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ORIGINAL RESEARCH ARTICLE

Evidences of induced resistance in tomato against *Alternaria solani*: An investigation

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ABSTRACT

Tomato is one of the major solanaceous vegetables, which has a unique place in the global vegetable market. Instead of being a high-value crop, there is still a need to do improvement in its potential against various biotic and abiotic stressors that adequately demolish its real yield. *Alternaria solani* (causing early blight disease) is designated as one of the fatal organisms that may reduce tomato crop yield by up to 80%. There were lots of methods, viz., chemical, cultural and biological suggested to overcome it. However, chemical strategies are much in vogue, but they have several negative consequences for human health and the ecosystem. Enlightening this issue, the efficacy of various treatments, viz., chemical fungicides (Amistar Top®, Nativo®, and Contaf®), biochar and fungal bioagent (*Trichoderma viride*) was assessed under both *in vivo* and *in vitro* conditions. Induced resistance is mediated by several regulating pathways, like salicylic acid and jasmonic acid. These mediating pathways manipulate different physiological processes like growth and development, stress tolerance, and defence mechanisms of the plant. The assessment of results revealed that among all treatments biochar at 3.25% by weight consistently displayed remarkable effectiveness against the early blight infection by triggering resistance and improving the overall performance of tomato plants. This result is attributed to improved soil health, fastening mineralization as well as absorption processes, and boosting the plant's immunity with the use of a higher concentration of biochar. Hence, it could be recommended for the overall improvement of tomato crop and its sustainability.

Keywords: early blight; tomato; induced resistance; production; sustainability

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1. Introduction

Tomato (*Solanum lycopersicum* L.; 2n = 2x =24), originally from the Andean region of South America, is a widely cultivated plant found all around the globe[1]. It is widely cultivated as a cash crop, making a substantial contribution to employment and income generation[2–4]. It served as a "functional food" that has a high nutritive value (rich source of lycopene, antioxidant, sugar, dietary fibers, ascorbic acid) and can thrive in diverse environments ranging from tropical to temperate climates. Due to its extensive utilization and nutritional benefits, there is a strong demand for both fresh market and processed tomato varieties. Meeting the ever-growing demand necessitates increased tomato production. However, cultivated tomatoes exhibit limited genetic diversity, stemming from rigorous selection and inbreeding throughout their evolution and domestication processes[5], rendering these varieties more susceptible to disease outbreaks. Nonetheless, the commercial tomato production faces impediments due to a multitude of fungal,

bacterial, viral, and nematode diseases[6]. Within the realm of fungal ailments, the early blight (EB) of tomato, caused by *Alternaria solani*[7] Jones and Grout, stands out as a soil-borne microorganism responsible for initiating leaf blight or early blight, collar rot, and fruit decay in tomatoes, spread through fungal spores[8]. *A. solani* reproduces asexually and persists in various forms, including conidia or mycelia, within diseased plant debris, seeds, soil, and alternate hosts, serving as primary sources of infection. Due to its short disease cycle, *A. solani* has the potential to cause multiple infection cycles[9]. Infections typically occur in warm and humid conditions. Conidia germinate within a temperature range of 8 °C–32 °C, particularly in cool and humid environments with adequate moisture, forming germ tubes[10]. These germ tubes can penetrate host tissues directly or gain entry through stomata or wounds, leading to infection. Lesions become visible approximately 2–3 days after infection, contingent on environmental factors, leaf age, and cultivar susceptibility. Spore production commences 3–5 days after lesion appearance[11]. Generally, an extended period of moisture is necessary for spore production, although spores can also form during alternating wet and dry conditions. Initially, conidiophores develop on damp nights and subsequently produce spores or conidia on another wet night following daylight and dry spells. These conidia are then rapidly dispersed by wind and rain splash, initiating new disease cycles in other healthy parts of the same plant or affecting different plants[9,10].

EB or leaf blight, manifests as dark, small, necrotic lesions on the leaf surface that tend to coalesce and form concentric patterns, resembling a target. These lesions are surrounded by yellow rings[11]. Initially, the disease appears on the lower, older leaves, which are more susceptible due to their lower sugar content and reduced glycoalkaloid levels, such as solanine, chaconine, and solanidine[12,13]. As the plant matures, the disease progresses upwards. As the disease advances, it leads to significant defoliation, which elevates the plant's respiration rate while reducing its photosynthetic rate. This defoliation also exposes the fruits to the sun, causing sunscald. This, in turn, results in diminished fruit quality and substantial yield loss. Collar rot, on the other hand, is characterized by initial dark and sunken lesions on the stem, which later expand to form lens-shaped lesions with concentric rings resembling those found on leaves. In young seedlings, lesions at ground level encircle the stem, damaging the vascular system and creating "collars"[14]. Infections on fruit cause dark, sunken, leathery, and purple lesions at the stem-end. Infected fruits tend to drop prematurely, and even those that reach maturity become unsuitable for the market[15].

EB disease has become exceedingly destructive, leading to annual yield losses of approximately 80%[16]. In severe instances, the disease can lead to complete defoliation, particularly in regions characterized by heavy and moist precipitation, high humidity, and elevated temperatures. During the interaction between *A. solani* and its host, several enzymes are produced, including cellulases and proteases. These enzymes play a crucial role in breaking down the host cell wall. Additionally, *A. solani* secretes gelatin methyl galacturonase, which aids in host colonization and contributes to its growth, development, survival, and pathogenicity[17]. Recent discoveries have identified the secretion of extracellular serine proteases and metalloproteases by *A. solani*, suggesting their involvement in phytopathogenicity[18,19]. *A. solani* is known to produce a variety of phytotoxic metabolites, including alternariol, altersolanol A, altertoxin, macrosporin, and solanapyrone[20]. These metabolites can have detrimental effects on plant health and contribute to disease development. The disease significantly hampers crop production by inducing premature defoliation and substantial losses in both the quality and quantity of fruits[21]. Factors contributing to the disease's proliferation include dense planting, high precipitation, and prolonged leaf wetness periods[22]. Effectively managing this disease presents a considerable challenge[23]. Failure to manage this disease can result in a reduction in crop yield[24].

