



Original Contribution

**ALLELOPATHIC EFFECT OF *EUCALYPTUS GLOBULUS*' ESSENTIAL OIL
ON SEED GERMINATION AND SEEDLING ESTABLISHMENT OF
AMARANTHUS BLITOIDES AND *CYNODON DACTYLON***

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ABSTRACT

To understand the allelopathic effect of essential oil extracted from leaves of *Eucalyptus globulus* against two weeds, the experiments were laid out as a randomized complete design with factorial layout in two stages (laboratory and greenhouse) in khoy-Iran, during growing season of 2010. Studied factors were Eucalyptus essential oil concentrations (0.5, 2, 3.5 and 5 nl/ml—in laboratory and 0.25, 0.5, 0.75 and 1 v/v—in greenhouse) and weeds which included '*Amaranthus blitoides*' and '*Cynodon dactylon*'. Results indicated that, with increasing of eucalypt oil concentration under laboratory and greenhouse conditions, Germination percentage, Germination rate, Radicle length, Plumule length, Seedling height, Primary root length and Primary pedicle length, significantly decreased. Among the weed species tested, the highest inhibitory effect of essential oil was observed in '*A. Blitoides*'. Under laboratory condition, Eucalypt oil concentration decreased seedling height in '*A. Blitoides*' from 61.65 to 37.92 mm, whereas in pot study, eucalypt oil greatly suppressed the seedling height of '*A. Blitoides*' from 34.67 to 22.17 mm. Findings suggest that Eucalypt volatile essential oil possess strong inhibitory potential against weeds that could be exploited for weed management and can be recommended as a biological herbicide with the aim of weeds control.

Key words: Allelopathy; biological herbicide; eucalypt essential oil; weed.

INTRODUCTION

Increasing population and developing demand for agricultural products provoke scientists to think more about efficient methods to increase the efficiency of agricultural plants. Subsequently, one of these methods is to control weeds. The interference of weeds in the agricultural fields reduces the quality and the quantity of the agricultural product and causes huge economic loss to farms. Weeds can be controlled through several means such as mechanical, chemical and biological methods

(1). However, chemical methods involving the use of synthetic herbicides widely used as global herbicide, reached to 28 billion US dollars in 2002; in other words, 47% of the total agrochemicals use in the World (2, 3). America reported 15 million dollars annual weeds damage (4). After being discovered in 1950s, herbicides were used extensively. Unfortunately, their indiscriminate use during the last three decades has resulted in various toxicological effects on the environment and living organisms including human. Furthermore, there are reports of fast evolving Weed biotypes with herbicidal resistance (2). These problems promoted efforts to product new herbicides with environmental coexistence and desirable herbicide action (5, 6). Thus, a large number of natural products are being tested as possible bioherbicides. Among

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the natural plant products, volatile Essential oil as one of the main medicinal plant and the constituents of aromatic plants, are being examined for production of biologic herbicides (7, 8). Essential oil neither stays long in soil nor penetrates underground water and never causes environmental poisoning (9). Formerly, vegetable oils, which assimilate fatty acids in structure, have been used as commercial herbicide (10). Essential oil can be used to control weeds in organic farming systems (11). Effect of *Eucalyptus globulus*' essential oil on *Triticum aestivum*, *Zea mays*, *Raphanus sativus*, *Cassia occidentalis*, *Amaranthus viridis* and *Echinochloa crus-galli* have been proved as their radicle length, plumule length, germination percentage and germination rate decreased under the effect of essential oil concentration. At pot condition, the chlorophyll content and respiratory activity of these weeds also decreased under the effect of eucalypt oil concentration and the leaves are necrotized (12). *Amaranthus blitoides* and *Cynodon dactylon* are dominant weeds in Iran and most part of the world (13). *Eucalyptus globulus* is a long and ever green tree from Myrtaceae family. Its height ranges between 45 to 65 meters and has at least 1% (volume/weight) essential oil (14). The *Eucalyptus globulus*' essential oil extracted from its leaves by codistillation method (15). Milhau et al. (16) recognized 17 major compounds in *Eucalyptus globulus*' essential oil in analyzing by gas chromatography (GC-MS). Among these compounds, 1,8-cineole (78.9%), *p*-cymene (7.4%), α -pinene (3.4%) and trans-pinocarveole (9.3%) had the highest rate respectively (17). To identify an herbicide, with natural structure and environment compatibility, *Eucalyptus globulus*' essential oil was examined to produce biological herbicide. Therefore, the present investigation was undertaken to assess the phytotoxicity of eucalypt oil against two kinds of weeds with a view to explore them as a bioherbicide for weed management. Finally, this research can be used to increase the quality and quantity of agricultural products especially by decreasing the toxicological effects on the environmental and living organisms including human.

