



Mitigation of Flood Stress in Mazamort Pepper Variety through Manganese Application

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ARTICLE INFO

Research Article

Received : 04.10.2024
Accepted : 26.10.2024

Keywords:

Flooding
Pepper
Abiotic Stress
Manganese
Physiology

ABSTRACT

This study was conducted to investigate the effect of Mn application on the resistance of pepper plants exposed to flood stress. The study was conducted in a climate-controlled room at Siirt University, utilizing the Mazamort three-lobed pepper variety as plant material. In the climate chamber (19 m²), conditions were established at 24±1°C during the day and 18±1°C at night, with a light/dark photoperiod of 16/8 hours. The growing medium consisted of a 2:1 (v) mixture of peat and perlite. Four treatment groups were established: control, flood stress, 2.5 mg/L manganese (Mn), and flood stress combined with 2.5 mg/L Mn. Sixty-day-old Mazamort pepper plants were subjected to continuous flooding and manganese application at each watering. The duration of flood stress was set at 0 days (control) and 10 days. The experiment was designed using a randomized complete block design with three replications, each containing 10 plants. Parameters evaluated at the end of the study included visual assessment, plant height, stem diameter, leaf number, leaf fresh and dry weight, leaf moisture content, root fresh and dry weight, root moisture content, chlorophyll content (SPAD value), ion leakage, relative water content (RWC), and turgor loss. The highest plant height was observed in the 2.5 mg/L manganese treatment (45.82 cm), while the greatest stem diameter was recorded in the control group. The highest leaf number (30.60) and SPAD value (35.34) were also noted in the control group. RWC was highest at 96.90% in the 2.5 mg/L manganese treatment. The maximum turgor loss was 5.606% in the control group, and the highest ion leakage (17.880%) was observed in the 2.5 mg/L manganese treatment. It was concluded that manganese application mitigated the negative effects of flood stress on various parameters; however, it did not fully restore the values to control group levels.

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Introduction

Seasonal rainfall and flooding are recognized as natural phenomena in many ecosystems; however, they can lead to significant crop losses in agricultural contexts. In arid regions, both water scarcity and excess water act as major stress factors for plants (Nishiuchi et al., 2012; Erol, 2024). Climate change and increasing flood risks necessitate a deeper understanding of marginal crop cultivation and water tolerance in vulnerable areas. During flooding events, changes in soil concentrations of oxygen (O₂), carbon dioxide (CO₂), reactive oxygen species (ROS), and ethylene have been observed (Perata et al., 2011). Specifically, standing water in the soil for extended periods can lead to complete oxygen depletion, resulting in anoxia, while partial oxygen deficiency in roots is referred to as hypoxia (Drew & Lynch, 1980). Such conditions can cause significant damage to plant roots, prompting various morphological and physiological responses to the hypoxia induced by flooding.

Flood-sensitive plant species can develop adaptive structures, such as adventitious roots and aerenchyma, to cope with these conditions (Zhou et al., 2012; Akhtar & Nazir, 2013). However, the capacity for adaptation varies among plant species. For example, significant reductions in photosynthetic capacity have been observed in water-sensitive plants (Liao & Lin, 2001; Yavuz, 2024). During flooding, soil fills with water instead of air, which restricts oxygen uptake and leads to serious respiratory problems. The oxygen deficiency caused by flooding primarily affects the roots, hindering root development and potentially leading to root death. Plant responses can vary based on the timing of flooding, duration of soil saturation, and the plant species involved. Prolonged flooding often results in common adaptations such as the formation of adventitious roots and aerenchyma (Bradford & Hsiao, 1982; Campos et al., 2009; Haupt-Herting & Fock, 2000; Yavuz, 2024).

Oxygen deficiency can lead to nutrient deficiencies in plants, adversely affecting both root and shoot development. Reduced photosynthetic capacity may further increase plants' susceptibility to flooding. Decreased stomatal opening and the inhibition of specific plant hormones can negatively impact plant and leaf expansion. To survive in low-oxygen conditions, plants increase the production of ethylene in the root zone (Yavaş & Ünay, 2016). Consequently, the lack of oxygen in the root zone results in adverse outcomes that significantly affect plant growth (Dias-Filho & Dos Santos Lopes, 2011).

Plants may attempt to generate energy through anaerobic respiration by rapidly increasing protein synthesis. However, in flooded soils, gas diffusion rates decrease, leading to a reduction in root respiration—one of the earliest responses of plants to oxygen deficiency (Rajhi, 2011; Akhtar and Nazir, 2013). Oxygen deficiency weakens root growth by halting secondary metabolism and root development. Moreover, toxic compounds such as iron (Fe), manganese sulfate ($MnSO_4$), and ammonia may accumulate in the soil (Visser et al., 1996).

