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Research Article

EFFECT OF FOLIAR APPLICATION OF METHANOL AND ETHANOL ON YIELD COMPONENTS AND ESSENTIAL OIL CONTENT OF DRAGONHEAD (*DRACOCEPHALUM MOLDAVICA* L.)

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ABSTRACT
Dragonhead (*Dracocephalum moldavica* L.) is an annual pharmaceutical plant belongs to family Lamiaceae which is a widely used remedy for treatment of pain and gastrointestinal disturbances due to its high essential oil contents. A randomized complete block design with 12 treatments and three replications was carried out. Treatments were the application of four levels of methanol (10, 20, 30 and 40 % v/v), four levels of ethanol (10, 20, 30 and 40% v/v) three levels of 1:1 mixture of ethanol and methanol (5, 15 and 25% v/v) and control (spraying with distilled water). Results showed that yield, essential oil content and its composition affect by the application of ethanol and methanol. The highest dry matter yield (10454 kg/ha) and essential oil content (47.7 kg/ha) produced by applying ethanol/methanol solution at 25% v/v. The highest Geranyl acetate (42%), geraniol (27 %) and neral (25%) produced by the same treatment too.

Keywords: *Dry Matter Yield, Geranyl Acetate, Geraniol, Neral*

INTRODUCTION
The use of plants for medicinal purposes enhanced globally and herbal remedies, are in great public demand. World health organization released guidelines and instruction in order to enhancing the use of herbal medicines (Gabler, 2002).
Dragonhead (*Dracocephalum moldavica* L.) is an annual, herbaceous plant belonging to the *Lamiaceae* family with blue to purple and sometimes white flowers (Omidbaigi, 2005). It originated in southern Siberia and off the slopes of the Himalayas. The vegetative parts of the plant are aromatic. The active substances of the vegetable organs of this plant have medicinal properties and are tranquillizing and appetizing. It's essential oil has antioxidant activity (Dastmalchi *et al.,* 2005). Producing high dry material is the key of higher plant yield. Plant dry mater accumulation is the result of CO2 assimilation during photosynthesis process. Thus crop yield increases by enhancing CO2 fixation rate. Applying substances such as methanol, ethanol, propanol, butanol and amino acids like glycine and glutamate on C3 plants, improved their growth productivity (Haakana *et al.,* 2001). Ethanol and methanol are natural compounds which produce with most of plants. They produce at leaf elongation stage due to pectin demethylation of cell walls and emit to the air (Obendorf *et al.,* 1990). According to Nonomura and Benson (1992) plants which treated with methanol, showed higher turgor, higher growth rates and consequently gave higher yield than the control plants. Phenological stage of plants accelerates by foliar application of ethanol and methanol, which resulted in early maturity and lower water demand (Nonomura and Benson, 1992). Research showed that plant biomass enhance by the application of methanol (Nonomura and Benson, 1992; Hemming and Criddle, 1995). Foliar application of methanol resulted in higher production and lower water requirement in warm and dry conditions (Nemecek-Marshall *et al.,* 1995; Fall and Benson, 1996). Nonomura (1997) stated that the most important role of methanol is preventing and reducing the effects of environmental stress. Lazr *et al.,* (2013) investigated the effect of foliar methanol and ethanol on different traits of *Plantago psylliumI.* Results showed that the highest and lowest leaf length and chlorophyll content produce by applying 30% v/v methanol and control treatment respectively.
Khosravi *et al.,* (2011) studied the effect of foliar application of methanol and ethanol on yield and quantitative characteristics of *Echinacea Purpurea*. Methanol and ethanol stimulate biomass production as a carbon source and result in higher dry matter accumulation of *Echinacea Purpurea*.

In another research the effect of ethanol and methanol on photochemical traits of *Melissa officinalis* was measured. Biosynthesis of essential oils of *Melissa officinalis* significantly affect by the application of ethanol and methanol (Khosravi *et al.*, 2012).

MATERIALS AND METHODS

An experiment was conducted at south east of Iran at 2013 growing season. The site located at 59° 26′ N and 35° 32′ E with 2130 m altitude with 320 mm annual precipitation. Seeds of *Dracocephalum moldavica* varietySZK-1 with a Hungary origin were planted.

Land ploughed, disk harrowed and prepared for sowing. A randomized complete block design with 12 treatments and three replications was carried out. Plots with 2 m length and 1.5 m width arranged for each treatment. Treatments were the application of four levels of methanol (10, 20, 30 and 40 % v/v), four levels of ethanol (10, 20, 30 and 40% v/v) three levels of 1:1 mixture of ethanol and methanol (5, 15 and 25% v/v) and control (spraying with distilled water).

Seeds planted are rows at late march. The first methanol and ethanol treatments were applied 20 days after planting. Further foliar applications were made once every 15 days. Foliar application was made to run-off stage.

