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Larvicidal activity of neem and karanja oil cakes against mosquito vectors, *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Anopheles stephensi* (L.)

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Abstract: *Larvicidal effect of neem (Azadirachta indica) and karanja (Pongamia glabra) oil cakes (individuals and combination) was studied against mosquito species. Both the oil cakes showed larvicidal activity against the mosquito species tested. The combination of neem and karanja oil cakes in equal proportion proved to have better effect than the individual treatments. The combination of the two oil cakes recorded an* LC_{95} *of 0.93, 0.54 and 0.77% against the mosquitoes, Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi respectively. The increase in efficacy of the combination treatment over individuals in all the mosquito larvae tested was found to range about 4 to 10 fold in terms of* LC_{50} *and 2 to 6 fold in terms of* $LC_{95.}$

Key words: *Karanja, Neem, Oil cakes, Mosquito larvae*
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Introduction

The best way to keep the mosquitoes at check is larviciding (Shanmugasundaram *et al.*, 2001). In view of this, an eco-friendly approach to control mosquito larvae is warranted. Several chemical insecticides are being indiscriminately used to control mosquito larvae (Bansal and Singh, 2006). As a result mosquitoes have developed resistance to the wide variety of chemicals used against them. Keeping an unpolluted and hazardless environment in mind, a number of plant products have been evaluated against mosquito larvae. Among them, neem (*Azadirachta indica*) and karanja (*Pongamia glabra)* are the two effective botanicals commonly used for controlling the insect pests. Informations are in glut for oil formulations of the above plants but not for the oil cakes (end product after oil extraction), particularly their combinations against mosquito larvae. The present investigation throws light on the efficacy of neem and karanja oil cakes and their combination against mosquito larvae.

Materials and Methods

Ten gram of locally available oil cakes was finely powdered and 100 ml of tap water was added (10% w/v) gradually in increment amounts. The mixture was then vigorously shaken for 10 minutes to obtain a uniform suspension and the resultant suspension was decanted. From the decanted stock solution, different concentrations of test solution were prepared by adding known quantity (ml) of stock solution. 100 ml of each test solution was transferred into the 150 ml plastic cup containing mosquito larvae. For combination treatments, equal proportions (50:50) of neem and karanja oil cakes were powdered and blended thoroughly and then solutions were prepared as mentioned above.

Larval bioassay was carried out separately for each test product *viz*., neem oil cake, karanja oil cake and combination of neem and karanja oil cakes in equal proportion. Non-replicated pilot studies were initially conducted with a wide range of concentrations to find out the activity range of the oil cakes. Based on the results obtained from the pilot study, doses were fixed (with a geometric factor of 2.0) for main experiment with four replications to attain 10 to 95% mortality for estimating the LC_{50} and LC_{95} values of the test products. Twenty five mosquito larvae of fourth instar (laboratory reared) were used for each replications including untreated control. For neem oil cake, following concentrations *viz.*, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4% were evaluated against all the three mosquito species. Similarly the mosquito larvae were exposed to the following karanja oil cake concentrations *viz.*, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2%. The concentrations of combination of neem and karanja oil cakes *viz.*, 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64 and 1.28% were evaluated against mosquito species. The temperature of the test room was maintained at 25-28°C with a photoperiod of 12 hr L: 12 hr D (WHO, 2005). After 24 hr, observation on larval mortality was recorded and the data were subjected to probit analysis (Finney, 1971) using NCSS 2000 statistical package. Increase in efficacy (Fold increase) was calculated by the following formula:

$$\text{Fold increase} = \frac{LC_{50} \text{ or } LC_{95} \text{ of Individual treatment}}{LC_{50} \text{ or } LC_{95} \text{ of Combination treatment}}$$

Results and Discussion

The results of the study are presented in Tables 1-4. In the present investigation, it was clearly revealed that the combination of Neem and Karanja oil cake was found to excel their individual's effects against all the mosquito larvae tested. The products exhibited an LC_{50} of 0.06, 0.038 and 0.048% against the larvae of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* respectively. Similarly, the LC_{95} was observed as 0.93, 0.54 and 0.77% respectively. However, the neem and karanja oil cake when

Table - 1: Data on larval mortality of neem and karanja oil cakes against different mosquito species

Concentrations (%)	Mortality at 24 hr (%)*		
	Culex quinquefasciatus	*Aedes aegypti*	*Anopheles stephensi*
Neem oil cake			
0.05	0.00	0.00	0.00
0.1	11.00 (1.91)	19.00 (1.91)	10.00 (1.15)
0.2	25.00 (3.42)	36.00 (0.00)	33.00 (1.91)
0.4	44.00 (1.63)	68.00 (1.63)	48.00 (3.26)
0.8	60.00 (3.27)	83.00 (1.91)	63.00 (1.91)
1.6	73.00 (3.42)	90.00 (2.58)	80.00 (3.65)
3.2	88.00 (3.27)	100	98.00 (1.15)
6.4	100	-	100
Untreated control	0.00	0.00	0.00
Karanja oil cake			
0.025	0.00	2.00 (0.29)	0.00
0.05	10.00 (2.00)	19.00 (1.91)	14.00 (1.15)
0.1	28.00 (1.63)	42.00 (2.58)	35.00 (3.00)
0.2	48.00 (1.63)	60.00 (1.63)	52.00 (2.83)
0.4	60.00 (3.27)	79.00 (2.52)	65.00 (3.78)
0.8	79.00 (2.52)	93.00 (2.52)	83.00 (1.91)
1.6	86.00 (2.58)	100	96.00 (1.63)
3.2	100	-	100
Untreated control	0.00	0.00	0.00

