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Effect of Salinity Stress on the Morphological and Physiological Characteristics of Peppermint under Greenhouse Condition

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ABSTRACT

Peppermint is a rich source of valuable compounds with therapeutic, coloring, preservative and other uses for humans. Peppermint is used in food, perfumery and medicine all over the world. Higher plants experience salt stress due to an excessive buildup of sodium chloride; salinity impacts plants through osmotic stress and toxicity. Salinity impacts every major activity in plants, including growth, photosynthesis, lipid metabolism, protein synthesis, energy production, and germination through biomass and seed formation. The experiment was carried out in the research farm of agriculture faculty of Lorestan University, Iran. The aim of the current research was to examine the effect of different levels of salinity (0, 50 and 100 mM) on the morphological and physiological characteristics of peppermint in the greenhouse. The result showed that salinity stress at 50 and 100 millimole (mM), decreased plant height (11.76%, 23.53%), number of leaves (25.11%, 33.92%), leaf surface (24.76%, 56.37%), crown diameter (14.15%, 25.47%), root length (20.61%, 31.58%), leaf fresh weight (14.47%, 30.47%), leaf dry weight (1.16%, 12.69%), fresh weight of stem (10.78%, 21.11%), dry weight of stem (16.32%, 37.76%), chlorophyll a (46.08% and 61.09%), Chlorophyll b (32.48% and 41.65%), total chlorophyll (40.84% and 53.59%) and carotenoid (34.67% and 67.02%) compared to the control conditions respectively. Hence, increase in salinity concentration at 50 and 100 mM, increased the amount of malondialdehyde (MDA) (37.06% and 73.63%), leaf proline (301.50% and 382.09%) and essential oil (100.40% and 80.32% compared to control. The results of this study showed that, the salinity stress had a significant effect on the morphological, physiological and biochemical characteristics of peppermint. The results showed that, increases in the salinity stress, significantly increased the amount of electrolyte leakage, production of essential oil, malondialdehyde and proline. Moreover, increase salinity levels in irrigation water, caused reduction in growth characteristics of peppermint. It is to be concluded that, Peppermint is a semi-salt resistant plant, cultivation of peppermint in the medium saline soil is recommended.

Keywords: Greenhouse, Morphological Characteristics, Peppermint, Salinity Stress, Physiological Characteristics

INTRODUCTION

Mentha piperita L., the scientific name for peppermint, is a herbaceous, perennial plant that is used worldwide in fragrance, medicine, and food for human protection. It is a member of the Lamiaceae family. (Mckay and Blumberg, 2006). The valuable works of the above-mentioned, that plants are related to the compounds in its essential oil, which are widely used in the pharmaceutical industry of food products (Roodbari et al., 2013). The purpose of peppermint cultivation, in addition to obtaining aerial parts is to use its extract and essential oil, which is increasing day by day in its commercial importance for changing the taste of medicines, edibles, soft drinks, and cosmetic products (Mousavi et al., 2010).

Peppermint is considered one of the medicinal plants of the ancient world, this is customarily utilized across the majority of the world (Keifer et al., 2007). The people of ancient Egypt (one of the ancient civilizations that were advanced in the field of medicine), planted the peppermint and used the leaves of that for digestion. On the other hand, ancient Greek and Roman people used peppermint for indigestion. In the 18th century in Europe, the mentioned plant was special importance for the treatment of stomach and menstrual disorders (Tyler et al., 1988).

Leaf is the most valuable economic part of peppermint, which 99% of the essential oil is synthesis and stored in the secretory hairs on the surface of the leaf (Croteau and Wildung, 2005). Numerous studies show that, the amount of elements in peppermint leaves is much higher than the amount of vitamins. Potassium, calcium, magnesium, iron, manganese, zinc and copper are the most important elements which found in the dry leaves of peppermint (Zimmna and Piekos, 1988). The speed of the leaf initiation of the plant is constant until the pre-flowering stage, and it includes the appearance of three pairs of new leaves every week or the appearance of one pair of leaves every 2-3 days (Turner et al., 2000). Three different types of hairs may be found on peppermint leaves: non-glandular hairs (multi- and single-celled, found in greater density in the major vein area and in lesser numbers on the lower surface of the leaf). Glandular hairs are peltate and captate (Askari et al., 2016).

