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OPTIMIZING SUNFLOWER YIELD IN LEAD CONTAMINATED SOIL VIA PGPR INDUCED ANTIOXIDANT DEFENSE MECHANISM

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Abstract
Lead (Pb) is a toxic heavy metal that can have detrimental effects on plant growth and development. Sunflowers are effective in bioremediation, utilizing their extensive root systems to absorb and remove heavy metals and other contaminants from soil and water. PGPR (plant growth promoting rhizobacteria) can enhance plant growth and reduce heavy metal accumulation by sequestering metals in the rhizosphere, thus minimizing their uptake by plants. The present study aims to investigate the efficacy of PGPR in mitigating lead (Pb) stress in sunflowers for which a pot experiment was conducted. The trial was performed according to factorial CRD (Completely Randomized Design) with four replications. Four treatments were used in this trial, L1P1(No Lead + No PGPR), L1P2 (No Lead + PGPR), L2P1 (Lead stress + No PGPR) and L2P2 (Lead stress + PGPR). Half seeds of five sunflower cultivars, FH-825, FH-721, Agsun5270, HSF-350 and T-40318 were inoculated with PGPR and sown in plastic pots containing nutrient rich loam. Lead stress @ 200ppm was applied 37 days after seed sowing. Plants were sampled 21 days after application of lead stress. Seed treatment with PGPR significantly enhanced dry weight of shoot and root, total achene weight per plant and 100 achene weight. Additionally, it increased catalase, SOD, peroxidase activities, total soluble proteins, total phenolic content, ascorbic acid levels. This enhancement suggests that PGPR-treated sunflowers were better equipped to handle oxidative stress induced by Pb compared to non-treated plants. Additionally, PGPR application significantly reduced lipid peroxidation levels, as shown by lower malondialdehyde content in PGPR treated plants under Pb contaminated soil. Among the sunflower varieties tested, T-40318 exhibited robust performance and showed increased tolerance to Pb stress. In contrast, Agsun-5270 cultivar was extremely sensitivity to Pb stress but performed best upon PGPR application compared to all other sunflower cultivars. Therefore, it can be concluded that sunflower cultivars sensitive to Pb stress can even perform better by improving morpho-biochemical attributes, especially antioxidant defence mechanism upon application of PGPR and help in mitigating Pb stress. Hence there is a dire need for incorporation of sustainable strategies, like inoculation of crops seeds with PGPR to improve crop resilience and food security in metal contaminated soils and different agro-climatic conditions.

Key word: Sunflower; PGPR Inoculation; Lead Stress; Antioxidants; ROS; Yield

Introduction

Various biotic and abiotic stresses caused devastating effects on cereal crop production (Lalarukh *et al*., 2022). Heavy metal pollution of soil is caused by anthropogenic activities including inappropriate industrial and farming procedures. Lead after arsenic is the most hazardous metal upon accumulation in arable land along with nutrient deficiency is a major threat to agriculture (Chandwani *et al*., 2023). Lead disrupts vital plant activities, it is a non-essential element that poses serious global hazards to plant development and production (Ayub *et al*., 2024: Khan *et al*., 2021). Excessive levels of lead led to oxidative stress, resulting in detrimental effects on the development and growth of plants because of the disruption of the cellular components and lipid peroxidation in plants (Dwivedi *et al*., 2024; Sharma *et al*., 2020; Ahmed *et al*., 2021). Lead poisoning may damage membranes, proteins, and DNA within cells, which can ultimately lead to the death of the plant (Singh *et al*., 2020). Lead exposure affects the ultrastructure of the chloroplasts, reducing the production of chlorophyll, and impeding electron transport, which prevents C_3 cycle enzymes in plants (Rani *et al*., 2024).

Applying bioremediation methods including the use of beneficial bacterial consortium to eliminate lead from polluted soils is critical for tackling lead toxicity. Techniques such as rhizofiltration and phytoremediation have significantly reduced lead contamination in soils (Kunsah *et al*., 2024). Plant growth-promoting rhizobacteria (PGPR) use in agriculture is one of the existing effective approaches due to their potential ability to enhance plant growth and yield under stressful conditions (Chang *et al*., 2024). Bioremediation strategies using beneficial microbes are gaining popularity due to their eco-friendly properties (Tonelli *et al*., 2024). Akram *et al*. (2022) reported that PGPR application may boost antioxidant defenses and improve membrane stability in sunflower plants upon exposure to heavy metal stress. Furthermore, in stressful circumstances, certain rhizobacterial strains have demonstrated the ability to stimulate root growth, which enables better nutrient and water uptake in wheat (Lalarukh *et al*., 2022). Wheat treated with PGPR inoculation showed a 41.2% increase in antioxidant enzyme activity, synthesis of phenolic compounds in maize by 28.5%, and reduction in oxidative stress (Ramesh *et al*., 2023; Kumar *et al*., 2020). Plant growth-promoting compounds including auxins, cytokinins, and gibberellins are produced by PGPR, which increases plant growth and production (Khan *et al*., 2020). PGPR also produces antibiotics, which suppress soil-borne pathogens and protect plant roots (Singh *et al*., 2020). PGPR penetrates the rhizosphere of plants, promoting mycorrhizal network formation, and improving the structure of soil hence, promoting nutrient absorption (Sharma *et al*., 2020).

Sunflower is a vital crop for the world's economy and ranked fifth among crops that are crucial for assuring food security (Lalarukh & Shahbaz, 2020; Santos *et al*., 2023). Sunflower has several health advantages due to its high level of biologically active substances like phenolics and flavonoids (Martinez *et al*., 2022). Sunflower is an essential source of biofuel and cooking oil (Gupta *et al*., 2022). Sunflowers are highly effective in extracting heavy metals from contaminated soil through phytoremediation (Kumar *et al*., 2020). Heavy metals are stored in vacuoles and membrane-bound organelles, where they are complexed with organic acids and other compounds, reducing their toxicity in plants (Mondal *et al*.,2022). However, it is assumed that plant growth-promoting rhizobacteria can improve the antioxidant defense mechanism of sunflower plants and protect them from the detrimental effects of lead stress. Therefore, the present research aimed to study modulation in lipid peroxidation and antioxidant defense mechanism of sunflower in response to PGPR seed treatment and its effect on dry weight and yield-related attributes.

