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MODULATORY EFFECTS OF BIOCHAR AND WOOD VINEGAR ON *SCLEROTIUM ROLFSII* COLONIZATION AND MULTIPARTITE INTERACTIONS OF *TRICHODERMA* SPP. IN AGRICULTURAL SOIL: A REVIEW

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ABSTRACT

Rhizosphere colonization is the critical first step for soilborne pathogens and beneficial microbes alike. Biochar, a porous by product of biomass pyrolysis, creates a favorable microhabitat in the rhizosphere, enhancing microbial activity and suppressing pathogens like *Sclerotium rolfsii*. Unlike sterile soils, unsterilized soils better reflect natural conditions, supporting dynamic microbial interactions. *Trichoderma* spp. notable biocontrol agents, exhibit antagonism toward pathogens and promote plant growth, especially when supported by biochar amendments. Tripartite interactions among *Trichoderma*-plant-pathogen reveal synergistic mechanisms of biocontrol and systemic resistance. Formulations combining biochar and microbial agents represent a sustainable strategy for disease management and soil health improvement.
***Keywords*:** Biochar, Wood Vinegar, *Sclerotium rolfsii*, Multipartite Interactions, *Trichoderma* spp.

Introduction

Rhizosphere colonization is initial step in the pathogenesis of soilborne pathogens. For successful colonization, a microbe must efficiently compete for the nutrients available in the rhizosphere. Biochar, a charcoal-like substance produced from the pyrolysis of biomass, significantly influences the rhizosphere the narrow region of soil directly influenced by plant roots. While not a direct food source for microbes (Bonanomi *et al.*, 2015; Kolton *et al.*, 2011), its porous structure provides a unique habitat for beneficial microorganisms. This creates a refuge, protecting them from predators and fostering a favorable environment for their growth and activity (Lehmann *et al.*, 2011). This enhanced microbial activity within the rhizosphere can improve nutrient cycling, enhance nutrient uptake by plants, and suppress the growth of certain soilborne pathogens (Bonanomi *et al.*, 2015). Furthermore, biochar's ability to improve soil structure, water retention, and aeration within the rhizosphere can enhance root growth, leading to increased plant vigor and overall productivity.

Studies on Microbial growth in their natural habitat (unsterilized soil)

Plants in both natural and agricultural settings are constantly exposed to a wide variety of microorganisms, leading to the colonization of roots and the rhizosphere. This process is thought to involve a complex web of simultaneous and dynamic interactions.

Sterilization of soils alters key properties, such as nutrient dynamics, organic matter breakdown, and microbial interactions (He & cui 2009). Some of the nutrients being heat labile get denatured during sterilisation process. Disruption of soil structure and elimination of competition skew results. Complementary approaches, including non-sterile conditions, ensure accurate assessment of microbial functions.

Ekundayo *et al*., 2018 studied that non-sterile soil enhances okra growth and yield compared to sterile soil, despite taller plants in sterile conditions. The presence of soil microbiota in non-sterile soil supports nutrient cycling and disease suppression, emphasizing its ecological importance. Sterile soil lacks these benefits, making it unsuitable for maximizing productivity.

Effect of biochar and wood vinegar amended soil on colonization of *Sclerotium rolfsii*

Biochar is a heterogeneous material produced through pyrolysis, a thermal process that occurs at temperatures between 200°C and 900°C under limited oxygen conditions. It is derived from various organic materials like municipal waste (Mitchell *et al*., 2013), crop residues (Yuan *et al*., 2011), wood (Spokas and Reicosky, 2009), sewage sludge (Mendez *et al*., 2012), manure (Uzoma *et al*., 2011), and also animal bones (Vassilev *et al*., 2013) and is widely used for soil improvement, carbon sequestration, and environmental remediation. The International Biochar Initiative defined biochar as "a solid material obtained from the thermo-chemical conversion of biomass in an oxygen limited environment" (IBI, 2012). Biochar can be effective against both airborne (e.g. *Botrytis cinerea*, different species of powdery mildew) and soilborne pathogens (e.g. *Fusarium* spp., *Rhizoctonia solani, Phytophthora* spp.) Bonanomi *et al*., 2015. Five different mechanisms have been proposed by Bonanomi *et al*., 2015 to explain biochar disease suppression: (i) modification of soil quality in terms of nutrient availability and abiotic conditions such as liming effect (ii) induction of systemic resistance in host plants (iii) enhanced abundance and activities of beneficial microbes, including mycorrhizal fungi (iv) direct fungitoxic effect of biochar (v) sorption of allelopathic, phytotoxic compounds that can directly harm plant roots and thus promote pathogen attacks.

