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Ameliorative Effect of Indole Acetic Acid on *Zea mays* Grown Under the Simultaneous Stress of Cr (VI) and *Macrophomina phaseolina*

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ABSTRACT

The present study aimed to investigate the ameliorative effect of Indole Acetic Acid (IAA) on *Zea mays* plants grown under simultaneous stress of hexavalent chromium Cr (VI) and *Macrophomina phaseolina.* The plants were treated with 1% IAA and subjected to Cr (VI) (2000 and 4000 ppm) and *M. phaseolina* stress for 30 days. The results revealed that the application of IAA significantly improved the growth parameters and photosynthetic pigments, however, the antioxidant enzyme activities increased in stressed plants compared to the control. Furthermore, IAA treatment reduced the stress of Cr (VI) in the roots and shoots of the plants. These findings suggest that IAA protects *Z. mays* plants grown under the simultaneous stress of Cr (VI) and *M. phaseolina* by enhancing their physiological activities, thereby reducing the toxic effects of Cr (VI) and *M. phaseolina*.

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INTRODUCTION

Maize (*Zea mays* L.) is an annual cereal crop that belongs to Poaceae family. It is considered, one of the most important cash crops all over the world. Among all cereals crops, *Z. mays* occupied the 3rd rank after *Triticum aestivum* L. and *Oryza sativa* L. Maize crop offers wide health benefits as it is a chief source of minerals, vitamins high fiber, and antioxidant (Murdia *et al*., 2016). With all of its benefits, this crop is affected by biotic and abiotic factors, resulting in annual heavy yield loss. Biotic factors include various diseases usually caused by bacteria, fungi, and viruses, whereas loss due to fungal disease is more dominant up to 44 percent (Bashir and Malik, 2018). Among these, a disease “charcoal rot” caused by a notorious fungus *Macrophomina phaseolina* (Tassi) Goid. is of utmost importance in reducing crop yield (Nabeel, Javed & Mukhtar, 2018). *M. phaseolina* is a soil-borne fungus widely spread worldwide and affects at least 500 species of plants in more than 100 families (Marquez *et al*., 2021). Furthermore, they also described that diseases such as charcoal rot, seedling blight, and stem and root rot are caused by this fungus. The main infectious source of *M. phaseolina* is micro-sclerotia, which are resistant enough and hence can last in the soil for up to 10-15 years. Micro-sclerotia are circular, oval, or elongated masses of solidified contagious mycelium that are at first light brown before maturing to a more profound shade of brown to dark. Several germinating hyphae from the sclerotia can infect the host plant's roots (Shang *et al*., 2024).

On the other hand, heavy metal (HMs) pollution of aquatic bodies is now a major concern on a global scale (Aziz *et al*., 2023). Maize, as the primary cereal crop grown across various soil types globally, faces significant threats to its productivity due to contamination by heavy metals in soils (Anjum *et al*., 2017). The most widely utilized heavy metal is chromium (Cr), which finds extensive use in industries including metallurgy, transportation, leather, electroplating, etc. Particularly, chromium (VI) induces substantial reductions in plant growth, development, and yield formation. Stunted growth, wilting of foliage, leaf chlorosis, and severe damage to root and shoot growth are the most prominent effects of chromium toxicity in plants (Sharma *et al*., 2020). When present in the soil, they persist for extended periods, causing harmful impacts on both the environment and human health (Münzel *et al*., 2023). Despite these external effects, heavy metals alter many physiological processes, nutritional status of minerals, rate of photosynthesis and respiration, enzymatic processes, and many biochemical processes. However, on exposure of plants to toxic concentrations of heavy metals, more production of reaction oxygen species (ROS) is considered the most prompt effect so far. Heavy metals interact with various components in the electron transport chain thereby altering its activity and leading to ROS generation mainly in chloroplast and mitochondria. With the rise in ROS level, the membrane potential gets imbalanced further inducing leakage of ion channels, lipid peroxidation, and destruction of macromolecules. Moreover, the toxicity of heavy metals liberating ROS on subcellular organelles may alter depending upon certain factors like duration of stress, developmental stage, dose/concentration of particular heavy metal, and plant organ involved (Mansoor *et al*., 2023).

