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**Insecticidal Effects and Chemical Composition of Lemongrass and Peppermint Essential Oils Against the Coconut Mealybug, *Nipaecoccus nipae* (Maskell), (Hemiptera: Pseudococcidae)**

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**ABSTRACT**

Plant essential oils have been found to exhibit pesticidal activity against many insects including mealybugs. The present study was conducted to evaluate the efficiency of different concentrations of two essential oils namely, lemon grass (*Cymbopogon citratus* (DC. Stapf.)) (Poales: Poaceae), and peppermint (*Mentha piperita* L.) (Mentha: Lamiaceae) on the populations of nymphs and adults of the mealybug *Nipaecoccus nipae* (Hemiptera: Pseudococcidae) under laboratory conditions after 24, 48 and 72 hrs. Additionally, analyzes and determines the chemical constituents of the two essential oils using GC-MS analysis. The results showed that the essential oils of lemongrass and peppermint recorded highly toxic effects against *N. nipae* after 72hrs. with lethal concentrations LC<sub>50</sub> values at 5.69 and 4.95 ppm for nymph populations, respectively. In the case of *N. nipae* adults, the LC<sub>50</sub> values were 6.45 and 2.99 ppm for lemongrass and peppermint essential oils after the same time, respectively. At the highest concentration (10000ppm), the percentages of *N. nipae* nymphs and adults mortality recorded the highest mortality percentage (100%) for lemongrass and peppermint essential oils after 72hrs. of treatment. Mealybug mortality increased with the increase in exposure time and oil concentration. Both essential oils were proved to be very toxic to *N. nipae* nymphs and adults. The GC-MS analysis results showed that twenty-two compounds were identified in the lemongrass essential oil representing 99.39% of essential oil compositions. These compounds mainly consisted of geranial (41.23%) and neral (34.51%) as major constituents. Other major compounds were geraniol (6.01%), Geranyl acetate (5.48%), while nineteen chemical constituents representing 99.99% of the *M. piperita* essential oil and the most abundant constituents of were menthol (36.94 %), menthone (22.62%), menthyl acetate (10.55%), and menthofuran (9.18%) and others are minor components such as  $\beta$ -pinene and  $\beta$ -caryophyllene. These monoterpenes compounds present in lemongrass and peppermint essential oils may responsible for the highly insecticidal activities against *N. nipae*. Our studies suggest that the lemongrass and peppermint essential oils can be useful as a botanical insecticide for an IPM strategy for mealybugs and protect crops against their attacks.

## INTRODUCTION

Mealybugs (Hemiptera: Pseudococcidae) are small, soft-bodied, sap-sucking insects that feed on nearly 149 plant species and nearly 246 families of different plants. They are serious agricultural pests and are thought to be the second-largest family of scale insects (Hemiptera: Coccoidea), with over 2,000 species and 300 genera. These mealybugs caused damage that effects several field crops (Millar *et al.*, 2002 and Gullan and Cook, 2007). *Nipaecoccus nipae* (Maskell), known as the coconut mealybug, is a new polyphagous pest of economically significant agricultural crops that affects 80 genera of plants and 43 families of plants (Ben-Dov, 1994). It attacks a variety of fruit and ornamental crops, including avocados, bananas, citrus, cocoa, coconuts, figs, guavas, mangoes, oil palms, orchids, pawpaws, pineapples, and grapes. It is widely distributed and has been found in America, Europe, Asia, Oceania and Africa (Ben-Dov, 1994 and Miller *et al.*, 2007). Due to the variety of the hosts that the coconut mealybug, *N. nipae* have a number of names, but coconut mealybug is the most frequent (Espinosa *et al.*, 2009). Yellowing, vigour loss, and discoloration of the roots at the place of feeding, followed by the drying out of such roots, are damage indicators of this pest (Mohan *et al.*, 2022). Due to the waxy substance mealybugs create, which offers protection from many insecticides, management can be challenging. Mealybugs are small and their colonies are frequently underground or under the bark of their host plants (Daane *et al.*, 2006), which reduces the effectiveness of any water-based pesticide solutions. As a result, mealybugs are difficult to control (Franco *et al.*, 2009). Chemical control methods for mealybugs have been inefficient nor sustainable (Tandon and Lal 1980). Insecticides lead to numerous problems including insecticide resistance. Additionally, the use of chemicals has been related to environmental contamination, harmful effects on non-target organisms, and the establishment of insecticide resistance in numerous populations of mealybugs (Franco *et al.*, 2009). Due to this circumstance, efforts to find and identify environmentally safe pest management approaches to control this pest have increased. Botanical pesticides that are naturally occurring and derived from floral sources are called phytochemicals. Due to their successful usage in plant protection as potential biocontrol agents, phytochemicals are botanicals that naturally occurring insecticides (botanical insecticides) (Ramya and Jayakumararaj 2009). In the context of integrated pest management programmes, using natural substances that can affect the physiology of insect pests is a possible alternative to chemical insecticides (La Pergola *et al.*, 2017 and Abdellaoui *et al.*, 2019). The purpose of this study is to investigate the effects of lemon grass (*Cymbopogon citratus* (DC. Stapf.) (Poales: Poaceae), and peppermint (*Mentha piperita* L.) (Mentha: Lamiaceae) essential oils (EOs) on the mealybug *N. nipae* under laboratory conditions by using spray technique method. Additionally, gas chromatography-mass spectrometry analysis was used to determine and identify the chemical composition of these two essential oils.

