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MICROBIAL SEED PRIMING MODULATES PHYSIO-BIOCHEMICAL RESPONSES OF *ORYZA SATIVA* L. UNDER POLYETHYLENE GLYCOL-INDUCED DROUGHT STRESS

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ABSTRACT
Drought stress is a major constraint to rice production, adversely affecting seed germination, seedling establishment, growth and physiological functions. Microbial seed biopriming offers an environmentally sustainable approach for enhancing crop resilience under water-deficit conditions. The present study was conducted to evaluate the potential of selected plant growth-promoting bacterial inoculants for improving seed germination, seedling growth, water status, membrane stability and antioxidant defence in rice under polyethylene glycol (PEG 6000)-induced osmotic stress. Seeds of rice cultivars CO 53 and TKM 15 were bioprimed individually with 20% *Azospirillum lipoferum*, *Bacillus megaterium*, *Paenibacillus mucilaginosus* and *Pseudomonas chlororaphis* and compared with hydroprimed and untreated seeds. The treated seeds were evaluated under control conditions and PEG-induced osmotic potentials of −4 and −6 bar. Germination, seedling growth, vigour indices, dry matter production, relative water content (RWC), electrolyte leakage and antioxidant enzyme activities were assessed. Increasing osmotic stress progressively reduced germination, root and shoot growth, seedling vigour and dry matter production in both cultivars. However, microbial biopriming substantially alleviated the adverse effects of osmotic stress. Among the treatments, *Azospirillum lipoferum* consistently recorded superior performance, producing the highest germination percentage, seedling growth, vigour indices and dry matter production under both moderate and severe stress followed by *Bacillus megaterium.* In CO 53, *A. lipoferum* recorded 85 and 77% germination at −4 and −6 bar, respectively, compared with 73 and 64% in the untreated control. Similarly, in TKM 15, germination reached 79 and 74% under −4 and −6 bar, respectively, compared with 69 and 61% in the untreated control. The treatment also maintained higher RWC and reduced electrolyte leakage, indicating improved cellular hydration and membrane stability. Furthermore, *A. lipoferum* enhanced catalase, peroxidase and superoxide dismutase activities under increasing osmotic stress, suggesting improved antioxidant defence and reactive oxygen species scavenging. Overall, the findings demonstrate that microbial seed biopriming, particularly with *A. lipoferum*, effectively improves early-stage drought resilience in rice by sustaining seedling growth, water status, membrane integrity and antioxidant defence under PEG-induced osmotic stress.

KEYWORDS: *Azospirillum lipoferum*, microbial seed biopriming, PEG 6000, seed germination, antioxidant enzymes, osmotic stress

1. INTRODUCTION

Agricultural production has increasingly relied on chemical fertilizers and pesticides to improve crop growth and productivity. However, the excessive and indiscriminate use has resulted in soil pollution, deterioration of soil health and potential risks to human health. At the same time, increasing population and anthropogenic activities have contributed to climate change and global warming, exposing crops to various abiotic stresses such as drought, salinity, heat and temperature fluctuations. Among these, drought is a major constraint to sustainable crop production. Drought-induced oxidative stress causes excessive accumulation of reactive oxygen species (ROS), disrupting cellular metabolism and physiological processes. It can reduce photosynthetic pigment synthesis, alter osmoprotectant accumulation such as proline, impair cell membrane stability, restrict plant height and ultimately reduce crop productivity. Therefore, the adoption of sustainable crop management practices offers considerable potential for improving soil fertility, plant health and crop resilience under adverse environmental conditions.

Rice (*Oryza sativa* L.) is one of the world's most important cereal crops and plays a major role in global food and nutritional security. It is cultivated on approximately 165 million hectares worldwide, with annual paddy production exceeding 780 million tonnes. Rice is particularly important in Asia, where nearly 90 per cent of global rice production and consumption occurs, contributing significantly to food security, nutrition and poverty alleviation (Das *et al*., 2024). India is the second-largest producer and one of the leading exporters of rice,

highlighting its importance to national food security and the global rice trade. However, rice productivity is constrained by several abiotic stresses that adversely affect germination, seedling establishment, vegetative growth, flowering, panicle initiation, grain filling and final yield.

Among these stresses, drought is particularly detrimental to rice as it restricts water availability and disrupts important physiological and biochemical processes. It reduces plant biomass by decreasing leaf number and size, restricting root development and limiting nutrient uptake (Ullah *et al*., 2019; Khan *et al*., 2021). Drought also reduces cellular turgor, inhibits cell expansion and elongation, and impairs photosynthetic efficiency, resulting in poor germination, reduced seedling establishment and lower crop productivity (Hussain *et al*., 2019). Terminal drought during the reproductive and grain-filling stages is especially damaging due to reduced photosynthesis, accelerated leaf senescence and disruption of source–sink relationships, which ultimately restrict grain development and sink capacity. Hence, effective seed enhancement technologies are required to improve seed performance, early establishment and crop productivity under changing environmental conditions.

Seed priming is a pre-sowing treatment in which seeds are partially hydrated under controlled conditions to initiate early germination processes without radicle emergence. Ibrahim 2016; Sheteiwy *et al*. 2017). Microbial seed biopriming is an environmentally sustainable approach that uses beneficial microorganisms to improve seed quality, germination and stress tolerance. The association of microorganisms with seeds during priming can promote early microbial colonization, enhance seedling vigour and stimulate plant defence mechanisms, thereby improving establishment under adverse conditions (Baez-Rogelio *et al*., 2017).

Plant growth-promoting bacteria (PGPB) enhance plant growth through direct and indirect mechanisms, including the production of phytostimulatory metabolites and phytohormones, improved nutrient availability and enhanced nutrient uptake. Their association with plant roots can also promote root development and activate physiological and biochemical mechanisms involved in abiotic stress tolerance (Kour *et al*., 2020). Thus, PGPB-based seed biopriming represents a promising strategy for improving crop establishment and resilience while supporting sustainable agricultural production.

Considering the potential of beneficial microorganisms for improving seed quality and stress resilience, the present study was undertaken to evaluate the effects of microbial seed biopriming with *Azospirillum lipoferum*, *Bacillus megaterium*, *Paenibacillus mucilaginosus* and *Pseudomonas chlororaphis* on seed germination, seedling growth, vigour characteristics and biochemical attributes of rice under PEG induced drought stress. The microbial biopriming treatments were compared with hydroprimed and untreated seeds to identify effective microbial inoculants for improving seed quality early seedling establishment and physiological performance in rice under stress condition.