Various agrochemicals have been used by farmers to eradicate the fungal pathogens, but these chemicals are non-environmental friendly and non-biodegradable. These chemicals entered into the food chain and caused deteriorated effects in living organisms. From this perspective, a bio-friendly and bio-degradable approach is required for disease control (Correa *et al*., 2009). In this regard, phytohormones can be used as an alternative to agrochemicals to eradicate fungal and heavy metal contamination. Within the realm of phytohormones, IAA (Indole acetic acid), serving as an auxin, plays a crucial role as a plant hormone in regulating diverse aspects of plant growth and development (Duca *et al*., 2014). Indole acetic acid plays a critical role in maize to acclimatize to numerous environments by mediating growth, nutrient allocation development, and defense in plants (Yue *et al*., 2021). Plant cell division, elongation, and cell differentiation are regulated by the signaling molecule imposed by IAA, in a concentration-dependent mechanism (Sugawara *et al*., 2015). Auxin regulates a wide range of developmental processes, including apical dominance in collaboration with cytokines, but its most notable impact on cell elongation is the traditional physiological Auxin response (Christian *et al*., 2006). However, still inadequate literature is available for revealing the potential role of phytohormones in regulating fungal and several HM stress-responsive stimulating components. The present study was designed to manage the charcoal rot disease caused by *M. phaseolina* and chromium metal contamination by the application of Indole acetic acid in maize plants.

MATERIALS AND METHODS

Aseptic conditions were maintained utilizing a variety of equipment, including a Laminar air flow cabinet, glassware, and sterilized and contamination-free media, for the maintenance and correct growth of cultures. Maize Afghoi SG-2002 seeds were obtained from Punjab Seed Corporation in Sahiwal, Pakistan. The seeds were immersed in distilled water for an entire night to break dormancy. *Macrophomina phaseolina* (accession number: FCBP-PTF-1156), a pathogenic fungus, was acquired from the First Fungal Culture Bank of Pakistan at the Institute of Agriculture Sciences, University of the Punjab, Lahore, Pakistan. The fungus was blended with double distilled water to create a mycelial suspension. The solution of 1% indole acetic acid was prepared by adding 1 g of indole acetic acid in 100 mL of double distilled water. Moreover, potassium dichromate salt was used for the preparation of Cr(VI) concentrations i.e., 2000 and 4000 ppm.

Experiment

The experiment was carried out with different tenth treatments i.e. T_1: Control, T_2: (Cr (VI))+2000 ppm), T_3: (Cr(VI) + 4000 ppm) T_4: (M.P), T_5: (Cr (VI) 2000 ppm + M.P), T_6: (Cr(VI) 4000 ppm + M.P), T_7: (Cr (VI) 2000 ppm + IAA), T_8: (Cr (VI) 4000 ppm + IAA), T_9: (M.P+IAA), T_{10}: (Cr (VI) 2000 ppm + M.P + IAA), T_{11}: (Cr (VI) 4000 ppm + M.P + IAA). Different growth, morphological, physiological, and biochemical changes in plants were recorded on the 30^{th} day after seed germination.

Physiological and biochemical traits

Ethanol (10 mL of 80%) was used to homogenize 0.5 g of fresh leaf material, which was then centrifuged for about

20 minutes at 2000 ppm. The absorbance of the supernatant was then measured at 470, 645, and 663 nm (Lichtenthaler, 1987). Fresh leaf material weighing 0.5 g was homogenized in around 10 mL of phosphate buffer to determine the total protein content. It was then centrifuged for 20 minutes at 2000 rpm. A test tube containing 0.1 mL of supernatant, 0.9 mL of distilled water, and 1 mL of reagent C was agitated for 10 minutes. The tube mentioned above was filled with around 0.1 mL of reagent D, which was then incubated for 30 minutes. The absorbance was then measured at 670 nm (Lowry *et al*., 1951). For peroxidase activity, the reaction mixture contained phosphate buffer (pH 7.0), enzyme extract, 5.33% pyrogallol, and 0.5% H_2O_2. The samples were measured for absorbance at 420 nm after incubation for 5 min at 25 °C. To measure the catalase content, about 1gm of plant material was homogenized with 10mL of 0.1M phosphate buffer and then centrifuged at 4°C for 10 minutes. A total of 1 mL of supernatant was obtained, to which 1 mL of 0.1M phosphate buffer and 1 mL of 0.01 M H_2O_2 were added. It was incubated at 20 °C for 5 minutes. It was then titrated against 0.005 N $KMnO_4$ after being added 10ml of 1% H_2SO_4. The pink color appeared as the titration's end point (Kumar and Khan, 1982).

