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Phytotoxic Effects of Essential Oil from *Eucalyptus lehmanii* against Weeds and its Possible Use as a Bioherbicide

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ABSTRACT
A study was undertaken to assess the bioherbicidal activity of essential oil extracted from Eucalyptus lehmanii against five weeds species, Sinapis arvensis, Diplotaxis harra, Trifolium campestre, Desmazeria rigida and Phalaris canariensis. Emergence and seedling growth were significantly reduced in a dose-response bioassay conducted in Petri dishes on Whatman filter paper impregnated with eucalypt oil. Generally, the shoot length was inhibited more as compared to the root length and the inhibitory effect was greatest in S.arvensis followed by D.harra and least in T.campestre. Post-emergence application of eucalypt oil on 4-week-old weed plants caused visible injury (1and 3days after spray) ranging from chlorosis to necrosis to complete wilting of plants. Plants sprayed with higher concentrations of eucalypt oils were desiccated and wilted in appearance. In addition, a significant reduction was observed in chlorophyll content in leaves of weeds sprayed with eucalypt oil. Among the sprayed test weeds, the greatest reduction in chlorophyll content was observed with S.arvensis followed by D.harra and T.campestre. It is concluded that volatile essential oils from E.lehmanii possess weed-suppressing ability and could be used as a bioherbicide for the future weed management programs.
***Keywords**: Eucalyptus lehmanii, Growth inhibition, Visible injury, Chlorophyll content, Bioherbicide.*

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INTRODUCTION

Weeds invasion in agricultural field's results in enormous economic losses and low quality crop yields [1]. Globally, a huge amount of money is spent every year to control them. Whereas control of weeds can be achieved through different means as chemical, mechanical, biological and cultural, the use of synthetic herbicides is widespread and provides an efficacious process. Sadly, the use of synthetic herbicides may damage the environment and human health, and is also leading to enhancing herbicidal resistance among many weed species. Therefore, efforts to develop alternative means of weed control, which are eco-friendly, cost effective and bio-efficacious are needed [2]. In this way, efforts to use natural plant products for the effective weed management are being made [3-4-5]. Natural products are biodegradable and possess novel molecular target sites different from synthetic herbicides. Volatile essential oils, natural plant products, and their constituents have attracted much attention owing to their phytotoxicity, their allelopathic property and relatively quicker degradation in the environment [5-6-7-8-9]. Terpenoids, especially monoterpenes and sesquiterpenes, are the major essential oils components and are often responsible for their inhibitory activity. Besides, it was reported lesser vegetation surrounding purple sage (*Salvia leucophylla* Greene), owing to the presence of volatile compounds such as terpenes [10]. It was reported that the essential oil from *Eucalyptus tereticornis* inhibits the seedlings growth of lentil *(Lens culinaris Medik.*). The essential oils from *Eucalyptus globulus* inhibit the growth of mung bean (*Phaseolus aureus L.*), barley (*Hordeum vulgare L.*) and oat (*Avena sativa L.*) [6]. Also, it was demonstrated that the volatile oil from *E.citriodora* and Tasmanian blue gum inhibits the germination and seedling growth of ragweed parthenium (*Parthenium hysterophorus L.*). Thus, these could be used for weed management [11]. The present study was conducted to explore the phytotoxicity of *Eucalyptus lehmanii* essential oil against some weed species with a view to exploit them for the future weed management.

MATERIAL AND METHOD

Plant material : Leaves were collected in April 2014 from E.lehmanii trees acclimated in Korbous arboreta (located in Nabeul, northeast of Tunisia, with a sub-humid bioclimatic stage). The E.lehmanii essential oils were extracted by hydrodistillation of 100g of dried leaves for 4h according to the standard method described in the European Pharmacopoeia. Hydrodistillations were performed in triplicate. The yield in essential oil was expressed in % (v/w) of the dry material.

Chemical characterization of the oil: The chemical composition of the extracted essential oil was determined by gas chromatography-mass spectroscopy (GC-MS).

