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Quality and Quantity of Dill Essential Oil as Influenced by Foliar Application of Polyamines

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ABSTRACT

Dill *(Anethum graveolens* L.*)* is a popular herb of the Apiaceae family, widely used in medicine and food industry. The aim of this study is to measure the effects of polyamines on the percent and the compounds of the dill essential oil. This study was conducted in three replications in a Randomized Complete Block Design (RCBD). The treatment compounds were made up of polyamines (spermine, spermidine, and putrescine) at 50, 100 and 150 mg/l concentrations, respectively. The foliar application of polyamines was performed in three replications in the beginning of the flowering stage. It was repeated 14 days apart. Our results demonstrated that the maximum values of dill essential oil (3.58%) were obtained by application of 100 mg/l of putrescine. The dill essential oil compounds include: α-phellandrene (4.03%) was obtained by application of 50 mg/l of putrescine treatment, and the maximum values of limonene (41.63%) were obtained by application of 50 mg/l spermidine treatment. but, the highest values of carvone and trans- dihydrocarone (61.96%) and (5.76%) were observed in the control treatment. The dill essential oil compounds are generally affected by the cultivar, plant type, harvest time, geographical factors, and type of fertilizers. It is also vital to mention that among polyamines, spermidine and putrescine (50 mg/l) at the lowest concentration had the highest influence on the value of the main compounds of dill essential oil.

Keyword: *Anethum graveolens* L., polyamines, essential oil, carvone

INTRODUCTION

Dill *(Anethum graveolens* L.*)* is an important aromatic and medicinal herb and essential spice. The main compounds of dill essential oil are obtained from leaves, stems, flowers, fruits, and seeds. This annual herb belongs to the Apiaceae family and is native to Western Asia and the Mediterranean [1].

Dill is used in traditional medicine [2] and contains essential oils, protein, fat, carbohydrates, carotenoids and minerals [3]. Essential oil produced varies in different tissues of dill [4]. Levels of plant essential oil are affected by climate conditions, harvest time and application of fertilizers making various food elements available to plants which increase production and change the composition of the essential oil [5]. Furthermore, researchers have found that the essential oil properties and its components depend on genetics, climate conditions and topography [6, 7]. Dill essential oil compounds were made up of carvone, limonene, α-phellandrene, dill apiole, p-cymene and linallol, which the highest essential oil compounds contain, α-phellandrene and carvone [8, 9], while presence of carvone causes aroma of the seed [8].

The seed of dill contains carvone, and its leaves and stems contain limonene. The peak in, α-phellandrene occurred immediately after pollination and then decreased in all parts of the plant except leaves, whereas that in limonene occurred when the seeds were about half their full size [10]. The plant's essential oil possesses antioxidants [11] and shows antimicrobial activity [12]. It is necessary to use the best fertilizers to increase the efficiency of medicinal plants because of the usage of micro-fertilizers on the quantity and quality of essential oils and the positive effects of medicinal herbs on human life [6, 7].

Polyamines are low-molecular-weight aliphatic polycations essential for average eukaryotic and prokaryotic growth. The diamine putrescine, the triamine spermidine, and the tetramine spermine are ubiquitous in plant cells, while other polyamines are of more limited occurrence [13]. Polyamines are present in all plant cells, reproductive tissues, and organs such as roots, stems, leaves, and flowers. These compounds are found in seeds, pods, tubers, meristems, wood, phloem, and parenchymal tissues [14]. Researchers found that polyamines bear a positive physiological pH range, so they play essential roles in physiological activities. Polyamine is attached to molecules

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with negative charges to prevent their destruction, like DNA, proteins and phospholipids, membrane oils and pectin polysaccharides. Polyamines also play an essential role in the phosphorylation of lipids and pre-transcriptional changes. The antioxidant and polycationic properties of polyamines contribute to the stability and integrity of the cell membrane. Polyamines with ethylene have a precursor called -S-adenosylmethionine with ethylene, which competes with ethylene synthesis. Another effect of them as anti-aging and anti-stress compounds are related to their effect in preventing free radicals. These compounds are considered electron donors. They also have positive charges to create free radicals to prevent the accumulation of these harmful compounds that accelerate aging and create cell tension. [15].

By considering the positive effects of polyamines on plants' quantitative and qualitative performance, we will study the effect of foliar spraying with different levels of spermine, spermidine and putrescine on the percent and compounds of dill medicinal plant essential oil.

