Role of high shear mixing in improving stability and bio-efficacy of botanical oil in water formulation for early stage mosquito eradication

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ABSTRACT

Neem based formulations conventionally being used as dustable powders, wettable powders, emulsifiable concentrates etc for controlling insects and mosquitoes. These formulations are prepared by mechanical mixing or low shear mixing processes. Among these formulations solvent based EC formulation is more common in use, though it have many drawbacks like phyto-toxicity, flammability, environmental contamination and dermal toxicity. Along with these drawbacks stability of the active ingredient is the most concerned problem, as the active ingredients are unstable in solvent based formulations. Neem oil based oil in water emulsion formulation (EW) formulation may be a safer alternative to EC formulation. In the present study, composition and process technology involved in Neem EW formulations were optimized. Different types of the EW formulation has already been formulated, but no investigations were made to prove the influence of high shear mixing, turbulence flow, time duration on the stability of formulation and its bio-efficacy. The main objective of the study is to determine the effect of turbulence stirring duration on droplet size and emulsion stability. The 60 min turbulence mixing at 3600 rpm decreased the particle size from 6.7 to 1.2 μm. The prepared formulation stability was further confirmed by different analytical techniques like HPLC and FTIR. The 60 min high shear turbulence stirring enhanced the bio-efficacy in terms of 99 % mortality after 24 h at concentration of 500 ppm of 10 EW.

1. Introduction

Now days, mosquito prevalence is increasing worldwide and has becomes a vector for spreading many harmful pathogenic diseases (Kulkarni and Biradar, 2017). For eradication of this ever increasing mosquito problem by synthetic pesticides will exhaust the environment. Non judicious use of these pesticides now exhaust the environment and harmful towards non-target organisms. Owing to the concern of human and environment safety. A safe, effective and environment friendly pesticides alternates are required to combat with mosquito problems in a greener way. Many botanical pesticides have been replaced the existing synthetic pesticides. All the botanical pesticides are enriched with several bioactive compounds or phytochemicals viz. alkaloids, flavonoids phenolic and terpenoids (Phuagphong et al., 2015). Therefore, botanical pesticides could be effectively replacing the presently available synthetic pesticides. Neem, a crucial plants loaded with bioactive compounds can be effectively used against 200 insect pests without harming the environment and non-target organisms (Rimpi et al., 2010). Azadirachtin (C35H44O16), a tetranortriterpenoid most significant active compound in neem which has wide range of properties such as growth inhibition, oviposition deterrent, and repellent (Sundaram et al., 1995).

Conventional formulations such as dustable powders, wettable powders, emulsifiable concentrates, solutions, etc. could cause problems like eco-toxicity, dermal toxicity and residual toxicity (Slavica et al., 2012a, b) Due to these reasons conventional formulations are being improved to develop new low risk, environmental friendly botanical based formulations. Coherently, adjuvant technologies improved particularly to convene the requirements of user as well as ecological protection or for better bio-efficacy and long term persistence of the active ingredient. Consequently, environment friendly water based formulations like oil-in-water emulsions (EW), aqueous suspension concentrates (SC), and aqueous capsule suspensions (CS) etc. are the new replacement for conventional pesticide formulations. These formulations increases the efficacy of products through a proper selection of balanced components in the formulation (Gasic and Oreskovic, 2006; Knowles, 2005).

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Emulsifiable concentrate (EC) is a most commonly used known formulation for botanical pesticides. EC formulations are a blend of active ingredients along with organic solvent, and one or more surfactants. Aromatic paraffin, solvents such as xylene and sovesso are most common solvents for EC formulations (Chin et al., 2012). Due to organic solvents, these formulations cause many negative impacts to environment and users like dermal toxicity to the applicator and pollute the environment (Zhao et al., 2009). In current years a huge requirement to replace solvent ECs with oil-in water (O/W) emulsions, technically known as EWs. Harmful effects of EC can overcome to a greater extent by emulsion in water (EW) formulation. In emulsion in water (EW), an active ingredient is dispersed as fine oil-phase droplets in water and solvent used in very low amount. As EW formulations are water based systems so, they are not only environmentally suitable but also less toxic to applicator as well as to plants, safer to transport and free from inflammability problem due to high flash point (Malqueen, 2005). Neem based Emulsion in water formulation have emulsion stability problem. In Neem based Emulsion in water (EW), emulsion droplet agglomerate and lead to phase separation which results in decrease of emulsion stability and reduced the bio-efficacy. Hence, emulsion stability is very important for good bio-efficacy. Scientist has developed many types of emulsions but no study is related to the stability enhancement by long term high shear mixing of oil in water emulsion.

