Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 3 [12] November 2014: 193-196

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

Investigating the Effects of Methanol on Biochemical Indices of Lavender

Hamid Reza Bagheri[1*], Ali Reza Ladan Moghadam[2], Hossein Afshari[1]
1. Department of Horticulture, Damghan Branch Islamic Azad University, Damghan, Iran.
2. Department of Horticulture, Garmsar Banch Islamic Azad University, Garmsar, Iran.
[1*]-Email:hamid_130654@yahoo.com

ABSTRACT
A study was done in 2012 to investigate the effects of methanol spray on biochemical traits of lavender. This experiment was carried out using one-way ANOVA. Treatments included 3 levels of methanol 0 (control or c), 10% methanol 10% (M10), methanol 20% (M20), and methanol 40% (M40). The application of methanol spray was done five times, once a week with one week interval. Regarding the comparison of means tables, the highest of total chlorophyll of M20 treatment was 379 mgg^{-1}fw. With respect to chl a and chl b, M20 treatment had the highest level with 246 and 133 mgg^{-1}fw, respectively. In addition, both M10 and M20 accounted for the highest of carotenoids. With respect to the leaf sugar soluble, M20 showed the highest amount with 196.5 μgg^{-1} fw. Regarding the analysis of variance table, a significant difference was observed in all measured factors at statistical level of 1%. Therefore, it can be concluded that the application of methanol on the measured traits was effective, in that concentrations of 10% and 20% had stimulating effects. On the other hand, the concentration of 40% did not show significant difference between experimental and control groups. Moreover, the concentration of 20% was the most effective treatment.
***Keywords**: Lavender, Methanol, Biochemical traits*

Received 11.08.2014 Revised 27.09.2014 Accepted 01.11. 2014

INTRODUCTION

Lavender (scientific name *Lavandula officinalis* L) is a perennial, soilage, and evergreen plant from lamiaceae family [14]. Its main root is long and wooden with several dense branches. It can uptake moisture from depth of 3 to 4 meters. It is native to Europe with a thick and brown stem [21]. The leaves are opposite, narrow, long, spear-like, and dark green covered in indumentum [1,3] with special scent [3,14]. Vegetative body of the plant has a pleasant smell from its essential oil [6]. Essential oil are produced and stored in the leaves and flowers of lavender and in certain cavities of it. In comparison to the leaves, flowers produce higher quality of essential oil, whose amount (between 0.5 to 1.5 percent) depends on the type of plant and climatic conditions. Lavender has analgesic, antispasmodic and calming effects [5, 18, 20]. Flavonoids (pigments), tannins, and coumarin can be found in the essential compounds of aerial organs of lavender [4, 8].
Methanol is considered as the simplest of alcohols. It increases the yield of plants and is the first prerequisite for achieving high yield at high dry matter production level, since about 90% of the plant dry mass is because of photosynthetic assimilation of CO_2. In result, increasing the rate of CO_2 fixation can be suitable for boosting crop production capacity [7,15]. The most important advantage of consuming these compounds is to prevent and reduce photorespiration caused by stresses induced to crops (7,15, 10). It was first reported in the 90s that the application of methanol solutions on aerial parts of crops causes higher yield, accelerates ripening, decreases stress, and reduces water requirement (19). Regarding that 25% of plant's carbon is consumed by photorespiration, the application of methanol spray can minimize photorespiration [25]. That is because methanol is absorbed in plant and rapidly metabolized to CO^2 in plant tissue [23], which is due to smaller size of methanol rather than CO^2. Rajala et al (1998) attributed reduced photorespiration in the methanol treated plants to rapid oxidation of methanol to CO^2, its combination with ribulose-1,5-bisphosphate carboxylase, and reduced oxygen competition.

Nadali et al. [26] reported increased yield via the application of methanol spray on sugar beets. One way to increase CO_2 concentration in the plants is using such compounds as methanol, ethanol, propanol, and butanol, as well as using amino acids like glycine, glutamate and aspartate. Studies by Nonomura and Benson (1992) showed that methanol caused increased yield in plants treated with this material. They reported that the application of methanol on aerial parts of crops increases the yield, accelerates ripening, decreases the effect of drought stress, and reduces water requirement. They referred to the action of methanol as an inhibitor of photorespiration pathway. An investigation by Heming et al (1995) showed that increased carbon conversion efficiency and thermal metabolic rate in pepper and tomato were due to exposure of leaf tissue to methanol.

