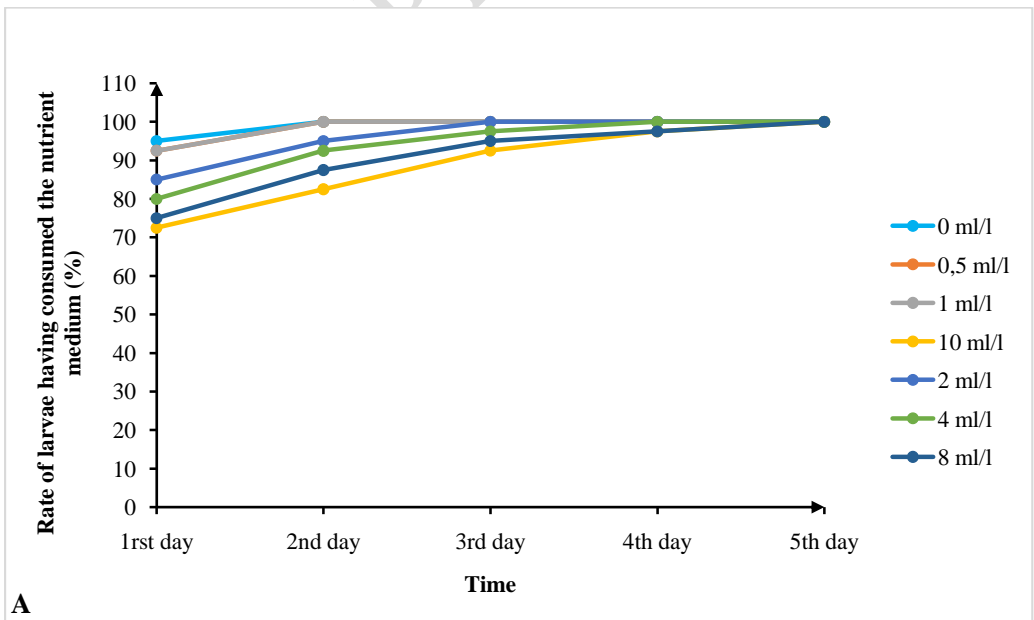


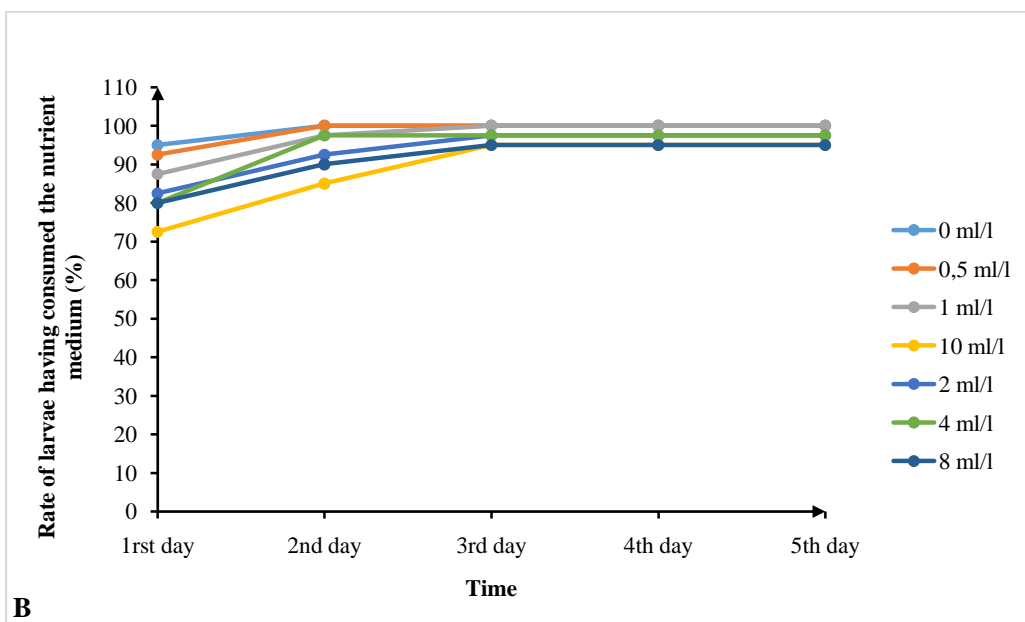


A : On pieces of cane stalk

B : On artificial nutrient medium

Figure 5: Changes in the rate of 28-day-old larvae that consumed nutrient media treated with the biopesticide ASTOUN 50 EC





A : On pieces of cane stalk

B : On artificial nutrient medium

Figure 6 : Changes in the rate of 28-day-old larvae that consumed nutrient media treated with the biopesticide NECO 50 EC

Larval palatability as a function of age, nutrient medium and biopesticide

The rate of *Eldana saccharina* larvae consuming the treated nutrient media varied significantly with age. The highest rate was recorded for 28-day-old larvae (98.75%). Larvae aged 14 and 21 days fed less on the treated nutrient media, with rates of 95.10 and 95.18% respectively. Thus, the older the larvae, the more indifferent they were to the treatments (table 3).

