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Biochar led induced resistance to control *Fusarium oxysporum* f.sp. *lycopersici*

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Abstract
Bio-char, a highly carbonaceous organic byproduct of pyrolyzed organic wastes which have shown a great significance in agriculture. It has been used in inducing resistance in plants by stimulating the activity of PR-proteins and phenolic compounds, stimulates the physical characteristics and chemical compositions of soils helping to prevent the numerous soil borne pathogen. It has been used against *Fusarium oxysporum* f.sp. *lycopersici* (FOL). Biochar is added in soil as an amendment with different concentrations (ranging 0.5%-5% w/w) to induce resistance into tomato plants. It has been seen that Biochar induces the resistance with increase in the level of total phenols by 64.44% and soluble proteins upto 91.51% over control at 3% conc. Biochar has increased the phenols content upto 2.22 mgCE/gFW and proteins content upto 27.98 mgBSA/gFW at 3% conc. Under *in vitro* analysis biochar inhibits the mycelium percentage by 15.62% at 3% concentration.

Keywords: Bio-char, pyrolysis, vesicular-arbuscular mycorrhiza, *Trichoderma harzianum*, induced resistance, *Fusarium oxysporum* f.sp. *lycopersici* (FOL)

Introduction

Tomato (*Solanum lycopersicon*) is the edible, fruit/berry which is often red in color. The plant belongs to family Solanaceae (Bergougnoux, 2014) [1]. Tomato is the day-neutral plant which means the flowering is not been hampered by altered sunlight conditions (Osnato *et al*., 2022) [11]. Tomato is being consumed in so many ways including sauce, as an ingredient in many dishes, raw, salads and for some other purposes as well (Wu *et al*., 2022) [20]. Tomato is grown under various atmospheric conditions *viz*., Tropical and Sub-tropical in different parts of the world. Tomato is known as a culinary fruit because it has very low sugar amount than any other culinary fruits. India ranks second in the production of tomato worldwide after China with an annual production of 19.4 million tonnes (Gulati *et al*., 2022) [6]. Tomato has been widely grown in various parts of the world as it is the primary food as it is been used in various edible products. These include China, India and United States as the three leading producers in the world. During 2018, the worldwide production of tomato was total of 170.8 million tonnes. China contributes total 31% of production throughout the world followed by India and United States. In European Nations tomato crop accounted for 23% of world production. The global export values for tomato crop worth 88 billion USD during 2018 (Heuvelink, 2018) [3].

In India tomato crop is sown over an area of 260.4 thousand hectare. In different states under different conditions (open field and greenhouse). The leading state (in area) in India is Chhattisgarh with an area of 38.8 thousand ha. Followed by Madhya Pradesh with 37.0 thousand ha., Haryana with 14 thousand ha., Uttar Pradesh with 12.8 thousand Ha., Tamil Nadu with 12.7 thousand ha., Himachal Pradesh with 1.3 thousand ha., last year according to report of Horticulture Department of India (2019). The leading state with maximum production is Andhra Pradesh (3352.74 tonnes) followed by Madhya Pradesh (2192.97 tonnes), Karnataka (1996.47 tonnes) sharing 18.07%, 11.82% and 10.76% share in total agriculture production of tomato throughout India. The Punjab state ranks 17th position with respect to production with 185.87 tonnes of production with share of 1% of total tomato production in country (Gulati *et al*., 2022) [6]. Tomato is about 95% water, 4% carbohydrates and <1% of proteins. According to USDA nutritional report about tomato it has been shown that one tomato (62-100 g) contains 58.6 g

water, 2.6 g sugars, 1.5 g fibers, 0.5 g protein, 11.5 g calories, 8.8 g carbohydrates, 1.0 g fat respectively. Also it contains various vitamins *viz.*, Vitamin A 516 IU, Vitamin C 7.9 mg, Vitamin K 4.9 mg, Folate 9.3 mg, Choline 4.2 mg, and Niacin 0.4 mg. It contains various minerals essential for human health in various amounts *viz.*, Calcium 6.2 mg, Iron .02 mg, Magnesium 6.8 mg, Phosphorus 14.9 mg, Potassium 147 mg, Sodium 3.1 mg, Zinc 0.1 mg and Fluoride 1.4 mg. Similarly one tomato also contains various fatty acids *viz.*, Total Omega-3 fatty acids 1.9 mg and Total Omega-6 fatty acids 49.6 mg (Waheed *et al*., 2020) [17].

