Research Report

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Botanical Based Safe and Nontoxic Mosquito Impregnated Gel

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Journal of Mosquito Research, 2019, Vol.9, No.3 doi: [10.5376/jmr.2019.09.0003](https://doi.org/10.5376/jmr.2019.09.0003)

Received: 06 Mar., 2019

Accepted: 28 May, 2019

Published: 16 Aug., 2019

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Preferred citation for this article:

Iqbal N., Kumar N., Agrawal A., and Kumar J., 2019, Botanical based safe and nontoxic mosquito impregnated gel, Journal of Mosquito Research, 9(3): 18-24(doi:[10.5376/jmr.2019.09.0003](https://doi.org/10.5376/jmr.2019.09.0003))

Abstract Mosquito problem is the major problem in our country. There are many pesticide formulated products available in the market to compete the mosquito generating problems. The major drawback of these products is that they are not safe for human beings so, there is urgent requirement of safe and effective formulation. The aim of this study is to develop an effective, safe, and slow release formulation against mosquito control. In the present study, neem microemulsion was converted into microemulsion gel by using polymeric gel matrix Carbopol-934. Microemulsion formulation is the thermodynamically stabilized formulation and globular size is in nano range. These characteristic features of microemulsion will remain same in the gel phase. Size and zeta potential of microemulsion gel are (400 ± 6) nm and -10.0 mV, respectively. Syneresis, spreading coefficient value are in the range of stabilized gel formulation and suitable for impregnating mosquito net. Neem microemulsion based gel impregnated net gives 90% repellency against mosquitoes.

Keywords Microemulsion; Impregnated net; Spreading and repellency

Background

Mosquito problem are the main causative agents for many dangerous diseases that will threaten productivity and economy development of any country. Mosquitoes are the most favorable vector for the transmission of different destructive diseases (Lengeler, 2004). In every 10 years, mosquito transmits a new pathogen for new disease and sudden occurrence will cause serious and unmanageable problems.

There are various mosquito control insecticide formulations available in the market like mosquito coil, cream, aerosol sprays etc.. These formulations have been effectively controlled the mosquito problem since many years but recently some acute and chronic problems are seen in human beings. Insecticide treated Nets (ITN) can significantly reduce the individual direct exposure risk to harmful insecticides and effectively decrease the transmission of mosquito borne diseases (Howard et al., 2000). ITN are not only create physical barrier from mosquito biting, but also give prolonged duration competence by means of killing and repellency of mosquito species.

ITN are most commonly impregnated by Permethrin and Piperonyl Butoxide pesticides, but these pesticides prenatal exposure cause lowering the mental development of the children (Megan et al., 2011). Many studies reveals that Permethrin are also responsible for endocrine disruption of human beings (Kabir et al., 2015). The toxic effect to human beings can be reduced by introduction of plant based mosquito repellent for impregnating mosquito nets. These nets will be non-toxic in all respect like inhalation, ingestion, or direct contact to skin or eye.

Present study is based on impregnation of mosquito net by polymeric encapsulated neem nanogel for prolonged repellency towards mosquito species.

1 Results and Discussion

1.1 Microemulsion size, zeta potential and Poly- dispersive index

Microemulsion thermodynamic stability is mainly depending upon the globular size in the nano range. Zeta sizer is mainly used for the measurement of globular size of Microemulsion. Neem ME mean globular size was (200 ± 4) nm and size of neem ME based gel is (400 ± 6) nm. The increased size in gel might be due to gelling matrix

carbopol. Polydispersive values were measured as 0.23, low value signifies that dispersion of neem oil is homogeneously distributed in the ME formulation (Moghimpour et al., 2013).

Zeta potential reading of micro emulsion and micro emulsion gel was $-(10.2 \pm 4)$ mV and -10.0 mV respectively. Higher negative values indicate that the micro emulsion gel is stabilized formulation.

1.2 Phase separation

Micro emulsion is the thermodynamically stable system so even after centrifugation no phase separation was seen as commonly occurs in emulsion system (unstable system). Hence, no separation further confirms that the system is highly stabilized.

There was no any phase separation was seen on micro emulsion system and after dilution with water it remains a homogenous stable system. Any type of crystallization was not observed even after extended period of storage.

1.3 Physical appearance

Neem microemulsion gel was off white coloured creamy, smooth in texture and glistening appearance and homogenous and uniformly mixed.

