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RESEARCH ARTICLE

NANOEMULSION FORMULATION USE FUL AS A NEW TOOL FOR MOSQUITO CONTROL

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ABSTRACT

Mosquitoes play a vital role in the spread of vector borne diseases and their management has gained great importance. Plant extracts have been studied for their mosquitocidal activity against various vectors. The present study was found on the larvicidal and pupicidal activity of *Lantana camara (L)* essential oil and *azadiractin* loaded novel nanoemulsion formulation for the control of vector mosquitoes. Nanoemulsion formulations of double emulsion type- W/O/W. *Lantana camara (L)* essential oil and *azadiractin* loaded nanoemulsion formulation. Five different concentrations (5, 10, 15, 20,25ppm) were prepared and the insects (*Culex quinquefaciatus* and *Aedes aegypti)* were treated with it. The mortality percentage and lethal dose (LC50) were calculated. The study clearly reduced the repellent activity of the oil emulsion *(Lantana camara (L))* and *azadiractin* loaded nanoemulsion formulation had potent biocidal activity on vector mosquitoes.

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INTRODUCTION

Insect vectors, especially mosquitoes are responsible for spreading serious human diseases like malaria, Japanese encephalitis, yellow fever, dengue Zika and filariasis. Most of the mosquito control programmes target the larval stage in their breeding sites with larvicides, whereas adulticides may only reduce the adult population temporarily (Elhag *et al.,* 1999, 2001). Plant essential oils have been suggested as an alternative source for insect control, because due to its less side effects and easy biodegradable nature in the environment. Nanoemulsions are emulsions with droplet size on the order of 100 nm. A typical nanoemulsion contains oil, water and an emulsifier. The addition of an emulsifier is critical for the creation of small sized droplets as it decreases the interfacial tension i.e., the surface energy per unit area, between the oil and water phases of the emulsion. The emulsifier also plays an important role in stabilizing nanoemulsion through repulsive electrostatic interactions and steric hindrance. (Mason *et al* 2006). Nanomaterials are being used for a wide variety of applications due to its varying properties on scaling down from bulk size to nanometer size (10-9m).

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Nanotechnology and nanoparticle based products are increasing nowadays in various fields of science, engineering, and medicine (Tomaszewska-Grzedaa *et al.,* 2005). Zinc nanoparticle is currently used under use in a variety of fields due to its uniqueness and attractiveness in their properties (Kamaldeep *et al.,* 2012) which makes them to be a promising element in various fields such as automobiles, optical electronics, textiles, medicine, cosmetics drug delivery and cosmetics (Becheri *et al.,* 2007). This study was aimed to assess the larvicidal and pupicidal effect of nanoemulsion coated plant oil. The toxicity of the oils was also tested against larvae and pupae of *Culex quinquefasciatus* and *Aedes aegypti.*

MATERIALS AND METHODS

Collection of Plant materials and preparation of extracts

The Leaves of the *Lantana camara* were collected from in and around sattaparai hill station palani taluk, in Dindigul District, Tamil Nadu India. From the leaf, 1 kg powdered was macerated with 3.0 L of ethanol sequentially for a period of 72 h and filtered. The yield of the *Lantana camara* crude extract was produced by ethanol (21.5 g). The extracts were concentrated at reduced temperature on a rotary vacuum evaporator and stored at a temperature of 4 °C. One gram of the plant residue was dissolved in 100 ml of acetone (stock solution) considered as 1 % stock solution.

From this stock solution, concentrations were prepared ranging from (100-500 ppm), respectively.

Collection of mosquitoes

The mosquitoes of *Culex quinquefasciatus* and *Aedes aegypti* were collected from National Institute for Communicable Disease (NICD), Mettupalayam, Coimbatore, Tamil Nadu, and India. The mosquitoes were collected without exposure to any insecticide in and around Coimbatore district, India at different breeding habitats with the help of 'O' type brush. These mosquitoes were brought to the laboratory and were transferred to 18 x 13 x 4 cm size enamel trays containing 500 ml of water and kept for larval hatching.

Preparation of Nanoemulsion

Based on the screening of different formulation of nanoemulsion preparation and the most potent nanoemulsion obtained (Ostertag et al., 2012) using 75% (w/w) of water, 15% (w/w) of essential oil and 10% (w/w) of Tween20. The nanoemulsion was prepared with composition of essential oil and Tween20 were stirred at 1000 rpm using magnetic stirrer for 15 min. Followed by the stirring drop by drop water was added at a flow rate of 5 ml/min. This mixture was stirred at 1200 rpm for 30 min. The final product of nanoemulsion was stored under room temperature.

