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MITIGATING LEAD TOXICITY IN *TRITICUM AESTIVUM* L. THROUGH *ASPERGILLUS TERREUS* INOCULATION: A PROMISING BIOREMEDIATION STRATEGY

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Abstract

The study aimed to assess pre-characterized endophytic plant growth promoters *Aspergillus terreus* SQU14026 ($1x10^8$ conidia per ml in distilled water) for their potential in alleviating lead contamination's toxic effects on wheat cultivars exposed to varying lead concentrations. *A. terreus* exhibited the highest MTC value (3000 ppm) for Pb2+. Wheat cultivar grains were inoculated in a greenhouse experiment with endophytic fungal spores. The effect of grain inoculation treatment on mitigating lead ions' adverse effects on morphological and physiological parameters, lead accumulation, and translocation in wheat cultivars' different plant parts was evaluated at two stages (after 60 days of sowing and at harvest stage). The spore inoculation treatment enhanced all morphological and physiological parameters of wheat grown in lead-free soil compared to non-inoculated controls. Using lead-tolerant *A. terreus* to inoculate wheat plants alleviated the harmful impact of lead and enhanced their growth and physiological attributes under lead-contaminated conditions, in contrast to treatments without inoculation. Lead contamination negatively affected the growth parameters and physiological features of plants at all concentrations of lead stress. Applying inoculants to wheat grains reduced the translocation of lead ions from the root to the shoot and then to the grains.

Key words: Endophytes, Wheat cultivars, Lead toxicity, Plant growth promoters.

Introduction

The widespread contamination of land with heavy metals, some of the most dangerous inorganic contaminants, has resulted from the use of biosolids, chemical treatments, and agricultural amendments, as well as pollutants from municipal trash incineration, automobile exhaust, and metal mining residues.

Lamhamdi *et al*., (2011) reported that heavy metal pollution has a countereffect on plant growth and poses a high risk to animals and humans. Lead contamination in the environment has developed due to industrialization, urbanization, fertilizers with heavy metal components, and automotive exhaust (Adriano, 2001). For plants, lead is not an essential element; however, they are able to consume it if it is available in their surroundings. Lead is considered as a toxic environmental pollutant that poses as a danger to all living organisms. The accumulation of lead in plants was negatively associated with plant growth morphological characters. Lead accumulation was linked with increased lipid peroxidation and soluble protein concentrations, as well as enhanced esterase activity (Adriano, 2001).

Microorganisms can significantly improve the effectiveness of phytoremediation. by facilitating the transfer of metals from the soil to root cells, aiding in bioaccumulation or transferring metals from roots to shoot tissues. Additionally, they can improve phytoextraction or stabilize metal presence in the soil (Ma *et al*., 2013). Due to their numerous mechanisms that can break down complex molecules, influence the absorption of heavy metals by biological systems, and decompose chemical contaminants, Endophytes are regarded as a highly promising strategy to addressing the challenges posed by heavy metal pollution (Li, *et al*., 2012). Microorganisms that enhance plant growth (PGPMs) are more advantageous in remediation than any chemical alternates; this is because the metabolites produced by the microorganisms are environmentally friendly and have lower toxicity (Rajkumar, *et al*., 2012). Microorganisms that enhance plant growth (PGPMs) were found to be resistant to metal, boost plant development, diminish metal toxicity, and fixate metals in the soil. This resulted in the creation of innovative restoration methods and microbe-assisted phytoremediation strategies (Ma, *et al*., 2011).

Microbes that produce phytohormones modify the internal hormone production of plants (Sorty *et al*., 2016) causing the shape of roots to change and plants develop resistance to stress produced by toxic metals.

In the aforementioned circumstances, employing mycorrhizal fungi and microorganisms that promote plant growth enhances plant development (Ashraf *et al*., 2017). It provides an environmentally responsible solution to safeguard plants from stressful situations (Kumar & Verma, 2018). These microorganisms are involved in various activities, such as preventing the penetration of metals, efflux volatilization, EPS preservation, metal complexity, and enzymatic detoxification. Additionally, these plant-associated microorganisms enhance plant health and progress by lowering ethylene concentrations, generating growth regulators like ACC deaminase and IAA, and suppressing disease. (Glick, 2010). Thus, this research aimed to explore the possible effects of the symbiotic relationship between the endophytic fungus *Aspergillus terreus* SQU14026 and wheat (*Triticum aestivum*) on the growth and yield of wheat plants, along with the accumulation and movement of lead in various plant components (roots, shoots, and grains) in artificially polluted soil with lead.

Material and Method

Organism: The pre-characterized endophytic plant growth hormones (GA and IAA) producer *Aspergillus terreus* SQU14026 Ghoniemy *et al*., (2020) was assessed for plant growth promotion to lead stress by utilizing wheat cultivar as a test crop.

Determination of Pb^{2+} maximum tolerance concentration (MTC): The dilution method was used to ascertain *Aspergillus terreus* SQU14026's tolerance to Pb^{2+}. Metal ion was added to Czapek Dox-Medium at amounts ranging from 1 to 4000 parts per million. The dishes received 8-mm agar plugs containing young fungal colonies, previously cultivated on Dox-medium. Each concentration was tested in triplicate, in addition to a control without any metals, were performed. The inoculation dishes were kept at 28±2°C for at least a week. The influence of heavy metals on the growth of the isolates was evaluated by measuring the colony expansion radius (in millimeters) and comparing it to the control group (medium without metal). This comparison was used to establish the Minimum Tolerated Concentration (MTC).

Plant growth regulators (indole-3-acetic acid and gibberellic acid) in response to varying metal ion concentrations: The examined fungus was tested for its ability to generate plant growth regulators (indole-3-acetic acid and gibberellic acid) at various Pb^{2+} concentrations (200, 600, and 1000 ppm). The filtrate was calibrated in accordance with Shindy *et al*., (1973) and Sarwar *et al*., (1992) respectively, for the measurement of indole acetic acid (IAA) and Gibberellic acid. Indole acetic acid and GA standard curves that had already been prepared were used to calculate the data (Udaguwa *et al*., 1961).

Greenhouse experiments: In a fenced area at the Faculty of Science (girl's branch), Al-Azhar University, Cairo, Egypt a pot experiment was carried out in the winter of 2020–2021 to examine the impact of fungal endophytic inoculation of wheat grains on the growth, certain metabolic constituents as well as yield components under lead contamination. Grains were sowed in ceramic pots. (diameter 37 cm, height 39 cm).

Each pot had 20 kg of used soil. The soil's physical and chemical characteristics, along with its lead ion concentration are listed in (Table 1). The wheat *(Triticus aestivum* cultivar Sakha 93) grains utilized in the study were supplied by the Crop Field Institute at the Agriculture Research Centre. NPK fertilizer was administered to the plants at a dosage of 80 kg nitrogen, 30 kg phosphorus (as P_3O_5), and 15 kg potassium (as K_2O) per feddan. The fertilizers were added as potassium sulphate (20.5%), ammonium nitrate (33.5%), and superphosphate (15.5%), respectively. The nitrogen was applied in three doses: one time prior to planting and the other two every three weeks, as advised by the Agriculture Ministry, superphosphate and potassium sulphate were added prior to sowing. The soil utilized for conducting this experiment was sourced from agricultural land in Kafr El-Sheikh governorate.

Table 1. Some physical, chemical properties and lead ions in the used soil.

Particle size %	Sand	69.25
	Silt	10.50
	Clay	20.25
Soil texture		Sandy loam soil
Ph		6.88
EC mm hos/cm		0.62
Soluble cations meq/L	Ca^{++}	13.7
	Mg^{++}	6.23
	Na^{+}	7.74
	K^{+}	3.98
Soluble anions meq/L	CO_3^{-}	0.00
	HCO_3	2.22
	Cl^{-}	1.74
	SO_4^{--}	0.33
Organic carbon %		2.00
Organic matter %		3.44
Pb^{2+}		0.004 µg/g

Various concentrations of lead were introduced in the form as a solution of nitrate at rates of 200, 600, and 1000 mg/ kg of soil, alongside a control group that did not receive any additional heavy metals. The soil was treated with heavy metal before planting.

