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Research Article

ALLELOPATHIC IMPACT OF ESSENTIAL OIL OF *TAGETES MINUTA* ON COMMON AGRICULTURAL AND WASTELAND WEEDS

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ABSTRACT

Objective: *Tagetes minuta* (Family Asteraceae) is an aromatic plant possessing volatile essential oil. *T. minuta* oil possesses medicinal and insecticidal properties as antiheminthic, nematicidal, bactericidal, antiviral, fungicidal, and insecticidal. However, it has been explored for herbicidal potential in very few reports. The aim of this study is to find out its allelopathic potential against common wasteland and agricultural weeds.

Methods: The various agricultural and wasteland weeds were selected for laboratory growth studies. Oil was applied in solution form using an emulsifier.

Results: Growth of all test weeds was inhibited by *T. minuta* oil; however, the effect was maximum in *Amaranthus tricolor* with complete inhibition at 1 µl/ml, and *Echinochloa crus-galli* was least affected with complete inhibition at 5 µl/ml concentration of *T. minuta* oil.

Conclusion: *T. minuta* oil offers great potential for effective weed management in agricultural as well as wasteland areas.

Keywords: *Tagetes minuta*, Essential oil, Allelopathy, Solution form, Wasteland, Agricultural Weeds.

INTRODUCTION

Tagetes minuta L. is an aromatic plant commonly known as stinking roger, Khakibush, wild marigold, Khali weed, Mexican marigold [1], etc. It is one of the most important species of genus *Tagetes* known to yield highest amount of essential oil [2]. *T. minuta* is large annual herb growing over 1 m with erect branched stems; green pungent, glabrous, and pinnately compound leaves with serrated leaflets. Inflorescence is terminal shortly stalked corymb having yellowish green flower heads. Fruits are black flattened spindle-shaped achenes [3,4]. The plant is native to South America and is often found growing in disturbed areas during succession [5] and in areas with loose soil [6]. *T. minuta* has made alien introduction to many parts of the world including Asia, Africa, United States, Europe, and New Zealand as a weed [7]. In India, it was introduced for its essential oil [8]; however, it escaped cultivation and started growing abundantly at high altitudes and disturbed sites of deforestation or forest fires [9]. The plant has been known to acquire weedy habit competing with many economically important crops, *viz., Zea mays*, *Oryza sativa*, and beans [3].

An exhaustive study on the antibacterial, antifungal, insecticidal, acaricidal, nematicidal, and insecticidal properties of *T. minuta* essential oil has been documented by number of researchers [10-18]. *T. minuta* oil also possesses therapeutic and antioxidant properties [19,20]; however, very few researchers have tested essential oil of this plant for weed management. Recently, we have reported allelopathic potential of *T. minuta* oil against *Chenopodium murale* L., *Phalaris minor* Retz., *Amaranthus viridis* L., and *Cassia occidentalis* L. [21,22] where oil was used in volatile form. However, for foliar or soil application, it has to be applied in solution form. In view of this objective, this study was conducted to explore allelopathic potential of *T. minuta* oil on few agricultural (*Amaranthus tricolor*, *A. viridis*, *Echinochloa crus-galli,* and *P. minor*) and wasteland (*Bidens pilosa* and *Chenopodium murale*) weeds.

METHODS

Collection of plant material and extraction of oil

Above-ground flowering shoots of *T. minuta* were collected from various locations around Solan (Himachal Pradesh, India). The plant specimen was identified from herbarium of IHBT, Palampur (Voucher PLP2537). These were hydrodistilled in Clevengers apparatus for 2 hrs. Oil was collected in glass vials and kept in refrigerator in dark till used.

Procurement of weed seeds

The seeds of agricultural and wasteland weeds, *viz*., green amaranth (*A. viridis* L.), vegetable amaranth (*A. tricolor* L.), hairy beggars tick (*B. pilosa* L.), little seed canary grass (*P. minor* Retz.), nettle leaf goosefoot (*C. murale* L.), and barnyard grass (*E. crus-galli* [L.] Beauv.) were collected from wildly growing stands of these weeds in Panjab University Campus, Chandigarh and agricultural fields in adjoining areas.

