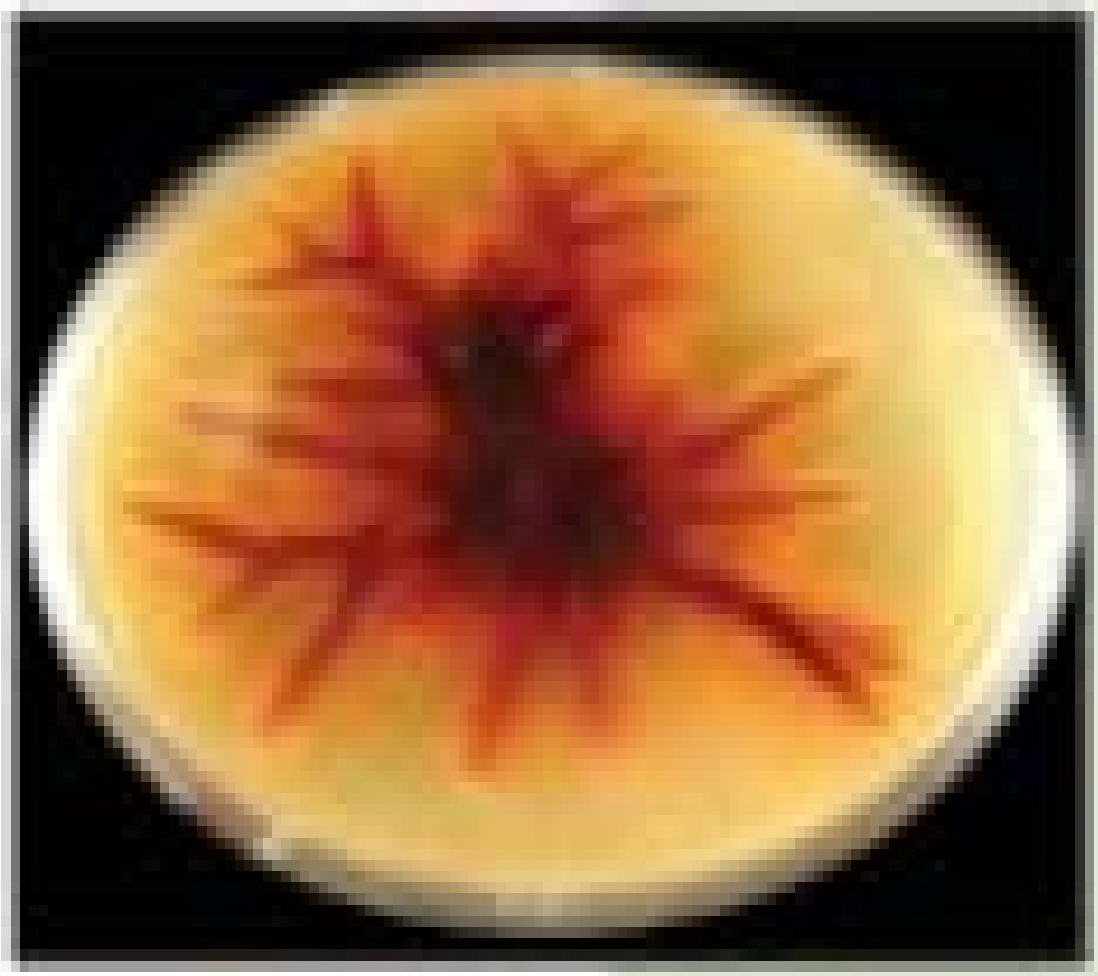

Journal of Applied BioSciences

Volume X (XX)
January 2020
ISSN

Journal of Applied Biosciences 129: 13029 -13038

ISSN 1997-5902

Effectiveness of neem seed oil (*Azadirachta indica* A. Juss: Meliaceae) on *Syllepte derogata* Fabricius, Lepidoptera: Pyralidae

Haffizou GANDA[1*], Euloge C. TOGBÉ[2], Thomas A. HOUNDÉTÉ[3], Elisabeth T. ZANNOU BOUKARI[4], Mohamed GOGAN[5], Gustave D. DAGBÉNONBAKIN[6] & Dansou K. KOSSOU[7]

[1] Laboratory of Agricultural Entomology (LEAg), Faculty of Agronomic Sciences, University of Abomey-Calavi, 03 BP 2819 Cotonou, Republic of Benin: gandahaffiz@gmail.com

[2] Laboratory of Crop Biology, Faculty of Agronomics Sciences, University of Abomey-Calavi, 03 BP 2819 Cotonou, Republic of Benin: euloge.togbe@yahoo.fr

[3] Agricultural Research Center for Cotton and Fibers, National Institute of Agricultural Researches of Benin (CRA-CF/INRAB), BP 143 Bohicon, Republic of Benin: houndetet@yahoo.fr

[4] Laboratory of Agricultural Entomology (LEAg), Faculty of Agronomic Sciences, University of Abomey-Calavi, 03 BP 2819 Cotonou, Republic of Benin: ezannou2@yahoo.fr

[5] Faculty of Agronomy and Environment Sciences, Catholic University of West Africa, Benin (FSAE/UCAO), 04 BP 928 Cotonou, Republic of Benin: mendosmoo@yahoo.fr

[6] National Institute of Agricultural Researches of Benin (INRAB), 01 BP 884 RP, Cotonou, Republic of Benin: dagust63@yahoo.fr

