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Allelopathic Potential of the Essential Oil of Wild Marigold (*Tagetes minuta* L.) Against Some Invasive Weeds

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Abstract: *Tagetes minuta* is an aromatic plant native to Tropical America. It exhibits wide range of biological activity against insects, nematodes, microbes including medicinal properties. It also creates nuisance for agricultural land. This may be attributed to its allelopathic properties. Therefore, the present study investigated the allelopathic potential of volatile oil of *T. minuta* on other invasive weeds - *Chenopodium murale* L., *Phalaris minor* Retz. and *Amaranthus viridis* L. It was observed that the volatile oil of *T. minuta* significantly reduced the germination, growth, chlorophyll content and respiratory ability of recipient weeds in a dose dependent manner. Mitotic studies revealed a complete arrest of mitotic activity in cells of treated root tips of *Allium cepa* with various **aberrations** like distorted, trinucleolated and binucleated cells. Thus, it can be concluded that the volatile oil of *T. minuta* shows allelopathic potential on other plants and this property could be further explored for weed management.

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1. Introduction

Weeds constitute an integral component of many ecosystems. Besides being reservoir of many vulnerable genes, they are a threat to agro-ecosystems as well. Allelopathy offers an important tool for selective biological weed management through production and release of allelochemicals from leaves, flowers, seeds, stems and roots of plants (Weston, 1996). The most promising method to overcome synthetic herbicides is by using environmentally benign natural plant products released from allelopathic plants. These biologically active substances with phytotoxic potential have been searched these days using highly advanced chemical identification procedures (Dayan et al., 2000). Among natural plant products, volatile essential oils constitute the most effective bio-herbicide agents on account of high phytotoxicity (Singh et al., 2003) and quick biodegradation in the environment (Tworkoski, 2002). However, if aromatic plants which are being explored for weed management are weeds (Kong et al., 1999, 2006; Barney et al., 2005 and Batish et al., 2007), this serves to a dual purpose, by controlling weeds using weeds. Among such plants with bioactive oils, one is *Tagetes minuta* (Family Asteraceae).

T. minuta (wild marigold) is a plant native to South America and severely noxious in over 35 countries (Holm et al., 1997). In some parts of East Africa, it has been reported as infesting 10% of maize fields and particularly severe in low-growing crops such as beans (Holm et al., 1997). It has been reported as a contaminant in wool crop in South Africa (Wells et al., 1986). Many workers have reported bioactivity of its essential oil against microbes (Hetheyli et al., 1986; Senatore et al., 2004; Ali et al., 2014; Shirazi et al., 2014), insects and pests (Saxena and Srivastava, 1973 and Kyarimpa et al., 2014) and used in aromatherapy, perfumery industry etc. (Hulina, 2008). *T. minuta* was recently reported as a useful underutilized plant of family Astercaceae (Sadia et al., 2013). Chemical characterization of its volatile components showed presence of 27 compounds that constituted 92% of essential oil of aerial parts (Meshkatalsadat et al., 2010). But in spite of such a rich phytochemistry, very little has been done to explore phytotoxic potential of its oil against weeds. Therefore, present study was undertaken to

assess the phytotoxicity of *T. minuta* oil on common agricultural weeds *Chenopodium murale* L., *Phalaris minor* Retz. and *Amaranthus viridis* L.

2. Material and Methods

T. minuta oil was extracted from aerial parts of the species by hydro-distillation using a clevenger's apparatus. The material was collected from Solan and adjoining places of Himachal Pradesh, India (30°55'0" North, 77°7'0" East). For the present study, seeds of *P. minor*, *A. viridis* and *C. murale* were collected from wildly growing strands in and around Panjab University, Chandigarh, India (30°45'34" North 76°45'59" East).

2.1 Germination and Growth Studies

To test the inhibitory effect of the donor species *T. minuta* on weeds, 15 seeds of each recipient weed (after imbibition) were placed on Whatman no. 1 filter paper moistened with 8 ml of distilled water. Oil was applied in various amounts (0.25, 0.5, 1, 2 and 4 mg 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. Temperature was 25 ± 2°C for *A. viridis* and 10 ± 2°C for *P. minor* and *C. murale*. Relative humidity was 80% and irradiance was 150 µmole m^{-2} sec^{-1}. After seven days germinated seeds were counted and seedling length and dry weight were measured.

