

# Effects of Methanol Sprays on Essential Oil and Phenol Yields and Vegetative Characteristics of Lavender

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**Abstract** – An experiment in the form of single factor ANOVA was conducted to study the effects of spraying methanol on essential oil yield and vegetative characteristics of the medicinal plant lavender. The treatments included three levels of methanol (Zero percent or C, the control), 10% (M10), and 20% (M20), and 40% (M40) methanol. A Clevenger-type apparatus extracted the Essential oil. Results showed the highest essential oil yield (1.4%) the maximum wet and dry leaf weight (4.2 and 16 grams per plant, respectively), and the tallest plants (24.56 centimeters) were obtained in the M20 treatment. There were significant differences among the treatments in the length and width of leaves, with the largest leaf length and width belonging to the M20 treatment. The maximum leaf surface areas were observed in the M10 and M20 treatments with 124.6 and 128.6 square centimeters, respectively. The highest dissolved leaf phenol content ( $171.5\mu\text{g}\cdot\text{g}^{-1}\text{fw}$ ) was also observed in the M20 treatment. Therefore, it can be concluded that methanol application increased essential oil yield and improved the measured vegetative features, that the M20 treatment had greater stimulating effects compared to the other treatments, and that the effects of the M40 treatment were not significantly different from those of the control.

**Keywords** – Essential Oil, Lavender, Methanol, Vegetative Growth.

## I. INTRODUCTION

Lavender (*Lavandula officinalis* L.) belongs to the Lamiaceae family and the *Lavandula* genus [22, 14], and is a small, evergreen, perennial, and ligneous tree that grows densely with woody and vertical branches that are without leaves on the lower parts. The main root is long and woody with many dense branches. This plant is a native of Europe [14]. The thick stems are brown and herbaceous and sometimes reach a length of up to one meter. The leaves are opposite, long and narrow, dark green, and covered with pubescence [1, 2, 14]. The foliage has a pleasant smell that results from the presence of essential oil [5]. The very fragrant violet-blue flowers grow in clusters on the tips of stems [17]. The essential oil is produced and stored in leaves, flowers, and special cavities, and the essential oil of the flowers is of better quality compared to that of the leaves [23]. Lavender has analgesic, anti-spasmodic, and tranquilizing properties [3, 19, 21]. The essential oil is obtained by distillation of flowering twigs, especially by distillation of flowers, and contains more than 40 different compounds the most important of which are linalyl acetate, cineole, linalool, terole, and borneol. There are also compounds such as butyric acid, propionic acid, and valeric acid, free linalool,

flavonoid (pigments), tannin, phenolic compounds, and coumarin in lavender essential oil [4, 7].

Methanol, the simplest of the alcohols, increases crop plant yield. The first requirement for achieving high yield per unit area is the production of large quantities of dry matter because about 90% of the dry weight of plants is contributed by assimilation of  $\text{CO}_2$  through photosynthesis. Therefore, increasing the rate of  $\text{CO}_2$  fixation can be useful for raising production capacity of crop plants [6, 15]. The most important benefit of these compounds is the inhibition, and reduction, of photorespiration induced in stressed crop plants [6, 9, 10, 15]. It was first reported in the 1990s that application of methanol solutions on aerial parts of crop plants increased yield, accelerated maturity, reduced the effects of stresses, and lowered their water needs [20]. Considering 25% of plant carbon is used in photorespiration, photorespiration can be minimized with methanol spray [25]. The reason for this is that plants absorb methanol and rapidly metabolize it to carbon dioxide in their tissues because methanol molecules are small compared to  $\text{CO}_2$  molecules [24]. Rajala et al (1998) attributed the reduction of photorespiration in plants treated with methanol to the rapid oxidation of methanol to carbon dioxide, to  $\text{CO}_2$  reaction with ribulose-1, 5-bisphosphate carboxylase, and to reduced competition exhibited by oxygen. Makhdoom et al (2002) reported that spraying cotton with methanol in arid regions of Pakistan increased plant height and cottonseed yield. Mauney and Gerik(1994) reported that applying methanol on cotton in the Unites States increased cotton yield by 56 percent, and that leaf surface area and leaf diameter also increased with methanol application. Hernandez et al. (2000) reported that spraying with methanol increased stem length, leaf surface area, stem dry weight, and the number of florets of sunflower plants. Ramirez et al. (2006) reported that methanol spray increased wet weight of tobacco plants. They showed that methanol delayed leaf senescence and increased photosynthetic activities in leaves, which led to higher yields. Nadali et al (2010) reported methanol application increased sugar beet yield. One strategy for increasing  $\text{CO}_2$  concentration in plants is to use compounds such as methanol, ethanol, propanol, butanol, or to apply substances like the amino acids glycine, glutamate, and aspartate [27]. Nanamura and Benson (1992) showed that methanol increased yields of crop plants treated with it. They stated that application of methanol on the aerial parts of crop plants increased their yields, accelerated their maturity, reduced the effects of stresses, and lowered crop water requirements. They said methanol influenced the

photorespiration pathway and disrupted it. Research by Heming et al (1995) suggested that when pepper and tomato leaf tissues were exposed to methanol, their efficiency in converting carbon and the thermal effects on their metabolic rate increased. Increased photosynthetic efficiency and greater plant resistance to stresses are very important, especially in medicinal plants. Researchers have expressed that application of methanol can influence plant growth and metabolism, and that determination of optimal concentration of applied methanol is important too.

