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Laboratory evaluation of the larvicidal effect of cashew balm and three vegetable oils based on the insecticidal plant's *Tephrosia purpurea*, *Ricinus communis*, and *Thevetia neriifolia* for the management of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) populations.

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Abstract

Market gardeners use synthetic pesticides in an unreasonable and uncontrolled way to protect their plots from insect pests. With the aim of finding alternative control methods to synthetic products, the efficacy of Cahew Nut Shell Liquid (CNSL) cold-applied Cahew Nut and three vegetable oils based on Tephrosia purpurea, Ricinus communis, and Thevetia neriifolia plants was compared to that of a synthetic insecticide (Lambda-cyhalothrin) and to that of a botanical reference pesticide (Topbio). Two experiments were carried out in this study. In the first experiment, the products were tested on the L2 stage of Helicoverpa armigera, with doses of 50, 25, 10, 5, 3, and 1% of each of the plant products. In the second experiment, the same plant products were tested on the L3 stage of the same insect with the same doses of the first experiment, compared to the reference control Lambda-cyhalothrin at doses of 10, 5, 3, 2.1, and 0.5% in both experiments. Variables measured were larval mortality, pupae formation, and adult emergence. The LD50s were determined according to the Cox regression model. The results showed that vegetable oils and cashew balm are promising biopesticides for the management of H. armigera populations. Mortality and pupae formation rates varied with dose. For all products used, regardless of the larval stage of H. armigera, a significant difference was observed between the applied doses. Cashew balm and the three vegetable oils are positioned as alternatives to synthetic pesticides that can be used in market gardening.

Keywords: *Helicoverpa armigera, vegetable oil, lethal dose, biological control.*

I. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an annual plant of the family Solanaceae. Originally from Southwestern America (Ranc, 2010), it is now cultivated worldwide for its fruits, which play an important role in human nutrition. The nutritional importance of the tomato fruit lies in its richness in nutrients such as essential amino acids, vitamin C, lycopene, and β-carotene. Tomatoes are grown in many countries of the world and in various climates, including relatively cold regions through the development of protected crops (FAO, 2007). It is the world's most-produced vegetable, ahead of watermelon and cabbage, but behind potatoes and sweet potato, the latter two being considered more like starchy foods (FAO, 2010). The WHO (2002) estimates that sufficient consumption of these fruits would reduce the incidence of heart disease by

31%, stroke by 11%, and gastrointestinal cancer by 20% to 30%. According to statistics from the Food and Agriculture Organization of the United Nations, world tomato production in 2007 amounted to 126.2 million tons for an area of 4.63 million hectares, an average yield of 27.3 tons per hectare (FAO, 2008). These figures include only marketed production and do not include family production, which is significant in some regions. To ensure food security, the development of market gardening remains a solution approach for most populations in African cities (Mondédji *et al.* 2014). In Benin, the rural sector employs more than 70% of the active population and remains an essential factor of economic growth (CIRAD, 2005). Agriculture remains one of the main drivers of economic growth in Benin, with an average contribution to the growth of 0.9% between 2007 and 2009 (CPRS 2007-2009). Urban and peri-urban agriculture has evolved following the urban population explosion (Assogba-Komlan *et al*., 2007). This agricultural sub-sector re-groups market gardening and the breeding of small ruminants and poultry. Vegetable production is developing on the outskirts of large African cities and contributes to food security. Tomato cultivation takes place in all regions of Benin, on the plateaus, in the alluvial plains, in the valleys, and in the lowlands. Because of its domestic production, Benin cannot cover its food needs in market garden products. Benin continues to import much of its consumption of market garden produce from neighboring countries such as Burkina Faso and Nigeria (PSRSA, 2009). Tomatoes have the potential to earn foreign exchange. It has been identified as one of the priority agricultural speculations to be promoted in Benin in the Government's Programme of Action. However, it faces many constraints and also generates nuisances that limit its sustainability. Nuisances linked to market garden production have been documented in several tropical African countries (Cissé *et al*., 2003; Akogbéto *et al*., 2005; Obopile *et al*., 2008; Williamson *et al*., 2008). Among the constraints hampering tomato production is the lack of pest control. The fruit worm *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) attacks tomato crops, preferably during the flowering period. Females lay eggs on the first leaf below the flo-eral bunches, then, from the 3rd instar onwards, the larvae enter the fruit to feed, the caterpillars eating only part of the fruit and then attacking another (Torres-Vila et al. 2003). Singh (1975) showed that eggs are preferentially deposited on the underside of young leaves. Several fruits are attacked during the larval life, usually on the same bunch (Poitout & Bues, 1979). The fruit is depreciated by the moth due to the presence of a gallery, and there is also loss of yield due to fruit drop and the development of rots. Indeed, vegetable production in tropical Africa is dependent on the use of chemical pesticides (Akogbéto *et al*., 2005; Obopile *et al*., 2008; Williamson *et al*., 2008; Ahouangninou *et al*., 2011). While the use of pesticides in agriculture is useful for optimizing yields in cash and vegetable crops, there are a number of considerations that must be taken into account to minimize health and environmental impacts. The study by Sæthre *et al.* (2011) also found pesticide residues in vegetables traded in markets in southern Benin. The impact of pesticides on the environment can result in significant economic losses. During phytosanitary treatments, the portion of pesticides that penetrates the soil by leaching can be harmful to soil microflora and particularly earthworms, which play an important role in maintaining soil fertility. Pesticides can be harmful to the antagonists (competitors, predators, and parasites) of target pests.

Alternative control methods that present less risk exist. These include the use of locally produced plant extracts such as *Azadirachta indica* (A.Juss) (Meliaceae), *Hyptis suaveolens* (L.) (Lamiaceae), and *Carica papaya* (L.) (Caricaceae). The introduction of these biopesticides into vegetable production systems is to control crop pests while respecting ecological principles, human health, and the environment. Alternative control methods make the best use of local resources, improve product quality, reduce production costs, and would help to increase productivity and thus improve producers' incomes (Adétonah, 2007). This is why we were interested in vegetable oil extracts from the seeds of three plant species, namely: *Tephrosia purpurea* (L.) Pers. (Fabaceae), *Ricinus communis* (L.) (Euphorbiaceae), *Thevetia neriifolia* (L.) (Apocynaceae), and cashew balm, known for their biocidal properties in the management of H. armigera on tomato. This study assessed the larvicidal properties of CNSL, *T. purpurea*, *R. communis*, *T. neriifolia*, compared to Topbio and Lambda-cyhalothrin (Pyrethroid) on *H. armigera* populations in the laboratory. Pest susceptibility tests were carried out for each of these biopesticides. Several doses of each botanical pesticide were tested on *H. armigera* larvae, and the effects of the botanical pesticides on insects that survived the applied doses were observed at the pupae stage and then at the butterfly stage.

