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Optimization of Process Parameters to Formulate Nanoemulsion by Spontaneous Emulsification: Evaluation of Larvicidal Activity Against *Culex quinquefasciatus* Larva

Vijayalakshmi Ghosh · Amitava Mukherjee · Natarajan Chandrasekaran

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Abstract Nanoemulsions are colloidal dispersions of oil and water with droplet size in the range of 10–100 nm. They are optically transparent stable systems which require less surfactant concentration than microemulsion. Basil oil (extracted from *Ocimum basilicum*) nanoemulsion was formulated by spontaneous emulsification method and was stabilized by non-ionic surfactant Tween20. Process parameters like surfactant concentration, stirring speed, and holding temperature were optimized to obtain nanoemulsion with minimized droplet size and these factors demonstrated positive correlation on nanoemulsion droplet size and stability. BT20/11/800/75 nanoemulsion (oil system=basil, surfactant=Tween20, oil to surfactant mixing ratio 1:1 vol/vol, rpm=800, pre-heating temperature of organic phase=75 °C) showed lowest droplet size of 28 nm and greater stability. Transmission electron micrograph image showed the spherical morphology of BT20/11/800/75 nanoemulsion droplets. Formulated BT20/11/800/75 nanoemulsion was evaluated for larvicidal activity against *Culex quinquefasciatus*. Basil oil nanoemulsion exhibited dose-dependent mortality of *C. quinquefasciatus* larva which increased with increase in exposure time. Lethal concentration (LC_{50}) value decreased from 70 to 3 mg/L^{-1} with increase in exposure time from 1 to 5 h. Further increase in exposure time did not show any significance reduction in LC-50 value. Histopathological staining revealed the structural and functional changes in nanoemulsion-treated larva. Epithelial cells are swollen and showed distorted morphology. Disintegration of epithelial membrane and peritrophic membrane upon nanoemulsion treatment resulted in leakage of mid-gut lumen contents.

V. Ghosh · A. Mukherjee · N. Chandrasekaran (✉)
Centre for Nanobiotechnology, VIT University,
Vellore 632014, Tamil Nadu, India
e-mail: nchandrasekaran@vit.ac.in

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1 Introduction

Mosquito as a vector of disease causing virus and parasites have become one of the most challenging problems to public health especially in tropical and subtropical countries. Mosquitoes are the important arthropods which play predominant role as vector for the transmission of dreadful diseases dengue fever, filariasis, Japanese encephalitis (JE), malaria, schistosomiasis, and yellow fever [1, 2]. The control of mosquitoes worldwide is done primarily on sustained applications of synthetic larvicides i.e., organophosphates (e.g., temephos, fenthion, etc.) and insect growth regulators (e.g., diflubenzuron, methoprene, etc.). These larvicides are found to be very effective in mosquito larval control, but the repetitive use of these larvicides has encouraged several health and environmental concerns by outbreaks of other pest species and development of resistance [3]. These concerns have highlighted the need for development of new strategies to control mosquito larva.

Essential oils are volatile substances of natural origin found in a wide variety of plants. These plant essential oils have found applications as flavoring agent in food industry, as antimicrobials in pharmaceutical industry; as odorants and fragrances in cosmetics; and as insecticides and insect-repellent in agriculture and health [4]. The plant essential oils as insecticide/insect-repellent have drawn much attention owing to its broad spectrum of activity against wide insect species and less mammalian toxicity.

Basil (*Ocimum basilicum*) is an annual herb and belongs to the Lamiaceae family. It is used as culinary herb and grows

worldwide. *O. basilicum* essential oil is reported to have antibacterial, antidiarrheal, anti-inflammatory, antioxidative, antiulcer, blood-sugar lowering, and chemo-preventive property [5]. These applications of basil oil pertain to the presence of wide range of bioactive volatile compounds in it, e.g., eugenol, linalool, methyl eugenol, estragole, etc. The composition and oil yield of these bioactive compounds in basil essential oils depend on geographical distribution and seasonal variation and the plant part from which oil is extracted [6].

Nanoemulsions are colloidal dispersion of oil and water having droplet size in the range of 10–100 nm [7]. The very low droplet size of the nanoemulsion makes them physically stable and lesser turbid than the conventional systems. The droplet size of nanoemulsion is much smaller than the wavelength of light which results in weak scattering of light making the nanoemulsion system optically transparent [8]. The smaller droplet size consequences in a greater reduction in the gravitational force and prevents creaming or sedimentation of the nanoemulsion during prolonged storage. The small emulsion droplets also impede their coalescence by preventing the surface fluctuations as these nanometric range droplets are non-deformable. They have been increasingly used as a delivery system for lipophilic bioactive compounds because the smaller droplet size of nanoemulsion enhances the bioavailability of these bioactive compounds.

Spontaneous emulsification is one of the low energy methods of emulsion development in which an emulsion is spontaneously formulated when the constituent phases are mixed together at a specified temperature. Using this method, the droplet size of the emulsion can be varied by changing the process parameters i.e., compositions of the constituent phases, temperature, pH, ionic strength, stirring speed, etc [9]. It has been postulated that the physicochemical mechanism behind the formation of emulsion by spontaneous emulsification is the movement of a water-miscible surfactant (i.e., the surfactant with a higher hydrophile-lipophile balance; usually more than 16) from the organic phase (containing oil and surfactant) into the aqueous phase [10].

