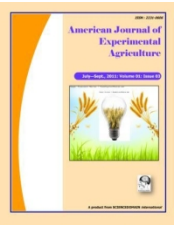

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Toxicity, Antifeedant and Growth Regulating Potential of Three Plant Extracts against the Desert Locust *Schistocerca gregaria* Forskal (Orthoptera: Acrididae)

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Authors' contributions

This work was carried out in collaboration between the two authors. Author EMB performed the different experiments, the statistical analysis, wrote the protocol, and the first draft of the manuscript. Author HAFE designed, advised, evaluated the data and finalized the manuscript for publication. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The main objective of this investigation was to assess the toxicity and growth regulating potential of *Jatropha* (*Jatropha curcas* L.), neem (*Azadirachta indica* A. Juss) and argel plant, (*Solenostemma argel* Hayne) against the desert locust, *Schistocerca gregaria*. The effect on fecundity, feeding behavior and egg hatchability was also studied. Argel of 5% concentration induced a significantly less (56%) antifeedant effect on desert locust nymphs compared with 79.62 and 78.92% for neem and *Jatropha* oils respectively. Significant mortality of 40.54 and 43.39% was recorded, 7 days after treatment, in insects treated with 10% concentration of neem and *Jatropha* oils respectively compared to 15.19% in the control. Argel resulted in the lowest mortality of 20.70% which was not significantly different from the control. There was a significant difference between treatments for the time it took surviving nymphs to moult to the next instar. Nymphs in the control group took significantly less time (11.3 days) to develop from the 5th instar to the 6th instar than those treated with neem and *Jatropha* oils where they took 17.5 and 16.5 days respectively. Argel was not

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significantly different from the control. *Jatropha* oil significantly reduced the fecundity of females developed from nymphs treated in the 3rd instar by 42.2% compared with 58.54% due to neem treatment. Argel caused no significant effect on fecundity of treated insects. Both *Jatropha* and neem oils resulted in 99.71% egg un-hatchability while argel had no significant effect. Of the three test products, only *Jatropha* and neem oil have shown growth regulating effect.

Keywords: Jatropha oil; neem; Solenostemma argel; desert locust; nymphal mortality.

1. INTRODUCTION

Locusts and grasshoppers are major economic pests of crops and grasslands throughout the world's dry zones [1]. The desert locust *Schistocerca gregaria* (Forskal) has been a most serious crop pest in many countries of Africa and Asia [2]. It is characterized by a remarkable phase polyphenism, a form of phenotypic plasticity in which the expression of numerous physiological, morphological and behavioral traits occurs in response to changes in local population density [3]. During the 2003-2004 desert locust campaigns and throughout the last invasion areas, control teams applied some 13 million liters of mainly organophosphorus pesticides to roughly 13 million ha of land [4]. Current locust control operations are mainly based on organophosphorus pesticides as a result of the ban on the use of organochlorine especially dieldrin [5]. Rembold [6] adverted to the rapidly increasing insect tolerance against any type of neurotoxic insecticide; and all insecticides given their wide spectrum of action, undoubtedly had substantial side-effects on the non-target fauna [7]. A preventive control strategy for locusts was developed in the 1960s' and was recommended by FAO and applied by national and regional locust control units [8]. The strategy requires monitoring ecological conditions and locust populations in outbreak areas and conducting preventive treatments against the first gregarious locusts. Due to the regular application of this strategy, the frequency and duration of desert locust invasion were reduced since 1960s [9]. Peveling et al. [10] showed that, there was no evidence of serious side-effects of alternative control agents (microbial insecticides) on epigeal arthropods when compared with conventional ones. Mycopesticides, particularly *Metarhizium anisopliae* should have a role in an integrated control strategy alongside classic insecticides [11]. It is now available in locust control market and part of FAO [12] list of products recommended for locust control. Other potential alternative is the use of the pheromone phenylacetonitrile (PAN) which inhibits pheromonal communication among gregarious hoppers and induces stresses that lead to high mortality [13]. Various researches on the effect of botanical bio-pesticides including neem on desert locust have been or are being carried out as alternatives to the currently used harmful pesticides [14]. These botanicals are still at the experimental stage as far as locust control is concerned and large scale production is problematic with difficulties in the registration of variable products limiting adoption [15]. Accordingly, the use of such products may be aimed at protecting crops locally [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 [16]. The extracts of *Jatropha* showed nematicidal, fungicidal [17], molluscicidal [18] effects. It also exhibited insecticidal activities against moths, butterflies, aphids, bugs, beetles, flies, and cockroaches [19]. Toxicity of *J. curcas* seeds is attributed to several components, including saponins, lectins (curcin), phytates, protease inhibitors, curcalonic acid and phorbol esters [20]. The