At flowering stage, 10 random plants selected for measuring plant height and number of stesm. Biomass and essential oil content measured by harvesting total plot for each treatment. Plants harvested at final flowering stage. Samples dried at room temperature and dry weight recorded.

The essential oils of D. moldavica aerial parts were obtained by steam distillation by a Clevenger apparatus.

Identification of the oil components was performed by a GC and a GCMS- gas chromatograph. Data analyzed using SAS software. Comparison between means carried out applying Duncan’s multiple range test.

RESULTS AND DISCUSSION

Plant Height

Results showed that plant height significantly affect by treatments ($p<0.01$) (table 1). The highest plant height (82 cm) produced by applying 25% v/v ethanol-methanol. The lowest plant height (59.9 cm) produced by applying 10% v/v ethanol (figure 1).

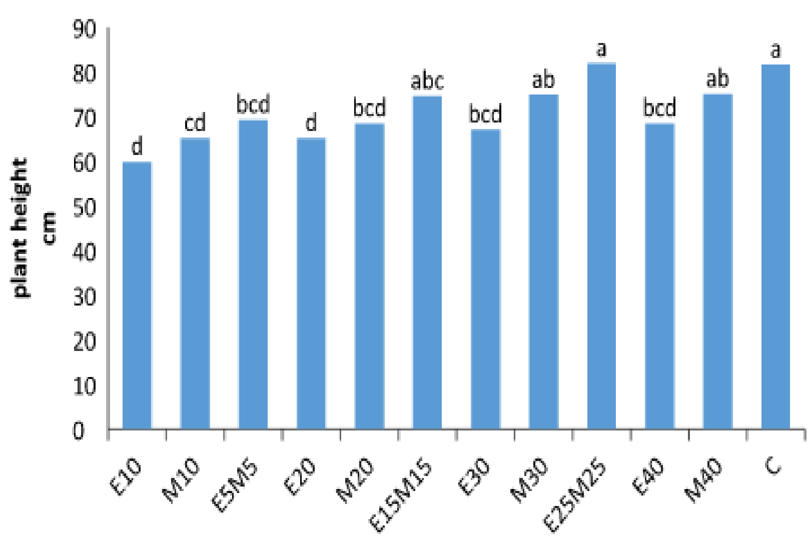


Figure 1: Plant height as affected by different foliar applications
E, M and EM for ethanol, methanol and ethanol/methanol and C for control treatment

Table 1: Results of analysis of variance of measured traits under different treatments

Source of variation	Degree of freedom	Plant height	Number of stems	Biomass yield	Essential oil content	Essential oil yield
Block	2	4.3	0.7	1045914	0.001	26.7
Treatment	11	137**	8.7**	3527297**	0.006**	19.01**
Error	22	6.5	0.6	420120	0.0005	0.16
Total	35	__	__	__	__	__

** and ** significant at 5 and 1 probability levels*

Number of Stems

Analysis of variance showed that number of stems per plant affect by foliar application ($p<0.01$) (table 1). The highest (17.4) and lowest (11.6) number of stems produced by 25% v/v ethanol/methanol and 10 % v/v ethanol respectively (figure 1). The results supported by Ramadan and Omran (2005).

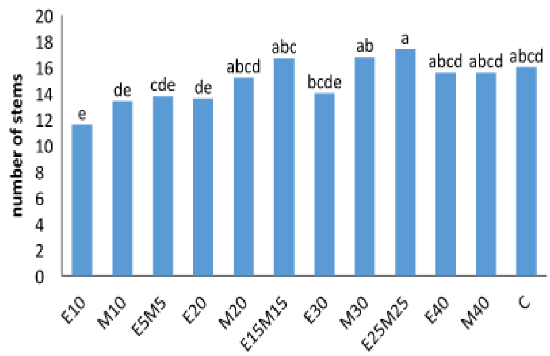


Figure 2: Number of stems as affected by foliar application
E, M and EM for ethanol, methanol and ethanol/methanol and C for control treatment

Biomass yield/ ha

There was significant different between foliar treatments in respect of biomass yield ($P<0.01$) (table 1). The highest (10454 kg/ha) and lowest (7110 kg/ha) biomass yield produced by 25% v/v thanol-methanol and10% ethanol respectively (figure 3).

The positive effect of methanol application on biomass production may be due to abundant CO_2 supply from methanol as suggested by Hemming *et al.*, (1995). Methanol can be utilized to inhibit photorespiration, thus increasing the photosynthetic productivity (Hemming *et al.*, 1995).