Culex quinquefasciatus Say (Diptera: Culicidae) is one of the most important vectors of filarial parasite *Wuchereria bancrofti* that causes lymphatic filariasis. *C. quinquefasciatus* mosquito transmits *W. bancrofti* more commonly in islands of the Indian Ocean, the East African Coast, and Zanzibar [11]. *C. quinquefasciatus* preferably bites at night and is highly anthropophilic. Swift industrialization and urbanization devoid of ample drainage facilities have led to amplified dispersal of the mosquito and its larvae in blocked open drains; polluted ponds and lakes; and wet pit latrines.

Very few reports are available on larvicidal activity of plant essential oil-based nanoemulsion. The surfactant concentration used in the earlier reported nanoemulsions are more than the oil concentration and the LC-50 value of the neem oil nanoemulsion was reported to be 11.75 mg/L^{-1} [12]. In the present study, we have optimized process parameters to develop basil oil nanoemulsion with less surfactant concentration by spontaneous emulsification method. Selected nanoemulsion formulation was tested for its larvicidal activity against *C. quinquefasciatus.* Histopathological staining was done to find out the structural deformities in the larval tissue sections upon nanoemulsion treatment.

2 Materials and Methods

2.1 Chemical Reagents

Basil oil (extracted from *O. basilicum*) and Tween20 (polyoxyethylene (20) sorbitan monolaurate; molecular formula = $C_{58}H_{114}O_{26}$; hydrophile-lipophile balance, HLB= 16.7) were obtained from Sigma Aldrich, India. Estragole is the major bioactive compound in the basil oil that occupies 88 % of the total peak area of GC-MS chromatograph [13]. Double distilled water (Cascada Bio Water, Pall Corporation) was used for all the experiments throughout the study.

2.2 Nanoemulsion Preparation

Nanoemulsion was prepared using basil oil, non-ionic surfactant Tween20 (HLB-16.7), and water by spontaneous emulsification method. Nanoemulsion was formulated in two steps: First, organic phase was prepared by mixing oil and surfactant. Second, the organic phase was added drop wise to water by stirring the system magnetically. Optimization of process parameters like surfactant concentration, stirring speed, and holding temperature was done to study the effect of these parameters on nanoemulsion droplet size and stability.

2.2.1 Effect of Surfactant Concentration

Nanoemulsion was prepared from different surfactant concentration keeping the concentration of basil oil constant i.e., 6 % vol/vol. Oil and surfactants were mixed in different ratios (1:1, 2:1, 3:1, 4:1, and 5:1 vol/vol) to form the organic phase. The organic phase was then added to water by magnetically stirring the system at 800 rpm.

2.2.2 Effect of Stirring Speed

The influence of stirring speed on nanoemulsion droplet size was examined by adding the organic phase (oil and surfactant) to water (25 °C) while magnetically stirring the system at varying speeds (200, 400, 600, 800, and 1,000 rpm).

2.2.3 Effect of Holding Temperature

The effect of holding temperature on nanoemulsion droplet size was investigated by incubating the organic phase (oil and surfactant) at different temperatures (25, 50, 75, and 90 °C) for 10 min prior to addition to water at 25 °C.

2.3 Droplet Size Distribution of Nanoemulsion

Droplet size of formulated nanoemulsions determined using a particle size analyzer (90 plus; Brookhaven Instruments Corporation, USA). Droplet size analysis was done by dynamic light scattering (DLS) technique. The nanometric range droplets undergo a Brownian motion in the emulsion system for which the intensity of the scattered light fluctuates which can be measured by DLS technique. The droplet radius (R) is calculated using Stokes-Einstein equation (Equation 1) [14]:

$$D = \frac{kT}{6\eta R} \quad (1)$$

where D is translational diffusion coefficient; k is Boltzmann's constant; T is absolute temperature, and η is the viscosity of the medium.

2.4 Stability

Stability of all the nanoemulsion developed by spontaneous emulsification was investigated by centrifuging at 10,000 rpm for 30 min. All the nanoemulsion formulations were stored at room temperature and observed for any physical instability like flocculation, creaming, or sedimentation.

2.5 Larvicidal Activity

2.5.1 Dose-Response Kinetics

The larvicidal effect of the basil oil nanoemulsion was evaluated according to the WHO procedures and guidelines for testing mosquito larvicides [15]. Third-instar and early-fourth-instar larvae of *C. quinquefasciatus* were treated with different concentration of nanoemulsion, and control larvae were kept in water without any treatment. According to the WHO protocol, 20 larvae of *C. quinquefasciatus* were transferred by means of droppers to the beaker containing 200 ml of water. After adding the mosquito larvae to the beaker, basil oil nanoemulsion were added in different concentrations separately. The beakers containing the mosquito larvae were then kept at room temperature of 25 °C and a photoperiod of 12 h light followed by 12 h dark (12 L:12 D). Food was not supplied to the larva throughout the study as the maximum exposure period is 24 h. Larval mortality of the basil oil nanoemulsion was monitored after different interval of exposure time. All the test studies were performed with three replicates. Dead larvae were identified as the larva which fails to move upon probing with a needle in their siphon/cervical region. The larval mortality was corrected according to Abbott's formula (Equation 2) [16]:

$$\text{Mortality}(\%) = \frac{X-Y}{X} \times 100 \quad (2)$$

where X denotes the survival (%) in the untreated control group and Y denotes the survival (%) in different treated groups.

