Nat. Volatiles & Essent. Oils, 2022; 9(3): 65-78

NVEO
Natural Volatiles & Essential Oils

The Effect Of Biofertilizers And Different Drying Method On The Quality And Quantity Of Lemon Balm (Melissa Officinalis) Essential Oil

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Abstract:

The aim of this study was to apply mycorrhiza and azotobacter biofertilizers to increase the yield and recognize the most proper method for drying Lemon Balm to conserve the quality and quantity of active ingredients. The transplants were treated with mycorrhiza and azotobacter before beding transferred to the main field. The drying process was carried out subjected to sun, shading, oven (35 and 55 oC), and microwave (Output power of 100, 300, 900 w), and the quality and quantity properties were measured. Variance analysis showed that Mycorrhiza and Azotobacter significantly affect the quantitative traits, including fresh weight and dry weight, plant height, leaf number per plant, 10 leaf area, and the essential oil percentage ($P<0.05$). Drying methods significantly affect the essential oil percentage, appearance, Geraniol, Neral, Citronella, Caryophyllene Oxide, Citronellal, Beta-Caryophyllene, Geranyl acetate, and Geraniol ($P<0.01$). Mycorrhiza increased the Lemon Balm's essential oil percentage. Appearance, essential oil percentage, and the effective ingredients of the essential oil, such as Geranial (citral a), citronella, and caryophyllene oxide, and drying in the shades exhibited a better performance compared to the other drying methods (drying under the sun, in the oven, and in the microwave) ($P<0.05$). For Geranial, Neral and Citronella drying under the sun and in the oven proved to be the best methods, respectively. Increasing the oven temperature negatively affects the Lemon Balm's essential oil. It could be suggested to use mycorrhiza in order to improve the Lemon Balm yield. Additionally, saving time and expenses are among the other benefits of rapid drying methods, such as a low-temperature oven and the low-output power of the microwave.

Keywords: Lemon Balm, biofertilizer, drying, effective ingredient, packaging.

Introduction

Lemon Balm or Balm mint, with the scientific name of Melissa officinalis, belongs to the Lamiales order and the Lamiaceae family. It is native to South-Central Europe and the Eastern Mediterranean Basin. All vegetative parts are used for pharmaceutical applications (Moradkhani et al., 2010). Growth is highly dependent on solar radiation, while there is still a slight growth in the shadow (Janina, 2003). When plants grow in semi-sunny conditions, they produce more leaves and habitats in comparison with fully sunny conditions. Lemon Balm can grow rapidly in the range of 15 to 35 °C of temperature and needs 500 to 600 mm of perception during the growth season; otherwise, it should be irrigated. The water demand reduces when a deep root system is created (Moradkhani et al., 2010). Lemon Balm contains protein biosynthesis inhibitors for cancer cells (Adjorjan and

Buchbauer, 2010), which corresponds to oil, flavonoids, and phenolic acids, such as Rosmarinic and Caffeic acids (Constantine, 2007), phenylpropanoid heteroids, and triterpenes (Mencherini et al., 2007). Meftahizade et al. (2010) reported that the main ingredient of the Lemon Balm oil (about 96%) included Citral (Geranial and Neral), Citronellal, Geraniol, Beta-pinene, and Beta-caryophyllene. In another study, Abdellatif et al. (2014) evaluated the chemical composition and antibacterial activity of the Lemon Balm oil. They introduced 63 chemical compositions in the Lemon Balm oil, whose main composition is Geraniol (44.2%), Neral (30.2 %) and Citronellal (6.3%). Lemon Balm can be used as a pharmaceutic material to protect food and increase durability (Bisht et al., 2012). Recent studies confirmed the efficacy of the Lemon Balm extract for the topical treatment of herpes viral lesions, according to new applications (Schultz and Hansel, 1998). In Spain, Lemon Balm leaves are added to the salad dressing, and its fresh leaves are mixed with the common tea to make a pleasurable drink (Parameswari et al., 2009). The chemical composition of Lemon Balm produced a small amount of it on the plant. Therefore, we considered increasing the biomass and secondary metabolites. Along these lines, several researchers stated that adding different organic compounds to the soil improves the plant's yield, growth, and chemical composition (El Sherbeny et al., 2005). Tahmasebi Omran et al. (2015) reported that using biofertilizers resulted in improving some traits, such as the height, canopy extent, germination count, leaf length, and total dry weight. Mafakheri et al. (2016) studied the biological and chemical fertilizer that affects the Lemon Balm's essential oil content. Using 30% of vermicompost increased the oil content significantly. After harvesting, some moisture remains in the components of the plant, which can cause some interactions that can change, decompose, and destroy the effective ingredient materials of plants (Singh et al., 2015). One of the most commonly used and effective operations for post-harvesting physiology of medicinal herbs is drying the collected vegetation organs (medicinal). However, the drying procedure severely changes the quality and quantity of the effective ingredients of medicinal plants, especially essential oils (Singh et al., 2015). Drying consisted of removing the moisture through evaporation to a specific threshold in order to store products for a long time and cut off enzymatic microorganisms and yeast activity (Azizi et al., 2009). Different factors such as temperature, the drying procedure, and the proper time for drying affects the active ingredients of medicinal herbs. As a matter of fact, the drying method is related to the moisture content of plant organs, for example, rapid and complete drying of medical herbs containing essential oils, induced color, and essence preservation. Sometimes, it became evident that the drying process increased the essential oil yield in certain medical plants (Asekan et al., 2007). Rapid drying and lower energy output prevents the loss of essential oils (Venskutonis, 1997). Thus, regarding the importance of medical properties of Lemon Balm, the aim of the present study was to find the best combination of Mycorrhiza (fungal fertilizer) and Azotobacter (bacterial fertilizer) biofertilizer and recognize the most proper drying method for Lemon Balm in order to preserve the quality and quantity of effective materials during storage.

