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RESEARCH ARTICLE

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Natural Insecticidal Activity of *Mentha piperita* (Peppermint) Oil Nanoemulsion against Agricultural Pest *Aulacophora foveicollis* (Red Pumpkin Beetle) for Environmental Sustainability

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ABSTRACT

Background: Red pumpkin beetle, *Aulacophora foveicollis* (Coleoptera: Chrysomelidae), is a serious agricultural pest of cucurbit plants. Chemical pesticides have been in practice for many years to control the pests. Recently, the plant essential oils were formulated as nanoemulsion, have gained interest for their effectiveness and eco-friendly nature. The current study was aimed to assess the efficacy of *Mentha piperita* nanoemulsion against an agricultural pest *A. foveicollis.*

Methods: *M. piperita* essential oil was analyzed by GC-MS technique. Ulrasonication method was used to formulate the nanoemulsion. The stable nanoemulsion was investigated and characterized by DLS. The efficacy of the nanoemulsion was screened against the pest of cucurbits, *A. foveicollis* at different concentrations and compared with its own bulk emulsion.

Result: In GC-MS technique, menthol was found to be the major component with the highest percentage of 16.47 and the nanoemuslion was prepared in different ratios comprising of peppermint oil, surfactant, acetone and water. The stable nanoemulsion was investigated and characterized by DLS with mean droplet size of 10.84nm, PDI was 0.1 and zeta potential was -45 mv, altogether proves to be the good stability of nanoemulsion. The Bioassay was conducted for about 96 hours and the highest mortality was recorded in the nanoemulsion than the bulk emulsion. The resulted LC_{50} value of nanoemulsion is 22.38% v/v is found to be effective than the LC_{50} value of bulk emulsion is 87% v/v, respectively. Thus, from the results obtained the nanoemulsion was found to be highly significance than that of the bulk emulsion. So, nanoemuslion may be used as an eco-friendly agent against the agricultural pest management.

Key words: *Aulacophora foveicollis,* Bulkemulsion, Essential oil, Insecticidal activity, *Mentha piperita*, Nanoformulation.

INTRODUCTION

Red pumpkin beetle, *Aulacophora foveicollis* Lucas is a polyphagous pest of cucurbits plants and inflicts damage of about 30-100% yield loss (Gupta and Verma, 1992 and Doharey, 1983). The larvae feed on the root tissues and destroy the newly developed seedlings and the adult beetles feed voraciously on leaves, flowers and flower bud. The damage is severe because the beetles are difficult to control (Narayanan and Batra, 1960). However, this pest occurs throughout the year and damages the crop severely mostly at seedling stage (Rajak, 2001).

Essential oils are compounds found in aromatic plants as a mixture of volatile components, were present as secondary metabolite in plants (Bassole *et al*., 2011). This essential oil was found to act as repellent, fungicidal, insecticidal and growth -reducing effects on insects (Pooja singh *et al*., 2012 and Gaurav *et al*., 2017). The essential oil of *Mentha piperita* are used in cosmetics, aromatherapy, perfume industry, *etc.* and are also known to have the antiviral, antibacterial, antifungal and insecticidal activity (Saharkhiz *et al*., 2012 and Samarasekera *et al*., 2008). Therefore, the formulation of essential oils into nanoemulsion is developed for its efficient and potential application in different fields (Fernandes *et al*., 2014 and Sugumar *et al*., 2014) and also due to its decreased in particle size of essential oils from original to nano-size (Anjali *et al*., 2012). This naturally occurring components from the plants were considered to be very essential source and may be used as an alternate for synthetic pesticides for the insect-pest management (Rattan, 2010). The aim of our study was to evaluate the chemical constituents present in the *M. piperita* essential oil and then to formulate nanoemulsion by

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ultrasonication method and to assess the insecticidal activity of *M. piperita* oil nanoemulsion against the agricultural pest of cucurbit plants, *A. foveicollis*.

MATERIALS AND METHODS

Materials

Peppermint oil, Tween 80, acetone and distilled water was used for the preparation of nanoemulsion.

Insect collection and rearing

Insects were collected with in the vicinity of Madras Christian College during the early morning from the cucurbitaceous plants. Insects were maintained in laboratory, at room temperature, 33°C with relative humidity 67 ± 1%. The fresh leaves of cucurbit were provided to the insects daily early in the morning. The research duration was conducted from august 2019 to February 2020.