Peppermint is anti-convulsant and anti-cough that drinking a cup of hot tea soothes the chest. Peppermint relieves stomach pain and is effective in treating of colds and flu (Fleming, 2004). Peppermint has an aromatic essential oil in its vegetative organ, which has a cool and slightly spicy taste. The aerial parts of peppermint have an average of 1 to 1.5% essential oil. Peppermint essential oil contains compounds such as menthyl acetate, menthol, menthone and a small amount of cineole and other terpenes. Essential oil is also in the form of a colorless or pale yellow liquid and has a specific and strong smell and a nasty taste. Peppermint essential oil has been used as a flavoring agent in the production of various food products, and studies have shown significant antioxidant, antibacterial, antifungal, and antiviral effects of this essential oil. According to the conducted research, the primary ingredient in peppermint essential oil is menthol. Menthol is used as a stomach booster, lowering body temperature during fever, anti-cough and vomiting, and an effective antiseptic in lung inflammation. Menthol is also used in the production of liqueurs, sweets, food industry, cosmetics, perfumery, toothpaste, mouthwashes and pharmaceutical products (altadin lozenges, alicom coated tablets, plant gel granules and mentagel gel). In addition, menthol is an important antiseptic compound that has very effective antibiotic effects (Mahmoud and Croteau, 2003).

Around the world, salinity stress is thought to be the most significant factor influencing the decline in agricultural, horticultural, and medicinal crop output, after drought (munns, 2005). In the whole world, more than 397 million hectares of land are saline, and probably the following areas have increased salinity due to improper irrigation and removal of their native vegetation. Plants that experience salinity stress undergo biochemical alterations and physiological reactions that impact all phases of life, including photosynthesis and plant growth and development (Kumar, 2005).

Plants respond to salt stress in a highly diverse and intricate way. The effects of growth inhibition by salinity can be caused by changes in the balance of plant hormones due to stress. Typically, a rise in soil salinity results in a reduction in plant development and output. Additionally, salinity has an impact on all of the major functions of the plant, including energy production, lipid metabolism, and protein synthesis. Thus, it has an impact on every phase of a plant's existence, from germination to seed production. Plants that are under salinity stress, the lack of proper turgorescence of cells and the consumption of more synthesized substances to deal with the stress, the shortening of the plant's growth period and also the stress escape mechanisms, all can prevent the normal development of cells. And finally reduce the height of the plant. The amount of water accessible for plants is decreased by salinity because it lowers the soil solution's osmotic potential. As a result, plants face drought stress (Sreenivasulu, 2007).

During salinity stress, plants adjust their osmosis using organic compounds such as proline and carbohydrates. The mentioned compounds partially provide suitable conditions for continued growth and photosynthesis for plants. Biochemical studies show that a number of organic compounds (compatible solutions) accumulate in plants under salt and drought stress. Accumulated compounds do not interfere in their chemical processes and the following compounds can be mentioned as soluble carbohydrates (mannitol, sucrose, raffinose and oligosaccharide) and nitrogenous compounds (proline amino acid and glycine-betaine). When plants are stressed, adaptation chemicals play a crucial function in enhancing osmotic control (Good, Zaplachiniski, 1994).

More than other non-living environmental factors, salinity and drought have an effect on agricultural productivity (Zhang et al., 2006). Primary stress has consequences that become noticeable in a matter of seconds or minutes, but if the duration of stress is longer, Secondary stress is manifested by indirect effects and injuries (Parida and Das, 2005). Salinity affects all metabolic activities of plants and causes changes in the structure, shape and anatomy of plants. In actuality, a few of these modifications are adaptations that increase the plant's resistance to salt stress. But most of the alterations that have been noticed are indicators of salt damage (Azarnivand and Ghorbani, 2007).

