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Nanoemulsion: A Green initiative for pest management

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Abstract

Brinjal or eggplant is a very important crop (vegetable) of sub-tropics and tropics of India. This plant is highly infested by, Henosepilachna vigintioctopunctata (Coleoptera: Coccinellidae). It is a polyphagous pest in nature, the adult and grub stages cause severe damage to the plants by feeding on its leaves, flowers and vegetables and create a heavy economic loss. Though the beetles could be controlled using synthetic pesticides, indiscriminate use of pesticide in the field causes problems such as pest resistance, environmental and health hazards etc. These pave way for the development of effective ecofriendly pest control measures. Plant based essential oils are used against these insect pests, as they constitute a rich source of bioactive components and reported to have many biological properties. Recently, essential oils were formulated as nanoemulsion and was developed for their effectiveness and eco-friendly nature. In this study, the oil in water (O/W) nanoemulsion of Mentha piperita were prepared by ultrasonication method at different concentrations and examined for the stability study. The stable nanoemulsion was characterized by DLS and was reported to consist with a mean droplet size of 10.84 nm, PDI was 0.1 and zeta potential was -45 mv which proved the good stability of the nanoemulsion. To assess the efficacy of the formulated nanoemulsion, and bulkemulsion, it was screened against the adult beetles, Henosepilachna vigintioctopunctata at different concentrations for 96 hours and the resulted LC_{50} value of nanoemulsion was 15.84 % which was found to be very effective than the LC_{50} value of bulkemulsion, which was 60.25 %. Hence from the results obtained, Mentha piperita nanoemulsion may be used as an organic pesticide because of its higher efficacy against the adult beetles, Henosepilachna vigintioctopunctata.

Keywords: Brinjal plant, Bulkemulsion, Essential oil, Henosepilachna vigintioctopunctata, Mentha piperita, and Nanoemulsion.

1. Introduction

Brinjal or eggplant (*Solanum melongena* Linn) is one of the most important vegetables crops grown all over India (Sarker *et al*., 2006) and it is well known for its nutritive value. Eggplant is highly infested by many insect-pests, but the major pest that attack the plant is *Henosepilachna vigintioctopunctata* (Coccinellidae: Coleoptera). It is the most destructive and polyphagous pest that leads to the heavy economic yield loss (Ali *et al*., 2017, Anam *et al*., 2006 & Rahaman *et al*., 2008, Dhamdhare *et al*., 1995). Both, the grubs and the adult beetles causes damage to the plants and infestation begins just after the hatching of eggs (Murata *et al*., 1994). The grubs were feed on the lower epidermal layer of the leaves and the adult beetles feed on the upper surface by scrapping the leaves (Imura and Ninomiya, 1978) [1-5].The synthetic pesticides were used to manage the pests (Samanta *et al*., 1999; Das *et al*., 2002) but there is always a growing concern regarding the adverse effects, due to the indiscriminate use of the pesticides in the field, such as contamination of pesticides residues in the food, pest becomes resistance and environment pollutions (Ogendo *et al*., 2008 & Kranthi *et al*., 2002). Recently, studies have reported the use of the plant essential oils, and their bioactive chemical constituents may be considered as an alternate to synthetic insecticides (Rajendran and Sriranjini, 2008), Since these essential oils consist of the different biological activity, such as antibacterial, antioxidant, insecticidal, etc. (Irshad *et al*., 2011 and Kumar *et al*., 2012). Although, the essential oils consist of various properties, there are also certain limitations which are due to their volatile and hydrophobic nature. So, these problems may be overcome by recent trends, such as formulating essential oils into nanoemulsion, due to their transparency, solubility in water and also less amount of essential oil is required (Qian *et al*., 2012) [6-11]. An attempt was made to formulate the *Mentha piperita* essential oil as nanoemulsion. Because, the genus *M. piperita* is commonly known as peppermint, it is a cultivated natural hybrid of two species, *Mentha* aquatic L and *Mentha spicata* L. (Saller, 2004) and it is cultivated worldwide (Foster, 1996). The *M. piperita* essential oil is known to have antimicrobial (Chraibi *et al*., 2016), antioxidant (Singh *et al*., 2015) and insecticidal (Kumar *et al*., 2011) activity. The present study aims to evaluate the insecticidal activity of *M. piperita* essential oil nanoemulsion against the serious agricultural pest, *H. vigintioctopunctata* (adult beetles). [12-16].

2. Materials and Methods

2.1 Materials: *M. piperita* essential oil, Tween 80 (surfactant), Acetone and distilled water were utilized for the entire formulations.

2.2 Methods: Insects were collected early morning within the vicinity of the Madras Christian College by hand picking method from the brinjal plants. Before the treatment, Insects were maintained at room temperature around 33°C with 67 ± 1% relative humidity and the fresh leaves were provided to the beetles.

