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

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Research Article

Enhancing rhizosphere bacterial activity against bacterial wilt of tomato (*Ralstonia solanacearum*) using biochar

Subhan Ali, Rabia Rasheed, Muzamil Qazi, Umair Rafiq, Hira Akhtar
Institute of Plant Protection, MNS- University of Agriculture Multan, 61000, Pakistan.

ABSTRACT

Tomato is a plant of tropical origin and belonging to the *Solanaceae* family, and it provides the body with dietary fibers, minerals, vitamins, and essential proteins. It is grown globally, and its production is prone to bacterial wilt, which is caused by the disease agent *Ralstonia solanacearum.* Bacterial wilt can result in substantial damage to tomato production, leading to losses in yield and economic losses to farmers. The rhizosphere, which is the part of soil where plants penetrate their roots deeply in contact with the soil, contains many microorganisms that generate metabolites that act as chemical signals for motile bacteria to migrate to the surface of the roots and fix nitrogen for plant growth and development. The objective of this study was to explore biochar in the management of bacterial wilt of tomato disease caused by *R. solanacearum.* The experiment consisted of three parts to discern effects of different isolates of *R. solanacearum,* the influence of biochar with various concentrations on plant height and fruit yield, and the effects of biochar amendment with rhizosphere bacteria on plant height, fruit and disease. The result indicated that the most aggressive *R. solanacearum* was isolated from Chiniot district, and the 1 % biochar concentration exhibited the highest value when the plant height, the number of fruits, and the disease reduction were considered. Biochar can supplement the population of rhizosphere anti quorum quenching bacteria against *R. solanacearum*.

Keywords: *Ralstonia solanacearum*, Tomato, Biochar, Disease management.

OPEN ACCESS

Correspondence
Subhan Ali
sa99344@gmail.com

Article History
Received: June 29, 2023
Accepted: August 22, 2023
Published: September 23, 2023

INTRODUCTION

Tomato (*Lycopersicon escylentum Mill.*) is an edible berry of plants belongs to *Solanaceae* family. Tomatoes are an excellent source of lycopene, vital amino acids, dietary fibers, iron, phosphorus, minerals, and vitamins A, B, and C (Khokhar, 2013). This is a tropical crop with a short growing season that is farmed worldwide. It had its origins in Peru. Beginning in the early 19th century, the British brought tomatoes to the Indo-Pak area. Global tomato production leaders are China, India, the United States, Turkey, Egypt, Iran, Italy, Brazil, and Spain, in that order. Annual fresh tomato production is estimated to be 159 million tons worldwide. To make tomatoes the most processed produce, around ¼ of their total production is consumed in the industry. 530 thousand tons of tomatoes are produced in Pakistan each year. The crop is grown in all Pakistani provinces on 50,000 hectares, with a meager national yield of 10.1 tons/ha (Khokhar, 2013). The reason for Pakistan's low tomato yield is a disease outbreak. The nematodes, viruses, bacteria, and fungus that cause these disorders. One of the most severe bacterial disease infections infecting plant vascular bundles is bacterial wilt caused by *Ralstonia solanacearum* (Derib et al., 2013). Every region in the globe that grows tomatoes has a high prevalence of the disease. The disease limits the amount of tomatoes that may be successfully cultivated by causing a yield loss of 15–100%. Besides other significant crops like bananas, peanuts, and ginger, the disease also impacts crops including eggplant, tomato, potato, and pepper.

The disease often affects tomato plants in China during the flowering and early fruit phases, and there is currently no effective treatment for it (Choudhary et al., 2018). Bacterial wilt in tomatoes initially manifests in a few younger leaves during the warmest portion of the day. Early leaves develop symptoms, and the plant as a whole begins to wilt that day. The pith and cortex turn brown, and the stem takes on a deeper shade of brown. There is a discoloration that is brownish, first in the vascular system (Kumar et al., 2017).

