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Effects of Rhizospheric *A. terreus* upon Growth, Phytohormone Homeostasis, and Antioxidant Responses in *B. Juncea* L. under Arsenic Stress: A Multivariate Approach

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ABSTRACT

Background. Agricultural soil contamination by arsenic (As) is a severe environmental problem and affects soil-plant systems which can pose risk to food safety by trophic transfer. Inoculation with microorganisms can be used as a tool to enhance plant performance under metal(loid) stress; however, the physiological and biochemical mechanisms by which rhizospheric fungi affect plant responses to arsenic are poorly understood, especially in *Brassica juncea.* In the current study, the rhizosphere isolate of fungus *Aspergillus terreus* was evaluated as bioinoculant to reduce arsenic phytotoxicity in *B. juncea*, an oilseed crop with agronomic and phytoremediation significance.

Methods. A total of thirteen treatments were employed, comprising the control treatment, four arsenic treatments (30-120 ppm), four treatments of arsenic + *A. terreus,* and four treatments of *A. terreus.* The number of plants that were used to maintain three uniform plants per pot was determined for each treatment (3 replicates) as biological replicates. Growth and biochemical characteristics of the plants were determined, while the variations among the different treatments and responses due to biomass were evaluated using PCA and structural path analysis.

Results. Dose-dependent phytotoxicity occurred on exposure to arsenic, which caused a 46.2% decrease in shoot length and 64.7% decrease in dry biomass at 120 ppm ($p < 0.001$). *A. terreus* inoculation was able to reverse this toxicity, with an increase of 7.1% in shoot length and 9.5% in fresh biomass at 30 ppm, as compared to controls. Further, 96.5% of agronomic variance and 92.2% of biochemical variance respectively was explained with clear separation among treatments. The correlation of phytohormones with biomass was high ($r > 0.90$) and those for phenols and proline were high negative ($r < -0.90$). Path analysis results showed that protein synthesis ($r = +0.19$) and phytohormonal regulation ($r = +0.63$) are the main drivers of biomass ($R^2 = 0.967$).

Conclusion. The phytohormonal and antioxidant metabolic regulation by *A. terreus* in *B. juncea* represents a viable and cost-effective approach for bioremediation of As-contaminated agricultural soils.

Keywords: arsenic toxicity, *A. terreus*, *B. juncea*, phytohormones, phytoremediation

Highlights

- Under As-stress, *A. terreus* showed a significant effect on reducing growth inhibition

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and enhancing biomass production in *B. juncea*.

- Increased levels of phytohormones (IAA and GA_3) and protein buildup along with decreased oxidative stress markers showed that fungal inoculation provided stress tolerance.
- Through multivariate and path analyses, phytohormonal regulation and protein synthesis were found to be the key processes that influenced biomass recovery and suggested the potential of *A. terreus* in sustainable bioremediation of As-contaminated soils.

1. INTRODUCTION

Heavy metal pollution of agricultural soils has become one of the most urgent global environmental issues of the 21st century, posing a direct threat to world food security and the health of the ecosystem [1, 2]. Arsenic (As) is a highly toxic metalloid. It is extremely harmful to the health of the plants and poses serious risks to the safety of human food. Compared to vital micronutrients, As has no biological role in higher plants; it is likely absorbed through phosphate transporter because of the chemical resemblance between arsenate and phosphate, resulting in disruption in cellular energy metabolism, release of reactive oxygen species (ROS), inhibition of root development, and the inhibition of chlorophyll synthesis [3, 4]. Anthropogenic activities, such as mining, industrial discharge, utilization of As-based pesticides, and the use of contaminated groundwater in tending irrigation, are continuing to mount, increasing the global burden of As contamination [5, 6].

B. juncea L. (Indian mustard) is an important oilseed crop, widely cultivated for its edible oil production and several other uses in agriculture and industry; additionally, it is a better candidate for heavy metals phytoremediation because of its high biomass production rate and deep root penetration, as well as due to its intrinsic ability to store metals in the aboveground tissues [7]. This species has been widely used to investigate its tolerance mechanisms to Cd, Pb, Zn, Cu, and metalloid As, as it provides a good model to study plant-microbe interactions under metal stress conditions [8, 9]. Although *B. juncea* shows promising phytoremediation, it is significantly affected at high As levels. Moreover, it is necessary to identify the biological mechanisms such as antioxidant defense, metal sequestration and detoxification, osmotic adjustment, and phytohormonal regulation, to help it become more tolerant of the heavy metal and also increase its biomass in stress situations [10, 11].

Rhizospheric fungi has received substantial interest as a biological agent promoting plant growth and reducing metal and metalloid stress [12]. Among them, aspergillus species, especially *A. terreus*, has shown an excellent competency in metal biosorption, synthesis of phytohormones (gibberellins, auxins, abscisic acid), dissolution of phosphates, and systemic stress tolerance induction in the host plant [13-15]. *A. terreus* secretes a collection of secondary metabolites and extracellular enzymes that bind metal ions, decrease their bioavailability, and increase the growth of the colonized plants with nutrient uptake and antioxidant defense [16-18]. Growth-promoting and metal-contaminating effects of *A. terreus* in cereal crops were reported in several recent studies, yet its role in Brassicaceae species, especially under As-stress, remains underexplored.

To date, the majority of research on fungal-mediated heavy metal tolerance is based on univariate statistics, where a single parameter is considered at a time and the interconnectedness between plant stress responses might be underestimated [19]. The novelty of the present study is the combination of rhizospheric *A. terreus* inoculation with a comprehensive multivariate evaluation of *B. juncea* responses for achieving a gradient of arsenic stress. This study investigates plant growth, phytohormonal regulation, as well as pigments and metabolites involved in stress responses in a single study and integrates these responses through PCA, correlation analysis, regression modeling, structural path analysis, and cluster analysis–a combination of studies that have not been undertaken before. This holistic approach gives a new insight into the biochemical features most strongly correlated with biomass responses in the presence of both arsenic and fungus [20].

The current study aims (i) to assess the effectiveness of *A. terreus*, obtained as a by-product from the rhizosphere of *Parthenium hysterophorus* L., in alleviating arsenic toxicity in *B. juncea*; (ii) to determine the modulation of phytohormone balance, photosynthetic pigments, and defense metabolites under different conditions of arsenic and fungal inoculation; and (iii) to use multivariate statistical tools (PCA, correlation heatmap, regression modeling, structural path analysis, and cluster analysis) to fully describe the plant response and identify key biochemical pathways related to arsenic stress.

2. METHODOLOGY

2.1 Fungal Culture Preparation, Identification, and Isolation

A. terreus was isolated from *Parthenium hysterophorus* L. rhizosphere soil in heavy metal contaminated areas near Charsadda, Khyber Pakhtunkhwa, Pakistan (Figure 1). The rhizosphere samples of the soil were diluted in series (10^{-1} to 10^{-1}), plated on PDA (potato dextrose agar) enriched with 100 mg/L of streptomycin (which was added to prevent the growth of the bacteria), and incubated for 72 hours at 28°C. By sub-culturing on brand new PDA plates, morphologically different colonies were purified. Molecular identification was performed using ITS rDNA sequencing (ITS1-5.8S-ITS2 region) with primers ITS1 and ITS4 [21]. BLAST analysis against the NCBI GenBank database (GenBank Accession No. KT310979.1) confirmed the isolate as *A. terreus* (99.8% sequence identity). The fungus was maintained on PDA slants at 4°C.

