Original Research Article

Multiplication of arbuscular mycorrhiza on chickpea and their co-inoculative impact with *Trichoderma sp.* on chilli wilt

Abstract:

Fusarium wilt has emerged as a serious problem in all chilli growing areas of India. Due to difficulties in disease management and lack of stable genetic resistance in chilli cultivars, arbuscular mycorrhiza and *Trichoderma* were used to manage the disease. Mycorrhizal fungi will be raised and maintained on chickpea in earthen pots. Further experiment was conducted and Plant height, Root length, Dry weight of root and shoot, Mycorrhizal colonization (Phillips and Hayman method), Sporocarp number (Gerdemann and Nicolson method), SPAD chlorophyll content (SPAD meter) and NPK content was observed. The present investigation deals with the beneficial effect of *Glomus intraradices* with *Trichoderma harzianum* on chilli wilt under pot experiment. In soil (Chickpea) sporocarp population of *Glomus intraradices* (5283) in 100 g soil and mycorrhizal colonization of *Glomus intraradices* (87 per cent) was observed. Chilli plant showed a significant increase in the Plant height, Root length, Dry weight of root and shoot, SPAD chlorophyll content and NPK content as compared to control. The results reveal that mixed *Fusarium oxysporum* + *Glomus intraradices* + *Trichoderma harzianum* inoculation contribute best growth and development of chilli plant under pot experiment at 90 days after transplanting.

Keywords: Mycorrhiza, *Fusarium oxysporum*, *Trichoderma*, wilt, *Glomus*

Introduction: Chilli (*Capsicum annuum* L.) is an important commercial and export-oriented crop in India (Reddy et al 2014). The major diseases affecting chilli productions are Anthracnose, Phytopthora, Leaf blight, Fusarium wilt, bacterial wilt, damping-off and root rot etc. Among these, Fusarium wilt caused by the *Fusarium oxysporum* has emerged as a serious problem in recent years (Sarita and Chugh, 2020). The major diseases affecting chilli productions are Anthracnose, Phytopthora, Leaf blight, Fusarium wilt, bacterial wilt, damping-off and root rot etc. Among these, Fusarium wilt caused by the *Fusarium oxysporum* has emerged as a serious problem in recent years. It causes wilt disease ~~on~~ numerous crop plants (Prabhukarthikeyan et al 2014). In 1809, the term Fusarium was introduced by Link. Leonian in 1919 first reported Fusarium wilt disease of chilli and named the pathogen ~~as~~ *Fusarium annuum*. In India, the most common species found associated with the chilli wilt of Fusarium are *Fusarium oxysporum* and *Fusarium solani*, while, in some parts of India *Fusarium moniliforme* and *Fusarium pallidoroseum* are found (Naik, 2006). Fusarium is a soil-borne fungus. Once a field is infested, the pathogen may survive in the soil for many years. Mycorrhizal inoculation suppresses the incidence of wilt and root rot disease by 54 per cent and 64 per cent respectively (Kumar et al 2004) The fungus can be transported by farm equipment, drainage water, wind or animals, including humans. Fusarium is necrotrophic and typically soil-borne. Warmer and drier climates (>25^{0}C) favour the disease. Wilt is a highly damaging disease of chilli crop causing a significant reduction in yield because it blocks the xylem vessel and there is no uptake of nutrients and minerals by the plant which result in the death of ~~a~~ plant.

Material and methods:

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The present study entitled “Multiplication of Arbuscular mycorrhiza on chickpea and their co-inoculative impact with *Trichoderma* sp. on chilli wilt” was conducted in laboratories, screen house and research area of Department of Plant Pathology, CCS Haryana Agricultural University, Hisar during 2017-18. Hisar is located in the semi-tropical region of Western Zone of India with a latitude of 29°10’N, longitude 75°46’E and an elevation of 215.2 m above mean sea level. The materials and procedures used throughout the investigation are described in detail below.

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Maintenance of mycorrhizal Inoculum:

The experiment was conducted in screen house of Department of Plant Pathology, CCSHAU Hisar. The mycorrhizal fungi will be raised and maintained on chickpea in earthen pots This mixture of soil and root segments were used as inoculum. Plants were maintained as per agronomic practices of CCS HAU Hisar.

