IOCAG 2022

MDPI

Proceeding Paper

The Effect of Bio and Non-Bio Fertilizers on Biochemical and Morphological Characteristics of Peppermint (*Mentha piperita*) Under Different Irrigation Intervals †

Farideh Goshasbi [1,*] and Seyyed Mohammad taghi Tabatabai [2]

[1] Ph.D. crop physiology, Department of Agronomy, Shahrood University of Technology, Agricultural and Natural Resources Research and Training Center, Agricultural Research, Education and Extension Organization (AREEO), Yazd, Iran
[2] Assistant Professor, Head of Agricultural-Horticultural Research, Agricultural and Natural Resources Research and Training Center, Agricultural Research, Education and Extension Organization (AREEO), Yazd, Iran; email
* Correspondence: faridehgoshasbi@gmail.com
† Presented at the 1st International Online Conference on Agriculture—Advances in Agricultural Science and Technology (IOCAG2022), 10–25 February 2022; Available online: https://iocag2022.sciforum.net/.

Abstract: Since the global approach to the production of medicinal plants is effective in improving the quantity and quality of the material, it seems that the nutrition of these plants through the application of biological fertilizers in different environmental conditions is most in line with the production goals of medicinal plants. Therefore, in order to investigate the effect of bio and non-bio fertilizers on biochemical and morphological characteristics of Peppermint (*Mentha piperita* L.) under different irrigation intervals, an experiment in the form of a split-plot in a randomized complete design with 4 replications in the research farm of Agricultural and Natural Resources Research Center, Yazd, Iran was conducted in 2020. Experimental factors including three irrigation intervals: Control (every 7 days), every 12 days, every 17 days, and different levels of fertilizer including Control (without fertilizer application), *mycorrhizal* (Glomus), *Azperium brasilense*, *Pseudomonas fluorescens* and chemical fertilizer (NPK). The results showed that with increasing irrigation intervals, height decreased by 33%. Comparison of the mean of interaction effects of the treatments showed that the biological yield decreased by 81% compared to the control treatment. Also, the highest rate of biological yield was observed in the 7-day irrigation interval and mycorrhiza fertilizer. Comparison of the mean of the data showed that with increasing the irrigation interval, the percentage of essential oil increased. Also, the use of mycorrhiza had the highest percentage of essential oil (2.54%). Therefore, the use of bio-fertilizers, especially mycorrhiza which can greatly reduce the adverse effects of peppermint in dehydration stress conditions is recommended.

Keywords: fertilizer; irrigation; peppermint; yield; mycorrhiza

Citation: Goshasbi, F.; Tabatabai, S.M.t. The Effect of Bio and Non-Bio Fertilizers on Biochemical and Morphological Characteristics of Peppermint (*Mentha piperita*) Under Different Irrigation Intervals. *Chem. Proc.* **2022**, *4*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Published: date

1. Introduction

Peppermint (*Mentha piperita* L.), a perennial plant of the mint family, is one of the most widely used medicinal plants. Widespread cultivation in the world will expose the plant to non-living stress, including water deficit stress during the growing season. On the other hand, the quantity and quality of active ingredients of medicinal plants in addition to genotype, is also affected by environmental factors [4,18]. Water and fertilizer are the main factors determining the level of agricultural production in the world, in addition to which they also affect the quantity and quality of medicinal plants [5]. Drought stress and lack of irrigation are important environmental stresses that disrupt the function of stomata, photosynthetic system, degradation of proteins and enzymes and reduce leaf area, causing the fall of flowers and fruits and thus reduce yield [1,15]. It has been shown that bio- fertilizers have a lot of roles in improving the growth conditions of plants.The results of research have shown that the use of Azospirillium during the reproductive stage, especially the flowering stage, increases the activity of nitrogenase enzyme in the roots of inoculated plants. As a result, the availability of nitrogen for the plant increases the number of flowers and, consequently, increases grain yield [23]. Among phosphate-solubilizing microorganisms, Pseudomonas bacteria is one of the most important types of phosphate-solubilizing bacteria [20]. In addition to improving plant growth, mycorrhizal fungi also increase nutrient uptake. One of the most important elements that is actively and widely absorbed by mycorrhizae is phosphorus [7]. In recent years, mycorrhizal fungi have been used in many plants to combat dehydration and drought stress [24]. Mycorrhiza fungi are a type of bio fertilizer that has a peaceful coexistence with plant roots [12,16]. Therefore, this research was conducted with the aim of investigating The effect of bio and non-bio fertilizers on biochemical and morphological characteristics of Peppermint (Mentha piperita) under different irrigation intervals.

