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Biological Management of *Fusarium oxysporum* f. sp. *ciceris* an incitant of chickpea wilt by consortium of *Trichoderma asperellum* and *Pseudomonas fluorescens* under glasshouse conditions

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ABSTRACT: Chickpea wilt which is caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *ciceris* (Padwick) is the most deadly and prevalent disease in the world's chickpea growing regions. *Trichoderma* spp., a fungal bio-control agent and fluorescent pseudomonads, a bacterial bio control agent, have become popular in the biological management of plant diseases due to their adaptability and capacity to contain a large number of plant pathogens in a variety of target conditions. The study used efficient *Trichoderma asperellum* (TR-14) and *Pseudomonas fluorescens* (PF-19) combined with the suitable chemical cymaxanil + mancozeb in a glass house to decrease wilt disease. The results demonstrated that fungicide treatment, *i.e.*, seed treatment with cymoxanil + mancozeb at 3 g/kg seed followed by soil drenching with cymoxanil + mancozeb at 3g/l, resulted in a considerably lower disease incidence of 10.67%.

Keywords: Fusarium wilt, *Trichoderma asperellum*, *Pseudomonas fluorescens*, cymoxanil + mancozeb, per cent disease incidence.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an annual legume crop that belongs to genus *Cicer*; the tribe Cicereae, the family Fabaceae and the subfamily Papilionaceae. It is one of the first legumes to be farmed, with 7500-year-old remains discovered in the Middle East. After the common bean, it is the world's second most significant pulse crop, and it is produced all over the world for protein (20-22%), fibre, minerals, and -carotene. It also fixes nitrogen in the atmosphere (40 kg N ha^{-1}) and minimizes the requirement for nitrogen fertilizers.

Chickpea is susceptible to a variety of biotic and abiotic stressors found in various locations of the world. Diseases and insect pests cause major output losses in its cultivation, ranging from 50-100 percent in tropical areas to 5-10 percent in temperate areas (Van-Emden *et al*., 1988). Diseases are the most important biotic limitations to chickpea productivity, causing yield losses of up to 100%.

Wilt induced by an important soil borne pathogen *Fusarium oxysporum* f. sp. *ciceris* (Padwick) is regarded the most deadly and widespread disease among the fungus that harm chickpea (Haware, 1990). Wilt is a vascular disease that causes xylem browning or blackening due to melanin pigment. It is regarded the most deadly and widespread disease among the fungus that harm chickpea (Haware, 1990). Wilt is a vascular disease that causes xylem browning or blackening due to melanin pigment and disrupts water and nutrient transfer, causing the plant to wilt or die. Different chickpea cultivars wilt at different rates. Different chickpea cultivars exhibit wilting at different phases of growth, resulting in varying degrees of yield loss. Under extreme circumstances, the wilt infection can entirely destroy the crop, resulting in a 100% yield loss (Halila and Strange 1996; Navas-Cortes *et al*., 2000).

A worldwide outcry against synthetic pesticides has sparked interest in safer plant disease management methods such as biological control, genetically engineered crops, and resistant cultivars. Depending on the target organisms, several kinds of organisms, such as viruses, bacteria, fungus, and nematodes, are put to work in the biological control of plant diseases. *Trichoderma* spp., a fungal biocontrol agent, and fluorescent Pseudomonads, a bacterial biocontrol agent, have grown popular due to their adaptability and capacity to contain a large number of plant pathogens in a variety of target conditions. Chemical pesticides have been recommended as an alternative to using these microbial antagonists against plant diseases in agricultural crops (Pal and McSpadden 2006).

Trichoderma asperellum is a filamentous fungus that is an asexually reproducing ascomycete with a sexual teleomorph in the genus Hypocrea. It acts as an antagonist fungus for a variety of plant infections through a variety of ways. *Pseudomonas fluorescens* is a Gram-negative bacterium that belongs to the Phylum Proteobacteria, Class -proteobacteria, and family Pseudomonadaceae, which includes fluorescent Pseudomonads with antifungal properties.

Seeds and seedlings are protected from soil-borne and seed-borne inoculums using a combination of fungicides and suitable bio-agents in an IDM strategy (Dubey and Patil 2001). The use of bio-agents in conjunction with fungicides would result in disease suppression comparable to that achieved with higher fungicide use (Monte, 2001). By combining antagonists with synthetic compounds, the risk of resistance is reduced and the amount of fungicide used is reduced.

The current study used efficient *Trichoderma asperellum* and *Pseudomonas fluorescens*, as well as the suitable chemical cymoxanil + mancozeb, to manage chickpea wilt disease in a pot experiment.

MATERIAL AND METHODS

A. Isolation and maintenance of the pathogen (Fusarium oxysporum f. sp. ciceris)

Chickpea plants with typical wilt symptoms were taken from a chickpea field. The adhering dirt particles and other debris from the diseased stem area were thoroughly washed away with flowing tap water. The infected stem section was chopped into small 1 cm pieces and surface sterilised by soaking for a minute in a 1% sodium hypochlorite solution. To remove any traces of sodium hypochlorite, the stem parts were rinsed three times in sterile distilled water. The sterilised parts were inoculated into Potato Dextrose Agar (PDA) medium and cultured for 5 to 7 days at 28±1 °C. With the help of the PDA slants, the hyphal points growing from the infected portions were transplanted with the assistance of a sterilized needle. The pathogen was purified using the hyphal tip approach (Rangaswami, 1972) and the culture tubes were stored at 4 °C in the refrigerator.

