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Research Article

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Biostimulatory Effect of Biochar and Jeevamrut on the Potential of *Trichoderma harzianum* as a Growth Enhancer in Maize Seedlings

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Abstract

The adoption of biopesticides and biofertilizers is being encouraged worldwide as an ecologically sustainable and economically feasible alternative to harmful chemical inputs. The current study is based on one such fungal bioagent, *Trichoderma harzianum*, which plays an important role as a soil inhabiting biocontrol agent against various plant pathogenic microbes as well as a plant growth promoter. *T. harzianum* (Th azad, ITCC Acc No. 6796) acquired from Chandra Shekhar Azad University of Agriculture and Technology (CSAUA and T) Kanpur was used for application along with different combinations of biochar and jeevamrut as treatments for maize seedlings. Analysis and comparisons were done based on physiological and biochemical parameters like germination percentage, shoot length, root mass, number of leaves, chlorophyll content and total phenolic content (TPC) respectively. In addition to this, microbiological parameters like microbial population in rhizospheric soil and endophytic root infection potential. The results showed that the co-application of *T. harzianum* with jeevamrut and biochar in combination as soil application showed the best growth rates as well as plant health. Collectively, the three inputs resulted in 130% increase in germination, 59.1% increase in seedling length and 246.13% increase in seedling vigor over control. Further comparison with control indicated improved nutrient uptake along with 32.78% increase in chlorophyll content, 159.4% increase in TPC, enhanced root mass formation by 536.84% and promoted rhizospheric microbial activity, which in turn lead to a healthier plant biochemistry and better seedling development. The combined treatment showed a 130% increase in seed germination and 59.1% increase in seedling length over control. The study demonstrates these bio-inputs' potential for improving crop productivity through enhanced nutrient uptake and microbial activity.

Keywords

Biochar, Rhizospheric competence, Jeevamrut, Trichoderma, Bio-agent, Soil ecology

Introduction

The rise of biopesticides and biofertilizers in agriculture has ushered in a new era of sustainable and environmentally conscious practices. These bioagents are efficient as symbionts with other beneficial soil-borne microbes and have been found to utilize natural resources, demonstrating remarkable efficacy in crop nutrient management, control of plant pathogenic diseases, pest management, and promotion of plant growth and stress resistance. Among these bioagents, *T. harzianum*, a soil-borne fungal bioagent, has emerged as an effective tool for managing soil-borne diseases and enhancing overall plant health and root growth. *T. harzianum* is extensively present in agricultural and forest soils worldwide, and optimizing its potential using natural inputs is essential for maximizing its bene-

fits for crops, soil, and environmental well-being [1].

Indian indigenous technical knowledge offers various solutions and formulations that can be prepared to enhance the growth of soil microbes and create a favorable soil rhizospheric ecosystem for beneficial microbe-crop interactions, thereby boosting the population and vigor of these microbes. Jeevamrut, for instance, is a bioenhancer prepared from desi cow dung, desi cow urine, gram flour, jaggery, and a handful of healthy soil, fermented in water [2]. It is a simple and easily prepared solution using readily available raw materials, possessing a high potential to boost soil microbes due to its rich content of sugar, nitrogen, phosphorus, calcium, and other nutrients [3].

Another crucial factor for microbial growth in soil is the availability of carbon. Biochar has recently gained attention as an effective method to add carbon to the soil while also improving soil structure, moisture retention capacity, pH buffering, and nutrient availability for plant roots. Additionally, biochar provides a porous surface with a suitable microclimate for the growth of many soil microbes, including beneficial ones like Trichoderma, thereby enhancing their population and effectiveness. Agricultural stubble can be utilized as a cost-effective and sustainable resource for producing biochar through on-field kilns, offering a solution for agro waste management, and improving soil microbial activity [4].

Previous studies by Negi et al. [5] and Hossain et al. [6] have shown that both jeevamrut and biochar individually enhance beneficial soil microbiota when applied as inputs. This research aims to investigate the potential of Jeevamrut and biochar as a combined application and its impact on the activity of *T. harzianum* as a bioagent. Controlled environment pot trials were conducted on maize crops grown in sterilized soil, incorporating various combinations of *T. harzianum*, jeevamrut, and biochar. Several parameters such as germination percentage, seedling vigor, root mass, root length, leaf chlorophyll content, TPC, etc., were evaluated to assess the effects of different treatments.

