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Research Article

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Impact of Co-inoculation of Arbuscular Mycorrhizal Fungi with Biochar on Maize Crop

Pavan B Kumar[1], Diptanu Banik[1], Chandra Mohan Mehta[1*], Nishihara Eiji[2], Kazuyuki Inubushi[3], Shigeto Sudo[4], Sachiko Hayashida[5] and Prabir Kumar Patra[6]

[1]*Department of Agronomy, School of Agriculture, Lovely Professional University, Punjab, India*
[2] *Faculty of Agriculture, Tottori University, Tottori, Japan*
[3]*Department of Agricultural Chemistry, Faculty of Applied Biosciences, Tokyo University of Agriculture, Tokyo, Japan*
[4]*Institute for Agro-Environmental Sciences, National Agriculture and Food Research Organization, Tsukuba, Japan*
[5]*Research Institute for Humanity and Nature, Japan*
[6]*Japan Agency for Marine-Earth Science and Technology, Japan*

***Correspondence to:**
Chandra Mohan Mehta
Department of Agronomy,
School of Agriculture,
Lovely Professional University,
Punjab, India.
E-mail: chandra.18376@lpu.co.in

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Abstract

This research explores the influence of the co-inoculation of various types of biochar with arbuscular mycorrhizal fungi (AMF) on the growth and development of maize (*Zea mays*). Eight treatments were evaluated: T1 (control), T2 (AMF), T3 (rice straw biochar), T4 (rice husk biochar), T5 (sugarcane leaves biochar), T6 (rice straw biochar + AMF), T7 (rice husk biochar + AMF), and T8 (sugarcane leaves biochar + AMF). Growth parameters, such as the root dry matter, stem girth, plant height, whole dry matter content, root volume, and chlorophyll index, were measured. The results revealed that T7 (rice husk biochar + AMF) resulted in greater root dry matter, indicating significant root development in the treated plants. T4 (rice husk biochar) resulted in greater stem girth, leaf area, plant height, and chlorophyll index values. Additionally, T3 (rice straw biochar), T6 (rice straw biochar + AMF), and T8 (sugarcane leaves biochar + AMF) were statistically similar in terms of plant height, whole dry matter and root volume. These results demonstrate how biochar and AMF could be adopted to improve maize production and growth. Combining some forms of biochar with AMF enhances root formation while also increasing plant vigor, biomass accumulation, and the chlorophyll index. This study highlights the sustainable farming approaches, which indicates that more research is necessary to maximize the application of AMF and biochar in a wide range of soil types and environmental conditions.

Keywords

Biochar, Arbuscular mycorrhizal fungi, Maize, Co-inoculation, Plant growth

Introduction

The production of agricultural output is threatened by soil nutrient depletion and human-caused environmental climate change. Since the "green revolution," the beneficial effects of inorganic fertilizers have been effectively shown, and they have significantly contributed to increasing agricultural output and productivity over the past 50 years [1]. Fertilizer usage has been steadily increasing as a result of deteriorating soil quality. However, the use of too many inorganic fertilizers, particularly nitrogen, can have negative effects on the environment because of the faster mineralization of organic matter. This means that using inorganic fertilizers alone is not a long-term solution for increasing soil fertility and maintaining yields [2]. Consequently, it is essential to create alternatives to synthetic fertilizers to increase crop output without endangering soil health and agricultural ecosystems. The role of various soil organisms in ecosystem multifunctionality is ex-