EB can be managed through various approaches, viz., fungicidal treatments, cultural management, use of resistant plant varieties, etc. Among these, cultural practices and fungicidal treatment are the most commonly employed methods[14]. Cultural practices encompass various strategies, including maintaining a healthy field and crop vigor, implementing good sanitation measures, removing infected plant debris and volunteer weeds

from the vicinity of the field, practicing crop rotation, incorporating organic inputs like biochar, and reducing leaf wetness through soil-directed irrigation systems[15]. Biochar is an organic byproduct derived from the pyrolysis of various organic feedstocks that has a substantial surface area for providing habitat for various soil microorganisms. In agriculture, it has been utilized for soil reclamation and for inducing disease resistance in various plant species against destructive plant pathogens[25]. Incorporating biochar into the growing medium of crops such as tomatoes, sweet peppers, and strawberries has been shown to induce resistance against various pathogens and enhance production[26,27]. The effectiveness of biochar amendments has been proved significant 85% in various case studies and non-significant in 12% of cases[28]. To ensure maximum benefits and avoid deleterious effects, the dosage of biochar should typically range between 0.5% to 3% by weight, applied with localized placement[25]. However, it's important to note that cultural practices alone may not suffice for EB control, and as a result, various types of fungicides have been developed for managing EB in tomato crops.

Anand et al.[29] revealed that azoxystrobin was highly effective at 125 g a.i. ha^{-1} concentration against leaf blight disease of tomato and it reduced disease incidence 92.82% over control. They observed that increase in azoxystrobin concentration reduced the rate of disease progress and improved the production in treated plants. Aslam et al.[30] reported that Nativo 75% WG (0.25%) showed maximum (62.85%) percentage inhibition during the assessment of efficacy of different fungicides, plant extracts, and vitamins in EB disease of tomato under both *in vitro* and *in vivo* conditions. Dhaka and Choudhary[31] analyzed the *in vitro* efficacy of different fungicides against *Alternaria solani* and observed 78.17% mycelium growth inhibition using hexaconazole against *Alternaria solani*. Although, synthetic amendments, such as fungicides, have increased production quantities to some extent but they come at a cost to soil quality, human health, and the environment. Over-reliance on synthetic chemicals can lead to catastrophic consequences, deteriorating soil quality and compromising the quality of agricultural production. Residual synthetic chemicals can also result in the accumulation of heavy metals in the soil, which can be toxic to plants as well as soil microorganisms and fauna[25]. Fungicides can face limitations in high disease pressure, and such treatments may not be economically viable or environmentally sustainable[14]. For instance, a study conducted in North Carolina found that a total of 15 fungicide applications were necessary per growing season to effectively manage diseases of fungal origin[32]. Frequent fungicide use can also contribute to the emergence of new, fungicide-resistant isolates due to the substantial selection pressure exerted by these chemicals.

As an alternative approach for managing EB, the use of fungal bioagents has been explored and documented in various scientific studies. These bioagents operate by inducing resistance in plants, providing an alternative method for EB management. *Trichoderma* is among the most isolated and studied soil endophyte found in plant root ecosystem that has been long known for their antimicrobial activities against various phytopathogens along with the abilities to promote plant growth and development[33]. Various species of genus *Trichoderma* have been continuously exploited as bioagent in agriculture[34]. The role of *Trichoderma viride* and *T. harzianum* in the control of EB has been previously documented[35,36]. Sreenivasulu et al.[37] found *T. viride* at the rate 0.25% highly effective against *Alternaria solani* under *in vivo* and *in vitro* conditions in tomato. This antagonistic fungus act as mycoparasite against the phytopathogenic fungi, by secreting numerous hydrolytic enzymes, produced antibiotic compounds and different types of hormones including auxins and cytokinins which had a favourable impact on plant growth and development[38–40]. Thus, it gives competition to phytopathogens for space and nutrients[41], stimulates plant growth and do induce acquired resistance[42].

Several resistant sources have also been identified in wild tomato species like *S. habrochaites* (PI390513, PI390514, PI390662, PI126445, PI127827), *S. pimpinellifolium* (LA1921 and its BC4F4 progeny) and *S. peruvianum* (PE33, PI127829 LA1292, LA1365), and utilized to develop resistant lines in a resistance breeding program against EB disease[14]. However, due to high genetic variation, cross compatibility barriers with the cultivated ones, breaking of resistance with due course of time, polygenic inheritance of EB resistance,

alterations in pathogen strains, insufficient resistance in cultivated species, transferring of unwanted horticultural traits along with EB resistance feature have thwarted the effective breeding of EB resistance in tomato[16].

Upon breaching the physical barriers of the plant's biological structure, various immune defense systems are activated to combat invading pathogens within the plant's organs. Induced resistance refers to an elevated defensive capability in a plant that is triggered by specific environmental cues. This enhanced resistance is developed when the plant's defenses are preconditioned by prior infection or treatment, resulting in increased resistance to subsequent challenges by pathogens or parasites[43]. Based on the location within the host where local tissue immunity is stimulated and the type of microbes detected by the plant, these strategies are typically categorized as induced systemic resistance (ISR) and systemic acquired resistance (SAR)[44]. Systemic acquired resistance (SAR) is generally associated with increased levels of salicylic acid (SA) and the induction of pathogenesis-related proteins (PR proteins) in uninfected host tissues. This response can be triggered by prior exposure to virulent, avirulent, or nonpathogenic microbes, or artificially induced using chemicals[45,46]. In contrast, induced systemic resistance (ISR) can be initiated by nonpathogenic soil microbes. It primarily protects the host plant's shoots against necrotrophic pathogens and insects by modulating the biosynthesis of jasmonic acid (JA) and ethylene, along with their associated signal transduction pathways[44,47].

During the literature survey relevant to induced resistance against EB disease in tomato plants it was identified that a single approach can't be enough to control the infection. Consequently, there is a need to develop a novel formulation of the available management strategies (as explained in the previous paragraph) in such a way that their effective mode of action can enhance the potential of tomato crop to resist several biotic and biotic stresses and lead to improved production both qualitatively and quantitatively. By keeping this approach in mind, the proposed study was designed to assess the efficacy of various treatments, viz., chemical fungicides (Amistar Top®, Nativo®, and Contaf®), biochar and fungal bioagent (*Trichoderma viride*) under both *in vivo* and *in vitro* conditions to induce resistance against the EB disease of tomato.

