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Effect of nitrogen fertilizer and salicylic acid spraying on growth and volatile oil content of peppermint (*Mentha piperita* L.)

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Abstract

A field study was carried out at the Agricultural Research Station of the Agriculture College, Al-Muthanna University (located in southern Iraq) , during season growth 2022.The study aims to know the effect of two factors, first one : adding nitrogen at three levels (0, 75 and 150 Kg. N. ha^{-1}), second factor : spraying salicylic acid with three different concentrations (0, 100, 200 mg. SA. L^{-1}) and their interaction on growth and essential oil yield of peppermint (*Mentha piperita* L.). Results showed the significantly superiority of nitrogen fertilizer level 150 Kg. N. ha^{-1} in all studied characteristics : plant height, branches number, leaves number, fresh weight of the shoot , dry weight of the shoot, volatile oil content and volatile oil yield (46.92cm, 16.89 branch $plant^{-1}$,153.7 leaves $plant^{-1}$, 17.49 g plant, 2.66 g plant, 1.02% and 271.0 µL $plant^{-1}$, respectively. The peppermint plants sprayed with a concentration (200 mg SA. L^{-1}) were significantly superior in plant height, branches number, leaves number, shoot fresh and shoot dry weight (42.88cm, 13.52 branch $plant^{-1}$,129.9 leaf $plant^{-1}$, 12.97 g $plant^{-1}$ and 1.98 g $plant^{-1}$), respectively. While plants treated with concentration (100 mg. SA. L^{-1}) gave the highest significant values of volatile oil content and yield of volatile oil (0.96 % , 291.9 µL plant), respectively.

The interaction treatment (150 Kg. N. ha^{-1} , with 200 mg SA. L^{-1}) gave the highest rates for all the studied except yield of essential oil was a highest in treatment (150 Kg. N. ha^{-1} , with 100 mg SA. L^{-1}).

Key words *Mentha piperita* L. , Peppermint, Nitrogen fertilizer, Salicylic acid, Essential oil

Introduction

Peppermint (*Mentha piperita* L.) is a perennial herbal plant belonging to the Lamiaceae family, its cultivation is spread all over the world . Peppermint is one of the significant medicinal and aromatic plants, It represents the main exporter of flavored compounds , which can be used in treatment of many diseases ,

pharmaceutical industries and food products (Yazdani *et al.* 2002, Grieve 1999). Peppermint is planted primarily for the essential oil extracted from its leaves. The essential oil of the peppermint plant contains 50-60% menthol and 10-20% menthone, in addition to tannins , esters and flavonoids (Murray *et al.* 1988). It is used as a carminative, mild sedative, diuretic, antiemetic, antiseptic, antispasmodic, diaphoretic, digestive, chlorotic , antiviral, as well as using it in many mixture for treating of indigestion, colic , cold remedies and cough (Khare 2007). Peppermint oil is also used to improve the taste and flavor of many pharmaceutical preparations , such as solutions of toothpastes, mouthwashes and sterilization of the mouth, as well as in the manufacture of perfumes, sweet and chewing gum (Gupta 1991, Budavari *et al.* 1989). Chemical fertilization is one of the most important growth- limiting factor, and it depends on the characteristics of soil, type of crop and availability of nutrients (El- Gendy *et al.*2013). Singh *et al* (1995) reported that the biosynthesis of volatile oils is greatly influenced affected by several internal and external factors including fertilization and environmental conditions. Nitrogen is one of the most important nutrients that the plant cannot dispense with, as it is used in the construction of many organic compounds inside the plant, such as nucleic acids, amino acids , proteins, enzymes and others. Salicylic acid (2-hydroxybenzoic acid) is an organic phenolic compound with a hormonal effect in biosynthesizes, and has a role in responding to many biotic and abiotic stresses (Noreen *et al.* 2009, He *et al.* 2002). Salicylic acid can stimulate the effectiveness of alternative oxidase enzyme in the mitochondria, which contributes to the mechanism of alleviating the effect of stress (Raskin 1992, Vanlerberghe & McIntosh 1997). Salicylic acid has effected many physiological processes such as photosynthesis, growth, stomata closure and reduced transpiration, protein synthesis and cellular metabolism, in addition to its role in stimulating the plant's defense system against various diseases and induction of antioxidant synthesis (Pacheco *et al.* 2013). The effect of salicylic acid on the physiological processes of plants varies according to salicylic acid concentration, environmental conditions, developmental stage and species (Shraiy and Hegazi ,2009). The aim of this research is to improve the growth of peppermint plant (*Mentha pipeirta* L.) and increase its essential oil percentage by the of different levels of nitrogen fertilizer and different concentrations of salicylic acid and their interaction.