tremely important. Owing to their crucial ecological tasks, including nutrient cycling, litter decomposition, and soil fertility management, soil organisms have received much attention in recent years [3] and have been presented as safe and effective biofertilizers. AMF are the most widespread soil microorganisms that form symbiotic relationships with more than 80% of plants [4, 5]. AMF, which constitute a crucial part of soil microorganisms, are members of the phylum Glomeromycota. There are five orders (Archaeosporales, Diversisporales, Gigasporales, Glomerales, and Paraglomerales), 14 families, 29 genera, and more than 240 species within this phylum, which is further split into two classes: Archaeosporomycetes, Glomeromycetes [6]. In addition, they make up 20 - 30% of the total soil microbial biomass, create obligatory mutually beneficial interactions with 80% of vascular plants, and are essential components of the rhizosphere microflora in natural habitats. It is believed that AMF promotes the uptake of nutrients, particularly phosphorus, in nutrient-deficient environments. Fungi obtain carbohydrates for survival via exchange from the host [7-9]. Biochar is a carbon-dense substance generated by the pyrolysis of organic materials, including agricultural waste, at moderate temperatures (350 - 700 °C) in an oxygen-free environment. It is becoming more well known as a sustainable method for improving soil fertility and encouraging agricultural development while also contributing to climate change mitigation. It also has a wide range of uses, including the generation of heat and electricity, the purification of flue gases, metallurgical uses, usage in agricultural and animal husbandry, construction materials, and even medicinal applications. In recent years, it has grown in favor of replacing fossil carbon carriers in a number of these applications in an effort to minimize greenhouse gas emissions [10]. It has a superior porous exterior that is highly biochemically stable and has a high adsorption capacity for sorbing and releasing mineral nutrients due to its substantial specific surface area. Biochar can increase the amount of soil organic carbon, cation exchange capacity, soil porosity, and bioavailability of minerals such as potassium, calcium, magnesium, and phosphorus, which increase root growth and nutrient uptake. Additionally, the majority of the carbon in biochar remains in the soil for hundreds to thousands of years, making it an appealing alternative to inorganic fertilizers for soil amendment. AMF and biochar are well known for enhancing plant growth and soil health, but when used together, they frequently have synergistic benefits. AMF establish mutualistic connections with plant roots to facilitate the uptake of nutrients, especially phosphorus, into plants [11]. Biochar has also been shown to alter the diversity and composition of microbial populations in soil. Such modifications may have an impact on the soil structure or nutrient cycles, which affect plant development. Additionally, rhizosphere fungi and bacteria encourage plant development. The effects of organic amendments combined with biochar on the growth and yield of maize have not received much attention.

Materials and Methods

Experimental design

The pot experiment was conducted at the Experimental Research Greenhouse Facility of the Department of Agriculture, Lovely Professional University, Punjab, India. The average temperatures in the greenhouse ranged between 25 °C and 30 °C, with the relative humidity maintained at 70 - 80%. The experiment was conducted in a completely randomized design with a factorial arrangement to test the independent and interactive effects of biochar application and AMF inoculation. The design included 8 treatments: T1 (control), T2 (AMF), T3 (rice straw biochar), T4 (rice husk biochar), T5 (sugarcane leaves biochar), T6 (rice straw biochar + AMF), T7 (rice husk biochar + AMF), and T8 (sugarcane leaves biochar + AMF). Each experiment was replicated three times, for a total of 24 experimental units (pots). To minimize error and account for environmental variability in the greenhouse environment, each treatment was distributed at random.

Biochar preparation

Prior to the production of the biochar, all the materials were thoroughly dried. The biochar was made from rice straw, rice husk, and sugarcane leaves. The feedstock was carbonized over an open flame in a 48 cm high and 142 cm diameter stainless-steel tub to create biochar. A portion of the feedstock is burned during the autothermal process of open fire, which heats the remaining material and turns it into char. After being added to the open burn tank, the feedstock was ignited. Because the enzymes consume all of the oxygen, the feedstocks are carbonized below the flames, where it is absent, forming a pyrolysis zone.

Plant attributes

Plant height was measured from the base at the soil level to the base of the first leaf. Measurements were taken every week and recorded in centimeters (cm) to track the growth of the plants. By using ImageJ software, the leaf area was measured in cm^2 at significant growth stages to measure foliar development. Images of the leaves were taken and processed to estimate the total leaf area per plant. A SPAD chlorophyll meter (SPAD-502Plus) was used to measure the amount of chlorophyll three times along the leaf blade to account for variations in the distribution of chlorophyll, and the average of the readings was used. While the stem girth was measured, a Vernier caliper was used 5 cm above the soil surface, and the girth was measured periodically for stem development. While the root volume at the end of the growing season was assessed, the plants were carefully uprooted, the roots were thoroughly cleaned with water from the soil, and the volume was calculated via the displacement method and measured in cm^3.

