

Effect of Foliar Application Methanol on the Quality and Quantity of *Artemisia dracunculus* L.

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Research Article

Abstract

In order to study the effect of foliar application of methanol on the quality and yield of *Artemisia dracunculus* L., an experiment was carried out in a randomized complete blocks design with 3 replications in northern of Iran. In this experiment, treatment was considered in five levels of methanol (0) control (10, 20, 30 and 40 % V/V) in the form of spraying on the plant. The first stage of treatment was administered in April 2014 after planting the sprouts in the third week. The second stage of treatment took place in the middle of growing season during the sixth week within the planting and just before flowering phase and the third stage of treatment was at start of flowering phase (tenth week). The results of the Variance Analysis of this experiment showed that methanol had significant effect on all tested traits ($P < 0.01$ in Duncan's MRT). The highest fresh weight, dry weight, crown diameter, leaf number, stem length, total chlorophyll content, dry yield and essential oil content were obtained from the treatment with 40% V/V methanol. Given its importance in the food and pharmaceutical industries, *A. dracunculus* L. can be considered as an important plant. The use of methanol as a safe substance for human health, and the resultant high efficiency with respect to the plant make it necessary for the production of this plant.

Keywords: Methanol; *Artemisia dracunculus* L.; Foliar application; Yield.

1. Introduction

The *Artemisia dracunculus* L. from Asteraceae family is an herbaceous, aromatic, perennial plant with straight stems. The leaves are slender, long and linear [1]. Today, the aerial parts of this plant and the essential oil of *A. dracunculus* are used in the preparation of sauces, salads and as a food flavour, also has applications in the food, canning and perfume industries [2]. Due to its insecticidal, anti-fungal and anti-bacterial properties, this genus has great industrial value. The plant also has analgesic, sedative and mild hypnotic properties and has anticonvulsant effects. In addition, the ethanol essential oil of this plant has anti-diabetic properties

[3]. The essential oil content of plant is produced in leaves and two structures: secretory trichomes and secretory bags [4].

The ways that promote CO₂ incorporation in field crops can be used as efficient strategies for increasing the yield and biomass of field crops. In recent studies, the use of methanol as a carbon source for field crops has been increasingly suggested because plants can easily absorb the methanol on their leaves and use it as a carbon source in addition to the atmospheric carbon. Like natural methanol created in leaves due to Pectin Methylesterase (PME) enzyme in process of the cell wall expansion, use of sprayed methanol can also increase the production of Cytokinins (CK) and, consequently, the growth of plants, increases the yield, speeds up the fruition, reduces the effects of drought and decreases the required water [5].

It has been found that use of methanol solutions can increase the wet weight of Tobacco shrubs and the increase in the amount of dry matter produced by plants depends on the amount of consumed methanol. According to studies about the effect of methanol on the leaves in different plants, it has been recognized that this solution increases the effective area of leaf mainly by delaying the senescence of leaves on one hand and more production of IAA and CK Hormones by stimulating Methylotrophic bacteria on the surface of leaves [6]. The results of some of the researches on soybean showed that the grain weight, grain yield and number of pods in shrub of those plants that have been treated with methanol were significantly higher [7]. In case of sunflower, the foliar application of methanol increased the stem length, leaf area and shoot dry weight and the amount of florets, too [8]. During the recent research on the impact of the methanol and ethanol use *Echinacea angustifolia*, it was found that the greatest yield of morphological indexes of plant height, stem diameter, number of leaves in stem, leaf length, leaf width, root wet weight, wet weight, root weight, shoot dry weight, dry leaf weight, dry weight, root length and diameter of the Capitol occurred when a solution of 40% methanol was sprayed, while the highest yield of branches, root rhizomes, leaf area and the

amount of chlorophyll was obtained 30% methanol solution. Overall, in this study, spraying alcohol as a source of carbon and bio-stimulants can increase the biomass and yield of *E. angustifolia* [9].

