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Response of *Mentha longfolia* to water irrigation intervals and/or Mepiquat Chloride treatments

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Abstract
This investigation was carried out to examine the influence foliar applied of Mepiquat Chloride in mitigating the effects of water stress in *Mentha Longefollia* . The data showed that the herb growth , yield and essential oil percentage and yield as well as its chemical constituents were influenced by different levels of irrigation intervals (every 3 and 6 days). The results reveal that Mepiquat Chloride doses (0, 500, 1000 and 1500 ppm) reduced the effect of water stress on different yield characters and essential oil. The main components of essential oil are carvon, menthone and 1, 8 cineol.

Keywords: Mint, Mepiquat Chloride, Water stress, herb yield, essential oil

Introduction
As the lack of water is the major restricting factor to cultivation in arid regions, the search for drought resistant plant species is of great importance since it allows the increase of cultivated plants in areas with severe shortage of water.
However, water deficit is a limiting factor in production of many field crops [1,2] and water stress causes different morphological, physiological and biochemical changes including: leaf area reduction, leaf senescence and reduction in cell development [1], stomatal closure and photosynthetic limitation [1,3]. It appears that the effect of water stress on economic yields of medicinal plants which are mainly secondary metabolites, are somehow positive [4]. In many cases, a moderate stress could enhance the content of secondary metabolites.
A possible approach to minimize drought induced crop losses is the foliar application by plant growth regulators. Mepiquat Chloride (MC, 1, 1-dimethyl piperidinium chloride) is one of the most widely used as plant growth retardants. MC has been reported with several crop plants such as cotton [5], wheat [6, 7 and 8], ground nut [9] and rape varieties [10].
This investigation was, therefore, undertaken to examine the influence of foliar applied Mepiquat Chloride in mitigating the effects of water stress in *Mentha longefollia* .

2- Materials and Methods
2-1- Plant material, culture site and experimental design

This experiment was carried out on *Mentha Longfolia* at El-Adlya Experimental Field, Sharkia Governorate. A split plot design was used, with irrigation intervals as the main plot and mepiquat chloride (MC) levels as the sub-plots. The soil of experimental site was deep, well – drained sandy composing with 84.5% sand, 10.3% silt and 5.3% clay with pH 8.1 , EC 1.93 dS/m ,$CaCO_3$ 2.93, O.M. 0.31%. The available N,P and K were 0.11, 1.96 and 19.7 mg/100g . The experimental treatments were replicated four times. The net size was 2.70 x 5.00 m (13.50m^2). Two irrigation intervals (3 and 6 days) took place. Along with these irrigation intervals, four levels of Mepiquat Chloride (MC) [0, 500, 1000 and 1500 ppm]. Spraying of MC was carried out after 20 days from sowing and the 2nd and 3rd cuts after 20 and 40 days from the 1st one, respectively. Mint cuttings were planted during the 2nd week of May during both seasons (2011 and 2012). Concerning fertilizers application, all treatments received 15m^3 of compost + 300 Kg superphosphate during preparing the soil. Also, 100 Kg of ammonium nitrate + 50Kg potassium sulphate / Fed. were added after 30 days from sowing . After every harvest 75 Kg ammonium nitrate + 25 Kg potassium sulphate / Fed. will be added. Two hoeing were done to remove weeds from the crop.

2-2- Growth and yield assessments

The 1st, 2nd and 3rd harvests were carried out every three months (August, November and February). Plant heights (cm), herb fresh and dry weight (g/plant) were measured.

2-3- Volatile oil:

The percentages of volatile oil were determined in the fresh herb using 100 g samples for each cut per plant during both seasons. Water distillation of the volatile oil was as described in the British Pharmacopoeia [11]. The volatile oil obtained from the fresh herb was analyzed using Ds Chrom 6200 Gas Chromatograph equipped with a flam ionization detector for separation of volatile oil constituents. The analysis conditions were as follows:

- GC analysis were performed on HP 6890 GC
- Detector: FID (flame Ionization Detector)
- Column: HP5 fused silica Column(Size: 1=60m,Ø=0.25mm, stationary phase: macrogol 20 000 R [film thickness 0.25µm]).
- Oven program : The oven temperature program was initiated at 60°C , held for 1 min then raised up to 240°C at rate of 10°C /min
- Carrier gas: Nitrogen is used as a carrier gas
- Flow rate: 1.0 ml/min
- Injector temperature250°C
- Detector temperature 275°C
- Injection volume : 1µl of diluted oil in hexane solution (10%) .
- Splate ratio1:50

