Comparative account of allelopathic potential of essential oil of *Tagetes minuta* L. and its major component cis-β-Ocimene

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Abstract: The study aims to explore the chemical composition of volatile essential oil of *Tagetes minuta* and comparison of its allelopathic potential with cis-β-ocimene, a major component of oil. *T. minuta* L. is an exotic aromatic plant found growing in northern plains and western Himalayas in India. The water distilled essential oil from aerial parts of *T. minuta* (at flowering stage) was analyzed by GC-MS. The analysis revealed 27 compounds representing 95.73% of the oil. cis-β-Ocimene (44.56%), dihydrotagetone (28.52%), limonene (3.99%) and tagetone (7.42%) were its major compounds. A comparison of allelopathic effect of cis-β-ocimene was made with *T. minuta* oil using *Cassia occidentalis* L., a common weed, as test plant. Laboratory study included growth studies in the form of germination, seedling length and dry weight of test plant under both treatments i.e. *T. minuta* oil and cis-β-ocimene. The inhibitory effect of ocimene was more significant as compared to oil. However, highest concentration (20µg/cc) of both treatments was found to be inhibitory with respect to all parameters.

Key words: Allelopathy; *Cassia occidentalis*; cis-β-ocimene; essential oil; GC-MS; *Tagetes minuta*.

Introduction

*Tagetes* (Family Asteraceae) species are cultivated world over for its ornamental value and essential oil. Among these, *Tagetes minuta* L. is an important species to yield highest oil among all cultivated *Tagetes* species (Singh et al., 2003). It is traditionally used in folklore medicine for treating different ailments and is credited with several pharmacological and biological activities (Syamasundar & Rao 2013). It originated in South America but has spread throughout the world as a weed (Singh et al., 2003). In India, it is found growing along road-sides and wastelands in the regions of Northern plains and Western Himalayas including Solan, Himachal Pradesh, India and adjoining areas. Numerous reports have appeared on chemical composition of its essential oil (Chamorro et al., 2008; Brene et al., 2009; Meshkatalsadat et al., 2010; Shahzadi et al., 2010; Shirazi et al., 2014) but no information has been reported on the comparative allelopathic impact of *T. minuta* oil in relation to its major component cis-β-ocimene. Thus present study was designed to explore the allelopathic potential of oil in comparison to cis-β-ocimene on growth of *Cassia occidentalis* (Family Fabaceae), a weed species.

Materials and Methods

Plant collection and oil extraction

The plant was collected at flowering stage from Solan and adjoining places of Himachal Pradesh, India (30°55′N, 77°7′E). Aerial parts of plant (1Kg) were subjected to hydro-distillation for 2h using a Clevenger-type apparatus. Seeds of *C. occidentalis* were collected from wildy growing strands in and around Panjab University, Chandigarh, India (30°45′34″ North 76°43′59″ East).

Analysis of the essential oil and identification of its components

GC-MS analysis was carried out with QP2010 Mass spectrometer equipped with fused silica BP- 21 Column (30m × 0.25mm, 0.25 µm film thickness). The carrier gas used was helium. Injector temperature was set at 250°C. Oven temperature was programmed from 70°C, held isothermal for 4 min and then increased @ 4°C/min upto 220°C and held isothermal for 5 min. For mass spectrometer, ion source and interface temperatures were 200 and 250°C, respectively. Solvent cut time was 3.50 min and start and end m/z were 50 and 800 till the detection of ions completed at 46 min (end time).

Compound identification was based on computer matching of mass spectra using library search system of HP-5872 (Hewlett-Packard) consulting databases viz. Wiley 275 and NBS 75K libraries (Mc Lafferty, 1989), NIST 98 (Stein, 1990) and compilation by Adams (1995).