Fig.1. Photograph view of O/W emulsions of essential oil and *Azadirachtin* nanoemulsion preparation

Table 1. Two type of composition of nano-emulsion formulation preparation

S.No	Different Composition and their Ingredients	
	Compositions 1	Compositions 2
1	Distilled water	Distilled water
2	*Lantana* oil	*Lantana* oil
3	Emulsifier	Emulsifier
4	-	*Azadirachtin*

Table 2. The different percentages of water phase nano-emulsion formulations with different ingredients.

S.No	Name of the Ingredients	% Compositions of NEF W/W (gm)*	
		F1	F2
1	Distilled water	55	55
2	*Lantana* oil	25	20
3	Emulsifier	20	20
4	*Azadirachtin*	-	05
6	Total	100	100

*NEF indicates nanoemulsion formulations of (O/W) and W/O) in water phase system.

Maintenance of larvae

The mosquito larval culture was maintained in our laboratory at 27±2°C, 75-85% RH, under 14L: 10 D photoperiod cycles. The mosquito larvae were fed with dog biscuits and yeast at 3:1 ratio. The feeding was continued till the larvae were transformed into pupae.

Maintenance of pupae and adult

The pupae were collected from the culture trays and were transferred to plastic (12 cm X 12 cm) containing 500 ml of water. The plastic jars were kept in 90 X 90 X 90 cm sized mosquito containers (12 cage for adult emergence. The freshly emerged adults were maintained 27±2°C, 75-85% RH, less than 14L: 10D photoperiod cycles.

Larval/ Pupal toxicity test (Abbott's, 1925)

Laboratory colonies of mosquito larvae/pupae of *Culex quinquefaciatus*, and *Aedes aegypti* were used for the larvicidal/pupicidal activity of the NE. Twenty-five numbers of I to IV instar larvae and pupae were introduced into 500 ml glass beaker containing 249 ml of de-chlorinated water and 1 ml of desired concentrations of biopesticide. Larval food was given for the test larvae. At each tested concentration 2 to 5 trials were made and each trial consisted of three replicates. The larvae/pupae exposed to de-chlorinated water without biopesticide served as control. The control mortalities were corrected by using Abbott's formula.

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae /pupae}}{\text{Number of larvae /pupae introduced}} \times 100$$

Statistical analysis

All data were subjected to Analysis of Variance (ANOVA). LC_{50} and LC_{90} values and their 95% confidence limits were estimated by getting a probity regression model to the observed relationship between percentage mortality of larvae and logarithmic concentration of the substance.

The goodness of fitness of the model was tested using Chi-Square test AP value of less than 0.05 was considered as a significant departure of the model from the observations. In case of significant departure a heterogeneity factor was used to calculate the 90% confidence limit for LC_{50} and LC_{90}. All analysis was carried out using SPSS Software version 16.0

RESULT AND DISCUSSION

Mortality rate of C*ulex quinquefaciatus* and *Aedes aegypti* - I to IV instar larvae and pupae

A preliminary bioassay was conducted to test the efficacy of *Lantana camara* oil NE on larval insects and in the pupae of *Culex quinquefaciatus and Aedes aegypti*. The results presented in Table 3 and Table 4 shows that *Lantana camara* oil, were effective against the immature stages, of *A. agepyti* (dengue vector) and *C.quinquefaciatus (*Wucheria vector) respectively. The percentage of mortality was calculated for the immature stages of *A.aegypti*.

Table 3. Mortality analysis of Lantana camara oil NE on A.aegypti

Instars	Larval and Pupal mortality (%) (Mean ±S.D)					LC_{50} (LC_{90})	95% Confidence Limit		Regression equation	Chi square value (χ^2)
	Concentration (ppm)									
	10	20	30	40	50		LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)		
I	38.3±1.98	52.4±1.85	67.2± 1.72	81.8±2.03	94.6±3.13	18.183 (46.959)	14.406-21.169	42.633 53.154	X= 0.045 Y= -0.810	1.705*
II	31.2±1.16	44.5±1.73	59.1±3.13	73.6±2.05	85.7±2.07	23.337 (56.482)	19.718 26.402	50.689 65.209	X= 0.039 Y=-0.902	0.157*
III	26.8±1.16	39.89±2.25	47.95±1.48	64.48±1.00	72.4±2.15	29.731 (71.676)	25.851 33.573	62.227 87.648	X= 0.031 Y=-0.908	0.579
IV	19.5±2.52	28.3 ±2.71	38.8±0.74	53.6±1.62	61.6±3.77	38.943 (82.017)	34.943 44.267	70.387 102.269	X= 0.030 Y=-1.159	0.319*
Pupa	12.2±1.72	21.7 ±1.98	32.2±1.60	45.8±2.13	53.6±1.85	45.295 (85.991)	40.851 51.886	73.915 106.877	X= 0.031 Y=-1.426	0.592*

Mortality rates are means ±SD of five replicates. No mortality was observed in the control. Within each column means followed by the same letter(s) are not significantly different (P<0.05). LC50 lethal concentration that kills 50% of the exposed organisms, LC90 lethal concentration that kills 90% of the exposed organisms, LCL- lower confidence limit, UCL- upper confidence limit, χ2- Chi- square test, NS- not significant.