Table (1): Physical and Chemical Analysis of Compost

S.	Tests	Unit	Results	Soil & More Standard
Physical Analysis				
1	Bulk Density	kg/m^3	916	600 : 1000
2	Moisture Content	%	27.9	25 : 30
Chemical Analysis				
3	Electrical Conductivity (1:5)	dS/m	5.1	< 10
4	pH_w (1:5)		7.6	7 : 8
5	Total Organic Carbon	%	13.2	> 10
6	Total Organic Matter	%	22.7	> 17
7	Ash	%	77.3	60 : 80
8	Total Nitrogen	%	0.60	0.5 : 1.0
9	C /N Ratio	-	22.1	1 : 15
10	T.K	%	1.42	-
11	T.P	%	0.30	-

2-4- Statistical analysis

The obtained data of mean value of both seasons were statistical analyzed using analysis of variance (ANOVA) as described by Snedecor and Cochran [12] using the MSTAT-C statistical [13].

3. Results and discussion

3-1- Vegetative parameters:

Data tabulated in Tables (2 , 3 and 4) show that, decrement was detected in some the above – ground vegetative growth including plant height , fresh and dry weight of herb as a result of increasing intervals between irrigation during cuts except herb dry weight for the third cut . The highest mean values of these parameters were obtained due to the use of irrigation every 3 days. Irrigation intervals treatment had no significant effect on plant height and herb dry weight. According to the previous results, ElTahir et. al [14] reported that , this may be due to the vital roles of water supply at adequate amount for different physiological processes such as photosynthesis , respiration , transpiration, translocation, enzyme reaction and cell turgidity occurs simultaneously. Such reduction could be attributed to a decrease in the activity of meristemic tissues responsible for elongation. Moreover, increasing levels of water stress reduce growth and yield due to reduction in photosynthesis and plant biomass. Under increasing water- stress levels photosynthesis was limited by low

Co2 availability due to reduced stomata and mesophyll conductance. Drought stress is associated with stomatal closure and thereby with decreased Co2 fixation.

The same Tables indicated that, MC treatments decreased the most vegetative parameters for three cuts. Generally, the maximum mean values of vegetative parameters were obtained as a result of untreated plants. The plant height was decreased significantly in third cut only. Similarly, the herb dry weight in the three cuts recorded significant decreament. Meanwhile, the herb fresh weight appeared an stimulation with 1000 ppm MC at the second cut, and with highest dose of MC (1500 ppm) at third cut. In this connection , Shalaby and Zeinab [15] and El-Ashry and Shalaby [16] worked on faba bean and chickpea and indicated that growth retardants at low concentration induced endogenous growth parameters whereas at high concentration a remarkable decrease in the endogenous hormones was observed.

Concerning the combination between irrigation intervals and Mepiquit Chloride (MC), it can be noticed that these treatments had no significant effect on plant height and herb dry weight for the three cuts. During the 1st and 2nd cuts, it can be observed that the highest mean values of plant height (55.1 and 38.9 cm for 1st and 2nd cut respectively) were obtained as a result of water irrigation every 3 days without MC application. On the other hand, the maximum mean value of plant height during the 3rd cut (31.2cm) was obtained as a result of irrigation intervals every 3 days treated with MC at 500 ppm. The highest values of herb fresh and dry weight (151.9 and 16.9 g/ plant) during the 1st cut were obtained due to irrigate every 3 days + MC at 500 ppm. During the 2nd cut , the highest values due to the irrigated every 3days + MC at 1000 ppm or irrigated every 6 days + MC at 1000 ppm for herb fresh weight (123.8 g/ plant) while the maximum mean value of herb dry weight (13.0 g/ plant) was obtained as a result of irrigated plants every 3 days + MC at 500 ppm. The interaction between irrigated plants every 3 days + MC at 1500 ppm % showed the maximum mean values of fresh and dry weight of herb (121.4 and 12.5 g/plant) during the 3rd cut.