2. Materials and methods

The presented work was accomplished in two distinct trials; one was conducted at the research farm of Lovely Professional University, Phagwara, Punjab. The research site featured a characteristic sub-tropical climate, marked by scorching summers and prevailing winds from April to July. This was succeeded by a hot and humid rainy season, followed by cold winters spanning from December to January. Another experiment was conducted in the laboratory of the Department of Plant Pathology of Lovely Professional University, Punjab, during the year 2022.

2.1. Collection, isolation and identification of pathogen

Leaf samples displaying dark spots with concentric rings on older leaves and on adjacent areas that had turned yellow were collected from the tomato field established at Lovely Professional University's research farm in Punjab. These gathered samples containing *Alternaria solani* inoculum were transported to the laboratory for the purposes of isolation, identification, and further investigation.

The isolation of microorganisms was executed using the tissue segment technique[48]. The identification of microorganisms was confirmed by observing their morphological characteristics through the use of a compound microscope[49]. *A. solani* was distinguished by its septate, dark-colored mycelium, and the production of short, simple, upright conidiophores that carry single and dispersed chains of conidia in acropetal arrangements[50].

2.2. Inoculation of pathogen

Root dip method

The root dip inoculation technique was employed to the "20-day-old" seedlings. Spore suspension solutions (having concentration from 0.1 to 1 × 10^6 spores/mL) were prepared from "10-day-old" cultures which was previously isolated from refined potato dextrose agar (PDA) cultures[51,52]. Subsequently, the seedlings' roots were gently managed with a sterile scissors and immersed in tubes containing 30 mL of spore suspension for approximately 30 min. Following this, the inoculated seedlings were transplanted into pots. In contrast, the roots of control plants underwent treatment with distilled water.

2.3. Procurement of fungicides and its dosages

Three chemical fungicides, viz., Amistar Top® (Syngenta India Limited), Nativo® (Bayer CropScience Limited) and Contaf® (Tata Rallis India Limited) were collected and prepared at 25 SC (120 g a.i. ha^{-1})[29], 75% WG (0.25%)[30] and 5% EC (2 mL per litre)[31,53] concentration, respectively for the management of EB disease of tomato.

2.4. *In vitro* evaluation of fungicides, biochar and fungal bioagent

The feasibility of different chemical fungicides (Amistar Top® 25 SC, Nativo® 75% WG and Contaf® 5% EC), biochar (at the concentration of 2.25%, 2.75% and 3.25% by weight)[25] and fungal bioagent (*Trichoderma viride* at 0.25%) (as stated in **Table 1**) were verified *in vitro* utilizing poison food technique[54], and double culture method[55] against the pathogen. Mycelium growth of *Alternaria solani* and its percentage inhibition over control was measured using the formula; [(C – T)/C × 100] where "C" was the "mycelium growth in control" and "T" was "mycelium growth in respective treatment" (**Table 1**).

Table 1. *In vitro* evaluation of efficacy of different treatments on percentage inhibition of mycelium growth and *in vivo* evaluation on disease severity percentage against *Alternaria solani* infection in tomato plants.

Treatments		Mycelium diameter (cm)	Percentage inhibition of mycelium growth over control	Disease severity percentage	Percentage increase in disease severity over control
T_1	Amistar Top®	6.86^{cd}	14.16	48.77^{b}	17.61
T_2	Nativo®	7.08^{bcd}	11.45	28.59^{f}	51.7
T_3	Contaf®	7.35^{bc}	8.12	40.2^{c}	32.09
T_4	Biochar at 2.25%	7.22^{bcd}	9.66	31.47^{e}	46.84
T_5	Biochar at 2.75%	7.09^{bcd}	11.33	22.76^{g}	61.55
T_6	Biochar at 3.25%	6.75^{d}	15.62	10.09^{h}	82.96
T_7	*Trichoderma viride* at 0.25%	7.45^{b}	6.87	35.83^{d}	39.48
T_8	Control	8.1^{a}	–	59.2^{a}	–
	SEM	0.09	–	0.65	–

Different letters indicate significant differences at $p < 0.05$ according to the Duncan's multiple range tests.
[where, cm = centimeter; Amistar Top® = 25 SC (120 g a.i. ha^{-1}); Nativo® = 75% WG (0.25%); Contaf® = 5% EC (2 mL per litre)].

2.5. *In vivo* evaluation of fungicides, biochar and fungal bioagent

The assessment of various chemical fungicides, organic input, and the fungal bioagent was done *in vivo* during the rabi season of year, 2022. The previously pathogen-treated seedlings (upon reaching 1 month of age) were transplanted into the main field at a spacing of 60 cm × 45 cm. Soil applications of biochar, and fungal bioagent at their predetermined concentrations were done twice; first at the time of transplanting and second at 15 days after transplanting (DAT). Two foliar applications of each fungicide were applied after 40 DAT maintaining an interval of 15-days using a knapsack sprayer[29]. The percent disease intensity (PDI) was documented for each plot and assessed following two consecutive applications of fungicides, biochar, and

fungal bioagent at 15-day intervals. The assessment of EB infection incidence in tomatoes was conducted on 5 randomly selected plants from each plot, utilizing a 0–5 scale[56].

