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Trans-stadial effects and larvicidal potential of jatropha (*Jatropha curcas*) and castor (*Ricinus communis*) oils on *Spodoptera frugiperda* in laboratory

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RESUME

Over the past decade, outbreaks of the fall armyworm (*Spodoptera frugiperda*) have had devastating impacts on agriculture in Africa.

This study aimed to evaluate the insecticidal properties of jatropha and castor oils on *S. frugiperda*. The oils were specifically applied to eggs and tested for egg hatching, subsequent larval mortality, and pupae emergence. Thus, two bioassays were conducted in the laboratory. On one hand, the trans-stadial effect of insecticidal oil application on fall armyworm eggs was measured. On the other hand, direct application effect of the oils on fall armyworm's second instar larvae was determined. Four treatments (T1-4) were used for each assay in a complete randomized block design: T1: Water; T2: Water + Omo (laundry detergent); T3: Jatropha oil (1.25% v/v), and T4: Castor oil (1.25% v/v). For the trans-stage effect bioassay, batches of 10 eggs were sprayed with 1 mL of the treatment using a micropipette. The experimental eggs were monitored to the pupal stage, to calculate egg hatching, larval mortality, and pupation rate. On the direct insecticidal oil effect bioassay, batches of 10 second instar individuals were sprayed with 2.5 mL of each treatment. The larval mortality was recorded. The results showed that castor and jatropha oils had a trans-stage effect, resulting in significant larval mortality ($df = 3$, $p < 0.01$) and reduced pupation rate ($df = 3$, $p < 0.01$). Unexpectedly, larval mortality in the trans-stage assay was higher than that obtained by direct application of biopesticides on the larvae. This study suggests that an application of the jatropha or castor oils on the fall armyworm's egg stage is associated with high larvicidal effect.

Keywords: invasive species, insecticidal plant, early stage control, sustainable management.

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1. Introduction

Since its initial detection in sub-Saharan Africa in early 2016, the fall armyworm (*Spodoptera frugiperda* J.E. Smith), an invasive pest native to the tropical and subtropical regions of the Americas, has rapidly established itself as one of the most serious agricultural threats on the continent (Goergen et al., 2016).

The pest has a broad host range, with maize being its prime host. It caused a particularly devastating impact on maize production (Acharya et al., 2020; Overton et al., 2021; Kenis et al., 2022). As a consequence, this directly threatened food security and livelihoods across the region (Mlambo et al., 2024).

Therefore, the development and implementation of sustainable management strategies for the fall armyworm has received high priority across countries. Plant-based biopesticides remain one of the candidate options that are environmentally sound and accessible (Ekenwosu et al. 2021). They are affordable and largely accepted in the context of smallholder farmers. However, their efficacy to control fall armyworm is still uncertain. Among these, oils extracted from seeds of *Jatropha curcas* (jatropha) and *Ricinus communis* (castor) have exhibited insecticidal properties against a range of insect pests (Rioba et al., 2020; Fareed et al., 2024). Nevertheless, a significant gap exists in our understanding of their full potential against *S. frugiperda*. The majority of previous studies have focused on the direct effects of these oils on larval stages and nymphs at adulthood. To the best of our knowledge and on consulted literature, none of them have explored the potential trans-stadial effects of the insecticidal plant oils. Understanding these trans-stage effects is crucial for developing comprehensive and effective pest management strategies. Therefore, this study aimed at evaluating the insecticidal properties of jatropha and castor oils on *S. frugiperda*. The oils were specifically applied to eggs and tested for egg hatching, subsequent larval mortality, and pupae emergence. Furthermore, we examined larval mortality upon direct oil application on FAW larvae.

We hypothesized that early egg exposure to these plant oils disrupts the growth of the developing embryo, thereby affecting post-hatching survival and larval development. To test this hypothesis, we conducted two distinct bioassays. On one hand, the trans-stage effect of insecticidal oil application on fall armyworm eggs was measured. On the other hand, direct application effect of the oils on fall armyworm's second instar larvae was determined.

