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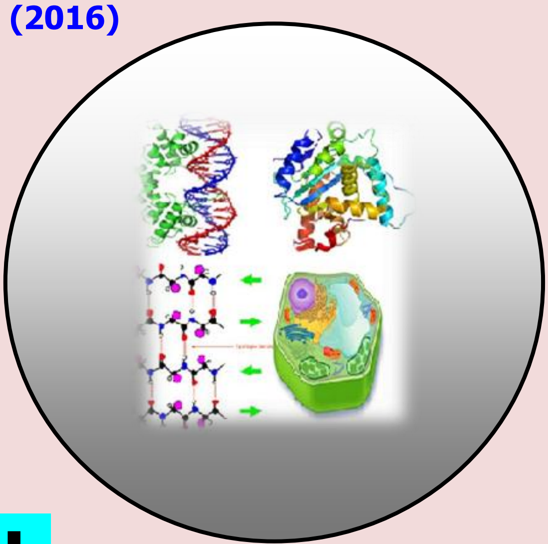

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ABSTRACTS

The present study was prepared an essential oil (Lantana camara) loaded nanoemulsion formulation (NEF) green pesticide in different percentage of essential oil, emulsifier, and water through mechanical stirring method. The investigated adult-mosquitocidal and antioxidant using nanoemulsion of essential oil loaded formulation. The anti-mosquitocidal property was evaluated under laboratory condition against adult dengue vector Aedes aegypti. adult-mosquitocidal effect of formulated nanoemulsion was contacted against A. aegypti. The oil in water (O/W) emulsion was prepared using with L.camara (essential oil), emulsifier and water. The nanoemulsion formulation was characterized by dynamic light scattering (DLS), UV–vis spectrum, Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The LD_{50} and LD_{90} values for adult mosquito were 11.947mg/cm2 and 47.716 mg/cm^2 respectively. Moreover, the antioxidant activity was carried out to examine the effect of nanoemulsion. The antioxidant activity of ascorbic acid (55.9%), glutathione (67.7%) and quercetin (48.6%) demonstrated respectively. This present investigation put in information about the use of green pesticide of nanoemulsion in the integrated vector management, allow us to suggest essential oil loaded nanoemulsion formulation as an effective bio-product to develop novel and bio-safety for control immature as well as adult mosquito control using nano-pesticides.
Keywords: Lantana camara, Essential Oil, Nanoemulsion, DLS, UV, FTIR, SEM, Adulticidal and Antioxidant.

INTRODUCTION

Mosquitoes are creating the threat to public health because of their transmiting diseases such as malaria, lymphatic filariasis, dengue, chikungunya, yellow fever, and Japanese encephalitis. The diseases are responsible for the mortality and, morbidity and aslo economic loss in the wordwide (Becker et al., 2003; Curtis, 1992; Fradin and Day, 2002; Jang et al., 2002).The current trend of nanotechnology has been considered a as newer area for the development ecofriendly pesticides, with natural products incorporated as active ingredients (Rawani et al., 2013; Angajala et al., 2014). The nanotechnology approach in the development of pesticides using nano method of repellent or water-soluble formulation of nanoemulsion (Nuchuchua et al., 2009). This type of stable formulation is constituted by two immiscible liquids and one or more surfactants, with small droplet size (Solans et al., 2005; Sole et al., 2012), clear or transparent appearance (Forgiarini et al., 2000). The nanoemulsion formulations are stable for a long time (Bouchernal et al., 2004). The stability depending on the individual system or mixture of ingredients, viscosity and droplet size make them better looking system for many industrial applications in the pharmaceutical industrial. It can also be used in cosmetics and in pesticide delivery systems (Sonneville- Aubrun et al., 2004). The developments of nanoemulsions are stable systems, and their stability depends on the method of preparation (Gutierrez et al., 2008). The approach is high emulsification, which engages high stirring, high-pressure homogenizers and low energy emulsification method has been developed by attractive of phase behaviour and properties to promote the formation of small droplets with constant vigorous stirring (Ostertag et al., 2012).

The preparation of nanoparticles in pharmaceutics is common, although the nanoparticles are prepared by a variety of methods such as solvent evaporation from emulsions and precipitation in aqueous solutions. The simple method reported for obtaining organic nanoparticles by a direct conversion of oil- in-water microemulsion droplets into particles by rapid evaporation of the liquids (Magdassi and Margulis-Goshen, 2007; Magdassi et al., 2008; Margulis-Goshen and Magdassi, 2009; Margulis-Goshen et al., 2009). Oil in water nanoemulsions have been described as delivery systems to solubilize lipophilic ingredients, being a method to encapsulate ingredients into droplets, with smaller size in comparison with conventional emulsions (McClements and Rao 2011; McClements 2011; Sagalowicz and Leser 2010; Solans et al. 2005; Mason et al. 2006). The newly, the generation of nanoemulsion based delivery systems has been reported. For example, Spinelli et al. (2010) obtain synthetic oil nanoemulsions with droplet sizes lower than 100 nm by ultrasounds. The research work are presented by Leong et al. (2009) and Kentish et al. (2008), edible oil nanoemulsions are produced by power ultrasounds.

