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Comparison of Antagonistic Activity of *Pseudmonas fluorescens* and *Trichoderma viride* against Selected Species of Fungal Pathogens

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Authors' contributions

This work was carried out in collaboration between both authors. The first author PSS performed the research work and wrote the initial draft of manuscript. The corresponding author VJ designed the research problem and corrected the final format of manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The antagonistic activities of *Pseudomonas fluorescens* and *Trichoderma viride* have been evaluated by a number of studies. This research work was aimed to compare the antagonistic activities of *Pseudomonas fluorescens* and *Trichoderma viride* against selected species of plant fungal pathogens *viz*, *Pythium aphanidermatum, Fusarium oxysporum, Alternaria alternata and Aspergillus niger.*
Study Design: *In vitro* assay of antifungal activity.
Methodology: Dual culture method is conducted to compare the antagonistic activities of the bio control agents like *Pseudomonas fluorescens* and *Trichoderma viride* against selected species of fungal pathogens *viz*, *Pythuim aphanidermatum, Fusarium oxysporum, Aspergillus niger* and *Alternaria alternata*. Three replicates were maintained for each treatment and mean percent

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inhibition of radial growth of pathogen in dual culture plate was recorded. Statistical analysis was done to know the significance of comparison.
Results: Out of the four fungal pathogens *Fusarium oxysporum* showed the highest inhibition of radial mycelial growth in the presence of both *Pseudomonas fluorescens* (49.41±0.4%) and *Trichoderma viride* (85.7±0.3%). *Aspergillus niger* recorded the least value. It was found that *Trichoderma viride* exhibited comparatively greater antagonistic activity compared to *Pseudomonas fluorescens.*
Conclusion: *In vitro* studies suggested that both the bio controls were effective against four fungal pathogens under the study and are promising biological control for *Fusarium oxysporum.*

Keywords: Pseudomonas fluorescens; Trichoderma viride; dual culture technique; percent inhibition.

1. INTODUCTION

Plant pathogens continue to reduce the availability of food resources on a global scale as well as to diminish the economic potential of greenhouse and nursery industry [1,2]. Fungi such as *Pythium aphanidermatum* (Edson) Fitzp*, Fusarium oxysporum* Schlecht. emend. Snyder & Hansen*, Alternaria alternate (*Fr.) Keissl and *Aspergillus niger* Van Tieghem are some of the major ones among them. *Pythium* species are worldwide in distribution [3] that attacks the cuttings, seeds, seedlings and all stages of the various crops causing significant losses to them. *Aspergillus niger* is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles and other decaying vegetation. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions and peanuts and is a common contaminant of food. It is ubiquitous in soil. They are geographically widely distributed and have been observed in a broad range of habitats because they can colonize a wide variety of substrates [4]. The soil-borne fungus, *Fusarium oxysporum* is the causal agent of vascular wilt, a disease that affects a large variety of economically important crops worldwide [5]. *Alternaria alternata* causes leaf spots and blight on a large variety of agricultural and horticultural crops such as tomato potato, carrot, cauliflower, broccoli, cabbage, pepper, beans, apple, peach and citrus species. Moreover, *A. alternata* can also attack a several weeds and ornamental plants [6].

The most common means to check the spread of these fungal pathogens is the use of chemical fungicides. But their frequent use leads to various environmental pollutions, health hazards and the elimination of several non-target beneficial fauna. The increasing awareness of common people about the detrimental effects of these toxic chemicals has emphasized the need for adopting an alternative method to tackle these problems. Biological methods of controlling these fungal pathogens have gained immense importance in the present scenario as they possess very little side effects.

Biological control as a concept and approach to the control the plant pathogens has been studied intensively over the past few decades. Antagonistic fungi especially *Trichoderma viride* and the bacteria *Pseudomonas fluorescens* have been widely used against a number of phyto pathogens [7]. In the view of above and the growing importance of biological control agents, the present study was carried out to compare the antagonistic activities of bio control agents like *Pseudomonas fluorescens* and *Trichoderma viride* against four selected phytopathogenic fungi such as *Pythium aphanidermatum, Fusarium oxysporum, Alternaria alternata* and *Aspergillus niger.*

2. MATERIALS AND METHODS

2.1 Collection and Maintenance of Pure Cultures

Pure broth culture of both *Trichoderma viride* and *Pseudomonas fluorescens* and all the fungal cultures namely *Pythium aphanidermatum, Fusarium oxysporum, Alternaria alternata and Aspergillus niger* used in the present study were supplied from the repository of the Department of Plant Pathology, Agriculture University, Vellanikara, Thrissur, Kerala, India. All the cultures were stored, sub cultured and maintained at Department of Botany, St. Thomas' College (Autonomous), Thrissur, Kerala, India.