The grains were placed in 95% ethyl alcohol for 5 minutes, then washed three times in sterile deionized water. Next, they were soaked in 1.5% sterile sodium hypochlorite for 10 minutes, followed by three rinses in sterile deionized water, and finally thoroughly rinsed with distilled water.

Fifty grams of wheat grains were exposed to 10 ml of a suspension containing endophytic fungal spores ($1x10^8$ conidia per ml in distilled water) for a period of two hours. Following this, the grains were dried using sterile tissues for 30 minutes. The processed seeds were subsequently planted in pots, with a soil depth of 4-5 cm. Tap water was used for watering until complete germination took place. Following germination, the seedlings were reduced to ten evenly spaced specimens per pot. Watering was carried out as required to keep the soil moisture level at 70% of its maximum capacity.

Physical and chemical analyses of soil: The soil samples, after drying in the air, were finely pulverized into powder and Filtered with a 2 mm mesh sieve and well combined in accordance with guidelines of Piper, (1947).

The soil's physical and chemical characteristics were assessed using standard procedures outlined by Page *et al*., (1982) and Clark *et al*., (1986). To determine pH, a suspension with a soil-to-water ratio of 1:2.5 (w/v), the solution was created and a pH meter was used to measure it. Electrical conductivity was gauged using a meter following Rachit *et al*., (2016) method. Organic carbon (OC) and organic matter percentage was quantified using Zeng *et al*., (2011) technique. Inductively coupled plasma spectrometry was used to assess the concentration of lead present in the soil, following Soltanpour & Schwab's (1977) approach.

Sampling: Plants were harvested at intervals of 60- and 130-days post-planting, samples of roots and shoots were gathered in order to represent the heading stage. The following analyses were conducted:

Growth measurements: Dry weight (g/plant), fresh weight (g/plant), shoot length (cm/plant), and leaf area (cm/plant) were measured.

Plant metal concentrations: To assess the levels of N, P, K, and Pb^{2+}, plant powder samples were subjected to acid digestion based on the method outlined by Jones, (2001).

Protein nitrogen levels were determined using an adapted micro-Kjeldahl method as outlined by Peach & Tracey, (1956). The phosphorus level measured according to Humphries, (1956). Flame photometry was used to estimate the Potassium concentration was measured by Flame photometry method (Rowell, 1994). The concentration of potassium, phosphorus, and nitrogen were represented as grams per 100 grams of dry weight, while Pb^{2+}concentration as µg /g of dry tissue weight. At the time of harvest, which was 130 days after planting the following measurements were determined, the spike's length, shoot length (cm), and weight of 100 grains.

During the heading stage, the experimental data was recorded as follow:

Photosynthetic pigments were quantified using the technique outlined by Metzner *et al*., (1965) and presented as mg/g of fresh weight. Proline levels were analyzed in accordance with methodologies by Bates *et al*., (1973) and reported as mg/g of dry weight. The total soluble sugar content was measured following the phenol-sulfuric acid methodology created by DuBois *et al*., (1956) and expressed as grams per 100 grams of dry weight. Peroxidase and catalase activities were assessed using modified methods from Kong *et al*., (1999) & Chen *et al*., (2000), with activity reported as µg /g of fresh weight. Lipid peroxidation was measured according to the methodolgy outlined by Zhou (2001).

Statistical analysis

Each parameter underwent statistical analysis, and comparisons were conducted using the least significant difference (LSD) test at a significance level of 5%. All statistical computations were executed utilizing the SAS software (SAS, 2004).

Results and Discussion

Soil characterization: Sandy loam soil was used, exhibiting a pH that ranged from slightly acidic to neutral (Table 1). Soil pH significantly influences the organic processes and metal solubility in both the soil and soil solution (Zeng *et al*., 2011). Additionally, the organic matter content greatly affects the soil's cation exchange capacity and buffering capacity, as well as its ability to retain heavy metals. Consequently, heavy metals in organic-rich soils are less bioavailable and more immobile compared to those in mineral soils (Olaniran *et al*., 2013).

In general, heavy metals are not readily available or mobile in soil, especially when the soil is rich in clay and organic matter (Rosselli *et al*., 2003). In the soil matrix, lead precipitates and has poor water solubility; it is an immobile metal that is commonly inaccessible. Depending on the characteristics of the soil, these elements may act alone or in combination, and they may alter how the lead in the soil behaves as well as how rapidly plants absorb it (Dede *et al*., 2012).

The endophytic fungus *Phoma* sp. GAH7, isolated from cucumber roots, was found to produce significant amounts of GA19, GA9, GA4, and GA3, which serve as GA controls (Hamayun *et al*., 2009). Similarly, the endophyte Paecilomyces variotii LHL, also found in cucumber roots, potentially increase plant growth by generating large amounts of GAs and IAAs. (Khan *et al*., 2012). Endophytic fungi are known to promote growth, and new discoveries on their methods imply that this resource merits more research in order to potentially find applications in agriculture.

Determination of Pb^{2+} maximum tolerance concentration (MTC) by *Aspergillus terreus* SQU14026: The fungus strain showed high Pb^{2+} tolerance with varying levels, as evident from the MTC value of the tested metal. The fungus exhibited the highest MTC value (3000 ppm) for Pb^{2+}.

Effect of different concentrations of Pb^{2+} on the production of plant growth hormones (GA and IAA) by *A. Terreus* SQU14026: Quantitative estimation of plant growth promotion (PGP) in *Aspergillus terreus* was tested at different Pb^{2+}concentrations. Indicated in (Fig. 1), the addition of lead metal ions, up to 600 ppm produced a non-significant decrease in both the growth of the endophytic fungus and hormone production. Certain endophytic fungi strains have great resistance to poisonous metals. Endophytic fungus can survive harmful metal concentrations in the mM range in culture (Domka *et al*., 2019).

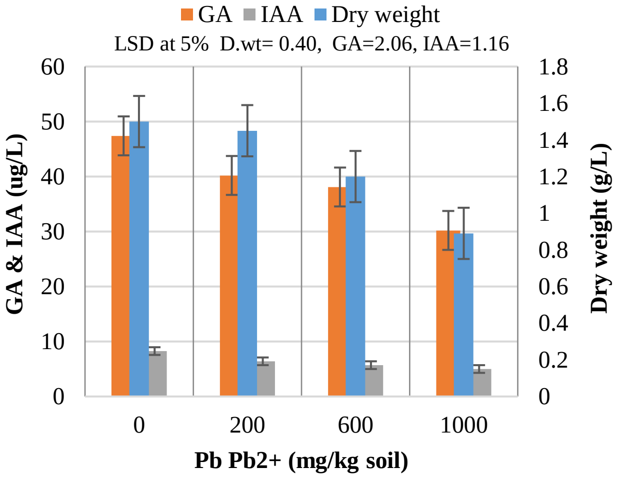

Fig. 1. Effect of different concentrations of lead on GA and IAA production by *Aspergillus terreus* SQU14026.

It is still unclear whether the metal-tolerant fungus helps the host plant growth in soils rich in metalliferous elements or if the toxic metal-tolerant plant acts as a haven for the fungus, enabling it to develop metal tolerance while being exposed to toxic metal stress.

In this context, the capacity to colonize plant tissues, along with metal tolerance, is crucial for endophytic fungi to adapt to metal toxicity. In a similar study, Sim *et al.* (2018) extracted exceptionally resilient fungal endophytes from Phragmites species to aid in wastewater treatment. Numerous fungi in this research demonstrated the ability to tolerate Cu, Zn, and Pb^{2+} concentrations above 200 mg/L.