Growth, photosynthesis, protein synthesis, energy production, and fat metabolism are all impacted by salt stress. Plants' ability to acquire water is restricted when soil salinity rises. The degree of growth inhibition brought on by salt stress is contingent upon the length and severity of the stress as well as the stage of development at which the stressed plant is growing. And it is possible that all the tissues and organs of the plant are equally affected by these stresses, do not react (Shani and Duddly, 2001). In the first step, there is a decrease in the expansion of the leaf surface, which causes the increase of the leaf surface to stop with the intensity of the stress. Growth starts again after the stress is removed. Carbohydrates are among all substances needed for cell growth, which are obtained through photosynthesis. In general, plants that are exposed to salinity, especially sodium chloride salinity, have lower photosynthesis rate (Parida and Das, 2005). Salt stress causes a reduction in the leaf surface (Savant and Korndorfer, 1999). Therefore, the objective of this study is to examine how salt stress affects the physiological and morphological traits of peppermint (*M. piperita* L.) grown in greenhouses.

MATERIAL AND METHODS

Research site and design

The experiment was conducted in the educational and research greenhouse of the Faculty of Agriculture of Lorestan University, Iran in 2022. This study aimed to find the effect of varying salinity levels on growth attributes (morphological and physiological) of peppermint (*M. piperita* L.). A fully randomized design with eight replications was used to conduct a factorial experiment. Three levels of 0, 50 and 100 mM salinity were applied. Therefore, 3 treatment combinations with 8 replications and a total of 24 pots of the experimental unit (plastic pots) were used.

Sample collection

Rooted rhizomes of peppermint were obtained from the Research and Technology Complex of Medicinal Plants of Lorestan Province. The mentioned rhizomes were cultivated in sterilized two-kilogram pots containing agricultural soil, animal manure and cocopeat in a ratio of 2:1:1. Pots were filled with 3 cm of washed gravel sand, and the rest of the pot volume was covered with a culture bed consisting of mixture of sterilized soil, cocopeat and manure.

Peppermint cultivation took place in the greenhouse in March 2022. Peppermint rhizomes were cultivated to a depth of 2-3 cm in pots. The pots were grown in the greenhouse with 12-13 hours of light (natural light), relative humidity of 50-60% and temperature of 25±5°C. Peppermint rhizomes were not subjected to salt stress until they were fully established and the rhizomes grew to the same size. The watering cycle of peppermint pots continued for three days until the end of the experiment period. In order to determine the amount of water used, the pots were irrigated with 200 cc and measured by human.

Application of salt stress

Applying salt stress was done by irrigation. In order to irrigate the pots, irrigation was prepared according to the plant's needs on the same day and the pots were watered immediately. In the first step, a 1 M solution of sodium chloride salt (molecular weight 44.58 g/mol) was prepared as a base. In order to generate the concentration of solutions needed for salinity, 58.44 grams of sodium chloride salt were dissolved in one liter of distilled water. Salt stress was applied after the establishment of peppermint rhizomes and at the 4-6 leaf stage. The watering process for each treatment (0, 50, and 100) was carried out every 3 days, 16 times until the end of experiment. In addition, in order to prevent the EC of the pots from increasing too much, they were washed with normal water at the rate of one liter for each pot in which salinity stress was applied and was done once every two weeks.

Data collection

Various data such as plant height, plant crown, number of leaves, root diameter, root length, root volume, and fresh and dried weights of the root; fresh and dry weight of leaf, number of internodes and internode length, fresh and dry weight of stem, relative water content, ion leakage, chlorophyll a, chlorophyll b and total, carotenoid content, proline content, malondialdehyde content, essential oil percentage, transpiration carbon dioxide under the chamber, stomata conductance and photosynthesis were collected at the end of experiment.