2.3 GC-MS analysis:

JEOL, GC MATE II (GC Model), quadruple double focusing detector. One microliter of the essential oil was injected in the split less mode of the injection port of the GC column. 220 °C for about 1 min then 10 °C min^{-1} was setup as an inlet temperature. The Helium gas act as a carrier gas at the constant flow rate of 1.0 mL/min. Total run time was about to 60 min. The interface temperature (GC to MS) was set to be at 250 °C. The MS was in a scam mode and the temperature was 250 °C. Ions are obtained by an electron ionization mode. Molecular ions (mass range) were monitored for the identification, which was set 50-600 m/z peak area denoted the relative percentage of constituents.

2.4 Preparation of nanoemulsion:

By using the peppermint oil, Tween 80, Acetone and distilled water, the oil in water (O/W) nanoemulsions were prepared at different concentrations by ultrasonication method. The standard concentration was fixed for the peppermint oil (10%, v/v) and acetone (6%, v/v) for all formulations. The formulations were investigated for stability test and characterized. For the bulk emulsion, the peppermint oil (10%, v/v), acetone (6%, v/v), Tween 80 (15%, v/v) and water (69%, v/v) was used for formulation. [17-23].

2.5 Characterization of nanoemulsion:

To check the stability of the formulations, it was expressed to different thermodynamic study such as centrifugation, heating-cooling cycle and freeze-thaw cycle. After passing the study, the stable emulsion was considered for characterization of the particle size. Dynamic light scattering (Model: Nanotrac Wave II; Make- Microtrac Inc, (USA) technique was used for the analysis of particle size distribution, polydispersity index (PDI) and zeta potential. So, prior to the measurements the emulsion was diluted using double distilled water (1:100) and equilibrated at 25°C for 1 min. the measurements have performed and the average droplet size was expressed as mean diameter and the surface charge of the droplets was determined by measuring the electrophoretic mobility at 25°C and the values of zeta potential were expressed in mV.

2.6 Bioassay:

The bioassay was carried out to evaluate the insecticidal activity of *M. piperita* essential oil nanoemulsion and bulkemulsion to check its effectiveness against the agricultural pest the adult beetles, *H. vigintioctopunctata*. Both the emulsions were screened against beetles, at different concentrations such as 10, 20, 30 & 40 % (v/v). Each treatment contains of 10 beetles and the fresh leaves (similar sizes) were chosen for the entire treatments. By leaf dipping method, the fresh leaves have been dipped into emulsions and allowed the leaves to air dried about a half an hour before the treatment. The entire treatment was carried out for about 24, 48, 72 & 96 hours respectively. So finally, for every 24 hours the leaves treated with the emulsions at different concentrations has been provided to the beetles of the treatment groups and the leaves treated with the acetone alone has been provided to the beetles of the control groups. Each test was triplicated and the mortality was recorded after every 24 hours of the treatments and the percentage mortality was calculated.[24-28].

2.7 Statistical analysis:

All the data has been subjected to the mean value and standard deviation. By using the probit analysis method, the LC_{50} values were calculated and the significance difference between the treatment were analysed with one way ANOVA and multiple comparison between the variable were determine with Turkey's post Hoc test ($P < 0.05$) using the software SPSS, version 24.

3. Results & Discussion

3.1 GC-MS analysis

In the present study, the chemical constituents of *M. piperita* essential oil were identified using GC-MS technique. There are 15 components were identified and the major component was found to be menthol with highest percentage 16.47% among the other constituents **(Table 1 & Fig 1)**. The identified major component is in good agreement with Hashimoto *et al*., (2016) and Malheiros *et al*., (2016). Gershenzon *et al*., (2000) reported that the essential oils mostly consist of monoterpenes. Prakash *et al*., (2015) reported that the essential oils and their bioactive components together is responsible for the biological activity, So the menthol present in essential oil may be responsible for the insecticidal activity against the insect pests. Bakkali *et al*., *(2008)*, Huang *et al*., (2002) & Suthisut *et al*., (2011) has also reported the major component of the essential oils determines the biological activity. Peeyush *et al*., (2011) reported the *Mentha* species exhibit the insecticidal and the repellent against the various insect pests. The factors such as species, environmental conditions, physiological, geographical, genetic factors and also the extraction procedures, vary the chemical compositions present in the essential oils (Pala-Paul *et al*., 2001, Santos-Gomes *et al*., 2001, Lalli 2010 & Park *et al*., 2016). [29-35].

