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EFFECT OF DIFFERENT PERIODS OF IRRIGATION AND ASCORBIC ACID ON GROWTH, YIELD AND QUALITY OF ESSENTIAL OIL OF *MENTHA PIPERITA* L. PLANTS

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ABSTRACT: The present study was carried out during two growing seasons (2019 and 2020) at the Nursery of Department of Floriculture, Ornamental Horticulture, Faculty of Agriculture, Alexandria University, Egypt to study the effect of irrigation intervals (4 days as control stress, 8 days as moderate stress and 12 days as severe stress) and ascorbic acid (AsA) at 0, 100, 150, 200, 250 mg l^{-1} on the growth, quantity and quality of the essential oil of *Mentha piperita* L. plants. The main results of this study indicated that most of the vegetative growth characteristics were increased when AsA was applied at 150 or 200 mg l^{-1} to the plants grown under control stress (irrigation interval at 4 days). AsA at 200 mg l^{-1} was more effective on leaves fresh weight than 150 mg l^{-1} which affects mostly the number of branches of the plants grown under moderate stress (irrigation interval at 8 days). Increasing irrigation interval to 12 days (severe stress) in addition to AsA at 200 mg l^{-1} decreased the harmful effects in most peppermint plants and leads to obtain high oil percentage and yield. Peppermint oil was analyzed according to GC analysis of fresh leaves grown under water stress and sprayed with ascorbic acid with different concentrations identified 9 components, the major component was menthol with an average of 34.55-52.67% during the three cuts. In general, there was a direct relationship between increasing irrigation intervals and oil components percentage (menthol, menthone and limonene). These results suggest that normal water deficit (4 days irrigation interval) in peppermint will be appropriate to enhance better growth and productivity. In addition, ascorbic acid treatment reduced the damaging action of water stress, especially at concentrations of 150 and 200 mg l^{-1}, so we can conclude that these concentrations are sufficient enough to support peppermint growth, leaves fresh weight and consequently maximum oil yield.

Key words: medicinal and aromatic plants, peppermint, *Mentha piperita* L., water stress, ascorbic acid, essential oil.

INTRODUCTION

Mentha piperita L. (Lamiaceae formerly Labiatae) is one of the famous well known and important medicinal plants which has a good reputation in the US, Australia, and Europe. United Kingdom, Holland, Spain, and Germany are the major markets (Ministry of Agriculture, 1999). It is considered to be a hybrid between *Mentha spicata* and *Mentha aquatic* (Foster, 1996). Peppermint is dicotyledon, rhizomatous, tender perennial sedge, herbaceous plant, 30 to 90 cm high, purplish or green, leaves ovate or oblong, coarsely serrate, flowers purplish.

Peppermint is considered as a medical and aromatic plant and extensively using for the medicinal and food product industries (Yazdani *et al.*, 2002). It is also used as a flavor in pharmaceutical and dental preparations, mouthwashes, cough drops, chewing gums, candies confectionery, alcoholic liqueurs, cosmetic and perfumery industries (Briggs, 1993). Moreover, mint has antibacterial, antifungal and insecticidal properties (Saljoqi *et al.*, 2006) and Lawrence (2007).

Peppermint oil is a colorless, pale yellow liquid, has a strong agreeable odor used for flavoring pharmaceuticals. According to the University of Maryland Medical Center (UMMC), peppermint oil has many properties that can help with cold and flu, nausea, sore throat cramps, indigestion, even a spastic colon, treating pain, headache and oral problems (Neeraj *et al.*, 2013). In this concern, there is a direct correlation between peppermint oils and enhanced memory (Moss *et al.*, 2008). In addition, in traditional medicine, peppermint and its essential oil have been used as antispasmodic, antiseptic remedies and in the treatment of cancers due to having promising radioprotective effects for cancer patients (Baliga and Rao, 2010).

Plants have developed physiological responses and ecological strategies to overcome water shortage through avoidance or tolerance water stress (Kramer *et al.*, 1995). FAO (2002) reported that in the last decades there has been a great interest in the adoption of water-saving strategies in horticulture, due to the competition for water resources with other sectors and water scarcity. In fact, water is one of the most considerable compounds on the ground and $^{2}/_{3}$ of the ground-level was surrounded by water, but in most parts of the world, the lack (shortage) of water is a critical factor that affects the production of the agricultural products (Redy *et al.*, 2003). Also, Mancosu *et al.* (2015) informed that water stress is one of the major limiting factors for agriculture and food safety worldwide, and this is agreed with Bañon *et al.* (2006) who informed that water limitation has an impact on plant growth.

One of the techniques used to increase plant tolerance to stress and alleviate the problem is using ascorbic acid (AsA) as a foliar spray, it is white powder that dissolves in water with the molecular formula $C_6H_8O_6$, AsA participates in a variety of processes, including photosynthesis, cell wall growth and cell expansion, resistance to environmental stresses (Galal *et al.*, 2000; Smirnoff and Wheeler, 2000 and Zhang, 2013). Ascorbic acid (AsA) as a factor that regulates various physiological processes controlling growth, development, cell division and stress tolerance (Hossain *et al.*, 2017).