2.5.2 Statistical Analysis

All the values were expressed as the mean±standard error (SE). LC-50 value was determined at 95 % confidence level ($P<0.05$) using probit analysis. Results having probability value <0.05 were considered as statistically significant.

2.5.3 Histopathology Staining

For histological studies, third-instar larvae of *C. quinquefasciatus* were treated with LC-50 of basil oil nanoemulsion after 5 h at 3 mg L^{-1}. The treated and untreated (control) larva were then dehydrated with series of graded ethanol (70 to 95 %). The dehydrated larva were mounted and sliced by the glass knife in the rotary microtome. These sliced larval sections were stained with haematoxylin-eosin (HE stain), and images were observed under an optical microscope.

3 Results

3.1 Effect of Surfactant Concentration on Emulsion Droplet Size

The effect of surfactant concentration on nanoemulsion droplet size was investigated by preparing nanoemulsions with fixed basil oil composition (6 %) and varying oil to surfactant mixing ratio (5:1 vol/vol, 4:1 vol/vol, 3:1 vol/vol, 2:1 vol/vol, and 1:1 vol/vol) at 25 °C. Highest droplet size of 921 nm was obtained with oil to surfactant mixing ratio of 5:1 (vol/vol) due to its lower surfactant concentration. Gradual decrease in the mean droplet diameter was observed with increase in surfactant concentration i.e., 734 nm for oil to surfactant mixing ratio of 4:1, 538 nm for oil to surfactant mixing ratio of 3:1, 230 nm for oil to surfactant mixing ratio of 2:1, and 85 nm for oil to surfactant mixing ratio of 1:1 (Fig. 1).

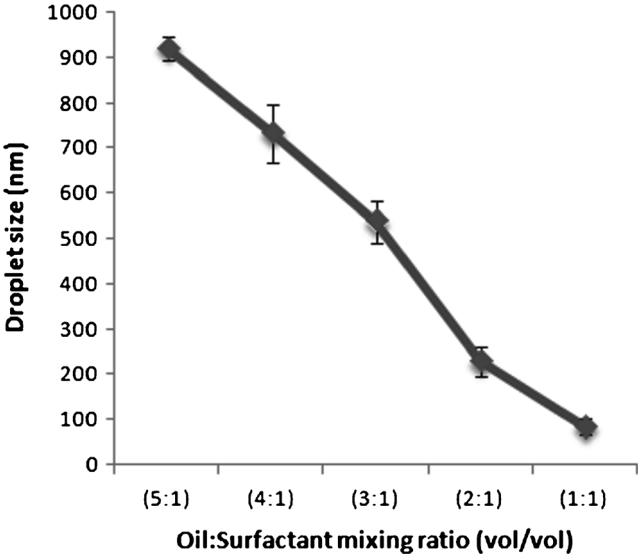

Fig. 1 Effect of surfactant concentration on nanoemulsion droplet size prepared by spontaneous emulsification method

3.2 Effect of Stirring Speed on Emulsion Droplet Size

The effect of stirring speed on the emulsion droplet size was examine by formulating different nanoemulsions with fixed oil composition (6 % vol/vol) and surfactant concentration (6 % vol/vol) and oil-Tween20 mixing ratio (1:1 vol/vol) at 25 °C. Organic phase (oil and water) was added to water stirred at varying speed (200, 400, 600, 800, and 1,000 rpm). Nanoemulsion with droplet size of 265 nm was obtained when the system was magnetically stirred at 200 rpm while adding organic phase to water. Decreasing trend in mean droplet size was observed with increase in stirring speed i.e., 237 nm (400 rpm), 155 nm (600 rpm), and 85 nm (800 rpm) (Fig. 2). When the stirring speed was further increased to 1,000 rpm, the reduction in droplet size was not significant.

3.3 Effect of Holding Temperature on Emulsion Droplet Size

Prior addition to water, the organic phase was incubated at different temperature (25, 50, 75, and 90 °C) for 10 min to study the effect of holding temperature on emulsion droplet size. Organic phase was sealed with thin film to avoid evaporation, and also organic phase volume was measured before and after heating to make sure that pre-heating did not cause oil phase evaporation. For the temperature study, other process parameters such as oil concentration (6 % vol/vol), surfactant concentration (6 % vol/vol), oil to surfactant mixing ratio (1:1 vol/vol), and stirring speed (800 rpm) were constant. Incubation of the organic phase at holding temperature of 25 °C for 10 min resulted in droplet size of 85 nm. Rise in holding temperature exhibited decreasing trend in droplet size i.e., 45 and 28 nm for holding temperature of 50 and 75 °C, respectively (Fig. 3). Significant reduction in droplet size was not observed when the holding temperature was further increased to 90 °C.

3.4 Effect of Droplet Size on Emulsion Stability

Table 1 shows the stability and visual appearance of all the basil oil nanoemulsions formulated by spontaneous emulsification method. Emulsion stability and optical transparency can be correlated with mean droplet size. Greater stability was observed with lower the droplet size. BT20/11/800/75 showed the greater stability for 3 months. The nanoemulsion with low droplet size (<100 nm) were optically transparent,