Prolonged flooding can induce morphological changes in plants and promote the formation of aerenchyma, facilitating the transfer of oxygen from the shoots to the roots and enabling oxygen respiration in the roots (Yavaş & Ünay, 2016). In flood stress conditions, the formation of intercellular spaces in the root cortex increases, alongside lignification, which can heighten susceptibility to root rot. Plants unable to access sufficient nutrients from the soil may destroy harmful cells in their root tissues, leading to increased node numbers and internode lengths, as well as early senescence and yield losses (Yavaş & Ünay, 2016; Ekanayake, 1998).

Climate change models predict an increase in the frequency of flooding globally, turning flood stress into a significant environmental threat for plants. Annual crop damages resulting from non-seasonal and severe flooding events are estimated to amount to billions of dollars in yield losses (Pucciariello et al., 2014). With a growing global population and intensified agriculture, a reduction in arable land per capita is anticipated (FAO, 2006). Many crops are sensitive to flooding, and even a few days of inundation can cause serious damage. Identifying traits that enhance flood tolerance is therefore critical (Perata et al., 2011).

Manganese (Mn) is an essential micronutrient that serves various physiological functions in plants (Lidon et al., 2004; Ducic & Polle, 2005; Humphrie et al., 2007). While manganese can exhibit both synergistic and antagonistic effects on the uptake of other nutrients, such as iron, zinc, and copper, excessive levels may lead to nutrient imbalances and toxicity (Marschner, 1998; Shenker et al., 2004). In Turkey, peppers represent a significant agricultural product with extensive consumption both fresh and processed (Anonymous, 2018). A substantial portion of pepper production is processed industrially, providing a vital source of employment in the agricultural sector (Güvenç, 2017; Güvenç, 2020).

This study focused on the Mazamort pepper cultivar, which was subjected to flood stress for two different durations: 0 days (control) and 10 days. The experimental treatments included a control group, partial flood stress, 2.5 mg/L manganese (Mn), and a combination of partial flood

stress with 2.5 mg/L Mn. The objective of the present study was to investigate the effects of manganese application on the morphological and physiological resistance of pepper plants exposed to flood stress.

Materials and Methods

The experiment was conducted in the climate chamber of the Plant Protection Department at Siirt University. The plant material used was the Mazamort three-lobed pepper variety, obtained from Sunagri Seed Company. This commercially available variety is suitable for both greenhouse cultivation and open-field production. The fruits are 10-12 cm long, crisp, sweet, uniformly shaped, dark green, and three-lobed, making them ideal for fresh consumption. The plant material was produced from seeds. In the climate chamber (19 m²), conditions were established at 24±1°C during the day and 18±1°C at night, with a light/dark photoperiod of 16/8 hours. A growing medium consisting of a 2:1 (v) ratio of peat to perlite was prepared for seed sowing and seedling cultivation. After transferring the prepared medium into 45-cell trays with an inner depth of 5 cm and outer dimensions of 33 cm x 54 cm, seeds were sown. Once the seeds germinated and the plants reached the 3-4 leaf stage, they were transplanted into larger pots. The transplanted pots had a top diameter of 160 mm, a height of 140 mm, and a volume of 2 liters.

Application of Flood Stress to Plants

After the seedlings adapted to their environment and overcame transplanting stress, flood stress was applied as a partial flooding treatment. The plants subjected to flood stress were placed in deep plastic containers, where they remained until the end of the treatment. Partial flooding stress was implemented by submerging the plants above the root collar. The treatments included control, 2.5 mg/L manganese (Mn), partial flooding, and partial flooding combined with 2.5 mg/L Mn. Manganese was applied as a foliar spray, while control plants received a foliar spray of distilled water. The duration of flood stress was set to 0 days (control) and 10 days. The treatments were determined based on findings from previous studies (Dere et al., 2019; Zhen et al., 2021). The experiment was designed with three replications, each consisting of ten plants. The plants were provided with a nutrient solution (in ppm) containing: N (150), P (50), K (150), Ca (150), Mg (50), Fe (5.0), Mn (0.5), B (0.5), Zn (0.05), Cu (0.03), and Mo (0.02). The pH and electrical conductivity (EC) of the nutrient solution were maintained within the range of 6.0-6.5 and 1.8-2.0 dS/m, respectively. The plants were exposed to flood stress for the designated periods, after which the specified measurements and observations were conducted.

Parameters to be Evaluated in the Experiment

Morphological Parameters

Visual Scale Assessment: A scale assessment was conducted to evaluate the damage resulting from flood stress in the plants. The damage was rated on a scale from 0 to 5 based on the condition of the plants (Kuşvuran, 2010):

- 0: No damage to pepper plants under flood stress
- 1: Yellowing and curling of pepper leaves

2: 25% necrosis and yellowing of pepper leaves
 3: 25-50% necrosis and observed leaf drop in pepper plants
 4: 50-75% necrosis in pepper leaves and onset of plant death

5: 75-100% necrosis in pepper plant leaves occurs rapidly and/or the plant death completely

Plant Height (cm)

The height of the plants was measured in centimeters from the root collar to the apex using a ruler (Dere et al., 2019).