The botanical based essential oils (EO) could be an alternative to chemical preservatives for extending the shelf life of food products industry. In fact, Eessential oil has shown not only in the antimicrobial activity against pathogens (Hammer et al. 1999). Moreover, they are often used as flavoring agents in several food products (Fernaroli 1995). The oil of lemongrass (*Cymbopogon citratus*) essential oil, apart from its appealing citric flavor, has shown to have a strong antimicrobial potential (Raybaudi-Massilia, et al. 2006), thus being susceptible for incorporation to food products. The use of essential oil in food products presents limitations. On the one hand, their intense and persistent aroma significantly affects the organoleptical properties and consumer acceptance of foods (Raybaudi- Massilia et al. 2008). Moreover, they may present toxicological effects at high doses (Burt 2004). Therefore, there is a need to reduce the concentration of essential oi incorporated to food products to avoid consumer rejection, toxicological effects (Sánchez-González et al. 2011). The eucalyptus is a diverse genus of flowering trees and shrubs in the Myrtle family, Myrtaceae. The oil extracted from eucalyptus leaves possesses allelopathic property and prevents insects from attacking it, thereby, acting as a natural pesticide (Brooker & Kleinig, 1990; Batish et al., 2008). In the fumigation activity (Papachristos & Stamopoulos, 2004) and repellency (Trigg, 1996), the efficiency of eucalyptus oil has been demonstrated (Kumar et al., 2012). Larvicidal activity of the eucalyptus oil nanoemulsion was compared with the bulk emulsion of the same. The nanoemulsion of the eucalyptus oil was found to be more effective than its bulk complement. The observed histopathological effects of the eucalyptus oil nanoemulsion on *Cx. quinquefasciatus* (Suresh Kumar et al. 2013).The larvae and pupae of *A. aegypti* showed a decrease in the protein concentration levels on exposure to extracts of the kernel of soapnut, Sapindus emarginatus results reported in the ealier study (Koodalingam et al. 2011).The antioxidant defense mechanisms present in the cell take part in a vital role in maintaining the physiological redox status and protect against the harmful effect of toxic drug metabolites and free radicals. The non-enzymic antioxidants like reduced glutathione (GSH), ascorbic acid (AA), and vitamin E among others and the enzymic antioxidants such as glutathione-*S*-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT) (Valko et al.,2006). The (QCT) is a flavonoid antioxidant which is ubiquitous in plants and plant food sources (Sikder et al., 2014). The Quercetin (QCT) and its derivative have been promoted in several studies as potent antioxidants (Magalingam et al., 2014). The present research work is preparation and use of nanoemulsion as an effective product of green nano-pesticide formularion with adding of suitable ingredients to improve the efficacy on target mosquito. Therefore, the enlightenment of the present research work was to discriminate the nanoemulsion formulation with incorporate the essential oil (*Lantana camara*) in terminology of droplet size distribution, UV, FTIR and SEM for comparison. The O/W phase was used for the preparation of emulsion to enhancing the bioactivity and stability of nanoemulsion.

The present investigation of larvicidal (I- IV instars), pupicidal,adulticidal activities was carried out against *A. aegypti* mosquito. The nanoemulsion formulation makes an alternative larvicide against *A. aegypti*. Also, the biochemical (enzymes) and antioxidant acivity was contacted using with nanoemulsion.

MATERIALS AND METHODS

Essential oil

The essential oil extraction from leaves of *Lantana camara* was performed by hydrodistillation using a Clevenger apparatus. According to the protocol for extraction of the essential oil used in this work were previously described (Fernandes et al., 2013). The extracted essential oil was stored in a dark glass bottle and kept at a temperature of 4°C until further used.

Test mosquito

The *Aedes aegypti* cultured in our department under the laboratory conditions (26±2 °C, 70–85 % R.H, 14:10 (L:D) photoperiod) and placed in 22×10×8 cm plastic containers containing 1000 mL of tap water for larval hatching. The Larvae were reared in the plastic containers and larvae fed with dog biscuits and yeast at 3:1 ratio (w/w). Water was changed every day. Breeding container was checked daily. The containers were kept closed with cloth to prevent emerged adults mosquito. Both larvae and pupae for experiments were collected daily from culture for assays (Ramar, et al., 2013).

Preparation of Nanoemulsion formulation

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 (Figure1). 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.

Characterization of Nanoemulsion

Measurement of particle size Distribution

The measurement of droplet size distributions of nanoemulsion was performed using spectroscopy. To obtain size measurement, the formulation was diluted with 0.5mL of 0.25-µm of water to remove the effect of thickness by the ingredient. Droplet size was reported as the average of four measurements (Fernandes et al., 2013).

UV–visible spectrum

UV–visible spectrum of nanoemulsion was recorded in time of reaction by UV–visible spectrophotometer. The formation nanoemulsion monitored in the range of 300–800 nm. nanoemulsion of essential oil surface resonance in between 300 and 800 nm which was very close to the calculated optical spectrum of various nanoparticles both in water and in vacuum by Creightonand Eadon (1991).

