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# Fabrication, characterization and bio-efficacy of volatile oil based nano bio-pesticide.

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**ABSTRACT**

Volatile oil based pesticides are gaining importance in the Integrated Pest Management (IPM) strategies as an alternative to the chemical pesticides. Due to the broad spectrum activity of these volatile oils on agriculture pests and domestic pests, we selected the volatile oils such as lemon grass, citronella and palmarosa; which was been formulated into a nano emulsion. A detailed characterization of the nano emulsion was undertaken. The bioefficacy of this volatile oil based nano emulsion was assessed on the chilli pests, *S.dorsalis* which showed an 86.4% reduction in the pest population during the study period.

**Keywords**: Volatile oil, bio efficacy, nano bio pesticide, nano emulsion, particle size, high energy emulsification

**INTRODUCTION**

It is well documented that the volatile oils of plant origin have pesticidal properties by means of repellency, antifeedancy, respiratory blocker, neurotoxin and heamotoxin to various insect pests. The usage of synthetic pesticides usually leads to serious environmental threat. Many plant volatile oils and their phytochemical constituents which is considered as reduced risk pesticides possess broad spectrum of insecticidal activites.

Plant-based extracts and volatile oils based bio insecticides formulations have emerged as attractive alternatives to synthetic insecticides in the recent two decades for insect pest management. These naturally occurring insecticides are derived from plants and contain a range of bioactive chemicals [5]. These bioactive chemicals act as repellents, attractants, antifeedants, respiratory blockers, disturbing the host plant selection, oviposition deterrence, ovicidal , adult emergence and larvicidal effect which is depending on the physiological characteristics of insect species as well as the type of host plant, plant extracts and volatile oils (EOs). [1,2,3]. The composition of volatile oils varies greatly and hence the volatile oils based formulations show multidimensional activity on the insect control. Volatile oils such as citronella and lemon grass are potent biochemical pesticide candidate. It is estimated that the volatile oil represent a market of USD 700.00 million with a total world production of 45,000 tons. Industries in the US are able to bring volatile oil-based pesticides to market in a shortened time period, as compared to the time taken in conventional pesticide launch [8].

Below table shows some Common volatile oils used in pesticide formulations.

**TABLE 1: List of common volatile oils used in pesticides formulation:**

| **Plant volatile oil** | **Botanical source (species, family)** | **Major constituent(s) (% by weight) [ref]** |
|---|---|---|
| Cinnamon oil | *Cinnamomum verum* (Lauraceae) | Cinnamaldehyde (55–76), eugenol (5–18) [3] |
| Citronella oil | *Cymbopogon winterianus* (Poaceae) | Citronellal (27–33), citronellol (10–16), geraniol (24–40) [3] |
| Clove oil | *Syzygium aromaticum* (Myrtaceae) | Eugenol (89) [3] |
| Eucalyptus oil | *Eucalyptus globulus* (Myrtaceae) | 1,8-Cineole (67–84) [3] |
| Lemongrass oil | *Cymbopogon citratus* (Poaceae) | Geranial (34–45), neral (5–51), myrcene (9–25) [3] |
| Mint oil | *Mentha* spp. (Lamiaceae) | Menthol (30–55), menthone (10–40) [3] |
| Orange oil | *Citrus sinensis* (Rutaceae) | *d*-Limonene (91–97) [3] |
| Peppermint oil | *Mentha piperita* (Lamiaceae) | Menthol (7–48), menthone (20–46) [4] |
| Rosemary oil | *Rosmarinus officinalis* (Lamiaceae) | 1,8-Cineole (52), α-pinene (10), camphor (9), α-pinene (8) [5] |
| Tea tree oil | *Melaleuca alternifolia* (Myrtaceae) | Terpinen-4-ol (35–48), α-terpinene (14–28) [6] |
| Thyme oil | *Thymus vulgaris* (Lamiaceae) | Thymol (50), *p*-cymene (33) [7] |

Though these volatile oil are effective in controlling the insect pests of agriculture, horticulture, household and stored products pests, utilization of the same have some limitations because of their high volatility, poor water solubility and susceptibility towards degradation. Hence to enhance improved water solubility, enhanced bio-efficacy, stability and controlled release, thereby expanding their applicability fabrication of nanoemulsion of these volatile oils is currently considered as a promising tool.

