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Influence of Mycorrhizal Fungi and Biocontrol Agents on the Management of Fusarium Wilt in Chilli (*Capsicum annum* L.)

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ABSTRACT

The most significant role of arbuscular mycorrhizal fungi (AMF) is enhancing disease tolerance in the host plant. Pathogens that infect the plant can cause significant crop losses, posing a serious threat to food security. To overcome this issue, the present study explores the use of biocontrol strategies involving the use of indigenous AMF in the control and combination with other fungi viz., *Aspergillus niger*, *Trichoderma viride*, and *Trichoderma harzianum.* The experiment was conducted using five inoculum combinations of indigenous AMF and other fungal species. Disease incidence and yield of the chilli plant were recorded as primary outcomes. Among the combinations tested, *AMF+Trichoderma harzianum* was the most effective in control, showing the greatest reduction in disease incidence (1.40±0.03%). Several parameters like shoot length, root length, number of leaves, leaf area, shoot and root fresh weight, shoot and root dry weight, Mycorrhizal Efficiency index (MEI), and Interactive Effect of fungi (IEF) were studied. Results indicated a decrease in disease incidence and a corresponding increase in chilli plant biomass. The highest biomass was recorded in the *AMF + T. harzianum,* followed by *AMF + T. viride*, *AMF+ Aspergillus niger,* respectively. The positive interaction between the host plant and microbial inoculants significantly enhanced plant growth and health.

Keywords: AM fungi, Chilli, *Fusarium* wilt, Biocontrol agents, Biomass Production

INTRODUCTION

Chilli (*Capsicum annuum* L.) is a widely cultivated commercial spice crop in India, valued for its pungent flavour, vibrant colour and distinctive aroma. However, chilli is vulnerable to infection by numerous fungal pathogens. The foremost diseases of chilli were bacterial wilt, damping-off and root rot, *Anthracnose*, Phytophthora blight, Leaf blight, and *Fusarium* wilt etc. Among these, the most serious problem in recent years was *Fusarium* wilt caused by *Fusarium* oxysporum *f. sp. capsici*. The productivity of the chilli was affected by many pathogens, but the *F. oxysporum* mainly affected the vascular wilt, causing losses up to10-50% globally and 10-80% in India (Bai *et al.,*2018). The major chilli-producing states in India are Andhra Pradesh, Bihar, Karnataka, Madhya Pradesh and Maharashtra (Anonymous, 2017). As of 2019, chilli was cultivated on approximately 388 thousand hectares, with an annual production of 3.737 million tonnes (Anonymous, 2019). In the wilt, pathogen primarily blocks the xylem vessel, severely disrupting nutrient uptake and water transport, which ultimately leads to reduced plant vigour and substantial yield. Muthuraj and jayashella (2005), who reported that a combination of Pseudomonas fluorescens, *Glomus mosseae*, and *Azospirillum brasilense* led to increased shoot and root dry weight in chilli, ultimately enhancing the productivity of *Capsicum annum*. Similarly, Sharma and Johri et. al. (2002) demonstrated that *fusarium* wilt of tomato was significantly controlled by *Glomus etunicatum,* followed by *G. mosseae,* which with the effectiveness of AM fungi observed in our study. Pandey and Pandey (2005) reported that the effect of the five different interactions of the pathogenic fungi *Fusarium solani, Sclerotium rolfsii, Pythium aphanidermantum, Rhizotonia solani* and *Macrophonia phaseolina* with the biocontrol agents such as *T. koningii* and *Bacillus subtilis* and *T. harzianum* resulted in 100% improvement in the growth of chilli and brinjal. Treatments involving *T. koningii* and *Bacillus subtilis* and *T. harzianum* also showed (76.7% improvement). Mishra et. al. (2004) reported that *T. virens* showed an antagonistic effect to *Fusarium oxysporum* in the wilt of corn. The wilt chickpea was controlled by *T. viride* and *T. harzianum* (Bhat *et al.,*2003). Naraghi et. al. (2007) reported that a 10-20% reduction in the wilt diseases of cotton by using the AM fungi as a biofertilizer. AM fungi play an important role in the enhancement of the vigorous growth of the tomato plant (Chen *et al.,*2018).