Fourier transformed infrared (FTIR) spectroscopy

Characterization of essential oil loaded nanoemulsion was done using FTIR analysis. The carbonyl group in nanoemulsion formualtion was confirmed according to method (Bar et al., 2009).

Morphology of nanoemulsion by scanning electron microscopy (SEM)

The scanning electron microscopy (SEM) was used to analyze the morphology of the nanoemulsion particles. The nanoemulsion samples were processed as described () after dilution 200 fold with distilled ionized water. Samples were placed on the carbon-attached Vinylec Films, negatively stained with 10% phosphotungstic acid, allowed to stand 5 minutes for drying and placed in the vacuum chamber for 30 minutes before analysis.

Adulticidal activity

An adulticidal activity was carried out by WHO protocol (WHO, 1996). The selected nanoemulsion essential oil impregnated filter paper was prepared in adding of 2 ml of emulsion formulation. The different doses viz., 10,20,30,40 and 50mg/cm^2 were studied. The fixed doses of 2 ml of formulation were applied on Whatman no. 1 filter papers (size 12 x15 cm^2); control papers were also maintained. Two to five days old sugar fed mosquitoes (N=20) adult mosquitoes were exposed on treated paper for 1 hour and every 5 minute intervals mosquitoes were recorded. After one-hour knocked down mosquitoes was transferred into recovery test tubes for mortality observation at 24 hour. Five replicates were run at a time with control. The reference insecticide was used with 0.05 % deltamethrin impregnated paper as a comparison. Lethal dose (LD_{50} and LD_{90}) were calculated by probit analysis using SPSS software.

Antioxidant activity

The ABTS.+ stock solution was prepared by mixing 5ml of 7mM (2,2'-azino-bis- (3-ethylbenzothiazoline –6-sulfonic acid) of ABTS radical scavenging activity of the extract was measured following method (Rice Evans *et al* .,1997). The stock solution was diluted to give absorbance of at 830 nm. The standard stock solution of ascorbic acid, glutathione and quercetin was prepared in acetone. The standard antioxidants were added to the diluted ABTS.+ solution in ethanol and the absorbance analysis was taken at room temperature. A suitable solvent blank was run in each assay and the experiments were carried out in three readings. The total antioxidant activity was calculated using the following equations.

$$\text{Total antioxidant activity (\%)} = \frac{\text{O.D ABTS +- O.D Sample}}{\text{O.D ABTS +}} \times 100$$

Statistical analysis

All data were subjected to Analysis of Variance (ANOVA). The LC_{50} and LC_{90} and LD_{50} and LD_{90}, values and their 95% confidence limits were subjected to Probit analysis. LC_{50} and LC_{90} and LD_{50} and LD_{90}, were calculated using the method by Finney (1971). 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. The probability level of $P<0.05$ was used for the significance of differences between values.

RESULTS

Particle size and distribution of nanoemulsion

Nanoemulsion was prepared with different percentage of composition. The results of average droplet size (nm) of emulsion formulation. This emulsion technique used to influence the combination of nanoemulsion droplet size of unify. The emulsion droplet size distribution was noticed depending on the percentage of water used in the nanoemulsion. The present results of nanoemulation confirmed that average droplet size was 418.4 ± 17 nm of droplet size is shown (Figure 2). The present results showed the significant decrease in the sizes of nanoemulsion formulation. The mean droplet size 18.4 ± 0.004), which is in concurrence with the formation of nanoemulsions were observed in the measurements. These mean droplet size of this formulation was monitored (Figure 2), indicating small droplet size of manipulation that this formulation presented a bluish reflection, characteristic for nanoemulsions.

UV analysis

The UV spectroscopy is an essential technique to verify the stability of metal nanomulsion in the development of formulation. The change of the color in nanoemulsion was noticed in image when the formulation was incubated (Figure 3). The nanoemulsion displays the color in solution due to the surface plasmon resonance occurrence. Intensity of whitish colour increased with proportion to the incubation period. The absorption spectrum of the incubated solution at the ranging from 300 to 800nm revealed a peak at 300nm.

Fourier transformed infrared (FTIR) spectroscopy analysis

The support of results of particle size and distribution and UV of nanoemulsion, additional study was carried out by FT-IR analysis to identify the compound formation and interface of major functional groups involved in the nanoemulsion formulation (Figure 4). The broad absorption band noticed at 3415 cm. The broad band indicates that the nanoemulsion in crystalline nature. The weak band near 1641.42 cm was allocated to bending sensations of H O H bonds are present in the analysis. These observations present the evidence for the presence of hydroxyl group. It performs as reducing and stabilizing agents during the arrangement of emulsion. The combination bands rough in the region 4000–1000 cm assigned to C O stretch vibrations and O C O symmetric and a symmetric stretching vibrations of organic segment nearby the nanoemulsion of oil loaded formulation.The FTIR measurements were carried out to identify the possible biomolecules responsible for the stabilization of the nanoemulsion.