2. MATERIALS AND METHODS

Certified seed of rice (*Oryza sativa* L.) variety CO53 was obtained from the Department of Rice, Tamil Nadu Agricultural University (TNAU), Coimbatore and TKM15 was obtained from the Rice Research Station Tirur. The Pure cultures of *Azospirillum lipoferum*, *Bacillus megaterium*, *Paenibacillus mucilaginosus* and *Pseudomonas chlororaphis* were obtained from the Department of Agricultural Microbiology, TNAU, Coimbatore. The seed physiological parameters and biochemical analysis were carried out in the High Equipped Laboratory at Department of Seed Science and Technology, TNAU, Coimbatore.

2.1. Biopriming of paddy seeds with microbial inoculants

Surface sterilization for paddy seeds with 2% sodium hypochlorite (NaOCl) solution for 2 mins, followed by 80 % ethanol for 2 min. The surface-sterilised seeds were rinsed with distilled water to remove residual sterilizing agents. The seeds were then primed with different microbial combinations, each containing bacterial population density of 10^8 CFU ml^{-1}. The seed solution ratio was 1:1 and seeds were soaked for 12 hrs and were shade dried for 12 hrs to regain their initial moisture content. The treatment details are as follows: Control dry seeds (T_0), Hydropriming (T_1), 20% *Azospirillum lipoferum* (T_2), *20 % Bacillus megaterium* (T_3), 20% *Paenibacillus mucilaginosus* (T_4), 20% *Pseudomonas chlororaphis* (T_5). Each microbial inoculant was evaluated compared with hydroprimed and untreated control seeds.

2.2. Effect of microbial inoculants on seedling growth and vigour

The bioprimed seeds were studied under low potential conditions (-4 and -6 bar) by using polyethylene glycol 6000 in the laboratory

Ψs = (-1.12x10-2)xC-(1.18x10-4)xC+(2.67x10-4)xCxT+(8.39x10-7)xCxT where Ψs is the osmotic pressure (Bar), C is the concentration of polyethene glycol 6000 (g kg-1 H2O) and T is the temperature (°C) (20).

The germination test was conducted in the germination room maintained at 25 ± 1°C and 95 ± 2% relative humidity (RH) and subjected to germination with the guidelines of International Seed Testing Association, 2019. The speed of germination was calculated using the formula said by Maguire, (1962) as below:

Speed of germination = [G1/1]+[G2/2]+[G3/3]+[G4/4]+[G5/5]+[G6/6] ..[G14/14] (Eqn. 1)

Here, G1, G2, … G14: Number of emerged seedlings and 1, 2, … 14: Number of days after sowing

Germination Percentage (ISTA, 2019)**:** Germination percentage (GP)= Number of normal seedlings / Total number of seeds sown×100 (Eqn.2)

Vigour Index: The seedling vigour index I was calculated according to Abdul Baki and Anderson (1973) and seedling vigour index II was calculated based on the formula below.

Vigour index (SVI-I) = Total seedling length × Germination percentage (Eqn. 3)

Vigour index (SVI-II) = Total dry matter production × Germination percentage (Eqn. 4)

2.3. Determination of enzyme activity

Relative water content of leaves was estimated as described by Barr and Weatherley (1962). Relative water content (RWC) was determined using 14-day-old seedlings. Approximately 25 leaf discs were collected, and their fresh weight (FW) was recorded. The discs were floated in distilled water for 4 h to obtain turgid weight (TW), followed by oven drying at 80°C for 48 h to determine dry weight (DW). Relative water content was calculated using the following formula

Relative Water Content % = Fresh Weight- Dry Weight / Turgid weight – Dry Weight x 100

Electrolyte leakage was determined as described by Wu *et al*.,2017 .0.5 g of fresh leaf tissue were collected at 14 DAS. The samples were immersed in 10 mL of distilled water and incubated in a water bath for 2 hrs at 32°C. The initial electrical conductivity (L_1) of the solution was measured using an EC meter. The samples were then autoclaved at 121°C for 20 min to achieve complete electrolyte release, cooled to room temperature, and the final electrical conductivity (L_2) was recorded. Electrolyte leakage was calculated using the following formula

Electrolyte leakage (%) = L_1/L_2 x 100

Catalase (CAT) activity was determined by the following method described by (Aebi,1983). Fresh leaf tissue (0.5 g) was homogenized in 5 mL of 50 mM potassium phosphate buffer (pH 7.0) under ice-cold conditions. The homogenized sample was centrifuged at 15,000 rpm for 20 min at 5°C, and the resulting supernatant was used as the enzyme extract. The reaction mixture comprised 1.5 mL of 50 mM potassium phosphate buffer, 1.2 mL of 12.5 mM H_2O_2, and 0.3 mL of the enzyme extract. The decrease in absorbance due to H_2O_2 decomposition was recorded at 240 nm for 1 min at 15 s intervals using a UV–Vis spectrophotometer. Catalase activity was calculated from the rate of H_2O_2 decomposition using a standard curve prepared with known H_2O_2 concentrations and expressed as µg H_2O_2 reduced g^{-1} fresh weight min^{-1}.

Catalase activity = Difference in titre value / Volume of sample pipetted out x 1/15

Peroxidase (POD) activity was determined using the standard method described by Malik and Singh, 1980. Fresh leaf tissue (500 mg) was homogenized in 5 mL of 0.25 M Tris buffer (pH 6.0), and the homogenized was centrifuged at 10,000 rpm for 10 min. The resulting supernatant was used as the enzyme extract. The reaction mixture consisted of 0.4 mL of enzyme extract and 0.5 mL of 0.5% (w/v) aqueous pyrogallol solution. The mixture was incubated at 25°C for 10 min, after which the reaction was terminated by adding 0.5 mL of 5% (v/v) H_2SO_4. Absorbance was recorded at the start of the reaction (0 min) and after 10 min using a UV–Vis spectrophotometer. Peroxidase activity was calculated from the change in absorbance and expressed as units mg^{-1} protein min^{-1}.