Tomatoes can be sown on a wide range of soils from sandy to heavy clay with pH ranging from 6.0-7.0 in well drained soils. The best suitable temperature for tomato cultivation is 21-24 °C. During their cultivation practices under field and greenhouse conditions this crop has been attacked by various diseases and pests as well which cause huge economic losses to growers. These involves various bacterial, fungal and viral diseases and disorders as well *viz.*, bacterial wilt, Fusarium wilt, Alternaria leaf blight (early blight), Septoria leaf spot, late blight, leaf mold, tomato bud necrosis virus (TBNV), tomato spotted wilt virus (TSWV) etc. The fungal wilt or Fusarium wilt proves to be the most devastating of all these as it causes huge economic losses every year. The pathogen, FOL causes severe damage to the oldest leaves and causes permanent wilting of vascular tissues. The pathogen is soil borne by nature and favours hot and humid conditions for its wide spread. The symptoms apparently appear near the flowering or fruiting stages. Since, it is a vascular disease so its early detection is not possible by physiological means which lead to delay in its treatment. It causes near about 10-80% of yield losses every year (Singh *et al*., 2017) [15]. According to a research it has been seen that about 45.6% of tomato production has been reduced by Fusarium wilt in India (Ramyabharathi *et al*., 2012) [12].

The agent used for the management of disease in this research is a highly carbon based heterogenous product named as biochar. It is being used as a soil amendment and is produced by pyrolysis procedure to sustain its highly carbonaceous nature. Various agro based wastes can be used to prepare this product and can be used in soil to induce resistance in plants (Rasool *et al*., 2021) [13-14]. The various concentrations of biochar are used under field conditions to check its effects against pathogen at different intervals. Along with biochar various other agents has been also used *viz.*, vesicular-arbuscular mycorrhiza (VAM) and *Trichoderma harziarum* in order to compare its results with biochar (Elad *et al*., 2011; Jogaiah *et al*., 2018; Wang *et al*., 2022) [4, 8, 18]. Biochar is being considered as a hope for the future amendments in order to safeguard soil and crops.

Materials and Methods

Preparation and Application of Biochar (Wang *et al*., 2020) [19]

Table 1: Characteristics of biochar used in the management of FOL

Properties	Remarks
Feedstock material	Teak wood
Category of Biochar	Hard wood
TOC	72.5%
Biochar liming potential	2.5%
pH	8.52
EC	1.806 ds/cm

Application of Biochar

Biochar was applied with different concentrations *viz.*, 2%, 2.5%, 3%, 3.5%, 4%, 4.5% along with other biological agents to compare its results with them separately i.e. VAM, *T. harziarum* and APSA 80.

Collection of Samples for Isolation, Purification and Identification of Test Pathogen

The pathogen FOL was isolated from diseased samples collected from Lovely Professional University. The samples were collected from different locations of infected fields of tomato crop with FOL. The pathogen was purified as shown in figure and were identified under microscopic study for the presence of chlamydospores, macrospores and microspores as shown. The pathogen was than incubated at 25±2 °C for further studies. For inoculation of pathogen under field conditions to evaluate the effect of various treatments on disease development and disease resistance the spore suspension were prepared using 7 days old culture. The spore suspension was prepared at 2×10^6 spores/ml. The pathogen was inoculated in trials after 30 days of transplanting tomato plants in pots.

Total Soluble Proteins Evaluation

Proteins the macromolecules by nature and are formed by various types of amino acids linked with each other by polypeptide bonds. The total of 20 amino acids is linked with each other in various groups to form different types of proteins depending upon the stage of plants and situations under which they are being formed. These proteins plays vital role in developing disease resistance against various pathogen in plants. These are proved to be very important for plants or any living organism to sustain life against any life endangering pathogens. The estimation of total soluble proteins was done according to the protocol mentioned by Lowry *et al.* (1951) [10]. In order to begin with total soluble protein estimation it required various reagents to be used in procedure. These reagents are required to get required results under suitable conditions in order to get good results. The various reagents used are the mixture of one or two chemicals together in proper concentrations. These reagents are:

Reagent A – 20% Sodium carbonate in 0.1N Sodium hydroxide solution

Reagent B – 0.5% Copper sulphate in 1% Sodium potassium tartrate

Reagent C – Alkaline copper (50 ml of solution A is mixed with 1 ml of Reagent B just before use)

Reagent D – Folin Ciocalteau Reagent

For the preparation of standard curve the standard bovine solution was prepared by mixing 50 mg Bovine serum in 50 ml of water and for that, to prepare working solution the 10 ml of standard solution was diluted in 50 ml of distilled water to prepare the working solution of 200 mg/ml protein solution. The samples were collected as same for the phenol estimation i.e. twice one was after 30 days of transplanting and another was after 60 days of transplanting (after 30 days of pathogen inoculation). The samples were collected for the different treatments and washed with distilled eater to remove soil. The leaves were dried on tissue paper and weighed about 0.5 g each for each treatment. The samples were than crushed using pestle mortar using protein extraction buffer. The crushed samples were then centrifuge at 10000 rpm for 20 minutes at 4 °C. For testing the samples different concentrations were used from each sample supernatant after centrifuge *viz.*, 25 ml, 50 ml, 75 ml, 100 ml. The volume made upto 1 ml in each test tube with distilled water. After that Reagent C were added in all the

samples along with FCR 0.5 ml in each test tube. The samples were then placed in dark area for 30 minutes to develop the blue color. After that the samples were taken under visible spectrophotometer to take the absorbance readings at 750nm. The final readings were taken as mgBSA/gFW.

Total Phenol Evaluation

Phenols or the phenolic content are the chemical compounds of organic chemistry. They consist of hydroxyl group attached to an aromatic hydrocarbon group. There are various classes of phenols named as polyphenols. Plants synthesize phenols of different types throughout their life cycle depending upon the stage o plants and situations for which they are being produced. N some conditions they are produced to stimulates the plant growth and in some instances they are produced under stress conditions *viz*., biotic and abiotic stresses in order to protect the plants under adverse conditions. The evaluation of phenols was done according to Bray and Thorpe (1954) [2] principle of phenol estimation. This estimation was based in the principle that under alkaline medium the total phenolic content in samples tends to react with phosphomolybdic acid in FCR. The procedure starts with the preparation of various reagents or chemicals required for the phenol estimation. These are 80% ethanol, Folin-Ciocalteau reagent, 20% sodium carbonate Na_2CO_3 (alkaline medium). Before starting the procedure standard catechol solution was prepared to prepare the standard curve for the estimation of phenols. The standard solution was prepared by mixing 100 g catechol in 100 ml water. After that working solution was prepared by diluting this solution 10 times with double distilled water. The estimation was twice during the trial. First observation was done after 30 days of transplanting just before the inoculation of pathogen and another after 60 days of transplanting i.e. after 30 days of inoculation of pathogen. The samples were brought to lab from different treatments for evaluation. The samples were weighed 50 g each and crush with pestle mortar using extracting buffer 80% ethanol 10 times the sample volume. The samples were centrifuged at 10000 rpm for 20 minutes and supernatant were extracted. The remaining pellets was again centrifuge with 80% ethanol for 4-5 times to extract all the phenols in supernatant and pellets were discarded in the end. All the extracted supernatant were mixed together for different sample and dried to remove the excess of methanol to get dried samples for extraction of phenols. The dried samples were weighed 0.5 g and mixed with 5 ml of double distilled water in a vial. Then different concentrations were pipette out for different treatments *viz*., 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1 ml. These concentrations were added in a test tubes and make up the volume upto 3 ml with double distilled water. After that 0.5 Folin reagent were added in each tube followed by addition of 2 ml of alkaline medium *viz*., 20% $Na2CO3$. The samples were mixed thoroughly for few minutes and observed under visible spectrophotometer for absorbance at 660nm. The final readings were noted as mgCE/gFW (For the preparation of standard curve various concentration were pipette out from working solution of catechol solution *viz*., 0.5 mg/ml,1mg/ml,1.5 mg/ml up to 5 mg/ml).

Statistical Analysis

The statistical analysis of the data was performed using analysis of variance followed by the least significant difference test according to Henson, R.N. (2015) [7]. It was done to determine the significant relationship between the various treatments and the control.