Stem Diameter (mm)

The stem diameters of the plants were measured in millimeters at the midpoint using a caliper (Dere et al., 2019).

Leaf Number (number/plant)

At the end of the treatments, the number of leaves on each plant was counted and recorded (Yılmaz, 2020).

Fresh Plant Weight (g)

The fresh weights of the plant samples collected at the end of the treatment were recorded in grams using a precision scale (Yılmaz, 2020; Altuntaş et al., 2020).

Dry Plant Weight (g)

The fresh weights of the plants were placed in paper bags and dried in an oven at 75 °C until a constant weight was achieved. The dry weight was then recorded in grams (Yılmaz, 2020; Altuntaş et al., 2020).

Moisture Content (%)

Moisture content was determined using the following formula based on the fresh and dry weights of the plants (Köksal et al., 2016):

$$MCwb = (FW - DW / FW) \times 100 \quad (1)$$

Where:

MCwb: Moisture content (%)

FW: Fresh weight of the root (g)

DW: Dry weight of the root (g)

Ion Leakage (%)

To measure ion leakage, 1 cm diameter leaf disks were placed in deionized water for 5 hours, and the electrical conductivity (EC1) was measured. The same disks were then incubated at 75 °C for 24 hours, and the EC (EC2) was measured again. Ion leakage was calculated using the following formula (Arora et al., 1998):

$$Ion\ Leakage = (EC1 / EC2) \times 100 \quad (2)$$

Relative Water Content of Leaves

At the end of the treatments, 1 cm diameter leaf disks were taken as samples of equal age and size. The fresh weights (FW) of these disks were recorded using a precision scale. The disks were then placed in petri dishes containing distilled water and allowed to hydrate for 4 hours. Turgor weight (TW) was then measured, followed by drying the samples in an oven at 70 °C for 24 hours to determine dry weight (DW) (Kılıç, 2023):

$$RWC(\%) = (FW - DW / TW - DW) \times 100 \quad (3)$$

Where:

FW: Fresh weight

TW: Turgor weight

DW: Dry weight

Turgor Loss (%)

Turgor loss was calculated based on the fresh and turgor weights of the leaf disks:

$$Turgor\ Loss(\%) = (TW - FW / TW) \times 100 \quad (4)$$

Where:

FW: Fresh weight

TW: Turgor weight

Chlorophyll Content of Leaves (SPAD)

At the end of the treatments, SPAD readings were taken for samples of the same age and size using a Minolta SPAD 502 meter (Minolta 502, Osaka, Japan), with three replicates (Daşgan et al., 2010; Dere, 2019). These readings were used to assess the green shade of pepper plants, which varies according to the chlorophyll content in the fourth leaves.

Statistical Analysis

The obtained data were subjected to analysis of variance (ANOVA) using the JUMP 7.0 software package. Means that showed significant differences were grouped using the LSD multiple comparison test (Jump, 2007).

Results and Discussion

The effects of the treatments on plant height were statistically significant (Figure 1; $p < 0.05$). This study examined the impact of various treatments on the height of the Mazamort pepper variety. The control group exhibited an average height of 45.08 cm. When a 2.5 mg/L manganese (Mn) treatment was applied, the plant height increased to 45.82 cm, indicating a slight enhancement in growth due to manganese. Conversely, under flooding stress, the plant height significantly decreased to 36.66 cm, demonstrating that flooding stress adversely affects plant growth. When Mn was applied in conjunction with flooding stress, the plant height measured 41.96 cm. These results suggest that Mn application partially alleviated the negative effects of flooding stress; however, it did not fully restore growth to the levels observed in the control group. Therefore, the influence of Mn on plant growth varies in the presence of water stress, highlighting the complex interactions between nutrient availability and abiotic stress conditions. Moderate waterlogging has been shown to significantly impair plant growth, leading primarily to stunting (Samad et al., 2001). For instance, in the case of *Sesamum indicum*, Mensah et al. (2006) demonstrated that plant height diminishes progressively with extended waterlogging duration. Furthermore, Collaku & Harrison (2002) reported that various wheat varieties and lines exhibited a marked reduction in height when subjected to waterlogging for 10, 20, and 30 days in controlled greenhouse conditions, reinforcing the notion that the impact of waterlogging is both duration-dependent and species-specific.

The effects of the treatments on stem diameter were statistically significant (Figure 2; $p < 0.05$). This study evaluated the impact of various treatments on the stem diameters of the Mazamort pepper variety. The control group exhibited an average stem diameter of 7.10 mm.