2.3 GC-MS analysis

GC-MS analysis was performed by using JEOL, GC MATE II (GC Model), quadruple double focusing detector. One microliter of the extract was injected in split less Mode in an injection port of GC column. The inlet temperature was set at 220°C for about 1 min then 10°C min^{-1}. Total run time was set up to 60 min. Helium gas was used as the carrier gas at the constant flow rate of 1.0 mL/min. The interface temperature (GC to MS) was set to be at 250°C.

MS was set in scam mode. MS quad temperature was 250°C. Ions were 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.

Preparation of nanoemulsion

The oil in water (O/W) nanoemulsion was formulated using peppermint oil, surfactant (Tween 80), acetone and distilled water. The concentration of peppermint oil (10%, v/v) and acetone (6%, v/v) was fixed for all the formulations. Initially, coarse emulsion was prepared by adding distilled water to organic phase containing oil and surfactant in different ratios using a magnetic stirrer, which was then subjected to ultrasonication. The formulated nanoemulsion was investigated for its stability and characterized by different techniques. Bulk emulsion was also formulated using peppermint oil (10%, v/v), acetone (6%, v/v), Tween 80 (15%, v/v) and water (69%, v/v). The general composition of the formulation is mentioned in the Table (1).

Characterization of nanoemulsion

Thermodynamic stability study: To prove the stability, formulated emulsion was subjected to different stress tests (Shafiq *et al*., 2007).

I. Centrifugation: The formulated nanoemulsions were centrifuged at 1500 rpm for 30 min and observed for phase separation, if any. It was triplicated.
II. Heating-cooling cycle: This was carried out by keeping the nanoemulsion at 40 and 4°C, alternating each temperature for 48 h. The cycle was repeated thrice.
III. Freeze-thaw stress: This was carried out by keeping the nanoemulsion alternatively at -21 and 25°C for each 48 h at each temperature. This cycle was also repeated thrice.

The formulation that passes through the thermodynamic stress tests was taken for the further characterization of studies.

pH measurement

The pH value was measured by using the digital pH meter, Model Ⅲ E. The electrode has immersed into the emulsion, using the calibrated pH meter the reading was noted and the measurement was repeated for three times.

Droplet size distribution, PDI and Zeta potential

The droplet size distribution (by volume) and polydispersity index (PDI) of peppermint oil nanoemulsion formulation was determined by dynamic light scattering using a Nanotrac Wave II; Make- Microtrac Inc, (USA). Prior to the measurement studies, emulsion was diluted using double distilled water (1:100) and the Samples were equilibrated at 25°C for 1 min. The measurements were performed and the average droplet size was expressed as the mean diameter. The surface charge of the nanoemulsion droplets was determined by measuring the electrophoretic mobility at 25°C and the values of zeta potential were expressed in mV.

Bioassay

The bioassay was carried out to evaluate the insecticidal activity of peppermint essential oil nanoemulsion and bulkemulsion for its efficacy against the adult beetles, *A. foveicollis*. The nanoemulsion and bulkemulsion was screened against the *A. foveicollis* at different concentrations such as 10, 20, 30 and 40% (v/v). Each treatment was consisting of 10 adult beetles, were placed into ventilated plastic box (7.75 x 6.87 x 3.50). Approximately, similar sizes of the fresh leaves of cucurbits were chosen for the entire

Table 1: Composition of the nanoemulsion formulations.

Materials % v/v	F1	F2	F3	F4
Peppermint oil	10	10	10	10
Acetone	6	6	6	6
Tween 80	6	12	18	24
Distilled water	78	72	66	60
Initial physical appearance	White emulsion	White emulsion	Translucent	Clear transparent
After stability study	Phase separation	Phase separation	Phase separation	Stable

treatment. By leaf dipping method, the fresh leaves were dipped in to the emulsions and the leaves were air dried for half an hour before the treatment. The entire treatment was setup for 24, 48, 72 and 92 hours respectively. Finally, for every 24 hours the leaves treated with the emulsions at different concentrations were provided to the beetles of treatment groups and the leaves treated with the acetone alone were provided to the beetles of control groups. Each test was performed in three replicates and the mortality was recorded after every 24 hours of treatment and the percentage mortality was calculated.

Statistical analysis

The data were subjected to mean, standard deviation and the values of LC_{50} were calculated by using the probit analysis method. The significance difference between the treatment were analyzed 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.