Materials and Methods

Seed collection and experimental layout:

An investigation on the impact of lead stress and PGPR on sunflower was conducted from February to June 2023 at Government College Women University Faisalabad at Botanic research section. Five sunflower cultivars achenes (FH-825, FH-721, Agsun-5270, HSF-350, and T-40318) were acquired from the oilseed research section of the Ayub Agricultural Research Institute in Faisalabad, Pakistan. Healthy sunflower achenes (300) from each cultivar were treated separately in glass beakers with PGPR (plant growth-promoting rhizobacteria). Each beaker carried 0.5 grams of sugar and 2 grams

of PGPR biofertilizer mixed with 50 milliliters of distilled water. The mixture was stirred well, and the seeds were soaked for half an hour. A PGPR suspension slurry was prepared by continuously stirring the mixture at 100 rpm for 30 minutes before planting. Lead @ 200ppm in the form of lead nitrate solution was added to the soil of half pots 37 days after seed sowing compared to other half (control) given no lead solution. The experiment followed a factorial CRD (Completely Randomized Design) statistical design with four repetitions. Data for various attributes was collected three weeks after applying lead stress at vegetative stage and yield at maturity. At the beginning, 10 sunflower seeds were planted in each plastic pots with uniform depth. Following the thinning process at the three-leaf seedling stage, the number of plants in each pot was six, with only the healthiest ones being retained. Leaves sample were collected 61 days after sowing to measure root and shoot dry weights, lipid peroxidation levels, and antioxidant enzyme activities. At the end of the experiment, yield attribute data were recorded.

Morphological attributes determination

The samples for the shoot and root dry weights were collected, put in paper envelopes, and dried for 48 hours at 70°C in an oven. The samples were weighed using an electronic scale after completely dried to a constant weight.

Biochemical analysis

Samples of fresh leaf (0.5 g) were crushed in cooled pestle mortar, mixed with 10 mL of 50 mM buffer (pH 7.8) at 4°C. The suspension was centrifuged at 12,000 rpm for 20 minutes, and the supernatant was collected and kept at -20°C for enzymatic antioxidant assays. The activities of catalase, peroxidase and SOD were determined on protein basis, providing a comprehensive assessment of activity of enzymatic antioxidant.

Superoxide dismutase activity was evaluated using the Giannopolitis & Ries method (1977). A mixture containing 250 µL of 50 mM phosphate buffer solution, 400 µl of distilled water, 100 µL of methionine, 50 µL of riboflavin, 50 µL of NBT, 50 µL of enzyme extract was taken in cuvette and exposed to a fluorescent lamp for fifteen minutes. Afterwards a UV-Vis spectrophotometer was used to measure absorbance of the mixture at 560 nm.

Catalase and peroxidase activities were analyzed using the method described by Chance & Maehly (1955). For catalase activity, 1 ml of H_2O_2 was added to 1.9 ml of 5.9 mM buffer solution in cuvette. The reaction was initiated by adding 0.1 ml of enzyme extract for two minutes, the absorbance changes were recorded every 20 seconds.

A combination of 750 µL phosphate buffer, 100 µL hydrogen peroxide (40 mM), and 100 µL guaiacol (20 mM) was placed in a cuvette along with 100 µL of enzyme extract for analysis for peroxidase activity. The variations in absorbance at 470 nm were recorded every 20 seconds for a duration of three minutes.

Bradford (1976) provided the method for determining the total soluble proteins (TSP). In a mortar and pestle, fresh leaf (0.5 gram) was chopped and combined with 10 mL of 50 mM buffer (pH 7.8). After centrifugation at 12,000 rpm for 20 minutes at 4°C, 0.5 ml of the supernatant was mixed with 1 ml of Bradford reagent in a test tube. Using a spectrophotometer, the mixture was incubated for 30 minutes at 32°C in darkness. The absorbance was subsequently measured at 595 nm. A standard curve using different concentrations of bovine serum albumin was created to measure the TSP content.

Using the technique outlined by Mukherjee & Choudhri (1983), the ascorbic acid content was determined. Using a mortar and pestle, freshly cut leaf tissue (0.25 g) was mixed in 10 ml of 6% trichloroacetic acid (TCA). It was subsequently centrifuged at 1000rpm for 10 minutes at 4°C. After adding 1 drop of a 10% thiourea solution to 4 ml of residue and 2 ml of 2% dinitrophenyl hydrazine, the entire mixture boiled in a water bath for 20 minutes. At 0°C, 5 ml of 80% sulfuric acid was added after the mixture quickly cooled on ice. To find the ascorbic acid concentration of the resultant combination. The absorbance was recorded at a wavelength of 530 nm.

The total phenolic content was determined using the Julkunen-Titto (1985) technique. After homogenizing 0.1 grams of fresh leaf samples in 2 ml of eighty percent acetone, the specimens were

centrifuged at 10,000 rpm for 15 minutes. At -20°C, the resultant supernatant was stored. 100 µL of the extract of leaves (supernatant) was blended with 2.5 ml of 20% Na_2CO_3, 0.5 ml of Folin-Ciocalteau phenol, and 2 mL of distilled H_2O in a test tube. After vigorously shaking for five to ten seconds, five milliliters of distilled water were added. The mixture was then allowed to react for twenty minutes before measuring the absorbance at 750 nm.

The hydrogen peroxide levels were determined using the technique delineated by Velikova *et al*. (2000). To be more accurate, 0.5 g of freshly crushed leaf material was mixed with 5 ml of 0.1% trichloroacetic acid (TCA), and the mixture was centrifuged at 12,000 rpm for 15 minutes. One milliliter of KI and half a milliliter of phosphate buffer were added to 0.5 milliliter of the resultant supernatant in a sample tube. After vortexing the suspension, the absorbance at 390 nm was determined.

The determination of the amount of malondialdehyde (MDA) in leaves was conducted using the protocol described by Carmak and Horst (1991). First, 10 milliliters of 0.1% Trichloroacetic acid were used to homogenize 0.5 gram of fresh leaf, which was subsequently centrifugated at 12,000rpm for 10 minutes. Four milliliters of 0.5% thiobarbituric acid in 20% TCA were added to one milliliter of the resultant supernatant. The mixture was cooled on ice after thirty minutes of incubation at 95°C in a water bath. Readings of absorbance were obtained at 600 and 532 nm.

Yield attributes

At the completion of the sunflower's life cycle, data was collected on various yield related parameters, including the Capitulum diameter, total weight of achenes plant^{-1}, the number of achenes plant^{-1}, and the 100 achenes weight. These parameters were recorded when the sunflower had fully matured and dried, indicating the end of the growth cycle.

Statistical Analysis

The final data was analyzed using Statistics 8.1 software through the factorial ANOVA test. Variance analysis assessed the effects of lead, cultivars, PGPR treatment, and their interactions on various parameters. Each factor's significance was represented by P-values, with ***, **, and * indicating significance levels of $p<0.001$, $p<0.01$, and $p<0.05$, respectively. Means were compared using the least significant difference (LSD) analysis ($p<0.05$).

