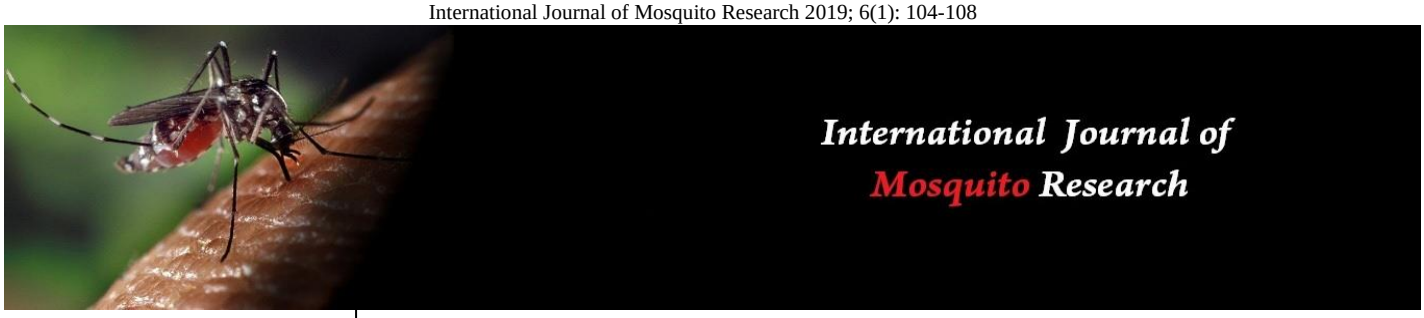

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2019; 6(1): 104-108

Received: 09-11-2018
Accepted: 13-12-2018

Nusrat Iqbal
Institute of Pesticide Formulation Technology, Gurugram, Haryana, India

Megha Pant
Centre for Rural Development and Technology, Indian Institute of Technology, Delhi, India

Saurabh Dubey
Centre for Rural Development and Technology, Indian Institute of Technology, Delhi, India

Nisha Sogan
National Institute of Malarial Research, Delhi, India

Neeraj Patanjali
Department of Agricultural Chemicals, Indian Agricultural Research Institute, 110060 Delhi, India

Satya Narayan Naik
Centre for Rural Development and Technology, Indian Institute of Technology, Delhi, India

Correspondence
Saurabh Dubey
Centre for Rural Development and Technology, Indian Institute of Technology, Delhi, India

Preparation of neem oil microemulsion using biodiesel waste for larvae control

Nusrat Iqbal, Megha Pant, Saurabh Dubey, Nisha Sogan, Neeraj Patanjali and Satya Narayan Naik

Abstract
Mosquito problem is the major problem worldwide. So, there is urgent requirement of some effective and safe product for resolve the mosquito problem. Neem has very good repellent properties against mosquitoes. Previous research shows that there are 46 types of emulsion formulations are present. But all are solvent or co-solvent based which makes the formulated product costly and not safe for environment as well as non- targets. In the present study Neem oil microemulsion process was optimized by using biodiesel waste as co-solvent which has no any toxic effect towards environment and non-target. Developed microemulsion has high physical and chemical stability. This stability was achieved by taking different concentrations of non-ionic surfactants in the process of emulsion formation. I-R results further confirm the stability by showing sharp band of neem oil at 1642, 2928 which did not alter in Microemulsion (ME) containing biodiesel waste. Biodiesel waste based microemulsion alone at concentration range of 40-280 ppm gave 20-100% mortality rates against *Aedes aegypti* larvae in time period of 24 & 48 h. The LC50 (Lethal dose) values were found to be56.985, 52.604, and 38.575 for neem oil microemulsion and 210.766 mg/L for biodiesel waste alone.