Lavender has many applications in various industries including pharmaceutical, cosmetics-health, and food industries, and there is a high demand for the compounds this plant contains. Therefore, extensive research is conducted on increasing the essential oil content of lavender, and that is why it was important to conduct this study.

### III. MATERIALS AND METHODOLOGY

This research was conducted at the greenhouse belonging to the Islamic Azad University of Garmsar in 2013. Uniform, herbaceous, rooted cuttings of lavender were used as the plant material. The six-month-old, 10-centimeter tall rooted lavender cuttings were planted in plastic pots. The experiment was conducted using the randomized complete block design with three replications. Plants were sprayed with three different methanol concentrations: Zero percent (the control or C), 10% (M10), 20% (M20), and 40% (M40). They were sprayed five times at weekly intervals. One month after the last spray, plants were harvested and analyzed.

#### *Measuring plant growth and yield*

Plant wet weights were measured by a digital balance. The samples were then dried in an oven at 70°C for 72 hours and reweighed using the digital balance. Leaf surface area was determined by millimeter graph paper, and plant height, length, and width were measured with a ruler. SPSS-11 software was used to analyze the collected information. The information was analyzed using the single factor ANOVA, and Duncan's test was employed for comparison of the means.

#### *Assessing soluble phenolic compounds in leaves*

Folin Reagent (yellow colored), which is available in commercial form, was needed for this assessment. This reagent must be kept away from light. To assess soluble phenolic compounds in leaves, one milliliter of the extract was added to one milliliter of the 50% Folin Reagent and two milliliters of saturated (21%) sodium carbonate and the mixture was left undisturbed for 10 minutes. Appearance of blue color indicated the reaction had taken place. Then the mixture was centrifuged at 12000 rpm for 15 minutes, and absorption rates at wavelength of 760 nanometers were read by a spectrophotometer. A mixture of one milliliter of the extract and one milliliter of the 50% Folin Reagent and two milliliters of saturated sodium carbonate was used as the blank sample. The equation of the standard curve was derived to determine the concentration of soluble phenolic compounds in leaves. A

phenolic compound named tannic acid was used as the standard compound. Using absorption rates and concentrations of the standard samples, the standard curve was drawn and used to calculate soluble phenolic compounds and, finally, concentrations of phenolic compounds were calculated, and reported, in micrograms per gram fresh weight ( $\mu\text{g g}^{-1}\text{fw}$ ).

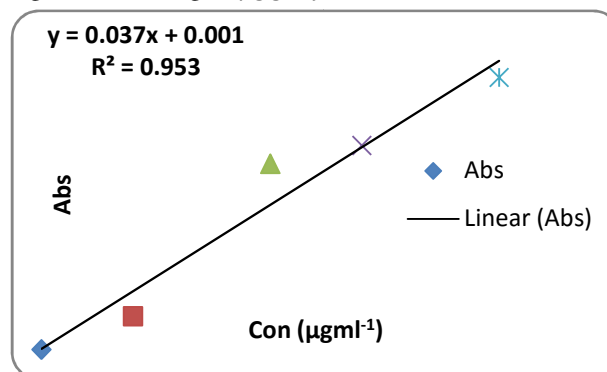


Fig.1. Standard curve for assessing phenolic compounds based on the folin method

The essential oil content was measured after grinding the sample with a Clevenger-type apparatus.

### IV. FINDINGS AND DISCUSSION

Results of this experiment conform to those presented by some researchers, to some of whom we will refer below. Makhdoom et al (2002) reported that cotton leaf surface area increased with methanol application, leaves became 20 to 50 percent thicker, plant height increased, and plant growth rose by 50 percent. Favero et al (1996), who worked at a large Finnish company that used methanol to increase growth, and improve quality, of roses planted in a greenhouse, stated that repeated spraying of methanol on roses reduced the number of applications of fungicides for controlling powdery mildew.