II. MATERIALS AND METHODS

A. Study environment

The experiments were conducted in the biopesticide laboratories of the IITA (Benin) and in the Laboratory of Applied Biology Research (LARBA) from September 2019 to February 2020. The ambient conditions of the room were 26 ± 0.5 °C and 65.5 ± 5 % RH.

B. Animal material

- *Helicoverpa armigera* larvae rearing

Method of breeding Helicoverpa armigera

For the rearing of *H. armigera*, larvae were collected from tomato fields at the four market gardening sites under study and at the tomato market gardening sites in Allada. They were reared in the laboratories of IITA-Benin on an artificial diet of beans, agar, maize, brewer's yeast, ascorbic acid, sorbic acid, honey, formaldehyde, and methyl p-hydroxybenzoate, developed by Teakle & Jensen (1985). During rearing, the laboratory was maintained at a temperature of 27 ± 1°C with a photoperiod of 12:12 h L: D.

C. Methods

a) Evaluation of the Effect of the treatment on the palatability of H. armigera

Larval appetence to different pesticide doses, i.e., 50%, 25%, 10%, 5%, 3%, and 1% for biopesticides and 10%, 5%, 3%, 2%, 1%, and 0.5% for the reference control (Lambda-cyhalothrin) synthetic chemical was tested in the first experiment and in the second experiment on tomato leaves exposed to stage 2 and 3 larvae of H. armigera. The leaf area consumed by the larvae after 24 hours was evaluated. Negative control was applied to distilled water. Each tomato leaf exposed to the larvae was removed after 24 hours and placed on graph paper. The consumed surfaces were reproduced on this paper and evaluated.

b) determination of the effects (mortality, repellent effect) of vegetable oil extracts, Topbio and Lambda-cyhalothrin on the larvae of the moth H. armigera

Treatments and doses applied

To determine the effect of the different extracts on stage 2 and 3 larvae of *H. armigera*, five botanical pesticides, and a treated control (Lambda-cyhalothrin) plus a negative control were used at different doses. For the first experiment, on L2 larvae, six doses were prepared as follows: 50%, 25% 10%, 5%, 3% and 1% for the botanical extracts and 10%, 5%, 3%, 2%, 1% and 0.5% for the reference control (Lambda). The doses were prepared with 30 mg of lecithin plus non-detergent soap in distilled water for each stock solution. The same rates of application of botanical and synthetic pesticides were repeated in the second experiment on stage 3 larvae. The negative control was applied with distilled water. A wetting agent was added to the different treatments tween 80.

c) Toxicity bioassays

Experiments were conducted in the laboratory to determine the response of the *H. armigera* moth to four botanical pesticides and two controls treated with Topbio and the insecticide lambda, plus a zero-dose negative control. Tests were conducted at a mean temperature of 26.78 ± 0.05°C and a mean humidity of 61.86 ± 0.13%. Second and third instar larvae of *H. armigera* were exposed to six types of treatments: *R. communis, T. purpurea*, cashew balm, *T. neriifolia*, Topbio, Lambda, and distilled water as a negative control. Second- and third-instar larvae were used because of their voracity (Parry, 1982). For this purpose, groups of 60 larvae of *H. armigera* previously fasted for 24 h were subjected to these treatments. For each dose, three replicates were carried out. Formulations were obtained by diluting these botanical pesticides and the chemical insecticide in distilled water. A wetting agent was diluted with Tween 80 at 3%. Because of the cannibalism peculiar to *H. armigera*, third instar larvae were individually placed in boxes (3.8 x 2.9 x 4.0 cm) with perforated lids for ventilation. Treatment consisted of feeding second and third instar larvae of *H. armigera* for 24 h on tomato leaves treated with 50%, 25%, 10%, 5%, 3%, and 1% of the botanical pesticides and 10%, 5%, 3%, 2%, 1% and 0.5% of the synthetic chemical insecticide (Lambda-cyhalothrin). These different doses were conducted in the first experiment on L2 larvae and in the second experiment on L3 larvae. For the control, larvae were fed with tomato leaves treated with distilled water. At the end of 24 hours, observations were made, and mortality rates were recorded.

D. Analysis of the data

The emergence rate was calculated based on the number of pupae formed in each treatment. Mean Survival Times (MST) were calculated using a Kaplan Meier survival analysis (SPSS, 1989-2007). For the various statistical analyses, R Core Team software (version 3.6.3-2020) was used. The generalized linear mixed-effect model was used to explain the mortality rate as a function of the products used and their concentrations. The mean mortality rate was subjected to an analysis of variance (ANOVA), using the SAS General Linear Model (GLM) procedure (SAS, 2002-2008). When F values were significant, treatments were compared using the SNK (Student-Newman-Keuls) test at the 5% probability threshold. The percentages of mortality were arc-sinus transformed to be subjected to an analysis of variance (ANOVA). The Log Rank (Mantel-Cox) test was used to compare MST. Pesticide efficacy was compared on the basis of mortality rate (i.e., 14 days cumulative mortality).

III. Results

A. Effect of three vegetable oils, cashew balm, Topbio, and the chemical insecticide Lambda-cyhalothrin on *H. armigera* larvae.

a) effect of treatments on the palatability of L2 and L3 larvae of H. armigera

Table I shows that the average surface areas consumed by a larva of H. *armigera* range from 14.25 ± 1.33, 8.31 ± 1.28, 58.23 ± 1.68, 47.56 ± 4.17 mm2 on L2 larvae to 31.25 ± 4.51, 19.74 ± 32.11, 67.21 ± 1.68,

80.04 ± 56.10 mm2 on L3 larvae respectively for cold CNSL, T. purpurea, *R. communis, T. neriifolia* at an application rate of 10% and 214.15 ± 11.33, 265 ± 15.77 mm2 on L2 and L3 larvae respectively (control treatment). Leaf areas consumed by H. armigera larvae are larger in control than in the treated leaves. The larval feeding behavior test showed that leaf consumption is dependent on the pesticide rate used. The higher the rate, the lower the consumption. Thus, for all botanical pesticides as well as the synthetic chemical pesticide, the largest leaf areas were consumed at the 1% rate. For the doses of 25%, 50% of the three vegetable oils, cold CNSL and 3% of Topbio as well as Lambda-cyhalothrin, either on L3 or L2 larvae, a phenomenon of inappetence was observed in some larvae which, despite fasting prior to the test, did not consume any part of the leaf. There was a significant difference between the areas consumed by *H. armigera* larvae, ($F = 36.55$; ddl = 5; $P = 0.0001$).