MATERIALS AND METHODS

The study was carried out at Islamic Azad university of Khoy (4 kilometer away from khoy) in 2010 cropping season. The volatile

essential oil from the leaves of *Eucalyptus globulus* were extracted by co-distillation method with water at 95 to 100 °C for 2 hours (2, 15). The experiment was conducted in laboratory (petri dish) and greenhouse (pot) conditions. The *Eucalyptus globulus*' essential oil effects on Germination percentage, Germination rate, Radicle length, Plumule length and Seedling height were determined in laboratory. In Both laboratory and greenhouse stages, the applied design was based on a completely randomized design by a factorial arrangement of 5×4×2 with four replications. In laboratory condition, the essence concentration consisted of 5 levels including: $a_1=0$ nl/ml, $a_2=0.5$ nl/ml, $a_3=2$ nl/ml, $a_4=3.5$ nl/ml and $a_5=5$ nl/ml (2). whereas, for greenhouse the eucalypt oil concentration levels were: $a_1=0$ v/v, $a_2=0.25$ v/v, $a_3=0.5$ v/v, $a_4=0.75$ v/v and $a_5=1$ v/v (18). In this study two kinds of weeds ($b_1=$ *Amaranthus blitoides* and $b_2=$ *Cynodon dactylon*), were investigated until seedling establishment. Some of the morphologic characteristics such as Germination percentage, Germination rate, Radicle length, Plumule length and Seedling height in laboratory condition and Primary root length, Primary pedicle length and Seedling height in greenhouse were evaluated.

The eucalypt oil concentration was estimated by hydro distillation of fresh leaves using Clevanger's apparatus, in the other hand; 500 g of the fresh leaves of eucalypt (*Eucalyptus globulus*.) trees were chopped and mixed with 400 ml distilled water in a 2 liter round bottom flask and fitted with condenser. The mixture was boiled in the distillation apparatus. After collecting 350 ml of distilled eucalypt oil from the nozzle of the condenser, distillation process was stopped, and then the resulted essential oil was dehydrated over anhydrous sodium sulfate and stored in a dark place at 4 °C for further use.

The seeds of *Amaranthus blitoides* and *Cynodon dactylon* were supplied from Pakan-Bazr Company in Isfahan. These seeds were counted then, equidistantly placed in 9 cm diameter Petri dishes as an experimental unit (30 seeds per Petri dish, four replicates per treatment) covered with two layers of moistened Whatman No. 1 filter paper. After adding different concentrations of eucalypt oil (A similar treatment with water only served as control), Petri dishes were sealed with

