Microbial-Mediated Valorization of Fly Ash Improves Groundnut Growth

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Abstract:

Integrating industrial waste with beneficial microorganisms offers sustainable solutions for agricultural development. This study investigated the combined effects of fly ash and *Bacillus cereus* on vascular growth in *Arachis hypogaea* L. Three treatments were used: control (unamended soil), T_1 (20% fly ash), and T_2 (20% fly ash + *B. cereus*). Morphological, anatomical, and biochemical analyses were conducted at 10-day intervals over 30 days. T_2 produced the best results, with shoot length (22.6 ± 0.2 cm), root length (13.6 ± 0.6 cm), and biomass significantly higher than both the control and T_1. Anatomical studies showed increased cambial activity, thicker cortex, and more advanced vascular differentiation in T_2. Proline levels increased notably in T_2 (3.20 µg/mL), indicating enhanced stress tolerance. *Bacillus cereus* effectively mitigated fly ash-induced stress while improving nutrient availability, emphasizing the potential of combining microbial inoculants with industrial by-products for sustainable crop growth and waste reuse.

1. Introduction:

Enhancing agricultural productivity sustainably is a pressing challenge driven by escalating global food demands and environmental limitations. Incorporating beneficial microbial inoculants with soil amendments has emerged as a viable and innovative approach to promote plant growth, enhance soil health, and strengthen ecosystem resilience. Among plant growth-promoting bacteria (PGPB), *Bacillus cereus* has emerged as a prominent microbial agent owing to its multifaceted role in promoting plant growth. It achieves this through nitrogen fixation, phosphate solubilization, phytohormone production, and synthesis of stress-mitigating compounds (Bhattacharyya & Jha, 2012; Kenneth et al., 2019).

Fly ash, a residual byproduct of coal combustion in thermal power plants, has long been associated with environmental challenges, primarily due to its extensive accumulation and potential ecological toxicity. However, its strategic use as a soil amendment in agricultural systems has gained increasing scientific interest. This is due to its composition, which is enriched with essential micronutrients such as iron (Fe), zinc (Zn), and manganese (Mn), providing a viable solution to address nutrient deficiencies in degraded soils (Varshney et al., 2022; Basu et al., 2009). Nonetheless, using fly ash in its raw form is constrained by inherent drawbacks, such as high alkalinity and potential heavy metal contamination. These issues call for innovative, integrated strategies to reduce risks and enhance their utility in agriculture (Kishor et al., 2010).

Combining microbial inoculants, such as *Bacillus cereus*, with fly ash-amended soil provides a synergistic approach to improve soil fertility while reducing potential toxicity.

Previous research has shown that microbial formulations can influence nutrient availability and reduce heavy metal stress, thus enhancing the benefits of fly ash application (Juwarkar & Jambhulkar, 2008). Additionally, microbial inoculants are known to enhance cambial activity and vascular tissue development, which are essential processes for efficient water and nutrient transport in plants (Kenneth et al., 2019).

This study examines the combined effects of *Bacillus cereus* and fly ash on the vascular development of *Arachis hypogaea* L. (groundnut), a vital oilseed crop widely grown in arid and semi-arid areas. The research highlights the importance of cambial activity and proline buildup as key physiological indicators of enhanced vascular growth and stress resilience. Although the role of proline as a stress regulator has been extensively documented (Ashraf & Foolad, 2007), its involvement in mediating the effects of microbial inoculants and soil amendments remains underexplored.

The integration of microbial biotechnology and advanced soil management strategies offers significant potential for achieving the twin goals of sustainable agricultural growth and environmentally friendly waste management. This study aims to elucidate the complex interactions between *Bacillus cereus* and fly ash that promote vascular development, thereby informing environmentally sustainable and resource-efficient methods to improve crop yields.

2. MATERIALS & METHODS

2.1 Study Area & Sampling:

The experiment was conducted at the Department of Biosciences at Veer Narmad South Gujarat University in Surat, Gujarat, India. The study used fly ash, agricultural soil, the *Bacillus cereus* strain, and *Arachis hypogaea* L. seeds as experimental materials.

Sampling:

Fly ash samples were collected from the Electrostatic Precipitator (ESP) units at the Ukai Thermal Power Plant in Songadh, Gujarat, to ensure purity and representativeness through multi-unit sampling. Seeds of *Arachis hypogaea* L. were sourced from the Agricultural Produce Market Committee (APMC), Songadh, Gujarat. Agricultural soil samples were collected from the Department of Biosciences at VNSGU and subsequently characterized for texture, pH, and nutrient content.