[7] Faculty of Agronomy and Environment Sciences, Catholic University of West Africa, Benin (FSAE/UCAO), 04 BP 928 Cotonou, Republic of Benin: kossoudansou@yahoo.com

***Corresponding author:** Haffizou Ganda, gandahaffiz@gmail.com; 03 BP 3232 Jéricho Cotonou, Bénin. Phone: 00229 97261936

Original submitted in on 31st May 2018. Published online at www.m.elewa.org on 30th September 2018 http://dx.doi.org/10.4314/jab.v129i1.6

ABSTRACT

Objective: Synthetic insecticides have long been used for cotton protection, resulting in pest resistance, toxicity and environmental pollution. Biopesticides have been suggested as alternatives to synthetic pesticides. Both field and laboratory experiments were conducted to evaluate the effectiveness of neem oil in controlling *Syllepte derogata* (Fabricius), a cotton phyllophagous pest.

Methodology and Results: In the field trials, effect of neem oil was compared to that of conventional insecticides; while in the laboratory direct larval immersion and leaf dip method using EMA SUPER 56DC and neem oil were tested. Decrease in damage by *S. derogata* for about 63 and 86% was recorded with neem oil and synthetic insecticides. In the laboratory, the mortality of *S. derogata* after 24 hours exposure to neem oil and Ema Super was significantly higher (2.5 to 100%) than that of the control. The mortality of larvae of *S. derogata* was positively correlated with the concentration of neem oil and exposure time. Lethal Concentration (LC50) after 24 hours exposure of larvae was respectively 4.03 10^4 ml/l and 51.13 ml/l for leaf dipping method and larval immersion.

Conclusion and application of results: Overall, these results showed the efficacy of neem oil in controlling *S. derogata*, as a biopesticide. This oil could also constitute a successful alternative to synthetic

pesticides. However, the effectiveness of neem oil appeared to be weakened by the rapid degradation of the active substances, azadirachtin in particular. Indeed, azadirachtin, the main active ingredient of neem is photo and heat labile. It easily degrades under high solar radiations and high temperatures, hence the need for stabilization.

Keywords: *Phyllophagous pest*, integrated pest management, leaf-dipping method, larval immersion, Lethal Concentration.

INTRODUCTION

Cotton is among the most important cash crops cultivated in the West African countries. This crop is ranked first in Benin in terms of acreage cultivated, production and its contribution to the Gross Domestic Production (Midingoyi, 2008). However, cotton is subject to many pest attacks. The incidence of those pests varies from one season to another depending on the prevailing conditions in each growing area (Togbé *et al.*, 2014a). Two categories of pests are dominant on this crop: phyllophagous pests that feed on leaves and carpophagous pests that feed on squares, flowers and bolls. Attacks of those pests are followed with a decrease in the yield when the crop is not adequately protected (Ochou *et al.*, 2012). To circumvent pest damages, farmers disregard the recommendations and increase the number of pesticides applied (Togbé, 2013). As a result of this indiscriminate use of pesticides, new problems emerge such as the development of pest resistance to pesticide, the intoxication of mammals, the destruction of non-target insects like pollinators, and the pollution of the environment (Sinzogan, 2006; Ochou *et al.*, 2012) Biopesticides appear then as the promising alternatives to address pest attack in cotton production. Among those biopesticides in use in cotton, spinozine is considered safe for human health and the environment and to a lesser extent indoxacarb and acetamiprid (Ton, 2006). Recently, leaf and seed extracts from *Azadirachta indica* (neem tree), *Hyptis suaveolens* (chan), *Carica papaya* (papaya tree) and *Nicotiana tabacum* (tobacco) have been also suggested. Among neem formulations, seed oil has gained interest due to its ability to control various pests of food crops, market gardening, fruits and vegetables and industrial crops like cotton on which such formulations appear to have a promising result (Kuklinski, 1998; Subbalakshml *et al.*, 2012). Testing the specific action of neem oil which has proved to be not harmful to humans, environment, and especially beneficial to the entomofauna has become a necessity (Nboyine *et al.*, 2012). The objective of this study was to assess the effectiveness of neem oil (*Azadirachta indica* A. Juss) on *Syllepte derogata* (Fabricius) in both field and laboratory.

MATERIALS AND METHODS

Laboratory rearing of S. derogata: *Syllepte derogata* caterpillars were collected from cotton untreated plots in two villages of Djidja, Central Benin: Aliou (7 ° 30'51.10 " North latitude and 1 ° 43'57.65" east longitude and Kpota (7°20'39.62" North latitude and 1°54'17.19' 'East longitude). These caterpillars were reared in transparent polystyrene boxes of 30 cm length and 8 cm height in the Laboratory of Entomology of Agricultural Research Center on Cotton and Fiber (CRA-CF), in Cana at Zogbodomey, Central Benin (7°7'52.48" North latitude and 2°5'19.11" East longitude). They were fed with fresh and healthy leaves of cotton. Sexing was performed after pupation. Adults (moths) emerged seven to nine days after pupae incubation. They were then coupled in cylindrical boxes (14 cm of diameter and 18 cm of height) containing cotton leaves and sugar water at 10%, and covered with net. After two to five days, eggs were laid on the compresses and leaves. These eggs were incubated in hatching boxes (10.6 cm of diameter and 8 cm of height) at a temperature of 27°C and a relative humidity located between 60% and 70%. The emerged larvae were placed on fresh cotton leaves in polystyrene boxes. Neonate larvae of first and second instars emerged and were used in laboratory trials.