Given the importance and role of vegetables and medicinal plants in various industries, greater biomass production and greater effective substance without using harmful ingredients are considered as necessities for sustainable production. The positive effects of the application of alcohol to achieve the desired objectives make the study of their effects on quality and yield of medicinal plants, especially *A. dracuncululus* L., a very special study in sustainable agriculture studies. Some evidences suggest improved quality of vegetable crops by spraying alcohol, yet few such studies of medicinal plants in Iran and other countries have been conducted. So appreciation of the effects of foliar application of methanol with respect to quality and yield of *A. dracuncululus* L. in sustainable systems and determination of the optimum conditions for cultivating it requires further study and research

2. Methods

This study aimed to investigate the effects of methanol on quality and yield of foliar application of *A. dracuncululus* L. in a randomized complete block design with three replications conducted in Kelardasht (situated at 36° 29'N and 51° 6'E) in northern Iran at the altitude of 1107 meters above sea in April 2014. In this experiment, the methanol treatment on *A. dracuncululus* L. was tested in five levels (0) control (10, 20, 30, and 40 percent V/V). Soil texture was loamy sand with pH 7.8. Seedlings were planted in plots with dimensions of 1.5 to 2.5 m, at 40 cm distances and 60 cm space between rows. To prepare the methanol solution with purity of more than 99.5% (Dr. Mojalali, Iran) twice distilled water was used. Determining the amount of alcohol and distilled water for each treatment was performed with volumetric flask. The foliar application was performed in three stages: the first stage of treatment was performed in the third week after cultivation when seedlings were fully anchored. The second stage of treatment took place in the middle of growing season or in the sixth week after cultivation and prior to flowering stage and finally, the third stage of treatments in time of flowering (tenth week). The foliar application of methanol was in form of sprayings on the plants without the solution being dripped to the ground or leaves being over-flown. Meanwhile, control plot was sprayed with distilled water. Weed mowing (by hand) and irrigation were done only when required. Two and a half months after being planted in prepared beds, plants were harvested and evaluated in terms of plant fresh weight, dry weight, stem diameter, leaf number, length, chlorophyll, dry yield and essential oil content.

2.1 Plant fresh weight

Plant fresh weight was measured by digital scale

(with an accuracy of 0.1 mg) and expressed in grams (g).

2.2 Plant dry weight

Plant was drying by Oven in 45°C then plant dry weight was measured by digital scale (with an accuracy of 0.1 mg) and expressed in grams (g).

2.3 Crown diameter

Crown diameter was measured at the stem contact area to soil by digital caliper.

Leaf number: Numbers of leaves of per plant were counting in harvesting time.

2.4 Stem length

Every stem was measured by a digital caliper and then the average size was obtained and recorded.

2.5 Total chlorophyll content

For evaluate the chlorophyll content 0.2 g samples prepared from middle part of the fourth leaf and were completely worn. Then 10 mL acetone (90%) was added and at the end, the absorbance was read at 470, 663 and 645 nm wavelengths by spectrophotometer (Optizen 3220UV) and chlorophyll content calculated by following formula (Arnon, 1967).

$$V=10$$

$$W=0.2$$

$$\text{Chlorophyll a} = [(12/7 \times A_{663}) - (2/69 \times A_{645})] V / (1000 \times W)$$

$$\text{Chlorophyll b} = [(22/9 \times A_{645}) - (4/69 \times A_{663})] V / (1000 \times W)$$

$$\text{Total Chlorophyll} = [(20.2 \times A_{645}) + (8.02 \times A_{663})] V / (1000 \times W)$$

2.6 Dry yield

Dry yield in per area unit of test (plots 1.5 in 2.5 m in order to 3.75 m²) was measured and by terms of kilograms per hectare was reported.

2.7 Essential oil content

In extraction of samples by water distillation method, 50 g of the *Artemisia dracuncululus* L. in 500 ml of distilled water was processed in Clevenger for 3 h and its percentage was measured. All data were analyzed using SPSS software and comparison of mean values was done at 1% and 5% level using Duncan's Multiple Range Test (MRT).

3. Results and Discussion

3.1 Plant fresh weight

The results of Variance Analysis showed that the fresh weight of *A. dracuncululus* L., when treated by

methanol, is significant at 1% Duncan test. The greatest amount of Plant fresh weight was associated with foliar application of 40% V/V methanol solution. In control experiment, the smallest amount of fresh weight was 5.99 g while this reached to 21.24 g after the treatment of 40% V/V methanol (Tables 1 and 2). In a study on tobacco plants, it was found that the largest tobacco leaf area was obtained through treatment of 40% methanol, hence consistent with results of this study [10]. It was found that the use of methanol can enhance the fresh weight of tobacco shrubs, and amount of dry matter produced by plants depends on the amount of consumed methanol. This shows that methanol can affect the assimilation of CO₂ in the plant [6]. The increase in fresh weight of plants under foliar application of methanol shows that this increase may be due to methanol preventing Photorespiration [11].