Analysis of morphological and physiological parameters: Effect of lead-tolerant PGPF *Aspergillus terreus* on shoot attributes and leaf area of wheat plants under lead toxicity.

The data illustrated in (Fig. 2) indicated that inoculating wheat grains with *A. terreus* spores led to an increase in all growth parameters, including shoot length, shoot fresh and dry weights, and leaf area by 8.6%, 21%, 17%, and 21% of plants grown under 1000mg Pb^{2+} in comparison to non-inoculated cultivars. Therefore, PGPF is regarded as one of the potential active components in formulations for both biofertilizers and mycofungicides. Plant growth-promoting fungi is shown to have favorable impacts on plant growth and development (Hamayun *et al*., 2009). By controlling the hormonal and nutritional balance, creating plant growth regulators, boosting the antioxidant system, and promoting resistance to phytopathogens, PGPM inoculation facilitates plant growth (Spence *et al.*, 2015).

Endophytic fungi are linked to several plant growth-promoting activities, like phosphorus (P^{3+}) solubilization and the production of phytohormones and siderophores (Chhabra & Dowling, 2017). For example, incorporating Fusarium spp. PPF1 and Aspergillus spp. PPA1 into soil substantially increased the amount of chlorophyll in Indian spinach and cucumber leaves, respectively (Islam *et al*., 2014). Enhanced leaf numbers and area can lead to a higher photosynthetic rate and greater net carbohydrate accumulation in plants. Metal toxicity, however, can negatively impact these parameters by binding to sulfhydryl groups in proteins, thus disrupting protein structure or function, or by displacing essential elements, resulting in deficiency effects (Jayasri & Suthindhiran, 2017).

Endophytic fungi significantly enhance plant growth by producing protective compounds and growth regulators, especially under stressful conditions. This includes synthesizing growth-promoting phytohormones such as auxins (Waqas *et al*., 2012), indole-3-acetic acid (Sukumar *et al*., 2013), gibberellins (Leitao & Enguita, 2016), and cytokinins. Additionally, endophytes improve nutrient uptake in plants (Shahabivand *et al*., 2019). Introducing plant growth-promoting microbes (PGPM) into the soil can further enhance plant growth by balancing hormones and nutrients, strengthening the antioxidant system, producing growth regulators, and increasing pathogen resistance (Spence *et al*., 2015).

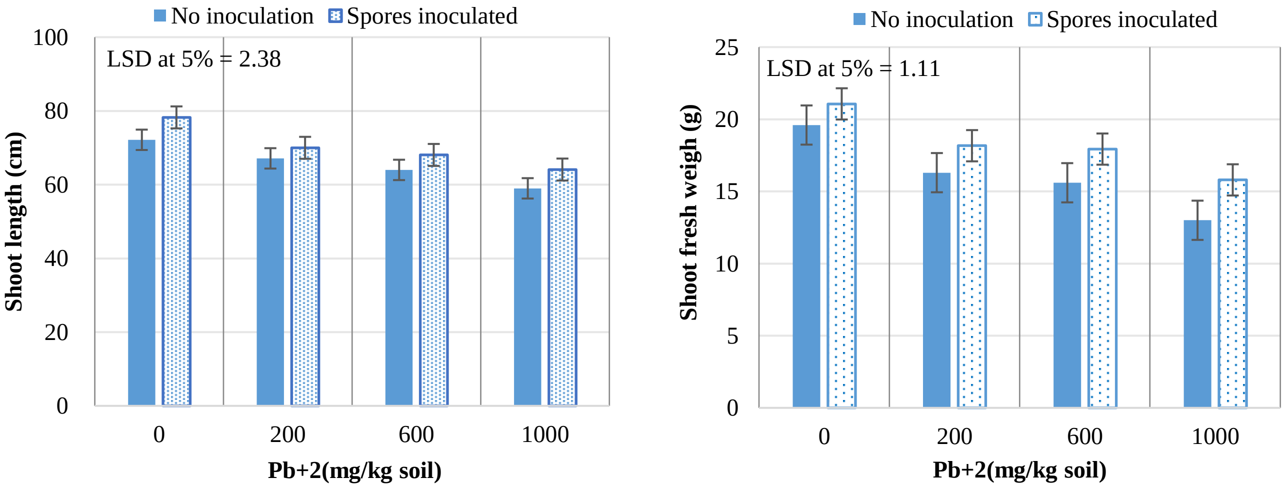

Fig. 2. Effect of lead tolerant PGPF*Aspergillus terrus*on shoot attributes and leaf area of wheat plants under lead toxicity.

Our findings revealed that all growth metrics diminished as Pb^{2+} concentrations increased. The data demonstrated a marked decline in wheat growth in lead-contaminated soil compared to wheat growth in non-inoculated treatments with lead. Elevated lead levels have been strongly linked to reduced germination and stunted growth in wheat plants (Mesmar & Jaber, 1991). This reduction is attributed to the uptake process, where wheat grains are most water-permeable at the end of the initial germination stage, leading to intense water absorption (Wierzbicka & Obidzińska, 1998).

Increased lead levels in the soil significantly reduced biomass (El Rabey & Zayed, 2005). Numerous studies show that introducing plant growth-promoting microorganisms to metal-stressed plants can mitigate the harmful impacts of heavy metal contamination. Most studies suggest that plants inoculated with endophytic fungi yield greater biomass under conditions of metal toxicity than those without such inoculation (Rozpądek *et al*., 2018). Similarly, the introduction of lead-tolerant bacteria (PGPR) has been found to reduce the advese effects of lead and enhance the growth and physiological health of wheat plants under lead stress. This enhancement is credited to siderophore production (Glick & Patten, 1999), phosphate solubilization (Yasmin & Bano, 2011), phytohormones (Humphry *et al*., 2007), as well as systemic defense mechanism activation against metal-induced phytotoxicity (Mishra *et al*., 2006). These combined factors lead to an overall increase in biomass production (Mishra *et al*., 2006).

Effect of lead-tolerant PGPF *Aspergillus terrus*on mineral content of wheat plants under lead toxicity: Fig. 3 presents how Pb contamination affects the absorption of macronutrients in both the roots and shoots wheat plants. The data indicates a significant decline in potassium (K), phosphorus (P), and nitrogen (N) uptake as the concentration of lead in the soil rises. The most pronounced reductions were observed in plants subjected to high lead levels (1000 mg/L). In the shoots of wheat, the decreases were 47%, 79%, and 52% for N, P, and K, respectively, compared to untreated plants. In the roots, the reductions were 68%, 78%, and 30% for the same nutrients. These findings align with previous research show that heavy metals in soil or nutrient solutions can alter how plants absorb nutrients, particularly cationic micronutrients such as Cu^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+}, which compete for the same absorption sites in roots (Zhang *et al*., 2014). The decreased nutrient uptake at higher heavy metal concentrations may be attributed to inhibited root growth, leading to reduced plant biomass and lower absorption of essential nutrients and water (Chatterjee & Chatterjee, 2000). Lead is known to hinder the absorption of various ions by blocking their entry points on roots (Godbold & Kettner, 1991), thus impeding their uptake. However, the substantial reduction in macro-element content observed in this study is not solely due to inhibition of ion absorption; it also suggests increased leakage of ions from the plants (Akinci *et al*., 2010). Additionally, there was a negative correlation between lead concentration in the soil and phosphorus uptake. Moreover, an inverse relationship was observed between soil lead levels and phosphorus absorption. Additionally, compared to lead-contaminated plants that were not inoculated, the results demonstrate that treating wheat grains with *A. terreus* spores significantly raised the macronutrient content in both shoots and roots across varied lead concentrations.

Nitrogen, phosphorus, and potassium content of shoots and roots increased by 35 and 68.4%, 14 and 48.4%,74 and 88.8%, respectively, under low lead contamination (200 ppm) in spore inoculated wheat plants in comparison to uninoculated plants. By speeding up effective germination and growth or improving the plant's ability to absorb nutrients, endophytic fungus may improve host fitness and competitiveness (Aly *et al*., 2011). Endophytic fungi enhance plant development during metal toxicity by aiding the plant's intake of water and nutrients, either directly by activating specific metal tolerance pathways or indirectly by subtly altering the host's metal tolerance (Rozpądek *et al*., 2018).