Volatile oil based nanoemulsions is one of the novel method in bio-pesticides formulation. In nanoemulsion formulation an emulsion plays a vital role through repulsive electrostatic interactions and steric hindrance. Nanoemulsions are prepared using two different methods viz., high energy method and low energy method. High energy emulsification method involves high pressure homogenization, high shear blending and ultrasonication. [9] Among this high energy emulsification system, ultrasonication is most efficient technique in developing stable nanoemulsion with smaller droplet size and low polydispersity index.

It has been evident from the previous works done in past 5 to 10 years that the use of volatile oil over other types of plant extract, is relatively high due to its well-known chemistry and terpenoid as a common constituent. Nanomaterial in the form of nano bio pesticides have a potential value due to is small size, big surface area and target modified properties. [4].

Nano-emulsions have gained popularity in recent times for their widespread usability in agriculture. Nano-emulsions are kinetically stable systems (droplet size range in the order of 100 nm) exhibiting multiphase colloidal dispersion with longer shelf life [10]. Due to their small size they enhance penetration, spreading and uniform distribution on the targeted area. Use of nano-emulsions formulation (water/non-ionic surfactant/ methyl decanoate) for the production of pesticide (β-cypermethrin) by phase inversion composition has been reported for commercial use. [11] The nano emulsion based pesticides formulation techniques were reviewed in detail by Priyanka and Prem (2023) [12].

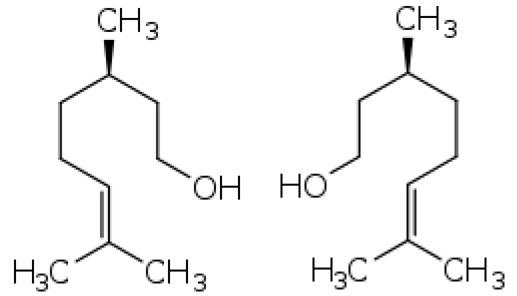
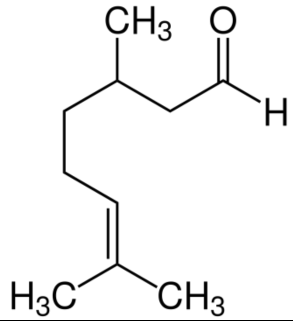
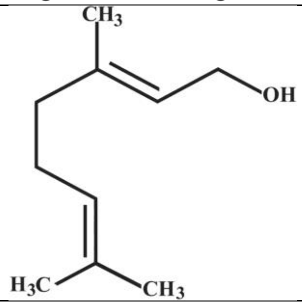
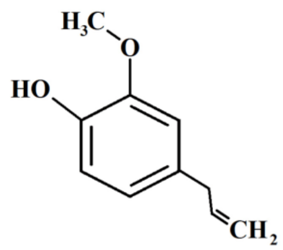
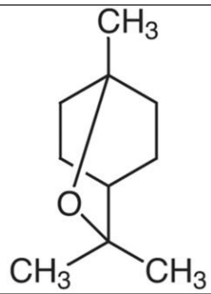
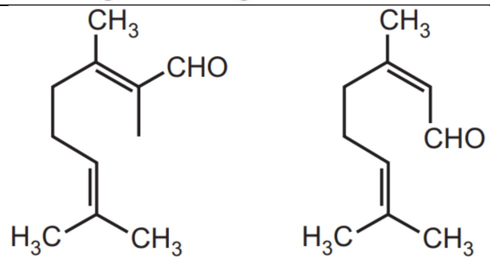
The formulation of selected volatile oils into a nano emulsion will give a new avenue to the biopesticides industry. Hence in the present study was carried out to fabricate, characterize and test the bioefficacy of a volatile oil based nano bio-pesticides.

