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Effect of Waterlogging at Different Growth Stages on Growth, Yield and Biochemical Characteristics of Brinjal (Solanum melongena L.)

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ABSTRACT

Waterlogging affects a variety of plants, including brinjal; however, little is known about the consequences of waterlogging on brinjal at various growth stages. A pot experiment was carried out on two brinjal cultivars, BARI brinjal 8 and BARI brinjal 11, to study the effects of waterlogging at various growth stages on plant growth, chlorophyll content, malondialdehyde (MDA) content, reducing sugar, proline, phenol, and fruit yield. The experiment was carried out using waterlogging treatments applied at the four-five-leaf and flowering stages, with standard management (no waterlogging) as a control. The negative effects of waterlogging on brinjal growth varied with waterlogging timing, with the greatest influence occurring during the flowering stage, followed by the seedling stage. BARI brinjal 8 was more susceptible to waterlogging than BARI brinjal 11. Waterlogged conditions reduced the chlorophyll content, ultimately lowering grain yield. Biochemical parameters such as proline, reducing sugar, phenol, and MDA concentration,

changed under waterlogging stress, with the change being more pronounced during the flowering stage. It was observed that, plants that received watering at the seedling stage recovered. However, during the flowering stage, waterlogging may cause morphological development to stall and hinder brinjal production from recovering.

Keywords: Brinjal, waterlogging, chlorophyll content, yield, reducing sugar.

INTRODUCTION

The number of waterlogging occurrences on croplands has grown globally in recent decades, owing primarily to more intense and unpredictable rainfalls caused by climate change (Hirabayashi *et al.*, 2013). Brinjal (*Solanum melongena* L.) is a hot-weather vegetable widely grown in tropical and subtropical climates around the world. It is a popular vegetable that is widely grown and consumed in Asian countries, particularly Bangladesh. Farmers typically cultivate their upland crops using traditional flooding irrigation methods that are widely used, resulting in excessive irrigation water use, increased surface runoff, deep percolation, water stagnation, and decreased aeration (Sarker *et al.*, 2019). Excess irrigation, rainfall, and inadequate water management can all contribute to waterlogging.

Waterlogging causes significant abiotic stress to plants. Globally, it is believed that waterlogging affects 10% of all irrigated land, potentially reducing crop productivity by up to 20%. Waterlogging disrupts plant growth and development, slows the growth process, and causes a major morphological response to stress (Ghobadi et al., 2017). The anaerobic environment created by waterlogging prevents aerobic respiration in the mitochondria and causes anaerobic respiration in the root system. Reactive oxygen species (ROS) build up as a result of the blockage of electron transport, the inability to make ATP through the aerobic pathway, and the quick energy crisis that can cause cell death (Le et al., 2016). According to Petrov et al. (2015), oxidative stress and ROS overproduction may be the common mechanism of phytotoxicity and the cause of damage to significant organic constituents of plant cells. Different enzymatic or non-enzymatic antioxidants, signaling mechanisms, and metabolites are present in plants to counteract the harmful effects of ROS (Ahammed et al., 2013). Short-term soil waterlogging can easily cause rapid biochemical changes, while long-term acclimation is more likely to include structural and morphological alterations such as the production of adventitious roots, hypertrophied lenticels, and aerenchyma (Yamauchi et al., 2018). Waterlogging has the greatest impact on several growth phases, including seedling, blooming, and fruiting. Crop damage from waterlogging at various stages affects production. Flowering and fruiting are key growth times for the crop, both in terms of soil moisture scarcity and excess (Reddi and Reddy, 2009).

However, crop tolerance to waterlogging varies from crop to crop, as does the duration of the waterlogged circumstances. Waterlogging caused by excessive rainfall during the rainy season is the most significant hindrance to brinjal cultivation in Bangladesh. The goal of this study was to assess the influence of waterlogging circumstances on brinjal development, yield, and biochemical properties, as well as to identify the crucial growth stage of brinjal under waterlogging stress.