Many studies discussed the importance of AsA and its effect as an antioxidant defense and have a regulatory role in promoting productivity on many plants via regulating cell division and photoprotection and regulation of photosynthesis and growth as reported by Halimeh *et al.* (2013) and Sarkar *et al.* (2016). Exogenous ascorbic acid (AsA) can be applied through root systems, during seed priming or using foliar-spraying (FS) treatment (Athar *et al.*, 2008). It is known that ascorbic acid, as well as irrigation, are two of the most important factors affecting medicinal and aromatic plants. However, no reports are available on the production of *Mentha piperita* under Egyptian conditions, concerning ascorbic acid (AsA) and irrigation requirements. In this concern, Koriesh (1989) studied the effect of irrigation rates on growth and flowering of rose cv. Baccara plants grown in sandy soil. Daily irrigation rates of 2, 4 or 6 litres/plant were applied. He reported that the flower yield increased using 4 litres/plant and root FW was inversely related to irrigation rate. Neeraja *et al.* (1999) studied the effect of four levels of irrigation on growth, yield and yield attributes of rabi onion (*Allium cepa* L.) in a sandy loamy soil. They found that the higher level of irrigation resulted in maximum plant height, more number of leaves per plant, maximum bulb length, bulb diameter, bulb weight and bulb yield. Mohamed *et al.* (2000) studied the effects of irrigation treatments on onion cultivars. They noticed that the total, marketable yields and average bulb weight, significantly increased, while total soluble solids (TSS) declined with increasing available soil moisture. Lal *et al.* (2002) studied the effects of irrigation levels on the growth and yield of onion cv. Hisar-2. They reported that plant height, number of leaves per plant, bulb size and bulb yield increased with increasing rates of irrigation. Hanson *et al.* (2003) investigated the response of garlic to different amounts of irrigation water. They observed that garlic yield was decreased linearly with the reduction of the applied water amount in sandy loam soil. Research applied on lemon balm (*Melissa officinalis* L.) using water deficiency (0, 12.5, 25.0, 37.5 and 50.0%) resulted that the highest essential oil ratio (0.16%) was at 50% water deficiency thus essential oil ratio was affected positively by increasing water deficit (Ozturk *et al.*, 2004). El-Gamal (2005) found that AsA has promote effect on vegetative growth, herb productivity and essential oil percentage of sweet basil plants grown under water deficit conditions. Khalil *et al*. (2010) sprayed AsA at 100, 150 and 200 mg l^{-1} on basil plants, the results indicated that all tested concentrations showed promote effect on growth, herb fresh and dry weight and oil percentage grown under water deficit stress. Zonouri *et al*. (2014) studied the effect of foliar spraying of ascorbic acid on chlorophyll a and chlorophyll b, and found that AsA with anti-oxidative properties prevents degradation of chlorophyll and indirectly increased in full irrigation treatments. So, the main objective of the present investigation was to evaluate the individual and combined effects of the application of ascorbic acid (AsA) as non-enzymatic antioxidants with water stress conditions via different irrigation intervals rates on the growth parameters, quantity and quality of the essential oil of *Mentha piperita* L. plants.

MATERIALS AND METHODS

The present work was carried out during the two growing seasons 2019 and 2020 at the Nursery of Department of Floriculture, Ornamental Horticulture and Landscape Gardening, Faculty of Agriculture, Alexandria University. Uniformed rooted cuttings of *Mentha piperita* L. plants with a length of 10-12 cm and with approximately 6-8 leaves each were used in a pot experiment. The cuttings have been transplanted in clay pots of 30 cm in diameter (one rooted cutting/pot) on 26th and 25th of March of the two seasons (2019 and 2020), respectively. The pots were packed with a medium of clay, sand and compost (2:1:1 v/v/v respectively) with soil texture sandy clay loam. The chemical analysis of the used medium indicated that it was containing 10, 5.84 and 203 ppm of N, P and K, respectively. The electrical conductivity (EC) was 4.8 dS m^{-1} with a pH value of 8.62. Plants were watered, as needed, regularly after planting for two weeks before starting irrigation treatments and spraying ascorbic acid (AsA).

Experimental treatments:

Two factors were involved in the present study, the first was intervals of irrigation, and the second was ascorbic acid. The three intervals of irrigation had been used 4 days as control stress, 8 days as moderate stress, 12 days as severe stress during growing seasons; a quantity of water (1 liter/pot) equivalent to 75% field capacity (FC) had been added to all planted pots in order to use a constant amount of water irrigation every time to retain water at different time intervals. In addition, foliar application of ascorbic acid with five concentrations (0, 100, 150, 200 and 250 mg l^{-1}) was applied four times during each season. The first spray was done after one month from the planting date, the second spray one month later and finally the third and fourth spray were added after one month of the first and second cuts respectively. Foliar application with ascorbic acid was added before irrigation time for each treatment interval and spraying took place till leaves run off early in the morning before dew time.

Experimental design:

This study was carried out using a split-plot design, the main plots including irrigation intervals 4, 8, 12 interval days and five concentrations of ascorbic acid 0, 100, 150, 200 and 250 mg l^{-1} were allocated to subplots with four replicates each replicate contained 15 treatment including control plants, and each treatment contained 3 plants as a plot. So, the total number of the plants used N= 3×5×4×3 = 180 plants for each season. Comparisons between means of the studied treatments were carried out using the least significant difference at 0.05 probability level ($L.S.D_{0.05}$) according to Gomez and Gomez (1984).

Growth characteristics:

The recorded vegetative growth data were; plant height (cm), number of leaves and leaves fresh weights (g).

Volatile Oil determination:

Volatile oil yield (ml):

Oil distillation has to be distilled the same day as harvested from peppermint fresh leaves (Aflatuni, 2005). The essential oil of fresh leaves was extracted by the hydro-distillation method (Novak *et al.*, 2002).

Volatile Oil percentage:

The amount of oil obtained from five plants was measured and oil percentage (%) was calculated according to Charles and Simon (1990). Volatile oil percentage was calculated using the following equation:

$$\text{Volatile oil \%} = \frac{\text{Oil volume in the graduated tube}}{\text{weight of leaves sample (g)}} \times 100$$

Volatile Oil Components:

Oil was analyzed in Horticultural Research Institute, Department of Medicinal and Aromatic Plants. Cairo. Using DsChrom 6200 Gas Chromatograph equipped with a flame ionization detector for separation of volatile oil constituents. The analysis conditions were as follows: the chromatograph apparatus was fitted with capillary column DB-WAX 122-7032 Polysillphenylene –siloxane 30 m × 0.25 mm ID × 0.25 mm ID × 0.25 µm film. Temperature program ramp increased with a rate of 13 °C /min from 60 °C to 220 °C flow rates of gases were nitrogen at 1 ml/min, hydrogen at 30 ml/min and 330 ml/min for air. Detector and injector temperatures were 280 °C and 250 °C, respectively. The obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of main components of volatile oil. The identification of components was based on a comparison of their relative retention times with those of authentic standards.