Peroxidase activity = Difference in OD value / 10 min x 1000/500 x 60

Superoxide dismutase (SOD) activity was estimated by following method described by Beauchamp and fridovich,1971. Fresh leaf tissue (0.5 g) from each treatment replication was homogenized in 10 mL of 15 mM potassium phosphate buffer using mortar and pestle. The homogenized sample was centrifuged at 1,000 rpm for 1 min at 4°C, and the supernatant was used as the enzyme extract. The reaction mixture consisted of 1.0 mL enzyme extract, 0.3 mL of 50 mM potassium phosphate buffer, 0.3 mL of 0.1 mM EDTA, 0.3 mL riboflavin (2 µM), and 0.3 mL nitroblue tetrazolium (NBT; 75 µM). A blank without NBT (Blank A) and a blank without enzyme extract (Blank B) were included. The reaction mixtures were illuminated under a 400 W light source for 15 min, and absorbance was recorded at 560 nm using a UV–Vis spectrophotometer. SOD activity was calculated based on the inhibition of NBT photoreduction and expressed as units mg^{-1} protein min^{-1}.

Superoxide dismutase activity = Test OD – Control OD/ Test OD x 100

3. RESULTS AND DISCUSSION

3.1. Effect of microbial inoculants on germination and seedling growth under PEG-induced stress

The effect of microbial inoculants on germination and early seedling growth of paddy varieties CO 53 and TKM 15 under PEG 6000-induced osmotic stress is presented in Tables 1–8. Increasing osmotic stress from M_0 (control) to M_1 (−4 bar) and M_2 (−6 bar) resulted in a progressive reduction in speed of germination, germination percentage, root length, shoot length, total seedling length, seedling vigour and dry matter production in both varieties. However, microbial inoculation substantially alleviated the adverse effects of PEG-induced stress, with *Azospirillum lipoferum* (T_2) consistently increasing all the physiological and seedling growth parameters.

In CO 53, the mean speed of germination decreased from 8.6 under M_0 to 6.1 under M_1 and 4.6 under M_2 (Table 1). T_2 recorded the highest speed of germination at all stress levels, with values of 9.6, 7.2 and 5.8 under M_0, M_1 and M_2 compared to uninoculated control (T_0) 6.8 under M_0, 4.6 and 3.6 under M_1 and M_2, respectively. A similar trend was observed for germination percentage. The overall mean germination declined from 86 per cent under M_0 to 79 and 71 per cent under M_1 and M_2, respectively. The uninoculated control recorded the lowest germination of 80, 73 and 64 per cent, whereas T_2 recorded the highest values of 90, 85 and 77 per cent under M_0, M_1 and M_2, respectively (Fig.1).

A similar response was observed in TKM 15 (Table 2). The mean speed of germination decreased from 7.9 under M_0 to 5.8 under M_1 and 5.0 under M_2. T_2 recorded the highest speed of germination, with values of 9.0, 6.9 and 5.7 under M_0, M_1 and M_2, respectively, compared with 6.3, 4.5 and 3.9 in T_0. Germination percentage also declined progressively with increasing osmotic stress, with mean values of 81.5, 74.2 and 67.3 per cent under M_0, M_1 and M_2, respectively. The highest germination was recorded in T_2 (87, 79 and 74%), while the corresponding values in T_0 were 76, 69 and 61 per cent. The decline in germination under increasing PEG-induced osmotic stress may

be attributed to the reduction in water potential, which restricts water uptake by the seeds and subsequently delays the metabolic events required for germination. PEG 6000 is widely used to impose controlled osmotic stress because it reduces the availability of water without readily penetrating the seed tissues. Similar reductions in germination and seedling growth under increasing PEG concentrations have been reported in rice, while appropriate seed-priming treatments have been shown to improve germination and seedling establishment under water-deficit conditions (Yuan *et al*., 2010; Muniyappan *et al.*, 2026).

The superior performance of T_2 may be associated with the plant growth-promoting properties of *A. lipoferum*. Beneficial rhizobacteria can improve seedling establishment through phytohormone production, particularly auxin, enhancement of nutrient acquisition and modulation of stress-responsive physiological processes. PGPR-mediated drought tolerance has also been associated with ACC deaminase activity, exopolysaccharide production, osmotic adjustment, antioxidant defence and modification of root architecture (Vurukonda *et al*., 2016).

PEG-induced osmotic stress markedly reduced root and shoot elongation in CO 53 (Table 3). The mean root length declined from 18.7 cm under M_0 to 15.9 cm under M_1 and 13.1 cm under M_2, while shoot length decreased from 9.4 to 7.6 and 6.5 cm, respectively. The reduction was greatest in T_0, where root length decreased from 17.0 cm under M_0 to 14.1 and 11.3 cm under M_1 and M_2, respectively. In comparison, T_2 maintained the highest root length, recording 20.2, 18.0 and 15.2 cm under M_0, M_1 and M_2, respectively. A similar trend was observed for shoot growth. T_2 recorded the maximum shoot length of 10.5, 8.7 and 7.6 cm under M_0, M_1 and M_2, respectively, compared with 8.2, 6.4 and 5.3 cm in T_0.

In TKM 15, T_2 similarly recorded the highest root length of 21.0, 19.2 and 17.4 cm and shoot length of 11.5, 9.6 and 8.2 cm under M_0, M_1 and M_2, respectively (Table 4). In comparison, T_0 recorded 16.5, 14.2 and 12.3 cm for root length and 7.5, 5.4 and 4.8 cm for shoot length under the respective stress levels. The greater retention of root growth in inoculated seeds is particularly important under water-deficit conditions because root system architecture plays a major role in water and nutrient acquisition and plant adaptation to drought (Ranjan *et al*., 2022). The improvement in root development following bacterial inoculation may be related to bacterial synthesis of auxin and other growth-promoting metabolites. PGPR can modify root architecture by stimulating lateral root development and root elongation, thereby increasing the effective root surface area available for water and nutrient acquisition. In addition, bacterial ACC deaminase activity and exopolysaccharide production may contribute to improved plant adaptation under drought conditions (Vurukonda *et al*., 2016).

The reduction in root and shoot growth under PEG-induced stress was reflected in total seedling length and vigour index I (Tables 5 and 6). In CO 53, mean total seedling length decreased from 28.2 cm under M_0 to 23.5 and 19.6 cm under M_1 and M_2, respectively. T_2 recorded the highest total seedling length at all stress levels, with 30.7, 26.7 and 22.8 cm under M_0, M_1 and M_2, respectively, compared with 25.2, 20.5 and 16.6 cm in T_0. A similar trend was observed for vigour index I. T_2 recorded the highest values of 2763, 2270 and 1756 under M_0, M_1 and M_2, respectively, whereas T_0 recorded 2016, 1497 and 1062. The advantage of T_2 was particularly evident under M_2, where vigour index I was approximately 65.3 per cent higher than that of the uninoculated control.