Pseudomonas fluorescens was sub cultured in King's B medium (Hi-Media laboratories, India). All other fungal cultures including *Trichoderma*

viride, Pythium aphanidermatum, Fusarium oxysporum, Alternaria alternata and Aspergillus niger was sub cultured in Potato Dextrose Agar (PDA, Hi-Media laboratories, India).

2.2 Antagonistic Activity of *Trichoderma viride*

About 5-days old culture mycelial disc (5 mm) from *Trichoderma viride* and test pathogen were placed on the PDA plate opposite to each other equidistant from the periphery and were incubated at 25℃. Petri dishes inoculated with fungal disc alone served as control. Three replications were maintained for each isolate. After 6 days of the incubation period, radical growth of pathogen was recorded and percentage inhibition was calculated in relation with control [8].

Percent inhibition (I) =(C-T)/C ×100

Where,

C= radial growth of mycelia of pathogen in control.
T= radial growth measurement of pathogen in the presence of antagonists.

2.3 Antagonistic Activity of *Pseudomonas fluorescens*

The antagonistic activities of *P. fluorescens* against fungal pathogens were tested by dual culture technique. Bacteria were streaked at one side of the Petri dish (one cm away from the edge) containing PDA medium. Mycelia disc (9 mm) from 7 day old PDA culture of fungal pathogen was placed at the opposite side of the Petri dish perpendicular to the bacterial streak respectively and incubated at 27+2℃ for 6 days. Petri dishes inoculated with fungal disc alone served as control. Three replications were maintained for each isolate. Observations on mycelial growth of test pathogen were recorded and percent inhibition of pathogen growth was calculated [9].

Percent inhibition (I) = C-T/C ×100

Where,

C= radial growth of mycelia of pathogen in control
T= radial growth of mycelia of pathogen in the presence of antagonists.

2.4 Statistical Analysis

Statistical analysis was performed with three replicates for each treatment. The data was subjected to analysis of variance (ANOVA) using Microsoft Excel version 2007 and significance of the two bio control agents were evaluated by F test (P=.05).

3. RESULTS AND DISCUSSION

The results of interaction between pathogen and bio control agent clearly indicated that *Trichoderma viride* exhibited inhibition of radial growth of all fungal pathogens studied (Fig. 2). Growth of pathogens was normal in the monoculture plates. Antagonistic activity of *Trichoderma viride* is presented in the Table 1. The maximum inhibition of radial mycelia growth was shown by *Fusarium oxysporum* (85.7±0.3%) This suggested that among the four selected pathogens *Trichoderma viride* was the most effective bio control against *Fusarium oxysporum.* It was reported that 8 isolates of *Trichoderma* spp were promising against *F. oxysporum*. They reduced the growth of pathogen by more than 60% within 6 days of inoculation. Two isolates of *Trichoderma viride,* TR19 and TR 22 were also found to be more promising against *F. oxysporum* and these isolates completely overgrew the pathogens and suppressed its growth within 7 days [10]. In the present study, least percent inhibition was recorded by *Aspergillus niger* (42.82 ±0.9%).The radial mycelial growth inhibition of *Pythium aphanidermatum* was found to be 62.8 ± 0.4%. This result was in confirmation with similar studies conducted by Vinit [11]. Formation of inhibition zone at the contact between *Trichoderma viride* and *Pythium aphanidermatum* in dual culture plate could be explained on the basis of production of volatile metabolites as well as production of cellular hydrolytic enzymes by *Trichoderma* spp [12]. The radial growth inhibition recorded by *Alternaria alternata was* 70.7±0.4%. *Trichoderma,* when used as biocontrol agent, act as an aggressive competitor and mycoparasite of the fungal pathogen and results in the disintegration of pathogen hyphae. Besides they may also produce antifungal phenolic compounds [13].

Pseudomonas fluorescens has marked significant inhibitory effect on the growth of selected pathogens in dual culture experiment (Table 2). The same technique has been adopted by many other workers [14,15]. Normal

