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Response of tomato growth and number of mycorrhizal spores applied with biochar on saline soil

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ABSTRACT

High evaporation in coastal areas leads to salt accumulation in the soil, elevating soil salinity. Tomato plants (Lycopersicum esculentum L.) are one of the ideal plants for evaluating saline soil amelioration strategies. Inadequate saline soil treatment is an obstacle for farmers in developing their agricultural practices. Therefore, saline soil remediation using biochar was conducted to reduce soil salinity levels. The study was conducted using a non- factorial randomized block design (RBD) with 4 replications and 4 treatments, namely B0 (control), B1 (50 grams of biochar), B2 (100 grams of biochar), and B3 (150 grams of biochar) with observation parameters of plant height, number of leaves, stem diameter, and number of arbuscular mycorrhizal (AMF) spores. The results of this study showed that biochar had no significant effect on plant growth and the number of spores in tomato roots. Therefore, based on the study's results, the use of biochar in tomato growing media did not significantly affect plant growth parameters or the number of AMF spores in the growing media. Various factors, including environmental conditions and interactions between the growing media and host plants, may influence this. Recommended that further research be conducted on the process of producing biochar using anaerobic pyrolysis or closed pyrolysis methods. This is important because anaerobic combustion can produce more stable biochar.

Keywords:
Biochar, Mycorrhizal, Saline soil, Tomato plants

1. Introduction

Climate change causes seawater intrusion and increased evaporation, thereby accelerating salt accumulation in the soil and leading to high salt levels —a significant obstacle to agricultural production in coastal areas [1]. According to data/research, high salinity can reduce crop yields by around 50% [2]. This condition can make it challenging to grow most horticultural crops, as it inhibits water absorption and nutrient uptake, thus affecting their growth [3].

Tomato plants (*Lycopersicum esculentum* L.) are one type of horticultural plant from fruit vegetable commodities that can grow up to 2 meters high. According to Zhahrah [4], tomato plants are bushy or shrubby, with round stems bearing fine hairs and fruit that varies depending on the variety. Tomato plants are among the most in-demand in the community. The community's increasing consumption of tomatoes has encouraged farmers to expand production, and according to data from the Central Statistics Agency (BPS), Indonesia's tomato production reached 1.15 million tons in 2024, a 0.79% increase from 2023. Therefore, due to its broad economic role and high sensitivity, tomatoes are an ideal model for evaluating saline soil amelioration strategies [5].

Suboptimal saline soil management is a barrier to farmers' development of agricultural practices. Frequent use of chemicals actually damages the soil, plants, and even the environment [6]. Therefore, appropriate, environmentally friendly

innovations for saline soil management are needed [7]. Using organic materials is an effective solution for saline soil because they are readily available and more environmentally friendly [8]. One organic waste that can be utilized is agricultural waste, which is used as a soil amendment.

Soil amendments are materials added to soil that can improve its physical and chemical properties, thereby aiding plant growth and development [9]. One such soil amendment is biochar [10]. Biochar is a charcoal-like substance produced by the oxygen-free combustion of waste, such as plant residues. Using biochar can improve soil quality, helping reduce the need for chemical inputs [11]. This is also supported by Bamawenewi et al. [12], who state that biochar can increase soil fertility and help retain air, thereby improving infiltration [13]. Using biochar can also increase soil organic matter, thereby boosting microbial activity [14]. Research on using biochar to improve growth and yields in cultivated plants is still limited, requiring more in-depth research [15].

A significant research gap also lies in the lack of studies on the effectiveness of biochar application combined with manure in reducing soil salinity. Furthermore, the interaction between biochar and manure in developing microbial activity in saline soils planted with tomatoes is still poorly understood. The use of biochar in soils with high salt content can increase microbial activity and enhance plant growth [16].

One of these is the spores of AMF, which can produce phosphatase enzymes that help break down soil phosphorus, making it more readily available to plant roots [17]. It also provides a solution to the insufficient availability of soil P for plant growth [18]. In addition, the collaboration between mycorrhizae and plants can increase plant growth, enhance drought resistance, assist in the production of plant growth regulators, and enhance plant resistance to pest attacks [19]. Therefore, this study quantified the growth response of tomato plants in saline soil with biochar application and analysed the number of mycorrhizal fungal spores in the roots of biochar-treated plants.

