Journal of
Agriculture and Biology
ISSN: 3007-1755 (Print), 3007-1763 (Online)

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Research Article

Green and Wood Waste Biochar Based Soil Amendment to Mitigate Early Blight Stress in Tomato

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ABSTRACT

The use of available resources for the benefit of both environment and human beings comes under sustainable agriculture. Application of chemical fertilizers and pesticides is against the sustainable management of agricultural resources. Biochar acts as alternative to chemical pesticides and fertilizers by promoting the disease suppression and enhancing plant growth. Current study examines the effect of different biochar concentrations on growth enhancement of tomato plants and availability of essential nutrients like potassium and phosphorus both in the absence and presence of *Alternaria solani* infection. Soil was amended with green and wood waste biochar (GWB). Both biochar concentrations (3 and 6%) effectively minimized disease progress while enhancing plant growth. However, 6% GWB performed better. Both root and shoot length increased in soil amended with 6% GWB. Both disease incidence and severity reduced drastically in soil amended with 6% biochar. Nitrogen contents increased by 13.07% under stress conditions. The disease incidence was 100% in un-amended soil while it was reduced to just 20% in soil amended with 6% GWB. There was significant drop (6%) in disease severity in 6% GWB amended soil. In short, soil amendment with biochar proved effect strategy for mitigation of early blight in to tomato by stimulating plant growth parameters and minimizing disease incidence. Biochar application is crucial for sustainable agriculture.

Keywords: Eco-protection, Soil amendment with organic matter, *Alternaria solani,* Biochar, Tomato

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Article History
Received: January 20, 2025
Accepted: May 29, 2025
Published: June 30, 2025

INTRODUCTION

To ensure environmental sustainability, there is requirement of developing energy sector on renewable sources of energy. Application of renewable energy resources will result in decreased emission of green-house gases in the environment. Due to high energy and adaptations, biomass expanded to many other renewable sources of energy (Digman et al., 2009). Previously, biofuel was produced in temperate regions by using starch as well as sugar containing crops. Temperate climates were rich in carbon because fossil fuels were used intensively as input for agriculture (Bruun and Luxhøi, 2008). Biofuel is carbon neutral after second and third generation (Mathews, 2008). The burning of biomass under anaerobic conditions is called pyrolysis. Products of pyrolysis include biogas, solid and liquid biochar. Biochar produced by pyrolysis is also called black gold. Soil sequesters the atmospheric carbon and enhances soil properties (Gaunt and Lehmann, 2008; Laird et al., 2010). Combined application of organic or chemical fertilizers along with biochar has been reported to enhance plant growth, nutrient availability and soil

biochar has been reported to enhance plant growth, nutrient availability and soil properties (Glaser et al., 2002). It has been proved that application of biochar enhanced the plant response to various abiotic and biotic stresses (Iswaran et al., 1980). Soil amendment with biochar has positive impact on agronomic parameters of crops (Lehmann et al., 2003). It has been suggested that biochar application along with organic matter significantly enhances properties of nutrient depleted soils (Hossain et al., 2010).

There is limited knowledge about the interaction of biochar with plants. Biochemistry of soil is greatly influenced by application of biochar. There is a variation in physical and chemical characteristics of biochar depending on feedstock of biochar and pyrolysis conditions (Keiluweit et al., 2010). Biochar from different feedstocks influence plant growth differently. Major mechanisms employed by biochar to increase crop productivity include enhanced cation exchange capacity of soil (Liang et al., 2006), higher nutrient and water retention, variations in soil microbial composition (Pietikäinen et al., 2000), and increase in soil pH (Rondon et al., 2007). These factors influence plant growth positively.

Tomato (*Solanum lycopersicum* L.) is considered as an essential component of human diet. Different types of antioxidants Vitamin C, Vitamin A and lycopene are present in soil. Due to presence of different antioxidants, consumption of tomato reduces risk of heart and skin diseases (Yashavantha Rao and Jayabaskaran, 2020).