Table 4. Mortality analysis of Lantana camara oil NE on C.quinquefaciatus

Instars	Larval and Pupal mortality (%) (Mean ±S.D)					LC_{50} (LC_{90})	95% Confidence Limit		Regression equation	Chi square value (χ^2)
	Concentration (ppm)									
	5	10	15	20	25		LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)		
I	49.4±1.85	65.8±1.72	79.2±2.56	92.4±2.15	100±0	5.913 (18.009)	3.778-7.485	16.380-20.250	X= 0.106 Y=-0.627	3.934*
II	42.5±1.73	58.8±1.46	68.0±1.41	82.8±1.72	93.2±2.13	7.723 (23.880)	5.292-9.507	21.457-27.499	X= 0.079 Y=-0.613	1.281*
III	37.2±2.13	49± 1.41	59.8±2.13	74± 1.41	81.8±1.72	10.330 (30.901)	7.766-12.264	27.021-37.367	X= 0.062 Y=-0.644	0.224*
IV	28.6±2.41	38.4 ±1.49	51.2±2.13	65±2.28	74.8±1.16	14.297 (34.629)	12.373 16.132	30.225 41.943	X= 0.063 Y=-0.901	0.116*
Pupa	21.7±2.27	32.2 ±2.13	43.4±1.85	57.8±1.72	67.8±2.56	17.388 (37.692)	15.577-19.504	32.766-45.939	X= 0.063 Y=-1.097	0.101*

Mortality rates are means ±SD of five replicates. No mortality was observed in the control. Within each column means followed by the same letter(s) are not significantly different (P<0.05). LC50 lethal concentration that kills 50% of the exposed organisms, LC90 lethal concentration that kills 90% of the exposed organisms, LCL- lower confidence limit, UCL- upper confidence limit, χ2- Chi- square test, NS- not significant.

Mortality rate was found to be increased as the concentration increased; for example, in the first instar stage at 10 ppm concentration, the larval mortality was 38.3%, whereas at 50 ppm concentration, it was increased to 94.6%. The pupal mortality was found to be increased to 53.6% at 50 ppm when compared to 10 ppm(12.2%). The median anti-larval potency (LC_{50}) of the *Lantana camara* oil NE on *A.aegypti* were found to be 18.18, 23.3, 29.7, 38.9, and 45.2 ppm for I, II, III, IV instar larvae and pupae, respectively. The anti-larval potency (LC_{50}) of the *Lantana camara* oil NE on *A.aegypti*. Were found to be 1.7, 0.15, 0.5, 0.3 and 0.5 for I, II, III, IV instar larvae and pupae, respectively (Dua *et al, 2010).*The results indicate that there is no much difference between the expected and the observed mortality.

The percentage mortality were also calculated for the immature stages of *C.quinquefaciatus* and treated with various concentrations (5-25 ppm) of *Lantana camara* oil NE Mortality and were also found to be increased as the concentration increased, In the first instar stage at 5 ppm, the larval mortality was 49.4%, whereas at 25 ppm, it was increased to 100%. The pupal mortality was increased to 67.8% at 25 ppm. The anti-larval potency (LC_{50}) of the *Lantana camara* oil NE on *C.quinquefaciatus* are found to be 5.913 ppm, 7.723 ppm, 10.330 ppm, 14.297 ppm, and 17.388 ppm for I, II, III, IV instar larvae and pupae, respectively. The median anti-larval potency (LC_{50}) of the *Lantana camara* oil NE on *C.quinquefaciatus* were found to be 3.934, 1.281, 0.224, 0.116 and 0.101 for I, II, III, IV instar larvae and pupae, respectively.

The obtained chi-square values states that there is no much difference between the expected and the observed mortality.

Conclusion

Nowadays, mosquitoes play a major role in transmitting serious dreadful disease. The essential oil obtained from the *Lantana camara* nanoemulsion oil showed effective larvicidal and pupicidal activity against important vectors such as dengue, yellow fever, and filariasis. Hence to conclude *Lantana camara* nanoemulsion oil can be effectively used to control the vectors *(Culex quinquefasciatus* and *Aedes aegypti)* and thereby prevent the spread of vector borne epidemic disease. In future this nanoemulsion oil can be utilized for the development of plant based pesticides.

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