insecticidal properties of neem were first observed in 1959, when it was noticed that neem trees in Africa were undamaged during a plague of locusts [21]. Most work has been focused on azadirachtin, a limonoid from the seeds of the Indian neem tree, *Azadirachta indica*. Neem acts both as potent antifeedant and insect growth regulator [22].

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 the Saudi Arabia [23]. It has many active compounds that have been shown to act as potent acute or chronic insecticides [24,25], antifeedant and insect growth regulators against a variety of insect species [26].

The present study was carried out with the objective of demonstrating the biological activity of *Jatropha* oil, neem oil and argel extracts against the desert locust *S. gregaria* and to determine the efficacy and potentials of using any of these three materials as choice candidates in the control of this insect pest.

2. MATERIALS AND METHODS

2.1 Insect Rearing

Desert locust eggs were obtained from the International Centre of Insect Physiology and Ecology (ICIPE), Port Sudan and mass reared in the insectary of crop protection department, University of Khartoum. Cages (1 mx1 mx0.35 m) fitted with wire mesh and a movable window made of cloth, to facilitate cleaning and feeding, was used for the purpose. The cages stood on poles one meter from the ground, 24 cups (10 cm x12cm) filled with moist soil are fitted in each cage for egg-laying. Locusts were fed on food plants such as *Pennisetum glaucum* (L.) R. Br. and *Heliotropium* spp. The hatching nymphs were removed from the cups (10 cm x12cm) to cages (1 m x1 m x0.7m) fitted with cloth and wire mesh and supplied with food. The 3rd and 4th instars of desert locust nymphs were used in the trials. Some of these nymphs were transferred into large cages (4 m x2 m x2m) made from steel and wire mesh and were kept for breeding and continuity of the colony.

2.2 Plant Materials

2.2.1 Jatropha

Fresh *Jatropha curcas* seeds were collected from EL-Damazein rain-fed areas in the Blue Nile State, Sudan, where the plant is grown on a large scale for the production of bio-fuel. The seeds were then cleaned, de-shelled and subsequently the kernels and hulls were separated manually. The kernels were dried under shade and grounded to fine powder, stored in glass vials until used.

2.2.2 Argel

Leaves of argel were obtained from retailer's stores in North Sudan (Elrobatab area). The leaves samples were cleaned, washed with water, dried, and milled into fine powder using laboratory mill.

2.2.3 Neem

Ripe neem seeds were hand-picked from mature disease-free trees in Shambat area, in Khartoum University campus. The collected seeds were de-pulped and thoroughly washed by hand to remove the pulp in order to disallow fungal growth. The seeds were then shade-dried on jute mats, ground to fine powder and used for different treatments.

2.2.4 Extraction

The powder preparation of each plantwas used to produce the crude oil extract in a soxhlet apparatus using 95% hexane (boiling point 65–70ºC). The hexane was evaporated at reduced temperature and pressure in a rotary evaporator and the obtained oil was stored at 4ºC for subsequent use in the different treatments.

