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Evaluation of Biocontrol Agents and Botanicals Against *Fusarium oxysporum* Under *in vitro* Conditions

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Abstract: ***The ongoing struggle to maintain sustainable crop production underlines a crucial turning point for global agriculture, especially in managing soil-borne diseases like Fusarium-induced damping-off. It is evident that conventional chemical control, while once effective, has become ecologically and economically untenable due to resistance development, ecosystem disruption, and health hazards. This study offers a refreshing perspective by combining biological control agents, organic amendments, and botanicals into a cohesive management framework. The integration of Trichoderma spp. and Pseudomonas spp. with organic carriers such as farmyard manure and vermicompost demonstrates how ecological interactions can be harnessed for disease suppression. This suggests that the success of biological interventions depends not only on antagonistic potential but also on their adaptability within the rhizosphere ecosystem. Laboratory and greenhouse trials further reveal that botanicals like Azadirachta indica, Eucalyptus globulus, and Ocimum basilicum possess notable inhibitory effects on Fusarium spp., emphasizing the untapped potential of plant-derived compounds. Taking this further, the study bridges microbiology, ecology, and agronomy to propose a resilient, environmentally responsible alternative to chemical fungicides, aligning well with modern principles of integrated pest management and sustainable soil health restoration.***

Keywords: Fusarium damping-off, biological control, Trichoderma, organic amendments, sustainable agriculture

1. Introduction

The global challenge of securing food production necessitates the adoption of resilient and ecologically sound agricultural practices. Despite significant advancements in crop yield, the vulnerability of crops, particularly during the critical seedling stage, remains a major bottleneck. Soil-borne plant pathogens are responsible for substantial quantitative and qualitative losses in agriculture worldwide, often attacking young plants before or immediately after emergence. Among these devastating agents, the genus *Fusarium* stands out as a pervasive and economically damaging threat, causing a suite of diseases including wilts, root rots, and, most notably for this study, damping-off.

Damping-off is an important disease world widely, which affects all agricultural and forestry crops, both in nurseries and fields. It may affect from 5 to 80% of the seedlings, thereby inducing heavy economic consequences for farmers (Lamichhane *et al.,* 2017). Damping-off disease leads to the decay of germinating seeds and young seedlings, which is one of the most important yield constraints both in nurseries and fields.

Historically, the primary line of defense against soil-borne diseases has been the application of synthetic chemical fungicides, often employed as seed treatments or soil drenches. While effective in the short term, this dependence has introduced a cascade of negative consequences that are now widely recognized. These include the rapid evolution of fungicide-resistant pathogen strains, environmental pollution affecting non-target soil and aquatic ecosystems, and serious health risks to farm workers and consumers due to chemical residues. Furthermore, repeated application of broad-spectrum chemicals disrupts the indigenous beneficial soil microflora, ultimately reducing the soil's natural suppressive capacity and leading to a cyclical reliance on chemical interventions. This ecological and economic unsustainability necessitates a pivot toward integrated and biologically driven control strategies.

This study investigates a holistic and synergistic approach to manage *Fusarium* damping-off by integrating three key components of sustainable agriculture: biological control agents (BCAs), organic amendments, and botanicals. The first component involves the selection and enrichment of antagonistic microorganisms, such as certain strains of *Trichoderma* or *Pseudomonas*. These BCAs function through multiple mechanisms, including mycoparasitism, antibiosis (secretion of growth-inhibiting compounds), and inducing systemic resistance (ISR) in the host plant, effectively creating a biological shield against the pathogen.

The success of BCAs in the field is heavily dependent on their delivery system and ability to colonize the rhizosphere. Therefore, the second critical component involves the use of farmyard manure (FYM) and vermicompost as organic carriers. These amendments are not merely inert carriers; they act as a nutritional substrate, enhancing BCA proliferation and survival. Moreover, the inherent qualities of vermicompost and well-decomposed FYM, rich in humic substances and beneficial native microflora, contribute to the development of a biologically suppressive soil environment, thereby enhancing seedling vigor and resilience.

1.1 Isolation of *Fusarium* spp.

Pathogen was isolated from wilt infested tomato plants collected from parts of Assam which is located in the North East region of India. Morphological identification of *Fusarium* isolates was done using conidial and hyphal structures. Molecular identification of *Fusarium* isolates was done by amplifying the internal transcribed spacer (ITS) region of the conserved ribosomal DNA using primers (ITS1 and ITS4) (Singha *et al.,* 2016)

The most prevalent pathogenic fungi in tomato plants were found to be *F.* solani and *R. solani*, the causal organisms of damping off and/or root rot diseases. These were isolated from naturally infected tomato roots and rhizosphere (Karima and Nadia, 2012).

From Uttar Pradesh, India, various isolates of *Fusarium* species were found in diseased tomato plant samples and were morphologically and molecularly identified. Pathogenicity test revealed that some of them were *F. oxysporum*. (Joshi *et al.*, 2013).

