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Insecticidal effect of *Jatropha curcas* L. Oil on *Spodoptera frugiperda* (Smith) (Lepidoptera:Noctuidae)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MT and KA designed, performed the study, MT and LK performed statistical analysis and wrote the first draft of the manuscript. Author MaT helped to set up trial and managed results discussion. All authors read and approved the final manuscript.

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ABSTRACT

The study was undertaken to contribute to the sustainable management of *Spodoptera frugiperda* Smith on maize. It aims to evaluate the insecticidal efficacy of *Jatropha curcas* L. oil on *S. frugiperda* larvae. Two concentrations of *J. curcas* oil (10 and 20ml.l^{-1}) were tested by ingestion on the six larval stages of *S. frugiperda* grouped into (L_{1-2} (3-5 days of age); L_{3-4} (6-8 days of age); L_{5-6} (>10 days of age)). The insecticidal efficacy of the oil was determined in the laboratory and the phytosanitary protection tests on maize were carried out in the field. In the laboratory, the concentration of 10 ml.l^{-1} with/without emulsifier caused a mortality rate of (87-92%) at stage L_{1-2},

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(51-58%) at stage $L_{3-4,}$ and (57- 68%) at L_{5-6} stage after 72 hours of ingestion. Concentration of 20 $ml.l^{-1}$ caused over 70% mortality whether applied with or without an emulsifier at all stages. Adult emergence was nil for L_{1-2} stages at 20 ml.l-1 and <10% for the other stages. Plots subjected to jatropha oil treatments (2$l.ha^{-1}$ and 4$l.ha^{-1}$ with or without emulsifier) were less infested like the plots treated with Emamectine benzoate (Emacot) compared to control plots untreated. The present results indicated that jatropha oil has insecticidal potential against *S. frugiperda*.

Keywords: Jatropha curcas oil; bio-insecticide; efficacy; management; Spodoptera frugiperda.

1. INTRODUCTION

Maize (*Zea mays* L.) is an important staple crop in West Africa [1]. The most produced cereal, maize, is used in human food, animal feed, and industrial uses. More than 70% of maize production is reserved for human consumption [2] in west Africa.

In Togo, maize is the main grain crop produced mainly for human and animal consumption. Yields obtained across Togo average 1.39 t/ha [3] against a potential yield of 5 t/ha (Ikenne) [4]. Maize cultivation experiences various factors that limit its yields such as the decline in soil fertility, poor cultivation practices, diseases, environmental stresses, and insect pests [5]. Pests, especially insects, incontestably play an important role in the loss of maize yield. On maize, lepidopteran stem borers and ear miners are the most damaging insects [6]. They are native pests and are responsible for 10 to 100% yield loss [7]. An exotic species, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), also appeared in Africa in 2016 [8]. A pest of economic importance, *S. frugiperda* attacks maize and various crops causing substantial damage [9]. These larvae feed on young tender leaves and sometimes on the ears in formation, which can cause more than a 10% loss of yield [10]. Faced with these scourges, chemical insecticides have been used to limit the spread of the pest [11]. However, although these chemical products have been effective [12], most of them constitute poisons for users, and the environment, and are inaccessible to producers due to its high cost [13].

Thus, it is important to find alternatives in terms of crop protection. The use of botanicals with pesticidal effects presents itself as a possibility in the fight against insect pests [14]. These botanical pesticides, commonly called biopesticides, have a real advantage due to their low persistence, their low toxicity for humans, and their mode of action on pests [15]. Botanical pesticides have been effective against maize insects in Africa [2]. Bruce et al. [16] demonstrated the effectiveness of neem products against the stem borers *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Agboka et al. [2] demonstrated that neem oil has deterrent effects on the egg-laying of *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae). Among these plants with proven biocidal properties, *Jatropha curcas* L. (Euphorbiaceae) has significant insecticidal qualities. Indeed, the work carried out by Solsoloy [17] proved the insecticidal potential of the oil of *J. curcas*. Also, the insecticidal effect of *J. curcas* oil has been demonstrated on *M. nigrivenella* [2], millet borers [18], stored foodstuffs ([17,19, 20]) and insect pests of cowpea in the field [21].

