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Jatropha (*Jatropha curcas*) and Argel (*Solenostemma argel*) extracts as an Oviposition inhibitor to Spiny Bollworm [*Earias insulana* (Lepidoptera: Noctuidae)]

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Abstract: **The study was carried out to evaluate the effect of aqueous and oil extracts of jatropha (*Jatropha curcas*) seeds and argel (*Solenostemma argel*) leaves on egg-laying of the spiny bollworm (*Erias insulana*). The study was carried out under laboratory conditions (25-30°C and 60-70% Relative Humidity) at the Department of Crop Protection, University of Khartoum. One concentration of the aqueous and oil extracts of each plant was tested in this study viz. 10% (w/v). The potential effects of the aqueous and oil extracts of tested plants at various doses were investigated on egg-laying of the spiny bollworm. The insecticide emamectin benzoate (proclaim®) at a rate of 75 g/100L was used as standard. Control sets of treated (n-hexane) and untreated also were included. The results showed that no significant difference (P≥0.05) was detected in the mean number of eggs laid by the spiny bollworm (SBW) on okra pods sprayed with emamectin benzoate (proclaim®) and those sprayed with 10% aqueous and oil extracts of jatropha seeds and argel leaves. Significantly low (P≤0.05) mean number of eggs was laid on pod sprayed with 10% aqueous extracts of jatropha seeds (4.5), and argel leaves (1.8) compared to that laid on the untreated pod (11.8). The study recommends the use of aqueous extracts of jatropha seeds at a concentration of 10% and argel leaves at a concentration of 10%. The study also recommends the use of aqueous extracts of jatropha and argel for their ease of preparation. The study suggested other work to validate these results under field conditions.**

Keywords: **Extract, Oil, Aqueous, Egg-laying, Sudan.**

I. INTRODUCTION

Earias insulana Boisduval (Noctuidae: Lepidoptera), commonly known as the spiny bollworm, is an important pest of cotton and okra in Sudan. It is difficult to identify the larvae of *Earias insulana*, therefore records often do not name the exact species of Earias found on crops. The recorded hosts are generally confined to the Malvaceous plants. Pearson [1] suggested that cotton is generally not the first choice as a host for Earias spp. A survey by Khidir et al. [2] found that okra was preferred towards the end of the cotton season when the cotton bolls were hard. The genus Earias is widely distributed in the Old World and Australasia, and some are pests of considerable importance in many of the cotton-growing countries of Africa and Asia. The spiny bollworm, *Earias insulana* has an extremely wide range and is found throughout most of Africa and the Mediterranean region and eastwards to India, China, and Southeast Asia [3].

Okra [*Abelmoschus esculentus* L. (Moench)] is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. World okra production in 2010 was 6.9 million tons, while in the Sudan okra production was estimated to be 2.6 million tons [4]. It is consumed by almost all the Sudanese people either as green immature pods (fried or cooked or in soup or stews) or sundried and ground into powder locally known as “wieka” which is used as an ingredient in the preparation of a favorable Sudanese “molah” [5].

J. curcas (physic nut) is a drought-resistant multipurpose shrub or a small tree belonging to the family Euphorbiaceae. It is a native of tropical America but now thrives in many parts of the tropics and sub-tropics in Africa, Asia and southern America [6]. The extracts of Jatropha showed nematicidal, fungicidal, Molluscicidal effects [7]. It also exhibited insecticidal activities against moths, butterflies, aphids, bugs, beetles, flies, and cockroaches [8]. Toxicity of *J. curcas* seeds is attributed to several components, including saponins, lectins (curcin), phytates, protease inhibitors, curcalonic acid and phorbol esters [9]. Argel plant, *S. argel* belongs to the family Apocynaceae and is indigenous to Africa. It is valued for its medicinal and aromatic qualities. It is widely distributed in Sudan and throughout North Africa (Egypt, Libya, and Algeria) and Saudi Arabia [10]. It has many active compounds that have been shown to act as potent acute or chronic insecticides, antifeedant and insect growth regulators against a variety of insect species [2], [11]-[12].

