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PHAGOREPELLENCY OF NEEM (*Azadirachta indica* A. Juss) OIL: ITS POTENTIAL USE IN THE MANAGEMENT OF THE MIGRATORY LOCUST, *Locusta migratoria manilensis* (Meyen)

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ABSTRACT

The study evaluated the phagorepellent properties of neem (*Azadirachta indica* A. Juss) oil as potential alternative or complement to synthetic insecticides for the management of migratory locust, *Locusta migratoria manilensis* Meyen. Adults and third instar nymphs were exposed to corn, sugarcane and napier grass terminals treated with 100, 50 or 25 ppm neem oil solution. The feeding intensity was rated after 24 and 48 h. In field simulation test, 1 m^2 cogon grass (*Imperata cylindrica*) patch was divided into three 30 x 100 cm strips and enclosed with 1 m^3 mosquito netting. The two outer strips were treated with 25, 50 or 100 ppm neem oil solution and the center strip was untreated. Treated plants consistently inhibited locust feeding at all concentrations and remained virtually untouched, whereas untreated plants were completely consumed. These findings can be used in an integrated pest management scheme, i.e. preventing damage to crop plants or weakening the locust population by starving and/or forcing them to migrate to non-crop areas.

Key words: Phagorepellent, neem oil, *Locusta migratoria manilensis,* integrated pest management

Abbreviations: ANOVA – Analysis of Variance, DM – Derris methanol, DMRT – Duncan's Multiple Range Test, FAO – Food and Agriculture Organization, IGR – insect growth regulator, IPM- Integrated Pest Management, JC – *Jathropa curcas*, NO – neem oil, ppm – parts per million, T – treated, TW – two way, U - untreated

INTRODUCTION

Locust plague has been recorded in the history of mankind as one of the most spectacular and feared pest problems worldwide. It is the most unpredictable pest occurrence in Africa and Asia, causing tremendous damage to both crops and natural vegetations (Krall, 1996). The United Nations Food and Agriculture Organization (FAO) has been in the forefront of instituting control programs against locust plague, particularly in Africa, for many years. Despite the best efforts of concerned national and international agencies, locust outbreaks continue to occur in regular or irregular intervals whenever environmental conditions are favorable for breeding and swarming.

Basically, there are two approaches to locust control: preventive (proactive) and plague treatment (reactive). Preventive control is more desirable but because of the generally inaccessible terrain of the breeding areas, requiring costly sustained and intensive monitoring, many locust-infested countries could hardly practise preventive control. Inevitably, reactive plague control had to be initiated against locust bands and swarms (Symmons, 1992).

In the 1950's, locust swarms were routinely decimated by massive and area-wide application of chlorinated hydrocarbon insecticides. Due to negative environmental impact, their use was eventually banned. Subsequently, newer rapidly-degradable synthetic insecticides were used, providing minimal population suppression and becoming more expensive eventually.

Considerable interest on ecologically friendly control agents also emerged during the late 1980's and early 1990's. The promising control agents identified were a) insect growth regulators (IGR); b) pheromones; c) microbials, particularly fungus of the genus *Metarrhizium,* and d) natural plant products or botanicals.

With the outbreak of migratory locust, *Locusta migratoria manilensis* (Meyen), in 1991 in the provinces of Pampanga and Tarlac, a search for botanicals to reduce synthetic pesticide application was initiated. Studies worldwide have elucidated the physiological action of plant-derived natural products particularly *azadirachtin* from the neem plant, *Azadirachta indica* A. Juss, which causes poisoning of larvae and adults, feeding deterrency or phagorepellency, oviposition inhibition and sterility (Kraus, et al. 1981, 1987; Zanno, et al. 1975). Neem has been recognized as a potential component of Integrated Pest Management (IPM) because of its exceptional phagorepellent activity on many insect species particularly those belonging to Order Orthoptera to which locust belongs (Abdul Kareem, 1981; Gahakur, 1995; Ladd et al, 1978, Sharma et al, 1984). Considering the potential of antifeedants in preventing crop damage, possible use of neem oil in an integrated management system was investigated by determining its effect on feeding intensity of locust on diverse graminaceous host plants.

