Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(4): 1769-1774

Received: 19-05-2017
Accepted: 20-06-2017

M Ramar
Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar, University, Coimbatore, Tamil Nadu, India

P Manonmani
Department of Biotechnology, PRIST University, Vallam, Thanjavur

P Arumugam
Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar, University, Coimbatore, Tamil Nadu, India

S Kamala Kannan
Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar, University, Coimbatore, Tamil Nadu, India

R Raskin Erusan
Department of Genetics, Dr.A.L.M. Post Graduate Institute of Basic Medical, Sciences, University of Madras, Chennai, Tamil Nadu, India

N Baskaran
Department of Chemistry and Bioscience, Srinivasa Ramanujan Centre (SRC), SASTRA University, Kumbakonam, Tamil Nadu, India

K Murugan
Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar, University, Coimbatore, Tamil Nadu, India

Correspondence
M Ramar
Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar, University, Coimbatore, Tamil Nadu, India

Nano-insecticidal formulations from essential oil (*Ocimum sanctum*) and fabricated in filter paper on adult of *Aedes aegypti* and *Culex quinquefasciatus*

M Ramar, P Manonmani, P Arumugam, S Kamala Kannan, R Raskin Erusan, N Baskaran and K Murugan

Abstract
The present study was contacted to prepared novel nanoemulsion formulations (NEFs) from the essential oil (*Ocimum sanctum*) as a nano-insecticidal formulation with different ratios of emulsifier, Stabilizer and water. The activities of knock down and adulticidal effect of the formulated nanoemulsion formulations was evaluated against *Aedes aegypti* and *Culex quinquefasciatus.* The oil in water (o/w) emulsions was prepared using with oil, emulsifier, Stabilizer and water. The larger droplet size decreased into a smaller size with increase in the concentration of emulsifier. The viscosity and thickness of the nanoemulsion decreased with increasing of concentration of emulsifier. Knock down (%) and KD_{50} and KD_{90} of the nanoemulsions against *Ae. aegypti* and *Cx. quinquefasciatus* more effect was recorded for F2 36.4±1.73, 48.2±2.64, 67.5±3.23, 85.4±1.45 and 98.2±3.43; and 42.3±2.73, 59.3±1.65, 78.4±0.14, 92.6±3.85 and 100±0.00 respectively. Also, KD_{50} and KD_{90} values were noticed for F2 7.01 and 29.94; 4.05 and 20.88, respectively. The adult mortality was recorded on *A. aegypti* and *C. quinquefaciatus* in F2 17.5±0.75, 22.5±1.25, 33.7±0.87, 52.5±1.50 and 71.2±1.25; 23.7±1.125, 30.2±1.25, 43.7±0.37, 61.2±2.185 and 82.5±1.75, respectively. The LD_{50} and LD_{90} values were observed in F2 28.60 and 69.82; 20.09 and 57.13, respectively. According to Knock down (1 h) and mortality (24 h) level, this nanoemulsion was showed potential insecticidal agent against *Aedes aegypti* and *C. quinquefaciatus* adult. The present study permitted the development of low-cost eco-friendly green Insecticidal nanoformulations with potential adulticidal activity, using a nano-technology based approach.

Keywords: *Ocimum sanctum*, essential oil, nanoemulsion, formulations, Knock down, adulticidal, *Aedes aegypti*, *C. quinquefaciatus*

1. Introduction

The research work of nano-emulsion has emerged as a potential alternative tool for both intravenous and dermal application. The nanoemulsions are oil-in-water dispersions having in different variety of droplet size [1]. The nanoemulsions are stable for a long time [2]. Furthermore, the stability depending on the individual system or mixture of ingredients, low viscosity and droplet size make them better looking system for many industrial applications, such as drug delivery systems in the pharmaceutical industrial. Also, it can also be used in cosmetics and in pesticide delivery systems [3]. The nanoemulsions are stable systems, and their stability depends upon the method of preparation [4]. The well-known approach is high emulsification, which engages high stirring, high-pressure homogenizers. The low energy emulsification method has been developed by taking of phase behaviour and properties to promote the formation of small droplets with constant vigorous stirring [5].