This research seeks to provide new insights on the overall insecticidal efficacy of jatropha and castor oils under an integrated FAW management framework.

2. Materials and Methods

2.1. Experimental conditions

The study was carried out at the Plant Health Science Platform (PHSP) hosted by the Unité de Recherche en Phytotechnie et Santé des Plantes (URPSP), under the Laboratoire des Agrosystèmes et Paysages Durables (LAPaD) of the Université Nationale d'Agriculture (UNA,Akpotokou campus). The experimental conditions in the laboratory fluctuated between 26 and 28 °C, between 65 and 70% relative humidity, and under a 12-hour light and 12-hour dark photoperiod regime.

2.2. Experimental insect and insecticidal plants

2.2.1. Spodoptera frugiperda eggs and larvae

The laboratory insect colony was established using parent fall armyworm larvae collected in maize fields in Akpotokou. Routine insect rearing protocol consisted of screening and breeding the collected larvae to pupal stage. The pupae were disinfected with 0.5% bleach and placed in plastic boxes (17 cm diameter and 9.5 cm height). Adults of *S. frugiperda* emerging from these pupae were fed with 10% honey solution and were monitored daily until death. The eggs laid by females were collected from oviposition boxes after mating experience. The eggs were incubated, and the hatched neonates continued the larval cycle, differentiating into six instars for a duration of 14 to 21 days. The larvae were fed with fresh leaves of sprouting maize. The experimental eggs and larvae used in the bioessays belonged to cohorts of the second generation (F2).

2.2.2. Insecticidal plant oils

Jatropha and castor oils used in the experiments were purchased from BioPhyto, an accredited biopesticide supplier in Allada (southern Benin).

2.2.3. Treatments and experimental procedure

For each oil, a 1.25% (v/v) aqueous solution was prepared by mixing 1.25 mL of *Jatropha curcas* or *Ricinus communis* oil with 0.2 g of Omo Klin detergent in 100 mL of water. The concentration 1.250% (v/v) is an equivalent dose of 2 l/ha of biopesticide, considering the manufacturer's recommendations. Omo Klin was chosen for its accessibility and low toxicity to CLA larvae compared to other adjuvants commonly used in Benin (Aniwanou et al., 2021). The 0.2 g of Omo Klin used was enough to mix the oils in the water. Four treatments (T) were used per bioassay in a complete randomized block design: T1: Water; T2: Water + Omo; T3: Jatropha oil; and T4: Castor oil. The treatments were applied to *Spodoptera frugiperda* eggs and second instar larvae. For the egg bioassay, batches of 10 eggs were sprayed with 1 mL of the oils using a micropipette. Treated eggs were placed in Petri dishes on a layer of absorbent paper. Larval bioassay consisted of batches of 10 individuals sprayed with 2.5 mL of each treatment. The experimental larvae were fed on fresh untreated leaves of sprouting maize. Ten replicates were organized for each of the two bioassays.

2.2.4. Data collection

The number of neonates hatching from experimental eggs was counted daily for five days to compute the hatching rate using the formula:

$$\text{Hatching rate (\%)} = \frac{\text{Number of neonate}}{\text{Total number of eggs treated}} x\ 100$$

The neonates were followed to the pupal stage, and larval mortality and pupation rate were calculated using the formulas:

$$\text{Larval mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae treated}} x\ 100$$

$$\text{Pupation rate (\%)} = \frac{\text{Number of pupae formed}}{\text{Total number of larvae treated}} x\ 100$$

Regarding the bioassay on the direct application of oils to the larvae, larval mortality was recorded daily over six days using the method described by Ammar et al. (2025). A larva was considered dead if it exhibited no response or was unable to ambulate when stimulated with a fine brush (Lourenço et al., 2018). The mortality rate was calculated using the same above-described formula:

$$\text{Larval mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae treated}} x\ 100$$

2.2.5. Statistical analysis

The analysis of variance (ANOVA) was performed to assess the effect of treatments on egg hatching rate, larval mortality, and pupation rate. The Tukey's Honest Significant Difference (HSD) test was used to compare means at the 5% significance level. All statistical analyses were carried out using R software (version 4.4.0).