Table 1. Mean square values from analyses of variance of data for antioxidants, Malondialdehyde (MDA), hydrogen peroxide (H_2O_2), total soluble proteins, dry biomass and yield related attributes of sunflower plants raised from PGPR treated plants.

Source of variation	df	POD	CAT	SOD	Ascorbic Acid
Lead (Pb)	1	214.04***	416.97***	5.41***	2.14***
Plant Growth Promoter Rhizobacteria (PGPR)	1	43.39***	119.34***	1.12***	0.61***
Cultivars (Cvs)	4	138.04***	174.74***	2.63***	1.42***
Pb × PGPR	1	0.09ns	0.10ns	0.05ns	0.02ns
Pb × Cvs	4	4.92*	4.03ns	0.03ns	0.07**
PGPR × Cvs	4	0.90ns	5.92*	0.006ns	0.02ns
Pb × PGPR × Cvs	4	0.56ns	1.23ns	0.03ns	0.006ns
Error	60	1.81	1.97	0.38	0.018
Source of variation	**df**	**Total Soluble protein**	**MDA**	**H_2O_2**	**Shoot dry Weight**
Lead (Pb)	1	0.0010***	1808.02***	30.90***	100.93***
Plant Growth Promoter Rhizobacteria (PGPR)	1	0.00031***	1695.07***	12.85***	43.54***
Cultivars (Cvs)	4	0.0022***	2439.27***	7.88***	150.88***

Pb × PGPR	1	0.000004ns	0.05ns	0.004ns	0.99ns
Pb × Cvs	4	0.00001ns	115.18**	0.52ns	0.44ns
PGPR × Cvs	4	0.00003ns	165.78***	0.38ns	3.11ns
Pb × PGPR × Cvs	4	0.000009ns	41.40ns	0.17ns	5.14**
Error	60	0.000009	28.23	0.26	1.40
Source of variation	**df**	**Capitulum diameter**	**Number of achene plant^{-1}**	**Total achene weight**	**100 achene weight**
Lead (Pb)	1	56.45***	408694.05***	353.30***	119.61***
Plant Growth Promoter Rhizobacteria (PGPR)	1	31.25***	199600.2***	198.32***	51.55***
Cultivars (Cvs)	4	109.51***	622768.16***	392.17***	68.18***
Pb × PGPR	1	0.31ns	6160.05ns	0.92ns	1.99ns
Pb × Cvs	4	0.7ns	864.89ns	5.04*	0.21ns
PGPR × Cvs	4	0.71ns	2288.73ns	8.79**	1.31ns
Pb × PGPR × Cvs	4	0.37ns	4871.52ns	4.38ns	0.27ns
Error	60	0.898	6560.52	1.97	0.80

* = p≤0.05, ** = p≤0.01, *** = p≤0.001, ns = non-significant df = Degrees of freedom; CAT = Catalase; POD = Peroxidase; SOD = Superoxide dismutase; H_2O_2 = Hydrogen peroxide; MDA = Malondialdehyde

Results

Antioxidants: Imposition of lead stress triggered the activity of peroxidase (POD) enzyme considerably (p≤0.001) in all sunflower cultivars compared to non-stressed plants (Table 1; Fig. 1). Under lead stress conditions, the sunflower cultivars demonstrated varied responses in terms of POD activity, ranked from lowest to highest percentage increase as follows: Agsun5270: 22.41%, FH-721: 23.06%, FH-825: 29.52%, HSF-350: 29.85%, T-40318: 29.95%. Treatment with PGPR led to a considerable increase (p≤0.001) in POD in all sunflower cultivars under lead stress as well as plants grown in control condition. The percentage increase in POD activity due to PGPR application varied among the cultivars, listed from lowest to highest as follows: FH-721: 7.78%, T-40318: 8.31%, FH-825: 9.97%, HSF-350: 11.82%, Agsun5270: 16.22%. Among all cultivars, T-40318 exhibited substantially highest increase in POD activity (p≤0.001) under control and lead stress. Substantial interaction between lead and cultivars (p≤0.05) was observed.

Lead stress caused increase in the activity of catalase (CAT) enzyme considerably (p≤0.001) in all sunflower cultivars compared to non-stressed plants (Table 1; Fig. 1). Under lead stress conditions, the percentage increase in CAT activity from lowest to highest values were as follows: Agsun5270: 48.39%, FH-721: 49.11%, HSF-350: 50.36%, FH-825: 54.07%, T-40318: 58.48%. PGPR treatment led to a substantial increase (p≤0.001) in CAT activity in all sunflower cultivars compared to untreated plants. The percentage increase in CAT activity due to PGPR application varied among the cultivars, listed from lowest to highest as follows: FH-721: 13.63%, HSF-350: 16.14%, T-40318: 14.45%, FH-825: 21.84%, Agsun5270: 30.32%. Overall, T-40318 exhibited the highest increase in CAT activity (p≤0.001) compared to all other sunflower cultivars. Considerable interaction between cultivars and PGPR treated plants (p≤0.05) was noticed.

Exposure to lead stress increased activity of superoxide dismutase (SOD) enzyme considerably (p≤0.001) in all sunflower cultivars compared to non-stressed plants (Table 1; Fig. 1). Under lead stress conditions, the sunflower cultivars demonstrated varied responses in terms of SOD activity, ranked from lowest to highest percentage increase as follows: Agsun5270: 17.47%, FH-721: 21.46%, FH-825: 24.19%, HSF-350: 30.06%, T-40318: 33.72%. Treatment with PGPR led to a significant increase (p≤0.001) in SOD activity in all sunflower cultivars under both lead stress and non-stress

conditions. The percentage increase in SOD activity due to PGPR application from lowest to highest values ranged in sunflower cultivars as: FH-721: 7.17%, HSF-350: 8.94%, FH-825: 9.28%, T-40318: 11.76%, Agsun5270: 10.03%. T-40318 cultivar exhibited substantially highest increase in SOD activity ($p \leq 0.001$) under non-stress and lead stress conditions.
Lead stress exposure caused highly significant rise ($p \leq 0.001$) in AsA (ascorbic acid) concentration in all sunflower cultivars compared to non-stressed plants (Table 1; Fig. 1) ranked from lowest to highest percentage increase as follows: Agsun5270: 25.56%, HSF-350: 27.83%. FH-825: 31.18%, FH-721: 39.13%, T-40318: 42.95%. PGPR application remarkably enhanced ($p \leq 0.001$) the leaf AsA activity in sunflower cultivars as compared to non-treated plants under lead stress. The percentage increase in AsA activity due to PGPR application varied among the cultivars, listed from lowest to highest as follows: FH-721: 6.77%, HSF-350: 10.09%, FH-825: 11.43%, T-40318: 12.35%, Agsun5270: 14.19%. T-40318 exhibited the highest increase in AsA activity ($p \leq 0.001$) both under control and lead stress conditions compared to all other cultivars. Strong interaction between lead and cultivars ($p \leq 0.01$) was observed.