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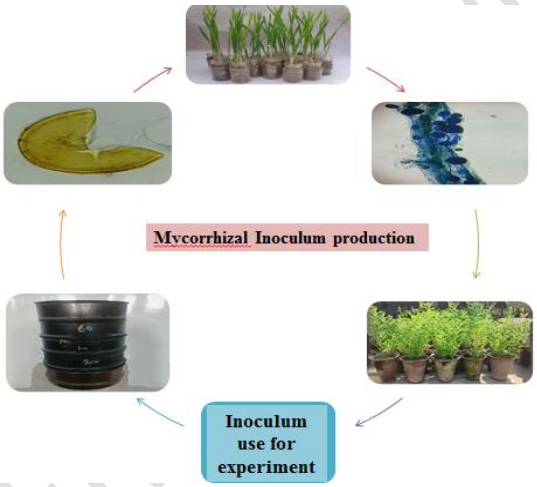
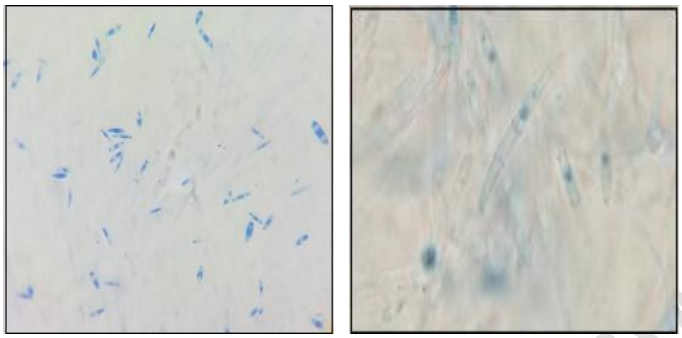


Fig: 1 Mass multiplication of mycorrhizal inoculum on chickpea

The experiment was conducted in Rabi season **(**27/11/2018) for the management of Fusarium wilt of chilli with bio-control agents in screen house of Department of Plant Pathology, CCSHAU Hisar. Seeds of Chilli cv. Pusa Jwala were sown in the nursery. Arbuscular mycorrhiza (100-150 spores and 1-1.5g roots) was multiplied in the pots and mixed in the upper 5 cm soil layer of pots. Captan (2.5g/lit of water) treatment was given by dipping the roots of seedlings in the solutions. Thirty days old seedling was transplanted in a 1 kg earthen pot. For each treatment, four numbers of sets were maintained and the statistical design was completely randomized design (CRD). Pots were watered regularly.



(A) **(B)**

Fig : 2 ***Fusarium oxysporum*** **f.sp.** ***capsici,*** **culture on PDA, front view (A), back view (B), microconidia (C) and macroconidia (D)**

1) Mycorrhizal colonization in roots

Mycorrhizal colonization was calculated by staining of roots by Phillips and Hayman (1970).

$$\textbf{Mycorrhizal colonization (\%)} = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Total number of samples assessed} \times \text{Maximum scale}}$$

2) Estimation of sporocarp in soil

Estimation of sporocarp in soil with the help of Wet Sieving and Decantation Technique was given by Gerdemann and Nicolson (1963).

Wilt intensity:

$$\textbf{Disease intensity} = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Total number of plants assessed} \times \text{Maximum scale}}$$

2) Nutrient estimation

a) Estimation of Nitrogen, phosphorous and potassium

For the estimation of nitrogen content, The Lindner method (1944) was adopted. For the estimation of phosphorous content, Vanadomolybdophosphoric yellow color method (Koenig and Johnson, 1942) was adopted. Potassium was determined in the acid digest of plant samples by using a flame photometer (Elico CL 361, India) by direct reading.

3) Chlorophyll content

The chlorophyll content of the plant was calculated by using the SPAD chlorophyll meter.

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Fig: 3. Estimation of chlorophyll content by using SPAD chlorophyll meter.