2.3 Nymphicidal Bioassay

Third instars nymphs were used to study the long–term impact of the test products on some important biological parameters of the desert locust. A 10% concentration of neem oil, Jatropha oil and argel extract was sprayed on the experimental nymphs using ultra-low volume sprayer. For each treatment, 60nymphs were used and the treated insects were allowed to dry before being transferred to rearing cages for further observation. Nymphs were checked every 24h post-treatment and mortality was recorded. Instar was determined by checking for shed exuviae. Recording of data continued until nymphs became adult or died. Nymphs were considered dead when they failed to respond to gentle touch with a fine camel hair brush. Corrected mortality was calculated according to Abbott's [27] formula as follows:

$$\text{Corrected mortality } \% = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) \text{x } 100$$

Where n = number of insects, T = treated, Co = control. The incidence of faulty development and deformities induced by the test products was also recorded.

2.4 Effects on Fecundity and Egg Hatchability

The fecundity experiments were conducted by selecting 10 pairs of freshly fledged adult males and females that survived treatment by the test product during the 3rd nymphal instar. The selected insects were then transferred to the mating and egg laying cage, 75 cm x 45 cm x 45 cm. The bottom board of the cage was made of plywood, which was divided into twenty holes to accommodate the plastic oviposition cups (7.5 cm diam., 11 cm deep). The plastic cups were filled with sandy soil for egg-laying. The sand was sieved using a 2 mm wire mesh; washed successively with distilled water, dried and heat-sterilized by baking in an oven at 150°C for 24h. The sterilized sand was moistened by addition of water to be attractive to females for egg laying. The effects of test products on fecundity were assessed on the basis of the number of egg-pods laid during the lifetime of the female. Number of eggs that successfully hatched in each treatment was also recorded and hatchability calculated.

2.5 Phago-deterrent Test

The antifeedant effects of the extracts at 5% against *S. gregaria* third instar nymphs were studied by mixing each extract in 250 g of wheat bran in a Petri dish. 10 3rd nymphs were used in each of the tests and replicated 4 times. Two sets of control were included, untreated and mixed with hexane only. The bran portions left in the dishes were weighted three and six days post-treatment and the amount of consumed food were estimated. The antifeedant index was calculated by the formula as adopted by Abivardi and Benz [28].

$$\text{Antifeedant index (AFI)} = \frac{\text{C} - \text{T}}{\text{C}} \times 100$$

Where:

C= Consumed wheat bran in control
T= Consumed wheat bran in treatment

2.6 Data Analysis

In each test, treatments were arranged in complete randomized design. Each treatment was replicated four times. Transformation of data was done using Arc Sine. Data were subjected to analysis of variance (ANOVA) using MINITAB Statistical software, version 13.30, Copyright 2000; Minitab Inc. and means were separated using the least significant difference (LSD). The Probit analysis was carried out to calculate regression equation [29], LT50, Lower fiducial limit, upper fiducial limit, time responses and Chi Square for the three test plants at a 10% concentration.

3. RESULTS

3.1 Effects of Test Products on Survival and Development

Significant mortality of 40.54 and 43.39% was recorded, 7 days after treatment, in insects treated with 10% concentration of neem and Jatropha oils respectively compared to15.19% in the control.Argel resulted in the lowest mortality of 20.70%, which was not significantly different from the control (Fig. 1). Table 1 illustrates the results of probit analysis responses of neem seed oil, Jatropha seed oil and argel extract. The results indicated that the neem and Jatropha oils were effective against 3rd instars nymph of *S. gregaria* as shown by the lower LT50 value of 117.84,135.95 respectively, compared to the value of argel which was 270.98. The responses of the two plants were more or less homogenous as indicated by their close LT50 /LT90 ratio and the slope. There was a significant difference between treatments for the time it took surviving nymphs to moult to the next instar. Nymphs in the control group took significantly less time (11.3 days) to develop from the 5th instar to the 6th instar than those treated with neem and Jatropha oils which took 17.5 and 16.5 days respectively. Argel was not significantly different from the control (Table 2). Jatropha and neem oils resulted in significant malformation effect on the test insects while argel proved to have no significant growth regulating effect (Fig. 2).