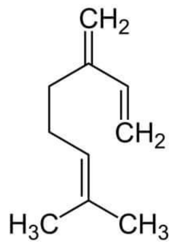
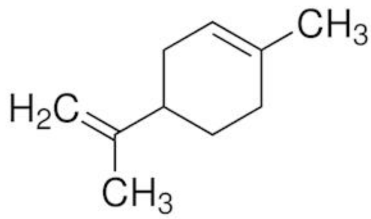
## MATERIALS AND METHODS

The volatile volatile oils Viz., citronella, lemongrass and palmarosa was procured from CIMAP, Lucknow. The solvent (Methanol), emulsifier (Tween-20), sticking agent (Poly Ethylene glycol) and surfactant (Oleic acid) was procured from SD Fine Chemicals. Carrier oil (Cold pressed Neem oil) was procured from country shop.

The volatile oils have the following compounds as in the table 1.

**TABLE 2: Chemical structure of various compounds in the 3 volatile volatile oils**

| Plant | Oil | Chemical structure |
|---|---|---|
| Citronella, Lemon Grass and Palmarosa | Citronellol |  |
| | Citronellal |  |
| | Geraniol |  |
| | Eugenol |  |
| | 1,8 Cineole |  |
| | Gerania and Neral |  Geranial (citral a) Neral (citral b) |

| | | |
|---|---|---|
| | Myrcene |  |
| | D limonene |  |

**Nanoemulsion preparation:**

Nanoemulsion was prepared using volatile oil, non-ionic surfactant Tween 20 (Hydrophile-Lipophile Balance (HLB) v alue-16.7) and water. Coarse emulsion was prepared by mixing oil, Tween 20 and water. Then the coarse emulsion was subjected to sonication using a Sonicator (Ultrasonics, USA) at a high frequency of 20 kHz and power output of 750 W. Energy input was given through sonicator probe with a probe diameter of 13 mm which generates strong disruptive forces and reduces droplet size of emulsion. Then to ensure the droplet size, this nano emulsion is then subjected to high shearing at an RPM of 3000 by the method explained by Priyanka and Prem (2023)[12]. The formulated nanoemulsions were then characterized.

Volatile oil + Solvent + Sticking agent ⟶ Solution A

Carrier oil + Emulsifier + Surfactant ⟶ Solution B

Solution A+ Solution B High Energy Emulsification ⟶ Nano Emulsion

**Characterization of Nano emulsion.**

**Analysis of chemical parameters:**

The volatile oil based nano emulsion was analyzed, for various physio-chemical parameters. To determine the free fatty acids, the ISO 5317:1983 method was used to analyse only the free fatty acids present in the crude oils. The Saponification value was estimated by AOCS Official method cd 3b-76 and the Acid value was analysed using AOCS official method cd 3d-63. AOCS official method cd 4-40 was used in determining the Hydroxyl value of the sample. The Iodine value was determined by using AOCS official method cd 1d-92. The analysis for free fatty acids was carried out through ISO 5317:1983. The content of the Sulphate ash was calculated by ISO 5809:1982 (en). The pH of the nano emulsion was checked by using ISO 4045:2018 and the water content was measured by ISO 1666:1996 (en) method. High performance liquid chromatography (HPLC) was used in determining Ricinoleic acid, Oleic acid and Palmitic acid.

**Analysis of physical parameters**

The various physical parameters were determined using standard methods. The colour, appearance and dispersibility were analysed visually. The Flavour score method was used in analysing the odour present in the volatile oil. Laser diffraction measure was used in calculating the droplet size. ASTM E537 – 20 was used in determining the Thermodynamic Stability of the sample. The density was measured using ASTM D792, ISO 1183 method. The ISO 3104 method was used in determining the viscosity of the volatile oil. To evaluate the accelerated Storage Stability ISO 16779:2015 method was used and Emulsion Stability was measured by DLS vs Time method.