## MATERIALS AND METHODS

### 1. Test Insect:

*Nipaecoccus nipae* (Maskell), a coconut mealybug, was the insect chosen for this investigation. It was collected from Poinciana trees (*Delonix Poinciana regia*) growing in Orman Garden, Giza Governorate. Randomly chosen samples were taken from each of the four cardinal directions (East, West, North and South). The leaves were placed in paper bags before being transported to the laboratory, where they were kept at a temperature of roughly 25±1°C °C and 65±1°RH (Relative Humidity). Taxonomy experts from the department of scale insects and mealybugs, Plant Protection Research Institute, Agriculture Researches

Center, Giza, Egypt. Nymphs and adult stages of the coconut mealybug were selected for the insecticidal activity of two essential oils formulations.

## 2. Plant Materials:

Two plant species, namely lemon grass, *C. citratus*) and peppermint, *M. piperita* L. were purchased from the herbal and vegetables market, in Cairo, Egypt in May 2022. Fresh aerial parts (leaves) of lemongrass and peppermint were used for essential oil extraction. The samples were identified at the Department of Botany, Faculty of Science, Cairo University, Egypt.

## 3. Extraction and Preparation of Formulated Essential Oils:

Essential oils were extracted by Cavalcanti *et al.*, (2004) method, and the leaves of lemongrass and peppermint were used in essential oils extraction. By hydrodistillation in Clevenger-type equipment (Winzer, Wertheim, Germany), for 4 hours at 100°C, essential oils were extracted from the fresh parts (200g weight of each sample in 400 ml of distilled water). The extracted oils were dried over anhydrous sodium sulphate and kept at 4°C until they were used in analysis and bioassay tests in airtight containers or dark glass tubes. Three concentrations of formulated oils from two plants were prepared by the emulsifier, Triton X-100.

## 4. Bioassay Technique:

The leaf spraying technique was used as a toxicity test for two natural oils (Lemongrass and peppermint) against nymphs and adults of the mealybug insect *N. nipae*. Poinciana tree leaves that weren't affected were chosen and put in Petri plates (9cm diameter, 1cm high by 3.5 cm diameter). *N. nipae* individuals were removed from the infected Poinciana leaves and placed in a container after being transferred to the laboratory, then ten nymphs and ten adults of mealybugs were placed on each leaf in Petri dishes. After that, each dish, leaf and *N. nipae* individuals were sprayed with 1ml for five seconds of concentrations 1000,5000 and 10000ppm of our two natural essential oils. The dishes were covered and allowed to dry at room temperature. For each treatment, three replicates were carried out. Petri dishes were used, which were then placed in laboratory conditions and examined for mortality after 24, 48, and 72 hrs. Control insects were sprayed with Triton-x100 alone.

## 5. Gas chromatography-mass Spectrometry Analysis of Lemongrass And Peppermint Eos:

An HP-5MS column measuring 30 metres, 0.25 millimetres inside diameter, and 0.25 millimetres thick was installed in the GC. Hydrogen was used in the analyses as the carrier gas at a flow rate of 1 ml/min with a split ratio of 10:1, an injection volume of 1 µl, and the temperature program as follows: 40 °C for one minute; 4 °C/min rise to 150 °C and hold for six minutes; 4 °C/min rise to 210 °C and hold for one minute. At 280 °C and 220 °C, respectively, the injector and detector were maintained. By utilizing an ionization energy of 70 eV, a spectrum range of m/z 50–900, and a solvent delay of 5.5 min, mass spectra were obtained. By comparing the spectrum fragmentation pattern with those found in the Wiley and NIST Mass Spectral Library data, the identification of various constituents was gained.

## 6. Statistics:

The mortality rate was calculated as a percentage, and the mortality was adjusted using the Abbott formula (Abbott, 1925). Calculating the LC<sub>50</sub> values was done using the LdP-line application. The means of all trials were compared using Duncan's Multiple Range Test (Duncan,1955) at P <0.05, and data from all experiments were statistically assessed using ANOVA.

## RESULTS AND DISCUSSION

### Toxicity of Plant Essential Oils to The Mealybug, *N. nipae*:

#### Response of Mealybug to Plant Essential Oils:

The two-plant species' essential oils (Lemongrass and peppermint) were tested for toxicity bioassays against nymphs and adults of *N. nipae*. Data on toxicity after 24,48 and 72 hrs., of exposure, are presented in (Tables 1 and 2, respectively).

The maximum mortalities of nymphs and adults of mealybug *N. nipae* after 24 hrs., of exposure, were recorded by the highest concentration of 10000ppm essential oil of lemongrass (86.67±0.00 and 90.00±3.30%, respectively) and peppermint essential oil (90.00±0.00 and 90.00±0.00%, respectively), while, after 48 hrs., the high mortalities of lemongrass and peppermint essential oils were (93.33±3.33 and 96.67±3.33 %) and (93.33±3.33 and 96.67±3.33 %), respectively with the same concentration. Both lemongrass and peppermint essential oils achieved the highest mortalities (99.00±0.58 and 99.00±0.58%) against nymphs and adults of mealybug *N. nipae* after 72 hrs., However, approximately, the essential oils of lemongrass and peppermint showed 100% mortality of the *N. nipae* nymphs and adults at the three tested concentrations 1000, 5000, 10000ppm after 72 hrs.. The minimum mortalities values of lemongrass and peppermint essential oils were exhibited by the lowest concentration (1000ppm) and recorded the same mortality percentages (60.33±5.77 and 60.00±5.77%, respectively) for nymphs and adults after 24 hrs. The results showed that lemongrass and peppermint essential oils presented considerably high insecticidal toxicity to the nymph and adults of the mealybug (*N. nipae*) with more than 90% mortality at the three examined concentrations (1000, 5000 and 10000ppm). Results on the toxicity effects of the essential oils against nymphs and adults of *N. nipae* revealed significant differences between the different concentrations. Increasing the concentrations from 1000 to 10000 increased the nymphicidal and adulticidal mortality range from 60-100 %. The highest mortality values were observed at the highest concentration 10000ppm. According to the results, both essential oils exhibited significant mortality of *N. nipae* nymphs and adults and in addition, this mortality response was concentration and time-dependent (Tables 1 and 2).