A biopesticide effect was observed on the palatability of *E. saccharina* larvae. This effect was significant only for 14-day-old larvae and for all ages combined (Table 4). The palatability of the larvae was higher on nitric media treated with the biopesticide NECO 50 EC than on those treated with ASTOUN 50 EC with 97.38 and 95.30 %.

A significant effect of the nutrient medium was recorded on the palatability of the larvae except at the age of 28 days. Larval palatability was higher on pieces of cane stem, with rates of 97.14 and 97.68% at 14 and 21 days of age respectively, compared with 93.04 and 92.68% on artificial medium. For all ages combined, the rate of larvae having consumed the treated media was higher on pieces of cane stem than on artificial medium, with 98.04 and 94.64% respectively (table 5).

Table 3: Rate of larvae consuming treated nutrient media as a function of age

Age of larva	Rate of larvae feeding (%)
14 days	95,10 ^b
21 days	95,18 ^b
28 days	98,75 ^a
Average	96,33929
C.V (%)	6,76000
Probability	0,000003

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In the table, values followed by different letters are significantly different at the 5% threshold according to the Newman-Keuls test. C.V. = Coefficient of variation

Table 4: Rate of larvae that consumed the treated nutrient media according to the biopesticides tested

Biopesticides	Rate of larvae feeding (%)			
	14-day-old larvae	21-day-old larvae	28-day-old larvae	Average
ASTOUN 50 EC	92,68 ^b	94,64 ^a	98,57 ^a	95,30 ^b
NECO 50 EC	97,50 ^a	95,71 ^a	98,93 ^a	97,38 ^a
Average	95,10	95,18	98,75	3,66
C.V (%)	7,98	7,46	3,36	6,76
Probability	0,000004	0,000004	0,000004	0,015212

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In the table, values in the same column followed by different letters are significantly different at the 5% threshold according to the Newman-Keuls test. C.V. = Coefficient of variation

Table 5: Rate of larvae having consumed the treated nutritive media as a function of the nutritive medium

Milieux nutritifs	Rate of larvae feeding (%)			
	14-day-old larvae	21-day-old larvae	28-day-old larvae	Average
Artificial nutrient medium	93,04 ^b	92,68 ^b	98,21 ^a	94,64 ^b
Piece of cane stem	97,14 ^a	97,68 ^a	99,29 ^a	98,04 ^a
Average	97,14	95,18	98,75	96,34
C.V (%)	7,98	7,46	3,36	6,76
Probability	0,000003	0,000003	0,000003	0,009898

In the table, values in the same column followed by different letters are significantly different at the 5% threshold according to the Newman-Keuls test. C.V. = Coefficient of variation

Percentage of repulsion of imagos by biopesticides

The repellent effect of the biopesticides ASTOUN 50 EC and NECO 50 EC on *E. saccharina* adults increased with the dose of the product. For ASTOUN 50 EC, it increased from 30 to 90 % and for NECO 50 EC from -5.41 % to 17.5 % when the concentration was increased from 0.5 to 10 ml/l (Tables 6). Also, for all doses tested, the biopesticide ASTOUN 50 EC had a more pronounced repellent effect than NECO 50 EC on *E. saccharina* adults (Tables 7).

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Laying rate in the treated compartment

The laying rate of *Eldana saccharina* females in the presence of NECO 50 EC changed from 48.73 to 46.73% with the increase in concentration from 0.5 to 10 ml/l. However, this decrease was not significant at the 5% level. In the presence of ASTOUN 50 EC, the laying rate of *Eldana saccharina* females in the treated compartment changed from 41.29 to 4.09% with the increase in concentration from 0.5 to 10 ml/l. The analysis of variance revealed a significant effect of the concentration on the spawning rate in the treated compartment. Thus, as the concentration of ASTOUN increased, the females moved further away and laid eggs in untreated areas (table 8). In comparison, the oviposition rate of *Eldana saccharina* females in a treated area was statistically lower in the presence of the biopesticide ASTOUN 50 EC than in the presence of NECO 50 EC from the dose of 2 ml/l (table 9).