Pot experiment

Topsoil (0 - 20 cm) was collected from the research field of Lovely Professional University. The soil was homogenized, sieved through a 2 mm mesh screen, autoclaved at 120 °C for two hours, and then examined for plant waste and stones. Standard polyethylene pots with a 2 kg soil-holding capacity were used. Each pot had a perforation at the base to facilitate proper drainage.

The soil used for this experiment was sterilized by autoclaving at 121 °C and 15 psi for 30 min to eliminate any native microbial or fungal populations, ensuring that the effects observed on AMF colonization and plant growth were solely

attributable to the treatments applied. This sterilization process was repeated twice to ensure complete elimination of the indigenous soil biota. After sterilization, the soil was air-dried and sieved through a 2 mm mesh before being mixed with the biochar amendments and AMF inoculum.

Pot filling and planting

Each pot had a volume of 2.0 kg, and 60 g of biochar (at the rate of 3% of total soil volume) was added to the treated pots. AMF was inoculated in the treated pots with a rate of 50 g per pot (at 2.5% of total soil volume). The maize seeds were treated with carbendazim and carbaryl (2 g of carbendazim were mixed with 200 milligrams of carbaryl per kilogram of seed) and planted at a depth of 2 cm (two seeds per pot). The pots were placed in a greenhouse under control conditions with an average temperature of 25 ± 2 °C and 12 h of daylight (Figure 1).



Figure 1: Variations in plant height. T1 (control), T2 (AMF), T3 (rice straw biochar), T4 (rice husk biochar), T5 (sugarcane leaves biochar), T6 (rice straw biochar + AMF), T7 (rice husk biochar + AMF), and T8 (sugarcane leaves biochar + AMF).

Irrigation and nutrient management

The pots were irrigated to provide suitable moisture levels throughout the trial period. No extra nutritional solution or fertilizer was added to the pots. In accordance with the experimental design, the only treatments given were the biochar and AMF inoculation treatments. This guaranteed that any changes in plant growth and soil health were attributable only to the impacts of biochar and AMF, without extraneous nutrient inputs impacting the findings. Irrigation was carefully regulated to minimize leaching and maintain constant moisture across all the treatments.

Results and Discussion

Impact of AMF and biochar on seed germination in maize

The first three days of the experiment revealed observable differences in the germination rates of the seeds across the various treatments (T1 - T8) (Figure 2). The rate of seed germination was relatively low on the first day. There was no discernible activity observed in T1 (control), T2 and T4 were better with germination rates that increased considerably and nearly reached 50%, whereas T6 and T7 were slightly better because their effects on germination were 33%, indicating that these treatments, which included either biochar alone or in combination with AMF substrates, quickly created an environment that was favorable for germination.

On the second day of germination, the percentage improved in all the treatments. Compared with the other treatments, T7 and T2 presented a percentage of 83% germination,

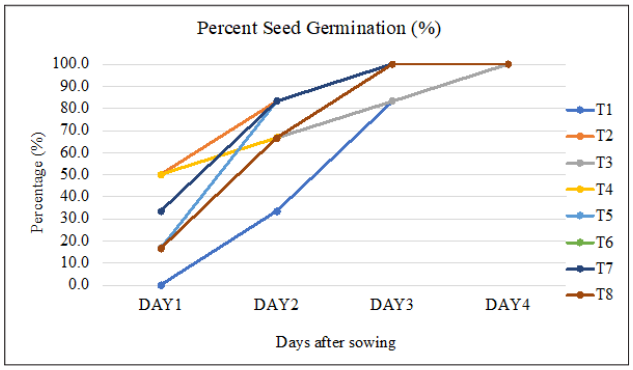


Figure 2: Impact of treatments on seed germination of maize.

followed by T6 80%. T3 and T4 also showed notable improvements, with values that were nearly 66%, indicating that the biochar treatments preserved some of their advantages. On the other hand, T1 resulted in the lowest amount of germination in the seeds compared with all the other treatments.

On the third day, almost every treatment reached 100% germination, except for T1 and T3, with a germination percentage of 83%. This suggests that the use of biochar and biochar-AMF combinations facilitated early and complete germination, and the treatment in which no amendments were administered (T1) resulted in slower germination. On the fourth day, all pots got 100% seed germination.