a film of paraffin and placed in a growth chamber (germinator) at 25 ± 2 °C temperature with a 16 h/8 h light/dark photoperiod, a photon flux density of approximately $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ and relative humidity of around 75% (12). After 24 hours, the germinated seeds were counted daily. At the end of 8th day; six samples were taken from each Petri dish and seedling height was measured. *Amaranthus blitoides* and *Cynodon dactylon* were cultivated in polypropylene pots (9 cm diameter and height) in greenhouse. The soil of the pots was mixture of soil and sand with 3:1 ratio. In each pot, 30 seeds of *Amaranthus blitoides* and *Cynodon dactylon* were placed and the seeds were covered with the thickness of 1 to 5 mm of soil, respectively. In the first irrigation, 5 ml of the prepared solution was sprayed on the pots (In the control pots 5 ml distilled water was sprayed) and they were kept in 29 ± 2 °C temperature with relative moisture of about 75 ± 5 . After 12 days, the sampling (6 samples of each pot) was done in seedling establishment stage (18). The measuring unit of length was millimeter and to obtain the dried weight, initially samples were kept in oven (70 degree of heat for 48 hours). The dried samples were weighed by an enclosed-scale with the sensitivity of 0.001 g. The data collected were analyzed statistically with MSTATC software, by using Fisher's analysis of variance (ANOVA) technique. Duncan's Multiple Range Test (DMRT) was applied to compare means of each trait at 1% probability.

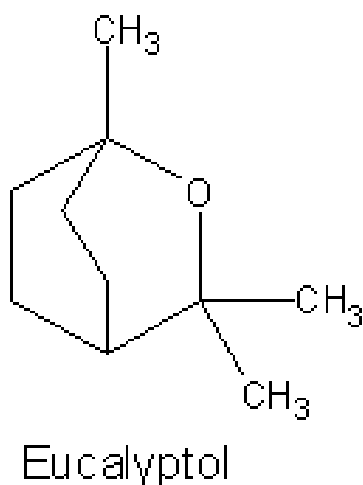


Fig. 1. The chemical formula of Eucalyptol (1, 8-cineole), the major material of *Eucalyptus globulus* (1).

RESULTS AND DISCUSSION

The obtained results of *Eucalyptus globulus*' essential oil effect on the germination and seedling establishment of two weeds (*Amaranthus blitoides* and *Cynodon dactylon*) indicated that the germination and growth of these two weeds decreased significantly under the effect of essential oil (**Table 1**). Means comparison with Duncan's Multiple Range Test revealed that treatments have highly significant difference ($p < 0.01$) (**Table 2 and 3**). The results showed that germination percentage and germination rate decreased by increasing the concentration of the essential oil and the highest germination percentage and germination rate was related to the control treatment. Germination rate and germination percentage were highly affected by interaction of weed $\times$ concentration and comparing to the *Cynodon dactylon*, the germination and growth speed in *Amaranthus blitoides* were more influenced and the highest effect was belonged to concentration of 5 nl/ml (**Table 1, 2 and 3**). Batish *et al.* (12) suggested that the *Eucalyptus globulus*' essential oils caused inhibition in germination and growth of *Triticum aestivum*, *Zea mays*, *Raphanus sativus*, *Cassia occidentalis*, *Amaranthus viridis* and *Echinochloa crus-galli*. However, the highest and the lowest allelopathic effect are related to *Amaranthus blitoides* and *Raphanus sativus* respectively. The results of these studies showed that the sensitivity of different species against allelopathic effects of essential oils can be different. The fact that, in a given species, germination is partly inhibited by a given essential oil does not imply that there is no normal germinated seed in the mentioned area (18). Results showed when the concentration of the essential oil increases; radicle, plumule and seedling height in Petri dish decreases (**Table 2 and 3**). In both weeds (*Amaranthus blitoides* and *Cynodon dactylon*) inhibition of germination and seedling establishment will be seen as radicle length > seedling length > plumule length, and in all of the investigated characteristics, the *Amaranthus blitoides* is inhibited more than *Cynodon dactylon* against *Eucalyptus globulus*' essential oils (**Table 2 and 3**). Muller *et al.* (19) and Muller (20) reported that, in the vicinity of aromatic shrubs, such as white leaf sage (*salvia leucophylla* Greene) or California sagebrush (*Artemisia Californica* Less), there was no