METHODOLOGY

Plant Materials and Cultivation

Two brinjal cultivars, BARI brinjal 8 and BARI brinjal 11 were used in this experiment. A pot culture experiment was conducted at the Horticulture farm of Sher-e-Bangla Agricultural University, Dhaka (41°49′ N and 123°33′ E) in February-June 2024. The Brinjal seeds were obtained from the Bangladesh Agricultural Research Institute (BARI) in Gazipur, Bangladesh. Seeds were sown in PVC tanks (1.2×0.6×0.6 m) using a soil combination and slow-release fertilizers. At 25 days after sowing (DAS), seedlings were transplanted to the maintained pot with recommended doses of fertilizer.

Experimental Design and Treatment

The experiment was set up using five replications and a completely random design. Three treatments were given to plants: (i) well-drained controls, which were watered daily and allowed to drain freely, (ii) waterlogged at an 'early' stage (4-5 leaf stage), and (iii) waterlogged at a 'late' stage (flowering stage). Early waterlogging, which coincided with the seedling stage, was applied to 35-day-old seedlings. Pots with holes were closed by filling them with tap water for 10 days, leaving 1-2 cm of water above the soil surface of the pots. After the waterlogging period finished, holes were opened to allow the water to drain, and plants were watered daily to field capacity until the experiment concluded, to assess their recovery. Late-waterlogging occurred during plant reproductive phases (flowering stage) and lasted 10 days, with recovery post-waterlogging also being tracked. The morphological, physiological, and biochemical characteristics were evaluated 10 days after the water logging stress and at the end of recovery.

Plant Height, Number of leaves and Leaf Area

From the base of the plant to the tip of the main stem, the height of each plant in each treatment was measured in centimeters, and a mean value was computed. Every plant in the treatment had its total number of leaves counted 10 days following water logging stress and at the end of recovery stage. Every leaf sample was measured for its greatest width (W) and length (L) using a ruler. The breadth was measured on the widest leaflet, and the length was calculated as the distance from the rachis's distal end to the first leaflet's insertion.

SPAD Value

Using a SPAD-502 chlorophyll meter (Minolta, Tokyo, Japan), the chlorophyll content of the first completely developed leaves was determined. The midpoint of the leaf lamina on both the treated and control plants was measured.

Measurements of Yield and Yield Traits

Yields per plant (g) were computed by averaging the harvests of all five plants in each treatment and replication to get the total. On each harvest day, the weight of the fruits (g) from each selected plant was recorded using an electronic top pan balance.

Reducing Sugar Content

The phenol-sulphuric acid method (DuBois *et al.*, 1956) was used to compute reducing sugars with minor adjustments to the test volume and wave length. After homogenizing 0.2 g of fresh leaf with deionized water, the extract was filtered. 0.4 milliliters of 5% phenol was mixed with 2 milliliters of the solution. The liquid was swiftly combined with 2 cc of 98% sulfuric acid. The

test tubes were left at room temperature for ten minutes before being immersed in a water bath heated to thirty degrees Celsius for twenty minutes to allow the color to develop. Next, the spectrophotometer was used to detect light absorption at 540 nm. The same procedure was used to prepare the blank solution, which is distilled water. The reducing sugar content was expressed as mg/g FW.

Determination of Proline Content

The leaf tissue's proline content was extracted and assessed using the Bates *et al.* (1973) method. Liquid nitrogen was employed in a mortar to ground fifty milligrams of fresh leaf material. After mixing the homogenate powder with 1 milliliter of aqueous sulfuric acid (3% w/v), it was filtered using Whatman #1 filter paper. The extracted solution was incubated for one hour at 95°C after being treated with an equal volume of glacial acetic acid and ninhydrin reagent (1.25 mg of ninhydrin to 30 mL of glacial acetic acid and 20 mL of 6 M H₃PO₄). Placing the reaction in an ice bath caused it to halt. Two milliliters of toluene were quickly added to the reaction mixture. After warming to 25°C, the chromophore was identified at 520 nm. L-proline was used as the standard.