In terms of frequency and functional significance, chlorophylls are the most important pigments existing in chloroplasts. There are two types of chlorophyll (i.e. a and b) in higher plants, which are accompanied with several "auxiliary" pigments. The auxiliary pigments include such carotenes as beta-carotene and xanthophyll, which are responsible for transferring energy to chlorophylls or have regulatory and protective roles.

Increased photosynthesis yield and enhanced plant resistance against stress are very important, especially with respect to herbs. This is suggested that the application of methanol can affect the growth and metabolism of the plants. In addition, determination of the optimal concentration is significant. Due to the vast application of lavender in different industries such as pharmaceutical, cosmetics and health food, and the high demand for its containing materials, a large amount of attempts is done to increase its essential oil and other effective matters. This is the important aspect of the present study.

MATERIALS AND METHODS

Plants were cultivated in plastic pots. Same cuttings were taken. The six-month lavender plants were treated three times in randomized complete block design. Lavender plants were treated with three different methanol densities including 0 (control or C), methanol 10% (M10), methanol 20% (M20), and methanol 40% (M40). Methanol spraying was done 5 times, each with one week interval. One month after the last treatment, samples were taken and analyzed.

In order to measure leaf chlorophyll content, Arnon's technique [2] was employed. A number of the leaves was removed and cut into pieces. They then were homogenized in a porcelain mortar with 80% cold acetone. After that, it was smoothed using Whatman filter paper. After smoothing with 80% acetone, all samples were homogenized (in terms of volume). Dilution of the extract was done using 80% acetone. Absorption of the solution was measured at 645 and 663 nm wavelengths for the estimation of chlorophyll. Finally, chlorophyll content was measured using below formula in milligrams per gram fresh weight ($mgg^{-1}fw$) or micrograms per gram fresh weight ($\mu gg^{-1}fw$).

Measurement of soluble sugars via Kochert method

For this, 0.2 g of fresh plant was first homogenized with 10ml 80% ethanol and then heated in water bath for 15 minutes. Tubes' contents were smoothed using filter paper. To remove chlorophylls, 0.5ml hydroxide barium 0.3 N and 5ml zinc sulphate (5%) were added. Above solution was centrifuged at 12000g for 15 minutes. Five samples were removed from supernatant of the solution. To each solution, 1 ml 5% phenol and 5ml concentrated sulfuric acid were added (the reaction is exothermic). Samples were placed at laboratory temperature for 30 minutes. Then, their absorption capacity was read using spectrophotometer at 485 nm. The monosaccharide glucose was used as the standard. Sample sizes were calculated using standard curve based on micrograms per gram fresh weight ($\mu gg^{-1}fw$).

For data analysis, SPSS11 was employed. Data were analyzed using single factor analysis of variance and Duncan test was used for grouping the treatments.

RESULTS AND DISCUSSION

Table 1: Analysis of Variance Traits

		Ms				
Sov	Df	Soluble sugars	Carotenoids	Total chl	Chl b	Chl a
Methanol	3	1670.56**	338.3**	6471.18**	719.02**	2876.08**
Error	8	3.698	19.500	12.000	13.333	5.333

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	Chl a ($mgg^{-1}fw$)	Chl b ($mgg^{-1}fw$)	Total chl ($mgg^{-1}fw$)	Carotenoids	**Leaf soluble sugar** (µgg-1 fw)
C	184^{c}	102^{c}	286^{c}	35^{b}	147.0833^{c}
M10	215^{b}	117.5^{b}	332.5^{b}	52.66667^{a}	173.25^{b}
M20	246^{a}	133^{a}	379^{a}	57.33333^{a}	196.5^{a}
M40	179.33^{c}	99.6667^{c}	279^{c}	39.33333^{b}	147.8333^{c}