2. Methods

2.1. Research Period and Location

This study was conducted over 3 months, from July to September 2025, at the Greenhouse, Faculty of Agriculture and Forestry, University Sulawesi Barat, Baurung, Banggae Timur Subdistrict, Majene Regency, West Sulawesi, at an elevation of 2,050 meters above sea level.

2.2. Research Procedures

2.2.1. Preparation of Planting Media and Cultivation Site

Planting media preparation involved collecting saline soil from the coastal area, 70 meters from the shoreline. The soil was then filled with 5 kg of 35 × 40 polybags, each containing 96 polybags. These were divided into two conditions. Condition 1, coded T (saline soil and biochar), comprised 48 polybags, and Condition 2, coded P (saline soil, biochar, and manure), comprised 48 polybags. The cultivation site was then constructed by constructing a bamboo frame and installing shade netting as the walls and roof.

2.2.2. Sowing

Seeding was done by soaking tomato seeds for 15–30 minutes, then preparing the seeding medium using soil and manure in a 1:1 ratio, mixing evenly, and placing it in perforated used glasses. Afterwards, planting holes about 1 cm deep in the planting medium, then inserting one tomato seed into each hole. The seeds were then covered with a layer of soil and watered thoroughly. Watering was intended to maintain the moisture of the planting medium so that the seeds can germinate well, without causing waterlogging.

2.2.3. Production and Application of Biochar

The biochar was produced by preparing rice husks, corn cobs, and coconut shells. Perform pyrolysis (combustion without oxygen). Biochar can be produced from rice husks using heat-resistant tools, such as wire-mesh chimneys with a diameter of 20–30 cm. The combustion process can be carried out on the ground surface. The wire chimney was placed on the ground. Then, husks piled around the chimney, so that it stands in the middle. Charcoal and wood were used as fuel. The combustion process took 3–4 hours, depending on the amount of material produced.

Biochar can be produced from coconut shells and corncobs using a simple pyrolysis method. The 2 × 2 m hole was dug, and coconut shells and corncobs were alternately burned in it until they formed evenly colored charcoal. This process takes 6–8 hours. Then they were doused with water to cool. The biochar was then meshed (3 mm) to produce the desired results and was ready for application to the growing medium. The biochar was applied by weighing 50 g for Treatment B1, 100 g for Treatment B2, and 150 g for Treatment B3. The treatments were then applied in the provided polybags according to the respective treatment. In this application, biochar treatment was applied at the predetermined dosage.

2.2.4. Transplanting

Seedlings were transferred to prepared planting medium/polybags after the plants were 21 days old in the nursery. Planting was carried out after the tomato plants had developed 3–4 true leaves, and the seedlings appeared healthy and free of pests and diseases. The tomato plants are ready to be transplanted. Planting holes were made in the polybags, and up to 96 polybags of plants were planted and then covered with soil. Plants were watered in the morning and evening. After transplanting, the plants were ready to observe growth and the number of AMF spores.

This experiment employed a non-factorial randomized block design (RBD) to investigate the impact of biochar type and dosage on tomato plant growth in the Greenhouse (polybag placement). Polybags were randomly placed within each block (row), with uniform spacing between them to minimise environmental impacts and enhance the validity of the research results.

2.2.5. Plant Growth Measurement and AMF Spore Estimation

Tomato plant growth observations began 7 days after planting (DAP) and were conducted three times over three weeks, using several parameters. Plant height was measured using a tape measure from the base of the stem to the tip of the tallest shoot. Stem diameter was measured using a vernier caliper below the base of the stem. The number of leaves was also counted manually. These observations were conducted two weeks after the tomato plants were transferred to polybags.

