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The Effect of Different Nitrogen Doses on Yield and Some Yield Components of *Melissa officinalis* subsp. L. *altissima* (Sibthr. et Smith) Arcang

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Abstract: *Melissa officinalis* subsp. L. *altissima* (Sibthr. et Smith) Arcang which belongs to Lamiaceae (mint) family is a perennial, herbaceous and essential oil bearing plant. Its essential oil and extract could be used as antimicrobial and antioxidant agent. However, there is a limited number of reports on agronomy and chemical contents of *Melissa officinalis* subsp. L. *altissima* (Sibthr. et Smith) Arcang. In this study, effect of five different nitrogen doses (0, 50, 100, 150 and 200 kg ha^{-1}) on some agronomic traits of *Melissa officinalis* subsp. L. *altissima* (Sibthr. et Smith) Arcang were examined in the conditions of Mediterranean climate in 2011 and 2012. All traits were significantly affected by the different nitrogen doses in the second year of the experiment. Higher nitrogen doses increased plant height, number of branches, fresh herbage yield, fresh leaf yield, dry herbage yield and dry leaf yield. On the other hand, the highest N dose (200 kg ha^{-1}) did not give the highest values in terms of plant height, number of branches, fresh herbage yield and dry herbage yield. In the second year, 150 kg ha^{-1} nitrogen treatment was appeared to be better than other treatments.

Keywords: Herbage, *Melissa officinalis* subsp. *altissima*, nitrogen, yield

1. Introduction

Melissa officinalis subsp. L. *altissima* (Sibthr. et Smith) Arcang (lemon balm) is one of the important culinary, aromatic and medicinal plant. It is a perennial herbaceous plant and belongs to Lamiaceae (mint) family. It was originated from Europe, Central Asia and Iran although it is cultivated in different parts of the world. Lemon balm has many health benefits due to its rich content of secondary metabolites (Dimitrova et al., 1993; Mimica-Dukic et al., 2004). *M. officinalis* essential oil is rich in aldehydes and terpenic alcohols which are attributed to therapeutic effects (Hener et al., 1995). The main components of the essential oil were found to be neral, geranial, citronelal and β-caryophyllene. Lemon balm extracts exhibited antioxidant properties due to phenolic acid components such as rosmarinic, protocatechuic, caffeic acids and their methyl esters (Tagashira and Ohtake, 1998; Mencherini et al., 2007). Some pharmacological properties have been attributed to rosmarinic acid as being antiviral and antioxidant (Hener et al., 1995; Carnat et al., 1998; Hohmann et al., 1999). Aqueous extracts of lemon balm have been used for treatment of Alzheimer's disease and regulation of the central nervous system (Akhondzadeh et al., 2003). Besides its medicinal properties, it is widely used as herbal tea and cosmetics.

There are three subspecies of *M. officinalis* namely subsp. *altissima* Arcangeli, subsp. *inodora* Bornm and subsp. *officinalis*. *M. officinalis* subsp. *altissima* is grown naturally in the Southern Europe, Balkans, Northern Africa, Caucasia and Turkey. The scent of *M. officinalis subsp. altissima* generally benefit from as nutritional, however its scent described as "fetid"

(Tucker and De Baggio, 2000). *Melissa officinalis* subsp. *officinalis* is the only one of three known subspecies that is in use today in pharmaceutical preparations. Knowledge about the two other subspecies (*inodora* and *altissima*) is limited.

Dawson et al. (1998) reported that essential oil of *M. officinalis* subsp. *altissima* comprised β-cubebene (39% of the oil) together with lesser amounts of other sesquiterpenes, including α- and β-copaene, β-bourbonene, β-caryophyllene, and germacrene-B and -D. The fragrance of the essential oil is green and woody (pine-like), with a floral (rosy) top note, probably attributable to the terpenes, terpineols and hexenol. The oil does not contain any geranial, neral, or citronellal typically found in *M. officinalis* L. subsp. *officinalis* (Dawson et al., 1998). Van De Berg et al. (1997) have identified the main components of the cultivated *M. officinalis* subsp. *altissima* as β-caryophyllene, germacrene D, sabinene and β-pinene whereas no citral or citronellal was detected. The oils of some *M. officinalis* subsp. *altissima* samples were characterized by the presence of a significant sesquiterpene fraction (53.8, 47.8%, respectively) with caryophyllene oxide and (*E*)-caryophyllene (Basta et al., 2005). Fialová et al. (2008) analysed the antioxidant phenolic compounds (total hydroxycinnamic derivatives, rosmarinic acid and flavanoids) in *M. officinalis* subsp. *altissima* extract and they were found that its extract was rich in total hydroxycinnamic derivatives and rosmarinic acid.