For culture filtrate preparation, *A. terreus* was inoculated into 250 mL Erlenmeyer flasks containing 100 mL Czapek liquid medium (glucose 10 g/L, peptone 10 g/L, KCL 0.5 g/L, $MgSO_4$ 0.5 g/L, and $FeSO_4$ 0.01 g/L in distilled water) and incubated at 30°C with orbital shaking at 120 rpm for 7 days. The culture was then filtered through Whatman No. 1 filter paper, while the cell-free filtrate and fungal biomass (spore suspension) were stored separately at 4°C until usage.

2.2 Plant Material and Experimental Design

The seeds of *B. juncea* L. cv. Pusa Bold were surface-sterilized with 1% sodium hypochlorite for 5 min, followed by three rinses in sterile distilled water. The experiment was conducted under greenhouse conditions at the Department of Botany, Bacha Khan University Charsadda, using a completely randomized design (CRD) with three replicates per treatment. Plastic pots (15 cm diameter) were filled with 200 g of autoclaved sandy loam soil. Arsenic stress

was imposed by irrigating pots with $Na_2HAsO_4 \cdot 7H_2O$ (sodium arsenate) solutions at 30, 60, 90, and 120 ppm, while *A. terreus* spore suspension was applied at 5, 10, 15, and 20 mL per pot mixed with the soil at sowing.

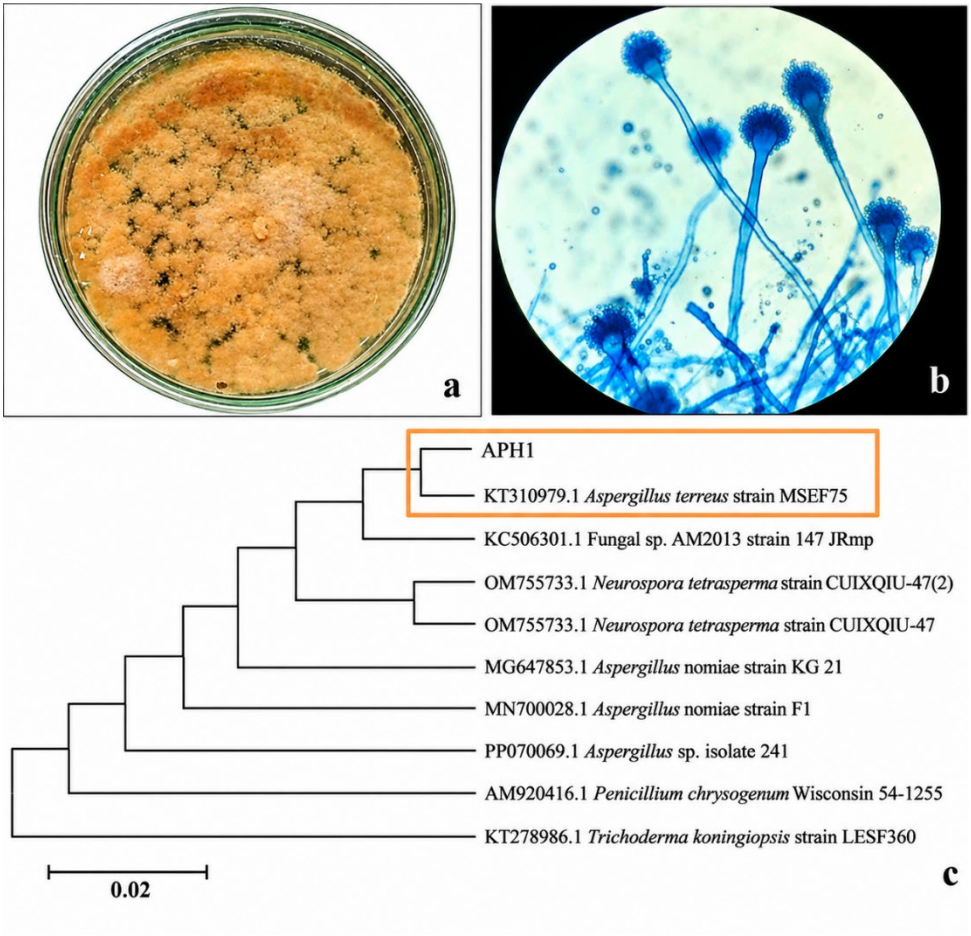


Figure 1. Colony Morphologies on (**a**) Macroscopic and (b) Microscopic Scale of Isolated Rhizospheric Fungi (c) Phylogenetic Tree based on ITS rDNA Sequences of the Rhizospheric Fungal Strain *A. terreus* was Constructed

The pot was defined as the experimental unit and three independent pots were used as biological replicates for each treatment. The three uniform plants grown in each pot were regarded as subsamples. The measurements taken were averaged to calculate one measurement per pot and the three pot-level measurements (n = 3) were used in statistical analysis. The thirteen treatments included T0 (control, distilled water), T1 (As-30 ppm), T2 (As-60 ppm), T3 (As-90 ppm), T4 (As-120 ppm), T5 (As-30 ppm + AT-5 mL), T6 (As-60 ppm + AT-10 mL), T7 (As-90 ppm + AT-15 mL), T8 (As-120 ppm + AT-20 mL), T9 (AT-5 mL), T10 (AT-10 mL), T11 (AT-15 mL), and

T12 (AT-20 mL). Arsenic treatments were prepared utilizing sodium arsenate. Five seeds were sown per pot. After germination, seedlings were thinned to three uniform plants per pot. Plants were kept in natural light for four weeks before being picked (12 hours of light, 25 ± 2°C). When combined treatment T5-T8 was used, the concentration of As and the inoculum dose of *A. terreus* both increased. This is because the present experimental design does not permit the effect of As concentration and fungal inoculum dose to be fully separated. When analyzing the responses obtained for the combined treatments, this co-variation has to be taken into account.

The *A. terreus* inoculum was applied volumetrically in experimental treatments as follows: 5 mL, 10 mL, 15 mL, and 20 mL. The spore density was not measured at the time of inoculation, while the inoculum density (spores/mL) was not calculated afterwards. In the future, standardisation of fungal inocula should be performed according to the number of spores, for instance, by hemocytometer.

2.3 Agronomic Parameters

Plants were plucked during harvest, 28 days post-sowing, and cleansed with distilled water. The measurements of shoot length (cm) were taken from the cotyledonary node to the apex of the shoot. Whereas, root length was measured from the shoot-root junction to the longest tip of the root. The root number was the sum of lateral roots and the main root per plant. An instant assessment of fresh weight (g) and long dry weight (g) of the product was conducted by oven-drying it at 70°C for 48 hours.