2.2 Bacterial Strain Isolation:

Three distinct compost formulations were created: CD (cow dung), CDM (cow dung enriched with microbial liquid formulation), and CDMF (cow dung supplemented with microbial liquid formulation and 20% fly ash). Bacterial strains were isolated from each compost matrix using standard serial dilution plating techniques, followed by preliminary morphological and biochemical characterization. Selected isolates exhibiting Bacillus-like traits were analyzed with Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF) for initial identification. The analysis identified *Bacillus cereus* DSM 31T with high confidence (Rank 1, +++), achieving a score of 2.39 against NCBI identifier 1396, confirming reliable species-level identification (Mellmann et al., 2008; Seng et al., 2009). This result indicates a strong protein profile match with the reference spectrum of *B. cereus* type strain. The detection of *B. cereus* in these compost matrices aligns with prior research documenting its natural presence in cow dung-based environments and its important role in organic matter breakdown and nutrient cycling (Liu et al., 2024; Radha & Rao, 2014). These findings support the potential development of *B. cereus*-based microbial inoculants from composted organic waste for sustainable agricultural applications.

2.3 Experimental Design:

Two treatment conditions were implemented and compared with a control group:

Treatment 1 (T_1): Soil amended with 20% fly ash

Treatment 2 (T_2): Soil amended with 20% fly ash and inoculated with *B. cereus* bacterial strain

2.3.1 Seed Germination:

Seed germination was conducted in sterile Petri dishes, with ten seeds placed on moistened cotton in each dish. Distilled water was applied on alternate days to maintain appropriate moisture levels. The Petri dishes were inspected daily for signs of fungal or other microbial infections. Once the seedlings reached an appropriate developmental stage, they were transplanted into pots for further experimentation.

2.3.2 Pot Experiment:

Table: 1 Tabular format shows Pot experiment

Treatments	Composition		
	Soil	FA	Inoculation of Bacteria
Control	100% (2000gm)	-	-
Treatment 1 (T_1)	80% (1600gm)	20% (400gm)	-
Treatment 2 (T_2)	80% (1600gm)	20% (400gm)	100 ml

These treatments were designed to assess the independent and combined effects of fly ash and bacterial inoculation on seedling growth relative to the untreated control. Each treatment group was established in triplicate to ensure statistical stability and accurate outcomes.

2.4 Morphological Growth:

Seeds of *Arachis hypogaea* L. were germinated under controlled conditions, and once the seedlings reached a suitable developmental stage, they were transplanted into pots for further experimentation. Plant growth and development were assessed by measuring plant height and biomass, and conducting anatomical analyses at 10-day intervals over 30 days. Each treatment was replicated in triplicate.

2.4.1 Plant Height:

Plant height was measured by determining the root and shoot lengths (in centimeters) to assess the plants' vertical growth and developmental processes.

2.4.2 Plant Weight:

Fresh and dry weight measurements were used to determine plant biomass. For fresh weight determination, plants were carefully uprooted, cleaned, and surface-dried with filter paper before being weighed using an electronic balance. Fresh weight represents the total mass of plants at the time of harvest. For dry weight determination, uprooted plant samples were oven-dried at 70°C for 24 hours before weighing (Dash et al., 2015). Dry weight represents the structural biomass of plants after water removal.

2.5 Anatomical Study:

Root and stem samples were collected for anatomical analysis. Samples were prepared for microscopic inspection and stained with safranin to enhance contrast and visualization of cellular and tissue structures (Khilji et al., 2024). Key anatomical parameters examined included the number of vascular tissue layers, cortical cell thickness, and parenchyma cell area. Cambial development and differentiation were evaluated to assess growth dynamics and structural modifications.

2.6 Biochemical Test:

Proline Test:

Proline concentrations were quantified using the colorimetric method of Bates et al., (1973). Fresh plant tissue (approximately 0.5 g) was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and filtered using Whatman filter paper. The filtrate (2 mL), acid ninhydrin solution (2 mL), and glacial acetic acid (2 mL) were combined in a test tube. After incubation at 100°C for one h, the mixture was rapidly cooled in an ice bath to terminate the reaction. The chromophore was extracted with 4 mL of toluene by vigorous mixing for 15–20 s. The toluene phase containing the chromophore was carefully aspirated from the aqueous layer and allowed to equilibrate to room temperature. Absorbance was measured at 520 nm using toluene as the blank. Proline concentration was determined on a fresh weight basis using a standard curve and the following equation: (Bates, 1973; Mwadzingeni et al., 2016; Rady et al., 2016):

$$Proline\ content\ (\mu g\ per\ gm\ of\ fresh\ leaf\ tissue) = \frac{[(\mu g\ proline/ml) \times (ml\ toluene)\ /115.5\ \mu g/\mu mole]}{(g\ sample)/5}$$

where; 115.5 g/mol is the molecular weight of Proline

3. RESULTS

3.1 Morphological Growth Parameters: Plant Height & Weight:

Table: 2 Tabular form represents the Effects of treatments on Morphological Growth Parameters of *Arachis hypogaea* L.