RESULTS AND DISCUSSION

Plant height/plant (cm):

Data presented in Table (1) showed that plant height was significantly influenced under normal stress condition (4 days) with 150 mg l^{-1} in 1st and 3rd cut achieving the highest plant height, the mean values were 23.79, 4.82 cm in the first season and 24.28, 4.48 cm in the second season, respectively. While applied 200 mg l^{-1} in 2nd cut induced significant increase in both seasons (15.28, 11.17 cm, respectively). These findings agreed with Mazher *et al*. (2011) on croton plants. Plants grown under moderate stress condition proved the highest plant height when interacting with 250 mg l^{-1} (21.91 cm) in 1st cut during the first season and (10.00 cm) in 2nd cut during the second season, at the same time the interaction of AsA with 200 mg l^{-1} during 2nd cut in the first season gained the best mean value 13.07 cm and with 150 mg l^{-1} in 3rd cut was significantly effective in both seasons (3.91,3.67 cm, respectively).

Concerning increasing water stress to a severe level (12 days) and foliar application with AsA, it was apparent that different concentrations showed no significant effect

Table 1. Effect of irrigation intervals, ascorbic acid treatments, and their interactions on plant height/plant (cm) of *Mentha piperita* plants during the two seasons 2019 and 2020.

Irrigation intervals (A)	First Season						Second Season					
	Ascorbic acid mg l^{-1} (B)											
	Cont.	100	150	200	250	Mean	Cont.	100	150	200	250	Mean
First cut												
4 days	19.65	20.52	23.79	22.48	22.16	21.72	20.42	21.13	24.28	21.08	20.86	21.55
8 days	15.12	16.76	18.02	18.66	21.91	18.09	17.61	18.45	20.47	20.73	19.25	19.30
12 days	14.16	16.72	17.49	18.08	19.12	17.11	14.39	16.94	18.64	20.78	17.47	17.64
Mean	16.31	18.00	19.77	19.74	21.06		17.47	18.84	21.13	20.86	19.19	
L.S.D $_{0.05}$	A= 0.83		B = 0.68		A x B = 1.18		A= 1.13		B = 0.73		A x B = 1.27	
Second cut												
4 days	9.20	9.89	11.58	15.28	10.50	11.29	8.47	8.64	8.81	11.17	9.84	9.38
8 days	8.92	9.56	10.86	13.07	11.05	10.69	7.02	7.19	8.06	8.75	10.00	8.20
12 days	6.50	8.89	9.36	11.67	10.03	9.29	6.83	6.58	7.08	8.60	7.68	7.36
Mean	8.21	9.45	10.60	13.34	10.53		7.44	7.47	7.98	9.51	9.17	
L.S.D $_{0.05}$	A= 0.41		B = 0.66		A x B = 1.14		A= 0.22		B = 0.45		A x B = 0.79	
Third cut												
4 days	2.92	3.14	4.82	3.47	3.25	3.52	2.92	3.23	4.48	3.34	3.12	3.42
8 days	2.67	3.08	3.91	3.17	2.90	3.15	2.43	3.00	3.67	3.25	3.08	3.09
12 days	2.22	2.84	3.48	3.07	2.91	2.91	2.38	2.57	3.25	2.92	2.81	2.79
Mean	2.60	3.02	4.07	3.23	3.02		2.58	2.93	3.80	3.17	3.00	
L.S.D $_{0.05}$	A= 0.12		B = 0.22		A x B = 0.38		A= 0.22		B = 0.18		A x B = 0.31	

L.S.D $_{0.05}$: Least Significant Difference at probability 0.05

on plants height in the 1st cut during the first season, although, AsA at 250 mg l^{-1} gave the higher plant height (19.12 cm) and was significant effective compared with untreated plants 14.16 cm. In addition, AsA at 200 mg l^{-1} in the 2nd cut produced for resulted in 11.67 cm and 150 mg l^{-1} showed the highest plant height in 3rd cut 3.48 cm in the same season. While in the second season the maximum significant promotion was detected at 200 mg l^{-1} in the 1st and 2nd cuts recorded 20.78 and 8.60 cm respectively. and 150 mg l^{-1} in the 3rd cut produced 3.25 cm.

Generally, deficit irrigation treatments produced changes in vegetative growth of peppermint plants which negatively affect plants height, whereas applied ascorbic acid (as a technique to face water stress on plants) affected positively on the studied character. The results obtained here confirm those of Hamad and Hamada (2005), Farahat *et al.* (2007) who stated that foliar application of AsA (50-200 ppm) caused significant increases in vegetative growth characters. Also, Khalil *et al*. (2010) sprayed AsA at 100,150 and 200 mg l^{-1} on basil plants and found promote effect on plants grown under water deficit conditions. Abo-Marzoka *et al.* (2016) sprayed ascorbic acid (AsA) at 200 ppm and obtained an increase in uptake of water and essential nutrients through adjusting cell osmotic potential, consequently affecting vegetative plants growth.

Number of leaves/plant:

The data in Table (2) indicated that the best treatment between irrigation intervals and AsA foliar application used at 4 and 8 days plus 150 mg l^{-1} in the 1st cut (363.25 and 346.50 in first season and 370.92 and 352.50 in second season). In the 2nd cut 150 mg l^{-1} was effective at 4 days in both seasons 470.67 and 536.83 while 200 mg l^{-1} was better at 8 days in both seasons (387.50 and 473.42). On the other side in the 3rd cut applied 200 mg l^{-1} at 4 days achieved the best results 210.32 and at 8 days (111.00) during the first season, however during the second season applied150 mg l^{-1} at 4 days produced

Table 2. Effect of irrigation intervals, ascorbic acid treatments, and their interactions on number of leaves/plant of *Mentha piperita* plants during the two seasons 2019 and 2020.