Number of works investigating the effects of nitrogen on the growth and yields of various plant species belongs to Lamiaceae family have been carried out (Kothari and Singh, 1995; Mitchell and Farris, 1996; Baranauskien et al., 2003; Karioti et al., 2003; Özgüven et al., 2006; Katar and Gürbüz, 2008; Sotiropoulou and Karamanos, 2010).

Melissa officinalis subsp. L. *altissima* (Sibthr. et Smith) Arcang has not been studied under cultivation and therefore, no agronomic treatments have not been reported so far. It is considered that sufficient use of chemical fertilizer increase yield and quality of aromatic plants. Nitrogen is used in crop cultivation because agricultural soils are often deficient in nitrogen. Nitrogen is one of the essential macronutrient elements required for plants and it is also a building block of protein in all parts of plant. Moreover it exists in the structure of chlorophyll molecule (Christensen and Peacock, 2000; Inugraha et al., 2014). To ensure adequate nitrogen supply to crops and prevent nutrient deficiencies, increasingly large amounts of inorganic N are employed (Shah, 2004). The aim of this study was to investigate the effects of different nitrogen doses on *M. officinalis* subsp. *altissima* with regard to yield and yield components and essential oil rate under cultivated condition.

2. Materials and Methods

Melissa officinalis subsp. L. *altissima* (Sibthr. et Smith) Arcang seeds were collected from the wild flora of Antalya (Serik-Akbas, altitude 220 m). This study was conducted in the experimental field of the Faculty of Agriculture, Akdeniz University located in Mediterranean Region of Turkey (33 m above sea level), in 2011-2012. This location was characterized by a Mediterranean climate with 1068 mm total rain fall, 18.7 °C mean air temperature, 13.6 °C minimum air temperature and 24.2 °C maximum air temperature.

Soil characteristics of the experimental field were clay loam, high in lime (15.1%), low in salt (0.022%), and high alkaline (pH 7.9). The layer of 0-30 cm soil had low concentrations of organic material (1.55%) and sufficient amount of nitrogen (0.118%). Available phosphorus content of the soil was low (51 kg ha^{-1}) and useful potassium content was high (1248 kg ha^{-1}).

Melissa officinalis subsp. L. *altissima* (Sibthr. et Smith) Arcang seedlings were grown in greenhouse condition and they were transferred to field with 50 cm and 30 cm row and intra-row spacing, respectively. In trials, 15 parcels were used in total and each parcel size was 10 m^2. The field trials have been set to be 2 meters space between each parcel and block and total area was 322 m^2. Field trials were designed according to completely randomized block design with three replications. Five different nitrogen doses [Control (0 kg ha^{-1}), 50 kg ha^{-1}, 100 kg ha^{-1}, 150 kg ha^{-1} and 200 kg ha^{-1}] in ammonium nitrate (33% N) form were applied to the experimental field. In addition, 40 kg ha^{-1} K_2O (as potassium sulphate) and 60 kg ha^{-1} P_2O_5 (as triple super phosphate) was applied to the soil in planting time. Drip irrigation system was used in the experiment and, in necessary situations weeding controls were done by hand. Plants were harvested at the flowering stage by cutting 10 cm above soil surface in 2011 and 2012. After determination of fresh herbage weights, samples were dried in an air-drying oven (Chi-Yeh Electric and Machinery Co., Taipei, Taiwan) at 35 °C and then dry weights were recorded. The plant height (cm), the number of branches (branches $plant^{-1}$), fresh herbage yield (kg ha^{-1}), fresh leaf yield (kg ha^{-1}), dry herbage yield (kg ha^{-1}), dry leaf yield (kg ha^{-1}) and leaf/stem ratio were used for agro-morphological characterization.

Dried leaves were distillated in Clevenger apparatus. For this study, 500 mL deionised water was added to 50 g of leaf samples and they were heated at 100 °C for 3 h. As a result, percentage of essential was determined for each sample.