Table (2) Effect of irrigation intervals or / and MC on growth and essential oil content of *Mentha Longefollia* during 1st cut (mean values of two successive seasons)

Irrigation intervals	MC ppm	Plant height (cm)	Herb F.W g/plant	Herb D.W g/plant	Essential oil %	Essential oil yield ml / plant
3 days	0	55.1	151.6	16.3	0.930	1.410
	500	54.6	151.9	16.9	1.140	1.732
	1000	52.2	148.9	16.7	0.950	1.415
	1500	46.2	150.1	16.5	0.990	1.486
Mean value		52.0	150.6	16.6	1.002	1.510
6 days	0	54.9	149.2	16.1	0.980	1.462
	500	54.6	149.5	16.4	0.980	1.465
	1000	51.4	146.6	16.4	0.980	1.437
	1500	46.3	146.5	16.2	0.990	1.450
Mean value		51.8	148.0	16.3	0.983	1.454
Mean value of : MC	0	55.0	150.4	16.2	0.960	1.444
	500	54.6	150.7	16.7	1.060	1.597
	1000	51.8	147.8	16.6	0.960	1.419
	1500	46.3	148.3	16.4	0.990	1.468
LSD at 5% For :	Irrigation	N.S	1.20	N.S	0.008	0.006
	MC	1.59	1.20	N.S	0.006	0.004
	Interaction	N.S	2.40	N.S	0.012	0.009

3-2- Essential oil content (%) and yield:

From the given data in Tables (2, 3 and 4) it can be noticed that irrigation intervals treatments had a significant effect on essential oil content (%) and yield (ml/plant). Accordingly, it can be stated that irrigation every 3 days was the most effective irrigation treatment for promoting the synthesis and accumulation of essential oil content and yield during 1st and 3rd cuts. The mean values of both characters were (1.002 % and 1.510 ml/ plant during 1st cut against 0.743 % and 0.896 ml/plant during 3rd one). In the contrary, irrigation every 6 days gave the maximum mean values of essential oil content (0.326%) and yield (0.397 ml/ plant) during 2nd cut. Similar results were observed for *Cympobogon flexuosus* under drought [17]. The reduction in essential oil content may be due to disturbance in photosynthesis and carbohydrate production under stress condition and suppression of the plant growth [18]. Reduction in oil content and compositional alterations in the essential oils as a consequence of drought has also been described in mints[19] , sweet basil [20] , dragon head [21],oregano [22] and lemon balm [23] . In *Artemisia annua*, Chalchat *et al.* [24] observed that water stress strongly depressed oil yield and plentiful irrigation raised it. Putievsky *et al.* [25] also reported that water stress had a negative impact on green yield and essential oil yield of geranium. Shabih *et al.* [26] reported that when moisture deficiency does not limit plant growth and survival, the production of secondary metabolites such as essential oil is even stimulated by limited stressful environments. In this experiment, one accession was used but based on growth retardation under drought stress conditions it seems that irrigation at optimum condition may promote greater essential oil biosynthesis in mint.

The response to Mepiquit Chloride (MC) on essential oil content (%) and yield of *Mentha longefollia* is presented in Tables (2, 3 and 4). It showed that a significant increment in mean values of essential content (%) and yield (ml/plant) occurred when plants treated with MC at 500 ppm during 1st and 2nd cuts. Mean values of essential content (%) and yield (ml/plant) treated with MC at 500 ppm were 1.060 % and 1.597 ml/plant for the 1st cut, 0.333% and 0.407 ml/plant for 2nd one. During the 3rd cut, it can be noticed that the highest mean values of essential content (%) and yield (ml/plant) were obtained as a result of MC at 1000 ppm where the mean values were 0.769 % and 0.911 ml/plant respectively. It could be suggested that growth regulator used in this study (MC) controlled the biosynthesis of essential oil from mevalonic acid through the main metabolic pathway. In support of this suggestion, Claudia et al.[27] concluded that the control of biosynthetic pathways leading to the production of specific metabolites as essential oils are controlled by enzymes, which in turn are mainly affected by growth regulators.

Table (3) Effect of irrigation intervals or / and MC on growth and essential oil content of *Mentha Longefollia* during 2nd cut (mean values of two successive seasons)

Irrigation intevals	MC ppm	Plant height (cm)	Herb F.W g/plant	Herb D.W g/plant	Essential oil %	Essential oil yield ml / plant
3days	0	38.9	123.3	12.6	0.285	0.351
	500	36.8	123.6	13.0	0.280	0.346
	1000	35.4	123.8	12.7	0.250	0.310
	1500	31.7	122.3	12.7	0.205	0.251
Mean value		35.28	123.3	12.8	0.255	0.314
6days	0	38.2	120.2	12.3	0.335	0.403
	500	37.8	121.3	12.9	0.385	0.467
	1000	34.7	123.8	12.9	0.385	0.477
	1500	30.4	121.8	12.8	0.200	0.244
Mean value		35.28	121.8	12.7	0.326	0.397
Mean value of :MC	0	38.6	121.8	12.5	0.310	0.377
	500	37.3	122.5	13.0	0.333	0.407
	1000	35.1	123.8	12.8	0.318	0.394
	1500	31.1	122.1	12.75	0.203	0.248
LSD at 5% For :	Irrigation	N.S	0.81	N.S	0.015	0.006
	MC	2.2	1.15	N.S	0.011	0.008
	Interaction	N.S	1.93	N.S	0.021	0.012