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Results :

The present study with the object of selecting an efficient AM fungus for inoculating chilli plants resulted in varied plant growth responses and management of chilli wilt disease. The effect of plant growth parameters (plant height, root length, dry shoot weight and dry root weight), Mycorrhizal colonization, sporocarp number, chlorophyll content and NPK content of chilli due to mycorrhiza alone and in combination with *Trichoderma harzianum* was found to be significantly ($p<0.05$) higher as compared to control. Among the treatments, *G. intraradices* + *T. harzianum* were significantly at par with each other. Data related to plant height was present in table 1 and plate 1. The statistical analysis revealed that plant height varied significantly depending on the different treatments and different dates of observations. It is evident from the table that triples inoculation *Fusarium oxysporum* + *Glomus intraradices* + *Trichoderma harzianum* significantly increases the plant height. Among all the treatments, maximum plant height was recorded in *F. oxysporum* + *G. intraradices* + *T. harzianum* (16.87 cm) followed by *F. oxysporum* + *G. intraradices* (14.56 cm), *G. intraradices* (14.07cm), *F. oxysporum* + *T. harzianum* (13.63 cm), *T. harzianum* (13.43 cm) and seedling dip with captan (11.07cm), *F. oxysporum* (11.03 cm) and minimum plant height was observed in control (10.93 cm) at 30 days after transplanting.

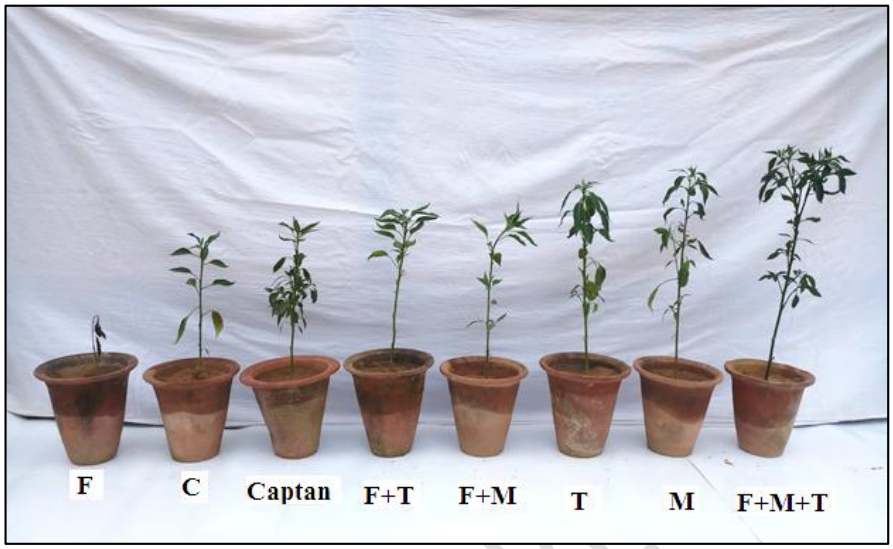


Fig: 4. Effect of different treatments on chilli plant (Where, F= *F. oxysporum*, T= *T. harzianum*, M = *G. intraradices* and C= Control)

Data related to wilt intensity was present in table 2. The maximum wilt intensity was found in *F. oxysporum* followed by *F. oxysporum*+ *T. harzianum*, *F. oxysporum* + *G. intraradices* and minimum in triple inoculation. The highest wilt intensity was *F. oxysporum* (100.00 per cent) followed by *F. oxysporum*+ *T. harzianum* (66.60 per cent), *F. oxysporum* + *G. intraradices* (60.00 per cent) and minimum in triple inoculation (43.00 per cent). The maximum per cent disease control (57 per cent) was recorded when triple inoculation *i.e. F. oxysporum* + *G. intraradices* + *T. harzianum* was done.

Data related to root length was present in table 3. The statistical analysis revealed that root length varied significantly depending on the different treatments and different dates of observations. Application of the *F. oxysporum* + *G. intraradices* + *T. harzianum* significantly increased the root length of chilli. Data were statistically analysed and found that the root length varied significantly at 30, 45, 60 and 90 days after transplanting. Among all the treatments maximum root length (11.9 cm) was recorded in *F. oxysporum* + *G. intraradices* + *T. harzianum* followed by *F. oxysporum* + *G. intraradices* (10.8 cm), *G. intraradices* (10.4 cm), *F. oxysporum* + *T. harzianum* (10.3 cm), *T. harzianum* (10.2 cm) and seedling dip with captan (7.8 cm,), control (7.7 cm) and minimum root length were recorded in *F. oxysporum* (7.3 cm) inoculated plants at 30 days after transplanting.