Randomized block design was carried out as a five-factorial experiment with three replications. The averages of data from each year were statistically analyzed using MSTAT analysis of variance. All values are expressed as mean values and standard deviation (SD) and it was analyzed with Least Significant Difference (LSD) test, involving triplicate analyses for each factorial. Samples were compared by LSD test ($p<0.05$).

3. Results and Discussion

Since *Melissa officinalis* subsp. L. *altissima* (Sibthr. et Smith) Arcang is a perennial plant, plant growth was slow in the first year and therefore plot yields were low. Also, essential oil was ignored in the first year due to insufficient plant development. On the other hand, plants were recovered and grown quite fast in the second year and produced sufficient amount of yield and essential oil.

Overall statistical analyses showed that there were significant differences between the years (2011 and 2012) for plant height, the number of branches, fresh herbage yield, fresh leaf yield, dry herbage yield, dry leaf yield except leaf/stem ratio. All the traits investigated in this research were affected by different nitrogen doses in the second year of the experiment (Table 1).

In terms of plant height, 2011 (78 cm) and 2012 (100 cm) were appeared to be statistically different. In 2011, various nitrogen doses did not reveal any significant differences in plant heights, since plant growth was very slow in the first year. On the other hand, plant heights were significantly affected by the different nitrogen doses in 2012. In this case, 150 kg ha^{-1} nitrogen application gave the highest average value (119 cm) of plant height and control treatment (0 nitrogen) gave the lowest value (71 cm). Plant heights increased significantly by the increasing nitrogen doses up to 150 kg ha^{-1}. According to mean of both years, 50, 100, 150 and 200 kg ha^{-1} doses were statistically placed in the same group with highest values (respectively 84, 92, 99 and 98 cm) and control treatment was the lowest one (Table 1). On the contrary, various nitrogen applications did not affect plant height of *Origanum vulgare* subsp. *hirtum* (Sotiropoulou and Karamanos, 2010).

Mean number of branches was significantly higher in 2011 (82 branch plant^{-1}) than 2012 (61 branch plant^{-1}). Although plants gave many branches in the first year, branches were weak. In 2011 and mean of both years, all nitrogen doses statistically took part in the same group. In the meantime, different nitrogen doses affected number of branches significantly in 2012 (Table 1).

The highest number of branches (69 branch plant^{-1}) was recorded from 150 kg ha^{-1} nitrogen dose and the lowest one was obtained from the control in 2012. The number of branches per plant increased significantly by increasing the nitrogen doses up to 150 kg ha^{-1} in 2012 (Table 1). Similarly, various nitrogen doses significantly affected branching of *Origanum vulgare* subsp. *hirtum*; 80 and 120 kg ha^{-1} nitrogen applications gave the highest branching (Sotiropoulou and Karamanos, 2010).

Fresh herbage yield was influenced significantly by different nitrogen doses in 2012 and mean of the years. As expected, mean fresh herbage yield in the second year (24.720 kg ha^{-1}) was significantly higher than in the first year (20.150 kg ha^{-1}). In 2012, fresh herbage yield risen gradually by increasing nitrogen doses up to 150 kg ha^{-1}; control treatment gave the lowest fresh herbage yield (17.330 kg ha^{-1}) and 150 kg ha^{-1} nitrogen treatment gave the highest fresh yield (28.700 kg ha^{-1}). However, 200 kg ha^{-1} nitrogen treatment reduced fresh herbage yield (27.760 kg ha^{-1}) when it was compared to 150 kg ha^{-1} N treatment. In the mean of the years, 50 kg ha^{-1}, 100 kg ha^{-1}, 150 kg ha^{-1} and 200 kg ha^{-1} nitrogen treatments were statistically placed in the same group with 22.660 kg ha^{-1}, 23.180 kg ha^{-1}, 23.650 kg ha^{-1} and 24.020 kg ha^{-1} fresh herbage yield. Control treatment gave the lowest mean value (18.640 kg ha^{-1}) (Table 1). In oregano, increasing nitrogen doses increased plant fresh weight compared to control treatment (Said-Al Ahl et al., 2009). Generally, the enhancing effect of N-fertilization on plant growth may be due to the positive effects of nitrogen on activation of photosynthesis and metabolic processes of organic compounds in plants which, in turn, encourage plant vegetative growth (Said-Al Ahl et al., 2009).