Through this work, the effectiveness of the toxic molecules contained in *J. curcas* oil has been tested on pests of stored foodstuffs (heevils) and those of crops in the vegetative phase (stem and pod borers, etc.). This study focuses on the toxic effect of *J. curcas* oil on crop pests in the vegetative phase and aims to determine the biocidal efficacy of the oil on the fall armyworm (FAW) *S. frugiperda*.

2. MATERIALS AND METHODS

2.1 Material

Six stages larvae of *S. frugiperda* grouped into (L_{1-2} (3-5 days of age); L_{3-4} (6-8 days of age); L_{5-6} (>10 days of age) were used for laboratory bioassays. The bio-insecticide *J. curcas* provided by International Institute of Tropical Agriculture (IITA) and synthetic insecticide, Emamectine benzoate (Emacot 50 GW) was used for testing.

2.2 Methods

2.2.1 Breeding of *S. frugiperda*

Larvae were collected from maize plants at the Lomé Agronomic Experimentation Station and brought back to the laboratory for rearing. They were fed on fresh, tender maize leaves until the pupa stage. These pupae were then transferred to the adult breeding cage until the emergence of the latter. Upon emergence, cotton soaked in honey and the foot of young corn plants were introduced into these breeding cages for resting support for adults and egg-laying for females. The breeding cage is a rectangular cage made of aluminum (80 cm high and 50 cm wide) with an opening on one side. The opening is closed by a canvas in the form of a sleeve through which the manipulations are made. The upper side is made of fine mesh canvas to allow ventilation. In order to obtain age-matched larvae for testing, the egg masses laid were collected from the cages and incubated in the boxes. After hatching, the larvae of the same day of hatching (larvae of the same age) were fed on fresh and tender maize leaves and kept in culture for the tests. These sheets were renewed every 24 hours. Based on estimates of larval development time by Pitre and [22], larvae were grouped into L_{1-2} stages; L_{3-4}; and L_{5-6} with 3 to 5, respectively; 6 to 8 and 10 to 14 days of age.

2.2.2 Preparation of aqueous emulsion of *J. curcas* oil

Two concentrations of aqueous emulsions (10 and 20ml.l^{-1}) with and without emulsifier are prepared. Thus, the quantity of oil indicated is introduced into a beaker containing a 1% (g.l^{-1}) soapy solution as an emulsifier for solutions with an emulsifier. For solutions without an emulsifier, the oil is just mixed with water. In both cases, the mixture is stirred manually using a glass rod in order to obtain a suitable emulsion.

2.2.3 Laboratory test

The assessment was made by ingestion under ambient conditions between 28°C and 30°C. For each treatment, a sample of 10 larvae of the different stages was used and repeated four times each. Maize leaves cut into 5 cm in diameter were immersed in the various preparations for 2 minutes. These leaves were then removed and dried in the open air for 5 minutes before being used for the larvae in breeding boxes provided for the tests. The larvae were previously left to starve for 6 hours before the introduction of treated leaves. The use of fasting larvae ensures that in contact with the treated leaves, they will feed.

2.2.4 Larval monitoring

The treated leaf was removed from the box after 24 hours and the larva was fed normally with the untreated leaves until it transformed into pupa and from the pupa into adult insects. Mortality data was collected 24 and 48 hours after application. Observations were continued until adults emerged. A larva is considered dead when no part of its body moves when lightly touched with a brush. Observations continued on surviving larvae until the formation of pupae and the emergence of adults. The number of dead larvae and emerged adults per treatment made it possible to assess the mortality rate and the adult emergence rate.

2.2.5 Field evaluation of *J. curcas* oil against FAW

Maize variety "Ikenne " was planted at the SEAL field station on a plot size of 3.2 m×2 m, with spacing of 80 cm between rows and 20 cm between plants. The maize plot was fertilized with NPK15-15-15, fifteen (15) days after sowing at the rate of 200 kg/ha and urea 45 days after sowing at the rate of 100 kg/ha.