In Sudan, the irrigated schemes comprise the main site of the annual application of large amounts of synthetic pesticides. The misuse of these chemicals created problems both man and animals. Moreover, their continuous application in the Gezira area was found to affect vital processes in the soil such as nitrification and respiration [13].

Botanical extracts have long been proposed as alternatives to synthetic insecticides for pest management with the promising result obtained [14]-[16]. Botanical extracts are eco-friendly, economical, usually target-specific, and biodegradable. Furthermore, they are cheap, readily available, and affordable; these are important qualities of pest control products for smallholder farmers in Africa [17].

The importance of the present study stems from the hazards posed by synthetic insecticides to the environment and human health. The present study was carried out with the objective of demonstrating the biological activity of both aqueous and oil extracts of jatropha seeds and argel leaves against the spiny bollworm and to determine the efficacy and potentials of using any of these materials as choice candidates in the control of this insect pest.

II. MATERIALS AND METHODS

This study was conducted at the Laboratory of Entomology, Department of Crop Protection, Faculty of Agriculture, University of Khartoum. The objective of this study is to investigate the effect of aqueous and oil extracts of jatropha (*Jatropha curcas*) seeds and argel (*Solenostemma argel*) leaves on the spiny bollworm (*Earias insulana*). The experimental work was carried out from November 2018 to February 2019 under controlled temperature (25-30°C) and relative humidity 60-70%.

A. Insect rearing

The larvae of the spiny bollworm (SBW) were collected from okra fruits, obtained from the Bahri Central Market, Khartoum State. The newly collected larvae were transferred each to a separate rearing plastic bowl (20 cm wide and 8 cm deep, Plate 1) and provided with okra fruits as food. The okra fruit in the rearing bowl was renewed daily until the pupation of the larvae. The pupae were kept individually in plastic bowls of the same dimensions until adult emergence. The newly emerged adults were sexed depending on the posterior end of the pupa according to Abdel Fattah (Personal communication). Pods of okra wrapped with muslin cloth were provided inside each cage for the deposition of eggs. These pods were renewed daily, and the deposited eggs were collected daily. The pods (wrapped with muslin cloth) containing the eggs were then transferred to plastic bowls (20 cm wide and 8 cm deep).

B. Insect eggs collection

Thirty newly emerged adult males and females were kept in four cages (27×19×14.5 cm) covered with the muslin cloth. Three small plastic cups, fitted with cotton wick impregnated with a 10% sucrose solution, were fixed in each cage as a food source for the adult moths. Three okra fruits, wrapped with the muslin cloth, were introduced into each plastic cage for egg-laying. The obtained eggs were divided into two groups, one was used for bioassay and the other was used for raising further generations of the insect.

C. Plant collection

Fresh jatropha (*Jatropha curcas*) seeds were collected from the Zalingei area in Central Darfur State. The seeds were then cleaned, de-shelled and subsequently, the kernels and hulls were separated manually. The kernels were dried, ground to a fine powder and stored in glass vials at 4 °C until used.

Leaves of argel (*Solenostemma argel*) were purchased from the Omdurman market. The dried leaves were ground, powdered, stored at 4ºC and protected from light for further use.

D. Preparation of the plant aqueous extract

The aqueous extracts of powder of *J. curcas* seeds and *S. argel* leaves were prepared by adding 25g of each powder to 250 ml of distilled water in a 500 ml conical flask. The mixtures were left to stand for 24 hours at room temperature, according to the method described by the Environmental and National Resources and Desertification Research Institute (ENRDRI). The mixtures were then thoroughly shaken by hand every 5-8 hours for 5-10 minutes within a period of 24 hours. The mixtures were then filtered using a clean muslin cloth. The filtrates (stock solutions, 25 w/v) were kept in the refrigerator at 4ºC for bioassay. Four concentrations (5%, 10%, 15%, and 20%) from each extract were prepared and used in the bioassay.