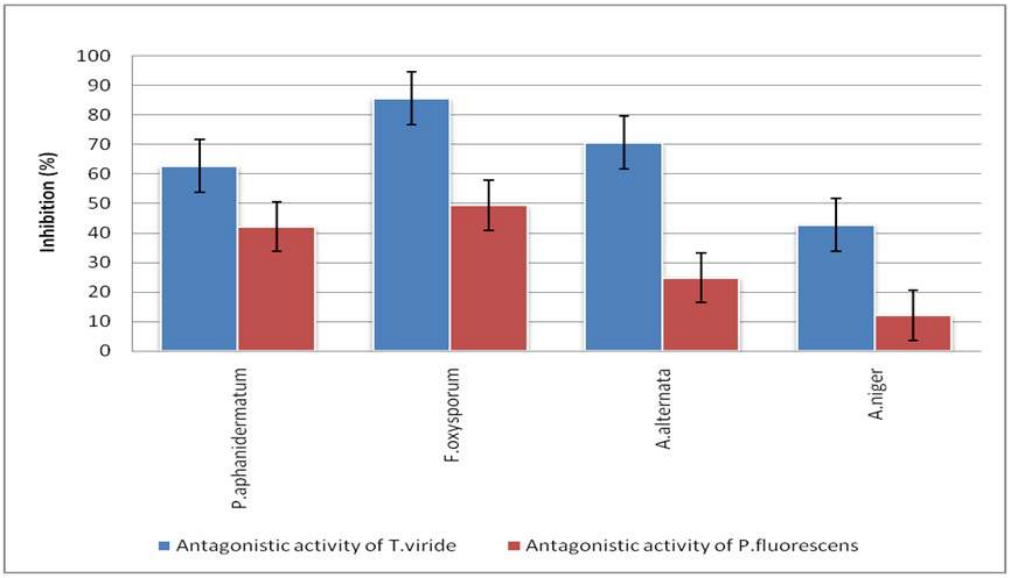

Fig. 1. Comparison of inhibition percentage (mean ± standard error) of *T. viride* and *P. fluorescens* against different species of fungal pathogens

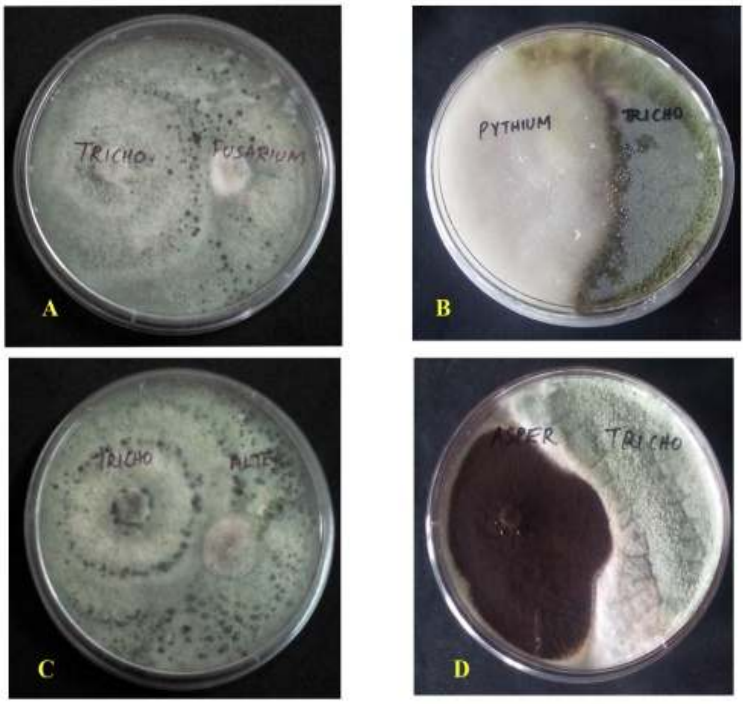

Fig. 2. Antagonistic activity of *Trichoderma viride* against A) *Fusarium oxysporum*, B) *Pythium aphanidermatum*, C) *Alternaria alternate, D) Aspergillus niger*

growth of pathogens can be seen in monoculture plates of each fungus. But the growth of pathogens becomes restricted in the presence of antagonist [Fig. 3]. A maximum percent inhibition of radial growth can be seen in *Fusarium oxysporum* (49.41 ± 0.4%) and is least in *Aspergillus niger* (12.17 ± 0.01%). *Pythium aphanidermatum* and *Alternaria alternata*

recorded 42.2±0.8% and 24.9±0.9% of percent inhibition respectively. The specific production of fluorescein and pyocyanin by the *Pseudomonas* bacterium against *Pythium* has been reported [16]. *Pseudomonas fluorescens* strains have greater potential to be used as bio control agents for the management of *Fusarium* spp. that caused fusarium wilt of tomato and flax [17]. It was documented that *P. fluorescens* isolates significantly inhibited the growth of *Fusarium oxysporum* f. sp. *cicer* in chick pea [18,19].