Materials and methods

The location of the project and cultivation

In this project, Lemon Balm was cultivated in Shahid Batuee Agro-Industrial Integration located in Hashmaytah Village, Neyshabour. For this pupose, 2*3 dimension plots were designed with 40 cm of distance as a safety margin. The given soil was under the cultivation of spring corn and summer watermelon last year. In order to reach the net performance of biological fertilizers, the addition of other fertilizers, such as chemical and animal fertilizers, was discarded. The Lemon Balm seeds were purchased from the Medicinal Plants Ecological Garden of the Ferdowsi University of Mashhad. The seeds were subjected to hydro priming treatment for 24h in ordinary

water. To expedite the experiment and, also, ensure an adequate plant, all of them were prepared as a transplant in the greenhouse. To grow transplants, plastic trays were used, and the seeds were placed in the cultivation tray on February 26, 2016. Maintenance and irrigation operations on the seedlings were performed regularly, and on April 8, 2016, they were transferred to the main field. The seedlings were planted 30 cm apart. Irrigation was applied on a regular basis in the first months and performed every four days. Weeding was carried out by hand in one stage on May 13th. Harvesting was also done by hand on 29th, 30th and 31st of June.

Biofertilizer treatment

The effects of Mycorrhiza (fungal biofertilizer) and Azotobacter (bacterial biofertilizer) on the quantity and quality of the Lemon Balm's effective ingredients were evaluated after harvesting. For the treatment with Azotobacter, when transferring the seedlings to the main field, they are placed in a 10% solution (200 cc solution of azotobacter fertilizer of the Dr. Bio brand added to 1.8 lit of water) for 5 minutes, then dried in the open air for 30 minutes, and then transferred to the mainland. In addition, for the treatment with Mycorrhiza transplants, before being transferred to the main plot, they were placed in an inoculation solution containing 100 g of Mycorrhiza powder by the Mycoroot brand for 5 minutes. This includes 300 g of the activated organ of a different Mycorrhiza arbuscular and 2 lit of water. Consequently, they are dried in the fresh air and transferred to the main field.

Lemon Balm drying

Various methods were used to dry the Lemon Balm produced by different methods, including drying in the sun, in the shadow, and using oven and microwave. In the sun-dried method, Lemon Balm was spread on the shadow fabrics of the treasury and in the ordinary sunshine of the experimental place. After reaching the proper moisture content, all were gathered and used for subsequent experiments. To dry in the shade, the crop was dispreaded in an experimental chamber with permanent air exchange through the windows on both sides and ambient temperature. Crops reach the desired humidity after three days. They are then collected for use in additional experiments. For oven drying, Lemon Balm was harvested for three days consecutively (similar weather) and transferred to the laboratory. For this purpose, the Lemon Balm crops were dried at 60 ^{o}C. To dry with the microwave, the Feller Microwave apparatus was used with an adjustable power in the range of 145-1450 W. To dry the samples, the device was set up on different power levels of 145, 290, and 870 W with air convections (in order to run produced steam out from the device compartments). For preventing excessive drying, a drying time was applied in the form of one-minute break times and after achieving the intended drought, the drying operation finished.

Measuring the quantitative and qualitative traits

The quantitative traits that were measured in the present study included the fresh weight, the dry weight, the plant height, the leaf number per plant, and 10 main leaves' areas. Immediately after harvesting, Lemon Balm was measured. For this aim, 10 bushes were selected randomly and picked. Then, they were weighted by digital scales with 0.1 g of precision. The shrubs stacked randomly to determine the fresh weight in the previous step were used to determine the dry weight, again. Thus, after drying the ten aforementioned bushes, the dry weight was recorded by digital scales with 0.1 g of precision. 10 plants height were measured by a paper ruler from the plant's collar up to the highest half of plant height with 1 cm of precision. Leaf numbers were counted and recorded by hand in three plants out of ten harvested bushes. For calculating the leaf area, 10 main leaves were selected and picked from the upper parts of the plant and placed on a white paper (A4). Photographs were taken