annual plants within a radius of 90 cm around and the presence of annuals was very limited within 2 to 6 m. The herbicidal action of essential oils from *E.citriodora* on *Parthenium hysterophorus* (kind of *Asteraceae*) was investigated and similar results in terms of Seedling height, Germination percentage, Germination rate, Plumule length and Radicle length were reported by Singh *et al.* (2). The obtained results of *E. globulus*' essential oil effect on the growth of two kinds of weeds including *Amaranthus blitoides* and *Cynodon dactylon* in pot studies supported the laboratory investigations and demonstrated that Eucalyptus' essential oil significantly decreases characteristics such as the length of primary root, pedicle and seedling height (**Table 4**). The highest decrease in primary root length, primary pedicle length and seedling height was that of 1 v/v concentration. The results showed that the highest amount of allelopathic effects was recorded in the seedling height, primary root length, and primary pedicle length, respectively (**Table 5**). Studying the weed×concentration interactions reveals that almost in all of the characteristics, *Amaranthus blitoides* are more influenced than *Cynodon dactylon* in pot

conditions, which is in accordance with the obtained results, on Petri dish (**Table 5 and 6**). Similarly, in both conditions, the allelopathic effects in radicle length were more than plumule length. According to an investigation, the growth of radicle in *T. aestivum*, *Brassica nigra* and *Amaranthus retroflexus* influenced by Pennyroyal (*Mentha pulegium*) essential oils, which have carvacrol effective material; was more limited than plumule growth and indicated that probable inhibition of the root growth is the effect of carvacrol prevention from much penetration in the soil (18). Also the obtained essential oil from umbelliferae seeds (i.e., Fennel, Dill and Cumin) on the germination of lettuce seed showed much allelopathic effect (21). Comparing the allelopathic effect in Both Petri dish (*in vitro* condition) and pot conditions, possibly, Eucalypt effective material (cineole) has the highest effects in chlorophyll part of the seedling. Different monoterpenes has much effect in different parts of a plant (22), and according to the less effectiveness of the primary root length in the pot to the seedling height comparing to Petri dish, it can be said that these essences are not stabilized in soil and it does not have environmental poisoning (9, 23).

Table 1. The mean squares of ANOVA for Radicle length Plumule length, Seedling height Germination percentage and Germination rate in Petri dish.

		Radicle	Plumule	Seedling	Germination	Germination
S. Q V	df	length	length	height	percentage	rate
C	4	74.724 **	38.834 **	307.933 **	1091.511 **	14.241 **
W	1	1258.884 **	2436.721 **	7249.556 **	64.262 **	106.048 **
C×W	4	17.131 **	24.545 **	95.875 **	329.792 **	4.284 **
Error	30	0.798	0.826	5.545	56.710	0.268
CV (%)		5.25	6.59	6.59	10.67	9.94

Note. *-p < 0.05; **-p < 0.01; NS-not significant (p>0.05); C-Concentration; W-Weed; C×W-represent interaction terms between the treatment factors.

Table 2. Individual effects of eucalypt oil concentration and kinds of weeds on some factors (Radicle length Plumule length, Seedling height Germination percentage and Germination rate) according to

Concentration	Kind of weeds	Radicle length (mm)	Plumule length (mm)	Seedling height (mm)	Germination percentage (%)	Germination rate (seed/day)
Control		20.98 a	17.50 a	43.72 a	82.78 a	6.781 a
0.5 (nl/ml) ¹		18.86 b	16.14 b	39.75 b	80.14 ab	5.963 b
2 (nl/ml)		17.4c	14.81 c	35.60 c	71.54 bc	5.463 b
3.5 (nl/ml)		14.78 d	12.99 d	31.02 d	63.96 cd	4.460 c
5 (nl/ml)		13.38 e	12.14 d	28.54 d	54.42 d	3.350 d
	<i>A. blitoides</i>	22.615 a	22.520 a	49.190 a	69.300 b	6.832 a
	<i>C. dactylon</i>	11.395 b	6.910 b	22.265 b	71.835 a	3.575 b

Duncan's Multiple Range Test in Petri dish.