In TKM 15, the calculated total seedling lengths were 32.5, 28.8 and 25.6 cm for T_2 under M_0, M_1 and M_2, respectively, compared with 24.0, 19.6 and 17.1 cm in T_0. The corresponding calculated vigour index I values were approximately 2828, 2275 and 1894 for T_2, compared with 1824, 1352 and 1043 in T_0. These results indicate that *A. lipoferum* was more effective in maintaining overall seedling growth under PEG-induced osmotic stress than the other bacterial treatments. The maintenance of seedling vigour following microbial inoculation may be attributed to the combined effects of improved nutrient availability, phytohormonal stimulation and enhanced stress adaptation. PGPR are known to influence root development and physiological processes involved in plant responses to drought, thereby helping plants maintain growth under water-limited conditions (Zhang *et al*., 2022, Abdelaal *et al*.,2022).

Vigour index II and dry matter production exhibited trends similar to those observed for seedling length and vigour index I (Table 7). In CO 53, mean vigour index II decreased from 10.44 under M_0 to 7.69 under M_1 and 6.19 under M_2. T_2 recorded the highest values of 12.15, 9.86 and 7.78 under M_0, M_1 and M_2, respectively, compared with 8.40, 5.91 and 4.80 in T_0. Dry matter production also declined progressively with increasing osmotic stress. The mean dry matter production decreased from 0.121 g under M_0 to 0.097 and 0.085 g under M_1 and M_2, respectively. T_2 maintained the highest dry matter production, recording 0.135, 0.116 and 0.101 g under M_0, M_1 and M_2, respectively, whereas T_0 recorded 0.105, 0.081 and 0.075 g.

For TKM 15 (Table 8), dry matter production was similarly highest in T_2, with values of 0.128, 0.117 and 0.106 g under M_0, M_1 and M_2, respectively, compared with 0.103, 0.084 and 0.074 g in T_0. The corresponding vigour index II values, calculated from the available germination and dry matter data, were approximately 11.14, 9.24 and 7.84 in T_2 under M_0, M_1 and M_2, respectively, compared with 7.83, 5.80 and 4.51 in T_0. The greater retention of dry matter under stress suggests that bacterial inoculation helped sustain metabolic activity and biomass accumulation despite the reduced osmotic potential. Similar observations have been reported for seed priming under drought stress, where primed seeds exhibited improved germination, seedling growth and antioxidant defence compared with unprimed seeds (Yuan *et al*., 2010).

Among the treatments, *A. lipoferum* (T2) consistently recorded the highest speed of germination, germination percentage, root length, shoot length, total seedling length, vigour indices and dry matter production under both control and stress conditions. This response indicates the superior effectiveness of *A. lipoferum* in promoting early seedling establishment and maintaining growth under PEG-induced osmotic stress. The beneficial effect of *A.*

lipoferum may be associated with its multiple plant growth-promoting mechanisms, including phytohormone production, particularly indole-3-acetic acid, improvement of root system architecture, enhanced nutrient acquisition and modulation of stress-responsive mechanisms. PGPR-mediated drought tolerance involves several interconnected mechanisms, including production of phytohormones, ACC deaminase, exopolysaccharides and osmoprotectants, together with enhanced antioxidant defence and regulation of stress-responsive pathways (Ahmad *et al*., 2022). Therefore, the present results demonstrate that microbial seed biopriming, particularly with *A. lipoferum*, has considerable potential for improving early-stage drought resilience in paddy. Microbial inoculation can enhance plant tolerance to abiotic stress by stimulating the accumulation of osmoprotective compounds, including proline, exopolysaccharides and free amino acids. In addition, beneficial microorganisms contribute to improved water-use efficiency, better regulation of stomatal activity and enhanced root development, thereby helping plants maintain growth and physiological functions under stressful conditions (Chandrasekaran *et al*., 2017; Annadurai *et al*., 2020).

3.2 Effect of microbial inoculants on relative water content and electrolyte leakage under PEG-induced stress

The relative water content (RWC) and electrolyte leakage (EL) of CO 53 and TKM 15 paddy seeds were markedly influenced by PEG-induced osmotic stress and microbial inoculation (Tables 5 and 12). Increasing the severity of osmotic stress from M_0 to M_1 (−4 bar) and M_2 (−6 bar) progressively reduced RWC, whereas electrolyte leakage increased. However, microbial inoculation, particularly with *Azospirillum lipoferum* (T_2), substantially alleviated these adverse effects.

In CO 53, the mean RWC decreased from 74.1% under M_0 to 71.0% under M_1 and 68.2% under M_2 (Table 5). The lowest RWC was recorded in the uninoculated control (T_0), with values of 70.6, 66.8 and 64.2% under M_0, M_1 and M_2, respectively. In contrast, T_2 recorded the highest RWC at all stress levels, with values of 77.9, 75.2 and 72.1%, respectively. At severe osmotic stress (M_2), T_2 maintained an RWC of 72.1%, compared with only 64.2% in T_0. T_3 also performed well, recording 76.2, 74.1 and 70.9%, respectively, whereas T_4 and T_5 showed intermediate responses.

A similar response was observed in TKM 15 (Table 12). The calculated mean RWC declined from 73.8% under M_0 to 70.7% under M_1 and 68.0% under M_2. T_0 recorded the lowest RWC of 70.1, 65.7 and 63.6% at M_0, M_1 and M_2, respectively. In comparison, T_2 recorded the highest values of 77.1, 74.5 and 71.9%. Maintenance of higher RWC under osmotic stress is an important indicator of improved plant water status. Water deficit causes cellular dehydration, reduction in turgor and disruption of physiological processes. PGPR can contribute to maintenance of plant water status through several mechanisms, including modulation of root development, production of osmoprotective compounds and exopolysaccharides, regulation of stress-responsive pathways and improvement of water and nutrient acquisition.

The reduction in RWC was accompanied by an increase in electrolyte leakage. In CO 53, mean EL increased from 88.3% under M_0 to 90.5% under M_1 and 92.3% under M_2. The highest leakage was recorded in T_0, increasing from 90.7% under M_0 to 92.7% under M_1 and 94.4% under M_2. Conversely, T_2 exhibited the lowest EL at all stress levels, with values of 85.8, 88.7 and 91.7%, respectively.