2.2 Respiratory Ability

Respiratory ability was determined using 2,3,5-Triphenyl tetrazolium chloride following the method of Steponkus and Lanphear (1967). Fresh and uniform leaf discs (25 mg) from fully expanded leaves of treated and control plants were dipped in 1.5 ml of freshly prepared TTC solution (0.6% TTC along with 1.333% sodium succinate, w/v, prepared in 0.1 M phosphate buffer, pH 7.4). The test tubes were incubated at room temperature (25°C) for 18 h in dark. To extract the red formazan formed during incubation of leaves in TTC, 5 ml of 95% (v/v) ethyl alcohol was added to each test tube and kept in water bath at 60°C for 20 min. Optical density was measured at 530 nm and expressed as percent of untreated control.

2.3 Chlorophyll Content

Chlorophyll was extracted from recipient plant-leaves using dimethyl sulphoxide (DMSO) as per method of Hiscox and Israelstam (1979). 25 mg of fresh leaf tissue from treated and control seedlings was suspended in 4 ml of DMSO and incubated at 60°C for one hour. The extinction value of thus recovered chlorophyll was measured at dual wavelength of 645 and 663 nm. Amount of total chlorophyll was calculated using the equation described by Arnon (1949). The final expression of chlorophyll content was made as µg/mg tissue on dry weight basis, as suggested by Rani and Kohli (1991).

2.4 Mitotic Studies

For studying the effect of the oil on mitotic activity of plants, *Allium cepa* L. was used as a model system since its sensitive and cytological studies are easy to perform, thus giving information about the effects on cell division and chromosomes (Fiskesjö, 1985 and Grant, 1999). Onion bulbs were raised over water for rooting for three days in dark. Roots were treated (while intact) with solution of *T. minuta* oil (0.2% with 0.1 ml of Tween-20 which is equivalent to1.38 mg of *T. minuta* oil) and distilled water (in control) for 24 hours. Roots were excised, fixed and stored in 70% alcohol at 4°C until used for squash preparation, using acetocarmine. Root tip cells were observed under a bright field microscope (Getner, India, model 66475) for various cytological abnormalities such as sticky chromosomes, distorted, binucleate or multinucleolate cells etc.

2.5 Statistical Analysis

Experiment was conducted in a completely randomized design (CRD) with five replicates for each treatment, including control. The experiment was repeated twice and pooled mean data was presented along with standard deviation. The significance of the treatment was tested with respect to control by applying One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test using the statistical package of SPSS version 12. Besides, values of correlation coefficient (r) were also calculated between the parameter and amount of treatment.

3. Results

The volatile oil of *T. minuta* reduced germination in all test weeds (Fig. 1). Maximum reduction was observed in *C. murale* followed by *P. minor* and least in *A. viridis*. The response was concentration dependent. Germination was not affected at all at lower concentrations in *A. viridis*, but in both other weeds (*P. minor* and *C. murale*); correlation was highly negative (r = -0.892 & -0.988, respectively) and significant with increasing dose of treatment.