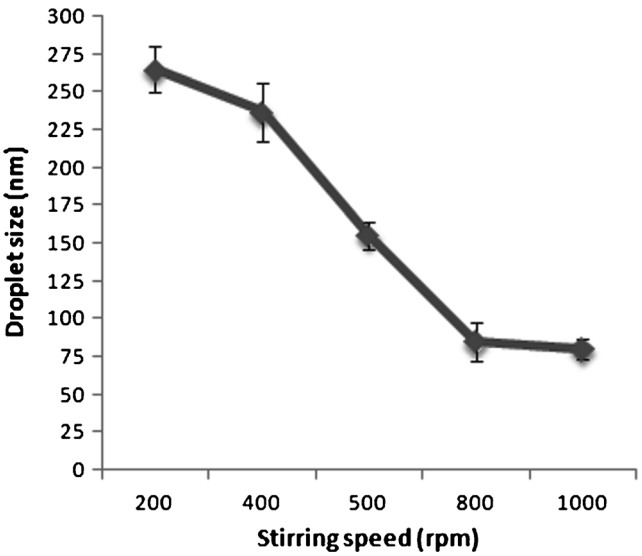

Fig. 2 Effect of stirring speed on nanoemulsion droplet size prepared by spontaneous emulsification method

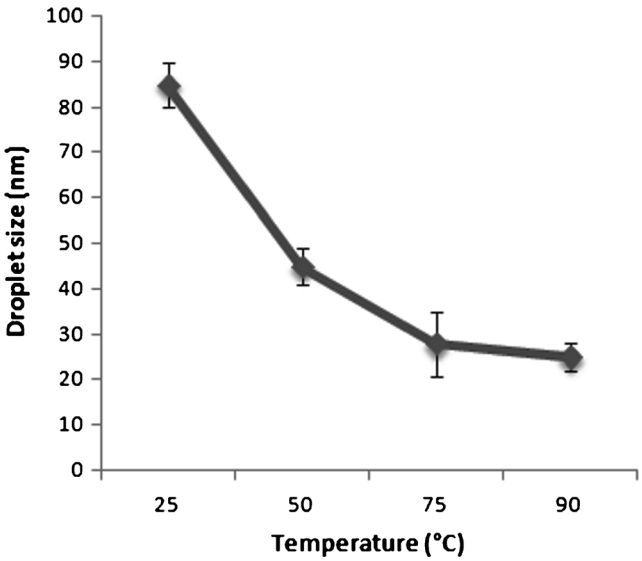

Fig. 3 Effect of holding temperature on nanoemulsion droplet size prepared by spontaneous emulsification method

Table 1 Stability of different basil oil nanoemulsion formulations

Formulation code	Oil to surfactant mixing ratio (vol/vol)	Stirring speed (rpm)	Holding temperature (°C)	Visual appearance	Stability (no. of days)
BT20/51/800/25	5:1	800	25	Turbid	1
BT20/41/800/25	4:1	800	25	Turbid	2
BT20/31/800/25	3:1	800	25	Turbid	5
BT20/21/800/25	2:1	800	25	Turbid	7
BT20/11/800/25	1:1	800	25	Transparent	24
BT20/11/1,000/25	1:1	1,000	25	Transparent	25
BT20/11/600/25	1:1	600	25	Turbid	4
BT20/11/400/25	1:1	400	25	Turbid	2
BT20/11/200/25	1:1	200	25	Turbid	1
BT20/11/800/50	1:1	800	50	Transparent	58
BT20/11/800/75	1:1	800	75	Transparent	90
BT20/11/800/90	1:1	800	90	Transparent	90

whereas the formulations with larger droplet size were turbid/milky-white in color.

Of all the formulated nanoemulsions, BT20/11/800/75 formulation showed greater stability and low droplet size. Figure 4 shows the droplet size distribution of BT20/11/800/75. Emulsion droplets were in the range of 10–35 with mean droplet size of 28 nm. The polydispersity index of this nanoemulsion formulation was found to be 0.102 indicating a very narrow droplet size distribution. Figure 5 shows the transmission electron micrograph of BT20/11/800/75 formulation. Nanoemulsion droplets were spherical in shape. It also provided additional information on droplet size.

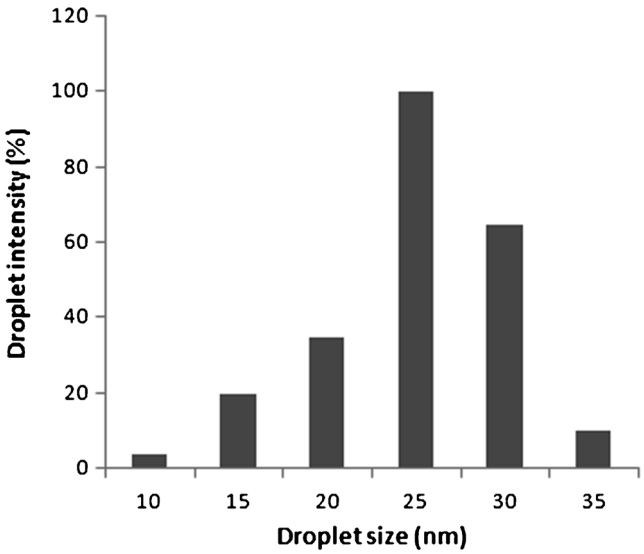

Fig. 4 Droplet size distribution of BT20/11/800/75 basil oil nanoemulsion prepared by spontaneous emulsification method using basil oil (6 %), Tween20 (6 %), and water at 800 rpm

3.5 Larvicidal Activity

Based on droplet size and stability, BT20/11/800/75 basil oil nanoemulsion formulation was selected for studying larvicidal activity.