E. Preparation of oil extracts

Jatropha seed oil extract;

To obtain the jatropha seed oil, 25g of seeds powder (prepared as above at the Pesticides Center in U of K) were defatted in a soxhlet apparatus using 250 ml of n-hexane (95% pure). The n-hexane was removed using a rotary evaporator apparatus at 40ºC. The extracted seed oil was kept in an amber glass bottle and stored in the refrigerator for subsequent application.

Argel leaves oil extract;

Twenty-five grams of argel leaves powder were used for oil extraction with 250 ml n-hexane (95% pure) using the soxhlet apparatus. The n-hexane was removed using a rotary evaporator apparatus at 40ºC. Extracted leaves oil was kept in an amber glass bottle and stored in the refrigerator for subsequent application.

F. Treatments and experimental layout

One concentration (10%) of the aqueous and oil extracts of each of jatropha seeds and argel leaves were prepared. These concentrations were compared with emamectin benzoate (proclaim®) at a rate of 75 g/100L. The treatments were applied by spraying method. The treatments were arranged in a Completely Randomized Design (CRD) with three replicates and the collected data were subjected to ANOVA using SAS software.

G. Effect of aqueous extracts of jatropha seeds and argel leaves on egg-laying by the spiny bollworm

Effect of aqueous extract of jatropha seed on egg-laying;

Two pods of okra, one was sprayed with a 10% aqueous extract of jatropha seeds and the other was sprayed with emamectin benzoate (proclaim®). Each pod was kept in a plastic bowl (20 cm wide and 8 cm deep). Ten percent of sugar solution was provided (in a plastic cup) in each bowl as adult food. A third bowl was included as a control in which the pod was left unsprayed (untreated control). One pair of the newly emerged adult male and female of the SBW was introduced into each plastic bowl. Each bowl was then tightly covered with muslin cloth fixed with a rubber band. The plastic bowls were arranged in a Completely Randomized Design. The treatments were replicated three times. The bowls were incubated at 25-30°C for 6 days. The number of eggs laid was counted every day for six days.

Effect of aqueous extract of argel leaves on egg-laying;

Two pods of okra, one was sprayed with a 10% aqueous extract of argel leaves and the other was sprayed with emamectin benzoate (proclaim®). Each pod was kept in a plastic bowl (20 cm wide and 8 cm deep). Ten percent of sugar solution was provided (in a plastic cup) in each bowl as adult food. A third bowl was included as a control in which the pod was left unsprayed. One pair of the newly emerged adult male and female of the SBW was introduced into each plastic bowl. Each bowl was then tightly covered with muslin cloth fixed with a rubber band. The plastic bowls were arranged in a Completely Randomized Design. The treatments were replicated three times. The bowls were incubated at 25-30°C for 6 days. The number of eggs laid was counted every day for six days.

H. Effect of oil of jatropha seeds and argel leaves on egg-laying by the spiny bollworm

Effect of oil of jatropha seeds on egg-laying;

Three pods of okra, one was sprayed with a 10% oil of jatropha seeds, one was sprayed with emamectin benzoate (proclaim®) and the third was sprayed with n-hexane (treated control). The pods were kept each in a plastic bowl as previously described. Ten percent of sugar solution was provided (in a plastic cup) in each bowl as adult food. A fourth bowl was included as a control in which the pod was left unsprayed. One pair of the newly emerged adult male and female of the SBW was introduced into each plastic bowl. Each bowl was then tightly covered with muslin cloth fixed with a rubber band. The plastic bowls were arranged in a Completely Randomized Design. The treatments were replicated three times. The bowls were incubated at 25-30°C for 6 days. The number of eggs laid was counted every day for six days.