R. solanacearum is able to persist in non-host soil for extended periods of time, and it is even resistant to crop rotation. Yield losses due to the disease might reach 90.62% (Dharmatti et al., 2009). Born in the soil, *R. solanacearum* exhibits a great degree of variation in its composite taxonomic features across all life stages, encompassing genetic, physical, physiological, and host range aspects (Genin and Boucher, 2004). Conversely, rhizobacteria that promote plant development interact with other microbes in the rhizosphere and with the roots of the plants they colonies, so providing a favorable impact. Certain PGPR are known to be antagonists of root pathogens, which may stop the onset of plant diseases (Tariq et al., 2017). The use of synthetic pesticides has significant risks, including the development of insect resistance, environmental pollution, deadly consequences, and residual contamination of organisms that are not intended targets (Tapondjou *et al*., 2002). To prevent the invasion of field crops, it is therefore imperative to develop safe substitutes for traditional pesticides. Studying local disease control approaches is becoming more and more important in order to update and revitalize their use (Shaaya *et al*., 1997).

The pyrolysis of biomass yields biochar, an agent for sequestering carbon (Hunt et al., 2010). It has a strong chance of reducing pollutants in the environment and enhancing soil fertility (Lehmann and Joseph, 2009). Adding biochar to the soil increases crop output, microbial growth, and agricultural productivity. higher P, K, C, and N concentrations in soil treated with biochar and higher potassium concentrations in plant tissues (Biederman and Harpole, 2012). By lowering the bioavailability of heavy metals, biochar mitigates the phytotoxic effects of soluble heavy metals (Beesley et al., 2010). In addition to these positive aspects, biochar prevents plant diseases by strengthening plant tolerance to external stimuli (Elad *et al*., 2010). Biochar mitigated the severity of disease in pepper and tomato plants infected with necrotrophic (*Botrytis cinerea*) and biotrophic (*Oidiopsis sicula*) fungus, respectively. In order to encourage systemic resistance in plants and mineral absorption, biochar amendment increases Mycorrhizae and Rhizobacteria colonization in the rhizosphere (Ennis et al., 2012). Along with its other advantages, biochar can therefore be employed to help plants develop tolerance against biotic and abiotic stressors.

MATERIALS AND METHODS

Collection of Diseased Samples

The tomato plant leaves containing typical symptoms of bacterial wilting of tomato were collected from the three different district chinniot, Faisalabad and Okara. The diseased leaves and branches specimens were brought to Disease Diagnostic Laboratory, Department of Plant Pathology, UAF for isolation, purification and identification.

Isolation, Purification and Identification

Simple isolation technique (Ricker and Ricker, 1936) was followed to obtain *Ralstonia solanacearum* Culture. Autoclaved NA medium was poured in petri plates under Flame hood chamber to avoid any sort of contamination. 20 mL NA medium was poured in each 9 cm size petri plate. The petri plates containing NA media were let to solidify for few minutes and then surface sterilized small pieces prepared as mentioned above were placed on NA medium and incubated at 25^{o}C. The petri plates were regularly observed to record the growth of *Ralstonia solanacearum.* For the Purification, pick the one colony of bacterial growth with yellow tips and streak on new nutrient agar plate. Petri plates were placed in incubator at 25^{o}C for 24 hours.

Pathogenicity Test

Tomato seedlings were grown in pots filled with sterilized soil. After that prepare the CPG liquid media. The process involves filling test tubes with 5ml CPG liquid media, flame looping them, and then selecting bacteria colonies from a pure culture plate. The bacteria are mixed in the liquid media, and the process repeats. The tubes are then shaken for 6-8 hours to prepare an inoculum, which is then inoculated into the test plants' root zone and observe the symptoms after 6-7 days.

Biochar Characterization

Ash contents in biochar

Carbon content of biochar was determined by loss on ignition technique (LOI) as described by Nelson and Sommers (1982). The application of NH4-acetate forced re-location technique was modified to determine the CEC of biochar (Gaskin et al., 2008).

Study Design and Treatments

The three replications of each treatment were used for the completely randomized design (CRD).