Phenolic Content Analysis

The phenolic content was determined using the Singleton et al. technique (Singleton et al., 1999). The leaves (250 mg) were homogenized with 85% methanol. The extract was centrifuged at $3000 \times g$ for 15 minutes to separate the supernatant. Folin-Ciocalteu reagent (2 mL) was added to each 2 mL of supernatant. Each test tube was filled with a 7.5% sodium carbonate solution (2 mL), and after 30-45 minutes, the absorbance was measured at 725 nm against a blank sample. Gallic acid was used to create a standard curve for determining total phenolic content.

Estimation of Lipid Peroxidation

The level of lipid peroxidation was assessed using a modified Heath and Packer (1968) approach, which measured MDA content, a result of lipid peroxidation.

Statistical Analysis

The data were analyzed using ANOVA in SPSS (Ver.17.0, SPSS, Chicago, IL, USA). Duncan's multiple range test was used to determine significant differences between treatments at the 0.05 level (P < 0.05).

RESULTS AND DISCUSSION

Response of Morphological Traits Under Waterlogging Stress

Waterlogging stress resulted in reduced growth in height, leaf number/plant, and leaf area compared to the control cultivars. The values of each morphological indicator were also lower in V_1 than V_2 , indicating that V_2 was less affected by waterlogging stress than V_1 . Moreover, the plants water logged at early stage (seedling stage) was less affected compared to the plants water logged at later stage (flowering stage). At 10 days after waterlogging, plant height of brinjal was significantly lower than that of control of early water logging plants in both cultivars i.e. BARI brinjal 8 (14.33 cm) and BARI brinjal 11 (17.6 cm) which had decreased by about 41.10% and 38.67% respectively (Table 1). At 10 days after waterlogging, number of leaves/plant of brinjal was significantly lower than that of control of early water logging plants

in both cultivars i.e. BARI brinjal 8 (2.66) and BARI brinjal 11 (4.33) which had decreased by about 60.06% and 51.98% respectively (Table 1). At 10 days after waterlogging, leaf area of brinjal was significantly lower than that of control of early water logging plants in both cultivars i.e. BARI brinjal 8 (106.83 cm²) and BARI brinjal 11 (136.67 cm²) which had decreased by about 67.23% and 61.70% respectively (Table 1). At the end of recovery, the difference of plant height, leaf number/plant and leaf area of the water logging and the control plants of BARI brinjal 8 were small, and the plant height, leaf number/plant and leaf area of the waterlogging of BARI brinjal 11 were lower than that of control. However, at 10 days after waterlogging, morphological parameters of both cultivars of brinjal were not taken as the plants of both cultivars were not withstand under water logging condition at flowering stage and died.

Table 1: Effect of waterlogging stress on brinjal cultivars of different morphological characteristics at different growth stage

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Tr	eatments	Plant height		Number of leaves		Leaf area		SPAD value	
		10	At	10	At	10	At	10	At
		DAW	recovery	DAW	recovery	DAW	recovery	DAW	recovery
			stage		stage		stage		stage
V_1	Control	24.33b	39.17b	6.66b	16.33b	326.0b	398.00b	52.15	51.70 a
								а	
	Seedling	14.33d	33.76c	2.66d	12.66c	106.83c	270.83d	46.33	49.53 b
	stage							b	
V_2	Control	30.33a	43.84a	8.33a	18.66a	356.33a	414.33a	51.45	52.53 a
								а	
	Seedling	18.6c	38.19b	4.0c	15.33b	136.67c	306.67c	47.13	50.17 b
	stage							b	
	LSD _{0.05}	3.41	3.56	0.57	1.39	29.46	32.19	2.49	2.10
	CV (%)	6.61	5.78	4.44	4.65	5.31	7.34	2.50	4.79

V₁ = BARI brinjal 8; V₂ =BARI brinjal 11; DAW = Days after waterlogging

The statistical analysis is two way ANOVA, and values followed by different letters within the same row are significantly different at P = 0.05 probability level.