Bonanomi *et al*., 2015 investigated the growth of *Fusarium oxysporum*, *Aspergillus niger*, *Penicillium italicum*, and *Rhizoctonia solani* on extracts from *M. sativa* and wood. The study found that these fungi thrived on *M. sativa* extracts but exhibited reduced growth on wood extracts, with biochar showing significant inhibition of microbial growth. The addition of simple carbon sources, such as potato dextrose broth, to biochar extracts partially or fully restored microbial growth on both *Medicago sativa* hay and wood biochar. These findings suggest that the inhibitory effects of biochar are mainly due to a lack of readily degradable carbon sources, while phenolic compounds have a lesser impact.

Jaiswal *et al*. (2015) reported that various types of biochar reduced damping-off caused by *Rhizoctonia solani* in *Phaseolus vulgaris*. However, both *in vivo* and *in vitro* experiments indicate that biochar has little to no direct inhibitory effect on *R. solani*.

Manasa *et al*., 2024 studied biochar's effects on groundnut stem rot caused by *Sclerotium rolfsii* and its influence on soil and microbial properties. Biochar (0%, 1%, 3%, 5%) indirectly suppressed the pathogen by reducing sclerotial body production. Treatments delayed disease onset and slowed progression, with genotypes ICGV 171002 and ICGV 181035 showing superior control at 3% and 5% concentrations. Field trials confirmed no significant difference between these concentrations but emphasized biochar's soil benefits, including improved nitrogen, phosphorus, potassium, organic matter, pH, and electrical conductivity. The findings highlight biochar's potential in disease management and soil fertility enhancement.

Gour and Sharma (2010) reported that *S. rolfsii* exhibited growth across a pH range of 4 to 9, with maximum growth at pH 6.0 (87.00 mm), followed by pH 5.0 (76.67 mm). The lowest growth occurred at pH 9.0 (28.67 mm) and pH 8.0 (40.33 mm). The optimum pH for growth lies between 6 and 7. Shridha *et al*., 2013 reported that pH 5.0 was found to be optimal for mycelial growth of *S. rolfsii*. For sclerotia production, pH 4.0 to 7.0 was most favorable. The maximum radial growth occurred at pH 6.5, followed by pH 6.0 and 7.0, while the highest sclerotial formation was observed at pH 7.0.

Bipartite interaction study

Any condition under which, or practice whereby, survival and activity of a pathogen is reduced through the agency of any other living organism (except man himself) with the result that there is a reduction in the incidence of the disease caused by the pathogen Garrett (1965).

***Trichoderma*-Pathogen interaction**

Biological control agents (BCAs) use various mechanisms to suppress pathogens that cause diseases are antibiosis, mycoparasitism, competition, induced systemic resistance, production of hydrolytic enzymes, siderophore production, volatile organic compounds production, biofilm formations. The mechanisms employed by biological control agents for managing plant diseases involve a combination of direct antagonism against pathogens and the enhancement of plant defenses. The effectiveness of BCAs often depends on factors such as environmental conditions, the specific pathogens involved, and the compatibility of the BCAs with the plant host. Utilizing BCAs contributes to sustainable agriculture by reducing reliance on chemical pesticides and fostering healthier ecosystems. The genus *Trichoderma* comprises mycoparasitic fungi like *Trichoderma harzianum*, widely recognized for their biocontrol properties against various plant diseases. These fungi can effectively combat both foliar pathogens like *Botrytis cineria* and soil-borne pathogens like *Rhizactonia solani* and *Sclerotium rolfsii*.

Naïma *et al*., 2004 studied Seventy *Trichoderma* spp. isolates from Morocco were tested against *Sclerotium rolfsii*. While 52% inhibited its mycelial growth moderately (45-55%), most (84%) had slight effects on sclerotia viability. Four *Trichoderma harzianum* isolates (Nz, Kb2, Kb3, Kf1) showed strong antagonistic activity and competitiveness in natural soil, making them promising biocontrol candidates.

An *in vitro* study by Padmaja *et al*., 2013 tested ten native *Trichoderma* isolates against *Sclerotium rolfsii*, finding eight isolates inhibited pathogen growth by 60% to 80%. The native isolates outperformed a commercial formulation, demonstrating potential for biocontrol applications against phytopathogens through their volatile and non-volatile compounds.

Swathi *et al*., 2015 the evaluation of two *Trichoderma* isolates, *T. harzianum* Th4 and *T. virens* Tv5 demonstrated distinct antagonistic properties against *Sclerotium rolfsii*. The isolate Th4 showed rapid radial growth, simultaneous lysis, and overgrowth on *S. rolfsii*, while Tv5 exhibited slower radial growth followed by subsequent lysis.