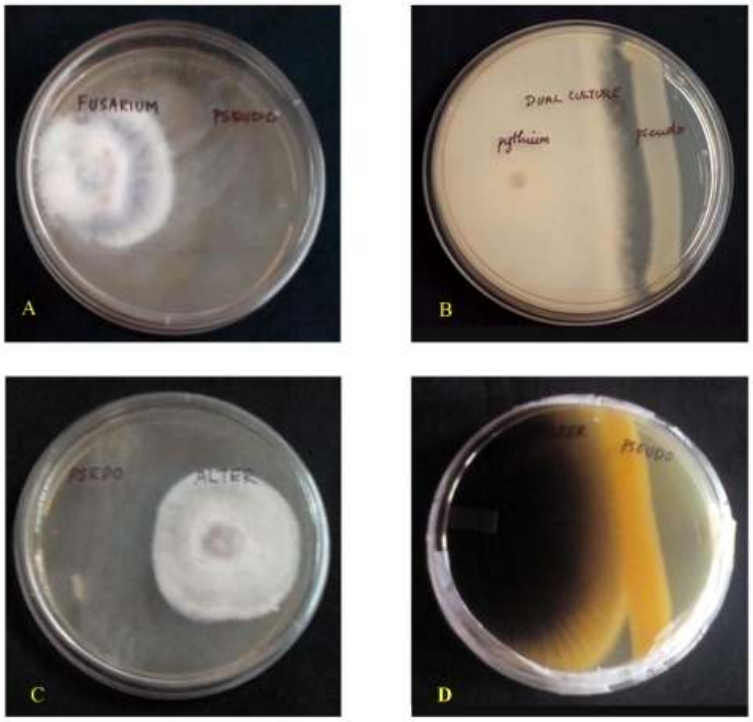

Fig. 3. Antagonistic activity of *Pseudomonas fluorescens* against A) *Fusarium oxysporum*, B) *Pythium aphanidermatum, C) Alternaria alternata, D) Aspergillus niger*

Table 1. Effect of *T. viride* on the percent inhibition of radial growth of fungal pathogens

Sl. no	Pathogens	Mean radial growth of mycelia in control (mm)	Mean radial growth of mycelia in dual culture (mm)	Mean percent inhibition (%)
1	*P. aphanidermtum*	59.7 ± 0.2	22.1 ± 0.4	62.8 ± 0.4
2	*F. oxysporum*	58.3 ± 0.9	8.4 ± 0.04	85.7 ± 0.3
3	*A. alternate*	25.3± 0.2	7.4± 0.04	70.7 ± 0.4
4	*A. niger*	58.3 ± 0.5	33.33 ± 0.4	42.82± 0.9

Values are expressed as mean ± standard error of three replicates

Table 2. Effect of *P. fluorescens* on the percent inhibition of radial growth of fungal pathogens

Sl. no	Pathogens	Mean radial growth of mycelia in control (mm)	Mean radial growth of mycelia in dual culture (mm)	Mean percent inhibition (%)
1	*P. aphanidermtum*	59.6 ± 0.2	33.8 ± 0.3	42.2 ± 0.8
2	*F. oxysporum*	58.3 ± 0.5	29.5 ± 0.2	49.41 ± 0.4
3	*A. alternate*	26.6 ± 0.5	20.0 ± 0.4	24.90 ± 0.9
4	*A. niger*	40.0 ± 0.4	40.0 ± 0.4	12.17 ± 0.01

Values are expressed as mean ± standard error of three replicates

A comparison of growth inhibition among the two bio control agents revealed that there is a significant difference in the radial mycelial growth inhibition of pathogens under study (F = 7.37, P = 0.03). Among the two bio control agents used, *Trichoderma viride* has a greater potential than *Pseudomonas fluorescens* to inhibit the growth of the four selected fungal pathogens (Fig. 1). Biological control of plant diseases is a result of different types of interaction among microorganisms and can occur through different mechanisms, which are generally classified as parasitism or predation, antibiosis, competition, lytic enzymes, and induced resistance [20]. An attractive feature of bio control strategy is that the population of pathogens developing resistance to antagonistic products by bio control agents is likely to be very slow [21].

4. CONCLUSION

From the present study it can be concluded that both bio control agents were effective for all the four fungal pathogens. Of these, *Fusarium oxysporum* showed the highest inhibition of mycelia growth in the presence of both *Pseudomonas fluorescens* (58.3%) and *Trichoderma viride* (86.16%). Least percent inhibition of radial mycelial growth was shown by *Aspergillus niger* in the presence of both bio control agents. Out of the two bio control agents tested namely, *Pseudomonas fluorescens* and *Trichoderma viride* it was found that *Trichoderma viride* exhibited comparatively greater antagonistic activity against the four fungal pathogens under this study. The present study was conducted in *in vitro* conditions. Further studies can be done on comparison of the bio control agents in a field, and first of all against *Fusarium oxysporum* on a valuable crop.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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