Response of Chlorophyll Content (SPAD Value) Under Waterlogging Stress

The value in chlorophyll content under waterlogging stress was lower than the control of two cultivars. Values of each chlorophyll content was also lower in V1 than V2, suggesting that V2 was less impacted by waterlogging stress than V1. Moreover, the plants water logged at early stage (seedling stage) was less affected compared to the plants water logged at later stage (flowering stage). At 10 days after waterlogging, chlorophyll content of brinjal was significantly lower than that of control of early water logging plants in both cultivars i.e. BARI brinjal 8 (46.33) and BARI brinjal 11 (47.13) which had decreased by about 12.56% and 9.17% respectively (Table 1). At the end of recovery, the difference of chlorophyll content of the water logging and the control plants of BARI brinjal 8 were small, and the plant height, chlorophyll content of the waterlogging of BARI brinjal 11 was lower than that of control. However, at 10 days after waterlogging, chlorophyll content of both cultivars of brinjal were not taken as the plants of both cultivars were not withstand under water logging condition at flowering stage and died.

Response of Biochemical Attributes Under Waterlogging Stress

Genetic variations exist in the biochemical factors that plants use to adapt to flooding and waterlogging conditions. Proline content showed variations in different stages on brinjal at water logged condition. After 10 days' water logging stress proline was lower at flowering stage compare to seedling and control stage. Highest proline content found in BARI brinjal 11 compare to BARI brinjal 8 cultivars at water logged condition. Moreover, the plants water logged at early stage (seedling stage) was less affected compared to the plants water logged at later stage (flowering stage). At stressed condition proline content was significantly higher at seedling stage (3.15 mg/g); (3.64 mg/g) respectively in both cultivars compared to flowering stage (2.05 mg/g); (2.25 mg/g) in both cultivars individually (Table 2).

MDA content showed variations in different stages on brinjal at water logged condition. After 10 days' water logging stress MDA was lower at flowering stage compare to seedling and control stage. Highest MDA content found in BARI brinjal 11 compare to BARI brinjal 8 cultivars at water logged condition. Moreover, the plants water logged at early stage (seedling stage) was less affected compared to the plants water logged at later stage (flowering stage). At stressed condition MDA content was significantly lower at seedling stage (4.43 mg/g); (4.11 mg/g) respectively in both cultivars compared to flowering stage (6.03 mg/g); (5.53 mg/g) in both varieties individually (Table 2).

Reducing sugar content showed variations in different stages on brinjal at water logged condition. After 10 days' water logging stress reducing sugar was lower at flowering stage compare to seedling and control stage. Highest reducing sugar content found in BARI brinjal 11 compare to BARI brinjal 8 cultivars at water logged condition. Moreover, the plants water logged at early stage (seedling stage) was less affected compared to the plants water logged at later stage (flowering stage). At stressed condition reducing sugar content was significantly lower at seedling stage (4.43 mg/g); (4.11 mg/g) respectively in both cultivars compared to flowering stage (6.03 mg/g); (5.53 mg/g) in both cultivars individually (Table 2).

Phenolic content showed variations in different stages on brinjal at water logged condition. After 10 days' water logging stress phenolic content was lower at flowering stage compare to seedling stage and control condition. The highest phenolic content found in BARI brinjal 11 compare to BARI brinjal 8 cultivars at water logged condition. Moreover, the plants water logged at early stage (seedling stage) was less affected compared to the plants water logged at later stage (flowering stage). At stressed condition phenolic content was significantly lower at flowering stage (2.32 mg/g); (2.56 mg/g) in BARI brinjal 8 and BARI brinjal 11 respectively, compared to seedling stage (3.44 mg/g); (3.76 mg/g) (Table 2).