The same pattern was observed in TKM 15. Mean EL increased from 87.7% under M_0 to 90.2% under M_1 and 92.2% under M_2. T_0 recorded 89.9, 92.0 and 93.9% under M_0, M_1 and M_2, respectively, whereas T_2 maintained the lowest EL at 85.0, 87.9 and 90.5%. Thus, the *A. lipoferum*-treated seeds showed comparatively greater membrane stability under PEG-induced stress. The increase in electrolyte leakage under increasing osmotic stress indicates deterioration of membrane integrity and increased cellular permeability. Excessive production of reactive oxygen species (ROS) under drought or osmotic stress can induce lipid peroxidation and membrane injury, resulting in greater leakage of cellular electrolytes. Conversely, reduced electrolyte leakage in microbial-inoculated seeds indicates better preservation of membrane stability. PGPR-mediated improvement in antioxidant defence has been associated with reduced oxidative membrane damage and lower electrolyte leakage under drought stress.

The superior performance of T_2 in maintaining RWC and restricting electrolyte leakage suggests that *A. lipoferum* was more effective than the other bacterial inoculants in maintaining cellular water balance and membrane integrity under PEG-induced osmotic stress. Similar mechanisms of PGPR-mediated drought tolerance have been associated with improved water relations, root development, osmolyte accumulation and activation of antioxidant defence systems. The increase in RWC in seedlings might be linked to microbial modulation of stomatal closure mechanisms by plant growth-promoting bacteria (PGPB) that helps in minimizing transpirational water loss (Vishnupradeep *et al*., 2022)**.** Seeds treated with rhizobium showed increased relative water content and improved water movement in drought stress compared to uninoculated control (Monisha, 2023).

3.3. Effect of microbial inoculants on antioxidant enzyme activities under PEG-induced stress

Osmotic stress resulted in a marked alteration in antioxidant enzyme activities in CO 53 and TKM 15. Catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) activities generally increased with increasing PEG-induced stress, with the highest activities observed in *A. lipoferum*-treated seeds. This indicates that microbial inoculation enhanced the antioxidant defence response of the seeds against stress-induced oxidative damage (Singh *et al*., 2020; Vurukonda *et al*., 2016)

In CO 53, CAT activity increased progressively with increasing stress severity (Table 6). The mean CAT activity increased from 1.43 under M_0 to 2.48 under M_1 and 2.78 under M_2. In the uninoculated control, CAT activity

increased from 1.12 under M_0 to 1.55 and 1.87 under M_1 and M_2, respectively. The highest CAT activity was recorded in T_2, with values of 1.67, 3.03 and 3.34 under M_0, M_1 and M_2, respectively. T_3 followed T_2 with 1.58, 2.87 and 3.12, while T_4 and T_5 recorded comparatively lower values.

The marked increase in CAT activity in T_2 under M_2 indicates enhanced capacity for detoxification of hydrogen peroxide under severe osmotic stress. Catalase plays an important role in the antioxidant defence system by decomposing hydrogen peroxide into water and molecular oxygen, thereby preventing its conversion into highly reactive hydroxyl radicals (Rahnama and Ebrahimzadeh, 2005; Singh *et al*., 2020). SOD, CAT and POX function together as important components of the enzymatic antioxidant system under drought and other abiotic stresses (Hasanuzzaman *et al.,* 2011 ;Vurukonda *et al*., 2016).

In TKM 15, CAT activity also showed a strong response to microbial inoculation (Table 13). T_2 recorded the highest CAT activity of 3.25, 3.03 and 3.34 under M_0, M_1 and M_2, respectively. T_3 recorded 3.02, 2.87 and 3.12, while T4 recorded 2.88, 2.64 and 2.87. In contrast, the uninoculated control recorded only 1.12, 1.42 and 1.87. Notably, T_2 maintained high CAT activity even under M_2, suggesting enhanced hydrogen peroxide-scavenging capacity. The relatively high antioxidant activity in microbial-inoculated seeds may represent an adaptive response that enables seedlings to maintain ROS homeostasis under osmotic stress. PGPR are known to stimulate antioxidant defence systems involving CAT, SOD and peroxidases, thereby reducing oxidative injury and supporting cellular metabolism during drought stress (Vurukonda *et al*., 2016; Gowtham *et al*., 2022)**.**

Peroxidase activity showed a pattern similar to CAT activity. In CO 53, POX activity in the uninoculated control increased from 1.22 under M_0 to 1.64 and 1.82 under M_1 and M_2, respectively. T_2 recorded the highest POX activity, increasing from 1.67 under M_0 to 2.22 under M_1 and 2.35 under M_2 (Table 6). T_3 recorded the next highest values of 1.58, 1.97 and 2.23, followed by T_4 and T_5. In TKM 15, T_2 again recorded the highest POX activity, with values of 1.74, 2.12 and 2.35 under M_0, M_1 and M_2, respectively (Table 13). The corresponding values for T0 were 1.32, 1.64 and 1.82. Thus, microbial inoculation, particularly with *A. lipoferum*, enhanced POX activity under both moderate and severe osmotic stress. Peroxidases participate in the removal of hydrogen peroxide and are important components of plant defence against oxidative stress. Increased POX activity under water-deficit conditions can contribute to detoxification of accumulated ROS and protection of cellular components. The greater POX activity observed in inoculated seeds therefore indicates activation of an enhanced enzymatic defence system.

SOD activity increased progressively with increasing osmotic stress in both varieties (Tables 7 and 14). In CO 53, the SOD activity of the uninoculated control increased from 1.44 under M_0 to 1.57 under M_1 and 1.68 under M_2. T_2 recorded the highest SOD activity, with values of 1.69, 1.75 and 1.86 under M_0, M_1 and M_2, respectively. The overall mean values across treatments increased from approximately 1.56 under M_0 to 1.65 under M_1 and 1.78 under M_2. A similar trend was recorded in TKM 15. T_0 showed SOD activities of 1.36, 1.57 and 1.68 under M_0, M_1 and M_2, respectively, whereas T_2 recorded 1.60, 1.75 and 1.86. The calculated overall means were approximately 1.48, 1.65 and 1.78 under M_0, M_1 and M_2, respectively. T_3, T_4 and T_5 also enhanced SOD activity relative to the uninoculated control, although their values were lower than those of T_2. The increase in SOD activity under PEG-induced stress is physiologically important because SOD represents the first enzymatic line of defence against superoxide radicals. It catalyses the conversion of superoxide radicals into hydrogen peroxide, which is subsequently detoxified by CAT and POX. Therefore, the simultaneous increase in SOD, CAT and POX observed in the present study indicates coordinated activation of the antioxidant defence machinery in response to osmotic stress. The consistently higher antioxidant enzyme activities in *A. lipoferum*-treated seeds suggest that this inoculant was particularly effective in stimulating the enzymatic defence system. PGPR-mediated activation of antioxidant machinery is an important component of induced systemic tolerance, enabling plants to maintain ROS homeostasis under drought stress.

