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A research on mosquito repellent and larvicidal activity of NEMCAM (*Azadirachta indica* and *Cinnamomum camphora*)

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Abstract

The present research focuses on development of NEMCAM (NEMCAM=Neem oil, *Azadirachta indica* and Camphor, *Cinnamomum camphora*) formulation and determining its mosquito repellent and larvicidal activity by various methods. Arena ring test was performed for prepared five different test formulations (TF-1 to TF-5) and compared with that of standard (Citronella oil). Distribution of mosquitoes was observed for 2hrs at a time interval of 10 minutes and % mortality rate was calculated. The results were tabulated and ANOVA was applied. NEMCAM Bio-Out was done and effectiveness of mosquito repellent activity was checked and compared with that of All-Out. By performing Ex-cito chamber test, % Mosquito repellency was calculated. Larvicidal activity was determined by observing larvae motility. Based on the results, it was fairly concluded that NEMCAM formulation possess mosquito repellent and larvicidal activity.

Keywords: arena ring test, NEMCAM bio-out test, ex-cito chamber test

Introduction

Mosquitoes transmit serious human diseases, causing millions of deaths every year. Use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. Many plants consist of volatile substances which repel insects^[1]. Repellency of plant essential oils--thyme, catnip, amyris, eucalyptus and cinnamon was tested against *Aedes albopictus*, *Aedes aegypti*. Plants like *Ocimum gratissimum*, *Clausena dentata*, *Eclipta prostrata*, *Tagetes erecta* have been reported to possess strong repellent activity against mosquitoes. Phytochemicals could be valuable weapons in the fight against mosquito-borne diseases^[2].

NEMCAM is a formulation developed using neem oil and camphor. Alone neem oil has mosquito repellent and larvicidal activity. But the potency of neem oil for mosquito repellent activity is increased if mixed with that of camphor, as camphor also possess mosquito repellent activity and due to its volatile nature it carries neem oil into air rapidly. So, synergistic effect is observed when neem oil and camphor is used. Various mosquito transmitted diseases are malaria, dengue, Zika fever, Japanese Encephalitis, Yellow fever etc.,^[3]. Most commercial repellents are prepared by using chemicals like DEET, dimethyl phthalate (DMP) and allethrin, and the most common mosquito repellents available in the market contain DEET. It has been reported that these chemical repellents are not safe for the public use^[4]. There has been much research on natural plant extracts both prior to and after the advent of synthetic repellents. To overcome the problem of development of resistance in insects, attention is being given to natural products because of their biodegradable nature^[5, 6]. A botanical phytochemical, with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, ovicidal, adulticidal and repellent properties^[7].

Materials and Methods**Materials**

Neem oil, camphor, citronella oil, ethanol, distilled water, weighing balance, measuring cylinder, petri plates, whatmann filter papers, beakers, empty All-out refill bottle, Arena jar, Ex-cito chamber. Alive mosquitoes were collected from garden; alive larvae were obtained from CSIR institute, Hyderabad.

Methods

Various methods opted were Arena ring test, NEMCAM Bio-out test, Ex-cito chamber test and Larvicidal activity determination.

a) Arena Ring Test

Different test formulations (TF-1 to TF-5) were prepared by taking different concentrations of neem oil and camphor (table-1). Different quantities of neem oil and camphor were measured and simply mixed with the help of stirrer. (Fig. 1 & 2) Citronella oil was taken as standard for mosquito repellent activity in this test. A Whatman filter paper disc was cut into two equal halves for both test and control. The test discs were treated with these formulations and air dried. The control disc was treated with distilled water and air dried. Both the discs (halves) were joined gently. (Fig. 3) On an average 11 ± 4 mosquitoes were released at the centre of disc and covered with lid of arena jar. The distributions of mosquitoes in the two halves were observed for every 10 minutes for two hours. (Fig. 4) The same above procedure was followed for standard and remaining different test formulations. The number of mosquitoes alive and dead at regular time intervals of 10 minutes for two hours was also checked and the results were tabulated. By applying ANOVA whether there was a significant difference between formulations or not was known. Percent mortality rate was calculated using the formula^[8,9]:

$$\% \text{Mortality Rate} = \frac{\text{No. of mosquitoes dead}}{\text{No. of mosquitoes taken}} \times 100$$

