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In-vitro Analysis of Inhibitory Potential of Fungicides and Biocontrol Agents against Vascular Wilt Pathogen, *Fusarium oxysporum* f. sp. *vasinfectum* infecting Cotton in Western U.P.

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ABSTRACT

Cotton yield production and productivity is significantly suppressed due to vascular wilt incited by *Fusarium oxysporum* f. sp. *vasinfectum* (FOV). The management of the wilt pathogen can be achieved by using various biocontrol agents, such as *Trichoderma* spp., *Pseudomonas* spp., and *Bacillus* spp., and fungicides, such as carbendazim, copper oxychloride, CM75% (carbendazim + mancozeb), propineb, mancozeb, vitavex, propiconazole and Amistar top. Among all the biocontrol agents, *T. harzianum, P. fluorescens*, *T. viride* and *T. atroviride* resulted highest inhibition (60-74%) of the mycelium growth of FOV was observed *in vitro*. The complete reduction (100%) in the mycelial growth of Fov was recorded with carbendazim, CM75%, propineb and mancozeb even at their used lowest concentration of 50 ppm among all the tested eight fungicides. The most significant biocontrol agents, including *T. harzianum, P. fluorescens*, *T. viride* and *T. atroviride* and fungicides, such as carbendazim, CM75%, propineb and mancozeb can be utilized in integrated disease management module for the best control of vascular wilt disease in cotton.

Key Words: Biocontrol agents, Cotton, Culture, Fungicides, *Fusarium* spp. Management.

INTRODUCTION

Cotton is one of the most significant fibre crops which is cultivating throughout the world (Cusser *et al,* 2016; Voora *et al,* 2020). The crop is mainly used for its natural fibre, oilseed, and animal feed (Chen *et al,* 2015; Zhang *et al,* 2019). The fibre production and productivity of the crop is hampered by numerous abiotic, biotic factors, and weeds which are causing significant suppression in the cotton yield (Pegg and Brady, 2002; Halpren *et al,* 2018; Hussain *et al,* 2024). Biotic factors includes; fungi, bacteria, virus and nematodes etc. (Kamburova *et al,* 2018; Tarazi *et al,* 2020), and all these pathogens are responsible for up to 30% losses in fibre yield (Tarazi *et al,* 2020). The most common soil borne, and foliar diseases of cotton are blackarm (*Xanthomonas campestris* pv. *malvacearum*), anthracnose (*Colletotrichum gossypii* or *C. capsica*), wilt (*Fusarium oxysporum* f. sp. *vasinfectum*), grey mildew (*Ramularia areola*), root-rots (*Rhizoctonia bataticola*), leaf curl (*Cotton leaf curl virus*), verticillium wilt (*Verticillium dahliae*), and leaf blight (*Alternaria macrospora*) (Ramod, 2016; Patel *et al,* 2021).

Among all these diseases, *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) is one of the most significant soil and seed borne pathogen that limits the fibre production and productivity of the crop (Armstrong and Armstrong, 1981; Atkinson 1892; Hillocks 1992; Sanogo and Zhang 2016; Zhu *et al,* 2022). The typical symptoms of wilt infected plants include cholorosis, yellowing, which is followed by leaf shedding, slow wilting, and in case of severe infection plant may die. One characteristic indication that is specific to fusarium

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wilt is a reddish-brown discoloration can be seen when cutting sections of stem and roots of the plant (Ayubov *et al,* 2024). Like other soil-borne pathogens, FOV can live in soil for many years without finding a suitable host plant, making it difficult to eradicate (Bani *et al*, 2018). Between 1953 and 2012, the wilt disease caused fiber yield losses that ranged from 0.19 to 1.36% (Blasingame and Patel, 2013) and in the United States, the National Cotton Disease Council had calculated an estimated 109,000 bales (227 kg or 500 lb) yield reduction was recorded in the year 2004 (Blasingame and Patel, 2005). Due to economic importance of the cotton crop and to reduce the yield losses caused by the wilt fungi, an experiment was conducted to evaluate the antagonistic or inhibitory effect of various biocontrol agents (fungal and bacterial) and fungicide on the mycelial growth of FOV.