Growth assay

Maize seedlings were collected 7 days after germination. Upon harvesting, the plants were uprooted, washed thoroughly under water, and dried with blotting paper. The roots and shoots were separated, and their length (cm) and fresh biomass (gms) were measured. The dry weight of both roots and shoots was then obtained by placing them in an oven at 80°C overnight.

Statistical Analysis

Statistic 8.1 was employed for statistical analysis. Moreover, the correlation was calculated by using Origin Pro.

RESULTS

Toxicity in maize plant

It was noticed that the maize plant (seedlings) grown under the stress of chromium at 2000 and 4000 ppm exhibited stunted growth with yellow leaves and a reduction in root growth as compared to control.

Morphological alterations in maize plant due to *M. phaseolina*

It was found that the maize plant infected with *M. phaseolina* showed typical symptoms of charcoal rot disease like brown-black charcoal rot appearance in the taproot or secondary roots. Moreover, the yellowing of leaves with stunted growth of seedlings was also noticed in the study in comparison with the control.

Growth parameter

Root parameters

The result showed that the root length, root fresh, and dry weight of maize seedlings when treated with Cr(VI) at 2000 and 4000 ppm significantly ($P \leq 0.05$) declined by 90-99% as compared to the control. Moreover, all said root parameters were significantly reduced by 80 to 90% when infested with *M. phaseolina* as compared to the control. Furthermore, in the simultaneous stress of Cr (VI) at 2000, 4000 and *M. phaseolina* significantly declined the root length, fresh and dry weight by 98 to 99% in comparison with the control. However, the soil application of IAA (1%) in individual stresses of both Cr (VI) (2000 & 4000 ppm) and *M. phaseolina* and the combined effect of both stresses significantly enhanced the root parameters by 3 to10 folds in comparison with their respective treatments (Figure 1 A to C).

Shoot parameters

The result showed that the shoot length, shoot fresh, and dry biomass of maize seedlings significantly ($P \leq 0.05$) declined by 45 to 95% as compared to control when treated with Cr(VI) at 2000-4000 ppm. Moreover, the shoot parameters of maize seedlings grown under the biotic stress of *M. phaseolina were* significantly reduced by 86% as compared to the control. Furthermore, the combined effect of both biotic and abiotic stresses significantly declined the tested parameters of maize seedlings up to 99% as compared to control. However, the soil amendment with 1% of IAA significantly profound the shoot parameters of the tested plant seedlings by 200 folds at Cr(VI) [2000 to 4000 ppm] stress, 84 folds (biotic stress), and 2000 folds (abiotic + biotic stress) as compared to their respective treatments (Figure 2 A to C).