Growth Parameters	Days	Tests		
		Control	T_1	T_2
Shoot Length (in cm)	10th Day	5.6 ± 0.4	6.2 ± 0.3	7.1 ± 0.4
	20th Day	14.3 ± 0.6	16.8 ± 0.1	17.2 ± 0.5
	30th Day	20.2 ± 0.2	21.1 ± 0.6	22.6 ± 0.2
Root Length (in cm)	10th Day	5.1 ± 0.2	6.0 ± 0.3	7.5 ± 0.4
	20th Day	8.7 ± 0.5	9.4 ± 0.8	10.8 ± 0.7
	30th Day	11.9 ± 0.3	12.8 ± 0.4	13.6 ± 0.6
Fresh Weight (in gm)	10th Day	1.51 ± 0.12	1.91 ± 0.08	2.14 ± 0.11
	20th Day	2.82 ± 0.09	3.12 ± 0.15	3.91 ± 0.07
	30th Day	3.63 ± 0.08	3.84 ± 0.13	4.23 ± 0.12
Dry Weight (in gm)	10th Day	0.32 ± 0.02	0.39 ± 0.06	0.45 ± 0.03
	20th Day	0.55 ± 0.06	0.64 ± 0.02	0.74 ± 0.07
	30th Day	0.81 ± 0.07	0.95 ± 0.04	1.18 ± 0.03

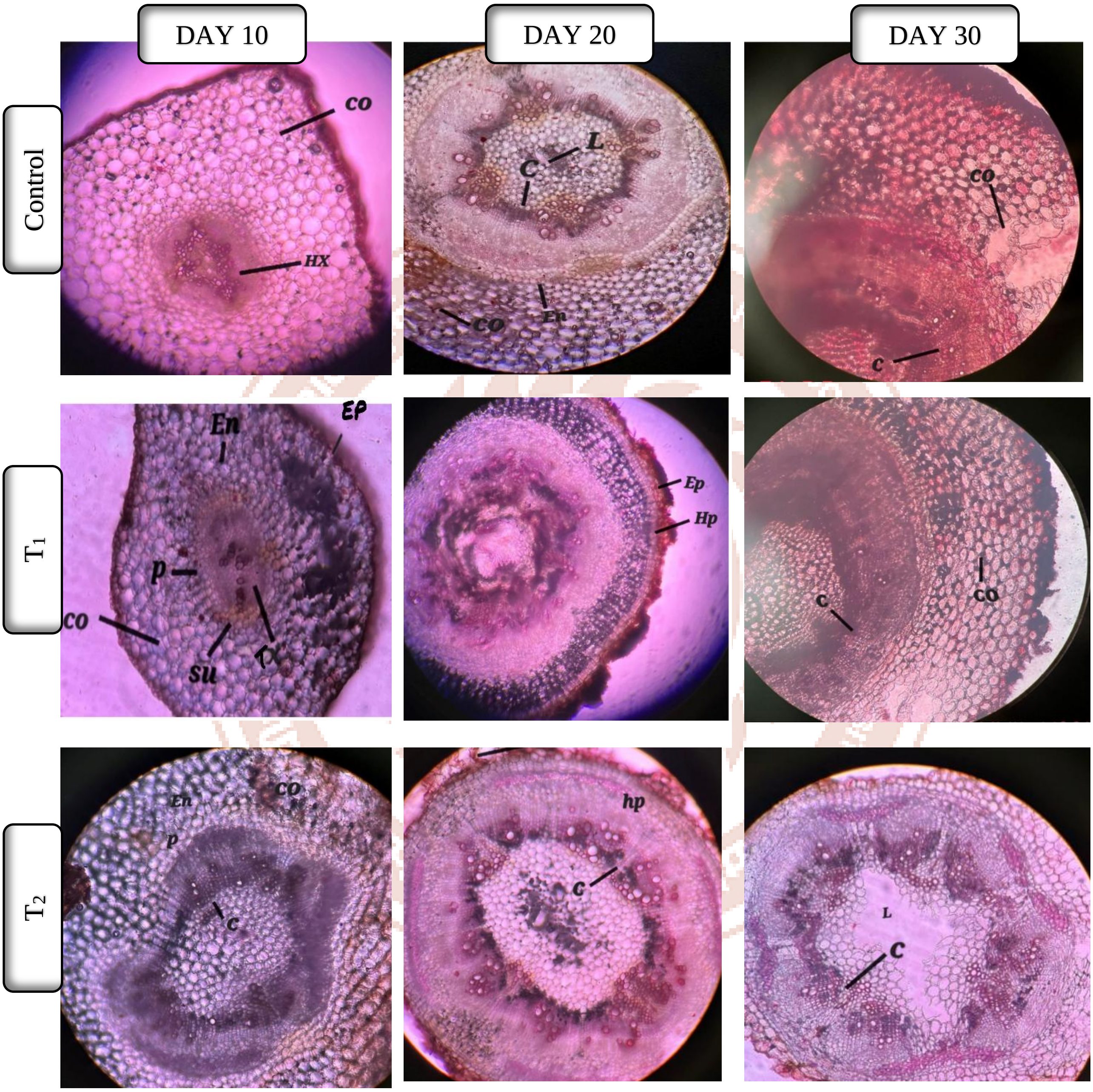
(Data presented as mean ± standard deviation, n = 3)