MATERIALS AND METHODS

The study was conducted at the at the Agricultural Research Station College of Agriculture , Al-Muthanna University, for the period from 15/February to15/June, 2022, to find out the effect of adding nitrogen fertilizer at three levels (0, 75 and 150) Kg. N ha^{-1} and spraying salicylic acid with three concentrations (0, 100 and 200) mg SA. L^{-1} on the growth of peppermint plant and it's a volatile oil. Before planting, the chemical and properties of the field soil were determined, and it was : sandy loam (sand 62%, silt 20%and clay 18%) with organic matter (1.5 %) , pH (7.3) , EC (4.7dsm), available nitrogen , phosphorous and potassium (8 , 11and 270) ppm , respectively. The field was divided into three sectors, and each sector contains 9 experimental units (1.5m × 1.5m) each of them contained three lines spaced at 30 cm . Underground stolons (3 cm long) were planted on 15/February (Al-Zyadi, 2019), in each line 15 cm apart. Triple super

phosphate fertilizer (100 Kg. P_2O_5.ha^{-1}) was added at once before planting . Salicylic acid concentrations were - sprayed in two batches (according to the specified concentrations), the first batch was sprayed - one month after the plants dawn from the surface of the soil, and the second batch was 15 days after the first batch. The plants were harvested on 15 June, 2022 (Al-Zyadi,2019). Five plants were randomly selected from each experimental unit from the inner lines of each of them, the traits were measured and estimated : plant height , number of leaves .plant , number of branches. plant , fresh weight of shoot , dry weight of shoot , percentage of volatile oil and yield of volatile oil. Essential oil content was estimated by the hydro distillation method described by Aflatuni (2005). After completing the process of air drying the vegetative parts of selected plants at a temperature of 40° C, and after grinding the dried plants, 25 g of powder from each experimental unit was taken separately and placed in a 1- liter flask, then mixed with 500 ml of water, , which was subsequently hydro distilled in Clevenger apparatus for three hours. The study was designed according to a randomized complete block design and was implemented as a factorial experiment with three replications. Analysis of variance (ANOVA) was extracted using GenStat software , means were calculated using least significant difference (LSD) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of nitrogen fertilizer

The results of Tables (1-7) indicated the significant superiority of nitrogen fertilizer in all the studied vegetative growth traits, as well as the percentage and yield of volatile oil, the plants fertilized at level (150 Kg N. ha) recorded the highest rates in plant height (46.92cm), number of branches (16.89 branch. plant), number of leaves (153.7 leaves. plant), shoot fresh weight (17.49 g .plant^{-1}), shoot dry weight (2.66 g. plant^{-1}), volatile oil percentage (1.02 %) and yield of volatile oil (271.0 µL. plant^{-1}) compared to the lowest rates found when the control treatment. The reason for the increase in the rate of plant growth by increasing the levels of nitrogen fertilizer may be due to the fact that nitrogen is essential for plant growth due to its important role in the synthesis of amino acids, proteins, enzymes and chlorophyll, which leads to increases plant branches (tab.2) and number of leaves (tab.3), thus, to increase the activity and efficiency of photosynthesis and promotion of plant growth. (Lawlor *et al*, 2004). Amino acids and enzymes have a key role in the biosynthesis of many compounds that are included in the composition of the components of volatile oils (Koeduka *et al*, 2006). Vegetative growth, volatile oil content and components of *Mentha piperita* and *Mentha arvensis* plants were increased by increasing nitrogen fertilization levels (Zheljazkov & Margina ,1996).

Effect of salicylic acid spraying

Data presented in Tables (1,2,3,4 and 5) showed the superiority of plants treated with salicylic at a concentration of 200 mg. SA. L^{-1} significantly, and the highest rates were recorded in plant height (42.88 cm.), number of branches (13.52 branch. plant), number of leaves (129.9 leaves. Plant^{-1}), shoot fresh weight (12.97gm. plant) shoot dry weight (1.98 g. plant^{-1}), the lowest rates were recorded in a control treatment. While the plants treated with concentration 2100 (mg. L^{-1}) SA. significantly superiority in essential oil percentage (0.96 %) (Table 6), and yield of essential oil (187.5µL.plant^{-1}).The significant effect of salicylic acid on the growth of peppermint and its volatile oil content, may be

attributed to important physiological roles that salicylic acid contributes to plant growth, stimulating the growth of chlorophyll, enzymes and increasing the absorption process of ions, or to its role in chloroplasts synthesis ,thus, increase the efficiency of photosynthesis (Hayat *et al*.2007). These results are in agreement with the findings of Al-Zyadi (2021) in his study on coriander plant.

Effect of the interaction

The data shown in Tables (1-7) confirmed the significant effect of the interaction between the two experimental factors for all studied characteristics. The interaction between the fertilizer level (150 Kg. N ha^{-1}) and salicylic spray at concentration 200 (mg. L^{-1}) SA. outperformed by giving it highest rates for all the studied vegetative growth characteristics, plant height, , number of leaves, number of branches and the fresh and dry weight of shoot . While the interaction between nitrogen fertilizer (150 Kg. N ha^{-1}) and salicylic spray 100 (mg. L^{-1}) SA. was significantly superior in percentage and yield of volatile oil.