**Table 1:** Insecticidal effect of lemon grass (*Cymbopogon citratus*) at three concentrations against nymph and adult of coconut mealybug *Nipaecoccus nipae* (Maskell)

Conc. (ppm)	Mortality (Mean%±SE)					
	24 hours		48 hours		72 hours	
	Nymph	Adult	Nymph	Adult	Nymph	Adult
1000	60.33±5.77 <sup>b</sup>	68.30±4.44 <sup>a</sup>	73.33±3.33 <sup>b</sup>	80.00±5.77 <sup>b</sup>	85.00±2.88 <sup>a</sup>	93.33±5.77 <sup>a</sup>
5000	70.33±0.33 <sup>b</sup>	75.00±2.88 <sup>a</sup>	85.00±2.88 <sup>ab</sup>	85.00±2.88 <sup>ab</sup>	90.00±5.77 <sup>a</sup>	96.67±3.33 <sup>a</sup>
10000	86.67±0.00 <sup>a</sup>	90.00±3.33 <sup>b</sup>	93.33±3.33 <sup>a</sup>	96.67±3.33 <sup>a</sup>	99.00±0.58 <sup>a</sup>	99.00±0.58 <sup>a</sup>
F	21.23	12.40	6.81	4.16	2.05	1.20
P	0.0074	0.0193	0.0515	0.1055	0.2435	0.3914

**Table 2:** Insecticidal effect of peppermint (*Mentha piperita*) at three concentrations against nymph and adult of coconut mealybug *Nipaecoccus nipae* (Maskell)

Conc. (ppm)	Mortality (Mean%±SE)					
	24 hours		48 hours		72 hours	
	Nymph	Adult	Nymph	Adult	Nymph	Adult
1000	60.00±5.77 <sup>c</sup>	70.00±0.00 <sup>b</sup>	73.33±3.33 <sup>b</sup>	80.00±3.33 <sup>a</sup>	95.00±2.88 <sup>a</sup>	93.33±3.33 <sup>a</sup>
5000	75.00±2.88 <sup>b</sup>	83.33±3.33 <sup>a</sup>	85.00±2.89 <sup>a</sup>	85.00±2.88 <sup>a</sup>	98.00±1.15 <sup>a</sup>	96.67±3.33 <sup>a</sup>
10000	90.00±0.00 <sup>a</sup>	90.00±0.00 <sup>a</sup>	93.33±3.33 <sup>a</sup>	96.67±3.33 <sup>a</sup>	99.00±0.58 <sup>a</sup>	99.00±0.58 <sup>a</sup>
F	20.25	28.00	17.50	1.90	0.84	2.00
P	0.0081	0.0044	0.0105	0.2630	0.4952	0.2500

Data in Table (3) demonstrated that our results from the study showed that LC<sub>50</sub> of lemon grass was (515.21, 176.56 and 5.69ppm) and (239.22, 86.08 and 6.45ppm), for nymphs and adults after 24, 48 and 72 hrs., respectively and LC<sub>90</sub> was (32512.57, 7651.09 and 3957.00ppm) and (20193.84, 4842.59 and 566.00ppm), for the same two stages of mealybug after 24, 48and 72 hrs., respectively. While results in Table (4) investigated that, LC<sub>50</sub> of peppermint was (583.57, 58.76 and 4.95ppm) and (194.29, 53.17and 2.99 ppm), for nymphs and adults after 24, 48 and 72 hrs., respectively and LC<sub>90</sub> was (15412.82, 5514.00 and 350.82 ppm) and (11878.92, 3157.00 and 354.00ppm), for the same two stages of mealybug after 24, 48and 72 hrs., respectively. Results reported in Tables (3 and 4) showed that Lemongrass and peppermint EOs exhibited insecticidal activity against *N. nipae*. Peppermint EO was relatively more toxic with LC<sub>50</sub> and LC<sub>90</sub> values Tables (3 and 4). Also, the corresponding Mean LC<sub>50</sub> and LC<sub>90</sub> values of both nymphs and adults were (6.07and 2261.50ppm) and (3.97and 352.41ppm), after 72 hrs., respectively indicating that after three days (72 hrs) of application, peppermint exhibited a significant degree of efficacy as an insecticide Tables, (3 and 4).

**Table 3:** Toxicological values (LC<sub>50</sub> and LC<sub>90</sub> ppm) of Lemon grass essential oil on coconut mealybug *Nipaecoccus nipae* (Maskell).

Lethal Conc. (ppm)	24 hours		48 hours		72 hours	
	Nymph	Adult	Nymph	Adult	Nymph	Adult
LC <sub>50</sub>	515.21	239.22	176.56	86.08	5.69	6.45
Mean LC <sub>50</sub>	377.21		131.32		6.07	
LC <sub>90</sub>	32512.57	20193.84	7651.09	4842.59	3957.00	566.00
Mean LC <sub>90</sub>	26353.20		6246.84		2261.50	
Slope	0.7121	0.6653	0.7830	0.7323	0.4509	0.6595
X <sup>2</sup>	0.3097	0.3878	0.0767	0.4809	0.0351	0.0860

**Table 4:** Toxicological values (LC<sub>50</sub> and LC<sub>90</sub> ppm) of peppermint essential oil on coconut mealybug *Nipaecoccus nipae* (Maskell).