By studying the outcomes of these trials, this research aims to provide insights into the synergistic effects of jeevamrut and biochar in combination with *T. harzianum*, shedding light on their potential to enhance agricultural sustainability and productivity through improved soil microbial activity and crop performance.

Materials and Methods

Chemicals

The chemicals and reagents used in the study were of analytical grade. The talc powder utilized for formulation preparation was food-grade quality, sourced from Central Drug House (P) Ltd, New Delhi. The cow dung and urine were collected from a local cow shed at a farm situated in Phagwara, Punjab for preparing the bioenhancer solution jeevamrut. Additionally, gram flour and jaggery were purchased from local food-grade markets in the region. Wheat straw acquired as agricultural residue from farms around Lovely Professional University, Phagwara, Punjab grounds served as feedstock for biochar fabrication through an on-field pyrolysis kiln operation. The surface sterilant employed included ethanol (75%) obtained from Byahut Scientico, Jaipur and sodium hypochlorite (NaOCl) solution (5%) from Emplura, Gurgaon. Methanol (99%), sodium carbonate (7%), folin-ciocalteu (FC) phenol reagent (10%), and gallic acid standards (0 - 100%) were procured from Emplura, Gurgaon; Rankem, Gurgaon; LOBA Chemie Pvt. Ltd., Mumbai and Sigma Aldrich, Bangalore respectively. The microbial media used was prepared following standard sterile techniques as applicable. The chemicals sourced served as high purity ingredients for the experimental purposes outlined in this study.

Selection and mass multiplication of trichoderma

In this research study, *T. harzianum* (Strain code: Th azad, ITCC Acc. No. 6796, National center for biotechnology information, GenBank Accession no. KC800922) was obtained from CSAUA and T Kanpur for the purpose of mass multiplication. The method employed for mass multiplication was based on the protocol established by Papavizas et al. [7]. The molasses-yeast broth was utilized as the growth medium, consisting of 30 g of molasses and 5 g of brewer's yeast per liter of broth media.

To initiate the mass multiplication process, two bits measuring 5 mm each were excised from the obtained pure culture. These bits were then inoculated into 250 ml of broth medium. Subsequently, the inoculated broth cultures were placed in an incubator-cum-shaker, set at a temperature of 25 °C, and allowed to incubate for a duration of 15 days.

This methodology was employed to enable the efficient propagation and proliferation of *T. harzianum*, ensuring a sufficient biomass for subsequent experimental analyses.

Preparation of formulation with talc

The preparation of the talc-based formulation of trichoderma was carried out in accordance with the methodology outlined by Ramakrishnan et al. [8]. Initially, the trichoderma isolate, which had undergone mass multiplication in a molasses-yeast broth medium, was combined with sterile talc powder at a ratio of 1:2.

After thorough mixing, the mixture was subjected to a drying process under shade for a duration of one week. During this period, the powder was manually agitated daily to break up any clumps and ensure consistent drying throughout the formulation.

Once the shade drying was complete, the resulting powder formulation was carefully transferred into sterile polythene zip-lock bags. To maintain the viability and stability of the formulation, the bags were stored in a refrigerator at a temperature of 4 °C until they were ready for use. This method ensures the long-term preservation of the trichoderma isolate, allowing for convenient storage and subsequent utilization in experimental applications.

Preparation of jeevamrut

The procedure used to prepare jeevamrut follows the methodology described by Anjali [9]. The following ingredients were combined in a large plastic drum: 20 kg of desi cow

dung, 20 L of desi cow urine, 2 kg of gram flour (besan), 2 kg of jaggery, and a handful of healthy microbe-rich soil sourced from a nearby forest. Additionally, 200 L of water were added to the mixture, which was then thoroughly mixed.