Treatment application : Specific treatments were applied using a hand-held sprayer at two-week intervals starting 15 days after planting until the appearance of male inflorescences. To avoid the risk of contamination, different sprayers were used for each formulation. 2 and 4 l/ha of jatropha oil were used with or without an emulsifier for the treatments. The control plots were not sprayed. Emacot, a chemical insecticide was applied at the rate of 1g/l. Randomized Complete Block Design (RCBD) with four replications was used for the experiment. Phytosanitary treatments were made by foliar application, mainly targeting the cornea of the plant.

Before the first application of the product, the number of egg masses, larvae per plant and infested plants were collected. After each application, the same data were collected until the emergence of male inflorescences. At each collection, destructive samples were taken to assess leaf scores using the method described by Davis et al. [23]. Therefore, each infested leaf is assigned a percentage of screening by the pest relative to the total leaf area of the leaf. The numerical scale of Davis [23], was used to assess leaf damage ranging from 1 (no leaf damage) to 9 (severe leaf damage) which corresponds to the screening percentages.

2.3 Statistical Analysis

The mortality data were corrected by the formula of Abbot [24]. Shapiro–Wilk's and Levene's tests were used, respectively to test the normality and homogeneity of the data before any analysis. Data that does not follow a normal law or that is not homogeneous are subjected to statistical transformations to make them normal or homogeneous. Thus, percentage data such as adult mortality and emergence rate are transformed into Arsin√ (percent x/100) and those on the number of larvae are transformed by the logarithm (log (X+1). normal or homogeneous are subjected to the analysis of variance and the means separated with Tukey's multiple comparisons test at the 5% level. Data that are not normal or homogeneous are analyzed using the nonparametric Kruskal–Wallis. The ranks are discriminated by the "all pairwise" method All these analyzes were carried out by the SPSS 26.0 software.

3. RESULTS

3.1 Evaluation of the Effectiveness of *J. curcas* Oil in the Laboratory on *S. frugiperda*

3.1.1 J. curcas oil on S. frugiperda larvae mortality

The different doses of jatropha oil produced lethal effects on the larvae of *S. frugiperda*. Based on the results of the analysis of variance, the mortalities vary according to the larval stages and the doses. After 24 hours of ingestion, mortality is almost complete for treatment with the synthetic chemical insecticide (Emacot) at all larval stages (Fig. 1). On the other hand, treatments based on jatropha oil caused variable mortality for the different larval stages. For the early stages (stage L_{1-2}), mortality varied from 49 to 67%. Statistical analysis reveals a significant difference in mortality between the different treatments ($F= 53.69$; $P <0.001$). Mortality is < 50% when the product is prepared with an emulsifier at a dose of 10 $ml.l^{-1}$ and > 60% when the preparation is made without an emulsifier at a dose of 20 $ml.l^{-1}$.

The two jatropha oil preparations were more toxic to the first two instar larvae. On the L_{3-4} and L_{5-6} stage larvae of *S. frugiperda*, jatropha oil caused low mortality (8-23%) by ingestion with 10 $ml.l^{-1}$ regardless of the preparation. The dose of 20 $ml.l^{-1}$ with emulsifier had a lower lethal effect (48-45%), the same as the same dose without emulsifier (63-58%) on stages L_{3-4} ($F= 40.49$; $P <0.001$) and stages L_{5-6} ($F= 26.66$; $P <0.001$).

