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EVALUATION EFFECT OF BIOAGENTS ON *FUSARIUM* WILT OF COWPEA

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ABSTRACT

The study is focused on the effect of two species of *Glomus* and *Trichoderma* in order to control the wilt disease and plant growth of cow pea. *In vitro* condition, among the bioagents, *T. harzianum* produced the maximum inhibition zone of 18.20 per cent compared to the minimum of 7.30 per cent by *T.viride*. Soil application of *T. harzianum* biomass at 60 g/kg soil in infected soil effectively controlled the *Fusarium* wilt disease and ultimately improved growth of different characters of plant under glasshouse condition. With regard to observations, results showed maximum plant height of 40.35 cm and fresh shoot weight of 13.20 g in plants treated with pathogen in infected soil using biomass application of *T. harzianum*.

INTRODUCTION

Cowpea (*Vigan unguiculata* L. Walp) is an important legume crop grown both for grain and fodder. It constitutes one of the most important sources of protein to people in many countries of the world. It is grown in many regions of the world especially in semi - arid (Rachie and Roberts, 1974; Fery 1985; Claudius – Cole *et al.*, 2010). It is affected by several diseases, which do not let the plants to grow and yield to a best of genetic potential. Among various pathogens, fungi constitute an important group as they inflict damage to crop plant at different stages (Agrios, 2000). Among the fungal diseases, wilt caused by *Fusarium oxysporum* f. sp. *tracheiphilum* remains to be a challenging task in terms of management, since it is soil – borne in nature. It is distributed worldwide and is prevalent in arid, sub – tropical and tropical climate, especially in the areas with low rainfall (Fery, 1985 and 1990). Therefore, the present investigation is, however, design in a way to investigate efficacy of two species of *Glomus* and *Trichoderma* against *Fusarium oxysporum* f. sp. *tracheiphilum* causal agent of wilt on cow pea.

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MATERIALS AND METHODS

The pathogen and bioagents used in the present study were obtained from Shiraz, Agricultural Research Centre. One – week old culture of pathogen and bioagents maintained on PDA slants at 26±2ºC to use for the study.

In vitro assay

The antagonistic properties of bioagents were tested to their efficacy to inhibit growth of *Fusarium* wilt pathogen in dual culture on PDA medium (Dwivedi and Enespa, 2013). For this, the mycelial plug of 5 mm diam. from the margin of eight days old culture of bioagents and pathogen were placed, three plugs of pathogen at three equally distant places of the periphery region and one for bioagents at the center of the medium. Dual plates were incubated at 26±2ºC and the extent of interaction was observed and measured at time intervals. On the basis of this assay the most effective bioagent will be selected for glasshouse experiment.

Preparation of infected pot

Efficacy of different bioagents to control wilt disease was tested after application and sowing of cowpea seeds into artificially made *Fusarium* infested soil described by Nene

et al., (1981) with suitable modification. The earthen pots (25 cm diam.) were filled with autoclaved soil (soil, sand and organic manure in ratio of 3:1:1). Pure culture of pathogen inoculated into autoclaved cowpea flour sand medium (cowpea flour 20 g, sand 80 g, water 20 ml) in 250 ml conical flask and incubated at room temperature for 20 days. Green cowpea aerial parts were chopped into small pieces, autoclaved and mixed with *Fusarium* infested medium by 1: 1 ratio. This mixture was incorporated into pot soil up to 15 cm depth. Then 15 seeds of susceptible var. were sown into this soil and after 90 days all the infected and healthy plants were removed. Wilted plants were chopped into small pieces and incorporated into the same pot soil was repeated twice. Then the pots were used to test the efficacy of different bioagents to control *Fusarium* wilt of cowpea.

Seed coating with bioagents

Cowpea seeds washed with sterilized water were air dried and dipped into 2% carboxy methyl cellulose (CMC) solution for 2 min. The seeds were taken from CMC solution and rolled over the different bioagents spores on Petri – plats and the bioagents coated seeds were sown in infected pot.

Biomass production of bioagents

The mycelial plug of eight days old culture of bioagents grown on PDA medium were put into sterilized maize meal sand medium (maize meal 20 g, sand 80 g, water 15 ml) in 250 conical flasks and incubated at room temperature for 18 days. Before application into soil the required amount of bioagents biomass was mixed with sterilized field soil by two times.

Determination of per cent diseased plant

From each treatment, plants were gently uprooted and the roots were washed thoroughly with tap water to remove adhering soil particles. The percentage of plant diseased was calculated by following formula:

Disease plant (%) = number of plant infected/number of plant examined × 100

Statistical analysis

Observation was recorded after 90 days of sowing and analyzed statistically using One – way ANOVA followed by Duncan's Multiple rang test (Duncan, 1955).