MATERIALS AND METHODS

Feeding Repellency and Toxicity of Plant Oils and Extract

These botanical materials were initially evaluated for feeding repellency and toxicity by spraying 10,000 ppm or 1% solution of neem oil, *Jathropa curcas* oil, and *Derris elliptica* methanol extract on different graminaceous host plants (napier, corn, sugarcane, sorghum, rice) of the migratory locust and on broadleaf non-host crops (soybean and mungbean).

In a "no choice test," plant terminals of napier, corn, sugarcane, and potted 25 days old rice, sorghum, soybean, and mungbean were treated with 1% of the three botanicals, separately confined in a 55 x 55 x 60 cm aluminum screen cage and offered to 50 (25 males & 25 females) field-collected 3rd instar locust nymphs. Untreated control was provided in separate cages for each host plant. Treatments per host plant were replicated three times and the mortality and feeding intensity per host plant were taken after 24 and 48 h access feeding regimen. The feeding intensity was quantified by counting the entirely consumed leaf blades divided by the total number of undamaged and partially eaten leaf blades. Leaves nibbled on slightly were considered undamaged for clarity of quantification of the feeding intensity.

Response to Neem Oil-treated Graminaceous Hosts

To establish the feeding response to graminaceous host plants, three species, namely, napier, corn and sugarcane, treated with 1,000 ppm neem oil solution were tested. Thirty adult locusts (15M & 15F) which developed from field-collected nymphs were released in each 55 x 55 x 60 cm cage provided with leaf terminals of the neem oil-treated host plants for 24 and 48 h access feeding. Untreated control for each host plant was confined in a separate cage offered to the same number of adult locusts. The feeding intensity and mortality were assessed after 24 and 48 h access feeding time.

The initial concentration of 1% (10,000 ppm) was found rather high, hence concentration was lowered to 100, 50, & 25 ppm to define a more realistic or appropriate neem oil dosage. Three neem oil-treated young sugarcane and corn terminals (with 8 leaf blades each) and untreated control (3 replications) were separately placed in 55 x 55 x 60 cm cages. The mortality and feeding intensity of thirty (15M & 15F) laboratory reared 3rd instar locust nymphs were assessed after 24 and 48 h access feeding.

The second set of feeding trial or discrimination test was conducted to confirm the phenomenon of phagorepellency observed earlier, i.e. by spraying half of the total leaf blades of hosts corn and sugarcane terminals (6 leaf blades/ terminal) with concentrations of 100, 50 or 25 ppm neem oil and offering them to 30 (15M & 15F) 3rd instar locust nymphs in 55 x 55 x 60 cm cages with 3 replications. Mortality and feeding intensity or gustatory response were taken 24 and 48 h later. Mortality data were included as a parameter to grossly assess whether death was due to the toxicity of neem oil or to starvation.

Field Effectiveness of Neem Oil as Antifeedant

A simulated field trial was conducted to evaluate phagorepellent property exhibited by the neem oil in the screen house. A one-meter square patch of young cogon grass (*Imperata cylindrica*) ca. 0.5 m tall was enclosed with 1 x 1 x 1 m mosquito netting. Each cogon grass patch was divided into three 30 cm x 100 cm strips. The two outer strips were treated with either 100, 50, or 25 ppm neem oil and the center strip kept untreated. Each treatment was replicated three times. One hundred 3rd instar nymphs and 100 adult locusts were released in each one-cubic meter enclosure containing ca 188-200 mature cogon grass tillers. Locust mortality and feeding intensity were taken after 24 and 48 h access feeding.

All data generated per trial were statistically analyzed by Two-way Analysis of Variance (TW- ANOVA) and Duncan's Multiple Range Test (DMRT). The control data in all tables were automatically included in the software analysis.