MATERIAL AND METHODS

This experiment was conducted at the Research Farm and Laboratories of the Islamic Azad University, Isfahan (Khorasgan) branch, Iran (latitude 32° 40' N, longitude 51° 48' E). The analysis of soil's chemical and physical properties is presented in Table 1.

Table 1 Physical and chemical characteristics of the soil of the test site.

Soil texture	Silt (%)	Sand (%)	Clay (%)	SP (%)	Nitrogen (%)	Gypsum (%)	Lime (%)	Organic Carbon (%)	pH	Ec (ds/m)
Silty Clay	46	10	44	59	0.06	0.025	38.4	0.55	7.5	5.8

Experimental Materials

Seed samples of the plant were planted in the plots of 2×1 meters with ten rows and 0.5 meters apart. Seeds were planted after plotting and other necessary operations. Plants were irrigated in the drip irrigation system. Weeds were controlled physically, and no herbicides were applied. The treatment compounds were made up of polyamines (spermine, spermidine, putrescine) at 50, 100 and 150 mg/l concentrations, respectively, and control treatment (without foliar spraying) were performed in three replications. The foliar application of leaves was performed at the beginning of the flowering stage and every 14 days apart. The experiment was conducted at dusk because polyamines are sensitive to the light. At full physiological maturity, 1 m^2 middle rows of each plot were harvested to determine the sample.

Essential Oil Extraction

The samples cut from the plant were dried in shadow to keep the quantity and quality of their essential oil. Then, in each replication, a 60-gram sample was selected. The essential oils were extracted with water distillation method by Clevenger type apparatus for 4 hours (16).

GC and GC/MS Analysis

Essential oils were injected into a gas chromatography system (GC) connected to Trace MS Model mass spectrometer (GC-MS) to analyze the oil samples and identify their constituents. The gas chromatography system (Aglinet Technology), 7890A Model with HP-5, a column 30 m long with a 0.32 mm internal diameter and 0.25 µm of statistic phase thickness. The temperature was 60 °C. The temperature was raised to 21 °C and 24 °C with gradients of 3 °C and 2 °C, respectively. The experiment was done in 60 minutes. The ionization was 70 eV, electro ionization, and the ion source temperature was 320 °C. The connected line and injection part temperatures were 28 °C and 280 °C, respectively. Helium carrier gas created by the process of decay had a flow rate of 1.1 mm/min, at the rate of 1:50 and was between 50 and 480 in mass points. The oil standard constituents were identified by comparing obtained spectrums with mass spectrums obtained from Wiley and NIST libraries [17, 18].

Data Analysis

Relative percent of each compound was calculated from chromatography by considering the area under the curve. The statistical design was completely randomized blocks design (RCBD). Data were subjected to analysis in SPSS. Duncan's multiple range test identified the mean of data comparison. It was done in 1% and 5% levels.

RESULTS AND DISCUSSION

Finally, 30 dills seed essential oil compounds were identified from each treatment. The main compounds in highest values were: α-phellandrene, limonene, tras-dihyarocarvone, Carvone and Dill Apiole (Table2).

Table 2 Dill essential oil compounds in the GC/MS Analysis.

Row	Name (%)	Compounds (%)	RI[a]
1	α-Thujene	0.03	925.9
2	α-Pinene	0.18	932.9
3	Camphene	0	947.9
4	Sabinene	0.03	972.9
5	β-Pinene	0.03	976.9
6	Myrcene	0.28	990.8
7	α-Phellandrene	4.67	1004
8	α-Terpinene	0.02	1018
9	p-Cymene	0.22	1026
10	Limonene	24.09	1028
11	β-Phellandrene	0	1030
12	(Z)-b-Ocimene	0.01	1.37
13	Benzene acetaldehyde	0.01	1043
14	(E)-b-Ocimene	0	1047
15	γ-Terpinene	0.01	1057
16	p-Cymenene	0.11	1089
17	n-Nonanal	0.05	1104
18	trans-Limonene oxide	0.05	1137
19	Dill ether	0.43	1185
20	cis-Dihydro carvone	0.9	1196
21	trans-Dihydro carvone	0.89	1203
22	cis-Carveol	0.09	1228
23	Unknown	0.16	1238
24	Carvone	38.92	1243
25	(2E,4E)-Decadienal	0.02	1316
26	cis-Carvyl acetate	0.01	1363
27	Germacrene D	0.02	1482
28	Myristicin	1.54	1520
29	Dill apiole	22.95	1624
30	Apiole	0.01	1679