Means not sharing a common letter in a column differ significantly at 0.01% level of probability; ¹nl/ml–nanoliter/milliliter.

Table 3. Interaction effects of eucalypt oil concentration and kind of weeds on Radicle length Plumule length, Seedling height Germination percentage and Germination rate, according to Duncan's Multiple Range Test in Petri dish.

Concentration	Kind of weeds	Radicle length (mm)	Plumule length (mm)	Seedling height (mm)	Germination percentage (%)	Germination rate (seed/day)
Control	<i>A. blitoides</i>	28.58 a	27.58 a	61.65 a	91.23 a	9.238 a
	<i>C. dactylon</i>	13.38 f	7.425 e	25.80 e	74.32 bc	4.325 d
0.5 (nl/ml)	<i>A. blitoides</i>	25.17 b	24.98 b	55.15 b	79.97 ab	7.387 b
	<i>C. dactylon</i>	12.75 fg	7.300 e	24.35 ef	80.30 ab	4.050 d
2 (nl/ml)	<i>A. blitoides</i>	22.75 c	22.73 c	49.47 c	69.63 bc	7.425 b
	<i>C. dactylon</i>	11.33 gh	6.900 e	21.72 efg	73.45 bc	3.500 de
3.5 (nl/ml)	<i>A. blitoides</i>	19.30 d	19.42 d	41.75 d	60.53 c	5.695 c
	<i>C. dactylon</i>	10.25 hi	6.550 e	20.30 fg	67.40 bc	3.225 de
5 (nl/ml)	<i>A. blitoides</i>	17.27 e	17.90 d	37.92 d	45.15 d	3.925 d
	<i>C. dactylon</i>	9.475 i	6.375 e	19.15 g	63.70 c	2.775 e

Means not sharing a common letter in a column differ significantly at 0.01% level of probability.

Table 4. The mean squares of ANOVA for Primary root length, Primary pedicle length and Seedling height in pot.

S. Q. V	df	Primary root length	Primary pedicle length	Seedling height
C	4	32.884**	8.436**	117.619**
W	1	1.849 ^{ns}	85.849**	132.132**
C×W	4	3.220*	0.188 ^{ns}	10.585**
Error	30	0.858	0.863	1.175
C.V (%)		6.45	11.70	4.18

Note. *— $p < 0.05$; **— $p < 0.01$; NS—not significant ($p > 0.05$); C—Concentration; W—Weed; C×W—represent interaction terms between the treatment factors.

Table 5. Individual effects of eucalypt oil concentration and kinds of weeds on some factors (Primary root length, Primary pedicle length and Seedling height) according to Duncan's Multiple Range Test in pot.

Concentration	Kind of weeds	Primary root length(mm)	Primary pedicle length(mm)	Seedling height(mm)
Control		17.29 a	9.225 a	31.39 a
0.25 (v/v) ¹		15.15 b	8.512 a	27.55 b
0.5 (v/v)		14.30 b	8.125 ab	25.92 c
0.75 (v/v)		12.94 c	7.162 cb	23.13 d
1 (v/v)		12.7 c	6.675 c	21.63 e
	<i>A. blitoides</i>	14.135 b	9.405 a	27.740 a
	<i>C. dactylon</i>	14.565 a	6.475 b	27.105 b

Means not sharing a common letter in a column differ significantly at 0.01% level of probability; ¹v/v—volume/volume.

Table 6. Interaction effect of eucalypt oil concentration and kind of weeds on Primary root length, Primary pedicle length and Seedling height, according to Duncan's Multiple Range Test in pot.