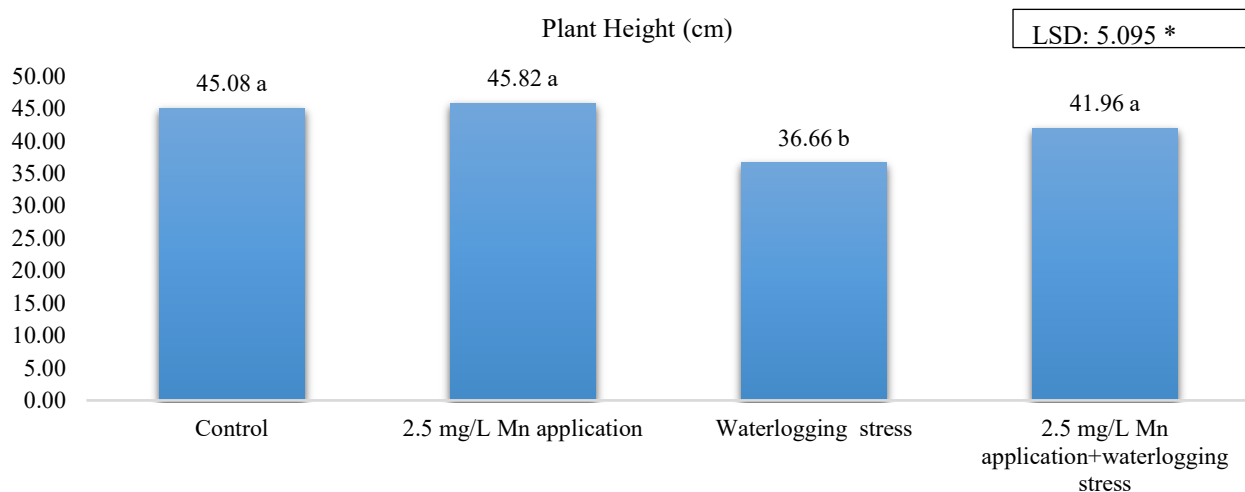


Figure 1. Effect of Manganese Application on Height of Pepper Plants under Waterlogging Stress Conditions

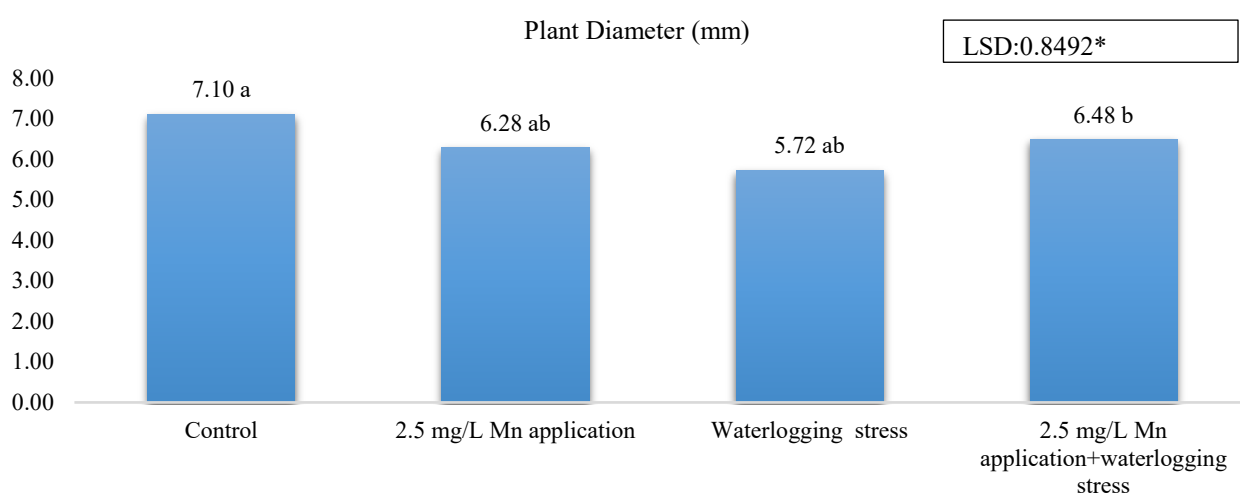


Figure 2. Effect of Manganese Application on Diameter of Pepper Plants under Waterlogging Stress Conditions

Following the application of 2.5 mg/L Mn, the stem diameter was measured at 6.28 mm, indicating that Mn application resulted in a reduction in stem diameter. Under flooding stress, the stem diameter further decreased to 5.72 mm, demonstrating the negative impact of flooding stress on plant growth. When Mn was applied in conjunction with flooding stress, the stem diameter was measured at 6.48 mm. These results suggest that Mn application partially mitigated the negative effects of water stress; however, it did not restore the stem diameter to the levels observed in the control group. Therefore, the effects of Mn on stem diameter vary in the presence of water stress, underscoring the complex interactions between nutrient application and abiotic stress conditions. This aligns with Villarreal-Navarrete et al. (2014), who reported that waterlogging stress negatively affected root growth parameters, including root collar diameter, in *Physalis peruviana*. Although they noted that waterlogging could initially increase root collar diameter in pepper plants, prolonged flooding led to a significant reduction. This highlights a complex relationship between water stress and plant growth, similar to your findings on the Mazamort pepper variety. Both studies underscore the detrimental effects of flooding on plant structural growth. Our results further emphasize how Mn application can influence these

parameters, though not entirely counteracting the adverse effects of water stress, reflecting the intricate dynamics between nutrient management and abiotic stress in agriculture.

