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# Insecticidal activity and chemical composition of plant essential oils against cotton mealybug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae)

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**Abstract**

Ten plant essential oils were extracted and tested for their toxicity against the adult females of cotton mealybug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) under laboratory conditions. Mortality varied according to the essential oil type and the delivered dose (ppm). The most remarkable toxic essential oils after 24h and 72h of treatment were *Thymus vulgaris* followed by *Mentha longifolia,* and *Cyperus articulates* essential oils. The $LC_{50}$ values were 29.03, 34.32 and 54.69 ppm, respectively after 24h while after 72h of treatments were 15.04, 24.93 and 29.21 ppm, respectively. Gas Chromatography-Mass Spectrometry was used to analyze the most potent essential oils and identify the most active ingredients.

**Keywords:** Essential oils, cotton mealybug, *Phenacoccus solenopsis, Thymus vulgaris, Mentha longifolia, Cyperus articulates,* gas Chromatography-Mass spectrometry

## 1. Introduction

The cotton mealybug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) is an invasive polyphagous and rapid spread pest attacking cotton, vegetables, ornamentals and other plants [1, 2]. The infested plants remain stunted and produce fewer bolls of a smaller size; the leaves turn yellow then dry up and eventually fall off. *P. solenopsis* produces honeydew which induces the development of sooty mold fungus and hinders the photosynthetic process [1-3].

The synthetic chemicals control of mealybugs is not favorable as a result of its harmful impact on human health and the environment also it threats the beneficial non-target organisms [3]. In addition, the water proof waxy secretion of the cuticle over the *P. solenopsis* body and its cryptic habitats in plants, mealybugs developed resistance due to injudicious use and continuous exposure to insecticides [4].

Therefore, environmentally friendly chemical control methods have received great attention by various scientific groups and found to be necessary to accomplish the control of *P. solenopsis* [4, 5]. Plant essential oils may be a secure alternative approach than synthetic pesticides for *P. solenopsis* control because of their effectiveness and versatility [6-8]. The volatility and chemical diversity of its constituents make them a potential sources of insect control agents [8].

This study was directed to assess the insecticidal activity of ten essential oils extracted from locally available Egyptian plants against *P. solenopsis*. Chemical investigation of the most highly active essential oils was carried out to identify its bioactive constituents.

## 2. Materials and Methods

### 2.1 Plant material

*Thymus vulgaris* L. (Lamiaceae) (Whole plant), *Artemisia absinthium* L. (Asteraceae) (Seeds), *Pluchea dioscoridis* L. (Asteraceae) (Leaves), *Cyperus articulatus L.* (Cyperaceae) (Tubers), *Mentha longifolia* (L.) Huds. (Lamiaceae) (Leaves), *Anethum graveolens* L. (Apiaceae) (Aereal parts) and *Lantana camara* L.(Verbenaceae) (Leaves) were collected from Mansoura University farm while *Zingiber officinale* Rosc. (Zingiberaceae) (Roots), *Elettaria cardamomum maton* (Zingiberaceae) (Seed pods) and *Syzygium aromaticum* (L.) Merr. & Perry (Myrtaceae) (Buds) were bought in the herbal markets of Mansoura (Egypt).

### 2.2 Essential Oils Extraction
The freshly aromatic plant's parts were subjected to hydro-distillation for 4 h in a Clevenger-type apparatus in order to extract their vaporizing essential oils, which were then dried over anhydrous sodium sulphate and stored in dark glass tubes under refrigeration (4 °C) until use.

### 2.3 Gas Chromatography-Mass Spectroscopy analysis of essential oils
GC-MS analysis of the volatile fractions was performed on a Varian GC interfaced to Finnegan SSQ 7000 Mass selective Detector (SMD) with ICIS V2.0 data system for MS identification of the GC components. The column used was DB-5 (J&W Scientific, Folosm, CA) cross-linked fused silica capillary column (30 m. long, 0.25mm. internal diameter) coated with poloy dimethyl-siloxane (0.5µm. film thickness). The oven temperature was programmed from 50 °C for 3 min., at isothermal, then heating by 7 °C /min. to 250 °C and isothermally for 10 min., at 250 °C. Injector temperature was 200 °C and the volume injected was 0.5µl. Transition-line and ion source temperature were 250 °C and 150 °C, respectively. The mass spectrometer had a delay of 3 min. to avoid the solvent plead and then scanned from m/z 50 to m/z 300. Ionization energy was set at 70 eV. (Agriculture Research Center, Dokki, Cairo).