The physiological and biochemical responses obtained in the present study demonstrate that increasing PEG-induced osmotic stress adversely affected seed water status and membrane stability while simultaneously inducing antioxidant defence responses. Reduction in RWC and enhancement of electrolyte leakage under M_1 and M_2 were accompanied by increases in CAT, POX and SOD activities. These responses indicate that increasing osmotic stress imposed both dehydration and oxidative challenges on the developing seedlings.

Microbial inoculation considerably modified these responses. Among the treatments, *A. lipoferum* (T_2) consistently recorded the highest RWC, lowest electrolyte leakage and highest CAT, POX and SOD activities in both CO 53 and TKM 15. The higher RWC coupled with lower electrolyte leakage in T_2 suggests improved maintenance of cellular hydration and membrane integrity, whereas the higher activities of SOD, CAT and POX indicate enhanced capacity for ROS detoxification. These two responses are physiologically interconnected: maintenance of cellular water status can reduce the severity of stress-induced metabolic disturbance, while an efficient antioxidant system can limit ROS-mediated membrane damage. PGPR can contribute to these responses through several mechanisms, including phytohormone production, ACC deaminase activity, osmolyte accumulation, exopolysaccharide production, modification of root architecture and stimulation of antioxidant defence (Shultana *et al*.,2022). The present findings indicate that *A. lipoferum* was the most effective bacterial inoculant among those evaluated for improving physiological water status, maintaining membrane integrity and activating antioxidant defence under PEG-induced osmotic stress. The enhanced antioxidant response observed in inoculated seeds, together with improved RWC and reduced electrolyte leakage, provides physiological evidence for improved tolerance to osmotic stress during early seedling establishment. These findings complement

the improvements observed in germination, root growth, shoot growth and seedling vigour, supporting the potential of *A. lipoferum* seed biopriming as a promising approach for improving early-stage drought resilience in paddy.

CONCLUSION

The present study demonstrated that microbial seed inoculation significantly enhanced osmotic stress tolerance in paddy cultivars CO 53 and TKM 15 under PEG 6000-induced stress. Inoculation with 20% *Azospirillum lipoferum* (T_2) consistently improved speed of germination, germination percentage, root and shoot growth, seedling vigour and dry matter production under both moderate (−4 bar) and severe (−6 bar) osmotic stress. The treatment also maintained higher relative water content and reduced electrolyte leakage, indicating improved cellular hydration and membrane stability in stressed seedlings. The enhanced physiological performance of *A. lipoferum*-treated seeds was further supported by increased activities of antioxidant enzymes, particularly catalase, peroxidase and superoxide dismutase, suggesting improved ROS-scavenging capacity and protection against oxidative damage under PEG-induced stress. The results indicate that microbial seed inoculation with *A. lipoferum* can serve as a promising, simple and environmentally sustainable approach for improving early-stage drought resilience and seedling establishment in paddy. However, further evaluation under soil-based and field drought conditions is required to validate its effectiveness and determine its practical potential for large-scale agricultural application.

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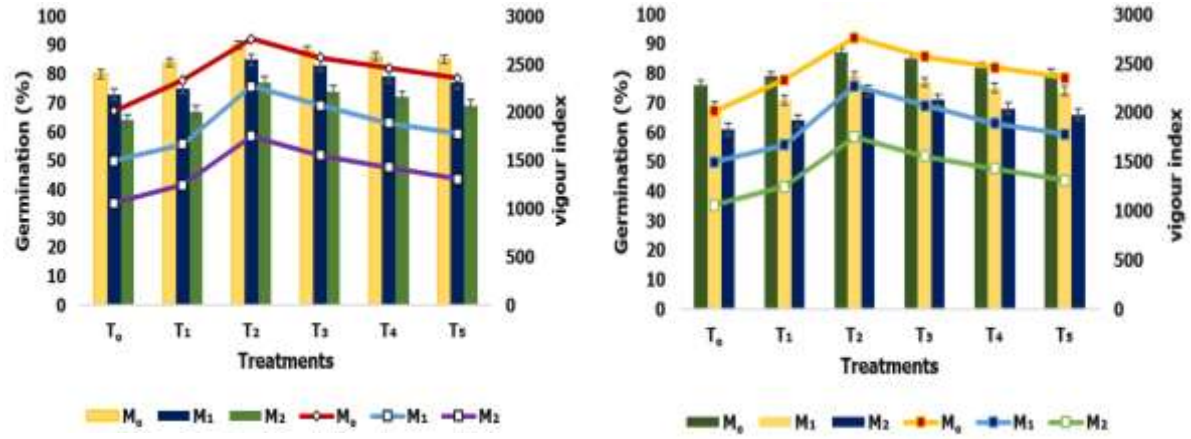

Fig. 1 Effect of microbial inoculation on seed germination and Vigor Index of paddy varieties viz., CO53 and TKM 15

Table 1 Effect of microbial inoculants on speed of germination and germination % in CO 53 paddy seeds under PEG induced stress

	Speed of germination			Germination %		
Treatment	**M_0**	**M_1**	**M_2**	**M_0**	**M_1**	**M_2**
T_0	6.8	4.6	3.6	80	73	64
T_1	8.3	5.9	4.1	84	75	67
T_2	9.6	7.2	5.8	90	85	77
T_3	9.3	6.8	5.3	88	83	74
T_4	9.2	6.3	4.9	86	79	72
T_5	8.7	6.0	4.3	85	77	69
Mean	8.6	6.1	4.6	86	79	71
	T	**M**	**TxM**	**T**	**M**	**TxM**
SEd	0.093	0.066	0.162	1.069	0.756	1.852
CD	0.190	0.134	0.329	2.169	1.534	3.757

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*, T_3- 20% *Bacillus megaterium*, T_4- 20% *Paenibacillus mucilaginosus*, T_5- 20% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).

Table 2 Effect of microbial inoculants on speed of germination and germination % in TKM 15 paddy seeds under PEG induced stress

	Speed of germination			Germination %		
Treatment	**M_0**	**M_1**	**M_2**	**M_0**	**M_1**	**M_2**
T_0	6.3	4.5	3.9	76	69	61
T_1	7.4	5.0	4.7	79	71	64
T_2	9.0	6.9	5.7	87	79	74
T_3	8.6	6.5	5.4	85	77	71
T_4	8.2	6.2	5.2	82	75	68
T_5	7.9	5.9	4.9	80	74	66
Mean	7.9	5.8	4.9	82	74	67
	T	**M**	**TxM**	**T**	**M**	**TxM**
SEd	0.078	0.055	0.136	0.995	0.675	1.655
CD	0.159	0.112	0.276	1.938	1.370	3.357

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*, T_3- 20% *Bacillus megaterium*, T_4- 20% *Paenibacillus mucilaginosus*, T_5- 20% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).