Impact of AMF and biochar on the growth and development of maize

Compared with the control, co-inoculation with biochar and AMF resulted in considerable improvements in maize growth parameters. The effects of biochar and AMF treatments on root and shoot growth, plant height and total dry matter accumulation are examined below.

Impact of AMF and biochar on plant height

The application of biochar had a substantial effect on the plant height data recorded at intervals of 15, 30, 45, and 60 days, revealing significant differences in growth across the treatments. After 15 days after sowing (DAS), the plants in the T4 treatment ($13.43^{a} \pm 1.01$) were significantly taller, whereas those in the T1 treatment ($8.67^{b} \pm 2.58$) were the lowest. This early variation suggests that biochar application may be useful for promoting early plant development. T4 continued to grow better after 30 DAS (23.83 ± 0.75), followed by T7 (20.83 ± 2.80) and T6 (20.63 ± 1.76), which further revealed how biochar can enhance the soil structure and nutrient availability to support plant growth. On day 45, compared with the other treatments, T7 (rice husk biochar + AMF) resulted in considerable improvements in plant height. A significantly greater plant height was recorded at T7 ($44.87^{a} \pm 0.16$), and the lowest was observed at T1 ($32.9^{f} \pm 0.28$), as shown in figure 3.

At 60 DAS, this trend continued, where T7 ($58.67^{a} \pm 2.43$) was highest and T1 ($43.70^{d} \pm 0.5$) was significantly lower than those of the other treatments. Similarly, T7 plants surpassed those in the other treatments in terms of height, indicating that biochar and AMF act in combination to promote plant development, as shown in figure 3. The control plants (T1)

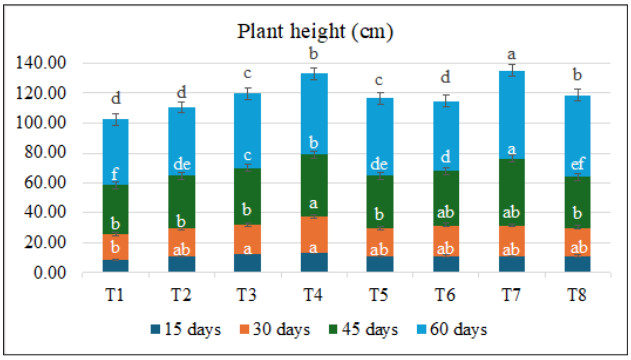


Figure 3: Impact of treatments on plant height at 15, 30, 45, and 60 DAS. Different lowercases indicate significant differences between treatments ($p < 0.05$).

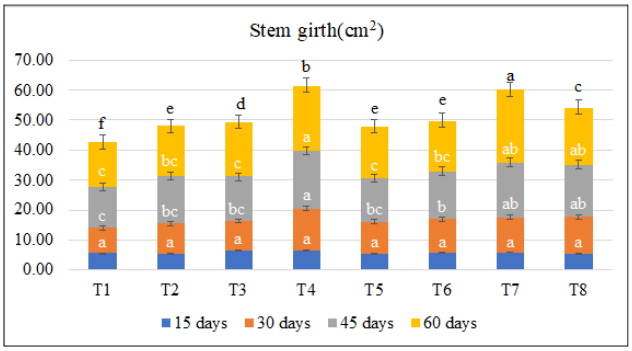


Figure 4: Impact of treatments on stem girth at 15, 30, 45, and 60 DAS. Different lowercases indicate significant differences between treatments ($p < 0.05$).

presented the lowest plant height, which was probably caused by the unfavorable soil structure and inadequate nutrient availability, which prevented root growth.