Mycorrhizal is a mutualistic non-pathogenic symbiotic association between a fungus and the root system of the plants. The AM fungi enhance the host plant's ability to thrive under adverse soil conditions by increasing the root surface area and absorption of mineral nutrients. The survival of the root surface area and absorption of mineral nutrients. The survival of the AM fungi solely depends on the host plant; hence, it is known as an obligate biotroph. In addition to improving nutrient uptake, AM fungi provide bio-protection against many soil-borne pathogens, including bacteria, fungi, and parasites. These soil-borne pathogens are often difficult to manage using conventional fungicidal methods (Chaubey *et al.,*2014). Hence, the present study demonstrates that a combination of the mycorrhizal inoculum with compatible beneficial fungi offers a promising biocontrol strategy against *Fusarium* wilt in chilli plants.

MATERIALS AND METHODS

Pot Experiment

The experiment was carried out in a pot under the greenhouse. The AM fungal inoculum was prepared by using the funnel technique and the pot culture method. Other fungal cultures were prepared under laboratory conditions. The five different combinations of the AM fungi and other fungi viz., *Aspergillus niger*, *Trichoderma viride* and *Trichoderma harzianum* were studied in this experiment. The treatment combinations are given below.

T1: Control (Infected with *Fusarium* wilt)

T2: *Arbuscular Mycorrhizal Fungi* (Control)

T3: *Arbuscular Mycorrhizal Fungi + Aspergillus niger*

T4: *Arbuscular Mycorrhizal Fungi + Trichoderma viride*

T5: *Arbuscular Mycorrhizal Fungi + Trichoderma harzianum*

Fungal pathogen

The wilt pathogen, *F. oxysporum,* was isolated from a chilli plant showing typical wilt symptoms. The fungus was purified by using the single hyphal tip method on Potato Dextrose Agar (PDA) medium, following the procedure described by Rangaswamy (1997) and was maintained under laboratory conditions at 30°C.

Preparation of the fungal culture

The fungi like *Trichoderma harzianum*, *T. viride* and *Aspergillus niger* were used to evaluate the combined effect of AM fungi and other biocontrol agents. The cultures of these biocontrol fungi were prepared and identified under laboratory conditions. 2cm diameter of disc of *A. niger*, *T.viride* and *T. harzianum* were used from one week old culture of fungi. The 2cm diameter disc of each fungus was homogenised into 100 mL of sterile distilled water in a conical flask. Flasks were shaken until the whole fungal disc dissolved into the water. This suspension was then applied to the pot experiment.

Mass multiplication

Isolated spores have a very short lifespan; hence, they are used for identification the genus level. Morpho-taxonomy plays a crucial role in accurate identification. detailed study of AM fungal spores required a large quality of monocultures of each species. Hence, the mass multiplication of indigenous AM fungal spores was carried out using the funnel technique and pot culture technique as described by Rodrigues (2006) and Habte and Osario (2001).

Arbuscular mycorrhizal fungal spores for the experiment.

An AM fungal spore isolated from the rhizosphere soil by using the wet sieving and decanting method by Gerdemann and Nicolson (1963). The spore was rinsed sterile distilled water and surface disinfected using 2% Chloramine-T. These sterilized spores were then multiplied using the funnel technique. For the bulk production of AM fungi, the funnel technique was employed effectively.

Preparation of different combinations of AM fungi and pathogenic fungi for the pot experiment.

