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ORIGINAL ARTICLE

Effect of salicylic acid and Zinc on Enzymatic characteristics peppermint (*Mentha piperita* L.) quality under drought stress

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ABSTRACT
Drought stress is one of the major constraints on crop production and food security worldwide. Salicylic acid (SA) is involved in the response to abiotic stress. Zinc (Zn) is one of the essential micronutrients and plays an important role in a wide range of processes and growth factors. Therefore, the present study aimed to investigate the effect of salicylic acid and zinc on the physiological characteristics, growth and menthol of peppermint in drought stress conditions. This experiment was conducted as asplit-plot factorial experiment based on randomized complete blocks design was used with three replications during the 2016 growing seasons at the research farm of Islamic Azad University, Varamin Branch, Tehran, Iran.The irrigation regimes including optimum irrigation (when evaporation reached 50 mm, using evaporation pan class "A"), moderate drought stress (70 mm), and severe drought stress (90 mm) as main plots.While zinc sulfate levels i.e. control (not used), spreading zinc with a ratio of 2 and 4 g/L tow time and salicylic acid containing two levels Comtel (No use salicylic acid) and 1 mM salicylic acid was used as a sub factor.The results of this study showed that the biological yield was significantly ($P < 0.01$) affected by drought stress and zinc sulfate. The greatest biological yield ratio of 992.8 Kg/ha was recorded for optimum irrigation and use 4 g/L zinc sulfate. The interaction between drought stress and salicylic acid showed that menthol had the highest amount in treatment of salicylic acid consumption and optimum irrigation and the value was 15.9%.
Keywords: Salicylic acid, drought Stress, Menthol, Zinc and Peppermint

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INTRODUCTION

Peppermint (*Mentha piperita* L.), a member of Lamiaceae family, is an important medicinal plant that has many useful properties, that is why there is always extensive research to find medicinal products and medicinal substances of the plants [14]. Abiotic stresses such as drought are among the most challenging threats to agricultural system and economic yield of crop plants [14]. Drought stress usually leads to reductions in crop yield, which can result from many drought-induced morphological, physiological, and metabolic changes that occur in plants [7, 1]. The role of salicylic acid (SA) as a key molecule in the signal transduction pathway of abiotic stress responses has already been well described [11, 18]. SA treatment prevented lipid peroxidation while the peroxidation increased in control plants. The results showed that exogenous SA can alleviate the damaging effect of long term drought stress by decreasing water loss and inducing the antioxidant system in the plant having leaf rolling, alternative protection mechanism to drought [12]. Singh & Usha [22] reported that SA treated plants showed, in general, a higher moisture content, dry mass, carboxylase activity of Rubisco, superoxide dismutase (SOD) activity and total chlorophyll compared to those of untreated seedlings. Zn plays a role in alleviating wheat plant drought stress by Zn-mediated increase in photosynthesis pigment and active oxygen scavenging substances, and reduction in lipid peroxidation. Furthermore, Zn fertilizer could regulate multiple antioxidant defense systems at the transcriptional level in response to drought [15, 11]. Hegazy *et al.* [10] reported that Zinc spraying on Medicinal Lamiaceae plant gave the best results of plant height, number of branches, herb fresh weight, essential oil % and total flavonoids % in the vegetative stage than control treatments. Nasiri *et al*. [17] reported that foliar application of Zncan considerably improve the flower and essential oil yields of chamomile, particularly if these micronutrients were applied together at both stages of stem elongation and flowering. Said & Omer [20]) reported that the treating coriander plants with 200 ppm iron in the vegetative stage and with 200ppm zinc + 200 ppm iron in the flowering stage produced the highest values of essential oil content and total carbohydrate percent. The present study was conducted to investigate whether exogenous application of zinc (Zn) and salicylic acid (SA) could regulate the activities of antioxidant enzymes and increasing the quantity and quality of Peppermint (*Mentha piperita* L.) on the under drought stress condition.

MATERIAL AND METHODS

The study was conducted in research farm of Islamic Azad University, Varamin Branch, Tehran, Iran in during the 2016 growing seasons. Varamin region has an arid to semi-arid climate with average annual precipitation of 251 mm.

Table 1. Physical-chemical properties of the soil.