Endophytic fungi play an essential role in enhancing plant water and nutrient uptake. Heavy metals frequently disrupt the root absorption of essential elements such as iron (Fe), phosphorus (P), magnesium (Mg), potassium (K), calcium (Ca), and zinc (Zn), as well as their metabolic functions, resulting in inhibited plant growth (Ouzounidou *et al*., 2006). Notably, the use of the endophytic fungus Glomus intraradices G72 has been observed to enhance nutrient uptake significantly (Rouphae *et al*., 2010).

Effect of lead-tolerant PGPF *Aspergillus terrus* on catalase, peroxidase, and lipid peroxidation of wheat plants under lead toxicity.

The findings depicted (Fig. 4) demonstrate a notable increase in catalase (CAT) and peroxidase (APX) activities, accompanied by heightened lipid peroxidation, with rising Pb^{2+} concentrations. The results further reveal that elevated Pb^{2+} levels in the soil significantly enhance CAT and APX activities, as well as increase malondialdehyde (MDA) levels in plant leaves, compared to the untreated control group of plants free from contamination. Under stressful conditions, reactive oxygen species (ROS) such as OH^-, H_2O_2, and O_2 are commonly produced. Reactive oxygen species have potent oxidizing properties that enable them to attack all types of biomolecules. These oxygen species are intermediates that appear during the successive reduction of O_2 to H_2O_2 (Panda, 2008).

Polyunsaturated lipids degraded in cellular membranes (lipid peroxidation that causes loss of membrane state) by Pb^{2+} stress-induced overproduction of ROS as a result, generating MDA, a biomarker that is used to assess the level and severity of oxidative stress (Del Rio *et al*., 2005). Several plant species, including *Morus alba* (Qin *et al*., 2017) and *Acalypha indiac* (Venkatachalam *et al*., 2017), showed elevated MDA concentration under Pb^{2+}-stressed conditions. Superoxide dismutase, CAT, and APX activity were noticeably increased in inoculated and non-inoculated plants under Pb^{2+} treatment. These results similar those noted for *Najas indica* (Wang *et al*., 2012). Stress or certain substances, including metals, often cause plants to overproduce reactive oxygen species (ROS). By altering lipids, proteins, photosynthetic pigments, and other cellular organelles, this process ultimately leads to plant death by causing lipid peroxidation and membrane damage (Zorov *et al*., 2014).

An excess of reactive oxygen species production can diminish ATP levels, leading to a decline in respiration rates that ultimately retards plant growth. To counteract ROS and inhibit oxidative damage, plants have evolved a variety of antioxidative defense mechanisms that protect cells and tissues from harm and dysfunction (Li *et al*., 2013). Among these antioxidants are ascorbate peroxidase (APx), glutathione reductase, catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (GPx), and peroxidase (POX) (Kasote *et al*., 2015). These antioxidants mitigate oxidative stress by scavenging ROS (Malecka *et al*., 2009) and protect cellular structures from oxidative harm (Ashraf & Foolad, 2007).

The studies performed by (Waller *et al*., 2005) showed a positive effect on the catalase and peroxidase activity of wheat colonized with *Aspergillus terreus* compared to the control plants under salt stress conditions. Additionally, Zhang *et al*., (2016) reported elevated levels of catalase and peroxidase in wheat seedlings infected with *Trichoderma longibrachiatum* compared to those without endophytic fungi under stress conditions. Plant growth-promoting fungi (PGPF) stimulate both enzymatic and non-enzymatic antioxidants, boosting the production of these compounds and safeguarding the plants from further harm (Ahmad *et al*., 2015).

Effect of lead-tolerant PGPF *Aspergillus terreus* on photosynthetic pigments of wheat plants under lead toxicity: Data recorded in (Fig. 5) demonstrate that, fungal inoculation of wheat plants resulted in a considerable increase in photosynthetic pigment; chlorophyll a, b, and carotenoids under lead treatments. Additionally, data indicated that non-inoculated plants had a gradual decline in photosynthetic pigments in comparison to inoculated plants, the highest recorded value of chlorophyll content was at 200 mg Pb^{2+}, while the lowest values of carotenoid and chlorophyll a and b were at 1000 mg lead treatment.

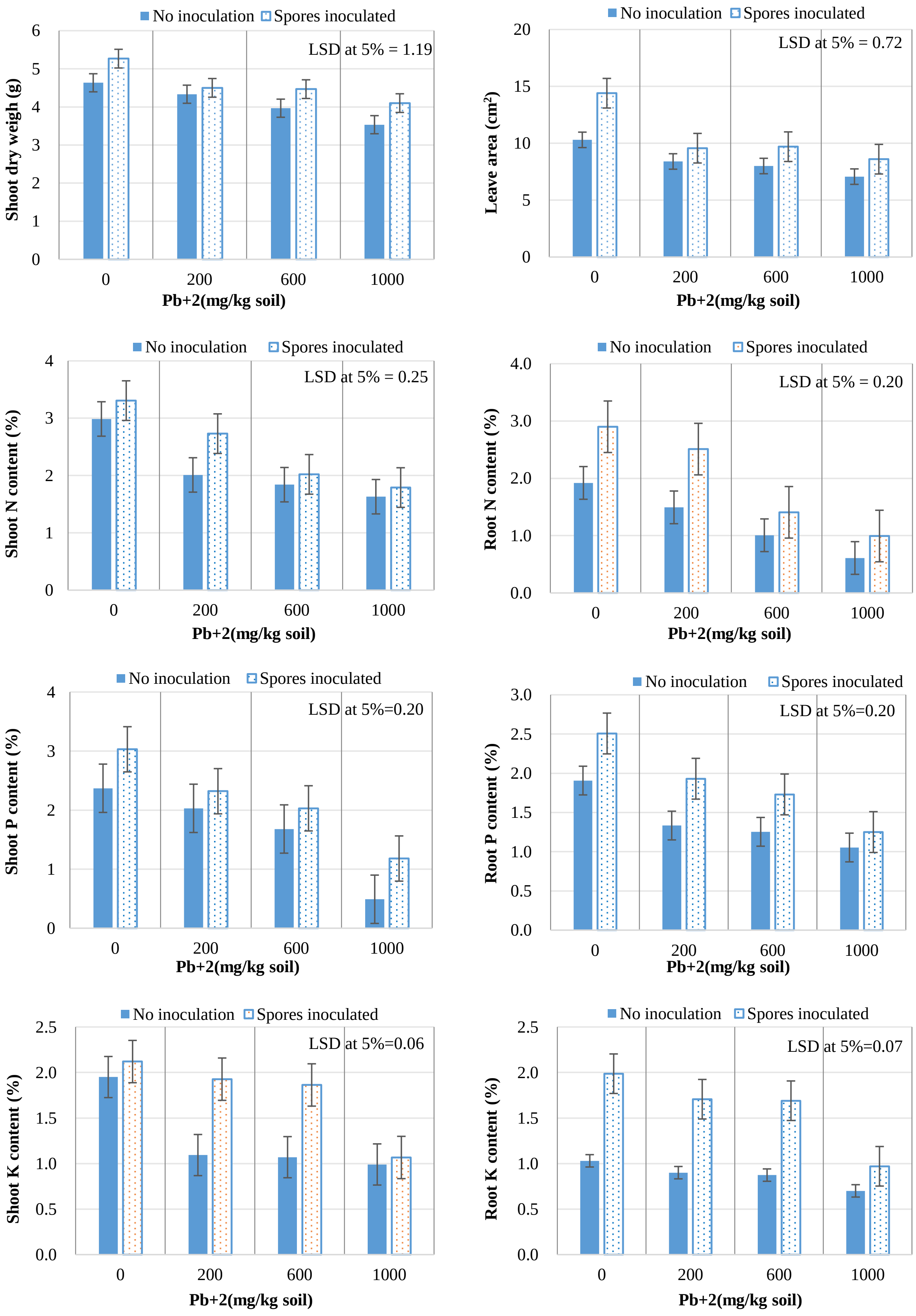

Fig. 3. Effect of lead tolerant PGPF *Aspergillus terrus* on mineral content of wheat plants under lead toxicity.