Results

The pathogen was identified on various bases after getting its pure culture in a Petri plate. The microscopic studies and morphology of mycelium growth indicates the presence of FOL in plates. The microscopic studies proved the presence of chlamydospores (branched and at intercalary positions), macrospores (curved and septate) and microspores (round and small). The analysis proved the identification parameters as given by Snyder and Hansen (1945) [16] in their monograph on *Fusarium* fungi.

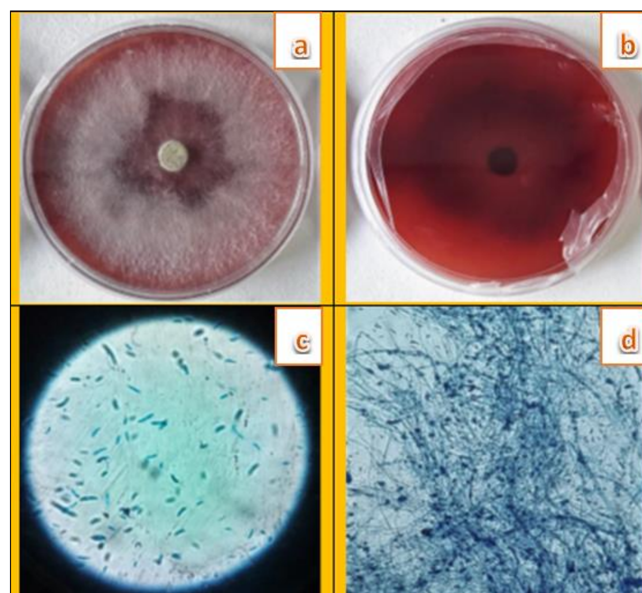


Fig 1 (a-d): Identification of FOL.

Pink color background due to mycelium mat ensuring the presence of FOL (a-b) and presence of numerous small curved and septate macrospores and chlamydospores confers FOL microscopically (c-d).

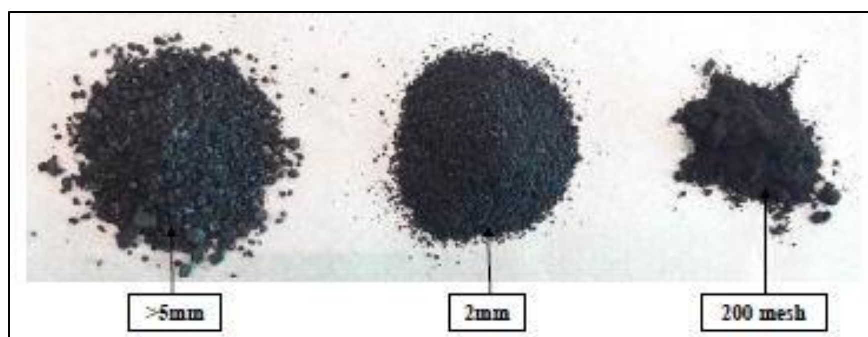


Fig 2: Different mesh size particles of biochar.

For actual evaluation of induced resistance it has to be necessary to evaluate these two parameters for basic. The evaluation was done twice as discuss earlier. It has been found that the content of total soluble proteins was increased significantly with the use of various concentrations of Biochar and other agents as well. The T_3 showed the highest rate of increase in the content of soluble proteins as it was at 13.68 mgBSA/gFW (after 30 DAT) but as it increased after the inoculation of pathogen in the soil as after 60 DAT and it was at 27.98 mgBSA/gFW. It showed the highest rate of increase than normal or control as of 91.51% as compare to all other treatments.

As compare to others the least increase was showed by T_5 as it was at 14.19 mgBSA/gFW after 30 DAT but after 60 DAT no increase has been seen as it was at 15.70 mgBSA/gFW. This treatment showed the least increase in its soluble protein content as of 7.46% only than control treatment. Other treatments *viz*., T_2, T_5 and T_6 also showed some rate of change in their total phenol content as of 75.77%, 81.93% and 63.99% respectively.

As similar in case of total phenol contents in plants. It has been found that T_3 showed the highest rate of increase in their total phenol content as of it was 1.09 mgCE/gFW at 30 DAT and after 60 DAT it was at 2.22 mgCE/gFW. This showed the rate of increase by 64.44% than normal or control treatment. The other treatments also showed the increase at some extent *viz*., T_1, T_2, T_4, T_5, T_6 and T_7 showed 40.74%, 51.85%, 57.03%, 53.33%, 42.96% and 51.85% respectively.