Effect of neem oil toxicity on field infestation of S. derogata: *The* experiment was carried out using a randomized complete block design with four replicates

in each village (Aliou and Kpota) on plots of 10 m × 10 m. The distance of 2 m was observed between two plots within the same block while blocks were separated by a distance of 5 m. Each plot had twelve rows separated with 80 cm distance while the intercrops distance on each row was 40 cm. Five treatments were tested : control with no insecticide, conventional based on synthetic pesticides and three neem oil doses (1; 1.5 and 2 l/ha). Neem oil was obtained using a cold-press-extraction. The azadirachtin content was determined at 0.087 µg/ml using High Performance Liquid Chromatography (HPLC). Six fortnight treatments were applied during the growing phases starting from the forty five (45) days after emergence (DAE) (Mathess *et al.*, 2005). In the conventional based treatments: Ema Super 56 DC (Emamectin benzoate 24 g/l + Acetamiprid 32 g/l) was applied for the first two treatments; Profenofos + Cypermetrin 440G / ESWL (Profénofos 400 g/l + Cypermetrine 40 g/l) for the third and fourth applications and Cotton Plus ™ 88 EC (Cypermetrin 72 g/l + Acetamiprid 16 g/l) for the fifth and sixth applications at the doses of 1 l/ha. All the sprays were brought to 10 litres per hectare using water as solvent. Field scouting was performed weekly before spraying. Twenty plants were sampled along the diagonals (10 plants/diagonal) in each plot to check the presence and damages by *S. derogata* by examining top and bottom faces of the leaves and counting the number of plants infested by *S. derogata*. The infection rate was obtained using the ratio number of plants attacked by the total number of plants scouted:

$$\text{Rate of infestated plants} = \frac{\text{Number of plants attacked by } S.\ derogata}{\text{Total number of plants scouted}} \times 100$$

Laboratory studies of the effect of neem oil toxicity on the mortality rate and weight of S. derogata: Effect of neem oil on 2nd instar larvae of 25 to 35 mg was conducted using a completely randomized block with four replicates and eight treatments (control without insecticide, synthetic insecticide EMA SUPER 56 DC at the dose of 50 ml/l; neem oil at 50, 100, 150, 200, 250 and 300 ml/l). Two methods of exposure such as direct larvae immersion in neem oil and larvae exposure to leaves previously dipped in neem oil and used as food substrate. Under each method, 10 larvae were used per replicate. Fresh cotton leaves were brought from fields and washed thoroughly with tap water and completely air dried and used for the tests. The leaf dipping method consisted of immersing leaves in solutions for 30 seconds and allowing it to air-dry (Mamoon-Ur-Rashid *et al.,* 2011). Leaves dipped only in soapsuds served as control. Each larvae was exposed to three treated leaves in Petri dish of 9 cm diameter. This method aimed at assessing the effect of neem oil on the intake ability of larvae. To study the effect of neem oil on the larvae by contact, larvae were dipped directly in the various solutions during 30 seconds and then removed and reared in Petri dishes containing three fresh healthy leaves (Wondafrash *et al.*, 2012). Number of dead larvae in the Petri dishes was recorded after 24, 48, 72, 96, 120 and 144 hours of exposure. Weights of larvae before treatment and after 3 and 6 days exposure were determined.

Data analysis: Analysis of Variance (ANOVA) was performed and means were compared using the test of Student Newman and Keuls at 5% P value. *Syllepte derogata* infection rate were analysed using GLM procedure (SAS 9.2, SAS Institute, Cary, NC, USA) including treatment and village as fixed factors. Blocks nested within villages were considered as random factors. In laboratory, effects of neem oil on *S. derogata* larvae were evaluated as percentages of cumulative daily mortality. For each concentration-mortality and concentration-larvae weight experiment, data were analysed using a PROC ANOVA procedure of SAS with treatment as the only factor. Statistical analyses were carried out using computer software Statistical Analysis System Version 9.2 (SAS v.9.2). "Dose Létale 50 Windows - Version 2.0" (WINDL50 V.2.0) was used to determine Lethal Concentration (LC50) of neem oil on *S. derogata*. Lethal time LT50 was determined using Minitab 14.

RESULT

Effect of neem oil on the infestation of S. derogata: The effect of treatments on the infestation by *S. derogata* did not vary significantly with villages ($p = 0.383$). However, the infestation by *S. derogata* was significantly reduced by various insecticides ($p < 0.001$). The highest reduction of infestation by *S. derogata* was recorded with synthetic insecticides (86.01%), followed by that of neem oil at 2.0 l/ha (74.40%), neem oil at 1.5 l/ha (64.29%) and neem oil at 1.0 l/ha (51.19%) (Table 1).