With respect to the combination between irrigation intervals and MC application, it was apparent that, treatments had a significant effect on essential oil content (%) and yield (ml/plant) during all cuts with some exceptions. During the 1st cut the maximum mean values of essential oil content (1.14%) and essential oil yield (1.732 ml/plant) were obtained as a result of the combination between irrigation every 3 days with foliar application of MC at 500 ppm. At the 2nd cut , the combination treatment between irrigation interval every 6 days with MC at 500 ppm or 1000 ppm gave the same maximum mean values of essential oil content (0.385%) , while the highest value of oil yield (0.477 ml/plant) was obtained due to irrigate every 6 days + MC at 1000 ppm. During the 3rd cut , the highest significant mean value of oil content (0.786 %) was observed with the combination treatment between irrigation intervals every 6 days + MC at 1000 ppm while , the highest mean value of oil yield (0.936 ml/ plant) was obtained as a result of the combination treatment between irrigation intervals every 3 days +MC at 1500 ppm.

Generally, the increment of essential oil yield (ml/plant) as a result of different treatments may be due to the increase of essential oil content (%) and / or herb fresh weight. From the same Tables (2 , 3 and 4), it can be

concluded that the highest values of essential oil content and yield were obtained during the 1st cut while the 2nd one gave the lowest values. These erratic yield responses to MC are probably due to many reasons.
Amounts and timing of application of the chemical depend on plant size, growth rate, and changing growing conditions caused by unpredictable weather after the chemical application.

Table (4) Effect of irrigation intervals or / and MC on growth and essential oil content of *Mentha Longefollia* during 3rd cut (mean values of two successive seasons)

Irrigation intervals	MC ppm	Plant height (cm)	Herb F.W g/plant	Herb D.W g/plant	Essential oil %	Essential oil yield ml / plant
3days	0	30.9	115.9	12.2	0.723	0.838
	500	31.2	120.1	12.4	0.725	0.871
	1000	31.0	120.9	12.1	0.752	0.909
	1500	31.3	121.4	12.5	0.771	0.936
Mean value		31.1	120.6	12.3	0.743	0.896
6days	0	30.1	119.6	12.3	0.709	0.848
	500	30.1	119.2	12.4	0.718	0.856
	1000	29.1	116.0	12.1	0.786	0.912
	1500	27.8	119.3	12.4	0.603	0.719
Mean value		29.28	118.5	12.3	0.704	0.834
Mean value of : MC	0	30.5	119.8	12.4	0.716	0.843
	500	30.7	119.7	12.1	0.722	0.864
	1000	30.1	118.5	12.45	0.769	0.911
	1500	29.6	120.4	12.3	0.687	0.828
LSD at 5% For :	Irrigation	1.10	1.10	N.S.	0.041	0.004
	MC	N.S	1.50	N.S.	0.062	0.001
	Interaction	N.S	2.12	N.S.	0.088	0.005

3-3-Chemical composition of essential oil:

The essential oil composition varies according to cuts and / or different treatments and was characterized by a high percentage of oxygenated compounds ranged from 43.995 to 95.71%.The components of the essential oil in herb for different treatments during 3 cuts were shown in Tables (5, 6, 7, 8, 9 and 10). The identified components ranged from 8-16 compounds representing about 63% - 97.99% as a result of different treatments during 3 cuts. Carvon was identified as the major compound in the different treatments ranging from 24.65 % - 76.17%. Menthone, the second main component, ranged 8.49% - 30.26 % in the essential oil followed with 1-8 Cineol which was identified as the third main constituent in the essential oil and its relative percentage accounted for 4.23% - 20.72%. The fourth one is β-Pinene which ranged from 0.08 – 4.47 %. In this respect, chemical composition of the essential oil of mint herb is very variable depending on the habitat and climate where the species grow. Forty five constituents were identified in the essential oil of *M. longifolia* from Turkey, with the cis-epoxy piperitone, pulegone and piperitenone oxide as main components, and studied oil exhibits strong antimicrobial activity [28]. In the essential oil of wild mint from South Africa, 31 components were identified. Menthone (50.9%), pulegone (19.3%) and 1,8- cineole (11.9%) were the main ingredients of the oil [29]. Analysis of oil of *M.longifolia* from Italy and Israel revealed piperitenone oxide as the main component, while the essential oil from Sinai contained 1, 8-cineole (28.8%), piperitone oxide (15.4%) and piperitone (13.8%) [30]. There is more than 70% of pulegone in the oil that grows in the desert of Jordan [31]

