

Encapsulation of neem and karanja oil mixture for synergistic as well as larvicidal activity for mosquito control

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This study presents larvicidal effect of neem (*Azadirachta indica*) and karanja (*Pongamia glabra*) oil encapsulated calcium alginate beads (individuals and combination) against mosquito species (*Aedes aegypti*). Studies were conducted by combining these two oils with and without converting into alginate based encapsulation formulation. Combination (30% neem oil and 70% karanja oil) was found to be more effective than any other combination. The combination of neem and karanja oil recorded an LC_{50} of 3.1 g/l against *A. aegypti*. Mortality also increases in terms of concentration of active ingredients (20-77%) after 48 h. FTIR studies indicate that there is no chemical interaction between oils or oil and polymer.

Keywords: Formulations, Karanja oil, LC_{50} , Mosquito larvae, Neem oil, Synergistic

Introduction

Repeated use of synthetic pesticides for insect-pest control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance¹, undesirable effects on non-target organisms and fostered environmental and human health concern². Bio pesticides may be considered as the best alternatives to these synthetic long persistent chemical pesticides for their control. Neem (*Azadirachta indica*) along with karanja (*Pongamia glabra*) is known for insecticidal activity³. Neem oil has been used in controlling plant pests and diseases^{4,5}. Karanja oil is used in pharmacy and in agriculture. Oilcake, when applied to the soil, has pesticidal value, especially against nematodes and also improves soil fertility^{6,7}. Spray-drying is the most commonly used method to encapsulate oil, and the wall materials are usually carbohydrates, proteins and gums^{8,9}. Oil-to-wall weight ratios normally range from 0.1 to 1.0, although 0.2 to 0.5 are more common, and the oil content could reach up to 50% of the total weight of final product^{10,11}. Controlled release formulations are known for increasing the activity of a longer period of time along with minimization of environmental damage of pesticide in the environment^{12,13}.

This study presents synergistic insecticidal effects of the combination of neem and karanja oils to reduce dosage along with application cost, in order to develop effective, safe and economic formulation for controlled release formulations using alginate based biodegradable polymer.

Experimental Section

Neem and karanja seed oils of technical grade were obtained from IIT Delhi. Sodium alginate (SA), calcium chloride (CC) and tween-80 of AR grade were purchased from Merck, New Delhi.

Preparation of Calcium Alginate Beads of Neem and Karanja Oils

Homogenous solution of SA was prepared using distilled water by gentle heating and constant stirring¹⁴, allowed cooling down at room temperature (RT) and then neem and karanja oils were added in different ratio. Resultant solution was then added dropwise into 2% aqueous CC solution using 25 ml syringes with constant stirring. Calcium alginate (CA) beads containing neem and karanja oils were formulated (Table 1) during this process, filtered and washed with distilled water. Sizes of beads were measured using 12 cm vernier callipers¹⁵.

Larvicidal Bioassay

Larvicidal bioassay of formulations with different amount of karanja and neem oil alone and in combination

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Table 1—Reaction parameters in preparation of calcium alginate beads (sodium alginate conc., 4%w/v; calcium chloride conc., 2 %w/v; beads weight, 6.46 g)

Formulation code	Neem oil / karanja oil conc. g	Beads size mm
NK I	0.5 neem oil + 2.5 karanja oil	1.84
NK II	1.0 neem oil + 2.0 karanja oil	1.92
NK III	1.5 neem oil + 1.5 karanja oil	1.86
NK IV	2.0 neem oil + 1.0 karanja oil	1.88
NK V	2.5 neem oil + 0.5 karanja oil	1.90
NK VI	3.0 neem oil	1.91
NK VII	3.0 karanja oil	1.93

NK, Neem and karanja oil

were performed on late 3rd and early 4th instar larvae of urban *Aedes aegypti*, a common vector of dengue haemorrhagic fever and yellow fever. For each experiment, 10 larvae were released in 250 ml glass beakers containing distilled water (100 ml). A mixture of dog biscuit and yeast powder (3:2) was added as a nutrient and supplemented for larvae. All experiments were performed in triplicate. Larval mortality was monitored after 24 h and mortality% was calculated as¹⁶