Table 1: Test Formulations

S. No	Test formulations	Neem oil quantity(ml)	Camphor Quantity(g)
1	TF-1	7	0.3
2	TF-2	9	0.4
3	TF-3	11	0.5
4	TF-4	13	0.6
5	TF-5	15	0.7

b) NEMCAM BIO-OUT Test and it's comparison with ALL- OUT

Tests were undertaken to determine the effectiveness of the NEMCAM bio-out, with that of ALL-OUT(standard).All Out empty refill bottle was cleaned with detergent water and wiped properly so that there was no moisture present in it.TF-3 was taken, to this 10 ml of rising solvent(ethanol) was added. The refill bottle was closed. Our NEMCAM Bio-out repellent was compared with all-out in three separate test sessions. During each session, the two repellents were tested. The effectiveness of Bio-out was compared with All out(test site-author room) by plugging both in electrical socket and checking the time taken for the disappearance of mosquitoes. (Fig. 5 & 6) After every session, the repellent was taken out from the electrical socket and time taken for reappearance of mosquitoes was checked. Behavior of the mosquitoes was estimated approximately^[10].

c) Ex-cito chamber test

The ex-cito chamber method is a modified custom method to observe the mosquito behaviour change in the form of moving away from the treated area with NEMCAM Bio-out. The box is made with one front and exit panel occupied with single escape portal. (Fig 7) It is build up with screened inner chamber, and door cover. The NEMCAM Bio-out was plugged into electrical socket inside by supplying electricity socket inside chamber with the help of electric wire. The behaviour of mosquito was observed in term of number of escaped mosquitoes to another space and remaining mosquitoes inside the chamber, filled with treated product. The observation is recorded after 10-30 min exposure. The

test was conducted in daylight and repeated for two times. The percentage of mosquito repellency was calculated using the formula:

$$\% \text{ Mosquito repellency} = \frac{(\text{NES} + \text{NDE})}{(\text{NEX})} \times 100$$

Where

NES = number of mosquitoes escaped,

NDE = number of mosquitoes dead and

NEX = number of mosquitoes exposed.

The results were collected and average was taken^[11].

d) Larvicidal activity determination

In the larvicidal assay, larvae of mosquitoes were exposed to TF-5 (TF-5 was added to the water-100ml) in the beaker. A control was also maintained without adding any TF, thereby just adding larvae to 100 ml of water taken in a beaker. (Fig 8) 10 ± 2 larvae per concentration were used. The larvae were fed with dry yeast powder (25mg/100 ml).The motility of larvae of test and control beaker was observed after 24hrs^[12].

Results and Discussion

a) Arena ring test

For standard (citronella oil) % mosquito repellency was found to be 100 at 100min. For TF-1, TF-2, TF-3, TF-4 and TF-5 % mosquito repellency were found to be 60% at 120 min, 77.7% at 120 min, 90% at 120 min, 100% at 100 min, 100% at 180 min respectively (Table-2). From these observations and Graph-1 it can be concluded that NEMCAM possess mosquito repellent activity.

Table 2: Arena ring test-percent mortality rates at different time intervals of different formulations

S. No	Time(min)	Citronella oil	TF-1	TF-2	TF-3	TF-4	TF-5
1.	10	0	0	0	0	0	0
2.	20	0	0	0	0	28.5	33.3
3.	30	30	0	11.1	30	28.5	50
4.	40	40	20	11.1	30	35.7	50
5.	50	40	20	22.2	30	35.7	58.3
6.	60	50	30	33.3	40	57.1	66.6
7.	70	50	30	33.3	50	57.1	66.6
8.	80	80	30	44.4	50	64.2	100
9.	90	80	40	44.4	50	71.44	-
10.	100	100	40	55.5	80	100	-
11.	110	-	40	66.6	90	-	-
12.	120	-	60	77.7	90	-	-