In early 90s a significantly reduction of tomato was observed in countries like UK, USA, India etc. In 1936 Saregiannis noted that a fungus is behind this issue and first time isolated this pathogenic fungus *Alternariasolani*. With the ability to cause up to 80% of tomato output losses, *A. solanai* is a soil-dwelling fungus that is airborne and known to be among the most devastating diseases.

Tomato disease is characterized by tiny, dark brown bull eye spots with concentric ring patterns that spread over the entire leaf as the disease worsens. Once a sufficient temperature range of 27–32°C, humidity of 50–70%, and host plant are present, the pathogen can overwinter as conidia or mycelia in plant waste or soil and provide a source of inoculum. Different management approaches such as use of resistant tomato varieties, chemical fungicide application and rotation with non-host crops (Madden et al., 1978; Sherf, 1986).. For the management of early blight of tomato, fungicide application has become a standard. From transplanting to harvesting, fungicides are applied regularly (Yazici et al., 2011). Application of chemical fungicides increases the cost, causes environmental hazards and pathogen resistance to this chemical (Miller and Miller, 2004).

Modern world is moving towards as alternative environmental friendly disease management methods. Different studies shown that use of biochar can be a source of disease management in many crops Soil amendment with biochar decreases nutrient leaching while enhancing soil pH. It has been reported that soil treatment with biochar enhanced uptake of calcium and magnesium (Major et al., 2010). Enhanced nitrogen and phosphorus contents along with biofortification of crops with zinc has been reported.

Current study was design to obtain the following objectives A) Characterization and the effect of biochar made from green and wood waste with different concentration on the growth of tomato plant, B) To estimate the impact of biochar on the disease incidence and severity of tomato induce by *A. Solanai*, C) *In vitro* antifungal potential of green and wood waste biochar against *A. Solanai*. It is anticipated that the study's findings would advance the control of plant diseases through organic technologies and achieve the goals of sustainable agricultural practices.

MATERIALS AND METHODS

Alternaria solanai Culture Acquisition

Alternaria solanai culture was requested from First Fungal Culture Bank of Pakistan (FCBP), under accession no. FCBP-PTF-831. Fungus was multiply and pure colonies were obtained on (Potato dextrose agar) PDA media plates.

Plantation Material

Tomato (cultivar Roma) seeds were procured from the local market in Lahore Punjab, Pakistan. Surface sterilization of seeds was performed before cultivation for this seeds were soaked in 2% commercial bleach (3.8% NaOCl) solution for 5 mints and seeds were cultivated in nursery tray. After 15-20 days of seed sowing, nursery was planted to pots, present in the greenhouse. Pots were filled with soil amended with different concentrations of biochar and compost.

Soil Preparation and Experiment Arrangment

The green and wood waste biochar (GWB) was prepared by pyrolyzing agricultural green and wood waste at 450℃. Compost used in this study was provided by the department of plant pathology (DPP), university of the Punjab, Lahore. Table 1 below describes the physical and chemical properties of soil, compost, and biochar produced from the mixture of green and wood waste.

Table 1. Characterization of Green and wood waste biochar (GWB), soil and compost

Parameter	Soil	GWB	Compost
Nitrogen (%)	0.04	0.74	1.40
Phosphorus (%)	1.99	0.59	0.30
Potassium (%)	1.67	0.48	0.33
Carbon (%)	1.10	55.64	38.44
CEC (mcq/100g)	115	11.85	-----
EC (mS/cm)	0.4	1.43	1.12
Organic matter (%)	0.614	55.88	16.90
pH	7.99	9.22	7.44

---- Parameter were not checked

Soil was amended with biochar and compost for cultivation of tomato. Mixture of green and wood waste biochar (GWB) with two concentrations 3 and 6% v/v was utilized in this study. Treatment combination used in the experiment were: a) soil, b) soil + 3% GWB, c) soil + 6% GWB. All the treatments were inoculated (+*A. solanai*) or un-inoculated (−*A. solanai*) with *Alternaria solanai*. Each treatment comprises of 5 replicates, one pot with one plant.