2.6. Evaluation of soil pH and electrical conductivity (EC) (dS/m)

Prior to assessing the soil pH and EC parameters, a thorough evaluation of various properties of different treatments was conducted to gain insight into the characteristics of the product used to induce resistance in tomato plants. Randomly soil samples (~600 g) were collected from the experimental site and air dried at the room temperature. The samples were then grinded, sieved and weighed to 100 g. A known volume of distilled water (1:1) was added to the sample in a container and stirred for few minutes. Soil-water suspension was allowed to sit for 1 h to ensure that pH equilibrates. After some times, the pH was measured using calibrated pH meter (Systronics pH system 361)[57]. For measurement soil EC the previously air-dried samples were sieved to obtain a homogeneous soil texture from which 100 g weighed and mixed with an equal volume of distilled water in the ratio 1:1. This prepared mixture was vigorously shaken to create a soil-water suspension and then allowed to equilibrate for around 30 min. After that the electrical conductivity of the equilibrated suspension was measured using an electrical conductivity meter (Systronics EC conductivity 7DS meter 308) in deciSiemens per meter (dS/m)[58]. The soil samples were brought twice first, at the beginning (before application of treatments) and second, at the end (after the field trial got over) for the analysis of pH and EC in the lab.

2.7. Total soluble solids (°Brix)

The measurement of Total Soluble Solids (TSS) was determined using a digital refractometer in °Brix. Under this, unripe, red ripe and over ripe tomato samples from each treatment were taken into the consideration and the value was noted.

2.8. Biochemical analysis

Biochemical analysis was performed by analyzing the total soluble protein and total phenol content for the confirmation of development of disease resistance. The estimation of total soluble proteins and total phenols were analyzed as per the methodology suggested by Lowry et al.[59] and Bray and Thorpe[60], respectively. The assessment of total phenols and total soluble proteins was checked two times (at 30 and 60 days after transplanting) with the aid of spectrophotometer at a wavelength of 765 nm and 595 nm.

2.9. Statistical analysis

Statistical analysis was performed using R-Studio. The results represent the averages from three replications of each treatment. The data underwent one-way analysis of variance (ANOVA), and mean comparisons were conducted using Duncan's multiple range tests at the significance level of $p < 0.05$. Differences at $p < 0.05$ were considered to be statistically significant.

3. Results and discussions

3.1. Identification of *A. solani*

Confirmation of *A. solani* identification was based on its morphological characteristics[7]. Microscopic examination revealed that the conidiophore colour ranged from olivaceous brown to brown, and the conidia exhibited a smooth, tapering to a bill-like shape form, flexuous or straight, elongated, measuring 160 to 300 µm in length, with 8–10 transverse and 2–4 longitudinal septa.

3.2. *In vitro* evaluation of fungicides, biochar and fungal bioagent

As per the results shown in **Table 1** maximum (15.62%) percentage of mycelium growth inhibition of

Alternaria solani and minimum (6.75 cm) mycelium diameter were observed in T_6 (biochar at 3.25%) treatment over control. Rasool et al.[61] reported comparable results in their study, which showed that as the concentration of biochar, specifically wood biochar (6%) and green waste biochar (6%) in PDA, increased, the efficiency of inhibiting fungal radial growth of *A. solani* also increased. Notably, green waste biochar (6%) exhibited a significantly higher level of mycelium growth inhibition, reaching 38.74%.

3.3. *In vivo* evaluation of fungicides, biochar and fungal bioagent on disease severity

The minimum (10.09%) disease severity percentage was observed in T_6 (biochar at 3.25%), followed by T_5 (biochar at 2.75%) whereas maximum (59.2%) was observed in control. In contrast maximum (82.96%) percentage increase in disease severity over control was observed in biochar at 3.25% (T_6) (**Table 1**). This outcome can likely be attributed to the fact that biochar application enhances interactions between soil microbes and plants, rather than directly releasing fungitoxic compounds[28]. This enhancement provides tomato plants with the potential to better withstand early blight (EB) stress[62]. Similar findings were reported by Harel et al.[63], in which they demonstrated that biochar application triggers resistant mechanisms, either through systemic acquired resistance (SAR) or by inducing systemic resistance, to reduce pathogen infection. Another contributing factor could be that biochar stimulates the growth and activity of plant growth-promoting microorganisms (PGPMs), including rhizobacteria, mycorrhizal fungi, and other endophytic fungi like *T. viride*. These microorganisms, in turn, play a crucial role in protecting against pathogens through mechanisms such as nutrient and space competition, direct parasitism, and antagonism via the production of secondary metabolites[64].

3.4. Soil pH and electrical conductivity (EC) (dS/m)

Assessing soil pH and EC provided insight into how different treatments influenced the physical and chemical attributes of the soil. As per the results stated in **Table 2**, it was observed that the pH showed highest (2.09%) variation in T_2 (Nativo®) than other treatments. Whereas EC was reported with the highest (53.72%) rate of change in T_5 (biochar at 2.75%) than initial EC values of soil. It might be due to the basic nature of the treatment and higher exchange of ions between soil and the employed treatment. The application of biochar had a positive impact on the chemical, physical, and biological properties of the soil, resulting in an increase in electrical conductivity (EC). These findings are consistent with previous research conducted by Guo et al.[65], Ali et al.[66] and Ud Din et al.[67], which also reported similar improvements in soil characteristics due to biochar application.

Table 2. *In vitro* evaluation of different treatments on percentage increase of soil pH and EC over control and total soluble solids (°Brix) present at unripe, red ripe and over ripe stages in tomato plants.

Treatments		Percent increase over control		Total soluble solids (°Brix)		
		Soil pH	Soil EC	Unripe	Red ripe	Over ripe
T_1	Amistar Top®	0.59	43.43	2.33^{c}	3.2^{b}	3.53^{b}
T_2	Nativo®	2.09	49.64	3.13^{ab}	3.27^{b}	3.73^{ab}
T_3	Contaf®	0.59	46.41	2.93^{bc}	3.49^{b}	3.8^{ab}
T_4	Biochar at 2.25%	0.48	46.23	3.67^{a}	3.67^{ab}	4.07^{ab}
T_5	Biochar at 2.75%	0.36	53.72	3.07^{ab}	3.73^{ab}	4.27^{a}
T_6	Biochar at 3.25%	0.24	53.61	2.93^{bc}	3.47^{b}	3.69^{b}
T_7	*Trichoderma viride* at 0.25%	0.83	10.62	2.67^{bc}	3.53^{b}	3.87^{ab}
T_8	Control	–	–	3^{abc}	4.2^{a}	4.05^{ab}
	SEM	–	–	0.13	0.10	0.08

Different letters indicate significant differences at $p < 0.05$ according to the Duncan's multiple range tests.
[where, pH = potential of hydrogen; EC = electrical conductivity; Amistar Top® = 25 SC (120 g a.i. ha^{-1}); Nativo® = 75% WG (0.25%); Contaf® = 5% EC (2 mL per litre)].