from the sheet and then the total main leaf area was computed by Adobe Photoshop image processing software. in order to assess the essential oil percentage in Lemon Balm samples, 100 g of leaves were isolated and the essential oil extraction was performed by the Cleungridge method in 4 hours. Dimethyl sulfoxide Emulsifier (DMSO) was used as a solvent to prepare essential oils, and the best solubility was obtained for the essence (Kalemba and Kunicka, 2003). Then, in sterile tubes, 20 ml of the essence were poured separately, and in the next stage, 280 ml of the DMSO solution were added. Essential oils were stirred by the shaker to obtain a transparent solution. The essential oils were analyzed and detected by the GC/MS apparatus. The carrier gas was Helium 99/99 and the injection one was Spilit type. The injection was 1ml and the gas flow rate was adjusted in 1ml/min. The 1ml essential oil was injected into the GC/MS device. Results obtained by this system were recognized through calculating Quartz Indicies and referring to natural ingredient Encyclopedia. Measuring the total ash performed in accordance with the Iranian National Standard 1197 (for spices and condiment, total ash measurements). For measuring the insoluble ash in the acid, the obtained ash was dissolved in 20 ml of Hydrochloric acid and boiled for 10 min over the gentle flame inside the hood. The solution was cooled and leached by the filter paper without any ashes. The filter paper was rinsed by the distilled water three times to thoroughly get free from acids. Then, the filter paper with the remaining ash was placed in the pre-weighted crucible and, again, burned at 550 $^{\circ}$C for 2 hours. The crucible is brought out of the furnace, and the remaining ash was recorded as insoluble ash weight.

Data analysis

The whole data analysis is performed in completely randomized design by the SAS software 9/1. The means comparison were carried out by the Multi-tail Duncan test in 95 % levels of probability. The required charts were depicted by the help of the Excel software.

Results and findings

Results obtained by the variance analysis of quantitative traits, such as fresh and dry weight, plant height, leaf number, 10 leaves areas, as well as the essential oil percentage of the Lemon Balm treated with different biofertilizers represented in table 1. The effect of treatment on the dry and fresh weight and leaf number per planted was significant in 5 % ($P<0.05$) and in 1% ($P<0.01$) for plant height and essential oil percent and there were not any significant effects for 10 leaves' areas ($P>0.05$). The replication effects were not significant for any quantitative characterization and measured an essential oil percentage. The minimum and maximum variations of the coefficient were related to the leaf number per plant and the essential oil percentage, respectively. Table 2 represented the results obtained by the variance analysis of the essential oil percentage, appearance, Geranial, neral, Citronella, Caryophyllin oxide, Citronellal, Beta-Caryophyllene, Geranyl acetate, and Geraniol of Lemon Balm dried with different procedures. The treatment mean square for the essential oil percent, appearance, Geranial, neral, Citronella, Caryophyllin oxide, Citronellal, Beta-Caryophyllene, Geranyl acetate, and Geraniol was significant in 1% ($P<0.01$). The replication effect was not significant in any of the measure traits ($P>0.05$) except Geranial ($P<0.05$). The variation coefficient in different characteristics ranged between 2.59 to 9.93%. It it worth mentioning that the lowest and the highest variation coefficients belong to Geranial and appearance, respectively.

Table 1. the means square obtained by the quantitative traits variance analysis and the essential oil percentage of Lemon Balm influenced by a biofertilizer.

SOV	df	Means squer					
		Fresh weight	Dry weight	Plant height	Leaf number per plant	10 leaves area	Essential oil percentage
treatment	2	540.34 *	79.00 *	98.77 **	1200.12 *	925.45 ns	0.004 **
rep	2	17.36 ns	4.33 ns	8.11 ns	69.78 ns	205.46 ns	0.00 ns
error	4	60.66	5.38	3.25	114.34	153.77	0.00
CV(%)	-	7.53	8.44	6.20	12.47	7.09	0
R^2(%)	-	82	88	94	85	78	1

ns, * and ** represented insignificant, significance in 5% and significance in 1% respectively.

Table 2. the means square obtained by the variance analysis of the essential oil percentage, appearence and the essential oil composition.

SOV	df	Means comparison									
		Essential oil percentage	appearance	Geranyl)Citral a(	neral) Citral b(	Citronellal	Caryophyllin oxide	Citronellal	Beta-Caryophylline	Geranyl acetate	Geraniol
treatmet	6	0.002 **	2.66 **	24.424 **	37.71 **	0.116 **	10.48 **	0.540 **	2.746 **	0.10 ** 4	0.98 8 **
rep	2	0.000 ns	0.00 ns	3.57 *	2.28 ns	0.000 ns	0.012 ns	0.031 **	0.252 ns	0.00 4 ns	0.04 1 ns
error	12	0.000	0.41	0.57	0.78	0.001	0.135	0.003	0.065	0.00 3	0.08 4
CV(%)	-	3.76	9.93	2.59	3.97	6.92	3.58	8.37	8.35	9.30	7.18
R^2(%)	-	96	76	99	96	97	97	99	95	94	85

ns, * and ** represented insignificant, significance in 5% and significance in 1% respectively.