2.4 Biochemical Analyses

Photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) were extracted from 0.1 g fresh leaf tissue homogenized in 80% acetone, centrifuged at 1000 rpm for 5 min, and quantified spectrophotometrically at 663, 645, and 480 nm, following Arnon [22]. Phytohormones, including gibberellic acid (GA_3, at 254 nm) and abscisic acid (ABA, at 263 nm), were determined using the methanol–chloroform–ammonium hydroxide extraction method described by Kettner and Dörffling [23]. Indole-3-acetic acid (IAA) was quantified using the Salkowski reagent method at 540 nm [24]. Salicylic acid (SA) was measured by using the $FeCl_3$ method at 540 nm [25]. Soluble protein content was estimated using the Lowry method at 650 nm [26]. Proline content was determined using the acid-ninhydrin assay at 520 nm [27]. The amount of total soluble sugar in the phenol sulphuric acid method was determined at 420 nm [28]. The Folin-Ciocalteu method was used to determine total phenolics at 760 nm and presented as mg gallic acid equivalents/g fresh weight [29].

2.5 Statistical Analyses

Python (v3.11) was used to analyze all the data alongside the SciPy, scikit-learn, and pandas libraries. One-way Analysis of Variance (ANOVA) was conducted to determine the legitimate variance of treatments ($p < 0.05$), followed by Tukey-HSD post-hoc test of mean differentiation. The results were displayed in the form of compact letters on bar charts. Agronomic and biochemical data were standardized (z-scored) to perform a Principal Component Analysis (PCA), which was used for dimension reduction and visualization of treatment patterns. Pearson correlation coefficients were computed for all pairwise variable combinations and visualized as a lower-triangular heatmap. Simple linear regression was used to model the relationships between key agronomic and biochemical variables, while multiple linear

regression was applied to identify the strongest biochemical predictors of shoot length. Using four mediating biochemical pathways phytohormones, protein content, photosynthetic pigments, and stress metabolites, a structural path analysis model was built to measure both the direct and indirect impacts of arsenic stress and *A. terreus* inoculation on plant development. Standardized path coefficients (β) were estimated using multiple linear regression on z-scored composite variables, while the overall model fit was assessed by the coefficient of determination (R^2). Hierarchical cluster analysis (Ward's method, Euclidean distance) was performed on the standardized treatment means across all 16 parameters. All figures were prepared at 300 dpi resolution.

3. RESULTS

3.1 Effects of As-Stress and *A. terreus* on Agronomic Parameters

Arsenic stress significantly ($p < 0.001$) impaired all agronomic parameters of *B. juncea* in a concentration-dependent manner (Figure 2). One-way ANOVA revealed highly significant F-values for all traits: shoot length ($F = 28.2$, $p < 0.001$), root length ($F = 30.9$, $p < 0.001$), root number ($F = 46.7$, $p < 0.001$), fresh weight ($F = 46.0$, $p < 0.001$), and dry weight ($F = 69.8$, $p < 0.001$). The Tukey post-hoc test separated treatments into distinct homogenous groups. At 120 ppm, the shoot length decreased from the control measurement of 18.2 ± 1.2 cm to 9.8 ± 0.8 cm, representing a 46.2% decline. Similarly, root length, as well as fresh and dry weight, decreased by 53.8%, 63.2%, and 64.7% respectively at the maximum dosage of arsenic. Root number was reduced from 12.0 ± 0.9 to 5.8 ± 0.5 at 120 ppm.Inoculation with *A. terreus* (T9 T12) improved all parameters of growth significantly above the control level. *A. terreus* AT-10 mL (T10) provided the best growth in terms of shoot length (22.1 ± 1.6 cm, increase 21.4) and fresh weight (3.62 ± 0.3 g, increase 27.0). Importantly, *A. terreus* inoculation partially recovered the growth of As-stressed plants: at level As-30 ppm + AT-5 mL (T5), shoot length was equalized to 19.5 + 1.4 cm (107.1% of control) and fresh weight rose to 3.12 ± 0.2 g (109.5% of control). At the fullest As dose (120 ppm), fungal inoculation increased shoot length and dry weight by 15.3% and 33.3% respectively in comparison to As-120 ppm.

3.2. Effects on Photosynthetic Pigments

Under arsenic stress, the concentration of chlorophyll a significantly decreased, from 1.52 ± 0.09 mg/g (control) to 0.89 ± 0.07 mg/g at 120 ppm, representing a 41.4% reduction. At the highest As concentration, a similar trend was seen for carotenoids (43.2% reduction) and chlorophyll b (47.4% decrease) (Figure 3). Fungal inoculation alone increased chlorophyll a levels by 10.5–13.2%, as compared to the control. The infusion of *A. terreus* markedly restored pigment levels in combination treatments: at As-30 ppm + AT-5 mL, chlorophyll a reached 1.61 ± 0.12 mg/g, surpassing control values by 5.9%.

3.3. Phytohormone Modulation

Following As exposure, all assessed phytohormones showed a substantial decrease ($p < 0.001$) (Figure 3). GA_3 levels decreased from 1.42 ± 0.10 (control) to 0.42 ± 0.04 µg/g at 120 ppm (70.4% reduction). Whereas, SA, IAA, and ABA levels showed comparable dose-dependent decreases.