RI[a]=Retention index

The highest limonene amount (41.63%) was observed (50 mg/l) in spermidine and the lowest (16.87 %) in control treatment. Also, the highest amount of α-Phellandrene and Dill Apiole which observed in (50 mg/l) in Putrescine treatment, were (4.03%) and (19.01%), respectively. The highest carvone, (61.96%) and trans dihyarocarvone (5.67%) amounts were observed in control and their lowest value, (38.67%) and (3.27%) were observed in (150 mg/l) putrescine and (50 mg/l) Spermidine treatment (Table 3). Carvone is the most abundant compound in dill essential oil. Its level is different in the flowering and seed-filling stages [19]. The maximum and minimum levels of carvone in essential oil compounds reported (73.61%) and (1.68%), respectively [20].

Mostafavi and et al. studied the (*Lavandula angustifolia*) and found that the substance resulting from the catabolism has a significant role in proline synthesis, not in the biosynthesis of trines. They claimed that Polyamines had no critical role in the plant's essential oil [21]. On the contrary to earlier studies, the results showed that growth regulators and essential oil compounds had a positive effect. However, the polyamine

treatments had little effect on the essential oil compound [22, 23]. Hence, the researcher found a significant relationship between the main compounds of essential oil and the treatment with polyamides (24). They found that the foliar application of the putrescine on basil *(Ocimum basilicum L.)* increased the amount of Thymol but decreased parasimoun and ˠ-Terpinene compared to the control treatment. Thymol, parasimoun and ˠ-Terpinene are progenitors and intermediate compounds, but putricine decreased the amounts of these compounds to increase the thymol [25].
The increment in oil yield might be due to the increase in vegetative growth, nutrients uptake or changes in leaf oil gland population and monoterpenes biosynthesis (26). Because polyamine, ethylene, putrescine, and spermidine have a similar precursor for the generation of SAM, the biological activity of ethylene is antagonistic with polyamines, and the production of one is deterrent to the other (15). The amount of essential oil in the leaves was increased by a high-concentration spray of putrescine solution. This could be related to the influence of putrescine on the strength of leaf cell membranes. (27). A direct relationship between total PAs. and K and inverse relationship between PAs. and Na as well as between K and Na were reported by Previous researchers (28), and they recorded that PAs. as spray application increased some nutrients elements particularly K, which was found to serve a vital role in photosynthesis by directly increase growth and photosynthetic pigments, and hence CO_2 assimilation (29). The increments in minerals uptake (N, P and K) by PAs. treatment, the promotive effect of PAs. treatment on plant growth productivity as well as on plant chemical composition may due to their effect on many metabolic and physiological processes (30). PAs. have possibly increased activities of metabolic processes in plant. Accordingly, physiological performance of such plants was improved, as manifested by increased efficiency of roots in absorbing macronutrients from the soil (31). Moreover, levels of plant essential oil are affected by climatic conditions, harvest time and extraction time [32]. The rise in temperature and solar radiation has negative effects on the chemical compounds of the plant, so it decreases the essence, vitamins and other compounds [33]. Researchers believe that dill essential oil's content and composition differ widely with the part where the essential oil is distilled from, harvesting date, cultivar, growth conditions, geographical origin, maturity status and storage conditions [34]. Genetic factors, climatic conditions, and cultural practices are some possible causes for this diversity in dill essential oil [35]. In an experiment, analysis of essence of 18 local dill mass (in Iran) showed that the values of dill essence vary between 0.3% in Maragheh and 0.2% in Khoramabad. Values of carvone and dill apiole vary between 31.3% (Tehran) and 60.8% (Taghmaie) and between 0.2% (Brigand) and 31.9% (Tehran), respectively. Values of trans-Dihydrocarvone vary between 3.6% Khomein paeenband and 14.5% of brigand and 6.6% Khoramabad, and finally, values of Dihydrocarvone vary between 0.3% and 4.3% for Qom [35].
In another experiment, the application of putrescine 20 mg/L significantly increased essence percentage in *(pelargonium graveolens* L.*)*, but in 10 mg/L and 40 mg/L concentrations of putrescine it decreased greatly. Citronellol and Linallol were the main components of essence had a significant increase in 20 mg/L putrescine treatment, but the increased value of Geraniol was a little [36]. The results of a research showed that polyamines in the highest and lowest concentrations had less function in comparison to the optimum concentration. The negative effects of high-concentration polyamines show that a defined level of endogenous polyamines is an essential part of seed growth and germination (37). Additionally, polyamine in high and low concentrations is toxic and clean, respectively. These biophysical effects are related to membrane structures and nucleic acids [38]. The exogenous polyamines increase the function of oils in aromatic plants, but they may affect the accumulation and composition of essential oils differently [24]. The results showed spermine treatments could not obtain the highest values of the main components. The lowest value of α-Phellandrene, 2.73%, was observed (50 mg/L) in spermine treatment, but the value of limonene and dill apiole in (50 mg/L) spermine treatment increased by19.1%, 1.1% in comparison to the control treatment. In fact, the increase in the concentration of spermine causes an increase in α-phellandrene and trans-Dihydrocarvone (Table 3).
In comparison to spermine, spermidine and putrescine had a great effect on the value of α-Phellandrene, limonene and dill aopiol at the minimum concentration. The highest values of α-Phellandrene (4.03%) and dill aopiol (19.01%) were in 50 mg/l the Putrescine treatment, and by comparing them to control treatment; a (1.3%) growth in α- Phellandrene and (1.8%) in dill apiole was observe. Moreover, the maximum level of limonene 41.63% was in 50 mg/l spermidinem, in comparison to the control treatment, it had a 2.4 % growth. Polyamines had affected