The dominant components of essential oil of wild mint herb in Vojvodina are menthone, isomenthone and 1,8-cineole, and the oil exhibits a strong antimicrobial and significant fungicidal effect [32]. Wild mint from Croatia contains carvone, piperitenone oxide, limonene and β-caryophyllene as the main ingredients [33]. Piperitone oxide was found as the main component in nine populations of *M. longifolia* essential oils from Greece [34].
Concerning, the effect of irrigation intervals on essential oil composition was shown in Tables (5, 6 and 7). It can be noticed that the mean values of carvon were (53.66 % and 52.12 % for the 1st cut), (66.12% and 74.17% for the 2nd cut) and (57.12% and 45.22% for the 3rd one) as a result of irrigation interval every 3 and 6 days, respectively. So, the maximum mean value of carvon was obtained as a result of irrigation interval at 3 days during the 2nd cut. Meanwhile, irrigation 3days intervals gave the highest mean values of carvon during 1st and 2nd cuts. On the other hand, the mean values of menthone as a second main component were (22.59% and 23.06% in the 1st cut), (15.35% and 9.83 % in the 2nd cut) and (25.57% and 19.73% for the 3rd one) as a result of

irrigation interval 3 days and 6 days, respectively. Thus, Menthone reached its maximum mean value (25.57%) as a result of irrigation at 3 days interval during the 3rd cut. The changes in essential oil composition occurring at different irrigation intervals are likely due to the changes of the activity of the related biosynthesis enzymes in response to drought [35,36].The formation of monoterpenes is catalyzed by terpene synthesis whose activity is mediated by developmental and stress- related programs [36].

Table (5) The chemical composition of essential oils of herb *Mentha longifolia* as affected by irrigation intervals and Mepiquet Chloride during 1st cut (during the 2nd season)

Irrigation Intervals	3 days					6 days				
MC ppm	0	500	1000	1500	Mean	0	500	1000	1500	Mean
A - Thujene	0.88	0.98	0.53	0.81	0.80	0.79	0.87	0.89	1.25	0.95
A – Pinene	1.13	1.23	0.37	1.05	0.95	1.09	1.19	1.01	1.30	1.15
B – Pinene	2.29	3.31	1.65	2.71	2.49	2.81	2.92	2.52	3.49	2.94
Myrcene	0.00	0.33	-	-	0.08	0.00	-	-	0.63	0.32
Limonene	0.00	0.13	-	-	0.04	0.00	-	-	-	-
1,8 Cineol	12.03	15.93	7.75	10.23	11.49	10.23	11.85	9.09	12.18	10.84
Menthone	19.83	21.39	24.97	24.17	22.59	21.03	15.63	27.39	28.19	23.06
Menthol	0.99	1.44	0.46	1.26	1.04	0.83	0.79	0.48	1.78	0.97
Cis-Isopulegone	-	-	-	-	-	0.00	-	-	-	-
Terpinene-4-ol	0.25	0.32	1.34	0.43	0.59	0.28	0.38	0.78	0.46	0.48
Cis dihydrocarvon	0.00	0.65	0.51	-	0.39	0.00	-	-	-	--
Carvon	49.30	50.66	56.13	58.56	53.66	53.21	60.90	50.78	43.59	52.12
Piperitone	0.78	0.89	1.06	0.68	0.85	0.43	0.53	0.55	0.25	0.44
Dihydroedulon	0.00	0.14	0.52	-	0.22	-	-	-	-	-
Piperitenone oxide	0.12	0.24	-	-	0.18	-	-	-	-	--
B- borarbonene	0.00	0.09	-	-	0.05	-	-	-	-	-
Transcaryophyllene	0.00	0.26	0.37	-	0.21	-	-	0.52	0.25	0.39
Total Iden. Com.	87.60	97.99	95.66	95.9	94.29	90.7	95.06	94.01	93.37	93.29
HydrocarbonCompounds	4.3	6.33	2.92	4.57	4.53	4.69	4.98	4.94	6.92	5.38
Oxygenated Compounds	83.3	91.66	92.74	95.33	90.76	86.01	90.08	89.07	86.45	87.90