Ayyandurai *et al*., 2022 studied Six *Trichoderma* spp. were evaluated against *Sclerotium rolfsii* using dual culture and paired plate assays. *T. longibrachiatum* T (SP)-20 showed the highest inhibition (84.44%), followed by *T. asperellum* T(AR)-10. Both effectively reduced mycelial growth via microbial VOCs, including plant growth-promoting compounds, highlighting *T. longibrachiatum's* strong biocontrol potential.

Trichoderma-Plant interaction

Biochar amendments can indeed stimulate the growth and activity of various beneficial microbes in the soil, which helps protect plants from pathogen attacks. These microbes include bacteria, fungi, and other microorganisms that promote plant health by outcompeting pathogens, producing antimicrobial compounds, or inducing plant defense mechanisms. The porous structure of biochar provides a favorable habitat for these beneficial microbes, enhancing their ability to colonize the soil and the plant's rhizosphere, thus contributing to a more resilient and disease-resistant crop. Both mycorrhizal fungi and bacteria that are beneficial to plants are able to effectively exploit biochar porous structure (Downie *et al*., 2009) to find refuge from predators like protozoans, mites, collembolan and nematodes (Warnock *et al*., 2007).

Multiple studies have demonstrated that biochar applications can enhance the presence of mycorrhizal fungi (Warnock *et al*., 2007), promote the growth of plant-beneficial microbes (Graber *et al*., 2010; Kolton *et al*., 2017) and increase microbial biomass (Liang *et al*., 2010), accompanied by shifts in microbial community function.

Novak *et al*., 2012 evaluated nine biochars' effects on soil-water storage. Biochars (≥500°C) enhanced surface area, nutrients, and water retention, particularly switchgrass biochars in Norfolk soil. They improved pot-holding capacity, reduced bulk density, and increased moisture retention in Ultisols and Aridisols, confirming biochar's potential to enhance soil-water storage.

Jaiswal *et al*., 2017 Biochar amendment to soil suppressed *Fusarium* crown and root-rot in tomatoes while enhancing plant growth. It reduced *Fusarium* root colonization and promoted beneficial microorganisms. Biochar increased microbial taxonomic and functional diversity, activity, and carbon-source utilization, contributing to disease suppression and improved plant performance, as supported by 16S rRNA sequencing.

The study conducted by Sani *et al*., 2020 demonstrated that co-application of *Trichoderma* and biochar increased tomato yield by 101.45% and 11.33% compared to half-dose and standard N-P-K treatments, respectively. This was accompanied by a significant increase in mineral content, antioxidants, and total soluble solids. The synergistic effect of *Trichoderma* and biochar in enhancing soil health, nutrient uptake, and plant growth offers a promising sustainable approach to tomato cultivation.

Tripartite(*Trichoderma*-Pathogen-Plant) interaction study

Biocontrol strategies against fungal plant pathogens involve complex interactions between plants, pathogens, and microbial communities.

Trichoderma, a genus of fungi widely used as biocontrol agents, exhibits antagonistic activity against pathogens through mechanisms like the production of secondary metabolites and cell wall-degrading enzymes (Dennis and Webster, 1971). Studying the tripartite interaction between *Trichoderma*, the plant, and the pathogen is crucial to understand the mechanisms underlying their beneficial association. Researchers employ various approaches, including genomics, proteomics, and metabolomics, to identify the molecular factors involved in mutual recognition and signaling between these organisms. This knowledge will facilitate the development of improved strategies for utilizing *Trichoderma* as biocontrol agents in sustainable agriculture.

Macías-Rodríguez *et al.* (2018) research investigated the interaction between *Trichoderma atroviride* and tomato plants, focusing on the role of root-exuded carbohydrates in facilitating *Trichoderma* colonization and biocontrol of *Phytophthora cinnamomi*. The study found that the composition of root exudates changed during *Trichoderma* colonization, with sucrose appearing only during this stage. These sugars likely provided a nutritional source for *Trichoderma*, enhancing its growth and enabling it to outcompete *P. cinnamomi* for resources, ultimately suppressing the pathogen.

Mukherjee *et al.* (2019) recently reported the development of a mutant strain of *Trichoderma virens* (G2). Following extensive field evaluations including replicated micro-plot trials, on-farm demonstration trials, and large-scale trials in farmers' fields across multiple locations in India the formulation based on this mutant, named TrichoBARC, was tested for managing collar rot (*Sclerotium rolfsii*) in chickpea. The results consistently showed enhanced seed germination, reduced seedling mortality, and improved plant growth and yield. Additionally, TrichoBARC-treated chickpea plants, as well as several vegetables, exhibited enhanced growth, increased pod development, and earlier flowering by 7–10 days.

