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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 wasteland 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; Breme *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 wasteland weed.

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°55'0" North, 77°7'0" East). Aerial parts of plant (1Kg) were subjected to hydro-distillation for 2h using a Clevenger-type apparatus. Seeds of *C. occidentalis* were collected from wildly growing strands in and around Panjab University, Chandigarh, India (30°45'34" North 76°45'59" 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

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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 µmole 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.

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

No	Compound	RRI	%	No	Compound	RRI	%
1	β- Myrcene	1162	0.09	15	Linalool	1548	0.08
2	α-Phellandrene	1165	0.06	16	*trans*-Caryophyllene	1582	0.81
3	dl-Limonene	1199	3.99	17	α-Humulene	1654	0.36
4	β-Phellandrene	1205	0.05	18	*cis*-Ocimenone	1685	1.36
5	*cis*-β-Ocimene	1237	44.56	19	Germacrene- D	1692	0.42
6	*trans*-β-Ocimene	1253	0.45	20	*trans*-Ocimenone	1703	1.63
7	n-Octanal	1289	0.10	21	Bicyclo-germacrene	1717	1.07
8	Dihydrotagetone	1309	28.52	22	Carveol	1822	0.05
9	α-Pinene oxide	1358	0.12	23	Piperitenone	1905	0.17
10	allo-Ocimene	1373	1.68	24	Spathulenol	2109	0.13
11	Artemisia ketone	1448	0.08	25	Cedrol	2321	0.16
12	Myroxide-Z	1465	0.12	26	n-Tetracosane	2393	1.09
13	*cis*-Tagetone	1506	3.85	27	n-Hexacosane	2661	1.18
14	*trans*-Tagetone	1508	3.55				
				Total Identified Compounds	95.73 %		

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	Concentrations (µg/cc)						
		0	0.625	1.25	2.5	5	10	20
Percent Germination	Tm	98.3 ± 2.9	95.0 ± 5.0 ns	91.65 ± 2.9 ns	90.0 ± 0.0 ns	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 ns	11.72 ± 0.28 ns	12.47 ± 0.12*	11.89 ± 1.30 ns	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/seedling)	Tm	7.85 ± 0.33	7.70 ± 0.79ns	7.74 ± 0.49 ns	8.28 ± 0.24 ns	8.36 ± 0.48 ns	6.89 ± 0.54 ns	5.99 ± 0.19**
	Oc	7.85 ± 0.33	6.20 ± 0.12**	6.06 ± 0.17**	7.66 ± 0.19 ns	7.72 ± 0.15 ns	8.23 ± 0.11 ns	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

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 Oliviera *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 for first time. However, the effect of other oil compounds in relation to *cis*-β-ocimene, need to be explored in future.

Conclusions

The study concludes that *T. minuta* oil and *cis*-β-ocimene are strongly allelopathic against *C. occidentalis*. However, the effect of *cis*-β-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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