GC Analysis: GC Analysis was carried out with a Hewlett-Packard 6890 apparatus equipped with FID and an intermediately polar Supelco SPB-20 cap. Column (30m×0.32 mm i.d., film thikness 0.25 µm).The oven temp. was programmed isothermal at 35°C for 1 min, rising from 35 to 250°C at 5°/min, and then held isothermal at 250°C for 3 min; injector temp., 250°C; detector temp., 280°C; carrier gas, N2 (1.2 ml/min). The injected volume was 1 µl (10% essential oil in purified hexane). The relative concentration was determined using the software HP Chemstation, which allowed assimilating the percentages of the different compounds. Retention indices (RI) were determined according to the retention times (tR) of a series of n-alkanes (C9-C28).

GC/MS Analysis: The essential oils were analyzed with a Hewlett-Packard 5890 series II apparatus equipped with a 5972 mass-selective detector and an intermediately polar Supelco SPB-20 cap. Column (30m*0.32mm i.d., film thickness 0.25 µm). He was used as the carrier gas. The operating conditions of the mass spectrometer were: ionization voltage, 70 eV; ion source, 230°C. The GC anal. Conditions were as described in GC Analysis [12].

Compound Identification: The identification of the compounds was based on the comparison of their RI and mass spectra with those of principal constituents by means of the NBS75K.L. and Wiley 275 databases and with literature data [13].

Dose-response studies

Seeds of all test species: *Sinapis arvensis, Diplotaxis harra, Trifolium campestre, Desmazeria rigida and Phalaris canariensis* were collected locally from agricultural fields on Ousseltia (located in Kairouan, centreast of Tunisia, with arid bioclimatic stage). These were surface-sterilized with sodium hypochlorite (0.1%, w/v) for 2 min, washed under running tap water (for 5min) followed by distilled water and stored for further use. Dose-response studies were conducted under laboratory conditions to determine the effect of eucalypt oil on growth of test weeds. Briefly, 10 seeds of all these test plants were germinated in Petri dishes (15cm diameter) on a filter paper wetted with 7ml of distilled water. To test the inhibitory effect of eucalypt oil, different amounts of oil were loaded on the inner side of cover of Petri dish (0.25, 0.5, 0.75 and 1µl/ml) after spacing the seeds on the base and then sealed immediately with tape. Control was kept without loading essential oil. For each concentration, five replicates were maintained. All the Petri dishes were kept in a growth chamber maintained at 16/18h light/dark period at 25±2°C temperature. After 7 days, the number of seeds that germinated was counted, and their root and shoot lengths were measured. The percents of inhibition of germination, root and shoot lengths were calculated from the following equation: Inhibition (% of control) = (100-(sample extracts/control) ×100) [14].

Field study

To test the herbicidal potential of the essential oil from *E.lehmanii* under field conditions, experiments were conducted in the greenhouse. Seeds of all test species were sown manually in 15cm pots. For this, 1200g of garden soil was taken in each pot and five seeds of *S.arvensis, D.harra, T. campestre, D.rigida* and *P.canariensis* were sown in each pot. Pots were placed in experimental house with natural light conditions (Temperature 21°C, Humidity 32%, Sunshine7hj-1) and irrigated daily. When the plants were 4-week-old, they were spray treated with 25, 50, 75 and 100µl/ml solution of eucalypt oil (or distilled water to serve as control) in such a manner that each plant received 6ml of treatment solution. For each treatment five replications were maintained. One- and 3-days after spray (DAS), the treated test weed plants were examined for visible injury levels in terms of percent chlorotic and necrotic areas.

Estimation of chlorophyll content: Chlorophyll content was measured using a chlorophyll content meter CCM-200 (SPAD). The chlorophyll content meter provides instantaneous measurements which can be done in the field under normal conditions. All obtained data can be downloaded to a computer for additional analyses using the software and data cable of the CCM-200.

Statistical analyses: All data obtained from seed germination, seedling growth, visible injury and chlorophyll content assays of test species were expressed as mean values and were, on the condition of significant ANOVA, analyzed by means of multiple comparison SNK tests in order to investigate if significant differences existed between eucalypt oil concentrations and test species. Values of $p \leq 0.05$ were considered significantly different.