Preparation of Soil for Experiment

Air dried soil was collected from the field and sieved with the help of 2mm size mesh. The objectives of sieving of soil were to make soil suitable for the homogeneous mixing of biochar in it and to remove any sort of plant debris, roots and weeds. The sieved soil was collected on a polythene sheet for further use in filling of pots

Amendment of soil with Biochar

150 kg of sieved soil was weighed and 2.5 kg biochar was amended in it. Thus the ratio of biochar in soil was set 1.5%w/w. Biochar and soil were mixed thoroughly for 3 times so that soil may be equally amended with biochar

Filling of Soil in Pots

Untreated sieved soil and biochar amended soil was weighed and 2 kg of soil was filled in each pot according to plan of experiment.

Tomato Nursery

Healthy, about 3 week old tomato nursery of variety "Rooma" was taken from Ayub Agriculture Research Institute (AARI), Faisalabad.

Isolation of Rhizosphere Bacteria

Islolation of rhizosphere bacteria from soil samples. Samples are collected from different areas District Chiniot, Faisalabad and Okara. Isolation of bacteria done by serial dilution method.

Prepare the Inoculum of Rhizosphere Bacteria

Prepare the CPG liquid media. Then Prepare inoculum of rhizosphere bacteria in CPG liquid media. 10 test tubes are filled with 5ml CPG liquid media. After that flame loop then allow it to cool then pick the rhizosphere bacteria colony from pure culture plate which already prepared and dip in the CPG liquid media test tube allow to mix the bacteria colony in liquid media. After that again flame loop then allow to cool and pick another bacteria colony and repeat this procedure. 10 test tubes of rhizosphere bacteria are prepared. On mechanical shaker these test tubes are shaking 6-8 hourse so that bacteria inoculum are prepared.

Inoculation of Rhizosphere Bacteria

After 3 weeks of Transplanting of nursery inoculated with Rhizosphere bacteria. With the help of blue tips About 5ml inoculum was inoculated in root zone of plant.

Prepare the Inoculum and Inoculation

For the preparation of *R. solanacerum* inoculum prepare in specific media which is TTC (triphenyl tetrazolium chloride). TTC helps distinguish *R. solanacearum* from contaminants.

After 4 weeks of Transplanting of nursery inoculated with *R. solanacearum.* About 5ml inoculum was inoculated in root zone of plant with the help of pipette and control pots will remained untreated.

Data recording

Data was collected after 2nd day of inoculation according to disease rating scale. Percent disease index was calculated by using formula:

$$\text{Disease Severity Index} = \frac{\text{Sum of all disease rating}}{\text{Total no. of rating x maximum disease grade}} \times 100$$

Statistical Analysis

Data was recorded and analyzed statistically using statistical analysis software Statistix 10. All the possible interactions and effects were analyzed using ANOVA test and means were compared by LSD test at 5% significance level (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

The study was carried out to examine the pathogenicity of many *R. solanacearum* isolates in the first experiment. Gather disease samples from several locations in Chinniot, Okara, and Faisalabad. *R. solanacearum* isolation, purification, and identification on a particular medium (TTC). Following that, the pathogenicity test was carried out and the findings were recorded using a disease evaluating scale. Three Faisalabad F1, F2, F3 isolates, three Okra O1, O2, O3 isolates, and four Chinniot C1, C2, C3, C4 isolates. The isolate with the highest percentage of disease cause, C1 48%, was obtained from Chinniot. The effects of varying biochar concentrations on plant height and fruit were examined in the second experiment. Three different concentrations of biochar—1%, 2%, and 3%—were utilized in this experiment to see which was best for plant height and fruit production. In comparison to 2% and 3% concentrations, plant height

and fruit yield are maximized when 1% biochar is mixed into the soil. Effect of rhizosphere bacteria-enriched biochar amendment on plant height, fruit, and disease in the third experiment.