Effect of oil of argel leaves on egg-laying;

Three pods of okra, one was sprayed with 10% oil of argel leaves, one was sprayed with emamectin benzoate (proclaim®) and the third was sprayed with n-hexane. The pods were kept each in a plastic bowl (20 cm wide and 8 cm deep). Ten percent sugar solution was provided (in a plastic cup) in each bowl as adult food. A fourth bowl was included as a control in which the pod was left unsprayed. One pair of the newly emerged adult male and female of the SBW was introduced into each plastic bowl. Each bowl was then tightly covered with muslin cloth fixed with a rubber band. The plastic bowls were arranged in a Completely Randomized Design. The treatments were replicated three times. The bowls were incubated at 25-30°C for 6 days. The number of eggs laid was counted every day for six days.

I. Statistical analysis

The collected data were transformed using Arc Sine transformation. Data were subjected to the Analysis of Variance (ANOVA) and the means were separated using the Least Significant Difference (LSD). The probability of 0.05 or less was considered significant (SAS 2004).

III. RESULTS

A. Effect aqueous extracts of jatropha seeds and argel leaves on egg-laying by the spiny bollworm

Effect aqueous extract of jatropha seeds on egg-laying by the spiny bollworm;

No significant difference ($P \geq 0.05$) was detected in the mean number of eggs laid by the SBW (2.1) on okra pod sprayed with the synthetic insecticide (proclaim®) and the mean number of eggs laid (4.5) on okra pod sprayed with a 10% aqueous extract of jatropha seeds. The untreated pod, however, has received a significantly high ($P \leq 0.05$) number of eggs (11.8) compared to those sprayed with 10% aqueous extract of jatropha seeds and the synthetic insecticide (proclaim®) (Table 1).

Effect aqueous extract of argel leaves on egg-laying by the spiny bollworm;

No significant difference ($P \geq 0.05$) was detected in the mean number of eggs laid by the SBW (2.1) on okra pod sprayed with the synthetic insecticide (proclaim®) and the mean number of eggs laid (1.8) on okra pod sprayed with a 10% aqueous extract of argel leaves. The untreated pod, however, has received a significantly high ($P \leq 0.05$) number of eggs (11.8) compared to those sprayed with 10% aqueous extract of argel leaves and the synthetic insecticide (proclaim®) (Table 1).

B. Effect of oil extracted from jatropha seeds and argel leaves on egg-laying by the spiny bollworm

Effect of oil extracted from jatropha seeds on egg-laying by the spiny bollworm;

No significant difference ($P \geq 0.05$) was detected in the mean number of eggs laid by the SBW (2.1) on okra pod sprayed with the synthetic insecticide (proclaim®) and the mean number of eggs laid on okra pod (2.8) sprayed with a 10% oil extracted from jatropha seeds. The untreated pod, however, has received a significantly high ($P \leq 0.05$) number of eggs (11.8) compared to those sprayed with 10% oil extracted from jatropha seeds and the synthetic insecticide (proclaim®). The pod sprayed with n-hexane has received a significantly high ($P \leq 0.05$) number of eggs (7.1) compared to those sprayed with the synthetic insecticide (proclaim®) and a 10% oil extracted from jatropha seeds (Table 1).

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Effect of oil extracted from argel leaves on egg-laying by the spiny bollworm;

No significant difference (P≥0.05) was detected in the mean number of eggs laid by the SBW (2.1) on okra pod sprayed with the synthetic insecticide (proclaim®) and the mean number of eggs laid (3.0) on okra pod sprayed with a 10% oil extracted from argel leaves. The untreated pod, however, has received a significantly high (P≤0.05) number of eggs (11.8) compared to those sprayed with the synthetic insecticide (proclaim®) and a 10% oil extracted from argel leaves (Table 1). The pod sprayed with n-hexane has also received significantly high (P≤0.05) number of eggs (7.1) compared to those sprayed with the synthetic insecticide (proclaim®) and a 10% oil extracted from argel leaves.