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Scanning Electron Microscopy (SEM)

SEM photographs were taken with JSM 5600 Scanning Microscope (Japan) to study morphology and surface structure of beads at required magnification at RT. Beads were deposited on brass hold and sputtered with a thin coat of gold under vacuum. Acceleration voltage used was 20 kV with secondary electron image as a detector¹⁷.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were used to detect chemical interaction between neem oil, karanja oil, SA and CC by taking four samples [sample 1, CA beads without active ingredient; sample 2, neem oil loaded with CA beads; sample 3, karanja oil loaded with CA beads; and sample 4, mixture (1:2) of neem and karanja oils loaded with CA beads]. Beads were crushed with KBr and pellets were formed under hydraulic pressure (600 kg/cm²).

Data Analysis

LC₅₀ and LC₉₅ values (conc. that caused 50 and 95% larval mortality, respectively) were determined by



a)



b)



c)

Fig. 1—SEM photographs of calcium alginate (CA) beads encapsulated by: a) neem oil; b) karanja oil; and c) neem + karanja oils

log-probit regression using SPSS 10.0 for Windows/ Microsoft Excel programme.

Results and Discussion

SEM Analysis of Beads

Cracks on surface can clearly be seen on neem oil (Fig. 1a) and karanja (Fig. 1b) oil encapsulated CA beads. External surface of beads containing mixture of neem and karanja oils (Fig. 1c) appeared smoother as compared