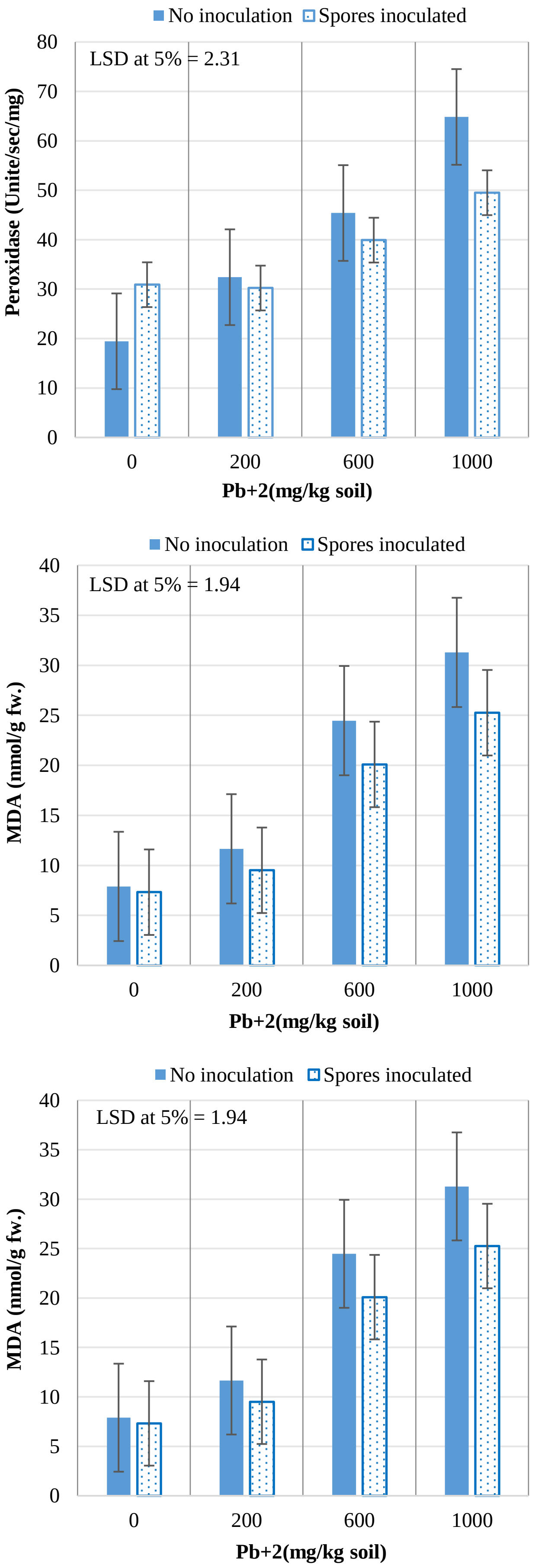

Fig. 4. Effect of lead tolerant PGPF *Aspergillus terrus*on catalase, peroxidase and lipid peroxidation of wheat plants under lead toxicity.

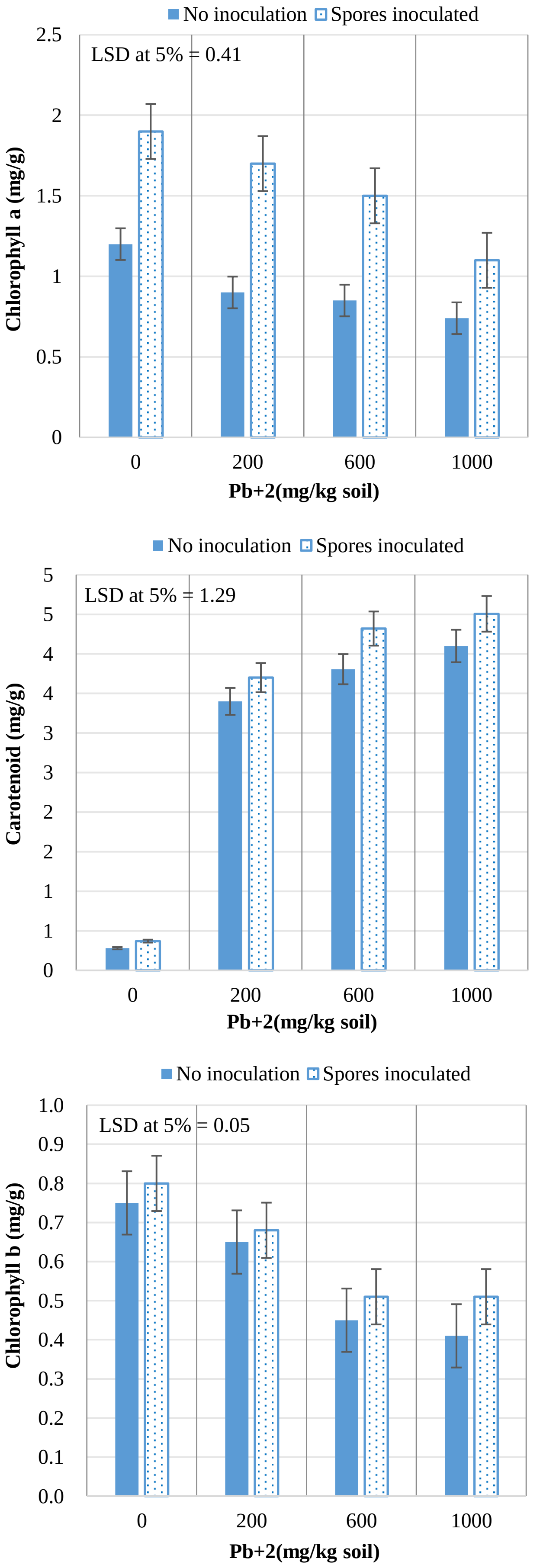

Fig. 5. Effect of lead tolerant PGPF *Aspergillus terreus* on photosynthetic pigments of wheat plant under lead toxicity.

Lead poisoning, in particular, can interfere with photosynthesis, one of the plant processes most negatively impacted by Pb^{2+} toxicity, especially at high dose. With Pb^{2+} treatment, the peroxidation of chloroplast membranes brought on by an increase in reactive oxygen species formation may cause a slowdown in the accumulation of photosynthetic pigments (Srinivasan *et al*., 2014).

Lead may inhibit the absorption of essential nutrients for photosynthetic pigment, including iron, magnesium, potassium, and calcium (Gopal & Rizvi, 2008). This leads to a disruption of photosynthesis as lead displaces divalent cations. Carotenoids are crucial for protecting the photosynthetic system of plants from oxidative damage by neutralizing reactive oxygen species. A reduction in carotenoid levels can detrimentally affect Photosystem II (PSII) because it causes regression in the D1 protein, hindering chlorophyll synthesis. Bacteria known for promoting plant growth and enhancing nutrient absorption by solubilizing phosphate and facilitating essential chemical transfers may also bolster the production of photosynthetic pigments crucial for the light-harvesting complex and its assimilation of light energy (Tanaka *et al*., 1998). Research has shown that *Azotobacter chrococcum*, when exposed to *Zea mays* plants supplemented with Cu and Pb^{2+}, led to improvements in chlorophyll content (Ahmad *et al*., 2015).