Once the ingredients were well blended, the drum was covered with a jute sack and placed in a cool and dry location, away from direct sunlight. The mixture was stirred twice a day for a duration of 5 to 10 min, always in a clockwise direction. This stirring process facilitates the fermentation and maturation of the jeevamrut. After a period of 5 days, the fermentation process was deemed complete, indicating that the jeevamrut was ready for use as an organic fertilizer.

Preparation of biochar

In this research, wheat straw obtained as an agro residue was utilized to prepare biochar. The straw was first subjected to a cleaning process to ensure its purity and freedom from contaminants. Subsequently, the cleaned wheat straw was air-dried to remove excess moisture. Once sufficiently dried, the wheat straw was crushed into pieces measuring 1 - 2 inches in length using a shredder.

To produce biochar, carbonization of the crushed wheat straw was carried out in an on-field kiln. The carbonization process was conducted at temperatures ranging between 400 - 500 °C for a duration of 2 - 3 h. This temperature range is crucial for the conversion of the biomass into stable carbonaceous material. After the carbonization process was completed, the resulting biochar was allowed to cool down to room temperature. To maintain its quality and prevent degradation, the biochar was carefully stored in airtight containers.

Experimental set-up and treatments

The experimental trial was conducted in a controlled environment within a germination chamber. The chamber was maintained at a temperature of 25 ± 1 °C and a relative humidity of 75 ± 5% with natural sun-lit lighting.

For the experimental design, a total of 8 treatment groups were established, including one control group. Each treatment group consisted of 5 replications. In each replication, 5 maize seeds were planted in individual grow bags with a diameter of 5 inches. The soil used in the grow bags was sterilized through triple autoclaving. This process involved subjecting the soil to triple autoclaving at a pressure of 150 psi and a temperature of 121 °C for a duration of 30 min once every day, for three consecutive days.

The 8 treatment groups applied in the experiment were as follows: T0 - control (no additional treatment), T1 - 2 g trichoderma talc formulation, T2 - 5 g biochar, T3 - 5 ml jeevamrut, T4 - 2 g trichoderma talc formulation + 5 g biochar, T5 - 2 g trichoderma talc formulation + 5 ml jeevamrut, T6 - 2 g trichoderma talc formulation + 5 g biochar + 5 ml jeevamrut, and T7 - 5 g biochar + 5 ml jeevamrut.

On day 14 after sowing, measurements for germination and seedling vigor were recorded. At this point, the seedlings in each grow bag were thinned out, ensuring that only 2 seedlings remained in each bag for further observations. On day 15 after sowing, a second dose of 5 ml jeevamrut was applied to all applicable replications, based on the specific treatment group.

Measurement of germination percentage, height, and leaf count

The germination percentage of the maize seeds was determined on day 7 after sowing and it indicates the proportion of seeds that have successfully sprouted. On day 14 after sowing, the height of the seedlings and the number of leaves were measured to assess the growth and development of the maize plants. The measurement of seedling height was taken from the soil surface to the highest point of the arch of the uppermost leaf, with its tip pointing downwards. This measurement provides an indication of the vertical growth of the seedlings.

Seedling vigor, an important parameter in assessing plant health and early growth, was determined by calculating the product of the average germination percentage and average shoot height of the seedlings and then dividing the result by 100. This method is based on the approach described by Abdul-Baki and Anderson [10].

$$Seedling\ Vigor = \frac{(Average\ Germination\ Percentage \times Average\ Shoot\ Height)}{100} \quad (1)$$

The leaf count was conducted by starting from the lowest leaf and counting to the last leaf that is arched over with its tip pointing downwards. Leaves that were standing straight up, were not included in the leaf count.

Relative chlorophyll content (Soil plant analysis development (SPAD) meter)

To assess the relative chlorophyll production and overall leaf health, a SPAD - 502 meter was employed on day 30 after sowing. SPAD meter readings provide a relative measurement of chlorophyll content in plant leaves, which serves as an indicator of their photosynthetic activity and overall physiological condition.

For each seedling, five readings were taken from different leaves using the SPAD - 502 meter. These readings were then averaged to obtain the SPAD value representing the chlorophyll content for that specific treatment. By comparing the SPAD values among the different treatments, the relative chlorophyll production and, subsequently, the efficiency of photosynthesis in each treatment group was evaluated.