Table 1: Mean number of plants infested by *Syllepte derogata* (N ± SE) during the 2014-2015 seasons based on the mean of 12 observation on 20 plants

Villages	N ± SE
Aliou	3.25 ± 0.61 a (0.34)
Kpota	2.77 ± 0,56 a (0.31)
Fisher F	**0.63^{ns}**
Treatments	N ± SE
Control	6.72 ± 1.14 A (0.51)
Conventional	0.94 ± 0.27 D (0.18)
NO 1l/ha	3.28 ± 0.17 B (0.36)
NO 1.5l/ha	2.40 ± 0.15 BC (0.31)
NO 2l/ha	1.72 ± 0.63 CD (0.24)
Fischer F	**21.51*** **

*** p<0.001, NO = neem oil

Means followed by the same letter within a column are not significantly different from each other at p<0.05 according to Student Newman and Keuls test.

The values in brackets represent the means transformed with 2Arcsin√ (n / 100) (Dagnelie, 1998) (n represents the actual values of the percentage of infected plants).

Effect of neem oil on the mortality of S. derogata larvae: The effect of the various treatments on the mortality of *S. derogata* larvae in leaf dipping experiment was significantly different (p<0.001). Hundred percent (100%) mortality were recorded after 24 hours with the population of *S. derogata* fed with leaf dipped in Ema Super 56DC while no mortality was recorded with larvae population in the control, as well as with population exposed to the various concentration of neem oil (Table 2). Mortality rate (2.50% to 5.00%) of *S. derogata* larvae exposed to leaf dipped in neem oil were similar to that of soapsuds treatment (0%) but significantly lower than that of leaf dipped in Ema Super 56 DC (100%) 24 hours after exposure. After 24, 48, 72 and 96 hours exposure to leaf dipped in treatment solutions, the mortality of population of *S. derogata* larvae recorded with Ema Super 56DC, was significantly higher than that of leaf dipped in various doses of neem oil (Table 2). However, the mortality rate after 120 hours exposure of larvae of *S. derogata* to leaf dipped with neem oil at 250 ml /l (85%) and 300 ml/l (87.5%) and those after 144 hours exposure at 100; 150; 200; 250 and 300 ml/l doses (respectively 80%; 90%; 87.5%; 97.5% and 97.5%) were not significantly different from that recorded with Ema Super 56 DC. As far as the direct immersion was concerned, the effects of various treatments on the mortality rate of *S. derogata* were significantly different (p<0.001). Hundred percent mortality of larvae were recorded with Ema Super 56DC 24 hours exposure, while mortality rate of 55.0%; 52.50%; 47.50%; 55.0%; 67.5% and 65.05% were recorded respectively with neem oil at 50, 100, 150, 200, 250 and 300 ml/l . There was no significant difference between the rates of mortality recorded at different times with the various doses of neem oil. The rates of mortality with neem oil concentrations were lower than that recorded with Ema Super 56DC after 24, 48 and 72 hours of treatment. The rate of mortality with Ema Super 56 DC (100%) and neem oil (82.5% to 97.50%) after 96, 120 and 144 hours exposure were not significantly different (Table 2).

Table 2: Effect of neem oil and Ema super 56 DC on the percent mortality of *Syllepte derogata* (M ± SE) at different intervals.

Methods	Treatments	M ± SE (%)					
		24h	48h	72h	96h	120h	144h
Leaf dipping	Control	00±00b	00±00c	00±00d	00±00f	00±00e	2.50±2.50c
	ES 5%	100±00a	100±00a	100±00a	100±00a	100±00a	100±00a
	NO 5%	2.50±2.50b	05±2.89c	12.50±2.50cd	20±4.08e	37.50±8.54d	50±12.25b
	NO 10%	2.50±2.50b	15±6.45bc	22.50±8.54bcd	40±4.08d	62.50±4.79c	80±4.08a
	NO 15%	2.50±2.50b	12.50±4.79bc	30±10.80bc	50±7.07cd	72.50±7.50bc	90±5.77a
	NO 20%	05±05b	15±6.45bc	30±7.07bc	62.50±6.29bc	80±4.08bc	87.50±4.79a
	NO 25%	05±05b	17.50±7.50bc	35±05bc	67.50±4.79b	85±05ab	97.50±2.50a
	NO 30%	05±2.89b	30±7.07b	50±10.80b	70±4.08b	87.5±2.50ab	97.50±2.50a
	Fisher F	121.84***	36.66***	18.84***	48.18***	42.27***	37.46***
Direct larval immersion	Control	00±00c	00±00c	00±00c	00±00c	00±00c	05±2.89b
	ES 5%	100±00a	100±00a	100±00a	100±00a	100±00a	100±00a
	NO 5%	55±2.89b	65±2.89b	72.50±4.79b	82.50±4.79b	82.50±4.79b	90±5.77a
	NO 10%	52.50±6.29b	65±2.89b	75±2.89b	82.50±2.50b	92.5±4.79ab	100±00a
	NO 15%	47.50±6.29b	65±05b	72.50±2.50b	82.50±4.79b	90±4.08ab	95±05a
	NO 20%	55±9.57b	75±2.89b	85±2.89b	90±00ab	97.50±2.50ab	97.50±2.50a
	NO 25%	67.50±7.50b	75±8.66b	85±05b	90±4.08ab	92.50±4.79ab	95±05a
	NO 30%	65±9.57b	77.50±4.79b	85±6.45b	92.50±2.50ab	97.50±2.50ab	100±00a
	Fisher F	18.76***	44.86***	65.87***	108.57***	91.18***	87.02***

*** p<0.001

Means followed by the same letter within a column are not significantly different from each other at P<0.05 according to Student Newman and Keuls test.