The main aim of the present study, to develop neem water based formulation (Emulsion, oil in water (EW)) and enhancement of the stability of neem EW formulation by using high turbulence mixing along with surfactants and polymers. The most important aim of this study is the application of neem EW in mosquito control by eradicating the early stages of mosquito in breeding aquatic bodies. This study is very significant for improving the stability of Neem oil emulsion in water for efficient and eco-friendly mosquito larvae control in different aquatic stages of mosquito in breeding aquatic bodies. This study is very significant for improving the stability of Neem oil emulsion in water for efficient and eco-friendly mosquito larvae control in different aquatic bodies. This formulation technique is very helpful for indoor and outdoor applications and gives prolonged period of mosquito control only after one spray application.

2. Result and discussion

2.1. Finalized composition of neem EW formulation

Neem EW was developed with 5% and 10% w/w of Neem Oil. Inert ingredient was optimized as per the wt of active ingredient for improved stability. Percent composition of optimized EW formulations is as shown in Table 1.

Neem 10 EW (F2) was taken as optimized formulation for further physico-chemicals and bio-efficacy evaluation experiments.

2.2. Physico-chemical analysis

2.2.1. Active ingredient

Main active ingredient of Neem oil is Azadirachtin. The antifeedent and growth inhibiting activities of azadirachtin has been studied in different insect pests (Govindachari et al., 1996). Azadirachtin is very unstable in water and decomposed 50% in water after 14 days of storage (Yongqing et al., 2004). In neem EW formulation rate of degradation was minimized in longer duration of high shear mixing. Maximum degradation observed in 15 min stirred sample i.e. 30 % degradation and very less in 60 min stirred samples i.e. only 5–10%. It confirms that oil in water surface active agents is continuous and uniformly distributed over the smaller droplets and protects the active ingredient from degradation. In 60 min stirred samples droplet size was very small. So, the rate of degradation of azadirachtin reduced sequentially from 15 min stirred sample to 60 min stirred samples (Table 2).

The HPLC chromatograms of 15 min and 60 min stirred samples before and after storage are shown in Figure 1. The study proves that in finer droplet size active ingredients also stabilized and prevent degradation.

2.2.2. pH

Neem oil active ingredient is highly stable in neutral and unstable in alkaline and acidic conditions (Jarvis et al., 1998). The pH value of the prepared emulsions was near to neutral pH value i.e. 6.9. The pH value remain the same after 14 days of storage in 60 min stirred sample (Table 2). Neutral pH shows that Neem EW is the stabilized formulation for azadirachtin content. Time duration of stirring cause no change in pH of the dispersing medium.

2.2.3. Stability

Emulsion in water are kinetically stable systems by various emulsifiers (Gasic et al., 2012). Physical and chemical parameters were checked at 0 °C and 54 °C for 7 days and 14 days respectively in 60 min stirred sample. Physical stability was improved by viscosity inducers like xanthan gum, waxes etc (Zhang et al., 2016). These viscosity inducers reduced the phase separation, creaming and sedimentation in emulsion system. Physical stability parameters are on the basis of phase separation, creaming or sedimentation or pH change during storage (Table 2).

2.3. Droplet size distributions in neem EW formulation

The droplet size plays a significant role in emulsion properties like stability, dispersion in water medium and long persistence on applied surface (Aichele et al., 2016). Results shown in Table 3 indicates that with increase in stirring time under high shear mixer at 3600 rpm, there is decrease in droplet size. Droplet size in 15 min stirred sample is 6.7 microns which is reduced to 1.2 microns in 60 min stirred sample. Above 45 min, no further reduction of droplet size was observed.