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Scanning electron microscopy (SEM)
The morphology of the essential oil loaded nanoemulsion was studied by using scanning electron microscopy (SEM). The results are obtained from SEM showed that the nanoemulsion is crystalline in nature with triangular shape and the average particle size of 100-10 nm (Figure 5).

Thesis reveals that nanoemulsion is polycrystalline particles showing unequal form with thin particle size distribution and there was no agglomeration was noticed. The present results gives clearly an idea that nanoemulsion is enclosed by a layer of organic biomaterial derived from oil loaded nanoemulsion which contain surface functional OH groups and it actively participate in reduction of nanoemulsion.

Adulticidal activity
The adulticidal activity was conducted to test the efficacy of nanoemulsion on adult mosquito of *A.aegypti*. The percentage of mortality was calculated for the adult of dengue vector, *A.aegypti* treated with nanoemulsion using different doses (10, 20, 30, 40 and 50mg/cm^2) of emulsion. The mortality was increased as the dose increased; the adult mortality was 48.2% at 10 mg/cm^2, whereas at 50 mg/cm^2 dose, it was increased to 92.5% (Table 2) respectively. The considerable mortality was found after the treatment of nanoemulsion impregnated paper for the important adult vector mosquito. The LD_{50} and LD_{90} values (Table 2) of the nanoemulsion treated paper found to be effective against *A. agepyti* (LD_{50} = 11.947mg/cm^2 and LD_{90} = 47.716 mg/cm^2) respectively. The significant mortality was observed after the treatment of emulsion formulation against the vector mosquito of *A. agepyti.* There was no mortality recorded in the control assay. X^2 value was significant at the $p \leq 0.05$ level.
Antioxidant activity
The antioxidant activity by absorbance spectrum of ABTS. + at dose defendant method results were present (Figure 6). A range of concentrations of each standard were read at 1min interval up to 15min. The results revealed that every part of were effectively completed in 1-2 minutes. As a result, point in time 1 and 3 min selected for interpretation the absorbance after preliminary mixing of the reactants in reading. The activity for standards of ascorbic acid (55.9%), glutathione (67.7%) and quercetin (48.6%) showed inhibition at 50 mM concentration respectively.

DISCUSSION
The prospective study with *copaiba oleoresin* performed by Xavier-Junior et al. (2012) using different blends of surfactants indicated that value of 14.8 for the oil phase, which is in agreement with our results. The oil phase is one of most important parameters that considered during the development of nanoemulsions (Schmidts et al., 2010; Fernandes et al 2013). The determined by calculating of the surfactant or mixture of surfactants, which allows achieving an emulsion with minimum droplet size, among a set of prepared emulsions (Rodriguez-Rojo 2012, Fernandes et al., 2013; 2014; Costa et al., 2014). The arrangement and stability of the reduced nanoemulsion in the clear solution was observed by UV spectrophotometer analysis and the nanoemulsion showed surface of absorption bands at 430 nm (Vidhu et al., 2011). The earlier reported by Sathishkumar et al., (2009) stated that maximum absorbance occurred at 435 nm due to presence of silver particle. This peak area increased with the increase in reaction time. The reason for this type of behavior could be due to the reduction of silver ion by the biomolecules in accordance with similar to earlier researchers (Huang et al., 2007). It is generally recognized that UV–Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions (Shrivastava and Dash, 2010). This is similar to the surface plasmon vibrations with characteristic peaks of silver nanoparticles arranged by chemical reduction (Kong and Jang, 2006). The silver nanoparticle exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Krishnaraj *et al.,* 2010). The AgNPs were observed to be stable in solution and showed little aggregation. In addition, the plasmon bands are broadened with an absorption tail in the longer wavelengths, which may be due to the size distribution of the particles (Ahmad *et al.,* 2003). The figure 4 represents the FTIR spectrum of nanoemulsion showed peaks at 3415 to 1641cm^{-1} also some peaks decreased. These peaks are indicate that the carbonyl group and amino acid residues and silver nanoparticles to prevent agglomeration (Sathyavathi, 2010). When the nanoparticles form in solution, they must be stabilized against the forces of attraction which may cause coagulation. The surfactant and polymers may cause electrostatic barriers or purely electrostatic barriers around the particle surface and may thereby provide stabilization (Mulvaney, 1996).