Lethal Conc. (ppm)	24 hours		48 hours		72 hours	
	Nymph	Adult	Nymph	Adult	Nymph	Adult
LC <sub>50</sub>	583.57	194.29	58.76	53.17	4.95	2.99
Mean LC <sub>50</sub>	388.93		55.96		3.97	
LC <sub>90</sub>	15412.82	11878.92	5514.00	3157.00	350.82	354.00
Mean LC <sub>90</sub>	13645.87		4335.50		352.41	
Slope	0.9013	0.7175	0.6498	0.7225	0.6927	0.6182
X <sup>2</sup>	0.2625	0.0264	0.2230	0.1153	0.0020	0.00370

Three plant essential oils, including *C. citratus* and *M. piperita*, have been studied by Brahmi *et al.*, (2022) for their insecticidal efficacy and behavioural and neurophysiological effects against the vine mealybug *Planococcus ficus*. Lethal concentrations LC<sub>50</sub> values for *C. citratus* and *M. piperita* were found to be 17.01 and 26.27 µl.L<sup>-1</sup> air, respectively, in bioassays of EOs against vine mealybug adults. The essential oil from *C. citratus* was the most effective in changing the behavioural response of treated mealybugs, which becomes hyperactive and disoriented, in both topical and fumigant bioassays. Acetylcholinesterase (AChE) activity significantly decreased after exposure to lemongrass essential oil (EO), which is indicative of fatal neurological consequences. The outcomes demonstrated that the EOs of *C. citratus* and *M. piperita* had insecticidal efficacy against *P. ficus*. *C. citratus* EO has LC<sub>90</sub> values of 0.01 µl.cm<sup>2</sup>, making it considerably more toxic. Their research showed that *C. citratus* and *M. piperita* EOs had a toxic effect on *P. ficus* adults in both contact and fumigant bioassays. In the study carried out by Sawsan *et al.* (2018), the percentage of *Phenacoccus solenopsis* mortality was recorded at 100% for lemongrass at high concentration (40µl/l), whereas it recorded 90% for lemon grass at 400

ppm of AgNPs after 72 hours of treatment. Both lemon grass essential oil and AgNPs-lemon grass have been shown to be extremely efficacy in *P. solenopsis* females. Similar findings were investigated by Saad *et al.* (2021), who found that after three days of treatment, lemongrass *C. citratus* and rosemary *Salvia rosmarinus* were the most toxic essential oils to the third instar nymph of *P. solenopsis* Tinsley. Values of the LC<sub>50</sub> were 3102.591 and 3323.293 ppm, respectively and LC90's of (13226.041 and 17074.043 and) ppm, respectively. while, the LC<sub>50</sub> values after seven days for *C. citratus* followed by *S. rosmarinus* were 680.073 and 740.591 ppm, respectively and LC90s of (4020.773 and 4226.784) ppm, respectively. Lemon grass essential oil at 3µl/L air with an LC<sub>50</sub> of 1.58 µL·L<sup>-1</sup> air was found highly toxic against the mealybug *Pseudococcus jackbeardsleyi* (Hemiptera: Pseudococcidae) with higher than 80% mortality (Pumnuan and Insung, 2016). In another research, El-Sonbaty *et al.*, (2018) found that lemon grass plants caused a 100% mortality rate against the female of *P. solenopsis* at high concentrations (40µl/l). According to Muhammad *et al.* (2017), less potent botanicals can be used to control the cotton mealybug, *P. solenopsis* Tinsley, without any harmful effects on natural enemies. According to the findings of Habibpour *et al.* (2020), the pure *Mentha longifolia* L. and *M. piperita* L. EOs (F: Lamiaceae) had LC<sub>50</sub> values of 113.49 ppm and 129.74 ppm, respectively, on *P. solenopsis* after 48 hours. Also, Karamaouna *et al.* (2014), exhibited that, the paraffin oil used as a reference product against the vine mealybug *P. ficus* was found to be less or equally toxic effects of peppermint essential oil.

#### **Chemical Composition of Essential Oils (EOs):**

GC/MS analysis was used for identified and determined the chemical composition of EOs obtained from *M. piperita* and *C. citratus* leaves (Tables 5 and 6).

#### **Chemical Composition of Peppermint Essential Oil (EO):**

The extracted essential oil from peppermint leaves was identified and quantified by GC/MS, and the obtained chemical constituents are represented in Table (5) and Figure (1). The chemical composition of peppermint oil, as shown, in Table (5), Nineteen components were identified in peppermint essential oil that represented 99.99% of the total volatile constituents, and the principal components were menthol (36.94%) and menthone (22.62%). Further identified compounds were menthyl acetate (10.05%), menthofuran (9.18%), 1,8-cineole (4.84%), Iso-Menthol acetate (4.24 %), β-Caryophyllene (2.30%) and neo-menthol (1.78%). Peppermint EO consisted of high amounts of menthol (36.94%), menthone (22.62%) and menthyl acetate (10.05%) which altogether represented about 70% of oxygenated monoterpene compounds identified.