3.5.1 Dose-Response Kinetics

Basil oil nanoemulsion BT20/11/800/75 demonstrated dose- and time-dependent killing of mosquito larva. The study was carried out using different concentration (5, 10, 25, and 50 ppm) of basil oil nanoemulsion to check the larvicidal efficacy in different time interval (1, 2, 3, 4, 5, and 6 h). Of the total nanoemulsion (BT20/11/800/75), 5 ppm resulted in 50 % larval mortality in 4 h, whereas 10 ppm of BT20/11/800/75 achieved 50 % larval mortality only in 3 h. Increase in the nanoemulsion concentration to 25 and 50 ppm resulted in

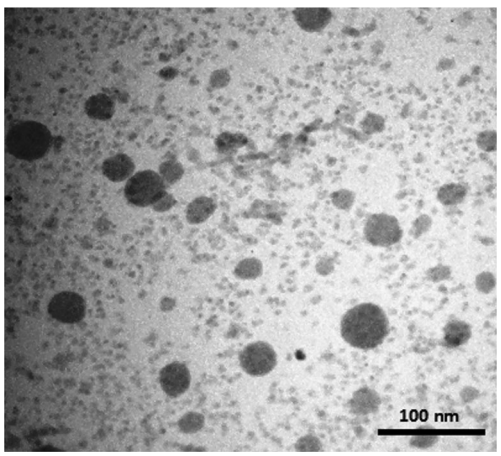

Fig. 5 Transmission electron micrograph showing the spherical droplets of BT20/11/800/75 basil oil nanoemulsion

complete loss of larval viability in 6 and 5 h, respectively (Fig. 6). No significant difference was observed between larval viability after 6 and 24 h in all the treatment groups. Suitable control groups were maintained by treating with Tween20, and result showed that all the larvae were live (result not shown) indicating that Tween20 is not an effective larvicidal agent and the larvicidal activity was only due to basil oil.

3.5.2 Determination of LC-50 Value

Toxicity effect was also reported as LC-50 representing the concentrations in parts per million with 50 % larvae mortality rate at different exposure period. LC-50 value of BT20/11/800/75 formulation decreased with increase in exposure time (Fig. 7). LC-50 value of BT20/11/800/75 nanoemulsion was found to be 70 mg/L^{-1} after 1 h of treatment against *C. quinquefasciatus* larva. With increase in exposure time to 2, 3, 4 and 5 h, the LC-50 values reduced to 60, 10, 5, and 3 mg/L^{-1}, respectively. Results pertaining to LC-50 was obtained using probit analysis software and revealed that the values were significant, based on 95 % (P<0.05) confidence limit. ANOVA and Student's *t* test analysis were also found to be significant. Further increase in exposure did not show any significant reduction in LC-50 value of BT20/11/800/75 nanoemulsion (result not shown).

3.5.3 Histopathology Staining

A series of longitudinal and cross sections of abdominal and thoracic regions of *C. quinquefasciatus* larva was examined by staining with hematoxylin/eosin (HE stain). This study served in finding out structural and functional deformities in *C. quinquefasciatus* larva upon treatment with LC-50 of BT20/11/800/75 nanoemulsion i.e, 3 mg/L^{-1} for 24 h. *C. quinquefasciatus* larva morphology can be divided into

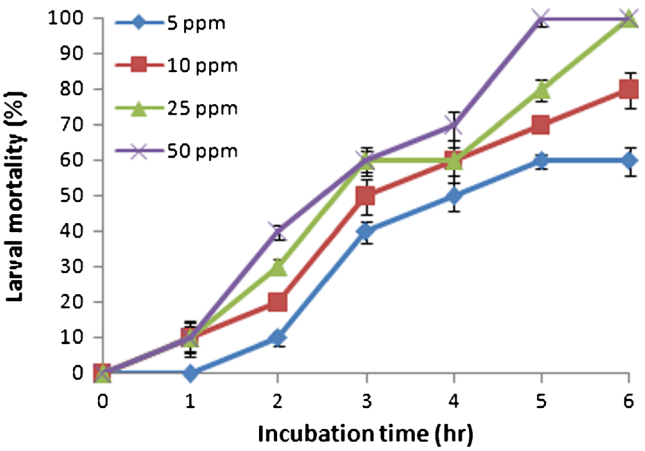

Fig. 6 Dose-response kinetics of BT20/11/800/75 basil oil nanoemulsion against *Culex quinquefasciatus* larva

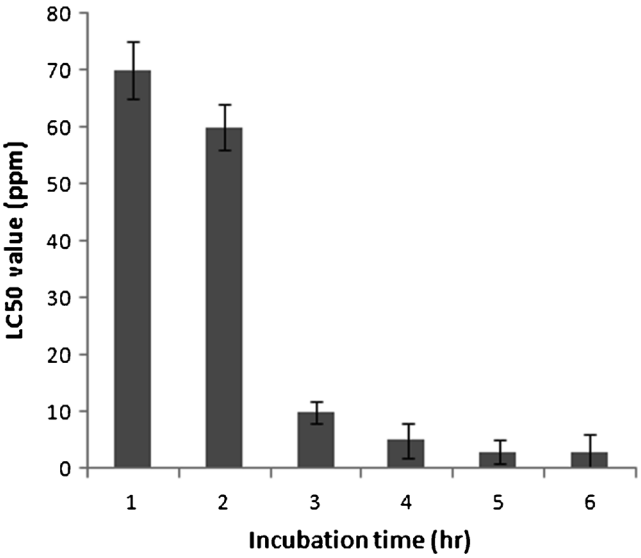

Fig. 7 LC 50 values of BT20/11/800/75 basil oil nanoemulsion against *Culex quinquefasciatus* larva at different interval time