Inoculation of *Alternaria solanai*

Distilled water was poured in the pure culture of *Alternaria solani*. Upper surface of culture was rubbed with spatula to harvest fungal spores in water. Prepared suspension was filtered through 3 layers of Whatman (grade 40) filter paper. Using hemocytometer, conidial concertation was set to 1×10^6 conidia/mL Leaves were rubbed with sand paper to rupture their upper surface. Inoculation was performed on this ruptured leaf surface by spraying conidial suspension over leaf surface in green house conditions (Zheng et al., 2015).

Plant Assay

When tomato plant reached its maturity, the plants were removed from the pots and placed in fully labeled envelops. Plant parameter like plant height, root and shoot dry weight was recorded (Awan et al., 2018).

NPK Estimation in Tomato Plant

A juicer machine was used to grind the dried tomato leaves to be used for nutrient (NPK) analysis. Leaf samples were crushed to powdered form and stored in labeled envelops.

To determine the nitrogen proportion, the materials were digested. To get the best results, 0.51 g of the sample was digested with 9.9 mL H_2SO_4 at 420 ± 2℃ for two hours. K_2SO_4 and $CuSO_4$ were employed as catalysts in a 9:1 ratio. Total phosphorus and potassium in plant material were assessed using the ICARDA manual wet-digestion process. The concentration of P was measured using a spectrophotometer, while the concentration of K was obtained using a flame photometer (Islam et al., 2017).

Disease Assessment

Tomato stems were split up to determine disease severity. For a particular plant, percentage of discolored vascular tissue to total length of stem (cm) was calculated. For assessment of disease incidence and severity, following formula was used for each treatment comprising 5 replicates (Akhtar et al., 2019):

$$Disease\ incidence\ (\%) = (Number\ of\ infected\ tomato\ plants\ /\ Total\ number\ of\ plants) \times 100)$$

Following formula was used for determination of disease incidence:

$$Disease\ incidence\ (\%) = (Length\ of\ infected\ stem\ /\ Total\ length\ of\ stem) \times 100$$

In-vitro* Effect of Biochar on *Alternaria solanai

On PDA plates, the inhibitory effects of green and wood waste biochar were investigated *in vitro* with regard to *A. solani* growth and inhibition. Prior to being added to PDA, biochar was sieved using a 100 µm sieve. Before autoclaving, the growth medium was supplemented with GWB at two different concentrations (3 and 6% v/v). Growth medium was then added to 90 mm Petri dishes and allowed to solidify at room temperature. Then, using a sterile cork borer, six mm diameter agar plugs of the fungal culture's actively developing portions (which were five days old) were extracted and put in the middle of the dishes. For six days, the infected Petri plates were incubated at 23 ± 2 °C. The colony diameter of five randomly arranged duplicates was averaged to determine the radial growth of *A. solani* (mm) per treatment.

Radial growth of *A. solani* was determined in different media such as control (C), amended (A) and amended media (A) to calculate the % growth inhibition using following formula:

$$Growth\ Inhibition\ \% = \frac{C - A}{A} \times 100$$

Statistical Analysis

Data was analyzed using statistix (version 8.1) software. Percentage values were converted prior to analysis. For the study, however, the combined data from the experimental repetitions was utilised. Tukey's HSD test was used to compare the means at the P ≤ 0.05 probability levels.

RESULTS

Plant Assay

Shoot and Root length

Figure 1 shown that addition of green and wood waste biochar significantly affects tomato plant shoot and root length. The maximum increase of shoot and root length (79.1 and 19.8 cm respectively) was noted in 6% biochar amended treatment (S+6%GWB) in the absence of pathogen (-*A. solanai*). While under the pathogen stress (+*A. solanai*) minimum shoot and root length was observed soil only treatment without any biochar amendment.