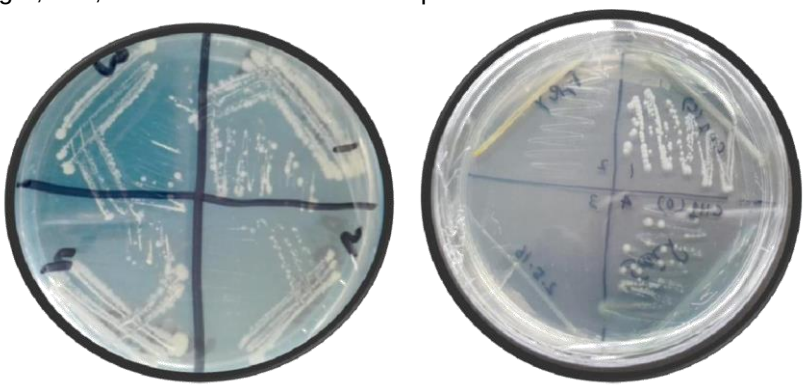

Figure 1. Purification of *R. solanacearum* on nutrient agar media plates.

To Assess the Pathogenicity of Several Isolates

The study analyzed the virulence of different *R. solanacearum* isolates in various treatments, revealing statistically significant results. The results showed that F1 treatment caused the highest wilting percentage (48%), followed by F2 (35%), F3 (15%), O1 (42%), O2 (18%), and O3 (25%). In comparison to other isolates, the chiniot C1 isolate was the most virulent and caused the greatest amount of wilting on tomato plants. Control plants had the lowest proportion of withering, which was 7%.

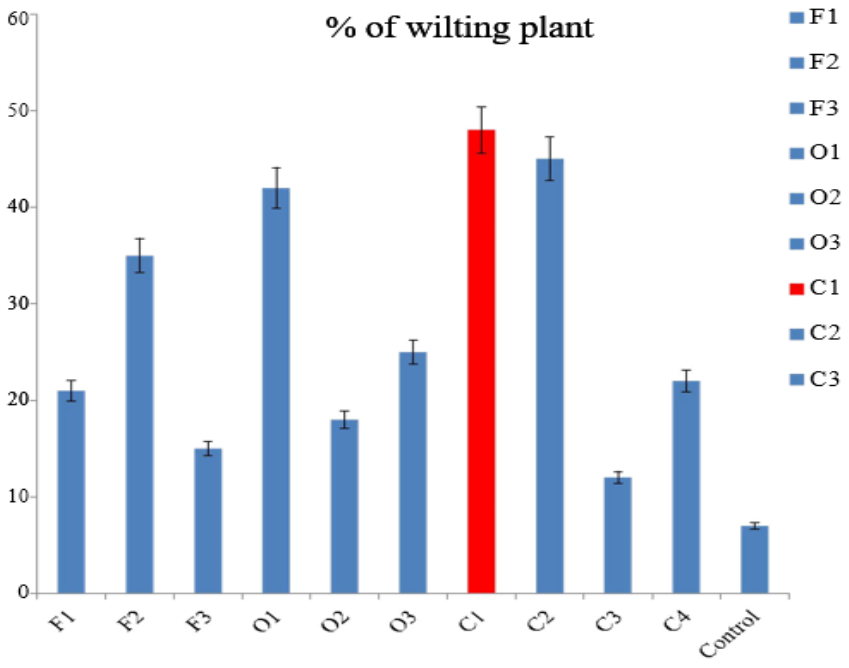

Figure 2. The pathogenicity of several *R. solanacearum* isolates.

To determine the impact of varying biochar concentrations on the quantity of fruits

The study found that different concentrations of biochar significantly affected the number of fruits in tomato plants. The best results were observed with the 1% concentration while the 2% and 3% concentration had no effect. As for LSD mean comparison, 1% biochar concentration showed the highest number of fruits in tomato plants whereas 2% and 3% had no significant effect. These findings imply that biochar is a positive admixture in tomato plants.