Our results are consistent with other studies; the research of Reddy *et al.*, (2019) studied the chemical constituents from the areal parts of *M. piperita* L. essential oil, and results showed that 19 chemical constituents identified that together represented 100% of the essential oil. The major components include menthol (36.02%), menthone (24.56%), menthyl acetate (8.95%), and menthofuran (6.88%); while others are minor. Menthol (40.7%) and menthone (23.4%) represent the main constituents of the essential oil from peppermint, with the other constituents being menthyl acetate, 1,8-cineole, limonene, β-pinene and β-caryophyllene (Schmidta *et al* 2009). The principal constituents of the peppermint leaf essential oil including menthol (44.39 and 39.07%), menthone (15.36 and 14.99%), menthofuran, (10.27 and 9.56%) 1,8-cineole, (5.81 and 7.96%) and menthyl acetate (4.78 and 6.10%) in fresh and shade peppermint leaves, respectively. According to essential oil, the chemical components primarily belonged to the class of oxygenated monoterpenes (72.34–86.41%) (Beigi *et al.*, 2018). The main components from peppermint essential oil were menthol (45.34 and 29.38%), menthone (16.04 and 16.88 %), menthofuran (8.91 and 11.38 %), cis-carane (8.70 and 14.39% and), 1,8-cineole (4.46 and 9.45%), neo-menthol (4.24 and 2.37%), in the hydrodistillation and HS/SPME methods and β-Pinene (2.26%), α-

Pinene (1.55%), limonene (2.22% trans-caryophyllene (2.76%), germacrene-D (1.41%), and neoisomenthyl acetate (1.02%) were the minor components (Taherpour *et al.*, 2017). Mahboubi and Kazempour (2014), reported that the main components of peppermint essential oil are menthol (36.9%), menthone (28.8%), menthyl acetate (4.54%), and 1,8-cineole (3.75%) in (99.8%) of the oil. Menthol (39.3%), menthone (25.2%), menthofuran (6.8%), and menthyl acetate (6.7%) are the main chemical compounds that comprise the majority of peppermint essential oil (93.4%) (Bassolé *et al.* 2010). Several studies also agree with our results where menthol and menthone were the most abundant constituents in peppermint EO (Sokovic *et al.*, 2007, de Sousa *et al.*, 2010 and Oh *et al.*, 2014).

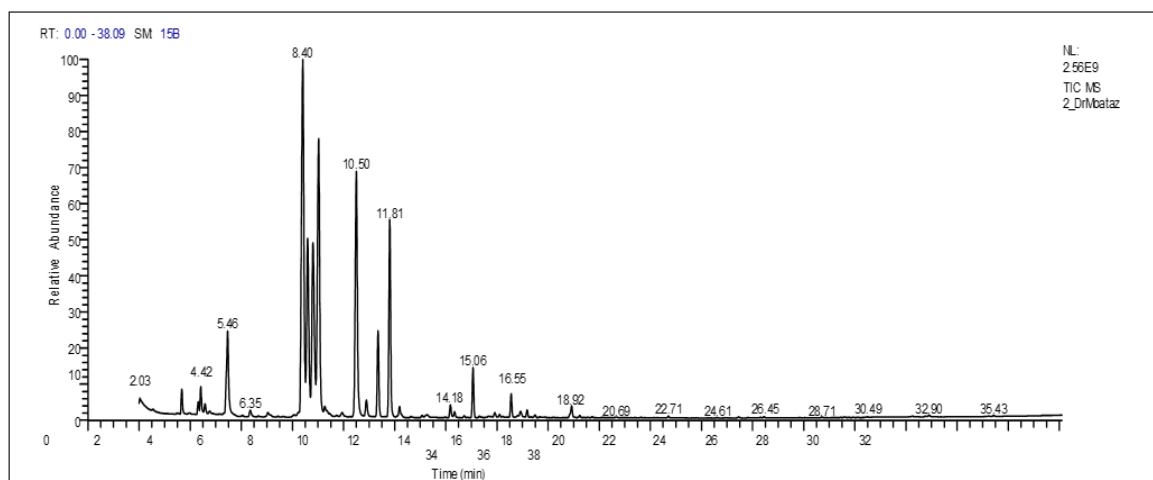
**Table 5:** GC-MS analysis of the essential oil of *peppermint, Mentha piperita* plant.

No.	RT	Compound Name	MW	Area (%)	MF
1	3.67	$\alpha$ -Pinene	136	1.03	C <sub>10</sub> H <sub>16</sub>
2	4.31	Sabinene	136	0.49	C <sub>10</sub> H <sub>16</sub>
3	4.41	$\beta$ -Pinene	136	1.14	C <sub>10</sub> H <sub>16</sub>
4	4.58	Cis-ocimene	136	0.39	C <sub>10</sub> H <sub>16</sub>
5	5.46	1,8- Cineole	154	<b>4.84</b>	C <sub>10</sub> H <sub>18</sub> O
6	8.41	Menthone	154	<b>22.62</b>	C <sub>10</sub> H <sub>18</sub> O
7	8.59	Menthofuran	150	<b>9.18</b>	C <sub>10</sub> H <sub>14</sub> O
8	8.80	Neo-menthol	156	<b>1.78</b>	C <sub>10</sub> H <sub>20</sub> O
9	9.03	Menthol	156	<b>36.94</b>	C <sub>10</sub> H <sub>20</sub> O
10	10.50	p-Menth-1-en-3-one	152	1.05	C <sub>10</sub> H <sub>16</sub> O
11	10.89	Pulegone	152	0.87	C <sub>10</sub> H <sub>16</sub> O
12	11.35	Iso-Menthol, acetate	198	<b>4.24</b>	C <sub>12</sub> H <sub>22</sub> O <sub>2</sub>
13	11.81	Menthyl acetate	198	<b>10.05</b>	C <sub>12</sub> H <sub>22</sub> O <sub>2</sub>
14	12.19	Isomenthyl acetate	198	0.50	C <sub>12</sub> H <sub>22</sub> O <sub>2</sub>
15	14.18	$\alpha$ -Bourbonene	204	0.57	C <sub>15</sub> H <sub>24</sub>
16	15.06	$\beta$ -Caryophyllene	204	<b>2.30</b>	C <sub>15</sub> H <sub>24</sub>
17	16.55	Germacrene D	204	1.06	C <sub>15</sub> H <sub>24</sub>
18	17.18	$\beta$ -Elemene	204	0.32	C <sub>15</sub> H <sub>24</sub>
19	18.92	Caryophyllene oxide	220	0.62	C <sub>15</sub> H <sub>24</sub> O
<b>Total identification</b>				<b>99.99</b>	
<b>Total monoterpenes</b>				<b>3.05</b>	
<b>Total oxygenated monoterpenes</b>				<b>92.07</b>	
<b>Total Sesquiterpenes</b>				<b>4.87</b>	