After 48 hours of ingestion, the dose of 10 $ml.l^{-1}$ with emulsifier caused a mortality < 40% on L_{3-4} (36%) and L_{5-6} (28%) stage larvae while on L_{1-2} stage larvae it caused 77% mortality (Fig. 2). More than 80% mortality was recorded at all stages with the dose of 20 $ml.l^{-1}$ without emulsifier. Statistical analysis reveals a significant difference between the oil-based products and the controls at all stages. Furthermore, at stage L_{1-2}, the oil-based preparations without emulsifier 10 $ml.l^{-1}$ and 20$ml.l^{-1}$ had practically identical toxic effects. The statistical analysis also shows that the dose of 20$ml.l^{-1}$ without emulsifier has more effect on the larvae of stages L_{1-2} (98%) than L_{3-4} stages (87%) and L_{5-6} stages (84%).

In sum, jatropha oil showed different toxicities on *S. frugiperda* larvae. All larval stages showed a mortality of 8 to 59% after 24 hours of ingestion for preparations with an emulsifier and 18 to 67% for those without emulsifier. After 48 hours of ingestion, mortality increased from 28 to 87% with an emulsifier and from 44 to 98% for preparations without emulsifier. Furthermore, mortality increased with the concentration of *J. curcas* oil. After 48 hours of ingestion, stages L_{1-2} (on average 76% mortality due to preparations with emulsifier and 92% due to those without emulsifier) were more impacted by the various oil-based treatments followed by stages L_{5-6} (on average 58% mortality due to preparations with emulsifier and 70% due to those without emulsifier). Indeed, preparations with an emulsifier caused between 74 and 77% mortality while those without emulsifier caused a mortality of 85 to 98% at these stages. The L_{3-4} stages, which are the most voracious, recorded on average 53 and 66% mortality respectively to preparations with emulsifier and those without emulsifier.

3.1.2 J. curcas oil on S. frugiperda adult emergence

Little or no emergence of *S. frugiperda* adults is observed under conditions where the larvae are exposed to leaves treated with J. curcas oil (Fig. 3). Jatropha oil with emulsifier at a dose of 10$ml.l^{-1}$ allowed the emergence of 25% of adults for L_{3-4} stage larvae; L_{5-6} and 2.5% for those at stage L_{1-2}. Preparations without low dose emulsifier resulted in 7.5; 15 and 2.5% emergence of adults respectively for stages L_{1-2}; L_{3-4} and L_{5-6}. The high dose without emulsifier impeded the emergence of adults at practically all stages, whereas that with the emulsifier only impeded the emergence of adults at the L_{1-2} stages. In general, emergence was zero with the application of Emacot for all stages. On the other hand, with jatropha oil, the emergence of adults was very low or even non-existent (<10%) at the L_{1-2} stage (df=6; $\chi 2$ =23.26; P<0.001), varying from 2.5 to 25% at stage L_{3-4} (df=6; $\chi 2$ =22.94; P<0.001). At stage L_{5-6}, certain concentrations hindered the emergence of adults (df=6; $\chi 2$ =22.96; P<0.001).