3.5. Total soluble solids (TSS) (°Brix)

The analyzed results of TSS (**Table 2**) revealed that unripen tomato samples showed highest (3.67) TSS in biochar at 2.25% (T_4) whereas, the red ripe and over ripe tomato samples showed highest concentrations of TSS as 3.73 and 4.27 in T_5 (biochar at 2.75%), respectively. The observed increase in the accumulation of soluble solids in tomato fruits at various harvest stages following biochar application may be attributed to the enhancement of physiological and biochemical attributes of the tomato plants. These findings align with previous studies conducted by Cao et al.[68], Hameeda et al.[69] and Ud Din et al.[67].

3.6. Total phenols and total proteins (mg BSA/g FW)

The evaluated results of total phenols and total proteins are presented in **Table 3** which showed the highest (78.94%) percentage increase in the level of total phenols in T_2 (Nativo®) over the control. The phenol contents increased from 1.14 mg BSA/g FW (before inoculation of pathogen) to 2.04 mg BSA/g FW (after inoculation of pathogen) when the Nativo treatment employed to tomato plants. It may be attributed to the efficacy of the treatment by means of inducing the resistance through manipulating the level of total phenols. The minimum (10.72%) percent increase in the level of total phenols was observed in the control. These results were in agreement with the findings of Aslam et al.[30].

Table 3. Differences between field and pot culture experiments in total phenols (mg BSA/g FW) and total protein contents (mg BSA/g FW) along with their percentage increase over control in tomato plants.

Treatments		Total phenols (mg BSA/g FW)			Total proteins (mg BSA/g FW)		
		Field sample	Pot sample	Percent increase over control	Field sample	Pot sample	Percent increase over control
T_1	Amistar Top®	1.12^{a}	1.91^{a}	70.53	2.93^{ab}	3.12^{ab}	6.48
T_2	Nativo®	1.14^{a}	2.04^{a}	78.94	2.99^{ab}	3.43^{a}	14.71
T_3	Contaf®	1.31^{a}	2.13^{a}	62.59	3.07^{a}	3.57^{a}	16.28
T_4	Biochar at 2.25%	1.44^{a}	2.28^{a}	58.33	3.1^{a}	3.72^{a}	20
T_5	Biochar at 2.75%	1.49^{a}	1.9^{a}	27.52	3.12^{a}	3.76^{a}	20.51
T_6	Biochar at 3.25%	1.51^{a}	1.93^{a}	27.81	3.01^{a}	3.35^{a}	11.29
T_7	*Trichoderma viride* at 0.25%	1.16^{a}	1.91^{a}	64.65	3.16^{a}	3.21^{ab}	1.58
T_8	Control	1.12^{a}	1.24^{b}	–	2.47^{b}	2.51^{b}	–
	SEM	0.06	0.10	–	0.07	0.14	–

Different letters indicate significant differences at $p < 0.05$ according to the Duncan's multiple range tests.
[mgBSA/gFW = milligram Bovine Serum Albumin per gram of fresh weight; Amistar Top® = 25 SC (120 g a.i. ha^{-1}); Nativo® = 75% WG (0.25%); Contaf® = 5% EC (2 mL per litre)].

The results (presented in **Table 3**) of total proteins revealed that T_5 (biochar at 2.75%) showed the highest (20.51%) percent of increase in the level total soluble proteins. It was noticed that initially (before inoculation of pathogen) the total protein content was 3.12% and later (after inoculation of pathogen) it reached to 3.76% when the particular treatment was applied. While the minimum (1.58%) increase in the level of total proteins was observed in T_7 (*T. viride* at 0.25%). The probable reason behind it that treatments containing varying concentrations of biochar were notably more effective in increasing the total soluble protein content in tomato plants, which in turn suppressed pathogenic symptoms. This effect could be attributed to the improved biochemical characteristics and the enhanced movement of nutrients and minerals within the plant structure, ultimately leading to increased levels of total proteins and other secondary metabolites. These elevated content levels played a role in reducing early blight (EB) stress in tomatoes. Similar results have been reported by Harel et al.[63] and Bonanomi et al.[64].

3.7. Root and shoot length (cm)

The application of various treatments affected the growth and development of tomato plants precisely.

The results shown in **Table 4** revealed that biochar at 3.25% (T_6) treatment reported with maximum increase in their root and shoot length. The maximum root length and shoot length was measured as 30.66 cm and 32.1 cm, respectively. While Amistar Top® (T_1) was noticed with the minimum (23.46 cm) root length and minimum shoot length (29.6 cm) was observed in control. Similar results were reported by Ud Din et al.[67] in their study involving individual biochar application. They observed an increase in agronomic traits of tomato plants ranging from 8% to 26% when compared to the control group. This improvement in agronomic traits can be attributed to the enhanced root length facilitated by biochar, which enabled the plants to absorb more nutrients and water uptake even under stressed conditions. Furthermore, it played a role in alleviating the detrimental effects resulting from plant-pathogen interactions, ultimately enabling the plants to perform optimally.

Table 4. *In vivo* evaluation of different treatments on root length (cm), shoot length (cm) and total yield (kg) against *Alternaria solani* in tomato plants.

Treatments		Root length (cm)	Shoot length (cm)	Total yield (kg)
T_1	Amistar Top®	23.46^{c}	30.76^{a}	7.36^{b}
T_2	Nativo®	28.53^{ab}	31.43^{a}	7.56^{b}
T_3	Contaf®	27.46^{ab}	29.93^{a}	7.16^{b}
T_4	Biochar at 2.25%	28.06^{ab}	30.1^{a}	7.46^{b}
T_5	Biochar at 2.75%	28.03^{ab}	31.7^{a}	7.8^{ab}
T_6	Biochar at 3.25%	30.66^{a}	32.1^{a}	8.26^{a}
T_7	*Trichoderma viride* at 0.25%	26.06^{bc}	30.5^{a}	7.5^{b}
T_8	Control	26.6^{bc}	29.6^{a}	5.63^{c}
	SEM	0.78	0.32	0.27

Different letters indicate significant differences at $p < 0.05$ according to the Duncan's multiple range tests.
[cm = centimeter; mgBSA/gFW = milligram Bovine Serum Albumin per gram of fresh weight; Amistar Top® = 25 SC (120 g a.i. ha^{-1}); Nativo® = 75% WG (0.25%); Contaf® = 5% EC (2 mL per litre)].