Root mass and root length measurements

On day 30 after sowing, the maize seedlings were carefully uprooted, and the roots were soaked in water for a duration of 30 min to loosen the soil particles adhering to them. Subsequently, they were washed under running water to ensure thorough cleaning.

After washing, the roots were soaked in water again and gently stirred to remove any remaining debris. The soaking water was drained and replaced until the water became clear, indicating that the roots were free from contaminants. To prepare the roots for further analysis, they were spread on blotting paper to facilitate surface drying. Root mass was then calculated based on the wet weight of the roots after the surface drying process. This calculation provides an indication of the total weight of the roots, considering the moisture present in the roots.

Additionally, the length of the longest root was measured as an indicator of root development. Only the radicle roots, which are the primary roots emerging from the seed, were considered for the length measurement. Nodal roots, which arise from the stem nodes, were not included in the analysis, in line with the methodology established by Freschet et al. [11].

Endophytic test

To assess the extent of endophytic infection by trichoderma, the protocol outlined by Manias et al. [12] was followed. After the roots were washed and cleaned, surface sterilization was performed using a series of treatments. First, the roots were dipped in 75% ethanol for a duration of 2 min, ensuring complete immersion. This step helps eliminate any external contaminants present on the root surface. Subsequently, the roots were rinsed three times with distilled water to remove any residual ethanol. Next, the surface-sterilized roots were immersed in a 5% NaOCl solution for 2 min. NaOCl acts as a disinfectant, targeting potential endophytic microorganisms residing within the root tissue. After the treatment, the roots were again rinsed three times with distilled water to remove any traces of NaOCl.

Following the surface sterilization process, the roots were cut into segments measuring 1 cm in length. These segments were gently crushed using a sterilized glass rod to release any endophytic microorganisms present within the root tissue. To isolate and cultivate the endophytic microorganisms, the crushed root samples from each treatment were placed on separate petri plates containing potato dextrose agar media. Four root segments were placed on each petri plate. The plates were then incubated at a temperature of 25 °C for a duration of 5 days, allowing the endophytic microorganisms to grow and form visible colonies.

This protocol enables the assessment of endophytic infection by trichoderma within the maize root tissue and by isolating and cultivating the endophytic microorganisms, the study helps to characterize the presence and abundance of trichoderma within the roots, shedding light on their potential role as endophytes and their extent of interaction with the maize plants under the different treatments applied.

Determination of TPC

To determine the TPC of the leaves, the FC reagent test was employed, as described by Ainswort and Gillespie [13]. A sample of 0.1 g fresh leaf tissue was macerated in 10 ml of methanol to extract the phenolic compounds present in the leaves. The mixture was allowed to rest for 1 h to ensure proper extraction. After the resting period, the leaf extract was subjected to centrifugation at 13,000 rpm for 10 min. The supernatant, containing the methanolic leaf extract, was carefully collected for further analysis.

In a separate container, 5 ml of 10% FC reagent and 4 ml of 7% Na_2CO_3 were combined. To initiate the reaction, 1 ml of the previously collected methanolic leaf extract was added to the reagent mixture. The contents were shaken well to ensure proper mixing and incubated at a temperature of 40 °C for a duration of 30 min to ensure optimum conditions for reaction with the FC reagent. Following the incubation, the absorbance of the reaction mixture was measured using a UV-visible spectrophotometer at a wavelength of 760 nm. A blank, containing all the reagents except the plant extract, was also measured as a reference. To obtain the TPC values, the absorbance readings of the plant extract were compared to a standard curve. The standard curve was constructed using known concentrations of Gallic acid, a commonly used phenolic compound. By correlating the absorbance values of the plant extract to the corresponding concentrations of gallic acid, the TPC of the leaves was determined.

Analysis of data

The collected data from the trials was subjected to statistical analysis using one-way analysis of variance (ANOVA) to assess any significant differences among the treatment groups. After performing ANOVA, Tukey's honestly significant difference (HSD) post hoc test was applied to identify specific differences between the treatment groups and determine which treatment groups significantly differ from each other after considering the overall variability in the data. For this statistical analysis, the SPSS software (statistical package for the social sciences) was employed. The compiled data, along with the results of the ANOVA and Tukey's HSD test, were carefully examined and analyzed to draw conclusions.