Keywords: neem oil, microemulsion, biodiesel waste, *Aedes aegypti* larvae, contact toxicity, Karin jin

1. Introduction
The Neem (*Azadirachta indica*) is an evergreen tree of India belongs to family Meliaceae. Neem is an important phytochemical source and plays a very significant role in household as well as agricultural pest management [1]. Neem (*Azadirachta indica*) has exceptional medicinal as well as insecticidal properties [2, 3]. Main extracted product from neem seed kernel is the neem seed oil (NSO). There are approximately 100 biologically active compounds present in the extracted NSO. The tetraterpenoids are the main constituents of NSO which include mainly Azadiractin, Nimbin, Salannin, and Azadiradione. NSO has been extracted by different extraction methods. NSO extraction process is first initiated in India by small producers but at present other countries are also involved in producing NSOs. Therefore, neem constituents may possibly vary in different geographical originated NSOs.
In addition to this pre- and post-harvesting factor also affect the constituents of neem oil [4]. NSOs have been formulated into many ways to remove the error generated by various factors and give desired and consistent bio-efficacy results.
As *Azadiractin* is highly unstable and the main active constituent of NSOs therefore, a stable formulation is very much required to stabilize the *Azadiractin* content for longer period of time. Microemulsion systems are thermodynamically stable, transparent and isotropic dispersed mixture of oil, water and surfactants and in combination with co-surfactant and having dispersed droplet size in between 5 and 100 nm [5, 6]. Microemulsions due to very small size and low interfacial intension were recognized by pharmaceutical researchers as a very good delivery system of drugs. Natural oil based microemulsion technology could provide a very potent mode for making mosquito repellents products [7].
Mosquito control is the major problem worldwide. They are vectors of many deadly diseases like dengue, malaria, chikangunia, filariases and many other diseases [8].About half of the world's population is on high risk. Therefore, effective methods are required to solve the mosquito generated problems. Repellents plays a very important role in preventing the mosquito transmitted diseases to humans.

Neem oil has very good repellent properties against mosquitoes. There are 46 types of neem oil emulsions was prepared by using different combination of emulsifiers and co-solvents and their larvicidal effects has proved against *Anopheles gambiae* [9]. Presently alcohol based co-solvents are used in micro-emulsion but these alcoholic solvents make the formulation costly. These alcoholic solvents can successfully be replaced by 10 % biodiesel waste generated as liquid by volume i.e. only 10 kg glycerol is produced from 100 kg biodiesel and rest is discarded as waste liquid [10]. In future this biodiesel waste creates disposal problem which results into many harmful effects on environments [11]. In India biodiesel production is by two main crops of non-edible oils- karanja (*Pongamia pinnata*) and jatropha (*Jatropha curcas*). Therefore, crude glycerol needs to be converted into more valuable products [12].

The aim of present paper is to prepare neem oil microemulsion by using biodiesel crude waste as co-solvent or carrier to make the formulation economical, stabilized and more effective.

2. Materials & Methods

Neem oil from Gogia Chemicals, Noida, India and Biodiesel waste (Karanja oil) from I.I.T Delhi, Polysorbate 80 (Tween 80), and Span 80 from Merck India Limited.

2.1 Test Insect Culture

The *Aedes Aegyptii* test culture was collected from National Institute of Malaria Research (NIMR) Delhi, India. The culture was kept in laboratory under conditions i.e. at temperature 27±2 ◦C, 75-85% relative humidity under 14:10 h (light/dark) photo period. The culture was maintained under inert conditions i.e. free from any pathogens, insecticides or repellents. Life cycle of mosquito under these conditions was completed in 3-4 weeks. Larval stage was fed on finely crushed dog biscuits and yeast extract in 3:1 ratio. Water was changed every day to remove the debris produced by casting of skin from larval stage to pupae stage. Next pupae stage was transferred from tray to a beaker which contain water and then this beaker was placed in screened cages (30 × 30 × 30 cm dimension) [13]. The beaker was positioned in this cage till adult emergence. After adult emergence the adults were reared in glass/plastic cages (30 × 30 × 30 cm dimension). The adult colony was supplied with 10% sucrose solution and intermittently fed blood of restrained rabbits. Eggs of mosquito were collected by a suitable ovitrap after 72 h and then transfer to enamel tray. All the developmental stages from larvae to adults were maintained to conduct the experimental trials.

2.2 ATR-FTIR analysis

FT-IR was performed by Bruker alpha ATR-FTIR spectrophotometer using the attenuated total reflectance (ATR) technique. The infrared (IR) spectra tell the compatibility of interactions by different ingredients presents in prepared neem Micoemulsion. The infrared (IR) Spectra were recorded and values are expressed as λ max cm^{-1}.

2.3 Preparation of Microemulsion

Microemulsion was prepared by two ways first is by diluting water / surfactant mixture with oil and in second by diluting oil/surfactant (Table 2) mixture with water [14]. Microemulsion by these combinations was prepared spontaneously at 30 °C.