3. Results

3.1. Trans-stadial effect of castor and jatropha oils application on fall armyworm eggs

3.1.1. Egg hatching

Figure 1 shows the fluctuation of egg hatching rate over the five days of observation. Less than 25% of the eggs hatched during the first two days after the application of the treatments. However, this rate increased significantly for all treatments at 3 days post-oil application, reaching 100% for the jatropha oil-treated eggs (Fig. 1). At the end of the observation period, i.e., 5 days after application, 100% egg hatching was attained in the eggs treated with Jatropha oil and Water (T3 and T1). The egg hatching was lower in the Water + Omo (T2) and Castor oil (T4) treatments, with rates of 98 ± 2 and 96 ± 2.2%, respectively. However, there was no significant egg hatching difference between treatments ($df = 3$, $p = 0.195$).

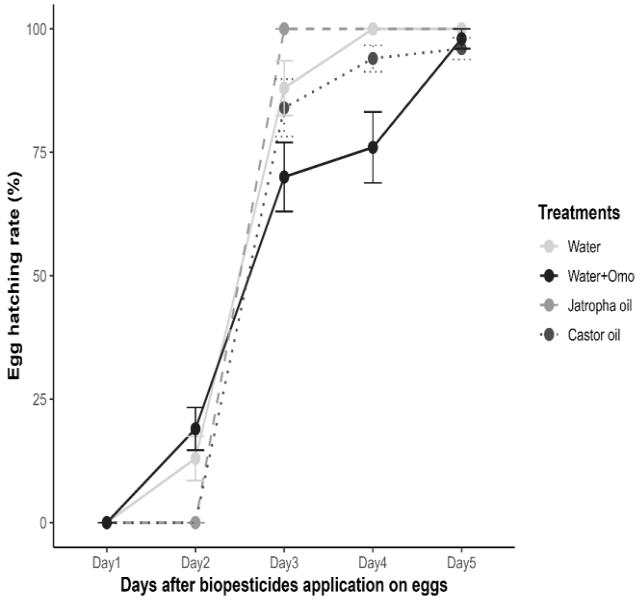


Figure1: Temporal evolution of egg hatching rate

3.1.2. Larval Mortality

The results showed that treatments had a significant effect on larval mortality from treated eggs (df = 3, $p < 0.01$). The larval mortality rates in the Water + Omo (T2) and Castor oil (T4) treatments were not significantly different from those in the Water (T1) treatment, but the mortality rate in the Jatropha oil (T3) treatment was significantly higher than T1 (Fig. 2). The Jatropha oil (T3) treatment caused the highest larval mortality rate.