1.4 pH value

pH was very important for stability of the active ingredient in microemulsion system. When any formulation which is alkaline or acidic in pH more chance of the degradation of the active ingredient. Microemulsion gel pH was record 6.8 i.e neutral it incanted that the active content is stable in the microemulsion gel formulation.

1.5 Viscosity

Viscosity is very important factor for easy spreading of the gel formulation. Viscosity of the neem ME gel was 24.76 Pa s due to gelling matrix cabopol (Chen et al., 2006).

1.6 Syneresis test

Syneresis is related to the structure and compaction of the gel which is controlled by the polymeric matrix. Syneresis results from the contraction of the gel without undergoing any evaporation of the fluid in microemulsion. This process occurs due to inter attractive forces of the gel matrix and direct the gel permeability and viscosity change due to gel compaction (Dewhurst et al., 1999). Syneresis was calculated by above mentioned formula and it was only 1% which mean compaction was more due to the inter attractive forces between Neem ME and polymeric gelling matrix carbopol.

1.7 Spread ability

Spread ability is the measure of lubricity directly related to friction coefficient. Rate of spreading depends upon viscosity, type of solvent used volatile or non-volatile in the formulation system (Duggin, 1996). When a gel takes less time to spread it means the gel has high spreadability and viscosity (Dantas et al., 2016). Uniform application of the gel formulation is highly depends upon the good spreadability and ideal for impregnation for any surface like mosquito net impregnation, dress impregnation etc., and give good activity against mosquito attack.

The microemulsion based gel formulation showed slight variation in spreadability values during storage at various conditions i.e. 0 degree, 54 degrees and 26 degrees. The spread ability of the neem microemulsion showed slight variation and acceptable limits during storage (Table 1).

Table 1 Spread ability of neem based microemulsion gel

Temperature	0°C	54°C	25°C
Spreading coefficient (gm.cm/sec.)	15.16 $\pm$ 0.19	14.65 $\pm$ 0.33	12.34 $\pm$ 0.22

Note: Results expressed as relative std. deviation (RSD) significant differences ($p < 0.05$) (mean $\pm$ S.D, n= 3)

Variation in temperature condition slightly changes the spread ability coefficient. In pure neem based gel by viscosity is fluctuated in different temperature conditions due to fatty acid contents and changed the spread ability beyond the acceptable limits and results into unstable gel formulation. This problem is successfully overcome in

Neem ME. In Neem ME, Surfactants and co surfactants was added and results into uniform spread ability at various temperature condition (Fontana et al., 2011).

1.8 Neem oil content

The uv- vis % transmittance was remain the same i.e. 98.63 ± 1.36 % in microemulsion gel as in microemulsion system. This shows that there was no degradation of the active ingredient during transformation to micro emulsion gel.

1.9 Release of active ingredient

The release of the active ingredient from the gel has good linearity as compared to microemulsion system. In gel, active ingredient rate of diffusion over period of time is constant and start decreasing gradually but in microemulsion rate of release is gradually decrease day by day. Rate of release suggested that the drier the impregnation with thick film shows extended period of activity against mosquito. By addition of carbopol act as thickener as well as gelling matrix which enhance the activity of neem microemulsion (Aiyalu et al., 2016).

Percentage release was maximum up to initial 10 days then it was constant up to 20 days (Table 2).

Table 2 Percentage release of azadirachtin from neem formulation from the initial concentration

0 d	2 d	4 d	6 d	8 d	10 d
10%	25%	40%	45%	55%	58%

1.10 Accelerated stability studies

Accelerated Stability testing of the gel at 54°C for 14 days demonstrate that the ME based microemulsion gel formulation physical appearance, viscosity, Syneresis remain same after 14 days storage period. Spreadability of the formulation remain same and uniform as freshly prepared gel. After storage p H values remain same as before i.e. 6.5-6.8. No significant change in active ingredient content analysis by HPLC remains same after 14 days storage period.

1.11 Repellency test of mosquito on neem microemulsion gel impregnated net

Repellency test was performed to evaluate repellency of mosquito against microemulsion based impregnated gel. Two sides of cage were hanged by treated net of dimensions 25 x 25 x 25 cm and two sides were left untreated and one upper side left for observing the repellency behavior of the mosquitoes. Mosquitoes were starved for four hours before exposing into the cage. 50 mosquitoes were put into the cage. Experiment of repellency was done in triplicates: three cages and each cage supplied with 50 mosquitoes. Observations were taken in every 10 minutes interval for 1 h (Table 3; Table 4; Figure 1; Figure 2).