Table 1. Mean leaf areas (X±ES) of tomato leaves consumed in 24 hours by L2 larvae of *H. armigera* as a function of treatment.
Experiment I

Doses (%)	**Treatments Larval stage: L2 (n = 20, r = 3)** CNSL cold	Oil *T. purpurea*	Oil *R. communis*	oil *T. neriifolia*	TopBio	Lambda cyhalothrine
50	00 ± 00 e	00±00 e	00±00 e	00±00 e	00±00 e	-
25	00 ± 00 e	00±00 e	00±00 e	00±00 e	00±00 e	-
10	14,25± 1,33 d	8,31±1,28 d	58,23±1,68 c	47,56±4,17c	00±00 e	00±00e
5	21,04 ±2,39d	17,11±7,58d	77,01±7,89 c	80,45±1,31c	00±00 e	00±00e
3	48,70 ± 31,08 c	40,08±8,7 c	132,55±6,44 b	144,11±21,5b	14,38±4,31 d	41,12±1,5c
2	-	-	-	-	-	30,5±1,5c
1	101,19 ± 22,7 b	85,21±7,13 c	178,99±5,71a	198,14±9,5 a	132,01± 9,78 b	40,78±45,7c
0,5	-	-	-	-	-	107,3±12,01b
0	214,15 ± 11,33 a	214,15 ± 11,33 a	214,15 ± 11,33 a	214,15 ± 11,33 a	214,15 ± 11,33 a	214,15 ± 11,33 a

Means in the same column, followed by different letters, are significantly different at the 5% threshold (ANOVA followed by the Student-Newman-Keuls test). ES= Standard Error

Table 2. Mean leaf area (X±ES) of tomato leaves consumed in 24 hours by L3 larvae of H. armigera as a function of treatment.
Experiment II

Doses (%)	**Traitements Stade larvaire : L2 (n = 20, r = 3)** CNSL cold	Oil *T. purpurea*	Oil *R. communis*	Oil *T. neriifolia*	TopBio	Lambda cyhalothrine
50	00 ± 00 e	00±00 e	00±00 e	00±00 e	00±00 e	-
25	00 ± 00 e	00±00 e	00±00 e	00±00 e	00±00 e	-
10	31,25± 4,51 c	19,74±32,11 d	67,21±30,7 c	80,04±57,44c	00±00 e	00±00e
5	45,62 ±5,57c	34,44±23,61c	89,27±7,25 c	88,50±6,43c	13,02±23 d	00±00e
3	60,88 ± 4,06 bc	54,02±42,80 c	156,71±8,04 b	131,07±50,02b	47,78±7,63 c	58,99±74,15c
2	-	-	-	-	-	78,25±14,25c
1	122,45 ± 12,73b	105,57±91,13 b	195,09±55,211a	222,08±10,90 a	165,01± 52,4b	104,03±45,13bc
0,5	-	-	-	-	-	120,42±66,05bc
0	265,34 ± 15,77 a	265,34 ± 15,77 a	265,34 ± 15,77 a	265,34 ± 15,77 a	265,34 ± 15,77 a	265,34 ± 15,77 a

Means in the same column, followed by different letters, are significantly different at the 5% threshold (ANOVA followed by the Student-Newman-Keuls test). ES= Standard Error

b) Variation in dose-fatality response in L2 and L3 stage larvae of H. armigera

Exposure of *H. armigera* larvae to tomato leaves treated with different rates of the biopesticides and the synthetic pesticide for 24 hours resulted in mean morbidity rates as presented in (Figure 1). Mortality of *H. armigera* larvae exposed to increasing doses of Lambda-cyhalothrin and Topbio varied according to the pesticides used. All pesticides used cause dose-dependent mortality (Figure 2). The insecticide lambda is the most toxic and kills all larvae treated at a dose of 5% or more. The biological insecticide Topbio is the second most effective product and kills all treated larvae from an application rate of 10%. This result also applies to L3 larvae, but a slightly higher mortality rate is recorded on L2 larvae than on L3 larvae. Thus, the biological activity of pesticides towards larvae is not only related to the product but also to the rate used.

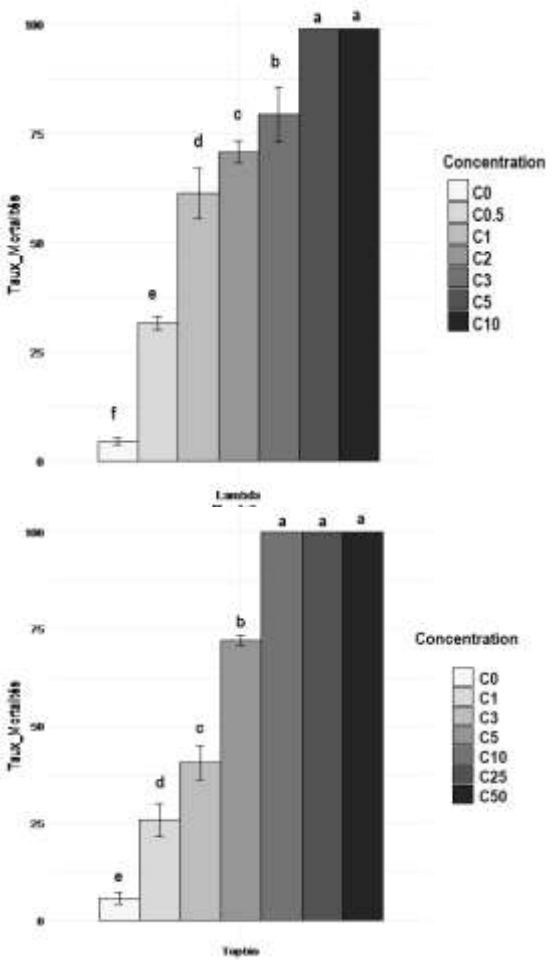

Figure 1: The mortality rate of L2 larvae of *H. armigera* after application of different doses of Lambda-cyhalothrin on the right and Topbio on the left