MF=Molecular formula

MW= Molecular weight

RT=Retention time



**Fig. 1:** GC/MS Chromatogram of *peppermint, Mentha piperita*, essential oil.

### Chemical Composition of Lemongrass Essential Oil (LEO):



Table (6) and Figure (2) showed the GC-MS analysis of lemongrass essential oil which showed the presence of twenty-two compounds, representing 99.39% of the total oil composition.

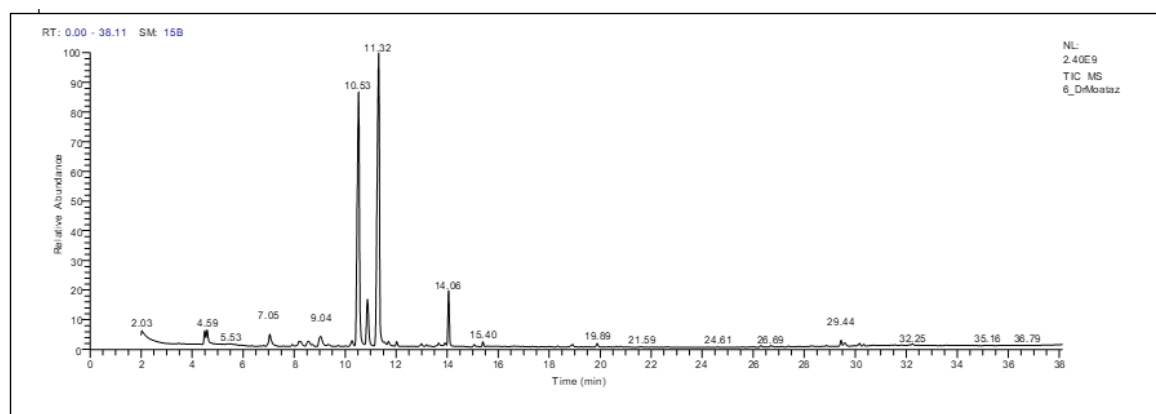
**Table 6:** GC-MS analysis of the essential oil of *Lemongrass, Cymbopogon citratus plant*

No.	RT	Compound Name	MW	Area (%)	MF
1	2.02	Hexane	86	0.65	C <sub>6</sub> H <sub>14</sub>
2	4.49	Sulcatone (6-methyl-5-Hepten-2-one)	126	1.31	C <sub>8</sub> H <sub>14</sub> O
3	4.59	$\alpha$ -Myrcene	136	<b>1.59</b>	C <sub>10</sub> H <sub>16</sub>
4	7.04	Linalool	154	<b>1.49</b>	C <sub>10</sub> H <sub>18</sub> O
5	8.19	trans 4,5-Epoxyarene	152	0.41	C <sub>10</sub> H <sub>16</sub> O
6	8.26	(R)-(+)-Citronellal	154	0.39	C <sub>10</sub> H <sub>18</sub> O
7	8.53	cis-Verbenol	152	0.39	C <sub>10</sub> H <sub>16</sub> O
8	8.58	trans-Verbenol	152	0.45	C <sub>10</sub> H <sub>16</sub> O
9	9.04	Isogeranial	152	<b>1.76</b>	C <sub>10</sub> H <sub>16</sub> O
10	10.27	Citronellol	156	0.75	C <sub>10</sub> H <sub>20</sub> O
11	10.53	Neral (Citral B)	152	<b>34.51</b>	C <sub>10</sub> H <sub>16</sub> O
12	10.87	Geraniol	154	<b>6.01</b>	C <sub>10</sub> H <sub>18</sub> O
13	11.32	Geranial (Citral A)	152	<b>41.23</b>	C <sub>10</sub> H <sub>16</sub> O
14	11.70	Isoneral	152	0.44	C <sub>10</sub> H <sub>16</sub> O
15	12.02	Geranyl formate	182	0.49	C <sub>11</sub> H <sub>18</sub> O <sub>2</sub>
16	13.66	Bornel	168	0.38	C <sub>11</sub> H <sub>20</sub> O
17	14.06	Geranyl acetate	196	<b>5.48</b>	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>
18	15.40	trans-Caryophellene	204	0.50	C <sub>15</sub> H <sub>24</sub>
19	19.89	$\alpha$ -Elemol	222	0.50	C <sub>15</sub> H <sub>26</sub> O
20	29.44	Linoleic acid, methyl ester	294	0.63	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>
21	29.58	Phytol	296	0.48	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>
22	30.17	7-Hydroxy-6-Keto-Drimanol	254	0.30	C <sub>15</sub> H <sub>26</sub> O <sub>3</sub>
<b>Total identification compounds</b>				<b>99.39</b>	
<b>Total monoterpenes compounds</b>				<b>1.49</b>	
<b>Total oxygenated monoterpenes compounds</b>				<b>93.53</b>	
<b>Total diterpenes</b>				<b>0.48</b>	
<b>Total Sesquiterpenes compounds</b>				<b>1.00</b>	
<b>Total other compounds compounds</b>				<b>2.89</b>	