Fig 1: Neem Oil



Fig 2: Camphor

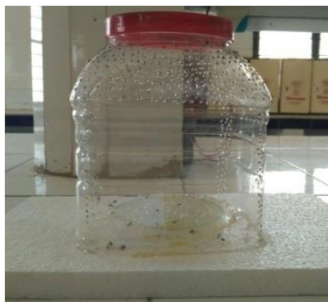


Fig 3: Arena jar

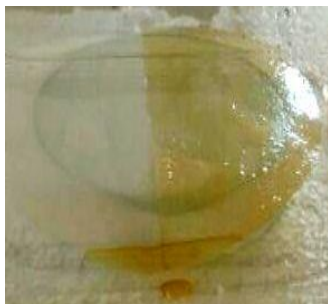


Fig 4: Whatmann filter paper treated with test formulation

Statistics application

One way ANOVA was applied for observations of arena ring test for different TF based on number of mosquitoes dead at different time intervals.

Table 3: ANOVA table for the observations of arena ring test for total number of mosquitoes dead at different time interval

TIME	TF-1	TF-2	TF-3	TF-4	TF-5
10	0	0	0	0	0
20	0	0	0	4	4
30	0	1	3	4	6
40	2	1	3	5	6
50	2	2	4	5	7
60	3	3	5	8	8
70	3	3	5	8	8
80	3	4	5	9	12
90	4	4	8	10	-
100	4	5	8	14	-
110	4	6	9	-	-
120	6	7	9	-	-
SUM	31	36	54	67	51
Average	2.58	3	4.5	5.58	4.25
Standard deviation	1.88	2.29	3.03	4.39	3.37
Y=239					

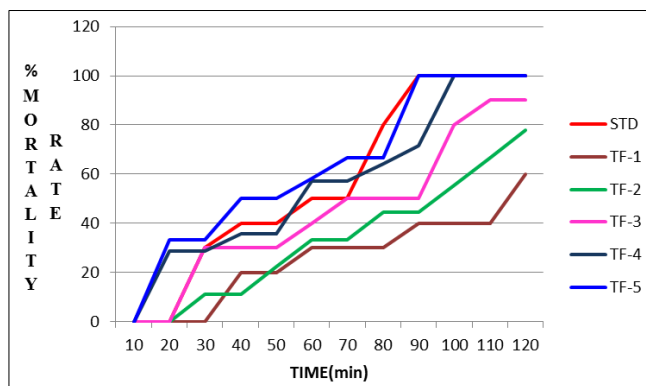
Various parameters calculated

- Correction Factor=952.01
- Total Sum of Squares(TSS)=672.99
- Formulations Sum of Squares/Mean Sum of Squares(FSS)=69.9
- Error Sum of Square(ESS)=TSS-FSS=603.9

Table 4: Overall ANOVA table (Arena ring test)

Source	Degree of Freedom	Sum of Squares	Mean Sum of squares	F-calculated value	P-value at 1%
Formulation	5-1=4	69.9	17.4	17.4/10.9=1.59	0.188
Error	60-5=55	603.09	10.9		
Total	60-1=59				

At 1% level of significance as P-value=0.188 < 1, null hypothesis is rejected. Therefore significant difference was found between different TF. (i.e. with increase in concentration there was increase in mosquito repellent activity).



Graph 1: Arena ring test-time vs % mortality rate

b) Nemcam Bio-Out test and it's comparison with all out

From observations in table-5&6, it can be concluded that NEMCAM was effective towards mosquitoes.