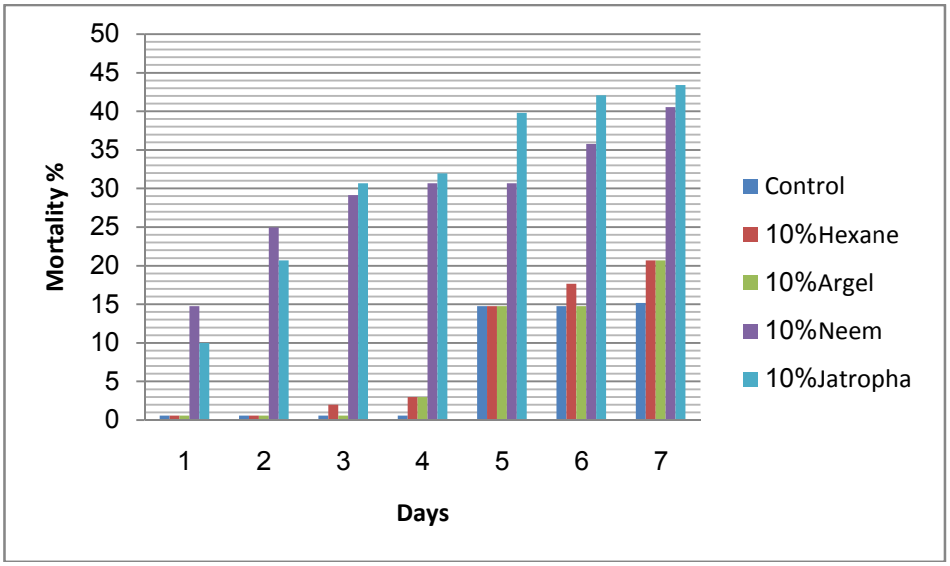

Fig. 1. Mean % mortality induced by 10% concentration of *Jatropha* oil, neem oil and argel extract on the 3rd instar nymphs of *S. gregaria*

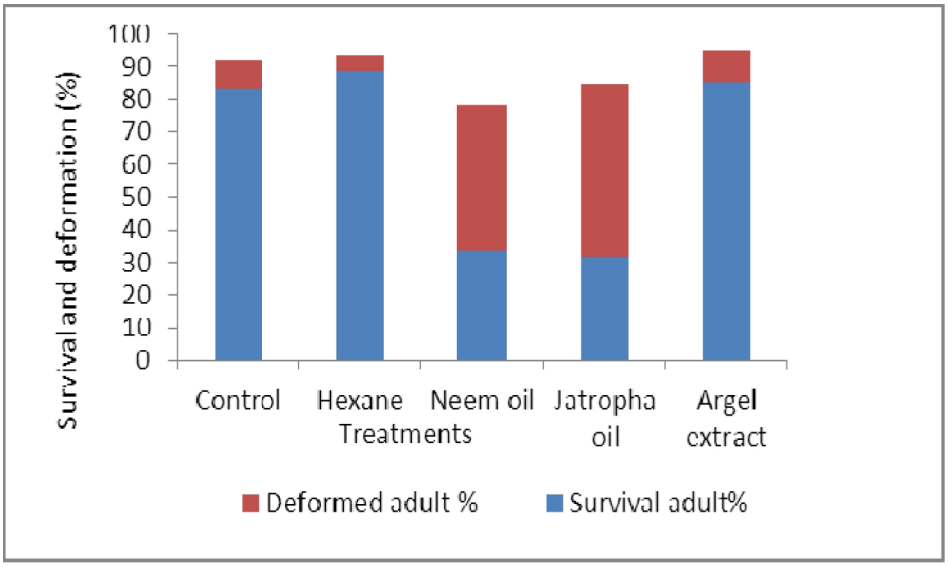

Fig. 2. Percentage of survival and deformed *S. gregaria* adults developed from 3rd instar nymphs treated with argel extract, Jatropha and neem oils

Table 1. Log time response of *S. gregaria* 3rd instar nymphs exposed to 10% concentration of argel extract, Jatropha and neem oils