RESULTS AND DISCUSSION

Feeding Repellency and Toxicity of the Two Oils and an Extract

The initial test with 10,000 ppm (1%) spray solution neem oil (NO) exhibited significant phagorepellent effect compared to derris methanol (DM) extract and *Jathropa curcas* (JC) oil after 24 and 48 h access feeding time, as shown by the means presented in Table 1. Feeding intensity of third-instar nymphs was much lower on treated graminaceous host plants such as corn, sugarcane, napier, sorghum and rice compared to the untreated control. Feeding on NO-treated graminaceous hosts (napier, corn, sugarcane, sorghum, and rice) intensified after 48 h, indicating that extreme starvation forced the locusts to feed. Treated and untreated mungbean and soybean plants were virtually untouched or with minimal feeding, if at all, confirming that locust is primarily graminovorous.

Table 1. Feeding response of 3rd instar field-collected migratory locust nymphs to host and non-host plants treated with 10,000 ppm (1%) solution of neem oil, (NO) *Jathropa curcas* (JC) oil or derris methanol (DM) extract.

PLANTS	HOURS AFTER EXPOSURE											
	24 Hours						48 Hours					
	% Feeding Intensity*			% Mortality			% Feeding Intensity			% Mortality		
	NO	JC	DM	NO	JC	DM	NO	JC	DM	NO	JC	DM
Napier	35.80 (84.39)**	88.23 (60.61)	33.33 (60.71)	9.33 (10.67)	6.67 (10.00)	23.33 (0.00)	44.44 (100.00)	97.05 (100.00)	59.25 (100.00)	25.33 (26.34)	8.34 (15.00)	23.33 (11.67)
Corn	3.74 (98.04)	94.44 (73.73)	11.11 (96.67)	14.33 (5.33)	0.00 (0.00)	5.00 (0.00)	7.47 (100.00)	100.00 (88.89)	33.33 (100.00)	32.66 (18.66)	0.00 (3.33)	5.00 (0.00)
Sugarcane	8.14 (62.84)	100.00 (77.78)	41.94 (100.00)	11.33 (4.33)	0.00 (0.00)	1.67 (0.00)	69.14 (87.35)	100.00 (88.89)	67.73 (100.00)	21.66 (14.00)	0.00 (0.00)	20.00 (0.00)
Sorghum	10.83 (89.28)	100.00 (100.00)	41.94 (100.00)	0.00 (36.67)	0.00 (7.00)	10.83 (3.33)	10.83 (100.00)	100.00 (100.00)	100.00 (100.00)	0.00 (54.67)	1.67 (7.00)	19.00 (11.60)
Rice	34.29 (100.00)	37.70 (26.79)	24.46 (100.00)	31.33 (36.67)	6.06 (7.00)	16.67 (3.33)	52.98 (100.00)	68.59 (100.00)	33.70 (100.00)	50.00 (54.67)	9.33 (7.00)	25.00 (11.60)
Soybean	0.00 (16.18)	12.74 (14.67)	3.70 (11.43)	0.00 (0.00)	0.00 (0.00)	1.67 (0.00)	2.47 (20.59)	12.74 (24.00)	6.48 (21.43)	5.00 (0.00)	3.33 (0.00)	6.67 (13.33)
Mungbean	0.00 (7.74)	4.17 (26.79)	1.06 (18.99)	0.00 (0.00)	0.00 (0.00)	53.33 (0.00)	2.34 (7.74)	8.34 (44.44)	1.06 (31.65)	16.67 (5.00)	0.00 (0.00)	81.66 (35.00)
Feeding Means	13.25c	62.46a	22.50b	9.47b	1.81c	16.97a	27.09c	69.53a	43.07b	21.62b	3.23c	25.80a

Means in a column followed by the same letters are not significantly different by DMRT analysis.