RESULTS

Morphological and physiological characteristics

The results of this study was showed that, by applying salinity at 50 and 100 mM, morphological characteristics such as plant height (11.76%, 23.53%), number of leaves (25.11%, 33.92%), leaf surface (24.76%, 56.37%), crown diameter (14.15%, 25.47%), root length (20.61%, 31.58%), root volume (24.07%, 37.04%), root diameter (23.05%, 46.81%), internode number (6.89%, 20.69%), internode length (14.52%, 21.77%), leaf fresh weight (14.47%, 30.47%), leaf dry weight (1.160%, 12.69%), stem fresh weight (10.78%, 21.20%), stem dry weight (16.32%, 37.76%), root fresh weight (12.68%, 21.71%), root dry weight (33.10%, 57.61%) and relative water content (18.30%, 20.87%) decreased compared to the control. Which shows the negative effects of salinity stress about peppermint's development and growth (Table 1).

Table 1: Effect of salinity stress on the morphological characteristics of peppermint

Salinity stress	Plant Height (cm)	Number of leave	Leaf area (cm^2)	Crown diameter (cm^2)	Length of internode (cm)	Number of internode	Dry weight of leaf (gr)	Dry weight of stem (gr)	Dry weight of Root (gr)
Control	29 a	69.17 a	550.91 a	15.62 a	3.38 a	7.83 a	1.50 a	1.46 a	2.72 a
50 mm	26.49 b**	58.41 b**	413.24 b**	13.75 b**	3.05 b**	7.33 a*	1.30 b**	1.02 b**	1.81 b**
100 mm	23.29 c**	50.75 c**	268.36 c**	11.67 c**	2.66 c**	6.33 b**	1.13 c**	0.76 c**	0.98 c**

** and * are significant at the level of 1 and 5% probability

Table 2. Effect of salinity stress on the morphological and physiological characteristics of peppermint.

Salinity stress	Length of root (cm)	Root diameter (cm)	Root volume (cm^3)	Fresh weight of leaves (gr)	Fresh weight of stem (gr)	Fresh weight of roots (gr)	Relative water content (%)	Stomata conduction (mmol m^{-2} s^{-1})	Photosynthesis (µmol/m2/s)
Control	31.33 a	5.77 a	15 a	5.96 a	4.26 a	7.05 a	81.37 a	245 a	15.40 a
50 mm	25.54 b**	4.06 b**	11.42 b**	5.01 b**	3.78 b**	5.87 b**	70.73 b**	201.08 b**	12.09 b**
100 mm	22.42 c**	3.14 c**	9.46 c**	4.13 c**	3.08 c**	4.33 c**	66.44 c**	162.93 c**	9.83 c**

** and * are significant at the level of 1 and 5% probability.

In this study, applying salinity stress at 50 and 100 mM, the characteristics of chlorophyll a (46.08% and 61.09%), chlorophyll b (32.48% and 41.65%), total chlorophyll (40.84% and 53.59%) and carotenoids (34.66% and 67.02 %) were decreased compared to the control. On the other hand, in this research, it was observed that with increasing salinity concentration at 50 and 100 mM, the amount of essential oil of peppermint (100.4% and 80.32%), MDA (37.06% and 73.63%) and proline content in leaves (301.50% and 382.09%) increased compared to the control, respectively. As a result of the reduced water potential in the root environment, plants under salt stress actually produce more proline (Table 3).

Table 3. Effect of salinity stress on the production of essential oil, chlorophyll, carotenoid, leaf proline and rate of transpiration of peppermint.