Effect of lead-tolerant PGPF *Aspergillus terreus* on the carbohydrates content of wheat plants under lead toxicity: Data in (Fig. 6) displayed a non-significant increase in carbohydrates content in stressed wheat plants in compares to the control. Additionally, the data demonstrated a considerable increase in total carbohydrates in the shoots of wheat plants inoculated with fungal spores under various concentrations of lead. This outcome demonstrates that the heavy metal pollution led to a buildup of carbohydrates. The primary constituents of accumulated solutes are sugars, which are stored in seeds and play a role in regulating cell osmotic balance (Bewlay & Black, 1994). Numerous studies have documented the accumulation of carbohydrates under diverse abiotic stresses in cereals and temperate grasses, particularly during reproductive development, when long-term storage of carbohydrates takes place (Meier & Reid, 1982). Various plant tissues exhibit an increase in sugar accumulation responding to different environmental stresses (Prado *et al*., 2000). Apart from their role in maintaining osmotic balance, soluble sugars aid plants in storing sufficient amounts of carbohydrates to sustain their basal metabolism under difficult circumstances (Dubey & Singh, 1999).

Effect of lead-tolerant PGPF *Aspergillus terreus* on the total protein content of wheat plants under lead toxicity: According to data in Fig 6, treatment with lead led a significant decrease in protein content in wheat plant shoots compared to control (uncontaminated plants). Additionally, results showed that when plants were grown in lead-contaminated soil and given fungal spore inoculations, the content of protein steadily increased. In this regard, Kevresan *et al*., (2001) investigated how various concentrations of lead affected the metabolism of proteins and nitrogen in an immature pea plant. They found that the metabolism of nitrate and protein was decreased by lead pollution. Due to its tendency to bind to sulfhydryl groups in proteins, lead may be damaging since this might lead to the alteration of protein structure or disrupt their biological function Morelli & Scarano, (2004) and Hossain & Komatsu, (2013).

The alterations caused by heavy metals lead the protein to lose its useful functions and also break the stabilizing bonds associated with their tertiary structure (Chrestensen *et al*., 2000).

Deteriorated proteins that are unable to revert to their original conformations are broken down by the ubiquity protein degradation process (UPS) (Liu & Howell, 2016). Heavy metals impair enzyme structure and function by either altering protein structure or replacing essential elements in high concentrations. Heavy metals have the ability to alter the structure of plasma membrane embedded proteins such as H^+ ATPases, leading to disruptions in membrane permeability and thus loss of function. Moreover, their toxicity triggers an oxidative stress cascade by generating reactive oxygen species, which can adversely affect various cellular components and consequently impact plant tissues (Sajedi *et al*., 2010). Inoculation of wheat cultivar grains with *A. terreus* spores significantly increased the total protein content and enhanced the levels of all macro-nutrients in both roots and shoots of wheat cultivars under varying lead concentrations in comparison to non-inoculated plants contaminated with lead. Among these essential elements, nitrogen is particularly noteworthy as a fundamental component of protein molecules. Endophytic fungi contribute to enhancing the competitive abilities and fitness of their hosts by promoting germination and growth rates or facilitating the absorption of essential nutrients (Aly *et al*., 2011). In line with this, Rouphael *et al*., (2010) observed that the use of the endophytic fungus *Glomus intraradices* G72 improved nutrient uptake.

Effect of lead-tolerant PGPF *Aspergillus terreus* on proline content in wheat plants under lead toxicity: As lead concentrations increased in both inoculated and non-inoculated wheat plants, proline levels in the plants' shoots increased significantly (Fig. 6). Specifically, in inoculated plants, the proline content exceeded that of non-inoculated plants by 30.5% when exposed to 1000 mg of lead. Proline acts as osmolytes and free radical scavenger (Kishor & Sreenivasulu, 2014) the beneficial function of endophytes in promoting the manufacture of proline endophyte supported by Saddique *et al*., (2018). Numerous plant species subjected to metal stress have been observed to accumulate proline (Sharma and Dietz, 2006). Proline may function as a metal chelate, a free radical scavenger, and an inhibitor of lipid peroxidation. Additionally, Proline accumulation might aid in improving cellular osmotic pressure (Perez-Alfocea *et al*., 1993) and act as a source of energy and nitrogen after the removal of stress (Chandrasekhar & Sandhya Rani, 1996).

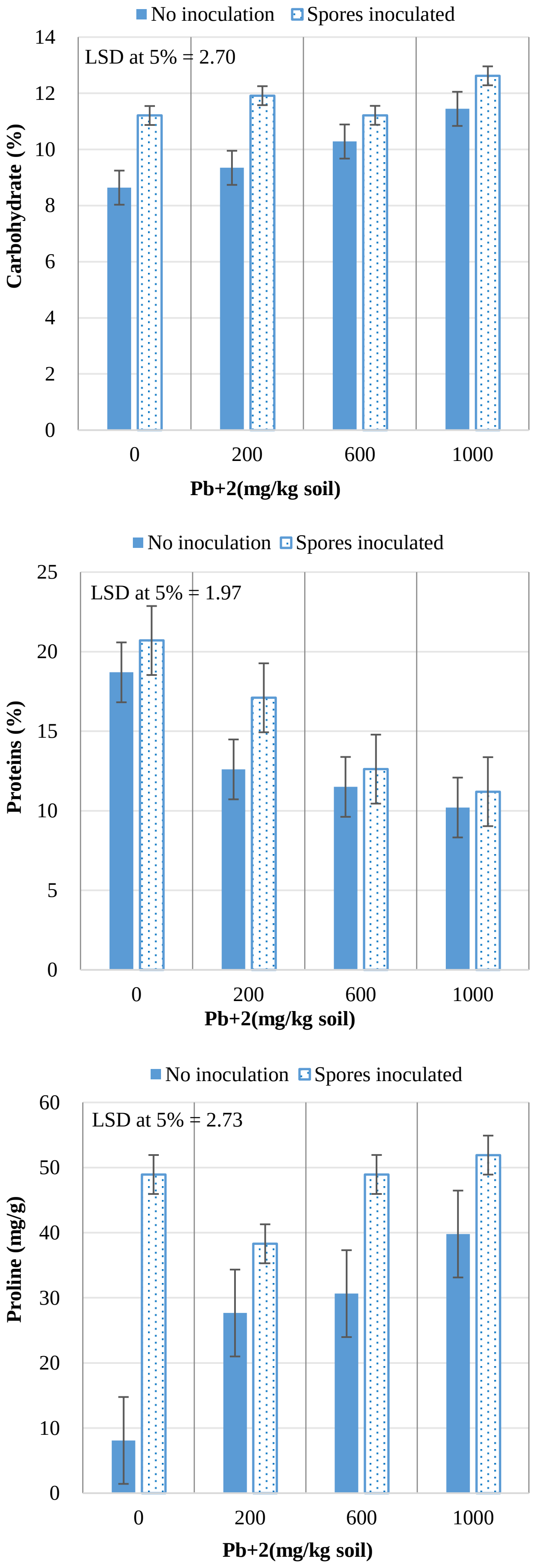

Fig. 6. Effect of lead tolerant PGPF *Aspergillus terreus*on carbohydrate, protein and proline of wheat plant under lead toxicity.

