Int. J. Phytopathol. 12 (03) 2023. 235-246 DOI: 10.33687/phytopath.012.03.4494

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Available Online at EScience Press
International Journal of Phytopathology
ISSN: 2312-9344 (Online), 2313-1241 (Print)
https://esciencepress.net/journals/phytopath

EFFICACY OF COMPOST AND BIOCHAR TO IMPROVE PLANT GROWTH UNDER GREENHOUSE TRIAL

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ARTICLE INFO

Article History
Received: July 02, 2023
Revised: November 19, 2023
Accepted: November 22, 2023

Keywords
Basidiomycota
Abiotic factors
Potato
Rhizoctonia solani
Solanum tuberosum

ABSTRACT

Potato (*Solanum tuberosum* L.), being the most cultivated tuberous crop worldwide, is threatened by various biotic and abiotic factors. Among fungal pathogens, *Rhizoctonia solani* is the most virulent and widely distributed soil-borne fungi of phylum Basidiomycota which cause severe yield losses to 30-40% around the globe. Several management practices have been adopted to overcome the yield losses inflicted by this fungus. Use of chemicals and fumigants are not encouraged due to health and environmental concerns. Present study was undertaken to develop alternate eco-friendly methods to manage this disease. A greenhouse experiment through CRD was performed with treatments application of compost, biochar and NPK on potato variety Desiree. Treatments and pathogen inoculum were applied at the time of sowing. Plant germination was recorded after two weeks of sowing. Data of growth parameters (Plant height, no. of leaves, shoot weight, root weight, root length, no. of tubers, tuber weight, tuber size) were taken at the end of experiments before harvesting the crop. Combined treatment application of biochar (5%) + compost (10%) + NPK was found to be the best to suppress *R. solani* and increased plant height, number of leaves, shoot weight, root weight, root length and various tuber parameters. On the basis of our results biochar and compost are suggested commercially to improve plant growth and suppress pathogens.

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most popular and consumed nutritious crop of family *Solanaceae* all over the world and in Pakistan (Abbas *et al.*, 2012). History revealed that Potato was introduced in India in 17^{th} century (Anwar *et al.*, 2015). Fungi plays a tremendous role in potato yield loss by causing foliar, tuber and soil borne diseases. *Rhizoctonia solani* is the most devastating pathogen of potato among all soil borne fungi (Ahmad *et al.*, 1995) which cause black scurf, root rot, stem canker and damping off diseases complex of potato crop (Bains *et al.*, 2002). This fungus was first reported on potato by Julius Kuhn about 150 years ago. *Thanatephorus cucumeris* (Teleomorph) is sexual stage of *R. solani* which is responsible for infection (Lehtonen *et al.*, 2008). This fungus survives in the form of sclerotia in soil and requires optimum temperature of 20-25 ^{0}C and moisture 20% for growth.

Temperature and soil moisture are essential for disease development (Lacey and Magan, 1991). This fungus belongs to phylum *Basidiomycota* and it's spores are barrel shaped and mostly found in clusters (Saifuddin and Sheikh, 2016). Mycelial hyphae are hyaline initial but turned brown as they mature. Hyphae are septate and branched at right angled 90^0 (Tsror and Peretz-Alon, 2005). This fungus survives on plant debris as saprophytic fungi in the form of vegetative mycelium called sclerotia. In the meantime, when hyphae of *R. solani* receive root exudates from the host plant then sclerotia grows to the plant roots and infect the plant. Sclerotia in soil and on tubers persist as inoculum for the next seasons (Dubey *et al.*, 2009). This is important soil borne fungi which is responsible for severe diseases of vegetables, beans, and grasses (Sneh *et al.*, 1991). *R. solani* produced symptoms on both above and below ground plant parts of potato. The most obvious and characteristics symptoms are brown to black sclerotia on potato tubers known as black scurf of potato (Carling and Leiner, 1986). Sclerotia are black, irregular shaped resting spores of *R. solani* that are present on the tuber surface with the lump of soil.

Above ground symptoms include brown lesions on collar region of the plant that leads to stem canker and stem girdling, yellowing and leaf curling, root detrition and falling off plant (Baker, 1970). Integrated disease management practices are adopted worldwide to manage this pathogen (Alptekin, 2011). Most commonly used methods are cultural and chemical control. Cultural practices should also be adopted to avoid pathogens such as crop rotation (Larkin *et al.*, 2017) and use of disease-free seed. Tuber seed with sclerotia is the primary source of inoculum. Use of certified seed is the best way to avoid this problem (Daami-Remadi *et al.*, 2008). Many chemicals are used to overcome the yield losses due to *R. solani.* Most commonly used fungicides Dithane-M, Mancozeb and Captan are used for the seed treatments to avoid seed borne pathogens (Olaya *et al.*, 1994). Fungicides Moncut and Quadris applied in furrow at planting also overcome the disease losses (Wharton *et al.*, 2007).