The least of increase in total phenol content was seen in T_9 as of it was at 1.09 mgCE/gFW after 30 DAT and at 1.45 mgCE/gFW after 60 DAT. This showed the least of increase by 7.40% only than control treatment. The rate of increase in other treatments was directly proportional to the type of treatments used for treating the plants. The induction of resistance was directly seen from all these parameters.

Along with these a correlation was setup with disease incidence and amount of total soluble proteins and phenols in order to understand the phenomenon of inducing resistance in plants. The various doses of treatments was plotted in graph to understand the rate of increase in these parameters.

Table 2: Level of total soluble proteins and total phenols after 30 and 60 days of transplanting

Serial No.	Treatments	Total soluble proteins (30 DAT) (mgBSA/gFW)	Total soluble proteins (60 DAT) (mgBSA/gFW)	%increase over control	Total phenols (30 DAT) (mgCE/gFW)	Total phenols (60 DAT) (mgCE/gFW)	%increase over control
1.	T_1 (2%)	14.19	24.04±0.96	64.54%	1.10	1.90±0.32	40.74%
2.	T_2 (2.5%)	13.80	25.68±0.68	75.77%	1.09	2.05±0.12	51.85%
3.	T_3 (3%)	13.68	27.98±1.84	91.51%	1.09	2.22±0.04	64.44%
4.	T_4 (3.5%)	13.51	27.10±1.45	85.48%	1.09	2.12±0.08	57.03%
5.	T_5 (4%)	14.07	26.58±1.38	81.93%	1.10	2.07±0.13	53.33%
6.	T_6 (4.5%)	13.24	23.96±1.04	63.99%	1.12	1.93±0.27	42.96%
7.	T_7 (VAM)	13.50	25.83±0.83	76.79%	1.09	2.05±0.25	51.85%
8.	T_8 (*T. harziarum*)	14.07	19.99±2.23	36.82%	1.10	2.01±0.29	48.88%
9.	T_9 (APSA)	14.19	15.70±8.24	7.46%	1.09	1.45±0.85	7.40%
10.	T_{10} (cntrl)	12.76	14.61±9.55	-	1.09	1.35±0.95	-
SE(M)		0.14	0.99		0.003	0.09	