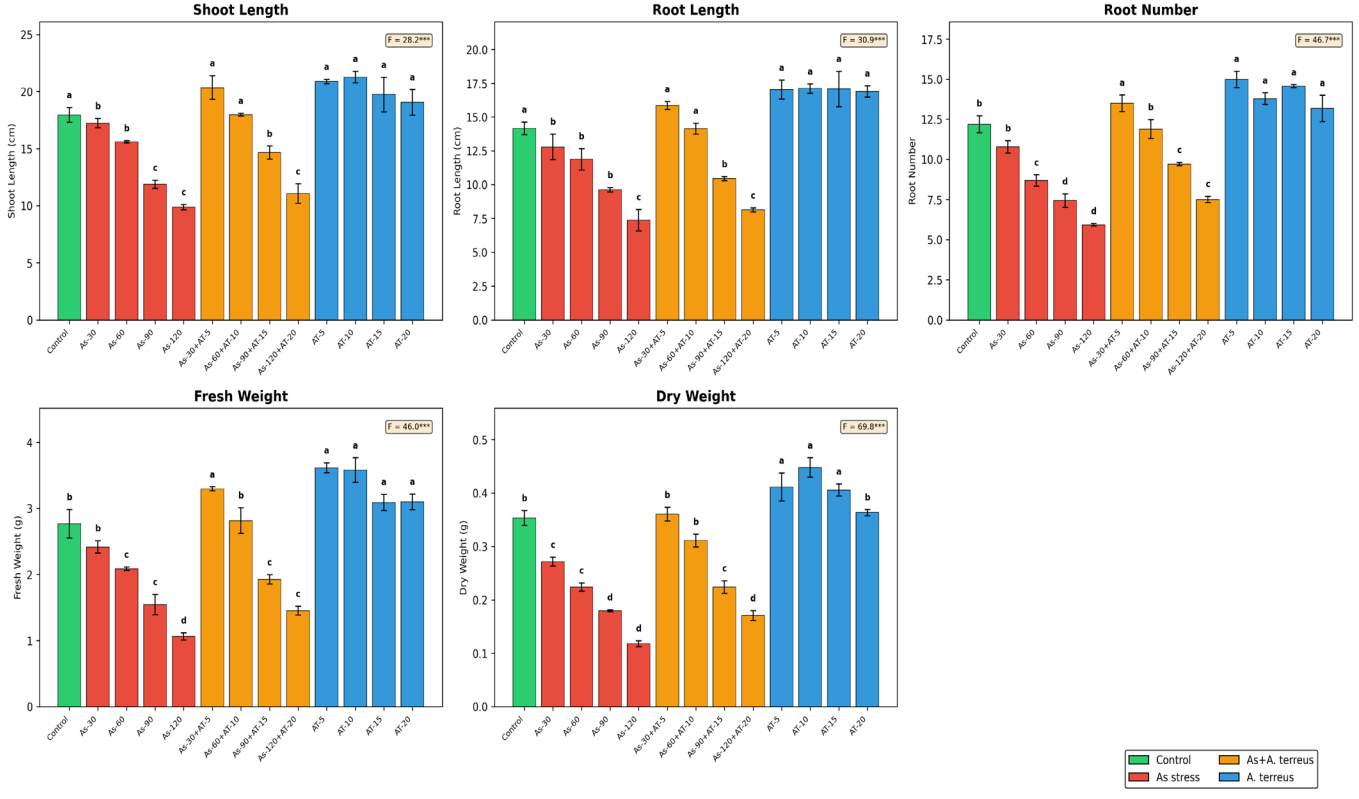


Figure 2. The Effects of Arsenic Stress and *A. terreus* Inoculation on *B. juncea's* Shoot Length, Root Length, Root Number, Fresh Weight, and Dry Weight

Note. Means ± SEM ($n = 3$) are shown by bars. Different letters in the aforementioned bars indicate statistically significant differences between treatments (Tukey HSD, 0.05). The given SPL F-statistics have significant values (***$p < 0.001$). As stress (red), As plus *A. terreus* (orange), *A. terreus* alone (blue), and control (green) are the colors

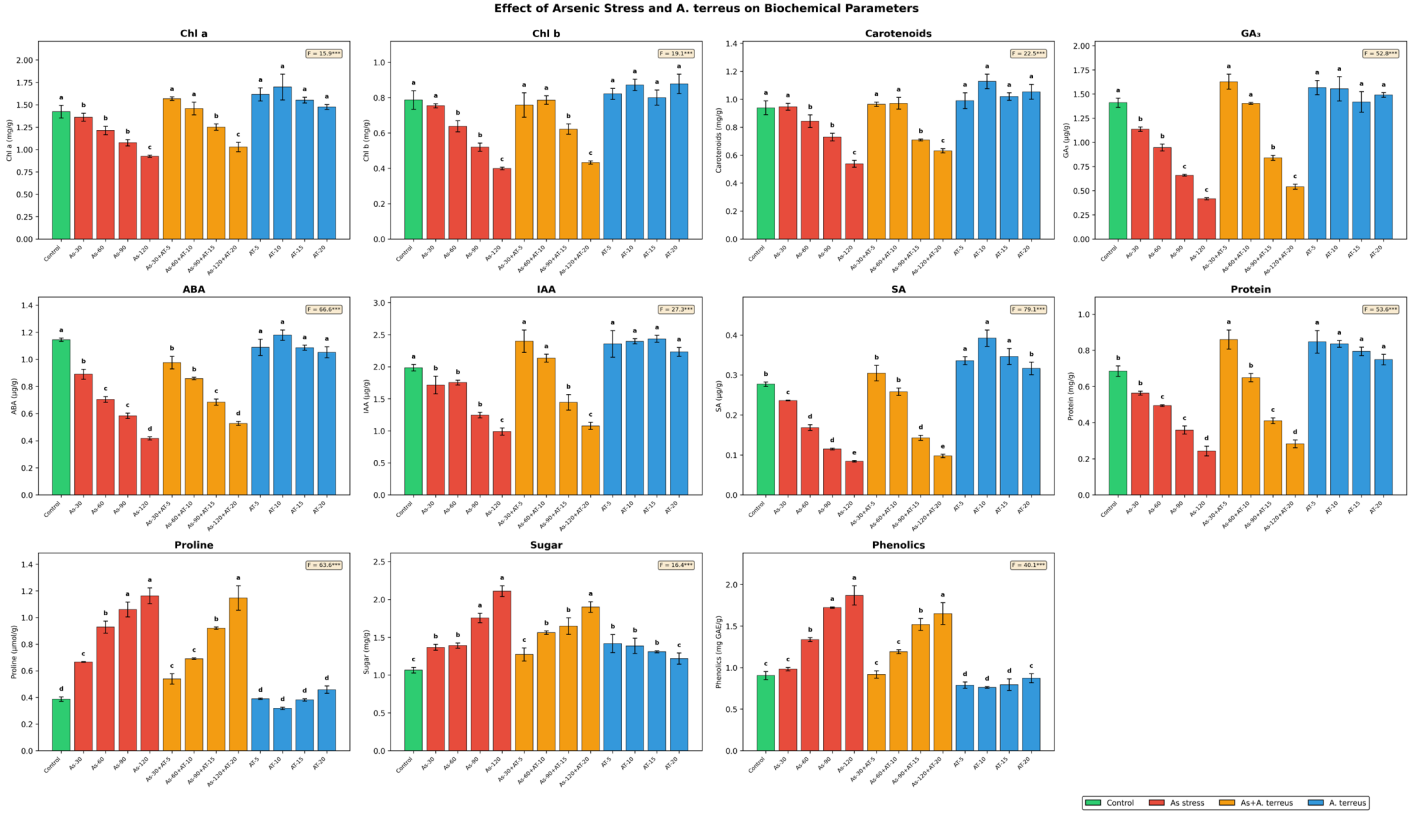


Figure 3. Impact of *A. terreus* Inoculation and Arsenic Stress on *B. juncea* Biochemical Indicators (Photosynthetic Pigments, Phytohormones and Metabolites)

Note. Bars represent means ± SEM ($n = 3$). Different letters above bars denote statistically significant differences (Tukey's HSD, $\alpha = 0.05$). ANOVA F-statistics with significance levels (***$p < 0.001$) are displayed for each parameter.