This pathogen inflicted 30-40% yield losses of potato in temperate regions and 19-34% losses in sub-tropical regions from all over the world (Banville, 1989). Use of fungicides to manage this pathogen is unsafe and costly. Certain ecofriendly approaches like nutrients, organic amendments, composts, biochar, plant extracts and oils are used as an alternative to chemical control against *R. solani* (Pawar and Thaker, 2006). The current study is based on the use of eco-friendly treatments like compost and biochar. Compost is a heterogeneous organic material prepared from animal manures, greenhouse waste, vegetable waste, bark solids and grape marcs are strongly capable of inhibition of soil borne pathogens (Hoitink *et al.*, 1996) which is effective against many soils borne fungal pathogens like *Rhizoctonia* spp, *Pythium* spp, *Fusarium* spp, *Phytophthora* spp and *Sclerotium* spp (Noble and Coventry, 2005). It is enriched with carbon, nitrogen, and micronutrients which fulfill the nutrient deficiency of plants in soil (Yogev *et al.*, 2006). Many types of organic based composts such as hardwood bark, vermi compost and municipal wastes are used against root rot pathogens (Hoitink *et al.*, 1991). In potato fields, compost is used as a remedy to manage several soils borne diseases such as *Verticillium dahlia*, black scurf caused by *Rhizoctonia solani*, common scab caused by *Streptomyces scabiei* (Larkin *et al.,* 2011). Chicken manure compost is suppressive against *Pythium aphanidermatum* by increasing soil bacterial population upto 68.4% (Nelson and Boehm, 2002). Many types of composts such as vermi composts, hardwood bark (Nelson *et al.*, 1983), animal manure, organic greenhouse and household waste are quite effective against *R. solani* (Santos *et al.*, 2011). Another study showed that cattle manure compost in furrow application was found best in reducing the disease incidence and severity of black scurf disease on potato (Tsror *et al.*, 2001). Biochar is carbon enriched source prepared from the pyrolysis of organic compounds wood bark, crop residues, municipal wastes, greenhouse waste, animal manure (Kammann *et al.*, 2015). Biochar plays important role to attain sustainable environment because of long term availability of carbon compounds to the soil that maintains soil fertility (Lehmann, 2007). Now a day biochar is a good organic source to manage yield losses and reduce disease incidence caused by soil borne pathogens (Bonanomi *et al.*, 2015). Biochar is helpful in reduction of greenhouse gas emissions and soil pollution and it maintains soil fertility level by nutrients uptake and addition of beneficial microorganisms (Lehmann, 2007). Bonanomi *et al.* (2015) reported that biochar is effective against *F. oxysporum* f. sp. *asparagi*, *F. oxysporum* f. sp. *radices-lycopersici*, *Pythium aphanidermatum*, *Phytophthora cinnamomi, Phytophthora cactorum*, and *R. solani*. Eucalyptus wood biochar and greenhouse waste biochar were found best to decrease disease incidence of damping off caused by *R. solani* on cucumber (Jaiswal *et al.*, 2014).

The objective of this study is to determine an environmentally friendly way to suppress *R. solani* as an alternative to noxious chemicals because there are concerns related to chemical application regarding soil fertility, human health and environmental pollution. Chemicals have adverse effects on both the environment and living organisms, leading to the destruction of nature. Additionally, their use contributes to pathogen resistance to various pesticides. Consequently, there is a pressing need to discover eco-friendly approaches for pathogen management in order to safeguard the natural environment and shield life from the harmful consequences of chemical substances (Ghorani *et al*., 2008). Considering the harmful nature of chemicals, this study aims to investigate the effectiveness of compost and biochar in enhancing plant growth in a greenhouse trial.

MATERIALS AND METHODS

Sample Collection, Isolation and Pathogenicity of *R. solani*

Diseased samples were collected during the survey in different areas of Punjab Pakistan. The samples were kept in polythene bags and stored at 4 ^{0}C. The samples were processed for the isolation of *R. solani* for further experiments. Potato dextrose agar media was prepared for the isolation of *R. solani* and autoclaved at 121 ^{0}C. Diseased samples were washed under tap water and disinfected with 2% sodium hypochlorite for 2-3 minutes and again washed with sterilized water (Goswami *et al.*, 2010). Infected portions from tuber samples were removed with the help of scalpel and placed on PDA plates and wrapped. Isolated plates were kept in incubator at 25^{0}C (Das *et al.*, 2014). After two days white mycelial growth was observed on PDA plates. For purification, a single spore technique was performed with inoculating needle and spore was placed on autoclaved PDA plates. These plates were kept at 25^{0}C for fungal growth for 3 days. After growth morphological characteristics of tested fungi were observed under compound microscope by following key lines of Barnett and Hunter (1972). The pathogenicity test of *R. solani* was checked out on potato tubers. For this purpose, healthy potato tubers were washed under tap water and disinfected with 2% NaOCl. *R. solani* plugs were taken from pure culture and placed on potato tubers with the help of inoculating needle (Zhang *et al.*, 2014). Potato tubers were placed in plastic boxes, wrapped and placed at 25^{0}C for 3 days.

Efficacy of Compost and Biochar with NPK Level against *R. solani*

Greenhouse pot experiment was conducted to evaluate the effect of poultry waste compost and sugar waste biochar with single dose of N:P: K to inhibit fungal pathogen. *R. solani* was multiplied on wheat grains for the inoculum. For this purpose, 100g of wheat was washed under tap water, air dried and autoclaved at 121^{0}C for 20 minutes (Table 1, 2). After autoclaved, 4-5 *R. solani* plugs from 1 week old culture were placed in wheat contained flasks and kept at 25^{0}C for two weeks (Balali *et al.*, 1995). Fungal growth was obtained within two weeks. Potato seeds after surface sterilization were grown in pots filled with sterile sandy loam soil and wheat inoculum 20g/kg (Sabet *et al.*, 2013). Following treatments with three replications were applied at sowing in CRD design.

Table 1. Table represents description of treatments compost, biochar and NPK along with their concentrations.