Salinity stress	Length of root (cm)	Root diameter (cm)	Root volume (cm^3)	Fresh weight of leaves (gr)	Fresh weight of stem (gr)	Fresh weight of roots (gr)	Relative water content (%)	Stomata conduction (mmol m^{-2} s^{-1})	Photosynthesis (µmol/m2/s)
Control	31.33 a	5.77 a	15 a	5.97 a	4.26 a	7.05 a	81.37 a	245 a	15.40 a
50 mm	25.54 b**	4.06 b**	11.42 b**	5.01 b**	3.78 b**	5.87 b**	70.73 b**	201.08 b**	12.09 b**
100 mm	22.42 c**	3.14 c**	9.49 c**	4.13 c**	3.08 c**	4.33 c**	66.44 c**	162.93 c**	9.83 c**

** And * are significant at the level of 1 and 5% probability.

DISCUSSION

It is evident from table 1 that, the leaf area decreased due to salt stress. Salt stress with concentrations of 50 and 100 mm decreased the leaf area of plant. Salinity raises the amount of energy needed to keep the cell functioning normally, which leaves less energy available for development requirements. Therefore, plants in saline conditions are generally weaker and have smaller leaves than normal plants. The reduction in leaf area as a result of salt; the reduction in leaf number as a consequence of reduced photosynthesis; or the reduction in leaf size as a consequence of decreased turgor pressure (Acosta-Motos *et al*., 2017). The finding of the study indicates that the plant growth was reduced by salt stress. Reduced plant growth under salt stress may result from a reduction in the plant's energy reserves, which are impacted by a decline and disruption in biological and metabolic processes (Crepsi and Ghaliba, 2000). These findings were in line with the findings of this research. Additionally, the findings of previous investigations (Savant and Korndofer, 1999) demonstrated that plants' leaf area decreases under salt stress, which was in line with the findings of this research.

It is revealed from the table 1 that the number of leaves decreased due to salt stresses. According to the study by (Ashrafi and Rezainejad, 2017), salt stress causes to lose both leaf number and leaf area of lisianthus.

The result of the study indicates that the morphological characteristics such as crown diameter, length of internodes, number of internode, dry weight of leaves and dry weight of stem and roots of the plant has decreased due to salt stresses. According to the findings of (Talebi *et al*., 2013), there is a considerable drop in the quantity and area of leaves, root length, fresh and dry weight of the stem and root, and total chlorophyll when salt levels raised.

Salt stress affected different morphological and physiological characteristics of the plant. The length of root, root diameter, root volume, fresh weight of stem, fresh weight of root, relative water content, stomata conduction and photosynthesis are decreased significantly (table. 2). In comparison to the control treatment, the findings of Abdul mohammadi and Omidi, (2016) study on the Lisianthus plant demonstrated that salt stress significantly reduced plant height, fresh and dry weight of aerial organs, and fresh and dry weight of roots. Furthermore, salinity stress reduced the height, stem diameter, relative water content, surface area, and number of leaves in the aromatic geranium plant, according to the findings of Hasanvand and Rezainejad's (2016).

Considering that the increased salinity caused the leaf area to decrease, it is considered that the amount of light received and as a result of net photosynthesis and both the buildup of dry matter and the aerial part's dry weight—which is the total of the dry weights of the stem and leaf—are decreased. Stress affects physiological functions like photosynthesis, which lowers crop growth and output. (Yamagoshi and Blomwald, 2005).

Salt stress in this study resulted in a reduction in root volume. From table 3 it is cleared that by increasing the level of salinity, root volume was decreased significantly. The plant's root is the most susceptible component as it is constantly exposed to salt, which alters the plant's ability to absorb water and how efficiently it uses it, among other factors and processes that are impacted by salt stress (Sanchez-Blanco *et al*., 2014). Root length and salinity have an inverse relationship. The characteristic of selective absorption in the root, similar to a filter, controls the passage of ions and provides the optimal ratio of sodium and potassium elements for optimal activities (Santos, 2004).

The physiological traits of the peppermint plant (wet and dry weight of leaves, stems, and roots) reduced as salinity stress increased from 50 to 100 mM, indicating that salinity stress had a detrimental impact on growth and several physiological traits. Salinity decreases water absorption by raising the concentration of soluble salts in the rhizosphere. More recently, it has been shown to decrease cell division and elongation in the growth region by raising the environment's osmotic potential. The experiments' findings demonstrated that the fresh and dry weight of the peppermint plant's roots and stem reduced when it was subjected to salt stress (table 2).