RESULTS

The growth of *Fusarium* wilt pathogen was inhibited due to all the four bioagents used in this experiment (Table 1). *Trichoderma harzianum* exhibited the maximum antagonistic activity causing an inhibition zone of 18.20 per cent, which is far from other bioagents. Maximum growth of bioagent was documented in case of *G. mosseae* by 73.33 per cent, while, minimum was observed in case of *G. intraradices* by 66.70 per cent. Maximum per cent growth of pathogen was recorded when inoculated along with G. *intraradices*, but, minimum growth per cent was noticed in case of *T. harzianum.* On the basis of statically analyzation, per cent inhibition zone of pathogen in case of *T. harzianum* was highly significant compared to the other bioagents. Insignificant effect of per cent inhibition zone was observed when the pathogen exposed to *G. intraradices* and *G.mosseae*. Insignifacant antagonistic effect of different bioagents was documented in case of per cent growth of pathogen using *G. intraradices* and *T. viride*. The same trends of result also noticed for *G. mosseae* and *T. harzianum*. However, these finding are highly significant to each other. Only in case of *T. harzianum* using dual culture inoculation, the per cent growth of pathogen and per cent inhibition zone was significant statically (Table 1).

Table 1. *In vitro* efficacy of bioagents against *Fusarium* wilt of cowpea [a]

Bioagents	Growth (cm)		Inhibition zone (per cent)
	Antagonist	Pathogen	
Glomus mosseae	$73.33^{a,1}$	$12.75^{b,2}$	$13.92^{b,3}$
Glomus intraradices	$66.70^{b,1}$	$23.10^{a,2}$	$10.20^{b,3}$
Trichoderma harzianum	$68.48^{b,1}$	$13.32^{b,2}$	$18.20^{a,2}$
Trichoderma viride	$70.60^{a,1}$	$22.10^{a,2}$	$7.30^{c,3}$

[a]Data are average of five replicates. Different alphabets in column and numeric in rows represent insignificant difference at $p \leq 0.05$ employing DMRT (Duncan 1955).

It is evident under glasshouse condition showed response of tested plant treated with *T. harzianum* a known bioagent, was much better in both treatments i.e. seed coating and biomass application as compared to plants treated alone with *Fusarium* wilt pathogen either in infected soil or natural soil (Table 2). However, the growth parameters of treated plants were higher in case of biomass application of *T. harzianum* compared to seed coating application. Highest growth characters were recorded when the bioagent applied as a biomass at 40 g/kg soil in natural soil. The maximum length of root was recorded in treated plant with bioagent as soil application @ 40 g/kg sown in natural soil by 29.90 cm, while, the minimum of 2.20 cm was noticed in untreated seeds sown in infected soil (Table 2).

Similar trends of results also recorded in case of percentage of disease plant in both treatments. Regarding to seed coating treatment of *T. harzianum*, the diseased plant was recorded 40% which was statically significant compared to untreated plants. In contrast, when plant treated with bavistin at 2 g/kg seed recorded of disease plant was insignificant statically. When the plants treated with bioagent at 60 g/kg soil as biomass application, all the parameters of tested plant highly increased except per cent diseased plant as compared to untreated plants. In the other side, using 40 g/kg soil of *T. harzianum* as a biomass application also decreased per cent diseased plant and increased other parameters, however, the data was statically significant except in case of plant height and per cent disease plant (Table 2). As comparing of untreated seeds sown in infected soil with untreated seeds in natural soil the data clearly showed statically significant in almost all the plant characters studied in this experiment. In different treatments of study the plant height was statically significant except in case of soil application of *T. harzianum* biomass at two level of dosage (Table 2).

DISCUSSION

As we know, various disease management methods have been implemented to combat and eradicate pathogenic fungi. These include regulatory, cultural, physical and chemical methods. All these methods are effective only when employed well in

Table 2. Glasshouse evaluation of *T. harzianum* on plant growth of cowpea infected by *Fusarium* wilt [a]