The effects of the treatments on leaf number were found to be statistically insignificant (Figure 3; $p < 0.05$). The highest leaf count was observed in the control group, with an average of 30.60 leaves. Following the application of 2.5 mg/L Mn, the leaf count decreased to 28.00, indicating that Mn application led to a reduction in leaf number. Under flooding stress, the leaf count further dropped to 27.00, demonstrating the adverse impact of flooding stress on the plant's leaf structure. When Mn was applied in conjunction with flooding stress, the leaf count measured 25.60. These results suggest that Mn application partially alleviated the negative effects of water stress; however, it did not restore leaf number to the levels observed in the control group. Therefore, the effects of Mn on leaf number vary in the presence of water stress conditions. Understanding these effects of Mn on plant growth and development, particularly under stress conditions, is crucial and highlights factors that should be considered in agricultural practices. This aligns with the findings of Nakayama & Komatsu (2008), Samad et al. (2001), Rao et al. (2002), Else et al. (2009), and Yetisir et al. (2006), all

of whom reported decreased leaf numbers in various crops due to flooding. Similarly, Çelik (2010) and Çelik & Turhan (2011) found that excess water reduced leaf counts in pigeon pea. Our research supports the broader consensus that flooding adversely affects leaf number across multiple species, reinforcing the idea that stress conditions negatively impact plant growth. Additionally, our findings on Mn suggest a potential for mitigation, although not enough to fully restore leaf numbers, highlighting the complexity of plant responses under stress and the importance of considering these factors in agricultural practices.

The effects of the treatments on the SPAD values of the plants were found to be statistically significant (Figure 4; $p < 0.001$). The SPAD value for the control group was determined to be 35.34. Following the application of 2.5 mg/L Mn, the SPAD value decreased slightly to 33.82, indicating that Mn application resulted in a minor reduction in chlorophyll content in the plant leaves. Under flooding stress, the SPAD value significantly declined to 28.74, demonstrating that flooding stress considerably reduces chlorophyll content in the plant leaves. When 2.5 mg/L Mn was applied alongside flooding stress, the SPAD value measured 31.08. These results suggest that Mn application alleviated the negative effects of water stress, although it did not restore the SPAD values to those observed in the

control group. Consequently, the effects of Mn on SPAD values are contingent upon the presence of water stress conditions. These findings provide insights into the potential impacts of Mn use and water stress on plant productivity and chlorophyll content in agricultural practices. Huang et al. (1994) found that Bayles & Savannah wheat varieties subjected to flooding for 17 days experienced reduced photosynthesis and chlorophyll content. Other studies (Olgun et al., 2008; Zheng et al., 2009; Li et al., 2011) reported that chlorophyll content in wheat leaves did not change significantly immediately after flooding, but differences became apparent in the period following stress. These findings highlight that plant responses to flooding may vary based on species, growing conditions, and duration of flooding. This comparison reveals that both studies emphasize the detrimental impact of flooding on chlorophyll content. Additionally, our findings suggest that Mn application can partially mitigate these negative effects. However, the immediate decline in chlorophyll content observed in our study contrasts with literature indicating that significant changes may occur later. Overall, your research contributes to the understanding of how Mn interacts with flooding stress, pointing to the need for further investigation into its potential benefits in various species under different stress conditions.

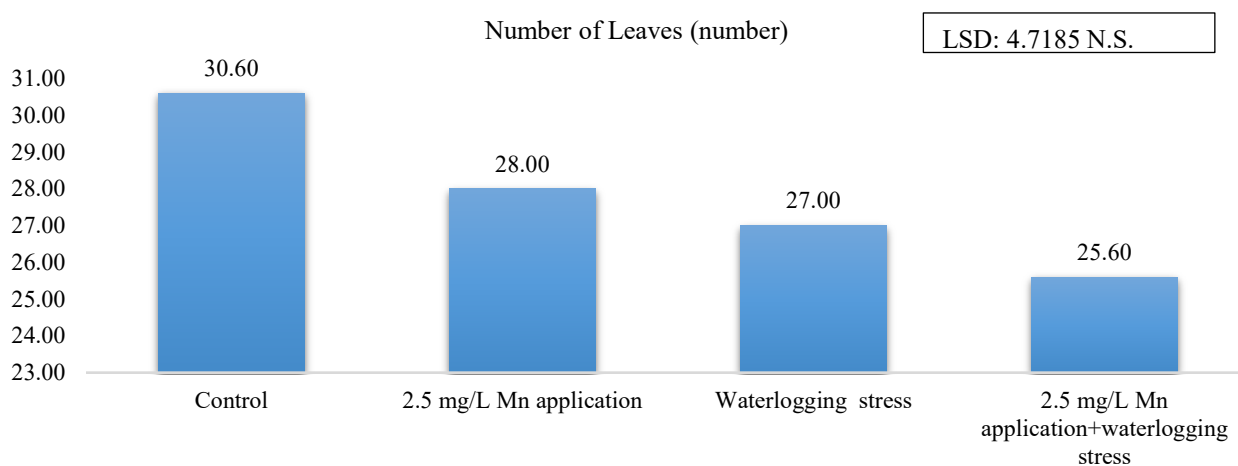


Figure 3. Effect of Manganese Application on Leaf Number of Pepper Plants under Waterlogging Stress Conditions