RESULTS AND DISCUSSION

GC-MS analysis of *M. piperita*

The *M. piperita* essential oil were analyzed by GC-MS. As a result, the 15 main constituents were identified and the major bioactive compound was found to be menthol with the highest percentage (16.47%), followed by Pulegone (15.56%), D-Limonene (11.16%) (Table 2 and Fig 1). Based on the literature surveys, the menthol was found to be the main component of *M. piperita* essential oils Iscan *et al*. (2002) and Sokovic *et al*. (2009) and reported to have the analgesic, anti-inflammatory, and antimicrobial properties Kamatou *et al.* (2013). The chemical constituents of *M. piperita* oil investigated in our study were mostly monoterpenes. The quantity and type of the monoterpenes present in the essential oil also indicates the improvement of the insecticidal activity of the naturally produce essential oil Chauhan *et al*. (2018).

Characterization of nanomeulsion

After passing the stability stress, the stable nanomeulsion (Table 1 and Fig 2) were analyzed for characterization. The pH of the formulated nanoemulsion was found to be 5.58. Morsi *et al*. (2014) measured the pH of acetazolamide nanoemulsion and was found to be 4.9 to 5.5, which indicates to be non-irritant for eye.

The particle size was analyzed for the mean droplet diameter were found to be 10.84 nm with the 0.1 PDI value. Anjali *et al.,* 2012 have already reported the effectiveness of *A. indica* nanoemulsion against the *C. quinquesfasciatus,* larvicidal activity is due to the particle size of the nanoemulsion.

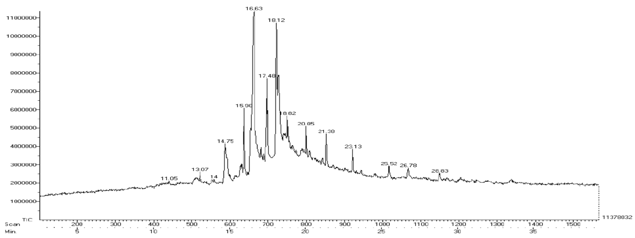

Fig 1: Chromatogram of peppermint oil.

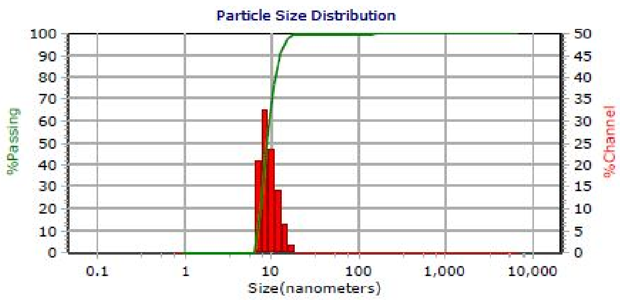

Fig 2: Particle size distribution by DLS

Table 2: Components of peppermint oil.

R. Time	Name of the compound	Molecular formula	Area %
11.05	Patchoulane	$C_{15}H_{26}$	3.07
13.07	β- Farnesene	$C_{15}H_{24}$	3.77
14	α-cis-ocimene	$C_{10}H_{16}$	3.19
14.75	E-1,6-undecadiene	$C_{11}H_{20}$	5.00
15.98	Caryophyllene	$C_{15}H_{24}$	1.88
16.63	Menthol	$C_{10}H_{20}O$	16.47
17.48	D-Limonene	$C_{10}H_{16}$	11.16
18.12	Pulegone	$C_{10}H_{16}O$	15.56
18.82	Menthone	$C_{10}H_{18}O$	8.21
20.05	(+)-Camphene	$C_{10}H_{16}$	7.36
21.38	Ethyl farnesoate	$C_{17}H_{28}O_2$	6.77
23.13	Aromadendrene	$C_{15}H_{24}$	5.58
25.52	Copaene	$C_{15}H_{24}$	4.23
26.78	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	4.09
28.83	n-Hexadeconoic acid	$C_{16}H_{32}O_2$	3.66

PDI value represents the non-uniformity in particle size, Hoeller *et al*. (2009) have reported the lesser the PDI value provides the good stability of the nanoemulsion.

Shi *et al.* (2017) has already reported that the zeta potential values greater than + or – 25 mV, indicates the good stability of the particles. Therefore, the zeta potential was found to be-45 mV in the present research, which indicates the good stability of the nanoemulsion. Over all, the characterization of the nanoemulsion showed a better particle size with low PDI values, zeta potential and pH values, which proves the good stability of the nanoemulsion. Further, the nanoemulsion was also physically observed for six months in the room temperature, for any phase separation. Hence, no phase separation occurred which also indicates the good stability of the nanoemulsion.