Data related to mycorrhizal colonization was present in table 4. The statistical analysis revealed that mycorrhizal colonization varied significantly depending on the different treatments and different dates of observations. The maximum mycorrhizal colonization was found in *G. intraradices* and minimum in *G. intraradices* + *F. oxysporum*+ *T. harzianum* (41.0, 45.3, 49.3 and 52.0 per cent at 30, 45, 60 and 90 days after transplanting respectively)**.** The maximum sporocarp number (Table 5) was found in *G. intraradices* followed by *G. intraradices* + *F. oxysporum* and minimum in *G. intraradices* + *F. oxysporum*+ *T. harzianum.* Sporocarp number in soil was calculated for the estimation of mycorrhizal

population. The highest sporocarp number was observed in *G. intraradices* (81.67, 98.00, 119.00 and 141.33 at 30, 45, 60 and 90 days after transplanting respectively) followed by *G. intraradices + F. oxysporum* (52.67, 76.67, 85.67 and 105.67 at 30, 45, 60 and 90 days after transplanting, respectively) and minimum in *G. intraradices + F. oxysporum+ T. harzianum* (46.00, 65.67, 72.00 and 85.33 at 30, 45, 60 and 90 days after transplanting, respectively).

It is evident from (Plate1) the effect of different treatments on NPK content of chilli shoot at 90 DAT. Among all the treatments maximum NPK content was recorded in *F. oxysporum + G. intraradices + T. harzianum* (1.21 per cent N, 0.61 per cent P and 1.85 per cent K) followed by *G. intraradices* (1.19 per cent N, 0.52 per cent P and 1.61 per cent K), *T. harzianum* (1.18 per cent N, 0.44 per cent P and 1.45 per cent K), *F. oxysporum + G. intraradices* (1.16 per cent N, 0.56 per cent P and 1.80 per cent K), *F. oxysporum + T. harzianum* (1.14 per cent N, 0.45 per cent P and 1.56 per cent K), captan (1.12 per cent N,0.39 per cent P and 1.43 per cent K) and minimum in control (1.11 per cent N, 0.36 per cent P and 1.41 per cent K) at 90 days after transplanting (Fig. 1,2 and 3).

Table 1: Effect of arbuscular mycorrhiza with *Trichoderma sp.* on the plant height of chilli plant

Plant height(cm) (Days after transplanting)					
Treatments	**30 Days**	**45 Days**	**60 Days**	**90 Days**	**Mean**
F. oxysporum + G. intraradices	14.56(3.95)*	18.53(4.42)	23.37(4.94)	31.67(5.72)	**22.03(4.79)**
F. oxysporum + T. harzianum	13.63(3.83)	18.40(4.40)	22.33(4.83)	30.40(5.60)	**21.19(4.71)**
F. oxysporum + G. intraradices + T. harzianum	16.87(4.23)	23.40(4.94)	26.13(5.21)	36.17(6.10)	**25.64(5.12)**
G. intraradices	14.07(3.88)	19.47(4.52)	25.03(5.10)	33.53(5.88)	**23.15(4.91)**
F. oxysporum	11.03(3.47)	0.00(1.00)	0.00(1.00)	0.00(1.00)	**2.76(1.94)**
T. harzianum	13.43(3.80)	19.17(4.49)	24.43(5.04)	33.00(5.83)	**22.51(4.74)**
Captan	11.07(3.47)	12.07(3.62)	15.77(4.09)	20.17(4.60)	**14.77(3.95)**
Control	10.93(3.45)	12.00(3.61)	15.63(4.08)	19.77(4.56)	**14.58(3.92)**
Mean	**13.20(3.76)**	**15.38(3.88)**	**19.09(4.29)**	**25.59(4.91)**	
CD at 5% level	**Days**= 0.024 **Treatments** =0.034 **Days × Treatments** = 0.068				

* Figures in parenthesis are square-root transformed value

Table 2: Effect of different treatments on wilt intensity of chilli

Treatments	Wilt intensity at 90 DAT	Per cent disease control
F. oxysporum* + *G. intraradices	60.00	40.0
F. oxysporum* +*T. harzianum	66.60	33.4
F. oxysporum* +*G. intraradices* + *T. harzianum	43.00	57.0
F. oxysporum	100.00	-

Table 3: Effect of arbuscular mycorrhiza with *Trichoderma sp.* on the root length of the chilli plant