RESULT AND DISCUSSION

Chemical characterization of the *E.lehmanii* essential oil

The essential oil was obtained by boiling hydrodistillation of *E.lehmanii* leaves, which gave an oil of 3.2% yield. The chromatogram obtained by gas chromatography coupled with mass spectrometry indicated that *E.lehmanii* essential oil shared a high proportion of the 1,8-Cineole (58.3%) and a relatively high mean percentage of α-Pinene (17.3%).The α-Thujene represent (15.5%) of the total *E.lehmanii* essential oil (Table.1). The presence of 1,8-cineole as the major compound in *E.lehmanii*, α-pinene and α-Thujene representing high proportion is in agreement with earlier studies[12].

Growth studies under laboratory conditions

The phytotoxicity of *E.lehmanii* essential oil was evaluated on germination, seedling growth and chlorophyll content of weeds by different assays. The germination of all the test weeds was significantly reduced. In general, a dose-response relationship was observed and the emergence was reduced with the increasing in amount of *E.lehmanii* essential oil. At 0.25µl/ml *E.lehmanii* essential oil, there was no significant effect on germination of test species, except in *S.arvensis* and *D.harra,* where 100% decrease was observed (Fig.1). However, at 1µl/ml *E.lehmanii* essential oil, 0% emergence was observed in *P.canariensis* and *D.rigida* and 6.25% in *T.campestre* (Fig.1a).

Not only emergence, even the seedling growth measured as root and shoot length was significantly reduced except at 0.25µl/ml *E.lehmanii* essential oil. At 0.5µl/ml *E.lehmanii* essential oil6 to 93 % reduction was observed in root length of tested weeds. The reduction was greater with increasing amount of *E.lehmanii* essential oil (Fig.1b et c). At highest concentration (1µl/ml), the maximum inhibition in root length was observed in *P.canariensis* and *D.rigida* (Fig.1b).Likewise, the shoot length of test weeds was significantly reduced in response to *E.lehmanii* essential oil, but with varying degrees of susceptibility. Also, the shoot growth was further reduced when eucalypt oil concentration increased. In general, the inhibitory effect was greater on shoot growth than on root growth (Fig.1b, c).

The observations made in the present study are parallel to earlier studies documenting the growth inhibitory activity of aromatic plants, including *Eucalyptus* species and their essential oils. For example, volatile oil (0.12-0.30 mg/ml) from *Eucalyptus citriodora* reduced seedling growth and dry weight accumulation in *Cassia occidentalis, Amaranthus viridis* and *Echinochloa crus-galli* by ≥ 50% [15]. It was demonstrated that essential oils from *Rosmarinus officinalis, Thymus vulgaris* and *Satureja montana* (at 500ppm) severely reduced germination and seedling growth of weedy species such as *Chenopodium album, Portulaca oleracea* and *E.crus-galli* [16].

TABLE 1: CHEMICAL COMPOSITION OF THE ESSENTIAL OILS FROM *E.LEHMANII*

N°	Compounds	RI	Area (%)	Identification
1	α-Thujene	922	15.5	RI, MS
2	α-Pinene	930	17.3	RI, MS
3	β-Pinene	975	0.1	RI, MS
4	β-Myrcene	980	0.2	RI, MS
5	Camphene	1011	0.3	RI, MS
6	ρ-Cymene	1015	3.4	RI, MS
7	γ-terpinenene	1069	0.5	RI, MS
8	para-Cymenene	1071	1.5	RI, MS
9	α-Terpinolene	1089	0.4	RI, MS
10	Pinocarvone	1122	0.1	RI, MS
11	Neryloxide	1137	Tr	RI, MS
12	Borneol	1150	1.1	RI, MS
13	Terpinene-4-ol	1163	0.1	RI, MS
14	α-Terpineol	1176	1.3	RI, MS
15	Fenchylacetate	1203	tr	RI, MS
16	*trans*-Carveol	1221	0.3	RI, MS
17	Carvarol	1228	0.3	RI, MS
18	Linalylacetate	1240	tr	RI, MS
19	Carvacrol	1279	0.2	RI, MS
20	1,8-Cineole	1282	58.3	RI, MS
21	Aromadendrene	1434	0.1	RI, MS
22	α-Humulene	1468	0.8	RI, MS
23	Calamenene	1502	0.2	RI, MS
24	δ-Cadinene	1517	0.1	RI, MS
25	β-Eudesmol	1362	0.2	RI, MS
26	Viridiflorol	1475	0.1	RI, MS