2. Materials and Methods

This experiment was performed as a split plot in the form of a randomized complete block design with 4 replications and in 2020 in the research farm of Yazd Research Center, Iran.

The results of chemical analysis of field soil are presented in Table 1.

Table 1. Analysis of soil texture and physical and chemicals characteristics of sample soil.

Soil Texture	EC ($dS.m^{-1}$)	(pH)	Sand (%)	Clay (%)	Loam (%)	OC (%)	K (ppm)	P (ppm)	N (%)
Sandy loam	2.05	7.66	75	10	15	0.339	19.08	14.8	0.029

In this experiment, water deficit stress treatment was applied using irrigation interval, which includes: control treatment (every 7 days), mild stress (every 12 days) and severe stress (every 17 days) as the main factor and biological fertilizers. Azospirillium, Glomus (mycorrhiza), Pseudomonas, NPK fertilizer and control treatment (without fertilizer application) were considered as secondary factors. Use of mycorrhiza bio-fertilizer was done in the form of sand inoculum. Pseudomonas and Azosporillium bacteria were used by immersing the roots in them. NPK fertilizer was also used in granular form. After preparing the suitable substrate, blocking and preparing the plots and in the 11-leaf stage, the seedlings were transferred to the field. The sub-plots had dimensions of 4 m in length and 3.5 m in width. Each experimental unit consisted of 4 planting rows with a spacing of 70 cm between rows and 50 cm on the row. After transplanting, the first irrigation in the field was done by flood. When the plants were fully established (14–15 leaves), water deficit treatment was applied by drip irrigation method and adjustment of irrigation frequency and according to the plan map, the volume of irrigation water was the same for all treatments each time (with the meter contract from The same volume of water was used). Water deficit continued until the plants were fully flowering and the plants were harvested at full flowering stage.

2.1. Measurement of Plant Height and Biological Yield

Sampling was done at the time of full flowering. The plants were harvested by removing the marginal effects and after drying the samples as dry shade and at room temperature of 18 to 22 °C, the dry weight of each sample was measured. Finally, the obtained values were calculated in terms of grams per plant. Several plants were selected by removing the marginal effects and the height of the tallest branch was measured with a ruler.

2.2. Assessing the Percentage of Essential Oil

After harvesting and drying the plants, first the mill and then the weight and finally the essential oil was extracted. Essential oil extraction was performed in the laboratory using water distillation by an essential oil extractor (Clevenger). Water distillation is used to separate water-insoluble substances (such as essential oils). Using this method, it is easy to extract essential oils from the desired plants. Finally, the extracted essential oil was poured into small glass containers (previously weighed and the empty weight of the glass was recorded) and weighed with an electric scale. The weight of essential oil was recorded in grams and calculated as a percentage.

2.3. Data Analysis

Finally, the obtained data were statistically analyzed using SAS statistical software (V9.4) and Excel¬ software was used to draw the graphs. Mean comparisons were performed using LSD test at 5% probability level.

3. Results and Discussion

3.1. Height

The results of data analysis show a significant effect of irrigation interval on height ($p < 0.05$) (Table 2).

Table 2. Analysis of variance of Height, Biological yield and percentage of Essential oil in treated Mentha with bio and non-bio fertilizers under different irrigation interval.

		Mean Square		
Source of Variance	**df**	**Height (cm)**	**Biological Yield (kg/plant)**	**Essential Oil (%)**
Repetition (R)	2	4.25	103.62 ns	0.0010 ns
(irrigation interval) (A)	2	123.89 *	97268.88 **	0.059 **
Fertilizers (B)	4	6.48 ns	18605.18 **	0.0055 **
irrigation interval * Fertilizers	8	6.39 ns	18036.88 **	0.00017 ns
Error	24	6.98	270.33	0.00025
C.V. (%)		11.87	6.35	4.69

ns, *, **: non-significantly difference and significantly differences at 5 and 1% of probability levels, respectively.