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Table 6: Percentage of repulsion of *E. saccharina* imagos by the biopesticide ASTOUN 50 EC and NECO 50 EC

Concentration	Percentage of repulsion	
	ASTOUN 50 EC	NECO 50 EC
0,5 ml/l	30,00 a	-5,00a
1 ml/l	45,00 b	7,50 a
2 ml/l	60,00 c	10,00 a
4 ml/l	67,50 c	12,50 a
8 ml/l	87,50 d	12,50 a
10 ml/l	90,00 d	17,50 a
Average	63,33	9,17
C.V (%)	0,39	3,33
Probability	0,000000	0,026070

In the table, averages in the same column followed by a different letter are statistically different at the 5% threshold according to the Newman-Keuls test.

Table 7: Percentage of repulsion of *E. saccharina* imagos by the biopesticide ASTOUN 50 EC and NECO 50 EC

Product	Percentage of repulsion					
	0,5 ml/l	1 ml/l	2 ml/l	4 ml/l	8 ml/l	10 ml/l
ASTOUN 50 EC	30,00 a	45,00 a	60,00 a	67,50 a	87,50 a	90,00 a
NECO 50 EC	5,00 b	7,50 b	10,00 b	12,50 b	12,50 b	17,50 b
Average	12,5	26,25	35	40,00	50,00	53,75
C.V (%)	2,47	1,09	0,97	0,92	0,93	0,8
Probability	0,007183	0,001187	0,000108	0,000071	0,000007	0,000001

In the table, averages in the same column followed by a different letter are statistically different at the 5% threshold according to the Newman-Keuls test.

Table 8: Rate of eggs laid in the compartment of the rearing box treated with the biopesticides ASTOUN 50 EC and NECO 50 EC

Concentration	Rate of eggs laid in the treated compartment	
	ASTOUN 50 EC	NECO 50 EC
0,5 ml/l	41,29 a	48,73 a
1 ml/l	37,15 b	48,18 a
2 ml/l	16,95 b	45,17 a
4 ml/l	9,76 b	45,49 a
8 ml/l	4,69 b	49,25 a
10 ml/l	4,09 b	46,73 a
Average	18,99	47,26
C.V (%)	78	57
Probability	0,000028	0,999180

In the table, averages in the same column followed by a different letter are statistically different at the 5% threshold according to the Newman-Keuls test.

Table 9: Comparative effect of the biopesticide ASTOUN 50 EC and NECO 50 EC on the oviposition rate of *E. saccharina-E. saccharina* females

Egg-laying rate in the treated compartment						
biopesticides	0,5 ml/l	1 ml/l	2 ml/l	4 ml/l	8 ml/l	10 ml/l
ASTOUN 50 EC	48,73 a	48,18 a	45,17 a	45,49 a	49,25 a	46,73 a
NECO 50 EC	41,29 a	37,15 a	16,95 b	9,76 b	4,69 b	4,09 b
Average	45,01	42,67	31,06	27,63	26,97	25,41
C.V (%)	71	57	75	120	102	114
Probability	0,614703	0,327538	0,003342	0,011174	0,000007	0,000125

In the table, averages in the same column followed by a different letter are statistically different at the 5% threshold according to the Newman-Keuls test.

DISCUSSION

The palatability of *E. saccharina-E. saccharina* larvae decreased significantly with increasing concentration of the biopesticide ASTOUN 50 EC. Similarly, this biopesticide had a very marked repellent effect on *E. saccharina-E. saccharina* adults and considerably reduced the oviposition rate of females in the treated compartments. These results indicate that the biopesticide ASTOUN 50 EC has a repellent effect on *E. saccharina-E. saccharina* larvae and adults. This justifies the fact that *E. saccharina-E. saccharina* larvae feed faster on control nutrient media than on treated nutrient media as well as the effects observed on adults. Our results are similar to those of [13] who showed that the essential oils of *Cymbopogon citratus* and *Ocimum canum* have an antiappetitive effect on *Cylas puncticollis* Boheman resulting in reduced consumption of sweet potato roots compared to the control. After 5 days of observation, a rapid increase in the rate of larvae having consumed the treated nutrient media was observed. This progressive feeding of larvae on treated nutrient media is thought to be due to either to the larvae's accommodation to the odour emitted by the biopesticides or to the loss of the latter's repellent effect over time. The latter would be attributable to the high volatility of the active ingredients in the biopesticide ASTOUN 50 EC. As indicated by the work of several authors [14, 15, 16], the essential oil of *Cymbopogon citratus*, the main component of the biopesticide ASTOUN 50 EC, is highly volatile, which severely limits its repellent activity over time. In the presence of NECO 50 EC, the palatability of the larvae was lower than that of the control only on the first day after culturing and for the highest concentrations.