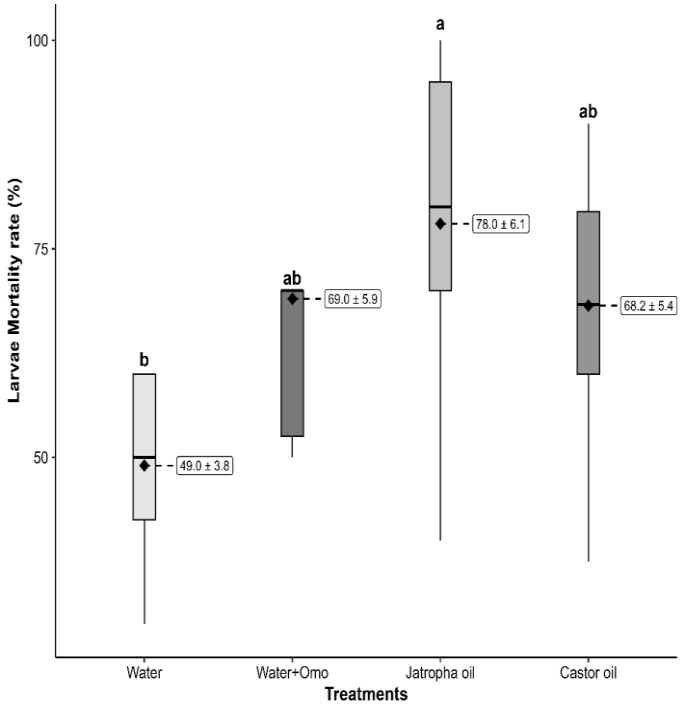


Figure2 : Larvae mortality rate per treatment

3.1.3. Pupation rate

Treatments had a significant effect on the pupation rate (df = 3, $p < 0.01$). The first pupae were observed 19 days after oil application on eggs for the Water (T1), Water + Omo (T2), and Castor oil (T4) treatments (Fig. 3). At the end of pupation formation, pupation rates ranged from 22 ± 6.1% (T3, Jatropha oil) to 51 ± 3.8% (T1, Water). The pupation rate obtained with Jatropha oil (T3) was significantly lower than that obtained with the Water treatment (T1) (Fig. 4). The pupation rate on the Water + Omo (T2) treatment was not significantly different from that on the Water (T1) treatment. Jatropha oil was more effective than castor oil.