MATERIALS AND METHODS

Isolation of *Fusarium oxysporum* f. sp. *vasinfectum*

Ten plants of cotton showing wilt disease symptoms were collected from the cotton fields. Sterilized poly bags were used to collect the plant samples and taken to the laboratory. To isolate wilt fungus, FOV, the small pieces (2-5mm) were cut from the stem and root of sample plant. After being surface sterilized for 30 seconds in 2.5% NaOCl (vol/vol), the pieces were rinsed two or three times with distilled water. The parts were put in a Petri plate with solidified potato dextrose agar (PDA) after being soaked on sterilized tissue paper. The inoculated plates were kept at 25±2°C in a BOD incubator. To identify *F. oxysporum* f. sp. *vasinfectum*, the fungal colonies that had grown on the plates were examined under a microscope (Updhayay and Rai, 1992; Gilman, 2001).

Dual culture test of biocontrol agents and wilt fungus

Antagonistic activity of nine isolates of bio-agents *viz*., *T. virens* ITC-477, *T. viride* AMUTVRD-13, *Trichoderma harzianum* AMUTHZM-21, *T. atroviride* AMUTATVRD-27, *T. longibrachiatum* AMUTLNG-23, *T. asperellum* AMUTA-1, *T. hamatum* AMUTHMT-19, *Pseudomonas fluorescens* AMUPF-7 and *B. subtilis* AMURBS-7 against *F. oxysporum* f. sp. *vasinfectum* (FOV7) was evaluated using the dual culture technique (Zivkovic *et al,* 2009). Two mycelial discs (5 mm dia.) of each FOV7 and biocontrol fungi were placed on the solidified PDA, 3 cm apart from each other in a Petri plate. The plates inoculated with *F. oxysporum* f. sp. *vasinfectum* FOV7 without BCAs served as control. Every treatment was kept in five replicated plates. After inoculation, the plates were kept at 25±2 °C for two weeks. On PDA-containing Petri plates, the bacterial antagonists were streaked 3 cm from the pathogen's mycelial disc. The plates were then incubated for 7 days at 35±2 °C and monitored. The radial growth of the biocontrol agents and *Fusarium* colonies and inhibition zone were measured.

Calculating the minimum inhibitory concentration (MIC) of fungicides against mycelial growth of *Fusarium oxysporum* f. sp. *vasinfectum*

Eight fungicides, enlisted in Table 2 were evaluated by poisoned food technique against *Fusarium oxysporum* f. sp. *vasinfectum* (FOV7) (Dhingra and Sinclair, 1985). The sterilised medium was mixed with stock solutions of the fungicides in a liquid state until the concentration of active ingredients reached 50, 100, 150, and 200 ppm. The mediums were poured in Petri plates (20 ml/ plate). After allowing the PDA in the plates to solidify, a mycelial disc of FOV7 (9 mm diameter) was positioned in the middle. A control (without fungicides) was maintained. Each treatment was maintained with five replicate plates. The plates were incubated at 25±2 °C. On the seventh day, the colonies' diameter was measured, and the following formula was used to determine the percentage of fungal growth inhibition:

$$PI = \{(C - T)/C\} x100$$

Where,

C = the test pathogen's growth (mm) in control;

T = the test pathogen's growth (mm) in the amended medium.

Table 1. *In vitro* efficacy of biocontrol agents in inhibiting of radial growth of *Fusarium oxysporum* f. sp. *Vasinfectum.*

Treatment	Radial growth (mm)		Percent inhibition over control (%)
	F. oxysporum* f. sp. *vasinfectum	**Biocontrol agents**	
Control (FOV7 alone)	90[a]	--	--
T. harzianum AMUTHZM-21	24.1[c]	65.9[a]	73.3[a]
T. virens ITC-477	31.1[b]	58.9[b]	65.5[bc]
T. viride AMUTVRD-13	28.4[bc]	61.6[b]	68.4[ab]
T. hamatum AMUTHMT-19	34.6[b]	55.4[c]	61.6[cd]
T. longibrachiatum AMUTLNG-23	34.7[b]	54.3[c]	60.4[d]
T. asperellum AMUTA-1	30.9[b]	59.1[b]	65.7[bc]
T. atroviride AMUTATRVD-27	29.5[bc]	60.5[b]	67.2[b]
*P. fluorescens*AMUPF-7	28.4[bc]	61.6[b]	68.5[ab]
*B. subtilis*AMUBS-7	29.7[bc]	60.3[b]	66.7[b]
LSD at P≤ 0.05	3.746[*]	2.044[*]	2.908[*]
F values of Treatments (df=8)	227.9[*]	212.9[*]	466.5[*]

Each value is a mean of five replicates. Values within a column followed by different alphabets are significantly different at P≤0.05 according to Tukey's test

RESULTS AND DISCUSSION

The nine biocontrol agents (fungi and bacteria) and eight fungicides were screened *in vitro* conditions to evaluate their effectiveness against *F. oxysporum* f. sp. *vasinfectum* (FOV7).