thoracic and abdominal regions. Figure 8 shows the longitudinal section of *C. quinquefasciatus* larva. Gut is further subdivided into fore-gut, mid-gut, and hind-gut. Figure 8 shows the longitudinal section and Fig. 9 shows the cross section of *C. quinquefasciatus* larva. Control/untreated *C. quinquefasciatus* larva shows that it consists of a layer of epithelial cells (EP) that rest on basement membrane. This layer of epithelium is separated from the food bolus (FB) by a layer called peritrophic membrane (PM) (Figs. 8a, c and 9a). Treatment with BT20/11/800/75 nanoemulsion resulted in deformities of epithelial cell in fore-gut (Fig. 8b), mid-gut, and hind-gut regions (Figs. 8d–f and 9b–d). Dead larva shows complete disintegration of epithelial membrane and peritrophic membrane which would have resulted in leakage of mid-gut contents (Figs. 8d and 9b).

4 Discussion

Basil oil nanoemulsion was stabilized by non-ionic surfactant Tween20 as emulsifier that reduces the interfacial tension at oil/water interface and also facilitates stearic stabilization. Hydrophile-lipophile balance (HLB) value of the surfactants play important role in determining the type of emulsion. High HLB value surfactants aid in formulating oil-in-water nanoemulsion, whereas low HLB value of surfactants formulate water-in-oil surfactants. Tween20 was preferred to be used as emulsifier as it has a high HLB value of 16.7. Also, being non-ionic, in nature, Tween20 stabilizes the emulsion through stearic stabilization by repelling the bulky hydrophobic groups of the surfactant. Polysorbates are low-molecular weight emulsifiers when compared to polymeric surfactants;

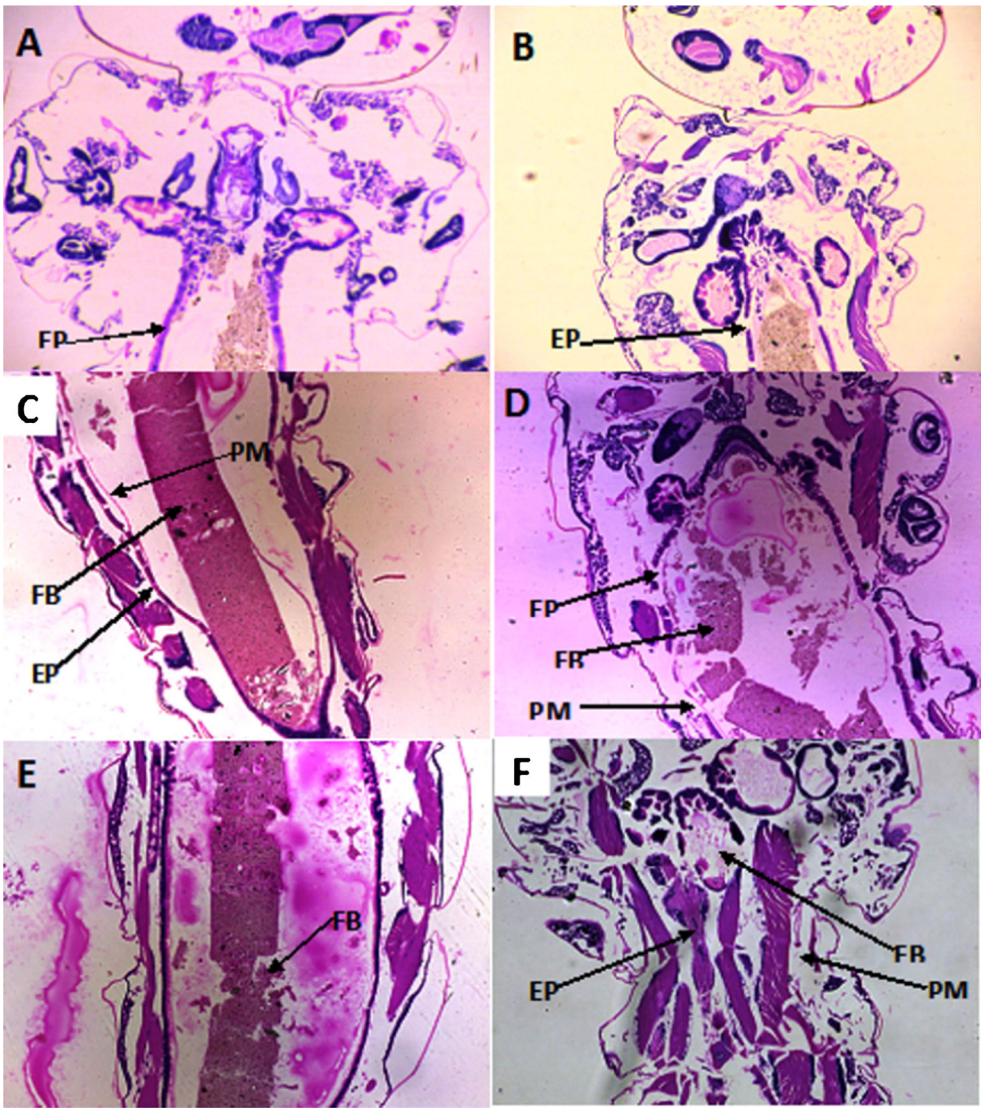

Fig. 8 Longitudinal section of fore-gut region of control/untreated (**a**) and fore-gut region of BT20/11/800/75 basil oil nanoemulsion treated (**b**); mid-gut region of control/untreated (**c**) and mid-gut region of BT20/11/800/75 basil oil nanoemulsion treated (**d**, **e**, and **f**) *Culex quinquefasciatus* larva. Epithelial membrane (*EP*), food bolus (*FB*), peritrophic membrane (*PM*)

hence they get adsorbed onto oil droplets more efficiently and minimize emulsion droplet size.