The morphological growth of *Arachis hypogaea* L. was assessed under three experimental conditions (Control, T_1, and T_2) at 10-day intervals. The data presented in Table 2 indicate significant enhancement in plant growth parameters with fly ash (T_1) and combined fly ash-*Bacillus cereus* treatment (T_2) compared to the control group. The highest growth metrics were recorded in T_2, including shoot length (22.6 ± 0.2 cm), root length (13.6 ± 0.6

cm), fresh weight (4.23 ± 0.12 g), and dry weight (1.18 ± 0.03 g), which consisted of soil amended with 20% fly ash and inoculated with *Bacillus cereus*.

3.2 Anatomical Changes:

3.2.1 Root Anatomy:



(EP- Epidermis; HP- Hypodermis; CO- Cortex; Cu- Cuticle; En- Endodermis; P- Pericycle; Su- Suberin; Pi- Pith; C- Cambium; Tx- Tetrarch xylem)

Fig. 1 Root Anatomy of the *Arachis hypogaea* L. plant in different test conditions

Anatomical analysis of *Arachis hypogaea* L. roots at 10, 20, and 30 days revealed significant variations in root tissue organization, vascular development, and structural adaptations.

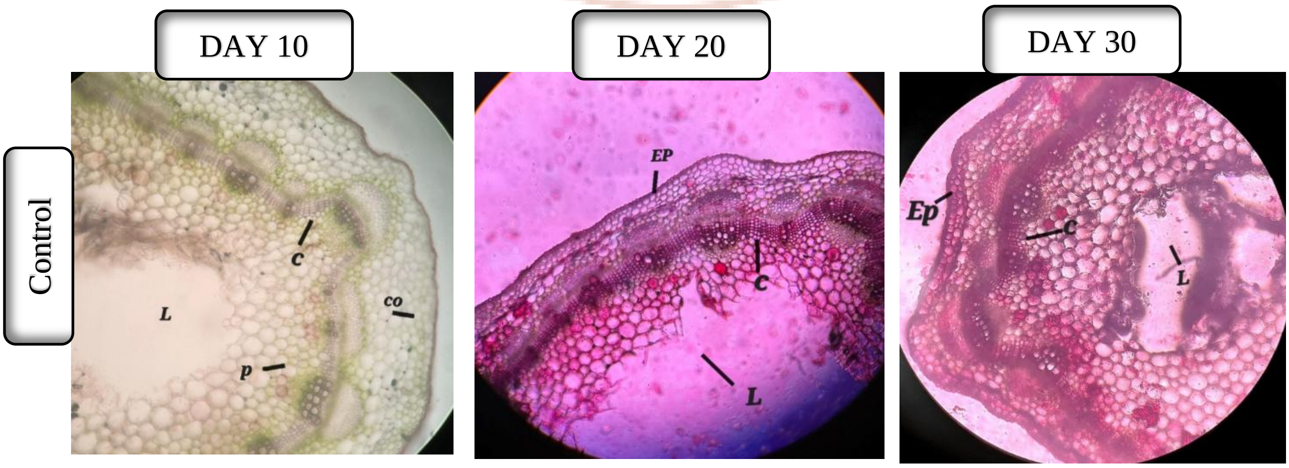
In the control treatment, root anatomy was typical, with an intact epidermis (EP), hypodermis (HP), and cortical cells (CO) on Day 10. Moderate vascular differentiation was observed, including tetrarch xylem (Tx) and cambium (C). By Day 20, cortical cell thickness increased, and vascular tissues showed enhanced lignification, marking the initiation of secondary growth. By Day 30, secondary thickening was prominent, with well-differentiated xylem, phloem, and enlarged pith cells (Pi), though overall root growth remained limited under standard soil conditions.