A study by Umadevi & Anandraj 2019 investigated defense responses in black pepper plants primed with *Trichoderma harzianum*, a biocontrol fungus, followed by infection with *Phytophthora capsici*, a fungal pathogen. Three scenarios were analyzed: black pepper with *T. harzianum*, black pepper with *P. capsici*, and all three together. Proteins uniquely present in the three-way interaction were identified as "*Trichoderma*-induced resistance proteins." These included 18 reactive oxygen species-related proteins and 22 defense-related proteins, suggesting their role as potential markers. The study also found enhanced ethylene synthesis, isoflavonoid pathway activation, and lignin synthesis. Notably, early systemic resistance was observed in leaves as early as 72 hours after root priming with *Trichoderma*.

Development of modulator-based *Trichoderma* formulation

Studies showed that a superior formulation increases microbial activity after being inoculated into the host plants at a quicker rate and increases their quantity in soil (Arora *et al*., 2010). The ability to deliver nutrients, an easily-changeable pH, the utilisation of an appropriate low-cost raw material in adequate amounts and availability are some fundamental characteristics of a successful formulation (Catroux *et al*., 2001; Herrmann and Lesueur, 2013).

Zaidi and Singh, 2004 *Trichoderma harzianum* (TH) and *Pseudomonas fluorescens* (PsF) are widely used biocontrol agents for managing plant diseases. A major challenge in biological control is the lack of efficient methods for mass multiplication and delivery. Both organisms multiply effectively on cow dung or farmyard manure (FYM) under laboratory conditions. A simple system involving the addition of TH and PsF into decomposed FYM under shade or compost pits at 25–32°C ensures sufficient population growth. Colonized FYM enhances disease protection and improves crop growth compared to untreated FYM. This technology has gained acceptance among farmers, especially in Uttaranchal and Uttar Pradesh.

Devi and Paul, 2008 The population dynamics of *Trichoderma harzianum* (JMA-4), a biocontrol agent against pea root rot wilt complex, were studied under varying moisture, temperature, and pH conditions. Maximum population (71.0×10^6 cfu/g soil) occurred at 35% moisture, followed by 30% moisture. The optimal temperature for growth was 25°C, with higher temperatures (30–35°C) inhibiting growth. The highest population (142.3×10^6 cfu/g soil) was recorded at pH 6.6, followed by pH 5.4. Alkaline pH levels were detrimental to the growth of *T. harzianum*, indicating it thrives best in slightly acidic to neutral conditions.

Niranjana *et al*., 2009 study focused on mass-multiplying *Trichoderma harzianum* and *Pseudomonas fluorescens* using agricultural waste products like wheat bran, rice bran, paddy straw, and neem cake. Ten isolates of each biocontrol agent were isolated from pigeonpea rhizosphere soil and evaluated for their ability to improve seed quality and inhibit *Fusarium udum* growth. Isolates 4 (*T. harzianum*) and 3 (*P. fluorescens*) were selected for mass multiplication, with boiled rice bran proving to be the most effective growth medium. Talc and sodium alginate formulations of the biocontrol agents successfully reduced fusarium wilt and improved seedling emergence under greenhouse conditions.

A study by Postma *et al*. (2013) demonstrated that biochar derived from animal bones can serve as an effective carrier for beneficial biocontrol agents. Scanning electron microscopy showed that bacteria such as *Pseudomonas chlororaphis*, *Bacillus pumilus* and *Streptomyces pseudovenezuelae* were able to extensively colonize the porous structure of the biochar. When this microbe-enriched biochar was introduced into the soil, it successfully suppressed diseases caused by *Fusarium oxysporum* f. sp. *lycopersici* and *Pythium aphanidermatum* in tomato plants. The porous architecture of the biochar creates a favorable habitat for these microbes, enhancing their survival and ability to combat plant pathogens while supporting overall plant health.

The antimicrobial activity of wood vinegar from cocoa pod shells against *Candida albicans* and *Aspergillus niger* was studied by Desvita and Faisal, 2022. Wood vinegar derived from cocoa pod shells exhibits antimicrobial properties against *Candida albicans* and *Aspergillus niger*. The study found that higher concentrations of wood vinegar resulted in increased inhibition zone diameters, ranging from 6 to 6.14 mm, indicating its potential as a natural antimicrobial agent.

Debode *et al*., 2024 showed biochar exhibited variable efficacy as a *Trichoderma* carrier. While a subset of biochars, such as B49_450_fax, maintained *Trichoderma* viability, a significant proportion exhibited a substantial decline in fungal populations, with some biochars experiencing a reduction of over 100-fold. These findings suggest that biochar properties, including porosity, surface area, and nutrient content, play a critical role in influencing *Trichoderma* colonization and persistence. Optimizing these factors, such as increasing the specific surface area or incorporating additional nutrients, is crucial for maximizing the potential of biochar as a sustainable carrier for biocontrol agents.

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