Irrigation intervals (A)	First Season						Second Season					
	Ascorbic acid mg l^{-1} (B)											
	Cont.	100	150	200	250	Mean	Cont.	100	150	200	250	Mean
First cut												
4 days	263.09	287.00	363.25	324.92	290.42	305.73	282.50	305.17	370.92	326.58	313.42	319.72
8 days	240.75	253.09	346.50	305.50	262.33	281.63	240.33	254.50	352.50	300.53	287.58	287.09
12 days	204.83	227.17	241.33	291.17	248.33	242.57	211.17	223.42	240.83	253.33	292.55	244.26
Mean	236.22	255.75	317.03	307.20	267.03		244.67	261.03	321.42	293.48	297.85	
L.S.D $_{0.05}$	A= 18.63		B = 18.30		A x B = 31.69		A = 27.52		B = 23.11		A x B = 40.06	
Second cut												
4 days	316.17	322.00	470.67	410.58	348.00	373.48	407.83	422.00	536.83	485.98	461.83	462.89
8 days	254.33	262.34	347.67	387.50	304.33	311.23	316.33	334.09	384.50	473.42	427.00	387.07
12 days	229.67	244.33	270.08	353.34	274.83	274.45	232.17	286.58	322.00	445.58	370.84	331.43
Mean	266.72	276.22	362.81	383.81	309.05		318.78	347.55	414.45	468.33	419.89	
L.S.D $_{0.05}$	A= 23.87		B = 20.78		A x B = 35.99		A = 17.69		B = 21.08		A x B = 36.51	
Third cut												
4 days	139.42	158.34	178.33	210.32	190.17	175.31	204.83	210.38	249.33	233.70	223.49	224.34
8 days	74.62	87.00	90.59	111.00	91.67	90.97	78.25	89.00	112.17	125.33	97.17	100.38
12 days	67.50	70.84	72.08	95.83	81.67	77.58	58.84	74.58	99.50	117.83	94.17	88.98
Mean	93.84	105.39	113.67	139.05	121.17		113.97	124.65	153.67	158.95	138.27	
L.S.D $_{0.05}$	A= 6.40		B = 5.57		A x B =9.64		A= 0.72		B = 4.15		A x B = 7.19	

L.S.D $_{0.05}$: Least Significant Difference at probability 0.05

249.33 and 200 mg l^{-1} at 8 days produced 125.33. Concerning plants grown under severe stress 12 days when applied AsA at 200 mg l^{-1}, it gave the highest mean values in the first season (291.17 in 1st cut , 353.34 in 2nd cut and 95.83 in 3rd cut), while during the second season applied AsA at 250 mg l^{-1} with the same irrigation interval gave the highest means values 292.55 in the 1st cut and 200 mg l^{-1} (445.58 in 2nd cut and 117.83 in 3rd cut). The same trend was achieved by Khalil *et al*. (2010) on basil plants.

Leaves fresh weight/plant (g):

Obviously, data in Table (3) indicated that the irrigation at 4 days treated with 150 or 200 mg l^{-1} in 1st cut proved the same effect on plants (22.38 and 23.93 g, respectively), in the 2nd cut applied 150 mg l^{-1} was the best concentration combined with the same irrigation interval (28.69 g) and 200 mg l^{-1} in the 3rd cut 21.36 g during the first season. However, in the second season, applied 150 mg l^{-1} gave the highest effect under the same level of stress 26.39, 33.84 and 24.75 g in the three cuts respectively. The highest mean values of leaves fresh weight/plant were obtained as a result of irrigation at 8 days combined with 200 mg l^{-1} in both seasons 21.86 and 22.44 g in 1st cut, 23.93 and 27.31g in 2nd, 15.11 and 14.96 g in 3rd cut. The same trend of results was achieved when applied irrigation interval at 12 days and interacted with 200 mg l^{-1} in the first season gave the highest effect on leaves fresh weight/plant (18.03, 21.64 and 13.65 g in the three cuts resp.). Concerning second season, results demonstrated that 250 mg l^{-1} with the same irrigation interval was the best interaction on plants in the 1st cut (17.66 g) while 200 mg l^{-1} was more effective in the 2nd and 3rd cut in the same season (24.08 and 13.45 g, respectively). It can be concluded that spraying plants with AsA under water stress recorded a significant effect in the three cuts during both seasons due to the vital role of AsA which is considered to be a regulator of cell division and a non-enzymatic complex which enables plants to defend against stresses (Shafiq *et al*., 2014).

Table 3. Effect of irrigation intervals, ascorbic acid treatments, and their interactions on leaves fresh weight per plant (g) of *Mentha piperita* plants during the two seasons 2019 and 2020.