NO = neem oil; ES = Ema Super; 5% = 50ml/l; 10% = 100ml/l; 15% = 150ml/l; 20%= 200ml/l; 25% = 250ml/l; 30% = 300ml/l.

The mortality recorded with neem oil increased gradually with the exposure time and the doses of treatments. Thus, with the leaf dipping method, the first 50% mortality were observed after 72 hours exposure with the dose of 300 ml/l; the mortality rate of 50% was reached with the other doses of neem oil after 144 hours exposure to treatments. With direct immersion, 50% mortality was recorded with various doses of neem oil after 24 and 48 hours exposure (Table 2).

Weight of larvae under treatments : In both experiments, average weight of *S. derogata* larvae after 72 and 144 hours exposure (HAE) showed significant difference ($p = 0.0003$ to $p<0.0001$) as far as treatments are concerned. The effect of neem oil on the average weight reduction of *S. derogata* in larvae immersion experiment and leaf dipping method was significantly higher after 72 and 144 hours exposure compared to the control (Table 3). After 72 hours exposure, the higher percentages (67.45% and 69.37%) of weight losses by *S. derogata* larvae were recorded from larvae dipped in neem oil respectively at the doses of 250 and 300 ml/l. The highest weight losses of *S. derogata* larvae were recorded in leaf dipping method after 144 hours exposure with 150; 200 and 250 ml/l solution (72.93; 72.49 and 72.22% respectively) (Table 3).

Table 3: Mean weight (W ± SE) and percent weight losses (WL) of *Syllepte derogata* larva at 72 and 144 hours after exposure (HAE) with leaf dipping and larval immersion methods

Treatments	0 HAE	72 HAE		144 HAE	
	W ± SE(mg)	W ± SE(mg)	WL (%)	W ± SE(mg)	WL (%)
Leaf dipping method					
Control	30.03±0.83a	72.99±3.49a	-	66.29±3.62a	-
NO 5%	29.77±0.24a	39.84±2.41b	45.22	30.24±3.97b	54.20
NO 10%	29.36±0.23a	34.06±2.13bc	52.90	21.17±5.58b	67.74
NO 15%	29.27±0.55a	34.27±1.62bc	52.55	17.74±00b	72.93
NO 20%	28.91±0.29a	30.94±0.29c	56.95	17.93±4.93b	72.49
NO 25%	29.73±0.30a	30.80±0.57c	57.63	26.85±00b	59.31
NO 30%	29.97±00a	29.78±0.78c	59.17	18.40±00b	72.22
Fisher F	0.48ns	50.54***		14.29***	
Larval immersion method					
Control	29,89±0,25a	75,89±0,31a	-	71,72±0,47a	-
NO 5%	29,70±0,26a	33,50±2,79b	55,75	34,90±2,30b	54,52
NO 10%	29,87±0,51a	25,51±1,09c	66,38	-	-
NO 15%	29,62±0,90a	26,92±0,68c	64,40	-	-
NO 20%	29,27±0,15a	25,55±1,26c	66,06	20,70±00c	66,90
NO 25%	29,34±0,38a	24,52±1,30c	67,45	19,70±00c	67,98
NO 30%	29,51±00a	23,13±1,68c	69,37		-
Fisher F	0,96ns	116.77***		298.93***	

*** p<0.001; ns: No Significant

Means followed by the same letter within a column are not significantly different from each other at P<0.05, Student Newman and Keuls test.
NO = neem oil, 5% = 50ml/l; 10% = 100ml/l; 15% = 150ml/l; 20%= 200ml/l; 25% = 250ml/l; 30% = 300ml/l.

Toxicological parameters of neem oil on S. derogata larvae after exposure*:* Toxicological study of neem oil on *S. derogata* larvae showed that the lethal concentration CL10, CL50 and CL90 recorded with the larval immersion method were lower than that recorded with leaf dipping method (Table 4). Under the leaf dipping method, the LC 50 and LC 90 were estimated respectively at 4.03 x 10^4% and 1.13 x 10^7% at 24 hours; while under larval immersion method, the LC 50 and LC 90 were respectively 5.11% and 1.11 x 10^4%. In direct larvae immersion method, the LC50 recorded after 24 hours exposure was 36 times higher than that recorded after 144-hours exposure (Table 4). In addition, the LC50 of neem oil calculated for a longer time of death (144h) was about 36 times lower than that recorded after 48h exposure with the leaf dipping method. The time required to kill 50% of the larvae (LT50) was estimated with leaf dipping at a concentration of 50 ml/l and was twice higher than that recorded with the concentration of 300 ml/l of neem oil (Table 5). With the direct larvae immersion method, regardless of the neem oil concentrations, the time necessary to kill 50% of the larvae was less than 24 hours.