Bioassay studies

The effect of oil on weeds was studied through laboratory bioassay using oil in solution form. Various concentrations of *T. minuta* oil (0.25, 0.50, 1, 2, 3, 4, and 5 µl/ml, v/v) were prepared by dissolving requisite amount of oil in distilled water with the help of emulsifier Tween-20 (0.1%, and v/v). About 15 seeds each of *E. crus-galli*, *B. pilosa*, 20 seeds each of *C. murale* and *P. minor,* and 50 seeds each of *A. viridis* and *A. tricolor* were used per Petri dish. Seeds of *P. minor* were pre-treated with concentrated sulfuric acid for 1 minute and washed several times with tap water before imbibition to remove acid completely. Imbibed seeds of all test plants except *A. viridis* and *A. tricolor* (used without imbibition) were spaced equidistantly on double layer of Whatman filter paper No. 1 moistened with 8 ml of respective concentration of oil solution. The Petri dishes were sealed immediately with double layer of adhesive tape and kept in growth chamber under standard conditions of 16/8 hrs, light/dark photoperiod, 25±2°C temperature, 75±2% relative humidity,

and 150 µmol/m^2/seconds photon flux density for summer plants (*B. pilosa*, *E. crus-galli*, *A. viridis*, and *A. tricolor*), whereas winter plants (*C. murale* and *P. minor*) were kept at temperature 10±2°C maintaining rest of the conditions similar to summer plants. A parallel control was kept without oil treatment and 5 replicates were maintained for each concentration. After 7 days, observations/measurements were made in terms of germination percentage, radical length, and plumule length.

Statistical analysis

The experiment was conducted in completely randomized block design and repeated twice. All the data were presented as mean values of both the experiments. 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

The effect of *T. minuta* oil on growth of test weeds was studied in the form of germination percentage, radical length and plumule length of seedlings grown under lab conditions. The % germination of control samples was recorded as 94.44±1.93, 97.78±1.92, 100±0, 96.67±5.77, 83.33±2.89, and 100±0% in *A. tricolor*, *A. viridis*, *B. pilosa*, *C. murale*, *E. crus-galli*, and *P. minor*, respectively (Fig. 1a). At lower concentrations, a dose-dependent decrease in germination was noticed in all test plants, which was statistically significant too except *P. minor*. None of the seeds of *A. tricolor* germinated at 1 µl/ml treatment whereas among other weeds maximum inhibition was observed in *C. murale* (65%) and *B. pilosa* (53.3%). At higher concentrations (2 µl/ml), the % germination declined to zero in *C. murale, A. viridis,* and *B. pilosa*. Least allelopathic effect on germination was observed in *E. crus-galli* where a reduction of 65% was observed at 4 µl/ml followed by complete inhibition at 5 µl/ml concentration. From the dose-response curves, inhibitory threshold values were calculated. Among agricultural weeds, half maximal inhibitory concentration value (IC_{50}) was maximum for *E. crus-galli* (3.36 µl/ml) and minimum for *A. tricolor* (0.47 µl/ml) (Table 1).

Similarly, reduction in radicle length was also observed in response to concentration of oil (Fig. 1b). Radicle length in control seedlings was 5.64±0.04, 3.04±0.02, 1.87±0.08, 3.49±0.06, 6.75±0.28, and 6.5±0.12 cm in *A. tricolor*, *A. viridis*, *B. pilosa*, *C. murale*, *E. crus-galli*, and *P. minor*, respectively. With treatment of 0.25 µl/ml, reduction in radicle length was nearly 23, 24, 26, and 16% in *A. tricolor*, *A. viridis*, *C. murale,* and *E. crus-galli*, respectively. At 0.5 µl/ml, radicle length in weeds *A. tricolor*, *A. viridis*, *B. pilosa*, *C. murale*, *E. crus-galli,* and *P. minor* was 1.89±0.04, 2.05±0.03, 2.13±0.03, 1.83±0.06, 5.39±0.05, and 5.21±0.23, respectively. At 1 and 2 µl/ml concentrations, most sensitive plant was *P. minor* (nearly 65% reduction) and least was *B. pilosa* (nearly 4% reduction). At 3 µl/ml, radicle length in *E. crus-galli* and *P. minor* was 2.42±0.08 and 0.67±0.03 cm, respectively. At 5 µl/ml concentration, none of the weeds germinated at all.