The AMF spores were then observed under a microscope in the laboratory. Observations were made by crushing the soil samples while still in clumps, placing them in a container, mixing them with 200 mL of water, and homogenising. The mixture was sieved using a graduated sieve with sieve sizes of 500 µm, 53 µm, 45 µm, and 38 µm. The final filtered sample (38 µm) was placed in a sample bottle by spraying it with a 60% glucose solution. The sample was placed in a centrifuge tube, centrifuged at 3,000 rpm for 3 minutes, and then decanted into the sample container. Samples were taken with a pipette and placed into petri dishes. A 15 ml and 20 ml sample were then observed using a binocular microscope at 4.5 magnification. The average number of spores per ml of inoculum was calculated. If the number of spores exceeded 2 per field of view, a 1 cm^2 grid was placed on an acetate sheet under the petri dish. A minimum of 40 squares was selected for counting, and the resulting spore count was multiplied by the number of spores per ml of inoculum to estimate the number of AMF spores separated from the soil and manure. The spore count was calculated by recording observations, then entering and processing the data in Microsoft Excel to obtain the spore count for each sample.

2.3. Data Analysis

The obtained data —plant height, stem diameter, number of leaves, and number of AMF spores— were analysed using analysis of variance (ANOVA) at the 95% confidence level, and the differences between treatments were tested using Duncan's Multiple Range Test in Microsoft Excel.

3. Results and Discussion

3.1. Plant Growth Observations

Plant height measurements (Table 1) show that 38 days after planting, treatment B2 produced the highest growth (94.63 cm), while treatment B1 showed lower results (88.75 cm). This indicates that biochar does not increase plant height. Research by El-Fawal et al. [20] also confirmed that although biochar can improve soil quality, it does not directly affect plant growth, especially if not combined with other materials. In addition, research by Agosti et al. [21] reported that adding biochar to cocopeat-based planting media resulted in only a slight difference in plant height, which was not significant compared to the control. Research by Suarez et al. [22] also found that applying biochar at various doses (0–20 $tons.acre^{-1}$) in sandy soil did not significantly affect tomato plant height. These results confirm that plant height increase is more strongly influenced by natural physiological factors than by biochar itself [23].

Table 1. Effect of biochar used on tomato plant height (cm)

Treatments	Plant Height		
	24 DAP	31 DAP	38 DAP
B0 (Control)	38.49	69.15	91.00
B1 (50 g)	38.15	68.09	88.75
B2 (100 g)	38.94	68.41	94.63
B3 (150 g)	34.29	64.02	86.88

Notes followed by the symbol "ns" in the same row indicate no significant difference according to ANOVA with a 95% confidence level, and DAP is days after planting.

Table 2. Effect of biochar use on the number of tomato plant leaves

Treatments	Number of Leaves		
	24 DAP	31 DAP	38 DAP
B0 (Control)	47.87^{ns}	90.5^{ns}	153.25^{ns}
B1 (50 g)	45.37^{ns}	85.13^{ns}	157.25^{ns}
B2 (100 g)	46.70^{ns}	91.25^{ns}	148.33^{ns}
B3 (150 g)	39.12^{ns}	79.38^{ns}	147.25^{ns}

Notes followed by the symbol "ns" in the same row indicate no significant difference according to ANOVA with a 95% confidence level, and DAP is days after planting.

Regarding leaf count (Table 2), B1 showed the highest number of leaves at 38 DAP (158.25 leaves), but control B0 was also almost equivalent (153.25 leaves), and the other treatments showed insignificant variations. Thus, the increase in leaf count cannot be directly attributed to biochar application. Research by Gelardi et al. [24] shows that in fertile soils, adding various types of biochar does not increase vegetative growth parameters, including leaf number, because the soil already has a high nutrient status. Paliaga et al. [25] also report that natural and enriched biochar do not cause significant differences in tomato shoot biomass, including leaf number, especially when other environmental factors, such as salinity, affect nutrient uptake. The formation of plastocron and phyllocron can also influence leaf formation in plants; the faster both are formed, the greater the number of leaves that will form [26].

Table 3. Effect of biochar use on tomato plant stem diameter (mm)

Treatments	Stem Diameter		
	24 DAP	31 DAP	38 DAP
B0 (Control)	4.61^{ns}	5.74^{ns}	6.63^{ns}
B1 (50 g)	4.74^{ns}	6.13^{ns}	6.98^{ns}
B2 (100 g)	4.80^{ns}	5.99^{ns}	6.91^{ns}
B3 (150 g)	4.49^{ns}	5.75^{ns}	6.88^{ns}

Notes followed by the symbol "ns" in the same row indicate no significant difference according to ANOVA with a 95% confidence level, and DAP is days after planting.