Concentration	Kind of weeds	Primary root length(mm)	Primary pedicle length(mm)	Seedling height(mm)
Control	<i>A. blitoides</i>	17.98 a	10.95 a	34.67 a
	<i>C. dactylon</i>	16.60 ab	7.500 de	28.10 c
0.25 (v/v)	<i>A. blitoides</i>	15.25 bc	9.975 ab	30.22 b
	<i>C. dactylon</i>	15.05 bc	7.050 def	24.88 d
0.5 (v/v)	<i>A. blitoides</i>	14.03 cde	9.525 abc	27.55 c
	<i>C. dactylon</i>	14.58 cd	6.725 def	27.30 de
0.75 (v/v)	<i>A. blitoides</i>	12.25 ef	8.757 bcd	24.08 de
	<i>C. dactylon</i>	13.63 cde	5.750 ef	22.17 ef
1 (v/v)	<i>A. blitoides</i>	11.18 f	8.000 cd	22.17 ef
	<i>C. dactylon</i>	12.98 def	5.350 f	21.08 f

Means not sharing a common letter in a column differ significantly at 0.01% level of probability.

Dudai *et al.* (18) in their study under laboratory and pot conditions were also reported allelopathic effect of 32 medicinal plants essential oil on the germination and primary growth of root and pedicle of *Amaranthus blitoides*, *Amaranthus palmeri*, *Euphorbia hirta*, *Sambucus nigra*, *Trifolium campestre* and *L. esculentum*.

The reduction of the germination percentage and germination rate caused by Eucalyptus' essential oil on the weeds of *Amaranthus retroflexus* and *Portulaca oleracea* showed identical results (1). The mechanism with which the Eucalyptus essence prevents germination was completely unknown. However, some researches show that volatile monoterpenes like cineole stop the mitosis (24, 25). The extracted essential oils from Cinnamon and red garden thyme (*Thymus vulgaris* L.) inhibits germination and growth of the eyes of potato (sprouts) by killing meristem cells (26). The growth of most onion cells which are cared by cineole stops at interphase stage, and a few of the cells will stop in the other mitosis phases (25). The plant poisoning of the Eucalyptus' essential oil in the

examined species can be resulted from the interference in the reduction of mitosis. It is also reported that by increasing the membrane permeability and destruction of cells and killing them by Electrolyte leakage, the essential oils inhibit germination and growth in other plants (27, 11, and 2). The Eucalypt oil is composed of the mixture of several monoterpenes, such as cineole, linalool and Citronellol which is known as allelochemicals materials. Singh *et al.* (28) suggested that citronellal is more effective in phyllode plants (broad leaf plants) than cineole. Likewise, cineole is more effective in frail leaves plants (25). The monoterpene compounds may have an activity similar to other allelopathic materials (29). Although solubility is a limited factor in poisoning of other plants and monoterpenes are usually active in fewer concentrations, it could be much soluble by surfactants such as paraffin oils (11, 20, and 30). It is reported that the formative monoterpenes of Eucalyptus essence by decreasing the amount of chlorophyll, are effective in plants photosynthesis (12). Abraham *et al.* (31) reported that the interference of monoterpenes in the

respiratory activities of plant can be the evidence of reduction in germination and growth. Moreover, Batish *et al.* (12) reported that the reduction of respiratory activities of a plant by monoterpenes reduces the amount of photosynthesis and disturbs the growth and germination as well. It is also reported that the allelopathic effects of monoterpenes were also stronger than that of the herbicide 2,4-D (22), in addition, this material inhibited the growth of phytopathogenic fungus (32).

CONCLUSION

According to obtained results of the present research, it can be concluded that the essential oils from *Eucalyptus* may have much poisonous effect on the *Amaranthus blitoides* and *Cynodon dactylon*, hence, it can be used extensively in natural productions of plants as biologic herbicide and this finding can be an important stage in stable agriculture. However, it needs further researches on herbicides mechanism of action to improve the possibilities of using them effectively in cultivated lands as bioherbicides in future.

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