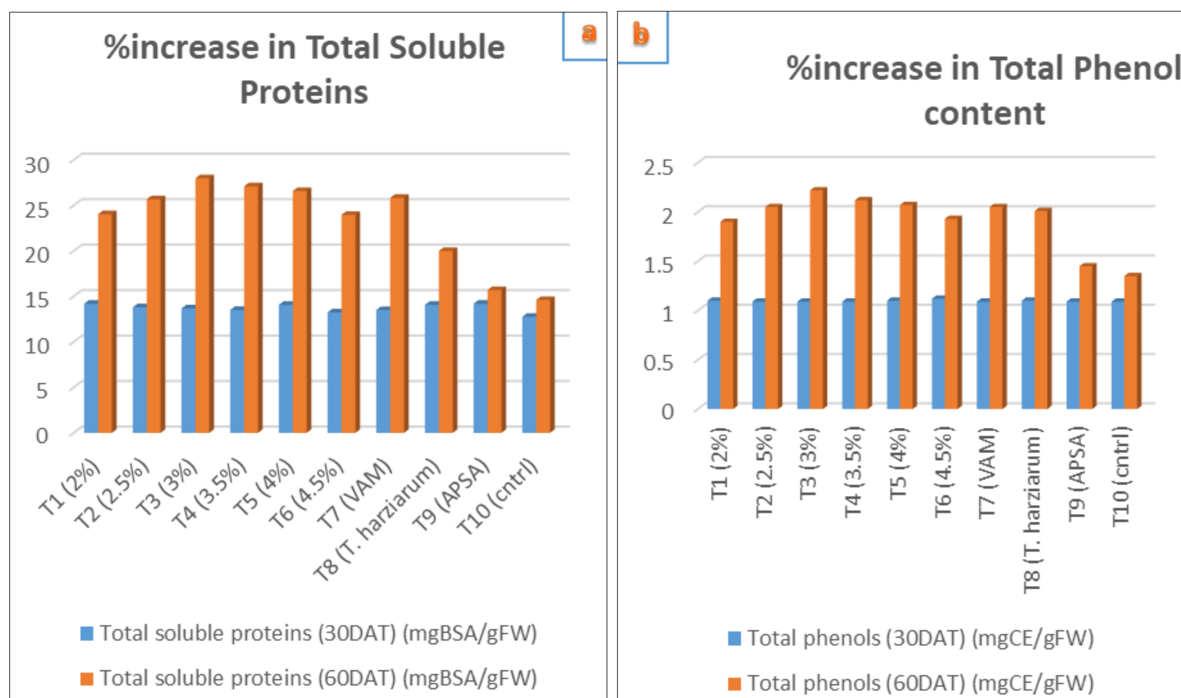


Fig 3 (a-b): Total soluble proteins and total phenols (mg/g) after 30 and 60 days of transplanting.

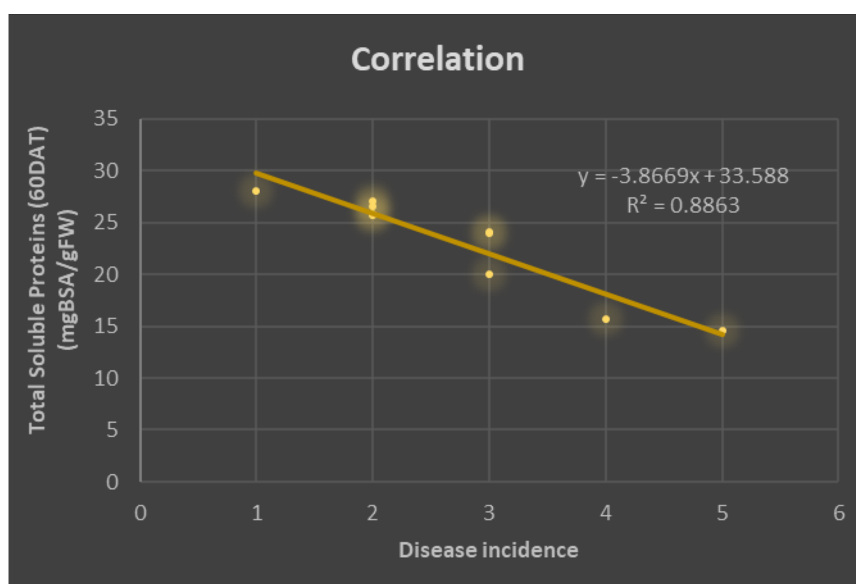
These graphs show the rate of change in total phenols and proteins content during the trials at different stages of plant growth. The different treatments showed the different rate of increase in their respective contents after a specific period of time as seen in these graphs.

The percentage increase over control has also been noticed with

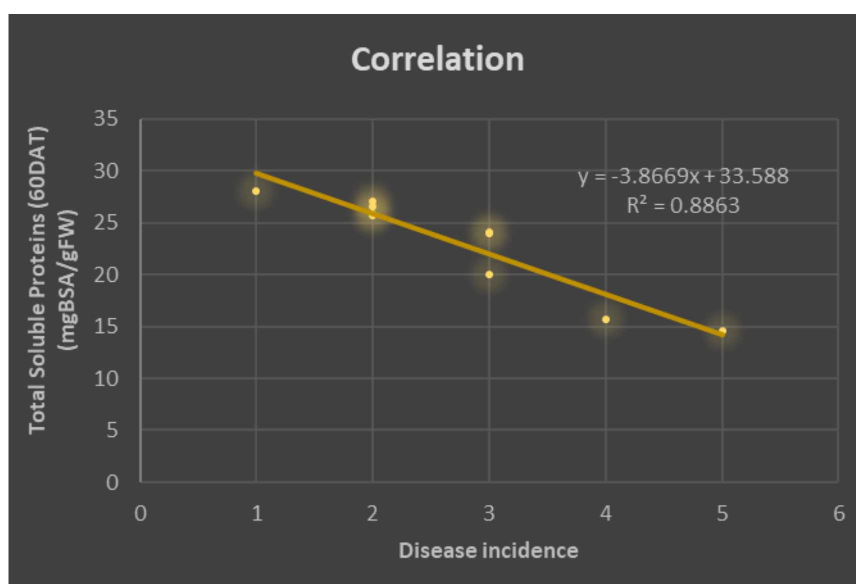
respect to other agents. It has been seen in the case of total soluble proteins two analysis were carried out at different interval of times to get data more significantly and accurately. The final analysis of total soluble proteins it was seemed that during their second analysis the rate of proteins was increased significantly in treatments with respect to biochar application and other agents as well.