T1	Control (0%)
T2	10% compost
T3	5% biochar
T4	10% compost + 5% biochar
T5	NPK
T6	10% compost + NPK
T7	5% Biochar + NPK
T8	10% Compost + 5% Biochar + NPK

Data was recorded after 90 days of planting. Growth parameters (Plant height, root length, root shoot weight, no. of tubers, size of tubers, and weight of tubers) were measured (Jaiswal *et al.*, 2014).

Statistical Analysis

Tukey's test was applied to evaluate the results according to the established protocol of Jaiswal *et al.* (2014).

RESULTS

Sample Collection and Isolation of *R. solani*

After 3 days of incubation fungal colony was observed. White mycelial growth was obtained which later on changed into brown color. Spores were seen in cluster form and oval or barrel in shape. The presence of septate hyphae with a right-angled appearance, along white mycelial growth, and brown lesions on tubers (Figure 1), provided confirmation of *R. solani's* pathogenic nature.

Table 2. Fig shows physiochemical properties of poultry waste compost and sugar waste biochar.

Features	Unit	Compost	Biochar
pH	-	8.2	5.5
Carbon	%	25	39
Nitrogen	%	1.15	0.90
C/N	%	19	30
Phosphorus P_2O_5	%	0.21	2.50
Potassium K_2O	%	0.10	0.39
Silicon	%	0.001	0.5
Color	-	Gray	Black
Commercial Name	-	Suraj	Kissan

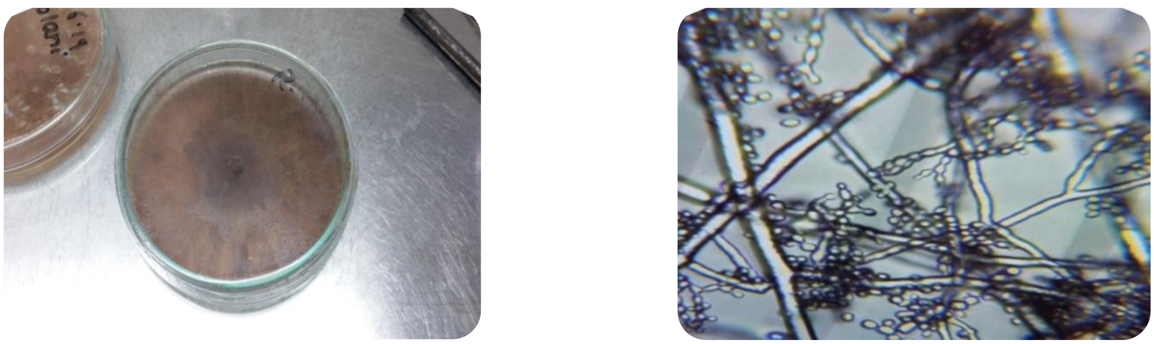

Figure 1. Mycelial growth and hyphae of *R. solani*.

Efficacy of Compost and Biochar with N: P: K Level against *R. solani*

Treatment's effect of compost and biochar along with single dose of N: P: K to inhibit *R. solani* under green house was observed. All treatments had inhibitory impact on *R. solani* and significant impact on plant health. Plant seedlings were observed after two weeks of sowing and plant germination % was recorded. Maximum plant germination 100% was recorded in T8 (10% compost + 5% biochar + N: P: K), 70% in T4 (10% compost + 5% biochar), 66% in T2 (10% compost) and T3 (5% biochar), 50% plant germination was shown by T7 (5% biochar + N: K) and T6 (10% compost + N: P: K) while minimum plant germination 44% and 33% was recorded in T5 (N: P: K) and T1 (control) as shown in Figure 2.

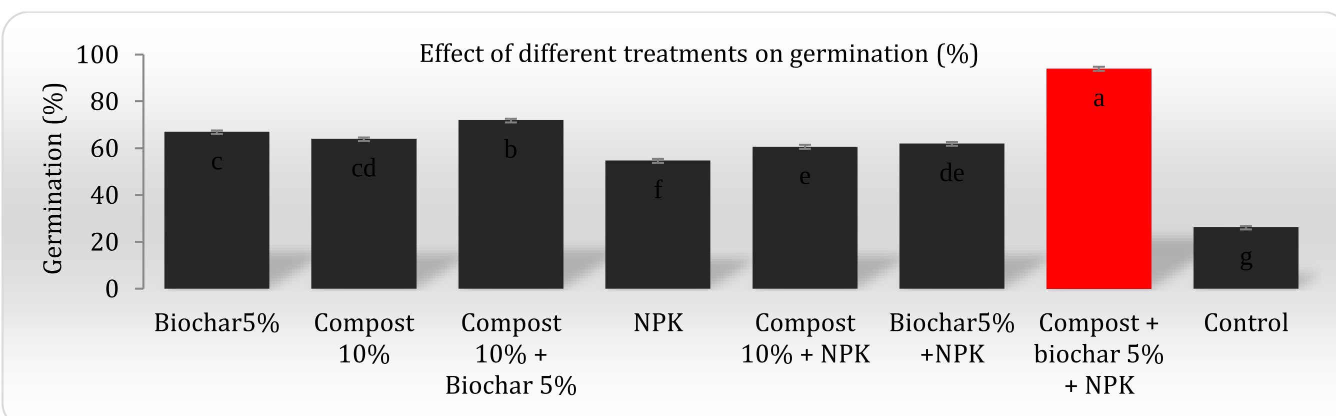

Figure 2. Figure shows the impact of different treatments on plant germination %.