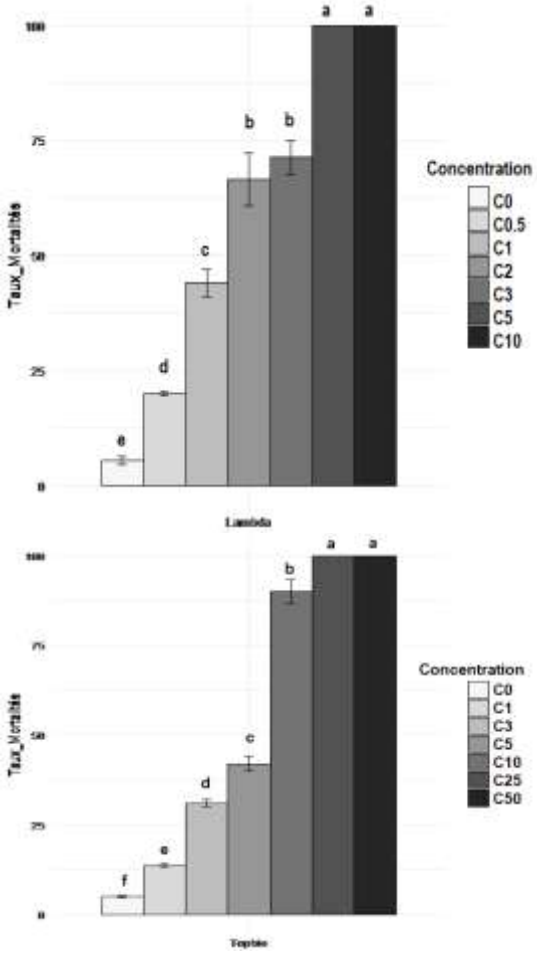

Figure 2: The mortality rate of L3 larvae of *H. armigera* after application of the different doses of Lambda-cyhalothrin on the left and Topbio on the right.

Mortality of *H. armigera* larvae exposed to increasing doses of vegetable oils varies according to the plants. All vegetable oils used cause dose-dependent mortality (Figure 3). Cold CNSL and *T. purpurea* oil recorded similar mortality at all application rates on L2 and L3 larvae. Application of the 50% and 25% rates resulted in 100% mortality on L2 and L3 larvae after 14 days of exposure to the products. The application of *T. purpurea* and CNSL oils cold applied to the 2nd instar larvae of H. armigera showed that mortality rates were higher compared to those obtained with the 3rd instar larvae. These rates were 5.41 ± 7.0%, 21.60 ± 4.3%, 26.40 ± 1.3%, 54.26 ± 9.1%, and 80.35 ± 15% for the control, 1%, 3%, 5%, and 10% oil *T. purpurea* respectively. on 2nd instar larvae and 6.45 ± 6.8%, 21.45 ± 7.9%, 33.18 ± 3.3% 58.23 ± 1.1% and 78.34 ± 10% cold CNSL for the control, 1%, 3%, 5%, and 10% dose, respectively. There was a significant difference between the 25% and 5% dose on L2 and L3 larvae treated with the botanical pesticides (Figures 3 and 4). There is also a significant difference between untreated controls and larvae treated at the 5% threshold. Thus, the biological activity of the vegetable oils used with respect to larvae is not only related to the plant from which they are. extracted but also to the dose used

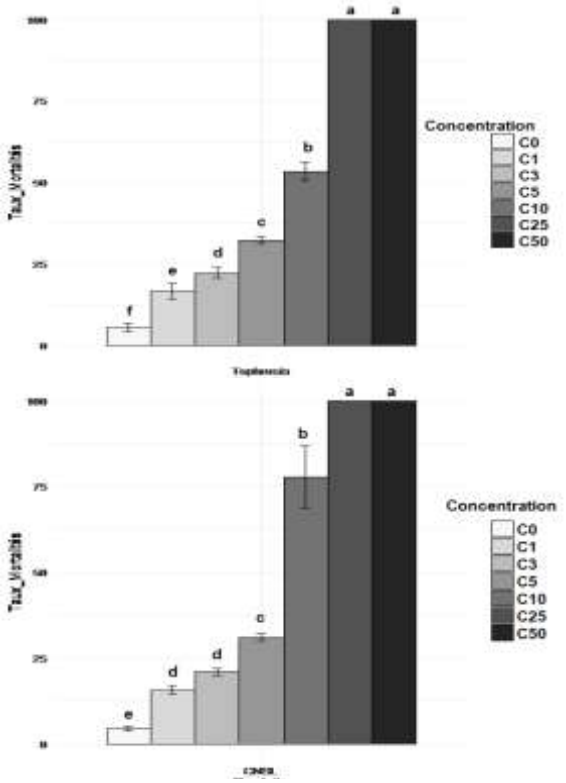

Figure 3: Mean mortality rate of L3 larvae of *H. armigera* after application of different doses of *T. purpurea* oil on the left and CNSL on the right.

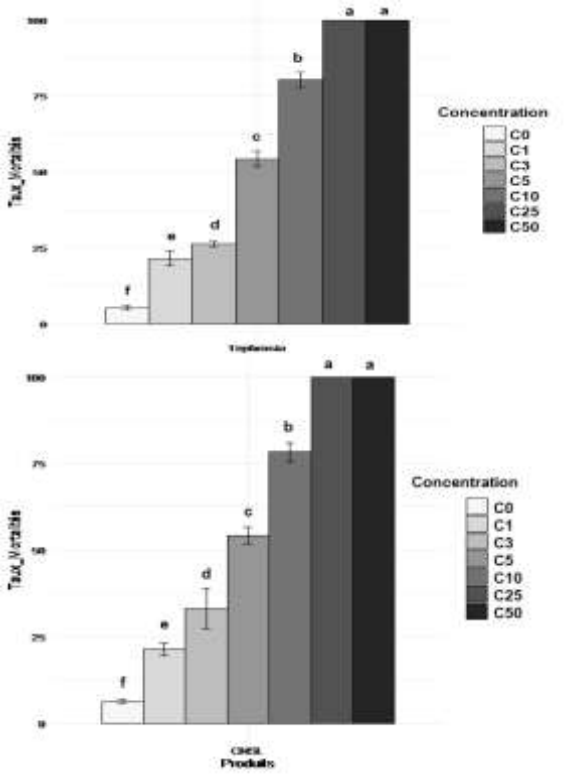

Figure 4: Mean mortality rate of L2 larvae of *H. armigera* after application of the different doses of *T. purpurea* oil on the left and CNSL on the right.

Mortality of *H. armigera* larvae exposed to increasing doses of vegetable oils varies according to the plants and the dose of pesticide used as well as the larval stage of the insect. All vegetable oils used to result in dose-dependent mortality (Figures 5 and 6). *R. communis* and *T. neriifolia* oil recorded similar mortality at all application rates on L2 and L3 larvae. Application of the 50% rate of these vegetable oils resulted in 100% mortality on L2 and L3 larvae after 14 days of exposure to the products. Application of 25% of *R. communis* and *T. neriifolia* oils on the 2nd instar larvae of *H. armigera* killed 100% of the exposed larvae after 14 days, which is not the case for the 3rd instar. R. communis oil and *T. neriifolia* oil caused similar mortality on the L2 and L3 larvae of *H. armigera*, the mortality rates of L2 larvae were higher compared to those obtained with the 3rd instar larvae. There was a significant difference between the highest doses, 50%, and 25% and the lowest doses 3% and 1% on treated larvae (Figures 5 and 6). The 50% and 25% doses of *T. neriifolia* and *R. communis* caused 100% mortality on L2 larvae after 14 days, while the cumulative mortality of these doses on the 3rd instar was 75, 57 ± 14%, and 83.76 ± 7.8%, respectively. There was also a significant difference between untreated controls and larvae treated at the 5% threshold. Thus, the biological activity of the vegetable oils used with respect to larvae is not only related to the plant from which they are extracted but also to the dose used.