**Droplet size determination**

The emulsion droplet size and size distribution was determined using particle size analyzer (Malvern-UK, 4700 model). Droplet size was analysed using dynamic light scattering (DLS) technique. Prior to all the experiments, the nanoemulsion formulations were diluted with water to get rid of the multiple scattering effects. The droplet size and the polydispersity index (PDI) of the formulated nanoemulsion were measured.

**Stability**

The emulsion developed by ultrasonic emulsification was subjected to centrifugation at 10,000 rpm for 0.5 h and the resistance of emulsion to centrifugation was studied. Thermodynamic stability was checked by storing the formulated emulsion at both refrigerator temperature (4 ºC) and room temperature (25 ºC) Kinetic stability was studied by storing the emulsion at room temperature for prolonged storage time. It was then observed for phase separation or creaming or cracking if any.

**Bioefficacy studies.**

The method of studying the bioefficacy of the volatile oil based nano emulsion against *Scirtothrips dorsalis* has been described in detail below.

The experiment was laid out in a randomized block design (RBD) with five treatments including the control and each treatment was replicated thrice. The individual plot size was 3m x 4 m, keeping row to row and plant to plant distance of 30cm and 10 cm, respectively. The recommended package of practices was followed to raise the crop.

**Bio pesticide and their application**

The volatile oil based nano emulsion was applied as a foliar spray. The spraying was done by using a pre-calibrated foot sprayer. There were 3 treatments of volatile oil based nano emulsion. T1 (2.00 ml/liter), T2 (2.25 ml/liter) and T3 (3.375 ml/liter). The comparator treatment (T4) was also done using a chemical pesticide. The study duration was 4 weeks. There was a spray of every week.

The observations on Chilli thrips population were recorded on five randomly selected and tagged plants on three leaves each from top, middle and bottom canopy of plants in each plot.

The observations on aphid population were recorded early in the morning by visual counting method. The data thus obtained were taken into consideration to calculate the percentage reduction in the population which was determined by applying a correction factor given by Henderson and Tilton (1995) [13] referring it to be a modification of Abbott (1925) [14] formula.

$$\text{Percentage reduction} = 100 \times 1 - \frac{Ta \times Cb}{Tb \times Ca}$$

Where,
Ta = Number of insects after treatment
Tb = Number of insects before treatment
Ca = Number of insects in untreated control after treatment.

**RESULTS**

Table 3 and 4 shows the results of important chemical and physical properties of formulated volatile oil based nano emulsion, which was been produced using high energy emulsification methods (HEE). This method has yielded a particle size of 65-75 nano meter r. The formulation is stable in terms of thermodynamic stability and accelerated storage stability.

**Table 3: Chemical characteristics of volatile oil based nano emulsion formulation:**

| Parameter | Method | Range | Result | Unit |
|---|---|---|---|---|
| Saponification value | AOCS Official method cd 3b-76 | 65 - 70 | 70 | mg |
| Acid value | AOCS official method cd 3d-63 | ≤ 2.0 | Conform | mg |
| Hydroxyl value | AOCS official method cd 4-40 | 65 - 78 | 66 | ml/g |
| Iodine value | AOCS official method cd 1d-92 | 25 - 35 | 30 | |
| Water | ISO 1666:1996 (en) | 5.0 - 10 | 5 | % |
| pH | ISO 4045:2018 | 5.0 - 7.0 | 6 | |
| Sulphate ash | ISO 5809:1982 (en) | ≤ 0.2 | Conform | % |
| Free fatty acids | ISO 5317:1983 | ≤ 1.0 | Conform | % |
| Ricinoleic acid | HPLC | ≤ 0.2 | Conform | % |
| Oleic acid | HPLC | ≤ 0.1 | Conform | % |
| Palmitic acid | HPLC | ≤ 0.1 | Conform | % |