Same as herbage yield, mean fresh leaf yield in the second year (12.550 kg ha^{-1}) was significantly higher than in the first year (10.200 kg ha^{-1}). However, different nitrogen doses were effective on fresh leaf yield only in 2012. The highest nitrogen dose (200 kg ha^{-1}) produced the highest fresh leaf yield (13.910 kg ha^{-1}) and control treatment produced the lowest fresh leaf yield (10.200 kg ha^{-1}). Other nitrogen doses (50, 100 and 150 kg ha^{-1}) were in the same statistical group (Table 1). Similar to our second year result, Katar

Table 1. Average values of some yield components in *M. officinalis* subsp. *altissima*

Nitrogen doses (kg ha^{-1})	Plant height (cm) 2011	Plant height (cm) 2012	Plant height (cm) Mean	Number of branches (branch plant^{-1}) 2011	Number of branches (branch plant^{-1}) 2012	Number of branches (branch plant^{-1}) Mean
0	77 ± 14	71 ± 6 c	74 b	91 ± 20	53 ± 10 c	72
50	77 ± 12	90 ± 1 bc	84 ab	79 ± 4	65 ± 17 ab	72
100	79 ± 7	105 ± 12 ab	92 ab	77 ± 13	65 ± 3 ab	71
150	78 ± 3	119 ± 7 a	99 a	81 ± 7	69 ± 8 a	75
200	80 ± 19	116 ± 20 a	98 a	81 ± 18	55 ± 12 bc	68
LSD$_{(0.5)}$	14.74	20.31	16.7	25.07	12.42	13.84
Nitrogen doses (kg ha^{-1})	**Fresh herbage yield (kg ha^{-1}) 2011**	**2012**	**Mean**	**Fresh leaf yield (kg ha^{-1}) 2011**	**2012**	**Mean**
0	19970 ± 319	17330 ± 170 c	18640 b	10620 ± 161	10200 ± 121 b	10410
50	22010 ± 804	23310 ± 491 b	22660 ab	10260 ± 303	12440 ± 226 ab	11350
100	19880 ± 216	26470 ± 255 ab	23180 a	10580 ± 103	13360 ± 66 ab	11970
150	18600 ± 114	28700 ± 139 a	23650 a	9660 ± 45	12810 ± 184 ab	11230
200	20280 ± 831	27760 ± 379 ab	24020 a	9900 ± 346	13910 ± 162 a	11910
LSD$_{(0.5)}$	731.95	473.08	487.35	334.57	316.05	212.99
Nitrogen doses (kg ha^{-1})	**Dry herbage yield (kg ha^{-1}) 2011**	**2012**	**Mean**	**Dry leaf yield (kg ha^{-1}) 2011**	**2012**	**Mean**
0	4980 ± 84	5130 ± 49 b	5060 b	2640 ± 13	3310 ± 40 b	2970
50	5840 ± 233	6860 ± 133 a	6350 a	2580 ± 64	4200 ± 90 ab	3390
100	5130 ± 53	8010 ± 66 a	6570 a	2680 ± 40	4230 ± 28 ab	3460
150	4810 ± 40	8420 ± 89 a	6620 a	2450 ± 17	4070 ± 74 ab	3260
200	5370 ± 205	8440 ± 117 a	6910 a	2540 ± 72	4540 ± 60 a	3540
LSD$_{(0.5)}$	200.98	159.46	138.65	77.20	122.51	65.24
Nitrogen doses (kg ha^{-1})	**Leaf/stem 2011**	**2012**	**Mean**		**Essential oil rate (%) 2012**	
0	1.14 ± 0 ab	1.44 ± 0 a	1.29 a		0.13	
50	0.92 ± 0 c	1.17 ± 0 ab	1.05 b		0.11	
100	1.17 ± 0 a	1.03 ± 0 bc	1.10 b		0.10	
150	1.09 ± 0 ab	0.84 ± 0 c	0.96 b		0.09	
200	1.00 ± 0 bc	1.01 ± 0 bc	1.00 b		0.06	
LSD$_{(0.5)}$	0.15	0.26	0.20			

Different letters between cultivars denote significant differences (LSD test, $p< 0.05$). Different letters between susceptible and resistant cultivars denote significant differences (LSD test, $p < 0.05$).

and Gürbüz (2008) obtained the highest fresh leaf yield from 120 kg ha^{-1} nitrogen treatment and the lowest fresh leaf yield from the control treatment in the second year of *M. officinalis* subsp. *officinalis*.