Depth (cm)	Ds.m (Ec)	pH	Clay (%)	SALT (%)	SAND (%)	TEXTURE	P (ppm)	K (ppm)	N (ppm)	OC (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)	B (ppm)
0 - 30	1.95	7.66	33	44	33	loamy clay	20	452	0.1	0.92	3.55	0.81	3.55	1.19	1.11

This experiment was conducted as a factorial split plat in randomized complete block design with three replications. This experiment has 3 factors including drought stress including optimum irrigation (when evaporation reached 50 mm, using evaporation pan class “A”), moderate drought stress (70 mm), and severe drought stress (90 mm) as main plots and salicylic acid spraying at control (not use) and 1mM molar levels and Zinc sulfate foliar application The values were control (not use), 2 and 4 g/L.The experimental site was plowed by moldboard plow, harrowed and divided into three blocks, each contained 24 plots. Each subplot was 2 m wide and 4 m long and consisted of four rows of Peppermint which were 0.4 m wide and 0.3 m long on each line. A gap of 2 meters was considered between adjacent main plots and the distance between replications was 3 m.Plots were planted on the June in 2016, respectively, at a row spacing of 0.3 m.After planting, plots were irrigated equally to facilitate growth. Irrigation regimes were applied when plants were completely established, by average. Plots were irrigated anytime evaporation reached the considered amount for each irrigation level (50, 70, and 90 mm evaporation from the surface of the evaporation pan).In this experiment, the traits (Superoxide dismutase (SOD), Catalase (CAT)Menthol and Biological yield) were investigated.CAT was measured from young and developed leaves and then with the method according to the method Upadhyaya *et al.,* [23] in a reaction mixture containing 46mM potassium phosphate buffer, pH 7.0, 0.03% H 2 O 2 and an enzyme extract. The decomposition of H2O2 was followed at 240 nm (1 EU / 1 mmol H_2O_2 decomposed in 1 min). Based on method Beauchamp & Fridovich [2] SOD activity was measured by the photochemical method, One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of nitro blue tetrazolium (NBT) reduction at 560 nm in the presence of riboflavin in the light. The reaction mixture contained 45mM potassium phosphate buffer, pH 7.0, containing 0.1mM EDTA and 13mM methionine, 0.17mM NBT in ethanol, 0.007mM riboflavin and enzyme aliquot. Blanks were kept in the dark and the others were illuminated for 15 min. Essential oil extraction and estimation Essential oil of peppermint were extracted by hydro-distillation with a modified Clevenger trap. The distillation equipment consists of a boiler, distillation still, condenser and receiver. 100 g herbage was crushed in electric grinder with distilled water. The pulverized mass was loaded in to the still for distillation. The condenser cooled the hot vapours received from the still. The jet of vapours consisting of steam and essential oil were cooled in the condenser tubes and condensate flowed out into the receiver. The oil being lighter than water and insoluble, floated on the top surface of water in the receiver and only the water got drained out. The oil was drawn off, the volume of oil was noted, and percentage of essential oil content measured in volume/100 g fresh weight basis [4]. In order to measure the biological yield in full flowering stage into in July 2016 peppermint 1 square meter in the middle of each plot were cut to 2 cm above the soil surface and separated and reported in terms of kilograms per hectare and plants were dried by oven at 75 ºC for 48 h and dry matter yield obtained. Analysis of variance was performed using SAS 9.4 (SAS 2014) and EXCEL and the means were compared using student T test at 5% probability level.

RESULTS AND DISCUSSION

Table 2. Analysis of variance for various studied traits of peppermint

Source	M.s				
	Df.	**SOD**	**CAT**	**Menthol**	**Biological yield**
Rep.	2	21.16ns	0.004ns	2.12ns	203609.7ns
Drought stress	2	73.84*	0.881**	16.22**	276114.1**
Error a	4	14.28	0.002	0.5	3142.4
Zinc	2	17.88ns	0.453**	59.28**	195013.7**
Drought * Zinc	4	24.67ns	0.069**	0.73ns	9290.3**
Salicylic acid	1	42.69*	0.931**	24.34**	101532.2*
Drought * Salicylic acid	2	8.60ns	0.004ns	15.68**	9549.8**
Zinc * Salicylic acid	2	15.37ns	0.080**	2.53ns	12058.2ns
Drought * Salicylic acid * Zinc	4	16.56ns	0.013**	0.54ns	2759.5ns
Total	30	6.44	0.003	1.94	3075.0
CV (%)	-	8.42	5.47	6.5	12.4
n.s. = Non-significant * = Significant at 5% level ** = significant at %1 level					

Results of combined analysis of variance indicated The interaction effects between drought stress and salicylic acid were significant at 1% besides the SOD, CAT, menthol and Biological yield of peppermint . The interaction effect between the drought stress and zinc sulfate were significant at 1% besides the SOD, CAT, menthol and Biological yield of peppermint. The Triple interaction effect of drought stress, salicylic acid, and zinc sulfate were significantat 1% besides the CAT (Table 2).