Relative water content

From Table 3 it is evident that salinity stress caused a decrease in the relative water content of the plant. This could be because the leaves had less access to water when under stress, or because the root systems' capacity to replace the water lost through transpiration was diminished because of the lower absorption level. This support the findings of (Smart and Bengham, 1974), who reported that access to water under stress conditions could affect the water content of the plant.

Chlorophyll

Chlorophyll is one of the most significant biochemical markers of a plant's health, leaf chlorophyll is influenced by nourishment and water availability (Daud *et al*., 2014). One of the key elements influencing a plant's ability to photosynthesize is its number of photosynthetic pigments, as they have a direct impact on the pace and volume of photosynthesis, and eventually the generation of biomass. Depending on the plant's genotype, one indicator of salinity stress in plants is a reduction in photosynthetic pigment size (Jan, 2005). Saline conditions can lead to a decrease in photosynthetic pigments due to various factors such as the breakdown of the chloroplast structure and photosynthetic apparatus (Niukiles and Nsilakaks, 2007).

Salinity also affects leaf anatomy and chloroplast substructures, therefore, photosynthesis is also affected by these factors (Perida and Des, 2005).

Malondialdehyde

Malondialdehyde is considered as a suitable indicator for membrane lipid peroxidation (Sofa *et al*., 2004). By the increase of salt concentration, the quantity of malondialdehyde increased, which can be said that salinity in this plant increased the permeability and peroxidation of lipids, which affected the strength and function of the membrane. Under normal circumstances, lipid peroxidation is a normal metabolic process that also happens, but under stressful circumstances, it intensifies (Jill and Tutija, 2010).

Proline

Proline protects proteins, controls the acidity of the liquid inside the cell, lessens oxidative stress, and slows down degradation in salt-stressed plants (Sadegh *et al*., 2020). It also plays a significant role in osmotic regulation, which lowers the toxicity of sodium element in plants (Wong *et al*., 2016). The plant actually tries to increase the proline in its leaves in order to control osmosis and preserve the water potential of the leaves because they are crucial to the synthesis of photosynthetic products. As a result, photosynthesis occurs even in stressful environments. As a chemical chaperone, proline really preserves the native shape of proteins and keeps enzyme compounds from changing from their normal state (Powell and Hesigawa, 1996). The results of recent studies showed that the increase in proline due to salinity stress has been reported by many researchers (Ashraf and Tufail, 1995; Zidan, 1995; Shitiva, 2007; Singh *et al*., 2000; Khosrovinejad *et al*., 2009).

Essential oil

It is evident from table 3 that the essential oil of the plant has increased by applying salinity. The rise in the percentage of essential oil could be the result of a reduction in leaf surface area. According to the (Charles and

Simon, 1990), modifications to the biosynthesis of essential oil during stress and reduction in leaf surface area could case the rise in the percentage of essential oil. Also, in two basil and mint plants, reports have shown that the high density of essential oil-secreting glands due to the reduction of the leaf surface due to stress conditions causes more accumulation of essential oil (Farzaneh *et al*., 2010). Furthermore Bintarit *et al*. (2011), stated that aromatic species have also shown a rise in essential oil content under mild salinity conditions, which is consistent with the findings of this study.

CONCLUSION

The study's findings indicate that peppermint's morphological, physiological, and biochemical properties were significantly impacted by the salt stress. Its effects were caused by the reduction of morphological traits like (plant height, number and surface of leaves, crown diameter, length of root, root diameter and root volume, number and length of internode), and physiological traits such as (wet and dry weight of leaf, stem, root; relative leaf water content, chlorophyll a, b and total; stomatal conductance, carotenoid, transpiration, carbon dioxide and photosynthesis). The study's findings indicate that as salinity stress increases, the amount of electrolyte leakage, percentage of essential oil, malondialdehyde and proline increased significantly. With the increase of salinity, the physiological and biochemical activities of the plant are transformed and it causes the growth and performance of the plant to stop and ultimately leads to the death of the plant.

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