Comparison of mean data showed that the highest plant height (23.62 cm) in control (every 7 days) and the lowest (15.65 cm) in every 17 days irrigation interval was observed. This means that with increasing irrigation interval, plant height decreased by 50.92% (Table 3).

Table 3. Means comparison of effect of irrigation interval on height and essential oil percentage in Mentha (*Mentha piperita*).

	Trait	
Treatment	**Height (cm)**	**Essential Oil (%)**
Control (each 7 days)	23.62 [a]	2.04 [b]
Each 12 days	15.68 [b]	2.053 [a]
Each 17 days	15.65 [b]	2.056 [a]

The reason for the decrease in plant height in water deficit conditions is the reduction of turgor pressure and consequently the reduction of cell division and enlargement compared to non-stress conditions [8]. Studies have shown that water deficit reduces height in plants. In *Fenugreek* [2], Basil [17,6], *Salvia officinalis*, *Yarrow*, *Plantago ovata*, *Pot marigold*, *Chamomile* [6], Giant Blue Hyssop [21] and *Moldavian dragonhead* [6] reports in this field.

3.2. Biological Yield

The results of data analysis show a significant interaction between fertilizer and irrigation interval on biological yield ($p < 0.01$) (Table 2). Figure 1 shows that biological yield decreased by 81% compared to the control treatment. The highest biological yield (460.99 g/plant) in control irrigation interval (every 7 days) and *mycorrhiza* fertilizer and the lowest (87.56 g/plant) in every17 days irrigation interval and Azospirillum fertilizer was obtained.

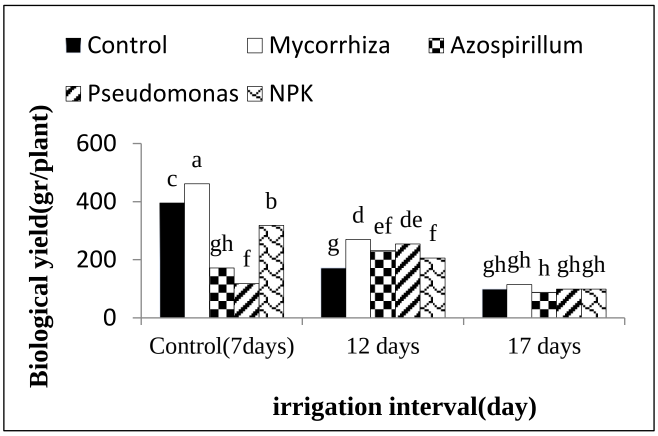

Figure 1. Comparison of the mean interactions of irrigation interval and biological and non-biological fertilizers on the biological yield of *Mntha piperita.*

Biological yield decreased under water deficit conditions. It seems that the main reason is related to the reduction of cell division in the vegetative growth stage and finally the reduction in height, number of lateral branches and biomass produced by the plant. Water deficit reduces the accumulation of dry matter in the plant and thus reduces biological yield [10]. Ref [19] while examining different soil moisture regimes on a type of mint concluded that increasing soil moisture significantly increased dry matter accumulation. Regarding the positive effect of application of fungal treatments on biological yield, it can be stated that mycorrhizal fungus through hyphae expansion and development of root system, provides more water absorption for the plant and after absorbing more water, more nutrients are absorbed. They lead to the production and accumulation of more dry matter in the plant [3,9]. Investigating the effect of water deficit on peppermint (*Mentha piperata* L.), it was reported that water deficit reduces the dry weight of leaves, stems and roots. Bio-fertilizers also provide plant nutrients in solution by producing soluble secretions and reducing acidity [14,22].

3.3. Percentage of Essential Oil

The results of data analysis showed a significant effect of irrigation interval and fertilizer on the percentage of essential oil ($p < 0.01$) (Table 2). Comparison of the mean of irrigation interval data showed that with increasing irrigation interval, the percentage of peppermint essential oil increased so that the highest percentage of essential oil (2.056%) in every17 days irrigation interval and the lowest (2.04%) was observed in control irrigation interval (every 7 days) (Table 3).