3. Bioefficacy of Neem oil microemulsion against *Aedes aegypti* larvae by contact toxicity bioassay

The efficacy studies of Neem oil microemulsion with biodiesel waste and biodiesel waste alone was checked against *Aedes aegypti* larvae. Selected doses of the microemulsion for contact toxicity bioassay were 40, 80, 120, 160, 200, 240, 280 & 320 ppm. Contact toxicity bioassay was performed in a tray and selected doses were applied by microapplicator.10 larvae were released in each tray containing different doses concentrations. After 24 & 48 h mortality was observed. Each assay of different concentrations was carried out in three replicates along with a control tray [15].

4. Results &Discussion

FTIR spectra of neem oil, biodiesel waste as an active ingredient, surfactant, co-surfactant and water as an inert ingredient were recorded. Microemulsion was prepared by physical mixing of the neem oil and biodiesel waste as an active ingredient along with surfactant, co-surfactant and water as an inert ingredient [14] in oil in water Microemulsion formulation. Characteristics sharp band of neem oil (1642, 2928) did not alter (Fig.1) the formation of Microemulsion [18] there by indicating absence of chemical interaction between oils and other inert ingredients [16].

4.1 Discussion for insecticidal bioassay

The effect of Neem oil microemulsion, with biodiesel waste as a solvent or carrier and LC50 values were determined. The neem microemulsions with biodiesel waste were divided into eight groups i.e. F1 to F8 (Table2). The amount of biodiesel waste in microemulsion was 1.0 % w/w by weight (Table 1). Biodiesel waste was used to solubilise neem oil in disperse phase. Biodiesel waste alone has insecticidal activity and was observed against larvae (Table 3). The LC50 values of biodiesel alone were 236.70 and 210.766 mg/L at time period of 24 & 48 h and percent mortality values were 20-70 (Table 3). When Biodiesel waste or BDW was used in the microemulsion (ME) (Table 2) as disperse phase for formulation [16]. The LC50 values were decreased i.e. 127.797, 86.313 & 78.778 mg/L (Table 3) and mortality also increased i.e. 100% for F2, F5 & F7 after 24 hours at 320 ppm (in case of mortality) (Fig. 2). The variation of the toxicity of the microemulsion, with biodiesel waste against larvae is shown in Figure 2. This graphical representation of Fig.2 shows that biodiesel wastes in microemulsion were more lethal and effective. Effectivity of the formulation F7, F5 & F2 increases with time i.e. after 48 hours mortality became 100% at 280 ppm and LC50 values were 56.078, 52.604 & 38.575 mg/L respectively. Table.1 shows that biodiesel waste contains some mono-, di- and tri- glycerides, free fatty acids, alcohol, Glycerol and methyl ester [12], and when neem oil is solubilized in biodiesel waste; the biodiesel waste reduces the surface tension between the neem oil and water by making the surfactant monomer into miceller form which results into decreasing the contact time with water and stabilize the microemuslsion further [17]. Presence of karinjin [18] in the biodiesel by-product, which is generated after transesterification of Karanja oil shows insecticidal properties against pest [17]. In the presence of karinjin, biodiesel waste alone shows increase in percentage mortality and decrease in LC50 with increase in concentration w.r.t. time. The use of biodiesel waste makes the emulsions more environmental

friendly and user friendly as compared to conventional petroleum based solvents. In the presence of Biodiesel waste, neem oil emulsion i.e. F2, F5 & F7 (Table 2) achieves 100% mortality after 48 hours. At 280 ppm concentration of 4, 10 & 14% neem oil microemulsion, activity was the same after 48 hours (Table 3). It was clear after mortality results that there is no requirement to make higher concentration of neem oil microemulsion with biodiesel waste i.e. F5, F6, F7 & F8 (Table 2). F2 formulation (Table 3) also attains the same target i.e. 100% mortality after 48 hours and biodiesel waste alone has its own toxicity against mosquito larvae (Table 3).

