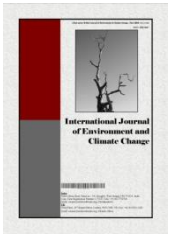

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Multiplication of Arbuscular Mycorrhiza on Chickpea and their Co-inoculative Impact with *Trichoderma sp.* on Chilli Wilt

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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, integration of arbuscular mycorrhiza and *Trichoderma* were used to manage the disease. Different mycorrhizal fungi were raised and maintained on chickpea in earthen pots. A 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 were investigated. The present investigation deals with the beneficial effect of *Glomus intraradices* and *Trichoderma harzianum* on chilli wilt under greenhouse. 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 revealed that mixed *Fusarium oxysporum* + *Glomus intraradices* + *Trichoderma harzianum* inoculation contribute best growth and development of chilli plant under greenhouse experiment at 90 days after transplanting.

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1. INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important commercial and export-oriented crop in India [1]. The major diseases affecting chilli production are Anthracnose, Phytopthora, Leaf blight, Fusarium wilt, bacterial wilt, damping-off and root rot. Among these, Fusarium wilt caused by the *Fusarium oxysporum* has emerged as a serious problem in recent years [2]. It causes wilt disease on numerous crop plants [3]. In 1809, the term Fusarium was introduced by Link. Leonian in [4,5] 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 [6]. 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 [7,8]. The fungus can be transmitted 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 [9]. 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 plants.

2. MATERIALS AND METHODS

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^{o}10'N, longitude 75^{o}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.

2.1 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. These pots were filled with 5 kg sterilized river sand. In upper 5 cm soil layer put one hundred g of mycorrhizal inoculum which contain about 450-500 chlamydospore and root bit and then ten seeds of wheat or pearlmiellet were sown and watered regularly. Hoagland's nutrient solution was applied @ 10 ml/pot after every 30 days of transplanting. After 90 days shoot portion of plant were cut at soil level and left the soil in pots to air dry. The soil was crumbled and cut the rootlets into 1 cm segments. This soil was used as a mycorrhizal inoculum.

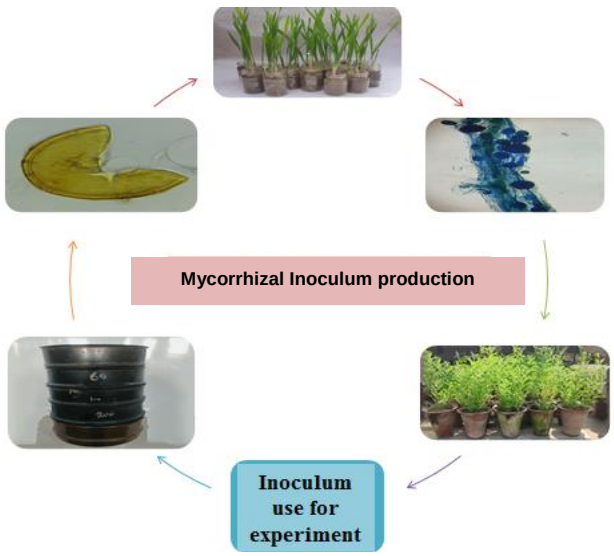


Fig. 1. Mass multiplication of mycorrhizal inoculum on chickpea

The experiment was conducted in Rabi season **(**27/11/2018) for the management of Fusarium wilt of chilli with bio-control agents in greenhouse 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).

2.2 Evaluation of Mycorrhizal Colonization in Roots of chilli

Mycorrhizal colonization was calculated by staining of roots by Phillips and Hayman [10].

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

2.3 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 [11].

2.4 Wilt Intensity

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

2.5 Nutrient Estimation

2.5.1 Estimation of Nitrogen, phosphorous and potassium

For the estimation of nitrogen content, The Lindner method [12] was adopted. For the estimation of phosphorous content, Vanadomolybdophosphoric yellow color method [13] was adopted. Potassium was determined in the acid digest of plant samples by using a flame photometer (Elico CL 361, India) by direct reading.