**Table 4: Physical characteristics of volatile oil based nano emulsion formulation**

| Parameter | Method | Result | Unit |
|---|---|---|---|
| Appearance | Visual | Golden Yellow viscous liquid | Visual |
| Colour | Visual | Golden yellow | Visual |
| Odour | Flavour score | Typical aromatic | Flavour |
| Droplet size | Laser Diffraction | 65-75 | r, nm |
| Thermodynamic stability | ASTM E537 - 20 | Stable | Visual |
| Dispersibility | Visual | B | Visual |
| Density (25 °C) | ASTM D792, ISO 1183 | 1.06 | g/ml |
| Viscosity (25 °C) | ISO 3104 | 680 | mPas |
| Accelerated Storage stability | ISO 16779:2015 | Stable | Stable |
| Emulsion stability | DLS vs Time | Stable | Stable |

Table 5 shows the particle size distribution of the nano emulsion during the HEE process. It was observed that, there was a gradual development of nano particles and nano emulsion from macro to micron to sub micron to nano. It took 50 minutes to form a 100% nano emulsion with a particle size distribution of 65% of 20 nm; 25% of 40 nm and 10% of 60 nm.

**Table 5: Particle size distribution at various stages of High Energy Emulsification (HEE) formulation**

| S. No. | HEE reaction time (Minutes) | Particle Size (Range) |
|---|---|---|
| 1 | 0 | Macro |
| 2 | 5 | Macro |
| 3 | 10 | Micron |
| 4 | 15 | Micron to Sub Micron |
| 5 | 20 | Sub Micron |
| 6 | 25 | Sub Micron to Nano |
| 7 | 30 | Nano particles 50% |
| 8 | 35 | Nano particles 75% |
| 9 | 40 | Nano Emulsion 40%<br>20 nm – 10%<br>40 nm – 25%<br>60 nm – 65% |
| 10 | 45 | Nano Emulsion 70%<br>20 nm – 30%<br>40 nm – 40%<br>60 nm – 30% |
| 11 | 50 | Nano Emulsion 100%<br>20 nm – 65%<br>40 nm – 25%<br>60 nm – 10% |

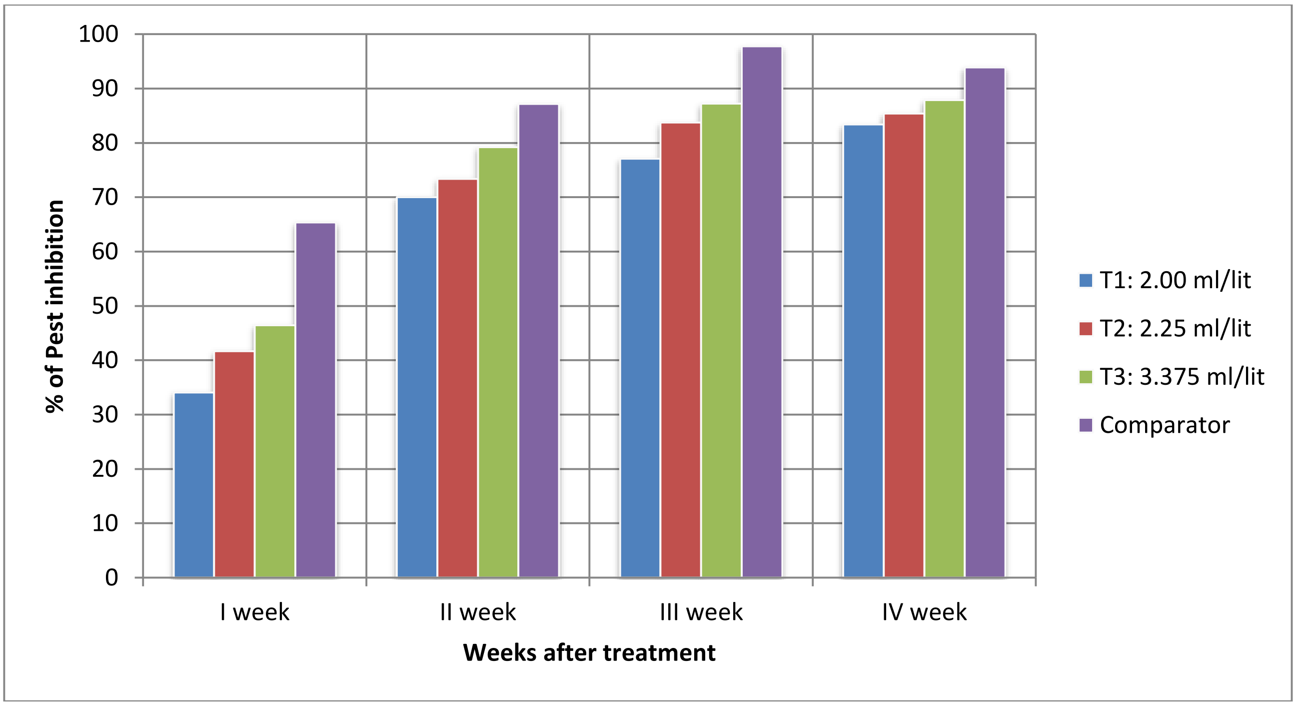
The bioefficacy of the volatile oil based nano emulsion is presented in table 6 and graph 1.