Table 4: Toxicological parameters of neem oil after 24h, 48h, 72h, 96h, 120h and 44h of Exposure 1% = 10 ml/l

Exposure duration (hour)	Leaf dipping							Direct larval immersion						
	LC 50 (%)	confidence limits of LC 50 (%)		LC 90 (%)	confidence limits of LC 90 (%)		Slope	LC 50 (%)	confidence limits of LC 50 (%)		LC 90 (%)	confidence limits of LC 90 (%)		Slope
		Upper	Lower		Upper	Lower			Upper	Lower		Upper	Lower	
24	4.03x104	6.243x10 -36	1.00x1038	1.13x107	1x 10+38	1x 10-38	0.52	5.11	1.00x1038	1.00x10-38	1.11x104	2.457x 10+20	5.004x 10-13	0.38
48	1.79x102	4.464	7.161x103	4.62x103	2.69310 +11	7.914x 10-5	0.91	1.26	1.00x1038	1.00x10-38	4.44x102	1.418x 10+34	1.393x 1029	0.50
72	4.04x101	1.190x10 1	1.368x102	3.95x102	2.950x 10+4	5.302	1.29	7.54x1 0-1	1.00x1038	1.00x10-38	7.90x101	7.861x 10+30	7.935x 10-28	0.63
96	1.43x101	2.352	8.719x101	7.41x101	1.518x1 0+2	3.613x 10+1	1.79	2.24x1 0-1	1.00x1038	1.00x10-38	2.68x101	1.00x 10+38	1.00x 10-38	0.62
120	7.16	2.094x10 -1	2.447x102	3.41x101	1.128x 10+2	1.030x 10+1	1.89	5.98x 10-1	2.538x1029	1.410x10-30	9.99	5.573x1 0+19	1.791x 10-18	1.05
144	4.99	2.450	7.357	1.69x101	2.370x 10+1	1.247x10+1	2.42	1.43x1 0-1	3.410x101	5.960x10-4	3.87	2.016x 10+1	7.412x 10-1	0.89

Table 5: Lethal time (LT50) of neem oil on larvae of *Syllepte derogata*

Neem oil Concentration (ml/l)	Regression equation		LT50 (hour)	
	Leaf dipping	Direct larval immersion	Leaf dipping	Direct larval immersion
50	Y = 35.1 + 2.30X	Y= - 168 + 3.38X	150.1	1
100	Y = 28.5 + 1.50X	Y= - 114 + 2.55X	103.5	13.5
150	Y = 28.0 + 1.30X	Y= - 105 + 2.51X	93	20.5
200	Y = 24.7 + 1.27X	Y= - 132 + 2.59X	88.2	-2.5
250	Y = 23.4 + 1.18X	Y= - 254 + 4.01X	82.4	-53.5
300	Y = 12.4 + 1.26X	Y= - 200 + 3.29X	75.4	-35.5

Y= Lethal time and X= mortality recorded after treatment

DISCUSSION

Sensitivity of S. derogata larvae to neem oil*:* In field, the application of various doses of neem oil led to a reduction in *S. derogata* infection indicating that neem oil was not only toxic in laboratory bioassays but were also effective in the field. In fact, the principal active ingredient responsible of neem oil toxicity is Azadirachtin. Azadirachtin is known to be active against nearly 550 insect species including Coleoptera, Dictyoptera Diptera Heteroptera, Lepidoptera, Orthoptera, Thysanoptera etc. (Mehaoua *et al.*, 2013). The results also showed that higher was the dose, higher was the larval mortality in laboratory bioassays and lower was the infestation rate in field. Similar results were observed with the carob moth, *Ectomyelois ceratoniae*, *Plodia interpunctella* and *Musca domestica* (Rharrabe *et al.*, 2008; Chougourou *et al.*, 2012; Mehaoua *et al.*, 2013). This study concludes that azadirachtin has a dose-mortality effect with a significant increase in larval mortality and effectiveness when the dose increases. The percentage of larvae killed by neem oil after 144 hours exposure was significantly higher than that killed in 24 hours for both methods, suggesting a delayed toxicity with neem oil. Such an observation was already made by Martinez and Van Emden (2001) who concluded that neem products have a high response time. The mortality rates recorded with direct larval immersion method were higher than that observed with the leaf dipping method. The highest mortality obtained 24 hours after treatment in the leaf dipping experiment was 7.5%, whereas in direct larval immersion experiment, the lowest mortality rates within the same time frame was 47.5%; the highest was 85%. In fact, azadirachtin is known for its dual action (contact and ingestion) which justified its acute and chronic toxicity (Poland *et al.*, 2006). The rapid death of larvae can be explained by the direct contact effect (Mehaoua *et al.*, 2013), showing that the effect of ingestion is not necessarily required for the insect death (Bitten and Blackwell, 1993) Greater toxicity was observed in larval immersion compared to larvae exposure in leaf-dipped method. These study findings contrast with the work by Wondafrash (2007) conducted on *Helicoverpa armigera.* In this study, larval mortality was higher in square dip study than in the larval immersion experiment. He explained his findings by the short exposure time (20 seconds versus 30 seconds in the current study) resulting likely in the low absorption of the active ingredient that might be required to affect the treated larvae. Laboratory results were confirmed by field observations, which recorded the reduction of 51.19% to 74.40% in infestation by *S. derogata.* Similarly, the works by Togbé *et al.* (2014b) indicated a decrease of 29.58% to 44.19% in infestation by this phyllophagous pest using neem oil at 1 l/ha. This suggests the possible variation in the effectiveness of neem oil according to the experimental areas. In fact, their field experiments were carried out in three districts (Kandi, N'dali and Djidja) while ours was conducted only in Djidja.