* Feeding Intensity = $\frac{\text{No. of fully consumed leaf blades}}{\text{No. of undamaged and partially eaten leaf blades}}$ - x 100

**

( ) untreated control

Mortalities attributable to NO, JC and DM toxicity were relatively minimal. DM treatment resulted in statistically higher mortality than NO and JC treatments taken after 24h access feeding time. After 48 h access feeding on graminaceous hosts, mortality on NO-treated hosts varied relative to the host plants (Table 1). Mortality was apparently induced by starvation. On JC treated host plants, feeding intensity after 24 h ranged from 37.70 to 100 % and on DM treated 11.11 to 41% among the graminaceous hosts. Feeding was strongly inhibited by NO on the hosts offered as compared to JC and DM and mortality observed was due to starvation. On broadleaf crops (soybean, mungbean) which are known non-preferred hosts, feeding was very slight. At 48 h access feeding, NO-treated host plants maintained comparatively strong phagorepellency and showed higher level of feeding inhibition followed by those treated with DM and JC. In all cases, feeding intensity on treated mungbean and soybean was extremely low. All graminaceous host plants treated with DM or JC were either completely or almost completely consumed, while treated or untreated soybean and mungbean were only slightly fed upon, indicating non-host status. Phagorepellency under field condition may force locust to seek untreated host plants whereby NO-treated crops may be spared from damage. This exploratory data provided strong evidence that neem oil has a strong phagorepellent property against locust but with negligible toxicity. *Jathropa* oil and Derris extracts possess only slight phagorepellent property.

Feeding Response of Locust to Neem Oil-treated Graminaceous Hosts

Subsequent tests were focused on three graminaceous hosts (napier grass, corn and sugarcane) treated with 1% NO to confirm the initial exploratory data (Table 2). At 24 and 48h after exposure, the third-instar locusts consumed only 4.41 to 7.75% and 5.50 to 10.00% of the leaf blades, respectively, but the untreated control were completely consumed after 48 h. Feeding on treated plants increased only slightly after 48 h access which was apparently influenced by the initial inhibitory impact of neem oil during the first 24 h feeding. The number of dead insects increased after 48 h access feeding period on napier and corn apparently due to starvation as a result of inhibited feeding but not on sugarcane for unknown reason.

As mentioned earlier, 1% or 10,000 ppm solution NO was deemed rather high, hence the phagorepellency of 100, 50 and 25 ppm was evaluated on corn and sugarcane. There was no feeding at the three lower doses (Tables 3 & 4), while minimal feeding was observed on corn and sugarcane sprayed with NO at 10,000 ppm (Table 1). Probably, the batch of locusts used in this test of higher doses were more active or stronger than those used at lower doses of NO.

Table 2. Feeding response of third-instar locust nymphs to three graminaceous host plant species sprayed with 10,000 ppm neem oil solution after 24 and 48 h access feeding periods.

Host Plant	Percent Feeding Intensity		Percent Mortality	
	24h	48h	24h	48h
Napier	7.75 (60.61)	10.00 (100.00)	2.60 (3.33)	10.56 (4.10)
Corn	4.41 (100.00)	9.00 (100.00)	7.00 (2.66)	25.00 (5.50)
Sugarcane	4.50 (100.00)	5.50 (100.00)	5.66 (2.66)	5.66 (2.66)

( ) unsprayed control

Table 3. Feeding response of third-instar locust nymphs after 24 h access feeding period* on corn and sugarcane terminals sprayed with 100, 50 or 25 ppm neem oil.

Concentration (ppm)	% Feeding Intensity		Percent Mortality	
	Corn	Sugarcane	Corn	Sugarcane
100	0.00	0.00	8.89c	12.22a
50	0.00	0.00	11.11b	5.55b
25	0.00	0.00	18.89a	6.67b
Control	100.00	100.00	0.00d	0.00c

* Thirty 3rd-instar nymphs per treatment replicated 3X
Means in a column followed by the same letter are not significantly different by DMRT.

Table 4. Feeding discrimination of third-instar locust nymphs gauged after 24h access feeding time on corn and sugar cane with one half of the total number of leaf blades per terminal treated with 100, 50 or 25 ppm neem oil (T) and the other half untreated (U).