Table (6) The chemical composition of essential oils of herb *Mentha longifolia* as affected by irrigation intervals and Mepiquit Chloride during 2nd cut (during the 2nd season)

Irrigation Intervals	3 days					6 days				
MC ppm	0	0.5	1.0	1.5	Mean	0	0.5	1.0	1.5	Mean
A – Thujene	0.31	0.37	0.29	0.30	0.32	0.31	0.35	0.00	0.24	0.23
A – Pinene	0.54	0.55	0.46	0.46	0.50	0.42	0.50	0.93	0.39	0.56
B – Pinene	0.97	1.28	1.02	1.02	1.07	0.73	1.14	0.08	0.82	0.69
Myrcene	0.00	-	-	-	-	0.12	0.27	0.44	-	0.28
Limonene	0.00	-	-	-	-	0.00	-	-	-	-
1,8 Cineol	4.31	4.42	5.25	4.95	4.73	4.42	4.45	4.82	4.79	4.62
Menthone	18.31	19.60	12.70	10.79	15.35	8.91	8.49	12.21	9.71	9.83
Menthol	0.93	2.60	0.76	0.56	1.21	0.59	0.55	0.68	2.73	1.14
Cis-Isopulegone	0.00	-	-	-	-	0.00	0.00	0.00	0.64	0.16
Terpinene-4-ol	0.98	1.02	1.39	1.26	1.16	1.32	1.61	1.19	1.27	1.35
Cis dihydrocarvon	0.00	-	-	0.00	-	0.00	0.00	0.00	0.00	0
Carvon	53.92	61.97	72.73	75.84	66.12	73.03	75.12	74.16	76.17	74.62
Piperitone	0.52	0.52	0.42	0.45	0.48	0.41	0.40	0.43	0.40	0.41
Dihydroedulon	0.00	-	0.00	-	-	0.00	-	-	0.00	-
Piperitenone oxide	0.31	0.49	0.41	0.44	0.41	0.00	0.34	-	0.00	0.09
B- borarbonene	0.00	-	0.00	-	-	0.00	-	-	0.00	
Transcaryophyllene	0.53	0.68	0.56	0.56	0.58	0.42	0.62	0.49	0.79	0.58
Total Iden. Com.	81.63	93.50	95.99	96.63	91.94	90.68	93.84	95.43	97.95	94.47
HydrocarbonCompound	2.35	2.88	2.33	2.34	2.48	2	2.88	1.94	2.24	2.26
Oxygenated Compound	79.28	90.62	93.66	94.29	89.46	88.68	90.96	93.49	95.71	92.21

According to the effect of MC treatments on essential oil composition, it is clear from Tables (8, 9 and 10) that MC treatments increased carvon percentage comparing with control (untreated). The maximum mean value of carvon (55.78%) during 1st cut was obtained as a result of MC at 500 ppm while at 2nd and 3rd cuts, MC at 1500 ppm gave the highest values (76.01% and 55.26 %, respectively. Concerning the effect of MC application on relative percentage of Menthone, it was observed that these treatments increased this component during 1st and 3rd cuts while at the 2nd cut there is no clear effect. So, the maximum mean value of menthone was 26.18% during the 1st cut which was obtained as a result of MC at 1000 ppm or 1500 ppm. During the 2nd cut, the highest mean value of menthone (14.05%) was obtained as a result of MC at 500 ppm. MC at 1000 ppm gave the best mean value of menthone (28.42%) in the 3rd cut. Moreover, for 1, 8 cineol relative content (%), MC treatments increased this constituent during the three cuts. It can be noticed that, MC at 500 ppm gave the highest mean value of 1,8 cineol (11.85 and 13.60 %) during 1st and 3rd cuts respectively. On the other hand, during the 2nd cut the maximum mean of this component was 5.04% as a result of Mc at 1000 ppm. In this respect, Kim et al. [37] and Li et al. [38] indicated that exogenous applications of that hormone can influence the production of compounds present in essential oil by gene regulation, promoting an increment in the number of transcripts of enzymes linked to metabolic pathway of those compounds.

