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Studies on essential oils. Part 35: chemical and biocidal investigations on *Tagetes erecta* leaf volatile oil

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ABSTRACT: Chemical investigations on the leaf essential oil of *Tagetes erecta*, performed by HPLC, GC and GC–MS techniques, showed the presence of 26 components, accounting for 89% of the total oil. The major constituents were (*Z*)-β-ocimene (42.2%), dihydrotagetone (14.3%), (*Z*)-tagetone (8.3%), limonene (7.3%), (*E*)-ocimenone (6.1%) and (*Z*)-ocimenone (5.3%). The biocidal investigations showed that the oil possessed a significant but limited and dose-related antifungal and insecticidal activity. The oil showed 100% mortality of white termite (*Odontotermes obesus* Rhamb.) at a dose of 6 µl/Petri-plate after 24 h of exposure, while at lower doses and shorter exposures it showed diminished mortality rates. The oil only partially affected the mycelial growth of any of the tested fungi. Thus, the leaf oil of this plant, which is rich in (*Z*)-β-ocimene, has a statistically significant antifungal and insecticidal activity.

KEY WORDS: *Tagetes erecta*; marigold; essential oil; chemical composition; (*Z*)-β-ocimene; antifungal activity; insecticidal activity

Introduction

Tagetes erecta Linn. (Family Compositae), commonly known as 'marigold', is widely cultivated in the northern plains of India during the winter season. Marigold flowers are a rich source of a natural yellow to orange dye, helenien (a dipalmitate ester of a xanthophyll), which is in high demand by national and international companies. The flowers are used in processed food, confectionery, drugs, pharmaceuticals and in the poultry industry.[1,2] The leaf extract of *T. erecta* possesses good nematicidal activity[3,4] against *Meliadogyne arenaria, M. hapla, M. javanica* and a root-knot nematode, as well as pharmacological[5] responses on frogs, guinea-pigs, rabbits and dogs. The leaf extract of this plant also affects[6] the higher spore population and mycorrhizal colonization of *Meliadogyne incognita* and *Glomus fasciculatum*, respectively. The incorporation of leaves of *T. erecta* in soil decrease the *Nitrosomonas* population.[7] The leaf of *T. erecta*, on steam distillation, gives a volatile oil which possesses low antifungal activity[8] against *Aspergillus niger*, *A. flavus* and *Fusarium oxysporum* and also showed repellent activity[9] against *Tribolium castaneum*, a stored grain pest. Hausen *et al.*[10] isolated and identified three contact allergens, viz. butenyl bithiophene, α-terthienyl and hydroxy-tremetone.

Several synthetic pesticides have been formulated, and are available on the market, which can control various microbial diseases but they are dangerous to human and host plants, cause adverse effects and have residual toxicity. Recently, some natural plant products have been found to be effective sources of chemotherapeutic agents and to provide renewable sources of useful antifungals, having biodegradable nature and devoid of side effects. Hence, there is a strong need to develop natural and safe biopesticides. The chemical composition of the leaf volatile oil from *T. erecta* is quite meagre.[11–14] As part of our on-going research programs,[15–21] we have undertaken chemical, antifungal and insecticidal investigations on the leaf volatile oil and the results are reported in this communication.

Experimental

Plant Material and Isolation of the Oil

The leaves of *Tagetes erecta* were collected from Kushinagar, India, in July 2000. A voucher specimen has been deposited in the herbarium of the Faculty of Science,

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D.D.U. Gorakhpur University, Gorakhpur. The leaves were cut into small pieces and washed with distilled water, and the volatile oil was obtained by hydrodistillation using a Clevenger apparatus.[22] The oil thus obtained was dried over anhydrous sodium sulphate and stored under refrigeration at 4 °C.

Chemical Investigation of the Volatile Oil

The chemical composition of the oil was investigated by HPLC, GC and GC–MS, as described below.

HPLC

The leaf volatile oil was subjected to HPLC analysis using a DATALAB 3103 UV-VIS detector coupled with a 3101 pump and an Alltech Econosil C-18 column (250 mm × 4.6 mm; particle size, 5 μm). About 25 μl oil (without dilution) were injected using methanol: water (9 : 1) as the mobile phase. The flow rate was fixed at 1 ml/min and the chromatogram was recorded at 254 nm.

GC

The oil was subjected to GC analysis by using a Hewlett-Packard 5890 Series II gas chromatograph, equipped with flame ionization detector (FID) and two HP fused silica columns (A and B). Column A was an HP-5 (5% phenylmethylsilicone, 30 m × 0.32 mm i.d., film thickness 0.25 μm). The injector and detector temperatures were maintained at 250 °C and 270 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 1.2 ml/min. The amount of sample injected was 0.1 μl (split mode). The oven temperature was programmed as follows: 60 °C for 5 min, rising to 140 °C (at 1 °C/min, then 140–270 °C at 10 °C/min), then held at 270 °C for 5 min. Column B was an HP-innowax (30 m × 0.53 mm, film thickness 1.0 μm). Injector and detector temperatures were 250 °C and the same amount of sample was injected in split mode; carrier gas, nitrogen; flow rate, 2.5 ml/min. The oven temperature program was: 60 °C for 5 min, rising to 140 °C at 1 °C/min, then 140–240 °C at 10 °C/min, then held at 240 °C for 5 min.