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After that, the rate of larvae feeding on the treated nutrient media increased very rapidly to equal that of the control in most cases. Thus, ~~like the~~ biopesticide ASTOUN 50 EC ~~and~~ , NECO 50 EC has a repellent effect against ~~E. saccharina~~ *E. saccharina* larvae. However, its action is very limited in time. These results are in line with those of [17], who demonstrated that *Ocimum gratissimum* L., whose essential oil is the main component of NECO [4], is a repellent plant against the lepidopterans *Hellula undalis*, *Spodoptera littoralis* and *Plutella xylostella*. In addition, several laboratory and field studies have demonstrated the insect-repellent and/or insecticidal effect of the NECO biopesticide against a wide range of stock predators, fungal and bacterial agents and borers [4, 5, 18, 19, 20].

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Comparative analysis of these two biopesticides in terms of their effect on the palatability of ~~E. saccharina~~ *E. saccharina* larvae and repellency of adults revealed significant differences. The biopesticide ASTOUN 50 EC had a more marked repellent effect on larvae, ~~and~~ imagoes and greatly reduced the oviposition rate of ~~E. saccharina~~ *E. saccharina* females than the biopesticide NECO. Thus, the biopesticide ASTOUN 50 EC was more insect repellent than NECO 50 EC on ~~E. saccharina~~ *E. saccharina* larvae and adults. This difference in efficacy could be explained by the difference in composition of these two biopesticides. ~~Indeed~~, the biopesticide ASTOUN 50 EC includes neral, α -citral, sulcatone, oxygenated monoterpenes and β -myrcene [8] whereas NECO 50 EC has Thymol, Gamma-Terpinene and Eugenol as active ingredients [9]. This result is consistent with those of [6] who demonstrated a difference in the larvicidal effect of topical application of the essential oils of *Zingiber officinale*, *Cymbopogon citratus* and *Ocimum gratissimum*.

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Conclusion

This study is part of the search for means and methods to control *Eldana saccharina*, the main sugarcane stalk borer in Côte d'Ivoire. The effect of two biopesticides, ASTOUN 50 EC and NECO 50 EC, was tested on the palatability of the pest's larvae and adults. The results showed that these two biopesticides had an antiappetitive effect on larvae and a repellent effect on ~~Eldana saccharina~~ *Eldana saccharina* adults. However, the biopesticide ASTOUN 50 EC was the most effective. It is highly repellent to adults, considerably reduces the oviposition rate of females in treated areas and is highly antiappetizing. This biopesticide could be tested in the field for effective use against ~~Eldana saccharina~~ *Eldana saccharina* in sugar cane.

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References

1. Kouamé D. K., Péné C. B. and Zouzou M., 2010. Assessment of varietal resistance of sugarcane to the African tropical stem borer (~~Eldana saccharina~~ *Eldana saccharina* Walker) in Côte d'Ivoire. *Journal of Applied Biosciences* 26: 1614-1622.