Treatment T_3 with biochar concentration at 3% showed the highest degree of change in soluble proteins. With time after the inoculation of pathogen in pots the levels of proteins changes significantly. The levels increase in other treatments as well with respect to concentration of biochar used. The least of increase was seen in case of T_9 in which very little increase was seen in total soluble proteins as compared to other agents.

A similar thing seems to be happened with total phenol contents in plants. The total contents was seems to be changed or increased with respect to biochar concentrations in different treatments. The most of the increase was seem in T_3 plants which showed the most of change in total phenol contents. The changes were also noticed in other treatments as well *viz.*, T_7 and T_8 which were not inoculated with biochar, except with VAM and *T. harziarum.* The concentration of phenols was changed from their normal concentration upto certain extent with induction of resistance in plants.



A



B

Fig 4 (a-b): Correlation between total soluble proteins and total phenols (mg/g) and disease incidence after 60 days of transplanting.

To understand this phenomenon better a correlation was set up between total soluble proteins and disease incidence. The correlation gives the r2=0.8863 and regression line y=-3.8669x+33.588. The correlation gives us the idea that with increase in total soluble proteins the disease incidence become reduced and vice versa. With such similar case scenario another correlation was setup between total phenols and disease incidence with r2=0.8971 and regression line y=-0.2343x+2.5476. The rate of disease incidence changed with rate of change in total phenols.

The disease incidence became reduced with increase in total phenols and total soluble proteins with respect to induction of resistance in plants. Both the correlations proved to be very significant with respect to disease incidence and total proteins

and phenols induction in plants with respect to different concentrations of Biochar used in different treatments.

Many researchers have been working on plant growth, biochemical reactions after adding biochar in soil and in regard to the plant diseases. Graber *et al*. (2014) found that adding biochar to soil can improve plant vigour by increasing nutrient availability. Rasool, M. (2021) [13-14] also stated in the support of current research, he reported that Biochar contains a variety of organic acids, phenolics, and phytotoxic chemicals in minute amounts that might modify plant function, as well as influence PGPR and PGPF such as mycorrhizae and *Trichoderma* spp. Khalifa, W. (2015) [9] Incorporated biochar in soil and found that it increased phenoloxidase (PO) activity both in roots and leaves of infected and non-infected plants.

Conclusion

Biochar has been used to improve the soil physical and chemical properties along with the purpose to induce resistance in tomato plants against FOL. Biochar has been also seen to induce various level changes in potential of hydrogen (pH) and electrical conductivity (EC) levels of soil. The pH changed with respect to the concentrations of biochar used, and it can be seen that the pH will always be increased with more concentrations of biochar used. The Biochar seems to increase the production of soluble proteins and total phenols by significant percentages depending upon the treatments and their concentrations. These two parameters are the building blocks of any induced resistance studies. Any sort of fluctuations in these parameters determined that either the resistance has been induced or not. Biochar and other agents applied induced resistance in plants at significant levels. The percentage of total soluble proteins was seen to be increased up to 27.98% over control under concentration of 3% biochar. Same way total phenol content increased up to 57.03% in biochar treatments with 3% concentrations than control treatments. Various correlations theories also proved the same scenarios with regards to disease incidence. Biochar proved to be very effective in inducing resistance in tomato crop against Fusarium wilt under field conditions.