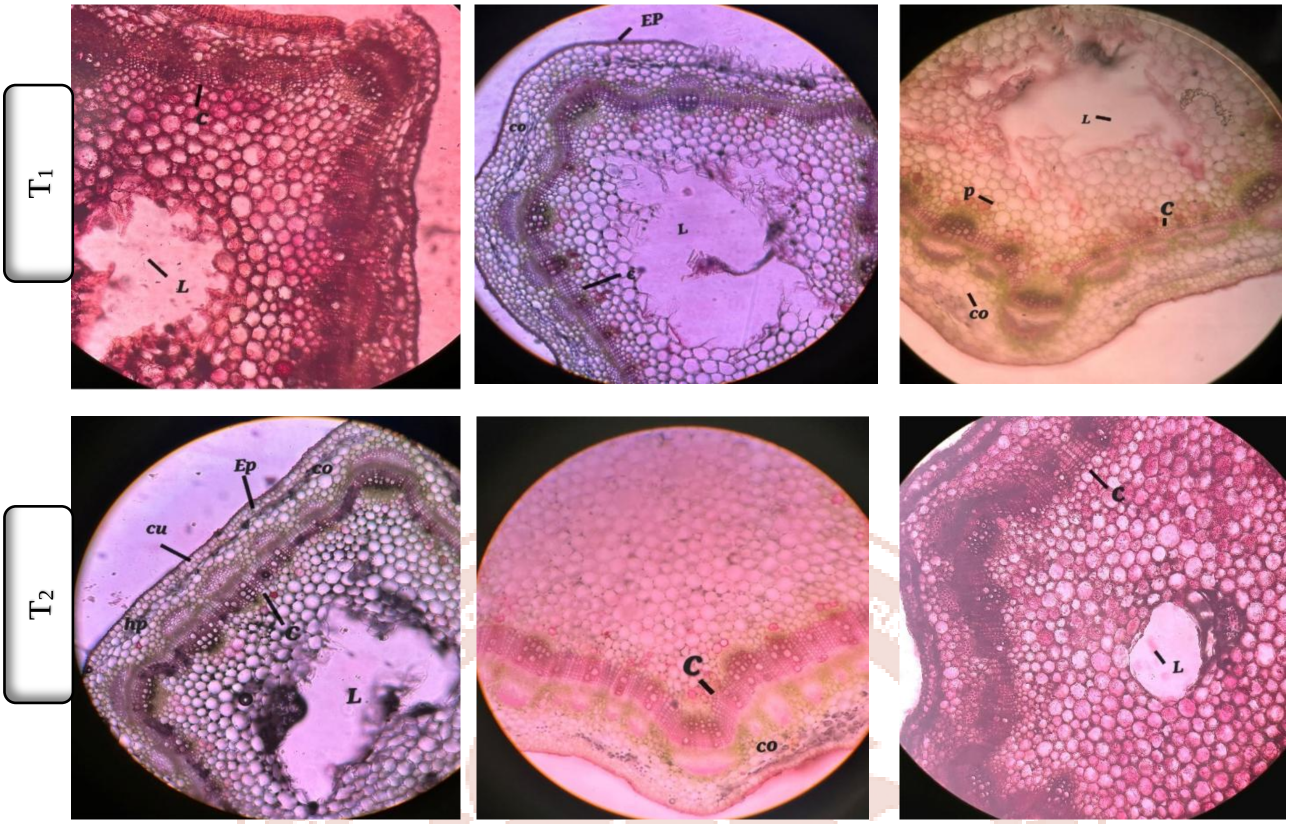
In Treatment 1 (T_1) (soil amended with 20% fly ash), root development improved compared to the control, despite minor stress indicators. At Day 10, cortical cells (CO) exhibited increased thickness with lignification near the endodermis. By Day 20, vascular tissues had better differentiation, and cambial activity increased, although the cortical parenchyma showed minor disorganization. By Day 30, the epidermis (EP) and hypodermis (HP) exhibited wear, but vascular tissues remained thicker, indicating moderate growth improvement under fly ash-induced stress.

In Treatment 2 (T_2) (soil amended with 20% fly ash and inoculated with *Bacillus cereus*), roots exhibited the most significant anatomical enhancements. At Day 10, cortical cells were notably thicker and well-organized, with intact epidermis (EP) and hypodermis (HP). By Day 20, vascular differentiation advanced, with a well-developed cambium (C) and thicker xylem walls. By Day 30, secondary growth was markedly enhanced, with broader cambial zones and thicker vascular tissues.

These results demonstrate that *Bacillus cereus* effectively mitigated fly ash-induced stress, promoting superior root growth and differentiation compared to the control and T_1. These findings highlight microbial inoculation as a promising strategy for improving plant growth in fly ash-amended soils.

3.2.2 Shoot Anatomy:





(EP- Epidermis; CO- Cortex; Cu- Cuticle; C- Cambium; L- Lysigenous cavity)

Fig. - 2 Shoot Anatomy of the *Arachis hypogaea* L. plant in different test conditions

Microscopic analysis of *Arachis hypogaea* L. shoot anatomy at 10, 20, and 30 days revealed significant differences in tissue structure. In the control group, the epidermis (EP) remained thin, cortical cells (CO) were consistently thick, and vascular tissues showed limited differentiation. Cambium (C) activity and secondary growth were moderate, with smaller lysigenous cavities (L) and less-developed parenchyma cells, indicating minimal adaptation.

In T1, cortical cells thickened, vascular bundles differentiated more extensively, and cambial activity increased, thereby enhancing secondary growth and transport. The lysigenous cavity enlarged, thereby improving gas exchange, but parenchymal improvements were limited relative to T2. In T2, the EP was well-defined, with a well-developed cuticle (Cu) that enhanced stress protection. Cortical cells thickened significantly, parenchymal cells expanded, and cambial activity was highest, promoting robust vascular differentiation. Enlarged lysigenous cavities improved transpiration and aeration.