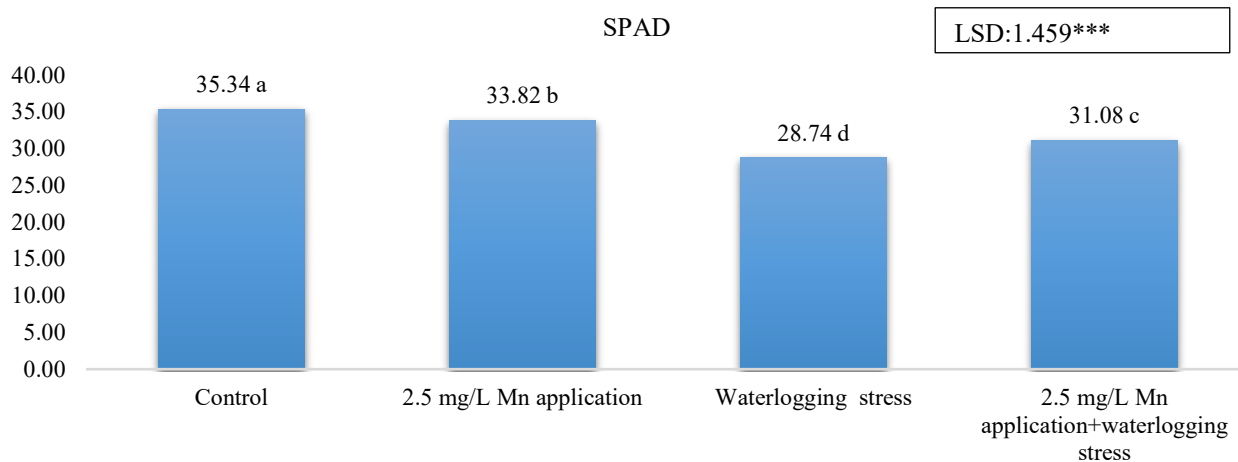


Figure 4. Effect of Manganese Application on SPAD Value of Pepper Plants under Waterlogging Stress Conditions

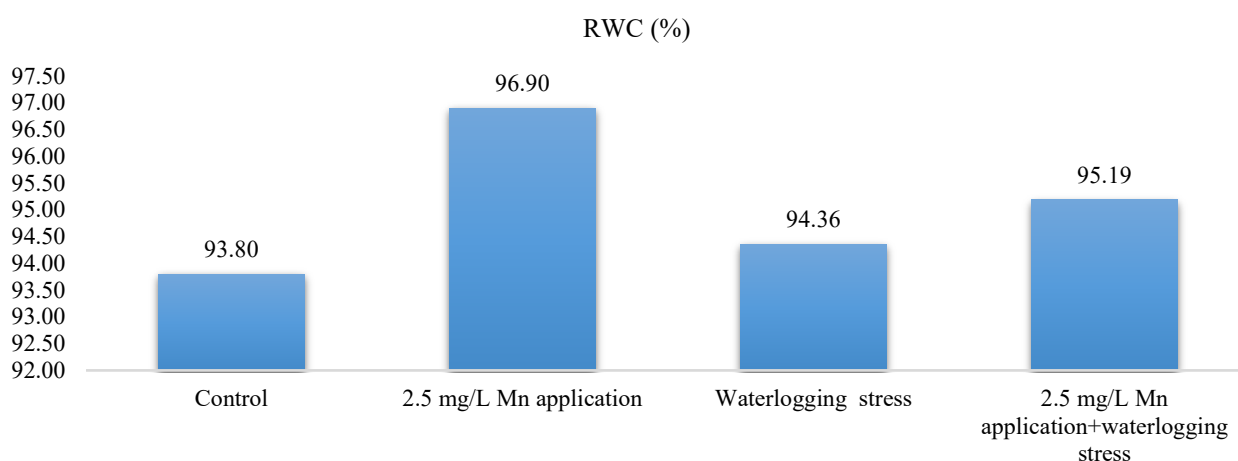


Figure 5. Effect of Manganese Application on RWC value of Pepper Plants under Waterlogging Stress Conditions