The FTIR peaks that are corresponding to aromatic rings, geminal methyls and ether linkages indicates the presence of flavones and terpenoids responsible for the stabilization of the AgNPs synthesized by the leaf extract of *Sesuvium portulacastrum* (Nabikhan, 2010). The SEM study, reaction mixtures was air-dried on silicon wafer solution. As a result, a coffee-ring phenomenon was observed. This is well recognized that when liquids that contain fine particles was evaporated on a flat surface area and the particles are accumulate along the outer edge and form typical structures (Chen and Evans, 2009). From the present study, it is evident that the AgNPs coalesced to nano-clusters when reaction mixtures were incubated and some nanoparticles aggregated. The average size was 30-65nm for synthesized silver nanoparticles using *H. musciformis*. This is same results reported that the particle size between 25 and 50nm in cubic structure synthesized by papaya fruit extract (Jain *et al.* 2009; Cataleya, 2006. The SEM images of AgNPs from *Emblica officinalis* were also predominantly spherical with average size of 16.8nm ranging from 7.5 to 25nm (Ankamwar *et al.,* 2005). The plant based essential oil nano-formulations have been developed to study their larvicidal effect, with nickel nanoparticles, silver nanoparticles, nanoemulsions reported in eelier research (Veerakumar et al., 2014**;** Sugumar et al., 2014). Larvicidal assessment of *copaiba oleoresin* has been studied against *Aedes aegypti*, the vector of dengue problem (Prophiro et al., 2012). The nanoemulsion showed significant larval and pupal mortality. The essential oils have been showing very effect against mosquitoes (Gillij et al., 2008). The results was observed that essential oils of many plants exhibited mosquito larvicidal activity (Phasomkusolsil & Soonwera, 2010). The botanical oils to form nanoemulsions could be effective as larvicidal agents (Anjali et al., 2012).The essential oils are mixtures of volatile substances and heating step would lead loss of substances. The study is relating to nano biotechnology of *R. officinalis* essential oil, we decided to test a method without heating, which proved to successfully produce a nanoemulsion. The earlier study with *R. officinalis* essential oil allowed determination of lethal value and attainment of an O/W nanoemulsion. The emulsification method involved in the process used mixing of the oil phase, constituted by essential oil and surfactant; in arrange to obtain small droplets (Fernandes et al., 2013). The adulticidal results from this study showed that essential oil nanoemulsion have potential as adulticidal against *Ae. aegypti* which is the important vector for dengue fever. The previous results of hexane fraction of *A. calamus* and *P. aduncum* and methanol fraction of *L. elliptica* possessed noticeable adulticidal activity and suggests that the active constituents are extracted in with respective solvents. All extracts had LC_{50} values those less than 0.20 mgcm-2 representing promising adulticidal properties. The value is reported for the well-known insecticidal plant *Azadirachta indica* (Zebitz, 1986) and various author reported for *Asimina triloba* (Mikolajcczak *et. al.*, 1988). Adulticidal studied by Jeyabalan *et. al.* (2003) have adulticidal effect of *Pelargonium citrosa* on *An. stephensi*, with LC_{50} and LC_{90} value of 1.56% and 5.22% respectively.These results also confirmed with parts of Rohani *et. al.* (1997) study which showed that *L. elliptica* and *P. aduncum* has potentials as adulticides but it's a new finding to record that *A. calamus* also has such property. Therefore these results should encourage further efforts to investigate the compounds that might possess higher adulticidal properties when isolated in pure form. The many plant extracts tested and have shown potential adulticidal effects. The study by Lee and Chiang (1994) showed larvicidal property of *Stemona tuberosa* , however no adulticide was detected. Choochote *et. al.* (1999) also tried to demonstrate the adulticidal property of *Kaempferia galanga,* it caused a knockdown effect after transferring to the holding tube, the mosquito recovered from the knockdown effect. The changes in metabolism and decreases in the enzyme activity of neem treated individuals may be expected to affect enzyme titers and activities (Schmutterer, 1990; Senthil Nathan et al., 2004, 2005).The change of LDH (lactate dehydrogenase) activities after treatment using neem limonoids toxin indicate that changing in the physiological balance of the midgut might affect these enzymes (Senthil Nathan et al., 2004). According to earlier studies, it is shown that the protein concentration of larvae decreases on treatment with nanoemulsion formualtion that is in consistency with the results of our present study. The activity of glutathione reductase in *Morinda citrifolia* fruit extract was observed in the oxidized/ min/g sample. The results are reported that the methanol extract of *Oxalis corniculata* was found to have higher activity of glutathione reductase (Badrul Alum *et al.,* 2011).The improvement of quercetin can be evaluated trolox equivalent antioxidant capacity of Carotene than that of ascorbic acid and glutathione (Arnao et al., 2001) and it is observed in the present study. The scavenging activity of NEF was equivalent to ascorbic acid, glutathione and quercetin. lg/ml. Scavenging activity reported in eliear study using of aqueous extract of *Mentha spicata* was equivalent to quercetin (Kumar and Chattopadhyay (2007). The scavenging activity was observed in a concentration-dependent manner.

Table 1. The percentage adult mortality (mean ± SE) and lethal doses (LD_{50}, LD_{90}) of *Aedes aegypti* after 24 h with nanoemulsion formulation.

Mosquito species	Concentration (mg/cm^2)	Mortality (%) (mean ± SD)	LD_{50} ppm (*LFL-UFL*)	LD_{90} ppm (*LFL-UFL*)	χ^2
A.agepyti	10 20 30 40 50	48.2 ± 1.21^{a} 61.6 ± 1.72^{b} 72.2 ± 2.28^{c} 83.7 ± 1.04^{d} 92.5 ± 3.32^{e}	11.947 (5.524-16.293)	47.716 (42.433-55.949)	0.419*

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). The LD_{50} lethal dose that kills 50 % of the exposed organisms and LD_{90} lethal dose that kills 90 % of the exposed organisms, LCL lower confidence limit, UCL upper confidence limit. Chi-square value followed by an asterisk is significant (heterogeneity factor used in calculation of confidence limits) (P<0.05)
The reference insecticide deltamethrin (0.05 %) was used and 100% adult mortality noticed.