Treatment	Plant height (cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Disease plant (%)
			A				
A_1	$29.90^{c,2}$	$7.73^{c,3}$	$10.31^{c,3}$	$3.72^{d,4}$	$3.72^{c,4}$	$1.65^{b,5}$	$40.00^{b,1}$
A_2	$19.96^{d,2}$	$7.40^{c,3}$	$10.68^{c,3}$	$3.40^{d,4}$	$2.84^{c,d,4}$	$1.13^{b,5}$	$37.50^{b,1}$
			B				
B_1	$39.00^{b,1}$	$8.26^{c,3}$	$15.22^{b,2}$	$5.33^{c,4}$	$5.74^{c,d,4}$	$2.17^{b,5}$	$6.30^{c,3,4}$
B_2	$40.35^{b,1}$	$13.74^{b,3}$	$20.10^{a,2}$	$9.00^{b,4}$	$13.20^{a,3}$	$4.28^{a,6}$	$5.70^{c,5}$
			C				
C_1	$7.12^{f,2}$	$2.20^{e,2,3}$	$2.79^{d,2}$	$1.27^{e,3}$	$1.10^{e,3}$	$0.19^{c,d,3,4}$	$56.50^{a,1}$
C_2	$14.72^{e,1}$	$6.30^{d,3}$	$9.02^{c,2}$	$3.24^{d,4,5}$	$2.57^{c,5}$	$0.59^{c,6}$	$4.30^{d,4}$
C_3	$12.80^{e,1}$	$4.50^{c,3}$	$6.10^{c,d,2}$	$3.70^{d,3,4}$	$2.00^{d,4}$	$0.50^{c,5}$	$5.60^{c,2,3}$
C_4	$59.80^{a,1}$	$29.90^{a,2}$	$27.17^{a,2}$	$12.30^{a,4}$	$16.50^{a,3}$	$5.80^{a,5}$	$2.10^{e,6}$

[a]Data are average of five replicates. A= Seed coating; A_1= Treated seed in infected soil; A_2= Bavistin (2g/kg) treated seed in infected soil; B= Soil application of *T. harzianum* biomass; B_1= 40 g/kg soil in infected soil; C= Chick; C_1= Untreated seed in infected soil (Control); C_2= Treated seed in natural soil; C_3= Untreated seed in natural soil; C_4= Biomass applied 40 g/kg in natural soil. Different alphabets in column and numeric in rows represent insignificant difference at $p \leq 0.05$ employing DMRT (Duncan 1955).

advance as precautionary measure (Sharma, 1996 and Kata, 2000). Once a disease has appeared, these methods become impractical / ineffective. In that situation, chemical control offers a good choice to grower to control the disease. Chemical pesticides have been in use since long and they provide quick, effective and economic management of plant diseases. However, in recent past, it has been realized that use of chemical in agriculture is not as beneficial as it was visualized. Chemical pose serious health hazards to an applicator as well as to a consumer of the treated material. In addition to target organism, pesticides also kill various beneficial organisms. Their toxic forms persist in soil and contaminate the whole environment (Hayes and Laws, 1991). Increasing awareness of humankind towards the ecosystem and environment has made a marked shift from synthetic materials to bio – products. Fungi constitute a major group of bioagents against various kinds of pests (Papavizas, 1985; Biswal, 1992; Alguacil *et al.*, 2011; Dwivedi and Enespa, 2013). *Trichoderma* spp. is known not only a bioagents for disease control but also as an agent which enhanced the growth of the plant by providing soil nutrition in soluble forms for absorption by the roots resulting in better of plants (Pathak *et al.*, 2007).

Mycorrizal fungus was very efficient and successful in inhibition of wilt of *Fusarium* (Clavet *et al.*, 1992 and 1993; Yedidia *et al.*, 2001; Kadam *et al.*, 2005; Chandanie *et al.*, 2009; Martinez – Medina *et al.*, 2010; Singh *et al.*, 2013). Garcia - Garrido (2009) indicated that arbuscular mycorrhiza acts as a root covering which ultimately reduced the space of root region, resulting of increasing of biomass of plant. Further, he also noticed that the yield of treated plant was significant to uninoculated plants. As the results revealed irrespective to the crops, when arbuscular mycorrhiza fungi along with *T. harzianum* were assessed, better growth in almost all parameters of chickpea plant observed. They have also reported that between two species of *Glomus* i.e. *mosseae* and *fasciculatum*, former one was the most effective against *Fusarium* wilt of tested plant (Singh *et al.*, 2010). Similar results were obtained when chickpea plants were inoculated with *G. intraradices* + *Pseudomonas alcaligenes* + *Bacillus pumilus* as biocontrol agents for the control of root rot disease (Jat and Bhardwaj, 2005). Dabbas *et al*. (2008) indicated that green manuring + neem cake + seed treatment by *T. viride* gave highest germination, lowest plant mortality and highest seed yield of pea. The same trends of result also reported by Linderman (1992) who meantioned that the vesicular – arbuscular mycorrhiza can play antagonistic interaction with different soil borne pathogens without any side effect to other benefit microorganisms and environment. Garmendia *et al.* (2005) observed that the pepper plants when treated with mycorrhizal fungi along with *Verticillum* wilt, the mortality and wilting were at the lowest level and simultaneously increase the different parameters of tested plant including height, fresh and dry weight and yield of crop, as compared to inoculated plant alone with pathogen. On the basis of present study the bioagents of fungi, it can be exploited for future plant disease management programs.

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