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2. Goebel F. R. and Way M. J., 2003. Investigation of the impact of *Eldana saccharina* (Lepidoptera: Pyralidae) on sugarcane yield in field trials in Zululand. Poceeding of South Africa Sugarcane Technologists Association, 77: 256-265.
3. Assefa Y., Conlong, D. E. and Mitchell A., 2006. Phylogeography of ~~*Eldana saecharina*~~ *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Annals of the Entomological Society of France 42 (3-4): 331-338.
4. Kassi, M.F., Badou, J.O., Tonzibo, F.Z., Salah, Z., Bolou, A.B.B., Camara, B., Amari, E.G.D. N.-L. & Koné, D., 2014. Antifungal potential of the essential oil of *Ocimum gratissimum* in the biological control of banana black streak disease caused by *Mycosphaerella fijiensis* morelet (mycosphaerellacea). African Agronomy, 26 (2): 1 - 11.
5. Kobenan K. C. Tia V. E., Ochou G. E. C., Kouakou M., Bini K. K. N., Dagnogo M., Dick A. E. and Ochou O. G., 2018. Comparison of the insecticidal potential of the essential oils of *Ocimum gratissimum* L. and *Ocimum canum* Sims on *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae), insect pest of cotton in Ivory Coast. European Scientific Journal edition 14 (21): 1857 – 7881.
6. Ouattara D., Kouamé K. D., Johnson F., Kassi K. F. J. M., Yao K. J.-E., Yao K. and Koné D., 2022. In-vitro Biocontrol of Sugarcane Stem Borer (~~*Eldana saecharina*~~ *Eldana saccharina* Walker) with Essential Oils from Aromatic Plants. Asian Research Journal of Agriculture, 15 (4): 161-169.
7. Kouamé K. D., Kassi K. F. J. M., Kouamé K. G., Kouassi K. V., Dove J. H., Ng Cheong L. R., Zouzou M., 2018. Soil moisture management and mulch impact on sugarcane yields under irrigated and rainfed conditions in Côte d'Ivoire. Journal of Biodiversity and Environmental Sciences, 12 (5): 381-390.
8. Kobenan K. C., Kouakou B. J., Bini K. K. N. Kouakou M., Dick A. E. and Ochou O. G., 2019. Effects of the Essential Oils of *Ocimum gratissimum* L. and *C. citratus* Stapf on the Growth and Production Parameters of Cotton in Côte d 'Ivory. European Journal of Scientific Research. 154 (1), 21-35.
9. Minader (Ministry of Agriculture and Rural Development), 2018. List of pesticides approved and authorized in Côte d'Ivoire as of March 15, 2018. Directorate of Plant Protection, Control and Quality, 100 p.
10. Kla Y. F., 2012. Identification of insects (parasitoids and others) resulting from the breeding of caterpillars and pupae of ~~*Eldana saecharina*~~ *Eldana saccharina* Walker at the Zuénoula Integrated Agricultural Unit. End of studies dissertation in Engineering at the Ecole Supérieure d'Agronomie de Yamoussoukro (INPHB). 69 p.

11. Sotondji F. A., Djihinto C. A., Kobi D. K. O., Dannon E., Zodome G., Adjou E., Chougourou D. C., Soumanou M. M., 2020. Laboratory evaluation of the larvicidal effect of cashew balm and three vegetable oils based plants of *Tephrosia Purpurea*, *Ricinus Communis* and *Thevetia Neriifolia* to control populations of *Plutella Xylostella* L. 1758 (Lepidoptera, Plutellidae). *European Scientific Journal*, 16 (24), 29-59.
12. McDonald L. L., Guy R. H. and R. D., Speirs., 1970. Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored product insects. *Marketing. Res. Rep. No. 882*. Washington: Agric. Res. Service, US. Department of Agriculture, 183 p.
13. Tia V. E., Cisse M., Douan G. B. and Kone A., 2019. Comparative study of the insecticidal effect of the essential oils of *Cymbopogon citratus* DC and *Ocimum canum* Sims on *Cylas puncticollis* Boheman, a sweet potato weevil. *Int. J. Biol. Chem. Sci.* 13(3): 1789-1799.
14. Fradin M. S., Day J. F., 2002. Comparative efficacy of insect repellents against mosquito bites. *New England Journal Medicine*, 347: 13-8.
15. Thorsell W., Mikiver A., Tunon H., 2006. Repelling properties of some plant materials on tick *Ixodes ricinus* L. *Phytomedicine*, 13: 132-4.
16. Katz T. M., Miller J. H., Hebert A. A., 2018. Insect repellents: historical perspectives and new developments. *Journal of American Academy of Dermatology*, 58: 865-71.
17. Yarou B. B., Silvie P., Komlan F. A., Mensah A., Alabi T., Verheggen F., Francis F., 2017. Pesticidal plants and protection of market gardening in West Africa (bibliographic summary). *Biotechnology, Agronomy, Society and Environment*, 21 (4), 288-304.
18. Camara B, Koné D, Kanko C, Anno A. and Aké S., 2007. Antifungal activity of the essential oils of *Ocimum gratissimum* L., *Monodora myristica* (Gaaertn) Dunal and two synthetic products (Impulse and Folicur) , on the mycelial growth and in vitro spore production of *Deightoniella torulasa* (SYD.) ELLIS. *Revue Ivoirienne des Sciences et technologies* (9): 187 - 201
19. Tano D. K. C., Tra B. C. S., Kouassi K. A., Ossey C. L. and Soro S., 2019. Impacts of *Podagrica decolorata* Duvivier 1892 attacks on okra cultivation and control of these adults using the biopesticide NECO 50 EC (Daloa, Ivory Coast). *Journal of Applied Biosciences* 143: 14692 – 14700.
20. Kouassi A. M., Ouali N'Goran S.-W. M., Akesse E. N., Coulibaly A., 2022. Evaluation of the performance of an anti-insect net in an integrated control system against the main pests of cabbage in Korhogo, northern Ivory Coast. *Journal of Applied Biosciences* 177: 18424 – 18433.