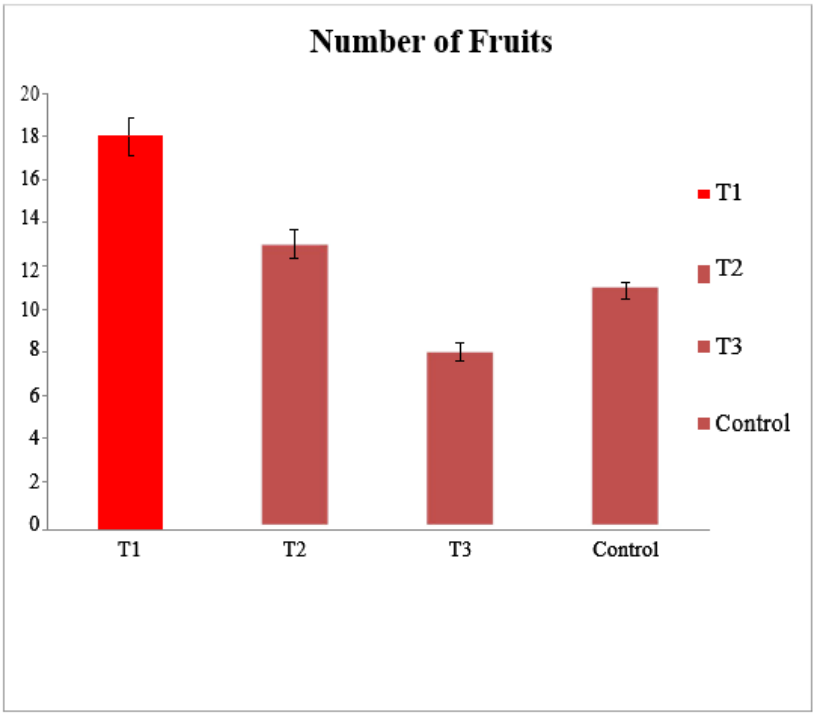

Figure 3. The Impact of Various Biochar Concentrations on the Number of Fruits.

To Determine how Different Biochar Concentrations Affect Plant Height

The research showed that various biochar concentrations had a significant impact on tomato plant heights. A control test showed us the highest efficiency, with 62 cm of height increasing in T1 under 1% of concentration. The T2 samples at 2% indicated a curve height of 52 cm and the T3 samples at 3% showed a flange height of 38 cm. The control plants were 45 cm in height. The outcome is that biochar at 1% was the highest in terms of plant height improvement.

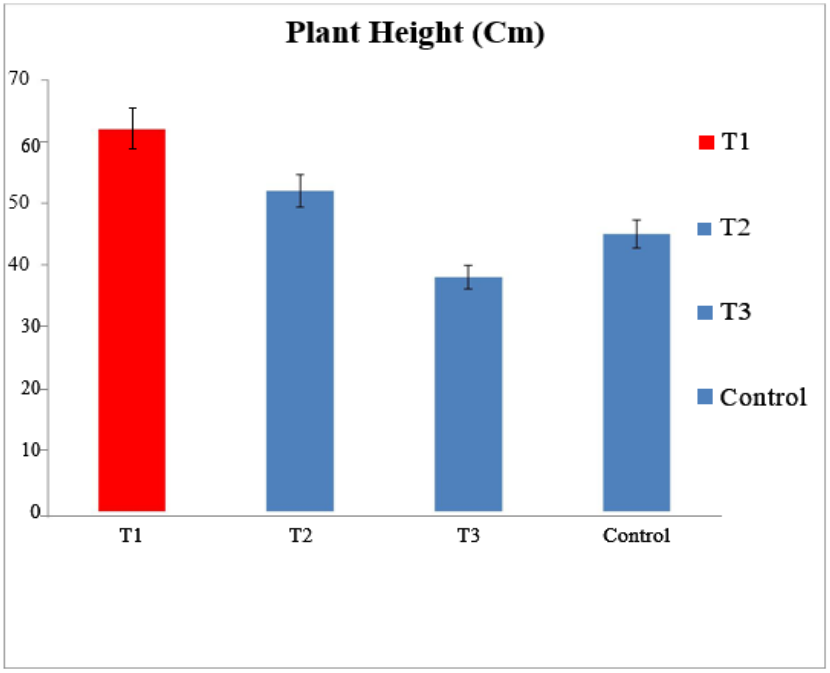

Figure 4. Plant Height Affected by Different Biochar Concentrations.