Sunflower plants' exposure to Lead stress showed significant increase ($p \leq 0.001$) in amount of phenolics compared to non-stressed plants Under lead stress conditions, the sunflower cultivars demonstrated varied responses in terms of phenolics activity, ranked from lowest to highest percentage increase as follows: Agsun5270: 26.33%, FH-825: 28.32%, FH-721: 29.32%, HSF-350: 33.17%, T-40318: 35.11%. PGPR treated plants led to a considerable increase ($p \leq 0.001$) in phenolic contents in all sunflower cultivars compared to nontreated plants under lead stress. The increase in ascending order was as follows: FH-721: 6.99%, HSF-350: 11.11%, FH-825: 15.61%, T-40318: 12.69%, Agsun5270: 17.35%. T-40318 exhibited a highly significant ($p \leq 0.001$) overall increase in phenolic content among all sunflower cultivars. High interaction between lead stress and cultivars ($p \leq 0.01$) was noticed.

Total Soluble Proteins: Imposition of Lead stress showed considerable reduction in TSP (total soluble proteins) in all sunflower cultivars ($p \leq 0.001$). The percentage values for TSP from lowest to highest reduction were: T-40318: 14.28%, HSF-350: 17.86%, FH-721: 21.05%, FH-825: 25%, Agsun5270: 29.27%. Application of PGPR remarkably ($P \leq 0.001$) enhanced the TSP in sunflower cultivars as compared to nontreated plants under lead stress (Table 1; Fig. 1).. PGPR showed increase in TSP as: FH-721: 11.76%, T-40318: 12.19%, HSF-350: 14.81%, FH-825: 14.28%, Agsun 5270: 17.14%. Among all these cultivars, T-40318 showed the highest increase in TSP ($p \leq 0.001$) both under lead stress and non-stressed conditions.

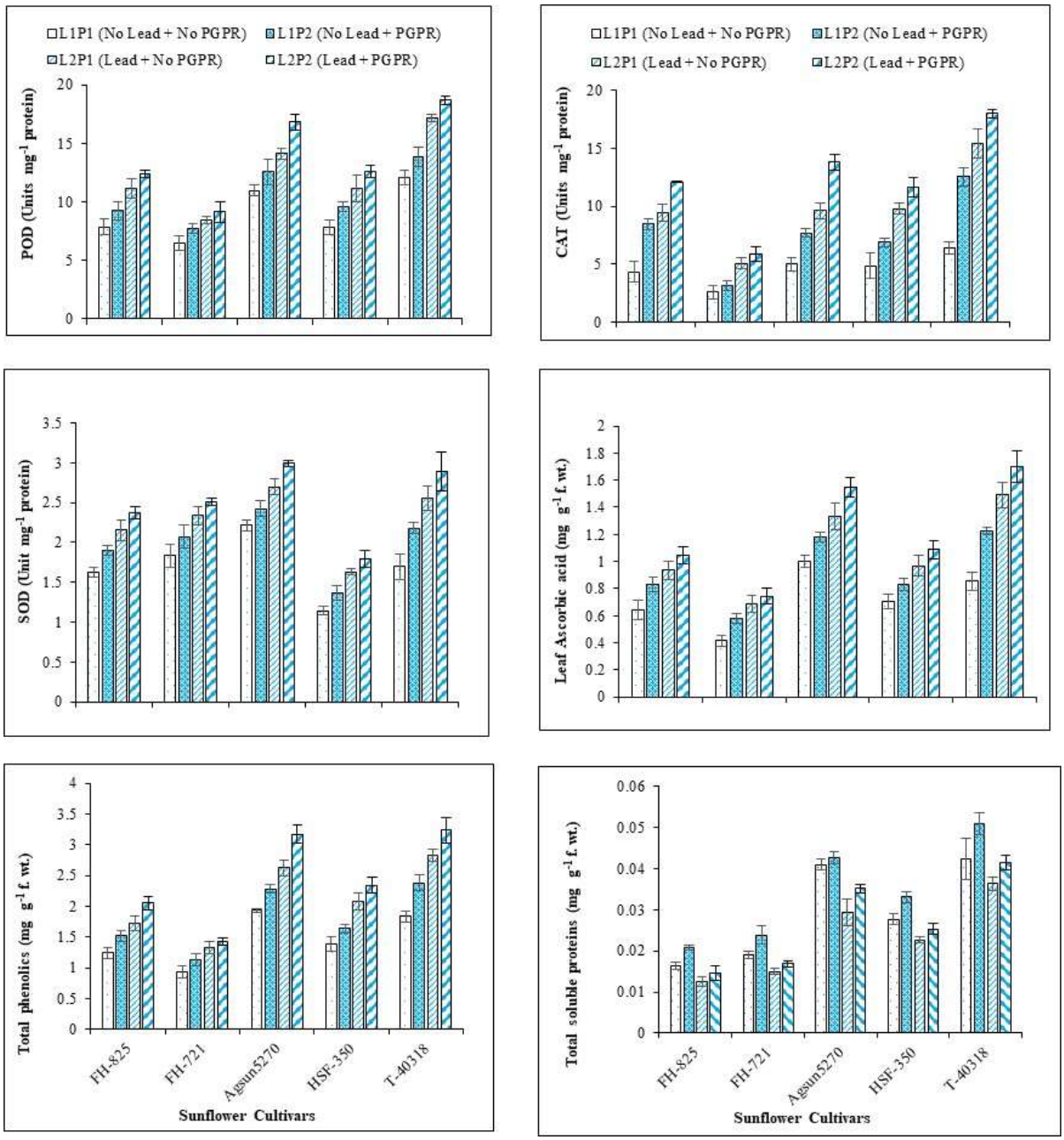

Fig. 1 Antioxidant activities and total soluble proteins of sunflower plants raised from PGPR treatment under lead stress and control conditions.

Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2): Lead stress showed highly ($p \leq 0.001$) significant but variable response in production of malondialdehyde (MDA) content in sunflower cultivars (Fig. 2; Table 1). Varieties exhibiting the highest increase in MDA under lead conditions, listed from lowest to highest: FH-721: 14.66%, HSF-350: 17.53%, FH-825: 19.09%, Agsun5270: 28.53% whereas, Cv.T-40318 showed 4.83% decrease in MDA content in lead stress compared to non-stressed plants. Among the varieties, Agsun-5270 revealed the most marked increase (28.53%), signifying its limited ability to tolerate lead stress. Conversely, T-40318 demonstrated the decrease (4.82%) in MDA content, suggesting greater resilience to lead stress. PGPR treated plant caused substantial reduction ($p \leq 0.001$) of MDA content. The percentage decrease in MDA content due to PGPR application varied among the cultivars: Agsun 5270: 8.48%, HSF-350: 15.11%, FH-825: 16.00%, FH-721: 17.85%. Whereas Cv. T-40318 showed 9.34% increase in MDA content in PGPR treated plants under lead stress. Among all sunflower Cvs. T-40318 exhibited overall least ($p \leq 0.001$) production of MDA content. Strong interactions were observed between cultivars and lead stress ($p \leq 0.01$) and between cultivars and PGPR ($p \leq 0.001$).

Obtruding lead stress substantially increased ($p \leq 0.001$) hydrogen peroxide (H_2O_2) level in all cultivars. Varieties exhibiting the highest increase in level of hydrogen peroxide under lead conditions, listed from lowest to highest: T-40318: 11.22%, FH-721: 20.17%, FH-825: 21.95%, HSF-350: 22.31%, Agsun5270: 28.50%. Among the varieties, Agsun-5270 revealed the most marked increase (28.50%), signifying its less ability to tolerate lead stress. Conversely, T-40318 demonstrated the least increase (11.22%) in hydrogen peroxide level, suggesting greater resilience to lead stress. PGPR treated plant caused substantial reduction ($p \leq 0.001$) of hydrogen peroxide levels as Agsun 5270: 3.37%, FH-825: 16.66%, HSF-350: 18.01%, FH-721: 18.02% whereas T-40318 showed increase in H_2O_2 level to 4.99%. Among all cultivars, T-40318 exhibited least hydrogen peroxide ($p \leq 0.001$) production under both lead stress and non-stressed conditions (Fig. 2; Table 1).

Dry biomass: Lead stress drastically reduced ($p \leq 0.001$) dry weight of shoot in all sunflower cultivars (Fig. 2; Table 1). Under lead stress conditions, the sunflower cultivars demonstrated varied responses in terms of shoot dry weight, ranked from lowest to highest percentage reduction as follows: T-40318: 13.97%, FH-825: 17.61%, FH-721: 16.93%, HSF-350: 18.52%, Agsun5270: 35.24%. PGPR application considerably ($P \leq 0.001$) enhanced shoot dry weight in sunflower cultivars as compared to nontreated plants under lead stress condition as listed from lowest to highest percentage increase: FH-721: 16.27%, FH-825: 16.82%, HSF-350: 13.77%, T-40318: 16.16%, Agsun 5270: 36.48%. Among all cultivars, T-40318 exhibited substantially highest increase in shoot dry weight ($p \leq 0.001$) under control and lead stress. Furthermore, the interaction among lead stress, PGPR, and sunflower cultivars was significant ($p \leq 0.01$). Imposition of lead stress caused ($p \leq 0.001$) substantial reduction in root dry weight in all sunflower cultivars ranking from lowest to highest percentage reduction as follows: T-40318: 14.14%. HSF-350: 16.14%, FH-825: 19.42%, FH-721: 22.91%, Agsun5270: 36.68%. PGPR treated plant resulted in a considerable increase ($p \leq 0.001$) in dry weight of root under lead condition in all sunflower Cvs. listed from lowest to highest percentage increase as: FH-721: 4.37%, T-40318: 9.07%, HSF-350: 9.12%, FH-825: 22.06%, Agsun 5270: 22.22%. T-40318 sunflower cultivar showed the highest increase in root dry weight ($p \leq 0.001$) both under lead stress and non-stressed conditions (Fig. 2; Table 1).

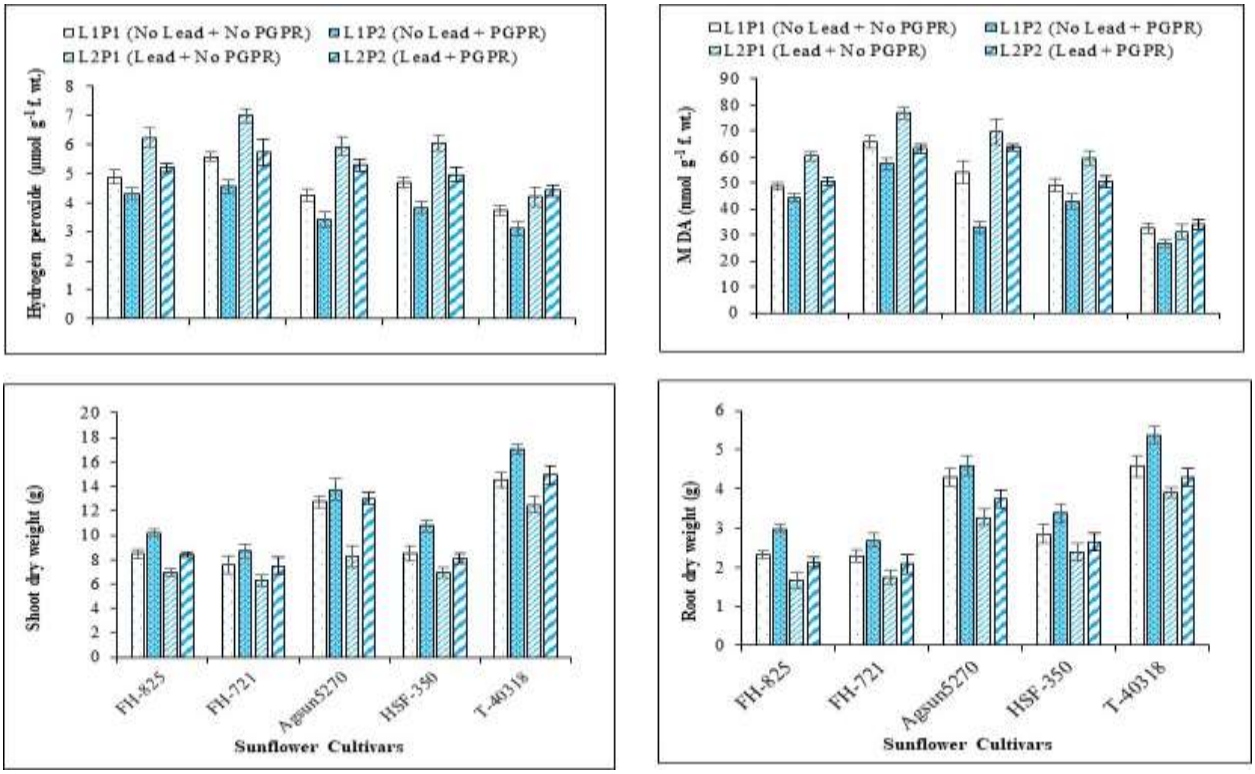

Fig. 2. Leaf malondialdehyde (MDA), hydrogen peroxide (H_2O_2), shoot and root dry weight of sunflower plants raised from PGPR treatment under lead stress and control conditions.