Growth study

To test the inhibitory effect of *T. minuta* oil and cis-β-ocimene on *C. occidentalis*, 20 seeds of weed (after imbibition) were placed on Whatman no. 1 filter paper moistened with 8 ml of distilled water. *T. minuta* oil and cis-β-ocimene were applied in various amounts (0.625, 1.25, 2.5, 5, 10 & 20µg/cc per Petri
dish) on lid of Petri dishes and sealed immediately with parafilm. A similar treatment with water served as control. For each treatment five replicates were placed in a completely randomized design in growth chamber, maintained at 16/8-hour light/dark period and temperature 25 ± 2°C. Relative humidity was 80% and irradiance was 150 μmol m⁻² sec⁻¹. After seven days, germinated seeds were counted and seedling lengths and dry weights were measured. cis-β-Ocimene of analytical grade was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Statistical Analysis
For each treatment (including control), five replicates were kept in a completely randomized manner. Data were subjected to one-way analysis of variance followed by separation of treatment means from the control at p < 0.01 and 0.05 applying post-hoc Dunnett’s Test using SPSS PC software.

Results and Discussion
GC-MS analysis of hydro-distilled aerial parts of T. minuta revealed 27 compounds accounting 95.73% of oil (Table 1). Major constituents of oil were identified to be cis-β-ocimene (44.56%), dihydrotagetone (28.52%), limonene (3.99%) and tagetone (7.42%) (Table 1). Considerable variation in the composition of volatile oil within this species has been reported in many previous reports (Mohamed et al., 2002; Senatore et al., 2004; Moghaddam et al., 2007) which have been attributed to different climatic & geographical conditions, stage of harvesting and method of distillation of oil.

The allelopathic effect of direct contact of C. occidentalis with the T. minuta oil and cis-β-ocimene revealed that both treatments reduced overall growth of test seedlings, however, growth inhibition was more significant with cis-β-ocimene (Table 2). There was reduction in germination percentage of C. occidentalis in dose dependent manner. Seedling lengths of test plant also decreased with maximum inhibition of 75.82% at 20 μg/cc cis-β-ocimene treatment. Dry weight changes were not much apparent for most of concentrations, however, at low concentrations, it decreased significantly thereafter increased in dose dependent manner. Though the exact mechanism for inhibition of germination and growth remains unknown, a number of reports indicate that essential oils and their constituent terpenes inhibit mitotic activity and reduce mitotic index (Romagni et al., 2000; Nishida et al., 2005) which may be the possible reason of suppressed growth.

The inhibitory effect of essential oils and their components on other plants have been reported by many workers (Scrivanti et al., 2003; Singh et al., 2006; de Oliveira et al., 2014; Miranda et al., 2015; de Oliveira et al., 2016; El-Gawad 2016). The present study provided an instance of allelopathic inhibition of C. occidentalis by T. minuta oil and cis-β-ocimene.