B. Mass multiplication and inoculation of F. oxysporum f. sp. ciceris

For large replication of the pathogen, sorghum grains were employed. 200 g sorghum grains were soaked in a 2% sucrose solution for 16 hours before being placed in a 500 ml conical flask and autoclaved for 45 minutes at 121.6 °C. Under aseptic circumstances, each flask was seeded with a mycelial plug (1cm) from a pure culture of *F. oxysporum* f. sp. *ciceris* and cultured at 28±1 °C for 20 days. The flasks were shaken on alternate days to ensure that the pathogen grew uniformly. The resulting enormous culture was used to make sick pots. The pathogenicity test was conducted using a 20-day-old inoculum of *F. oxysporum* f. sp. *ciceris* grown on sorghum grains. An autoclave was used to sanitize the soil at 1.1 kg/cm^2 (121.6 °C) pressure for 30 min, then mixed with a giant pathogen culture at 50 g/kg soil and placed in 2 kg earthen pots. The inoculum was not added to the control group.

C. Mass multiplication of T. asperellum

The efficient *T. asperellum* (TR-14) isolated from chickpea rhizosphere was grown on potato dextrose broth (potato: 200 g; dextrose: 20 g; DW: 1000 ml) and the culture filtrate containing spore suspension was mixed with sterilised talc powder (1:2.5) and the talc based formulation was used at 5 g/kg for the study.

D. Mass multiplication of P. fluorescens

After four days of inoculation, the effective *P. fluorescens* (PF-19) isolated from chickpea rhizosphere was cultivated on King's B broth (peptone: 10 g; K_2HPO_4: 1.5 g; KH_2PO_4: 1.5 g; MgSO4: 1.5 g; glycerol: 10 ml; DW: 1000 ml) and the culture filtrate containing bacterial cells was mixed with sterilized talc powder (1:2.5) and the talc based formulation was used at 5 g/kg for the study.

E. Management of wilt disease in pot experiment

For the management study, talc powder formulations of *T. asperellum* (TR-14) and *P. fluorescens* (PF-19) were blended in equal proportion (5 g) to make consortium, which was used for seed treatment and consortium multiplied on FYM for soil application in pot culture. The experiment was carried out at the Department of Plant Pathology, College of Agriculture, Raichur. The potting mixture (red soil: sand: decomposed FYM at 1:1:1 w/w/w) was produced and autoclaved for one hour at 121 °C and 15 lbs pressure for two days in this experiment. Later, the potting mixture was poured into the 2 kg capacity earthen pots. These pots were then made sick by inoculating them with a 20-day-old pathogen inoculum produced on sorghum grains. The therapy combinations listed below were tested.

Treatment	Treatment details
T_1	Seed treatment with talc based consortium of *Trichoderma asperellum* + *Pseudomonans fluorescens* at 10 g/kg seed
T_2	Seed treatment with cymoxanil (8 %) + mancozeb (64 %) WP at 3 g/kg seed
T_3	Soil application of FYM enriched formulation of *Trichoderma asperellum* + *Pseudomonans fluorescens* at 2.5 kg in 250 kg FYM
T_4	Soil drenching with cymoxanil (8 %) + mancozeb (64 %) WP at 3 g/l water
T_5	Seed treatment with consortium of *Trichoderma asperellum* + *Pseudomonans fluorescens* at 10 g/kg seed followed by soil application of FYM enriched consortium of *Trichoderma asperellem* + *Pseudomonans fluorescens at* 2.5 kg in 250 kg FYM
T_6	Seed treatment with talc based consortium of *Trichoderma asperellum* + *Pseudomonans fluorescens* at 10 g/kg seed followed by soil drenching with cymoxanil (8 %) + mancozeb (64 %) WP at 3 g/l water
T_7	Seed treatment with cymoxanil (8 %) + mancozeb (64 %) WP at 3 g/kg seed followed by soil drenching with cymoxanil (8 %) + mancozeb (64 %) WP at 3 g/l water
T_8	Untreated control

Each treatment was maintained by sowing five seeds of the susceptible Annigeri-1 genotype per pot with an untreated control. The experiment was carried out three times with a completely randomised design (CRD). Treatment combinations were created and administered to ill pots. At 30, 45, 60 and 90 days, observations on total number of plants and number of wilted plants were made. The percent disease incidence was then determined using the formula below.