3.8. Total yield (kg)

The total yield of tomato plants improved by the different employed treatments (as presented in **Table 4**). However, the highest total yield (8.26 kg) was obtained with biochar at 3.25% (T_6) and the minimum (5.63 kg) was reported in control. The positive outcomes observed can likely be attributed to soil amendment with biochar, which contributed to increased soil fertility and resulted in improved crop growth, yield, and overall crop quality[62].

4. Conclusion

Plant resistance is influenced by a range of factors, including plant species, growth tendencies, crop load, physiology of the plant, etc. More importantly, the pathogen itself modifies its strains in due course of time. On the other side, the available resistant varieties/hybrids against the early EB obstruction haven't sufficiently combated the disease or it may happen that the resistance got broken in between. Tomato growers are now-a-days relying much upon fungicides against EB infection. This caused adverse impacts on soil and human health. By understanding the seriousness of this devastating disease, we have planned an approach consisting of different treatments (fungicides, biochar, and fungal bioagent) and implemented these against the pathogen to induce resistance in tomato plants. The assessment outcomes demonstrated that biochar exhibited significant efficacy in countering EB infection in tomato plants by triggering resistance. The utilization of biochar treatments not only brought about alterations in the plants' morphological traits but also enhanced the overall growth patterns of the plants. When contrasting various biochar concentrations with alternative inputs, it was noted that a concentration of biochar at 3.25% consistently displayed remarkable effectiveness. Therefore, the same can be recommended for improved production of tomato crop and its sustainability. Furthermore, we suggest that in addition to its use in sustainability of agriculture it could also be an alternative for carbon dioxide reduction techniques due to its carbon seizing ability which may favour in greenhouse gases reduction.

Author contributions

Conceptualization, AK; methodology, AK; investigation, TR; writing—original draft preparation, AK; writing—review and editing, TR, EG, SP and AKK; supervision, AK. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no competing interest for the proposed work.

References

1. Bauchet G, Causse M. Genetic diversity in tomato (*Solanum lycopersicum*) and its wild relatives. In: Caliskan M (editor). *Genetic Diversity in Plants*. Books on Demand; 2012. pp. 134–162.
2. Malherbe S, Marais D. Economics, yield and ecology: A case study from the South African tomato industry. *Outlook on Agriculture* 2015; 44(1): 37–47. doi: 10.5367/oa.2015.0195
3. Food and Agriculture Organization of the United Nations (FAO). *World Food and Agriculture—Statistical Yearbook*. FAO; 2021. doi: 10.4060/cb4477en
4. Nuwamanya AM, Runo S, Mwangi M. Farmers' perceptions on tomato early blight, fungicide use factors and awareness of fungicide resistance: Insights from a field survey in Kenya. *PLOS ONE* 2023; 18(1): e0269035. doi: 10.1371/journal.pone.0269035
5. Zhang LP, Khan A, Nino-Liu D, Foolad MR. A molecular linkage map of tomato displaying chromosomal locations of resistance gene analogs based on a *Lycopersicon esculentum* × *Lycopersicon hirsutum* cross. *Genome* 2002; 45(1): 133–146. doi: 10.1139/g01-124
6. Manda RR, Addanki VA, Srivastava S. Bacterial wilt of solanaceous crops. *International Journal of Chemical Studies* 2020; 8(6): 1048–1057. doi: 10.22271/chemi.2020.v8.i6o.10903
7. Ellis JB, Martin GB. Macrosporium solani E&M. *American Naturalist* 1982; 16: 1003.
8. Datar VV, Mayee CD. Assessment of losses in tomato yield due to early blight. *Indian Phytopathology* 1981; 34: 191–195.
9. Jones JP, Jones JP, Stall RE, Zitter TA. *Compendium of Tomato Diseases*. The American Phytopathological Society (APS) Press; 1991.
10. Kemmitt G. Early blight of potato and tomato. *The Plant Health Instructor* 2002. doi: 10.1094/PHI-I-2002-0809-01
11. Sherf AF, Macnab AA. *Vegetable Diseases and Their Control*. John Wiley & Sons; 1986.
12. Sinden SL, Obrien MJ, Goth RW. Effect of potato alkaloids on growth of *Alternaria solani*. *American Potato Journal* 1972; 49: 367.
13. Rotem J. *The Genus Alternaria: Biology, Epidemiology, and Pathogenicity*. The American Phtyopathological Society (APS) Press; 1994.
14. Foolad MR, Merk HL, Ashrafi H. Genetics, genomics and breeding of late blight and early blight resistance in tomato. *Critical Reviews in Plant Sciences* 2008; 27: 75–107. doi: 10.1080/07352680802147353
15. Chaerani R, Voorrips RE. Tomato early blight (*Alternaria solani*): The pathogen, genetics, and breeding for resistance. *Journal of General Plant Pathology* 2006; 72: 335–347. doi: 10.1007/s10327-006-0299-3
16. Adhikari P, Oh Y, Panthee DR. Current status of early blight resistance in tomato: An update. *International Journal of Molecular Sciences* 2017; 18(10): 2019. doi: 10.3390/ijms18102019
17. Shahbazi H, Aminian H, Sahebani N, Halterman D. Effect of *Alternaria solani* exudates on resistance and susceptible potato cultivars from two different pathogen isolates. *The Plant Pathology Journal* 2011; 27(1): 14–19. doi: 10.5423/PPJ.2011.27.1.014
18. Chandrasekaran M, Chandrasekar R, Sa T, Sathiyabama M. Serine protease identification (*in vitro*) and molecular structure predictions (*in silico*) from a phytopathogenic fungus, *Alternaria solani*. *Journal of Basic Microbiology* 2014; 54(S1): S210–S218. doi: 10.1002/jobm.201300433
19. Chandrasekaran M, Thangavelu B, Chun SC, Sathiyabama M. Proteases from phytopathogenic fungi and their importance in phytopathogenicity. *Journal of General Plant Pathology* 2016; 82: 233–239. doi: 10.1007/s10327-016-0672-9
20. Kasahara K, Miyamoto T, Fujimoto T, et al. Solanapyrone synthase, a possible Diels-Alderase and iterative type I polyketide synthase encoded in a biosynthetic gene cluster from *Alternaria solani*. *ChemBioChem* 2010; 11(9): 1245–1252. doi: 10.1002/cbic.201000173