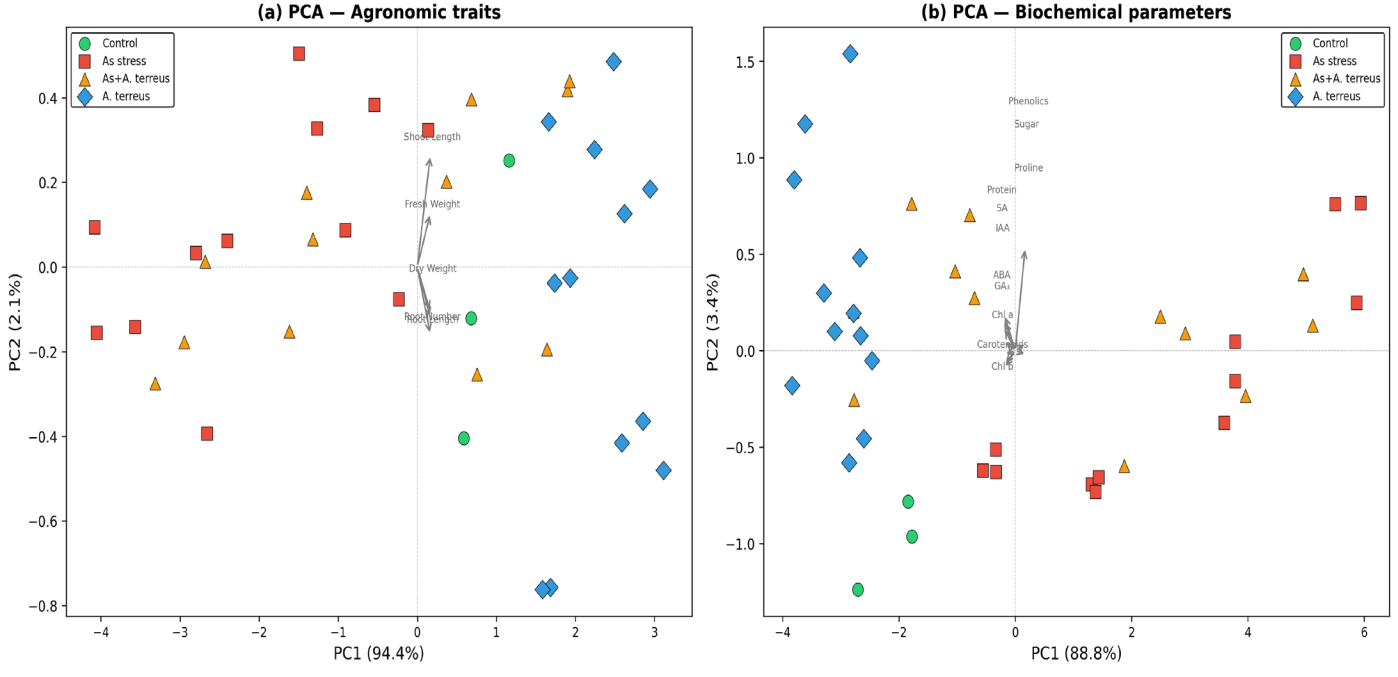


Figure 4. Principal Component Analysis (PCA) Biplots of (a) Agronomic Traits and (b) Biochemical Parameters of *B. juncea* under Arsenic Stress and *A. terreus* Inoculation

Note. Individual replicates (n = 39) are plotted; symbol shape and color denote treatment category. Loading vectors (gray arrows) indicate the direction and magnitude of variable contributions. Agronomic PCA: PC1 = 94.4%, PC2 = 2.1% (96.5% total). Biochemical PCA: PC1 = 88.8%, PC2 = 3.4% (92.2% total).

At the highest As concentration, IAA level dropped by 55.8%. In comparison to control levels, inoculation with *A. terreus* alone enhanced GA3 (14.1%), IAA (19.2%), and SA (35.7%) levels, indicating intrinsic growth-promoting activity. Fungal inoculation somewhat reduced the hormonal suppression caused by arsenic in combination treatments; IAA levels in T5 (As-30 + AT-5) reached 2.28 ± 0.15 µg/g, which was 9.6% higher than control values.

3.4. Defense Metabolites: Proline, Sugar, and Phenolics

Under As-stress, proline, soluble sugar, and total phenolics showed substantial ($p < 0.001$) increases in contrast to the diminishing trend of growth-promoting parameters (Figure 3). Proline content rose from 0.42 ± 0.03 µmol/g (control) to 1.25 ± 0.08 µmol/g at 120 ppm, representing a 197.6% increase. At the maximum As dosage, soluble sugar rose by 80.9% and total phenolics by 121.2%. Fungal inoculation attenuated these stress-induced accumulations: proline in T5 (As-30 + AT-5) was 0.55 ± 0.04 µmol/g, only 31.0% above the control, as compared to 61.9% above control in As-30 alone (T1). Instead of increased tolerance, this reduction of stress marker development in fungal-inoculated plants indicated less oxidative damage.

3.5. Principal Component Analysis

Treatment means were computed of the three biological replicates of each one of the 13 treatments and subjected to the PCA of agronomic traits (Fig. 4a). It revealed that the first two principal components explained 96.5% of total variance (PC1: 94.4%; PC2: 2.1%). PC1 was strongly loaded by all five agronomic variables and clearly separated treatments along a stress gradient, with *A. terreus*-only treatments clustering in the positive PC1 direction (high growth) and high As treatments in the negative direction (suppressed growth). Combined As + AT treatments occupied intermediate positions, confirming the ameliorative effect of fungal inoculation.

For biochemical parameters (Figure 4b), the first two PCs explained 92.2% of total variance (PC1: 88.8%; PC2: 3.4%). PC1 separated treatments based on the opposing contributions of growth-promoting biochemicals (chlorophyll, IAA, GA_3, protein; positive loadings) versus stress-responsive metabolites (proline, sugar, phenolics; negative loadings). The biplot revealed that the stress-responsive metabolite vectors pointed in the opposite direction of the growth-promoting parameters, consistent with the negative correlations observed in the heatmap analysis.

3.6. Correlation Analysis

The Pearson correlation heatmap (Figure 5) revealed distinct clusters of positively and negatively correlated variables. All agronomic parameters were strongly and positively correlated (r = 0.91–0.95, $p < 0.001$) and showed significant positive associations with photosynthetic pigments (r = 0.84–0.95) and phytohormones IAA, GA_3, ABA, and SA (r = 0.87–0.96). Conversely, proline, sugar, and phenolics exhibited a strong negative correlation with all growth metrics (r = −0.72 to −0.95) and a positive correlation among themselves (r = 0.79–0.95). This bipolar correlation pattern indicates that developmental pathways and stress response pathways remain incompatible when excessive as is present.