27	Palustrol	1562	tr	RI, MS
28	Caryophylleneoxide	1575	tr	RI, MS
29	Ledol	1585	0.1	RI, MS
30	(z)-2-heptenal	926	0.2	RI, MS
31	1-Octen-3-ol	959	0.1	RI, MS
32	Decane	1000	0.1	RI, MS
33	Nonanal	1081	0.1	RI, MS
34	2-phenylethanol	1119	0.1	RI, MS
35	Decanal	1182	Tr	RI, MS
36	Octylacetate	1191	Tr	RI, MS
37	Decanol	1253	Tr	RI, MS
38	Tricosene	2300	0.1	RI, MS
Yield (%)			3.2	
Total identified (%)			99.9	
Monoterpene hydrocarbons			36.2	
Oxygenated monoterpenes			61.4	
Sesquiterpene hydrocarbons			1.2	
Oxygenated sesquiterpenes			0.4	
Aliphatics compound			0.7	

R.I: Retention Index; MS: mass spectrometry; Tr: Trace (<0.1%)

Later, it was reported that eucalypt oil (at 0.2-5.0 nl/ml) reduced seed germination and seedling growth of *P.hysterophorus* by 56-100% [17]. The volatile oil from *Tagetes minuta* (at 100-1000ppm) was demonstrated that inhibited the emergence of weed species such as *Taraxacum officinale, Mikania cordifolia* and *Cynodon dactylon* [18]. Recently, it was reported that volatile oil from *Artemisia scoparia* (at 0.14-0.35mg/ml) inhibited radical emergence and seedling growth in *Cyperus rotundus* and *Phalaris minor* [19]. The growth inhibition observed on test plants may either be due to interactive effect of compounds in *E.lehmanii* oil. Allelopathy is the result of the simultaneous action of many compounds and often including compounds whose chemistry is different [20]. This ecological phenomenon is considered to be the major cause of dominance and successful colonization of a particular exotic species in invaded plant community [21-22].

