

MEYEROZYMA GUILLIERMONDII ALLEVIATES WATERLOGGING STRESS THROUGH THE MODULATION OF PHYTOHORMONE METABOLISM AND ANTIOXIDANT ENZYME ACTIVITIES IN ZEA MAYS L.

FAYAZ ASAD^{1,*}, SHAHIDA BIBI², NAHEED FAZAL², MUAZZAM ALI KHAN³, BEENISH ALI⁴, SABRINA SHAHID², IMTIAZ AHAMD¹ AND AIMA JAVED¹

¹Department of Botany, Bacha Khan University, Charsadda-24420, Pakistan

²Department of Botany, Women Campus Charbagh, University of Swat, Pakistan

³Department of Zoology, Bacha Khan University, Charsadda-24420, Pakistan

⁴Department of Geology, Bacha Khan University, Charsadda-24420, Pakistan

*Corresponding author's email: ayaz.asad79@gmail.com

Abstract

Crop growth and yield are severely hampered by waterlogging, particularly in agricultural regions of Pakistan. Several methods of waterlogging management in agricultural soils exist, however, beneficial microorganisms are one of the sustainable and environmentally friendly methods to reduce the negative effect of waterlogging on crop growth and productivity. In this work, we assessed the ability of the isolated rhizofungus *Meyerozyma guilliermondii* (*M. guilliermondii*) to reduce waterlogging stress in seedlings of *Zea mays* L. Three duplicates of each treatment control, waterlogged, *M. guilliermondii*, and waterlogged + *M. guilliermondii* were used in the fully randomized experiment. The findings demonstrated that under waterlogged stress, *M. guilliermondii* successfully colonized the roots of maize seedlings, increasing the dry biomass (06.7%), fresh weight (43.4%), length of shoot (36.1%), and root (24.1%) comparing to the control. Important biochemical indicators were also higher in inoculated seedlings than in control, including the level of chlorophyll “a” (66.1%), chlorophyll “b” (56.4%), lipid content (23.5%), protein (46.2%), soluble sugar (44.4%), and Indole-3-Acetic Acid (76.5%). Oxidative stress indicators such as proline level (54.3%), salicylic acid (11.5%); malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) were reduced together with a 311.7% increase in antioxidant enzyme activities, such as catalase, in comparison to the control, suggesting increased detoxification of ROS. According to these findings, *M. guilliermondii* improves maize resistance to waterlogging via regulating phytohormone levels and antioxidant defense. This approach provides a solution for sustainable waterlogging management in agriculture.

Key words: Waterlogged stress; *Zea mays* L.; Rhizofungus; Stress mitigation; Bio-stimulation; Phytoremediation; Sustainable agriculture

Introduction

Climate change and heavy precipitation have caused waterlogging condition in the low-lying areas, including Charsadda district, Khyber Pakhtunkhwa (KP), Pakistan (Hameed, 2017). Historically, the area received 555 mm of rain yearly (1991–2020). Recent climate studies show Charsadda has had a 10.5 mm per decade rise in annual rainfall over the last few decades. This matches province-wide rainfall increases and intensified downpour occurrences. Changed precipitation patterns promoted floods and waterlogging in low-lying areas. Waterlogging creating anaerobic conditions that damage plant roots, reduce nutrient uptake and finally impact the growth and yield of key crops such as maize (Wang *et al.*, 2023).

The balance of phytohormones in plants, particularly abscisic acid (ABA), which governs stomatal activities, may be altered in wet circumstances. This may result in diminished gas exchange and obstructed plant development (Muhammad *et al.*, 2022). Addressing these difficulties is essential for improving maize resilience and fulfilling global food demands and security. To provide local food security, maize, a vital

grain crop in KP, is extensively farmed in Charsadda using both irrigated and non-irrigated methods. Early growth and reproduction of maize are extremely vulnerable to damp circumstances. Overwatering may cause oxidative stress, root hypoxia, and nutrient absorption, which can reduce crop yields (Zhang *et al.*, 2026). To improve maize tolerance and production in waterlogged agro-ecosystems like Charsadda, waterlogging stress must be mitigated. Traditional approaches such as management of drainage, raised beds, planting dates, and region-appropriate cultivars have not reduced waterlogging, especially in protracted soil flooding. Therefore, there is an increasing need to investigate innovative, sustainable, and environment friendly biological approaches. Utilizing beneficial microorganisms, including rhizosphere fungi, has shown promise among these because of their ability to reduce abiotic stressors (Deveau *et al.*, 2018; Ali *et al.*, 2025; Shahid *et al.*, 2026). Nevertheless, their roles in alleviating stress induced by waterlogging remain significantly under-researched.

Microbial metabolites produced by fungi such as *M. guilliermondii* have induced nutrient availability and

stimulating plant defense mechanisms under various stress condition (Gomez-Garay *et al.*, 2024). Fungal genera such as, *Penicillium*, *Trichoderma* and *Aspergillus* species have been documented for their various role in degrading agricultural pollutants and increase plant tolerance to abiotic stresses by nutrient solubilization and enzymatic activity (Mohapatra *et al.*, 2022). Recent studies show that *M. guilliermondii* may elicit defense mechanisms under biotic stress, such as *Fusarium* crown rot in durum wheat (Kthiri *et al.*, 2021). As an aerobic yeast, it likely does not thrive in submerged or anaerobic conditions, such as waterlogged soils. The potential role in mitigating abiotic stressors such as waterlogging remains largely unexplored.

Rhizo-fungi, commonly utilize in fermentation and industrial processes, are also beneficial to agriculture because they solubilize phosphates and induced the nutrients uptakes (Ingle & Padole, 2017). This study investigates the ecological niche of waterlogged soils, an area that is underrepresented in microbial research. It also examines the ability of *M. guilliermondii* to enhance stress tolerance through phytohormonal regulation, stimulation of antioxidant enzymes, and improved root attachment. Prior studies have shown that rhizofungus mitigate the detrimental effects of abiotic stressors, improve plant resilience and enhanced nutrients availability in adverse conditions (CY *et al.*, 2018). Nevertheless, a thorough physiological and biochemical analysis to determine their effects in hypoxic stress situations still needs to be carried out.