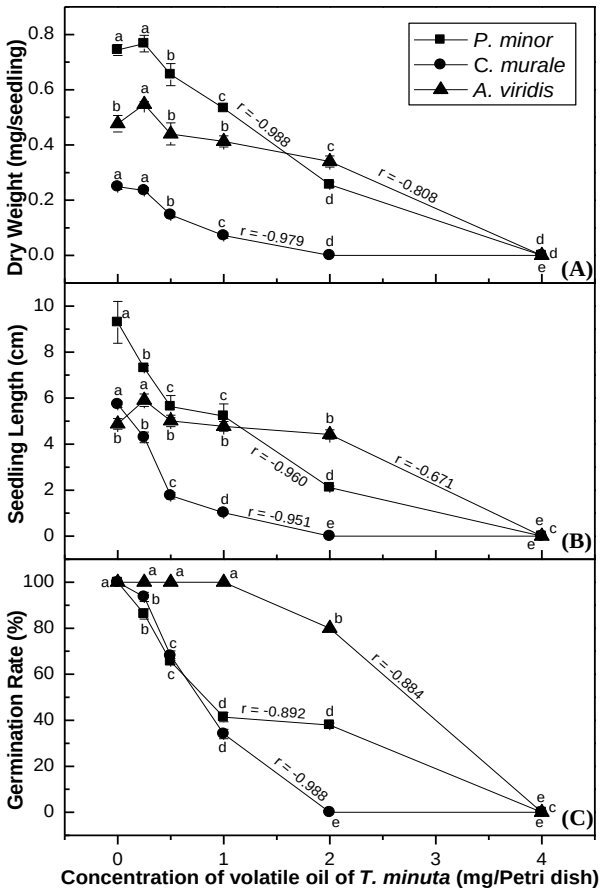

Figure 1. Effect of volatile oil of *T. minuta* on biomass (A), growth (B) and germination (C) of recipient weeds. Vertical Bars around each data point indicate standard deviation. All regressions were significant at $p \leq 0.05$.

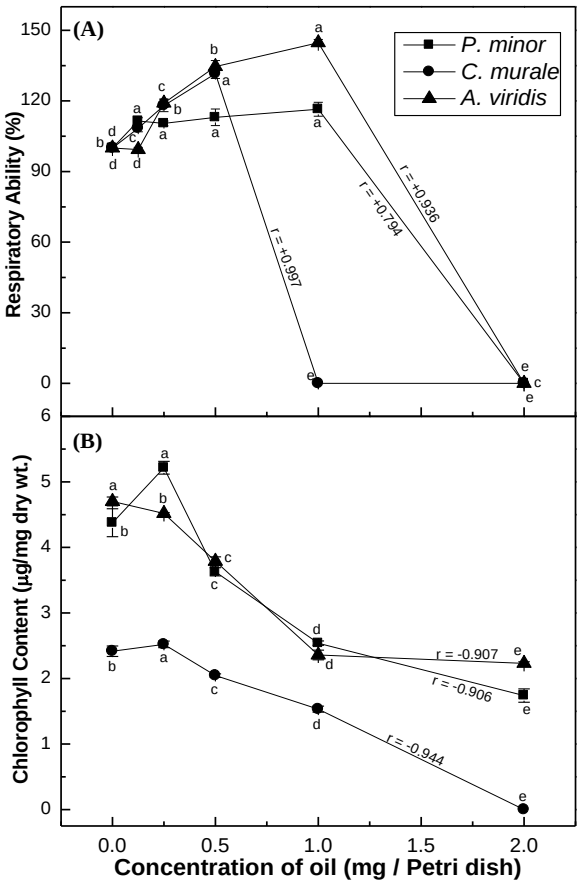

Figure 2. Effect of volatile oil of T. minuta on respiratory ability (A) and chlorophyll content (B) of recipient weeds. Vertical Bars around each data point indicate standard deviation. All regressions were significant at $p \leq 0.05$.

LC_{50} values for *C. murale* and *P. minor* were lower (0.761 and 0.822 mg/petridish respectively) than *A. viridis* (2.745 mg/petridish). Similarly, seedling length also followed this trend with maximum reduction in *C. murale* (Fig. 1). Dry weight decreased with increasing amount of oil except at lower concentrations. Minor enhancement in dry weight was observed in *A. viridis* at lowest concentration, however at higher concentrations, correlation was highly negative. However, respiratory ability enhanced in treated seedlings when compared to control, except for lower concentrations (Fig. 2). The enhancement was significant in *C. murale and A. viridis* but apparent changes in respiratory ability were not observed in treated *P. minor* seedlings. Chlorophyll content also declined in treated seedlings (Fig. 2), with clear symptoms of photo-bleaching in *P. minor*. However, at lower concentrations, chlorophyll content increased significantly in both *P. minor* and *C. murale*.

In mitotic study (Fig. 3), all stages of division viz. prophase, metaphase, anaphase and telophase were present in untreated root cells, whereas, in treated samples, all cells were arrested at interphase. Besides, certain cytological abnormalities occurred like distorted, binucleate cells and trinucleolate nuclei.