Data of plant growth parameters is mentioned below.

Plant Height

Analysis of variance mentioned significant results of treatments on plant height of potato. Results cleared that T5 combined effect of compost + biochar + NPK had maximum plant height 79 cm, T2 biochar + NPK had 77 cm, T6 compost + NPK had 69 cm plant height however T7 NPK alone was not much effective and increased plant height to 58 cm. While T4 compost + biochar, T1 biochar and T3 compost increased plant height to 66, 68 and 65 cm respectively. Compost and biochar in combination and alone had significant impact on plant growth and improved plant height as compared to T8 control with 45 cm (Figure 3).

Number of Leaves

Analysis of variance explained significant effect of treatments on number of plant leaves. Results stated that T5 compost + biochar + NPK was highly significant and increased number of leaves up to 96, T2 biochar + NPK and T6 compost + NPK increased leaf number to 78 and 75 respectively. T7 NPK alone was less significant and had 57 numbers of leaves. Treatment 4 compost + biochar, T1 biochar and T3 compost had 71, 70 and 67 number of leaves respectively. T8 control had the minimum number of leaves to 41 (Figure 4).

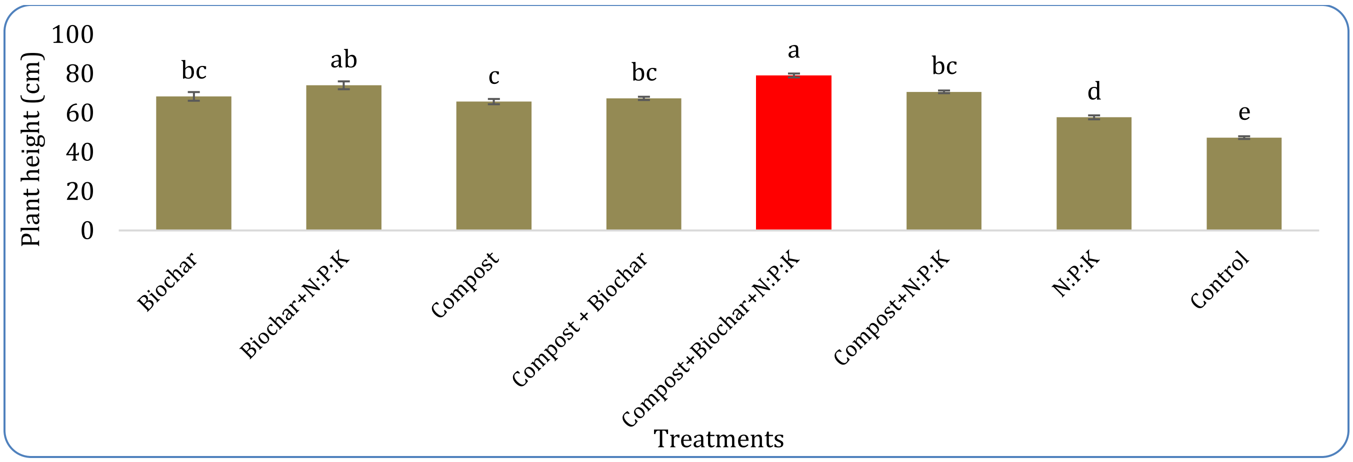

Figure 3. Fig shows the efficacy of 8 treatments control, 10% compost, 5% biochar, 10% compost + 5% biochar, NPK, 10% compost + NPK, 5% biochar + NPK, 10% compost + 5% biochar + NPK on plant height (cm). Error bars represent the difference between treatments.

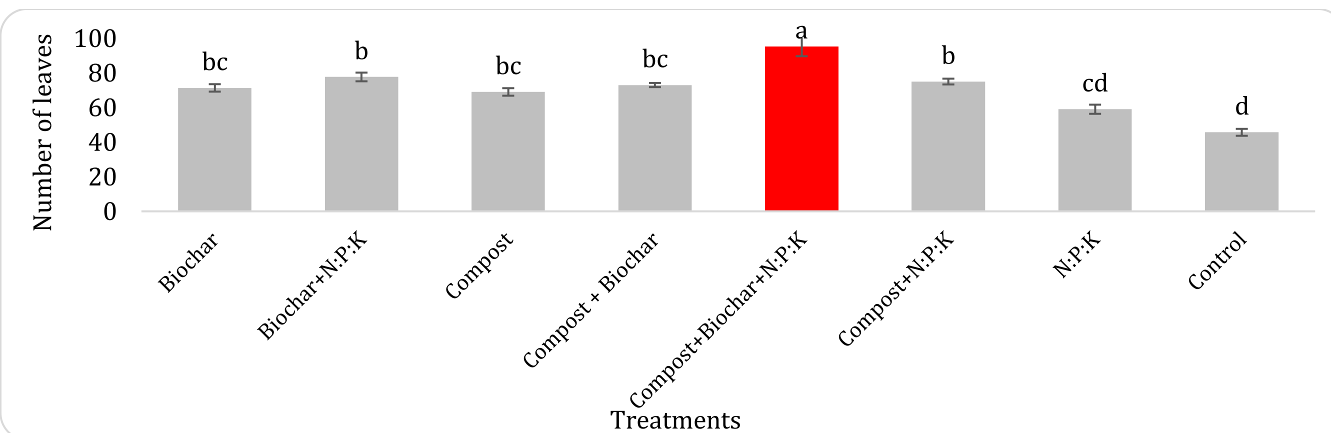

Figure 4. Fig shows treatment impact along with concentrations control 0%, compost 10%, biochar 5%, compost 10% + biochar 5%, NPK, compost 10% + NPK, biochar 5% + NPK, compost 10% + biochar 5% + NPK on number of plant leaves. Error bars represent the difference between treatments.