Therefore, the current investigation introduces a novel perspective by documenting the isolation of *M. guilliermondii* from a naturally wet habitat and assessing its impacts on maize by integrating agronomic, hormonal, and antioxidant parameters. The objective of this research was to examine the tolerance capacity of the rhizofungus *M. guilliermondii* under waterlogging stress and to determine its role in reducing the adverse effects of waterlogging on the growth of maize.

Materials and Methods

Isolation and purification of rhizospheric fungal strains: Plants were excavated from specific sites in the District Charsadda (34.13°N 71.83°E) in KP, Pakistan, using a spade, together with the close soil. Roots were agitated in the air to remove excess soil. Then roots were again agitated to gather the rhizosphere soil in a 1 L beaker containing sterilized distilled water. Fungi of the rhizosphere were then isolated using a serial dilution method (Hussain *et al.*, 2017), with dilutions ranging from 10.0^{-2} to 10.0^{-5} . To inhibit bacterial growth, 20 mL of streptomycin (2.5 mg/mL) enriched potato dextrose agar (PDA) was aseptically introduced into sterile petri plates. A spreader was used to apply an even layer of 100 μ L a portion from each dilution to the agar's surface. Following their Scotch tape sealing, the glass plates were then incubated at 28°C for three to seven days. After meticulous extraction from their petri plates, the fungal colonies were shifted to PDA slants and plates of PDA. Subsequently, they were accurately labeled and maintained in an incubator at $28 \pm 2^\circ\text{C}$ for duration of 7 days. For further utilization, the pure grown PDA slants were stored at 4°C.

Growth of isolated strains in Czapek media: To make Czapek medium, 10 g of glucose, 10 g of peptone, 0.5 g of potassium chloride (KCl), 0.5 g of magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), and 0.01 g of iron sulphate (FeSO_4) were mixed in 1000 mL of distilled water. We transferred 100 mL of this medium into each 500 mL flask and autoclaved it. We transferred a pure fungus culture from PDA medium into each flask under sterile conditions using a sterile inoculation loop. The flasks were then placed in a shaking incubator at 120 rpm for 7 days at 30 °C. The fungal biomass was separated by filtration and stored at 4°C for further analysis (Waqas *et al.*, 2014).

Molecular identification of rhizofungus: DNA was isolated from freezer-dried mycelium for molecular identification purposes (Mohapatra *et al.*, 2022). The DNA was extracted using the phenol/chloroform technique (Chi *et al.*, 2009). The fungus ITS1 and 4 sections of rDNA were amplified and sequenced using a conventional technique. The final consensus sequence was constructed by the alignment of both forward and reverse primers. Using NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>, retrieved on June 17, 2024), the consensus sequence was compared with nearly similar sequences in GenBank. The closely related hits were used to evaluate the phylogenetic relation of the ITS sequences of our isolate by MEGA-11 (Chun *et al.*, 2007; Tamura *et al.*, 2011).

Plant microbe interaction analysis: The research was conducted at Bacha Khan University, Charsadda (BKUC), Pakistan, under a naturally illuminated Greenhouse condition. Soil samples were collected from the University's agricultural field, having a pH of 7.79, an EC of 268 mS m^{-1} , and potassium (K), phosphorus (P), and nitrogen (N) concentrations of 113.41 mg kg^{-1} , 7.91 mg kg^{-1} , and 94.53 mg kg^{-1} , respectively. After surface sterilization with a 0.01% mercuric chloride solution and positioned the seeds of maize in plastic pots at a 3:1 seed-to-pot ratio and autoclaved them. One gram (g) of fungal biomass was thoroughly mixed with 100 g of sterile soil, and then added to 200 g soil was added per pot to provide uniform distribution. Each pot was 12 cm in diameter and contained viable maize seeds (Pak Afgoi Variety). Treatments were arranged in a completely randomized block design with three replications: (i) control, (ii) waterlogging stress for 9 days after the two-leaf stage, (iii) *M. guilliermondii* biomass application at 1 g per 100 g of soil, and (iv) combined waterlogging stress plus *M. guilliermondii* biomass application at 1 g per 100 g of soil.

Water logging stress was imposed by maintaining water surface of pots treated 15 days above soil surface 2-3 cm and this was maintained throughout the experimental period in controlled greenhouse environment (R.H. 60-70%, temperature 25-30°C). Water-table was measured daily during the experiment and watering was performed as required to keep the soil in water-saturated conditions. The inoculum of was grown in suitable growth media and checked for active growth and colony formation prior to use as microbial inoculant. The plants were harvested 60 days after sowing them for further analysis.

Morphological parameters of plants: Ten maize seeds were sown in each pot and after germination seedlings were carefully uprooted from their containers, immersed in a basin of water to eliminate residual soil, and then dried with blotting paper. A scale was used to determine the length of each plant. A digital balance was used to ascertain the fresh weight of each plant separately. The plant samples were oven-dried at $75 \pm 2^\circ\text{C}$ for 72h hours, and the final dry weight was recorded.

Determination of photosynthetic pigments: We used a pestle and mortar to pulverize 0.1g of fresh leaf tissue that was crushed in 2 ml of 80% acetone. The extract was centrifuged for 5 min at 1000 rpm. The final volume of the homogenate was adjusted to 7 mL using acetone. A Shimadzu UV-1800 spectrophotometer was used to measure the absorbance of carotenoids at 480 nm and 510 nm and chlorophyll a and b at 645 nm and 663 nm (Sestak *et al.*, 1971). Arnon (1949) methods were used to compute chlorophyll a and b such as:

$$\text{Chl } a \text{ } (\mu\text{g mL}^{-1}) = (12.25 A_{663} - 02.79 A_{647})$$

$$\text{Chl } b \text{ } (\mu\text{g mL}^{-1}) = (21.50 A_{647} - 05.14 A_{663})$$

$$\text{Total Chl } (\mu\text{g mL}^{-1}) = \text{Chl "a"} + \text{Chl "b"}$$

$$\text{Carotenoid } (\mu\text{g mL}^{-1}) = \frac{(1000 A_{470}) - (01.82 \text{ Chl } a) - (85.02 \text{ Chl } b)}{198}$$

Indole-3acetic acid and salicylic acid determination: The Salkowski reagent was utilized to determine the concentration of indole-3acetic acid (IAA) present in maize plants. The Salkowski reagent was prepared by combining 1 mL of ferric chloride with 50 mL of perchloric acid. We finely ground 0.50 g of fresh maize leaves using a mortar and pestle and subsequently placed them in 3 ml of ethanol for the examination of plant materials. Subsequently, the mixture underwent centrifugation at 10,000 rpm for duration of 15 minutes (min). A test tube containing 2 mL of Salkowski reagent and 1 mL of supernatant was maintained at room temperature for duration of 30 minutes. Optical density was recorded at 540 nm (Hussain *et al.*, 2018). The approach employed by Warriar *et al.* (2013) was utilized to quantify salicylic acid (SA).