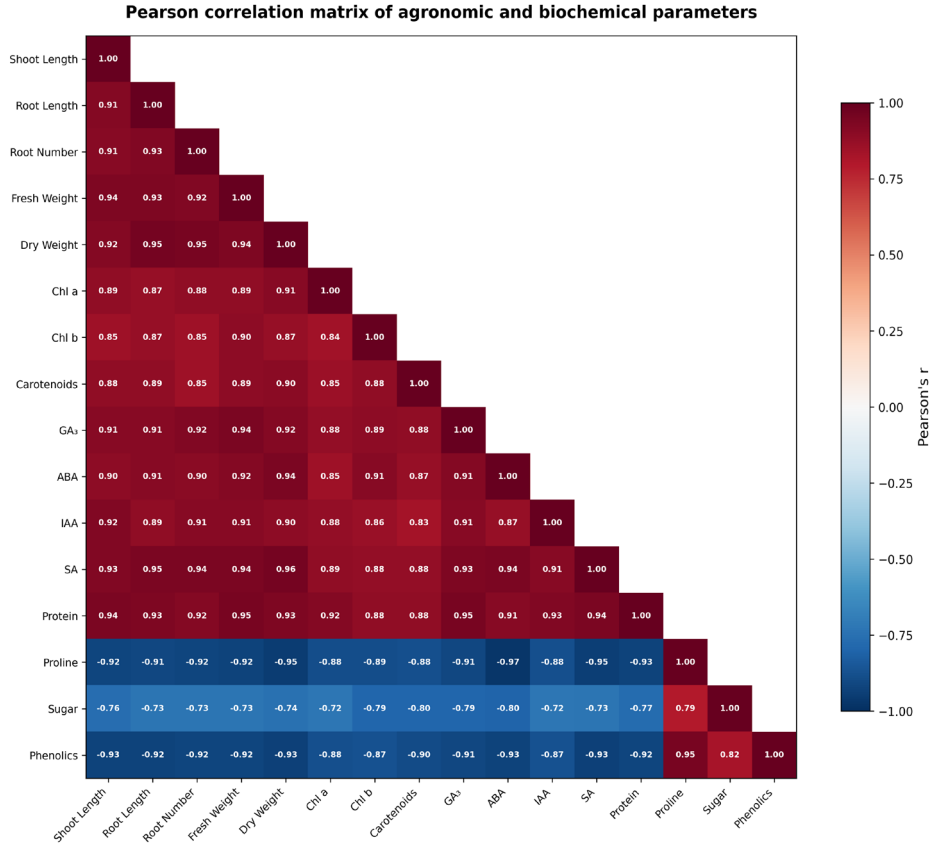


Figure 5. A Pearson correlation heatmap illustrating all the agronomic and biochemical traits evaluated in *B. juncea*.

Note. Pearson's r coefficients (n = 39). The lower triangle displays correlation values, with blue representing −1 and dark red indicating +1. All correlations with |r| > 0.50 were statistically significant at $p < 0.001$.

3.7. Regression Analysis

Linear regression analysis revealed very significant positive correlations between shoot length and chlorophyll a (R^2 = 0.784, $p < 0.001$) and between fresh weight and IAA (R^2 = 0.830, $p < 0.001$) (Figure 6). In contrast, a significant negative correlation was seen between dry weight and phenolics (R^2 = 0.867, $p < 0.001$), as well as between root length and proline (R^2 = 0.835, $p < 0.001$). In a multiple linear regression analysis, five biochemical predictors (Chlorophyll a, IAA, GA3, protein, and proline) explained 91.7% of the variance in shoot length (adjusted R^2 = 0.904, $p < 0.001$). IAA (β = 2.27) and protein content (β = 6.44) emerged as the most significant positive predictor. Whereas, proline emerged as the most substantial negative predictor (β = −3.52).

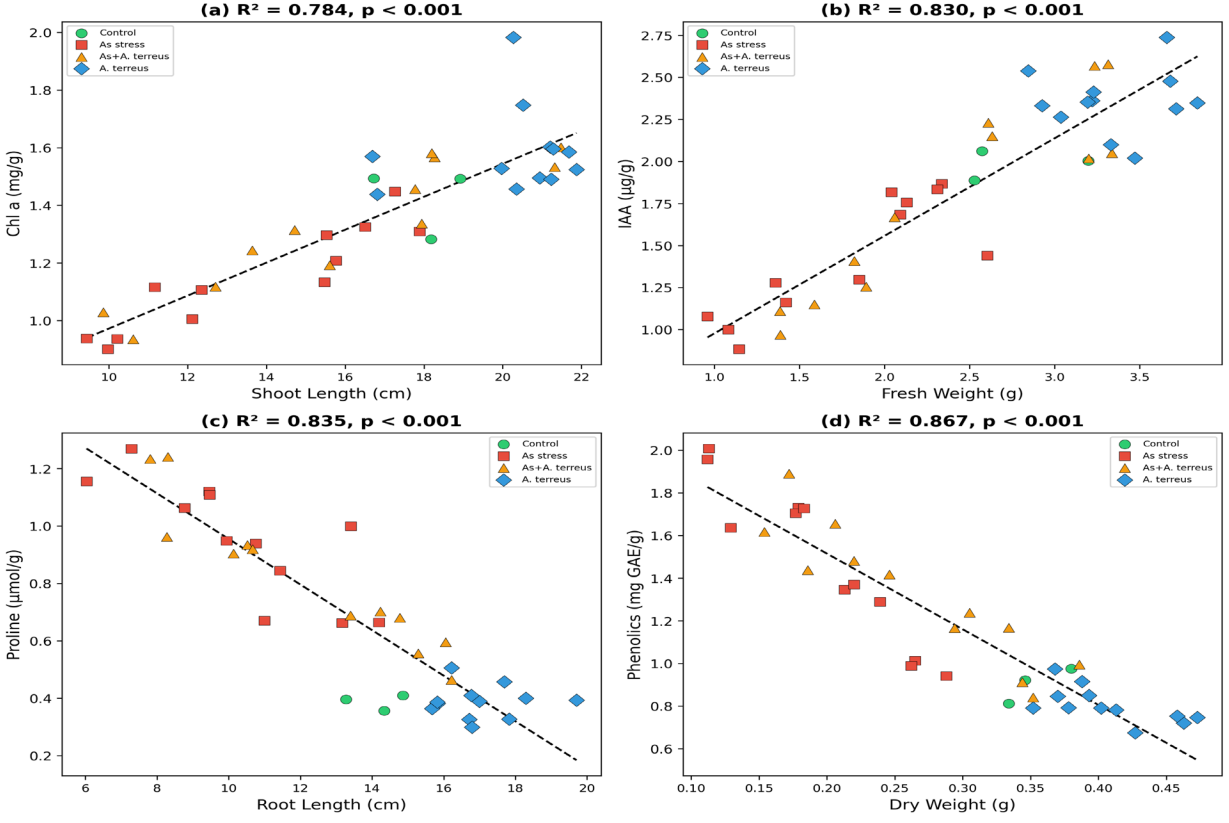


Figure 6. Key Agronomic and Biochemical Characteristics are Connected by the Following Linear Regression Research: (a) Length of Shoot vs Chlorophyll a (R^2 = 0.784); (b) Fresh Weight vs IAA (R^2 = 0.830); (c) Root Length vs Proline (R^2 = 0.835); and (d) Dry Weight vs Phenolics (R^2 = 0.867)

Note. dashed lines illustrate the degree of correspondence of the regression. At $p < 0.001$, all regressions were statistically significant.