The mean comparison results of the fresh and dry weight, the plant height, the leaf number per plant, the area of 10 leaves, and the essential oil percentage, as affected by the Mycorrhizal fertilizer (fungal biofertilizer) and Azotobacter (bacterial biofertilizer), with the control group was represented in Figures 1-4. Addition of Mycorrhiza and Azotobacter as fungal and bacterial biofertilizer induced a significant increase ($P<0.05$) in the fresh weight of Lemon Balm in comparison with the controlled treatment. Using Mycorrhiza and Azotobacter biofertilizer increased Lemon Balm's dry weight significantly in comparison with the controlled treatment ($P<0.05$). The least and highest means of Lemon Balm height was linked with the controlled and Azotobacter treatment, respectively. Mycorrhiza biofertilizer caused a significant increment in the height mean ($P<0.05$), which was equivalent to 27%. Azotobacter treatment also significantly increased the plant height in comparison with the controlled treatment, but it wasn't significant in comparison with the Mycorrhizal treatment ($P>0.05$). Adding Mycorrhiza and Azotobacter biofertilizer increased the mean of leaf number per plant. The average of

leaf area in 10 main leaves significantly increased in Mycorrhiza and Azotobacter treatments in comparison with the controlled one ($P<0.05$).

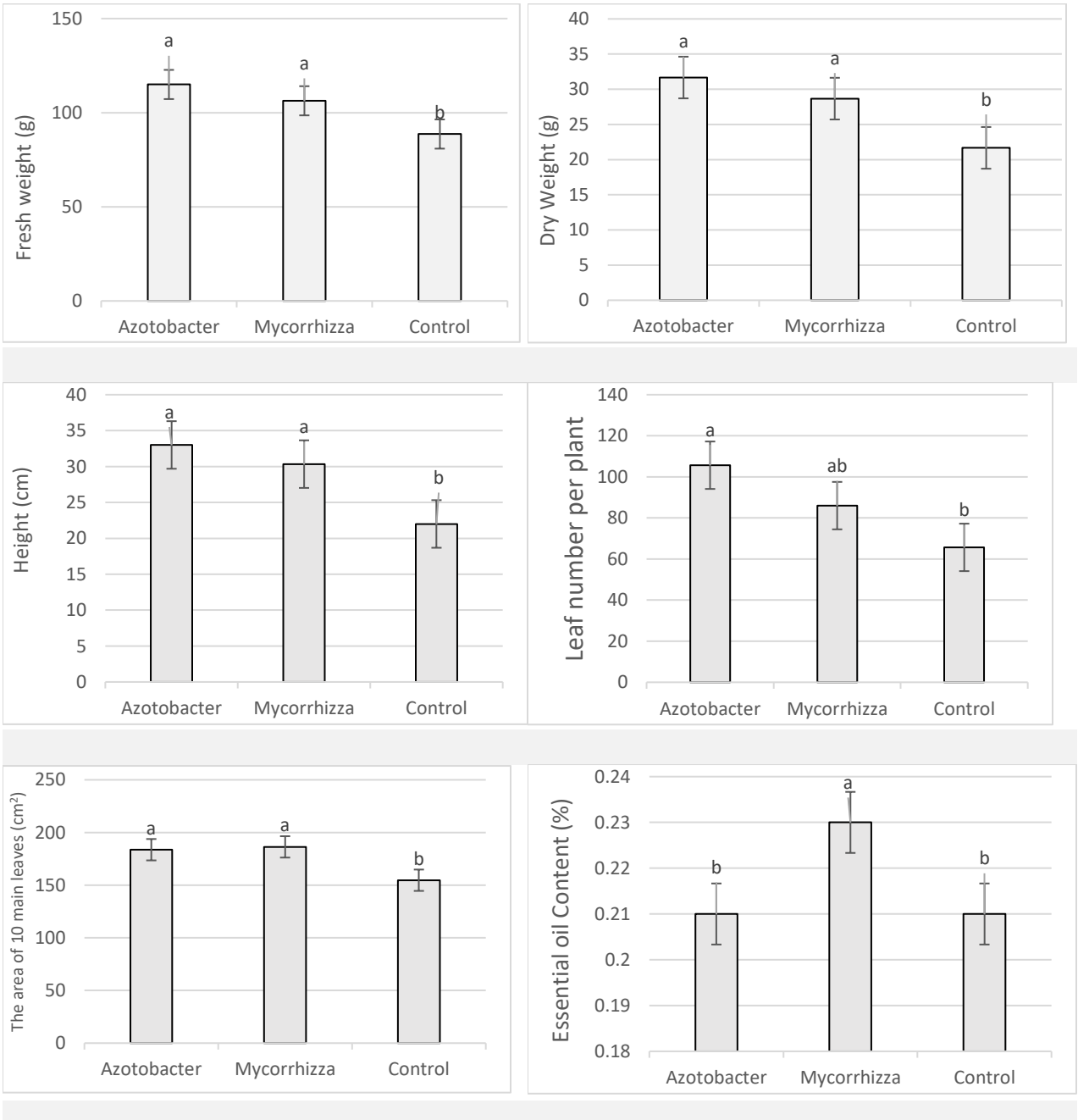
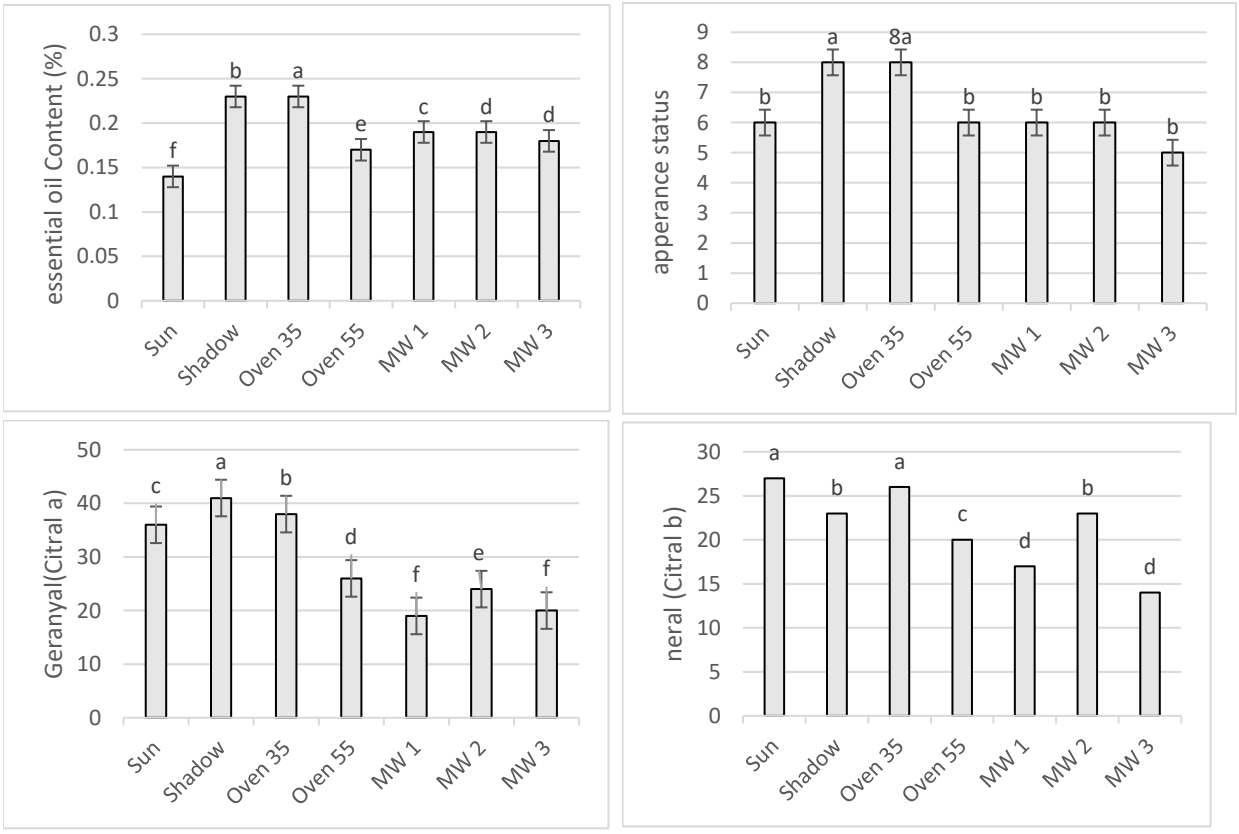