Measurement proline and sugar contents: Proline content was determined according to the method described by Bates *et al.* (1973). Freshly crushed 0.2 g of leaves in 5 ml of 3% sulphosalicylic acid solution was incubated for 24 hours at 5°C and centrifuged for 5 min at 4,000 rpm. Each sample included two mL of acid ninhydrin and plant supernatant extract in 10-mL test tubes. The ninhydrin reagent required 1.25g of acid. Ninhydrin was dissolved in 20 ml glacial acetic acid and 20 ml 6M phosphoric acid and heated in a water bath for 60 min. Samples were cooled and 4 mL of toluene was added in each sample. Toluene was isolated and the optical density at 520 nm measured using a dropper. Toluene was used as a blank.

A fresh maize leaf weighing 0.5 g was ground in 80% methanol. A 0.5 ml sample of this extract was centrifuged for 10 min at 3000 rpm with 9.5 ml of clean water. Following collection, 0.50 ml of the extracted leaf

supernatant and 01 mL of 80 percent phenol were combined, and the combination was left to incubate for duration of ten min. 5 mL of diluted H_2SO_4 was then added, and the entire mixture was left to incubate for an additional four hours. The optical density of the sample was measured at 420 nm (Lubna *et al.*, 2018).

Phenolic compounds and flavonoid component quantification: The total phenolic content of the maize samples was determined using a colorimetric method. After grinding 100 mg of fresh leaves in 02 milliliters of 80 percent ethanol, the resulting mixture was centrifuged for 30 min at 10,000 rpm. The supernatant was dissolved in 2 mL of distilled water. The mixture was also treated with 0.50 ml of Folin-Ciocalteu reagents and 2.0 ml of 20% Na_2CO_3 . The mixture was subsequently heated in a water bath at 40.0°C for duration of one to two min. The absorbance was detected at 650 nm after cooling. The flavonoid content of maize plant was quantified following the procedure of Van Handel (1985).

Measurements of lipids and catalase contents: The total lipid content in maize leaves was quantified. In 0.2 mL of methanol 0.2 g of fresh leaves were macerated with a chloroform ratio of 1:2. A 0.2 ml solution of leaf extract (0.73%) was formulated using 0.2 ml of NaCl. Subsequent to the creation of three layers, 100 μL of H_2SO_4 was added, and the lower lipid layer was extracted using a separator funnel. Following the agitation of the mixture, it was heated at 100°C for 10 minutes. 2.4 ml of vanillin reagent was supplied to the sample after it had cooled. An optical density measurement was performed at 490 nm once a pink tint appeared (Van Handel, 1985).

Employing the Luck methodology, the CAT content in maize was identified in the plant's leaves. Ten mL of phosphate buffer was used to triturate 0.2g of fresh maize leaves using a pestle and mortar. The solution was then centrifuged for 5 min at 10,000 revolutions per minute. An H_2O_2 -phosphate buffer of 3ml was mixed with 0.4 ml of leaf extract supernatant. The optical density was measured at 240 nm. The hydrogen peroxide served as the blank (Wheeler *et al.*, 1990).

Determination of ascorbic acid oxidase: The Oberbacher & Vines (1963) methodology was used to measure the concentration of Ascorbic Acid Oxidase (AAO) in maize leaves. Maize fresh leaves weighing 0.1g were crushed in 2ml of phosphate buffer and centrifuged for 5 min at 3000 rpm. A 3 ml solution containing 100 μL of leaf extract and 8.8 mg of ascorbic acid diluted in 300 ml of phosphate buffer was used. At 265 nm, the absorbance value was determined (Oberbacher & Vines, 1963).

Assessment of antioxidant enzyme: Enzyme extraction was performed by collecting 12-week-old *Z. mays* plants and promptly freezing the leaves (1 g) in liquid nitrogen to improve the grinding process efficiency. The frozen powder was homogenized in 2 mL of potassium phosphate buffer (pH 7) and then centrifuged at 12,000 g for 15 min at 4°C to eliminate suspended particles and acquire a clear supernatant. The supernatants were transferred into tubes and then kept at -20°C to create samples for enzymatic activity.

Photosensitivity utilizing riboflavin/nitroblue tetrazolium (NBT) was employed to assess the inhibition of blue diformazan production, facilitating the evaluation of SOD activity. Following three min of exposure to a 75W fluorescent light positioned 20cm from the reaction chamber, the absorption at 560nm was recorded. Fecht-Christoffers *et al.*, (2003) reported that SOD activity was quantified in micrograms of protein per milligram, where one unit corresponds to a 50% decrease in blue di-formazan formation. POD activity was evaluated by utilizing o-dianisidine as the substrate and measuring the absorbance rate at 470.0nm, following the methodology established by Chance & Maehly (1955). A solution was formulated for the catalase (CAT) experiment, incorporating 2.0mL of a 50.0mM potassium phosphate buffer at pH 7.0. The composition included 1 mM EDTA, 2.0% PVP, 0.05% Triton X-100, 1.0 g of oven-dried, finely crushed leaf powder, and 1.0 mM ascorbic acid. The mixture was centrifuged at 1000g for a duration of 20 min at a temperature of 4.0°C, following the methodology established by Aebi (1984). The resulting supernatant was then utilized to assess the enzyme activity of CAT.