Mosquitoes are the terrorize to public health because of they are transmit the deadly disease like malaria, lymphatic filariasis, dengue, chikungunya, yellow fever, and Japanese encephalitis [6&7].The various methods have been developed in globally level to reduce the occurrence of mosquito vector issues. The regularly used synthetic pesticide applications are highly efficient against the target species. This repeated application causes various threats through the persistence and accumulation of non-biodegradable toxic components in the ecosystem [8-9].

These low-energy techniques include self-emulsification, phase transition, and phase inversion temperature [10]. In this background, nanoparticles are usually produced in a "bottom-up" process. The mechanism of particle stabilization of emulsions and foams is commonly referred to as Pickering stabilization, in which particles of suitable wettability are effectively irreversibly adsorbed at the interface. Oil-in-water (O/W) or water-in-oil (W/O) emulsions can thus be produced depending on whether the particles are predominantly hydrophilic or hydrophobic [11&12].

The emulsions and foams can be stabilized not only by surfactants, but also by solid particles in the formulations. The subject of particle-stabilized dispersed systems has been provided [13], and particularly the stabilization of food foams by particles and the stabilization of both emulsions and foams by element in foods have been reviewed [11&12]. The type of food emulsions are homogenized and reconstituted milks (O/W) and (W/O) of margarines and fatty spreads [13], while surface-active molecules are also present. The emulsion based formulations containing tea tree, geranium, marjoram, rosewood; the mechanism of action of essential oils against microorganisms involves the interaction of phenolic compounds with the proteins in the cytoplasmatic membrane. The emulsion based formulation of water dispersible nano permethrin was prepared for larvicidal study. Nano permethrin was prepared using solvent evaporation of oil in water emulsion, which was obtained by mixing of two types of phases (organic and aqueous). The larvicidal studies were carried out against *Culex quinquefasciatus* [14].

The essential oils have a complex mixture of non-volatile and volatile compounds produced by aromatic plants as the secondary metabolites [15]. For example, in most oil in water emulsions proteins usually act as both emulsifiers and stabilizers, whereas the use of surfactants usually facilitates the formation, stabilization and controlled fat destabilization of food emulsions. The food industry is one of major industry that usage heavily on the use of emulsions and emulsifiers. The variety of products such as soft drinks, milk, cream, salad dressings, mayonnaise, soups, sauces, dips, butter and its examples of emulsions [16]. The common emulsifiers used in the food industry are amphiphilic proteins, polysaccharides, phospholipids and small molecule surfactants [17]. The nanoemulsions are frequently transparent or slightly dirty. This property is beneficial, making nanoemulsions for transparent products, such as clear beverages, sauces, soups, and syrups.

The nanoemulsion droplets mean that they have much better physical stability against gravitational separation, flocculation, and coalescence than conventional emulsions [18, 19 & 20]. The aqueous of *Pongamia glabra* and *Jatropha curcas* cakes after extracting oil to enhance the activity of *Eucalyptus globulus* as a pesticide by making a nanoemulsion for the control of *Tribolium castaneum*. The essential oils are encapsulated in suitable emulsion delivery systems. It can be incorporated into aqueous based foods and other products by mixing. Based on their droplet size, emulsions are divided into conventional nanoemulsions [18 & 20]. In the present study the use of nanoemulsions as potential use of nano-green insecticidal with suitable ingredients is enhance the promising activity. Hence, the present research work was to differentiate the nanoemulsions with fabricated essential oil in distribution for comparison. The two phases were used O/W and W/O emulsions for enhancing the bio-efficacy in emulsions.

2. Materials and methods

2.1. Extraction of essential oil

The fresh plant material (*Ocimum sanctum*) were collected in and around Coimbatore and washed with distilled water and dried under shade for 24 hours and extracted in steam distillation. The distillation period was about 8 hours in a Clevenger apparatus and the separated oil from the aqueous solution. The collected essential oil was transferred into a dark glass bottle and kept at a temperature of 4 °C until further used [21].

2.2. Test insects

The *Aedes aegypti* and *Culex quinquefasciatus* were cultured in our laboratory conditions (26±2 °C, 70–85 % R.H, 14:10 (L:D) photoperiod) and placed in 22×10×8 cm plastic containers with 1000 mL tap water. Larvae were reared in the plastic containers and larvae fed with dog biscuits and yeast at 3:1 ratio (w/w).The containers were kept closed with muslin cloth to prevent emerged adults mosquito outside. Larvae and pupae for experiments were collected daily from culture containers and separated to glass beakers for further assays [22].