Shoot Weight

Analysis of variance demonstrated significant impact of treatment on plant shoot weight. Results cleared that all treatments increased shoot weight, but maximum shoot weight 9.8 g was observed in T5 combined effect of compost + biochar + NPK. T2 biochar + NPK and T6 compost + NPK increased shoot weight up to 9.3 g and 9 g respectively, while T7 NPK was less effective than NPK combined with other treatments and had 7 g shoot weight. T4 compost + biochar, T1 biochar and T3 compost increased shoot weight 9, 8.9 and 8.5 g respectively. T8 control had minimum shoot weight of 5.8 g (Figure 5).

Root Weight

Analysis of variance showed significant effect of treatments on plant root weight. Results demonstrated that T5 compost + biochar + NPK had maximum root weight with 1.1 g, T2 biochar + NPK increased root weight 1.06 g and T6 compost + NPK had 1.05 g, T7 NPK had 0.8 g. T4 compost + biochar and T1 biochar alone had intermediate effect with 0.97 and 0.98 g respectively. T3 compost alone and T8 control had shoot weight up to 0.79 and 0.3 g respectively (Figure 6).

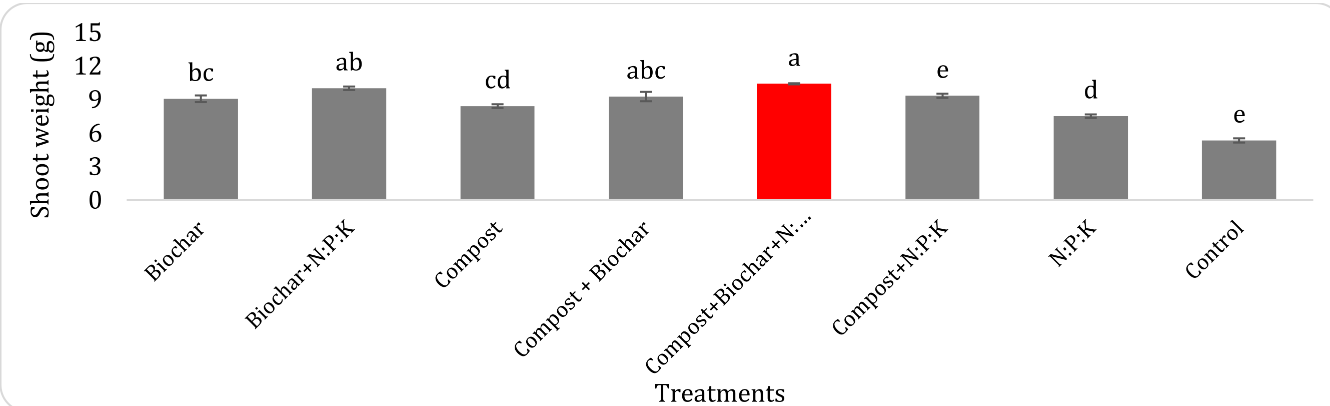

Figure 5. Fig represents the treatment efficacy of control, 10% compost, 5% biochar, 10% compost + 5% biochar, NPK, 10% compost + NPK, 5% biochar + NPK, 10% compost + 5% biochar + NPK on plant shoot weight (g). Error bars depict difference between treatments.

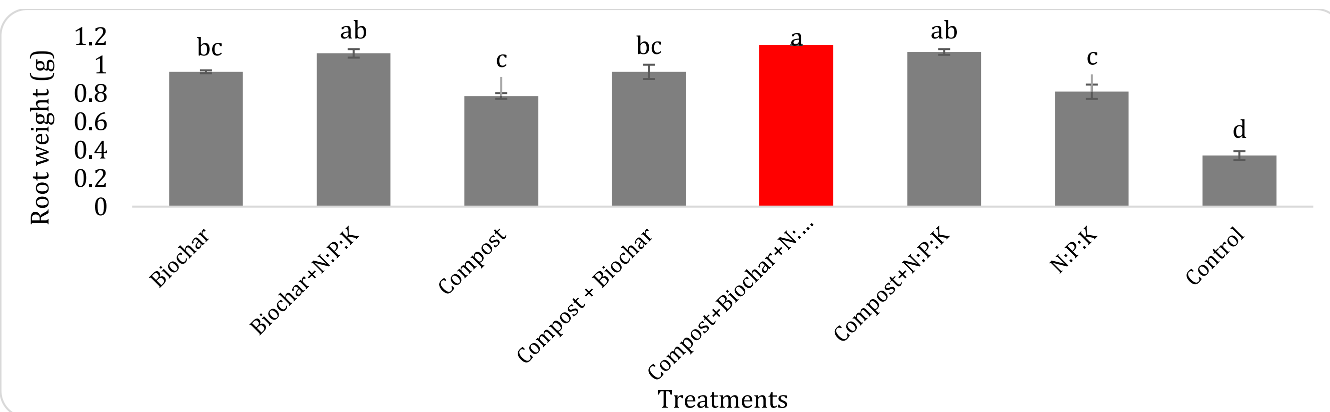

Figure 6. Fig shows the effect of treatments (control 0%, compost 10%, biochar 5%, compost 10% + biochar 5%, NPK, compost 10% +NPK, biochar 5% + NPK, compost 10% + biochar 5% + NPK) on plant root weight (g). Error bars represent the difference between treatments.