This formulation shows a good efficacy in controlling the chilli thrips, *S.dorsalis* at all the three dosages of treatments . A mean control of 66.12% on 2.00 ml per liter, 71.04% on 2.25 ml/liter and 75.19% on 3.375 ml/liter was recorded. The comparator chemical pesticide showed a mean control of 86.04% after the treatment and observation period of 4 weeks. All the three dosages of volatile oil based nano emulsion formulations showed a control 83.40%, 85.40% and 87.89% at 2.00 ml, 2.25 ml and 3.375 ml/liter respectively at the IV week of spraying and observations.

**Table 6:** Bio-efficacy of Volatile oil based nano emulsion formulation against Chilli thrips, *Scirtothrips dorsalis.*

| ***T. No*** | **Treatment** | **Dosage Ml/lit** | **% Bio-efficacy** | | | | **Mean (%)** |
|---|---|---|---|---|---|---|---|
| | | | I week | II week | III week | IV week | |
| *T1* | Volatile oil formula | 2.00 ml | 34.05 | 69.99 | 77.06 | 83.40 | 66.12 |
| *T2* | Volatile oil formula | 2.25 ml | 41.68 | 73.36 | 83.73 | 85.40 | 71.04 |
| *T3* | Volatile oil formula | 3.375 ml | 46.45 | 79.19 | 87.22 | 87.89 | 75.19 |
| *T4* | Comparator | 2.25 ml | 65.33 | 87.18 | 97.78 | 93.86 | 86.04 |

**Graph 1:** The bioefficacy of the volatile oil based nano emulsion



**DISCUSSION:**

Over the three decades of research on the interaction of plant natural products and insect plant interaction to develop bio insecticides, only two products are commercially successful viz., Azadirachtin based bio insecticides and plant volatile oils based bio insecticides. The plant volatile oils mainly work as a neurotoxin to insect pests and active even at the sub lethal dosages. These volatile oil based bio insecticides are used in niche agriculture practices, especially in organic food production.

Many research publications explains that the plant volatile oils and their phytochemicals constituents exhibit insecticidal activity via fumigant, contact, repellent, antifeedant, ovicidal, oviposition deterrent and larvicidal activity. These volatile oils also work as a neurotoxin by means of inhibiting or altering the neurotransmitters such as acetylcholine esterase (AChE) and octopamine or neurotransmitter inhibitor γ-amino butyric acid (GABA). Its also observed that these volatile oils alter the enzymatic systems such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POx), glutathione-S-transferase (GST) and glutathione reductase (GR)] and non-enzymatic [glutathione (GSH)] antioxidant defence systems.

Though, through the current study, the pest control potential of the volatile oil based nano formulation was ascertained, the real mechanism of action of this product needs further research and validations.

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