Long duration of stirring under high shear, remarkably change the emulsion droplet size. Internal oil phase is broken into small droplets by long period agitation. The short period agitation results into bigger droplets while long period agitation results into small droplets. Reduction up to 1.2 microns from 6.7 microns in 45 min stirred sample but beyond this very less reduction in droplet size, due to reaching of

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**Table 1. Percentage composition (w/w) of EW Formulations.**

| S.No. | Name of Ingredients Active or Inert                          | *F1(%)w/w) | *F2(%)w/w) |
|-------|-------------------------------------------------------------|------------|------------|
| 1.    | Neem oil                                                    | 5          | 10         |
| 2.    | Ethoxylated nonylphenol (Non ionic emulsifier)              | 2.5        | 2.5        |
| 3.    | Span 80 (Anionic emulsifier)                                | 2.5        | 2.5        |
| 4.    | Poly ethylene glycol (Anti freezing agent)                 | 3.0        | 3.0        |
| 5.    | Xanthan gum (Viscosity enhancer for gravitational stabilization) | 0.16       | 0.16       |
| 6.    | Butanol defoamer (for foaming reduction during processing and application) | 0.3        | 0.3        |
| 7.    | Distilled Water (bulk medium)                              | q.s        | q.s        |
|       | **Total**                                                   | 100        | 100        |

*F1 = 5% Neem EW.

*F2 = 10% Neem EW.
turbulence to maximum, beyond which further breakdown is insignificant (Issaka et al., 2010). Finer droplet sized emulsion systems have long term stability (Ghosh et al., 2015). The 60 min stirred sample has finer range of droplet size which is more stable after 14 days of storage as compared to less stirred samples (Figure 2).

2.4. Emulsion stability studies

Emulsion stability can be evaluated by emulsion turbidity. Emulsion turbidity depends upon the particle concentration and particle size of the emulsion (Song et al., 2000a, b). Lesser the size of droplet more will be the emulsion turbidity. Stabilized turbidity gives maximum emulsion stability. So, for maximum emulsion stability particle size of the emulsion should be minimum. Particle size was reduced from 6.7 to 1.2 μm by high shear mixing in 60 min (Table 3). Therefore, emulsion turbidity is high in 60 min stirred samples where, particle size was 1.2 μm. Emulsion stability result illustrated in (Table 4).

Emulsion physical appearance after dilution is shown in Figure 2. The results in Table 4, indicated that the sample prepared by 15 min stirring (having droplet size 6.7 μm) has minimum emulsion stability (78.8 % after 24 h). The emulsion stability gradually improved with stirring duration. The emulsion stability was maximum in 60 min stirred sample i.e 96.7% (Figures 4 and 5). Emulsion physical appearance after dilution is shown in Figure 2.

2.5. Role of turbulence flow during high shear mixing in emulsion stabilization

All the physic-chemical analysis shows that high shear mixing stabilized the Neem EW formulation. A fine droplet in emulsion gives the stabilized emulsion system. Turbulence mixing is a very important in emulsion drop breakup into fine droplets (Kolmogorov, 1949; Shinner and Church, 1960). In turbulence mixing larger eddies (flow structures) formed in high speed mixing which results into finer droplets. Hinze-Kolmogorov theory explains the utilization of turbulent eddies concept to define droplet size. Drop break –up occurs due to collisions of turbulent eddies of comparable size drops (Liao and Lucas, 2009). According to Kolmogorov theory smaller spectrum of eddy sizes created at higher velocity gradients (u’/x) and their kinetic energy dissipated into heat and unable to break the droplets. Larger eddies are energy bearing eddies and break the droplet into smaller drops. The finer droplet size is explained by Kolmogorov by two Eq. (1) (see Figure 3).

