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BIOHERBICIDAL POTENTIAL OF ESSENTIAL OIL EXTRACTED FROM LEAVES OF *MELALEUCA LEUCADENDRA* (L.) L. AGAINST SOME WEEDS

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ABSTRACT

The present work was carried out to explore the herbicidal potential of essential oil from the foliage of myrtaceous tree Melaleuca leucadendra (referred to as Melaleuca oil) against three weed species, Amaranthus viridis, Medicago sativa and Chenopodium album. In a laboratory bioassay, the volatile essential oil significantly decelerated the germination and growth of the test weeds and the effect obtained was concentration dependent (0.25–1.5mg/ml). Maximum retardation in the emergence and growth of these weeds was observed at 1.5 mg/ml concentration of oil rich foliage of test plant. Generally, both root and shoot length showed an inhibitory effect in a dose response bioassay. In addition, significant reduction was observed in chlorophyll content and cellular respiration rate of test weeds. At the highest dose of Melaleuca treatment (1.5 mg/ml), the chlorophyll content was reduced to around 84 % and cellular respiration rate was increased by ~ 163 % in C. album. Greatest reduction in chlorophyll content was observed in C. album followed by M. sativa and A. viridis. Thus, it is concluded that volatile essential oil possess weed-suppressing potential and holds a good promise for the future weed management programs.

Keywords: Herbicidal activity, Inhibition, Melaleuca leucadendra, Phytotoxicity, Weeds

I. INTRODUCTION

Weeds are the unwanted plants that grow at non-specific places and spread in a short span of time due to their high reproductive rate [1]. They compete with crops and reduce crop production and crop quality. Declining yield of crops due to weeds is a major problem and cannot be overlooked as they alone cause around 34 % of world's total crop loss [2] and 15–85 % of India's total crop loss [3]. For managing weeds, various control methods, including mechanical, chemical and biological, are applied. Among these, mechanical methods are labor intensive and expensive equipments are required, whereas biological methods are time consuming. Both these methods do not provide adequate management of weeds. Therefore, chemical methods are preferred worldwide as their action is fast and are easy to apply. However, the use of synthetic herbicides has many disadvantages, including environmental pollution, owing to their persistence and slow biodegradation [4]; contamination of soil and ground water [5]; ecological imbalance and biomagnification through food chain

causing harmful effects on animal and human health [6, 7]. Furthermore, the extensive use of synthetic herbicides over the time has resulted in the development of resistant biotypes. Globally, 249 species (144 dicots and 105 monocots) that have acquired resistance towards 160 different herbicides have been reported [8]. Thus, there is a great need to replace the presently used synthetic herbicides with some environmentally safe and eco-friendly chemicals. In this regard, plant-based chemicals can serve as the best option, as these are biodegradable and hence environmentally safe [9]. Additionally, natural chemicals show low mammalian toxicity, possess short half–life and are more economical and affordable [10]. Among the natural products, essential oils and their major components especially monoterpenes and sesquiterpenes are often responsible for their inhibitory activity against weeds. It was reported that essential oil extracted from the aromatic plants have been shown to be very promising and hold good potential as bioherbicides [11, 12, 13, 14, 15, 16, 17, 18, 19]. In view of phytotoxic activity and weed suppressing ability of volatile essential oils and their constituents against weeds, it is thus pertinent to study the aromatic plant to scrutinize their phytotoxic ability with an aim of using them for weed management.

M. leucadendra (family Myrtaceae), commonly called as Cajuput tree, is native to Australia and Southeast Asia. It is commonly cultivated in parks and gardens as an ornamental tree in all parts of world including India. It is moderately fast growing perennial evergreen tree and mainly occurs near rivers and coastal plains [20]. It has bright green foliage and weeping, soft and spongy lamellate bark. The inflorescence is terminal with dense flowers occurring in triads. The genus *Melaleuca* is used for various purposes such as timber, mulch and essential oil as a source of medicine [21]. Volatile essential oil of the plant are used to cure the acne problem [22], dandruff [23] and oral candidiasis [24]. Tea tree oil also possess antitermite [25], antibacterial [26], antiviral [27] and antifungal [28] activities. Researcher reported that bioactive potential of the leaf oil components exhibiting antioxidant, anti-hyaluronidase and antifungal activities [29]. However, studies pertaining to phytotoxicity of *M. leucadendra* essential oil against weeds are largely lacking. We, therefore, selected *M. leucadendra*, being an aromatic plant, for the present study to investigate phytotoxicity of its essential oils for weed management.