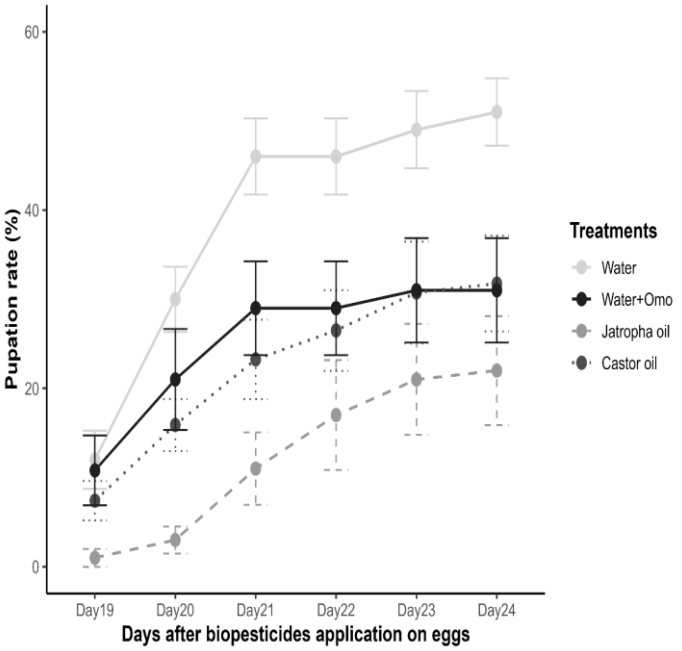


Figure3 : Temporal pupation

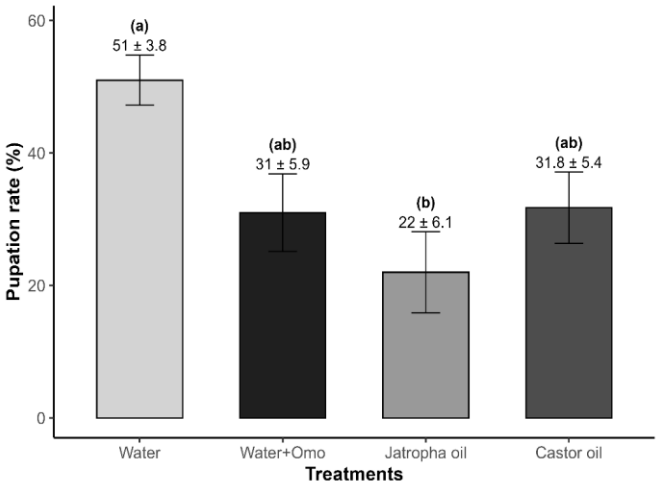


Figure4 : Final Pupation rate per treatment

3.2. Direct effect of castor and jatropha oils on FAW larvae

Figure 5 shows larval mortality rates after biopesticide application. The treatments had a significant effect on larval mortality (df = 3, $p < 0.05$). Larval mortality ranged from 14 ± 1.1 (T1, Water) to 25 ± 1.2 % (T3, Jatropha oil). Larval mortality on Jatropha oil (T3) was significantly higher than on the two controls (T1 and T2). The larval mortality rate obtained with Castor oil (T4) was not significantly different from that of the controls (T1 and T2). Jatropha oil (T3) was therefore the treatment with the highest larval mortality rate.

Figure 6 compares larval mortality when biopesticides are applied to eggs and when they are applied directly to larvae. This figure shows that there is a significant difference between these two insecticidal oil

application methods, and for any of the treatments considered (T3 and T4). The mortality of larvae from eggs treated with biopesticides was significantly higher than that obtained when the biopesticides were applied directly to the larvae (Figure 6).

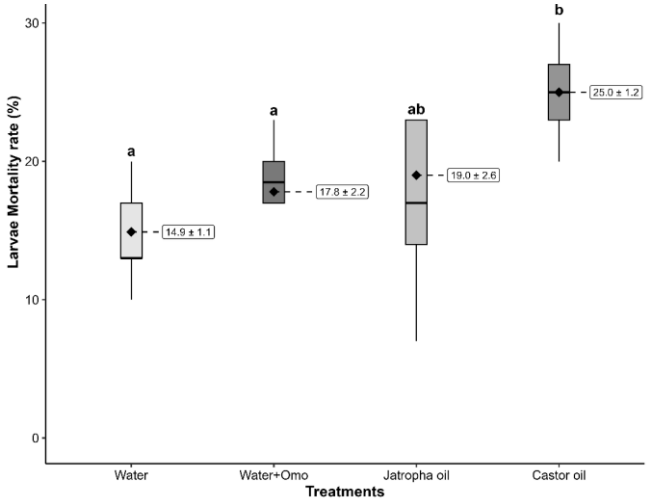
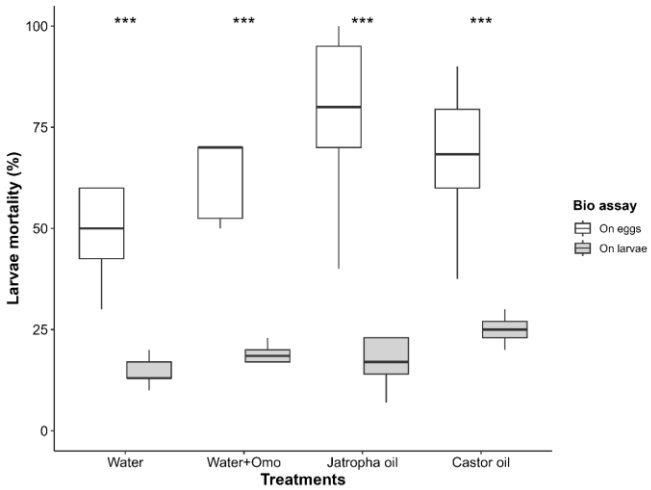


Figure5 : Larvae mortality rate per treatment



p value < 0.001 ~ "*", p value < 0.01 ~ "**", p value < 0.05 ~ "*", p > 0.05 "ns"**

Figure 6 : Comparison of larvae mortality (bioassay1 and bioassay 2)

4. Discussion

The present study evaluated the trans-stadial effect of jatropha and castor oil application on fall armyworm (FAW) eggs. We compared the larval mortality obtained on the trans-stage effect experiment with that obtained by direct application of the biopesticides to FAW larvae. The results showed that castor and jatropha oils had a trans-stage effect, resulting in larval mortality and reduced pupation rate. Also, larval mortality in the trans-stage assay was higher than that obtained by direct application of biopesticides on the larvae.