Table 3 Effect of microbial inoculants on root length and shoot length in CO 53 paddy seeds under PEG induced stress

	Root length			Shoot length		
Treatment	**M_0**	**M_1**	**M_2**	**M_0**	**M_1**	**M_2**
T_0	17.0	14.1	11.3	8.2	6.4	5.3
T_1	18.7	15.3	12.4	9.1	7.0	6.2
T_2	20.2	18.0	15.2	10.5	8.7	7.6

T_3	19.2	16.7	14.0	10.0	8.3	7.0
T_4	18.9	16.0	13.2	9.7	7.9	6.7
T_5	18.4	15.6	12.8	9.3	7.5	6.2
Mean	18.7	15.9	13.1	9.4	7.6	6.5
	T	**M**	**TxM**	**T**	**M**	**TxM**
SEd	0.212	0.150	0.367	0.083	0.058	0.144
CD	0.430	0.304	0.745	0.168	0.119	0.292

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*,, T_3- 20% *Bacillus megaterium*, T_4- 20% *Paenibacillus mucilaginosus*, T_5- 20% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).

Table 4 Effect of microbial inoculants on root length and shoot length in TKM 15 paddy seeds under PEG indusced stress

	Root length			**Shoot length**		
Treatment	**M_0**	**M_1**	**M_2**	**M_0**	**M_1**	**M_2**
T_0	16.5	14.2	12.3	7.5	5.4	4.8
T_1	17.6	15.1	13.2	8.6	6.3	5.5
T_2	21.0	19.2	17.4	11.5	9.6	8.2
T_3	19.4	17.5	16.2	10.7	8.8	7.4
T_4	18.6	16.6	15.8	10.0	7.9	6.9
T_5	18.0	15.9	15.2	9.7	7.1	6.2
Mean	18.5	16.4	15.0	9.6	7.5	6.4
	T	**M**	**TxM**	**T**	**M**	**TxM**
SEd	0.233	0.165	0.404	0.099	0.070	0.172
CD	0.473	0.334	0.820	0.202	0.142	0.349

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*,, T_3- 20% *Bacillus megaterium*, T_4- 20% *Paenibacillus mucilaginosus*, T_5- 20% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).

Table 5 Effect of microbial inoculants on total seedling length and vigour index 1 in CO 53 paddy seeds under PEG induced stress

	Total seedling length			**Vigour Index 1**		
Treatment	**M_0**	**M_1**	**M_2**	**M_0**	**M_1**	**M_2**
T_0	25.2	20.5	16.6	2016	1497	1062
T_1	27.8	22.3	18.6	2335	1673	1246
T_2	30.7	26.7	22.8	2763	2270	1756
T_3	29.2	25.0	21.0	2570	2075	1554
T_4	28.6	23.9	19.9	2460	1888	1433
T_5	27.7	23.1	19.0	2355	1779	1311
Mean	28.2	23.5	19.6	2417	1864	1394
	T	**M**	**TxM**	**T**	**M**	**TxM**
SEd	0.317	0.224	0.549	25.030	17.698	43.353
CD	0.642	0.454	1.113	50.768	35.899	117.909

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*,, T_3- 20% *Bacillus megaterium*, T_4- 20% *Paenibacillus mucilaginosus*, T_5- 20% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).

Table 6 Effect of microbial inoculants on total seedling length and Vigour Index 1 in TKM 15 paddy seeds under PEG induced stress

	Total seedling length			**Vigour Index 1**		
Treatment	**M_0**	**M_1**	**M_2**	**M_0**	**M_1**	**M_2**
T_0	24.0	19.6	17.1	1824	1352	1043
T_1	26.2	21.4	18.7	2070	1519	1197
T_2	32.5	28.8	25.6	2828	2275	1894
T_3	30.1	26.3	23.6	2559	2025	1676
T_4	28.6	24.5	22.7	2345	1838	1544
T_5	27.7	23	21.4	2216	1702	1412
Mean	28.1	23.9	21.5	2307	1785	1461

	T	M	TxM	T	M	TxM
SEd	0.283	0.200	0.490	20.782	14.695	35.996
CD	0.574	0.406	0.994	42.154	29.807	73.013

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*,, T_3- 20% *Bacillus megaterium*, T_4- 20% *Paenibacillus mucilaginosus*, T_5- 20% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).

Table 7 Effect of microbial inoculants on Vigour Index II and Dry Matter Production in CO 53 paddy seeds under PEG induced stress

	Vigour Index 2			Dry Matter Production		
Treatment	**M_0**	**M_1**	**M_2**	**M_0**	**M_1**	**M_2**
T_0	8.40	5.91	4.80	0.105	0.081	0.075
T_1	9.66	6.45	5.56	0.115	0.086	0.083
T_2	12.15	9.86	7.78	0.135	0.116	0.101
T_3	11.44	9.13	6.96	0.130	0.110	0.094
T_4	10.84	7.90	6.26	0.126	0.100	0.087
T_5	10.20	6.93	5.80	0.120	0.090	0.084
Mean	10.44	7.69	6.19	0.121	0.097	0.085
	T	**M**	**TxM**	**T**	**M**	**TxM**
SEd	0.106	0.075	0.184	0.0013	0.0009	0.002
CD	0.215	0.152	0.373	0.0022	0.0045	0.004

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*,, T_3- 20% *Bacillus megaterium*, T_4- 20% *Paenibacillus mucilaginosus*, T_5- 20% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).

Table 8 Effect of microbial inoculants on Vigour Index II and Dry Matter Production in TKM 15 paddy seeds under PEG induced stress

	Vigour Index 2			Dry Matter Production		
Treatment	**M_0**	**M_1**	**M_2**	**M_0**	**M_1**	**M_2**
T_0	7.83	5.80	4.51	0.103	0.084	0.074
T_1	8.69	6.46	5.12	0.110	0.091	0.080
T_2	11.14	9.24	7.84	0.128	0.117	0.106
T_3	10.46	8.47	6.75	0.123	0.110	0.095
T_4	9.76	7.65	5.92	0.119	0.102	0.087
T_5	9.20	6.88	5.41	0.115	0.093	0.082
Mean	9.51	7.41	5.92	0.116	0.099	0.872
	T	**M**	**TxM**	**T**	**M**	**TxM**
SEd	0.106	0.075	0.184	0.0013	0.0009	0.0022
CD	0.216	0.153	0.375	0.0026	0.0018	0.0046

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*,, T_3- 20% *Bacillus megaterium*, T_4- 20% *Paenibacillus mucilaginosus*, T_5- 20% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).