Also, among the fertilizer factor, the use of mycorrhiza had the highest percentage of essential oil (2.54%). Azospirillum and Pseudomonas fertilizers were second and third, respectively. The control fertilizer agent had the lowest percentage of essential oil (2.49%) (Table 4).

Table 4. Means comparison of effect of fertilizers on essential oil percentage in *Mntha piperita.*

Treatment	Trait
Fertilizer	**Essential Oil Percentage**
Control	2.49 [e]
Mycorrhiza	2.54 [a]
Azospirillum	2.52 [b]
Pseudomonas	2.51 [c]
NPK	2.50 [d]

It is thought that under conditions of water deficit stress, the production of active ingredients increases due to the prevention of intracellular oxidation. In basil, it was reported that with decreasing soil moisture, the percentage of essential oil increased [11]. After investigating the effect of mycorrhiza on quantitative and qualitative characteristics of peppermint, researchers reported that root colonization was improved by inoculation with this fungus and as a result, growth characteristics, dry matter yield and essential oil content of the plant increased compared to the control [13].

4. Conclusions

According to the results of this study, it is concluded that with increasing irrigation interval, plant height and biological yield decreased and the percentage of essential oil increased. The use of mycorrhiza also showed the highest percentage of essential oil and biological yield. Therefore, the use of bio-fertilizers, especially mycorrhiza can greatly reduce the adverse effects of this plant in conditions of water deficit. Therefore, the use of mycorrhiza is recommended in conditions of reducing the irrigation interval in Peppermint.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Acknowledgments: We would like to thank the member of Agricultural and Natural Resources Research and Training Center of Yazd, Agricultural Research, Education and Extension Organization (AREEO).

References

1. Jaleel, C.A.; Manivannan, P.; Sankar, B.; Kishorekumar, A.; Gopi, R.; Somasundaram, R.; Panneerselvam, R. Pseudomonas fluorescens enhances biomass yield and ajmalicine production in Catharanthus roseus under water deficit stress. *Colloids Surf. B Biointerfaces* **2007**, *60*, 7–11, https://doi.org/10.1016/j.colsurfb.2007.05.012.
2. Alhadi, F.A.; Jabr, M.; Yasseen, B.T. Water stress and gibberellic acid effects on growth of fenugreek plants. *Irrig. Sci.* **1999**, *18*, 185–190, https://doi.org/10.1007/s002710050061.
3. Augé, R.M. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **2001**, *11*, 3–42, https://doi.org/10.1007/s005720100097.
4. Baghbani-Arani, A.; Modarres-Sanavy, S.A.M.; Mashhadi-Akbar-Boojar, M.; Mokhtassi-Bidgoli, A. Towards improving the agronomic performance, chlorophyll fluorescence parameters and pigments in fenugreek using zeolite and vermicompost under deficit water stress. *Ind. Crop. Prod.* **2017**, *109*, 346–357, https://doi.org/10.1016/j.indcrop.2017.08.049.
5. Baghbani-Arani, A.; Modarres-Sanavy, S.A.M.; Mashhadi Akbar Boojar, M.; Mokhtassi, A. Bidgoli. Effect of application of zeolite and nitrogen fertilization on growth, seed yield and water productivity of fenugreek (*Trigonella foenum-graecum* L.) under drought stress conditions. *Ir. J. Crop Sci.* **2017**, *19*, 239–254.
6. Bazzazi, N.; Khodambashi, M.; Mohammadi, S. The effect of drought stress on morphological characteristics and yield components of medicinal plant fenugreek. *Isfahan Univ. Technol. -J. Crop Prod Proc* **2013**, *3*, 11–23.
7. Bolan, N. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant Soil* **1991**, *134*, 189–207, https://doi.org/10.1007/bf00012037.
8. Cabuslay, G.S.; Ito, O.; Alejar, A.A. Physiological evaluation of responses of rice (Oryza sativa L.) to water deficit. *Plant Sci.* **2002**, *163*, 815–827, https://doi.org/10.1016/s0168-9452(02)00217-0.