Yield: Lead stress showed highly substantial reduction ($p \leq 0.001$) in capitulum diameter in all sunflower cultivars (Table 1; Fig. 3). Under lead stress conditions, the sunflower cultivars demonstrated varied responses in terms of capitulum diameter, ranked from lowest to highest percentage reduction as follows: T-40318: 11.26%, FH-721: 14.28%, HSF-350: 14.73%, FH-825 17.85%, Agsun5270: 29.54%. The application of PGPR ($p \leq 0.001$) to sunflower cultivars under lead stress increased capitulum diameter compared to untreated plants under lead stress listed from lowest to highest percentage increase as: FH-721: 5.26%, T-40318: 15.75%, FH-825: 16.30%, HSF-350: 16.95%, Agsun 5270: 21.87%. T-40318 showed the highest increase in capitulum diameter under both lead stress and non-stressed conditions among all sunflower cultivars. Number of achenes plant^{-1} substantially reduce ($p \leq 0.001$) under lead stress in all sunflower as follows: T-40318: 6.77%, FH-721: 16.74%, HSF-350: 18.35%, FH-825: 18.62%, Agsun5270: 19.39%. PGPR treated plant caused a significant increase ($p \leq 0.001$) in number of achenes plant^{-1} in comparison to no PGPR given plants under lead condition as: FH-721: 4.66%, T-40318: 5.39%, FH-825: 10.21%, HSF-350: 14.56%, Agsun 5270: 17.22%. The number of achenes per plant was remarkably higher ($p \leq 0.001$) in T-40318 than all other cultivars (Table 1; Fig. 3). Lead stress showed highly substantial reduction ($p \leq 0.001$) in total achene weight in all sunflower cultivars, ranked from lowest to highest percentage reduction as follows: T-40318: 15.01%, HSF-350: 24.69%, FH-721: 26.25%, FH-825: 26.91%, Agsun5270: 37.59%. Treatment with PGPR caused a significant increase ($p \leq 0.001$) in total achene weight compared to nontreated plants in all sunflower cultivars under lead stress. PGPR application showed varying percentage increase in total achene weight values as: FH-721: 12.12%, T-40318: 14.51%, FH-825: 17.08%, HSF-350: 21.57%, Agsun 5270: 36.71%. Sunflower Cv. T-40318 showed maximum ($p \leq 0.001$) increase in total achene weight than all other sunflower cultivars. A significant interaction ($p \leq 0.05$) was observed between cultivars and Pb stress. Furthermore, a noteworthy interaction ($p \leq 0.01$) between cultivars and PGPR was noted in this attribute (Table 1; Fig. 3). Imposition of lead stress caused considerable reduction ($p \leq 0.001$) in 100 achene weight in all sunflower cultivars as follows: T-40318: 19.89%, FH-825: 22.46%, HSF-350: 22.89%, FH-721: 30.17%, Agsun 5270: 31.25%. PGPR treatment caused considerable increase ($p \leq 0.001$) in weight of 100 achene under both lead stress and non-stressed conditions. PGPR application varied among the cultivars, listed from lowest to highest increase in percentage values as: FH-721: 4.77%, FH-825: 12.96%, HSF-350: 17.2%, T-40318: 16.68%, Agsun 5270: 24.05%. Among tall sunflower cultivars T-40318 exhibited highest increase in 100 achene weight ($p \leq 0.001$) under control and lead stress (Table 1; Fig. 3).

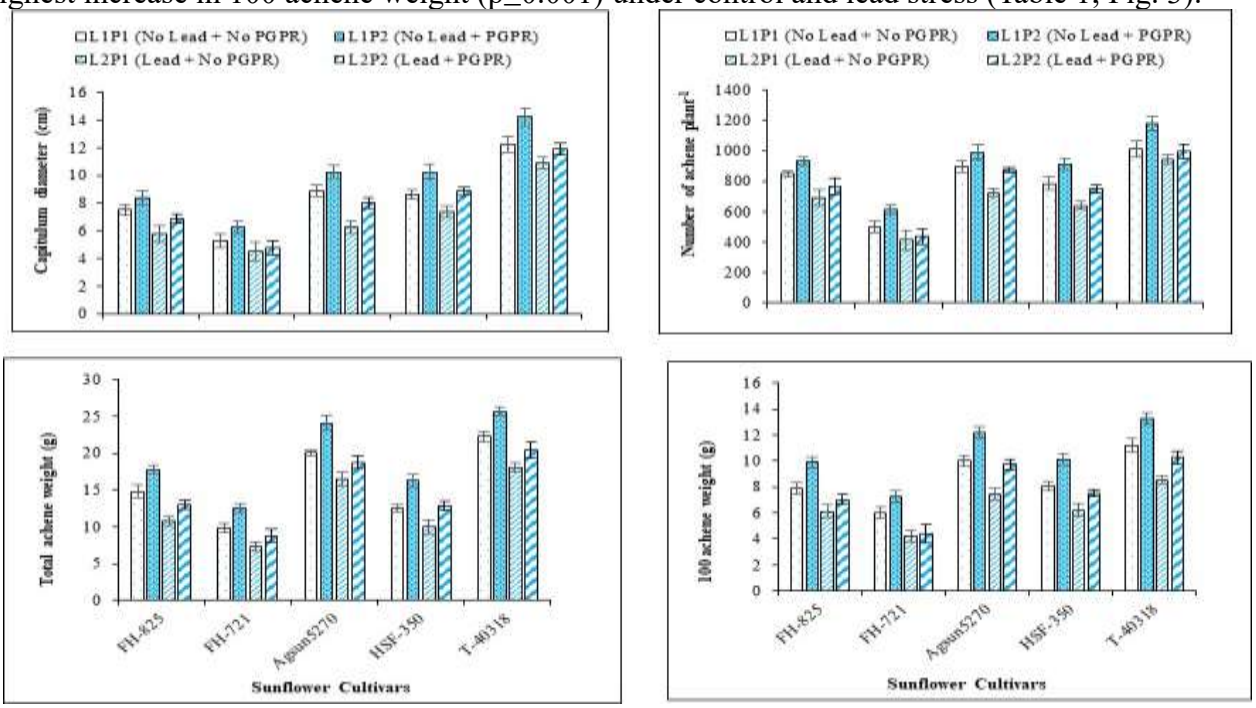

Fig. 3. Yield related attributes of sunflower plants raised from PGPR treatment under lead stress and control conditions.