2.3. Preparation of Nanoemulsion formulations

The nanoemulsion formulations (NEF) were prepared (Table1) with different ingredients (w/w) of *Ocimum sanctum* oil. The nanoemulsion formulations were prepared with slight modification as described [23]. The oil was dissolved in emulsifier at different proposition of phase systems (o/w or w/o). For the preparation of nanoemulsion, oil was dispersed in the aqueous phase under stirring technique for obtain emulsion. After the stirring of homogenization the produced oil in water or water in oil nanoemulsion was collected (Fig 1). All the nanoemulsion formulations samples were kept to room temperature.

2.4. Knock down and adulticidal bioassay

Ae. Aegypti and *Cx. quinquefasciatus* mosquitoes were selected for the testing of knock down and adulticidal activities. The Knock down and adulticidal bioassay was performed by WHO method [23]. Five different concentrations of the NEF of *O. sanctum* were applied on Whitman no. 1 filter papers (size 12 x15 cm2) as described earlier [24]. Control papers were treated without NEF under. The knock down and adulticidal activity of the NEF was evaluated at five concentrations (3.125 to 50 mg/cm2). 25 female mosquitoes (2-5 days old) were transferred into a plastic holding tube. Number of mosquitoes knocked down in the exposure tube was recorded at 3 min interval period till the last mosquito was knocked down. At the end of exposure period, mosquitoes were transferred to holding tube and kept for 24 h. Knock down (KD_{50} and KD_{90}) and lethal dose (LD_{50} and LD_{90}) values were determined using Probit analysis19. Mortality of mosquitoes was determined at the end of 24 h recovery period. Per cent mortality was corrected by using of Abbott's formula [25]. This present study was done from July to October, 2015 in our laboratory at department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu.

$$\text{Percent Adult mortality} = \frac{\text{Number of female adult mosquitoes died (at particular time \&dose)}}{\text{Number of female adult mosquitoes released}} \times 100$$

2.5. Statistical analysis

Knock down and adulticidal activities datas were analysed using one way ANOVA. The percentage mosquito Knock down and mortality data were subjected to Probit analysis. Knock down (KD_{50} and KD_{90}) and lethal dose (LD_{50} and LD_{90}) were calculated method [26]. A probability level of $P<0.05$ was used for the significance of differences between values.

3. Results

3.1. Knock down (%) and (KD_{50} and KD_{90}) activity

The knocks down activity were studied at 1 hr exposure using different concentrations with nanoemulsion formulations (NEF). Three different nanoemulsion formulations in five doses (3.125-50 mg/cm^2). The Tables 1 & 2 showed percentage of knock down and KD_{50} and KD_{90} (Knock Down) of *A. aegypti* and *C. quinquefaciatus* results for F1 36.4±1.73, 48.2±2.64, 67.5±3.23, 85.4±1.45 and 98.2±3.43 and 42.3±2.73, 59.3±1.65, 78.4±0.14, 92.6±3.85 and 100±0.00 for F2 24.3±0.38, 36.4±1.25, 45.6±1.71, 69.3±0.71 and 74.3±0.82 and 22.1±0.23, 41.3±1.35, 56.4±2.45, 71.1±0.81 and 82.7±0.72; and for F3, 16.2±0.21, 27.1±0.3, 34.5±2.43, 47.4±0.58 and 64.3±0.31 and 18.1±0.31, 33.3±0.56, 42.2±3.73, 52.4±0.53 and 68.1±0.52 and respectively. The KD_{50} and KD_{90} (Knock Down) values were 19.00 and 65.71; 14.66 and 54.39 for F1; 7.01 and 29.94; 4.05 and 20.88 for F2 and for F3, 32.29 and 82.81; 27.01 and 78.98 respectively. Among the three formulations, the F2 were more effective than the other two formulations on. The F1 and F2 nanoemulsion formulations showed considerable knock down activity on adult mosquitoes of *A. aegypti* and *C. quinquefaciatus*.