Table 1: Components of peppermint oil

S.No	R. Time	Name of The Compound	Molecular Formula	Area %
1	11.05	Patchoulane	$C_{15}H_{26}$	3.07
2	13.07	β- Farnesene	$C_{15}H_{24}$	3.77
3	14	á-cis-ocimene	$C_{10}H_{16}$	3.19
4	14.75	E-1,6-undecadiene	$C_{11}H_{20}$	5.00
5	15.98	Caryophyllene	$C_{15}H_{24}$	1.88
6	**16.63**	**Menthol**	$\mathbf{C_{10}H_{20}O}$	**16.47**
7	17.48	D-Limonene	$C_{10}H_{16}$	11.16
8	18.12	Pulegone	$C_{10}H_{16}O$	15.56
9	18.82	Menthone	$C_{10}H_{18}O$	8.21
10	20.05	(+)-Camphene	$C_{10}H_{16}$	7.36
11	21.38	Ethyl farnesoate	$C_{17}H_{28}O_2$	6.77
12	23.13	Aromadendrene	$C_{15}H_{24}$	5.58
13	25.52	Copaene	$C_{15}H_{24}$	4.23
14	26.78	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	4.09
15	28.83	n-Hexadeconoic acid	$C_{16}H_{32}O_2$	3.66

Table 2: Composition of the nanoemulsion formulations

Materials % v/v	PMF1	PMF2	PMF3	PMF4
Peppermint oil	10	10	10	10
Acetone	6	6	6	6
Tween 80	6	12	18	24
Distilled water	78	72	66	60
After stability study	**Phase separation**	**Phase separation**	**Phase separation**	**Stable**

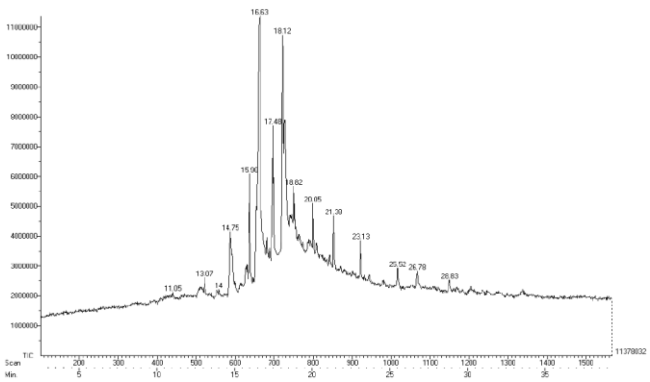

Fig 1 : Chromatogram of peppermint oil

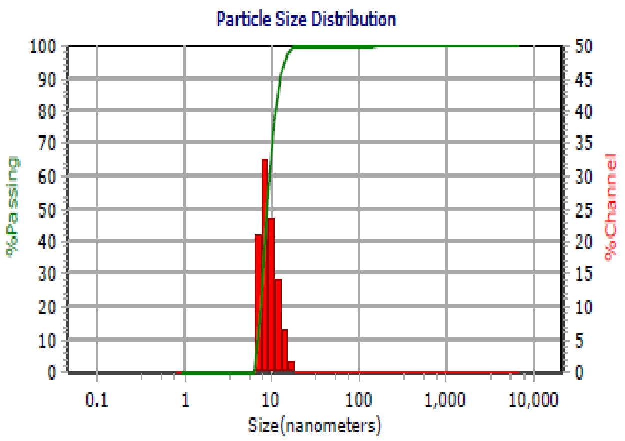

Fig 2: Particle size distribution by DLS

3.2 Characterization of Nanoemulsion

In the present study, the formulated emulsions after passing the thermodynamic stability tests, the stable emulsion was characterized with the particle size 10.84 nm, PDI value as 0.1 and zeta potential as -45 mV **(Table 2 & Fig 2)**. The droplet diameter less than 300 nm is considered to be as nanoemulsion (Sonneville *et al*., 2004, Nenaah, 2014 & Bordes *et al*., 2009). The PDI value less than 0.7 indicates to be broad distribution of the droplet size (Tang *et al*., 2013) and lesser the PDI value, the formulated nanoemulsion will consider to be more stable (Shinoda & Saito, 1969). The zeta potential value range above the -25 mV and +25 mV proves to be stable emulsion, while ranging within the values were consider to be unstable emulsion (Hertault *et al*., 2003). From the overall values, the formulated emulsion was found to be more stable and considered it as a nanoemulsion [32-37].