Table 1: Biodiesel waste composition

S. No.	Name of Ingredient	% of Ingredients
1.	Glycerol	40-50%
2.	Alcohol	0.2-1%
3.	Mono-, di-& tri- glycerides	1.5-3%
4.	Free Fatty Acid	1-2%
6.	Triglyceride Ester	0.1-0.2%
7.	Water	0.1-0.3%

Table 2: Preparation of Neem oil Microemulsion using Biodiesel by-product

S. No.	Formulation	Neem oil	Biodiesel waste	Surfactant + Co-surfactant	Propylene Glycol	Water
1.	F1	2	1	9 +2	10	Upto 100
2.	F2	4	1	11 + 2	10	Upto 100
3.	F3	6	1	13 + 2	10	Upto 100
4.	F4	8	1	15 + 2	10	Upto 100
5	F5	10	1	17 + 2	10	Upto 100
6	F6	12	1	19 + 2	10	Upto 100
7	F7	14	1	21+ 2	10	Upto 100
8	F8	16	1	23 + 2	10	Upto 100

Table 3: % Mortality and LC50 values of Neem Oil Microemulsion & Biodiesel waste alone

S. No.	Formulation Code	Concentration	% Mortality after 24 hours	% Mortality after 48 hours	LC 50 (mg/l) after 24 hours	LC 50 (mg/l) after 48 hours
1.	F2	40	20	50	127.797	56.098
		80	30	60		
		120	40	63.33		
		160	50	70		
		200	60	80		
		240	70	90		
		280	80	100		
		320	100	100		
2.	F5	40	30	50	86.313	52.604
		80	40	60		
		120	60	70		
		160	70	80		
		200	76.66	86.66		
		240	80	93.33		
		280	90	100		
		320	100	100		
3.	F7	40	30	60	78.778	38.575
		80	50	70		
		120	60	80		
		160	70	90		
		200	80	90		
		240	90	100		
		280	100	100		
		320	100	100		
4.	BDW	40	20	20	236.70	210.766
		80	20	30		
		120	30	30		
		160	30	40		
		200	40	50		
		240	50	50		
		280	53.33	53.33		
		320	70	70		