Infected tomato plant roots were gathered from tomato fields in Punjab province, Pakistan, where *F. oxysporum* f. sp. *lycopersici* (Fol) isolates were discovered. The disease index was computed after evaluation of 230 different tomato varieties and pathogen isolates combinations (Akram *et al.,* 2014)

From diseased tomato samples gathered from 10 different Indian states, 14 strains of *Fusarium* were found. The morphology and ITS rDNA sequencing used to characterise the fungal cultures showed that they belonged to *F. oxysporum* f. sp. *lycopersici* (Murugan *et al.,* 2020).

Four isolates of *Trichoderma* spp. and one pathogenic fungus responsible for tomato seedling damping-off disease are isolated and identified. Using the polymerase chain reaction (PCR) method, these isolates were molecularly identified by identifying the nucleotide sequences of the PCR-amplified products (Odeh *et al.,* 2021)

The occurrence of pathogenic *F. oxysporum* f. sp. *lycopersici* in wilted tomato seedlings was demonstrated by *in vitro* isolation and identification using suitable methodology (Ignjatov *et al.*, 2012).

The three soil-borne pathogens were isolated from previously planted tomato crop and identified as *F. oxysporum* f. sp. *lycopersici*, *Ralstonia solanacearum*, and *Sclerotium rolfsii*. Pathogen's pathogenicity was evaluated against the host plant, and discovered that tomato plants were susceptible to infection (Patil *et al.,* 2021)

Two pathogenic fungus, *F. solani* and *R. solani*, isolated and molecularly identified as being responsible for *Cucumis sativus* (cucumber) root rot and seedling damping-off disease. Moreover, *in vitro* testing and a greenhouse pot experiment were used to assess the effectiveness of *Pseudomonas fluorescens* and *Bacillus subtilis* (Al-Fadhal *et al.,* 2019)

In Ogbomoso, Nigeria, researchers isolated and tested the pathogenicity of fungus linked to tomato and eggplant seedlings affected with the damping-off disease. As a consequence, it was determined that five fungal isolates were linked to the damping off disease that affects tomato and eggplant seedlings. These fungi included *M. pheseolina*, *S. roffsii*, *R. solani*, *F. oxysporum*, and *F. solani* (OJO *et al.,* 2015)

1.2 *In vitro* testing of biocontrol agents for the control of *Fusarium* spp.

Trichoderma species were tested for their antagonistic impact on *F. oxysporum* f. sp. *lycopersici*. Pathogen had the smallest radial growth (20.93 mm) when treated with *T. koningiopsis*. It shows 60.45% growth suppression followed by *T. viride* (55.16%). *T. harzianum* was the least successful with a reduction of 51.44% (Kumar *et al.,* 2021)

In vitro, antagonists evaluated against *F.* oxysporum f. sp. *lycopersici* inhibited pathogen development. *T. harzianum* (52.33%) and *T. virens* (49.41%) were determined to be the most effective with the lower mycelial growth of pathogen (Gadhave *et al.,* 2020)

Seven *Trichoderma* spp. isolates (T1–T7) from Egypt were bioassayed *in vitro* for their ability to inhibit *F. oxysporum* f. sp. *lycopersici*. The results showed that the evaluated *Trichoderma* spp. exhibited varying percentages of inhibition against the tested pathogen, ranging from 51.83% (T6) to 66.80% (T7) (Sallam *et al.,* 2019).

T. viride was found to be superior among the fungal biocontrol agents as compared to the remaining three species, inhibiting the growth of the pathogen by 84.84 percent, followed by *T. harzianum* (72.54%), *T. hamatum* (69.93%), and *T. koningii* (61.49%), and among the two bacterial species, *B. subtilis* was found to be effective in inhibiting the growth of the pathogen by 79.25% as compared to *P. fluorescens* which showed 62.36% (Brujal *et al.,* 2021).

Bio-agents *T. viride*, *T. harzianum*, and *P. fluorescens* against *F. oxisporum* were examined *in vitro* where in *T. viride* gave the best results among all the treatments followed by *T. harzianum* (Pandey *et al.,* 2021).

T. viride, *T. harzianum*, *B. subtillis* and *P. fleuroscence* were evaluated against *F. udum*. *P. fleuroscence* was the most efficient with the highest mycelial inhibition (77.21%) of the testn pathogen, followed by *B. subtillis* with 73.48% and *T. harzianum* with 66.60% inhibition, and *T. viride* with mycelial inhibition of 65.95% (Arsia *et al.,* 2018).

T1- *T. harzianum*, T2- *T. viridae*, and T3- *Aspergillus niger* are the three bio-agents tested against *F.* oxysporum f. sp. *lycopersici in vitro. T. harzianum* showed the highest percent growth inhibition (52.60%), followed by *T. viride* (47.94%) and *A. niger* (38.90%) (Siddique *et al.,* 2019).