4. Discussion

Volatile essential oil of *T. minuta* showed inhibitory effect on seed germination. The degree of inhibition increased with oil concentration, leading to complete inhibition at the highest concentration (4 mg/Petridish). The highest value of LC_{50} in *A. viridis* indicates lesser susceptibility of this weed to *T. minuta* oil when compared to *C. murale* and *P. minor*. This is corroborated by the data of seedling length which was significantly reduced when compared with control except for lower concentrations. Decrease in dry weight of seedling is also in accordance with above results. In mitotic studies, no significant

change in cell size was observed but mitotic activity was found to be completely inhibited, thereby indicating direct effect of the oil on cell division. It has been reported that essential oils and their constituents inhibited cell division in growing root tips and interfered with DNA synthesis in growing meristems (Romagni et al., 2000 and Nishida et al., 2005). Moreover, essential oil imposed plant growth inhibition may be due to disruption of membrane integrity (Tworkoski, 2002 and Singh et al., 2009).

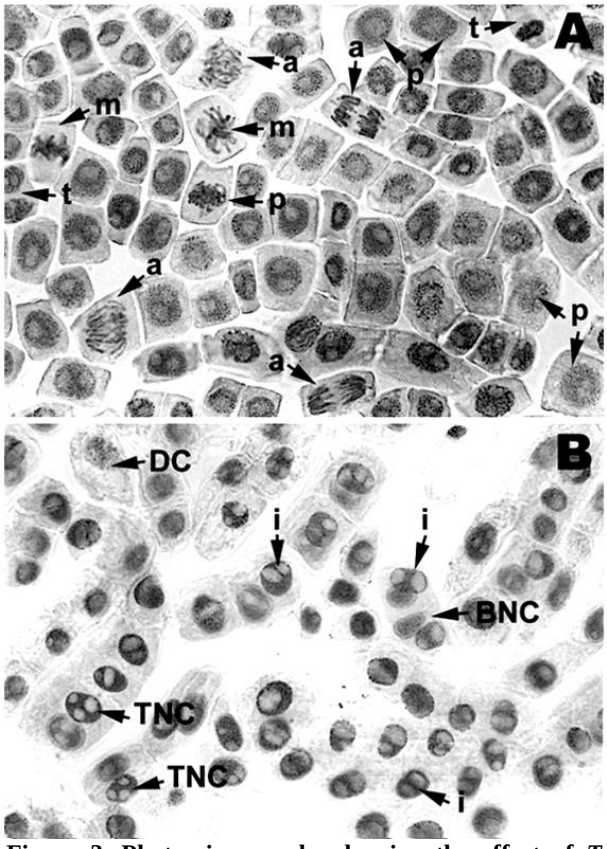

Figure 3. Photomicrographs showing the effect of *T. minuta* essential – concentration of 0.2% in Tween (B) (v/v) on the mitotic activity of onion root tip cells compared to control (A), at 40x. Arrows indicate different stages of mitosis and abnormalities in treated cells: i: interphase, p: prophase, m: metaphase, a: anaphase, t: telophase, TNC: trinucleolated cell, BNC: binucleated cell, DC: distorted cell.

Reduction in chlorophyll content in treated plants may be on account of increased degradation or decreased synthesis of metabolites involved. It is in agreement with earlier reports indicating negative effect of essential oils and monoterpenes on chlorophyll content (Batish et al., 2004; Zhou and Yu, 2006 and Kaur et al., 2011). In addition, enhanced rate of respiration is another qualitative marker that shows phytotoxic influence of the oil on recipient weeds. There are number of reports on increased respiratory activity in oil treated plants due to increased microbial growth or ROS generation (Misra et al., 1996; Vokou et al., 1984; Vokou and Margaris, 1988 and Singh et al., 2006). Though the inhibition of cellular metabolism is a highly complex process yet we may conclude from this study that unfulfilled demand for energy, perturbed cell division and scarcity of cellular photosynthate in treated plants resulted in complete inhibition in recipient weeds.

5. Conclusion

Thus, from the present study, it can be concluded that *T. minuta* oil causes retardation in seed germination and suppresses seedling growth by causing physiological changes that lead to altered chlorophyll content and respiratory ability. *T. minuta* oil therefore, can further be explored for bio-herbicidal potential in sustainable weed management programs. However, further studies in this direction are required. It is because of the fact that natural products need extensive exploration before their recommendation for practical use under natural field conditions for economical and safe practical use.

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Competing Interests

Authors declare that they have no competing interests.

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