Source of treatment	Lethal Time %		Fiducial limit		Chi-square	LT_{90}/LT_{50} Ratio	Slope	df.	Sensitivity
			lower	upper					
Argel extract	LT_{10}	161.00	142.87	185.39	1.034	1.68	0.176	2	2.29
	LT_{50}	270.98	224.29	394.50					
	LT_{90}	456.10	332.47	889.07					
Jatropha oil	LT_{10}	80.77	57.21	95.36	1.034	1.68	0.176		1.15
	LT_{50}	135.95	124.09	146.87					
	LT_{90}	228.82	198.04	307.45					
Neem oil	LT_{10}	70.01	45.98	85.50	1.034	1.68	0.176		1
	LT_{50}	117.84	102.30	128.37					
	LT_{90}	198.34	176.68	248.31					

LT_{10}: lethal time that kills 10% of the exposed nymphs
LT_{50}: lethal time that kills 50% of the exposed nymphs
LT_{90}: lethal time that kills 90% of the exposed nymphs
df: degrees of freedom

Table 2. Effect of argel extract, neem and Jatropha oils on nymphal development of the desert locust, *S. gregaria*

Treatment	Development duration (days)		
	3rd – 4th instar	4th – 5th instar	5th – 6th instar
Control	8.76[a]	10.16[a]	11.33[a]
Hexane	8.78[a]	10.50[a]	12.00[a]
Argel extract (10%)	8.78[a]	11.66[a]	*11.50[a]*
Neem oil (10%)	16.44[a]	15.33[b]	17.50[b]
Jatropha oil (10%)	16.41[a]	14.00[b]	16.50[c]
LSD	12.54	1.99	0.84
SE±	6.89	1.09	0.45

Third instar nymphs were treated and development was followed
The means followed by the same letter in the same column are not significantly different at (P=0.05) according to LSD.
LSD= Least Significant Different
SE± = Standard Error

3.2 Phago-deterrent Effect

The antifeedant effect of 5% concentration of neem oil, Jatropha oil and argel extract on the 3rd instar nymphs of the desert locust is shown in Table 3. Argel induced a significantly less (56%) antifeedant effect on desert locust nymphs compared with 79.62 and 78.92% for neem and Jatropha oils respectively. The antifeedant effect of both neem and Jatropha oils was similar and seems to last for at least 6 days after treatment.

Table 3. Antifeedant % induced byargel, neem and Jatropha oils on the 3rd nymphal instar of *S. gregaria*

Treatment	After 3 days	After 6 days
Control	-	-
Hexane	(54.72)[a] 42.30	(64.9)[a] 36.33
Argel extract 5%	(52.96)[a] 43.57	(56)[a] 41.54
Neem oil 5%	(76.45)[b] 29.06	(79.62)[b] 26.85
Jatropha oil 5%	(72.64)[b] 31.56	(78.92)[b] 27.83
LSD	14.59	12.24
SE±	8.02	6.73

The means followed by the same letter(s) in the same column are not significantly different at (P=0.05) according to LSD. The numbers between parentheses represent arc sine transformation data
LSD= Least Significant Different
SE+ = Standard Error

3.3 Effect on Fecundity and Egg Hatchability

The data presented in Tables 4 and 5 demonstrate the effect of the test products on fecundity and egg hatchability of females developed from nymphs treated in the 3rd instar. Jatropha oil significantly reduced the fecundity of females by 42.2% compared with 58.54% due to neem treatment. Argel caused no significant effect on fecundity of treated insects.