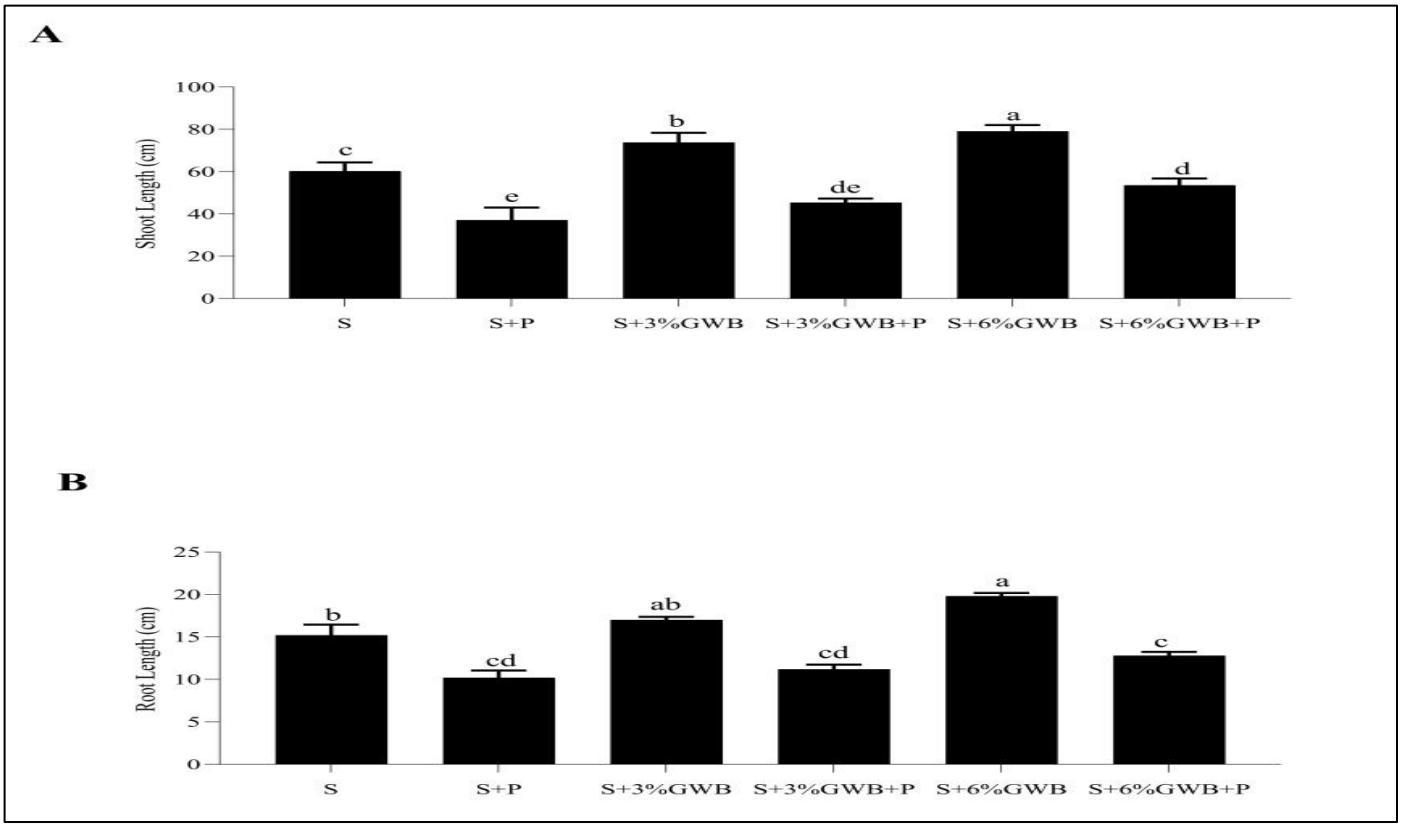

Figure 1. Effect of green and wood waste biochar (3 and 6% GWB) on tomato Shoot (A) and Root length (B). All values represent mean ± SE, Bars with different letters suggest significant differences as per Tukey's HSD test (P ≤ 0.05)

Stem and Root Dry weight

Minimum stem dry weight (1.05 g) was recorded in 3% GWB amended treatments under pathogen inoculation (Figure 2A). On the other hand, highest stem dry weight (3.03 g) was observed in treatment amended with 6%GWB in the absences of pathogen stress. While lowest root dry wright (0.4 g) was recorded in soil only treatment inoculated with *A. solanai* without any biochar amendment (Figure 2B).