Effect of biocontrol agents on the colonization of *Fusarium oxysporum* f. sp. *vasinfectum*

Antagonistic activity of nine BCAs isolates *viz., T. virens* ITC-477, *Trichoderma harzianum* AMUTHZM-21, *T. viride* AMUTVRD-13, *T. longibrachiatum* AMUTLNG-23, *T. atroviride* AMUTATVRD-27, *T. hamatum* AMUTHMT-19, *T. asperellum* AMUTA-1, *B. subtilis* AMURBS-7 and *Pseudomonas fluorescens* AMUPF-7 against *F. oxysporum* f. sp. *vasinfectum* AMURFOV7 was evaluated using dual culture technique. The biocontrol agents inhibited the colonization of *F. oxysporum* f. sp. *vasinfectum*, *in vitro*. However, substantial variation in the degree of inhibition was observed among the BCAs (60-74%). *T. harzianum* AMUTHZM-21, *P. fluorescens* AMUPF-7, *T. viride* AMUTVRD-13 and *T. atroviride* AMUTATVRD-27 were recorded highest effective and suppressed the growth of mycelial of wilt fungus by 73-69% (P≤0.05), followed by *B. subtilis* AMUBS-7 (67%), *T. asperellum* AMUTA-1 (66%), *T. virens* ITC-477 (65%), *T. hamatum* AMUTHMT-19 (62%) and *T. longibrachiatum* AMUTLNG-23 (60%) over control (Table 1).

In vitro effectiveness of fungicides against *Fusarium oxysporum* f. sp. *vasinfectum*

Eight fungicides *viz*., carbendazim, copper oxychloride, CM75% (carbendazim + mancozeb), propineb, mancozeb, vitavex, propiconazole and Amistar top were evaluated against *F. oxysporum* f. sp. *vasinfectum* (FOV7) at various concentrations (50, 100, 150 and 200 ppm) using poisoned food method to find the minimum inhibitory concentration (MIC) of each fungicide. As fungicide concentrations increased, resulting in the inhibition of fungal colonization usually. The cent percent reduction in the mycelial growth was recorded at 50 ppm of the carbendazim,

Table 2. Analysis of minimum inhibitory concentration (MIC) of various fungicides using poisoned food technique *in vitro*

Fungicide	Percent inhibition of *Fusarium oxysporum* f. sp. *vasinfectum*			
	50 ppm	100 ppm	150 ppm	200 ppm
Control (FOV7 alone)	00	00	00	00
Mancozeb	100[a]	100[a]	100[a]	100[a]
Copper oxychloride	69.39[b]	73.87[c]	76.89[c]	83.59[b]
Amistar Top	65.68[b]	69.40[c]	76.87[c]	78.35[c]
CM75% (Carbendazim+Mancozeb)	100[a]	100[a]	100[a]	100[a]
Vitavex	67.16[b]	88.80[b]	91.78[b]	97.02[a]
Carbendazim	100[a]	100[a]	100[a]	100[a]
Propiconazole	62.68[c]	72.39[c]	86.67[b]	73.14[d]
Propineb	100[a]	100[a]	100[a]	100[a]
LSD at P≤ 0.05	4.851	4.044	3.559	2.426
F values of Treatments (df=7)	395.9*	557.4*	713.5*	1563*

Each value is a mean of five replicates. Values within a column followed by different alphabets are significantly different at P≤0.01 and P≤0.05 according to Tukey's test. Significant at P≤0.01.

CM75%, propineb and mancozeb. The vitavex, copper oxychloride, Amistar top and propiconazole induced significant inhibition in the colonization of the fungus (Table 2).

CONCLUSION

The experiment has demonstrated that among nine isolates of BCAs, *T. harzianum AMUTHZM-21, P. fluorescens* AMUPF-7, *T. viride* AMUTVRD-13 and *T. atroviride* AMUTATRVD-27 showed highest antagonism against *F. oxysporum* f. sp. *vasinfectum* FOV7. Whereas *T. virens* ITC-477, *T. hamatum* AMUTHMT-19 and *T. longibrachiatum* AMUTLNG-23 were found relatively less suppressive to the wilt fungus. Among eight fungicides tested *in vitro* against FOV7, carbendazim, CM75%, propineb and mancozeb proved to be the most effective inducing 100% suppression of the wilt fungus at 50 ppm concentration and other four fungicides like Copper oxychloride, Amistar Top, Propiconazole and Vitavex are lesser effective at lower concentration.

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