on limonene, dill aopiol and α-flandrene. The most effects of polyamines on the volatile oils of the samples were on limonene, dill aopil and α- Phellandrene. They were (41.63% - 16.87%), (8.34% - 19.01%) and (2.73% - 4.03%), respectively.
In the spermidine application, the limonene values decreased by increasing concentration, but the value of carvone increased. In all putrescine treatments, the value of limonene increased with the increase in concentration, but the value of trans-dihydrocarvone decreased. Researchers found that the flavor of dill herb oil is due to α-Phellandrene, limonene and dill ether [39].
The highest value of α- Phellandrene is in dill leaves, the lowest levels are in flowers and the lowest values are in fruits [40]. This shows that the α- Phellandrene values increase during vegetative growth and decrease in the production phase. This study reported a minimal value of limonene in dill seed [34]. This study showed that the highest percentage of essential oil (3.58%) was observed in (100 mg/l) putrescine treatment. Then, the highest value of the essential oil (3.02%) was observed in (150 mg/l) putrescine treatment. The lowest value of the essential oil (1.97%) was observed in the control treatment. The value of essential oil increased by 1.8 % in the treatment of putrescine 100 mg/litre compared to the control plant. Recent studies showed the percentage of dill seed essential oil was between 0.2 and 4.6 [41].
Nitrogen and phosphorus are necessary to form terpenoid essential oil compounds [42]. An increase in the percentage of essential oil is related to the functions of polyamines, so it affects the compounds to increase the lifetime related to the production of terpenoids and other hydrocarbons in the essential oil of the medicinal basil plant and the effect of these compounds in the production of isoterpenoids [43]. In research conducted on chamomile *(Marticaria chamomile* L.*)*, the main components of its essential oil, including bisabolol oxide B, chamazulene, and bisabolol oxide A, increased after the application of spermidine. They were increased to 79.3%, 18.5%, and 83.1%, respectively [44]. Studies showed that the putrescine treatment on *(Thymus vulgaris L.)* was 0.2 mg/l, at the highest level, it reduced the harmful effects of dryness improved the percentage of essential oil [45]. In comparison to the control plant, applying 0.1 mg/l and 1 mg/l of putrescine increased the percentage of leaf essential oil in *(Menta piperita* L.*)* to 12.82% and 21.36% [46].

Table 3 The main components of dill essential oil as influence by foliar application of polyamines.