MF=Molecular formula

MW= Molecular weight

RT=Retention time



**Fig.2:** GC/MS Chromatogram of lemongrass, *Cymbopogon citratus*, essential oil.

The essential oil consists of monoterpenes (97.02%), sesquiterpenes (1.00%) and diterpenes (0.48%). Citral (75.74%), the main component of essential oil, consisted of 41.23% citral A (geranial) and 34.51% citral B (neral), followed by geraniol which was (6.01 %). Other components identified in oil were geranyl acetate 5.48%, isogeranial 1.76%,  $\alpha$ -myrcene 1.59%, linalool 1.49%, etc. The class of volatiles with the highest representation was oxygenated monoterpenes (93.53%). Neral and geranial were the most predominant of

them. The second-largest chemical class investigated was monoterpene hydrocarbons (1.49%). Generally, geranial and neral were reported or listed as the principal components of lemongrass essential oil elsewhere. These results are in agreement with several studies, Soliman *et al.* (2017) found 22 chemical components that represent 97.83% of the content of lemongrass essential oil. The majority of these chemicals were monoterpenes representing (96.37%), with only a little amount of sesquiterpenes (1.25) and diterpenes (0.21%) present. The major compounds were geranial (42.86%) and neral (39.83%) which were 79.69% of the total essential oil composition. Other major compounds were myrcene (8.05%), geraniol (3.22%), and cis-verbanol (1.84%). According to the study of Alwani *et al.*, (2017) essential oil of lemongrass consisted of 40 components, representing 99.035 % of the essential oil compounds with geranial (45.06%) and neral (33.10%) as major constituents. Also, the results of Masamba *et al.*, (2003) study reported that lemongrass oil contains 82% citral, which consisted of 41.67% geranial and 40.33% neral and 10% myrcene. In another study by Pino *et al.*, (2018) their results revealed that oxygenated monoterpenes were the highest class of volatiles (86%), which included the main components neral (33.2%) and geranial (39.8%). When Hanaa *et al.*, (2012) analyzed the constituents of lemongrass essential oil, they investigated that the main ingredients were geranial (31.5-39.9%), neral (30.1-35.5%), and myrcene (14.5-16.6%). Similar research carried out in Kenya revealed that the principal components of lemongrass essential oil were geranial (39.53%), neral (33.31%), and myrcene (11.41%) (Matasyoh, 2011). Many studies reported that citral content which is composed of 20 to 50% of geranial and 30 to 40% of neral were the main components of lemongrass chemical composition and in addition to other minor compounds such as isoneral, isogeranial, geraniol, geranyl acetate, citronellal, citronellol,  $\beta$ -myrcene,  $\beta$ -pinene ocimene,  $\beta$ -ocimene, linalool, caryophyllene, germacrene-D and *elemene* (Pandey *et al.*, 2003, Chandrashekar and Joshi, 2006, Al-Naqqash and Albazaz 2019 and Mukarram *et al.*, 2022). Citral is an isomeric combination of geranial and neral, and its concentration can serve as a quality indicator for LEO (Masamba *et al.* 2003, Hanaa *et al.*, 2012 and Muturi *et al.*, 2020).

Both essential oils' chemical constituents can generally be divided into three major chemical groups (oxygenated monoterpenes, monoterpene hydrocarbons, and sesquiterpene hydrocarbons). The average concentrations of the major chemical groups for peppermint and lemon grass essential oils, reported as percentages were presented in Tables (5 and 6). The results revealed that the chemical composition of the peppermint and lemon grass essential oils is characterized mainly by the presence of oxygenated monoterpenes (92.07 and 93.53%). Low levels of monoterpene hydrocarbons (3.05 and 1.49%), and sesquiterpene hydrocarbons (4.87 and 1.00%) are also presented. Due to the location, environment, harvesting season, and extraction technique, the percentages of the main components varied. Lemongrass and peppermint essential oils (Tables 5 and 6) showed a significant relative percentage of monoterpenes (95.02% and 95.12%), respectively. Insecticidal activities against *N. nipae* nymph and adults may be caused by the presence and high percentages of these monoterpenes. Among the monoterpenes components of lemon grass EO, geranial and neral showed the highest values. The components, menthol and menthone were the most important ones of peppermint EO. Our results indicated that mainly constituents of lemon grass EO (menthol and menthone) and peppermint EO (geranial and neral) may be the reason for the high potency of both oils to nymphs and adults of *N. Nipae*. These four compounds and other minor compounds such as geraniol and geranyl acetate, which are present in lemongrass and Menthofuran which is present in peppermint, were responsible for the insecticidal activity of both essential oils. Given their high volatility and ability to quickly penetrate insects while interfering with their physiological processes, plant essential oils are widely acknowledged as a significant natural source of monoterpenoids (Lee *et al.*, 2002,

Negahban *et al.*, 2006 and Kumar *et al.*, 2011). Although the exact mechanism of action of both essential oils and their constituent parts is still unclear, their toxic effects point to a workable option for the control of mealybug infestations. Due to the presence of terpenoids, the essential oils of lemongrass and peppermint exhibit neurotoxic effects on *N. nipae* and cause rapid insect mortality, as has been shown for other insects treated with plant essential oils (Tak *et al.*, 2017, Brügger *et al.*, 2019, De Souza *et al.*, 2019). This interpretation was supported by (Plata-Rueda, *et al.*, 2020 and Brahmi *et al.*, 2022) studies, which showed that the active ingredient in lemongrass, citral, and other minor components can regulate neuroreceptor activities, signal transduction, hormonal balance, membrane integrity, and cytotoxicity in insects. They can also slow down the activities of various neurotransmitters, including acetylcholine esterase and octopamine.