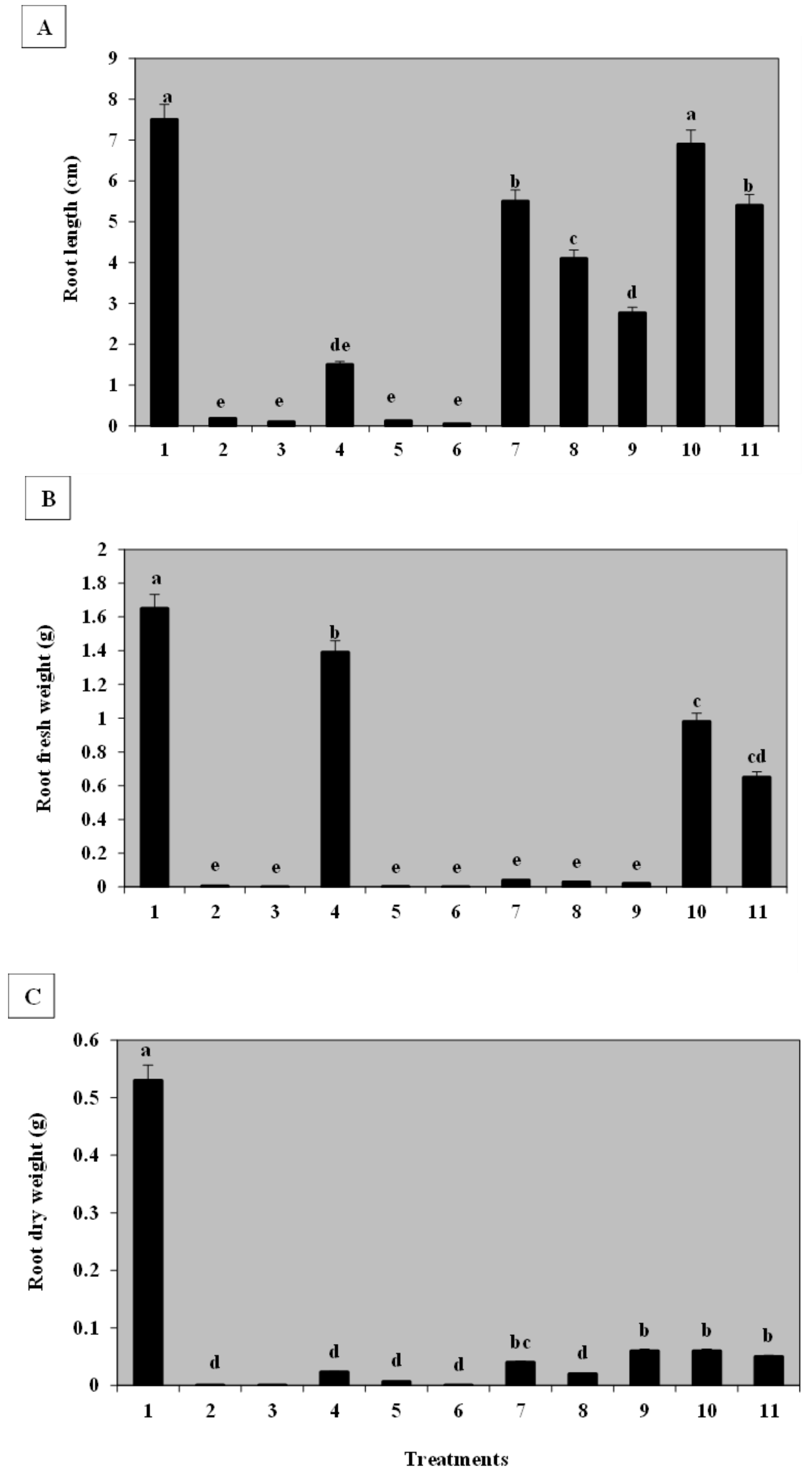

Figure 1 (A to C). Impact of indole acetic acid (IAA) on root parameters of maize seedlings grown under the stress of Cr (VI) and *Macrophomina phaseolina* (MP) after thirty days of seed germination. 1: control, 2: Cr(VI) 2000 ppm, 3: Cr(VI) 4000 ppm, 4: MP, 5: Cr(VI) 2000 ppm + MP, 6: Cr(VI) 4000 ppm + MP, 7: Cr(VI) 2000 ppm + IAA, 8: Cr(VI) 4000 ppm + IAA, 9: MP + IAA, 10: Cr(VI) 2000 ppm + MP + IAA, 11: Cr(VI) 4000 ppm + MP + IAA. Vertical bars represent the standard errors of means calculated from three replicates. Values labeled with different letters at their top indicate a significant difference (P≤0.05), determined by the LSD Test.