Table 2: Effect of waterlogging stress on brinjal cultivars of different biochemical attributes at different growth stage

Treatments		Reducing sugar (mg/g)	Proline (mg/g)	MDA content (mg/g)	Phenol (mg/g)
V	Control	2.29 e	4.24 b	3.04 e	4.01 b
V ₁	Seedling stage	4.13 c	3.15 d	4.43 c	3.44 d
	Flowering stage	5.14 a	2.05 f	6.03 a	2.32 f
	Control	2.23 e	4.61 a	2.57 f	4.69 a

	Seedling stage	3.73 d	3.64 c	4.11 d	3.76 c
V ₂	Flowering stage	4.81 b	2.25 e	5.53 b	2.56 e
	LSD _{0.05}	0.08	0.18	0.19	0.16
CV (%)		1.21	3.11	2.39	2.60

 V_1 = BARI brinjal 8; V_2 =BARI brinjal 11

The statistical analysis is two way ANOVA, and values followed by different letters within the same row are significantly different at P = 0.05 probability level.

Response of Yield and Yield Contributing Traits Under Waterlogging Stress

Compared to the control of two cultivars, the number of fruits, individual fruit weight, and yield/plant under waterlogging stress were all lower. Additionally, V₁ had lower values for each yield and yield contributing indicator than V₂, indicating that V₂ was less affected by waterlogging stress. Moreover, the plants water logged at early stage (seedling stage) was less affected compared to the plants water logged at later stage (flowering stage). After harvesting of all fruits, number of fruits/plant of brinjal was significantly lower than that of control of early water logging plants in both cultivars i.e. BARI brinjal 8 (7.33) and BARI brinjal 11 (10.33) which had decreased by about 21.50% and 18.40% respectively (Table 2). After reaping, individual fruit weight of brinjal was significantly lower than that of control of early water logging plants in both cultivars i.e. BARI brinjal 8 (78.33 g) and BARI brinjal 11 (86.33 g) which had decreased by about 15.47% and 13.67% respectively (Table 3). After harvesting of all fruits, fruit yield/plant of brinjal was significantly lower than that of control of early water logging plants in both cultivars i.e. BARI brinjal 8 (583.33 g) and BARI brinjal 8 (891.78) which had decreased by about 31.18% and 29.55% respectively (Table 3). At the end of recovery, the difference of plant number of fruits, individual fruit weight and yield/plant of the water logging and the control plants of BARI brinjal 8 were small, and the plant height, leaf number/plant and leaf area of the waterlogging of BARI brinjal 11 were lower than that of control.

Table 3: Effect of waterlogging stress on brinjal cultivars of different yield traits at different growth stage

amerent growth stage							
Treatments		Number of fruits	Individual fruit weight (g)	Yield (g/plant)			
\/	Control	9.33 b	92.67 ab	847.67 b			
V_1	Seedling stage	7.33 c	78.33 c	583.33 d			
\/	Control	12.66 a	100.00 a	1266 a			
V ₂	Seedling stage	10.33 b	86.33 bc	891.78 c			
LSD _{0.05}		0.99	9.71	86.22			
CV (%)		5.45	5.44	5.65			

 V_1 = BARI brinjal 8; V_2 =BARI brinjal 11

The statistical analysis is two way ANOVA, and values followed by different letters within the same row are significantly different at P = 0.05 probability level.

DISCUSSION

Brinjal plants are tough to grow in waterlogged soil. In a waterlogged state, the brinjal root becomes easily damaged and is unable to absorb nutrients from the soil. This study looked at how waterlogging during the four to five leaf and blooming phases affected brinjal growth,

yield, and biochemical characteristics. The varied waterlogging treatments lowered growth and yield to varying degrees; the greatest loss was observed when waterlogging occurred during the flowering stage. According to studies, waterlogging at the three-leaf stage in summer maize caused the most grain production loss (Ren *et al.*, 2017). In contrast to these observations in brinjal, the current study found that waterlogging during the flowering stage caused more damage to brinjal development and biochemical parameters than during the seedling stage.