In general, with an increase in concentration, plumule length also decreased (Fig. 2). In control, seedlings of broad-leaved weeds, *viz*., *A. tricolor*, *A. viridis*, *B. pilosa,* and *C. murale* and it was 1.61±0.16, 1.29±0.02, 2.69±0.20, and 2.03±0.21, respectively. With the treatment of 0.25 µl/ml, the % reduction observed in *A. tricolor*, *A. viridis*, *C. murale*, *E. crus-galli,* and *P. minor* was nearly 28, 16, 38, 3, and 11%, respectively. Promotory effect of oil was noticed in *B. pilosa*, with increase of 2% in plumule length at this concentration. In response to 0.5 µl/ml concentration of oil, maximum reduction was observed in *B. pilosa* and *A. tricolor* (nearly 67 and 65%, respectively). Similarly at

Table 1: IT and IC_{50} values for weeds and crops using solution form of oil treatment

Test plant	IT (µl/ml)	IC_{50} (µl/ml)
Agricultural weeds		
A. tricolor	0.25	0.47
A. viridis	0.25	1.25
E. crus-galli	0.25	3.36
P. minor	1	3.15
Wasteland Weeds		
B. pilosa	0.25	0.98
C. murale	0.25	0.49

B. pilosa: Bidens pilosa, C. murale: Chenopodium murale, P. minor: Phalaris minor, E. crus-galli: Echinochloa crus-galli, A. tricolor: Amaranthus tricolor,
IT: Inhibitory threshold, IC_{50}: Inhibitory concentration

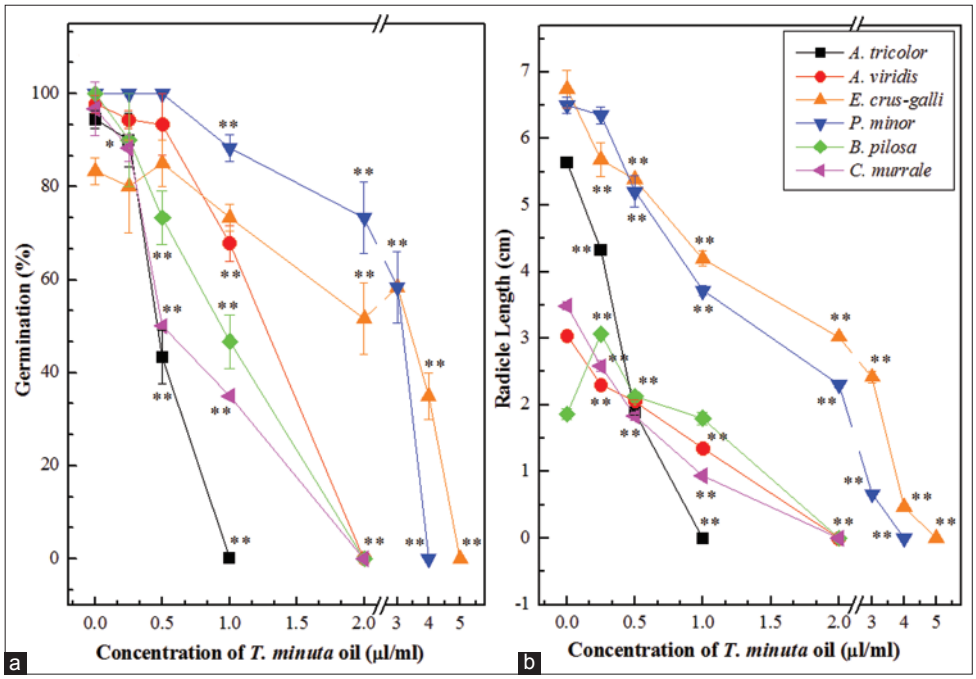

Fig. 1: (a) Effect of *Tagetes minuta* oil on germination and (b) radicle length of agricultural and wasteland weeds. Results are expressed as mean ± standard error (Y error bar); *.along each symbol in each figure represent significant difference at p≤0.05 and 0.01, respectively, applying Dunnett's test**