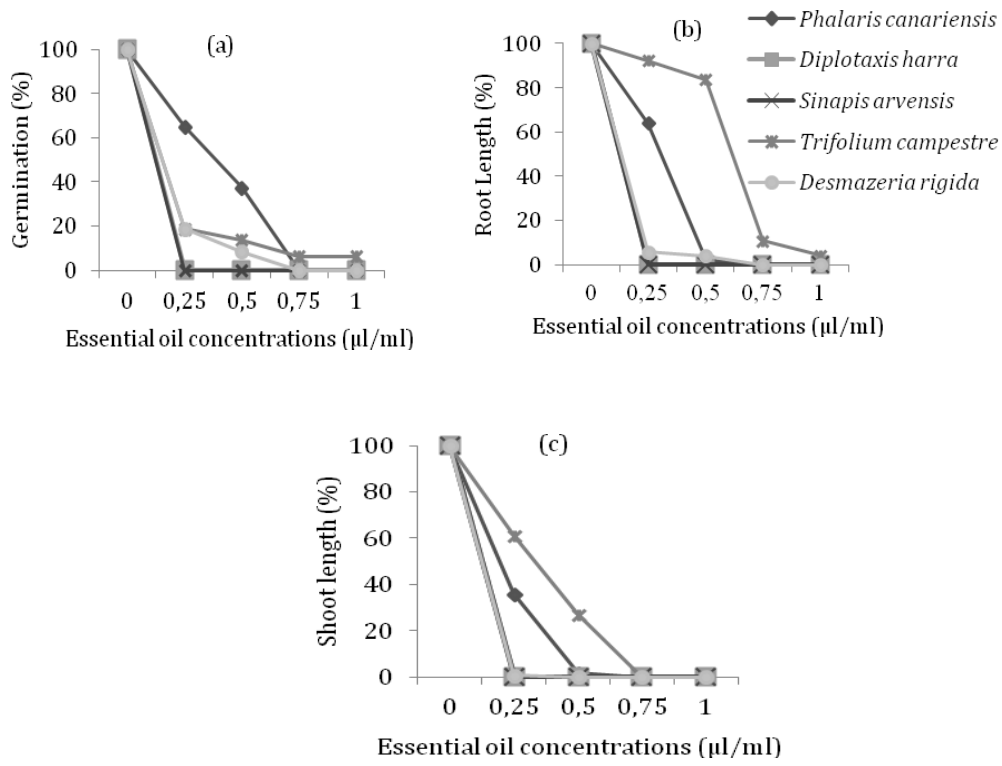

FIGURE1. THE EFFECT OF *E.LEHMANII* ESSENTIAL OIL ON GERMINATION PERCENT (a), ROOT (b) AND SHOOT LENGTH (c) OF TEST SPECIES MEASURED 7 DAYS AFTER SPRAY (7 DAS).

Eucalypt oil induces visible injury

The mature plants of test species were gravely damaged upon treatment with *E.lehmanii* oil and showed visible injury ranging from chlorosis to necrosis to even complete wilting of plants. In general, the visible injury symptoms observed 1-and 3-DAS treatment increased with increasing concentrations of eucalypt oil (Fig.2). The allelopathic effects of *E.lehmanii* oil further increased with time and weed plants were unable to recover. At 25 µl/ml *E.lehmanii* oil, the test weeds except *S.arvensis* did not show any sign of injury. However, in response to higher concentrations of *E.lehmanii* oil (≥50µl/ml), weed plants 1-DAS showed visible symptoms like chlorosis, necrosis, wilting and drying of leaves (Fig.2). On the other hand, 3-DAS with 100µl/ml eucalypt oil, 40-90% injury was observed in *P.canariensis, D.harra, T.campestre* and *D.rigida*. In *S.arvensis,* a complete death of plants was observed 3-DAS upon treatment with 100µl/ml.

These observations imply that *E.lehmanii* oil like other herbicides induces severe injuries in plants upon contact. Such observations are parallel to previously studies demonstrating that volatile oils and even their monoterpenes exhibit herbicidal activity [9]; [17]; [23]. It was reported that essential oils of *Satureja hortensis, T.vulgaris, Syzygium aromaticum* and *Cinnamomum zeylanicum* at 5 and 10% (v/v) sprayed on 12-week-old plants of *C.album*, *Ambrosia artemisifolia* and *Sorghum halepense* caused severe visible injury leading to plant death within 24h [9]. Likewise, it was noted death of 4-week-old plants of *P.hysterophorus* spayed with 75 and 100µl/ml *E.citriodora* volatile oil [17]. Finally, it was demonstrated that 5% essential oil from *E.citriodora* caused 50-80%visible injury in *C.occidentalis, A.viridis, P.minor* and *E.crus-galli* [15]; [23].