Root Length

Analysis of variance showed significance of treatments on plant root length. Results cleared that maximum root length 5.6 cm was observed by T5 compost + biochar + NPK and minimum root length 2.8 cm was recorded in T8 control. T3 compost alone increased root length up to 4.5 cm while T1 biochar and T4 compost + biochar improved root length up to 5 cm and 4.95 cm respectively. T7 NPK, T6 compost + NPK and T2 biochar + NPK increased root length up to 4.7, 5 and 5.01 cm respectively (Figure 7).

Number of Tubers

Analysis of variance described the significant impact of treatment efficacy on number of tubers. Results explained that the highest tuber production 5.5 was recorded in T5 compost + biochar + NPK and T4 compost + biochar increased 5 tubers/plant. T1 biochar and NPK and T2 biochar + NPK both had same tubers number 4.3, while T6 compost + NPK and T2 compost had number of tubers 3.9 and 3.8 respectively. Number of tubers were low in T7 NPK and T8 control with 2.2 and 1 respectively (Figure 8).

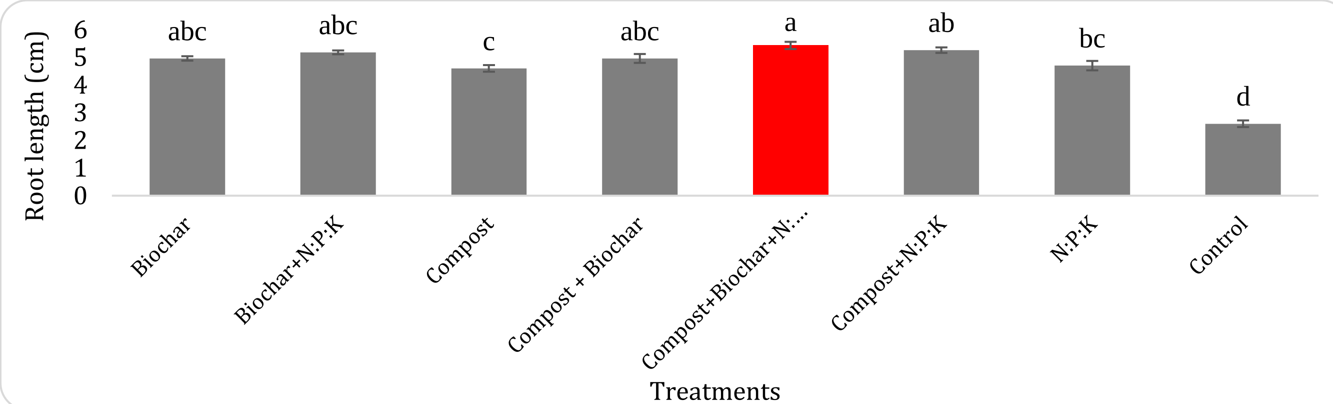

Figure 7. Fig explains treatments efficacy (control, compost 10%, biochar 5%, compost 10% + biochar 5%, NPK, compost 10% + NPK, biochar 5% + NPK, compost 10% + biochar 5% + NPK) on plant root length (cm). Error bars depict difference between treatments.

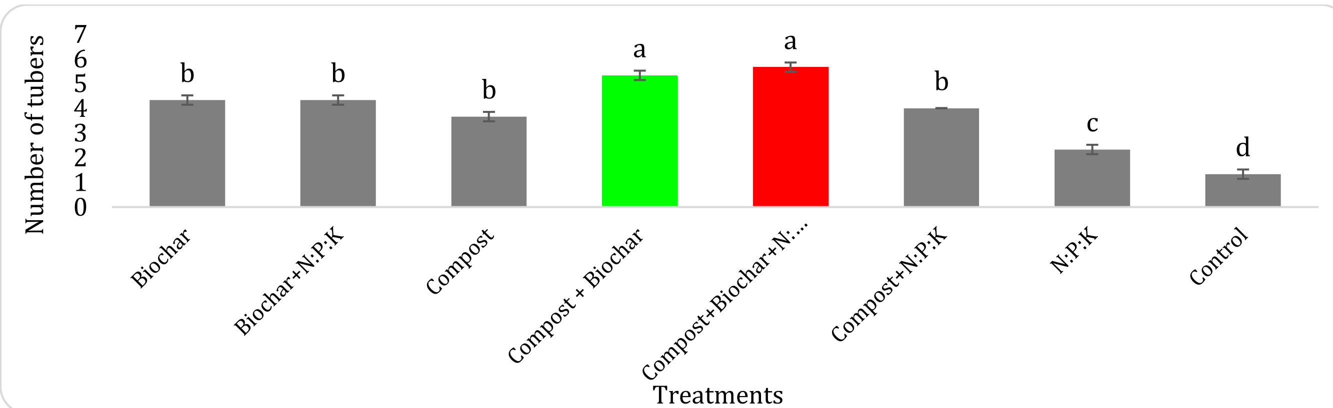

Figure 8. Fig represents impact of treatments along with concentrations control 0%, compost 10%, biochar 5%, compost 10% + biochar 5%, NPK, compost 10% + NPK, biochar 5% + NPK, compost 10% + biochar 5% + NPK. Error bars show the difference between treatments.