3.8. Cluster Analysis

Hierarchical cluster analysis categorized the thirteen treatments into three functionally separate categories (Figure 7). The control, *A. terreus*-only treatments (T9–T12), and As-30+AT-5 combination treatment were all categorized in Cluster I (orange), which exhibited circumstances that were conducive to almost optimal growth. Cluster II (green) received treatments with elevated levels of As (As-90, As-120) and the As-120+AT-20 combination therapy, which was found to be very stressful. In Cluster III (red), stress alone (As-30, As-60) and moderate combination therapy (As-60+AT-10, As-90+AT-15) exhibited moderate stress levels with little enhancement. This clustering pattern corroborates the PCA findings and illustrates the biological relevance of the treatment groups.

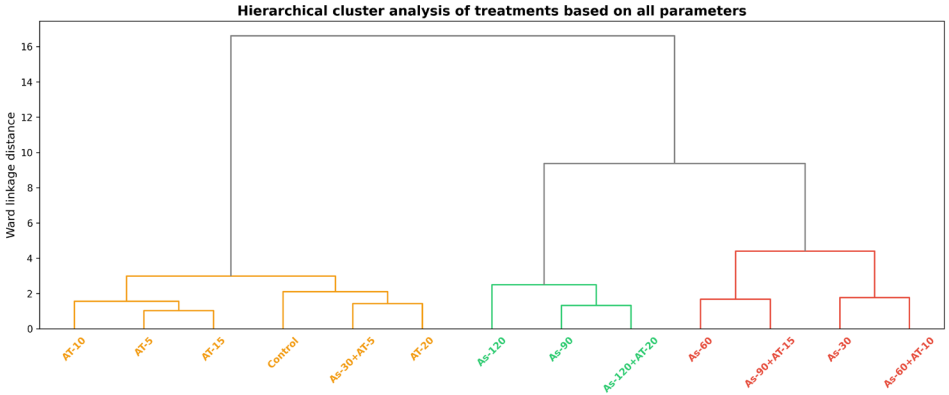


Figure 7. Hierarchical Cluster Analysis (Ward's Method, Euclidean Distance) of Thirteen Treatments using All Standardized Agronomic and Biochemical Traits
Note. There are three primary categories: optimal growth (Cluster I, orange), stress condition (Cluster II, green), and moderate stress with some enhancement (Cluster III, red).

3.9. Structural Path Analysis

The structural path analysis model was supplemented with four mediated biochemical pathway forming phytohormone composites (IAA, GA3, ABA, SA), protein levels, and photosynthesis-related pigments (chlorophyll a, b, carotenoid) to shed light on the effects of arsenic stress and *A. terreus* inoculations on plant growth (Figure 8). Approximately 96.7% of the variation in plant growth was accounted for by the comprehensive route model (R^2 = 0.967), suggesting an unusually robust model fit. However, there were only 39 observations and the number of relationships modelled was relatively large. Hence, the high R^2 value should be taken with a liberal pinch of salt and not as indicative of a very good fit. The results show a good explanation for the current experimental data set, although it may be partly due to large differences between the observations caused by the treatment and, therefore, needs to be verified using a larger independent data set.

As stress adversely impacted all growth-promoting mediators, including protein (β = -0.94), phytohormones (β = -0.93) and photosynthetic pigments (β = -0.91). Moreover, it substantially increased the accumulation of stress metabolites (β = 0.93). The inoculation of *A. terreus*, however, decreased stress metabolites (= -0.85) and showed a beneficial effect on phytohormones (β = 0.89) and pigments (β = 0.87).

Hence, *A. terreus* serves as a biological inhibitor to arsenic poisoning.

Phytohormones showed the most substantial beneficial influence on plant growth (β = 0.63, $p < 0.001$), followed by protein content (B = 0.19) and photosynthetic pigments (β = 0.15). Stress metabolites significantly impeded development by competing for metabolic resources, exerting a notable negative and direct effect on growth (β = -0.03). The path model indicating the hormonal pathway as the most influential (β = 0.63) corroborates multiple regression findings that identify protein and IAA as the primary predictors of shoot length, highlighting the essential function of phytohormone signaling in facilitating both A-toxicity and *A. terreus*-mediated growth enhancement.

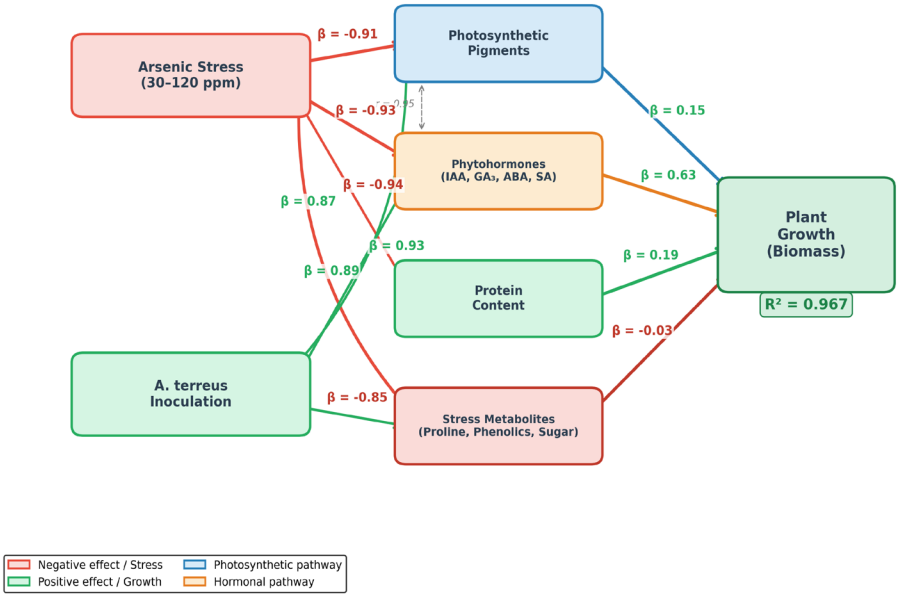


Figure 8. Structural Path Model of Arsenic Stress and Effects of *A. terreus* on Biochemical Pathways and *B. juncea* Growth ($R^2 = 0.967$)

4. DISCUSSION

A structural path assessment model showing how arsenic stress and *A. terreus* exposure directly and indirectly affect the development of *B. juncea* plants was developed. Exogenous factors (on the left) include arsenic stress (red) and fungi exposure (green). Biochemical pathways that connect things include pigments that help plants make food, plant hormones, protein levels, and stress metabolites. The endogenous variable (on the right) is the biomass composite of plant growth. Each arrow has a standardized path coefficient (β) on it. Green values represent positive impacts, while red values indicate negative effects. The model accounted for 96.7% of the variation in the development of plants ($R^2 = 0.967$).