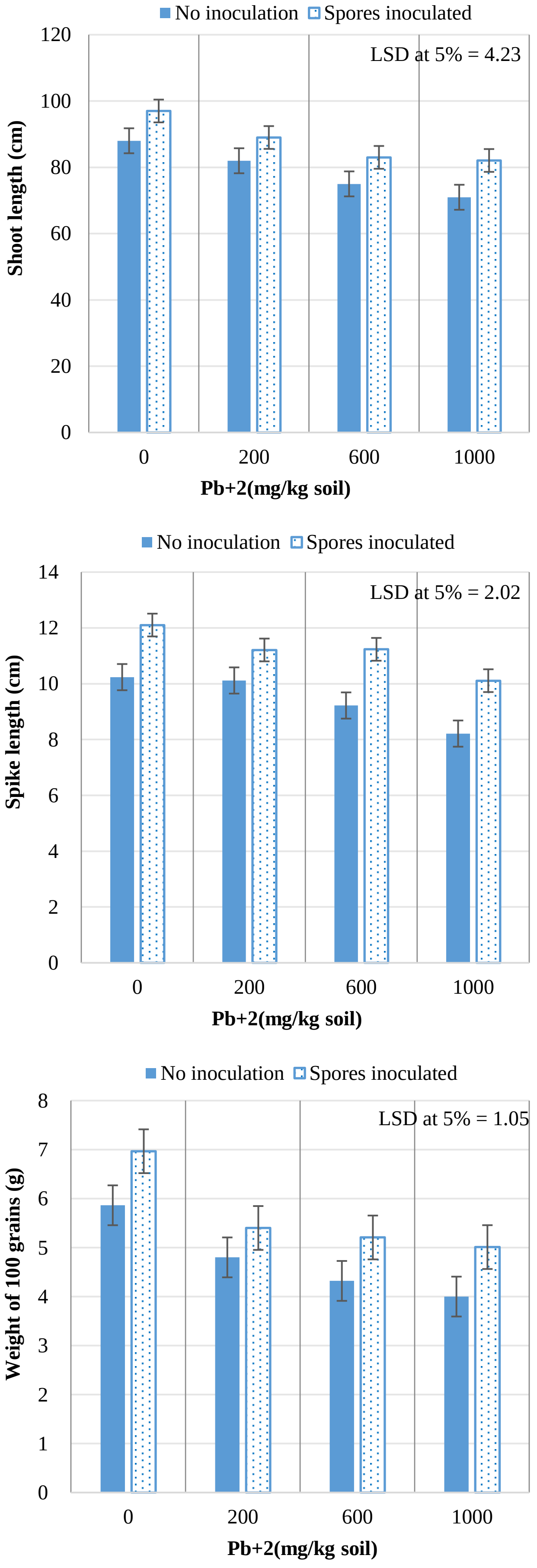

Fig. 7. Effect of lead tolerant PGPF *Aspergillus terreus* on some yield characters of wheat plants under lead toxicity (At harvest stage).

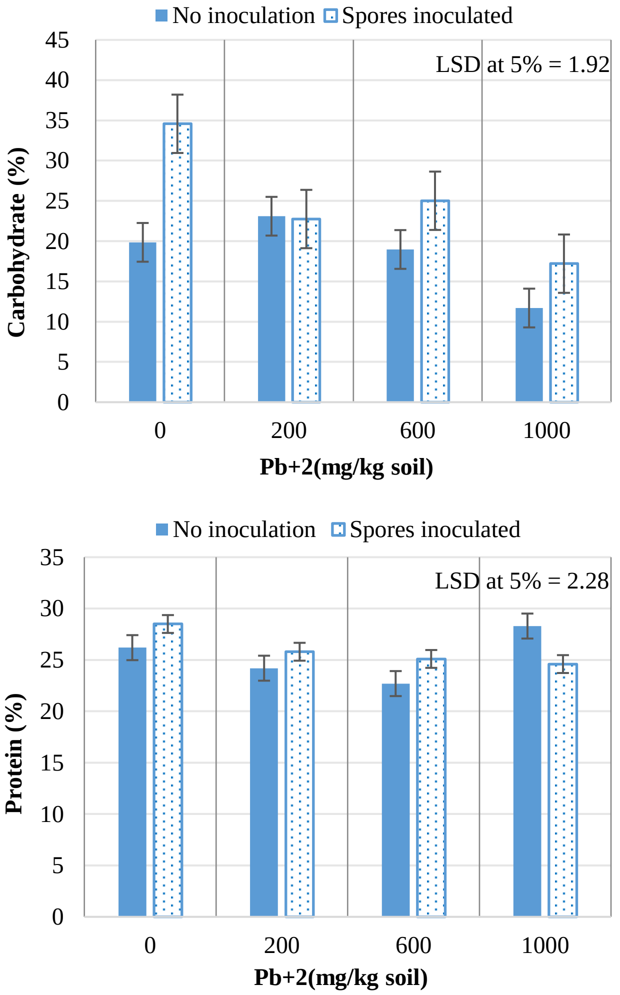

Fig. 8. Effect of lead tolerant PGPF *Aspergillus terreus*on carbohydrate and protein content of wheat(grains) under lead toxicity at harvest stage.

Effect of lead-tolerant PGPF *Aspergillus terreus* on some yield characters of wheat plants under lead toxicity: The impact of spore inoculation as well as various lead concentrations on the yield components of wheat plants were demonstrated by the results in (Fig. 7). These data showed that the length of spikes, weight of 100 grains, and shoot lengths at the harvest stage decreased with increasing lead concentrations by 19, 30, and 20%, respectively compared with untreated plant Lead had a negative impact on some yield components, most likely as a result of modifications to the metabolism and physiology of the plants compared to non-stressed plants.

Furthermore, data illustrated that the introduction of spores had a notably favorable impact on various aspects of wheat yield. This included increases of up to 23%, 20%, and 15% in spike length, weight of 100 grains, and shoot lengths during harvest, respectively, compared to untreated plants. Recent research has revealed that several fungi, including *Penicillium cyclopium, P. funiculosum, P. corylophilum, Rhizopus stolonifera, Aspergillus niger, A. flavus,* and *Fusarium oxysporum,* can synthesize plant-growth-promoting hormones such as gibberellic acid (GA) and indole acetic acid (IAA) (Asaf & Hamayun, 2018). These hormones play an essential role in enhancing crop development and mitigating the adverse effects of environmental stresses. Additionally, the mutually beneficial relationship between plants and non-pathogenic binucleate *Rhizoctonia* spp. has been shown to lead to increased yields in a variety of crops involving Chinese mustard, potato, carrot, lettuce, cucumber, cotton, radish, and wheat (Pascual *et al*., 2000; Musli & Hyakumachi, 2003; Jiang *et al*., 2015). These results show that plant growth promoting fungi (PGPF) hold significant potential for enhancing agricultural productivity.

Effect of lead tolerant PGPF *Aspergillus terreus* on carbohydrates and protein content of wheat grains under lead toxicity at harvest stage: The findings listed in (Fig. 8) showed how the application of *A. terreus under* lead toxicity affected the total protein and carbohydrates of the wheat grains at harvest stages. The total protein and carbohydrate contents of grains decreased by 13 and 40%in stressed wheat plants in comparison to uncontaminated one.

Fungal inoculation of wheat grains leads to increased amounts of total carbohydrates and protein compared to polluted, untreated plants. Lead pollution caused a decline in carbohydrates because of a direct correlation with chlorophyll content. Lead either inhibits the intake of essential elements such as Mg and Fe or lowers the activity of chlorophyllase in plants, which reduces the production of chlorophyll (Sharma & Dubey, 2005) Inoculation of wheat grains with *Aspergillus terrus* increased the levels of carbohydrates along with their levels of chlorophyll. In this connections, addition of *Aspergillus* spp. PPA1 and *Fusarium* spp. PPF1 to the soil greatly improved chlorophyll content and leaf area in the leaves of cucumber and *Indian spinach*, respectively (Islam *et al*., 2014).