Trichoderma species were isolated from rhizosphere soil of tomato plants. Higher antagonistic effectiveness against the pathogen was seen in the *T. atroviride* isolate. *T. atroviride* and *T. atroviride* shown antagonistic action against *F. oxysporum*, with inhibiting mycelial growth by 77.77% and 71.25% respectively (Yogalakshmi *et al.,* 2021)

Effectiveness of the *T. viride*, *A. niger*, and *T. harzianum* were tested against *F.* oxysporum f. sp. *lycopersici*. *T. harzianum* and *T. viride* showed the mycelial reduction 75.29% and 83.56% respectively (Jamil *et al.,* 2021).

The biochemical and molecular characteristics of 21 endophytic bacterial (*Bacillus*) strains were determined, and they were then dual culture-screened against *F. oxysporum* f. sp. *lycopersici*. Strain FZB24 showed the highest percentage of inhibition (48.3%) of *F. oxysporum* f. sp. *lycopersici* (Elanchezhiyan *et al.,* 2018)

A total of 136 bacterial strains from the tomato rhizosphere were tested for their antagonistic behaviour towards *Ascochyta* sp. and *F. oxysporum* f. sp. *lycopersici* (Fol). The top five strains with the highest inhibition efficiency were chosen. The results demonstrated that volatile chemicals slowed the growth of plant pathogens, with average inhibition rates of 55% for Fol and 17% for *Ascochyta*. For filtrates, inhibition rates against Fol and *Ascochyta* sp. were 33.01% and 33.74%, respectively (Rafanomezantsoa *et al.,* 2022)

Pseudomonas spp. isolated from tomato rhizosphere soil suppressed the development of phytopathogens that cause root rot and damping-off in tomatoes. There is increased plant population and rise in root dry weight under field conditions (Hammami *et al.*, 2013).

In vitro evaluation was done on the effectiveness of saprophytically competitive biocontrol agents against *Fusarium* spp. using the dual culture approach. The effectiveness of six biocontrol agents, including *T. viride, Gliocladium virens, T. harzianum, T. hamatum, A. niger,* and *P. fluorescens,* against *Fusarium* spp. was tested. With *T. viride, T. harzianum, T. hamatum, A. niger,* and *A. flavus* which were at per among themselves however the pathogen's growth was remarkably suppressed. *P. fluorescens* showed the least amount of inhibition (Singh *et al.,* 2014)

The study was conducted using a completely randomized design (CRD) with six treatments, *T. viride* at 2%, *P. fluorescens* at 2%, *T. harzianum* at 2%, neem leaf extract, datura leaf extract, each at 10%, and carbendazim at 0.2%. The effectiveness of these treatments against the fungal pathogen *F. oxysporum* was evaluated using the dual culture and poison food techniques on PDA medium with four replications. Results indicated that *T. viride* and *T. harzianum* establish a distinct zone of inhibition. Microscopic observation revealed that the hyphae of the *Trichoderma* fungi encircled and coiled around the hyphae of *F. oxysporum*. (Choudhary *et al.,* 2016)

1.3 *In vitro* testing of botanicals for the control of *Fusarium* spp.

Various plant extracts were evaluated against *F. oxysporum* f. sp. *dianthi in vitro* by using poisoned food approach. Neem leaf extracts showed the least pathogen development and had an outstanding inhibitory effect, reducing the pathogen's growth by 78.19% compared to control. In terms of merit, Eucalyptus extract came in second place (75.87%), followed by Ashoka extract (72.48%), *Calotropis* extract (65.22%), and others (Sunderrao *et al.,* 2017)

Using plant extracts from fifteen different plants, an *in vitro* antifungal experiment was performed against *F.* oxysporum f. sp. *Lycopersici* (Fol). The antifungal effect of the extracts at four different concentrations (10, 20, 40, and 60) on the mycelial growth of Fol was assessed. Out of fifteen plants—*Solanum indicum* (78.33), *Azadirachta indica* (75.00), and *Oxalis latifolia* (70.33) found to have the capacity to slow the growth of the Fol (Anil Kumar *et al.,* 2015)

The effectiveness of plant leaf extracts of *Azadirachta indica, Pongamia pinnata, Parthenium hysterophorus, Calotropis gigantia,* and *Annona squamosal* against *Fusarium* spp. was examined *in vitro*. *A. indica* leaf extracts notably had the lowest pathogen growth rate, followed by *P. pinnata, P. hysterophoras, C. gigantia,* and *A. squamosa* leaf extracts (Singh *et al.,* 2014)

Adhatoda vasica, Eucalyptus globulus, Lantana camara, Nerium oleander, and *Ocimum basilicum* were tested against *F.* oxysporum f. sp. *Lycopersici*. According to the findings, *O. basilicum* and *E. globulus* cold distilled water extracts were the most successful in preventing the development of pathogen (Isaac and Abu-Tahon, 2014).

Twelve different plant extract were prepared and evaluated against the pathogen in lab conditions. Out of tested plant extracts neem extract resulted the lowest mycelial growth at 8 percent followed by thorn apple (Poussio *et al.,* 2022).

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