Impact of AMF and biochar on stem girth

The stem girth (cm^2) of the plants was measured at 15 day intervals over 60 days, as shown in figure 4. On 15 DAS, stem girth did not significantly differ among the treatments, but at 30 DAS, the disparities in stem girth were more visible, with T4 ($14.13^{a} \pm 0.56$) markedly surpassing the majority of the other treatments. T1 ($8.63^{c} \pm 2.44$) was lowest at 30 DAS, whereas T7 ($11.80^{ab} \pm 1.28$) significantly increased at 30 DAS. At 45 DAS, the trends continued where T4 ($19.52^{a} \pm 37$) was highest, followed by T7 ($18.22^{ab} \pm 1.57$) and T1 ($13.50^{f} \pm 1.77$). Moderate growth was also observed in the middle-range treatments, T6 ($16.06^{b} \pm 1.40$) and T5 ($14.59^{bc} \pm 1.50$), as shown in figure 4. At 60 DAS, the application of biochar had a substantial effect on stem girth, and T7 (rice husk biochar +AMF) exhibited a considerable improvement, with the greatest increase in stem girth. The lowest stem girth was recorded in T1 ($15.1^{f} \pm 0.6$), and the highest results were obtained in T7 ($21.74^{b} \pm 0.9$) (Table 1). The control plants (T1) ($15.1^{f} \pm 0.6$) presented the lowest plant height and stem girth, which was probably caused by the unfavorable soil structure and inadequate nutrient availability, which prevented root growth and plant development

Impact of AMF and biochar on leaf area

Leaf area was measured via ImageJ software, which revealed that the greater leaf area in the T7 treatment ($53.02^{a} \pm 0.56$) than in the T4 treatment ($51.36^{a} \pm 1.37$), which resulted in a significantly larger leaf area than did the control T1 treatment ($36.84^{d} \pm 0.93$), This resulted in greater light interception and photosynthetic activity. The improvement was likely due to the role of biochar in enhancing water retention and nutrient availability. The control plants had the lowest leaf area, which was indicative of their decreased vigor and nutrient intake from the soil. However, the total dry matter accumulation in the plants treated with rice husk biochar and AMF was greater than that in control, with T7 (99.33^{a} 1.07) followed by T4 ($84.67^{b} \pm 1.24$) and T6 ($80.33^{c} \pm 0.65$), as shown in table 1. The root volume of maize plants was significantly impacted by the biochar and AMF combination T7 ($96^{a} \pm 3.20$), which had the largest root volume, which was noticeably greater than that of the other treatments, and the lowest root volume was recorded in the control T1 treatment ($42.67^{f} \pm 1.74$), which may have been due to soil compaction and reduced nutrient availability, which limited root expansion.

Impact of AMF and biochar on the internode length

The results from the internodal length measurements across different treatments (T1 - T8) show considerable variation. The treatments' average internodal lengths varied from 6.00 cm to 8.03 cm. T7 presented the longest internodal length (average of 8.03 cm) of all the treatments. Compared with the other treatments, T7 had a substantial growth-promoting effect. Significant internodal development was also observed at T3, with an average length of 7.36 cm, and at T6, it was 7.23 cm. On the other hand, T1 presented the shortest average internodal length (6.00 cm), suggesting that stem elongation was greater in this treatment. These variations suggest that T7, T3, and T6 contributed more favorably to long stem length, whereas T1 appeared to have the least effect. This difference in performance could be due to differences in nutrient uptake or biochar composition affecting the rate of growth.

Table 1: Effect of treatments on plant growth attributes like leaf area, chlorophyll index, root dry matter, root volume, and internodal length.

Treatments	Leaf area (cm^2)	Chlorophyll index	Root volume (cm^3)	Root dry matter (g)	Internodal length (cm)
Control (Soil)	$36.84^{d} \pm 0.93$	$38.39^{d} \pm 0.25$	$42.67^{f} \pm 1.74$	$42.67^{f} \pm 0.43$	$6.14^{d} \pm 1.43$
Control (Soil) + AMF (2.5%)	$46.39^{bc} \pm 0.90$	$39.39^{d} \pm 0.96$	$51.33^{e} \pm 1.94$	$51.33^{e} \pm 0.19$	$6.43^{c} \pm 1.54$
Rice straw biochar (3%)	$45.15^{c} \pm 0.81$	$40.29^{cd} \pm 1.20$	$73^{b} \pm 0.92$	$73^{b} \pm 1.24$	$7.35^{b} \pm 1.58$
Rice husk biochar (3%)	$51.36^{a} \pm 1.37$	$44.66^{a} \pm 0.16$	$69.33^{c} \pm 0.69$	$69.33^{c} \pm 2.90$	$6.62^{c} \pm 1.42$
Sugarcane leaves biochar (3%)	$44.36^{c} \pm 0.04$	$41.82^{bc} \pm 1.28$	$64^{d} \pm 0.81$	$64^{d} \pm 2.33$	$6.54^{c} \pm 1.25$
Rice straw biochar (3%) + AMF (2.5%)	$48.07^{b} \pm 1.26$	$42.16^{b} \pm 0.80$	$75.33^{b} \pm 2.44$	$75.33^{b} \pm 0.65$	$7.22^{b} \pm 1.21$
Rice husk biochar (3%) + AMF (2.5%)	$53.02^{a} \pm 0.56$	$46.09^{a} \pm 1.25$	$96^{a} \pm 3.20$	$96^{a} \pm 1.07$	$6.00^{d.}$ 1.12
Sugarcane leaves biochar (3%) + AMF (2.5%)	$45.34^{c} \pm 1.31$	$42.8^{b} \pm 1.20$	$67.33^{c} \pm 2.67$	$67.33^{c} \pm 1.41$	$8.02^{a} \pm 2.09$