Effect of lead-tolerant PGPF *Aspergillus terreus* on Pb^{2+} accumulation at root, shoot, and grain levels of wheat plants: As the concentration of external metals increased, the lead accumulation in the roots and above-ground parts of wheat plants rose in comparison to the non-inoculated plants. Fungal inoculation significantly enhances the absorption of Pb^{2+} at all concentrations tested. Moreover, roots exhibited higher metal accumulation compared to shoots and grains. At a Pb^{2+} concentration of 1000 mg/kg, the highest levels of Pb^{2+} were found in both the roots and aerial parts of wheat plants that had been inoculated with fungi. (400.05 and 86.02 µg/g dry weight, respectively), in contrast to non-inoculated plants (Fig. 9). Since roots serve as the primary interface for interacting with toxic metals, they absorb more Pb^{2+} than the shoots (Babu *et al*., 2015). Consequently, fungi have an essential role in transferring metals from roots to shoot tissues. There exists a strong correlation between the increased availability of metals to plants and microbial activity surrounding plant roots. Organic ligands and metabolites, including siderophores, organic acids, and different degrading enzymes, are produced by microbes, in which these enzymes have an influence on metal speciation and solubility (Rajkumar *et al*., 2012). This process can result in heightened metal uptake by plants. The microbial mobilization mechanism of metals via organic acids involves protons displacing metal cations at sorption sites, thereby dissolving minerals containing metals (Gadd, 2010). Acid phosphatases released by *Piriformospora indica* mobilize otherwise inaccessible organic phosphorus, enabling host plants to absorb sufficient phosphorus to support their growth (Aslam *et al*., 2019). The observed tolerance of metals by *Aspergillus terreus*, which enhances Pb^{2+} uptake in *Triticum aestivum* L. plants, aligns with these mechanisms. Additionally, in stressful environments, fungi produce plant growth regulators and protective compounds, including phytohormones like indole-3-acetic acid (Sukumar *et al*., 2013), gibberellins (Leitao & Enguita, 2016), auxins (Waqas *et al*., 2012), as well as cytokinins, while also facilitating nutrient absorption as endophytes (Shahabivand *et al*., 2012).

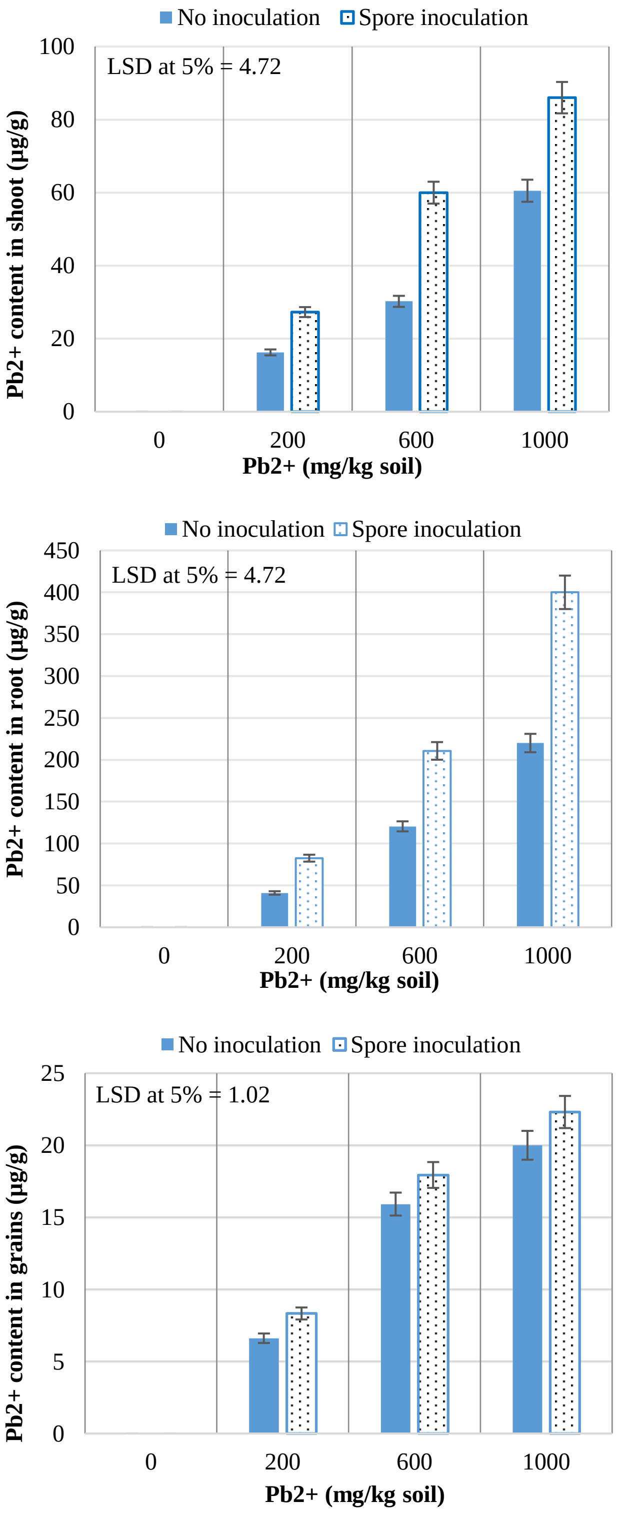

Fig. 9. Effect of lead tolerant PGPF *Aspergillus terreus* on Pb^{2+} accumulation at root, shoot and grains of wheat plants.

The availability of metals to host plants can fluctuate based on the type and concentration of metal influenced by fungal inoculation (Shahabivand *et al*., 2012). One reason for this trend might be the ability of fungal mycelium to extend through plant tissues, thereby binding more heavy metals.

The results are supported by the findings of Shi *et al*., (2017), which showed that Pb^{2+} availability in soil was significantly increased by *Penicillium* sp. and *Fusarium* sp. inoculation, and that higher Pb^{2+} concentrations were observed in *Brassica napus* shoots when in comparison to control treatments.

The results showed that, in an inoculated and non-inoculated wheat plants, only 27.51 & 9.1 and 21.5 & 9.58% of the accumulated lead in the root were translocated to the shoot and grains respectively under the highest lead treatment. Less than 10% of the Pb^{2+} absorbed by the roots was transferred to the grains, The data indicating comparatively limiting Pb^{2+} mobility by the wheat plant. Moreover, at varying Pb^{2+} concentrations in soil, roots strikingly sequestered more Pb^{2+} than shoot, suggesting a limitation on Pb^{2+} transport upward. According to reports, Pb^{2+} in *Raphanus sativus* is largely sequestered into the roots compared to the shoots (Wang *et al*., 2015). Thus, when maize plants were in symbiosis with the endophytic fungus *Gaeumannomyces cylindrosporus* they found that Pb^{2+} was predominantly maintained in the root system and the transfer factor (TF) was drastically decreased (Ban *et al*., 2017).

The transfer factor can be utilized to evaluate a plant's ability to facilitate the movement of heavy metals from roots to its harvested parts. Our results support the idea that Pb^{2+} preferentially accumulates in roots, as several investigations have shown (Geebelen *et al*., 2002).

Lead accumulates in roots by binding to cell wall ion-exchange sites and forming Pb^{2+} carbonates outside the cells. (Sharma & Dubey, 2005). Fungal mycelia serve a vital function as significant repositories for heavy metals, primarily by sequestering Pb^{2+} through ion chelation within the fungal cells or adsorption onto chitin in their cell walls. This process results in the accumulation of lead in the roots and restricts the translocation of Pb^{2+} from roots to shoots (Shahabivand *et al*., 2019). Functional groups like phosphoryl, carboxyl, and hydroxyl found in fungal cell walls serve as binding sites for the adsorption of heavy metal. Interestingly, these cell walls' carboxyl groups of glucuronic and galacturonic acids show a strong attraction for Pb2+, which restricts the metal's ability to pass through the apoplast. (Rudakova *et al*., 1988). Moreover, the endodermis's Casparian strip plays a crucial role in preventing Pb2+ from being transported from the endodermis to other plant tissues (Seregin & Kozhevnikova, 2008). In addition to these physical barriers, studies suggest that short-term exposure to heavy metals leads to poor translocation of Pb2+ from roots to new shoots (Utmazian *et al*., 2007). Lead can enter roots via ionic channels or transporters, but Pb2+ absorption requires the H+/ATPase pump to maintain a significant negative potential in the root's rhizodermal cells (Wang *et al*., 2007).

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review the manuscript; Hend A. El-khawaga, Abeer E. Mustafa, Abeer S. Meganid, Inas M M Abou El-Enain, Asmaa M. Radwan.

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