CONCLUSION

We conclude from this study, which was conducted in southern Iraq and under the conditions of the city of Samawah, that fertilizing the peppermint (*Mentha piperita* L.) with nitrogen fertilizer at the level (150 Kg. N ha^{-1}), and spraying it with salicylic acid at the concentration (100 mg SA. L^{-1}) were the most appropriate and best to give the highest content and yield of essential oil.

Table 1**.** Effect of nitrogen fertilizer and salicylic acid spraying on plant height of peppermint (cm.)

N. fertilizer (Kg N. ha^{-1})	Salicylic acid (mg. L^{-1})			
	0	100	200	Mean
0	30.13	34.46	35.76	33.45
75	39.26	43.40	45.00	42.56
150	45.88	47.00	47.88	46.92
Mean	38.88	41.6	42.88	
L.S.D. 0.05	N. fertilizer	Salicylic acid	Salicylic acid× N. fertilizer	
	1.54	1.54	2.68	

Table 2**.** Effect of nitrogen fertilizer and salicylic acid spraying on branches number of peppermint (branch. $Plant^{-1}$)

N. fertilizer (Kg N. ha^{-1})	Salicylic acid (mg. L^{-1})			
	0	100	200	Mean
0	5.18	6.99	7.55	6.67
75	12.44	14.22	15.00	13.88
150	15.33	17.33	18.00	16.89
Mean	10.98	12.85	13.52	
L.S.D. 0.05	N. fertilizer	Salicylic acid	Salicylic acid× N. fertilizer	
	0.64	0.64	1.12	

Table 3. Effect of nitrogen fertilizer and salicylic acid spraying on number of leaves of peppermint (leaf. Plant^{-1})

N. fertilizer (Kg N. ha.)	Salicylic acid (mg. L^{-1})			
	0	100	200	Mean
0	60.8	76.4	85.7	74.3
75	125.1	138.1	142.0	135.1
150	143.1	156.0	162.0	153.7
Mean	109.6	123.5	129.9	
L.S.D. 0.05	N. fertilizer	Salicylic acid	Salicylic acid× N. fertilizer	
	5.37	5.37	9.31	

Table 4. Effect of nitrogen fertilizer and salicylic acid spraying on shoot fresh weight of peppermint (g. plant)

N. fertilizer (Kg N. ha.)	Salicylic acid (mg. L^{-1})			
	0	100	200	Mean
0	5.28	6.48	7.00	6.25
75	11.50	13.05	13.53	12.43
150	16.36	17.74	18.37	17.49
Mean	11.04	12.43	12.97	
L.S.D. 0.05	N. fertilizer	Salicylic acid	Salicylic acid× N. fertilizer	
	0.80	0.80	1.39	

Table 5. Effect of nitrogen fertilizer and salicylic acid spraying on dry weight of shoot of peppermint (g. plant)

N. fertilizer (Kg N. ha^{-1})	Salicylic acid (mg. L^{-1})			
	0	100	200	Mean
0	0.78	0.98	1.06	0.94
75	1.70	1.95	2.09	1.91
150	2.47	2.70	2.80	2.66
Mean	1.65	1.88	1.98	
L.S.D. 0.05	N. fertilizer	Salicylic acid	Salicylic acid× N. fertilizer	
	0.13	0.13	0.22	

Table 6. Effect of nitrogen fertilizer and salicylic acid spraying on essential oil content of peppermint (%)

N. fertilizer (Kg N. ha^{-1})	Salicylic acid (mg. L^{-1})			
	0	100	200	Mean
0	0.70	0.82	0.68	0.73
75	0.85	0.98	0.86	0.90
150	1.00	1.08	0.98	1.02
Mean	0.85	0.96	0.84	
L.S.D. 0.05	N. fertilizer	Salicylic acid	Salicylic acid× N. fertilizer	
	0.052	0.052	0.091	

Table 7. Effect of nitrogen fertilizer and salicylic acid spraying on essential oil of peppermint (µL. plant)

N. fertilizer (Kg N. ha^{-1})	Salicylic acid (mg. L^{-1})			
	0	100	200	Mean
0	54.6	79.4	72.1	68.7
75	144.5	191.1	179.5	171.7
150	246.7	291.9	274.4	271.0
Mean	148.6	187.5	175.3	
L.S.D. 0.05	N. fertilizer	Salicylic acid	Salicylic acid× N. fertilizer	
	21.75	21.75	37.68	

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to the lack of rain, high temperatures and high evaporation-transpiration, which leads to the difficulty of managing soil and wate