Results from this experiment are in accordance with those of some other researchers Methanol treatment increased chlorophyll content of tobacco leaf [25]. Chlorophyll content of wheat, oath and grape leaves was also significantly increased after spraying methanol [23,24]. There are reports maintaining that the application of methanol spray increases drought resistance in plants at hot climates [25]. According to the report by Rajala et al (1998), methanol metabolism leads to increased production of sugar in the leaves, causing increased inflammation pressure, assimilation rate, and growth of treated plants. Spraying solution on pepper shrubs increases chlorophyll content, too [23]. Theodoridou et al [29]suggested that the application of methanol on plants increases intracellular contents as well as ratio of intracellular chlorophyll a to chlorophyll b. Increasing trend of chlorophyll content is positively correlated with up to 30% (by volume) increase of methanol concentration. After that, the more methanol volume (in percent) increases, the more chlorophyll concentration decreases [12]. Mirakhori *et al* [16] suggested that up to 21% increase of methanol volume increases chlorophyll content; while, higher than that decreases chlorophyll. With spraying different methanol concentrations on peanut plant and measuring chlorophyll content of the leaf, using chlorophyll meter, they reported that the highest amount of chlorophyll was observed in the leaf treated with 20% methanol (by volume) [1]. With spraying methanol, CO_2 is obtained from rapid oxidation of methanol on the plant and can compete with oxygen by succeeding in absorption at the RuBisCO (25). Results of the present study indicate that the application of methanol causes increased soluble sugar and phenol in the leaf, as well as essence level (in percent). This can increase water uptake and Turgor pressure. Increased photosynthesis can lead to increased synthesis of secondary metabolites, such as essential oils and different phenolic compounds. Favar et al [9], in a large Finnish company where methanol was used to enhance the growth and quality of the roses cultivated in the greenhouse, suggested that frequent use of methanol on roses decreases the need for fungicide to control powdery mildew. According to the reports by Zebik *et al* [27], increased concentration of CO_2 can neutralize the effects of environmental stresses. Nadali *et al* [17] have reported increased yield via methanol spray on sugar beets. Studies by Nonomura and Benson [19] showed that methanol caused increased yield in plants treated with this material. They reported that the application of methanol on aerial parts of crops increases the yield, accelerates ripening, decreases the effect of drought stress, and reduces the need for water. Studies by Heming *et al* [11] showed that adequate amount of CO_2, produced by spraying methanol, changes the photorespiration pathway from a catabolic reaction to an anabolic one. Investigations by Nanomura and Benson [19] depicted that after methanol spray, it is essential to expose the plant to the light for inducing photorespiration; otherwise, the plant will suffer from foliar damages. Lee *et al* [13] reported increased level of photosynthesis in sugar beets treated with methanol and suggested that methanol increases the plant yield to 10%.

CONCLUSION

The main goal of the present study is to investigate the effect of methanol on biochemical traits. Regarding the results from this study, methanol largely increased the factors measured in this research and could increase all factors. Moreover, using 20% and 10% methanol (by concentration) has had the greatest stimulating effect. However, increasing the concentration to 40% showed no significant difference comparing with control treatment. In addition, the most effective concentration was 20% methanol treatment.

REFERENCES

1. Afsharypuor, S & Azarbayejany, N. (2006). Chemical constituents of the flower essential oil of Lavandula officinalis chaix. From Isfahan (Iran). Iranian Journal of Pharmaceutical Sciences 2. 3: 169-172.
2. Arnon, D. (1949). Plant physiology. 24: 1-15.
3. Adam, K.L. (2006). Lavender production, products, markets, and entertainment farms. A Publication of ATTRA: National Sustainable Agriculture Information Service. USA.
4. Barazandeh, M.M. (1999). C*hemical Decomposition of Iranian and French Lavender Essential Oils Using Capillary Gas Chromatographic Method*. Research Institute of Forests and Rangelands.
5. Blumenthal, M., Busse, W.R, & Goldberg, A. (1998). The compelet commission E Mongraphs: therapeutic Guide to Herbar Medicines boston, MA: Integrative medicine communications. 159-60.