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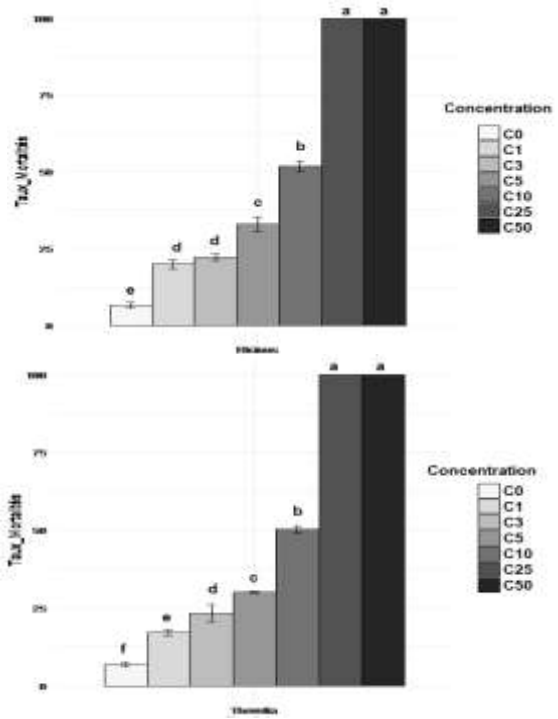

Figure 5: Mean mortality rate of L2 larvae of *H. armigera* after application of different doses of oil from *R. communis* on the left and *T. neriifolia* on the right.

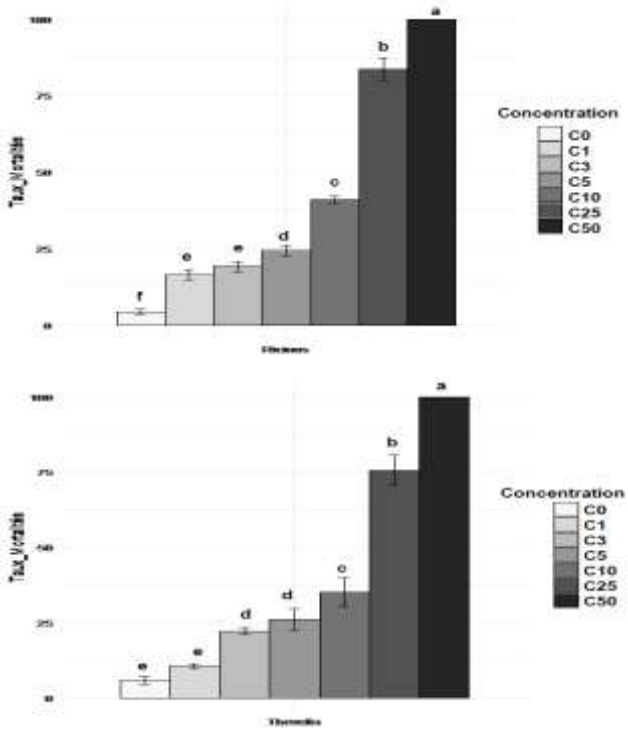

Figure 6: Mean mortality rate of L3 larvae of *H. armigera* after application of different doses of oil from *R. communis* on the left and *T. neriifolia* on the right.

c) Results of biological tests with pesticides used on product-dose-time interaction on exposed larvae

Tables 4 and 5 show that there is an interaction between products and dose on the one hand and between dose and time on the other. Finally, there is also an interaction between product-dose and time. From these two tables, we can see that the products and doses have a significant effect on larval mortality as a function of the duration of exposure to the products. Compared to stage 2 larvae (Table 4), a higher concentration is required to control stage 3 larvae (Table 5). This implies that the insecticidal effect observed depends on the extracts, the dose, and the larval stage.

Table 3: Product-dose-time interaction on L3 larvae of H. armigera (Analysis of Deviance Table (Type II Wald chi-square tests).

	Chisq	Df	Pr(>Chisq)	
Products	226.158	5	2.2e-16	***
Doses	763.954	1	2.2e-16	***
Time	58.982	1	1.591e-14	***
Products: doses	268.251	5	2.2e-16	***
Products: Weather	171.832	5	2.2e-16	***
Doses :Time	128.648	1	2.2e-16	***
Products : Doses :Time	28.420	5	3.012e-05	***

Table 4: Product-dose-time interaction on L2 larvae of H. armigera (Analysis of Deviance Table (Type II Wald chi-square tests

	Chisq	Df	Pr(>Chisq)		
Products	196.121	5	2.2e-16	***	<0.001
Doses	275.317	1	2.2e-16	***	<0.001
Time	12.036	1	0.0005217	***	<0.001
Products: doses	108.582	5	2.2e-16	***	<0.001
Products: Weather	89.173	5	2.2e-16	***	<0.001
Doses :Time	330.329	1	2.2e-16	***	<0.001
Products : Doses :Time	55.714	5	9.308e-11	***	<0.001

Figure 7 shows the curve of the products used as a function of the doses applied and time. The synthetic chemical Lambda-cyhalothrin has a significant effect on the mortality of exposed larvae compared to organic products: Topbio is slightly more effective than other vegetable oils and CNSL in cold conditions. The promising oils are those of *T. purpurea* and cold CNSL on L2 and L3 larvae of *H. armigera*. The oil of *R. communis* and *T. neriifolia* showed similarity on exposed larvae; compared to the Topbio biological reference control, there was a significant difference at the 5% threshold.