### Conclusions and Recommendations:

Phytochemicals encourage local initiatives to increase the scope of pest control because they are secure, readily available year-round, and affordable. Agriculturists can employ such an effective formulation of the two tested essential oils for extensive field research to completely eradicate mealybug and scale insects. This study investigated the chemical composition of two plant essential oils (lemongrass and peppermint) and tested their insecticidal on nymphs and adults of *N. Nipae* under laboratory conditions. Our findings revealed that in spray bioassays, lemongrass and peppermint, essential oils exhibited a toxic effect against *N. Nipae* nymphs and adults. The compositional analysis of two oils revealed that menthol and menthone, the major components of peppermint essential oil, and general and neral, the major components of lemongrass essential oil, have toxic and sublethal effects on *N. nipae* and can be used as an alternative to synthetic chemical insecticides. This study shows the potential of lemongrass and peppermint essential oils as an insecticide IPM approach to manage *N. nipae* and in order to reduce the environmental damage caused by pesticides, more studies should be carried out on lemongrass and peppermint essential oils to increase their effectiveness in controlling mealybugs.

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## ARABIC SUMMARY

التأثير الأبادى والتركييب الكييميائى لزييتى نبات حشيشة الليمون ونبات النعناع الفلفى ضد حشرة بق جوز الهند الدقيقى  
*Nipaecoccus nipae*((Hemiptera : Pseudococcidae)

ردينه احمد حسن، سحر على عطية، سالم محمد عواد وأحمد عبد المنعم الحفنى  
1- فرع المبيدات - قسم الحشرات الاقتصادية - كلية الزراعة - جامعة القاهرة - الجيزة  
2- معهد بحوث وقاية النباتات- مركز البحوث الزراعية-الدقى-الجيزة- مصر

وجد أن للزيوت النباتية تأثير ابادى على الحشرات ومن ضمنها البق الدقيقى. أجريت الدراسة الحالية لتقييم كفاءة التركيزات المختلفة من زيتى نبات حشيشة الليمون ونبات النعناع الفلفى على تعداد حوريات وبالغات حشرة بق جوز الهند الدقيقى تحت الظروف المعملية بعدد 24، 48 و 72 ساعة. وتهدف هذه الدراسة ايضا الى تحليل التركيب الكييميائى لهذين الزيتين بواسطة جهاز GC-MS analysis. واطهرت النتائج أن زيت حشيشة الليمون أعلى سمية من زيت النعناع الفلفى عند أعلى تركيز (10000 جزء فى المليون). أيضا أظهرت هذه النتائج أن زيتى حشيشة الليمون والنعناع الفلفى سجلت اعلى سمية ضد حشرة بق جوز الهند الدقيقى مع قيم التركيزات النصف مميتة وكانت ( 10.94 & 15.01 جزء فى المليون) و (12.91 & 16.01 جزء فى المليون) للحوريات وبالغات لكلا الزيتين على التوالى فى نفس الوقت. اوضحت هذه النتائج ان زيت حشيشة الليمون اعلى سمية من زيت النعناع الفلفى. سجلت النتائج أن أعلى نسب موت (100%) للحوريات وبالغات عند أعلى تركيز (10000 جزء فى المليون) لكلا من زيت حشيشة الليمون وزيت النعناع الفلفى وكانت بعد 72 ساعة من المعاملة. تزداد نسبة الموت بزيادة تركيز الزيت ووقت تعريض. ثبت ان كلا الزيتين شديدين السمية لحوريات وبالغات هذه الحشرة. بينت تحاليل جهاز GC-MS analysis بوجود اثنى وعشرون مركب تم تعريفها لنزيت حشيشة الليمون تمثل 99.39% من مركبات ذلك الزيت، من تلك المركبات الرئيسة Oxygenated monoterpenes بنسبة (93.53%) وتحتوى اساسا على geranial (41.23%) و neral (34.51%) كمحتويات اساسية، وتحتوى على مركبات اخرى رئيسية geraniol (6.01%) و Geranyl acetate (5.48%)، وايضا اوضحت النتائج بوجود تسعة عشر مركب كييميائى بزيت النعناع الفلفى تمثل 99.99% من محتوى ذلك الزيت ومعظم المركبات المتوفرة هى menthol (36.94%)، menthone (22.62%)، menthylacetate (10.55%) و menthofuran (9.18%) ومكونات اخرى صغيرة منها على سبيل المثال  $\beta$ -pinene و  $\beta$ -caryophyllene. نعزو هذه النتائج الى وجودها بنسبة عالية نسبيا فى زيتى كلا من حشيشة الليمون والنعناع الفلفى. لذلك تكون هذه المركبات هى المسؤولة عن النشاط الابادى ضد حشرة بق جوز الهند الدقيقى. اقترحت دراستنا ان زيتى حشيشة الليمون والنعناع الفلفى مفيدة كمبيدات نباتية فى استراتيجية IPM لبق جوز الهند الدقيقى وحماية المحاصيل منها.