Table 5: NEMCAM Bio-Out Observations (Disappearance of mosquitoes)

S. No.	Time taken for disappearance of mosquitoes (minutes)			
	Trial-1	Trial-2	Trial-3	Average Time
All Out	30	25	30	28.3
NEMCAM	32	33	31	32

Table 6: NEMCAM Bio-out Observations (Reappearance of mosquitoes)

S. No.	Time taken for reappearance of mosquitoes(minutes)			
	Trial-1	Trial-2	Trial-3	Average Time
All Out	35	40	50	41.6
NEMCAM	38	38	36	37.3

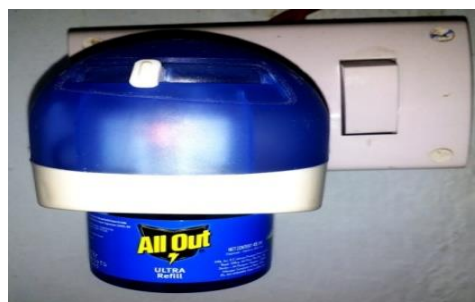
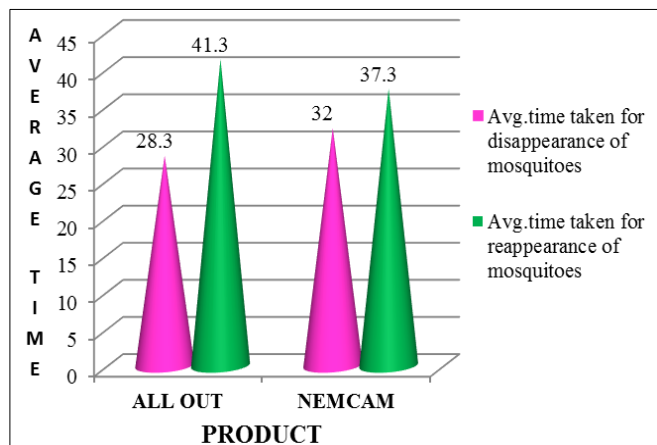


Fig 5: All Out



Fig 6: NEMCAM BIO-Out



Graph 2: Bio out test-time vs product

c) Ex-cito chamber test

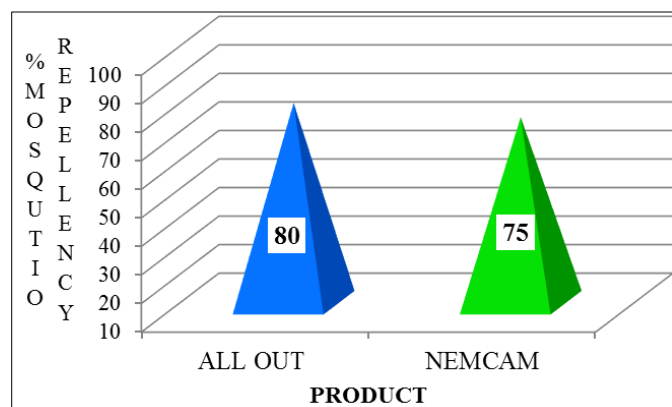
- % Mosquito repellency for All-out and NEMCAM was found to be 80% and 75% respectively.

Table 7: Ex-cito chamber test-observations

Product	NEX	NES	NDE	% Mosquito repellency
All-Out	15	8	4	80
NEMCAM	12	6	3	75



Fig 7: Ex-cito chamber



Graph 3: Ex-cito chamber test -Product Vs %Mosquito repellency

d) Larvicidal Activity

After 24 hrs it was observed that there was motility in control beaker and there was no motility of mosquito larvae in beaker treated with test formulation.



Fig 8: Control beaker (water) with larvae



Fig 9: Treated Solvent beaker with larvae

Conclusion

After performing various evaluation tests for mosquito repellent and larvicidal activity, it was found that NEMCAM formulation possess mosquito repellent and larvicidal activity. By performing arena ring test it was found that with increase in the concentrations of NEMCAM mosquito repellent activity was increased and 100% mortality rate was obtained with TF-4 and TF-5. By performing NEMCAM Bio-Out test, it was found that the effectiveness of NEMCAM Bio-Out was closer to that of All Out. By Ex-cito chamber test, it was found that % mosquito repellency of All Out was closer to that of NEMCAM. By observing motility of larvae, before and after treatment with NEMCAM formulation and comparing with that of control, it can be concluded that NEMCAM possess larvicidal activity. Hence we recommend that NEMCAM is a safe and effective mosquito repellent possessing larvicidal activity. Efforts can be taken further to popularize this natural remedy as a mosquito repellent, as it is an economical, easily available, side effect free, effective formulation.

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