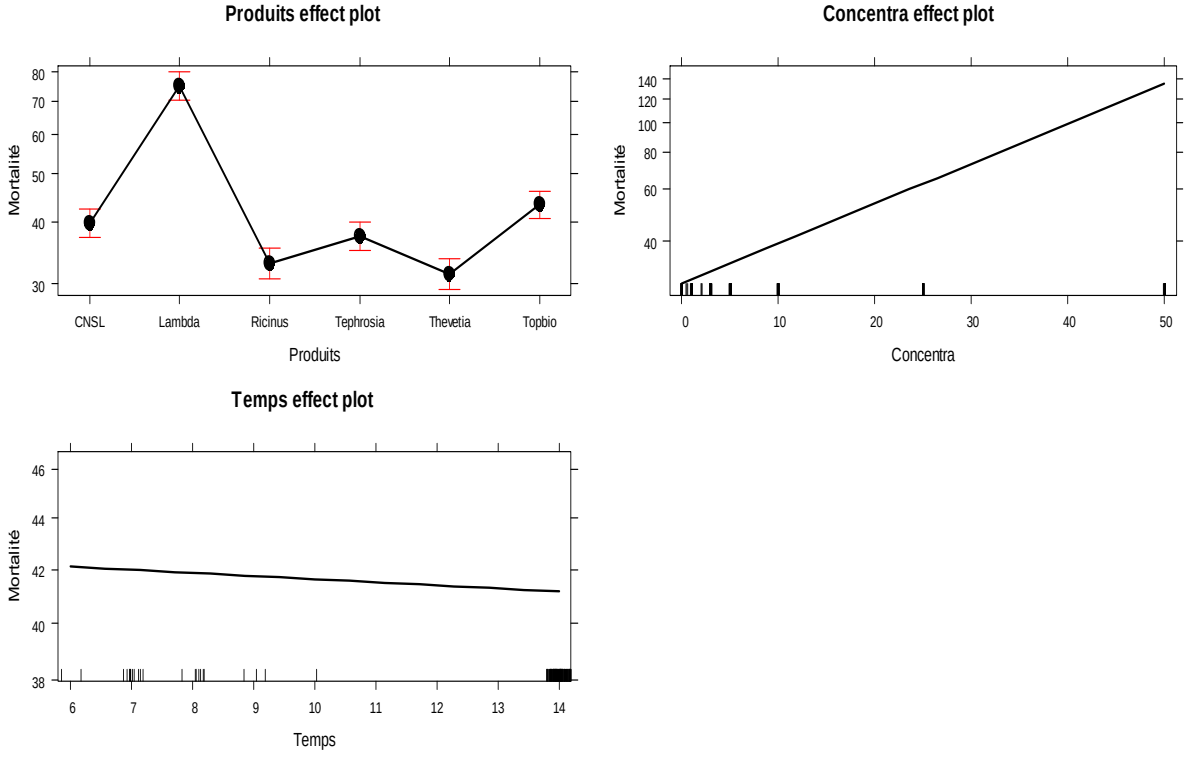

Figure 7: Dose product curve and time on L3 larvae of H. armigera

The results of the analysis of the insecticidal Effect of vegetable oil extracts, Topbio, and lambda-cyhalothrin on L2 larvae reveal that the mortality rate has a very high significant variation according to the pesticides used, the doses, the larvae, and the time considered on the one hand ($P < 0.001$) (Figure 8). Promising oils are those of *T. purpurea* and cold CNSL on L2 larvae of *H. armigera*. *R. communis* and *T. neriifolia* oil showed similarity on exposed larvae, these oils did not have enough effect on larval mortality of *H. armigera*, compared to the biological

reference control Topbio, there was a significant difference at the 5% threshold. Ranking the insecticidal effect of biological pesticides according to their efficacy allowed us to place Topbio, *T. purpurea*, and CNSL cold in the first group and *R. communis* and *T. neriifolia* in the second group. So there is a significant difference between the products used at the 5% threshold. The synthetic chemical Lambda-cyhalothrin reference control was more effective than the biological products on L2 larvae.