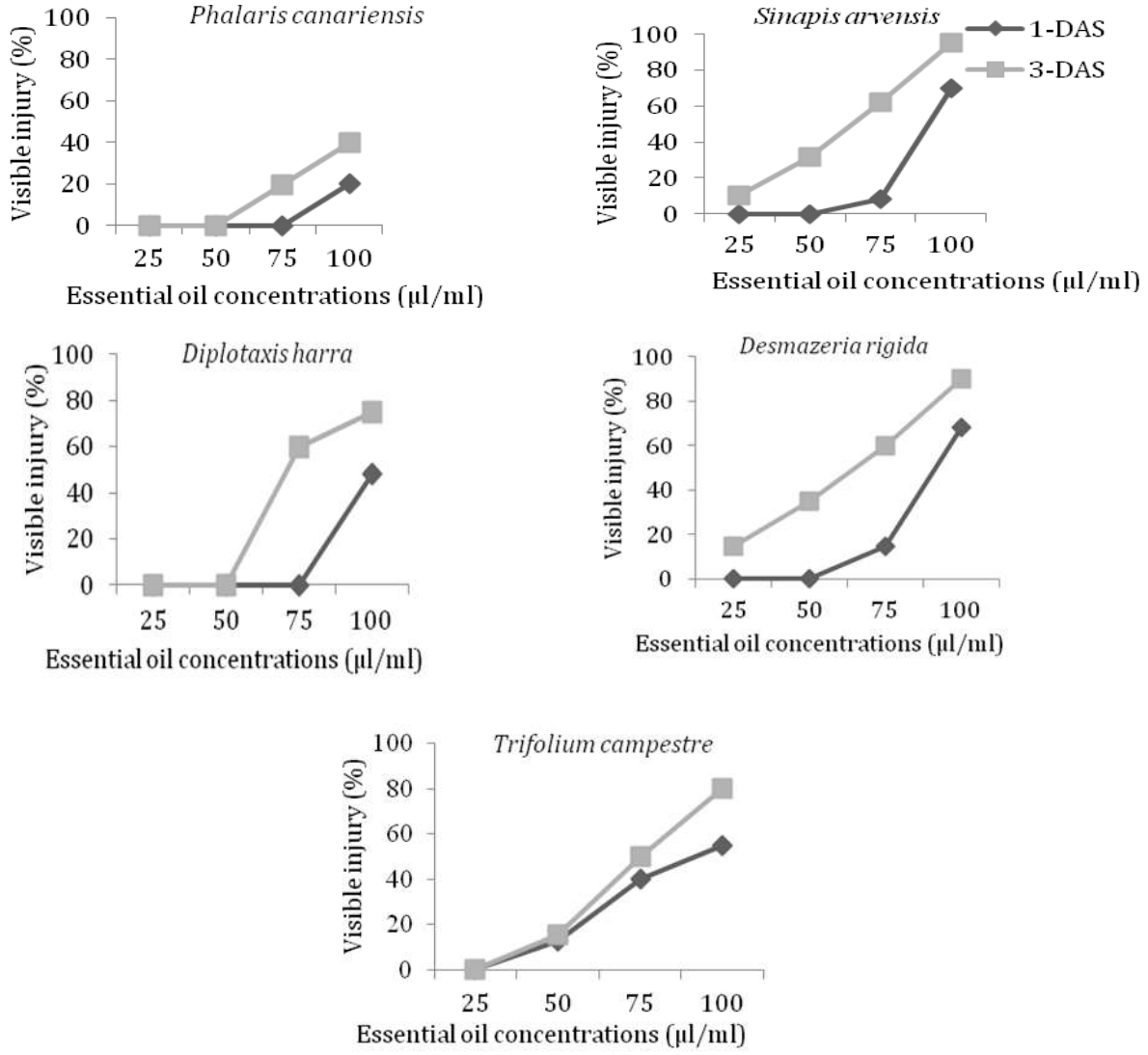

FIGURE2. EFFECT OF E.LEHMANII ESSENTIAL OIL ON VISIBLE INJURY LEVELS IN 4-WEEK-OLD PLANTS OF TEST SPECIES MEASURED 1-AND 3-DAYS AFTER SPRAY (1-DAS. 3-DAS).

Eucalypt oil affects the chlorophyll content

A significant reduction was observed in chlorophyll content in leaves of weed plants sprayed with *E.lehmanii* oil. Upon treatment with 25µl/ml, 1-DAS, the chlorophyll content was reduced for all species test. The chlorophyll content declined upon spray with higher concentrations of eucalypt oil (Fig.3). The greatest inhibition in chlorophyll content was observed in *S.arvensis* 1-DAS with 100µl/ml eucalypt oil. On the contrary, in other weed plants, the reduction in chlorophyll content was greatest 3-DAS. In response to 100µl/ml eucalypt oil, 3-DAS, the inhibition in chlorophyll content was greatest in *S.arvensis* (95%) followed by *D.harra* (88%), *T.campestre* (87%), *D.rigida* (78%) and *P.canariensis* (50%) (Fig.3). The observed loss in chlorophyll content is in agreement to earlier studies reporting that volatile oils reduce chlorophyll content and consequently interferes with photosynthetic activity of the plants [24]; [15]. The yellowing of weed leaves upon eucalypt oil spray may be the secondary effect due to decrease in chlorophyll content.