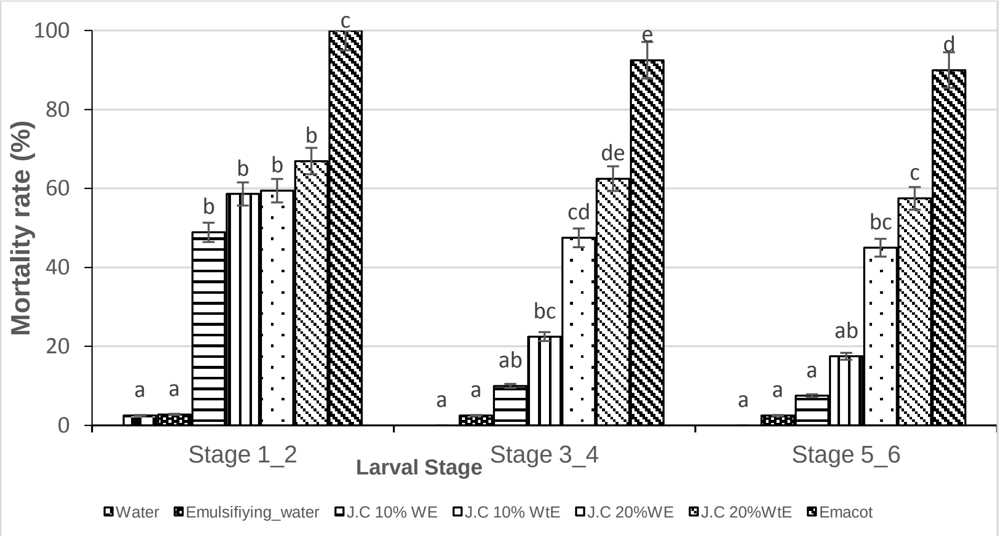

Fig. 1. Mortality rate of larvae of different larval stages of *S. frugiperda* subjected to different formulations based on jatropha oil after 24 hours

The significant differences between the treatments are indicated by different letters (Tukey's test at the 5% threshold). J.C : J. curcas ; WtE : without emulsifier; WE : with emulsifier

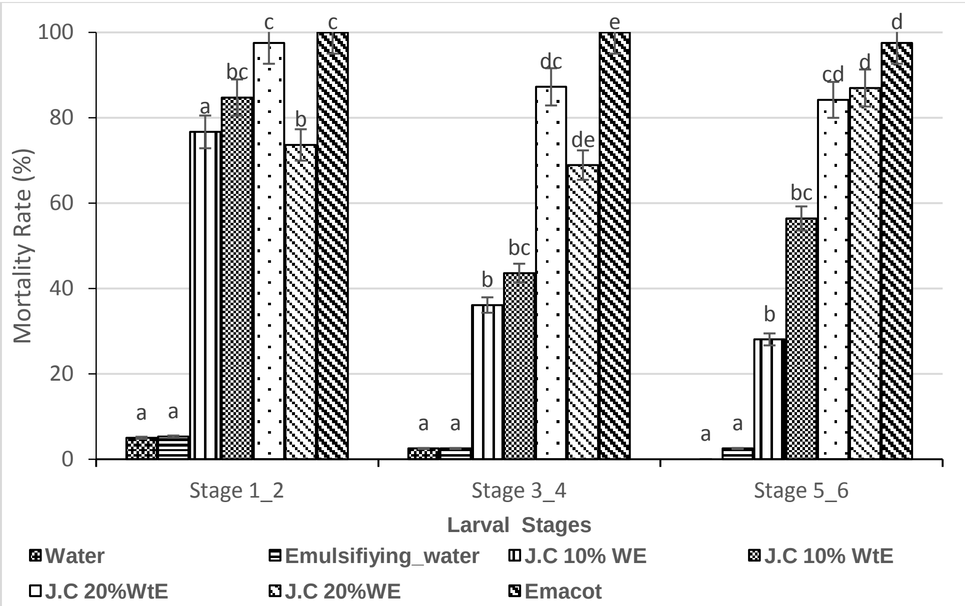

Fig. 2. Mortality rate of larvae of different larval stages of *S. frugiperda* subjected to different formulations based on jatropha oil after 48 hours

The significant differences between the treatments are indicated by different letters (Tukey's test at the 5% threshold). J.C : J. curcas ; WtE : without emulsifier; WE : with emulsifier