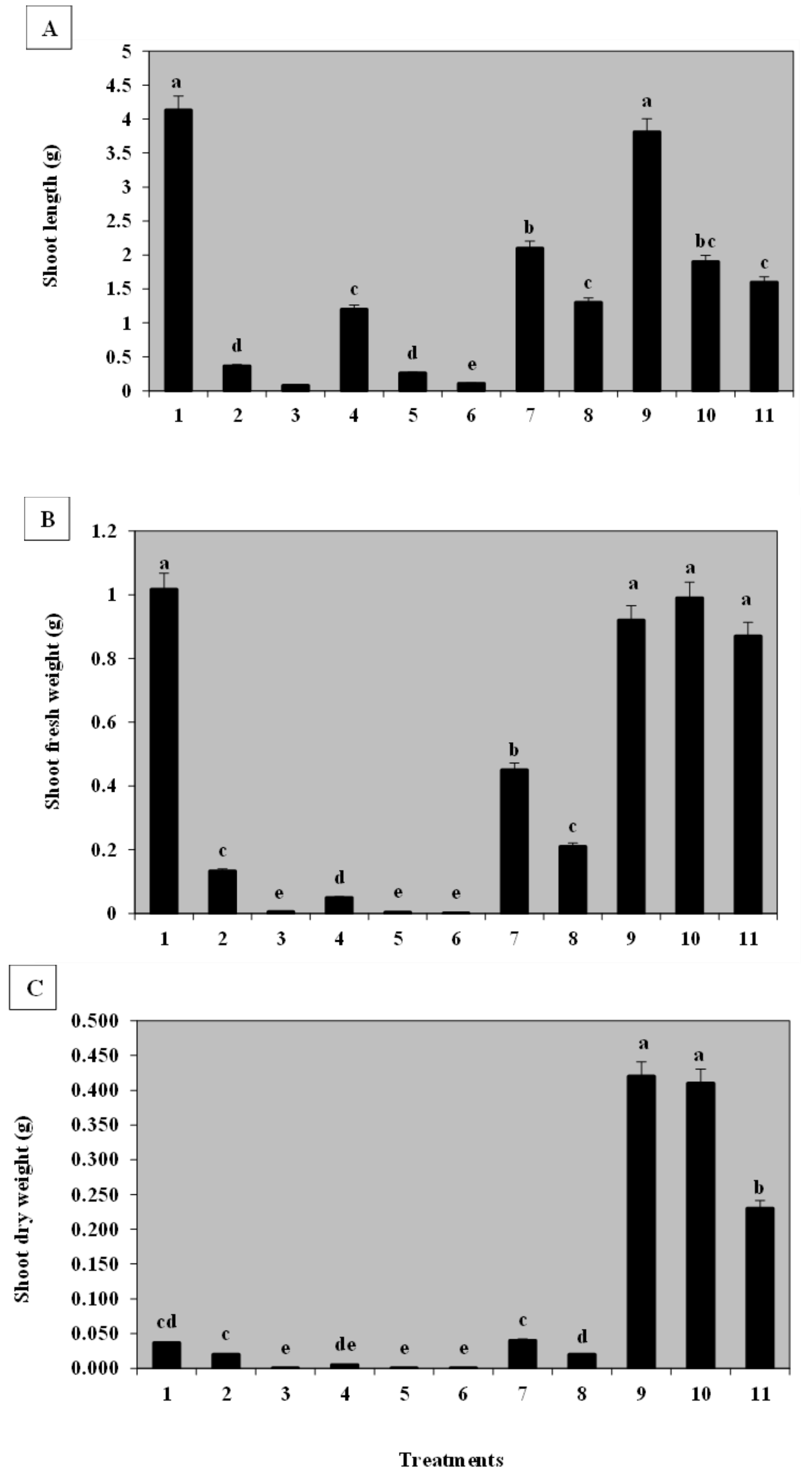

Figure 2. Impact of indole acetic acid (IAA) on shoot parameters of maize seedlings grown under the stress of Cr (VI) and *Macrophomina phaseolina* (MP) after thirty days of seed germination. 1: control, 2: Cr(VI) 2000 ppm, 3: Cr(VI) 4000 ppm, 4: MP, 5: Cr(VI) 2000 ppm + MP, 6: Cr(VI) 4000 ppm + MP, 7: Cr(VI) 2000 ppm + IAA, 8: Cr(VI) 4000 ppm + IAA, 9: MP + IAA, 10: Cr(VI) 2000 ppm + MP + IAA, 11: Cr(VI) 4000 ppm + MP + IAA. Vertical bars represent the standard errors of means calculated from three replicates. Values labeled with different letters at their top indicate a significant difference (P≤0.05), determined by the LSD Test.

Physiological and biochemical traits

Total chlorophyll content

The results revealed that the abiotic stress of Cr(VI) at 2000 to 4000 ppm significantly (P≤ 0.05) inhibited the chlorophyll content in maize seedlings by 60 to 84% as compared to control. Moreover, soil infested with *M. phaseolina* significantly declined the said content by 53% in comparison with control. Furthermore, simultaneous stresses (biotic and abiotic) significantly dropped the photosynthetic pigment of maize seedlings by 84 to 98% over control. However, the incorporation of 1% IAA in soil increased the level of tested content in maize seedlings by ~ 4 folds (abiotic stress), up to 4 folds (biotic stress alone), and up to 40 folds (abiotic + biotic stress) as compared to their respective treatments (Figure 3 A).

Total protein content and enzyme activities

It was noticed that the total protein content, catalase and peroxidase activities in maize seedlings were significantly (P≤ 0.05) more profound (~2 folds) in both stresses i.e., Cr(VI) (2000 and 4000 ppm) and *M. phaseolina* single and combined as compared to control. However, the application of 1% IAA significantly decreased the total protein content and enzymes (catalase and peroxidase) activities in tested plant's seedlings both in single stress and as well as in combined stresses by 12 to 20% and 30 to 40%, respectively as compared to their corresponding treatments (Figure 3 A to D).