3.2 Plant dry weight

The results of variance analysis showed that, under the influence of consumption of methanol, the dry weight of *A. dracunculus* L. is significant at 1% Duncan test. The greatest amount of plant dry weight was associated with foliar application of 40% V/V methanol. In case of control experiment, the least amount of dry weight was 2.67 g, while in treatment of 40% V/V methanol it reached to 7.78 gm and differences between all treatments were significant (Tables 1 and 2). What was found in this study was consistent with another study on *E. angustifolia* where the largest dry matter of *E. angustifolia* was obtained when treated with 40% methanol solution, and methanol increased the plant dry weight. However, our observations do not correspond with the results of a study on *Capsicum amuum*. During that study it was observed that the greatest dry weights of leaf and stem in *C. amuum* occur in the treatment of 10% methanol, and any higher density than 10% decreased the production volume of tested indexes. The reason for such a disparity in results may be related to type of plant, environmental conditions

and the procedure [9]. According to the researchers, the use of methanol on cotton and tomato plants increased the dry weight of aerial parts and root [12,13]. Indeed, the most important factor for increase in leaf dry weight in foliar application of methanol is the increase in biomass and carbon fixation because greater methanol concentration enhances the carbon dioxide in the leaf and transfer in leaf stomata [14].

3.3 Crown diameter

The results of Variance Analysis showed that, under the influence of consumption of methanol, the Crown diameter of *A. dracunculus* L. is significant at 1% Duncan test. The greatest amount of Crown diameter was associated with foliar application of methanol 40% V/V. So that from treatment of control, the least obtained amount of Crown diameter was 1.52 mm, while in treatment of 40% V/V methanol, it reaches to 5.95 mm, and differences between all treatments were significant (Tables 1 and 2). Findings of this study were consistent with another study on *E. angustifolia*: the largest stem diameter of *E. angustifolia* corresponded with treatment with 40% concentration of methanol [9]. Also, 25% methanol increases the stem diameter of soybean shrubs [11]. Observations show that, under the effect of methanol solutions sprayed on aerial parts, greater growth and yield of plants is due to the effects of methanol as an inhibitor of Photorespiration. In a study that examined alcohol spraying on the *Melissa officinalis*, it was found that the greatest impact on crown diameter was related to the 40% methanol [15].

3.4 Leaf number

According to the results of the table of Variance Analysis, it is determined that application of methanol at 1% level has a significant effect on the leaf number. Based on a test for comparing means in an analysis of variance, it is found that the leaf number was affected by methanol so that in treatment of control the lowest leaf number was 78.78 and the

Table 1. Results of variance analysis treatments on examined characteristics of *A. dracunculus* L.

S.O.V	df	Fresh weight	Dry weight	Crown diameter	Leaf number	Stem length
Block	2	2.83 ^{ns}	0.601 ^{ns}	2.254 ^{ns}	1.751 ^{ns}	3.087 ^{ns}
Methanol	4	238.64 ^{**}	166.627 ^{**}	107.717 ^{**}	151.383 ^{**}	121.861 ^{**}
Error	8	1.33	0.22	0.259	2088.298	24.756
CV%		8.21	17.56	16.98	19.17	11.93

^{**}, Significant at 0.01 level, ns: non significant.

Table 2. Compare of examined characteristics Average in *A. dracunculus* L.

Treatment	Fresh weight	Dry weight	Crown diameter	Leaf numbe	Stem length	Total chlorophyll content	Dry yield	Essential oil content	Essential oil Yield
0	5.99 ^d	2.67 ^e	1.52 ^d	78.78 ^d	23.44 ^d	0.4 ^d	110.28 ^e	0.5 ^e	0.55 ^e
10	10.37 ^c	3.81 ^d	2.00 ^{cd}	108.67 ^d	32.33 ^c	0.95 ^c	157.16 ^d	1.13 ^d	1.8 ^d
20	15.78 ^b	4.6 ^c	2.31 ^c	165.44 ^c	36.22 ^c	1.65 ^b	189.66 ^c	1.73 ^c	3.25 ^c
30	16.89 ^b	6.24 ^b	3.19 ^b	296.78 ^b	45.11 ^b	1.71 ^b	257.4 ^b	2.03 ^b	5.21 ^b
40	21.24 ^a	7.78 ^a	5.95 ^a	541.78 ^a	71.33 ^a	2.65 ^a	321 ^a	2.62 ^a	8.51 ^a