II. MATERIAL AND METHODS

2.1 Plant material: *M. leucadendra* is a perennial plant and grows in Chandigarh and its adjacent places. The plant material was collected from the Botanical garden of Panjab University Campus, Chandigarh, India.

2.2 Extraction of oil: Essential oil was extracted from leaves of the plant *M. leucadendra* by steam distillation process through Clevenger's Apparatus. Plant material (1 kg) was separated and mixed with distilled water (2 L) in a round bottom flask fitted with a condenser. The essential oil released from the material was collected from condenser and dried over anhydrous sodium sulphate. It was stored at 4 $^{\circ}$C till further use.

2.3 Collection of seeds: The seeds of the selected test weeds, *Amaranthus viridis* L. (green Amaranth), *Medicago sativa* L. (alfalfa) and *Chenopodium album* L. (lamb's quarters) were collected from the wild places of the Panjab University campus, Chandigarh and from the agricultural fields on the outskirts of Chandigarh. The test weeds were selected as they are economically important, troublesome and commonly found in agriculture fields.

2.4 Seed germination and growth of test plant: Viable and healthy seeds of the test weeds were scarified individually with 2 % sodium hypochlorite for 2 min followed by rinsing with distilled water and finally imbibed in distilled water for 24 h. For their treatment in solution form, various concentrations (0.25–1.5 mg/ml) were prepared by dissolving oil in water with the help of Tween-20. According to literature, petri dishes of 15 cm were lined with two layers of Whatman No. 1 filter circle and was moistened with 10 ml of different oil concentrations [30]. After 7 days of treatment, growth of test weeds was measured in terms of percent germination and seedling growth (root length and shoot length) under laboratory conditions.

2.5 Estimation of Chlorophyll content: Chlorophyll was extracted in Dimethyl Sulphoxide from the leaves of the test plants [31]. Its amount was measured at dual wavelength of 645 and 663 nm on Shimadzu UV–1800 double beam spectrophotometer against DMSO as blank [32], and calculated on dry weight basis [33].

2.6 Estimation of Cellular Respiration Rate: Cellular respiration was determined indirectly from fresh tissue using 2, 3, 5- triphenyl tetrazolium chloride (TTC) [34]. The absorbance value was read at 530 nm on Shimadzu UV–1800 double beam spectrophotometer using ethyl alcohol as blank. It was calculated on dry weight basis and expressed as percent over control [35].

2.7 Statistical Analysis: The statistical analysis of data was done using one-way analysis of variance followed by separation of treatment means from the control by using *post hoc* Tukey's test at $p \leq 0.05$ significance level using software program SPSS (Version 16.0).

III. RESULTS AND DISCUSSION

The phytotoxicity of *M. leucadendra* essential oil was tested on seed germination, seedling growth, chlorophyll content and respiration rate of *A. viridis*, *M. sativa*, *C. album*, important weeds in cultivated areas in agriculture. The germination of all the test weeds was reduced in a concentration-dependent manner. At lower concentration (0.25 mg/ml) of oil, minimum inhibition in germination of test weeds (11 % in *M. sativa*, 12 % in *C. album* and 18 % in *A. viridis*) over control were noticed (Table 1). The reduction was greater with increasing amount of *M. leucadendra* essential oil. At highest concentration (1.5 mg/ml), maximum inhibition was noticed in *M. sativa* (68.14 %) followed by *C. album* (64.91 %) and *A. viridis* (44.92 %) over the control.