Conc. (ppm)	Percent Feeding Intensity				Percent Mortality			
	Corn		Sugarcane		Corn		Sugarcane	
	U	T	U	T	U	T	U	T
100	100.00	0.00	100.00	0.00	0.00	8.89a	0.00	2.22b
50	100.00	0.00	100.00	0.00	0.00	2.22b	0.00	5.55a
25	100.00	0.00	100.00	0.00	0.00	0.00c	0.00	1.44b
Control	100.00	100.00	100.00	100.00	0.00	0.00c	0.00	0c

Means in a column followed by the same letter are not significantly different by DMRT

The levels of phagorepellency were identical for both corn and sugarcane (0.00% feeding) after 24 h at all doses and any subsequent mortality must have been mainly due to starvation. Feeding was completely inhibited after 24 h access time, justifying dose reduction to 100, 50, and 25 ppm.

Data in Table 4 show that locust nymphs were able to discriminate NO-treated (T) from untreated (U) leaves. Whereas the untreated leaves were completely consumed (Fig. 1, side B) after 24 hr, the 100 and 50 ppm treated leaf blades were untouched or unfed upon (Fig. 1, side A). Few locusts died on the treated leaves but significantly higher mortalities were observed on corn and sugarcane treated with 100 ppm than 50 ppm. The untreated corn or sugarcane leaves were completely consumed but the treated counterparts were completely untouched. Natural mortalities were relatively minimal. All leaves of the untreated plants (control) were consumed and no locust mortality was observed (Table 1).

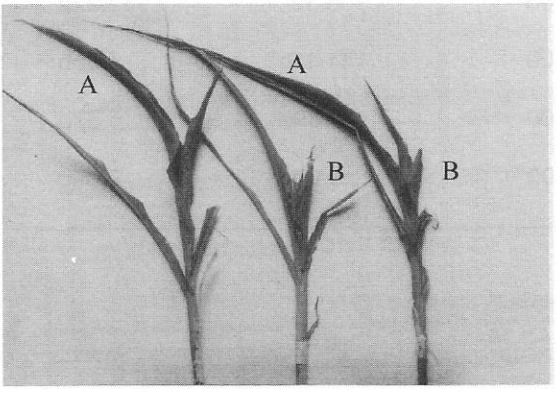

Fig. 1. Sugarcane terminals showing the leaves (left side, A) sprayed with 100 ppm neem oil intact and the untreated leaves (right side, B) heavily fed on when offered to 3rd instar locust nymphs for 24 h access feeding.