Figure 1. The image of oil in water based emulsion (O/W) nanoemulsions after preparation.

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Table 2. The percentage Egg hatchability (mean ± SE) of *Aedes aegypti* after 24 h with nanoemulsion formulation.

Mosquitocidal treatment	Target organism	**Egg hatchability (%)**					
		Control	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL
Nanoemulsion formulation	*Ae. aegypti*	100±*0.00 a*	54.4±*2.86*	45.8±*2.06*	32.5±*2.30*	21.5±*2.70*	NH

Values were means ± SD of five replicates
Different letters indicated significant differences (ANOVA followed by Tukey's HSD, *P*<0.05)
NH = no hatchability

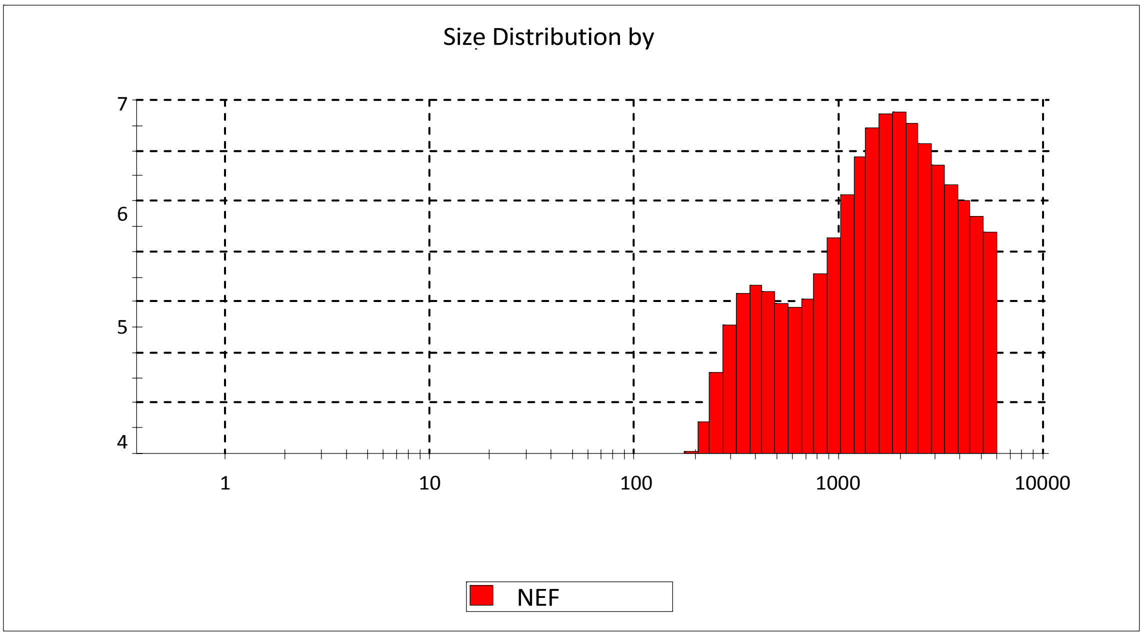


Figure 2. Particle size distribution of a nanoemulsion.