### 2.4 Test Insect
Mealybug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) was collected from infested Cotton plants (*Gossypium barbadense var*. Giza 86) at the field of Aga distract, Dakahalia governorate, Egypt during the fieldwork of the authors in summer 2016. The mealybug was identified at Scale Insect Department, Plant Protection Research Institute, Agric. Res. Center, Giza, Egypt as *P. solenopsis.* The mealybug was transferred to the laboratory and sprouting potatoe tubers were used as a host plant for its rearing. Gravid females of *P. solenopsis* were inserted in sprouting potatoes. Each sprouted potato was infested with an adult female and observed daily [9].

From the reared culture, newly hatched crawlers of *P. solenopsis* were placed on each sprouted potato before being confined in a carton cylindrical box of 8 cm long and 12 cm diameter. The carton boxes were kept at 30 ˚C and 60±5 % R.H. Daily examination for the morphological changes were recorded and monitored until adult emergence [9].

### 2.5 Bioassay
A total of ten *P. solenopsis* adult females at the same stage were selected randomly from the colonies and carefully transferred to a cotton leaf using camel hair brush and placed in a culture Petri dish (9 cm in diameter) and prepared for the essential oil treatments. Each treatment was replicated three times in addition to control. All essential oils were formulated as an emulsion in water containing 0.3% triton X-100. Five diluted concentrations series of each essential oil were prepared and tested immediately after preparation.

The Petri dishes containing the cotton leaf with *P. solenopsis* were sprayed with 1 ml aqueous solution of the essential oils. Spraying was carried out using a small volume hand atomizer. The excess run off solution was removed from the Petri dishes immediately after spraying, and the dishes were then covered with the lids bearing the ventilation holes to prevent vapor accumulation. The same procedure was followed for the control group, which consisted of water with 0.3% Triton X-100) [7].

Mortality percentages were determined after 24h and 72h of initial application and corrected by using Abotts formula [10] and they are statistically analyzed to estimate $LC_{50}$, $LC_{90}$ and slope values according to Finney [11]. Toxicity index was computed for different essential oils by comparing these materials with the most effective one using Sun's equation [12].

## 3. Results and Discussion
Synthetic insecticide applications have resulted in problems with the environment, human health and threat non-targeted organisms. Therefore, bio-insecticides of botanical sources such as essential oils have posted as an alternative to the synthetic one in agriculture. Recently, there has been a great interest in evaluating and studying essential oils as botanical insecticides, since its bioactive constituents are biodegradable into nontoxic products and potentially suitable for use in integrated management programs [8].

Ten plant essential oils of seven different families were extracted and examined for their insecticidal activity against adult females of mealybug, *P. solenopsis* (Tinsley). The susceptibility of *P. solenopsis* adult females to the tested essential oils table (1) showed that *T. vulgaris*. exhibited a high degree of efficiency as insecticide after 24h and 72h of initial application followed by *M. longifolia, C. articulates, Z. officinale, S. aromaticum, E. cardamomum, A. graveolens, P. dioscoridis, L. camara* essential oils while *A. absinthium* showed the lowest active essential oil. $LC_{50}$ and $LC_{90}$ values obtained from probit analysis for mortality values after 24h and 72h of each applied essential oil are given in table 1.

From the above mentioned data the tested essential oils can be classified according to their insecticidal action in to two groups the first group included the most effective essential oils of *T. vulgaris, M. longifolia* and *C. articulates,* while the second group represented *Z. officinale, S. aromaticum, E. cardamomum, A. graveolens, P. dioscoridis, L. camara* and *A. absinthium* essential oils.