3.2. Adulticidal (LD_{50} and LD_{90}) activity

The adulticidal activity was recorded after 24 hr in different concentrations with impregnated papers with NEF. The results showed adult mortality (%) and lethal doses (LD_{50} and LD_{90}) (Tables 1&2) on *A. aegypti* and *C. quinquefaciatus* for F1 8.74±0.87, 13.7±1.35, 27.5±0.37, 38.7±0.37 and 58.7±1.75; 12.5±1.50, 22.2±0.37, 31.3±0.75, 43.7±1.25 and 65.0±0.51; for F2 17.5±0.75, 22.5±1.25,33.7±0.87, 52.5±1.50 and 71.2±1.25; 23.7±1.125, 30.2±1.25, 43.7±0.37, 61.2±2.185 and 82.5±1.75 and for F3, 2.5±0.21, 7.5±0.75, 14.7±0.35, 23.7±0.33 and 33.7±0.81; 8.75±1.25, 13.7±1.62, 21.2±0.87, 32.5±0.86 and 48.7±2.37 respectively. The LD_{50} and LD_{90} values were 39.36 and 81.14; 33.99 and 78.46.39 for F1; 28.60 and 69.82; 20.09 and 57.13 for F2 and for F3, 63.02 and 115.73; 48.36 and 99.04, respectively. In the adulticidal activity F2 formualtion were more effective on *C. quinquefaciatus* than the *A. aegypti*. The F1 and F2 formulations showed moderate activity on adult mosquitoes of *A. aegypti* and *C. quinquefaciatus*. The chi-square values were not significant at $P<0.05$ level.

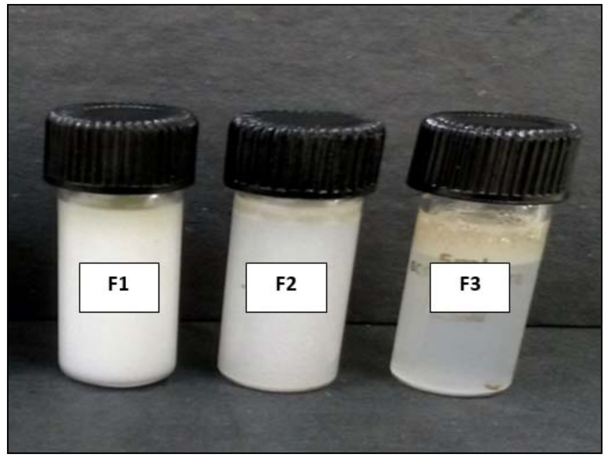

Fig 1: Nanoemulsion formulations after preparation. Emulsions: F1 (O/W); F2 (O/W) and F3 (W/O).

Table 1: The different percentages of water phase nano-emulsion formulations with different ingredients.

S. No	Type of Ingredients	% Compositions of NEF (w/w)*		
		F1	F2	F3
1	Distilled water	55	50	45
2	*Ocimum sanctum*	30	25	20
3	Emulsifier	10	15	20
4	Stabilizer	05	10	15
5	Total	100	100	100

*NEF indicates nanoemultion formulations.

Table 2: The knock down (%) and knock down (KD_{50}, KD_{90}) of *Aedes aegypti* with NEF formulations*.

NEF	% Knock Down (Mean ± SD) (mg/cm^2)					$KD_{50}KD_{90}$ and 95% Fiducial limits (Upper and Lower)		Regression equation	x^2
	3.125	6.25	12.5	25	50	(LFL UFL)	(LFL UFL)		
F1	36.4±1.73	48.2±2.64	67.5±3.23	85.4±1.45	98.2±3.43	7.01 (26.010-9..356)	29.94 (26.010-35.809)	$y = 0.559 + 0.002x$	3.59 *n.s.*
F2	24.3±0.38	36.4±1.25	45.6±1,35	69.3±0.71	74.3±0.82	19.00 (-6.28-47.11)	65.71 (41.59-342.71)	$y = 0.274 + 0.003x$	12.6 *n.s.*
F3	16.2±0.21	27.1±.0.3	34.5±2.43	47.4±0.58	64.3±0.31	32.29 (27.353-39.107)	82.81 (68.98-106.327)	$y = 0.253 + 0.003x$	4.72 *n.s.*

*NEF- Nanoemultion formulations.
Values are mean ± SD (No. of replicates = 5); Control-No Knock down
Mosquitoes were exposed for 1 h and mortality was recorded after 24 h period.
KD_{50}= Concentrations required to knock down 50% of the population exposed.
KD_{90}= Concentrations required to knock down 90% of the population exposed.
LFL - Lower Fiducidal Limit, UFL - Upper Fiducidal Limit, x2 – Chi-square value,
*Significant at P < 0.05 level.