Results and Discussion

Germination and seedling studies

Germination percentage was recorded 7 days after sowing. As indicated in table 1, the lowest germination percentage of 40 ± 6.32% was observed in the control. Application of Trichoderma alone enhanced the germination percentage to 60 ± 10.95%, like the results obtained by Anjum et al. [14] which showed that under laboratory conditions, wheat seeds treated with trichoderma isolate BDF22 showed 87% germination compared to 72.7% in control after 48 h. Similarly, an increase in germination was observed with addition of biochar to 44 ± 7.48% and to 64 ± 11.66% with the addition of jeevamrut as individual inputs. These findings were in accordance with the experiments of Das et al. [15] and Rathore et al. [16] respectively. The combination treatment of trichoderma + biochar + jeevamrut resulted in the highest germination rate of 92% (± 4.90), indicating a synergistic effect of the combined treatments.

Regarding seedling height, the control exhibited the lowest average height of 7.92 ± 1.00 cm. The treatment with trichoderma, trichoderma + biochar, and trichoderma + jeevamrut showed significantly increased seedling heights of 11.52 ± 0.64 cm, 11.66 ± 1.10 cm, and 11.06 ± 1.02 cm, respectively. These results align with the results of Doni et al. [17] which suggest that the addition of trichoderma to the soil promotes seedling growth and development. However, the highest seedling height of 12.60 ± 0.61 cm was observed in the case of combined application of trichoderma, biochar, and jeevamrut.

Seedling vigor, which reflects the overall health and robustness of the seedlings, was significantly enhanced over control (3.36 ± 0.78) with trichoderma, biochar, jeevamrut, and their combinations. However, the treatment with trichoderma

Table 1: Effect of treatments on growth parameters like germination percentage, seedling height and vigor, leaf number and root mass of maize.

Treatments	Germination (%)	Seedling height (cm)	Seedling vigor	Leaf no. (Average)	Root mass (g)
Control	40 ± 6.32^{c}	7.92 ± 1.00^{c}	3.36 ± 0.78^{c}	3.0 ± 0.32^{a}	0.38 ± 0.08^{b}
Trichoderma	60 ± 10.95^{b,c}	11.52 ± 0.64^{a,b}	7.17 ± 1.74^{b,c}	4.0 ± 0.32^{a}	1.05 ± 0.22^{b}
Biochar	44 ± 7.48^{c}	8.02 ± 0.65^{c}	3.69 ± 0.83^{c}	3.0 ± 0.45^{a}	0.93 ± 0.11^{b}
Jeevamrut	64 ± 11.6^{b,c}	9.76 ± 0.54^{b,c}	6.31 ± 1.37^{b,c}	3.6 ± 0.24^{a}	1.21 ± 0.11^{b}
Trichoderma + biochar	60 ± 8.94^{b,c}	11.66 ± 1.10^{a,b}	7.28 ± 1.64^{b,c}	3.8 ± 0.20^{a}	1.19 ± 0.29^{b}
Trichoderma + jeevamrut	84 ± 7.48^{a,b}	11.06 ± 1.02^{a,b}	9.53 ± 1.61^{a,b}	3.8 ± 0.20^{a}	1.35 ± 0.24^{b}
Biochar + jeevamrut	64 ± 7.48^{b,c}	11.02 ± 0.50^{a,b}	6.99 ± 0.75^{b,c}	3.6 ± 0.51^{a}	1.04 ± 0.21^{b}
Trichoderma + biochar + jeevamrut	92 ± 4.90^{a}	12.60 ± 0.61^{a}	11.63 ± 0.98^{a}	4.0 ± 0.32^{a}	2.42 ± 0.37^{a}

Note: The average (± standard error) values of growth parameters of maize seedlings. Values having different superscript in the columns for the same variety are significantly different under the limit of $p < 0.05$.