Treatment	RT[a]	α-Phellandrene	RT	Limonene	RT	Trans-dihydrocarvone	RT	Carvone	RT	Dill apiol
Spermine 50 mg/l	1002	2.73	1029	32.3	1204	3.52	1242	46.32	1625	12.6
Spermine 100 mg/l	1003	3.13	1029	33.02	1204	3.65	1242	46.13	1625	11.28
Spermine 150 mg/l	1003	3.75	1029	32.59	1204	4.65	1242	44.65	1625	11.69
Spemidine 50 mg/l	1002	3.74	1029	41.63	1204	3.27	1242	40.18	1625	8.69
Spemidine 100 mg/l	1003	3.69	1029	37.53	1204	4.64	1242	43.07	1625	8.34
Spemidine 150 mg/l	1003	3.67	1029	32.91	1204	3.44	1242	44.4	1625	12.63
Putrescine 50 mg/l	1002	4.03	1028	26.65	1204	4.32	1242	40.2	1625	19.01
Putrescine 100 mg/l	1002	3.45	1029	32.15	1204	3.85	1242	45.56	1625	12.52
Putrescine 150 mg/l	1002	3.85	1029	35	1204	3.79	1242	38.67	1625	15.66
Control	1003	2.93	1027	16.87	1204	5.76	1242	61.96	1625	10.55

RT= Retention time

By increasing the synthesis of growth accelerators such as oxygen, gibberellin and cytokinin, putrescine accelerates the growth of the plant and increases vegetative performance, so it increases the function of the essential oil in the plant [47]. *(Valerian officinalis* L.*)* treated by polyamines, the essential oil function, spermidine, putrescine and spermine increased by 18%-20%-19%, respectively. Putrescine was the most effective. The improvement of essential oil because of polyamines is related to root growth [48]. Putrescine application improves the value of essential oil in *(Mattiola incana* L.*)* Using growth regulators is an excellent way to increase the quality of medicinal plants economically [49]. The highest value of the essential oil components in (*Salvia officinalis*) was 1.8cineol, α-thujon and β-thujon, which was obtained with 1.5 putrescine under 20% irrigation 60% available soil water depletion (ASWD). The highest concentration of camphor using 0.2 mM putrescine and distilled water was 20% and 60% ASWD [50]. Using putrescine in (*Marticaria chamomile* L.) in optimal humidity conditions led to a 38% increase in essential oil content, but it increased only 4% in dry conditions. A crucial essential oil compound was basibolone oxide A. The treatment with putrescine had a positive effect on it, so its concentration increased to 87% in favourable humid conditions [51].
The main essential oil compounds are terpenioid compounds, and nitrogen is their precursor. Putrescine plays an important role in increasing the absorption of some food components like nitrogen and has an essential role in the synthesis of sugars and carbohydrates, causing a significant increase in the function of the essential oil compared to the control [25]. The use of different concentrations (50 and 100 mg/L) of putrescine on the medicinal plants *(Nepta Cataria)* in vitro culture causes an increase in the production of fineroots and the production of rosmarinic acid. The highest production of fineroots and rosmarinic acid was obtained in 50 mg/L putrescine treatment [52]. These research findings were similar to the results of the current research on the positive effects of putrescine on dill essential oil. El- Ghorab et al. [53] pointed out that in 200 mg/L putrescine treatment, the value of hexadecanoic acid in the essential oil of *(Dianthus caryophyllus* L.*)* increased strongly. By applying 400 mg/L putrescine, a great reduction in hexadecannoic acid was observed. They also noted that the change in the components of clove oil was caused by putrescine and its synergetic. This change resulted from an increase in the biosynthesis of terpenes or the indirect effect of the metabolism of carbohydrates. Growth regulators affect oil performance. It is due to their effect on the enzymatic terpenoid biosynthesis [54]. They can also affect essential oil production through their effect on plant growth, biosynthesis and the number of storage structures in it [55].

CONCLUSION

Generally speaking, this study shows that the use of putrescine and spermidine polyamines at the lowest concentration had the most excellent effect on the main components of the essential oil, such as dill aopiol, limonene and α-Phellandrene. The highest value of essential oil was observed in 100 mg/litre of the putrescine treatment. On the other hand, the highest value of carvone and trans-dihydrocarone was obtained in the control treatment. The essential oil levels are affected by climate, harvest time, and the use of different types of fertilizers, which make different elements available to the plant and cause an increase in production or changes in the composition of essential oils; therefore, the use of polyamines can cause an increase and can change the number of main compounds and the percentage of dill essential oil.

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