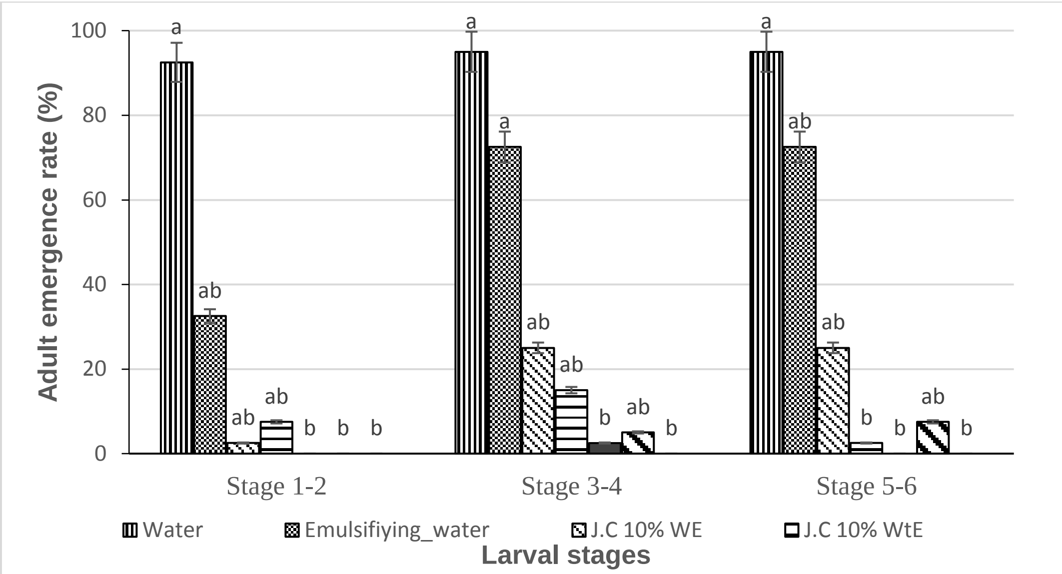

Fig. 3. Emergence rate of *S. frugiperda* adults of different larval stages subjected to different formulations based on jatropha oil

The significant differences between the treatments are indicated by different letters (Tukey's test at the 5% threshold). J.C : J. curcas ; WtE : without emulsifier; WE : with emulsifier

3.2 Field Evaluation of the Effectiveness of *J. curcas* Oil in the Station on *S. frugiperda*

3.2.1 Effect of treatments on the evolution of the population of *S. frugiperda*

Population development of *S. frugiperda* larvae under the effect of different treatments is presented in Table 1. Fewer larvae were observed on the plots treated with Emacot and *J. curcas* oil compared to the plots without treatment (F= 6; P<0.001) but no significant difference is observed between concentrations of the oil.

3.2.2 Effect of oil on infestation, leaf damage of *S. frugiperda* and maize grain yield

The infestation of the different plots by *S. frugiperda* was variable. It is more pronounced on the plots without treatment with 48% of infested plants (Fig. 4a). This is less than 20% on plots that have received Emacot for treatment (15%). Regarding treatments with *J. curcas* oil, preparations made without emulsifier (respectively 35 and 30% at doses of 10 and 20 ml.l-1) recorded a different infestation rate from that whose preparations are made with an emulsifier (respectively 22 and 24% at doses of 10 and 20 ml.l-1). In general, the statistical analysis reveals a significant difference (df = 5; F = 3.43; P = 0.024). Between the different doses and the different formulations of the oil, no difference is observed. The severity of *S. frugiperda* on maize plants varied between plots with product application and those without product application. The results obtained show that the damage in general is low for all the treatments (2 ≤ score ≤ 3) according to the Davis scale. Statistical analysis indicates no significant difference between the *J. curcas* oil treatments and those of Emacot for the level of foliar damage. On the other hand, the difference in damage levels is significant between the control and the plots subjected to insecticide applications ($\chi 2$ = 40.56; P < 0.001; Df= 5).

Oil treatment influenced maize kernel production compared to controls (Fig. 4b). The yield obtained under treatments where the product is applied with an emulsifier (yield ≤ 3 t.ha-1) is practically identical to that of Emacot. Analysis of statistical performance of different treatments was significant (F = 10.11; df = 5; P < 0.001). Effective in controlling the pest, jatropha oil without emulsifier was not efficient in obtaining yields. Thus, low yields were obtained under oil treatments without emulsifier (<2.5 t/ha). The yields obtained under Emacot and *J. curcas* oil with emulsifier were statistically identical at the 5% threshold.