Figure 1. the means of the comparison of qualitative traits such as the fresh weight, the dry weight, the plant height, the leaf number per plant, the area of 10 leaves, and the essential oil percentage of Lemon Balm as affected by different biofertilizers.

The mean comparison results of the essential oil percentage, the appearance status, Geranial and Neral, Citronellal, caryophyllene oxide, Citronella, Beta-caryophyllene, Geranyl acetate, and Geraniol are represented in Figure 2, as affected by different drying methods. The highest essential oil content was linked with drying Lemon Balm in the oven at 35 °C. The mean of the essential oil percentage in different drying procedures signaled a significant difference, except drying in the microwave with the power of 300 and 900 W ($P<0.05$). The maximum and minimum appearance status corresponded to the oven drying treatment in the microwave at 35 °C and 100 W, respectively. The mean of the appearance status was not significant between the two drying methods of shadow drying at 35 °C in the oven ($P>0.05$). The highest and lowest Geranyl means were linked with the shadow and 100 W microwave treatments, respectively. The highest means for neral was related to the sun-drying and the 35 °C oven treatments without any notable differences with each other, but they showed an incredible difference with other drying methods ($P<0.05$). The highest Citronelol mean was associated with the oven-dried treatment in 35 °C and the comparison of the means was noteworthy in comparison with other treatments ($P<0.05$). The highest and the least average of caryophyllene oxide was related to 300 and 900 W microwave, respectively, with remarkable differences ($P<0.05$). The highest and lowest average of Citronellol were observed in the treatment with the 55 °C oven and the sun-drying treatments, respectively. The highest amount of Beta-caryophyllene belongs to the sun-drying method, which is notably different from other drying methods (except drying in 35°C) ($P<0.05$). There were minor differences between the Geranyl acetate means linked with the sun-drying, shadow and the 55°C oven treatments ($P>0.05$).