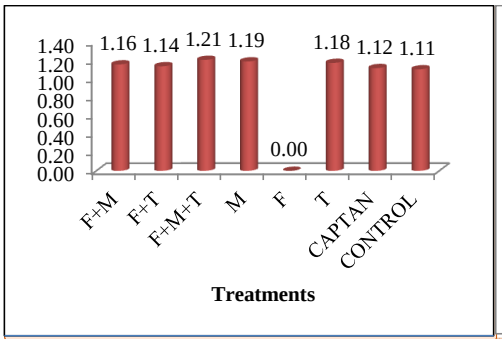
Root length(cm) (Days after transplanting)					
Treatments	**30 Days**	**45 Days**	**60 Days**	**90 Days**	**Mean**
F. oxysporum* + *G. intraradices	10.8(3.4)*	11.2(3.5)	12.0(3.6)	13.3(3.8)	**11.7(3.6)**
F. oxysporum* + *T. harzianum	10.3(3.4)	10.4(3.4)	11.3(3.5)	12.3(3.6)	**11.17(3.4)**
F. oxysporum* + *G. intraradices* + *T. harzianum	11.9(3.8)	14.8(4.0)	16.3(4.2)	16.6(4.2)	**14.9(4.0)**
G. intraradices	10.4(3.4)	12.5(3.7)	13.1(3.8)	14.0(3.9)	**12.5(3.5)**
F. oxysporum	7.9(2.9)	0.0(1.0)	0.0(1.0)	0.0(1.0)	**1.9(1.4)**
T. harzianum	10.2(3.3)	12.1(3.6)	12.5(3.7)	13.7(3.8)	**12.1(3.5)**
Captan	7.8(3.0)	8.8(3.1)	9.4(3.2)	10.2(3.3)	**9.0(3.2)**
Control	7.7(2.9)	8.4(3.1)	9.3(3.2)	10.1(3.3)	**8.9(3.1)**
Mean	**9.5(3.2)**	**9.9(3.2)**	**10.6(3.3)**	**11.4(3.4)**	
CD at 5% level	**Days =** 0.042 **Treatments** = 0.06 **Days × Treatments** = 0.12				

Table 4: Effect of arbuscular mycorrhiza with *Trichoderma sp.* on the mycorrhizal colonization (%) of the chilli plant

Mycorrhizal colonization (%)(Days after transplanting)					
Treatments	**30 Days**	**45 Days**	**60 Days**	**90 Days**	**Mean**
F. oxysporum* + *G. intraradices	43.3(6.6)	47.0(6.9)	53.3(7.3)	64.7(8.1)	**52.0(7.2)**
F. oxysporum* + *G. intraradices* + *T. harzianum	41.0(6.5)	45.3(6.8)	49.3(7.0)	52.0(7.2)	**46.9(6.9)**
G. intraradices	51.7(7.2)	58.0(7.6)	65.7(8.1)	82.7(9.1)	**64.5(8.0)**
Mean	**17.0(3.1)**	**18.8(3.3)**	**21.0(3.5)**	**24.9(3.7)**	
CD at 5% level	**Days**= 1.05 **Treatments** =1.49 **Days × Treatments** = 2.98				

Table: 5: Effect of different treatments on sporocarp in the soil

Sporocarp number (Days after transplanting)					
Treatments	**30 Days**	**45 Days**	**60 Days**	**90 Days**	**Mean**
F. oxysporum* + *G. intraradices	52.67(7.33)*	76.67(8.81)	85.67(9.31)	105.67(10.32)	80.17(8.94)
F. oxysporum* + *G. intraradices* + *T. harzianum	46.00(6.85)	65.67(8.16)	72.00(8.54)	85.33(9.29)	67.25(8.21)
G. intraradices	81.67(9.09)	98.00(9.95)	119.00(10.95)	141.33(11.93)	110.00(10.48)
Mean	22.54(3.53	30.04(3.99)	34.58(4.22)	41.54(4.57)	
CD at 5% level	**Days**= 0.064 **Treatments** =0.09 **Days × Treatments** = 0.181				