Mean dry herbage yield in 2012 (7.370 kg ha^{-1}) was higher than in 2011 (5.230 kg ha^{-1}) and difference between the years was found be important. In 2011, there was no important variation between the treatments. In 2012, all nitrogen doses were statistically placed in the same group except the control treatment which gave the lowest dry herbage yield. In the mean of the years, despite 200 kg ha^{-1} nitrogen treatment gave the highest dry herbage yield (6.910 kg ha^{-1}), there were no statistically important differences from 50 kg ha^{-1} (6.350 kg ha^{-1}), 100 kg ha^{-1} (6.570 kg ha^{-1}), 150 kg ha^{-1} (6.620 kg ha^{-1}) and 200 kg ha^{-1} (6.910 kg ha^{-1}) nitrogen treatments. Control treatment gave the lowest mean value (5060 kg ha^{-1}) (Table 1). Similar to our result, Azizi et al. (2009) reported that nitrogen increased the dry weight of *Origanum vulgare*. Sotiropoulou and Karamanos (2010) found that 80 kg ha^{-1} nitrogen application gave the highest dry herbage yield in *Origanum vulgare* subsp. *hirtum*.

In case of mean dry leaf yield, difference between 2012 (4.070 kg ha^{-1}) and 2011 (2.580 kg ha^{-1}) was found be important. However, nitrogen doses significantly affected dry leaf yield only in 2012. In that experimental year, 200 kg ha^{-1} nitrogen treatment gave the highest dry leaf yield (4.540 kg ha^{-1}) and control treatment gave the lowest one (3.310 kg ha^{-1}); and differences were appeared to be statistically important. Other treatments (50, 100 and 150 kg ha^{-1}) were in the same statistical group (Table 1). In *M. officinalis* subsp. *officinalis*, the highest dry leaf yield was obtained from 120 kg ha^{-1} nitrogen treatment in the second year (Azizi et al., 2009).

Leaf/stem ratio revealed quite different pattern from other agro-morphological characteristics. There was no important variation between the experimental years. On the other hand, leaf/stem ratio was significantly affected by different nitrogen doses in 2011, 2012 and mean of the years. In the first year, 100 kg ha^{-1} nitrogen dose gave the highest value (1.17) and 50 kg ha^{-1} nitrogen dose gave the lowest value (0.92). In the second year, the highest and lowest values were obtained from control treatment (1.44) and 150 kg ha^{-1} nitrogen dose (0.84). In mean of the years, the highest value (1.29) was recorded from control treatment and followed by 100 kg ha^{-1} nitrogen dose (1.10) (Table 1). Katar and Gürbüz (2008) obtained the highest fresh leaf rate from 120 kg ha^{-1} nitrogen treatment in the second year of *M. officinalis* subsp. *officinalis*.

When essential oils were evaluated, while essential oils could not be obtained in the first year, they were obtained in second year. Essential oil rates were negatively influenced by the nitrogen doses. The highest essential oil rate (0.13%) was recorded from the control treatment and the lowest rate (0.06%) was obtained from the highest nitrogen dose (200 kg ha^{-1}). Actually, essential oil rates were gradually decreased by increasing nitrogen doses (Table 1). In *M. officinalis* subsp. *officinalis*, essential oil rates were varied between 0.27-0.30% depending on the nitrogen doses (Katar and Gürbüz, 2008). Sotiropoulou and Karamanos (2010) found no significant effects of different nitrogen doses on essential oil rates of *Origanum vulgare* subsp. *hirtum*. The essential oil content of *Origanum vulgare* was not affected by the nitrogen levels in the first year of the experiment. However, higher nitrogen levels decreased essential oil content in the second year (Azizi et al., 2009). Sodre et al. (2012) utilized the six types of the fertilization of manure and mineral and they showed that the changing the relative proportion of some resulting compounds such as neral, geranial, and citronellal.

4. Conclusions

In conclusion, different nitrogen doses affected all traits significantly in the second year of the experiment. Higher nitrogen doses increased plant height, number of branches, fresh herbage yield, fresh leaf yield, dry herbage yield and dry leaf yield when they were compared to the control treatment (no nitrogen). On the other hand, the highest N dose (200 kg ha^{-1}) did not give the highest values in terms of plant height, number of branches, fresh herbage yield and dry herbage yield. In the second year, 150 kg ha^{-1} nitrogen treatment was appeared to be better than other treatments. Essential oil rates were negatively influenced by the nitrogen doses. Therefore as increasing nitrogen dose, the rate of essential oils decreased.

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