+ jeevamrut + biochar exhibited the highest seedling vigor, i.e., 11.63 ± 0.98. This indicates that when applied together, this combination treatment positively influences the physiological condition of the seedlings.

The results obtained for germination, seedling height and vigor when all three inputs were treated as co-application was not only statistically significant over control, but also statistically significant over individual applications of trichoderma, jeevamrut or biochar alone. These results were consistent over all the replications and hence the beneficial effects of using Trichoderma in combination with jeevamrut and biochar was found in enhancing germination, growth, and overall seedling health. The significance of these results for different samples were obtained at the level of $p < 0.05$.

In a study conducted by Devakumar et al. [18], it was reported that jeevamrut, a natural agricultural input, contains a variety of beneficial microorganisms such as nitrogen fixers, phosphorus solubilizers, actinomycetes, and fungi. These microorganisms play a significant role in enhancing plant growth and development. Jeevamrutha also contains essential micro and macronutrients required for plant growth. The presence of growth-enhancing substances in jeevamrut has been observed to have a positive impact on seed germination percentage, resulting in improved outcomes.

The same parameters show higher when applied along with biochar than when applied alone due to the enhancing effect of biochar on microbes added to the soil by jeevamrut as presented by Palansooriya et al. [19]. According to Li et al. [20], improvements in soil properties, including an increase in soil pH, soil-water retention capacity, and nutrient availability (such as carbon, nitrogen, phosphorus, potassium, magnesium, and calcium), can promote the growth of microbes in soil. According to Jaafar et al. [21, 22], and Ye et al. [23], the highly porous nature and large surface area of biochar create favorable habitats for these microbes. Amanullah and Khan [24], reported that biochar further enhanced the growth and physiology of maize when coupled with Trichoderma as compared to Trichoderma alone.

Seedling growth characters

The leaf number, representing the foliage development of the seedlings, was counted on the 14th day after sowing and it was observed that there was no significant effect by any of the treatments. All treatments, including the control group, had an average leaf number between 3 and 4 as shown in table 1, suggesting that these treatments do not have a significant impact on leaf production in the seedling stage of maize.

The lowest root mass of 0.38 ± 0.08 g was observed in control with higher results of 1.05 ± 0.22 g, 0.93 ± 0.11 g and 1.21 ± 0.11 g obtained for individual treatments of trichoderma, biochar and jeevamrut respectively as comparable to results obtained by Barbosa et al. [25], Liang et al. [26] and Shivran et al. [27] respectively. The combined treatment of trichoderma, biochar, and jeevamrut showed the highest effect on root growth with a root mass of 2.42 ± 0.37 g, which was significantly different from all other treatments and 6.3 times higher than control. This result suggests that the application of trichoderma, biochar, and jeevamrut together effectively promotes root growth and biomass accumulation in the plants, more than the application of any of these treatments individually. The significance of these results for different samples were obtained at the level of $p < 0.05$.

Biochemical parameters

The results of biochemical analysis of the experiment are presented in table 2, which shows the effects of different treatments on the relative chlorophyll content and phenol levels in the plants. The data is presented as mean values with corresponding standard errors and groups of significantly similar results.

Relative chlorophyll content was measured using a SPAD meter and an average reading of 28.92 ± 0.89 was observed in the control group. Among the treatments, trichoderma-treated plants exhibited a significantly higher reading of 34.06 ± 2.17 compared to the control. Similarly, the treatments with jeevamrut, trichoderma + biochar, and trichoderma + jeevamrut also resulted in significantly increased readings of 33.30 ± 0.77, 33.96 ± 1.67, and 38.18 ± 1.11 units, respectively. The treatment with trichoderma + biochar + jeevamrut showed the highest chlorophyll content of 38.40 ± 2.12 units, indicating a synergistic effect of the combined treatments.

As for the TPC, the control group exhibited a phenol level

Table 2: Effect of treatments on biochemical parameters including relative chlorophyll content and TPC of maize leaves.