Therefore, applying compounds (such as methanol) that can increase carbon dioxide concentration in plants stabilizes yields of crop plants. One strategy for increasing  $\text{CO}_2$  concentration in plants is application of compounds such as methanol, ethanol, propanol, butanol, and of substances like the amino acids glycine, glutamate, and aspartate [27]. Methanol, the simplest of the alcohols, is oxidized by oxygen, with the help of sunlight, and is converted to water and carbon dioxide. Methanol increases yields of crop plants, and the first requirement for achieving high yield is to have a large quantity of dry matter produced per unit area because about 90% of the dry weight of plants is contributed by assimilation of  $\text{CO}_2$  through photosynthesis. Therefore, increasing the rate of  $\text{CO}_2$  fixation can be useful in raising production capacity of crop plants [6, 15]. Nanamura and Benson (1992) showed that methanol increased yields of crop plants that were treated with it. They stated that methanol application on the aerial parts of crop plants increased yield, accelerated maturity, reduced effects of drought stress, and lowered crop plant water requirements. They said

methanol influenced and disrupted the photorespiration pathway. In another experiment, spraying with methanol increased stem length, leaf surface area, stem dry weight, and the number of florets in sunflower plants [11]. Spraying with methanol increased leaf surface area, and this raised photosynthesis rate and, finally, resulted in greater yield of cottonseed [15].

According to reports by Zebic et al (1999), increased CO<sub>2</sub> concentration can offset effects of environmental stresses. Nadali et al (2010) reported methanol spray increased sugar beet yield. Nanamura and Benson (1992) showed that methanol increased yields of crop plants sprayed with it. They stated that applying methanol on the aerial parts of plants increased yield, accelerated maturity, reduced the effects of drought stress, and lowered crop plant water requirements. Heming et al (1995) demonstrated that sufficient CO<sub>2</sub>, provided through the application of methanol, changed the photorespiration pathway from a catabolic reaction to an anabolic one. Nanamura and Benson (1992) showed that, after spraying with methanol, plants must be exposed to sunlight for the induction of photorespiration; otherwise, they might suffer leaf damages. Lee et al (2006) reported methanol spray increased photosynthesis in sugar beet leading to a 10% increase in yield.

## V. CONCLUSION

The main purpose of this research was to study the effects of spraying methanol on the yield of essential oil, on the content of phenolic compounds, and on vegetative features of lavender. Results showed that spraying with methanol, especially at the concentration of 20%, stimulated plant growth. Increased wet weight, dry weight, leaf surface area, plant height, soluble phenolic compounds in leaves, and essential oil yield in samples sprayed with methanol showed that methanol stimulated plant growth. However, the greatest plant stimulation was observed in the M20 treatment, while the M40 treatment did not exhibit considerable effects. Possibly, stimulation of photosynthesis stimulated production of compounds and, hence, leaf surface area, wet weight, and dry weight increased.

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**Table 1: Analysis of Variance Traits**

sov	df	MS								
		Leaf fresh mass	Leaf dry mass	Total dry weight of leaf	Height	Leaf length	Leaf width	Leaf area	phenol	Total fresh weight of leaf
Methanol	3	.004**	.000**	.813**	17.641**	1.431**	.509**	171**	1267.17**	15.790**
Error	8	.000	9.000E-06	.029	.691	.045	.008	7.250	26.625	.400

Ns, \*, \*\*: non significant, significant at  $p < 0.05$  and  $p < 0.01$ , respectively

**Table 2: Mean values of different traits in different treatment groups and comparison of the means based on Duncan test**

T	Leaf fresh mass (g)	Leaf dry mass (g)	Total fresh weight of leaf (g plant <sup>-1</sup> )	Total dry weight of leaf (g plant <sup>-1</sup> )	Height(cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (mm <sup>2</sup> )	Leaf soluble phenol ( $\mu\text{g g}^{-1}\text{fw}$ )	Essential oil (%)
C	0.21 <sup>c</sup>	0.07 <sup>d</sup>	10.6 <sup>d</sup>	3.02333 <sup>d</sup>	18.83333 <sup>c</sup>	3.16 <sup>b</sup>	3.96 <sup>c</sup>	112 <sup>b</sup>	125 <sup>c</sup>	0.8166 <sup>c</sup>
M10	0.2533 <sup>b</sup>	0.083444 <sup>b</sup>	13.2 <sup>b</sup>	3.816667 <sup>b</sup>	22.16667 <sup>b</sup>	3.6233 <sup>a</sup>	4.333333 <sup>b</sup>	124.6667 <sup>a</sup>	148.25 <sup>b</sup>	1.1166 <sup>b</sup>
M20	0.3 <sup>a</sup>	0.092889 <sup>a</sup>	16 <sup>a</sup>	4.256667 <sup>a</sup>	24.56667 <sup>a</sup>	3.7766 <sup>a</sup>	4.866667 <sup>a</sup>	128.6667 <sup>a</sup>	171.5 <sup>a</sup>	1.4366 <sup>a</sup>
M40	0.236667 <sup>b</sup>	0.076111 <sup>c</sup>	12 <sup>c</sup>	3.49333 <sup>c</sup>	20.66667 <sup>b</sup>	3.216667 <sup>b</sup>	4.033333 <sup>c</sup>	116.6667 <sup>b</sup>	132.5 <sup>c</sup>	0.9936 <sup>c</sup>