The vegetative growth of brinjal decreased with waterlogging and increased gradually after waterlogging was relieved which was consistent with the findings of (Luo et al., 2007). Waterlogging at the seedling stage caused a considerable decrease in these features in both cultivars, with V₁ seeing a more severe decline than V₂, according to data from the current trial. However, when both cultivars were waterlogged during the flowering period, they were unable to fully recover and did not exhibit any regrowth. These findings are consistent with those of De San Celedonio et al. (2014), who found that waterlogging was most detrimental to wheat and barley during the time between the start of stem elongation and anthesis stage. Our present study of investigation showed an increase in reducing sugar content varying under different water logging stages. In this study, the maximum accumulation of reducing sugar was found at the flowering stage for both cultivars with V₁ showing more increase than V₂. This suggests that the flowering stage was more susceptible than the seedling stage of brinjal to waterlogging. Dalai et al. (2021) reported an increase in reducing sugar level in leaf of sunflower under waterlogging stress. According to Herzog et al. (2016), under such circumstances, the leaves produce more sugar than they consume. Moreover, restricted root systems make it more difficult for the roots to move phloem, which causes photoassimilates to accumulate in the leaves and, eventually, excessive sugar production (Pais et al., 2022).

While drought stress enhanced phenolic content in *D. antarctica* (Zamora *et al.*, 2010), however, phenolic content was decreased under water logging stress in *D. Antarctica* shoot (Park *et al.*, 2019). Inconsistent with these observations in brinjal, the present study demonstrated that phenolic content was decreased under water logging stress (Table 2). Most phenolic substances are extremely potent scavengers of hydroxyl and peroxyl radicals, and they can stabilize lipid peroxidation (Yamasaki *et al.*, 1997). Proline also possesses antioxidant characteristics, which can increase cell stability by preserving the redox balance and decreasing lipid peroxidation (Liu *et al.*, 2021). In our results, phenolic content was decreased in both waterlogging stage compared to control. However, the decreasing trend was more pronounced at flowering stage compare to seedling stage.

Waterlogged circumstances have been shown to drastically reduce the amount of chlorophyll in leaves in earlier research, particularly in sensitive plant species like cotton (Zhang *et al.*, 2021), maize (Ren *et al.*, 2023), and peanut (Sharma *et al.*, 2022). Changes in cell membrane structure under stress cause a decrease in the amount of chlorophyll (Cao *et al.*, 2015). According to Yi *et al.* (2008), stress also raises ROS and MDA, which speeds up the breakdown of chlorophyll and lowers its total concentration. ROS was also linked to a decrease in photosynthetic enzyme activity and chlorophyll content, which led to oxidative membrane damage and the buildup of MDA (Zheng *et al.*, 2017). Our findings showed that water logging increased MDA content in two brinjal cultivars (BARI brinjal 8, BARI brinjal 11), implying that water logging has an impact on membrane integrity and consequently membrane degeneration

(Yu *et al.*, 2015). Changes in chloroplast shape under waterlogging were linked to increased active oxygen content (Luan *et al.*, 2018) and a compromised protective enzyme system (Bin *et al.*, 2010). According to this study, waterlogging destroys cell integrity, reduces the activity of photosynthetic enzymes, lowers chlorophyll content, and eventually leads to a drop-in photosynthesis. Waterlogging during the blossoming stage resulted in serious damage.

CONCLUSION

The study found that waterlogging significantly reduced brinjal growth, yield, and biochemical characteristics. Brinjal was the most sensitive to waterlogging at the flowering stage, followed by four to five-leaf stages. Between the cultivars of the study, BARI brinjal 8 was more sensitive to waterlogging than BARI brinjal 11. Waterlogging stress affected plant growth and development, with a decrease in chlorophyll content. Biochemical analyses revealed changes in various parameters, such as proline, phenol, malondialdehyde (MDA), and reducing sugar. Meanwhile, other characteristics indicate that the flowering stages were the most responsive to water logging stress.

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