GC–MS

The oil was subjected to GC–MS analysis using a Hewlett-Packard HP 6890 Series GC fitted with a Hewlett-Packard Mass Detector (Model 5973) and a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm). The injector, GC–MS interphase, ion source and selective mass detector temperatures were maintained at 270 °C, 280 °C, 230 °C and 150 °C respectively. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The oven temperature was programmed as follows: 60 °C for 5 min, rising to 140 °C at 1 °C/min, then 140–270 °C at 10 °C/min, then held at 270 °C for 5 min.

Identification of Components

The chemical constituents have been identified by comparing their mass spectra with NBS 75 K library and/or by co-injection with authentic samples when available. The results are reported in Table 1.

Insecticidal Activity

The oil was screened for its insecticidal activity against white termite (*Odontotermes obesus* Rhamb.) using 80 mm glass Petri-plates. The required dose of oil (0.5, 2, 3 and 6 μl/Petri-plate) was soaked in a piece of filter paper (10 mm diameter) and pasted on the inner surface of the cover of the Petri-plate. A group of 10 termites, along with 10 g of soil and small pieces of sugarcane stem, were placed inside each Petri-plate. To ensure sufficient aeration inside the Petri-plates, a constant gap was maintained in between each pair of Petri-plates by inserting a small piece of filter paper. Similarly, a control experiment was also done by pasting a blank filter paper

Table 1. Chemical composition of *Tagetes erecta* leaf oil

No.	Components	RI[a]	Percentage[b]
1	Ethyl-2-methyl butanoate	857	0.8
2	2-Methyl-1-butyl acetate	883	0.2
3	α-Pinene	941	tr
4	Camphene	953	tr
5	Sabinene	975	0.3
6	β-Pinene	980	0.1
7	Myrcene	993	0.1
8	Octanal	1000	0.1
9	α-Phellandrene	1007	0.3
10	α-Terpinene	1020	tr
11	*p*-Cymene	1028	0.1
12	Limonene	1030	**7.3**
13	(*Z*)-β-Ocimene	1040	**42.2**
14	(*E*)-β-Ocimene	1050	0.4
15	Dihydrotagetone	1055	**14.3**
16	γ-Terpinene	1064	tr
17	Terpinolene	1090	tr
18	Linalool	1098	0.2
19	*allo*-Ocimene	1131	0.6
20	(*Z*)-β-Ocimene oxide	1140	0.5
21	(*E*)-Tagetone (*trans*-tagetone)	1149	0.4
22	(*Z*)-Tagetone (*cis*-tagetone)	1155	**8.3**
23	(*Z*)-Ocimenone (*cis*-ocimenone)	1231	**5.3**
24	(*E*)-Ocimenone (*trans*-ocimenone)	1240	**6.1**
25	*trans*-Caryophyllene	1420	0.7
26	Bicyclogermacrene	1495	1.0

[a] Retention index.

[b] Percentage taken from capillary GC (column HP-5 and HP-Innowax) traces with FID when available or directly from the GC–MS percentage of total ion current peak.

disc (without oil) on the same surface of the Petri plate. In order to investigate the insecticidal nature of the oil, the termite revival was observed after transferring them to a fresh Petri-plate. Similarly, the insecticidal efficacy of the oil was also compared with two commercial synthetic insecticides (without dilution), viz. endosulphan 35% (Thiodan) and chlorpyriphos 20% (Primoban-20). Testing of similar doses of synthetic insecticides was also undertaken and the results for vapour action of the essential and synthetic oils were campared.

Antifungal Activity

The antifungal activity of the oil against seven pathogenic fungi (viz. *Fusarium moniliforme, F. oxysporum, Colletotrichum falcatum, Trichothecium roseum, Curvularia palliscens, Aspergillus niger* and *A. terreus*) was tested using the inverted Petri-plate method.[23] The fungal cultures were maintained in oatmeal agar medium. For antifungal studies, the tested doses of oil (2, 4 and 6 μl/Petri plate) were deposited on presterilized filter paper discs (15 mm diameter) pasted on the inner surface of the Petri-plate cover and placed in inverted position. Similarly, a control experiment was also run simultaneously using a blank filter paper disc, and the fungitoxicity was recorded in terms of percentage mycelial inhibition (see Table 3), which was calculated according to the formula:

$$\% \text{ mycelial inhibition} = \frac{dc - dt}{dc} \times 100$$

where, dc and dt are average diameters of the mycelial colonies in the control and treated sets, respectively.