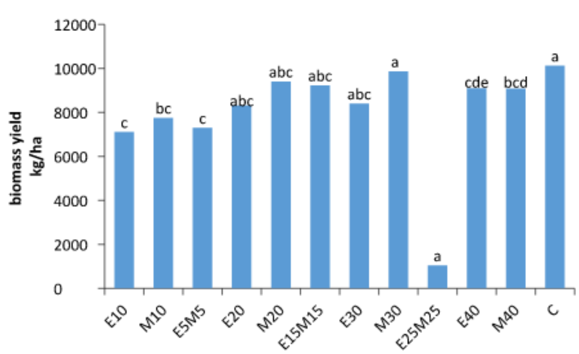
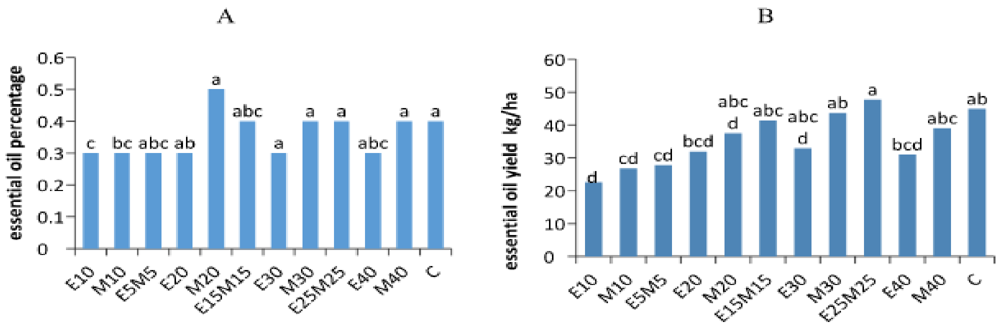


Figure 3: biomass yield as affected by foliar application
E, M and EM for ethanol, methanol and ethanol/methanol and C for control treatment

Essential Oil Percentage and Yield

The percentage of essential oils affected by treatments (p<0.01) (table 1). The highest (45%) and lowest (31%)essential oil content produced by 30% v/v methanol and 10% v/v ethanol respectively (figure 4).
The yield of essential oil affect by treatments (p<0.01) (table 1). The highest essential oil yield (47.7 kg/ha) produced by foliar application of 25% v/v ethanol-methanol. The lowest essential oil (22.5 kg/ha) produced by 10% v/v ethanol (figure 4).



A **B**

Figure 4: Essential oil percentage (A) and yield (B) as affected by foliar application E, M and EM for ethanol, methanol and ethanol/methanol and C for control treatment

Exogenous methanol application incorporates to synthesis of methyl groups of molecules, such as serine, methionine and phosphatidylcholine. Metabolic pathways related to plant growth and development (*e.g.* the content of amino acids) affects by exogenous application of methanol affect, too (GOUT *et al*., 2000).

Chemical Composition of Essential Oil

Gas chromatography showed that the amount of geranyl acetate, geraniol, geranial, neral, neryl acetate and n-decane was different in different treatments.

Table 2: Effect of different treatment of chemical composition of essential oil

Code	**Compound**	**E10**	**E20**	**E30**	**E40**	**M10**	**M20**	**M30**	**M40**	**E.M5**	**E.M15**	**E.M25**	**C**
1	n-decane	1	1	1	1	1	1	1	1	1	1	1	1
2	neral	19	23	19	19	22	18	19	21	21	21	22	25
3	geraniol	16	15	15	16	18	17	12	18	18	18	19	17
4	geranial	16	15	13	16	16	15	27	16	18	16	17	15
5	neryl acetate	4	2	6	7	9	7	3	4	4	7	6	9
6	geranyl acetate	39	42	42	38	31	40	35	35	35	35	30	30

E, M and EM for ethanol, methanol and ethanol/methanol and C for control treatment

The highest geranyl acetate (42%) and geraniol (19%) content observed for applying 15% v/v ethanol-methanol (table 2). Applying 5% v/v ethanol-methanol resulted in lowest geranyl acetate (30%) and geraniol (12%) content (table 2).
The highest (27%) and lowest (13%) geranial content produced by 30% and 10% v/v methanol respectively (table 2).

The highest (25%) and lowest (18%) neral content produced by applying 15% and 5% v/v ethanol-methanol while the highest (9%) and lowest (2%) neryl acetate content produced by applying 10% methanol as well as control treatment and 20% v/v ethanol respectively (table 2).
Amount of n-decane content was equal for all treatments (table 2).

Conclusion

Results showed that yield and essential oil percentage of *Dracocephalum moldavica* L. significantly affect by applying ethanol and methanol. The highest biomass production and essential oil yield produced by applying 25% v/v ethanol-methanol solution. The highest gerayl acetate, geranial and neral content produced by this treatment too. Thus applying the above solution is advisable in *Dracocephalum moldavica* L. production in order to gain higher quantity and quality.

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