**Table 1:** Toxicity of plant essential oils against adult females of *P. solenopsis* after 24h and 72h of treatment.

| Essential oils | 24 h | | | | | 72 h | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $LC_{50}$ (ppm) and confidence limits at 95% | $LC_{90}$ (ppm) and confidence limits at 95% | Slope ± SE | $X^2$ | Toxicity index | $LC_{50}$ (ppm) and confidence limits at 95% | $LC_{90}$ (ppm) and confidence limits at 95% | Slope ± SE | $X^2$ | Toxicity index |
| *A. absinthium* | 2287.87<br>913.04 73697.86 | 39596.26<br>5708.16 11326.2 E+4 | 1.035±0.325 | 0.72 | 1.27 | 1414.29<br>616.54 30982.99 | 40897.0<br>5395.10 22122.1 E+4 | 0.877±0.280 | 0.28 | 1.06 |
| *S. aromaticum* | 137.08<br>37.55 254.48 | 4113.29<br>1213.17 64568.3 E+1 | 0.868±0.280 | 0.17 | 21.18 | 54.32<br>8.73 101.42 | 1157.83<br>492.68 22628.54 | 0.965±0.292 | 1.37 | 27.69 |
| *C. articulatus* | 54.69<br>6.17 109.01 | 2012.09<br>699.56 12630.4 E+1 | 0.819±0.260 | 0.02 | 53.08 | 29.21<br>1.28 66.97 | 900.83<br>398.50 19094.03 | 0.861±0.277 | 0.13 | 51.51 |
| *A. graveolens* | 188.71<br>68.61 350.54 | 3716.36<br>1218.55 27913.9E+1 | 0.990±0.310 | 0.04 | 15.38 | 115.48<br>40.81 203.02 | 2896.91<br>1002.56 89930.76 | 0.916±0.259 | 0.20 | 13.03 |
| *Z. officinale* | 59.57<br>8.93 113.11 | 1958.16<br>710.73 83192.25 | 0.845±0.260 | 0.66 | 48.73 | 41.22<br>4.15 83.90 | 1162.78<br>494.40 23300.33 | 0.884±0.270 | 0.26 | 36.50 |
| *E. cardamomum* | 179.55 | 8144.99 | 0.774±0.235 | 0.39 | 16.17 | 58.63 | 1079.80 | 1.013±0.291 | 0.70 | 25.66 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 85.44 370.91 | 1830.96 23000.0 E+2 | | | | 10.79 107.40 | 506.26 11653.18 | | | |
| *L. camara* | 960.62<br>481.98 7999.09 | 24034.16<br>4156.61 15601.1 E+3 | 0.917±0.272 | 3.18 | 3.02 | 400.85<br>233.16 1227.27 | 11100.416<br>2514.48 18765.7 E+2 | 0.889±0.255 | 5.22 | 3.75 |
| *M. longifolia* | 34.32<br>2.95 71.08 | 650.95<br>319.09 7006.04 | 1.003±0.309 | 0.74 | 84.57 | 24.93<br>2.28 47.38 | 168.15<br>108.84 483.48 | 1.546±0.488 | 0.06 | 60.35 |
| *P. dioscoridis* | 427.29<br>262.50 1087.30 | 7833.70<br>2227.88 309021.48 | 1.015±0.260 | 1.29 | 6.79 | 148.06<br>56.67 271.78 | 4929.68<br>1377.33 57202.3E+1 | 0.842±0.255 | 0.25 | 10.16 |
| *T. vulgaris* | 29.03<br>2.47 63.39 | 537.07<br>277.44 3493.15 | 0.884±0.276 | 0.22 | 100.00 | 15.04<br>0.47 39.17 | 234.31<br>132.36 730.96 | 1.075±0.329 | 0.83 | 100.00 |

The neurotoxic mode of action was reported by observing its symptoms when insect pests treated with essential oils or their constituents [13, 14]. The competitive inhibition of Acetyl Cholinesterase Enzyme (AChE) by monoterpenes have been previously reported [15, 16]. For this reason, the components of highly effective essential oils *T. vulgaris*, *M. longifolia* and *C. articulates* were chemically investigated using GC-MS technique figures (1-3) and tabulated in table 2.