\[ u' (x) = C \varepsilon^{1/3} \frac{x^{1/3}}{\rho^{1/3}} \]

\[ x = \text{eddy size} \]
\[ \rho = \text{mass density} \]
\[ C = \text{constant} \]
\[ \varepsilon = \text{energy dissipated per unit time} \]

Eddies generate large pressure change (Y) in liquid medium and cause droplet break up of diameter D(x). The turbulence generated by silverson during mixing causes uniform size and shape droplets which

| Stirring time (in minutes) | 15 | 25 | 35 | 45 | 60 |
|---------------------------|----|----|----|----|----|
| Active ingredient degradation (%) | 30 | 25 | 25 | 10 | 5 |
| pH                        | 6.7 | 6.8 | 6.8 | 6.7 | 6.9 |
| Emulsion analysis         | Phase Separation | Creaming | Slight Separation | Good dispersion | Highly stable |

Table 2. EW Stability analysis after 14 days of storage at room temperature.

| Time period of mixing (min) | 50 μm | d 90μm |
|-----------------------------|-------|--------|
| 15                          | 6.7   | 13.6   |
| 25                          | 1.6   | 2.8    |
| 35                          | 1.3   | 1.9    |
| 45                          | 1.3   | 1.8    |
| 60                          | 1.2   | 1.8    |

Table 3. Droplet size variation with high shearing mixing in varying time period.
results into stabilized emulsion system during long duration mixing (Figure 6). During high shear mixing two droplets interfacial tension is maintained by turbulence eddies due to which particle coalescence of droplets not occurs and stability of emulsion increased as per Eq. (2) (Acevedo-Malave, 2016).

\[
D(x) = C \epsilon^{-2.5} \gamma^{1.5} \rho^{1.5}
\]  

(2)

2.6. FTIR characterization

The FTIR spectra of neem oil and the two samples, fresh and stored EW formulations are described in Figure 7. FTIR spectra shows alkanes (C–H stretch at 2932.07 cm\(^{-1}\) and 2856.52 cm\(^{-1}\) along with C–C stretch at 1465.17 cm\(^{-1}\) ), aromatics (C–C stretch in ring at 1465.17 cm\(^{-1}\) ) esters (C–O stretches at 1748.32 cm\(^{-1}\), 1240.44 cm\(^{-1}\), 1099.37 cm\(^{-1}\)) and alcohols (3009.16 cm\(^{-1}\) ) have been identified in the oil. In case of the EW, corresponding peaks of neem oil which remain same are esters (C–O stretches at 1742.14 cm\(^{-1}\) ), alkenes (C–H stretch at 2919.45 cm\(^{-1}\) and 2852.77 cm\(^{-1}\) along with C–C stretch at 1466.21 cm\(^{-1}\) ). As the formulation is oil in water based emulsion, high frequency peak has been observed at 3255.17 cm\(^{-1}\) due to extensive hydrogen bonding due to presence of high concentration of water. At 1539.79 cm\(^{-1}\) peak is due to xantham gum polymeric structures. So, the neem oil constituents remain same in water based EW formulated product and no chemical reaction with inert takes place in the formulated EW formulation.

2.7. Anti larval activity of neem based 10% EW formulation

Neem is the most extensively used biopesticide in agriculture and household applications. Neem has azadiractin as main active ingredient which has many insectical properties like growth inhibitory, deterrenary, growth regulatory, larvicidal etc (Schmutterer, 1990). Neem in water based formulations make a stable emulsion with appropriate surfactant. Main criteria of stable emulsion are good dispersibility, spreading and fine droplet size. The stability parameters further improves the bio-efficacy of emulsion. The developed emulsion formulations in different concentrations of 100ppm, 200ppm and 500ppm gave maximum mortality i.e. 12.1 ± 0.92, 50.6 ± 0.85, 90.68 ± 0.78% respectively after 24 h in 60 min stirred formulation samples. After 1 week application of 60 min stirred sample gave maximum mortality was 55.7 ± 0.82, 95.69 ± 0.82, and 99.7 ± 0.76 in 100ppm, 200ppm and 500ppm respectively.