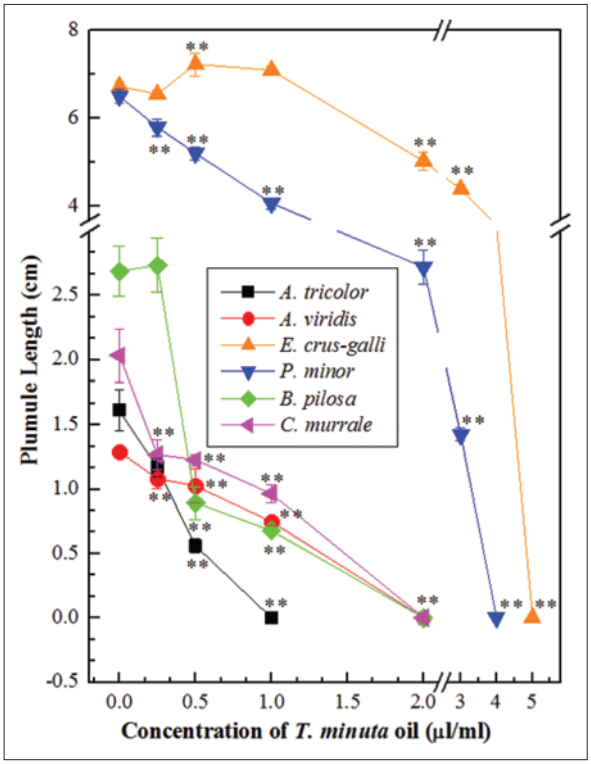

Fig. 2: Effect of *Tagetes minuta* oil on plumule length of agricultural and wasteland weeds. Results are expressed as mean ± standard error (Y error bar); **along each symbol in each figure represent significant difference at p≤0.01 applying Dunnett's test

1 µl/ml concentration, among all the target plants, maximum inhibition of plumule length was observed in *C. murale* (nearly 52%). At 2 µl/ml, *B. pilosa*, *A. viridis*, *A. tricolor*, *C. murale* were completely inhibited, and plumule length in *E. crus-galli* and *P. minor* was reduced by nearly 25% and 58%, respectively. At 4 µl/ml concentration, it was 3.28±0.07 cm in *E. crus-galli* leading to approximately 51.3% reduction.

Thus, allelopathic impact of *T. minuta* essential oil resulted in overall reduction of growth of test weeds, in general.

Germination and growth bioassays are primary tools for determining phytotoxic activity [23], and may detect potential allelopathic effects under controlled laboratory conditions. In this study, it is clear that *T. minuta* oil exerts allelopathic effect on various weeds. In general, a dose-dependent type of relationship between oil concentrations and germinating parameters of all test plants was apparent, indicating greater inhibition with increase in concentration. Similar observations that essential oil exerts inhibitory effect were also made by several other workers [21-29]. Since essential oils are composed of monoterpenes and/or sesquiterpenes, thus their inhibitory effect may be contributed by all or few major constituents either synergistically or additively. In this study, effect of volatile essential oil of *Tagetes* was species specific. Some weeds were affected to greater extent (*A. tricolor* and *A. viridis*) than others (*E. crus-galli*). The differential susceptibility of different plants may be attributed to their seed size [30] or due to their different genetic constitution. In relevance to observations made in this study, we may infer that *T. minuta* oil inhibits germination and growth of other plants, however, detailed study for determining the mode of action of oil with regards to biochemical, anatomical, and morphological changes needs to be done.

CONCLUSION

T. minuta oil possesses phytotoxicity toward test weeds. Radicle growth was affected more compared to the plumule. The effect of *T. minuta* oil varied from species to species with maximum allelopathic inhibition of *A. viridis* and *A. tricolor*.

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