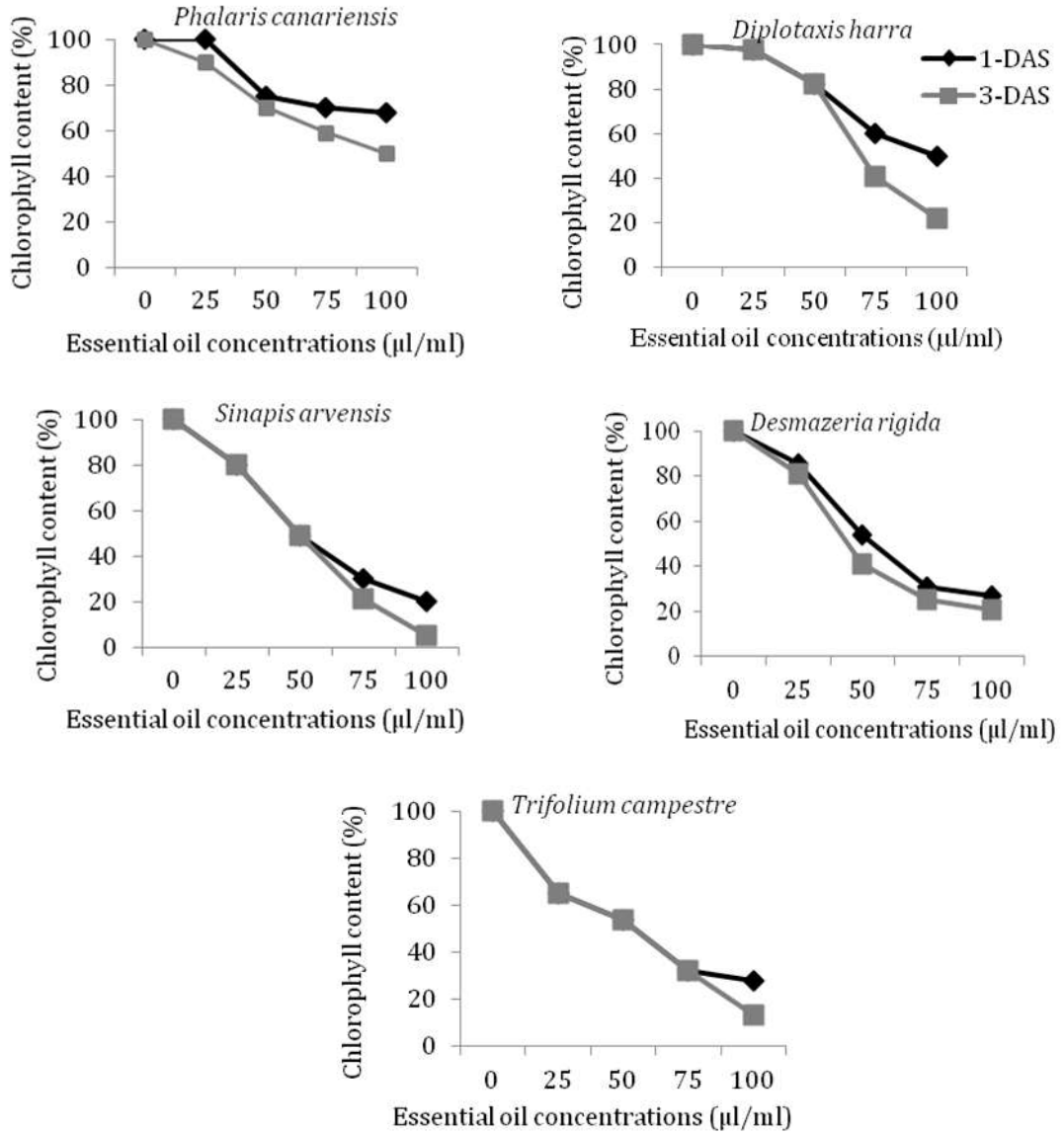

FIGURE3. EFFECT OF E.LEHMANII ESSENTIAL OIL ON CHLOROPHYLL CONTENT IN 4-WEEK-OLD PLANTS OF TEST SPECIES MEASURED 1-AND 3-DAYS AFTER SPRZY (1-DAS; 3-DAS).

CONCLUSION

From the present study, it could be concluded that eucalypt oil exhibits strong phytotoxicity activity against weeds. Therefore, it could be useful for developing natural herbicides. Furthermore, additional work is required to determine the mode of herbicidal action, determine the effect on non-target species and investigate formulations that may increase essential oil efficacy.

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CONFLICT OF INTEREST
None.

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