To determine the impact of a QQ bacterium-infused charcoal amendment on plant height

The experiment studied the influence of biochar amendment with QQ on plant growth 9 days after inoculation in various treatments. The results for the first treatment showed that biochar application at 1% led to a plant height of 72 cm. For

the second treatment, plant height was noted as 70 cm after application of quorum quenching bacteria. When tomato inoculum with *R. solanacearum* was applied in the third treatment, tomato plant growth was not observed as a result of bacterial wilt. And therefore 82 cm of plant height was achieved with biochar and quorum quenching bacteria applied in the fourth treatment and 45 cm of plant height was when biochar and R. solanacearum was used in the fifth treatment. These results suggest that the highest plant height occurred in cases where biochar with quorum quenching bacteria was used.

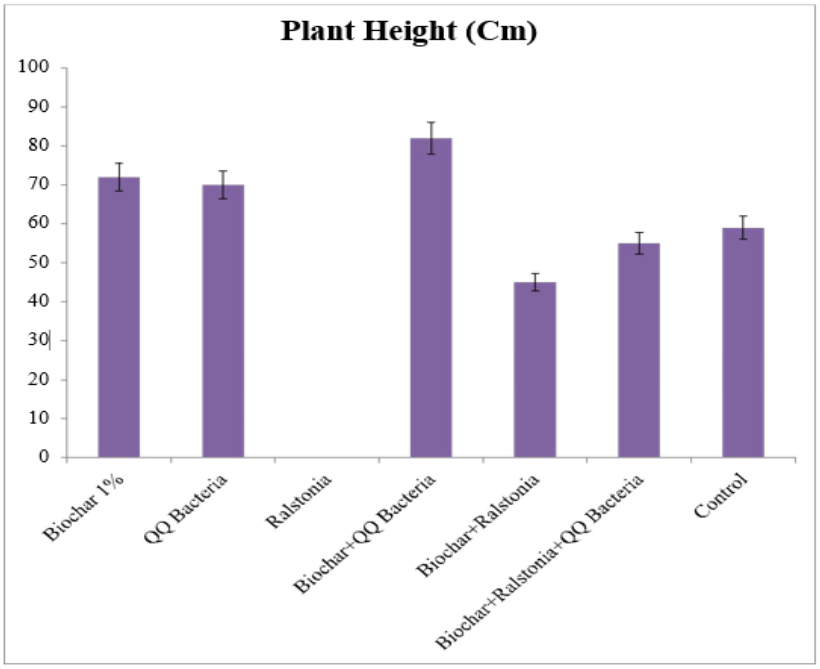

Figure 5. The impact of a QQ bacterium-infused charcoal amendment on plant height.

To determine the number of fruits affected by the charcoal addition containing QQ bacteria

This study analyzed the influence of biochar addition with QQ bacteria on the number of fruits after inoculation for 9 days in different treatments. There was a statistically significant contribution to the results. The maximum number of fruits was recorded when quorum quenching bacteria were applied along with biochar, while biochar only led to an increase in bacterial wilt. The biochar and the quorum-sensing inhibiting bacteria were utilized in various treatments, with the highest number of fruits being noted in the biochar with quorum-quenching bacteria. The results imply that the biochar with quorum quenching bacteria is the most successful in improving fruit yield increase whereas only *R. solanacearum* inoculum causes bacterial wilt and fruit loss.

To determine how plant disease is affected by the addition of QQ bacteria to biochar

The influence of QQ bacterium infected biochar on plant disease were studied after 9 days of inoculation under different treatments. To give you an idea, the results showed significant statistical significance. In the first treatment with biochar 1%, plant wilting was not observed, whereas in the second treatment with bacteria *R. solanacearum* quorum quenching and in the third treatment wilting was noticed. The biochar 1% with quorum quenching bacteria in the fourth treatment and *R. solanacearum* inoculum in the fifth treatment all showed wilting. The maximum percentage of wilting was observed when only the *R. solanacearum* inoculum was applied to tomato plants, resulting in bacterial wilt and all plants dying.