Efficacy of *Mentha piperita* nanoemulsion

In the present study, the stable nanoemulsion of *M. piperita* was screened for its efficacy against the serious agricultural pest *A. foveicollis* in different concentrations and also compared with the bulk emulsion of the same. From the above treatment, the highest mortality percentage was recorded at the 40% concentration of nanoemulsion with 86.6% v/v and bulkemulsion with 36.6% v/v respectively. The highest mortality occurred in nanoemulsion formulation is due to the small size of the droplet. While no mortality was observed in the control samples. It has been observed that the nanoemulsion formulation of peppermint oil is more effective with LC_{50} 22.38% than the bulk emulsion LC_{50} : 87% (Table 3 and Fig 3). Based on the results obtained, a significance increase in the mortality of *A. foveicollis* is also due to the increase in the concentration of nanoemulsion (df= 1, MS= 37.500, F= 13.235, p<0.022), the increase in ingredient reveals the high effectiveness, as reported by many researchers earlier such as Mojgan Heydari *et al.* (2019) and Anjali *et al.* (2012). The several parameters such as concentration of the formulation, preparation timing, exposure timing, insect species and method of application, plays an important role in the insecticidal efficiency of the nanoformulation Mojgan Heydari *et al.* (2019) and Anjali *et al.* (2012). Now-a-days, nanoemulsions where also used as botanical insecticides, such as the *Manilkara subsericea* essential oil formulated as nanoemulsion showed the insecticidal activity against *Dysdercus peruvianus* Fernandes *et al.* (2014). Hashem *et al*., (2018) reported that essential Oil of *Pimpinella anisum* nanoemulsion showed the insecticidal activity against *Tribolium castaneum*. Recently, the peppermint oil nanoemulsion was also reported to consist of aphicidal activity Mojgan Heydari *et al.* (2019). Therefore, the results obtained in the present work is compatible with Anjali *et al*. (2011) the nano-permethrin is more effective towards the *C. quinquefasciatus* larvae, than compared to the bulk-permethrin, it was demonstrated it is due to the size effect. In the current research work, *M. piperita* nanoemulsion has shown the high significance as insecticidal activity against the agricultural pest *A. foveicollis*, of cucurbit plants when compared to its bulkemulsion.

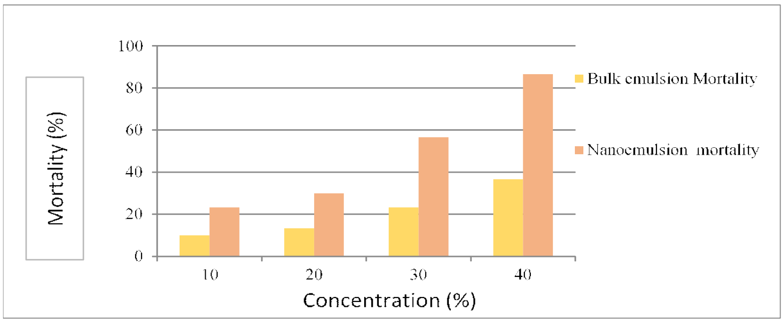

Fig 3: Comparative study between the *M. piperita* Nanoemuslion and Bulkemulsion, against the adult beetles, *A. foveicollis.*

Table 3: Bioassay of nano and bulk emulsion peppermint against adult beetle, *Aulacophora foveicollis.*

Bioassaty	Concentrations (V/V) (%)	24 HRS (%)	48 HRS (%)	72 HRS (%)	96 HRS (%)	Total mortality (%)	LC_{50}	R^2
Nanoemulsion	10%	13.33	10.00	0.00	0.00	23.33	22.38	0.9401
	20%	10.00	10.00	6.67	3.33	30.00		
	30%	13.33	6.67	20.00	16.67	56.67		
	40%	23.33	33.33	30.00	0.00	86.66		
Bulkemulsion	10%	0.00	3.33	6.67	0.00	10.00	87	0.9403
	20%	0.00	0.00	6.67	6.67	13.34		
	30%	0.00	10.00	6.67	6.67	23.34		
	40%	10.00	6.67	6.67	13.33	36.67		

The highest significant mortality was observed at 40% of nanoemulison. Confidence limit at 95%.

CONCLUSION

Nanotechnology is a boon for the development of modern techniques for the improvement in agricultural sector. Over all from the results obtained, the present study proved that the efficiency of formulated nanoemulsion of *M. piperita* ensures the higher efficacy as an insecticidal activity against the adult beetles, *A. foveicollis* when compared to its bulk emulsion under laboratory condition. Therefore, nanoemulsion may be used as an alternative of synthetic pesticides for the agricultural pest management because of its significant impact as insecticidal activity and environmental sustainability.

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