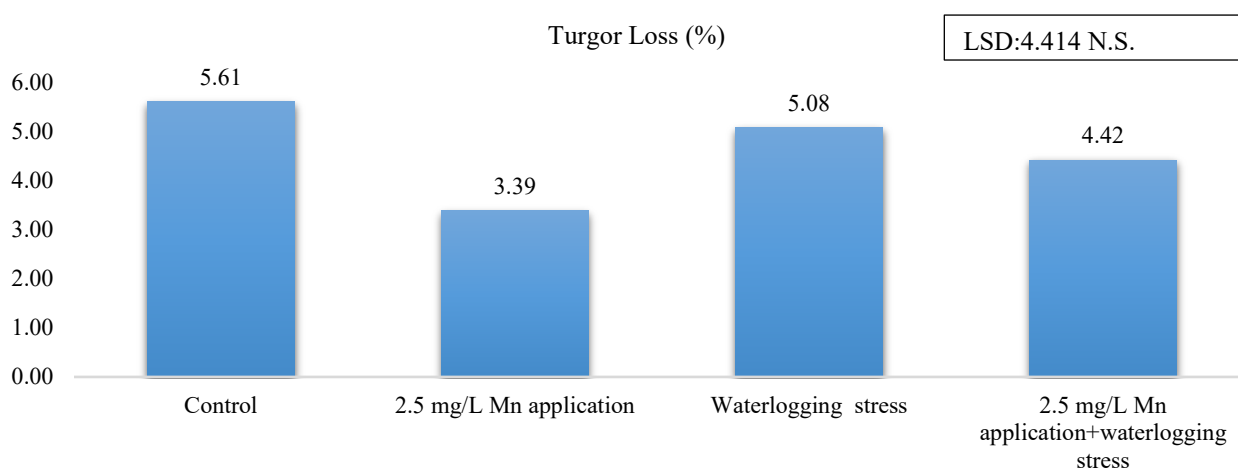


Figure 6. Effect of Manganese Application on Turgor Loss of Pepper Plants under Waterlogging Stress Conditions

The effects of the treatments on the relative water content (RWC) of the plants were not statistically significant (Figure 5; $p < 0.05$). The RWC value for the control group was determined to be 93.80%. Following the application of 2.5 mg/L Mn, the RWC value increased to 96.90%, indicating that Mn application enhances water content in the plant leaves. Under flooding stress, the RWC value decreased to 94.36%, suggesting that flooding stress reduces water content in the leaves. When 2.5 mg/L Mn was applied in conjunction with flooding stress, the RWC value was measured at 95.19%. These results indicate that Mn application mitigated some of the negative effects of flooding stress, although it did not fully restore RWC values to those of the control group. Thus, the impact of Mn on RWC values is influenced by the presence of water stress conditions. These findings contribute to our understanding of the potential effects of Mn use on plant water status and resilience in agricultural practices. These findings align with the results reported by Kozłowski (1984), which indicated that waterlogging caused stomatal closure and reduced leaf water potential and turgor ratio in various tree seedlings. Similarly, Ashraf & Rehman (1999) noted that waterlogging reduced leaf turgor potential, reinforcing the idea that water stress adversely affects plant water status. In contrast, Ahmed et al. (2002) found that, in mung beans, the leaf water potential of control plants did

not differ significantly after 8 days of waterlogging, suggesting variability in responses among different species. Overall, our research contributes to understanding how Mn can influence plant water status under stress conditions, paralleling the findings in the literature regarding the negative effects of waterlogging on water potential and turgor. These comparisons emphasize the complexity of plant responses to water stress and highlight the potential for Mn to enhance resilience in agricultural practices, albeit not to the extent of fully restoring optimal water content.

The effects of the treatments on plant turgor loss were not statistically significant (Figure 6; $p < 0.05$). In the control group of the rocket plant, a turgor loss of 5.606% was observed. Following the application of 2.5 mg/L Mn, the turgor loss decreased to 3.386%, indicating that Mn application reduces turgor loss in the plants. Under flooding stress, turgor loss increased to 5.075%, suggesting that flooding stress exacerbates turgor loss. When 2.5 mg/L Mn was applied in conjunction with flooding stress, the turgor loss was measured at 4.418%. These results indicate that Mn application mitigated some of the negative effects of water stress, although it did not fully restore turgor loss levels to those of the control group. Therefore, the effects of Mn on turgor loss vary depending on the presence of water stress conditions.