Determination of electrolyte leakage: Distilled water (DW) was utilized to clean the leaves of *Z. mays* and after the leaves was divided into five equal circular discs, the discs were shaken once every 5 min and put in test tubes with 10 mL of DW. The first measurement (EC_1) was assessed utilizing an EC meter after the samples were incubated for 24.0 hours. A second measurement (EC_2) was ready after the discs were heated to 120.0°C for 15 min in an autoclave. Following formula was used to calculate the final result as a percentage:

$$EC = \frac{EC_1}{EC_2} \times 100$$

Determination H_2O_2 and malondialdehyde (MDA) content: Maize leaves were used to extract H_2O_2 using the method outlined by Velikova *et al.*, (2000). Five hundred milligrams of fresh leaves were subjected to immersion in liquid nitrogen and then pulverized in a mortar with 0.10 percent TCA. After 15 minutes of centrifugation at 12000.00g, 0.50 mL of the supernatant was extracted. Following the pH adjustment to 7.0, 0.50 mL of a 10.00 mM potassium phosphate (KH_2PO_4) buffer was included. Following the addition of 1.00 mL of 1.00 M potassium iodide, the absorbance at 390.00 nm was used to determine the concentration of H_2O_2 . A KH_2PO_4 buffer and potassium iodide served as controls.

The thiobarbituric acid (TBA) reaction was used to determine the MDA concentration in compliance (Kong *et*

al., 2016). Using a previously cooled mortar and pestle, frozen materials were homogenized with 0.0010 percent (w/v) TCA. Centrifugation was performed at $15,000 \times g$ for 15 minutes. The resulting supernatant (01.00 mL) was heated to 95.0°C for 30 seconds before being mixed with 02.00 mL of 00.50 percent (w/v) TBA in 20.00 percent (w/v) TCA. The resultant solution was quickly chilled in ice bath. The absorbance of the supernatant was measured at 532.00nm after a final centrifugation at 10,000.0g for 10 minutes at 4.0°C. The data were corrected for nonspecific absorption by removing the absorbance at 600 nm.

$$MDA \text{ Conc. } (\mu\text{mol L}^{-1}) = 06.45 \times (A532 - A600) - 0.56 \times A450$$

MDA Conc. refers to the concentration of malondialdehyde, quantified in micromoles per liter.

Statistical analysis

All data obtained from these different parameters showed mean value $\pm$ standard deviation (SD) – three independent replications per treatment. Before statistical analysis data was evaluated for normal distribution using the Shapiro–Wilk test and for homogeneity of variance using Levene's test to assure the normal statistical assumptions for one-way ANOVA were met. The replicated data were processed by one way Analysis of Variance (ANOVA) software (SPSS v25.0). The mean separation was done at the same probability level using Duncan's Multiple Range Test (DMRT) when any significant difference was detected between the treatment means ($p < 0.05$). Mean comparisons were done using the DMRT; different lower case letters signifies statistically significant difference among the treatments; identical lower case letters means that they do not differ significantly. Asterisks (* - three stars) show levels of statistical significance and “ns” refers to a difference that was not significant ($p > 0.05$). Symbols are well explained in the figure legends. Processing of the graphs was done in OriginPro 2025.

Results

Identification and phylogenetic analysis of HBDP: The taxonomic status of the HBDP strain was determined through ITS rDNA sequencing and a phylogenetic examination. The obtained sequence results were compared with data from the Gen-Bank sequence database to determine the genus or species (the Gen-Bank Database Accession No. ON838586.1). Following a search for homology on Gen-Bank, a sequence of the HBDP (here after *M. guilliermondii*) Strain was identified at the species level, demonstrating 100% similarity with *M. guilliermondii* (Fig. 1).

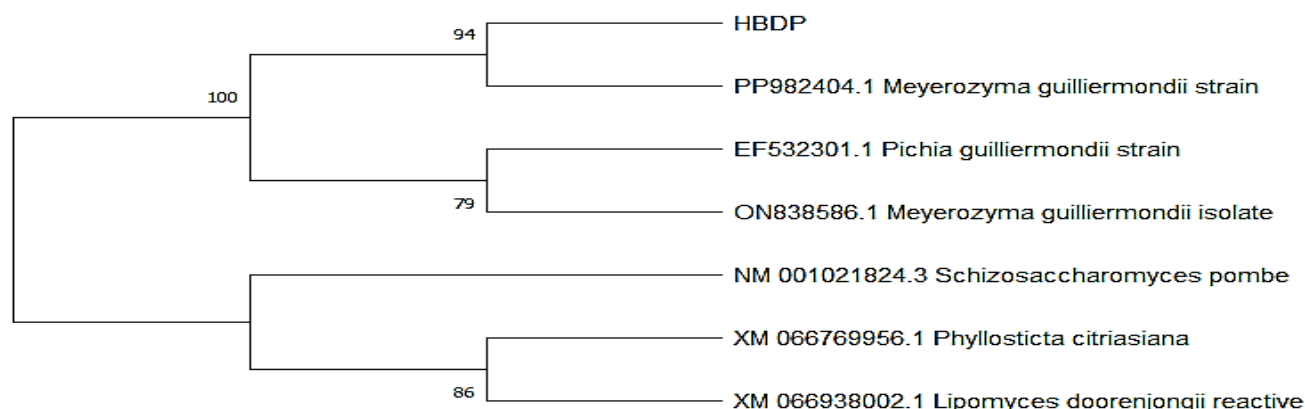


Fig. 1. Phylogenetic tree showing the relationship of the isolated fungal strain (HBDP) with closely related species based on ITS rDNA sequencing.