Effect of neem oil on the weight of S. derogata larvae: Neem oil reduced significantly the growth and development of *S. derogata* larvae compared to the control treatment. This effect on the growth was observed through the significant reduction in weight of larvae treated with neem oil in both methods. Similar result was observed with *Helicoverpa armigera* larvae and pupae (Wondafrash *et al.*, 2012). In addition, the reduction in weight increased with the doses of various neem products. This implies that higher concentrations led to higher weight losses. Larvae treated with neem oil did not evolved into pupae while the untreated control came into pupation between the 7th and 9th day after treatment. The weight reduction could be explained by the effects of azadirachtin on the morphology and physiology of larvae through direct contact and ingestion. The well-known effects of azadirachtin are antifeedant, repellent, deterrent and growth inhibition action (Grišakova *et al.*, 2006). Lynn *et al.* (2010) argued that azadirachtin inhibits or reduces the rate of the 20-hydroxyecdysone, the molting hormone in insects, which delays or stops insect growth and development. Martinez and Van Emden (1999) contended that azadirachtin does not influence the digestive capacity of the insect but reduces his ability to convert food consumed into nutrients. Ahmed *et al.* (2012) reported that the ingestion of food treated with NeemAzal (neem insecticide) led to the reduced weight of *Plutella xylostella* pupae. Neem oil has therefore an anti-growth and anti-development effect, which leads to the death of exposed insects.

Toxicological parameters of neem oil on S. derogata larvae after exposure: Lethal concentrations calculated for 50% mortality (LC50) in various times, showed that more time was required to obtain 50% mortality with different concentrations of neem oil in leaf dipping experiment than in larval immersion experiment. The LC50 decreased with exposure time. In other words, neem oil was less toxic to *S. derogata* larvae within a short exposure times, but becomes more toxic when larvae's exposure to biopesticide is

extended. Similar results were found by Mehaoua *et al.* (2012), Martinez-Tomas *et al.* (2009) and Hameed *et al.* (2012) with respectively *Ectomyelois ceratoniae* larvae, *Culex quinquefasciatus* larvae and *Tribolium castaneum*. Our results indicated a strong correlation between concentration, time and mortality.

CONCLUSION

Infestations of cotton leaves by larvae of *S. derogata* were reduced by neem oil spray in the field. In laboratory, the study of neem oil toxicity on *S. derogata* larvae showed that this oil was less toxic than Ema Super 56DC, a synthetic product that killed all the larvae within 24 hours exposure. Despite its relative low toxicity, neem oil delayed the growth of larvae of *S. derogata*, leading to a loss of the weight of larvae. Mortalities caused by neem oil were positively correlated with the dose and the exposure time of the larvae to the product. Similarly, the LC50 calculated were positively correlated with duration of larvae exposure to product. Neem oil could be considered as a promising insecticide to manage *S. derogata*. This could also constitute a successful alternative to synthetic pesticides.

ACKNOWLEDGMENTS

We acknowledge « Convergence of Sciences-Strenghtening Innovation System (CoS-SIS) program » and «Centre de Recherches Agricoles Coton et Fibre (CRA-CF) » in Benin for co-funding this study. The authors are also grateful to cotton growers and extension agents in Djidja for their support during the field work.

REFERENCES:

Ahmed N., Ansari M.S. and Hasan F., 2012. Effects of neem based insecticides on Plutella *xylostella* (Linn.). *Crop Prot*. 34: 18-24.

Anuradha A. and Annadurai R.S., 2008. Biochemical and molecular evidence of azadirachtin binding to insect actin. *Current Science* 95: 1588-1593.

Chougourou C.D., Dellouh P.L., Agbaka A., N'guessan K.R. et Gbenou J.D., 2012. Toxicité et effets répulsifs de certaines huiles extraites des plantes locales béninoises sur la mouche domestique *Musca domestica* L. (Diptera Muscidae). *Journal of Applied Biosciences* 55: 3953-3961.

Dagnelie P. A., 1998. Quantitative vision of biometry in developing countries. *Biom. Bull.* 5(1): 6-9.

Grišakova M., Metspalu L., Jõgar K., Hiiesaar K. and Poldma P., 2006. Effects of biopesticide Neem EC on the Large White Butterfly, *Pieris brassicae* L. (Lepidoptera, Pieridae). *Agronomy Research* 4 (Special issue): 181-186.

Hameed A., Freed S., Hussain A., Iqbal M., Hussain M., Naeem M., Sajjad A., Hussain H., Sadiq M.A. and Tipu A.L., 2012. Toxicological effects of neem (*Azadirachta indica*), Kanair (*Nerium oleander*) and Spinosad (Tracer 240 SC) on the red flour beetle (*Tribolium castaneum*) (Herbst.). *African Journal of Agricultural Research* 7 (4) : 555-560.

Kuklinski F., 1998.Test d'efficacité de l'huile de neem et du savon liquide contre les déprédateurs clés de culture cotonnière pluviale. Projet DPV/GTZ « *Promotion de la protection intégrée des végétaux à Madagascar*» : 242-249.

Lynn O.M., Song W.G., Shim J.K., Kim J.E. and Lee K.Y., 2010. Effects of Azadirachtin and Neem-based formulations for the control of sweet potato whitefly and root knot nematodes. J. Korean Soc. *Appl. Biol. Chem.* 53 (5): 598-604.