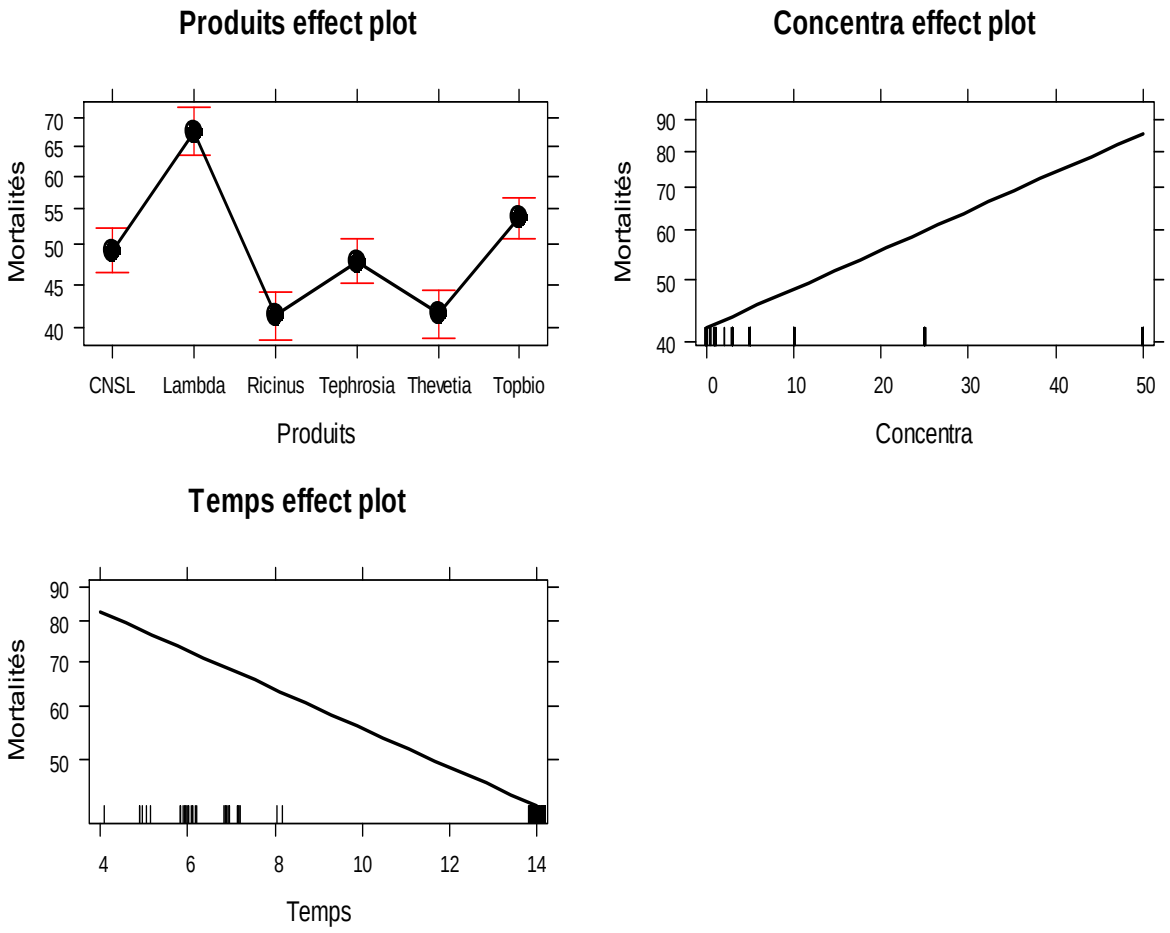

Figure 8: Curve of dose products and time on L2 larvae of *H. armigera*

B. Mean Survival Time of H. armigera L2 and L3 larvae

Compared to controls, the survival of L2 and L3 larvae of *H. armigera* was significantly affected by the pesticides used (F=641; D=13; P<0.0001 and F=784; D=13 P<0.0001 for L2 and L3, respectively). With the different doses: 50%, 25%, and 10% of the pesticides used, there was a progressive decrease in Mean Survival Time (MST) of the larvae of the controls towards the highest doses. This shows that the different doses of cold CNSL, *T. purpurea*, *T. neriifolia*, *R. communis*, and Topbio, considerably affect the life span of the larvae. The lowest MST was mainly recorded on L2 larvae with 4.0 ± 0.3 days (50% cold CNSL), 9 days less than the dead larvae of the control lot with MST of 13.1 ± 0.5 (untreated control). In general, regardless of the application rate, the MST of L2 larvae was lower than the MST of L3 larvae. From the third larval instar (L3) onward, MST became significantly higher even at the highest application rates (Tables 6 and 7). The fit of the data by the Cox regression model for the LD 50 tables was relatively good and is a function of the value of B. The higher the B value, the narrower the confidence intervals. The B values for all pesticides are positive, which explains why the dose-response relationship was very good for all pesticides used (Tables 6 and 7).

Table 6: Mean Survival Time (MST) (Days ± SE of Helicoverpa armigera L3) 15 days after application of the different doses of pesticides.

Focus	Traitements				
	CNSL	*T. purpurea*	Topbio	*R. communis*	*T. neriifolia*
Witnesses	13.2 ± 0,5 aA	13.5 ± 0,4 aA	13.2 ± 0,5 aA	13.7 ± 0,4 aA	13.5 ± 0,4 aA
1%	12.8 ± 0,5 aA	12.6 ± 0,5 aA	11.9 ± 0.6 aA	12.5 ± 0.6 aA	12.5 ± 0.6 aA
3%	10.8 ± 0.7 bA	10.9 ± 0.7 bA	10.0 ± 0.7 bA	10.5 ± 0.7 bA	11.2 ± 0.7 bA
5%	10.1 ± 0.7 bA	10.1 ± 0.7 bA	8.5 ± 0.7 cA	9.4 ± 0.7 bA	10.8 ± 0.7 bA
10%	8.0 ± 0.6 cA	8.2 ± 0.7 cA	8.3 ± 0.6 cA	9.5 ± 0.7 bA	10.3 ± 0.7 bA
25%	6.0 ± 0.3 dA	6.0 ± 0.3 dA	6.1 ± 0.4 dA	6.5 ± 0.6 cA	8.7 ± 0.7 cB
50%	4.8 ± 0.2 Ae	4.6 ± 0.3 eA	5.3 ± 0.4 eB	5.6 ± 0.4 cB	5.6 ± 0.4 dB

Different at the 5% threshold (Log Rank Test) Means in the same column followed by the same lowercase letter are not significantly different at the 5% threshold (Log Rank Test).

Table 7: Mean Survival Time (MST) (Days ± SE of Helicoverpa L2) 15 days after the application of different pesticide doses.

Concentration	Traitements				
	CNSL	*T. purpurea*	Topbio	*R. communis*	*T. neriifolia*
Témoins	13,1 ± 0,5 aA	12,9 ± 0,5 aA	13,2 ± 0,5 aA	12,9 ± 0,5 aA	13,8 ± 0,4 aA
1%	12,3 ± 0,5 aA	12,2 ± 0,5 aA	12,7 ± 0,4 aA	12,1 ± 0,6 aA	12,2 ± 0,6 bA
3%	10,4 ± 0,7 abA	10,4 ± 0,6 abA	9,3 ± 0,6 bA	9,7 ± 0,7 bA	10,8 ± 0,7 bA
5%	9,2 ± 0,6 cA	8,4 ± 0,6 cA	7,8 ± 0,6 cA	7,7 ± 0,7 cA	9,1 ± 0,7 cA
10%	5,9 ± 0,3 dA	4,9 ± 0,3 dA	5,5 ± 0,4 dA	7,7 ± 0,6 cB	8,6 ± 0,6 cB
25%	4,7 ± 0,3 eA	4,7 ± 0,3 deA	4,6 ± 0,3 deA	5,8 ± 0,4 dB	5,8 ± 0,4 dB
50%	4,0 ± 0,3 fA	4,4 ± 0,3 eB	3,9 ± 0,3 eA	4,9 ± 0,4 BeC	5,5 ± 0,4dC

The averages of the same line, followed by the same capital letter are not significantly different at the 5% threshold (Log Rank Test).

Means of the same column followed by the same lowercase letter are not significantly different at the 5% threshold (Log Rank Test).

Table 8: B-value estimation model resulting from Cox regression for the products used, including Wald coefficients.

Produits	**Stade *Helicoverpa***	**B**	**SE**	**Wald**	**ddl**	**Prob.**
Lambda-cyhalothrine	L3	2,378	0,489	41,674	1	0,000
Topbio	L3	1,895	0,425	32,410	1	0,000
T. purpurea	L3	1,478	0,397	27,801	1	0,000
Cold CNSL	L3	1,470	0,380	27,203	1	0,000
R. communis	L3	0,915	0,301	24,501	1	0,000
T neriifolia	L3	0,901	0,285	22,206	1	0,000

B = B value of the Cox regression; SE = Standard error; Wald = Wald coefficient; ddl = degree of freedom; Prob. = Probability

Table 9: B-value estimation model resulting from Cox regression for the products used including Wald coefficients

Produits	Stade *Helicoverpa*	B	SE	Wald	ddl	Prob.
Lambda-cyhalothrin	L2	6,245	0,875	48,258	1	0,000
Topbio	L2	4,105	0,621	37,784	1	0,000
T. purpurea	L2	3,078	0,415	32,987	1	0,000
CNSL	L2	3,041	0,401	30,541	1	0,000
R. communis	L2	2,520	0,321	25,470	1	0,000
T. neriifolia	L2	2,147	0,301	24,345	1	0,000

B = B value of the Cox regression; SE = Standard error; Wald = Wald coefficient; ddl = degree of freedom; Prob. = Probability