Five surface sterilised, seeds of chilli were sown 2 cm below the autoclaved soil in a plastic pot. These pots were placed outdoors under natural environmental conditions. Plants were waters regularly each week to maintain field capacity. No fertilisers were at any of the development stages. After 20 days, the chilli plants were treated with a suspension of the *Fusarium oxysporium* along with combination of the AM fungi and selected biocontrol fungi. After 90 days of treatment roots were checked for AM assessment, and various growth parameters were recorded. The basic biomass measurement was also carried out.

Growth indicators such as shoot length, Root length, dry weight of the shoot and root, mycorrhizal Efficiency index and the Interactive Effect of fungi (IEF)on the chilli plant were studied. Biomass was measured using the harvest method. The leaf area was measured by the disc method before harvesting the plants. Fifty leaf discs were randomly collected from the plant, and the remaining plant material was oven-dried. Leaf area was calculated using the formula described by Vivekanandan *et al.* (1972). The length of the shoot was measured from the surface of the soil. The plant's root length was measured by uprooting the plant. A digital balance was used for the measurement of the fresh weight of the shoot and root. For dry weight measurements, plant materials were oven-dried at 80^{0}C for 24 to 48 hours until a constant weight was achieved, then cooled and weighed. The mycorrhizal efficiency (MEI) index was measured using the formula provided by Bagyaraj (1994), which is based on dry weight.

$$\text{MEI}=\frac{\text{Wt. of Inoculated plant - Wt. of uninoculated plant}}{\text{Wt. of Inoculated Plant}}\times 100$$

The interactive effect of other fungi (IEF) with AMF was measured by using the biomass of an AMF-treated plant (dry weight) as a mycorrhizal control.

The interactive effect was measured by using the following formula.

$$\text{IEF}=\frac{\text{Wt. of AMF and other fungi treated plant - Wt. of AMF plant}}{\text{Wt. of AMF and other fungi treated Plant}}\times 100$$

Disease incidence (%): Disease incidence was assessed both before and after treatment with AM fungi and other biocontrol pathogens. The percentage of disease incidence was calculated using the formula provided by Rashed et. al. (2002).

$$\text{DI \%}=\frac{\text{No. of diseased leaves}}{\text{No. of total examined leaves}}\times 100$$

STATISTICAL ANALYSIS

The data collected from the experiment were statistically analysed the method by Mungikar, (1997).

RESULTS

Mycorrhizal Efficiency

The experiment conducted to control the disease of wilt in chilli using AM fungi and other fungi is summarized in **Table 1** and **Figure 1**. The yield of the chilli plants, and the impact of the AM fungi and other fungal treatment used in combination to control disease incidence are presented. The study on disease incidence control, Mycorrhizal Efficiency Index (MEI), and the Interactive Effect of Fungi (IEF). Additionally, increases in chilli productivity were evaluated through the various developmental growth indicators.

Disease incidence

The greatest reduction in diseases was seen in the combination of *AMF + Trichoderma harzianum* (1.40±0.03%) followed by *AMF + T. viride* (4.41±0.02%), and *AMF+ Aspergillus niger* (7.6±02.2%). Treatment with AM fungal alone significantly reduces *Fusarium* wilt incidence (14.5±04.4%) compared to the untreated control (88.8±12.2). The reduction in the disease incidence directly contributed to improved growth of the chilli plants, as demonstrated by various growth parameters.

Biomass production

The maximum shoot length was recorded in the combination of *AMF + Trichoderma harzianum* (72.38cm), followed by *AMF +T. viride* (69.4cm), *AMF+ Aspergillus niger* (53.2cm). The minimum shoot length was recorded in the infected plant (wilted) chilli plant (36.7cm). similarly, the highest root length was reported in the *AMF + Trichoderma harzianum* (25.1cm) followed by *AMF + T. viride* (21.2cm), and *AMF+ Aspergillus niger* (20.1). the lowest root length was reported in the wilted plant as compare to the AMF control and AMF+ other fungi treated plant (15.1cm).