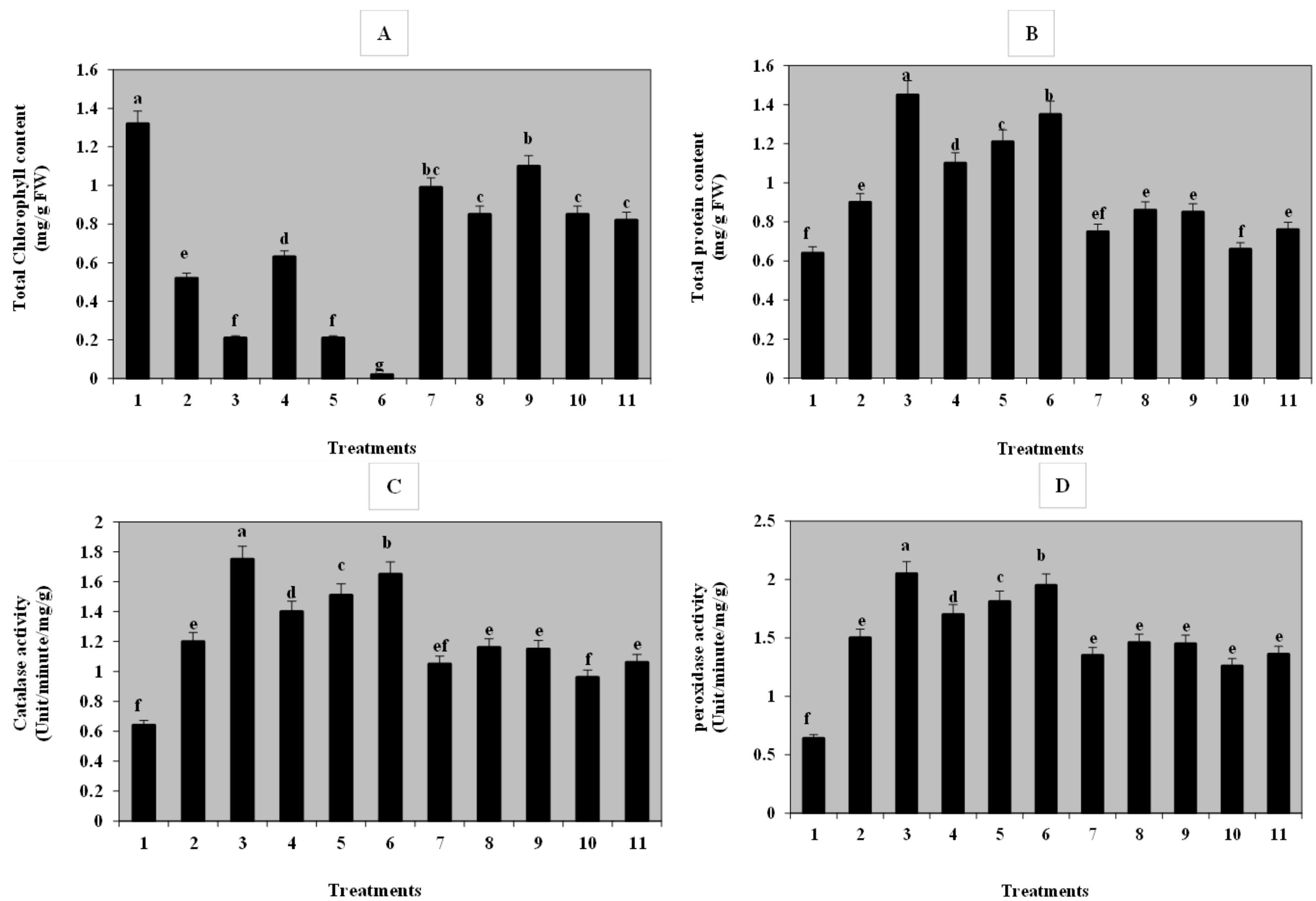

Figure 3 A to D. Impact of indole acetic acid (IAA) on physiological and biochemical attributes of maize seedlings grown under the stress of Cr (VI) and *Macrophomina phaseolina* (MP) after thirty days of seed germination. 1: control, 2: Cr(VI) 2000 ppm, 3: Cr(VI) 4000 ppm, 4: MP, 5: Cr(VI) 2000 ppm + MP, 6: Cr(VI) 4000 ppm + MP, 7: Cr(VI) 2000 ppm + IAA, 8: Cr(VI) 4000 ppm + IAA, 9: MP + IAA, 10: Cr(VI) 2000 ppm + MP + IAA, 11: Cr(VI) 4000 ppm + MP + IAA. Vertical bars represent the standard errors of means calculated from three replicates. Values labeled with different letters at their top indicate a significant difference (P≤0.05), determined by the LSD Test.