Overall, T_2 demonstrated superior anatomical development, highlighting the synergistic benefits of combined fly ash amendment and *B. cereus* inoculation for enhanced vascular efficiency, metabolic adaptation, and structural integrity in *Arachis hypogaea L*.

3.3 Proline Test:

Proline content in *Arachis hypogaea* L. was evaluated under three experimental conditions: Control, Test 1, and Test 2. Results are presented in Table 3 on the tenth, twentieth, and thirtieth days of treatment:

Table: 3 Proline content in µg/ml of *Arachis hypogaea* L.

Tests	**Proline Content (µg/ml)**		
	10th Day	**20th Day**	**30th Day**
Control	1.22	1.27	1.30
T_1	1.33	2.81	3.05
T_2	1.42	2.98	3.20

In the control group, proline content increased minimally over time, starting at 1.22 µg/ml on the 10th day and reaching 1.30 µg/ml by the 30th day. In comparison, significantly higher proline levels were observed in T_1 and T_2. Proline content in Treatment 1 increased from 1.33 µg/ml on the 10th day to 3.05 µg/ml by the 30th day, while Treatment 2 recorded the highest levels, rising from 1.42 µg/ml on the 10th day to 3.20 µg/ml on the 30th day. Figure 3 presents the mean proline content across the three groups, demonstrating a pronounced increase in proline levels for T_1 and T_2 relative to the Control group.

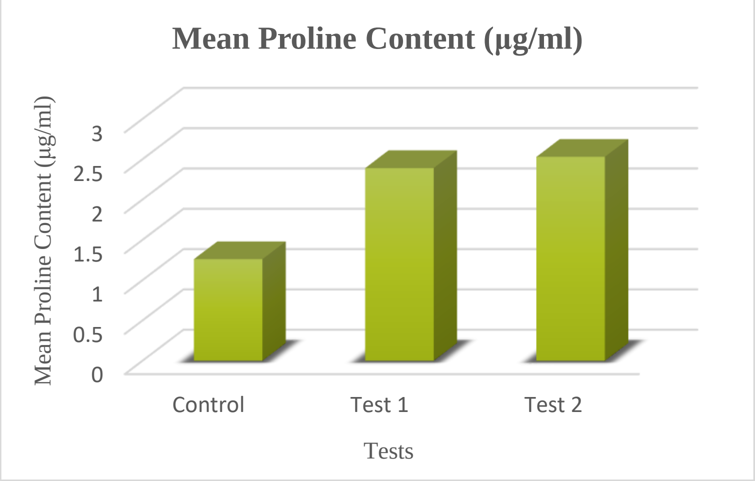


Fig: 3 Bar Chart Presentation of Mean Proline Content

The treatments in T_1 and T_2 may enhance stress resilience in *Arachis hypogaea L.*, potentially improving its growth and yield under challenging conditions. Furthermore, the progressive increase in proline content over time in T_1 and T_2 highlights a sustained response to the applied treatments.

4. DISCUSSION

The current study shows the combined effect of adding fly ash and inoculating with *Bacillus cereus* to boost vascular growth, cambial activity, and stress resistance in *Arachis hypogaea* L. Our results indicate that using both of these treatments together (T2) led to significantly better morphological, anatomical, and biochemical results than using fly ash alone (T1) or leaving the plants untreated. This suggests a dynamic interaction between microbial nutrient mobilization and the utilization of industrial waste.

The morphological parameters recorded in this study underscore the effectiveness of the combined treatment in promoting plant vigor. Treatment 2 exhibited the highest shoot length (22.6 ± 0.2 cm), root length (13.6 ± 0.6 cm), fresh weight (4.23 ± 0.12 g), and dry weight (1.18 ± 0.03 g) at 30 days, representing substantial improvements over both the control and T1 treatments. These findings align with recent observations by Zhao et al., 2024, who demonstrated that *Bacillus cereus* CGMCC 1.60196 significantly enhanced maize growth through extracellular hydrolytic enzyme activities that improved nutrient bioavailability. Similarly, Kulkova et al., 2023 documented that environmentally safe *B. cereus* strains promote biometric traits through indole-3-acetic acid (IAA) production, phosphate solubilization, and ACC deaminase activity—mechanisms likely operative in our experimental system. The superior performance of T2 over T1 can be attributed to *B. cereus*-mediated amelioration of fly ash-induced stress. While fly ash contributes essential micronutrients including Fe, Zn, and Mn (Varshney et al., 2022), its application may introduce alkalinity and potential heavy metal toxicity. Recent work by Pradhan et al. (2025) on rice cultivation with fly ash amendments revealed that microbial interventions optimize nutrient release while mitigating toxicity through biosorption and biotransformation mechanisms. Our results corroborate this model, as T2 plants exhibited robust growth without the stress-induced growth retardation occasionally observed in T1, particularly evident in root anatomical features at day 30.