The diameter of the plant stems (Table 3) also increased over the observation period. At 38 DAP, treatment B1 produced the highest stem diameter (6.98 mm), followed by

B2 (6.91 mm). Stem diameter observations showed a similar pattern: B1 had the largest stem diameter 38 days after planting (6.91 mm), but the control B0 was also competitive (6.63 mm). This slight difference indicates that biochar did not significantly affect stem diameter growth. A study by Adekiya et al. [27] found that applying bovine bone *biochar* at various doses did not substantially affect tomato stem diameter. Although there was an increasing trend compared to the control, statistical tests showed that the stem diameter values at doses of 10 and 20 tons.ha^{-1} were statistically similar, thus showing no significant difference. This can be explained because all treatments received NPK 15–1–15 base fertilizer, so the macro-nutrient requirements were already met.

Biochar plays a greater role in improving soil chemical properties [28] and increasing crop yield [29] and fruit quality [30], but its effect on stem diameter, a vegetative parameter, is relatively less apparent. Environmental factors and natural plant variation are also thought to mask differences between treatments [31]. Thus, it can be concluded that bovine bone biochar does not significantly affect tomato stem diameter.

3.2. Estimation of AMF Spores

In the fertilizer treatment group (Table 4), the data showed considerable variation. Treatment B1 recorded the highest number of mycorrhizal spores, 73.89 per 50 cm^3 of soil, followed by treatment B2 with 24.63 per 50 cm^3 of soil and B0 with 22.74 per 50 cm^3 of soil. These figures indicate that fertilizer can create conditions that support

Tabel 4. Estimation of the number of mycorrhizal spores on tomato plants (50 cm^3 of soil)

Treatments	Number AMF Spores	
	Fertilizer	No Fertilizer
B0 (Control)	22.74^{ns}	2.18^{ns}
B1 (50 g)	73.89^{ns}	0.22^{ns}
B2 (100 g)	24.63^{ns}	0.22^{ns}
B3 (150 g)	14.21^{ns}	0.22^{ns}

Notes followed by the symbol "ns" in the same row indicate no significant difference according to ANOVA with a 95% confidence level.

AMF growth, although different types of fertilizer or doses (represented by B0, B1, B2, and B3) have varying effects. It should be noted that treatment B3 showed the lowest spore count, only 14.21 per 50 cm^3 of soil, which may be due to an unsuitable fertilizer type or an excessive dosage, both of which inhibited spore growth. In contrast, the number of mycorrhizal spores was relatively low in the treatment group without fertilizer. Treatment B0 showed the highest spores at 2.1 per 50 cm^3 of soil, while treatments B1, B2, and B3 were relatively uniform. Research by Sarathambal et al. [32] found that soil without fertilizer had limited spores, while soil with fertilizer had spore counts ranging from 50 to 70.5. This was also stated in the study by Kabir et al. [33]. Using biochar can improve soil physical properties, but under some conditions, it can absorb water and nutrients, leading to nutrient deficiencies in plants. Then, in the study by Zhang et al. [34] conducted on corn plants in brown soil, soil without fertilizer had low spore counts (19.8 spores), while soil with fertilizer had higher counts (37.8 spores). This means fertilizers play an important role in

facilitating mutualistic symbiosis between mycorrhizae and host plants, and providing essential nutrients needed for reproduction [35].

4. Conclusion

The use of biochar in tomato growing media does not significantly affect plant growth parameters, including plant height, stem diameter, and leaf number. This finding is in line with previous studies that show that using biochar does not affect plant growth because it can be influenced by several factors, including the plant's natural physiology, environmental conditions, and soil fertility or soil with already high nutrient status. Furthermore, in assessing the number of AMF spores in tomato plants, biochar did not affect the number in tomato plant roots. This may be due to a lack of nutrients in the growing medium, preventing mycorrhizal fungi from developing. Based on the results of the research, it is recommended that further studies should be conducted on the process of producing biochar using anaerobic or closed pyrolysis methods. This is important because anaerobic combustion can produce more stable biochar with higher porosity than aerobic combustion, thereby potentially increasing its activity as a soil conditioner, especially in saline soils.

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