### Table 1: Chemical composition of the essential oil of T. minuta

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>RRI</th>
<th>%</th>
<th>No</th>
<th>Compound</th>
<th>RRI</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>β-Myrcene</td>
<td>1162</td>
<td>0.09</td>
<td>15</td>
<td>Linalool</td>
<td>1548</td>
<td>0.08</td>
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<tr>
<td>2</td>
<td>α-Phellandrene</td>
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<td>0.06</td>
<td>16</td>
<td>trans-Caryophyllene</td>
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<td>0.81</td>
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<tr>
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<td>α-Humulene</td>
<td>1654</td>
<td>0.36</td>
</tr>
<tr>
<td>4</td>
<td>ß-Phellandrene</td>
<td>1205</td>
<td>0.05</td>
<td>18</td>
<td>cis-Ocimene</td>
<td>1685</td>
<td>1.36</td>
</tr>
<tr>
<td>5</td>
<td>cis-β-Ocimene</td>
<td>1237</td>
<td>44.56</td>
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<td>Germacrene D</td>
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<td>trans-β-Ocimene</td>
<td>1253</td>
<td>0.45</td>
<td>20</td>
<td>trans-Ocimene</td>
<td>1703</td>
<td>1.63</td>
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<tr>
<td>7</td>
<td>n-Octanal</td>
<td>1289</td>
<td>0.10</td>
<td>21</td>
<td>Bicyclo-germacrene</td>
<td>1717</td>
<td>1.07</td>
</tr>
<tr>
<td>8</td>
<td>Dihydrotagetone</td>
<td>1309</td>
<td>28.52</td>
<td>22</td>
<td>Carveol</td>
<td>1822</td>
<td>0.05</td>
</tr>
<tr>
<td>9</td>
<td>ß-Pinene oxide</td>
<td>1358</td>
<td>0.12</td>
<td>23</td>
<td>Piperitenone</td>
<td>1905</td>
<td>0.17</td>
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<tr>
<td>10</td>
<td>allo-Ocimene</td>
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<td>1.68</td>
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<td>Spathulanol</td>
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<td>11</td>
<td>Artemisia ketone</td>
<td>1448</td>
<td>0.08</td>
<td>25</td>
<td>Cedrol</td>
<td>2321</td>
<td>0.16</td>
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<tr>
<td>14</td>
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<td>1508</td>
<td>3.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: RRI, relative retention indices calculated against n-alkanes, % calculated from FID data

### Table 2: Effect of T. minuta oil and cis-β-ocimene on growth of C. occidentalis

| Parameter                  | Treatment | 0.0625 | 1.25 | 2.5 | 5 | 10 |
|----------------------------|-----------|--------|------|-----|---|----|--------|
| Percent Germination        | Tm        | 98.3 ± 2.9 | 95.0 ± 5.0 | ** 91.65 ± 2.9 | ** 90.0 ± 0.0 | 86.65 ± 2.9 | 80.0 ± 5.0 | ** 55.0 ± 5.0 |
|                           | Oc        | 98.3 ± 2.9 | 90.0 ± 0.0 | 90.0 ± 0.0 | 86.65 ± 2.9 | 85.0 ± 0.0 | 60.0 ± 0.0 | 56.65 ± 2.9 |
| Seedling length (cm)       | Tm        | 10.71 ± 0.36 | 11.40 ± 0.56 | ** 11.72 ± 0.28 | ** 12.47 ± 0.12 | 11.89 ± 1.30 | 9.06 ± 0.86 | 6.96 ± 0.21 |
|                           | Oc        | 10.71 ± 0.36 | 8.14 ± 0.62  | 6.58 ± 0.15 | 6.23 ± 0.54 | 5.85 ± 0.22 | 5.11 ± 0.38 | 2.59 ± 0.23 |
| Dry wt. (mg/sc)            | Tm        | 7.85 ± 0.33 | 7.70 ± 0.79 | 7.74 ± 0.49 | 8.28 ± 0.24 | 8.36 ± 0.48 | 6.89 ± 0.54 | 5.99 ± 0.19 |
|                           | Oc        | 7.85 ± 0.33 | 6.20 ± 0.12 | 6.06 ± 0.17 | 7.66 ± 0.19 | 7.72 ± 0.15 | 8.23 ± 0.11 | 9.05 ± 0.28 |

Note: Tm: T. minuta oil; Oc: cis-β-ocimene; Values are means ± Standard deviation; *; ** indicate significant difference from controls at P < 0.05 and P < 0.01, respectively (in a row), applying Dunnett’s test; ns: non-significant

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for first time. However, the effect of other oil compounds in relation to cis-\(\beta\)-ocimene, need to be explored in future.

**Conclusions**

The study concludes that *T. minuta* oil and cis-\(\beta\)-ocimene are strongly allelopathic against *C. occidentalis*. However, the effect of cis-\(\beta\)-ocimene is more as compared to oil. Since it is the major component, its relative contribution towards overall allelopathy of *T. minuta* oil may be more but its interaction with other components present in oil needs to be further explored in relation to wide variety of weeds.

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**References**


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