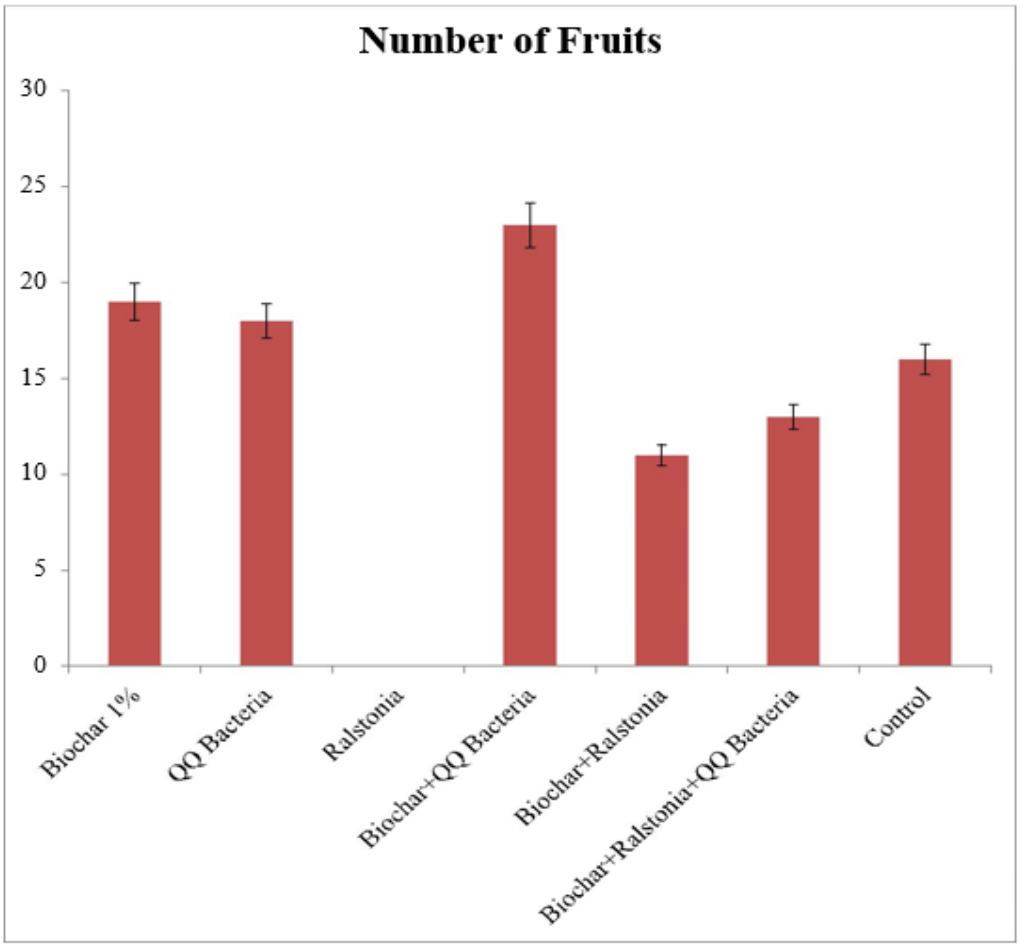

Figure 6. To determine the impact of adding QQ bacteria to charcoal on plant disease.