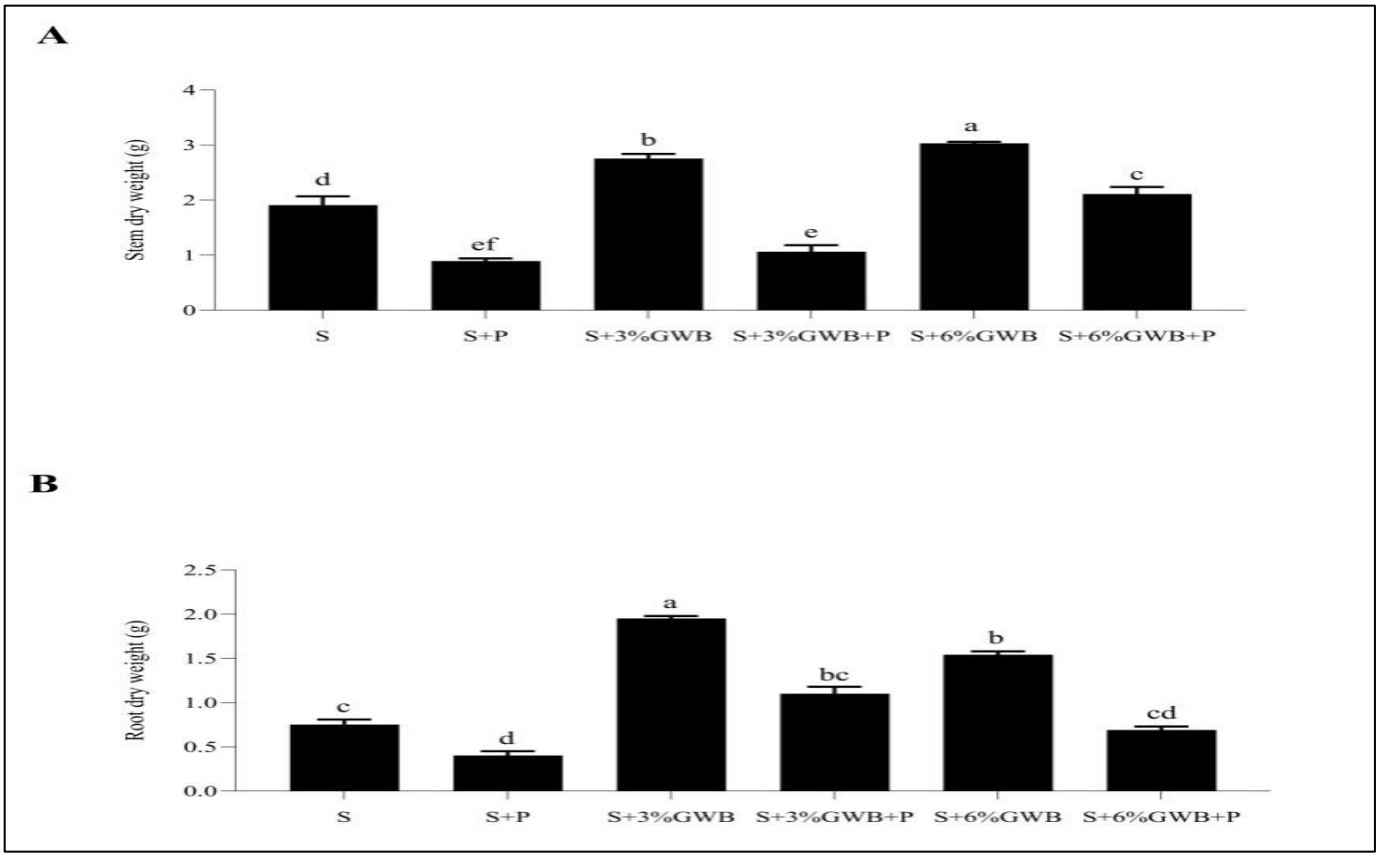

Figure 2. Effect of green and wood waste biochar (3 and 6% GWB) on tomato Stem (A) and Root (B) dry weight.