Table 1: Effect of *Melaleuca leucadendra* oil on percent germination (%) of test weeds

Concentration (mg/ml)	*Amaranthus viridis*	*Medicago sativa*	*Chenopodium album*
Control	100±1.20a (0)	100±2.45a (0)	100±3.04a (0)
0.25	81.92±1.41b (18.08)	88.23±4.24ab (11.77)	87.72±1.75a (12.28)
0.5	72.03±2.45c (27.96)	78.43±2.45b (21.57)	66.67±1.84b (33.33)
1.0	62.14±1.41d	53.92±6.84c	57.89±3.04b

	(37.85)	(46.08)	(42.1)
1.5	55.08±0.72d (44.92)	31.86±2.45d (68.14)	35.08±6.32c (64.91)

Data presented as mean ± SE.

Different alphabets represent significant difference at $p \leq 0.05$ after applying post hoc Tukey's test.

Values within parenthesis indicate percent decrease over control.

Table 2: Effect of *M. leucadendra* oil on root length (cm) of test weeds

Concentration (mg/ml)	*Amaranthus viridis*	*Medicago sativa*	*Chenopodium album*
Control	4.63±0.15a (0)	4.20±0.66a (0)	7.90±1.15a (0)
0.25	3.86±0.17b (17.73)	3.93±0.84a (6.35)	6.60±1.73b (16.45)
0.5	3.03±0.03c (35.46)	2.67±0.89b (36.51)	5.36±0.45c (32.06)
1.0	2.70±0.12cd (42.55)	2.10±0.57c (50)	4.76±0.67d (39.66)
1.5	2.31±0.06d (51.06)	1.76±0.45c (57.94)	4.21±0.56e (46.83)

Data presented as mean ± SE.

Different alphabets represent significant difference at $p \leq 0.05$ after applying post hoc Tukey's test.

Values within parenthesis indicate percent decrease over control.

Table 3: Effect of *M. leucadendra* oil on shoot length (cm) of test weeds

Concentration (mg/ml)	*Amaranthus viridis*	*Medicago sativa*	*Chenopodium album*
Control	4.20±0.58a (0)	5.53±0.89a (0)	5.31±0.57a (0)
0.25	3.41±0.12b (19.04)	4.53±0.82b (17.57)	4.43±0.12b (16.35)
0.5	2.92±0.57c (30.95)	3.96±1.02c (27.87)	4.03±0.31bc (23.89)
1.0	2.43±0.12d (42.06)	3.36±0.66d (38.78)	3.76±0.89c (28.93)
1.5	2.01±0.06e (52.38)	2.76±0.63e (49.69)	2.46±0.15d (53.45)

Data presented as mean ± SE.

Different alphabets represent significant difference at $p \leq 0.05$ after applying post hoc Tukey's test.

Values within parenthesis indicate percent decrease over control.

Not only emergence, even the seedling growth measured as root and shoot length was significantly reduced. At 0.25 mg/ml *M. leucadendra* essential oil, 6 to 18 % reduction was observed in root length of all the tested weeds over the control (Table 2). Further, the treatment of 1.5 mg/ml, *Melaleuca* oil reduced the root length by ~ 47 %, ~ 51 % and ~ 58 % in *C. album*, *A. viridis*, and *M. sativa*, respectively over the control (Table 2). Likewise, the shoot length of test weeds was significantly reduced in response to *M. leucadendra* essential oil. The shoot growth was further reduced when *Melaleuca* oil concentration increased. In *M. sativa*, shoot length was reduced by nearly 17 %, 28 %, 39 % and 50 % at 0.25, 0.5, 1.0, 1.5 mg/ml respectively over that of control (Table 3). The shoot length in *A. viridis* was also declined significantly upon exposure to different concentration of oil. It decreased by nearly 19 %, 31 %, 42 % and 52 % at 0.25, 0.5, 1.0, 1.5 mg/ml respectively over that of control (Table 3). In case of *C. album*, shoot length declined significantly by 16 %, 24 %, 29 % and 54 % at 0.25, 0.5, 1.0, 1.5 mg/ml respectively over that in the control (Table 3).