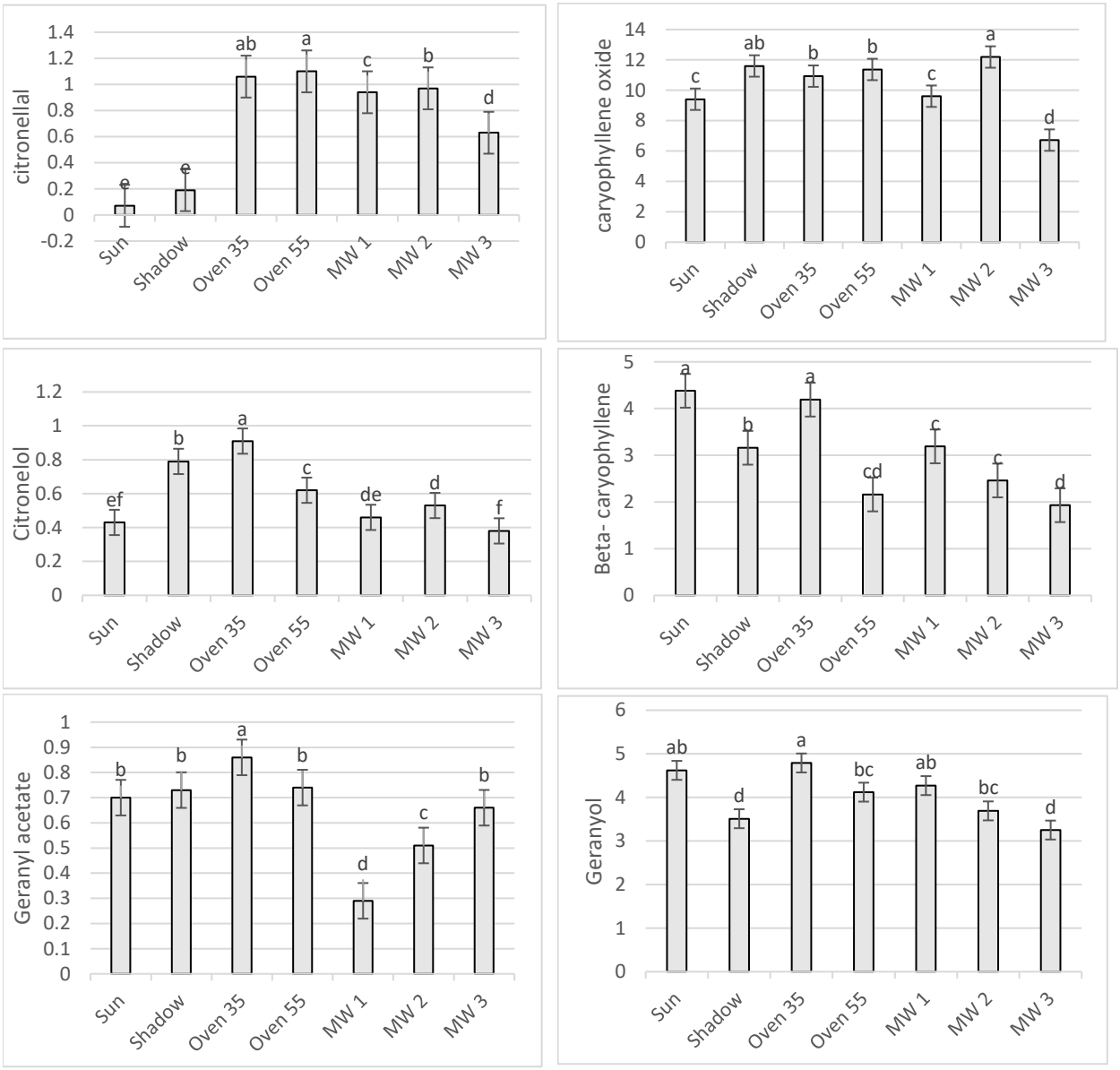


Figure 2. the mean comparison of the essential oil percentage, the appearance status, Geranial and Neral, Citronellal, caryophyllene oxide, Citronelol, Beta-caryophyllene, Geranyl acetate and Geraniol of Lemon Balm as affected by different drying methods.

The mean comparison of Lemon Balm's measured characteristics were evaluated as a group (sun-drying, shadow, oven, and microwave). The mean comparison results were presented in Table 3 for different drying methods. The highest essential oil percentage were obtained by drying in the shadow, remarkably different from the oven and microwave methods ($p<0.05$), but no noteworthy differences were observed by the sun-drying method ($p>0.05$). The appearance status showed an averagely better function in the shadow rather than others ($P<0.05$). The highest and lowest Geranial means were observed in the shade and microwave drying methods, respectively ($P<0.05$). For Neral means, there was not any notable difference between the sun-drying, shade, and oven methods ($P>0.05$). For drying in shade and oven, there was no significant difference for the Cetronelol means ($p>0.05$). The performance of the shadow-drying method was better than other methods for Cariofiline oxide in Lemon Balm. The difference was insignificant with the oven method, but it proved meaningful for the

sun and microwave methods (P<0.05). The least Cariofiline oxide mean belongs to the sun-base drying method. The best yield for Citronellal in Lemon Balm's essential oil was obtained by the oven-drying procedure, notably different from other drying methods (P<0.05). For Geranyl acetate means, no significant effects were observed in the sun-dried, shade and oven methods (P>0.05), while remarkable differences were observed with the microwave-drying method. The minimum mean of Geranyl acetate was obtained by the microwave procedure. The sun-drying and oven-drying procedures exhibited the highest values for Ganiol means, notably different from the shade and microwave methods (P>0.05).