References

1. Bergougnoux V. The history of tomato: from domestication to biopharming. Biotechnology Advances. 2014;32(1):170-189. https://doi.org/10.1016/j.biotechadv.2013.11.003.
2. Bray HG, Thorpe W. Analysis of phenolic compounds of interest in metabolism. Methods of Biochemical Analysis. 1954;27-52. https://doi.org/10.1002/9780470110171.ch2.
3. Costa JM, Heuvelink EP. The global tomato industry. In: Heuvelink EP, editor. Tomatoes. CABI; c2018. p. 1-26.
4. Elad Y, Cytryn E, Harel YM, Lew B, Graber ER. The biochar effect: plant resistance to biotic stresses. Phytopathologia Mediterranea. 2011;50(3):335-349. https://www.jstor.org/stable/10.2307/26556455.
5. Graber ER, Frenkel O, Jaiswal AK, Elad Y. How may biochar influence severity of diseases caused by soilborne pathogens? Carbon Management. 2014;5:169-183. https://doi.org/10.1080/17583004.2014.913360.
6. Gulati A, Wardhan H, Sharma P. Tomato, Onion and Potato (TOP) value chains. In: Gulati A, Wardhan H, Sharma P, editors. Agricultural value chains in India. Springer; c2022. p. 33-97. https://doi.org/10.1007/978-981-33-4268-2.
7. Henson RN. Analysis of variance (ANOVA). In: Brain Mapping: an encyclopedic reference. Elsevier; c2015. p. 477-481.
8. Jogaiah S, Abdelrahman M, Tran LSP, Ito SI. Different mechanisms of *Trichoderma virens*-mediated resistance in tomato against *Fusarium* wilt involve the jasmonic and salicylic acid pathways. Molecular Plant Pathology. 2018;19(4):870-882. https://doi.org/10.1111/mpp.12571.
9. Khalifa W, Thabet M. Biochar amendment enhances tomato resistance to some soil borne diseases. Middle East Journal of Agriculture Research. 2015;4:1088-1100.
10. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry. 1951;193(1):265-275.
11. Osnato M, Cota I, Nebhnani P, Cereijo U, Pelaz S. Photoperiod control of plant growth: flowering time genes beyond flowering. Frontiers in Plant Science. 2022;12:805635. https://doi.org/10.3389/fpls.2021.805635.
12. Ramyabharathi SA, Meena B, Raguchander T. Induction of chitinase and β-1, 3-glucanase PR proteins in tomato through liquid formulated *Bacillus subtilis* EPCO 16 against *Fusarium* wilt. Journal of Today's Biological Sciences: Research & Review. India. 2012;1(1):50-60.
13. Rasool M, Akhter A, Haider MS. Molecular and biochemical insight into biochar and *Bacillus subtilis* induced defense in tomatoes against *Alternaria solani*. Scientia Horticulturae. 2021;285:110203. https://doi.org/10.1016/j.scienta.2021.110203.
14. Rasool M, Akhter A, Soja G, Haider MS. Role of biochar, compost and plant growth promoting rhizobacteria in the management of tomato early blight disease. Scientific Reports. 2021;11(1):6092. https://doi.org/10.1038/s41598-021-85633-4.
15. Singh VK, Singh AK, Kumar A. Disease management of tomato through PGPB: current trends and future perspective. 3 Biotech. 2017;7:1-10. https://doi.org/10.1007/s13205-017-0896-1.
16. Snyder WC, Hansen HN. The species concept in *Fusarium* with reference to *Discolor* and other sections. American Journal of Botany. 1945;32(10):657-666. https://www.jstor.org/stable/2437621.
17. Waheed K, Nawaz H, Hanif MA, Rehman R. Tomatoes. In: Hanif MA, Khan MM, Nawaz H, Byrne HJ, editors. Medicinal Plants of South Asia: Novel Source for Drug Discovery. Elsevier; c2020. p. 631-644. https://doi.org/10.1016/B978-0-08-102659-5.00046-X.
18. Wang H, Hao Z, Zhang X, Xie W, Chen B. Arbuscular mycorrhizal fungi induced plant resistance against *Fusarium* wilt in jasmonate biosynthesis defective mutant and wild type of tomato. Journal of Fungi. 2022;8(5):422. https://doi.org/10.3390/jof8050422.
19. Wang L, Ok YS, Tsang DC, Alessi DS, Rinklebe J, Wang H, *et al*. New trends in biochar pyrolysis and modification strategies: feedstock, pyrolysis conditions, sustainability concerns and implications for soil amendment. Soil Use and Management. 2020;36(3):358-386. https://doi.org/10.1111/sum.12592.
20. Wu X, Yu L, Pehrsson PR. Are processed tomato products as nutritious as fresh tomatoes? Scoping review on the effects of industrial processing on nutrients and bioactive compounds in tomatoes. Advances in Nutrition. 2022;13(1):138-151. https://doi.org/10.1093/advances/nmab109.