Superoxide dismutase (SOD)

The results of this study showed that the main effect of drought stress and salicylic acid on SOD was significant, but other treatments had no effect on SOD (Table 2). Drought stress of 90 mm evaporation from the evaporation pan with the amount of SOD 28/29 (H_2O_2dec./min/mg protein) caused a 27% increase of this trait compared to 50 mm tension (stress) from a pan evaporated (Fig. 1). With the use of salicylic acid, SOD was obtained in the amount of 27.17 (H_2O_2dec./min/mg protein), which was 15% higher than non-consumption (Fig. 2). In relation to these results, Hassanein *et al.,* [8] showed that the SA and their combination resulted in great increases in the activity of SOD and CAT enzymes accompanied by great reduction in peroxidase (POX) and ascorbic peroxidase (APX) activities. Maximum increase of SOD and CAT was observed by treating plants with TU at 2.5mM for SA-pretreated plants either under normal irrigation (44% and 32%, respectively) or under drought conditions (57.8% and 47.8%, respectively).

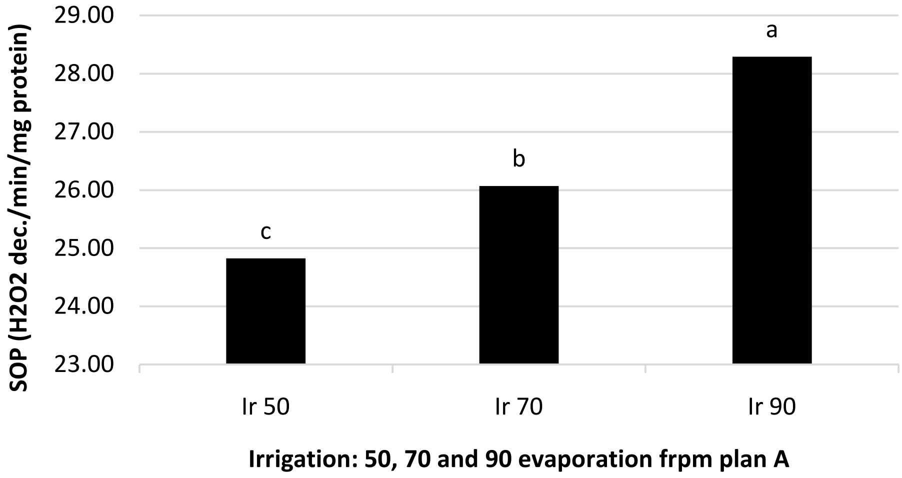


Fig 1. Main effect of drought stress on SOD

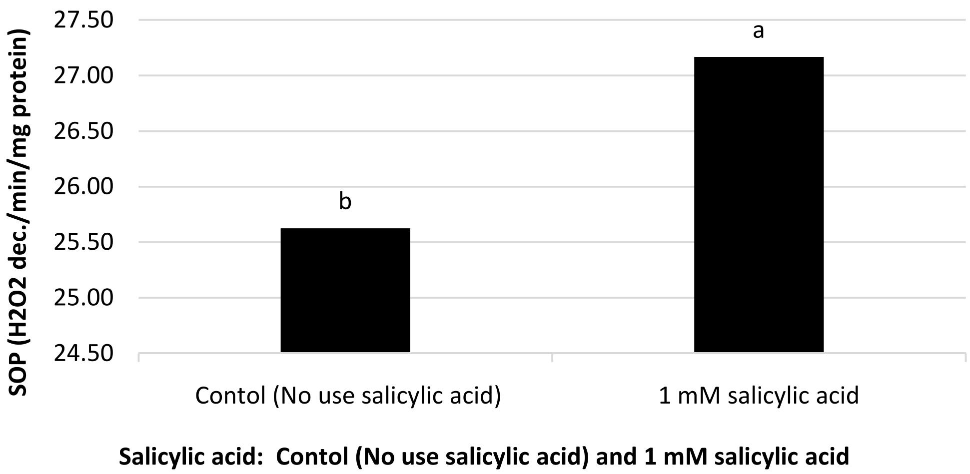
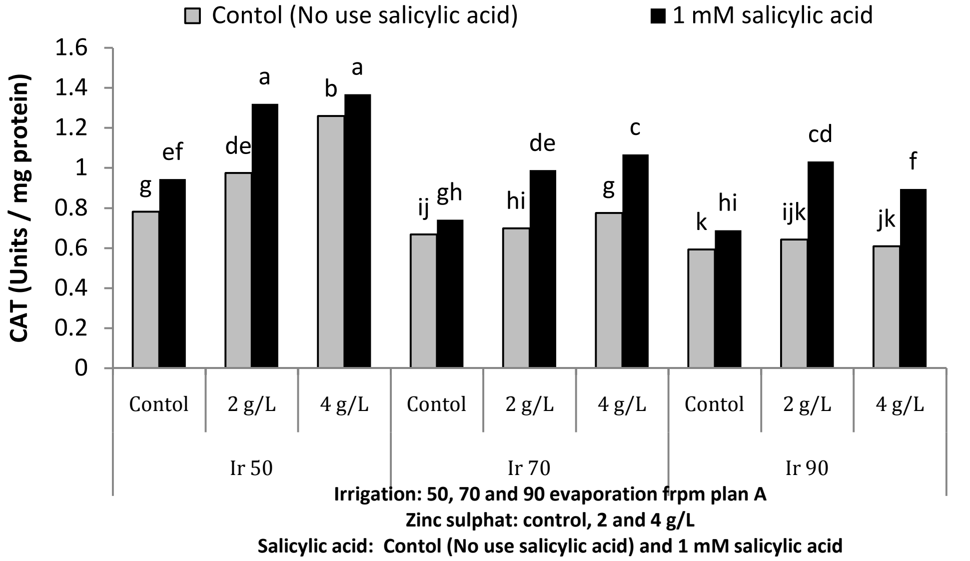


Fig 2. Main effect of salicylic acid on SOD

Catalase (CAT)

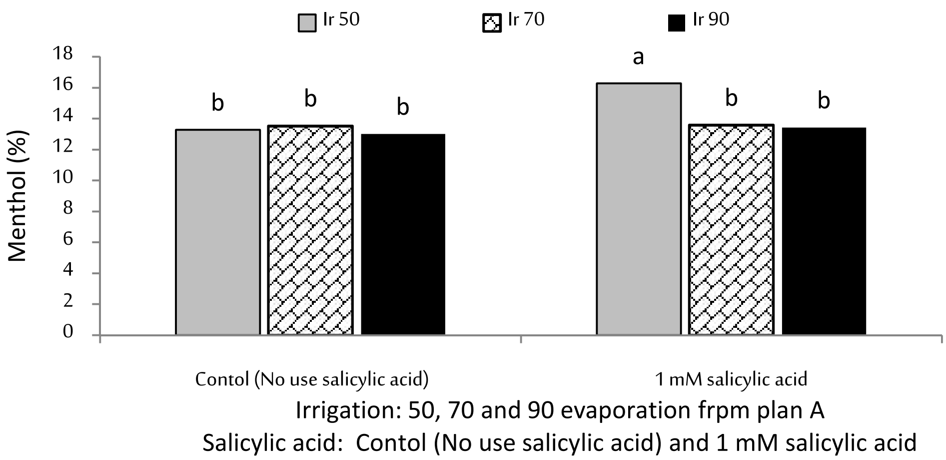