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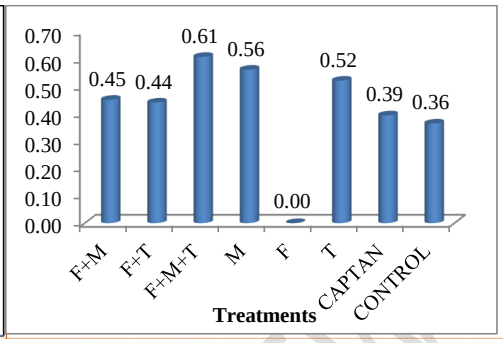

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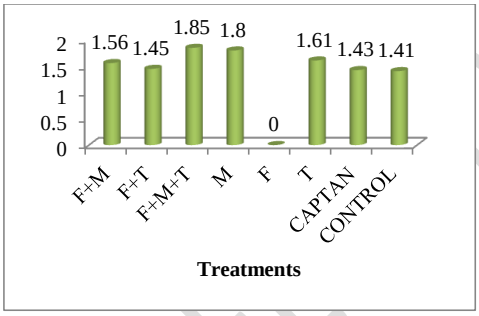

(C)

Plate: 1 Effect of different treatments on the NPK (A, B and C resectively) of chilli shoot

Discussion :

In the present study, *G. intraradices* and *T. harzianum* were applied against the soil-borne pathogen of chilli (*F. oxysporum*). Beneficial effects of both the biocontrol agents were seen under the pot experiment (Sarwade et al (2017). For this purpose, both the biocontrol agents were used with different combinations against chilli wilt. In the last few years, due to chilli wilt, 15 to 20 per cent yield losses in dry areas was reported by Siddiqui and Akhtar (2007). Further, Sarita and Chugh (2020) reported that maximum wilt intensity was recorded from the Fatehabad district (7.9 per cent), followed by Mahendragarh (7.3 per cent) and minimum from Hisar (5.2 per cent) during the cropping session 2017-18. When mycorrhiza was previously inoculated with fungal symbionts, it shows the increased resistance to fungal root rots and wilts (Jalali and Jalali 1991).

The results obtained in the present study are in agreement with the results of other researchers (Bagyaraj and Sreeramulu 1982; Sreeramulu and Bagyaraj 1986; Sarwade et al 2017; Pereira et al 2016). In the present study, it was found to be effective in inhibition of *F. oxysporum* with up to 57 % although the success rate varied among the different treatments (Table 2). The maximum per cent disease control (57%) was recorded when *F. oxysporum* + *G. intraradices* + *T. harzianum* were inoculated. Evidenced in

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root development, allowing an improvement in soil nutrient uptake by plants, just as was demonstrated by Duan et al (2015) and Watts-Williams et al (2015), they reported that the expression of phosphate (Pi) or nitrogen (N) transporter genes in roots of plants could be regulated by arbuscular mycorrhizal (AM) fungi, as responsible for growth in plants. However, Bodker et al (1998) reported that P-content alone was not sufficient for restricting the disease development.

Another study found stimulatory effects of AMF (*G. fasciculatum*), in the defence of tomato seedlings against *Fusarium oxysporum* f. sp. *lycopersici* (Raman et al 2001). In certain studies, the disease inhibition by AMF was connected to their ameliorative effects for plant nutrients especially for P-content (Caron et al 1986; Ozgonen et al 1999). Hassan Dar et al (1997) determined that the disease severity diminished in parallel to the reduction in the numbers of Fusarium propagules that are around the root rhizosphere colonized with AMF. Therefore, in the present study, we have presumed that the disease inhibition of AMF might not be completely related to the increase in P-content although there is a significant increase in P-contents and dry weights of roots. It has been thought that besides the plant nutrient uptake the competition for space and nutrients, changes in the root system, mycorrhizosphere effect and the activation of plant defence mechanisms are responsible for disease inhibition by AMF (Linderman, 1994; Azcon-Aguilar and Barea, 1996; Demir and Akkopru, 2005).

Conclusion:

This paper highlights the importance of using arbuscular mycorrhizal and *T. harzianum* as they help in the management of chilli wilt. In this study, *G. intraradices and T. harzianum* were used with different combinations for the management of chilli wilt and these successfully manage the disease as a mansion in the above tables. The arbuscular mycorrhizal with *T. harzianum* may represent a convenient alternative to chemicals and may offer economically and ecologically important advantages in sustainable or organic cropping systems.

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