The anatomical analyses revealed profound differences in cambial dynamics and vascular tissue organization among treatments, with T2 demonstrating the most advanced secondary growth characteristics. By day 30, T2 roots exhibited markedly enhanced cambial activity, characterized by broader cambial zones, well-organized cortical cells, and extensive vascular differentiation with thickened xylem walls. These observations suggest that *B. cereus* inoculation stimulates cambial proliferation and vascular differentiation beyond the baseline effects of fly ash amendment alone. Recent advances in understanding the regulation of the vascular cambium provide a mechanistic context for our observations. Wybouw et al., 2024 established that cambial stem cell maintenance and proliferation are orchestrated by complex hormonal networks, particularly involving auxin signaling pathways. The enhanced cambial activity observed in T2 plants may reflect *B. cereus*-mediated IAA production, which has been well documented among plant growth-promoting rhizobacteria (Kulkova et al., 2023). Endogenous auxin regulates the expression of key cambial genes, including WOX4 (WUSCHEL-RELATED HOMEOBOX4), which maintains stem cell identity in the cambium (Galibina et al., 2023). Furthermore, (Wang et al., 2022) demonstrated that brassinosteroid-mediated signaling coordinates cambium initiation and patterning, potentially synergizing with bacterial phytohormone production to amplify vascular development. The progressive increase in vascular tissue differentiation observed from day 10 to day 30 in T2 plants suggests sustained cambial activity throughout the experimental period. Lu et al., 2024 noted that cambial activity responds to both developmental cues and environmental signals through intricate gene regulatory networks. In our system, *B. cereus* may modulate these networks by producing signaling molecules that maintain cambial cell division while simultaneously promoting orderly differentiation into secondary xylem and phloem. The well-defined epidermis, developed cuticle, and expanded lysigenous cavities in T2 shoots indicate coordinated tissue maturation consistent with enhanced metabolic capacity and stress adaptation.

A critical aspect of the observed synergy involves *B. cereus*-mediated enhancement of nutrient mobilization from fly ash. Fly ash contains substantial quantities of silicon, phosphorus, potassium, and micronutrients, but these elements often exist in forms of limited bioavailability (P. Kumar et al., 2024). Narayanan et al., 2022 demonstrated that *B. cereus* strains possess multiple plant growth-promoting traits, including phosphate solubilization, siderophore production, and metal tolerance, enabling them to function effectively in amended soils. In our study, the superior performance of T2 suggests that *B. cereus* facilitated the transformation of fly ash-bound nutrients into plant-available forms through rhizosphere acidification, chelation, and enzymatic activities. Moreover, Kumar et al., (2024) recently reported that *B. cereus* MCC3402 exhibited arsenic resistance and biosorption capacity while simultaneously promoting rice growth under heavy metal stress. This dual functionality—detoxification and growth promotion—likely contributed to T2's enhanced performance in our experiment. By sequestering potentially toxic elements through biosorption and bioaccumulation, *B. cereus* may have created a more favorable rhizosphere environment, allowing plants to exploit the nutritional benefits of fly ash while minimizing exposure to harmful constituents.

The substantial elevation of proline content in both T1 and T2 treatments, reaching 3.05 μg/mL and 3.20 μg/mL, respectively, by day 30, represents a critical biochemical adaptation to fly ash amendment. Proline functions as an osmoprotectant, antioxidant, and signaling molecule during stress conditions (S. Kumar et al., 2022). The progressive accumulation of proline over 30 days suggests an active stress-response mechanism triggered by fly ash constituents, possibly including elevated salinity, alkalinity, or trace-metal presence. The slightly higher proline levels in T2 than in T1, despite superior growth performance, present an intriguing paradox that warrants mechanistic consideration. Das et al., 2024 recently elucidated that proline accumulation is intricately linked to ROS (reactive oxygen species) metabolism and stress signaling pathways. In our system, the elevated proline in T2 may reflect not distress but rather *B. cereus*-mediated priming of stress-defense mechanisms. This interpretation aligns with the "stress memory" concept, in which microbial inoculation triggers preemptive activation of stress-response pathways, thereby enhancing resilience without compromising growth (Andy et al., 2023). Furthermore, proline biosynthesis is energetically expensive, requiring coordinated regulation of carbon and nitrogen metabolism. The ability of T2 plants to maintain high proline levels while simultaneously achieving superior biomass accumulation suggests metabolic optimization facilitated by *B. cereus*. Enhanced nutrient availability, improved water relations, and potentially altered hormonal balance may have provided the metabolic capacity to support both growth and stress preparation. This integrated response reflects the multifunctional benefits of plant-microbe partnerships under challenging edaphic conditions.