Irrigation intervals (A)	First Season						Second Season					
	Ascorbic acid mg l^{-1} (B)											
	Cont.	100	150	200	250	Mean	Cont.	100	150	200	250	Mean
First cut												
4 days	15.28	18.05	22.38	23.93	20.00	19.92	17.49	21.88	26.39	25.04	24.20	23.00
8 days	13.51	15.41	17.29	21.86	18.96	17.40	14.72	18.80	19.58	22.44	20.84	19.28
12 days	11.34	12.02	13.75	18.03	14.60	13.95	11.43	12.60	13.01	15.82	17.66	14.10
Mean	13.37	15.16	17.80	21.27	17.85		14.55	17.76	19.66	21.10	20.90	
L.S.D $_{0.05}$	A= 0.81		B = 0.92		A x B =1.58		A= 0.55		B = 0.66		A x B =1.15	
Second cut												
4 days	20.58	22.66	28.69	26.65	24.68	24.65	25.09	27.29	33.84	30.30	28.54	29.01
8 days	18.59	19.74	20.74	23.93	21.66	20.93	19.29	22.48	24.83	27.31	23.73	23.53
12 days	13.45	15.46	18.48	21.64	19.33	17.67	16.07	18.01	19.35	24.08	21.13	19.73
Mean	17.54	19.29	22.63	24.07	21.89		20.15	22.59	26.00	27.23	24.46	
L.S.D $_{0.05}$	A= 0.95		B = 0.93		A x B =1.60		A=0.55		B = 0.98		A x B =1.69	
Third cut												
4 days	13.23	14.95	19.06	21.36	19.30	17.58	13.95	17.16	24.75	22.00	20.98	19.77
8 days	10.51	11.83	13.01	15.11	13.90	12.87	10.13	11.31	11.83	14.96	13.12	12.27
12 days	9.20	9.31	10.59	13.65	11.35	10.82	8.26	9.97	10.39	13.45	11.45	10.70
Mean	10.98	12.03	14.22	16.71	14.85		10.78	12.81	15.66	16.80	15.19	
L.S.D $_{0.05}$	A= 0.38		B = 0.94		A x B=0.63		A=1.09		B = 0.59		A x B =1.02	

L.S.D $_{0.05}$: Least Significant Difference at probability 0.05

These results are in agreement with that of Kordi *et al.* (2013) on *Ocimum basilicum*.

Volatile Oil percentage:

Evidently, data in Table (4) reveal the increment in volatile oil % resulting from using irrigation intervals and ascorbic acid concentration, data showed that during the first season applied 200 or 250 mg l^{-1} to plants grown under control stress (4 days) had the same effect on oil percentage in 1st cut (0.633 and 0.610%, respectively). On the other side applied 150 or 200 mg l^{-1} in the 2nd cut of the first season and 1st cut in the second season had no significant effect on oil percentage (0.682, 0.659% and 0.655, 0.622%, respectively), while applied 200 mg l^{-1} under the same level of stress had the maximum oil percentage in the plants in 3rd cut (0.579%). However, applied AsA at 150 mg l^{-1} in 2nd and 3rd cut in the second season achieved the highest means values (0.727 and 0.575%, respectively).

It was noticed also that the interaction between 8 days irrigation interval and AsA at 150 or 200 mg l^{-1} had the same effect during the first season in the 1st cut (0.546 and 0.572%, respectively) and 2nd cut as well (0.603 and 0.631%, respectively), while applied 200 mg l^{-1} in 3rd cut gave the highest oil percentage 0.518%, on the other hand, in the second season, applied 200 mg l^{-1} during 1st cut was the best interaction applied to mint plants (0.566 %), but during 2nd cut, AsA at 150, 200 and 250 mg l^{-1} proved no significant difference on oil percentage between them (0.530,0.616 and 0.574% respectively), in addition, AsA at 200 and 250 mg l^{-1} had the same effect on plants in the 3rd cut (0.485 and 0.448%, respectively).

As for plants grown under severe stress (12 days), it was obvious that applied 200 mg l^{-1} in the 1st cut in the first season recorded the maximum means values of oil percentage (0.514%), while applied 200 or 250 mg l^{-1} during 2nd cut had no significant difference between them (0.562 and 0.518%

Table 4. Effect of irrigation intervals, ascorbic acid treatments, and their interactions on volatile oil percentage of *Mentha piperita* plants during the two seasons 2019 and 2020.

Irrigation intervals (A)	First Season						Second Season					
	Ascorbic acid mg l^{-1} (B)											
	Cont.	100	150	200	250	Mean	Cont.	100	150	200	250	Mean
First cut												
4 days	0.395	0.477	0.571	0.633	0.610	0.537	0.519	0.547	0.655	0.622	0.559	0.580
8 days	0.330	0.395	0.546	0.572	0.479	0.464	0.369	0.405	0.473	0.566	0.513	0.465
12 days	0.298	0.311	0.408	0.514	0.462	0.399	0.313	0.344	0.388	0.420	0.454	0.384
Mean	0.341	0.394	0.508	0.573	0.517		0.400	0.436	0.505	0.536	0.509	
L.S.D 0.05	A = 0.025		B = 0.026		A x B = 0.045		A = 0.025		B = 0.027		A x B = 0.046	
Second cut												
4 days	0.425	0.549	0.682	0.659	0.597	0.582	0.554	0.592	0.727	0.668	0.652	0.639
8 days	0.382	0.483	0.603	0.631	0.512	0.522	0.439	0.489	0.530	0.616	0.574	0.530
12 days	0.279	0.270	0.453	0.562	0.518	0.416	0.420	0.426	0.471	0.552	0.509	0.476
Mean	0.362	0.434	0.579	0.617	0.542		0.471	0.502	0.576	0.612	0.578	
L.S.D 0.05	A = 0.023		B = 0.036		A x B = 0.062		A = 0.023		B = 0.032		A x B = 0.055	
Third cut												
4 days	0.298	0.328	0.445	0.579	0.504	0.431	0.477	0.498	0.575	0.529	0.485	0.513
8 days	0.269	0.334	0.416	0.518	0.446	0.396	0.323	0.375	0.405	0.485	0.448	0.407
12 days	0.269	0.296	0.465	0.480	0.396	0.381	0.295	0.312	0.335	0.430	0.411	0.356
Mean	0.278	0.319	0.442	0.526	0.449		0.365	0.395	0.438	0.481	0.448	
L.S.D 0.05	A = 0.024		B = 0.025		A x B = 0.043		A = 0.039		B = 0.025		A x B = 0.043	

L.S.D 0.05: Least Significant Difference at probability 0.05

respectively), as for the 3rd cut, data showed that 150 and 200 mg l^{-1} had the same effect on oil percentage (0.480 and 0.465%, respectively). On the other hand, during the second season, it was clear that 200 and 250 mg l^{-1} had the same effect under the same level of stress in 1st and 3rd cut (0.420 and 0.454 % and 0.430 and 0.411% resp.) except 2nd cut there was no significant difference between 150, 200 and 250 mg l^{-1}.