Treatments	Relative chlorophyll content (SPAD value)	TPC (mg/g of leaf tissue)
Control	28.92 ± 0.89^{d}	204.70 ± 5.94^{g}
Trichoderma	34.06 ± 2.17^{b}	352.64 ± 6.47^{d}
Biochar	$29.14 \pm 1.04^{c,d}$	236.05 ± 6.94^{f}
Jeevamrut	$33.30 \pm 0.77^{b,c}$	312.30 ± 4.04^{e}
Trichoderma + biochar	33.96 ± 1.67^{b}	385.33 ± 5.97^{c}
Trichoderma + jeevamrut	38.18 ± 1.11^{a}	444.37 ± 7.85^{b}
Biochar + jeevamrut	$31.32 \pm 0.79^{b,c,d}$	310.47 ± 3.89^{e}
Trichoderma + biochar + jeevamrut	38.40 ± 2.12^{a}	530.99 ± 7.47^{a}

Note: The average (± standard error) values of biochemical parameters - relative chlorophyll content (SPAD value) and TPC (mg/g of leaf tissue). Values having different superscript in the columns for the same variety are significantly different under the limit of $p < 0.05$.

of 204.70 ± 5.94 mg/g leaf tissue. Among the treatments, the trichoderma + biochar + jeevamrut treatment resulted in the highest phenol levels of 530.99 ± 7.47 mg/g, which was significantly different from all other treatments.

The results of this study indicate that the treatments have significant effects on the relative chlorophyll content and phenol levels in the plants. Chlorophyll is a vital pigment involved in photosynthesis, and its higher content indicates enhanced photosynthetic activity and overall plant productivity. The trichoderma treatment consistently resulted in significantly increased the relative chlorophyll content, suggesting its positive influence on photosynthetic efficiency and plant growth as supported by previous studies by Heflish et al. [28]. The combination treatments, particularly trichoderma + biochar + jeevamrut, showed synergistic effects on chlorophyll content. These combinations might have facilitated the provision of essential nutrients, growth-promoting substances, and beneficial microorganisms as well as a suitable micro ecosystem for these microorganisms to thrive, leading to an enhanced chlorophyll biosynthesis pathway and improved photosynthetic efficiency.

Phenols, on the other hand, are secondary metabolites that play a role in plant defense mechanisms against biotic and abiotic stresses. The treatment with trichoderma + biochar + jeevamrut resulted in significantly elevated phenol levels, indicating a potential activation of the plant's defense response. Elevated phenol levels can confer resistance against pathogens, herbivores, and environmental stressors and an enhanced phenolic content was observed by Ebrahimi et al. [29] in eggplant by addition of biochar as well as by Singh et al. [30] by addition of jeevamrut. Enhanced phenol production by plants on treatment with trichoderma was observed by Inayati et al. [31]. Further research is necessary to determine exactly the mechanism followed by the co-application of trichoderma, jeevamrut and biochar to enhance TPC in plants.

Conclusion

T. harzianum, a soil-originating biocontrol agent, has been shown to effectively suppress plant pathogens while promoting plant growth. When combined with jeevamrut, a liquid organic fertilizer containing beneficial microorganisms, and biochar, a carbon-rich material that enhances soil fertility, the co-application yielded remarkable results.

The physiological and biochemical parameters assessed, such as germination percentage, shoot length, root mass, number of leaves, chlorophyll content, and TPC, consistently demonstrated superior performance in the treatments involving the co-application of the three inputs. Furthermore, the co-application led to improved nutrient uptake, as indicated by increased chlorophyll content and TPC. Root mass formation was significantly enhanced and rhizospheric microbial activity was promoted, resulting in healthier plant biochemistry and better seedling development.

In conclusion, the co-application of *T. harzianum*, jeevamrut, and biochar as soil amendments has demonstrated significant benefits for maize seedlings in terms of growth rates and overall plant health. These findings support the notion that such a co-application offers a highly effective and sustainable approach to plant growth promotion and disease suppression. The adoption of such bio-based inputs holds great promise for reducing reliance on harmful chemical inputs in agriculture while ensuring improved crop productivity and environmental sustainability. Further research and field trials are warranted to explore the applicability and potential benefits of this co-application approach across different crop systems and agricultural contexts.

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Conflict of Interest

None.

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