Mamoon-ur-Rashid M., Khattak M.K., Abdullah K. and Hussain S., 2011. Toxic and residual ctivities of selected insecticides and neem oil against cotton mealybug, Phenacoccus solenopsis Tinsley (Sternorrhyncha, Pseudococcidae) under laboratory and field conditions. *Pak. Entomol*. 33 (2): 151-155.

Martinez S.M. and Van Emden H.F., 2001. Growth Disruption, Abnormalities and Mortality of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) Caused by Azadirachtin. *Neotropical Entomology* 30 (1): 113-125.

Martinez S.S. and Van Emden H.F., 1999. Sublethal concentrations of azadirachtin affect food intake, conversion efficiency and feeding behaviour of *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* 89: 65-71.

Martínez-Tomás S.H., Pérez-Pacheco R., Rodríguez-Hernández C., Ramírez-Valverde G. and Ruíz-Vega J., 2009. Effects of an aqueous extract of *Azadirachta indica* on the growth of larvae and development of pupae of *Culexquinquefa sciatus*. *African Journal of Biotechnology* 8 (17): 4245-4250.

Mathess A., Van den Akker E., Chougourou D. and Midingoyi S., 2005. Compétitivité et durabilité de cinq systèmes culturaux cotonniers. Deutsche Gesellschaft fûr Technishe Zusammenarbeit (Gmbh), Coopération Technique Allemande et Ministère de l'Agriculture de l'Élevage et de la Pêche Bénin : 206p.

Mehaoua M.S., Hadjeb A., Lagha M., Bensalah M.K., and Ouakid M.L., 2013. Study of the toxicity of azadirachtin on larval mortality and fertility of Carob Moth's female *Ectomyelois ceratoniae* (Lepidoptera, Pyralidae) under controlled conditions. *American-Eurasian Journal of Sustainable Agriculture* 7 (1): 1-9.

Midingoyi G.S.-K., 2008. Analyse des déterminants de l'efficacité de la production cotonnière au Bénin : cas des départements de l'Alibori et de l'Atacora. TFE en vue de l'obtention du diplôme de master complémentaire en économie et sociologie rurales. 77p.

Mordue A.J. and Blackwell A., 1993. Azadirachtin an update. *Insect Physiol.* 39: 903-924.

Nboyine J.A., Abudulai M. and Opare-Atakora D.Y., 2013. Field efficacy of neem (*Azadirachta indica* A. juss) based biopesticides for the management of insect-pests of cotton in northern Ghana. *Journal of Experimental Biology and Agricultural Sciences* 1 (4): 322-327.

Ochou G., Doffou M.N., N'goran E.K. and Kouassi P., 2012. Impact de la gestion de la résistance aux pyrethrinoïdes sur l'évolution spatio-temporelle des principaux lépidoptères carpophages du cotonnier en Côte d'Ivoire. *Journal of Applied biosciences* 53: 3831-3847.

Poland T.M., Haack R.A., Petrice T.R., Miller D.L. and Bauer L.S., 2006. Laboratory Evaluation of the Toxicity of Systemic Insecticides for Control of *Anoplophora glabripennis* and *Plectrodera scalator* (Coleoptera: Cerambycidae). *J. Econ. Entomol.* 99 (1): 85-93.

Rharrabe K., Amri H., Bouayad N. and Sayah F., 2008. Effects of azadirachtin on post-embryonic development, energy reserves and a-amylase activity of *Plodia interpunctella* Hübner (Lepidoptera, Pyralidae). *Journal of Stored Products Research* 44: 290-294.

Sinzogan A.A.C., 2006. Facilitating learning toward sustainable cotton pest management in Benin: the interactive design of research for development. PhD thesis. Wageningen University: 185p (2006).

Subbalakshmi L., Muthukrishnan P. and Jeyaraman S., 2012. Neem products and their agricultural applications. *JBiopest.* 5 (Supplementary): 72-76.

Togbé C.E., 2013. Cotton in Benin: Governance and pest management. Thesis submitted in fulfilment of the requirements for the degree of doctor at Wageningen University. 201p.

Togbé C. E., Haagsma R., Zannou E., Gbèhounou G., Déguénon J.M., Vodouhè S., Kossou D. and van Huis A., 2014a. Field evaluation of the efficacy of neem oil (*Azadirachta indica* A.Juss) and Beauveria bassiana (Bals.) Vuill. in cotton production. *J. Appl. Entomol.* DOI: 10.1111/jen.12174

Togbé C.E., Zannou E., Gbèhounou G., Kossou D. and van Huis A., 2014b. Field evaluation of the synergistic effects of neem oil with *Beauveria bassiana* (Hypocreales: Clavicipitaceae) and *Bacillus thuringiensis var. kurstaki* (Bacillales: Bacillaceae). *International Journal of Tropical Insect Science DOI : 10.1017/S1742758414000447*.

Ton P., 2006. Promouvoir la production plus durable de coton : Possibilités au Burkina Faso et au Mali. FAO, PNUE: 70p.

Wondafrash G.M., 2007. Effects of neem extracts on the feeding, survival, longevity and fecundity of African bollworm, *Helicoverpa armigera* (hubner) (Lepidoptera, Noctuidae) on cotton. Master thesis, Addis-Ababa University: 111p.

Wondafrash M., Getu E. and Terefe G., 2012. Neem, *Azadirachta indica* (A. Juss) extracts negatively influenced growth and development of African bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera, Noctuidae). *Academic Journal of Entomology* 5 (1): 22-27.