Generally, increase/decrease of essential oil percentage affected by water stress and ASA treatments may be due to increase/decrease of number of oil glands, or their size or both.

Volatile oil yield per plant (ml):

The results in Table (5) indicated that the interaction between irrigation intervals and ascorbic acid concentration, the results showed that applying irrigation at 4 days interact with 200 mg l^{-1} in 1st and 3rd cut in the first season were the best means values of oil yield (0.134 and 0.124 ml and using 150 mg l^{-1} in 2nd cut recorded (0.182 ml) compared with other concentrations, while the same irrigation interval with 150 mg l^{-1} induced a significant increase in oil yield in the three cuts in the second season 0.173,0.208 and 0.142 ml, respectively. In addition, applying 8 days irrigation interval and AsA at 200 mg l^{-1} gave the maximum oil yield/plant in both seasons 0.099 and 0.137 ml during 1st cut, 0.151 and 0.146 ml during 2nd cut, while in the 3rd cut, there was no change in oil yield/plant values 0.073 ml in both seasons.

Similar trend was observed with severe stress 12 days interact with AsA at 200 mg l^{1} obtained the maximum oil yield in three cuts in both seasons, 0.075 ml during 1st cut, 0.123, 0.113 ml during 2nd cut and 0.062 and 0.057 ml during 3rd cut, except 1st cut in second season, using 250 mg l^{-1} was the best concentration applied which produced 0.076 ml compared with 200 mg l^{-1} (0.066 ml).

Generally, the increment of essential oil yield (ml/plant) as a result of different treatments may be due to the increase of

Table 5. Effect of irrigation intervals, ascorbic acid treatments, and their interactions on on volatile oil yield (ml) per plant of *Mentha piperita* plants during the two seasons 2019 and 2020.

Irrigation intervals (A)	First Season						Second Season					
	Ascorbic acid mg l^{-1} (B)											
	Cont.	100	150	200	250	Mean	Cont.	100	150	200	250	Mean
	First cut											
4 days	0.060	0.078	0.111	0.134	0.122	0.101	0.091	0.120	0.173	0.156	0.135	0.135
8 days	0.045	0.055	0.081	0.099	0.077	0.072	0.054	0.076	0.092	0.127	0.107	0.091
12 days	0.034	0.037	0.051	0.075	0.063	0.052	0.036	0.044	0.050	0.066	0.076	0.054
Mean	0.046	0.057	0.081	0.103	0.087		0.060	0.080	0.105	0.116	0.106	
L.S.D $_{0.05}$	A = 0.006		B = 0.006		A x B = 0.010		A =0.007		B = 0.005		A x B =0.009	
	Second cut											
4 days	0.081	0.115	0.182	0.163	0.138	0.136	0.108	0.148	0.208	0.183	0.171	0.164
8 days	0.071	0.095	0.131	0.151	0.110	0.112	0.075	0.103	0.112	0.146	0.129	0.113
12 days	0.037	0.042	0.085	0.123	0.099	0.077	0.053	0.063	0.079	0.113	0.091	0.080
Mean	0.063	0.084	0.132	0.145	0.116		0.079	0.104	0.133	0.147	0.130	
L.S.D $_{0.05}$	A =0.006		B = 0.009		A x B =0.016		A =0.012		B = 0.009		A x B =0.016	
	Third cut											
4 days	0.039	0.049	0.085	0.124	0.098	0.079	0.066	0.085	0.142	0.116	0.102	0.102
8 days	0.028	0.040	0.054	0.073	0.062	0.051	0.033	0.043	0.048	0.073	0.059	0.051
12 days	0.025	0.028	0.053	0.062	0.042	0.042	0.024	0.031	0.034	0.057	0.047	0.039
Mean	0.031	0.039	0.064	0.086	0.067		0.041	0.053	0.075	0.082	0.069	
L.S.D $_{0.05}$	A =0.004		B = 0.005		A x B =0.009		A =0.008		B = 0.004		A x B =0.008	

L.S.D $_{0.05}$: Least Significant Difference at probability 0.05

essential oil percentage, leaves fresh weight, and/or number of leaves, and/or leaf area.

Volatile Oil Components:

According to GC analysis of essential oil of peppermint fresh leaves grown under water stress and sprayed with ascorbic acid with different concentrations, data presented in Tables (6-8) showed 9 components were identified during the first season in the three cuts.

The major components were menthol, menthone, 1,8-cineole, limonene, and menthyl acetate with different percentages depending on stress level and AsA concentration applied to the grown plants.

Data illustrated that menthol percentage ranged from 34.55-52.67% during the three cuts the highest percentage was obtained by treatment of severe stress at 12 days interact with ascorbic acid at 200 mg l^{-1} (45.36% in 1st cut, 52.67% in 2nd cut and 50.15 % in 3rd cut) followed by moderate stress at 8 days interact with the same ascorbic acid concentration (44.28% in 1st cut, 51.89% in 2nd cut and 48.76% in 3rd cut), however under control treatment data obtained the least menthol percentage was at the same concentration in the 1st cut (42.54%) and at 150 mg l^{-1} (50.08 in 2nd and 38.73% in 3rd cut).

Worthy to mention that menthol percentage in 2nd cut is higher than 1st and 3rd ones and this may be due to maturation of oil glands with age or genetic factors that play role in the hindrance of biosynthetic pathway resulting in accumulation of menthol or pulegone at the end product of terpene conversion (Tausif, 2015).

As for another major component in peppermint oil which is menthone, data indicated that the percentage ranged from 23.44-40.01%. The highest percentage was obtained by treatment of 4 days interact with 200 mg l^{-1} (33.92% in 1st cut, 36.06% in 2nd cut, 35.72% in 3rd cut), and under moderate stress, interact with 200 mg l^{-1} in 1st and 3rd

Table 6. Effect of irrigation intervals, ascorbic acid treatments, and their interactions on volatile oil components of *Mentha piperita* plants during season 2019 in the 1st cut.