Chlorophyll index

The chlorophyll index, measured via SPAD readings, was significantly greater in the biochar-treated plants than in the control plants. The T7 treatment had the highest chlorophyll index, indicating high photosynthetic activity. Compared with the control plants, the plants treated with biochar presented a considerable increase in the chlorophyll index, as measured via a SPAD meter. The T7 (46.09[a] 1.25) treatment resulted in the greatest chlorophyll index, suggesting increased photosynthetic activity. The control plants presented the lowest SPAD values, which may indicate low nutrient availability and reduced photosynthetic activity.

Discussion

The results of this study show that biochar, especially in combination with AMF, enhances the growth and physiological parameters of maize. Biochar improves the structure of the soil and nutrient availability and retention, increasing the water content required for the optimal growth and development of plants

The increases in the stem girth and plant height of the biochar-treated plants, which were recorded in the T4 (rice husk biochar) and T7 (rice husk biochar + AMF) treatments, were related to the improved physical structure of the soil with increased porosity but reduced bulk density, which promoted root growth and, in turn, increased nutrient and water availability [2]. Previous studies have reported that biochar improves the soil structure to support more effective root growth and greater plant health generally [12].

The improved effectiveness of the biochar + AMF interaction (T7) suggests synergistic interactions between biochar and mycorrhizal fungi, specifically the release and uptake of phosphorus. Phosphorus plays a key role in root growth, and plant productivity studies have shown that it can be released into higher availabilities in the soil by biochar. In addition, the contribution of AMF to increasing phosphorus uptake is even more meaningful and maximizes the efficiency of nutrient uptake while increasing plant growth [13]. The application of biochar markedly increased the diameter and volume of the roots. The T4 rice husk biochar application treatment was performed in parallel with the T7 rice husk biochar application with the application of AMF. Root diameter increases because biochar can improve the physical properties of soils by increasing porosity and reducing bulk density, thus providing space for root expansion and growth [14]. Improved soil conditions favor good root development but also increase nutrient uptake efficiency, hence contributing to further plant development.

The significant increase in the chlorophyll index, especially in the T7 treatment, suggested that biochar increased the availability of nitrogen, an element required for chlorophyll synthesis and photosynthetic efficiency. Studies have also reported greater nitrogen utilization efficiency by plants in biochar-treated soils, which is linked to improved physiological functioning in plants. The relatively large leaf areas observed in the biochar-treated plants, particularly those in T7 and T4, facilitated increased light interception, which is essential for photosynthesis and biomass production. The biochar capacity to retain water and provide a sustained nutrient supply likely underpins the increased leaf area, further increasing photosynthetic activity [15].

Conclusion

In conclusion, the application of biochar, especially rice husk biochar (T4) and its combination with AMF (T7), resulted in significantly high maize growth, indicating that biochar plays a major role in enhancing soil quality and plant productivity. In the present study, the treatments with biochar in combination with AMF improved the growth parameters, such as stem girth, plant height, leaf area, chlorophyll index, and total dry matter accumulation, with T7 having the most pronounced effects. Overall, biochar has good potential for sustainable agriculture, particularly when combined with AMF.

These findings suggest that biochar can address challenges such as poor soil fertility and compaction, improving plant growth and yield. Further research could focus on the long-term effects of biochar amendments on different crops and soil types.

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Conflict of Interest

None.

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