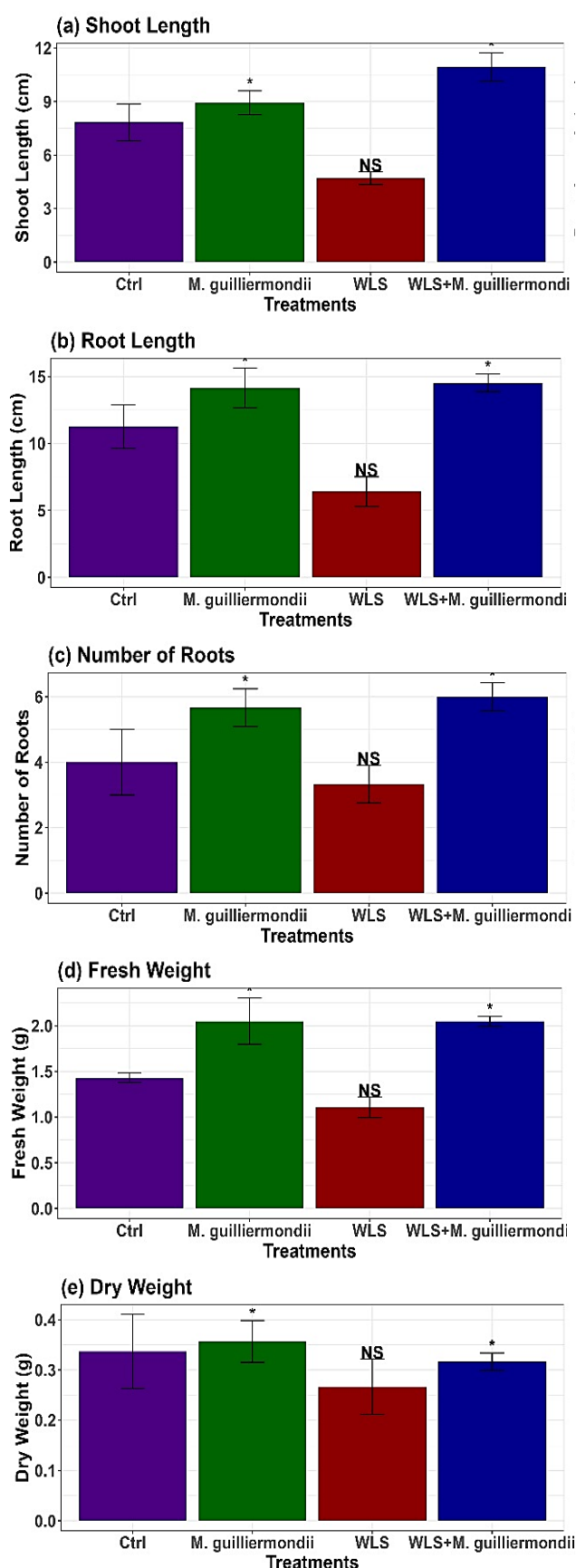


Fig. 2. Effect of *M. guilliermondii* (MG) on the shoot and root growth of *Zea mays* under Waterlogged Stress (WLS). "Ctrl" denotes the control group, while "NS" indicates non-significant results. Data is expressed as the mean $\pm$ standard deviation (SD) from three replicates; with each replicate consisting of 10 plants (this description is applicable to all the other figures).

Plant growth responses: The shoot and root lengths of *Z. mays* were determined throughout waterlogged treatments, in the presence or absence of *M. guilliermondii* (Fig. 2). Waterlogged stress reduces root and shoot length by 40.0%, indicating a negative impact on growth, however, the seedlings inoculated with *M. guilliermondii* strain under waterlogged conditions increases shoot length by 39.6% and root length by 24.1% (Figs. 2a and 2b), indicating a beneficial growth effect relative to the control group. Fresh and dry biomass accumulation was also negatively affected by waterlogging stress, declining by 22.6% and 21.6% respectively compared to the control (Figs. 2d and 2e). However, inoculation with *M. guilliermondii* increased fresh and dry biomass by 43.4% and 6.7% respectively, under waterlogged stress, compared to the control groups (Fig. 2).

Photosynthetic pigments: Under waterlogged conditions, the chlorophyll content of the seedlings decreased, compared to the control (Fig. 3). During the waterlogged condition, the significant decline in chlorophyll content occurred, with chlorophyll a by 84.8% and b by 61.4%, respectively. Conversely, when the seedlings were treated with the rhizofungus (*M. guilliermondii*), the chlorophyll a content inclined by 66.1% and b content by 56.4% in the seedlings, respectively, relative to the control group (Fig. 3; left panel). Waterlogged conditions had a negative impact on the protein and sugar contents in *Z. mays* seedlings (Fig. 3; right panel). Waterlogged stress reduced protein content by 8.6% and sugar by 25.0% respectively, then those in control plants. In contrast, the rhizofungus (*M. guilliermondii*) enhanced protein and sugar content in seedlings by 55.3% and 65.2%, respectively, compared to control plants. Although under waterlogging stress, seedlings linked with *M. guilliermondii* maintained elevated amounts of protein and sugar contents.

Phytohormones and metabolites: In the response of waterlogged stress, rhizofungus showed various defense dynamics including indole-3acetic acid (IAA; Fig. 4b), salicylic acid (SA; Fig. 4c), flavonoids (Fig. 4d) and electrolyte leaks (Fig. 4a). Water related-stress significantly affected the IAA and SA concentrations in *Z. mays* seedlings. Upon assessing the IAA and SA concentrations in each sample, it was found that *Z. mays* grown in a waterlogged medium exhibited a 37.9% reduction in IAA and a 58.8% decrease in SA respectively, compared to the control group. Conversely, relative to control plants, the rhizofungus-*M. guilliermondii* with water logged stress elevated the concentrations of IAA and SA in seedlings by 12.2% and 56.9%, respectively (Fig. 4).

Under waterlogged-stress, the flavonoids contents were declined by 58.7%, whereas seedlings treated with *M. guilliermondii* induced the levels of flavonoids in seedlings were elevated by 49.8%, compared to the control (Fig. 4). Furthermore, the membranes of the seedlings exhibited increased porosity during water stress, resulting in heightened electrolyte leakage relative to the control (Fig. 4). In this case, in comparison to the control, seedlings subjected to waterlogged treatment exhibited induced electrolyte leakage rates of 21.1%. However, fungus-associated seedlings reduced the electrolyte leakage significantly ($p < 0.05$) compared to the control seedlings (Fig. 4).