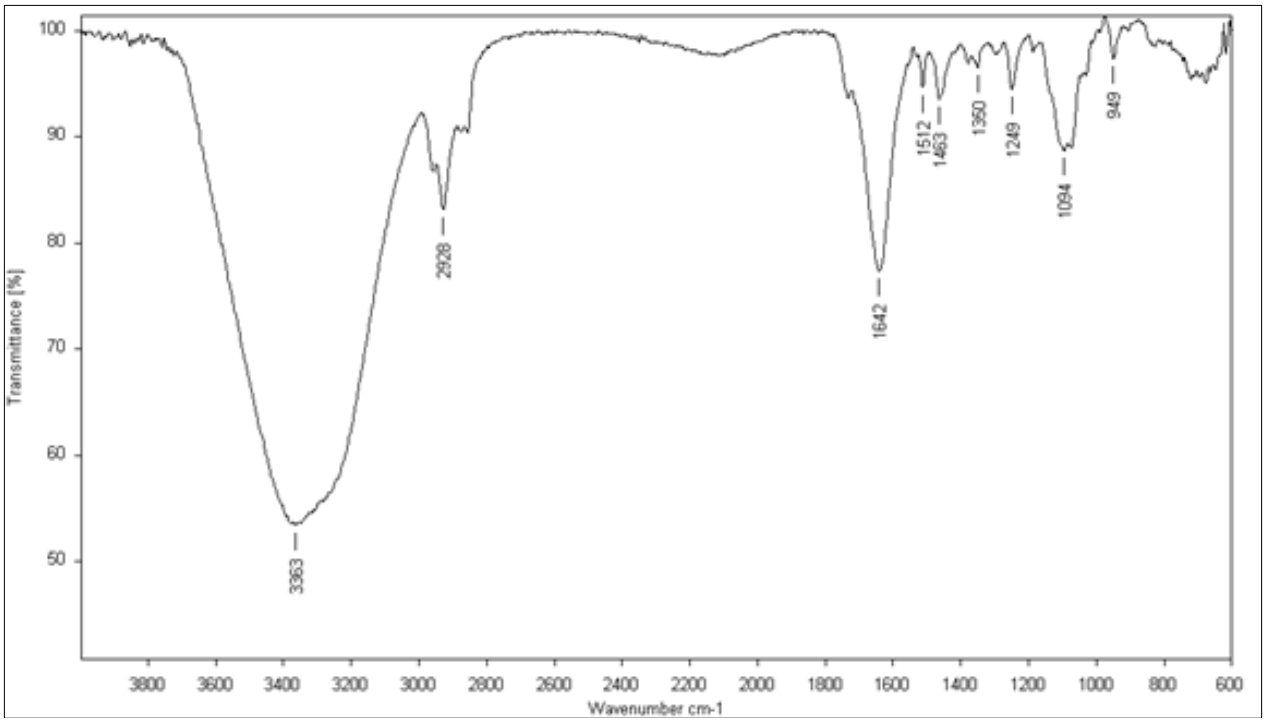

Fig 1: FTIR of neem oil Microemulsion using biodiesel waste

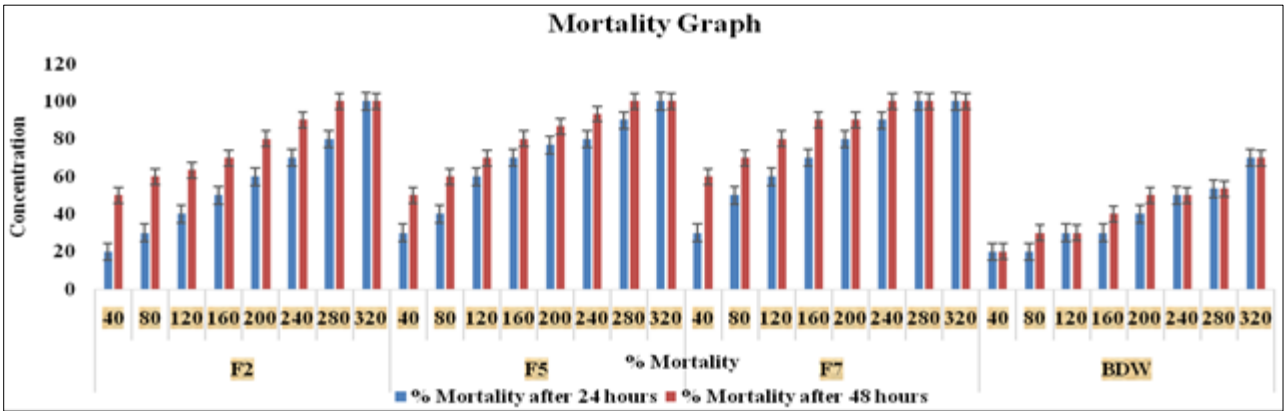

Fig 2: Graph between percent Mortality vs. Concentration in ppm

5. Conclusion

The paper greatly emphasizes on the efficient utilization of biodiesel waste or crude glycerol as a solvent or carrier or to replace the petroleum based hazardous, carcinogenic solvents to figure out environment friendly and cost effective product development work in new era. As Biodiesel waste creates disposal problem due to active constituents present in its own composition, hence it can be formulated with other ingredients and an efficient green product can be developed which is economical, sustainable and feasible. In stability aspects of other active ingredient like neem oil where active constituents of neem oil i.e. *Azadirachtin* is unstable in water based formulation, the above exploratory work play a major role to prepare stabilize the water based formulation by using biodiesel by-product and this kind of product used against household as well as agricultural pest for urban and also for rural sectors. This kind of work also makes the society aware of utilization of green waste in scientific manner and generates the small scale industry work for qualified unemployed people.

6. References

1. Schmutterer H. Properties and potential of natural pesticides from the neem tree *Azadirachta indica*. Annual Review of Entomology. 1990; 35:271-297. https://doi.org/10.1146/annurev.en.35.010190.001415.
2. Boadu K, Tulashie S, Anang M, Kpan JD. Production of natural insecticide from Neem leaves (*Azadirachta indica*), Pelagia Research Library Asian Journal of Plant Science and Research. 2011; 1(4):33-38.
3. Kumar VS, Navaratnam V. Neem (*Azadirachta indica*): Prehistory to contemporary medicinal uses to humankind. Asian Pac J Trop Biomed. 2013; 3(7):505-5. https://doi.org/10.1016/S2221-1691(13)60105-7.
4. Gahukar RT. Factors affecting content and bioefficacy of neem (*Azadirachta indica* A. Juss.) phytochemicals used in agricultural pest control: a review. Crop Prot. 2014; 62:93-99. https://doi.org/10.1016/j.cropro.2014.04.014.
5. Kayali I, Kaaein M, Qamlieh K, Wadah S, Ahmad W, Olsson U. Phase behavior of bicontinuous and water /diesel fuel Microemulsion using non-ionic surfactant combined with hydrophilic alcohol ethoxylates. J Disp