$$\text{Disease incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100$$

RESULTS AND DISCUSSION

A. Management of wilt disease in pot experiment

In a pot culture experiment, the *Trichoderma asperellum* (TR-14) and *Pseudomonas fluorescens* (PF-19) were examined by constructing several treatment combinations in a management trial. The results showed that seed treatment with cymoxanil + mancozeb at 3 g/kg seed followed by soil drenching with cymoxanil + mancozeb at 3 g/l around the infected plant (T_7) had a significantly lower disease incidence of 10.67%, while seed treatment with talc based consortium of *Trichoderma asperellum* + *Pseudomonans fluorescens* at 10 g/kg seed. The control group had the highest wilt incidence (69.67%) and the lowest yield (16.67 g). Seed treatment with a talc-based consortium of *Trichoderma asperellum* + *Pseudomonans fluorescens* at 10 g/kg seed resulted in a lower disease incidence (29.67%) when compared to soil application of an FYM-enriched formulation of *Trichoderma asperellum* + *Pseudomonans fluorescens* at 2.5 kg in 250 kg FYM (33.33%) with seed yields of 33.67 and 30.67 (Table 1 and Fig. 1).

Table 1: Management of wilt of chickpea in pot culture

Sr. No.	Treatment	Disease incidence (%)*	Reduction over control	Seed yield (g)*
1.	Seed treatment with talc based consortium of *Trichoderma asperellum* + *Pseudomonans fluorescens* at 10 g/kg seed	29.67 (33.00)	57.42	33.67
2.	Seed treatment with cymoxanil (8 %) + mancozeb (64 %) at 3 g/kg seed	23.33 (28.88)	66.51	36.67
3.	Soil application of FYM enriched formulation of *Trichoderma asperellum* + *Pseudomonans fluorescens* at 2.5 kg in 250 kg FYM	33.33 (35.26)	52.51	30.67
4.	Soil drenching with cymoxanil (8 %) + mancozeb (64 %) at 3 g/l water	17.17 (24.47)	75.36	39.33
5.	Seed treatment with consortium of *Trichoderma asperellum* + *Pseudomonans fluorescens* at 10 g/kg seed followed by soil application of FYM enriched consortium of *Trichoderma asperellum* + *Pseudomonans fluorescens at* 2.5 kg in 250 kg FYM	28.67 (32.67)	58.85	35.33
6.	Seed treatment with talc based consortium of *Trichoderma asperellum* + *Pseudomonans fluorescens* at 10 g/ kg seed followed by soil drenching with cymoxanil (8 %) + mancozeb (64%) WP at 3 g/l	13.50 (21.55)	80.62	41.67
7.	Seed treatment with cymoxanil (8 %) + mancozeb (64 %) at 3 g/kg seed followed by soil drenching with cymoxanil (8 %) + mancozeb (64 %) at 3 g/l	10.67 (19.06)	84.69	45.33
8.	Untreated control	69.67 (56.57)	-	16.67
S. Em ±		**1.02**	-	**0.38**
CD (P=0.01)		**4.21**	-	**1.56**

* Mean of three replications, yield per five plants

Figures in the parenthesis are arcsine transformed values

T_7: Seed treatment with cymoxanil (8%) + mancozeb (64%) WP at 3 g/kg seed followed by soil drenching with cymoxanil (8%) + mancozeb (64%) WP at 3 g/l water

T_6: Seed treatment with talc based consortium of *Trichoderma asperellum* + *Pseudomonans fluorescens* at 10 g/kg seed followed by soil drenching with cymoxanil (8%) + mancozeb (64%) WP at 3 g/l water

Fig. 1. Management of wilt disease in pot culture.

Seed treatment with cymoxanil + mancozeb at 3 g/kg seed followed by soil drenching with cymoxanil + mancozeb at 3 g/l (T_7) showed the greatest reduction in wilt (84.69%) over control, followed by 80.62 percent in seed treatment with talc based consortium of *Trichoderma asperellum* + *Pseudomonans fluorescens* at 10 g/kg (T_6) (Table 1).

According to Srivastava *et al*. (2010), the combination of fluorescent *Pseudomonas*, *Trichoderma* and arbuscular mycorrhiza provided much better control than uninoculated therapy in pot culture, reducing disease incidence by 74%. In comparison to treatment with a single bio-agent, Singh *et al*. (2013) concluded that using a consortium of compatible bio-agents will improve plant development and biological control of phytopathogens. According to Dubey *et al*. (2015), combining *T. harzianum* and *P. fluorescens* with Mesorhizobium dramatically reduced the incidence of wilt. According to Mahmood *et al*. (2015), seed treatment with *Trichoderma harzanium* followed by chemical drenching was the most efficient, reducing disease by up to 93.75 percent.

CONCLUSION

Chickpea wilt caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *ciceris* (Padwick) is the most prevalent disease. *Trichoderma* spp. and fluorescent pseudomonads have become popular in the biological management of plant diseases due to their adaptability and capacity to contain a large number of plant pathogens in a variety of target conditions. The present study helps in understanding the role of *Trichoderma asperellum* and *Pseudomonas fluorescens* in combination for the management of soil borne pathogen *Fusarium oxysporum* f. sp. *ciceris* as compared to the chemical management.

FUTURE SCOPE

The work was conducted in glass house, since there is a scope to carryout efficacy of *T. asperellum* and *P. flourescens* in consortium for the effective management of wilt of chickpea in the field conditions.

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Conflict of Interest. None.

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