NPK Analysis

Green and wood waste biochar based soil organic amendment significantly influence the nutrient content in tomato plants (Table 2). There was significant increase in nitrogen contents of tomato plants grown in soil amended with biochar under *A. solani* induced stress. Highest nitrogen contents (3.89 ± 0.01) were calculated in 6% GWB amended soil under *A. solani* induced stress.

While minimum nitrogen amount was recorded in soil only control without any biochar amendment.

In case of phosphorus (P) and potassium (K) content the maximum (0.31±0.02 and 1.44±0.01) amount of P and K was noted in soil with 3% GWB treatment under *A. solani* stress (Table 2).

Table 2. Effect of Green and Wood waste biochar (GWB) on the Nutrient content (NPK) in tomato plant. Data represent means ±SD, following different letters in superscript indicate differences as per Tukey's test.

Treatment	Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)
S	3.44±0.02^{d}	0.1±0.05^{d}	1.21±0.02^{c}
S+ *A. solani*	3.66±0.01^{c}	0.13±0.06^{c}	1.02±0.01^{d}
S+3% GWB	3.45±0.01^{d}	0.25±0.03^{b}	1.44±0.01^{a}
S+3% GWB+ *A. solani*	3.66±0.05^{c}	0.31±0.02^{a}	1.09±0.01^{d}
S+6% GWB	3.7±0.02^{b}	0.12±0.01^{c}	1.35±0.02^{b}
S+6% GWB+ *A. solani*	3.89±0.01^{a}	0.1±0.01^{d}	0.74±0.03^{e}

Disease Assessment

Response of tomato plants to *A. solanai* infection was rated from highly susceptible to resistant depending on soil composition. Disease response in soil without any biochar amendment was highly susceptible while it was resistant under 6% GWB amendment. Maximum disease severity (80%) and incidence (100%) was observed in soil only control while the minimum was recorded in soil amended with 6% GWB (Table 3).

Table 3. Effect of Green and Wood waste biochar (3 and 6%) on the Disease Incidence (DI), Disease Severity (DS) of tomato plant

Treatment	Disease Incidence (%)	Disease Severity (%)	Plant Response
S +*A. solani*	100	80	HS
S+3% GWB + *A. solani*	70	45	MS
S+6% GWB + *A. solani*	20	15	R

In-vitro* Effect of Biochar on *Alternaria solanai

In-vitro toxic effect of green and wood waste biochar (GWB) in PDA gainst *A. solani* is depicted in Table 4, while the PDA with no any additional additive served as a control group. Minimum inhibition (16.32%) of radial growth of *A. solani* was recorded in 6% GWB amended media. On the other hand, the inhibition (29.03%) of fungal growth was observed in 3% amended treatment.

Table 4. *In vitro* inhibition (%) and mycelium radial growth (mm) of *Alternaria solani* in control (un-amended), 3% and 6% amended PDA media

Treatments	Radial growth (mm)	Inhibition (%)
Control	86.3 ± 0.75^{a}	
GWB (3%)	61.2 ± 1.31^{c}	29.08 ± 2.25
GWB (6%)	72.2 ± 4.43^{d}	16.32 ± 2.11