The results showed that the main and mutual effect of all treatments was significant on CAT except for the effect of drought stress on salicylic acid (Table 2). The results showed that the highest amount of CAT was obtained in treatment of 4 grams of zinc per liter with salicylic acid consumption and in the treatment of 50 mm evaporation stress from the pan was 1.24 (Units/mg protein). The lowest amount was 0.64 (Units/mg protein), in the absence of zinc and salicylic acid and in the 90 mm evaporation stress from the evaporation pan. In all levels of drought stress, consumption of salicylic acid and zinc sulfate increased the amount of CAT (Fig. 3). According to these results Dianat *et al*., [5] showed that the drought stress significantly increased the amount of sugar, proline and the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and, peroxidase (POD). Singh & Usha [22] showed that the role of SA in regulating the drought response of plants and suggest that SA could be used as a potential growth regulator, for improving plant growth under water stress. SA protected against the stress generated by water and significantly improved the CAT, POX, and SOD. However, proline content and antioxidant enzymes increased under drought as well as under SA treatments [9]. Moghadam *et al.,* [16] showed that severe water stress decreased chlorophyll content, relative water content, zinc grain content, auxin and gibberellin, and it caused an increase in antioxidant enzyme activity and in the other hand, zinc foliar application was effective against the detrimental effects of water stress.