3.3 Toxicity against *H. vigintioctopunctata*

In our study, we screened the formulated nanoemulsion of *M. piperita* oil for its efficacy against the adult beetles of *H. vigintioctopunctata* at different concentrations and also compared with its own bulkemulsion. After the treatment of the 96 hours the highest mortality percentage was recorded at the 40 % concentrations, such as the nanoemulsion showed 96.6 % v/v and the bulkemulsion has showed the 40 % v/v respectively. While no mortality was observed in control sample. The nanoemulsion formulation of *M. piperita* oil is found to be more effective with LC: 15.84 % than its own bulk emulsion LC: 60.25 %, the highly significance results were based on the increase in the concentrations (df= 1, MS= 48.167, F= 72.250, p<0.001) **(Table 3 & Fig 3)**. From the above results, the highest mortality has observed in the nanoemulsion than the bulkemulsion, it is due to the small size of the particle already reported by many researchers (Anjali *et al*., 2011 & Sugumar *et al*., 2014). Mojgan Heydari *et al*., (2019) reported the various parameters during the formulations, shows the differences in the insecticidal efficiency of the nanoemulsion. Sugumar *et al*., (2014) has already reported the eucalyptus oil nanoemulsion ensures the higher effective against the *C. quiquefasciatus* larvae, than its bulkemulsion. Abouelkassem *et al*., (2015) has also reported that the jojoba oil nanoemulsion has showed a higher efficacy against the adult beetles, *Sitophilus oryzae* than its bulkemulsion. Therefore, the *M. piperita* nanoemulsion were found to be very effective against the adult beetle, *H. vigintioctopunctata* then the bulkemulsion and also in good agreement with the other researchers already reported. [37-42].

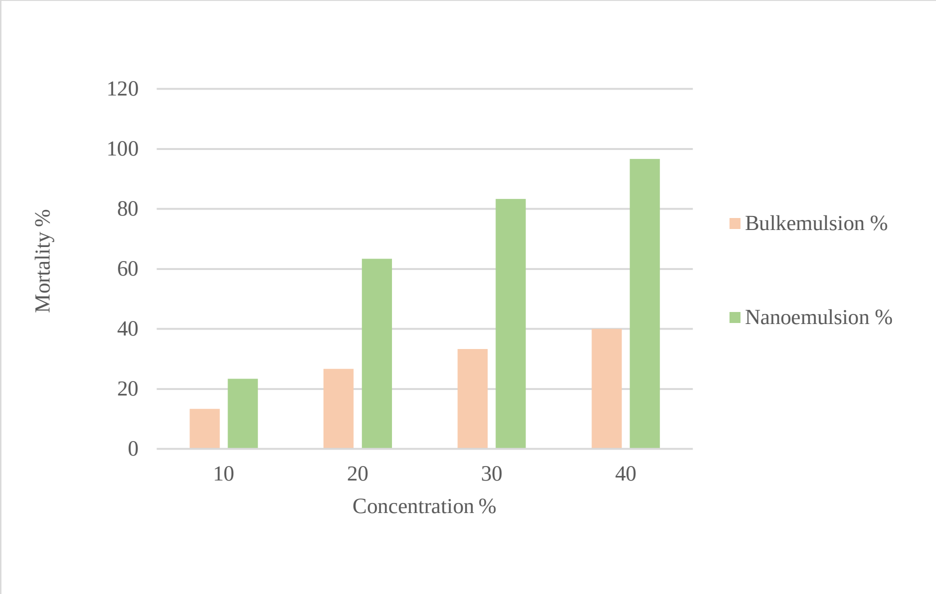

Fig 3: Comparative study between the M. piperita Nanoemuslion and Bulkemulsion, against the adult beetles, H. vigintioctopunctata.

Table 3: Bioassay of *M. piperita* oil, Nanoemulsion and bulk emulsion against the adult beetle, *H. Vigintioctopunctata.*

Bioassay	Concentrations (V/V) (%)	24 HRS (%)	48 HRS (%)	72 HRS (%)	96 HRS (%)	Total mortality (%)	LC_{50}	R^2
Nanoemulsion	10%	0.00±0.0	3.33±0.5	10.00±1.0	10.00±1.0	23.33±0.5	15.84	0.939
	20%	3.33±0.5	10.00±0.0	20.00±1.0	30.00±1.0	63.33±1.5		
	30%	13.33±0.5	20.00±1.0	26.67±0.5	23.33±0.5	83.33±1.5		
	40%	10.00±1.0	36.67±0.5	40.00±1.0	10.00±1.7	96.67±0.5		
Bulkemulsion	10%	0.00±0.0	0.00±0.0	6.67±0.5	6.67±0.5	13.34±0.5	60.25	0.966
	20%	3.33±0.5	0.00±0.0	6.67±0.5	16.67±0.5	26.67±1.1		
	30%	0.00±0.0	6.67±0.5	10.00±1.0	16.67±0.5	33.34±1.1		
	40%	3.33±0.5	6.67±0.5	3.33±0.5	26.67±0.5	40.00±1.0		

Conclusion

Hence, based on the results *M.piperita* nanoemulsion may be used as an alternative for the synthetic pesticides to control the agricultural pest *H. vigintioctopunctata* and also considered as safer to the non-target mammalian organisms, and also for environment sustainability.

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