Discussion

Heavy metal pollution in the environment has become a significant threat, likely caused by both natural factors and human activities affecting the environment (Zahid *et al*., 2024). To achieve the best plant growth and productivity, sustainable methods must be implemented to address these harmful contaminants (Rehan *et al*., 2023). Our study demonstrated that integrating PGPR (*Pseudomonas* spp, *Bacillus* spp, *Azola*, *Azotobacter* spp) into soil significantly alleviated lead toxicity in sunflower plants, promoting growth, enhanced stress tolerance, and better adaptability in lead-contaminated environments. Lead (Pb) is a toxic heavy metal that can have detrimental effects on plant growth and development. Exposure to lead increases the production of reactive oxygen species (ROS) in plants by impeding electron transport chains in chloroplasts and mitochondria. Furthermore, lead hampers antioxidant enzymes, intensifying oxidative stress and disrupting cellular functions. These factors significantly impact plant growth and physiology under stress conditions (Fu *et al*., 2023; Sharma *et al*., 2023). Antioxidant enzymes scavenge ROS and help as indicators of plant defense against stress (Shaheen *et al*., 2024). Enzymatic antioxidants including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) can defend plants from oxidative damage (Azeem *et al*., 2022). Improved resistance to abiotic stress is directly related to increased antioxidant activity (Lalarukh & Shahbaz, 2020a). Our study demonstrated a significant increase in activity of antioxidant enzymes (SOD, POD, CAT) and the levels of phenolics, and AsA (non-enzymatic) contents in sunflower under lead stress. Like our findings Singh *et al*. (2022) and Kumar *et al*. (2020) reported an increase in antioxidant activities in maize and wheat respectively. On the other hand, Sarkar *et al*. (2021) and Cheema *et al*. (2023) reported substantial decrease in antioxidants in chickpea and soybeans upon exposure to lead stress respectively. Superoxide dismutase (SOD) is a crucial enzyme that eliminates reactive oxygen species (ROS) and serves as the primary line of defense. SOD utilizes various substrates to donate electrons, converting the superoxide anion into hydrogen peroxide (H_2O_2). Additionally, enzymes such as ascorbate peroxidase (APX), catalase (CAT), and peroxidase (POD) break down H_2O_2 into molecular oxygen and water. This process also helps reduce lipid peroxidation and prevent membrane damage by converting lipid hydroperoxide into alcohol (Rajput *et al*., 2021). Total phenolics, the most abundant and significant class of secondary metabolites, play a crucial role in enhancing stress resistance (Salam *et al*., 2023). Phenolics can bind lead ions, forming complexes that reduce the ions mobility and toxicity (Magray *et al*., 2023). These powerful antioxidants can detoxify reactive oxygen species (ROS) and protect plants from adverse effects by modulating the phenylpropanoids pathway (Hatami, 2023). Ascorbic acid (AsA) is the most common antioxidant essential for various biological processes in plants (Chaturvedi *et al*., 2022). It primarily acts as a redox buffer, converting H_2O_2 to H_2O through the APX reaction, and quickly scavenging $O_2^{\bullet-}$, OH^-, and $^1O_2$ under abiotic stress (Liang *et al*., 2019). In this research, the treatment of PGPR notably boosted the antioxidant defense mechanisms of plants under lead (Pb) stress. The inoculation with PGPR seemed to reduce negative effects of lead stress by increasing the activity of key antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). This is consistent with findings from other research that show PGPR can trigger the upregulation of plant defense genes and enzymes responsible for ROS detoxification (Khan *et al*., 2023; Hossain *et al*., 2022). Zhou *et al*. (2024) found that PGPR treatment increased the levels of antioxidants in rice under heavy metal stress, leading to better growth and reduced metal toxicity. According to Sahoo *et al*. (2024) additionally, PGPR strains contribute to the reduction of Pb uptake by enhancing root development and altering soil microbial communities, which can decrease the bioavailability of toxic lead ions to plants. Moreover, the non-enzymatic antioxidants, such as ascorbic acid and phenolics, were found to be elevated in PGPR-inoculated plants, further supporting the enhanced antioxidative response. This finding suggests that PGPR-induced changes in antioxidant levels not only protect against oxidative stress but may also play a role in lead detoxification by reducing Pb accumulation in plant tissues. Our findings correspond with those of Qadir *et al*. (2024), who discovered that PGPR treatment raised antioxidant activity of enzymes in cultivars of sunflower. Our results also concur with those of Mhlongo *et al*. (2020), who found that PGPR-treated cotton plants showed higher amounts of proteins and phenolics. Alshaal *et al*. (2024) showed that sunflower cultivars exhibited different responses to

PGPR treatment and resilience to heavy metal stress. This highlights the potential of PGPR to mitigate lead stress in sunflowers and other crops, as demonstrated by research conducted on maize (Srivastava *et al*., 2023) and wheat (Rajendran *et al*., 2022). Our study highlights the essential role of enzyme activity as antioxidants in plant stress tolerance, in addition to phenolics AsA and protein content.

Total Soluble Protein (TSP) in plants represents the overall amount of proteins dissolved within the cells, crucial for metabolic processes, growth, and stress responses. In normal conditions, TSP is essential for enzyme activities, transport functions, and regulating various biochemical pathways (Bacher *et al*., 2022). Our results showed that total soluble protein decreased under lead stress condition. Lead toxicity frequently hampers protein synthesis and stability, resulting in lower TSP levels, which compromises crucial plant functions like enzyme activity and defense mechanisms. Moreover, lead accumulation causes oxidative stress, potentially damaging proteins through carbonylation and denaturation, further reducing TSP levels (Kou *et al*., 2022). Our results agreed with those obtained by Khan *et al*. (2020b) who described that lead stress highly reduced the protein content in *Brassica napus* plants and Lalay *et al*. (2024) observed reduction of protein content in lentil plants. PGPR inoculation boosts the total soluble protein content in plants by improving nitrogen uptake and assimilation (Sharma *et al*., 2022). Moreover, PGPR generates plant hormones and enzymes that promote protein synthesis, enhancing plant growth and productivity (Kour *et al*., 2024). Our research shows that PGPR boosts total soluble protein (TSP) levels in sunflower, aligning with previous studies on other crops. Ahemad and Kibret (2014) reported a 22.1% increase in TSP content in wheat due to PGPR inoculation compared to non-inoculated controls. Similarly, Kaushal and Singh (2017) observed a 15.6% rise in TSP content in soybean with PGPR application. These findings in sunflower are consistent, indicating that PGPR positively affects TSP content across various plant species.