Table 3. the mean comparison results of the qualitative and quantitative properties of Lemon Balm's essential oil in the sun-, shade-, oven-, and microwave- drying method groups.

properties	Drying method	mean	properties	Drying method	mean
Essential oil percentage	sun	0.146 c	Caryophyllene oxide	sun	9.410 b
	shade	0.220 a		shade	11.600 a
	oven	0.200 a		oven	11.150 a
	microwave	0.186 b		microwave	9.501 b
Appearance status	sun	6.333 b	Citronellal	sun	0.170 d
	shade	7.833 a		shade	0.190 c
	oven	7.000 a		oven	1.080 a
	microwave	5.778 b		microwave	0.846 b
Geraniol (Citral a)	sun	36.000 a	Beta – caryophyllene	sun	4.380 a
	shade	41.000 a		shade	3.160 b
	oven	32.000 b		oven	3.175 b
	microwave	21.000 c		microwave	2.526 b
Neral (citral b)	sun	27.000 a	Geranial acetat	sun	0.700 a
	shade	23.000 ab		shade	0.730 a
	oven	23.000 ab		oven	0.800 a
	microwave	20.000 b		microwave	0.486 b
Citronellal	sun	0.430 b	Geraniol	sun	4.613 a
	shade	0.790 a		shade	3.510 b
	oven	0.765 a		oven	4.455 a
	microwave	0.456 b		microwave	3.736 b

Discussion

In the current study, using mycorrhiza and azotobacter biofertilizers significantly improved the fresh and dry weight, the plant height, the leaf number per plant, and the area of 10 main leaves. There was not any noteworthy effects between mycorrhiza and azotobacter for the yield. The only difference was increasing Lemon Balm's essential oil by mycorrhizal application, while azotobacter does not indicate any significant differences in the essential oil percentage by comparison with the controlled treatment. In various studies, the beneficial

effects of biofertilizers on the yield and medical plant's essential oil was reported frequently (Cardoso and Kuyper, 2006; Engel et al., 2016). Mycorrhiza caused improvements in the physical properties of soil through the development of fungal hyphae, the chemical properties of it through increasing the nutrition adsorption, and the biological quality of it through the soil nutrition network (Cardoso and Kuyper, 2006). Mycorrhiza not only increased the plant growth and propagation, especially the root growth, but increased the plant's capability of growing in soil with a lack in the amount of phosphorus (Joshee et al., 2007). Gewaily et al. (2006) evaluated the Marjoram plant, which did not have any significant differences regarding the biomass between the treatments inoculated with phosphate solubilizing bacteria and the controlled one with nitrogen-fixing bacteria, such as azotobacter, which does not count as a phosphate-solubilizing component. In fact, azotobacter could produce and secrete bioactive materials in the plant root area, which induced root establishment. Azotobacter increased the water and nutrient absorbance and affected the plant yield and soil properties through fixing the biological nitrogen (Mamta, 2017). Some bioactive materials consisted of nicotinic acid, pantothenic acid, biotin, B-vitamin class, auxins, gibberellins, etc. (Mamta, 2017). The obtained results indicating the improving characteristics of Lemon Balm's medicinal plant are confirmed with other scientific results. Shokrani et al. (2012) reported the flower yield, dry matter, and essential oil improvements in Calendula officinalis. Moreover, the positive effect of azotobacter was stated on the yield and the yield component of Apium graveolense, which was consistent with our results. Azotobacter in the Rhizosphere area and the synthesis and elimination of some bioactive materials increased the root growth (Chen 2006). In our study, azotobacter did not affect Lemon Balm's essential oil percentage. It is worth noting that in different studies on medical plants, the positive effects of azotobacter were shown on the essential oil content results being inconsistent with the with the results of this research. Omar et al. (2008) stated that the nitrogen fertilizer induced Origanum syriacum and Ocimum americanum essential oil increment. Indeed, the seed inoculation with azotobacter improved the growth and the essential oil yield (Gomaa and Abou-Aly, 2001). In this study, higher adsorption of inorganic phosphorus by mycorrhizal plants, as a required element in essential oil biosynthesis, was reported to have influencing factor for increasing the essential oil percentage (Kapoor et al., 2002). Copetta et al. (2006), in a survey on basil, found that the increase in the essential oil by mycorrhizal fungi is due to the heightened number of secretory fluff in the main place for constructing the essential oil located in the base and center of the basil leaf. On the other hand, considering that essential oil constituents, such as Isopentenyl pyrophosphate and Dimethylallyl pyrophosphate need ATP and NADPH for synthesis, and phosphorous plays a critical role in these composition biosyntheses, the phosphorus presence is necessary to construct them (Loomis and Corteau, 1972).