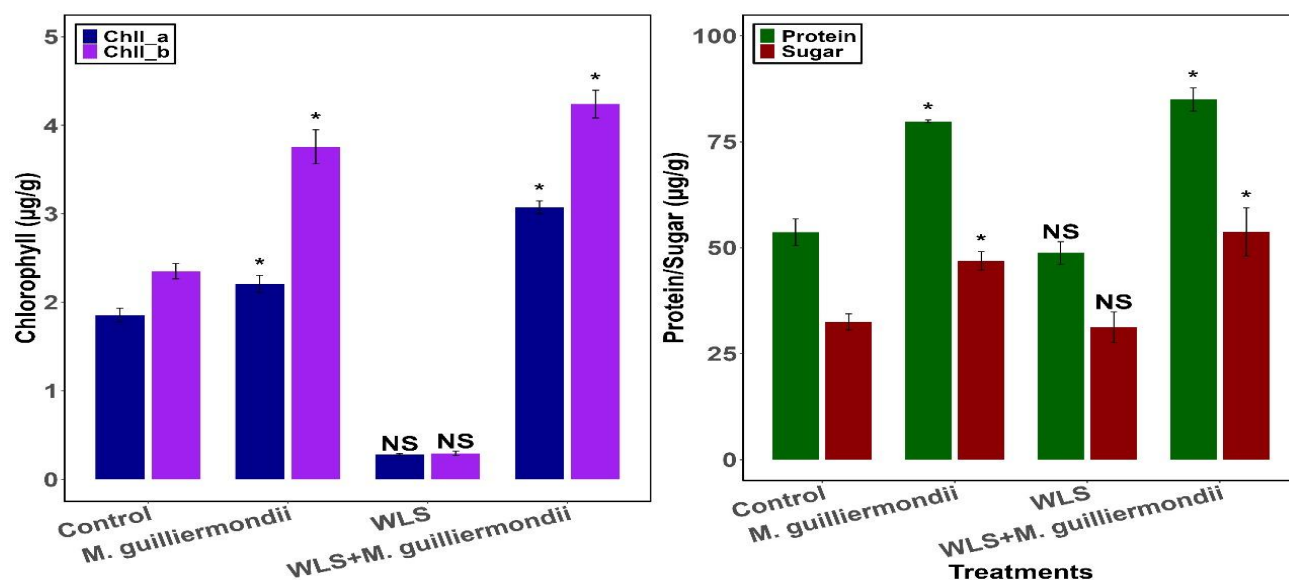


Fig. 3. Effect of *M. guilliermondii* inoculation on Chlorophyll “a” and “b”; protein and sugar contents in *Z. mays* seedlings under waterlogged stress.

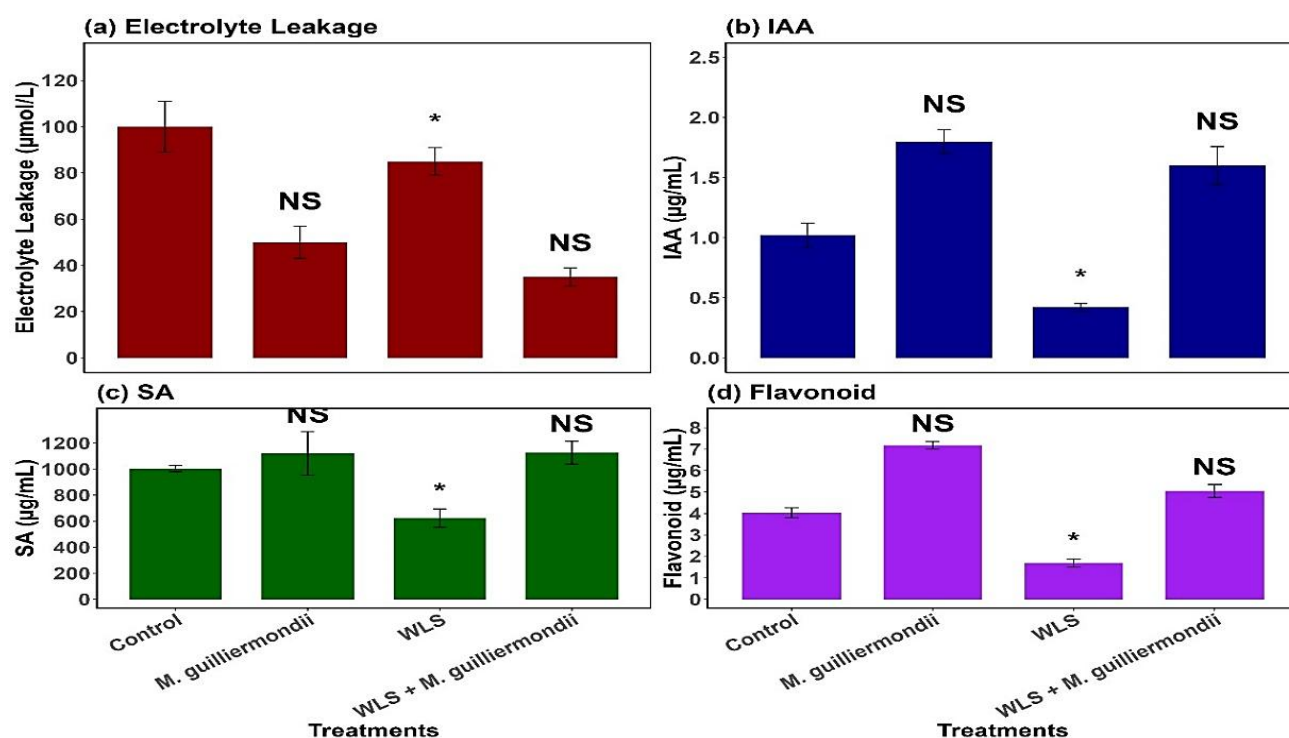


Fig. 4. Effect of waterlogged stress and *M. guilliermondii* inoculation on electrolyte leakage (a), Indole-3-acetic acid (b), salicylic acid (c) and flavonoid levels in *Z. mays* L.

Concentrations proline and lipid: Proline and lipid concentrations in *Z. mays* L. were assessed after exposing to waterlogged stress, both with and without treatment of *M. guilliermondii*. Proline concentration markedly induced whereas the lipid concentrations were reduced during the waterlogged stress by up to 21.70%, compared to control seedlings (Fig. 5). Compared to control plants, the rhizofungus (*M. guilliermondii*) enhanced proline and lipid content in seedlings by 53.33% and 11.17%, respectively.

Antioxidant enzymes and ROS markers: The production and concentration of many enzymes, including superoxide dismutase (SOD) peroxidase (POD) (Fig. 6, upper panel)

and catalase (CAT) (Fig. 6, lower panel) were affected when *Z. mays* was subjected to waterlogged stress. In the waterlogged treatment, the levels of SOD, POD, and CAT were elevated by 56.7%, 61.8%, and 44.0%, respectively, relative to the control group. While seedlings inoculated with *M. guilliermondii* exhibited a significant ($p < 0.05$) reduction in the measured parameters i.e. SOD and POD, in response to the waterlogged stress, decreasing the catalase (CAT) activity by 11.7% (Fig. 6), whereas some treatment combinations were not different from each other (non-significant). The endogenous hydrogen peroxide (H_2O_2) levels were 63.3% higher in waterlogged seedlings than in control groups (Fig. 6, lower panel).