DISCUSSION

The use of mycorrhizal fungal inoculum in combination with appropriate biocontrol fungi has proven to be an effective strategy for both the management of fungal diseases and the enhancement of crop productivity.

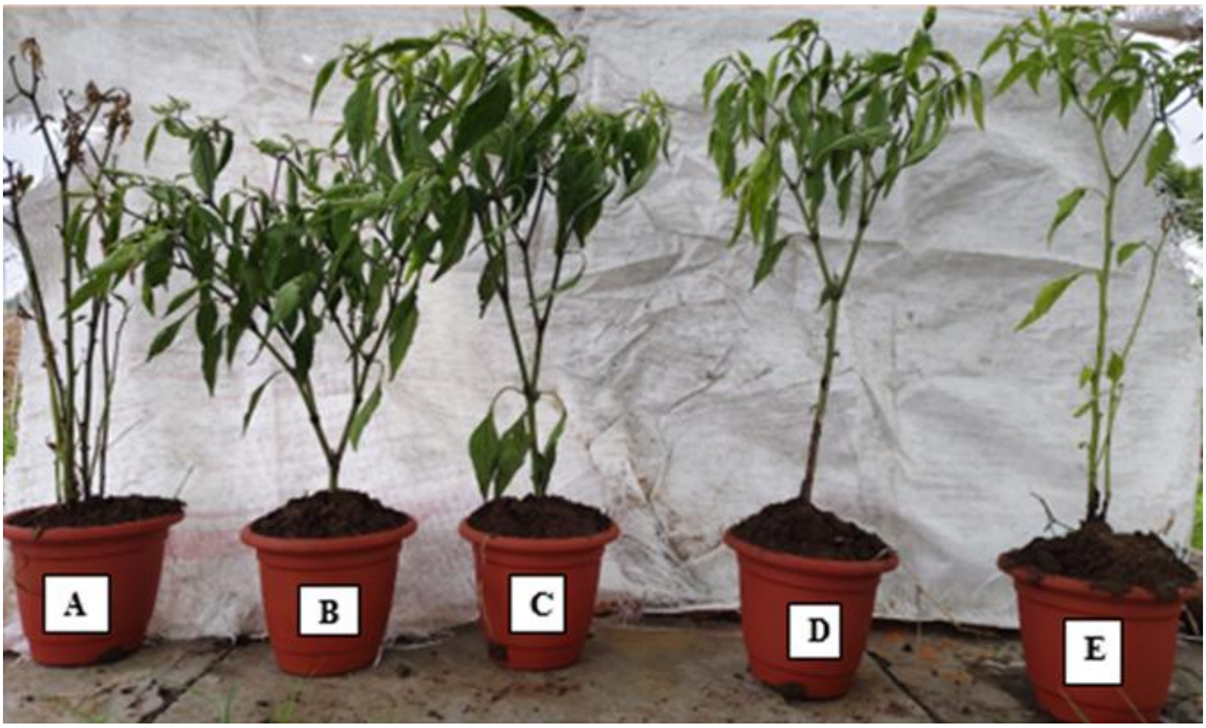

Figure 1: Interaction of the AM fungi and other fungi on the root and shoot of the wilted chilli plant. A, Wilted chilli plant with symptoms; B, Arbuscular Mycorrhizal fungal (AMF) control; C, AMF+*Aspergillus niger*; D, AMF+*Trichoderma viride*; E, AMF+*Trichoderma harzianum*