Table 1. Effect of J. curcas oil on *S. frugiperda* larva population fluctuation

Treatement	Number of larvae per plant (mean ± SE)
J.C 10% WE	0,37±0,04bc
J.C 10% WtE	0,40±0,05b
J.C 20%WE	0,38±0,04b
J.C 20%WtE	0,32±0,05bc
Emacot	0,24±0,06c
Control	0,58±0,03a
Df	5 ; 88
F	6,00
P	<0,001

Values in a column followed by different lowercase letters are statistically different at the 5% level (Tukey test).
J.C : J. curcas ; WtE : without emulsifier; WE : with emulsifier.
The density of S. frugiperda varies significantly with the date of collection (F = 10.85; P < 0.001). On the plots treated with J. curcas oil, the density is almost static at each collection.

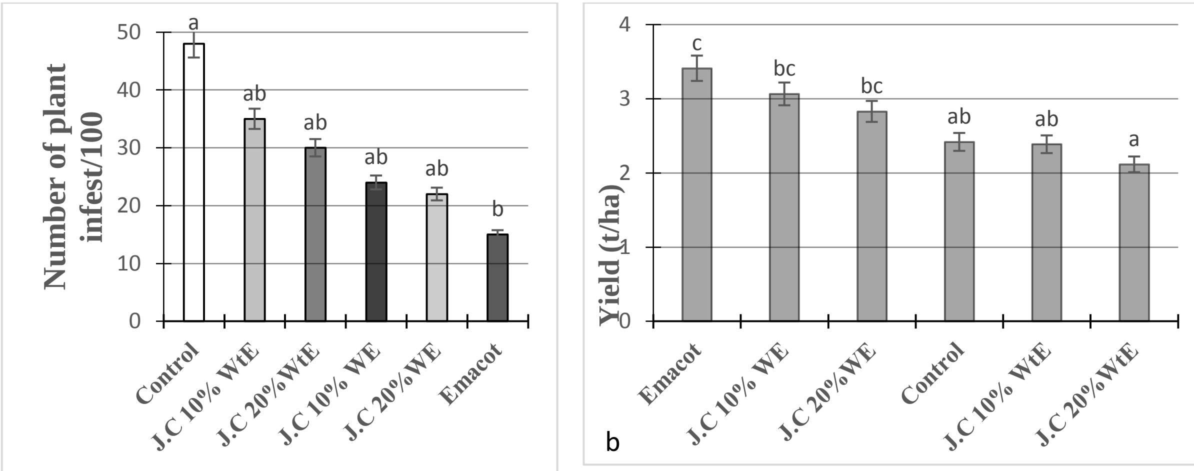

Fig. 4. *S. frugiperda* infestation rate (a) and Yield of grain maize (b) according to the different formulations based on jatropha oil. The significant differences between the treatments are indicated by different letters (Tukey's test at the 5% threshold)
J.C : J. curcas ; WtE : without emulsifier; WE : with emulsifier

In short, it should be noted that in the station, the oil of J. curcas had almost identical effects to that of emamectin benzoate (Emacot) but leaf burns were observed on the plots having received the oil, especially those of 4 l. ha^{-1}.

4. DISCUSSION

The results of the biological tests in the laboratory show that the various products tested had significant effects on the larvae of *S. frugiperda*. Emacot used at 1g.l^{-1} had more effect on the larvae and caused almost 100% mortality of the larvae at all stages after 48 hours of exposure. These high mortalities are explained by the insecticidal power and the mode of action of chemical insecticides on the larvae. Indeed, Emacot, a synthetic trans-laminar contact and systemic insecticide, induces an increase in cell apoptosis, DNA breaks leading to the rapid death of larvae [25]. Furthermore, Sisay et al. [12] in Ethiopia proved the toxicity of synthetic chemical insecticides on *S. frugiperda* larvae in the laboratory. In their study, out of nine chemical insecticides tested, five caused more than 90% mortality after 72 hours of application. In pest control in the Americas, the use of chemical insecticides has been an important option [26]. Thus, Belay et. al. [27] reported >60% mortality of *S. frugiperda* larvae after 16 h of application of Radiant (Spinetoram), Orthene (Acephate), and Larvin (Thiodicarb).