TABLE I: EFFECT OF 10% JATROPHA SEEDS AND ARGEL LEAVES EXTRACTS ON EGG-LAYING BY THE SPINY BOLLWORM AFTER 6 DAYS OF TREATMENT APPLICATION

	Oil extract		**Aqueous extract**	
Treatments	**Argel leaves**	**Jatropha**	**Argel leaves**	**Jatropha**
10% concentrations	(30.3) 3.0^{b}	(31.7) 2.8^{b}	(26.3) 1.8^{b}	(46.5) 4.5^{b}
Emamectin benzoate (proclaim®)	(27.2) 2.1^{b}	(27.2) 2.1^{b}	(27.2) 2.1^{b}	(27.2) 2.1^{b}
Untreated control	(80.2) 11.8^{a}	(80.2) 11.8^{a}	(80.2) 11.8^{a}	(80.2) 11.8^{a}
n-hexane (%) control	(53.6) 7.1^{a}	(53.6) 7.1^{a}	ND	ND
LSD	5.0	4.9	4.8	5.2

Means followed by the same letter are not significantly different at (P≤0.05) according to LSD.

Numbers in parentheses are actual means. ND ≡ Not applicable

IV. DISCUSSION

The increasing problems of pesticides, especially those associated with the large-scale use of broad-spectrum synthetic pesticides, have directed the need for effective, biodegradable pesticides with greater selectivity. They must be pest-specific, non-toxic to mammals, biodegradable, less prone to pest resistance, relatively less expensive and safe to non-target organisms [18].

The effect of the aqueous extracts of jatropha seeds and argel leaves was found comparable to that caused by the conventional insecticide (proclaim®). This result justifies the use of extracts of these plants instead of synthetic insecticides since they are readily available, cheap, environmentally-friendly and easy to use by farmers.

In this study, the adult insects were confined in the plastic bowls which contained treated pods of okra. This condition leaves no choice for the adult female to lay its eggs. It is known that laying females have to get rid of their load of eggs in a substrate suitable for larval feeding. If a suitable substrate is not found, it may deposit its load of eggs in any object irrespective of the fate of its offspring. In this study, the eggs laid on pods treated with the aqueous extracts of jatropha seeds and argel leaves may be explained as above. A field experiment will verify this opinion.

The effect of the plant extracts obtained in this study could be due to their inherent capacity of the chemical constituents found in their leaves and seeds to protect them from various herbivores. This could be in agreement with the results reported by El-Kamali [19] about the chemical constituents of argel leaves including acylated phenolic glycosides, namely argelin and argelosid, choline, flavonoids, monoterpenes, pregane glucoside, sitosterol, and a triterpenoid saponin. Similar results were obtained by Abdelbagi et al. [20] and Ahmed et al. [21] who used garlic oils of three garlic cultivars on eggs of cowpea beetle, *Bruchidius incarnatus* and *Callosobruchus maculatus*, they found the oils were significantly reduced number of eggs laid, also repot that the highest dose (10%) caused complete inhibition of egg laying.

The repellent action and volatiles of jatropha seeds and argel leaves could have resulted in the reduction of egg-laying by the SBW as presented in Tables 1. This phenomenon is also stated by Shelke et al. [22] who studied the effect of jatropha seed oil on potato tuber moth *Phthorimaea operculella*.

Finally, this study investigated the influence of jatropha seeds and argel leaves on the spiny bollworm due to their

availability, cheap and eco-friendly. The results showed that those plants have potential ovicidal and oviposition effect on SBW eggs. Therefore the jatropha seeds and argel leaves could serve as an alternative to conventional synthetic pesticides, notably in developing countries such as Sudan due to their mentioned merits.

V. CONCLUSION

Aqueous and oil extracts of jatropha seeds and argel leaves at a concentration of 10% have a comparable impact, in reducing the eggs laid by the spiny bollworm on okra pods, to that of the synthetic insecticide (proclaim®). The aqueous extract of jatropha seeds at 10% concentration was more effective in reducing the number of eggs hatched than the other concentrations.

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