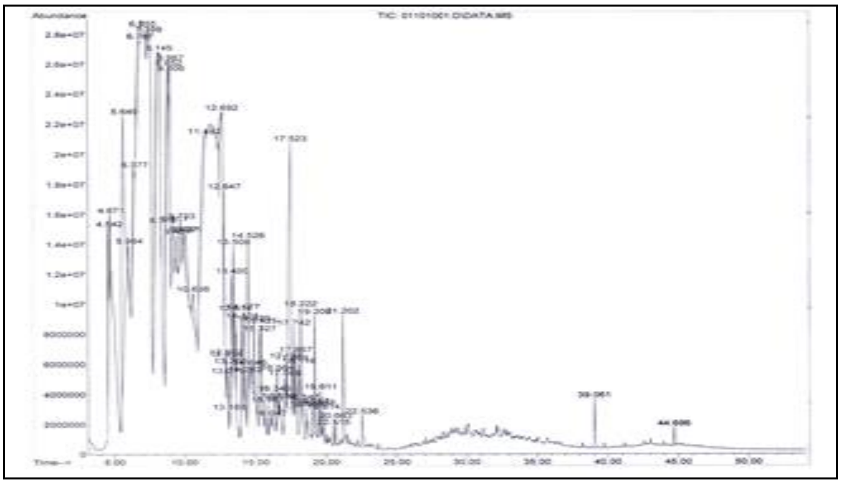

**Fig 1:** GC chromatogram of *T. vulgaris*

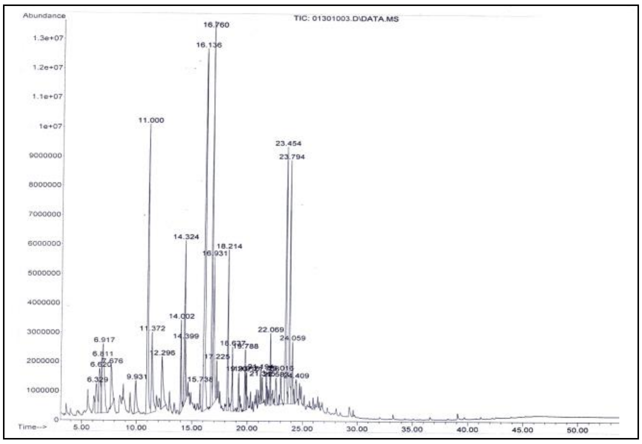

**Fig 2:** GC chromatogram of *M. longifolia*

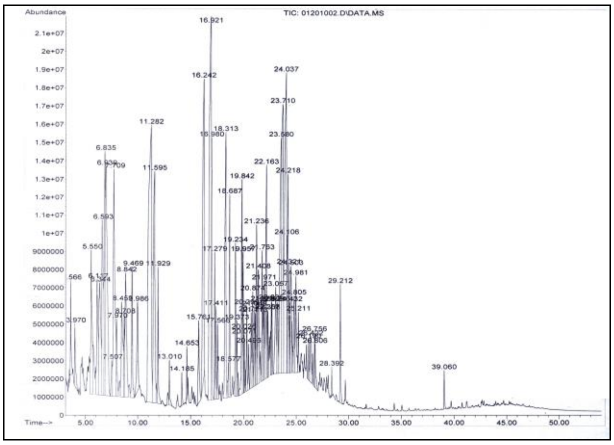

**Fig 3:** GC chromatogram of *C. articulates*

**Table 2:** Chemical constituents of the most active essential oils

| Component Name | R.T | Molecular Formulae | Molecular Weight | ***Percentage (%) Composition of the Essential oils*** | | |
|---|---|---|---|---|---|---|
| | | | | ***Thymus vulgaris*** | ***Mentha longifolia*** | ***Cyperus articulatus*** |
| ***Monoterpene hydrocarbon*** | | | | | | |
| α-Thujene | 4.54 | $C_{10}H_{16}$ | 136 | 3.23 | | |
| α-Pinene | 4.68 | $C_{10}H_{16}$ | 136 | 1.80 | | |
| α-Sabinene | 5.65 | $C_{10}H_{16}$ | 136 | 16.16 | | 1.69 |
| α-Phellandrene | 6.38 | $C_{10}H_{16}$ | 136 | 2.40 | 1.85 | 2.17 |
| α-Terpinene | 6.59 | $C_{10}H_{16}$ | 136 | | 2.39 | 2.65 |
| *o*-Cymene | 6.83 | $C_{10}H_{14}$ | 134 | | 1.34 | 2.10 |
| 1,5,8-*p*-Menthatriene | 6.92 | $C_{10}H_{14}$ | 134 | | 5.04 | |
| β-Terpinene | 6.94 | $C_{10}H_{16}$ | 136 | 1.89 | | 4.73 |
| γ-Terpinene | 7.67 | $C_{10}H_{16}$ | 136 | 5.74 | 2.07 | 3.63 |
| α-Terpinolene | 8.85 | $C_{10}H_{16}$ | 136 | 4.17 | | 1.55 |
| *p*-Cymenene | 9.00 | $C_{10}H_{12}$ | 132 | 0.46 | | |
| Total | | | | 35.85 | 12.69 | 18.52 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***Oxygenated monoterpenes*** | | | | | | |
| (Z)-Sabinene hydrate | 9.21 | $C_{10}H_{18}O$ | 154 | 1.82 | | 0.65 |
| trans-*p*-Menth-2-en-1-ol | 9.73 | $C_{10}H_{18}O$ | 154 | 1.77 | | 1.17 |
| 1-Terpineol | 9.99 | $C_{10}H_{18}O$ | 154 | | | 1.20 |
| 4-Terpinenol | 11.48 | $C_{10}H_{18}O$ | 154 | 3.40 | 10.10 | 8.00 |
| cis-Verbenone | 11.93 | $C_{10}H_{14}O$ | 150 | | | 0.92 |
| α-Terpineol | 12.69 | $C_{10}H_{18}O$ | 154 | 0.15 | 3.78 | 3.69 |
| (Z)-piperitol | 12.85 | $C_{10}H_{18}O$ | 154 | 1.62 | | |
| (E)-Carveol | 12.94 | $C_{10}H_{16}O$ | 152 | 0.11 | | |
| Linalyl acetate | 13.01 | $C_{12}H_{20}O_2$ | 196 | | | 0.29 |
| 2-Methyl-4-(1-methylethyl)-2-cyclohexenone | 13.16 | $C_{10}H_{16}O$ | 152 | 0.13 | | |
| Carvone | 13.40 | $C_{10}H_{14}O$ | 150 | 0.79 | | |
| Piperitone | 13.61 | $C_{10}H_{16}O$ | 152 | 0.76 | | |
| Bornyl acetate | 14.17 | $C_{12}H_{20}O_2$ | 196 | 0.74 | | |
| trans-Anethole | 14.28 | $C_{10}H_{12}O$ | 148 | 0.73 | | |