Table 9 Effect of microbial inoculants on Relative Water Content and Electrolyte Leakage in CO 53 paddy seeds under PEG induced stress

	Relative Water Content			Electrolyte Leakage		
Treatment	**M_0**	**M_1**	**M_2**	**M_0**	**M_1**	**M_2**
T_0	70.6	66.8	64.2	90.7	92.7	94.4
T_1	72.5	69.2	66.1	89.4	91.3	93.1
T_2	77.9	75.2	72.1	85.8	88.7	91.7
T_3	76.2	74.1	70.9	87.0	89.9	90.1
T_4	74.1	71.3	69.1	88.2	90.1	92.0
T_5	73.4	69.6	67.0	88.9	90.8	92.7
Mean	74.1	71.0	68.2	88.3	90.5	92.3
	T	**M**	**TxM**	**T**	**M**	**TxM**
SEd	0.828	0.586	1.435	1.206	0.853	2.090
CD	1.681	1.188	2.912	2.447	1.730	4.239

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*,, T_3- 20% *Bacillus megaterium*, T_4- 20% *Paenibacillus mucilaginosus*, T_5- 20% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).

Table 10 Effect of microbial inoculants on Relative Water Content and Electrolyte Leakage in TKM 15 paddy seeds under PEG induced stress

	Relative Water Content			**Electrolyte Leakage**		
Treatment	**M_0**	**M_1**	**M_2**	**M_0**	**M_1**	**M_2**
T_0	70.0	65.9	63.0	89.5	92.2	93.6
T_1	71.9	68.2	64.5	88.1	90.3	92.1
T_2	76.7	74.0	71.5	84.0	86.8	89.0
T_3	74.8	72.3	69.6	85.5	87.5	89.5
T_4	73.6	70.1	67.0	86.3	88.5	90.4
T_5	72.9	69.5	65.5	87.2	89.4	91.5
Mean	73.3	70.0	66.8	86.7	89.1	91.0
	T	**M**	**TxM**	**T**	**M**	**TxM**
SEd	0.866	0.612	1.501	1.080	0.763	1.871
CD	1.757	1.243	3.044	2.191	1.549	3.795

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*,, T_3- 20% *Bacillus megaterium*, T_4- 20% *Paenibacillus mucilaginosus*, T_5- 20% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).

Table 11 Effect of microbial inoculants on catalase and peroxidase in CO 53 paddy seeds under PEG induced stress

	Catalase			**Peroxidase**		
Treatment	**M_0**	**M_1**	**M_2**	**M_0**	**M_1**	**M_2**
T_0	1.12	1.55	1.87	1.22	1.64	1.82
T_1	1.36	2.43	2.90	1.30	1.83	2.08
T_2	1.67	3.03	3.34	1.67	2.22	2.45
T_3	1.58	2.87	3.12	1.58	1.97	2.23
T_4	1.47	2.64	2.87	1.49	1.82	2.12
T_5	1.40	2.39	2.62	1.40	1.69	1.87
Mean	1.43	2.48	2.78	1.44	1.84	2.09
	T	**M**	**TxM**	**T**	**M**	**TxM**
SEd	0.030	0.021	0.053	0.018	0.013	0.032
CD	0.062	0.043	0.107	0.038	0.027	0.066

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*,, T_3- 20% *Bacillus megaterium*, T_4- 20% *Paenibacillus mucilaginosus*, T_5- 20% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).

Table 12 Effect of microbial inoculants on catalase and peroxidase in TKM 15 paddy seeds under PEG induced stress

	Catalase			**Peroxidase**		
Treatment	**M_0**	**M_1**	**M_2**	**M_0**	**M_1**	**M_2**
T_0	1.05	1.47	1.89	1.11	1.48	1.79
T_1	1.21	1.87	2.30	1.28	1.68	2.00
T_2	1.55	2.50	2.90	1.60	2.10	2.55
T_3	1.46	2.39	2.76	1.52	1.88	2.39
T_4	1.35	2.26	2.58	1.44	1.75	2.23
T_5	1.30	2.10	2.32	1.33	1.70	2.11
Mean	1.32	2.09	2.45	1.37	1.76	2.18
	T	**M**	**TxM**	**T**	**M**	**TxM**
SEd	0.022	0.016	0.039	0.018	0.013	0.032
CD	0.046	0.032	0.080	0.037	0.026	0.065

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*,, T_3- 20% *Bacillus megaterium*, T_4- 30% *Paenibacillus mucilaginosus*, T_5- 40% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).

Table 13 Effect of microbial inoculants on superoxide dismutase and in CO 53 paddy seeds under PEG induced stress

Superoxide dismutase			
Treatment	**M_0**	**M_1**	**M_2**
T_0	1.34	1.57	1.78
T_1	1.47	1.70	1.89
T_2	1.69	2.10	2.47
T_3	1.60	1.98	2.35
T_4	1.57	1.80	2.21
T_5	1.52	1.75	2.00
Mean	1.53	1.81	2.15
	T	**M**	**TxM**
SEd	0.023	0.016	0.040
CD	0.047	0.033	0.082

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*,, T_3- 20% *Bacillus megaterium*, T_4- 20% *Paenibacillus mucilaginosus*, T_5- 20% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).

Table 14 Effect of microbial inoculants on superoxide dismutase in TKM 15 paddy seeds under PEG induced stress

Superoxide dismutase			
Treatment	**M_0**	**M_1**	**M_2**
T_0	1.26	1.54	1.78
T_1	1.33	1.71	1.89
T_2	1.69	2.10	2.47
T_3	1.57	1.94	2.34
T_4	1.49	1.85	2.23
T_5	1.40	1.79	2.10
Mean	1.45	1.82	2.13
	T	M	TxM
SEd	0.020	0.014	0.035
CD	0.041	0.029	0.071

The data are presented as the means ± standard errors of the means from triplicate samples. (T_0- Control, T_1- Hydropriming, T_2- 20% *Azospirillium lipoferum*,, T_3- 20% *Bacillus megaterium*, T_4- 20% *Paenibacillus mucilaginosus*, T_5- 20% *Pseudomonas chlororaphis*, M_0 – Control , M_1 – -4 bar, M_2 – -6 bar).