9. Casson, S.A.; Lindsey, K. Genes and signalling in root development. *New Phytol.* **2003**, *158*, 11–38, https://doi.org/10.1046/j.1469-8137.2003.00705.x.
10. Clarke, J.M.; Townley-Smith, F.; McCaig, T.N.; Green, D.G. Growth Analysis of Spring Wheat Cultivars of Varying Drought Resistance. *Crop. Sci.* **1984**, *24*, 537–541, doi:10.2135/cropsci1984.0011183x002400030026x.
11. Goshasbi, F.; Heidari, M.; Sabbagh, S.K.; Makarian, H. Effect of irrigation interval, bio and non-biofertilizers on yield components and some of biochemical compounds in Thyme (*Thymus vulgaris* L.). *J. Hortic. Plant Nutr.* **2020**, *3*, 51–68. https://doi.org/10.22070/HPN.2020.5084.1070.
12. Gogoi, P.; Singh, R.K. Differential effect of some arbuscular mycorrhizal fungi on growth of *Piper longum* L. (Piperaceae). *Indian J. Sci. Technol.* **2011**, *4*, 119–125. https://doi.org/10.17485/ijst/2011/v4i2/29946.
13. Gupta, M.; Prasad, A.; Ram, M.; Kumar, S. Effect of the vesicular–arbuscular mycorrhizal (VAM) fungus Glomus fasciculatum on the essential oil yield related characters and nutrient acquisition in the crops of different cultivars of menthol mint (Mentha arvensis) under field conditions. *Bioresour. Technol.* **2001**, *81*, 77–79, https://doi.org/10.1016/s0960-8524(01)00109-2.
14. Han, H.; Supanjani; Lee, K. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptáme and growth of pepper and cucumber. *Plant Soil Environ.* **2011**, *52*, 130–137, https://doi.org/10.17221/3356-pse.
15. Jaleel, C.A.; Gopi, R.; Panneerselvam, R. Growth and photosynthetic pigments responses of two varieties of Catharanthus roseus to triadimefon treatment. *Comptes Rendus. Biol.* **2008**, *331*, 272–277, https://doi.org/10.1016/j.crvi.2008.01.004.
16. Watababe, K. Coherency Preserving Feature Transformation for Semantic Segmentation. In Proceedings of the 2018 IEEE International Conference on Systems, Man, and Cybernetics (SMC), Miyazaki, Japan, 7–10 October 2018; pp. 1368–1373. http://dx.doi.org / 10.1109/SMC.2018.00239.
17. Khalid, K.A. Influence of water stress on growth, essential oil, and chemical composition of herbs [Ocimum sp.]. *Int. Agrophys* **2006**, *20*, 289–296.
18. Kheiry, A.; Tori, H.; Mortazavi, N. Effects of drought stress and jasmonic acid elicitors on morphological and phytochemical characteristics of peppermint (*Mentha piperita* L.). *Iranian. J. Med. Aromat. Plants Rese* **2017**, *33*, 268–280. https://doi.org/10.22092/IJMAPR.2017.106481.1783.
19. Ram, M.; Ram, D.; Singh, S. Irrigation and nitrogen requirements of Bergamot mint on a sandy loam soil under sub-tropical conditions. *Agric. Water Manag.* **1995**, *27*, 45–54, https://doi.org/10.1016/0378-3774(95)91231-u.
20. Narsian, V.; Patel, H.H. Biodiversity of phosphate solubilizing microorganisms in various rhizosphere soils of Bhavnagar district. *Asian J. Microbiol. Biotechnol. Environ. Sci.* **2006**, *8*, 201.
21. Omidbaigi, R.; Mahmoudi Sorestani, M. Effect of drought stress on some morphological traits, amount and yield of essential oil of Agastache foeniculum [pursh] kuntze. *J. Hortic. Sci. Iran* **2010**, *4*, 153–161.
22. Rademacher, W. Gibberellin formation in microorganisms. *Plant Growth Regul.* **1994**, *15*, 303–314, https://doi.org/10.1007/bf00029903.
23. Ratti, N.; Kumar, S.; Verma, H.; Gautam, S. Improvement in bioavailability of tricalcium phosphate to Cymbopogon martinii var. motia by rhizobacteria, AMF and Azospirillum inoculation. *Microbiol. Res.* **2001**, *156*, 145–149, https://doi.org/10.1078/0944-5013-00095.
24. Song, H. Effects of VAM on host plant in the condition of drought stress and its mechanisms. *Electron. J. Biol.* **2005**, *1*, 44–48.