Surfactant concentration played major role in determining droplet size. Increase in surfactant concentration resulted in lower droplet size [13, 17, 18]. This can be attributed to the fact that augmented adsorption of Tween20 molecules reduces the interfacial tension at oil/water interface facilitating the formation of smaller droplets. Due to its high hydrophile-lipophile balance (HLB) value, Tween20 molecules diffuse from the organic phase (oil and surfactant) to the aqueous phase and forms low droplet size emulsions.

Stirring speed also exhibited effect on emulsion droplet size. Increase in stirring speed of the magnetic stirrer facilitated formation of low droplet diameter. The applied mechanical energy through the magnetic stirrer would have distributed the different constituent phases of emulsion suggesting the requirement of mild agitation of oil, water, and surfactant during spontaneous emulsification [19].

Effect of holding temperature on nanoemulsion droplet size was studied by keeping the organic phase in varying temperature prior to the addition to water. Nanoemulsion droplet size decreased with increase in the holding temperature. This attributes to the fact that oil viscosity decreases with increase in temperature which would have facilitated the formation of emulsion with reduced droplet diameter [20].

In the present study, we have optimized the process parameters to obtain nanoemulsion with less surfactant concentration. Also the formulated nanoemulsion exhibited very low LC-50 value of 3 mg/L^{-1}. This result suggests that basil oil nanoemulsion is an effective larvicide at very low concentration. Hence, the approach is cost effective too as very small quantity of basil oil is required to kill mosquito larva.

Histopathological staining of larval tissue samples illustrated the damage to epithelial membrane, peritrophic membrane, and basement membrane of fore-, mid-, and hind-gut regions. The structural disintegration caused leakage of gut lumen

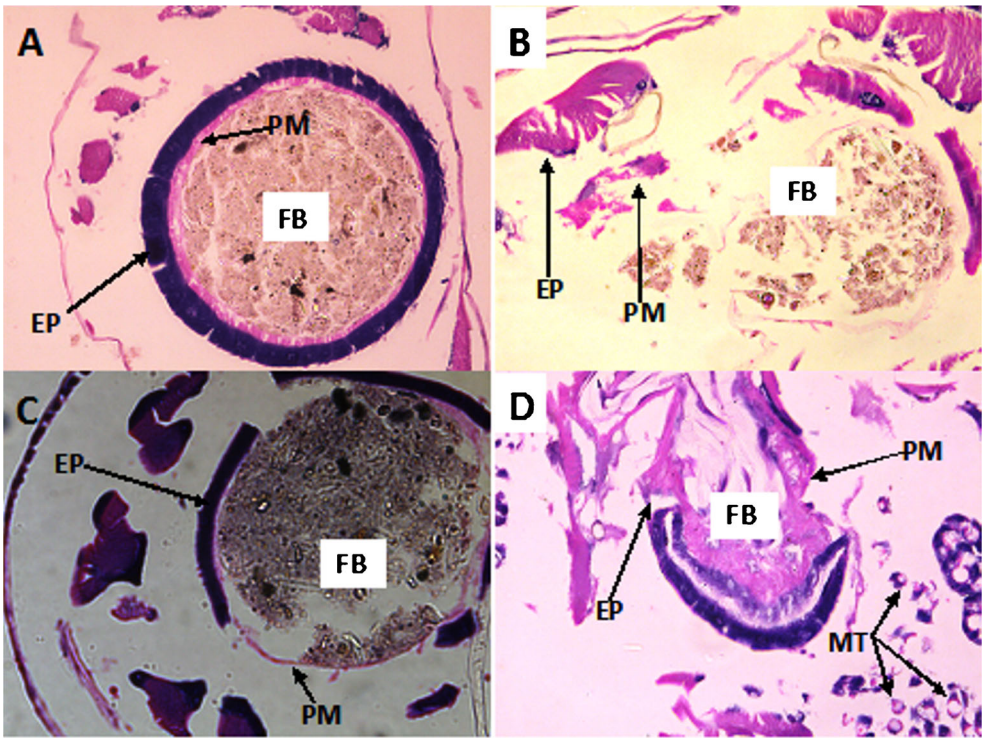

Fig. 9 Cross section of control/untreated (**a**) and BT20/11/800/75 basil oil nanoemulsion treated (**b**) *Culex quinquefasciatus* larva. Epithelial membrane (*EP*), food bolus (*FB*), peritrophic membrane (*PM*)

contents and also would have led to functional deformities in nanoemulsion-treated larva. hematoxylin/eosin stain (HE stain) is one of the popular methods of staining in histology. Hemalum is formed from complex of aluminum ion and hematin (the oxidation product of hematoxylin) and is the active ingredient in the hematoxylin/eosin staining solution. Hemalum has a blue/purple color and it stains nucleic acids. Eosin is the counter stain which is pink in color and stains proteins. When a tissue is stained with hematoxylin/eosin staining solution, then the nuclei are stained blue, but the cytoplasm along with the extracellular matrix stains pink [21]. The hematoxylin/eosin staining reveals ample information about the structural difference in control and treated tissue samples.