The results of Pearson Correlation (Figure 4 A) showed that the association between physiological attributes (total chlorophyll content and reducing sugar) × growth parameters (root, shoot length; root, shoot weight [fresh and dry]) showed positive, week to strong and significant to highly significant relationship. However, the correlation among biochemical traits (total protein content, catalase, and peroxidase activities) × growth parameters exhibited negative, weak to strong, and significant to highly significant association. Moreover, in the heat map the results revealed that the incorporation of IAA in soil increased the growth parameters in maize seedlings both in combined stress of heavy metal and *M. phaseolina* and in single stress Figure 4 B).

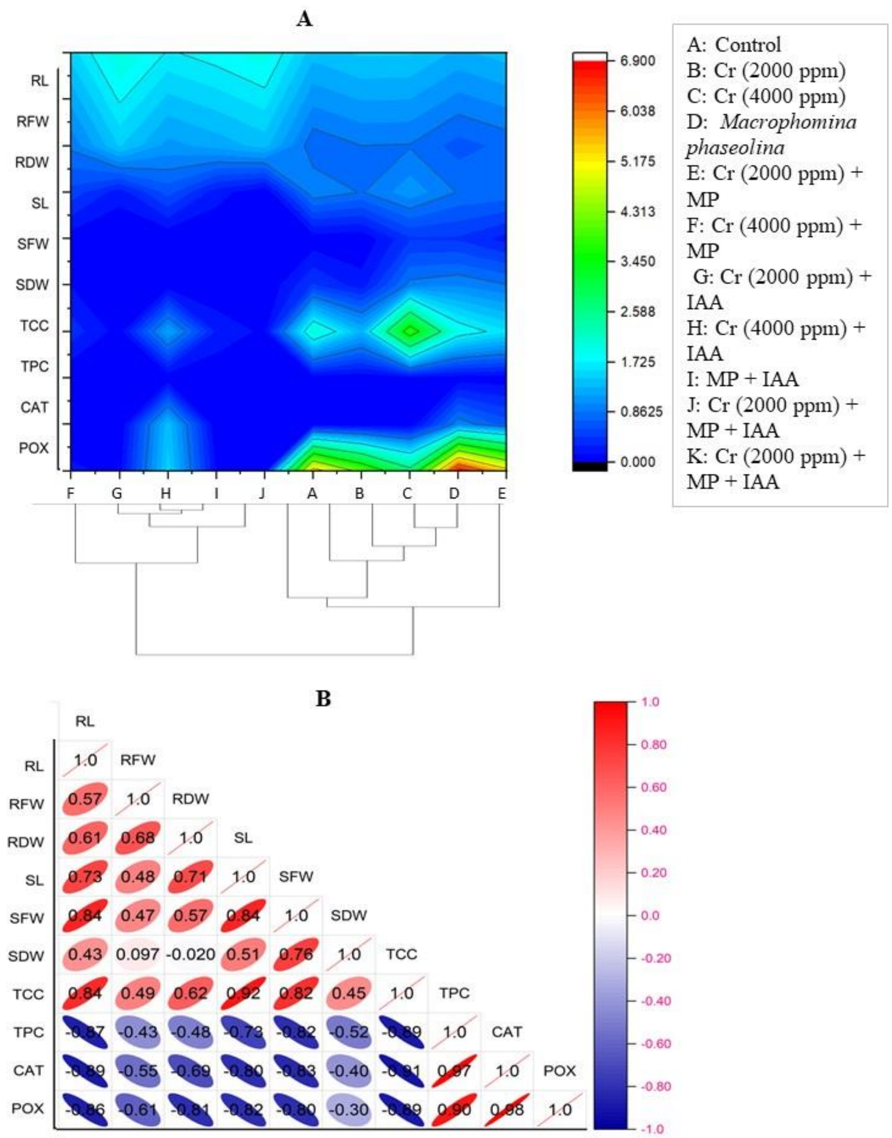

Figure 4. Heat map analysis (A) and Pearson correlation (B) among biochemical, physiological, and growth attributes in maize seedlings.