Table 3: The knock down (%) and knock down (KD_{50}, KD_{90}) of *C. quinquefasciatus* with NEF formulations.

NEF	**% Knock Down (Mean ± SD) (mg/cm^2)**					**$KD_{50}KD_{90}$and 95% Fiducial limits (Upper and Lower)**		**Regression equation**	**x^2**
	3.125	**6.25**	**12.5**	**25**	**50**	**(LFL UFL)**	**(LFL UFL)**		
F1	42.3±2.73	59.3±1.65	78.4±0.14	92.6±3.85	100±0.00	4..05 (1.353-6.031)	20.88 (18.025-25.350)	y =0.761 + 0.002x	2.94 *n.s.*
F2	22.1±0.23	41.3±1.35	56.4±2,45	71.1±0.81	82.7±0.72	14.66 (-25.13-36.24)	54.39 (34.18-332.12)	y =0.322 + 0.003x	16.4 *s.*
F3	18.1±0.31	33.3±.0.56	42.2±3.73	52.4±0.53	68.1±0.52	27.01 (14.069-57.355)	78.98 (52.11-244.41)	y =0.246 + 0.003x	8.04 *s.*

*NEF- Nanoemultion formulations.
Values are mean ± SD (No. of replicates = 5); Control-No Knock down
Mosquitoes were exposed for 1 h and mortality was recorded after 24 h period.
KD_{50}= Concentrations required to knock down 50% of the population exposed.
KD_{90}= Concentrations required to knock down 90% of the population exposed.
LFL - Lower Fiducidal Limit, UFL - Upper Fiducidal Limit, x2 – Chi-square value,
*Significant at P < 0.05 level.

Table 4: The adult mortality (%) and lethal dose (LD_{50} and LD_{90}) of *Aedes aegypti* with NEF formulations*.

NEF	**% Mortality at 24 h (Mean ± SD) (mg/cm^2)**					**LD_{50} LD_{90} and 95% Fiducial limits (Upper and Lower)**		**Regression equation**	**x^2**
	3.125	**6.25**	**12.5**	**25**	**50**	**(LFL UFL)**	**(LFL UFL)**		
F1	17.5±0.75	22.5±1.25	33.7±0.87	52.5±1.50	71.2±1.25	28.60 (24.68-33.451)	69.82 (60.039-84.925)	y =0.310 + 0.035x	4.08 *n. s.*
F2	8.74±0.87	13.7±1.35	27.5±0,05	38.7±0.37	58.7±1.75	39.36 (27.618-75.262)	81.14 (56.44-188.70)	y =0.306 + 0.003x	8.91 *s.*
F3	2.5±0.21	7.5±.0.75	14.7±0.35	23.7±0.33	33.7±0.81	63.02 (42.19-247.39)	115.73 (73.06-546.27)	y =0.243 + 0.038x	7.95 *s.*

*NEF- Nanoemultion formulations.
Values are mean ± SD (No. of replicates = 5); Control-No Knock down and No Mortality
Mosquitoes were exposed for 1 h and mortality was recorded after 24 h period.
LD_{50} = Lethal dose required to kill 50% of the population exposed.
LD_{90} = Lethal dose required to kill 90% of the population exposed.
LFL - Lower Fiducidal Limit, UFL - Upper Fiducidal Limit, x2 – Chi-square value,
*Significant at P < 0.05 level.

Table 5: The adult mortality (%) and lethal dose (LD_{50} and LD_{90}) of *C. quinquefasciatus* with NEF formulations*.