21. Holm AL, Rivera VV, Secor GA, Gudmestad NC. Temporal sensitivity of *Alternaria solani* to foliar fungicides. *American Journal of Potato Research* 2003; 80: 33–40. doi: 10.1007/BF02854554
22. Gondal AS, Ijaz M, Riaz K, Khan AR. Effect of different doses of fungicide (Mancozeb) against *Alternaria* leaf blight of tomato in Tunnel. *Journal of Plant Pathology & Microbiology* 2012; 3(3). doi: 10.4172/2157-7471.1000125
23. Pasche JS, Piche LM, Gudmestad NC. Effect of the F129L mutation in *Alternaria solani* on fungicides affecting mitochondrial respiration. *Plant Disease* 2005; 89(3): 269–278. doi: 10.1094/PD-89-0269
24. Mallik I, Arabiat S, Pasche JS, et al. Molecular characterization and detection of mutations associated with resistance to succinate dehydrogenase-inhibiting fungicides in *Alternaria solani*. *Phytopathology* 2014; 104(1): 40–49. doi: 10.1094/PHYTO-02-13-0041-R
25. Singh N, Kumar A. A review on bio-char: A byproduct of bio-wastes. *International Journal of Current Microbiology and Applied Sciences* 2020; 11: 1678–1691.
26. Elad Y, David DR, Harel YM, et al. Induction of systemic resistance in plants by bio-char, a soil-applied carbon sequestering agent. *Phytopathology* 2010; 100(9): 913–921. doi: 10.1094/phyto-100-9-0913
27. Elad Y, Cytryn E, Harel YM, et al. The biochar effect: Plant resistance to biotic stresses. *Phytopathologia Mediterranea* 2011; 50(3): 335–349.
28. Bonanomi G, Ippolito F, Scala F. A "black" future for plant pathology? Bio-char as a new soil amendment for controlling plant diseases. *Journal of Plant Pathology* 2015; 97(2): 223–234. doi: 10.4454/jpp.v97i2.3381
29. Anand T, Chandrasekaran A, Kuttalam S, Samiyappan R. Evaluation of azoxystrobin (Amistar 25 SC) against early leaf blight and leaf spot diseases of tomato. *Journal of Agricultural Technology* 2010; 6(3): 469–485.
30. Aslam HMU, Ikram A, Raza MM, et al. Efficacy of fungicides, botanicals and vitamins against early blight disease of tomato. *International Journal of Biosciences* 2018; 13(2): 138–146. doi: 10.12692/ijb/13.2.138-146
31. Dhaka S, Choudhary A. Efficacy of different fungicide against *Alternaria solani* caused early blight disease of tomato under *in vitro* condition. *International Journal of Agriculture, Environment and Biotechnology* 2022; 15(1): 61–65. doi: 10.30954/0974-1712.01.2022.7
32. Ivors KL, Louws FJ. *North Carolina Agricultural Chemicals Manual*. North Carolina State University; 2013.
33. Samuels GJ. *Trichoderma*: Systematics, the sexual state, and ecology. *Phytopathology* 2006; 96(2): 195–206. doi: 10.1094/PHYTO-96-0195
34. Chet I. *Trichoderma*: Application, mode of action, and potential as a biocontrol agent of soilborne plant pathogenic fungi. In: Chet I (editor). *Innovative Approaches to Plant Disease Control*, 99th ed. Wiley-Interscience; 1987.
35. Chowdappa P, Kumar SPM, Lakshmi MJ, Upreti KK. Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. *Biological Control* 2013; 65(1): 109–117. doi: 10.1016/j.biocontrol.2012.11.009
36. Sarkar S, Beura SK, Nandi A, et al. Management of early blight of tomato (*Alternaria solani* Ellis and Martin) by chemicals and biocontrol agents under field condition. *Journal of Mycopathological Research* 2016; 54: 81–84.
37. Sreenivasulu R, Reddy MSP, Tomar DS, et al. Managing of early blight of tomato caused by *Alternaria solani* through fungicides and bioagents. *International Journal of Current Microbiology and Applied Sciences* 2019; 8(6): 1442–1452. doi: 10.20546/ijcmas.2019.806.175
38. Bordenstein SR, Theis KR. Host biology in light of the microbiome: Ten principles of Holobionts and Hologenomes. *PLoS Biology* 2015; 13(8): e1002226. doi: 10.1371/journal.pbio.1002226
39. Vandenkoornhuyse P, Quaiser A, Duhamel M, et al. The importance of the microbiome of the plant holobiont. *New Phytologist* 2015; 206(4): 1196–1206. doi: 10.1111/nph.13312
40. Berg G, Rybakova D, Grube M, Köberl M. The plant microbiome explored: Implications for experimental botany. *Journal of Experimental Botany* 2016; 67(4): 995–1002. doi: 10.1093/jxb/erv466
41. Hjeljord L, Tronsmo A. *Trichoderma* and *Gliocladium* in biological control: An overview. In: Harman G, Kubicek C (editors). *Trichoderma* and *Gliocladium: Enzymes, Biological Control and Commercial Application*. CRC Press; 1998. Volume 2.
42. Bailey BA, Lumsden RD. Direct effects of *Trichoderma* and *Gliocladium* on plant growth and resistance to pathogens. In: Harman G, Kubicek C (editors). *Trichoderma* and *Gliocladium: Enzymes, Biological Control and Commercial Application*. CRC Press; 1998. Volume 2.
43. Van Loon LC, Bakker PAHM, Pieterse CMJ. Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology* 1998; 36: 453–483. doi: 10.1146/annurev.phyto.36.1.453
44. Pieterse CMJ, Zamioudis C, Berendsen RL, et al. Induced systemic resistance by benefcial microbes. *Annual Review of Phytopathology* 2014; 52: 347–375. doi: 10.1146/annurev-phyto-082712-102340
45. Vlot AC, Dempsey DA, Klessig DF. Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology* 2009; 47: 177–206. doi: 10.1146/annurev.phyto.050908.135202
46. Fu ZQ, Dong XN. Systemic acquired resistance: Turning local infection into global defense. *Annual Review of Plant Biology* 2013; 64: 839–863. doi: 10.1146/annurev-arplant-042811-105606
47. Walters DR, Ratsep J, Havis ND. Controlling crop diseases using induced resistance: Challenges for the future. *Journal of Experimental Botany* 2013; 64(5): 1263–1280. doi: 10.1093/jxb/ert026