Indeed, jatropha and castor oils did not inhibit the hatching of fall armyworm eggs. This suggests that the compounds in these two oils do not directly alter the embryonic viability of fall armyworm eggs. However, this may be due to the concentration used (1.5%), as studies by Komi et al. (2009) showed a concentration-dependent ovicidal effect of jatropha oil on the eggs of another maize lepidopteran pest, *Mussidia nigrivenella*. Using concentrations of 2.5%, 5%, 10 and 100%, these authors found a reduction in the hatching of the pest's eggs. Therefore, the concentration used in the present study may not be sufficient to kill the eggs and inhibit hatching.

A larval mortality from treated eggs was observed. This indicates a trans-stage effect, meaning that early exposure to the oils may affect the subsequent development of individuals. It can be concluded that the application of the active compounds of jatropha and castor oils to eggs resulted in metabolic disturbances after hatching.

Castor and jatropha oils induced a reduction in pupation rate. The pupation rate obtained on larvae from eggs treated with jatropha oil was lower. This showed that both jatropha oil and castor oil have an indirect effect on the pupae of *S. frugiperda*. Valdez-Ramírez et.al (2024) showed that jatropha extracts prolonged the pupation time of larvae and the weight of *S. frugiperda* pupae. Another study on the insecticidal effect of castor oil on *Spodoptera frugiperda* larvae in the laboratory showed that larvae treated with aqueous castor seed extract had a slightly longer development time and lower pupal viability, as well as certain morphological discontinuities in adults and pupae (Kombieni et al., 2023). These findings suggest that both oils may interfere with the normal metamorphosis of *S. frugiperda*, potentially through physiological disruptions during larval development.

The results showed that jatropha oil and castor oil have a significant effect on larval mortality in direct application. This is not new and corroborates with previous studies on *Spodoptera frugiperda*, *Spodoptera litoralis*, and *Bemisia tabaci* (Ali & Ibrahim, 2018; de Almeida Marques et al., 2014; Devappa et al., 2012). The larvicidal effect of the oils could be attributed to the presence of bioactive compounds, including saponins, flavonoids, diterpenes, and phorbol esters. These substances act by ingestion or contact, disrupting the digestive system or causing dehydration, thus contributing to insect control (Devappa et al., 2010; Rossi et al., 2012). The mortality obtained was, however, relatively low, suggesting an increase in dose for future studies.

Larval mortality rates were higher in the trans-stage assay than in direct application. This difference suggests a potential residual or transovarial effect of the oils that may affect post-embryonic development of exposed individuals from the egg stage. Such early action may affect larval growth as soon as they hatch by increasing their sensitivity and vulnerability. An application of the oils from the egg stage is worth explorable further to maximize the larvicidal efficacy of jatropha and castor oils.

The two bioassays carried out in the present study suggest that the two jatropha and castor oils tested have insecticidal properties against the fall armyworm. Nevertheless, it would be interesting to study the mode of action of these oils to better understand the mechanism underlying the findings on the egg-larval trans-stage effect. This will bring more insights on the significant difference observed between the mortality of larvae from eggs treated with the oils and that obtained by direct application on the larvae.

5. Conclusion

This study evaluated the trans-stadial effect of jatropha and castor oils applied to *Spodoptera frugiperda* eggs. The application method was compared to direct application to larvae. The oils were not ovicidal, but

they significantly killed the larvae after egg hatching. The findings need to be further investigated. More studies may also test different concentrations for enhanced optimization and understanding the mode of action of the active compounds. This work consolidates opportunities for affordable locally accessible biopesticide alternatives for a sustainable integrated management of fall armyworm in small-scale farms.

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