Weight of Tubers

Analysis of variance showed significant effect of treatments on tuber weight of plant. Results showed that maximum tuber weight was recorded in T5 compost + biochar + NPK and T4 compost + biochar with 80 and 79 g respectively. T2 biochar + NPK and T6 compost + NPK increased tuber weight to 70 g, T7 NPK alone had tuber weight 60 g, T1 biochar and T3 compost increased tuber weight up to 65 g as compared to T8 control with 51 g (Figure 9).

Tuber Size

Analysis of variance explained significant difference among treatments. Results concluded that maximum tuber size 57 mm was obtained in T8 compost + biochar + NPK and T4 compost + biochar. It was cleared that T8 and T4 were highly significant than remaining treatments. T7 biochar + NPK increased tuber size to 45 mm, while T6 compost + NPK, T4 biochar and T3 compost alone gave similar results with slight difference as 43, 43.3 and 43.4 mm respectively. T5 NPK had the least significant impact on plant tuber size with 42 mm and T1 control had minimum tuber size by 28 mm (Figure 10).

DISCUSSION

The pathogen, *Rhizoctonia solani* (*Thenatophorus cucumeris*) is a soil borne and most studied pathogen of potato (Kühn, 1858) because of genetic and morphological diversity. Present study results are similar to observations of (Desvani *et al.*, 2018) who stated that *R. solani* isolates from rice were rapidly grown on PDA. Growth was obtained at 3rd day of incubation with white color colony. White colony color turned in to brown when culture is being old. Branched hyphae were septate and curved at 90⁰. Another study found sclerotia in clusters and white or creamy in color and turned brown to dark brown as they mature (Gopireddy *et al.*, 2017).

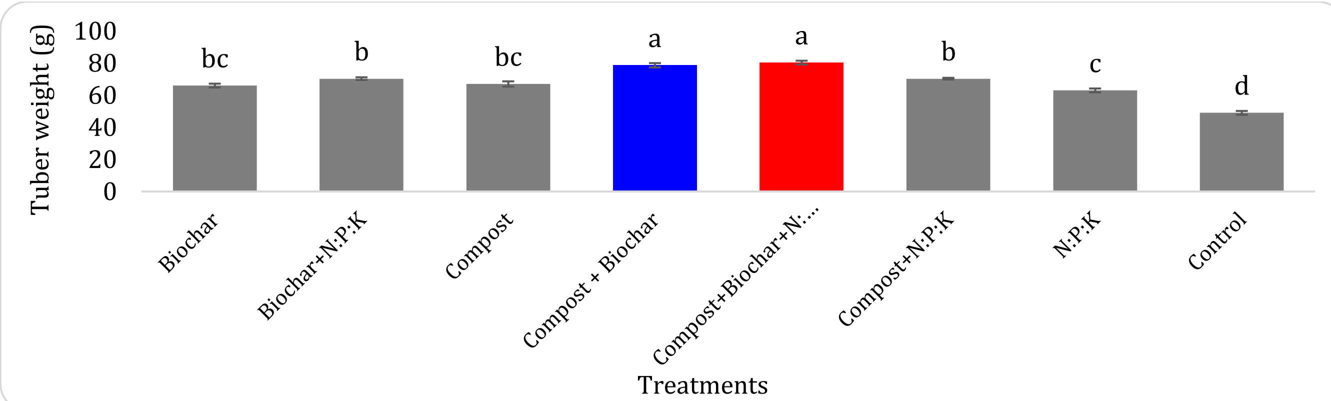

Figure 9. Fig represents impact of treatments control 0%, 10% compost, 5% biochar, 10% compost + 5% biochar, NPK, 10% compost + NPK, 5% biochar + NPK, 10% compost + 5% biochar + NPK on tuber weight (g). Error bars depicts difference between treatments.

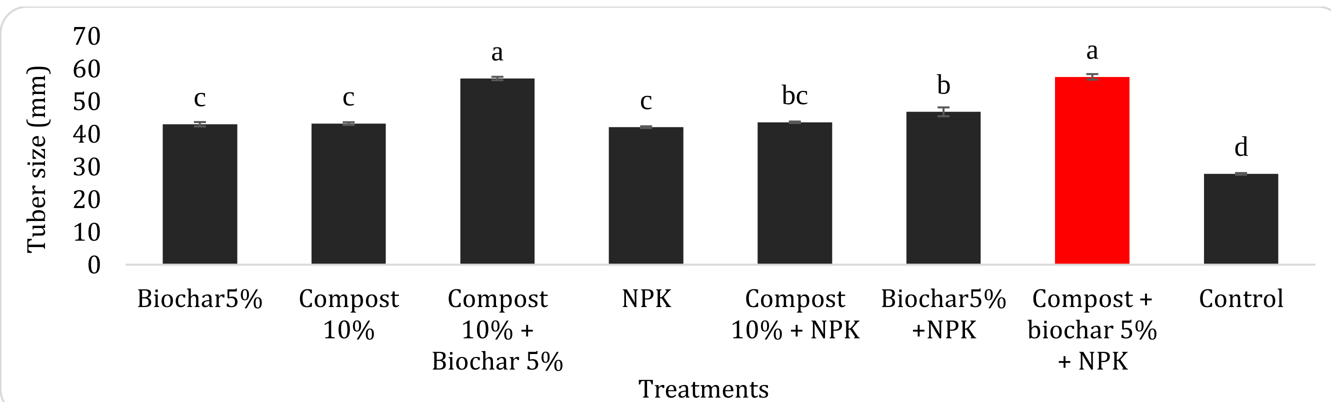

Figure 10. Fig shows the treatments effect with concentrations control 0%, compost 10%, biochar 5%, compost 10% + biochar 5%, NPK, compost 10% + NPK, biochar 5% + NPK, compost 10% + biochar 5% + NPK. Error bars explains difference among treatments.