Table (7) The chemical composition of essential oils of herb *Mentha longifolia* as affected by irrigation intervals and Mepiquit Chloride during 3rd cut (during 2nd season)

Irrigation Intervals	3 days					6 days				
MC ppm	0	500	1000	1500	Mean	0	500	1000	1500	Mean
A – Thujene	0.49	0.54	0.53	0.79	0.59	0.47	2.05	0.45	0.50	0.87
A – Pinene	0.60	0.67	0.60	0.94	0.70	0.50	2.39	0.60	0.64	1.03
B – Pinene	1.63	1.62	1.58	2.49	1.83	0.86	4.47	1.96	1.56	2.21
Myrcene	-	-	-	0.45	0.11	-	-	-	0.32	0.08
Limonene	-	-	-	-	-	-	9.89	-	-	2.47
1,8 Cineol	4.23	6.47	6.88	11.10	7.17	8.88	20.72	9.89	8.99	12.12
Menthone	26.31	27.78	26.57	21.62	25.57	10.55	12.89	30.26	25.21	19.73
Menthol	0.43	0.43	0.49	0.63	0.50	0.63	2.01	0.46	0.33	0.86
Cis-Isopulegone	-	0.00	-	-	-	-	-	-	-	-
Terpinene-4-ol	0.67	0.88	0.78	0.64	0.74	0.78	1.4	0.68	0.92	0.95
Cis dihydrocarvon	0.00	0.00	0.36	0.35	0.18	-	-	0.33	0.43	0.19
Carvon	56.31	56.57	59.19	56.39	57.12	40.51	38.07	48.17	54.13	45.22
Piperitone	0.53	0.77	0.44	0.29	0.51	0.51	2.00	0.43	0.26	0.8
Dihydroedulon	0.00	-	-	-	-	-	-	-	0.69	0.17
Piperitenone oxide	-	0.50	-	-	0.13	-	-	0.28	0.58	0.22
B- borarbonene	-	-	-	-		-	-	-	0.17	0.04
Transcaryophyllene	-	0.78	0.57	0.31	0.42	-	-	0.50	0.72	0.18
Total Iden. Com.	91.20	97.01	97.99	96.00	95.55	63.18	95.89	94.01	95.45	87.13
Hydrocarbon Compounds	2.72	3.61	3.28	4.98	3.65	1.83	18.8	3.51	3.91	7.01
Oxygenated Compounds	88.48	92.9	94.71	91.02	91.78	61.86	77.09	90.22	90.96	80.03

The combination treatments between irrigation intervals and MC application affected on the main constituents of essential oil are shown in Tables (5, 6 and 7). It can be noticed that carvon reached its maximum value (76.17%) as a result of the combination between irrigation intervals every 6 days and MC at 1500 ppm as compared with other treatments during 3 cuts, while the minimum relative percentage of this compound (38.07%) was determined for plants treated with the combination between irrigation every 6 days and MC at 500 ppm. However, at the 1st cut, it can be observed that the highest mean value carvon was 60.90% as a result of irrigation every 6 days + MC at 500 ppm followed by (58.56%) for plants irrigated every 3 days and sprayed with MC. For the 2nd cut, it can be noticed that the maximum mean value of carvon (76.17%) for plants irrigated every 6 days and sprayed with MC at 1500 ppm followed by 75.84% which obtained as a result if irrigation every 6 days + MC at 1500 ppm. During the 3rd cut, carvon reached its maximum value (59.19%) followed by (56.57%) as a result of the combination between irrigation every3 days + MC at 1000 ppm or at 500 ppm respectively. It is a great of interest to mention that, Menthone reached its maximum relative percentage (30.26%) as a result of the combination between irrigation every 6 days and MC at 1000 ppm while its minimum value (8.4%) was determined for plants treated with irrigation every 3 days without spraying with

MC. For 1, 8 Cineol, it is clear that the interaction between irrigation every 6 days and MC at 500 ppm resulted in the highest relative percentage (20.72%) in the 3rd cut. It is clear from the above results that the date of cuts or harvests played an important role for herb, essential oil yield as well as essential oil composition. In this connection amount and composition of essential oil is strongly dependent on developmental stage of the plant (ontogeny), and therefore harvesting time is one of the most important factors influencing mint oil quality.