The increasing plant height in this experiment might be linked with the azotobacter and mycorrhiza biofertilizer application, which resulted in plant growth stimulation synthesis and secretion as plant growth regulators, such as auxin, different amino acid discharge, diverse antibiotic, hydrogen cyanide, and siderophore. These compounds induced plant height by stimulating the plant growth and increasing internode length (Tilak et al., 2005). Bitarafan et al. (2017) reported the positive effect of mycorrhiza on the growth characteristic, the essential oil, and the garden Thyme yield, while mycorrhiza did not have any significant effect on Thyme height. For the plant appearance status, the essential oil percentage and its effective materials, including Geranial (Citral a) and Citronellol Caryophyllene oxide, the performance of drying in the shade was better than other drying methods (sun-drying, oven, and microwave), due to the highest amount of these materials obtained by this procedure with remarkable differences ($P<0.05$). Effective compounds, such as Neral, Citronella, and Jeniol showed better performances in the sun, the oven, and the sun, respectively. For the oven-drying method, increasing the oven temperature was observed to have negative effects on Lemon Balm's essential oil. The rate of moisture loss and the decrease in the moisture content is influenced by the water transduction from interior

layers to the plant surface. Rapid scattering of microwave radiation plays an important role in reducing the moisture content quickly, which determines the total quality of the plant (Hevia et al., 2002). In the oven method, increasing the oven temperature decreased Lemon Balm's essential oil content. Evidently, increasing the temperature decreased the amount of effective compounds due to the heightened water molecules movement to the plant surface, as well as increasing the transfer rate of aromatic compound molecules in organs during evaporation (Asekun et al., 2007). Cuervo and Hensel showed (2008) that a higher drying temperature caused intense changes in colour and reduced the ultimate crop quality and economical value. In the investigations of Khalid et al. (2008), the drying method did not affect the chemical composition of the essential oil. Their suggested that, for obtaining the highest amount of monoterpene, it is necessary to keep it fresh during the first and second time harvesting. Chan et al. (2000) observed that drying with the microwave (at 800 W), the oven (at 50°C), and the sun caused a severe reduction in the antioxidant and phenolic compounds in the dried leaf samples from four plants of the ginger family in comparison with the fresh leaf. Sefidkon et al. (2006) evaluated the summer savoury and showed that the highest essential oil percentages were obtained in the oven, shade, and sun-drying methods, respectively. The results observed by Ahmadi et al. (2008) linked with the higher Citronellol essential oil content in the shade-drying method was in line with our findings for Lemon Balm. In this study, the highest temperature for oven drying was determined to be 55 °C, while in previous studies, adverse effects were reported for higher temperatures in the oven for active ingredients of medicinal plants (Que et al., 2008). Paakkonen et al. (1990) stated that the plant responds differently to various drying conditions, packaging, and storage. Their results were different in Marjoram and Origanum majorana. As for drying by hot weather, the scent and taste of the sample were better than the freeze-drying method. Ghasemi et al. (2013) mentioned that the oven drying method was better than others, which was consistent with our results related to the active ingredient of Lemon Balm's essential oil. Mirahmadi et al. (2017) determined the main essential oil composition in the shade-dried and oven-dried methods in 35 and 55 samples as beta-caryophyllene, germanial, and gamma cadinene, respectively. Drying at 55 °C caused a reduction in Neral, Geranial, and Geranil acetate. The highest essential oil yield was observed in the oven-drying method at 35 °C, hence, recommended for drying Lemon Balm. In the current study, some active ingredients in the dried samples of Lemon Balm's essential oil decreased significantly in the oven at 55 °C. The active ingredient destroyed in dried samples are the ones reserved in leaves and stem (Zhang et al., 2007). Rahimmalek and Goli (2013) found that drying in a high-temperature oven resulted in losing several effective ingredients' contents in Thymes compared to the shade-dried and freshly harvested samples.

Conclusion

Using mycorrhiza (fungal) and azotobacter (bacteria) biofertilizer caused yield and yield component improvement in the Lemon Balm medical plant. Mycorrhiza increased Lemon Balm's essential oil significantly, while azotobacter did not affect the essential oil production. The highest essential oil percentage and content were obtained in the drying treatment in the shade, but due to the energy consumption, time-consumption, and requiring proper space for drying, we can benefit from rapid drying methods, such as low-temperature ovens and microwaves with less output power and reduced energy input, on the account of conserving herbal essential oil quality and cost efficiency. Indeed, changing an active ingredient in the Lemon Balm medical plant through drying processes showed that this plant is sensitive to the drying process. Therefore, regarding the results, it could be suggested to dry Lemon Balm in lower temperatures and microwaves with less output energy in order to prevent the reduction of the essential oil content and the main determinant factor of quality as affected by the evaporation of aromatic components.

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