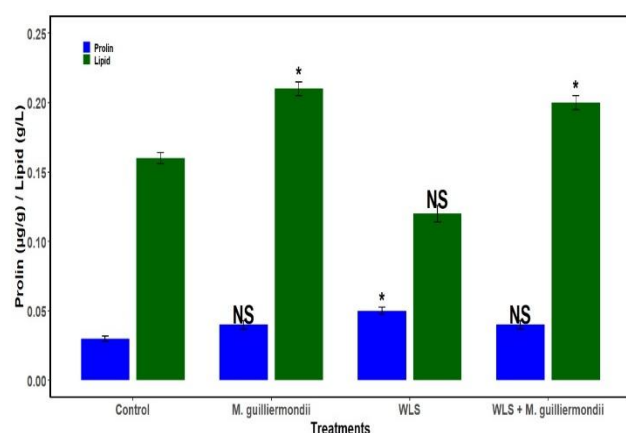


Fig. 5. Proline and lipid concentrations in *Z. mays* seedlings in response to waterlogged stress (WLS) in the with and without of *M. guilliermondii* (MG).

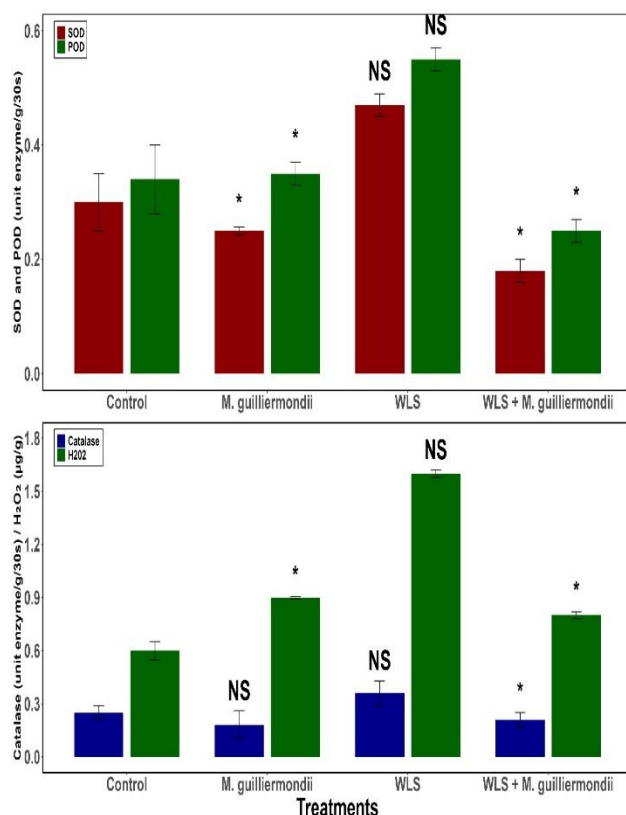


Fig. 6. Effect of *M. guilliermondii* inoculation on SOD, POD (a), and CAT and H₂O₂ (b) levels on *Z. mays* seedlings under waterlogged stress.

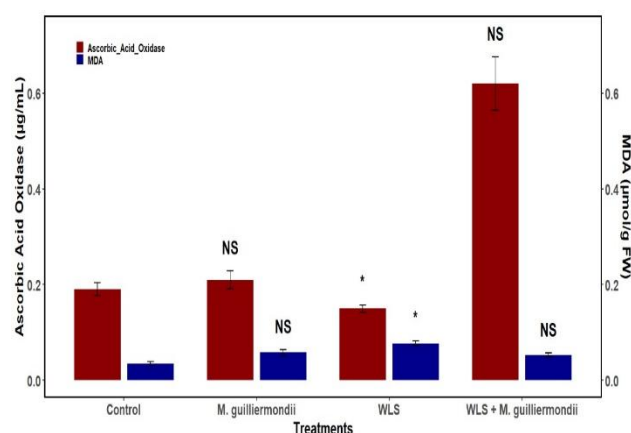


Fig. 7. Ascorbic acid oxidase (AAO) activity and malondialdehyde (MDA) contents in *Zea mays* seedlings subjected to waterlogged stress (WLS) with and without of *M. guilliermondii* (MG) inoculation.

Ascorbic acid oxidase and malondialdehyde contents:

During waterlogged-stress, the levels of ascorbic acid oxidase fell by 21.1%, whereas the inoculation of *M. guilliermondii* induced the ascorbic acid oxidase (AAO) level up to 68.4% relative to control seedlings. This showed that waterlogged stress seedlings associated with *M. guilliermondii* had elevated levels of AAO compared to control (Fig. 7). Similarly, maize seedlings, exposed to waterlogged stress, induced the levels of malondialdehyde (MDA), by 54.0%. In contrast to the control, seedlings inoculated with the rhizofungus *M. guilliermondii* exhibited reduced concentrations of MDA (40.0%). (Fig. 7).

Discussion

M. guilliermondii treatments potentially declined the negative influence of water related-stress (waterlogging) *i.e.*, enhanced the growth of roots and shoots (Figs. 2a,b), suggesting a potential to improve nutrient uptakes and water absorption independent of the availability of water (Guo *et al.*, 2023). This may be due to root colonization, which enhance nutrient intake, and increases the plant tolerance and capacity to thrive under harsh condition. The rhizofungus associated with plant root produce a large hyphal network, which induce the soil nutrients availability to plant roots and improves their growth (Martin and van Der Heijden, 2024). However, insufficient oxygen, as well as inadequate water or air, often results in reduced fungal activity and hyphal development. This may impede nutrient transport and reduce growth-promoting benefits. Therefore, rhizospheric fungus play a vital role in supporting plant growth in stressed environments by enhancing defensive metabolic pathways, and nutrient intake (Sonbol *et al.*, 2025). Similar to *Aspergillus niger* and *Penicillium bilaiae*, which have been shown to improve iron uptake and phosphorus solubilization in anaerobic environments (Odoni *et al.*, 2017; Raymond *et al.*, 2018), our findings suggest that *M. guilliermondii* could give multipurpose benefits via pathways for stress tolerance and nutrition mobilization. Several ecological activities, such as antagonistic enzyme activity, cytotoxic metabolite formation, and increased enzymatic activity to shield the maize plants from stress or unfavorable conditions (Cui *et al.*, 2024; Shahid *et al.*, 2025). *M. guilliermondii* is aerobic yeast and will not actively grow under anaerobic conditions, but may be present in the rhizosphere in micro-niches where oxygen is released by the roots. The positive effects of its activity can also be linked to early root colonization before flooding and/or the activity of bioactive metabolites which can increase the stress tolerance of the plants without the need for continuous fungal development.