The results shown in Table 5 and graph II, indicates that 500 ppm concentration of 60 min stirred sample of Neem EW is the effective concentration for efficient mosquito control applications. The long duration stirred samples have small particle size i.e. 1.8 μm which

![Figure 2. a. particle size distribution in 15 minutes stirred sample. b. particle size distribution in 60 minutes stirred sample.](image2)

![Figure 3. a. Turbidity in varying duration of high shearing mixing. b. Emulsion stability in varying duration of high shearing mixing.](image3)
reduced the agglomeration and dispersed homogeneously in aqueous solution for longer period of time which enhances the larval morality for long period of time (Farajollahi et al., 2012).

3. Materials and method

Location
Formulation development experiments and Bio-ef ficacy experiments conducted in Institute of Pesticide Formulation Technology, Gurugram.

Active Material and Inert Materials
Neem oil was purchased from SGS biotech Manesar, Gurugram, Haryana, India.
Xanthan gum (2%), Poly ethylene glycol (M/s S.D. Fine Chemicals Ltd.), Butanol (s.d fine limited), Nonyl phenol ethoxylate and Span 80 (M/s S.D. Fine Chemicals Ltd).

Instruments
Homogenizer (silverson l5M), HPLC (Model-Perkin Elmer Series 200 HPLC), Particle size analyzer (Malvern Instrument model system 2000), Turbidity meter (Model-Jain 2115), FTIR (Thermo Fischer spectrum).

3.1. Preparation of neem EW formulation

The EW-neem formulation was prepared by oil in water emulsification procedure under high shear mixing at 3000rpm. Neem oil was used as the oil phase, nonyl phenol Ethoxylate and Span 80 as emulsifiers, butanol as defoamer, and polyethylene glycol as antifreezing agent and xanthan gum as thickener as well as stabilizer in the formulations. Nonyl phenol was dissolved in aqueous phase, while neem seed oil and Span 80 in the oily phase. The oil phase was added drop by drop in aqueous phase with constant homogenization in silverson l 5M homogenizer at a speed of 13,500 rpm. After 10 min of homogenization xanthan gum was added in the formulation.

3.2. Physic-chemical analysis

The physico-chemical parameters mentioned in WHO & FAO manual for pesticide formulations (WHO & FAO, 2010) were analyzed by method no. 463/TC/M for active ingredient, method no. 75.3 for pH, method no. 39.3 for stability at 0 °C and method no. 46.3 for stability at elevate temperature of CIPC Handbook (CIPAC, 2007).

3.3. Chemical analysis

3.3.1. Active ingredient determination
The Active ingredient was determined in varying time interval high shear stirred Neem 10 EW samples (15, 25, 35, 45 and 60 min) by the HPLC (model-Perkin Elmer Series 200 HPLC) as prescribed in BIS method 14299: 1995. The operating conditions were as follows: stationary phase: reverse phase column C-18, run time-60 min, mobile phase: Acetonitrile/Water (35:65) at 1.2 ml/min, detector wavelength, 214nm, and injection volume is 10ul.

3.3.2. Acidity and alkalinity
The acidity or alkalinity of Neem EW samples, stirred for 15, 25, 35, 45 and 60 min was determined by the prescribed method in sec.13.5 of IS 6940: 1982. It should be below 0.5 % by mass as H₂SO or NaOH respectively.

3.3.3. Droplet size analysis
The droplet size distribution of EW samples in 15, 25, 35, 45 and 60 min high sheared samples was measured by using laser light scattering.
based particle size analyzer (Malvern, 2000) built-in He–Ne laser light source, and software series (Malvern 2000). Millipore water was used as dispersant. The neem EW samples were analyzed without sonication.

3.3.4. Emulsion stability studies by turbidity meter

Emulsion stability of 15, 25, 35, 45, and 60 min stirred samples was checked by the turbidity analysis. Emulsion generally destabilized by any

Figure 5. Emulsion stabilization by turbulence mixing.