Averages with the same in each column were non-significant difference at the level of 5 percent

maximum leaf number obtained in treatment of 40% V/V methanol was 541.78 and differences between all treatments were significant. Indeed, we observed a significant increase in the number of leaves after applying methanol (Tables 1 and 2). These results are consistent with previous results on *E. angustifolia* the highest number of leaves in *E. angustifolia* stem is related to foliar application of 40% methanol [9]. Studies show that the foliar application of methanol on tobacco increases the number of its leaves. It seems that this increase is the result of faster growth of the plant due to the foliar application of methanol. By increasing the plant growth, methanol promotes the uptake of nutrients from the soil. The resulting increase in photosynthesis as well as the greater amounts of photosynthetic material in growing leaves increase the number of leaves [10]. Methanol increases the turgor pressure in leaf cells that helps leaf grow and expand, too. Methanol can delay the senescence of leaves by modifying Ethylene production rate and this leads to greater photosynthetic activity of leaves and thus, an increase in the number of leaves [16].

3.5 Stem length

According to the results of the table of Analysis of variance, it is determined that application of methanol at 1% level has a significant effect on the stem length. Based on a test for comparing means in an analysis of variance, it was found that the stem length was affected by methanol: in treatment of control experiment, the lowest stem length was 23.44 cm, while the maximum length of stem obtained in treatment of 40% V/V methanol was 71.33 cm (Tables 1 and 3). The researchers stated that foliar application of 20% methanol increased the height of the safflower plant [17]. Foliar application of 30% methanol increased the height of *Calendula officinalis* L. so that the maximum height of 23.81 cm was obtained from methanol 30% application and the minimum height of 16.66 cm in treatment of control experiment [8]. In a study on *E. purpurea*, the greatest height occurred in foliar application of 40% methanol [9]. Researchers argue that apparently with increase in methanol consumption, the absorption of other nutrients such as nitrogen and phosphorus also increases, as does the production of GA20 and its conversion to GA1 in the plant. This has been determined that GA1 is the only active and effective Gibberellin responsible for increase in stem length; also, the stem elongation is corresponding with the metabolized GA20 on the GA1 path. By affecting the genes responsible for the synthesis of Gibberellin

Methanol affects the Gibberellin. Methanol attracts the areas near the nutrient stems end and also the hormone (such as cytokinin) involved in regulation and division of cell and thus, brings about increase in the stem height [10].

3.6 Total chlorophyll content

According to the results of the table of Analysis of variance, it was determined that treatment by methanol at 1% level has a significant effect on the total chlorophyll content. Based on a test for comparing means in an analysis of variance, it was found that the total chlorophyll content was affected by methanol: treatment of control and treatment of 40% V/V methanol showed the lowest total chlorophyll content (0.4 mg in per gram of fresh weight) and the maximum total chlorophyll obtained (2.65 mg in per g of fresh weight), respectively, indicating a significant differences between these treatments (Table 2 and 3). Foliar application of methanol on wheat plants increases the chlorophyll in leaves and promotes photochemical productivity, leading to greater photosynthesis and plant stomatal conductance which will have a considerable effect on grain yield [14]. In comparing *M. officinalis* and *E. angustifolia*, it was found that the synthesis of chlorophyll in *M. officinalis* increased in conjunction with increasing the concentration of hydro-alcoholic treatment of methanol up to 50%. In the *E. angustifolia*, however, the greatest chlorophyll biosynthesis occurred under hydroalcoholic treatment of methanol with concentration of 30% with higher concentrations of alcohol decreasing the chlorophyll biosynthesis [9]. An explanation for methanol impact on the amount of chlorophyll would be that, in foliar application, sprayed methanol quickly enters plant tissues and alters the carbon metabolism by entering the serine structure of plant, there by affecting the chlorophyll [10].

Dry yield: The results of the table of Analysis of variance determined that treatment of methanol at 1% level has significant effect on the dry yield. Based on a test for comparing means in an analysis of variance, it was found that the dry yield of *A. dracuncululus* L. was affected by methanol: in control experiment, the lowest dry yield was the 110.28 kg per hectare while the highest dry yield of 321 kg per hectare was obtained in the treatment of 40% V/V methanol, indicating significant differences between the treatments and considerable increase in the dry weight of aerial organ when applied by methanol (Tables 2 and 3). The first condition to achieve high

Table 3. Results of variance analysis treatments on examined characteristics of *A. dracuncululus* L.