Table 3 % repellency against mosquito up to 1 h after spraying of microemulsion on mosquito net

Mosquito Behavior	0 min	5 min	10 min	15 min	30 min	45 min	60 min
Landing (%)	67.5	55	42.5	30	20	15	7.5
Repellency (%)	32.5	45	57.5	70	80	85	92.5

Table 4 % repellency against mosquito of neem microemulsion gel up to 1 h after spraying on mosquito net

Mosquito Behavior	0 min	5 min	10 min	15 min	30 min	45 min	60 min
Landing (%)	50	30	25	12.5	2.5	0	0
Repellency (%)	50	70	75	87.5	97.5	99	99

Figure 1 Mosquito behavior after 1 hr on microemulsion sprayed net

Figure 2 Mosquito behavior after 1 hr on neem micro emulsion based gel sprayed net

Mosquito repellency was calculated by following formula (Table 5; Figure 3):

Mosquito Repellency (%) = No. of mosquitoes landing on treated net/ Total no. of mosquitoes in the cage × 100

Neemmicroemulsion is the stable isotropic solution. When it was spreaded on the mosquito net it was uniformly dispersed over the net, but persistence is not as in neem based microemulsion gel. Neemmicroemulsion gel is encapsulated in the polymeric coating so the effectiveness against the mosquito up to prolonged period of time in comparison to microemulsionspray.

Table 5 Comparison of % repellency of microemulsion and microemulsion

Time period	Total no. of mosquitoes	% repellency
Microemulsion gel –impregnated net		
0 h	10	92
2 h	10	70
3 h	10	50
5 h	10	20
Microemulsion- Impregnated net		
0 h	10	99
2 h	10	89
3 h	10	75
5 h	10	50

Figure 3 Mosquito behavior on neem gel impregnated net up to 5 h

2 Materials and Methods

Neem oil, Tween 80, lemon grass, Butanol, Carbopol, Triethylamine.

2.1 Preparation method of microemulsion

Microemulsion was prepared by adding oil phase with the help of dropper in aqueous phase under continuous stirring at 200 rpm. Aqueous phase contain 10 g of surfactant mixer in a 3: 2 ratio (Tween 80: Span 30), butanol 2 g, polyethylene glycol 1.5 g and 30 g water. Oil phase contain neem oil with lemon grass oil in 3:1 ratio. After adding oil phase the resultant emulsion solution was left for stirring on 200 rpm up to 1 h at room temperature. After homogenously mixing the two phases, final microemulsion solution was transparent in physical appearance.

2.2 Preparation method of microemulsion gel

Carbopol-934 was selected as gel matrix. 1g of carpool -934 was mixed in distilled water and left for swelling upto 1 h. Microemulsion was added in swollen carpool solution and mix homogenously. After complete mixing of microemulsion and carbopol, 10 g polyethylene glycol and 0.5 g of triethylamine was added drop wise to neutralize the acid residues due to carbopol. After addition of all the ingredients, the mixer was stirred vigorously by a glass rod till the stable gel formed.

2.3 Estimation of microemulsion stability

2.3.1 Globular size and zeta potential determination

The globular size and zeta potential determinations were done by Zetasizer (Malvern instrument). All the measurement was done at 25°C. 1 ml of sample was diluted with distilled water (Graf et al., 2009). All the measurement was done in triplicates.

2.3.2 Phase separation

Phase separation was checked by centrifugation at 15,000 rpm for 30 minutes (Yogeshwar et al., 2009).

2.4 Estimation of microemulsion based gel Physic-chemical analysis

2.4.1 Physical appearance

The microemulsion gel was examined visually for their appearance, color etc. Stability of gel was also observed by physical appearance of stable homogenous gel structure without any separation of oil and water phases.

2.4.2 pH determination

The Ph of the microemulsion gel was checked by the Ph meter. For measuring p H 1ml sample was diluted with distilled water and then Ph reading was taken at room temperature. All the measurements were done in triplicates (Bhanu et al., 2011).

2.4.3 Viscosity determination

Viscosity was determined at ambient temperature by Brookfield digital viscometer(S-62, model LVDV-E) with a viscometer having spindle rotating at 12 rpm (Nayak et al., 2005).