Figure 6. Neem oil along with neem EW FTIR.
type of sedimentation and creaming which results in reduction of turbidity. Emulsion stability percentage can be quantitatively estimated by turbidity measurement (Reddy and Fogler, 1981; Song et al, 2000a, b).

\[
\text{Emulsion stability(\%)} = \frac{\text{Final turbidity (NTU)}}{\text{Initial turbidity (NTU)}} \times 100
\]  

(3)

For emulsion stability studies, 0.2 ml EW sample was diluted with 99.8 ml standard water (342 ppm hardness) in 100ml measuring cylinder. The stopper was placed on the cylinder, and 10 inversions were given. Subsequently, the emulsion was allowed to stand undisturbed and emulsion turbidity was checked in 0, 4, 5, 24 h time interval by turbidity meter (Model-Jain 2115). The emulsion stability percentage was checked by the turbidity measurement data.

3.3.5. Storage stability

Storage stability of 15, 25, 35, 45 and 60 min stirred samples was checked as per the MT 39 method. For emulsion stability 100ml was checked by the turbidity measurement data. Emulsion stability percentage can be quantitatively estimated for type of sedimentation and creaming which results in reduction of turbidity. Emulsion stability percentage can be quantitatively estimated by turbidity measurement (Reddy and Fogler, 1981; Song et al, 2000a, b).

The FTIR measurements were done using Thermo Scientific™ Nicolet™ iS50 FTIR Spectrometer in the range of 4000–450 cm⁻¹. In the present study, the 15 % EW formulation was stored at 37°C for 14 days. FTIR characterization was done for pure and oil and neem EW.

3.3.7. Antilarval activity

The bioassay was conducted with the third instar larvae. The antilarval activity was checked in three concentration i.e. 1% (100ppm), 2% (200ppm), 5% (500ppm) along with the control. All the bioefficacy experiments were conducted in triplicates. 20 larvae were carefully released to glass jar and allowed to stand at room temperature for one hour. Larval mortality rates were recorded at 24 h, 48 h and then after 1 week.

3.3.7.1. Maintenance of larval culture. The Aedes aegypti mosquitoes were reared at Institute of Pesticide Formulation Technology, Gurugram in culture room. The culture room was simulated with natural climatic conditions of temperature and humidity i.e. 28 ± 2°C and 70 ± 5% respectively. Adult male and female mosquitoes in the ratio of 1male to 3 females were kept in culture cages of dimensions (30 cm × 30 cm X 30cm) with iron framed and covered with net. A plastic bowl as rearing container filled with water was kept inside the cage. Eggs were collected from the rearing container to moist filter paper. After collection, the eggs were again placed in cage for hatching. After 24 h larvae hatched and then first instar larvae was placed in culture trays of dimension (15 cm × 10 cm X 5 cm) along with water and larval diet. 3rd larval stage were collected for the experiment.

3.3.7.2. Larvicidal bioassay experiment. Larvicidal bioassay experimental studies were conducted with EW formulation on 3rd instar larvae of. Ten larvae were transferred into 100 ml glass beakers filled with 50 ml distilled water. Different concentrations viz. 1%, 2% and 5% of neem based 10% EW were taken in three replications along with other treatment and control. Ten larvae were carefully released to glass jar and allowed to stand at room temperature for one hour. Larval mortality% were recorded after 24hrs, 48 h and subsequently after 1 week in Neem EW samples. The experiments were carried out at standard laboratory conditions at temperature 26°C ± 2°C. The % mortality was calculated by using Abbott's formula (Abbott, 1985):

\[
\text{Mortality(%)} = \frac{\text{Initial turbidity (NTU)}}{\text{Final turbidity (NTU)}} \times 100
\]  

(3)

For emulsion stability studies, 0.2 ml EW sample was diluted with 99.8 ml standard water (342 ppm hardness) in 100ml measuring cylinder. The stopper was placed on the cylinder, and 10 inversions were given. Subsequently, the emulsions was allowed to stand undisturbed under. The stopper was placed on the cylinder, and 10 inversions were given. Subsequently, the emulsions was allowed to stand undisturbed der. The stopper was placed on the cylinder, and 10 inversions were given. Subsequently, the emulsions was allowed to stand undisturbed.