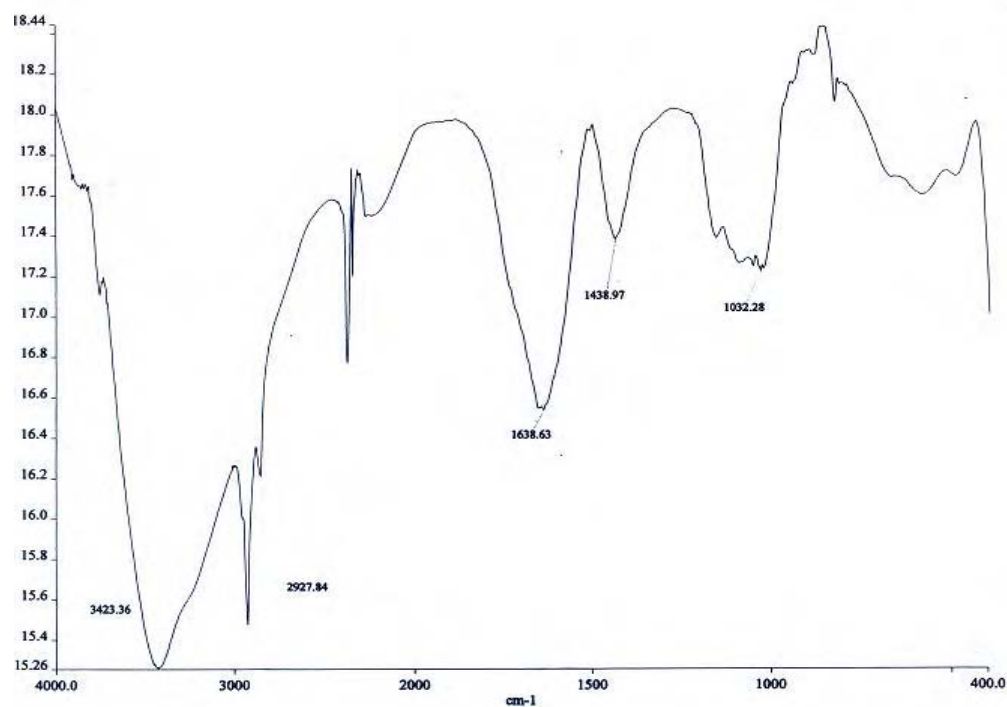


Fig. 2—FTIR spectra of calcium alginate (CA) without active ingredient

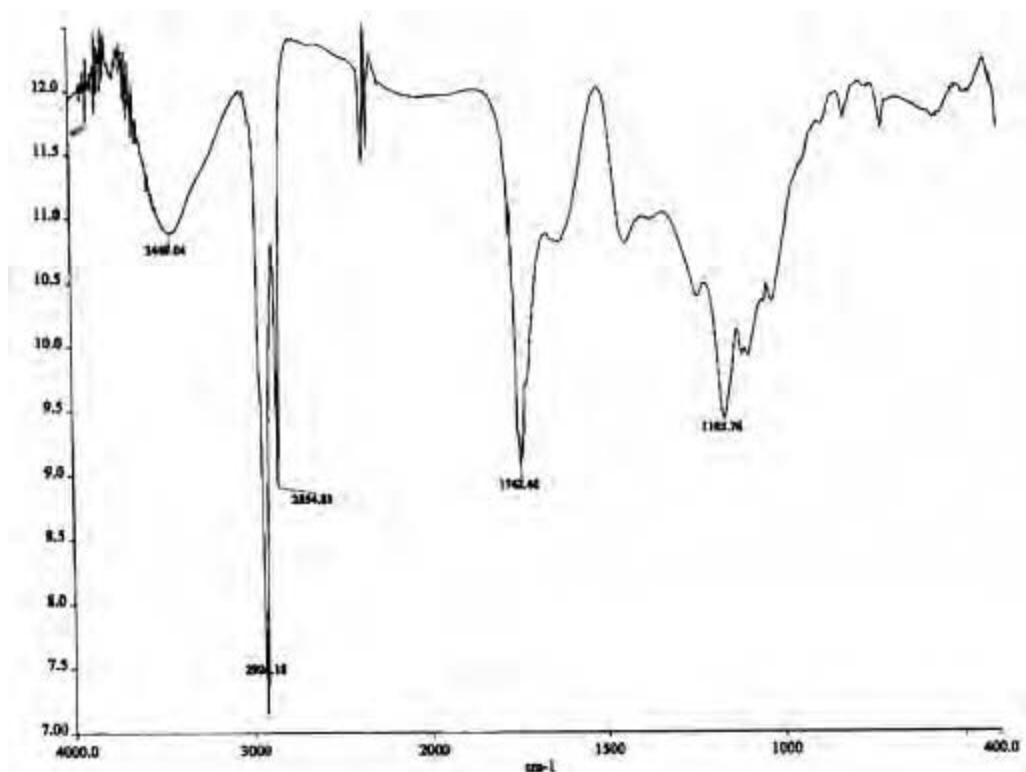


Fig. 3—FTIR spectra of calcium alginate (CA) encapsulated neem and karanja oils

to individual neem oil (Fig. 1a) and karanja oil (Fig. 1b) loaded CA beads. Surfaces of neem oil (Fig. 1a) and karanja oil (Fig. 1b) beads were more oily than beads

containing mixture of neem and karanja oil (Fig. 1c), may be because of oil release from the cracks surface of beads containing individual oil¹⁸.

Table 2—LC₅₀ values of neem and karanja oil (alone & in combination)

S. No	Formulation code	Neem & karanja oil g	Neem & karanja oil %	LC ₅₀ (Neem and karanja oil alone & in combination)
1	NK I	3.0	80% karanja oil + 20% neem oil	3.8
2	NK II	3.0	70% karanja oil + 30% neem oil	3.1
3	NK III	3.0	50% karanja oil + 50% neem oil	4.3
4	NK IV	3.0	80% neem oil + 20% karanja oil	4.9
5	NK V	3.0	70% neem oil + 30% karanja oil	4.7
6	Neem oil	3.0	100% neem oil	5.1
7	Karanja oil	3.0	100% karanja oil	4.0

NK, Neem and karanja oil

Table 3—Mortality (%) of *Aedes aegypti* with encapsulated neem, karanj and neem + karanja oil calcium alginate beads