Irrigation intervals	Ascorbic acid mg l^{-1}	α - Pinene	β - Pinene	Limonene	1.8 Cineole	Menthone	Menthol	Iso-menthol	Menthyl acetate	β - Caryophyllene	Total %	unknown
4 days	**Cont.**	0.36	1.56	5.72	1.12	23.44	35.21	1.92	3.42	1.14	73.88	26.12
	100	0.69	1.73	6.05	1.19	28.44	38.73	1.10	3.98	1.23	83.13	16.87
	150	0.72	2.09	6.36	1.29	29.67	39.77	1.79	4.24	1.38	87.31	12.69
	200	1.84	2.28	6.79	2.81	33.92	42.54	1.49	5.37	2.05	99.09	0.91
	250	0.87	1.96	6.54	2.02	32.46	41.21	2.08	4.37	2.11	93.63	6.38
8 days	**Cont.**	0.67	1.46	6.22	0.63	27.89	36.41	1.85	3.07	1.23	79.44	20.56
	100	0.72	1.50	6.34	0.64	30.58	38.68	2.00	3.72	1.26	85.44	14.56
	150	1.02	2.37	7.58	1.22	30.95	41.68	2.35	3.42	1.44	92.02	7.98
	200	1.18	2.06	6.90	1.09	34.32	44.28	2.25	4.51	1.58	98.17	1.83
	250	1.12	2.00	6.80	0.83	33.72	43.92	2.09	4.37	2.18	97.03	2.97
12 days	**Cont.**	0.86	2.48	6.52	1.12	27.84	41.77	2.11	3.12	1.29	87.10	12.90
	100	1.03	1.84	6.82	0.54	31.10	42.53	2.18	3.83	1.55	91.43	8.57
	150	0.61	1.71	6.82	0.89	32.32	43.98	2.32	3.59	2.01	94.24	5.76
	200	0.82	2.00	7.46	0.77	34.57	45.36	2.51	4.24	2.18	99.91	0.09
	250	0.76	2.18	7.16	0.66	33.74	45.19	2.31	3.44	2.28	97.72	2.28

Table 7. Effect of irrigation intervals, ascorbic acid treatments, and their interactions on volatile oil components of *Mentha piperita* plants during season 2019 in the 2nd cut.

Irrigation intervals	Ascorbic acid mg l^{-1}	α - Pinene	β - Pinene	Limonene	1.8 Cineole	Menthone	Menthol	Iso-menthol	Menthyl acetate	β - Caryophyllene	Total %	unknown
4 days	**Cont.**	0.86	1.56	4.87	0.32	30.14	42.63	1.29	1.22	1.91	84.79	15.21
	100	0.76	1.86	5.15	0.46	33.36	43.57	1.42	1.70	1.49	89.79	10.21
	150	0.67	2.04	5.58	0.51	34.62	50.08	2.08	2.16	1.99	99.73	0.27
	200	0.71	1.98	5.31	0.57	36.06	47.37	2.17	2.16	1.95	98.28	1.72
	250	0.78	1.87	5.27	0.42	34.48	45.24	1.24	2.16	1.65	93.10	6.90
8 days	**Cont.**	0.71	1.70	3.14	0.35	31.23	43.74	2.58	1.59	1.72	86.76	13.24
	100	0.94	1.35	3.38	0.42	32.68	45.74	2.45	2.06	1.33	90.34	9.66
	150	0.80	1.88	3.34	0.55	40.01	47.89	1.45	2.18	1.56	99.66	0.34
	200	0.83	1.73	3.86	0.75	35.68	51.89	1.12	2.76	1.27	99.89	0.11
	250	0.97	1.52	3.75	0.98	37.15	49.08	2.40	2.88	1.14	99.87	0.13
12 days	**Cont.**	0.65	1.39	3.04	1.01	31.89	46.15	1.61	2.04	1.10	88.87	11.13
	100	0.85	2.04	3.06	1.13	32.57	47.67	2.02	2.14	1.14	92.62	7.38
	150	1.17	1.76	3.90	1.01	35.26	48.67	2.74	2.43	1.16	98.10	1.90
	200	1.31	1.89	3.58	0.72	33.68	52.67	2.21	2.23	1.19	99.48	0.52
	250	1.15	1.89	3.55	0.67	33.82	51.67	2.54	2.52	1.66	99.45	0.55

Table 8. Effect of irrigation intervals, ascorbic acid treatments, and their interactions on volatile oil components of *Mentha piperita* plants during season 2019 in the 3rd cut.

Irrigation intervals	Ascorbic acid mg l^{-1}	α - Pinene	β - Pinene	Limonene	1.8 Cineole	Menthone	Menthol	Iso-menthol	Menthyl acetate	β - Caryophyllene	Total %	unknown
4 days	**Cont.**	1.37	1.09	5.06	1.03	26.43	34.55	2.03	5.01	1.66	78.22	21.78
	100	1.57	1.87	5.38	1.03	28.31	36.38	3.31	5.75	1.86	85.46	14.54
	150	1.83	1.68	6.98	1.99	34.63	38.73	3.60	4.79	2.02	96.24	3.76
	200	0.83	1.67	7.75	1.05	35.72	37.32	1.72	1.98	3.71	91.75	8.25
	250	0.80	1.39	5.55	0.64	33.37	35.28	1.98	3.92	2.50	85.44	14.56
8 days	**Cont.**	0.70	1.37	5.05	0.42	28.36	34.59	1.73	2.57	1.43	76.22	23.78
	100	0.74	1.48	5.46	0.46	29.13	37.13	1.82	2.68	1.45	80.35	19.65
	150	0.73	1.49	5.95	0.61	32.86	40.05	1.97	2.63	1.57	87.86	13.14
	200	0.63	1.24	4.11	0.56	34.86	48.76	2.28	3.38	1.93	97.76	2.25
	250	0.72	1.42	3.98	0.51	29.26	39.69	1.78	2.80	1.86	82.00	18.00
12 days	**Cont.**	0.79	1.24	5.02	0.62	29.67	35.59	1.47	4.88	1.11	80.40	19.61
	100	0.72	1.38	5.04	0.60	30.54	37.16	1.13	3.15	1.19	80.91	19.09
	150	0.76	1.31	5.03	0.61	32.76	41.06	1.30	4.01	1.56	88.40	11.60
	200	0.66	1.32	5.75	0.54	32.83	50.15	1.86	4.20	1.93	99.24	0.76
	250	1.36	1.46	5.47	0.63	32.80	39.62	2.05	3.04	1.44	87.87	12.13