| 1,4,4-Trimethyl-8-oxa-bicyclo[3.2.1]oct-6-en-2-one | 14.33 | $C_{10}H_{14}O_2$ | 166 | | 4.24 | |
| 4-Hydroxypiperitone | 14.40 | $C_{10}H_{16}O_2$ | 168 | | 1.45 | |
| Thymol | 14.93 | $C_{10}H_{14}O$ | 150 | 12.53 | | |
| Piperitenone oxide | 16.14 | $C_{10}H_{14}O_2$ | 166 | | 15.02 | |
| Geranyl acetate | 16.57 | $C_{12}H_{20}O_2$ | 196 | 0.92 | | |
| **Total** | | | | 25.47 | 34.59 | 15.92 |
| **Total Monoterpenes** | | | | 61.32 | 47.28 | 34.44 |
| ***Sesqueiterpene hydrocarbons*** | | | | | | |
| α-Copaene | 16.25 | $C_{15}H_{24}$ | 204 | | | 4.16 |
| β-Elemene | 16.68 | $C_{15}H_{24}$ | 204 | 0.20 | | |
| (-)-Cyperene | 16.92 | $C_{15}H_{24}$ | 204 | | 9.21 | 7.08 |
| α-Gurjunene | 17.09 | $C_{15}H_{24}$ | 204 | 0.52 | | |
| (-)-β-caryophyllene | 17.28 | $C_{15}H_{24}$ | 204 | 13.79 | 0.63 | 0.79 |
| α-Bergamotene | 17.74 | $C_{15}H_{24}$ | 204 | 1.18 | | |
| (+)-Aromadendrene | 17.87 | $C_{15}H_{24}$ | 204 | 0.97 | | |
| Valencene | 18.11 | $C_{15}H_{24}$ | 204 | 0.34 | | |
| α-Humulene | 18.22 | $C_{15}H_{24}$ | 204 | 1.67 | | |
| Rotundene | 18.32 | $C_{15}H_{24}$ | 204 | | 3.27 | 2.92 |
| (-)-α-muurolene | 18.64 | $C_{15}H_{24}$ | 204 | | 1.51 | |
| α-Amorphene | 18.69 | $C_{15}H_{24}$ | 204 | | | 1.32 |
| β-Cadinene | 19.06 | $C_{15}H_{24}$ | 204 | 0.69 | | |
| Bicyclogermacrene | 19.21 | $C_{15}H_{24}$ | 204 | 2.93 | | |
| Isolongifolene | 19.21 | $C_{15}H_{24}$ | 204 | | 1.06 | |
| Dehydroaromadendrene | 19.23 | $C_{15}H_{22}$ | 202 | | | 1.21 |
| α-Bulnesene | 19.37 | $C_{15}H_{24}$ | 204 | | | 0.45 |
| γ-Cadinene | 19.61 | $C_{15}H_{24}$ | 204 | 0.77 | | |
| δ-Cadinene | 19.81 | $C_{15}H_{24}$ | 204 | 0.52 | | |
| (-)-cis-calamenene | 19.84 | $C_{15}H_{22}$ | 202 | 0.33 | 1.46 | 1.62 |
| Calacorene | 20.30 | $C_{15}H_{20}$ | 200 | | | 0.57 |
| Cadala-1(10),3,8-triene | 20.73 | $C_{15}H_{22}$ | 202 | | | 0.66 |
| 1,4-dimethyl-3-(2-methyl-1-propene-1-yl)-4-vinyl-1-cycloheptene | 20.87 | $C_{15}H_{24}$ | 204 | | | 0.58 |
| **Total** | | | | **23.91** | **17.14** | **21.36** |
| ***Oxygenated sesqueiterpene*** | | | | | | |
| (-)-Caryophyllene oxide | 21.19 | $C_{15}H_{24}O$ | 220 | | 0.67 | 1.17 |
| 4,10(14)-cadinadien-8*β*-ol | 21.41 | $C_{15}H_{24}O$ | 220 | | | 1.24 |
| 4,10(14)-muuroladien-8*β*-ol | 21.87 | $C_{15}H_{24}O$ | 220 | | | 0.50 |