The larvicidal activity of the basil oil nanoemulsion can be attributed to the insecticidal property of estragole which is the major bioactive compound in the basil oil [13]. Eugenol and linalool [22, 23] also would have augmented the larvicidal activity of basil oil nanoemulsion that constitutes 7.8 and 4.2 % of the total peak area of GC-MS chromatograph, respectively [13].

5 Conclusion

Stable nanoemulsion was formulated using basil oil, Tween20, and water by spontaneous emulsification method. Surfactant concentration, stirring speed, and holding temperature demonstrated a significant effect on emulsion droplet size and stability. Selected basil oil nanoemulsion exhibited mosquito larvicidal activity against *C. quinquefasciatus* larva. The findings of the present study suggest that basil oil nanoemulsion as a potent larvicide for mosquito vector control.

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References

1. James, A. A. (1992). Mosquito molecular genetics: the hands that feed bite back. *Science, 257*, 37.
2. Gubler, D. J. (1998). Resurgent vector borne diseases as a global health problem. *Emerging Infectious Diseases, 4*, 442.
3. Yang, Y. C., Lee, S. G., Lee, H. K., Kim, M. K., Lee, S. H., Lee, H. S. (2002). A piperidine amide extracted from Piper longum L. fruit shows activity against Aedes aegypti mosquito larvae. *Journal of Agricultural and Food Chemistry, 50*, 3765.
4. Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods-a review. *International Journal of Food Microbiology, 94*, 223.
5. Sajjadi, S. E. (2006). Analysis of the essential oils of two cultivated basil (*Ocimum basilicum* L.) from Iran. *Daru, 14*(3), 128.
6. Hussain, A. I., Anwar, F., Sherazi, S. T. H., Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry, 108*, 986.

7. McClements, D. J. (2011). Edible nanoemulsions: fabrication, properties, and functional performance. *Soft Matter, 7*, 2297.
8. McClements, D. J. (2002). Colloidal basis of emulsion color. *Current Opinion in Colloid & Interface Science, 7*, 451.
9. Bouchemal, K., Briançon, S., Perrier, E., Fessi, H. (2004). Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimization. *International Journal of Pharmaceutics, 280*, 241.
10. Anton, N., & Vandamme, T. F. (2009). The universality of low-energy nano-emulsification. *International Journal Pharmaceutics, 377*, 142.
11. Pedersen, E. M., Kilama, W. L., Swai, A. B. M., Kihamia, C. M., Rwiza, H., Kisumku, U. M. (1999). *Bancroftian filariasis* on Pemba Island, Zanzibar, Tanzania: an update on the status in urban and semi-urban communities. *Tropical Medicine & International Health, 4*, 295.
12. Anjali, C. H., Sharma, Y., Mukherjee, A., Chandrasekaran, N. (2012). Neem oil (*Azadirachta indica*) nanoemulsion—a potent larvicidal agent against *Culex quinquefasciatus. Pest Management Science, 68*(2), 158.
13. Ghosh, V., Mukherjee, A., Chandrasekaran, N. (2013). Ultrasonic emulsification of food-grade nanoemulsion formulation and evaluation of its bactericidal activity. *Ultrasonic Sonochemistry, 20*, 338.
14. Tadros, T., Izquierdo, P., Esquena, J., Solans, C. (2004). Formation and stability of nanoemulsions. *Advances in Colloid and Interface Science, 108–109*, 303.
15. WHO guidelines for laboratory and field testing of mosquito larvicides. 2005. CDS/WHOPES/GCDPP/05.13.
16. Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology, 18*, 265.
17. Ghosh, V., Saranya, S., Mukherjee, A., Chandrasekaran, N. (2013). Antibacterial microemulsion prevents sepsis and triggers healing of wound in wistar rats. *Colloids and Surfaces B: Biointerfaces, 105*, 152.
18. Ghosh, V., Saranya, S., Mukherjee, A., Chandrasekaran, N. (2013). Cinnamon oil nanoemulsion formulation by ultrasonic emulsification: investigation of its bactericidal activity. *Journal of Nanoscience and Nanotechnology, 13*(1), 114.
19. Saberi, A. H., Fang, Y., McClements, D. J. (2013). Fabrication of vitamin E-enriched nanoemulsions: factors affecting particle size using spontaneous emulsification. *Journal of Colloid and Interface Science, 391*, 95.
20. Sahin, S., & Sumnu, S. G. (2006). *Physical properties of foods*. New York: Springer.
21. Fischer, A. H., Jacobson, K. A., Rose, J., Zeller, R. (2008). Hematoxylin and eosin staining of tissue and cell sections. *Cold Spring Harbour Protocols*. doi:10.1101/pdb.prot4986.
22. Champakaew, D., Choochote, W., Pongpaibul, Y., Chaithong, U., Jitpakdi, A., Tuetun, B., et al. (2007). Larvicidal efficacy and biological stability of a botanical natural product, zedoary oil-impregnated sand granules, against *Aedes aegypti* (Diptera, Culicidae). *Parasitology Research, 100*, 729.
23. Nour, A. H., Elhussein, S. A., Osman, N. A., Nour, A. H., Yusoff, M. M. (2009). A study of the essential oils of four sudanese accessions of basil (*ocimum basilicum* l.) against anopheles mosquito larvae. *American Journal of Applied Sciences, 6*(7), 1359.