Table 4. Fecundity of *S. gregaria* developed from 3rd nymphal instar treated with 10% argel extract, neem and Jatropha oils

Treatment	Average number of egg- pods/10 females			
	1st count	2nd count	3rd count	4th count
Control	(1.86)[a]	(5.04)[a]	(9.05)[a]	(10.95)[a]
	7.92	12.92	17.56	19.23
Hexane 10%	(0.57)[a]	(3.15)[a]	(7.30)[a]	(10.24)[a]
	0.1	10.31	15.68	18.72
Argel extract 10%	(1.86)[a]	(5.04)[a]	(7.16)[a]	(10.69)[a]
	7.92	12.92	15.56	19.09
Neem oil 10%	(0.57)[a]	(0.57)[a]	(3.15)[a]	(4.54)[b]
	0.1	0.1	10.31	12.25
Jatropha oil 10%	(0.57)[a]	(0.57)[a]	(3.15)[a]	(6.33)[b]
	0.1	0.1	10.31	14.54
LSD	2.24	3.64	5.55	4.31
SE+	1.63	2.41	3.68	2.99

The means followed by the same letter in the same column are not significantly different at (P=0.05) according to LSD. The numbers between parentheses represent arc sine transformation data.
LSD= Least Significant Different
SE+ = Standard Error

Table 5. Per cent hatchability of eggs laid by *S. gregaria* developed from 3rd nymphal instar treated with argel extract, neem and Jatropha oils

Treatment	Days		
	1	2	3
Control	47.50[a]	59.00[a]	34.52[a]
Hexane 10%	44.75[a]	58.75[a]	36.00[a]
Argel extract 10%	50.75[a]	60.00[a]	42.50[a]
Neem oil 10%	16.30[b]	2.52[b]	0.10[b]
Jatropha oil 10%	12.55[b]	5.50[b]	0.10[b]
LSD	19.16	14.63	13.74
SE±	12.71	9.70	9.11

The means followed by the same letter in the same column are not significantly different at (P=0.05) according to LSD. L.S.D. = Least significant difference.
SE+ = Standard Error

4. DISCUSSION

Jatropha and neem oils have demonstrated a remarkable effect on the 3rd instar nymphs of *S. gregaria* as toxic, antifeedant, and growth regulating compounds. However, argel seems to have no significant biological effect on the test insects, at least at the concentration tested. Many investigations were carried out on the toxicity, antifeedant effects, growth inhibition and abnormal development in various insects' including *S. gregaria* due to neem seed extracts and azadirachtin [21,30]. Their results were consistent with the findings reported in this study.

The antifeedant effect was manifested by differences in body weight of nymphs offered food treated with neem and Jatropha oils. This was in accordance with earlier findings of Champagne et al. [31], who reported, that azadirachtin is a potent antifeedant against the desert locust *S. gregaria* under a laboratory situation. Similar results were reported by Mordue et al. [32]. Nasiruddin & Mordue [33] reported that spraying of azadirachtin at low dose of 2 ppm onto barley seedlings infested by *S. gregaria* nymphs protected the plants. The repellent and antifeedant activity showed by Jatropha against *S. gregaria* during this study is in agreement with the results reported by Cobbinah and Tuani [34] who demonstrated the negative effect of Jatropha seed oil on survival and feeding rates of the variegated grasshopper, *Zonocerus variegatus* L. They also reported reduced damage by *Z. variegates* to cassava foliage after treatment with jatropha oil.Data concerning the biological effects of Jatropha oil on desert locust is scanty; however, good protection of cowpea seeds against *C. maculates* was achieved in storage by seed treatment with Jatropha seed oil [35]. Singh and Sushilkumar, [36] showed antifeedant activity against the termite, *Microcerotermes beesoni* caused by Jatropha oil at different dilution ranges from (1% to 20%). The maximum wood protection and weight loss by termites were obtained at the highest concentration of 20%. This persistency can be exploited as barrier treatment against hopper bands of the desert locust. Jatropha oil can also be integrated with the pheromone, Phenylacetonitrile (PAN) in the management of desert locust infestation, particularly in recession and before plague formation. However, field studies on evaluation of Jatropha toxicity to beneficial and non-target organisms require further investigation.

5. CONCLUSION

Crude Jatropha and neem oil extracts demonstrated a remarkable effect on feeding activity, growth, fecundity and finally fitness of the 3rd nymphal instar of *S. gregaria*. The test products could provide affordable and renewable source of botanical insecticide for tropical farmers to control desert locust infestation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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