NEF	**% Mortality at 24 h (Mean ± SD) (mg/cm^2)**					**LD_{50} LD_{90} and 95% Fiducial limits (Upper and Lower)**		**Regression equation**	**x^2**
	3.125	**6.25**	**12.5**	**25**	**50**	**(LFL UFL)**	**(LFL UFL)**		
F2	23.7±1.125	30.2±1.25	43.7±0.37	61.2±2.125	82.5±1.75	20.09 (16.71-23.68)	57.13 (49.57-68.44)	y =0.346 + 0.037x	2.92.*n s.*
F1	12.5±1.50	22.2±0.37	31.3±0.75	43.7±1.25	65.0±0.51	33.99 (29.39-40.200)	78.46 (66.71-97.24)	y =0.288 + 0.035x	4.56 *n.s.*
F3	8.75±1.25	13.7±.1.62	21.2±0.87	32.5±0.86	48.7±2.37	48.36 (41.22-59.80)	99.04 (81.88-128.94)	y =0.252 + 0.035x	3.51.*n s.*

*NEF- Nanoemultion formulations.
Values are mean ± SD (No. of replicates = 5); Control-No Knock down and No Mortality
Mosquitoes were exposed for 1 h and mortality was recorded after 24 h period.
LD_{50} = Lethal dose required to kill 50% of the population exposed.
LD_{90} = Lethal dose required to kill 90% of the population exposed.
LFL - Lower Fiducidal Limit, UFL - Upper Fiducidal Limit, x2 – Chi-square value,
*Significant at P < 0.05 level.

4. Discussion

The obtained results are concurring with earlier study of *R. officinalis* essential oil allowed determination of required value and achievement of an O/W nanoemulsion. The emulsification method involved in the procedure used heating of the oil phase, constituted by essential oil and sur-factant, in order to obtain small droplets [27]. The essential oils are complex mixtures of volatile substances andheating step would lead loss of substances. The studies are concerning nanobiotechnology of *R. officinalis* essentialoil, we decided to test a method without heating, which proved to successfully generate a nanoemulsion. The low energy method used in this study is based in a catastrophic phase inversion [28].The variation of insecticidal activity of the nanoemulsion of eucalyptus oil with and without the aqueous filtrate against *T. castaneum* after 2 months was observed. From this figure, it is clear that the toxicity of the nanoemulsion prepared using this filtrate as a continuous phase remains the same, but there is considerable decrease in insecticidal activity of the nanoemulsion formulations prepared using distilled water. The LC_{50} values of the formulations, i.e., F1, F2, F3, and F4 indicate that F4 was more toxic than F2 and F3, but F1 was not as toxic as F4, F3, and F2 because of the absence of aqueous filtrate as a continuous phase in the formulation [29]. The earlier study by Omoloa *et al*. (2005) [30] have reported the fumigant toxicity of essential oils from 15 species of

African plants against *Anopheles gambiae* in the laboratory. They reported that oils of 6 plant species viz., *Tarchonanthus camphoratus*, *Lippia javanica*, *Plectranthus marruboides*, *Tetradenia riparia*, *Lippia ukambensis* and *Conyza newii* were found to be relatively more toxic, with *C. newii* and *P. marruboides* showing the highest potency. Yang *et al.* (2005) [31] have studied the adulticidal activity of five essential oils against *Culex pipines*. They found that the Rutaceae oil obtained from *Citrus sinesis* was the most effective adulticidal treatment. Vartak and Sharma (1993) [32] have reported the knock-down effect of terpenoids of volatile oils against *A. aegypti* adult females.

Rajkumar and Jabanesan (2002) [33] reported the knocking down and killing effects of *Solanum aerianthum* D.Don. Leaf extracts against the mosquito *Culex quinquefasciatus* Say. Jeyabalan *et al.* (2003) [34] have reported the adulticidal effect of *Pelargonium citrosa* on *Anopheles stephensi*, with LC_{50} and LC_{90} values as 1.56% and 5.22% respectively. The obtained results are in agree with earlier study by Prajapati *et al*. (2005) [35] have studied 10 essential oils viz., *Cinnamomum zeylanicum*, *Cuminum cyminum*, *Cyperus scariosus*, *Curcuma longa*, *Juniperus macropoda*, *Ocimum basilicum*, *Rosmarinus officicinalis*, *Nigella sativa*, *Pimpinella anisum*, and *Zingiber officinale* for adulticidalt activity against three mosquito species; *Anopheles stephensi*, *Aedes aegypti* and *Culex uinquefasciatus.* The oil based nanoemulsion was prepared in three ratios by varying the volumes of oil, surfactant, and water. The ratios were 1:1, 1:2, and 1:3, respectively. The uniformity and stability of the nanoemulsion can be determined. The nanoemulsion is inversely proportional to the uniformity and stability of the nanoemulsion [36]. The seed and leaf extract of eucalyptus oil contain compounds that are toxic to mosquito larvae [37]. Eucalyptus oil is the oil distilled from the leaves of eucalyptus, a genus of the plant family Myrtaceae. In the present results are compatible with earlier study carried out in the repellent activity of eucalyptus oil was demonstrated against *Cx. quinquefasciatus* [37].