Table 8. The chemical composition of essential oils of herb *Mentha longifolia* as affected by Mepiquit Chloride (ppm) during 1st cut (during 2nd season)

Mepiquit Chloride ppm Constituents	0	500	1000	1500
A – Thujene	0.44	0.925	0.71	1.03
α – Pinene	0.96	1.21	0.69	1.175
B – Pinene	1.69	3.115	2.085	3.1
Myrcene	1.405	0.17	-----	0.315
Limonene	----	0.07	-----	-----
1,8 Cineol	6.015	11.85	8.42	11.205
Menthone	15.03	18.51	26.18	26.18
Menthol	11.01	1.115	0.47	1.52
Cis-Isopulegone	0.415	----	-----	-----
Terpinene-4-ol	0.125	0.35	1.06	0.445
cis dihydrocarvon	0.14	0.325	0.255	----
Carvon	24.65	55.78	53.455	51.075
Piperitone	26.995	0.71	0.805	0.465
Dihydroedulon	0.215	0.07	0.26	-----
Piperitenone oxide	0.06	0.12	----	----
B- borarbonene	----	0.05	----	----
Transcaryophyllene	----	0.13	0.445	0.125
Total Iden. Com.	43.8	96.525	94.835	94.635
Hydrocarbon Compounds	47.5	5.655	3.93	5.745
Oxygenated Compounds	43.995	90.87	90.905	90.89

Table 9. The chemical composition of essential oils of herb *Mentha longifolia* as affected by Mepiquit Chloride (ppm) during 2nd cut (during 2nd season)

Mepiquit Chloride ppm Constituents	0	500	1000	1500
A – Thujene	0.31	0.36	0.145	0.27
α – Pinene	0.48	0.525	0.695	0.425
B – Pinene	0.85	1.21	0.55	0.92
Myrcene	0.06	0.135	0.22	----
Limonene	-	-	-	-
1,8 Cineol	4.365	4.435	5.035	4.87
Menthone	13.61	14.045	12.455	10.25
Menthol	0.76	1.575	0.72	1.645
Cis-Isopulegone	---	----	----	---
Terpinene-4-ol	1.15	1.315	1.29	1.265
cis dihydrocarvon	----	----	----	----
Carvon	63.475	68.545	73.445	76.005
Piperitone	0.465	0.46	0.425	0.425
Dihydroedulon	----	----	----	----
Piperitenone oxide	0.155	0.415	0.205	0.22
B- borarbonene	----	----	----	---
Transcaryophyllene	0.475	0.65	0.525	0.675
Total Iden. Com.	86.155	93.67	95.71	97.29
Hydrocarbon Compounds	2.175	2.88	2.135	2.29
Oxygenated Compounds	83.98	90.79	93.575	95

Table 10. The chemical composition of essential oils of herb *Mentha longifolia* as affected by Mepiquit Chloride (ppm) during 3rd cut (during 2nd season)

Mepiquit Chloride ppm Constituents	0	500	1000	1500
A – Thujene	0.48	1.295	0.49	0.645
α – Pinene	0.55	1.53	0.6	0.79
B – Pinene	1.245	3.045	1.77	2.025
Myrcene	----	-----	-----	0.385
Limonene	-----	4.945	----	----
1,8 Cineol	6.555	13.595	8.385	10.045
Menthone	18.43	20.335	28.415	23.415
Menthol	0.53	1.22	0.475	0.48
Cis-Isopulegone	---	----	----	-----
Terpinene-4-ol	0.725	1.14	0.73	0.78
cis dihydrocarvon	----	-----	0.345	0.39
Carvon	48.41	47.32	53.68	55.26
Piperitone	0.52	1.385	0.435	0.275
Dihydroedulon	---	----	-----	0.345
Piperitenone oxide	---	0.25	0.14	0.29
B- borarbonene	---	----	----	0.085
Transcaryophyllene	----	0.39	0.535	0.515
Total Iden. Com.	77.19	96.45	96	95.725
Hydrocarbon Compounds	2.275	11.205	3.395	4.445
Oxygenated Compounds	75.17	84.995	92.465	90.99

Harvesting a crop early or late resulted in a low yield of leaves as well as the essential oil content because at an earlier or later stage of harvesting, the crop was immature or over mature resulting in a poor yield of herb and oil content [39]. Ulseth [40] found that the poorest oil quality was obtained from mints that were in a stage of early blooming or were not yet blooming at all. According to Clark and Menary [41], besides the timing of harvest, the numbers of harvests per year greatly influence yield, and composition of oil. The essential oil from the first harvest was richer in menthol than that of the second harvest. In the second harvest all the leaves were young with a higher menthone and lower menthol content [42]. According to Ram and Kumar [43], there was a difference between the yield and quality of essential oil in seven mint cultivars in the first and the second harvest. The concentration of carvone in Scotch spearmint was higher in oil of the first harvest compared with the second harvest [44].

Conclusion

It can be concluded that irrigation intervals every 6 days is very suitable for growth and production of *Mentha longfolia* plants at the same conditions.

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