The mortality rate related to *J. curcas* oil after 24 hours of ingestion was ≤ 50% at 10 ml.l^{-1} and > 50% at 20 ml.l^{-1} on all larval stages. These mortalities show that the product poisoned the larvae. The results of this study confirm those of other authors who used the extracts of *J. curcas* seeds for biological tests on insect pests. Indeed, [28] demonstrated in Mexico the insecticidal potential of *J. curcas* grain extracts on L_3 stage larvae of *S. frugiperda.* Ratnadass et al. [18] demonstrated that shredded *J. curcas* grains caused the death of the first stages of development of *S. calamistis* caterpillars. Abdoul et al. [21] demonstrated in the laboratory the effectiveness of *J. curcas* oil on the black bean aphid *Aphis fabae* Scop (Homoptera: Aphididae) after 24 hours of ingestion. These high mortality rates show the toxic nature of *J. curcas* oil. Indeed, the toxic nature of this oil is due to the presence of phorbol esters [29]. Phorbol esters are secondary metabolites of the family of diterpenes of the tigliane group [28] which act on biological membranes [14]. These insert into cell membranes and activate protein kinase (PKC) [30] leading to cell apoptosis [14]. At stages L_{3-6}, the mortality rate after 72 hours of ingestion is ≥ 50% at the dose of 10 $ml.l^{-1}$ and almost complete at the dose of 20 $ml.l^{-1}$. This proves that, 24 hours after ingestion, the effects of the product persist. These results are similar to those of Abdoul et al. [21] on *A. fabae* who showed that the mortality of these aphids increased up to 72 hours before stabilizing. According to these authors, mortality due to this oil increases with time. In addition, studies by Bourogâa [31] demonstrated the toxic effect of jatropha oil on Hemiptera. According to the author, this toxic effect can be explained by the presence of a high quantity of curcin (toxalbumin) in the seeds of *J. curcas*.

J. curcas oil with emulsifier affected the emergence of adults without being able to prevent it when the larvae were over 5 days old. Conversely, the oil of *J. curcas* without emulsifier hindered the emergence of adults which it inhibits almost for all stages. This may in part mean that *J. curcas* oil takes a while to be at its most effective. These results corroborate those obtained by Abdoul et al. [21] who found that the biocidal effect of *J. curcas* oil increases during the hours following application to reach a maximum level after 96 hours.

Just as in the laboratory, jatropha oil made it possible to reduce the evolution of the population of *S. frugiperda* larvae compared to the control plot without product application. It also made it possible to reduce the infestation and the level of damage practically in the same way as the chemical insecticide Emacot but without being able to improve the yield of grain maize. This reduction can also be explained by the toxic nature of the oil conferred mainly by the esters of phorbols. According to Devappa et al. [32] phorbol esters of the most toxic compounds in jatropha oil but which degrade very quickly and this degradation can be limited by adding an antioxidant. In this study, no antioxidant was added, so this reduction in the pest population would not be due solely to the effect of *J. curcas* oil but to other factors. However, similar reductions in the population of *M. nigrivella* in maize crops was observed in Benin treated with this oil [2]. Abdoul Habou [33] also showed that the same oil reduced the population of major cowpea pests in Niger and therefore, reduced the attacks of these pests. In Mali, crushed seeds of *J. curcas* resulted in a reduction in sorghum panicle bug damage [18]. Also, the 20 ml.l-1 concentration of oil corresponding to 4 l.ha-1 had effects almost identical to those of Emacot. Similar results were shown by Karka et al. [34] in Chad on cowpea.

5. CONCLUSION

It appears from the study that whatever be the method of preparation, the oil of *J. curcas* has toxic effects on the fall armyworm. The insecticidal effect of *J. curcas* oil is comparable to the reference insecticide (Emacot) on L_{1-2} stage larvae under laboratory conditions. In the station, the oil of *J. curcas* had almost identical effects to that of Emacot but burns on leaves were observed on the plots having received the oil, especially those of 4 $l.ha^{-1}$. Jatropha oil could be a good bio-insecticide in FAW management.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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