5. Conclusions

From the present investigation, a prominent result of nanoemulsion using with essential oil loaded formulation was notice. The NEF showed variation in knok down and adulticidal activity when tested by test tube such as filter paper impregnated method. Nanoemulsion formulation (F1) recorded 100 per cent knock down activity. The property of the nanoemulsion was related to the essential oil and with ingredient added in the nanoemulsion formulations. The activities such as knock down and adulticidal of nanoemulsions were evaluated by nanoemulsion (F1-F3) formulation. The nanoemulsion based essential oil fabricated as emulsion formulation and it enhanced adulticidal activity against *A. aegypti* and *Cx. quinquefasciatus* was observed. The current results support to the development new product of NEF from essential oil for the vector mosquito control. This is the important report on evaluated the anti- mosquitocidal activity of oil loaded nanoemulsion. It could be bringing a promising nano-insecticidal which can be used for the adult mosquito management.

6. Acknowledgments

The senior author would like to acknowledge University Grand Commission for the financial assistance through Post-Doctoral Fellowship (UGC-PDF-2014-15).

7. References

1. Solans S, Esquena J, Forigianini A, *et al.* Absorption and aggregation of surfactants in solution. In: Mittal KL, Dinesh OS. (Eds.), Nano-emulsion: Formation Properties and Applications. Marcel Dekker, New York. 2003, 525e554.
2. Bouchernal K, Brianeon S, Perrier E, *et al.* Nano-emulsion formulation using spontaneous emulsification, solvent oil and surfactant optimization. International Journal Pharmacology. 2004, 280-285.
3. Sonneville-Aubrun O, Simonnet JT, L'Alloret F. Nanoemulsions: a new vehicle for skincare products. Advance Colloid Interface Science. 2004, 145-149.
4. Gutiérrez JM, González C, Maestro A, *et al.* Nanoemulsions: new applications and optimization of their preparation. Current Opinion Colloid Interface Science. 2008; 13:245-251.
5. Ostertag F, Weiss J, McClements DJ. Low-energy formation of ediblenanoemulsions: factors influencing droplet size produced by emulsion phaseinversion. Journal Colloid Interface Science. 2012; 388:95-112.
6. Becker N, Petriae D, Zgomba M, *et al.* Mosquitoes and Their Control. Kluwer Academic Plenum Publisher, New York, 2003, 1-26.
7. Fradin MS, Day JF. Comparative efficacy of insect repellents against mosquito bites. New England Journal Medicine. 2002; 347:13-18.
8. Ghosh GK. Biopesticide and integrated pest management. APH. publishing corporation, New Delhi. 1991, 145-146.
9. Jang YS, Ahn YJ, Lee HS. Larvicidal activity of Brazilian plants against *Aedes aegypti* and *Culex pipiencs*. Diptera: Culicidae. Agricultural Chemistry Biotechnology. 2002; 45:131-134.
10. Izquierdo P, Feng J, Esquena J, *et al.*, The influence of surfactant mixing ratio on nano-emulsion formation by the pit method. Journal Colloid Interface Science. 2005; 285:388-394.
11. Murray BS, Ettelaie R. Foam stability: proteins and nanoparticles, Current Opinion Colloid Interface Science. 2004; 9:314-320.
12. Dickinson E. Food emulsions and foams: stabilization by particles, Current Opinion Colloid Interface Science. 2010; 15:40-49.
13. Binks BP, Horozov TS. Colloidal Particles at Liquid Interfaces, Cambridge University Press, Cambridge, 2009, (1).
14. Anjali CH, Sharma Y, Mukherjee A, Chandrasekaran N. Neem oil Azadirachta indica nanoemulsion - a potent larvicidal agent against Culex quinquefasciatus. Pest Management Science. 2012; 68:158-163.
15. Bakkali F, Averbeck S, Averbeck D, *et al.* Biological effects of essential oils e a review. Food and Chemical Toxicology. 2008; 46(2):446-475.
16. Guzey D, DJ McClements, Stability and properties of multilayer emulsions for application in the food industry, Advance Colloid Interface Science. 2006; 128:227-248.
17. McClements DJ. Food Emulsions: Principles, Practice, and Techniques, CRC Series in Contemporary Food Science, 2nd ed., CRC Press, Boca Raton., 2004.
18. McClements DJ, Rao JJ. Food-grade nanoemulsions: Formulation, fabrication, properties, performance, biological fate, and potential toxicity. Critical Reviews in Food Science and Nutrition. 2011; 51(4):285-330.
19. McClements DJ. Edible nanoemulsions: Fabrication,