Nature of Fungitoxicity

The nature of fungitoxicity of *T. erecta* leaf oil was determined using the method of Garber and Houston.[24] For this purpose, completely inhibited fungal mycelium of treated sets were aseptically excised, washed with sterilized distilled water and reinoculated in fresh Petri-plates containing 10 ml of the medium. These plates were again incubated and observed for revival of growth in the reinoculated Petri-plate.

Statistical Analysis

The statistical analysis was undertaken using one-way and two-way analysis of variance (ANOUA; Sokal and Rohlf[25]). The two-way ANOUA was used for determining the significant differences within different doses and durations in case of insecticidal investigation. However, for antifungal investigation, the one-way ANOUA was used to locate significant differences within different doses.

Results and Discussion

The chemical composition of the leaf oil from *T. erecta*, investigated by HPLC, GC and GC–MS techniques, showed the presence of 26 components, accounting for 89% of the total oil (Table 1). The major components are (*Z*)-β-ocimene (42.2%), dihydrotagetone (14.3%), (*Z*)-tagetone (8.3%), limonene (7.3%), (*E*)-ocimenone (6.1%) and (*Z*)-ocimenone (5.3%). Baslas *et al.*[11] reported linalool (26.8%), D-limonene (19.7%), tagetone (13.4%), ocimene (7.6%), β-phellandrene (5.0%), linalyl acetate (3.6%) as major constituents, while menthol, geraniol and dipentene were minor components of the oil. Recently, 27 components have been identified[14] in this oil, among which terpinolene, (*E*)-β-ocimene, piperitone and limonene were the principal constituents. These results are not comparable to ours.

Investigation of the oil for insecticidal activity against the white termite of sugarcane fields (Table 2) showed that it confers 100% mortality at 6 μl/Petriplate dose after 24 h of exposure, while at lower doses and shorter exposures, it showed disminished mortality rates. A comparison of the oil's insecticidal activity with two synthetic insecticides (Thiodan and Primoban-20) showed that the oil possesses lower activity than the synthetic compounds.

The antifungal investigation (Table 3) showed good activity against *Aspergillus terreus* and *Colletotrichum falcatum*, but the oil was less effective against *Trichothecium roseum*, *Fusarium moniliforme*, *Curvularia palliscens*, *Aspergillus niger* and ineffective against *F. oxysporum*. The results of the present antifungal investigation against *A. niger* and *F. oxysporum* are comparable to those obtained in earlier investigations.[8] Our results also showed that the leaf oil does not completely inhibit the mycelial growth of any of the fungi tested. The investigation on fungitoxicity showed that this oil possesses fungistatic behaviour against *F. oxysporum* and *A. niger* but fungicidal activity for other tested fungi.

In summary, it may be concluded that the leaf volatile oil of *Tagetes erecta*, which is rich in (*Z*)-β-ocimene, possesses statistically significant antifungal as well as insecticidal behaviour.

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Table 2. Insecticidal investigation of *T. erecta* leaf oil against white termite (*Odontotermes obesus* Rhamb.)

Oil/synthetic insecticides	Dose[a]	Mortality (%) at various exposure time (h)						
		1	2	3	5	7	12	24
Tagetes erecta leaf oil*	0.5	0	0	0	0	10	20	30
	2.0	0	0	0	40	60	70	80
	3.0	0	0	20	60	70	80	80
	6.0	0	0	40	70	70	90	100
Chlorpyriphos-20 (primoban-20)*	0.5	0	0	0	30	80	90	**100**
	2.0	0	10	50	80	**100**	**100**	**100**
	3.0	0	20	70	**100**	**100**	**100**	**100**
	6.0	0	30	**100**	**100**	**100**	**100**	**100**
Endosulfan-35% (Thiodan)*	0.5	0	0	10	20	60	80	90
	2.0	0	10	20	30	80	**100**	**100**
	3.0	0	10	20	40	80	**100**	**100**
	6.0	0	30	40	70	**100**	**100**	**100**
Control	—	0	0	0	10	20	20	30

[a] Dose of oil in µl/80 mm Petri-plate.
* Statistically significant ($p < 0.05$) when two way analysis of variance was used for locating significant differences within different doses and exposure times.

Table 3. Antifungal investigation on *T. erecta* leaf oil against pathogenic fungi

Name of fungus	Mycelial inhibition (%) at different doses[a] of the oil		
	2	4	6
*Fusarium moniliforme**	13	25	38
*Colletotrichum falcatum**	64	83	88
*Trichothecium roseum**	0	13	48
Fusarium oxysporum	0	0	0
*Culvularia palliscens**	0	31	49
Aspergillus niger	0	0	44
*Aspergillus terreus**	38	69	88

[a] Dose of oil in µl/80 mm Petri-plate.
* Statistically significant ($p < 0.05$) when single way analysis of variance was used for locating significant differences within different doses.

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