Despite *M. guilliermondii* inoculation, waterlogging stress dramatically decreased *Z. mays* leaf levels of chlorophyll (Fig. 3, left panel), but fungal injection restored photosynthetic pigments and plant development. The higher amount of chlorophyll after *M. guilliermondii* treatment indicated improved photosynthetic efficiency and improved stress-reduction strategies. Under waterlogged stress, fungal inoculations are known to increase the amount of chlorophyll contents. This might be because of many factors, including enhanced antioxidant defense and increased absorption of nutrients (Qin *et al.*, 2022). Comparable results were seen in maize treated with *A. terreus*, which preserved chlorophyll a

and b levels by upregulating glutathione and ascorbate peroxidase pathways (Sytykiewicz, 2016), suggesting a shared method among beneficial fungi. Similar increases in chlorophyll due to microbial inoculation under abiotic stress were observed in maize by Singh *et al.*, (2024). Moreover, *M. guilliermondii*-treated maize seedlings demonstrated a substantial induce in the synthesis of crucial secondary metabolites linked to stress, such as sugars, proline, and flavonoids (Seema *et al.*, 2023). It was reported that the rhizofungi such *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium funiculosum* and *Rhizopus stolonifer* potentially released proline, flavonoids, and phenols during the stress condition, which inducing plant defense and increase the plant growth (Ashraf and Foolad, 2007; Ahmad *et al.*, 2024).

An enhanced hormonal route was shown by the notable decrease in IAA (37.9%) and SA (58.8%), respectively (Fig. 4b,c) in maize during waterlogging stress (Fig. 4). While SA is essential for triggering defensive mechanisms, such as systemic acquired resistance (SAR), IAA is involved in the extension of roots and cell division. Increased SA levels cause the stomatal control and SAR signaling mechanisms to activate, improving gas exchange and plant resistance to pathogens (Yang *et al.*, 2024). The obtained data suggests that *M. guilliermondii* triggers signaling pathways which integrate both stress tolerance responses from biotic and abiotic sources. The suppression of these hormones under waterlogged conditions may compromise the plant's ability to mount effective defense responses and inhibit growth and development. The presence of soluble sugar molecules protects the cells yet directs antioxidant genetic responses along with ATP production during anaerobic energy production. Prior study confirmed that like *Trichoderma*-treated rice and *Bacillus subtilis*-treated maize with flood stress (Ruff *et al.*, 2024) the sugar content (65%) increased in maize seedlings. The carbohydrates produce energy and have osmoprotective properties, whereas flavonoids scavenge reactive oxygen species (ROS) produced in stressful conditions to provide an antioxidant effect. This demonstrated that *M. guilliermondii* acts to enhance plant defenses against osmotic and oxidative stress, hence raising the plant's total stress tolerance. Plant growth depend on the low levels of ROS in cells since these molecules help with cell division, differentiation, progression, intracellular signaling, and defense against pathogens. Higher levels of ROS have the potential to inhibit with vital physiological functions, leading to early aging and increased apoptosis. It also serves as a potential source of the enzymatic antioxidant defense system like CAT and ascorbic acid oxidase (Alici and Arabaci, 2016). This is consistent with earlier findings that *M. guilliermondii* treatment may lessen the consequences of waterlogging by preventing the production of ROS and strengthening the antioxidant system, which keeps ROS levels within reasonable bounds (Singh *et al.*, 2025).

Additionally, *M. guilliermondii* inoculation enhanced the activity of critical antioxidant enzymes that neutralized ROS and shield plant cells against oxidative stress, such as SOD, POD (Fig. 6; top panel), and CAT (Fig. 6; bottom panel) (Mittler, 2002). In maize plants, *T. harzianum* and *A. terreus* cause similar increases in antioxidant enzyme activity against hypoxic and wet conditions (Sutkoway *et al.*, 2024) proving a recognized microbial method for removing ROS. The analysis of the study indicated that the

activity of CAT and SOD enzymes were increased by approximately 44–61%, aligning with prior research on the scavenging mechanisms of fungal reactive oxygen species (ROS). Elevated MDA levels indicate that excessive ROS induces lipid peroxidation, resulting in membrane damage, electrolyte leakage, and impaired plant growth. Our findings demonstrate that *M. guilliermondii* significantly enhanced the activity level of antioxidant enzymes such as SOD, POD, and CAT, thereby mitigating ROS and protecting plant cells. Similarly, there is a correlation between increased ROS and elevated MDA and electrolyte leakage in maize seedlings subjected to waterlogging stress. ROS induce potassium leakage from ion channels, which may either lead to cell death or enhance the organism's stress response. The capacity of *M. guilliermondii* to regulate ROS and maintain MDA and electrolyte levels underscores its vital role in safeguarding maize during waterlogging conditions (Rani *et al.*, 2018).

Conclusion

This research demonstrates that *M. guilliermondii* increases maize's ability to endure waterlogged environments by boosting its biological and biochemical defenses. Both inoculated plants and waterlogged plants underwent growth parameter assessments, revealing that inoculated plants exhibited substantial enhancements in maize seedling development. The chlorophyll a and b contents were 66.1% and 56.4% higher than the control levels, respectively, while proline (54.3%), sugar (44.5%), and fat content (23.5%) were stimulated, indicating osmotic compensation. The waterlogged + *M. guilliermondii* treatment increased IAA and SA levels in the afflicted plants by 76.5% and 11.5%, respectively. In waterlogged maize, antioxidant enzymes SOD, POD, and CAT increased by 56.67%, 61.76%, and 44.00%, respectively. MDA was depleted by 40.00%, while H₂O₂ decreased by 63.33%. These findings suggest that *M. guilliermondii* has a high potential as a microbial investigator for reducing maize sensitivity to waterlogging circumstances. However, future research must conduct field testing to study *M. guilliermondii*'s field performance and survivability in a variety of environmental situations and soil types.

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