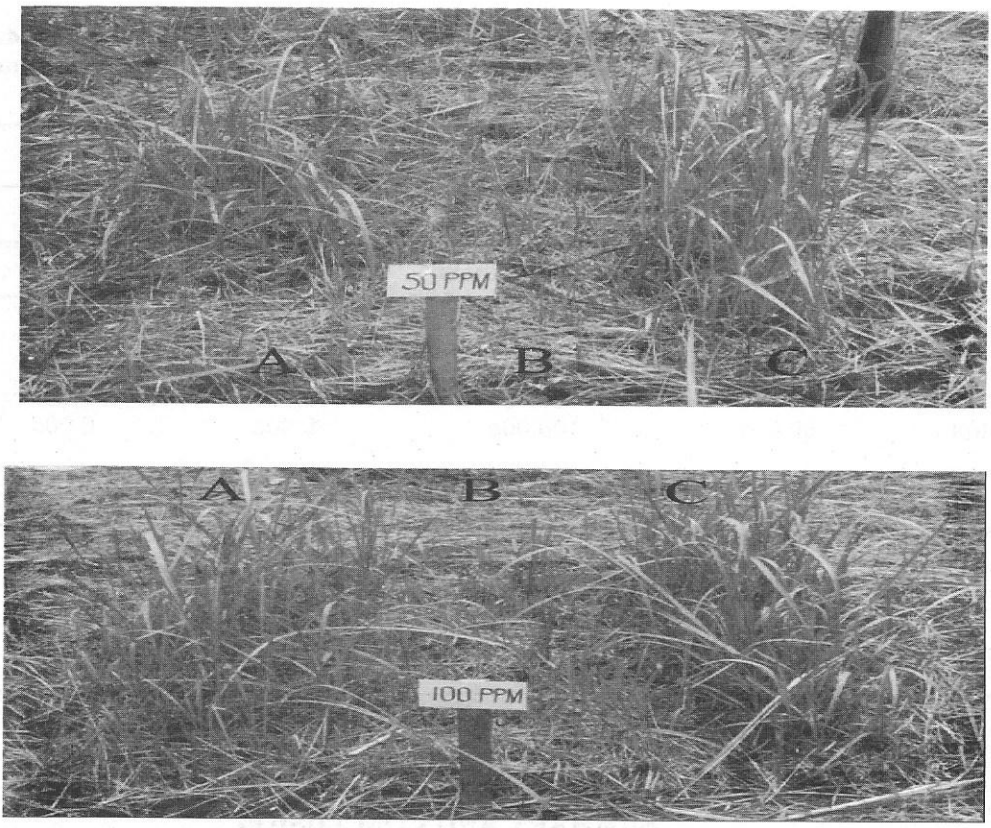

Figure. 2. Cogon grass (*Imperata cylindrica*) patches showing plants in the middle strips (B) wholly consumed by 3rd instar locust nymphs and those in the two outer strips (A&C) treated with 50 ppm and 100 ppm neem oil solution, respectively, not fed upon.

Field effectiveness of neem oil as antifeedant

Both 3rd instar and adult locusts did not feed on treated cogon after 24 h at the dosages tested (Fig. 2 and Table 5). There was a much higher mortality of the nymphs after 48 h at 50 and 100 ppm, indicating that the third instars were more prone to succumb to death than adults. Furthermore, mortalities were affected significantly by dose (i. e. 25 ppm was well tolerated by adults). There were significantly higher locust mortalities on cogon patches treated with high dose than on those applied with lower dose. Although the adult locusts fed on cogon sprayed with 25 ppm NO, no mortality was observed after 48 hours.

There was a strong possibility that mortality after 48 h was due to phagorepellency. The hunger stress and slight toxicity at the given dosage of neem oil resulted in more adverse effects leading to mortality of both 3rd instar and adult locusts.

Table 5. Feeding intensity and mortality of 3rd instar nymphs and adult locusts after 24 and 48 h access feeding on cogon grass sprayed with different concentrations of neem oil solution.

Conc. (ppm)	Percent Feeding Intensity		Percent Mortality	
	24h	48	24h	48
		Third Instar		
100	0.00b	0.00b	6.00a	30.50a
50	0.00b	0.00b	5.00a	16.50b
25	0.00b	100.00a	3.50b	8.00c
Control	60.00a	100.00a	0.00c	0.00d
		Adults		
100	0.00b	0.00c	0.00a	10.00a
50	0.00b	0.00c	0.00a	1.00b
25	0.00b	60.00b	0.00a	0.00c
Control	65.00a	100.00a	0.00a	0.00c

Means in a column followed by the same letter are not significantly different by DMRT

SUMMARY AND CONCLUSION

The expression of phagorepellency or feeding deterrency of neem oil was demonstrated after rigid serial evaluation processes. At the outset 10,000 ppm or 1% neem oil was compared with 10,00 ppm *Jathropa* oil and 1000 ppm derris methanol extract for antifeeding activity. Neem oil exhibited strong phagorepellency but the latter two botanicals showed very slight or insignificant antifeeding activity, thus subsequent tests were focused on neem oil.

The different laboratory evaluations (no choice test, choice test, discriminating tests) and field-simulated tests showed the phagorepellency of neem oil to the migratory locusts, *Locusta migratoria manilensis*, when applied on graminaceous host plants.

Inducing phagorepellency of host plants through neem oil application may provide graminaceous crops protection from feeding of locusts thereby avoiding damage. If crops are treated on time, locust swarms may be forced to migrate in search of other feeding grounds, leaving the treated crops. Since the locust will rather starve than feed on neem oil-treated plants, the insects may weaken physiologically, hence becoming more vulnerable to appropriate microbial or low toxicity and degradable synthetic pesticides.

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