cut recorded 34.32 and 34.86% and 150 mg l^{-1} in 2nd cut produced (40.01%), the same trend was applied by severe stress interact 200 in 1st and 3rd cut (34.57, 32.83%) and 150 mg l^{-1} in 2nd cut (35.26%).

Concerning limonene percentage, data ranged from 3.04-7.75% in the three cuts, the highest percentage was obtained at 4 days interact with 200 mg l^{-1} in 1st and 3rd cut (6.79 and 7.75%, respectively) and with 150 mg l^{-1} (5.58%) in 2nd cut, when applied 8 days interact with 150 mg l^{-1} in 1st and 3rd cut it produced 7.58 and 5.95%, respectively and 200 mg l^{-1} (3.86%) in 2nd cut. Under severe stress 200 mg l^{-1} in the 1st, 3rd cut obtained a higher percentage (7.46 and 5.75%, respectively), while 150 mg l^{-1} in the 2nd cut produced 3.90%.

The previous results were in harmony with the findings of Court *et al.* (1993) who stated that the amount of menthol increased as the plant matured, Dimandja *et al.* (2000) who found that the main volatile components identified in the essential oil of peppermint are menthol (33-60%), menthone (15-32%), menthyl acetate (2-11%), limonene (1-7%), β-caryophyllene (2-4%), and Anand (2003) who indicated that menthol is one of the chemical compounds of peppermint oil,

In addition, it was apparent that developmental and environmental factors are known to greatly influence the yield and composition of peppermint oil. Thus, oil yield and menthol content increase with leaf maturity, and a range of stress conditions such as light, temperature and moisture status tend to promote the accumulation of pulegone and menthofuran (Voirin *et al.*, 1990 and Brun *et al.*, 1991). Also, variation in essential oil compositions can occur as a result of differing soil conditions, altitude, climatic conditions, seasonal factors and other environmental features, leading in some cases to the evolution of different chemical variants or chemotypes (Heywood, 2002).

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تأثير فترات الرى المختلفة وحامض الأسكوبيك على النمو وإنتاجية وجودة الزيت الطيار فى نباتات النعناع الفلفلي

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أجريت هذه الدراسة في موسمي ٢٠١٩ و ٢٠٢٠ في مشتل الزهور ونباتات الزينة، كلية الزراعة، جامعة الاسكندرية، مصر وذلك لدراسة تأثير فترات الري (٤، ٨، ١٢ يوم) وخمسة تركيزات لحامض الأسكوربيك (صفر،١٠٠،١٥٠،٢٠٠،٢٥٠ مجم/لتر) على النمو الخضري وكمية ونسبة الزيت في نبات النعناع الفلفلي *Mentha piperita* L. وقد وُجد أن معظم خصائص النمو الخضري قد زادت عند الرش بحامض الأسكوربيك بتركيزات ١٥٠ أو ٢٠٠ ملجم/ لتر من حامض الأسكوربيك للنباتات المزروعة تحت ظروف الإجهاد المائي الطبيعي (الري كل ٤ أيام)، أما النباتات المزروعة تحت ظروف الإجهاد المائي المعتدل (الري كل ٨ أيام) كان تركيز ٢٠٠ ملجم/لتر حامض الأسكوربيك أكثر فاعلية على الوزن الطازج للأوراق و عدد الأفرع للنباتات في كلا الموسمين، كذلك زيادة مستوى الإجهاد إلى الإجهاد المائي الشديد (الري كل ١٢ يوم) والرش بتركيزات ٢٠٠ ملجم/لتر حامض الأسكوربيك قد أدى إلى تقليل الآثار الضارة في معظم نباتات النعناع مما أدى إلى إرتفاع إنتاج الزيت ونسبته المئوية. تم إجراء تحليل

كروماتوجرافى للزيت الطيار لنبات النعناع الفلفلي و التعرف على ٩ مكونات وأظهرت النتائج أن المنثول أهم المكونات للزيت الطيار لنبات النعناع الفلفلي والذي يتواجد بنسبة تترواح من ٣٤,٥٥ - ٥٢,٦٧٪ من إجمالي النسبة المئوية لجميع مكونات الزيت خلال الثلاث حشات لموسم النمو، و بملاحظة النتائج نجد أن هناك علاقة طردية بين زيادة فترات الري و نسبة مكونات الزيت (المنثول، المنثون، الليمونين). من النتائج السابقة يمكن التوصية بأن زيــادة فترات الري يزيد من التأثير الضار على نبات النعناع الفلفلي الذي أثبت انه نبات حساس لنقص المياه لذلك فإن أفضل فترة ري كانت كل ٤ ايام بالإضافة إلى أن أفضل تركيز تم إستخدامه من حامض الأسكوربيك رشاً على النباتات هو ١٥٠ و ٢٠٠ ملجم/لتر وذلك لزيادة قدرة النبات على مقاومة الإجهاد المائي خلال موسم النمو وبالتالي الحصول على أعلى جودة وأعلى كمية زيت وبذلك يتم تحقيق الهدف من الدراسة.