IV. Discussion

In general, the results obtained from the various experiments in the laboratory show that the botanical pesticides based on *T. purpurea*, *R. communis*, *T. neriifolia*, and the cold extraction cashew balm used are toxic to *H. armigera* larvae. These results corroborate studies on the larvicidal effect of different herbal extracts by (Sanda *et al*., 2006; Agboka *et al*., 2009). Larval mortality increased over time to reach dose-dependent peaks. As concentrations increase, so do larval mortality rates. With the L2 and L3 larval stages, the effects of the 3% and 1% doses of the pesticides used are significantly less than those generated by the 10% and 5% doses; similarly, the effects of the 10% and 5% doses of the pesticides used are significantly less than those generated by the 50% and 25% doses. The effects of 50% and 25% doses of vegetable oils and cold CNSL used are statistically equivalent. The emergence rate of L2 larvae from the low doses (1%, 3%, and 5%) is significantly lower than that observed with stage 3, probably due to the low quantity of product received in relation to their weight. The combination of parameters such as the very high mortality rate of larvae, and the short Mean Survival Time (MST) of larvae treated with *T. purpurea* and CNSL at the 25% and 10% application rate, make these vegetable oils an excellent candidate for the development of biological insecticides. These results confirm the work of Azonpkin *et al*. (2018), which showed that cold or hot CNSL is a potential candidate for the control of H. armigera in organic cotton from a low application rate. These results are also similar to those obtained by Akpo *et al*. (2017) using the same botanical pesticide on Anopheles gambiae mosquito larvae. However, a more detailed study of the chemical composition of these oils is needed in view of their large-scale use as a biopesticide. The 5% dose of the botanical pesticides used increased mortality in *H. armigera* compared to the control. These results are contrary to those of Lowery *et al*. (1993) on aphids, who showed that a dose of 1% neem oil resulted in a significant reduction of the aphid population compared to the control. *R. communis* oil had a significant effect on the L2 and L3 larvae of H. armigera tested at the different doses. The highest doses of *R. communis* oil, 50%, and 25% killed 100% of the L2 larvae of *H. armigera* after 14 days, only the 50% dose was able to eliminate 100% of these larvae on L3. There was a significant difference between the higher doses of 50% and 25% and the lower doses of 3% and 1% of the *R. communis* oil. The larvicidal effect of *R. communis* extracts had already been highlighted by (Olsnes, 2004; Kumar *et al*., 2007; Tounou *et al*.., 2011, on *P. xylostella*, by Aouinty et al. (2006) on four mosquito species, Culex pipiens (L.), Aedes caspius (Pallas), Culiseta longiareolata (Aitken) and Anopheles maculipennis (Meigen) Anani *et al*. (2004) and by Laghdaf and Ferji, (2005) on nematodes. The toxicity of the plant is due to the presence of ricin, a water-soluble substance, a glycoprotein concentrated in the endosperm seed. *T. neriifolia* is a plant of medicinal interest because of its toxic property (Garima and Amla, (2011). The insecticidal activity of the extracts has been proven by several authors in the agricultural field. Indeed, extracts from plant organs have shown biocidal activity on Callosobruchus maculatus (Mollah and Islam, 2007) and against adult maize weevils

(Wanyika et al., 2009) and on Anopheles gambiae mosquitoes (Akpo *et al.* 2017). The work carried out by Chougourou *et al*. (2012) also demonstrated the biocidal efficacy of oil extracted from seed kernels on Musca domestica larvae. Similar studies carried out in Tanzania have also shown the biocidal effect of extracts of T. purpurea on mosquito larvae (Mayunga, 2002; Touqeer *et al*., 2013). Indeed, the presence of botanical pesticides significantly reduced leaf consumption by the larvae. The areas of tomato leaves treated with different vegetable oil extracts consumed by *H. armigera* larvae were generally smaller than on the control leaves. The higher the concentration, the more inappetent the leaf is to larvae. In a study carried out by Anam *et al*. (2006), a significant reduction in the area of cowpea leaves treated with different concentrations of neem oil (0.25%; 0.5%; 1%; 2%; and 4%) consumed by Epilachna dodecastigma Wied larvae was observed in comparison with the control. Following a 24-hour treatment; (28,880 mm2) of leaves were consumed compared to 40,101 mm2 for the control. The observed anti-appetizer effects are, in fact, highly correlated with the sensory responses of chemoreceptors on insect mouthparts (Mordue *et al.* 1998). The position of these receptors (pits, mouthparts, mouth cavity) depends on the feeding behavior of the insect. However, in addition to feeding deterrence due to the inappetence of treated leaves, mortality and pupation of larvae are linked to molting disturbances. Effects on moulting are due to disturbances in the synthesis and release of ecdysteroids (moulting hormones) by inhibition of hormone release from the prothoracic glands (Mordue and Nisbet, 2000). In insects injected with azadirachtin prior to hormone production, the total blockade of the ecdysteroid was noted (Mordue (Luntz) and Nisbet, 2000). This resulted in the inhibition of insect development and then larval moulting and resulted in death. The Cox regression model used in the determination of the LD 50 of the pesticides used allowed an easy analysis of biopesticide bioassays. Models that consider time and dose effects appear to be more appropriate for assessing the efficacy of a pathogen or pesticide on the target (Robertson and Preisler, 1992). Cox regression models have been used to model the relationship between time, dose, and survival of pesticides infecting *H. armigera* larvae in lethal dose experiments. Among the vegetable oils tested on *H. armigera* larvae, the cold CNSL and *T. purpurea* yielded the highest B values (1.478, 1.470) and B values (3.078, 3.041) on stage 3 and 2, respectively, (and hence narrower confidence intervals (high and low CI) and lower LD50 values (B being the regression coefficient which is the relative risk (here instantaneous risk of death) associated with one treatment compared to another treatment). The adjustments to the B values of the biological pesticides and the reference control Lambda-cyhalothrin are all significant, indicating that the data show a significant effect/dose-response for the pesticides and confirming the good Cox regression for the latter on the young H. armigera larvae tested. Positive regression coefficients for both pesticides independently of the pests predicted increased pest mortality over time. Our results also show that for the same larval stage, the time taken to die is a function of the applied dose. Similarly, at a given time, the rate to be applied is a function of the larval stage; the more advanced the stage, the higher the rate to be applied. At 5 days after treatment with oil of T. purpurea, it takes 15.6% to kill 50% of stage 2 larvae versus 17.3% for L3 larvae, respectively.

Conclusion

From the results obtained, it appears that the effect/dose-response was significant for both larval stages of H. armigera used and for all pesticides used; the study also showed that larvae from the same population showed a sensitivity that differed according to the products used. Cold CNSL and T. purpurea appeared to be more effective than the other two vegetable oils used. There is indeed a correlation between the larval stage, the application rate of the product, and the average survival time of the larvae. An increase in rate is required to move from a younger to a more advanced larval stage. Of the two larval stages of H. armigera used, the L2 stage is the most vulnerable. Doses of 25% and 50% gave similar results allowing the lower dose to be chosen. From the results obtained, 25% and 50% rates can be tested in field trials to determine the application rate for the management of H. armigera in the field.

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