Fig 3. Interaction effect of the drought stress, salicylic acid and zinc sulphate on CAT



Menthol
The results of this study showed that the main effect of drought stress, main effect of zinc sulfate, main effect of salicylic acid and zinc interaction on salicylic acid on soluble sugars was significant, but other treatments had no effect on menthol (Table 2). Concerning the interaction between drought stress and salicylic acid, the results showed that menthol had the highest amount in treatment of salicylic acid consumption and 50 mm stress evaporation pan and the value was 15.9%. The minimum amount was obtained at the treatment of 90 mm stress and salicylic acid was not used. At all levels of stress, salicylic acid consumption has always been increased menthol (Fig. 4). In relation to these results, Bideshki & Arvin [3] showed that application of salicylic acid (SA) is an important signal molecule modulating plant response to water stress.. On the contrary, SA at 0.05mM spray reduced the harsh influences of water deficit resulted in improved growth and increased photosynthetic pigments as well as essential oil constituents. Also, Rezaei & Pirzad [19] showed that by increasing levels of drought stress, plant height, number of follicle per plant, grain, oil and essential oil yields of black seed significantly decreased. However, salicylic acid had a significant effect on decreasing severity of drought stress.

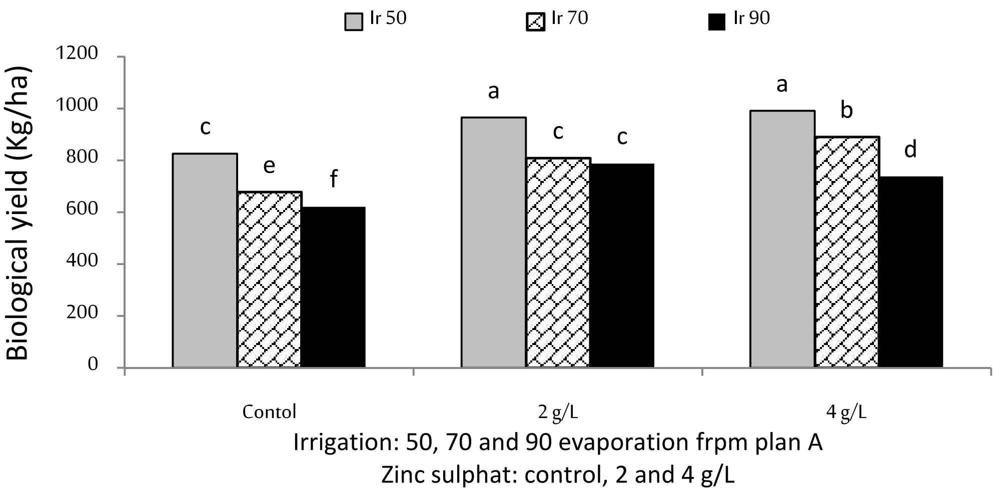
Fig 4. Interaction effect of the drought stress and salicylic acid on menthol



Biological yield
The results of this study showed that the interactions of drought stress in zinc sulfate and zinc interaction on salicylic acid on biological yield was significant but other treatments had no effect on biological yield (Table 2). Regarding the interaction between drought stress and zinc sulfate, the results showed that the