Table 5. Mortality % in different concentration of Neem 10 EW formulation in varying period of high shear mixing.

| Period of mixing (min.) | Replicates (N) | Mortality % in 100ppm | Mortality % in 200ppm | Mortality % in 500ppm |
|-------------------------|---------------|-----------------------|-----------------------|-----------------------|
|                         | After 24 h    | After 48 h            | After 1 week          | After 24 h            | After 48 h            | After 1 week          | After 24 h            | After 48 h            | After 1 week |
| 15                      | 3             | 16.42 ± 0.90ab       | 15.6 ± 0.83ab         | 30.66 ± 0.78cd       | 23.6 ± 0.89ab         | 46.6 ± 0.83ab         | 80.5 ± 0.82ab        | 75.6 ± 0.83ab       | 80.58 ± 0.70ab |
| 25                      | 3             | 16.35 ± 0.86ab       | 16.4 ± 0.84ab         | 45.7 ± 0.79           | 30.6 ± 0.79b          | 56.5 ± 0.85bc         | 85.7 ± 0.85b         | 74.6 ± 0.85b        | 81.6 ± 0.76b  |
| 35                      | 3             | 11.6 ± 0.91ab        | 16.8 ± 0.86ab         | 47 ± 0.76b           | 39.4 ± 0.89bc         | 65.8 ± 0.75b          | 90.5 ± 0.79b         | 86.75 ± 0.74ab      | 90.5 ± 0.79ab |
| 45                      | 3             | 12.1 ± 0.92a         | 17.3 ± 0.82a          | 55.7 ± 0.82           | 50.6 ± 0.85a          | 75.9 ± 0.80a          | 95.69 ± 0.82a        | 90.67 ± 0.78a       | 95.7 ± 0.74a  |
| Neem Baan EC            | 3             | 75.95 ± 0.54         | 80.8 ± 0.48           | 89.5 ± 0.66          | 0                     | 0                     | 0                     | 0                     | 0               |
| Control                 | 3             | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     | 0               |

Mean ± SE with same superscript letter are not significantly different at p<0.05 by DMRT.
4. Conclusion

The neem based oil in water emulsion formulation composition containing emulsifiers, anti-freezing agent, xanthum gum and defoamer was optimized for providing good emulsion. The result of this study indicate that stirring duration up to 1 h at 3600 rpm in the EW formulation had remarkable effect upon droplet size and thereby increase emulsion stability. As stirring time increases particle size of the emulsion decreases which results into stable emulsion formation. Long duration agitation gives very fine droplet size and excellent emulsion stability after accelerated temperature storage.

The oil water emulsion is environmentally friendly, solvent free neem formulation. Characterization of emulsion for its turbidity, pH, droplet size and emulsion stability by FTIR and HPLC analytical techniques, indicate the stability of the emulsion. The stable neem oil emulsion had anti larval activity i.e. 100% mortality after 48 h exposure. The present study will be very beneficial for developing water based a stable botanical formulation which is eco-friendly, economical as well as have good commercial value in organic farming.

Declarations

Author contribution statement

Nusrat Iqbal: Conceived and designed the experiments; Wrote the paper.
Natish Kumar: Performed the experiments.
Mahesh Kumar Saini, Saurabh Dubey: Analyzed and interpreted the data.
Amrish Agrawal, Jitendra Kumar: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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3.3.8. Statistical analysis (DMRT)

The results of mortality percentage were calculated by taking means of three replicates and then standard deviation was calculated by the replicates mortality percentage data. The data was further statistically analyzed (ANOVA-Analysis of variance) by using SAS 9.4 software. Significant differences between the treatment were also tested by Duncan’s multiple range test (DMRT).

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\text{% Mortality} = \frac{\text{test mortality} - \text{control mortality}}{100 - \text{control mortality}} \times 100
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