The anatomical enhancements observed in T2, particularly the development of thicker cortical cells, enlarged lysigenous cavities, and well-differentiated vascular tissues, represent structural adaptations that confer multiple functional advantages. The increased cortical thickness provides enhanced mechanical support and potentially greater storage capacity for metabolites and water. The expanded lysigenous cavities in shoots facilitate improved gas exchange, thereby supporting higher rates of photosynthesis and respiration—both critical for sustaining the observed increases in biomass production. Recent studies on vascular development emphasize the importance of coordinated tissue patterning for optimal plant function (Sun et al., 2024; Haas et al., 2022). The anatomical organization observed in T2 plants suggests that *B. cereus* inoculation promoted not merely quantitative increases in tissue formation, but qualitative improvements in tissue architecture. The well-organized arrangement of epidermal, cortical, and vascular tissues, coupled with appropriate lignification patterns, indicates developmental coordination consistent with hormone-mediated regulation of cell fate determination and differentiation. Moreover, the enhanced secondary thickening and vascular differentiation observed in T2 roots have important implications for nutrient and water uptake efficiency. Well-developed xylem vessels with thickened walls provide greater hydraulic conductivity while maintaining structural integrity under tension. This anatomical optimization likely contributed to the superior nutrient acquisition and growth performance of T2 plants, creating a positive feedback loop wherein improved vascular function supports enhanced cambial activity and further tissue development.

This study contributes to the growing body of evidence supporting integrated approaches to sustainable agriculture that combine biological and industrial resources. The successful utilization of fly ash, a major industrial waste product, addresses critical environmental challenges associated with the disposal of coal combustion residues. Hussain et al., 2023 recently highlighted that global research on fly ash has intensified, with particular emphasis on developing countries such as India, where both fly ash generation and agricultural land degradation are significant concerns. The synergistic benefits observed in T2 treatment suggest that microbial inoculation can transform fly ash from a marginally beneficial amendment (T1) into a highly effective soil conditioner (T2). This finding has practical implications for large-scale agricultural implementation. Huang et al., 2025 conducted a meta-analysis demonstrating that combinations of microbial inoculants with organic amendments consistently outperform single-component treatments across diverse cropping systems. Our results extend this principle to industrial waste products, thereby opening new avenues for circular-economy approaches in agriculture. However, translating these findings to field conditions requires careful consideration of environmental variability, dose optimization, and long-term impacts on soil health. (P. Kumar et al., 2024) employed machine learning approaches to identify optimal fly ash application rates (40 t ha^{-1}) in rice cultivation, demonstrating the value of data-driven optimization. Future research should explore dose-response relationships for the fly ash *B. cereus* combination across different soil types and climatic conditions to establish robust application guidelines.

While our study demonstrates clear synergistic benefits of combining fly ash with *B. cereus* inoculation, the precise molecular mechanisms underlying these effects warrant further investigation. Recent advances in plant-microbe interaction research, including transcriptomic, proteomic, and metabolomic approaches, could elucidate the signaling pathways and gene regulatory networks activated by this treatment combination (Mäkilä et al., 2023). Understanding how *B. cereus* modulates plant gene expression under fly ash stress would provide mechanistic insights applicable to other PGPR-amendment systems. Additionally, the temporal dynamics of cambial activation and vascular differentiation observed in this study suggest developmental programming worthy of detailed investigation. Lineage tracing studies combined with high-resolution imaging, as pioneered by (Wybouw et al., 2024), could reveal the cellular-level dynamics of cambial cell division and differentiation under microbial-amendment treatments. Such spatiotemporal resolution would advance our understanding of how environmental inputs translate into developmental outputs. Finally, the ecological dimensions of fly ash-microbe interactions in the rhizosphere merit a comprehensive study. Our experiment employed a single *B. cereus* strain, but natural soil harbors diverse microbial communities whose interactions may modulate treatment outcomes. Meena & Vishnuvardhan, 2021 emphasized the importance of integrated nutrient management strategies that consider microbial community dynamics. Future research should examine how *B. cereus* inoculation influences native soil microbiomes in fly ash-amended systems and whether synergistic interactions with indigenous microorganisms further enhance plant growth promotion.

5. CONCLUSION

This study demonstrates the synergistic effects of fly ash and *Bacillus cereus* inoculation in promoting growth and stress tolerance in *Arachis hypogaea* L. The results demonstrate that combined fly ash amendment and *Bacillus cereus* inoculation (T2) significantly enhanced plant growth, cambial activity, vascular development, and proline accumulation. The elevated proline content indicates an adaptive stress-response mechanism associated with fly ash amendment, enhancing plant defense mechanisms under environmental stress. This study provides a foundation for innovative approaches to sustainable agriculture that integrate waste valorization, environmental stewardship, and enhanced crop productivity.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the research, authorship, or publication of this article. Both authors have contributed collaboratively to the work, and the study's findings are independent of any commercial or financial interests.

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ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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