Figure 2: The fungi used in the interaction

In this study, chilli plants showed maximum root colonization (70%) when inoculated with AM fungi in combination with *T. harzianum*, while a lower colonization rate (56%) was recorded with AM fungi and *T. viride*. Meanwhile, maximum spore population was observed in the *AM fungi*+ *T. viride* treatment (85/50gm of soil). These improvements in colonization and spore density ultimately contributed to increased chilli yield by significantly reducing disease incidence. These findings align with those of Claudia et. al. (2009), who also reported improved crop performance associated with increased AM fungal activity. Amusat et.al. (2008) and Linderman (2000) previously reported that the AM fungi enhanced the growth of the chilli plant. Several species of *Trichoderma* (Erazo *et al.,* 2021) and arbuscular mycorrhizal fungi (AMF) (Devi *et al.,* 2022) have demonstrated effectiveness in managing Fusarium wilt caused by *Fusarium oxysporum* through biological control. Arbuscular mycorrhizal fungi (AMF) serve as effective biocontrol agents by boosting plant defence mechanisms against pathogens, improving nutrient uptake, and regulating microbial communities within the mycorrhizosphere (Vishwakarma *et al.,* 2022). Several studies have examined how different AMF species influence soil-borne diseases caused by diverse pathogens, focusing on the complex interactions among mycorrhizae, host plants, and pathogens using various analytical parameters (Song *et al.,*2015; Aljawasim, Khaeim & Manshood, 2020; Wu *et al.,*2021). While AMF inoculation has gained increasing attention, studies investigating its influence on the defence mechanisms of pepper plants against soil-borne pathogens especially *Fusarium* spp., remain limited (Rodriguez *et al.,* 2020; Coskun *et al.,* 2023).

Table 1: Effect of Arbuscular Mycorrhizal Fungi and other fungi on the growth of wilted Chilli plant in a pot experiment

Sr. No.	Treatment for chilli plant	Disease incidence (DI) (%)	Shoot Length (cm)	Fresh Shoot Weight (g)	Dry Shoot Weight (g)	Root Length (cm)	Fresh Root Weight (g)	Dry Root Weight (g)	No. of Leaves	Leaf Area (cm^2)	MEI	(%) IEF
1.	T1	88.8 ±12.2	36.7 ±14.1	18.3 ±12.1	5.23 ±2.1	15.1 ±3.1	3.43 ±0.04	1.00 ±0.02	009	442.5 ±52.0	000	000
2.	T2	14.5 ±04.4	48.9 ±12.2	21.4 ±12.1	7.21 ±2.1	19.3 ±3.1	4.98 ±0.03	1.93 ±0.02	048	988.2 ±60.0	24.47 ±12.0	000
3.	T3	7.6 ±02.2	53.2 ±32.1	32.2 ±14.1	8.00 ±2.1	20.1 ±3.3	6.23 ±0.03	2.00 ±0.04	052	1123 ±68.0	33.73 ±12.4	12.32 ±6.00
4.	T4	4.41 ±0.02	69.4 ±23.1	36.4 ±14.1	8.68 ±2.1	21.2 ±3.3	7.12 ±0.03	2.53 ±0.04	068	1893 ±70.0	41.63 ±14.0	22.75 ±12.0
5.	T5	1.40 ±0.03	72.8 ±21.1	38.2 ±14.1	9.10 ±2.1	25.1 ±3.2	8.14 ±0.03	3.10 ±0.02	071	2109 ±56.0	45.18 ±14.0	27.42 ±12.0

*(Values represent average of three sets of treatments, IP = Infected plant, MEI = Mycorrhizal Efficiency Index, IEF = Interactive Effect of Fungi). DI = Disease incidence. Values are means of three replications, standard error (±). T1 = Control (Infected with Fusarium wilt), T2 = Arbuscular Mycorrhizal Fungi (Control), T3 = *Arbuscular Mycorrhizal* Fungi + *Aspergillus niger,* T4 = Arbuscular Mycorrhizal Fungi + *Trichoderma viride,* T5 = Arbuscular Mycorrhizal Fungi + *Trichoderma harzianum.*

CONCULSION

Overall**,** the results of the present investigation demonstrate that the synergistic effect of AM fungi with biocontrol agents like *T. harzianum* and *T. viride* offers an effective approach to managing *Fusarium* wilt and improving chilli crop performance.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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