S. No	Calcium alginate beads g	Replicate I			Replicate II			Replicate III			Replicate (I, II, III: av. value)			Mortality %		
		N	K	N+K	N	K	N+K	N	K	N+K	N	K	N+K	N	K	N+K
1	0.10	0/10	0/10	3/10	1/10	2/10	4/10	2/10	4/10	6/10	1.00	2.00	4.33	10.00	20.00	43.33
2	0.15	1/10	3/10	4/10	1/10	4/10	5/10	2/10	6/10	7/10	1.33	4.33	5.33	13.33	43.33	53.33
3	0.20	1/10	3/10	6/10	2/10	5/10	7/10	2/10	6/10	7/10	1.66	4.67	6.67	16.66	46.67	66.67
4	0.25	1/10	4/10	6/10	2/10	5/10	7/10	3/10	7/10	9/10	2.00	5.33	7.33	20.00	53.33	73.33
5	0.30	0/10	6/10	8/10	2/10	7/10	9/10	3/10	9/10	9/10	2.33	7.33	8.67	23.33	73.33	86.67

N, neem oil; K, karanj oil; N+K, neem + karanj oil

FTIR Analysis of Beads

FTIR spectra of neem oil, karanja oil, alginate polymer, physical mixture of alginate polymer, neem oil and karanja oil containing CC as a cross-linking agent were recorded. Physical mixture was prepared using the same ratio of neem and karanja oil, alginate polymer along with CC used in the preparation of CC used in the preparation of CA beads of formulations prepared. Characteristics sharp peaks of neem oil (1746, 2854, 2924 cm⁻¹) and karanja oil (1705.24, 3070.02, 1452.39 cm⁻¹) did not alter the formation of beads, thereby indicating absence of chemical interaction between oils (neem oil and karanja oil) and SA polymer (Figs 2 & 3).

Effect of Encapsulated Neem and Karanja Oil Formulations Developed on Larvicidal Activity

Different encapsulated formulations of neem and karanja oil mixture and individually were evaluated against fourth instar larvae of *A. aegypti*. In all formulations evaluated, the total concentrations of oil encapsulated were constant (3%). From LC₅₀ of karanja oil (4.0 g/l) and neem oil (5.1 g/l), it is clear that neem oil individually is not much effective against larvae of *A. aegypti*. Keeping all the parameters constant, LC₅₀ of different

combinations of neem and karanja oil were evaluated. It can be seen (Table 2) that LC₅₀ of neem and karanja oil encapsulated formulation is more or less equivalent to LC₅₀ of neem oil except the combination (1:2) of neem and karanja oil, where LC₅₀ decreases to 3.1. The same experiment was repeated using fourth instar larvae of *Anophiles stiphensi* and the same combination at this particular ratio of neem and karanja oil was found to be more effective. In both the cases, larvicidal activity of the combination increases with respect to karanja and neem oil alone. These results indicate synergistic effect of combination of neem and karanja oil as far as larvicidal activities are concerned.

Activity of the combination formulation of neem and karanja oil developed, tested after 6 months, was found to be same as that of freshly prepared formulation. FTIR studies of the same 6 month old formulation also confirmed that there was no chemical interaction and no shifting of band.. Mortality rates (Table 3) of fourth instar larvae of *A. aegypti* at different concentration of active ingredients (neem and karanja oil), comparing with control, depend upon the active ingredients (neem and karanja oil concentration alone and in combination).

Whenever concentration of ingredients increases, mortality% also increases simultaneously. Larvae development was terminated and mortality rate increased with respect to the concentration of karanja oil.

Conclusions

Combination of neem and karanja oil can be used as a more effective larvicidal composition than the individual oils. Encapsulation formulations of the mixture of neem and karanja oil have shelf life more than 6 months and can be exploited for commercial potential. Such practice would reduce chemical burden on environment and also promote sustainable use of locally available bio resource by rural communities. Further studies may be directed towards enhancing efficacy of such oils with the use of potentiating/synergistic agents and their bio-efficacy evaluation in real field conditions.

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