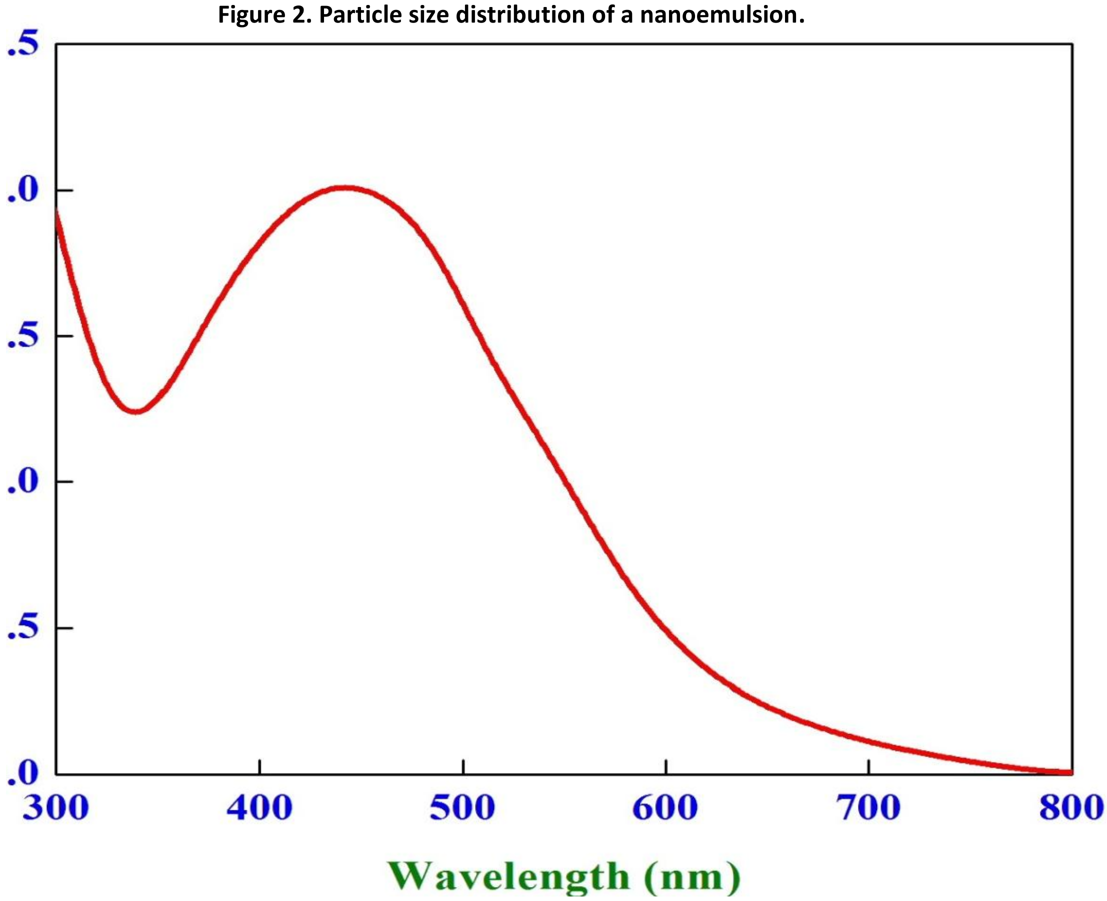


Figure 3. UV–vis spectra of essential oil loaded nanoemulsion.

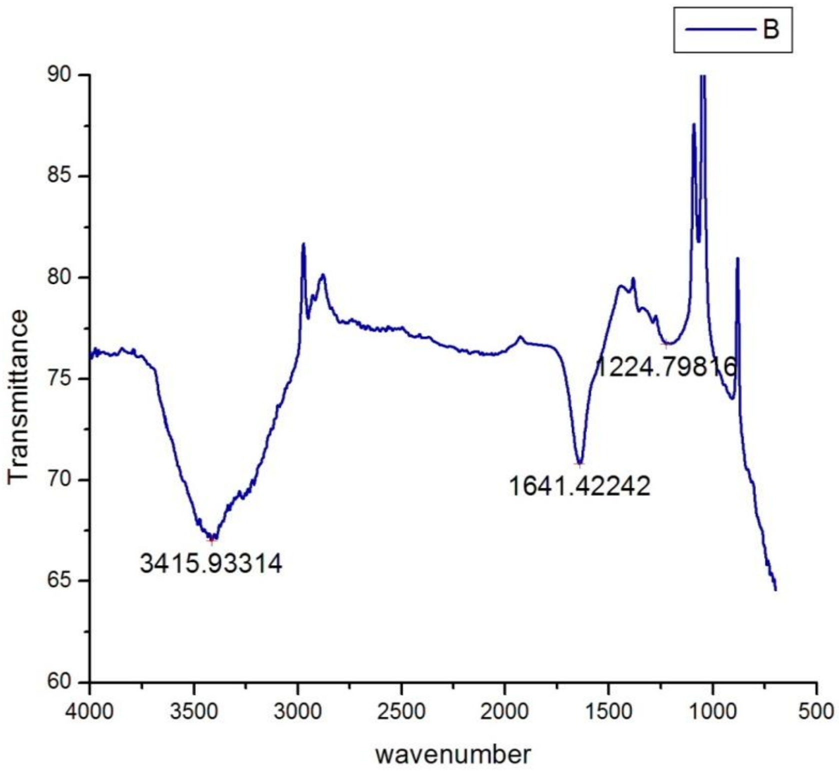


Figure 4. FTIR spectrum of nanoemulsion.