DISCUSSION

Stepping towards sustainable agricultural production, 21st century signifies the scientific reasons for positive and negative points of particular manufacturing, advertisement and making of policy for enhancing and minimizing the cost and unscheduled results of production in agriculture. Basic objective of sustainable agriculture is fulfilment of society requirements without disturbing the potential of future generations to fulfil their needs. The base of sustainable agriculture is the comprehensive knowledge of ecosystem. Sustainable agriculture aims to make earth eco-friendly. Utilization of biochar is best strategy to achieve sustainability in agriculture without harming the environment. Heavy doses of chemical pesticides and fertilizers is dangerous for environment and is not in suitable with requirements of sustainable agriculture. In 21st century, compost and biochar and efficient tools to obtain optimum crop yield without harming the environment. Application of biochar is an alternative strategy to enhance plant growth and to manage plant diseases. Efficient use of compost and biochar, enhances availability of essential nutrients and thus promotes plant growth parameters (Wood biochar and Green waste biochar). Various mechanisms induced by soil amendment with biochar are responsible for enhanced soil fertility, porosity, physical and chemical properties. Application of biochar causes reduction of disease severity and incidence. The ability of biochar to act as fertilizer (Lehmann and Rondon, 2006) and green sorbent (Ahmad et al., 2014) is verified by increased nitrogen and phosphorus in tomato by biochar utilization. The conversion of organic waste products into biochar is an eco-friendly approach of waste material recycling (Su et al., 2024). Application of biochar in cash crops along with compost enhances soil fertility and enhances plant growth (Antal et al., 2003; Gaskin et al., 2008). Combined application of compost and biochar in soil has been reported to enhance soil pH, cation exchange and water holding capacity of soil. Not only an improved yield but also activation of resistance in plants has been documented in biochar amended soil (Abbas et al., 2024).

Current study signifies the impacts of compost and biochar on physio-chemical properties of tomato plant wither in the absence or absence of *A. solani*. In response to biochar application, induced systemic resistance was observed by Elad et al., 2011. Current study shows that soil fortification with biochar enhances the availability of availability of nitrogen and phosphorus. A report by previous experiment confirms enhancement in root biomass after application of biochar (Ahmad et al., 2024). There is direct effect of biochar on *Alternaria solani*or in the from of modified response of plants. The presence of cation exchange site on biochar surface caused increase in CEC of soil (Domingues et al., 2020). Earlier research indicates that N- content increased in biochar amended soil. The presence of positively charged exchange sites, causes the biochar to retain phosphate ions in soil (Major et al., 2010). Alos, our findings suggest significant impact of biochar in minimizing disease severity. Earlier research also reported similar results (Saeed et al., 2023).

Application of biochar in soil nutrient context, facilitate better uptake by the plant and conserve water of soil (Waheed et al., 2024). In latest research, Abbas et al., 2024 reported significant enhancement of growth and reduced effect of

bacterial pathogens in chili plant upon application of leaf waste biochar. Future research should be focused on uncovering the mechanism behind induction of resistance in tomato plants against *A. solani* along with other economically significant pathogens. There is also a need to under the possible risk associated use of biochar due to various types of chemicals released by biochar in environment.

CONCLUSIONS

Treatment of soil with materials like biochar and compost play a significant role for environment and plants because the soil amendments minimize the need for using chemical fertilizers to boost plant growth and pesticides for management of plant diseases. Residues of chemical pesticides leave harmful impact on soil microbial population of beneficial microbes and humans. Use of organic amendments minimize reliance on the chemical fertilizers as well as pesticides. In conclusion, it can be said that soil amendment with biochar and compost has significant role in boosting plant growth. These amendments have shown promising effects for management of early blight of tomato induced by *A. solani*. Application of wood biochar resulted in sudden decrease in disease incidence and severity. Soil amended with both compost and biochar resulted in significant variations in nutrient contents of tomato plant. Findings of our research that soil amendment enhanced diseases resistance against *A. solani* and stimulated tomato growth.

Current research work focused on application of biochar and compost for management of *A. solani* induced disease. Soil amendment with green and wood biochar showed promising results by decreasing disease severity and incidence in tomato plants. Future research needs to be focused on management of diseases caused by *A. solani* on other members of Solanaceae family and studying genetic variations in plants in response to soil amend with biochar.

ACKNOWLEDGEMENTS

Not applicable.

AUTHOR CONTRIBUTIONS

All the authors contributed equally to this research.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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