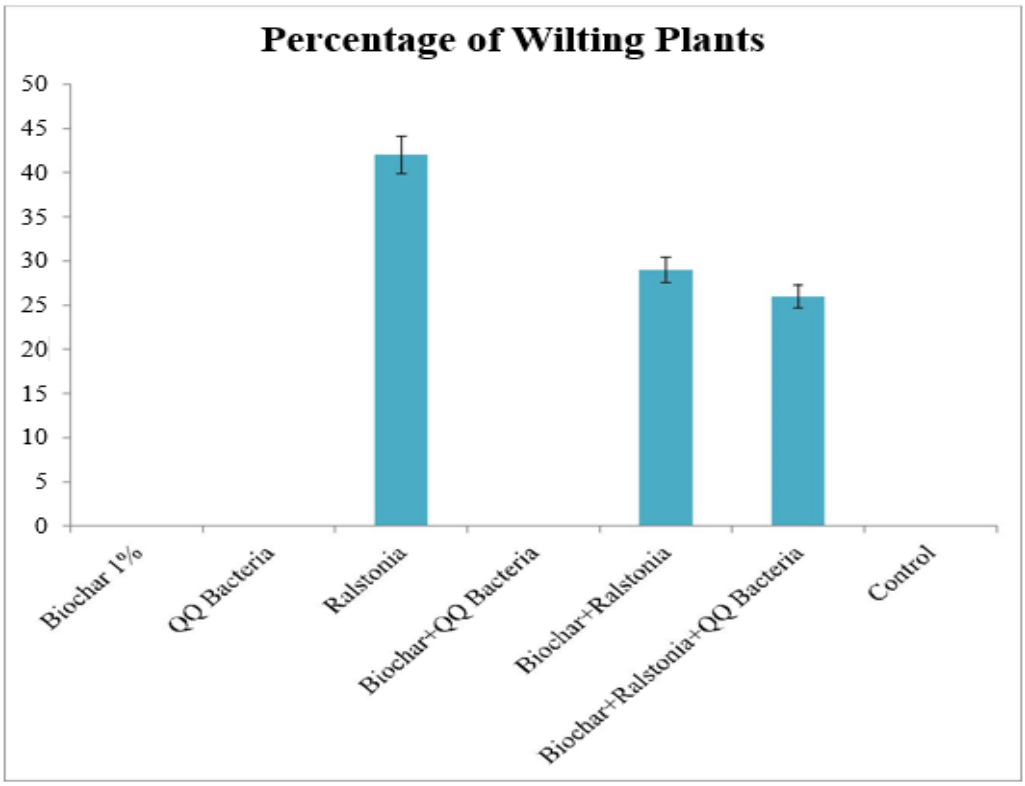

Figure 7. The impact of adding QQ bacteria to charcoal on plant disease.

The optimal concentration of biochar for plant height and fruit number is 1%, as demonstrated by previous experiments No. 1 and 2. C1 maximal withering cause isolate. For this experiment, take C1 isolates and 1% biochar. The highest plant height and fruit count compared to other combinations were achieved when biochar and rhizosphere bacteria were combined. The plants wilt and die when *R. solanacearum* is the only herb used. There is no wilting since all the plants are healthy when biochar, rhizosphere bacteria, and their combination are used. All plants are dead and not a single plant is alive while using the single *R. solanacearum* culture. Consequently, it was noted that the most pathogenic *R. sonalacearum* strain was discovered in the district of Chiniot. In terms of plant height, number of fruits, and disease control, 1% biochar content yields the best results. Rhizosphere quorum quenching bacteria can be more active against *R. solanecearum* by using biochar.

The study findings were in-line with the study on biochar application to soil infected by R. solanacearum found that biochar significantly reduced the disease severity of bacterial wilt, increased soil total organic carbon, total nitrogen, and C:N ratio and increased soil microbial population were observed which led to suppression of diseases and better plant health performance (Gao., et al., 2019). In another study, it was found that biochar from wheat straw eliminated the tomato bacterial wilt through changing the rhizosphere organic acid and amino acids (Tian et al. 2021). As a result, the role of biochar in preventing diseases was demonstrated. More importantly biochar had the capacity to suppress tomato early blight which proved its beneficial in the fight against the disease (Jin et al., 2022.

CONCLUSION

The result of the study indicates the application of biochar as a biological control of stem bacterial wilt caused by a phytopathogenic *R. solanacearum.* The experiment data demonstrated that 1% biochar application led to increases in the plant height, fruit production and disease control, especially with the presence of rhizosphere microorganisms. These data are shown in the form of the biochar effectiveness by the rhizobacteria in their combat with the pathogen and so, thereafter it brings about the improvement or change of the disease control methods aiming for sustainable plant health. The research paves the way of understanding biochar that is potential control method for tackling soil-borne diseases in tomato production and its further investigation and establishment may be possible in future studies and practices.

ACKNOWLEDGEMENTS

All authors contributed equally to this research.

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