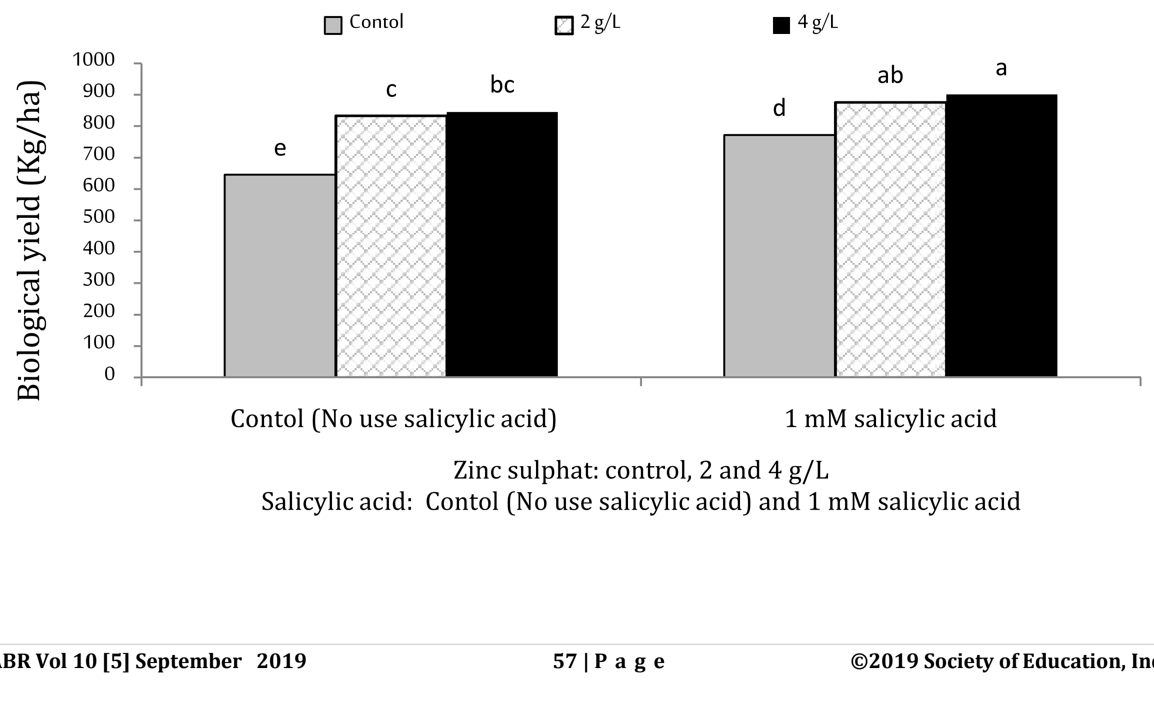
biological yield that was obtained in treatment of 4 gram zinc per liter and 50 mm evaporation from the evaporation pan was 973.8 Kg/ha, which was the highest. The minimum amount was obtained in non-zinc treatment and 90 mm stress evaporation from the evaporation pan. At all levels of stress, zinc sulfate has always been used to increase biological yield (Fig. 5).These results confirmed the findings of Shahri *et al*., [21] that reported the regarding the limitation of water resources, application of Zn could be used as a good strategy for yield sustainability of plant under drought stress. Comparisons of results in different levels of zinc sulfate spraying showed that biological yield was raised with increase of zinc sulfate spraying.

Fig 5. Interaction effect of the drought stress and salicylic acid on biological yield



Regarding the interaction between zinc and salicylic acid, the results showed that biological yield in the treatment of 1mM and 4 grams of zinc per liter of evaporation pan was 811.2 kg/ha, which was the highest. The minimum amount was also found in not using of zinc and salicylic acid. At all levels of zinc sulfate, salicylic acid consumption has always increased the biological yield (Fig. 6). Similar results were obtained by Gobarah *et al.,* [6] who that reported the foliar spraying with zinc had a significant effect on dry weight of leaves and stem per plant at 75 days after sowing. Increasing zinc level from 0.50 to 1.00 gm/L significantly increased the above-ground biomass. Foliar spraying with zinc encouraged the vegetative growth and increased the plant capacity for building metabolites.

Fig 6. Interaction effect of the salicylic acid and zinc sulphate on biological yield



CONCLUSION

In conclusion, The most quantity of the menthol obtained from the irrigation treatment of 50 mm evaporation from plan A along with the application of salicylic acid (15.9%). Furthermore, the application of Zinc fertilizer not only increased the SOD and CAT but also enhanced the of menthol. Thus, the application of salicylic acid on peppermint along with Zinc fertilizer enhanced the tolerance against abiotic stress and also increased the quantity and quality of peppermint. However, further research is needed to efficiently improve the yield in different areas.

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