Combined application of compost (10%), biochar (5%) alongwith NPK under greenhouse gave significant results. Present results of greenhouse experiment agreed to (Yogev *et al.*, 2006) who reported that combination of compost based on chicken manure and biochar prepared from jarrah wood and enriched with phosphate and iron improved vegetative growth and yield of *Brassica rapa* under screen house conditions in pots. It was stated that combined effect of compost and biochar with minerals doubled the action of biochar and improved soil quality, nutrient up take and carbon retention in soil. Biochar with compost and minerals increased soil humidity and electrical conductivity up to 26% and improved plant yield with height and weight and increased leaf area. Present project is quite similar to another study of (Khalifa, 1991; Khalifa and Thabet, 2015) who reported that 5% biochar is highly effective to reduce disease incidence of fusarium wilt and root rot caused by *F. oxysporum* and *R. solani* up to 85% and 80% respectively on tomato under controlled conditions of greenhouse. Biochar 5% increased plant biomass and plant vigor 140% and 139% in *F. oxysporum* inoculated plants and 105% and 100% in *R. solani* inoculated plants respectively. Another study of (Graber *et al.*, 2012) revealed that biochar has positive effect on plant vigor because biochar facilitated nutrients supply and promoted microbial activity in soil that has direct impact on plant physiological growth. According to (Graber *et al.*, 2012; Jaiswal *et al.*, 2014) biochar not only fulfilled nutrient requirement of plant and facilitate plant growth but it is also useful to suppress foliar and soil borne pathogens. Earlier studies demonstrated that biochar is an effective organic amendment against several soil borne pathogens as *F. oxysporum* f.sp. *asparagi* (Elmer and Pignatello, 2011), *Ralstonia solanacearum* (Nerome *et al.*, 2005), *Phytophthora cactorum* (Zwart and Kim, 2012), *Pythium aphanidermatum* and *R. solani* (Jaiswal *et al.*, 2014). In a greenhouse pot experiment, a study conducted by Silva et al. in 2020 demonstrated the significant effectiveness of biochar derived from eucalyptus, specifically when applied at a volume concentration of 5%. This treatment proved highly efficient in combatting Fusarium wilt in tomato plants, resulting in a notable reduction in disease incidence. Additionally, it fostered the growth of the plants by enhancing various parameters such as plant height, leaf count, stem thickness, and the mass of both roots and shoots. A recent study by Jin *et al.* (2023) found that using Helianthus tuberosus-derived biochar at 1% and 2% W/W reduced Fusarium wilt severity in tomatoes. This biochar also improved plant growth by boosting beneficial rhizosphere microbes like Pseudomonas sp. Present results suggested that biochar (5%) and compost (10%) alongwith NPK incorporation in potting mixture not only suppressed *R. solani* incidence but also improved vegetative growth (plant height, leaf number, shoot weight, root weight, root length) and reproductive growth (tuber number, tuber weight, tuber size). Biochar and compost are organic amendments that can increase soil fertility and soil quality and have a great tendency to improve plant growth and vigor. According to (Khalifa and Thabet, 2015) biochar (salix wood) increased tomato plant height up to 93% in *F. oxysporum* inoculated plant and 75% in *R. solani* inoculated plants in greenhouse trail. Biochar stimulated plant growth as plant height because of direct nutrient supply. The present study proposed that combined application of compost 10%, biochar 5% and NPK increased plant height up to 79 cm and number of leaves up to 96. These results are strongly related to (Khalifa and Thabet, 2015) who reported that biochar prepared from salix wood at 5% concentration increased number of leaves of tomato by 99% in *F. oxysporum* inoculated plants and 66% in *R. solani* inoculated plants under greenhouse conditions. In a controlled greenhouse experiment, Sales *et al.* (2020) discovered that using 10% and 20% conifer wood biochar by volume had several benefits. It helped maintain the soil's pH, enhanced nutrient absorption, and improved the growth of blueberry plants, specifically increasing their root and shoot weights (Sales *et al.*, 2020). Biochar enhanced nutrient supply to plants so that plant's physiological, histological and biochemical growth has been improved. These changes improved the plant growth in a manner of plant height, number of leaves, fresh weight of shoot, plant biomass.

Previous studies proved that biochar application along with fertilizer has a great potential to improve plant growth and yield and the present study has quite the same results.

CONCLUSION

The basic objective of this study was to develop alternative measures to chemical methods that are eco-friendly and harmless for human consumption. The present study used compost and biochar as organic amendments to *suppress R. solani*. These organic amendments are enriched with nutrients and beneficial microbes which can increase soil fertility, improved plant vigor and health and helped to reduce pathogen infection by inducing resistance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to publish the article.

AUTHORS CONTRIBUTIONS

Dr. Muhammad Usman Ghazanfar and Dr. Waqas Wakil conceived the idea, facilitated, guided and supervised the experiment. Ms. Irum Bilqees conducted field visits, carried out experiments and wrote initial draft while Dr. Waqas Raza co-supervised and contributed to finalizing the manuscript.

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