The observations made in the present study are in agreement to earlier studies reporting inhibitory effect of volatile oils from aromatic plants on germination and growth of weeds [36, 37, 38]. Recently, Grichi *et al*. [19] studied the phytotoxic effect of essential oil against weeds, and found complete inhibition of germination in *Sinapsis arvensis* and *Diplotaxis harra* at 1 µl/ml. A similar preferential inhibition in the root growth has been studied in earlier reports because of inhibition in cell proliferation in the root apical meristem or lowered root mitotic activity [39, 40]. The effect might be attributed either to high percent of main constituent or to synergy among different oil constituents.

Table 4: Effect of *M. leucadendra* oil on Chlorophyll content (µg/mg D.W.) of test weeds

Concentration (mg/ml)	***Amaranthus viridis***	***Medicago sativa***	***Chenopodium album***
Control	15.80±0.41a (0)	5.20±0.07a (0)	18.15±0.14a (0)
0.25	10.93±0.17b (30.80)	3.97±0.07b (23.49)	12.25±0.11b (32.52)
0.5	6.96±0.09c (49.28)	2.53±0.05c (51.37)	6.32±0.06c (65.20)
1.0	4.75±0.08d (69.91)	1.61±0.03d (69.10)	3.90±0.02d (78.51)
1.5	3.38±0.06e (78.58)	0.99±0.02e (80.80)	2.91±0.05e (83.98)

Data presented as mean ± SE.

Different alphabets represent significant difference at $p \leq 0.05$ after applying post hoc Tukey's test.

Values within parenthesis indicate percent decrease over control.

Further, the decline in total chlorophyll content was observed in all the test weeds in a dose-dependent manner. At highest concentration of 1.5 mg/ml of *Melaleuca* oil, the chlorophyll content was decreased by 78 %, 80 % and 84 % over the control in *A. viridis*, *M. sativa* and *C. album* respectively (Table 4). These observations are in

agreement with earlier studies reporting that volatile oils reduce chlorophyll content and consequently interferes with photosynthetic activity of the plants [30, 41, 42]. Although the exact cause of decrease in the total chlorophyll is not known, it could be either due to inhibition of chlorophyll synthesis or enhanced chlorophyll degradation or both [43, 44].

However, the cellular respiration was found to increase with an increase in concentration of oil. A significant enhancement in respiration was observed at 1.5 mg/ml concentration of oil in case of *A. viridis*, *C. album* and *M. sativa*. The increase was measured to be nearly 49 %, 60 % and 162 % in case of *A. viridis*, *M. sativa* and *C. album* compared to control (Table 5). Among the weeds, maximum increase was noticed in case of *C. album* followed by *M. sativa* and *A. viridis*. Such an observation is supported by a study demonstrating an increase in cellular respiration in response to *β*–pinene, possibly to meet the increased demand of energy by the plant in case of oxidative stress to meet the conditions for its survival [45].

Table 5: Effect of *M. leucadendra* oil on cellular respiration (%) of test weeds

Concentration (mg/ml)	***Amaranthus viridis***	***Medicago sativa***	***Chenopodium album***
Control	100±1.17a (0)	100±1.34a (0)	100±0.28a (0)
0.25	103.40±1.44a (3.40)	103.79±5.55a (3.79)	154.58±4.38b (54.58)
0.5	115.19±1.63b (15.19)	128.37±1.03b (28.37)	186.67±5.94c (86.67)
1.0	131.39±3.94c (31.39)	148.89±4.47c (48.89)	238.25±3.25d (138.25)
1.5	148.98±2.12d (48.98)	160.47±3.06c (60.47)	262.50±5.05d (162.50)

Data presented as mean ± SE.

Different alphabets represent significant difference at $p \leq 0.05$ after applying post hoc Tukey's test.

Values within parenthesis indicate percent increase over control

IV. CONCLUSION

The present study concludes that *Melaleuca* oil showed significant herbicidal and phytotoxic effects against all the test weeds and can be used as potential bio-herbicide in agro-ecosystem. Being biodegradable and ecofriendly, *Melaleuca* oil seems promising in future to be used in weed management programs.

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