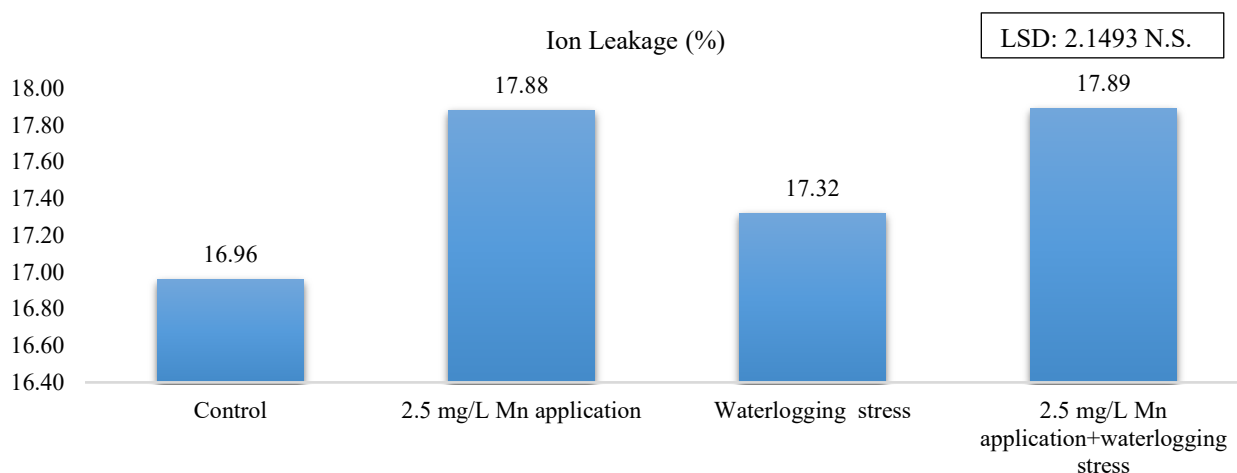


Figure 7. Effect of Manganese Application on Ion Leakage of Pepper Plants under Waterlogging Stress Conditions

These findings provide insights into the potential impacts of Mn use on plant water status and resilience in agricultural practices. This aligns with the findings of Ashraf & Rehman (1999), who reported that waterlogging reduced leaf turgor potential, reinforcing the idea that water stress negatively impacts plant turgor. The detrimental effects of waterlogging on plant water status and turgor, emphasizing how external stressors can significantly affect plant resilience. Our research adds a nuanced understanding by demonstrating that Mn can reduce turgor loss even under stress, though it does not fully counteract the effects of flooding. This interplay suggests that while Mn may enhance resilience in stressful conditions, further investigation is necessary to explore its full potential in agricultural practices.

In the control group, ion leakage was measured at 16.961%. Following the application of 2.5 mg/L Mn, the ion leakage value increased to 17.88%, indicating that Mn application raises ion leakage in the plants. Under flooding stress, ion leakage was recorded at 17.32%, suggesting that flooding stress also elevates ion leakage in the plants. When 2.5 mg/L Mn was applied alongside flooding stress, ion leakage reached 17.89%. These results demonstrate that while Mn application alleviates some of the negative effects of water stress, it does not fully restore ion leakage levels to those of the control group. Thus, the impact of Mn on plant ion leakage varies with the presence of water stress conditions. These findings enhance our understanding of the potential effects of Mn use on plant health and ion balance in agricultural practices. This is consistent with Aydoğan & Turhan (2012), who reported increased ion leakage rates in all green bean genotypes after waterlogging, noting that these rates were higher than those observed during recovery applications. Both studies highlight the adverse effects of waterlogging on ion leakage, demonstrating a common response across different plant species. Our findings expand on this by showing that while Mn can help mitigate some impacts of water stress, it still results in elevated ion leakage compared to control conditions. This suggests that the effects of Mn on ion balance are complex and influenced by stress conditions, underscoring the need for further research to optimize Mn use in agricultural practices for improved plant health and resilience.

Conclusion

This study evaluated the effects of manganese application and flooding stress on the growth and physiology of pepper plants. The impacts of manganese on plant height, diameter, leaf number, SPAD value, relative water content (RWC), turgor loss, and ion leakage were assessed in comparison to the control group. The effects of manganese on plant height varied depending on the presence of flooding stress. While the average height of plants in the control group was 45.08 cm, manganese application increased this value to 45.82 cm. However, under flooding stress, plant height significantly decreased to 36.66 cm. With the combined application of manganese and flooding stress, plant height was measured at 41.96 cm. These results indicate that manganese has a growth-promoting effect, although water stress partially mitigates this impact. Similarly, the assessment of plant diameter revealed that manganese application had a reducing effect compared to the control group. The average diameter in the control group was 7.10 mm, while this value dropped to 6.28 mm following manganese application. Under flooding stress, the diameter further decreased to 5.72 mm. However, after applying manganese alongside flooding stress, the diameter was recorded at 6.48 mm. These findings demonstrate that manganese reduces plant diameter, with flooding stress exacerbating this effect. Analysis of leaf number, SPAD value, RWC, turgor loss, and ion leakage also indicated that manganese generally exhibited reducing effects compared to the control group, while flooding stress intensified these effects. In conclusion, the influence of manganese application on growth and physiological parameters in pepper plants varies significantly under stress conditions such as flooding. These findings underscore the importance of carefully evaluating manganese use in agricultural practices and understanding its potential effects on plant health and productivity.

Declarations

Ethical Approval Certificate

There is no need for an ethics committee report for the study.

Author Contribution Statement

M.E.D.: Data collection, investigation, formal analysis, and writing the original draft.

S.D.: Project administration, supervision, conceptualization, methodology, review and editing.

Fund Statement

This study was supported by the TÜBİTAK 2209 University Students Research Projects Support Program.

Conflict of Interest

The authors have no conflicts of interest.

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