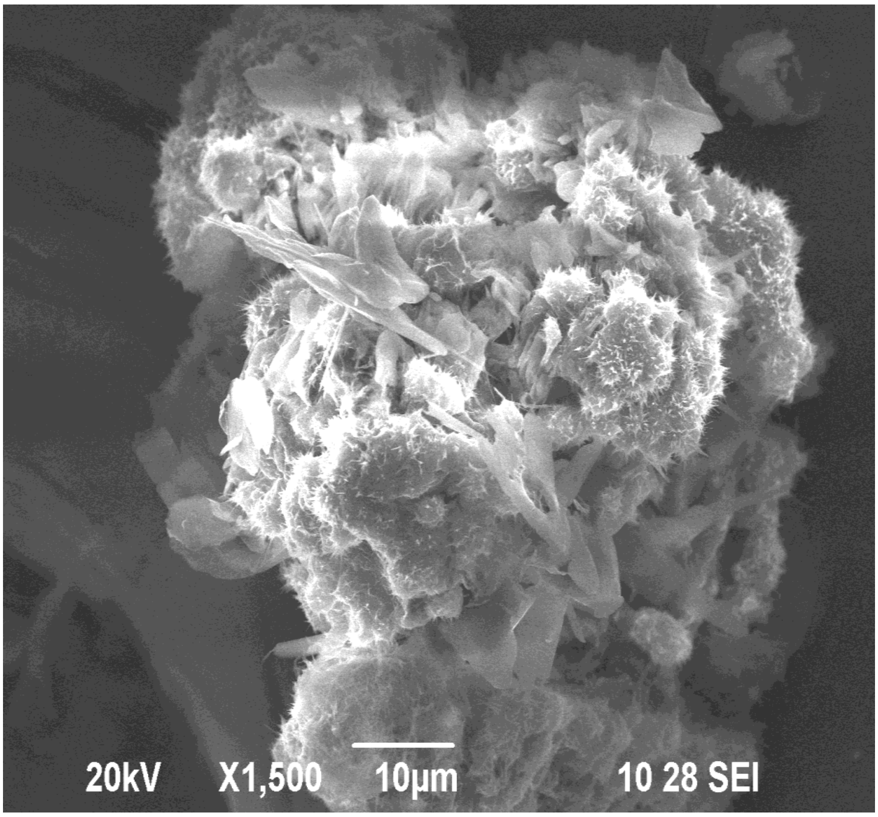
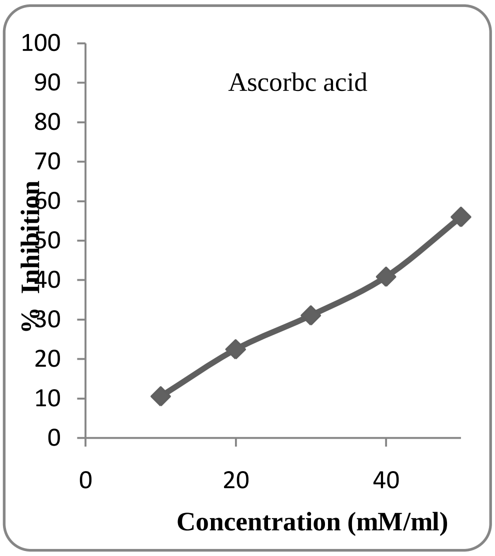
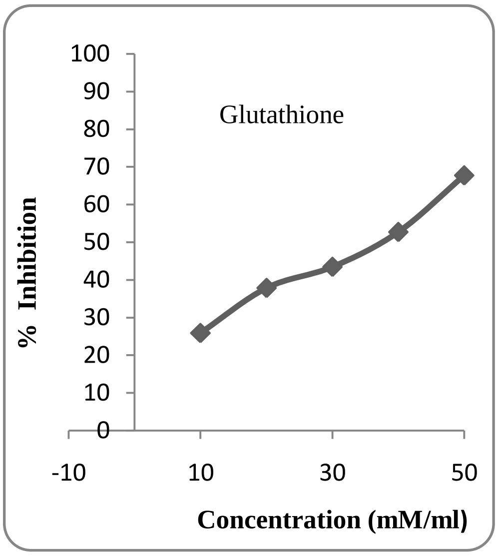


Figure 5. SEM of nanoemulsion.





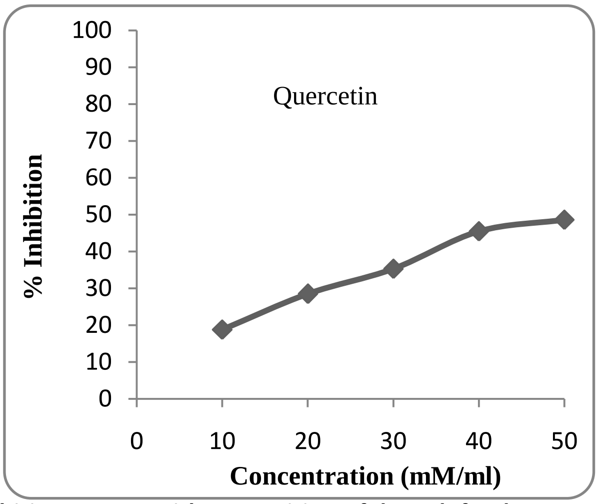


Figure 6. Percentage inhibition ABTS + with proposition of dose defendant concentration of standard antioxidants.

CONCLUSIONS

In this present investigation, a prominent result of nanoemulsion using with essential oil loaded formulation was noticed. There was considerable decrease in the droplet size distribution of nanoemulsion after formulation preparation. The property of the nanoemulsion was related to the essential oil and with ingredient added in the nanoemulsion formulations. The different activities such as adulticidal and antioxidant of nanoemulsions were evaluated by selected nanoemulsion formulation. The nanoemulsion based essential oil mediated formulation enhanced an adulticidal activity against *A. aegypti* was observed. The current results support the possible of nanoemulsion in the development of essential oil based a novel Nano-pesticide for the dengue vector mosquito control. This is the important report on assess the mosquitocidal and antioxidant activities for oil loaded nanoemulsion. Further, research needed using nano formulation on non target organisms and toxicity study will be evaluated. It could be bring a promising nano-pesticide which can be used for the mosquito control.

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