Sci Tech. 2015; 36(1):10-17. https://doi.org/10.1080/01932691.2014.886513.
6. Negi P, Singh B, Sharma G, Beg S, Raza K, Katare OP. Phospholipid Microemulsion based hydrogel for enhanced topical delivery of lidocaine and prilocaine: QbD based development and evaluation. Drug Deliv. 2016; 23(3):941-957. https://doi.org/10.3109/10717544.2014.923067.
7. Takase M, Zhao T, Zhang M, Chen Y, Liu H, Yang L *et al*. An expatriate review of neem, jatropha, rubber and Karanja as multipurpose non-edible resources and comparison of their fuel, engine and emission properties. Renew Sustain Ener Rev. 2015; 43:495-5. https://doi.org/ 10.1016/j.rser.2014.11.049
8. McKenzie N, Helson B, Thompson D, Otis G, McFarlane J, Buscarini T *et al*. Azadirachtin: an effective systemic insecticide for control of *Agrilus planipennis* (Coleoptera: Buprestidae). J Econ Entomol. 2010; 103(3):708-17. https://doi.org/10.1603/EC09305.
9. Okumu F, Knols BGJ, Ulrike FU. Larvicidal effects of a neem (*Azadirachta indica*) oil formulation on the malaria vector *Anopheles gambiae*. Malar J. 2007; 6:63. https://doi.org/10.1186/1475-2875-6-63.
10. Rastegari H, Ghaziaskar HS. Glycerol as the by-product of biodiesel production to value added monoacetin by continuous and selective esterification in acetic acid, J Indust Eng Chem. 2015; 21:856-861. https://doi.org/10.1016/j.jiec.2014.04.023.
11. Kong PS, Aroua MK, Daud WMAW. Conversion of crude and pure glycerol into derivatives: A feasibility evaluation, Renew Sustain Ener Rev. 2016; 63:533-555. https://doi.org/10.1016/j.rser.2016.05.054
12. Patanjali PK, Dubey S. An improved Agrochemical formulation, Patent Application no. 2391/Mum/2008, 2008.
13. Lucia A, Audino PG, Seccarini E, Licastro S, Zerba E, Masuch H. Larvicidal Effect of *Eucalyptus Grandis* Essential Oil and Turpentine and their Major Components on *Aedes aegypti* Larvae. J Am Mosq Control Assoc. 2007; 23:299-303. https://doi.org/10.2987/8756-971X(2007)23[299:LEOEGE]2.0.CO;2.
14. Singla M, Patanjali PK. Phase behaviour of neem oil based microemulsion formulations. Ind Crops and Products. 2013; 44:421-426. https://doi.org/10.1016/j.indcrop.2012.10.016
15. Shanmugasundaram RT, Jeyalakshmi MS, Dutt PB. Larvicidal activity of neem and karanja oil cakes against mosquito vectors, *Culex quinquefasciatus* (say), *Aedes aegypti* (L.) and *Anopheles stephensi* (L.). J of Environ Biol. 2008; 29:43-50.
16. Pant M, Kumar N, Singh O, Dubey S, Patanjali PK. Biodiesel waste based new generation formulation of Permethrin for cockroach control. JSIR. 2017; 76:184-185. http://nopr.niscair.res.in/handle/123456789/40644.
17. Verma M, Pradhan S, Sharma S, Naik SN, Prasad R. Efficacy of karanjinand phorbol ester fraction against termites, *Odontotermes obesus*. Int Biodeterior Biodegrad. 2011; 65:877-88. https://doi.org/10.1016/j.ibiod.2011.05.007
18. Pant M, Dubey S, Naik SN, Patanjali PK, Sharma S. Insecticidal activity of eucalyptus oil nanoemulsion with karanja and jatropha aqueous filtrates.Int J of Biodeter Biodegrad. 2014; 91:119-127. https://doi.org/10.1016/j.ibiod.2013.11.019.