properties, and functional performance. Soft Matter. 2001; 7(6):2297-2316.
20. McClements DJ. Nanoemulsions versus microemulsions: Clarification of differences, similarities and terminology. Soft Matter. 2012; 8(6):1719-1729.
21. Dua VK, Pandey AC, Singh R, Sharma VP, Subbarao SK. Isolation of repellent ingredients from *Lantana camara* Verbenaceae flowers and their repellency against *Aedes* mosquitoes. Journal Applied Entomology. 2003; 127:509-511.
22. Ramar M, Gabriel Paulraj M, Ignacimuthu S. Preliminary screening of plant essential oils against larvae of *Culex quinquefasciatus* Say Diptera: Culicidae. African Journal Biotechnology. 2013; 12(46):6480-6483.
23. World Health Organization. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides: diagnostic test. Geneva: WHO. 1981; WHO/VBC/81.807.
24. Dua VK, Alam MF, Pandey AC, Rai S, Chopra AK, Kaul VK, *et al*. Insecticidal activity of *Valeriana jatamansi* Verbenaceae against mosquitoes. Journal American Mosquito Control Association. 2008; 24:315-318.
25. Abbott WS. A method of computing the effectiveness of an insecticide. Journal Economic Entomology. 1925; 18:265-268.
26. Finney DJ. *Probit analysis*. Cambridge: Cambridge University Press, 1971.
27. Fernandes CP, Mascarenhas MP, Zibetti FM, *et al.* HLB value, an important parameter for the developmentof essential oil phytopharmaceuticals. Review Brarisil Farmacogn. 2013; 23:108-114.
28. Omolo MO, Okinyo D, Ndiege IO, Lwandeb W, Hassanali A. Fumigant toxicity of the essential oils of some African plants against *Anopheles gambiae* sensu stricto. Phytomedicine. 2005; 12:241-246.
29. Yang P, Ma Y, Zheng S. Adulticidal activity of five essential oils against Culex pipiens quinquefasciatus. Pesticide Science Society Japan. 2005; 30:67-72.
30. Vartak PH, Sharma RN. Vapour toxicity & repellence of some essential oils & terpenoids to adults of *Aedes aegypti* L. Diptera: Culicidae. Indian Journal Medical Research. 1993; 97:122-127.
31. Rajkumar S, Jebanesan A. Oviposition attractancy of *Solanum aerianthum* leaf extract for *Culex quinquefasciatus*. Journal of Experimental Zoology. 2002; 59:221-224.
32. Jeyabalan D, Arul N, Thangamathi P. Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, *Anopheles stephensi* Liston. Bioresource Technology. 2003; 89:(2):185.
33. Prajapati VV, Tripathi AK, Aggarwal KK, Khanuja SPS. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*. Bioresource Technology. 2005; 96:1749-1757.
34. Shinoda K, Saito H. The stability of O/W type emulsions as functions of temperature and the HLB of emulsifiers: the emulsification by PIT-method. Journal of Colloid and Interface Science. 1969; 30:258-263.
35. Elbanna SM. Larvaecidal effects of eucalyptus extract on the larvae of *Culex pipiens* mosquito. International Journal of Agriculture and Biology. 2006; 8:896-897.
36. Mandal S. Repellent activity of Eucalyptus and Azadirachta indica seed oil against the filarial mosquito Culex quinquefasciatus Say Diptera: Culicidae in India. Asian Pacific Journal of Tropical Biomedicine. 2011; 13:109-112.