4.1. Dose-dependent Arsenic Toxicity and Growth Inhibition

The resulting decrease in all agronomic parameters with increasing levels of

As (46.2% lowering the shoot length at 120 ppm) is consistent with other investigations on As phytotoxicity, especially in brassicaceous plants [7, 10, 30]. At high concentrations, arsenic disrupts phosphate metabolism, impairs auxin signaling, and prevents root cell differentiation, reducing the aerial (above ground) and subterranean (below ground) biomass [30, 31]. This decline in chlorophyll level (41.4% at 120 ppm) coincides with the data indicating that arsenic inhibits chlorophyll synthesis through the dehydratase of δ-aminolevulinic acid and the replacement of essential cofactors [32]. The mechanistic relationship between vegetative development and photosynthetic capability under arsenic stress is supported by the strong positive correlation between chloroplast a and length of shoot ($r = 0.89$).

4.2. *A. terreus* as a Bioinoculant for As-stress Mitigation

The ability of *A. terreus* to recover and boost growth above normal levels, especially at lower As quantities, shows that it may both stimulate growth and relieve stress. The 21.4% increase in shoot length with AT-10 mL alone and the restoration of growth to 107.1% of control values at As-30 ppm + AT-5 mL are consistent with the phytohormone-producing and metal-chelating properties of aspergillus species [16, 17, 33]. Partial restoration at higher As concentrations (15.3% improvement at 120 ppm) suggests that *A. terreus* inoculation would be the most effective in moderately contaminated soils (30–60 ppm As), where it can restore growth to or above control levels. Notably, inoculation with *A. terreus* also improved plant growth in the absence of As-stress, suggesting that some of the growth-promoting activity of the fungus is not specific to As-detoxification but is more related to general plant growth promotion.

4.3. Phytohormone Homeostasis and Stress Signaling

The As-induced suppression of GA_3 (70.4%), IAA (55.8%), ABA, and SA levels confirmed that As severely disrupts hormonal homeostasis in *B. juncea* [34, 35]. The restoration of hormonal levels (IAA and EAA) by the inoculation of *A. terreus*, especially in the combined treatment (T5) with slightly higher levels than in the control, revealed that fungal inoculation can be connected to better auxin status under As-stress; however, the provided IAA was not directly proven to come from fungal inoculation.

4.4. Defense Metabolite Accumulation as a Stress Biomarker

The inverse relationship between growth parameters and stress-responsive metabolites (proline, sugar, phenolics) is a hallmark of plant stress physiology [11, 13, 36, 37]. The 197.6% increase in proline at 120 ppm reflects osmotic adjustment and ROS scavenging, while the 121.2% increase in phenolics indicates the activation of the phenylpropanoid pathway [38, 39]. The application of *A. terreus* substantially decreased stress-related accumulations (proline increased by only 31.0% compared to control in T5, in contrast to 61.9% in As-30 alone), suggesting that fungal colonization diminished oxidative damage at the source.

4.5. Structural Path Analysis: Mechanistic Insights

The most effective mechanistic explanation of the results is provided by the structural path analysis model (Figure 8). The model determined that the overall impact of Asstress and *A. terreus* inoculation on growth response occurs via both direct and indirect channels, with the phytohormonal pathway ($\beta = 0.63$) being the most

significant mechanism by which plants react to growth. This study employs a quantitative methodology to suggested that arsenic is a primary disruptor of developmental hormonal signaling, rather than an inhibitor of photosynthesis (β = 0.15) or stress metabolite accumulation (β = -0.03). *A. terreus* stimulates development mostly via normalizing hormonal levels (β = 0.89) between the fungus-to-hormone axis), rather than by directly enhancing photosynthesis or reducing stress metabolites. The elevated model R^2 (0.967) indicates that the four biochemical pathways include almost all physiological variance contributing to the observed growth responses. This indicates that our measurement approach is comprehensive. The path-analytic findings have practical implications: bioinoculant strategies that regulate hormone levels (such as IAA- and GA3-producing fungi) may be more efficient in arsenic phytotransformation than those aimed at pigment restoration or antioxidant protection

4.6. Ecological Significance and Multivariate Integration

In order to provide a thorough understanding of the plant-fungus metal interactions that cannot be obtained with univariate analysis, this investigation employs a multivariate statistical technique that includes PCA, correlation heatmap, linear regression, route analysis, and cluster analysis. PCA condensed 16 measured variables into two orthogonal components, accounting for over 92% of the total variance in the biochemical data and above 96 percent in the agronomic data. The discernible discrimination in treatment observed in PCA space and the bipolar correlation structure, coupled with the primary hormonal pathway identified in the SEM and the three-cluster dendrogram, collectively elucidate a coherent biological narrative: *A. terreus* facilitates As-stressed *B. juncea* in altering its phenotype, thereby promoting growth and achieving hormonal equilibrium.

Our findings suggest the use of *A. terreus* as a bioinoculant for the treatment of Arsenic-contaminated agricultural soils. Additional study is required to assess the long-term efficacy of this plant-fungus symbiosis, quantify Arsenic levels in plant tissues, and elucidate the molecular processes behind the observed hormonal and metabolic changes. However, the results of this study must be interpreted in the light of a number of limitations. The sample sizes are relatively small, which may reduce the statistical power and generalizability of the results. The limited period of the experiments conducted may not be representative of the longer-term response of plants to Arsenic stress and the inoculation with *A. terreus*. Similarly, the experiment was conducted under controlled greenhouse conditions, which may not adequately represent the variability and complexity of field environments. Further, the concentration of arsenic in plant tissues and soil was not measured directly, and this resulted in limited conclusions about arsenic uptake, accumulation and detoxification mechanisms. Whereas, the non-factorial treatment design limits the ability to rigorously examine interactive and independent effects of arsenic and fungal inoculation. larger sample size, longer experimental periods, factorial design, field validation, and direct measurements of soil and tissue arsenic concentrations should thus be employed in future studies.

4.7. Conclusion

This study suggests that the rhizospheric fungus *A. terreus* effectively mitigates arsenic toxicity in *B. juncea* by restoring phytohormone homeostasis, enhancing photosynthetic capacity, and moderating stress-induced accumulation of defense

metabolites. The cohesive characteristics of plant stress responses were statistically validated by multivariate techniques, including PCA, correlation coefficient heatmaps, regression modeling, structural path analysis, and cluster analysis. Structural path analysis established the phytohormonal pathway ($\beta = 0.63$) as the primary mediator of growth responses, with the whole model accounting for 96.7% of the variance in biomass. This underscores the significance of hormonal control in both fungal-mediated stress alleviation and arsenic toxicity. The results confirm that inoculation with *A. terreus* might be a sustainable approach to enhance the growth and physiological responses of plants in arsenic contaminated environments. The arsenic remediation ability cannot be verified, however, without direct measurements of arsenic uptake and accumulation. Molecular characterisation of important signaling pathways, quantification of soil and tissue arsenic, and field scale validation should be a part of future studies.

Author Contribution

Aima Javed: Conceptualization, methodology, data collection, data analysis, visualization **Naveen Dilawar:** methodology, writing, reviewing, Analysis, **Tabassum Yaseen:** visualization, manuscript editing, **Imtiaz Ahmad:** Reviewing, editing and **Fayaz Asad:** data collection and original data analysis, validation.

Conflict of Interest

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

Data Availability Statement

Data supporting the findings of this study will be made available by the corresponding author upon request.

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