6. Dadman, B., Omidbeygi, R., & Sefidkan, F. (2007). Effect of nitrogen on essential oil of Mexican parsley. Iranian Medicinal and Aromatic Plants Res. 23 (4). 38: 484 – 91.
7. Downie, A., Miyazaki, S., Bohnert, H., John, P., Coleman, J., Parry, M., & Haslam, R. (2004). Expression profiling.
8. Fakhari, A.R., Salehi, R., Heydari, R., Ebrahimi, S.N., & Haddad, P.R.(2005). Hydrodistillation-headspace solvent micro extraction، a new method for analysis of the essential oil components of Lavandula angustifolia Mill. Journal of Chromatography. 9;1098 (1-2):8-14.
9. Faver, K.L., & Gerik, T.J. (1996). Foliar-applied methanol effects on cotton (Gossypium hirsutum L.) gas exchange.
10. Gout, E., Aubert, S., Bligny, R., Rebeille, F., & Nonomura, A.R. (2000). Metabolism of methanol in plant cells.
11. Hemming, D., & Criddle, R. (1995). Effects of methanol on plant respiration. Journal of Plant Physiology, 146: 193-198.
12. Jabbarzadeh, A.R., Safarzadeh, M.T., Gholizadeh, A., & Asimi, M.H. (2011). *The Effect of the Application of Methanol Spray on Some Physiological Traits of the Varieties of Tobacco Plants (Cooker 347)*. First National Conference on Modern Topics in Agriculture. Azad University of Saveh.
13. Lee, H.S., Madhaiyan, M., Kim, C.W., Choi, S.J., & Chung, K.Y. (2006). Physiological enhancement of early growth of rice seedling (Oryza sativa L.) by production of phytohormone of N2-fixing methylotrophic isolated. Bio. Fertil. Soils, 42: 402-408.
14. Lis-Balchin, M. (2002). Lavender: the Genus Lavandula. Taylor and Francis Publications. London. UR
15. Makhdum, M.I., Malik, M., Din,S.U., Ahmad, F., & Chaudhry, F.I. (2002). Physiology response of cotton to methanol foliar application. J. Res. (Sci.) 13:37-43.
16. Mirakhori, M., Paknejad, F., Ardakani, M., & Moradi, F. (2010). *Investigating the Effect of Drought Stress and Methanol Spray on Chlorophyll Fluorescence Parameters, Cell Relative Water Content and Chlorophyll Content of Soybean Leaf*. Iranian Journal of Agricultural Research, Vol 8, No 3, PP 531-541.
17. Nadali, I., Paknejad, F., Moradi, F., Vazan, S., Tookalo, M., JamiAl-Ahmadi, M., Pazoki, A. (2010). Effects of methanol on sugar beet (Beta vulgaris), Australian journal of crop science, 4(6):398-401.
18. Naturopathic Handbook of herbar for mulas herbar Research Publications the d. (1995): 1090.
19. Nonomura, A.M., & Benson, A. (1992). The path of carbon in photosynthesis: improved crop yields with methanol. Proc. Natl. Acad. Sci. U.S.A, 89: 9794-9798.
20. Ody, P. (1993). *The complete Medicinal Herbar*, Dorling kindersly New York., 73.
21. Omidbeigi, R.(2006). Production and processing of medicinal plants. Beh Nashr Pub Vol 1, 3.
22. Omidbeigi, R. (2010). *Production and Process of Herbs*. *Vol 4, P423*. Mashad: Astan Quds Razavi Publishers.
23. Rajala, A., Karkkainen, J., Peltonen, J., & Peltonen-Sainio, P. (1998). Foliar application of alcohols failed to enhance growth and yield of C3 crops. Ind. Crop. Prod. 7: 129-137.
24. Ramadant, T., & Omran, Y. (2005). The effects of foliar application of methanol on productivity and fruit quality of grapevine cv. Flame seedlees. Vitis Journal, 44, 11-16.
25. Ramirez, I., Dorta, F., Espinoza, V., Jimenez, E., Mercado, A., & Pen a-cortes, H. (2006). Effects of foliar and root applications of methanol on the growth of Arabidopsis, tobacco and tomato plants. J. plant Growth Regul, 25:30-44.
26. Safarzade Vishgahi, M.N., Normohamadi, G.H., Majidi Haravan, E., Rabiei, B. (2005). Effect of methanol on peanut Growth and yield (*Arachishypogaea* L.). J. Agric. Sci., 2005: 103-188.
27. Zbiec, I.I., Karczmarczyk, S., koszanski, Z. (1999). Influence of methanol on some cultivated plants. Folia Univ. Agic. Stetin., Agricultura, 73:217-220.
28. McGiffen, M. & Manthey, J.A. (1996). The role of methanol in promoting plant growth: a current. evaluation. Hort Sci. 31: 1092- 1096.
29. Theodoridou, A., Dornemann, D., & Kotzabasis, K. (2002). Light-dependent induction of strongly increased microalgal growth by methanol. Biochim. Biophys. Acta. 1573: 189–198.

CITATION OF THIS ARTICLE

Hamid Reza B, Ali Reza L M, Hossein A. Investigating the Effects of Methanol on Biochemical Indices of Lavender. Bull. Env. Pharmacol. Life Sci., Vol 3 [12] November 2014: 193-196