* Mean of four replications; Values in parentheses are standard errors

Table - 2: Data on larval mortality of neem + karanja oil cake combination against different mosquito species

Concentrations (%)	Mortality at 24 hr (%)*		
	Culex quinquefasciatus	*Aedes aegypti*	*Anopheles stephensi*
0.005	5.00 (0.48)	9.00 (0.63)	4.00 (0.41)
0.01	18.00 (2.58)	25.00 (1.91)	17.00 (1.91)
0.02	35.00 (3.00)	38.00 (2.58)	34.00 (2.58)
0.04	44.00 (2.83)	50.00 (1.15)	53.00 (1.91)
0.08	52.00 (3.65)	64.00 (1.63)	65.00 (2.52)
0.16	67.00 (1.91)	78.00 (2.00)	76.00 (2.31)
0.32	79.00 (1.91)	90.00 (2.58)	80.00 (1.63)
0.64	95.00 (1.91)	98.00 (1.15)	92.00 (1.63)
1.28	100	100	100
Untreated control	0.00	0.00	0.00

*Mean of four replications; Values in parentheses are standard errors

Table - 3: Data on LC_{50} and LC_{95} of neem and karanja oil cakes against mosquito larvae

Oil cakes	Mosquito species	LC_{50} (%)	LC_{95} (%)	Regression equation
Combination of neem and karanja (50:50)	*Culex quinquefasciatus*	0.06 ± 0.0098	0.93 ± 0.29	Y= 6.6857 + 1.3441 Log X
	Aedes aegypti	0.038 ± 0.0059	0.54 ± 0.18	Y= 7.0182 + 1.4157 Log X
	Anopheles stephensi	0.048 ± 0.0078	0.77 ± 0.25	Y= 6.8020 + 1.3669 Log X
Karanja	*Culex quinquefasciatus*	0.26 ± 0.039	2.33 ± 0.67	Y= 6.0064 + 1.7397 Log X
	Aedes aegypti	0.15 ± 0.02	0.86 ± 0.22	Y= 6.7564 + 2.1058 Log X
	Anopheles stephensi	0.21 ± 0.02	1.57 ± 0.41	Y= 6.2799 + 1.8692 Log X
Neem	*Culex quinquefasciatus*	0.56 ± 0.078	4.97 ± 1.41	Y= 5.4335 + 1.7396 Log X
	Aedes aegypti	0.29 ± 0.039	1.66 ± 0.39	Y= 6.1628 + 2.1822 Log X
	Anopheles stephensi	0.45 ± 0.03	3.15 ± 1.16	Y= 5.6729 + 1.9511 Log X

LC_{50} and LC_{95} = Concentration required to kill 50 and 95% of the test populations respectively; LC_{50} / LC_{95} ± Fiducial limits with 95 % confidence
Y = Probit mortality; X = Concentration

Table - 4: Increase in efficacy of neem and karanja oil cakes combination over individuals against mosquito larvae

Mosquito species	Fold increase of combination			
	Over karanja oil cake		Over neem oil cake	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}
Culex quinquefasciatus	4.33	2.50	9.33	5.34
Aedes aegypti	3.95	1.59	7.63	3.07
Anopheles stephensi	4.38	2.04	9.38	4.09

used individually, recorded an LC_{50} and LC_{95} ranging from 0.15 to 0.56% and 0.86 to 4.97% respectively against all the mosquito larvae. The increase in efficacy of the combination treatment over individuals in all the mosquito larvae tested was found to range from 3.95 to 4.38 fold for karanja oil cake and 7.63 to 9.38 fold for neem oil cake in terms of LC_{50}. Similarly the LC_{95} ranged between 1.59 and 5.34 fold in both the oil cakes for all the mosquito larvae tested (Table 4).

The performance of karanja oil cake was better than neem oil cake which could be revealed by the lower LC_{50} value exhibited by karanja oil cake against all the three mosquito larvae. Among the mosquito species, the larvae of *Ae. aegypti* were found to be more susceptible followed by *An. stephensi* and *Cx. quinquefasciatus* which was uniformly observed in all the treatments.

Though the crude oil fractions of neem and karanja were found to be effective against *Cx. quinquefasciatus* larvae (Shanmugasundaram *et al.*, 2001), the present study has proved that the oil cakes of the above plants are also equally effective in controlling mosquito larvae. Oil cakes are cheap, miscible with water and require no agitation before application as compared to oil fractions. Hence, the oil cake formulations are economically and practically feasible method to control mosquito larvae. Rao (1987) reported that neem cake powder applied @ 25, 50 and 100 g / m^2 was found to be effective against the larvae of *Cx. quinquefasciatus.* Sharma *et al.* (2005), Tonk *et al.,* (2006), studied by efficacy of plant leaves extracts with different extraction procedures against mosquito larvae. The present study revealed that the decanted cold extract of oil cakes is also effective in controlling mosquito larvae under laboratory condition. However, the efficacy of the oil cake extracts (decanted) under field condition is warranted.

From the present investigation, it is obvious that the performance of combined application of neem and karanja oil cake was better against the mosquito larvae than their individuals application. Though the individuals are good against mosquito larvae, the synergistic effect is well exhibited in this experiment. Use of the above products in alternation with the chemical insecticides may delay the development of resistance.

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