2.4.4 Syneresis test

This test is for measurement of any shrinkage of gel. In this first gel was kept in plastic tubes with a perforated base which is enclosed with what man filter paper. These plastic tubes were then kept in centrifuge tubes and centrifuged at 1 000 rpm for 10 minutes. After centrifugation filter paper and plastic tube content was weighted and syneresis calculated as (Bhumika et al., 2017):

$$\% \text{Syneresis} = \frac{\text{wt of filter paper after centrifugation} - \text{wt of filter paper before centrifugation}}{\text{Total weight of gel before centrifugation}} \times 100$$

Total weight of gel before centrifugation

2.4.5 Spread ability

For spread ability determination, by placing gel formulation on one point of one slide and then put another slide over the placed gel in such a way that the gel was sandwiched between two slides. The upper slide was slightly pressed by 100 g wt to form a thin layer between slides. After this weight was removed and fixed on one place. After 5 minutes 20 g weight was tied on upper slide cautiously and separated away the upper slide upto a distance of 7.5 cm (length of slide). The same procedure was repeated 3 times (Jain and Neal, 2007). Then spreadibility was calculated by this equation:

$$S = \frac{m \times l}{t}$$

Where, S- spread ability, m- Tied weight (20 g), l- Glass slide length (7.5 cm), t- Time taken (sec) to slip off the slide

2.4.6 Active ingredient determination

Active ingredient was determined spectrophotometric ally by taking absorbance reading at 400 nm. For absorbance measurement, 1 gm of gel was dissolved in 100 ml distilled water and left at room temperature for 5 hrs. The prepared dilution was filtered through filter paper and then absorbance was measured at 400nm along with blank sample.

2.4.7 Accelerated temperature Stability test

Neem oil loaded microemulsion gel was sealed in 15 mL glass vials and then stored at 54°C temperature for 14 days. Physical stability was estimated by visual assessment for physical alterations like oil or water separation or any precipitation. Chemical stability was evaluated by the absorbance measurement by UV- VIS spectrophotometer at λ_{max} 400 nm.

2.5 Mosquito repellency

2.5.1 Procedure for impregnation of mosquito net with microemulsion gel formulation

Four pieces of net having dimensions 30x30 cm were taken. Apply the prepared micro emulsion gel over the net. After uniform application allowed the net at room temperature for complete drying. In the same way micro emulsion formulation sprayed over the net, this was taken as reference. Mosquito repellency was checked on gel impregnated net and micro emulsion sprayed net along with control (untreated net).

2.5.2 Maintenance and rearing of mosquito culture

Mosquito culture was maintained at temperature of $26^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and 70%~85% relative humidity. Mosquitoes were kept in the iron framed cage covered by cloth of dimensions 30cmx30cmx30cm. Mosquitoes were supplied by the 1% glucose solution as energy source and water wetted cotton balls were kept in the cage as water source to satisfy their thirst. For proper development of gravid female mosquitoes they were fed on rabbit blood. Water container was also kept in the cage for laying egg over water surface. The eggs were kept undisturbed up to 48 h and after that the hatching will start and then eggs were transferred in other bowls filled with fresh water and with corn powder in the form of tablets.

3 Conclusion

Mosquito is extremely severe problem in India and if the problem remains same it will be unmanageable problem in future years. Mosquito is a major problematic insect out of all the household insects. Mosquito is the causative agent of many diseases like dengue, malaria, chickengunia etc. There are many mosquito control products are available in the market, but no control method or product is available which is botanical based and would give extended release against mosquito. Pesticide Impregnated net are available in the market but major drawback of this impregnated net formulation is that it gives negative effect on the brain cells and cause many mental disorders in new born babies. The present study gives totally safe and efficient product against mosquitoes for prolonged period of time. Neem oil micro emulsion based gel is used for impregnation of mosquito net. Micro emulsion gel has particles in the nano range and has very good spreading coefficient which results a uniform dispersion over the net surface. Polymeric gel marix makes it sticky and controlled release profile for prolonged period effectiveness. This micro emulsion based gel is the advancement of the micro emulsion formulation. Micro emulsion based gel is the highly stabilized formulation and can be used for textile impregnation in future and would be as effective as synthetic pesticide impregnation.

Authors' Contributions

NI (corresponding author) prepared the protocol and draft the manuscript, NK perform experiments and bioefficacy studies, AA analyzed the studies, JK approved the final manuscript. All authors read and approved the final manuscript.

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