DISCUSSION

It was noticed that the spore suspension when given to the soil infects the roots and lower stems of maize plants with stunted growth and wilting of maize seedlings. The same findings were noticed by Degani, Becher, and Gordani (2022). According to their findings, they described that when the *M. phaseolina* infects the plant roots it further leads to the degradation of vascular tissues and the production of characteristic dark lesions, which resemble charcoal. This fungal infection ultimately impairs the plant's ability to uptake water and nutrients, resulting in symptoms such as wilting, stunting, and reduced growth. Moreover, the exposure of Cr(VI) at 2000 and 4000 ppm significantly ($P \leq 0.05$) reduced the maize seedling height and biomass (fresh and dry) up to 90% as compared to control. According to the findings of Saud *et al*. (2022) phytotoxicity caused by direct or indirect Cr (VI) is a significant factor in the loss of seedling biomass and growth. The impact of chromium ions on plants varies and is dependent on the spectrum of their severity. Inhibiting the germination of plant seeds, slowing the growth of the plants' hypocotyl and epicotyl regions, and harming the chloroplast cell structures are the principal effects of the various frequent processes that take place during the plant's life cycle.

Furthermore, the findings revealed that due to the exposure of Cr(VI) at 2000 ppm or 4000 ppm either individually or in combination with *M. phaseolina* exhibited overall changes in the growth and physiology of the maize seedlings. Exposure to Cr(VI) at high concentrations, such as 2000 ppm or 4000 ppm, can inhibit the growth of maize seedlings. Chromium (VI) toxicity disrupts root development and reduces shoot elongation, resulting in stunted growth. Furthermore, the presence of *M. phaseolina* exacerbates these growth-inhibitory effects, as the fungal pathogen further damages the root system and impairs nutrient uptake (Rizvi and Khan, 2022). Moreover, maize seedlings exposed to combined Cr(VI) and *M. phaseolina* stress exhibit alterations in various physiological parameters. Increased levels of Cr(VI) induce oxidative stress in plant tissues, leading to the accumulation of reactive oxygen species (ROS) and lipid peroxidation. Concurrently, the presence of *M. phaseolina* activates plant defense mechanisms, such as the production of antioxidant enzymes (e.g., superoxide dismutase, catalase, peroxidase) to mitigate oxidative damage. Additionally, Cr(VI) exposure at high concentrations interferes with nutrient uptake and metabolism in maize seedlings. It disrupts the uptake of essential minerals such as nitrogen, phosphorus, and potassium, leading to nutrient imbalances and deficiencies. Also, the presence of *M. phaseolina* further compromises nutrient assimilation by damaging root tissues and inhibiting nutrient uptake processes (Atta *et al*., 2023).

However, the application of IAA on the maize seedling incorporated with single and both stresses of Cr (VI) (2000 and 4000 ppm) and *M. phaseolina* significantly enhanced the growth and physiological parameters in maize seedlings. Indole-3-acetic acid (IAA) is a naturally occurring auxin that plays a crucial role in regulating plant growth and development. Application of exogenous IAA has been shown to promote root elongation, shoot growth, and overall biomass accumulation in maize seedlings. Even under stressful conditions such as Chromium (VI) toxicity and fungal infection by *M. phaseolina*, exogenous IAA treatment can stimulate root and shoot growth, thus enhancing the overall growth performance of maize seedlings (Wiszniewska, 2021).

Moreover, exogenous application of IAA can modulate various physiological parameters in maize seedlings, leading to improved stress tolerance and performance. IAA treatment has been reported to enhance photosynthetic efficiency, chlorophyll content, and stomatal conductance, thereby increasing the photosynthetic rate and water use efficiency in plants (Mir *et al*., 2020). Additionally, IAA promotes nutrient uptake and assimilation, which can counteract the negative effects of Chromium (VI) toxicity and fungal infection on nutrient metabolism in maize seedlings. Furthermore, IAA treatment enhances the synthesis of stress-related proteins and antioxidants, such as superoxide dismutase (SOD) and catalase (CAT), which scavenge reactive oxygen species (ROS) and protect plant cells from oxidative damage. Moreover, IAA-mediated hormonal regulation can enhance the plant's ability to cope with stress by promoting stress tolerance and resilience (Srivastava and Srivastava, 2020).

CONFLICT OF INTEREST

The authors affirm that the research was conducted without any commercial or financial affiliations that could be perceived as potential conflicts of interest.

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