S.O.V	df	Total chlorophyll content	Dry yield	Essential oil content	Essential oil Yield
Block	2	0.478 ^{ns}	0.601 ^{ns}	4.187 ^{ns}	2.384 ^{ns}
Methanol	4	313.884 ^{**}	166.627 ^{**}	438.373 ^{**}	465.278 ^{**}
Error	8	0.021	373.724	0.014	0.189
CV%		9.84	9.33	7.38	17.07

^{**}, Significant at 0.01 level, ns: non-significant.

yield is the high production of dry matter per area unit since about 90% of dry weight of plants comes from the photosynthetic assimilation of CO₂. The methanol used on tricarboxylic Plants, especially those with high Photorespiration, can compensate some of losses of carbon fixation by photosynthesis, thereby increasing pure photosynthesis per unit area and enhancing the dry yield in tricarboxylic field crops [18]. Therefore, the ways that enhance plant photosynthesis can enhance plant yield as well [10].

3.5 Essential oil content, yield and compounds

Based on the results of the table of Analysis of variance, it was determined that the use of methanol at 1% level had a significant effect on the essential oil content and yield of *A. dracuncululus* L. Based on a test for comparing means in an analysis of variance, it was found that the essential oil content was affected by methanol: in treatment of control, the lowest essential oil content (0.5 mg) and the lowest essential oil yield (0.55 L/ha) were yielded while the greatest amount essential oil content (2.62 mg) and the greatest amount essential oil yield (8.51 L/ha) occurred in the treatment of 40% V/V methanol. In this way, significant differences were observed between all treatments (Tables 2 and 3). Major compounds were found to be Beta-phellandrene, Beta-ocimene, Terpinolene, Methyl chavicol, Trans anethol, Methyl eugenol, 6-phenyl-2, 4-hexadine (capillene). The highest percentages of Beta-phellandrene, Methyl chavicol, Methyl eugenol, 6-phenyl-2,4-hexadine (capillene) were (7.0%, 7.8%, 10.1%, 8.1%, respectively) from control of experiment, while the smallest percentage of those compounds (5.0%, 5.3%, 7.5%, 5.1%, respectively) were associated with foliar application of 30% V/V methanol. On the other hand, the greatest amount of Beta-ocimene, Terpinolene, Trans anethol were (10.4%, 15.0% ,15.7%, respectively) from foliar application of 30% V/V. In case of control experiment, the least amount of those compound were (7.6%, 10.5%, 11.5%, respectively) belongs to the control experiment.

The results of this study matched those of Yazdifar et al. who stated that the use of foliar application of methanol 30% in the *Calendula officinalis* increases the amount of essential oil up to 1.54% [8]. In another research, it was revealed that the use of methanol increases the yield of *Thymus vulgaris* [15]. In comparing *Thymus vulgaris* L. and *Dracocephalum moldavica* L. with the result of this study, it was found *T. vulgaris* L. essential oil compounds were increased at treatment of 50% Methanol, While in *D. moldavica* L. the highest Neral, Geraniol and Neryl acetate content observed from control experiment and applying 30% Methanol increased Geraniol [15,18].

Since the increase in production of secondary metabolites in medicinal herbs has a direct, but non-linear, association with the amount of dry matter of plants and considering that increase in the dry matter of *A. dracuncululus* L. generally occurs in the foliar application of alcohol, then it can be suggested

that use of methanol alcoholic treatment in the foliar application of *A. dracuncululus* L. justifies the larger use of this innovation to enhance field crop yield of this plant. In addition and because of complete metabolization of methanol to its components and its conversion to amino acids in plants, alcohol can be used as one of the satisfactory inputs used in organic or semi-organic farming systems [9].

4 Conclusion

In this study maximum yield achieved with foliar application of 40% V/V methanol. The reason, as stated earlier the alcohol has a nutritional role as a direct source and readily available for carbon. That alcohol bolsters the carbon assimilation and significant effect on the photosynthesis, also the use of methanol has a major role in increasing water efficiency. The greater photosynthesis improves all performance indices of plant. Therefore, to enhance the performance of fresh dry plant and essential oil content of *A. dracuncululus* L. recommend 40% methanol.

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