48. Rangaswamy G, Mahadevan A. Diseases of vegetables. In: Rangaswamy G, Mahadevan A (editors). *Disease of Crop Plants in India*, 4th ed. Prentice Hall of India Pvt. Ltd.; 1998. pp. 286–334.
49. Barnett HL, Hunter BB. *Illustrated Genera of Imperfect Fungi*, 3rd ed. Burgess Publishing Company; 1972.
50. Sivakumar T, Ravikumar M. *A Manual of Marine and Mangrove Fungi*, 1st ed. Darshan Publishers; 2021.
51. Herman R, Perl-Treves R. Characterization and inheritance of a new source of resistance to *Fusarium oxysporum* f. sp. *melonis* race 1.2 in *Cucumis melo*. *Plant Disease* 2007; 91(9): 1180–1186. doi: 10.1094/PDIS-91-9-1180
52. Karimi R, Owuoche JO, Silim SN. Inheritance of fusarium wilt resistance in pigeonpea [*Cajanus cajan* (L.) Millspaugh]. *Indian Journal of Genetics and Plant Breeding* 2010; 70(3): 271–276.
53. Sharma RL, Ahir RR, Sharma A, et al. Management of Alternaria blight (*Alternaria alternata*) of tomato through novel combined formulations of fungicides. *Research Square* 2022. doi: 10.21203/rs.3.rs-2079637/v1
54. Nene YL, Thapliyal PN. *Fungicides in Plant Disease Control*, 2nd ed. Oxford & IBH Publishing Company; 1979.
55. Morton DT, Stroube NH. Antagonistic and stimulatory effects of microorganisms upon *Sclerotium rolfsci*. *Phytopathology* 1955; 45: 419–420.
56. Hossain MT, Hossain SMM, Bakr MK, et al. Survey on major diseases of vegetable and fruit crops in Chittagong region. *Bangladesh Journal of Agricultural Research* 2010; 35(3): 423−429. doi: 10.3329/bjar.v35i3.6449
57. American Public Health Association (APHA). *Standard Methods for the Examination of Water and Wastewater*, 23rd ed. American Water Works Association; 2017.
58. Carter MR, Gregorich EG. *Soil Sampling and Methods of Analysis*, 2nd ed. CRC Press; 2007.
59. Lowry OH, Rosebrough NJ, Farr AL, Randall R. Protein measurement with the folin phenol reagent. *Journal of Biology and Chemistry* 1951; 193(1): 265–275. doi: 10.1016/S0021-9258(19)52451-6
60. Bray HG, Thorpe WV. Analysis of phenolic compounds of interest in metabolism. In: Glick D (editor). *Methods of Biochemical Analysis*. Interscience Publishers Inc.; 1954. Volume 1. pp. 27–52. doi: 10.1002/9780470110171.ch2
61. 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: 6092. doi: 10.1038/s41598-021-85633-4
62. Luigi M, Ariana Manglli A, Dragone I, et al. Effects of biochar on the growth and development of tomato seedlings and on the response of tomato plants to the infection of systemic viral agents. *Frontiers in Microbiology* 2022; 13: 862075. doi: 10.3389/fmicb.2022.862075
63. Harel YM, Elad Y, Rav-David D, et al. Biochar mediates systemic response of strawberry to foliar fungal pathogens. *Plant and Soil* 2012; 357: 245–257. doi: 10.1007/s11104-012-1129-3
64. Bonanomi G, Lorito M, Vinale F, Woo SL. Organic amendments, beneficial microbes, and soil microbiota: Toward a unified framework for disease suppression. *Annual Review of Phytopathology* 2018; 56: 1–20. doi: 10.1146/annurev-phyto-080615-100046
65. Guo X, Liu H, Zhang J. The role of biochar in organic waste composting and soil improvement: A review. *Waste Management* 2020: 102: 884–899. doi: 10.1016/j.wasman.2019.12.003
66. Ali MH, Khan MI, Bashir S, et al. Biochar and *Bacillus* sp. MN54 assisted phytoremediation of diesel and plant growth promotion of maize in hydrocarbons contaminated soil. *Agronomy* 2021; 11(9): 1795. doi: 10.3390/agronomy11091795
67. Ud Din MM, Khan MI, Azam M, et al. Effect of biochar and compost addition on mitigating salinity stress and improving fruit quality of tomato. *Agronomy* 2023; 13(9): 2197. doi: 10.3390/agronomy13092197
68. Cao Y, Gao Y, Li J, Tian Y. Straw composts, gypsum and their mixtures enhance tomato yields under continuous saline water irrigation. *Agricultural Water Management* 2019; 223: 105721. doi: 10.1016/j.agwat.2019.105721
69. Hameeda, Gul S, Bano G, et al. Biochar and manure influences tomato fruit yield, heavy metal accumulation and concentration of soil nutrients under wastewater irrigation in arid climatic conditions. *Cogent Food and Agriculture* 2019; 5(1): 1576406. doi: 10.1080/23311932.2019.1576406