Lead stress induced reduction in root and shoot dry weight is by impeding photosynthesis, disrupting nutrient absorption, and causing oxidative stress (Dalyan *et al*., 2020), which harms cellular components and reduces biomass (Chen *et al*., 2021). It also prevents cell division and elongation in roots and disrupts hormonal balances essential for growth (Singh *et al*., 2023). Our research showed that lead stress significantly reduced the dry weight of the shoots and roots. This is compatible with other sorghum research (Hussain *et al*., 2022), cotton (Ahmed *et al*., 2021), and rice (Khatun *et al*., 2021). More specifically, the T-40318 cultivar exhibits excellent resistance to lead stress, same as investigated by Pandey *et al*. (2023) in barley, but the FH 721 cultivar showed poor performance under lead stress, consistent with findings of Gupta *et al*. (2024) in millet. PGPR induced enhancement in root and shoot dry weight under lead stress might be by production of phytohormones such as indole-3-acetic acid (IAA) that stimulate root development, and by synthesizing ACC deaminase, which lowers stress-induced ethylene levels, thereby promoting plant growth (Gowtham *et al*., 2020). Additionally, PGPR enhance nutrient uptake by solubilizing phosphates and producing siderophores, thereby increasing the plant's resilience to heavy metal stress (Danish *et al*., 2021; Sagar *et al*., 2022; Pattnaik *et al*., 2021 and Cui *et al*., 2022). According to our research, PGPR greatly increased the shoots and roots dry weight of sunflower cultivars, which is similar to findings from other studies on wheat (Zafar *et al*., 2022; Singh *et al*., 2020), maize (Rajput *et al*., 2018), and soybeans (Cid *et al*., 2020).

When subjected to lead stress, plants suffer oxidative damage from an excess of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) (Shehzad *et al*., 2023). These ROS harm cellular structures, especially membranes, resulting in lipid peroxidation. Malondialdehyde (MDA) indicates this damage. Lead exposure disrupts the equilibrium of ROS scavenging mechanisms, heightening oxidative stress and hindering plant growth (Hasanuzzaman *et al*., 2024). Our results demonstrated that sunflower plants subjected to lead stress remarkably enhanced the MDA and H_2O_2 content by impairing antioxidant defense system that enhance ROS production and cause lipid peroxidation which produce H_2O_2 and MDA as an indicator of oxidative stress. Al-Turki *et al*. (2023) found that PGPR treatment enhanced the expression of antioxidant genes, increasing the activity of antioxidant enzymes, which in turn reduced superoxide levels and shielded the chloroplast from the harmful effects of reactive oxygen species (ROS).The results indicate that the PGPR may successfully

alleviate the adverse effects of drought and lead on sunflower plants, highlighting the potential of PGPR as a useful tool in environmentally friendly farming for a range of crops. In our investigation, we found that applying PGPR to sunflower under lead stress dramatically reduced the levels of MDA and hydrogen peroxide. This finding aligns with previous research demonstrating the protective qualities of PGPR against reactive oxygen species in plants (Singh *et al*., 2020; Zafar *et al*., 2022). The determined reduction in H_2O_2 levels aligns with that reported by Liu *et al*. (2024) in plants under abiotic stress that were given PGPR treatment. Jiang *et al*. (2019) and Ahmed *et al*. (2021), also reported the beneficial impact of PGPR on plant development and stress tolerance. Furthermore, Ali *et al*. (2022) discovered enhanced abiotic stress resistance in PGPR-treated plants, while Chen *et al*. (2021) demonstrated more growth and production in wheat plants under abiotic stress with PGPR treatment.

Our research demonstrated Lead stress diminishes sunflower yield by adversely impacting essential growth factors like capitulum diameter, total achene weight, and 100 achene weight. This reduction is mainly due to oxidative stress, which damages cellular structures (Alkio & Grimm, 2013). The oxidative damage interferes with nutrient and water absorption, impairs photosynthesis, and restricts reproductive growth, resulting in smaller flowers and lower seed production (Mosupiemang *et al*., 2024). These findings are consistent with research by Hussain *et al*. (2024), who reported significant reductions in sunflower seed weight under heavy metal stress. Likewise, Bakhoum *et al*. (2020) reported reduction in yield parameters of chickpea under lead stress. Our study demonstrated that the treatment of PGPR in sunflower cultivars causes considerable increases in capitulum diameter, total achene output per plant, total achene weight, and 100-achene weight. Similarly previous research showed that PGPR had positive effects on plant growth and development (Maqsood *et al*., 2021). On the other hand, lead stress significantly lowers these parameters, reflecting earlier findings on the negative impact of heavy metals on plant growth and development (Singh *et al*., 2020; Sharma *et al*., 2021). Our results align with those of Khalid *et al*. (2020), who observed higher achene production and capitulum diameter in sunflower cultivars treated with PGPR. The resistance of the T-40318 cultivar to lead stress that we found in our investigation aligns with the results of Roychoudhury & Tripathi (2020), who stated that certain sunflower cultivars have a comparable tolerance to heavy metal stress. Therefore different cultivars displayed varying levels of resistance to heavy metal stress and reaction to PGPR treatment which was confirmed by the differences in responses of sunflower cultivars to lead stress and PGPR treatment observed in our research.

Conclusion: PGPR seed treatment significantly increased dry biomass production and improved yields like 100 achene weight and total achene weight by mitigating the harmful effects of lead stress on sunflower plants. PGPR's function in regulating the system of antioxidants and lowering lipid peroxidation is probably responsible for this improvement. PGPR treated plants significantly enhanced the activities of the enzymatic antioxidant's catalase, superoxide dismutase and peroxidase, as well as elevated the non-enzymatic antioxidants level such as total phenolics and AsA. This led to a notable reduction in malondialdehyde (produced as the result of lipid peroxidation) and hydrogen peroxide levels (major stress indicators) in plants treated with PGPR. The sunflower cultivar T-40318 showed improved tolerance to lead stress and outperformed other cultivars. Therefore, growing sunflower cv. T-40318 in lead-contaminated soils is recommended, as PGPR seed treatment effectively protects sunflower plants from lead stress and enhances yield and biomass production. Moreover, from the findings of this research it is concluded that even the Pb stress sensitive cultivar Agsun-5270 showed outstanding performance in growth, yield and biochemical attributes when treated with PGPR. Further it is recommended that PGPR application in various oil seed, leguminous and cereal crops can be beneficial in improving crops resilience to different abiotic stresses and agro-climatic conditions.

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