| 3,5,6,7,8,8a-Hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-2(1H)naphthalenone | 21.98 | $C_{15}H_{22}O$ | 218 | | | 0.83 |
| (-)-Isolongifolen-9-one | 22.07 | $C_{15}H_{22}O$ | 218 | | 1.40 | |
| 8-oxo-9H-cycloisolongifolene | 22.16 | $C_{15}H_{22}O$ | 218 | | | 1.73 |
| Valerenal | 22.31 | $C_{15}H_{22}O$ | 218 | | | 0.38 |
| β-Elemenone | 22.64 | $C_{15}H_{22}O$ | 218 | | | 0.45 |
| 9,10-Dehydrofukinone | 23.79 | $C_{15}H_{22}O$ | 218 | | 6.05 | |
| lβ,7α,10β H-guaia-4,11(13)-dien-3-one | 24.04 | $C_{15}H_{22}O$ | 218 | | | 5.47 |
| Aristolone | 24.06 | $C_{15}H_{22}O$ | 218 | | 1.84 | |
| dehydro-cyclolongifolene oxide | 24.22 | $C_{15}H_{22}O$ | 218 | | | 1.57 |
| Patchoulenone | 24.32 | $C_{15}H_{22}O$ | 218 | | | 0.58 |
| 5,11-Epoxycadin-1(10)-ene | 24.41 | $C_{15}H_{24}O$ | 220 | | 0.94 | |
| (-)-Isobicyclogermacrenal | 24.98 | $C_{15}H_{22}O$ | 218 | | | 0.91 |
| **Total** | | | | | 10.9 | 14.83 |
| **Total Sesqueiterpene** | | | | 23.91 | 28.04 | 36.19 |
| ***Aromatic compounds*** | | | | | | |
| 2-Allyl-4-methylphenol | 12.30 | $C_{10}H_{12}O$ | 148 | | 2.83 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Methyleugenol | 17.20 | $C_{11}H_{14}O_2$ | 178 | 0.62 | | |
| 7-Methoxycoumarin-3-carboxylic acid | 26.19 | $C_{11}H_8O_5$ | 220 | | | 0.27 |
| Total | | | | 0.62 | 2.83 | 0.27 |

The qualitative and quantitative compositions of the most potent essential oils were analyzed (Fig. 1-3 and table 2) and the most abundant constituents of *T. vulgaris* were found to be α-sabinene (16.16%), (-)-β-caryophyllene (13.79%), thymol (12.53%) and γ-terpinene (5.47%). The *M. longifolia* essential oil mainly consisted of piperitenone oxide (15.02%), 4-terpinenol (10.10%) and (-)-cyperene (9.21%), while the major constituents of *C. articulates* essential oil were 4-terpinenol (8.00%) and (-)-cyperene (7.08%).

*T. vulgaris* essential oil (table 2) showed a significant relative percentage of monoterpenes (61.32%) than *M. longifolia* (47.28%) and *C. articulates* (34.44%), indicating that their presence may responsible for the highly insecticidal properties against *P. solenopsis* adult females. Our finding is in agreement with those obtained by Abdelgaleil *et al*., (2009) [16] who suggested that AChE may be a target for monoterpenes.

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