2.5.2 Chlorophyll content

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

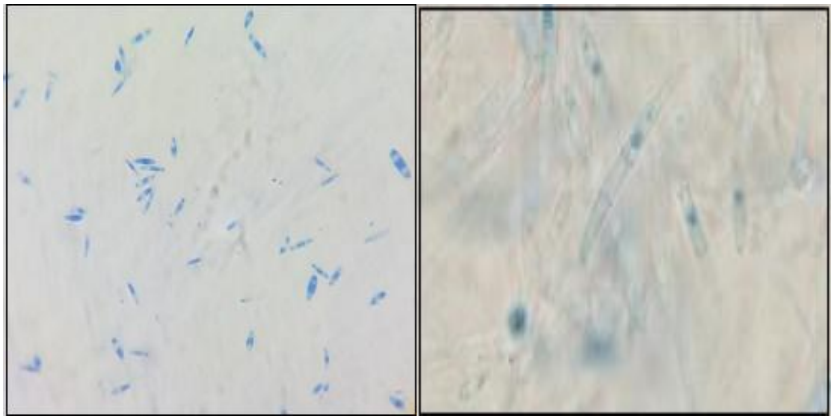
3. RESULTS

The present study with the object of selecting an efficient AM fungus for inoculating chilli plants resulted in promotion of 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 has been shown 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), control (11.03 cm) and minimum plant height was observed in *F. oxysporum* (10.03 cm) at 30 days after transplanting.

Data related to wilt intensity as above 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 as above 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.



(A) **(B)**

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

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

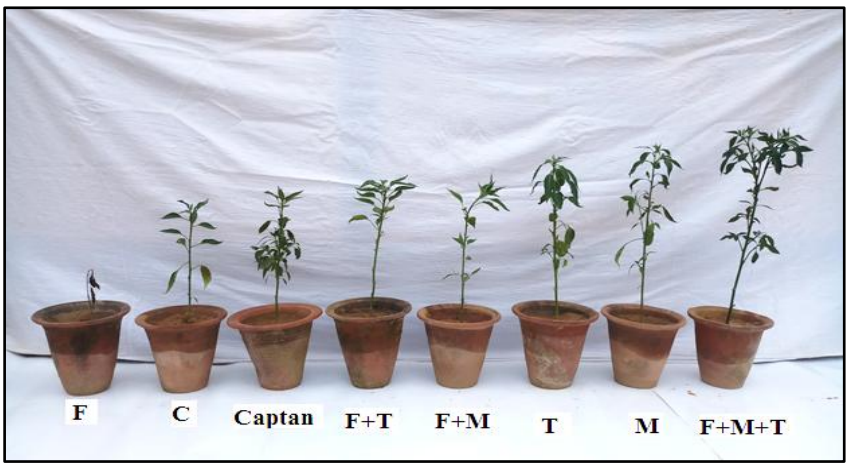


Fig. 4. Effect of different treatments on chilli plant (Where, F= *F. oxysporum*, T= *T. harzianum,* M = *G. intraradices* and C= Control)

Data related to 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 (Figs. 1,2 and 3).

Data was statistically analysed and found that the chlorophyll content varied significantly at 30, 45, 60 and 90 days after transplanting (Table 6). When *F. oxysporum + G. intraradices + T. harzianum* were inoculated, the maximum chlorophyll content was 27.0, 29.96, 31.40 and 36.36 at 30, 45, 60 and 90 DAT respectively followed by *F. oxysporum + G. intraradices* (27.00, 29.96, 31.40 and 36.36 at respective observation periods) and minimum in *Fusarium oxysporum* (16.46, 0.00, 0.00 and 0.00) inoculated plants.

4. 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. [14]. 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 [15]. Further, Sarita and Chugh [2] 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 [16].

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	10.03(3.47)	0.00(1.00)	0.00(1.00)	0.00(1.00)	2.57(1.60)
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	7.3(2.9)	0.0(1.0)	0.0(1.0)	0.0(1.0)	1.8(1.3)
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				

The results obtained in the present study are in agreement with the results of other researchers [17, 18, 14, 19]. 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 root development, allowing an improvement in soil

Table 6. Effect of different treatments on chlorophyll contant (SPAD) of chilli

Days/ Treatments	30 Days	45 Days	60 Days	90 Days	Mean
F. oxysporum + G. intraradices	27.00(5.3)*	27.06(5.3)	28.16(5.4)	30.20(5.6)	27.2(5.3)
F. oxysporum + T. harzianum	23.43(4.9)	26.86(5.3)	27.90(5.4)	28.40(5.4)	26.45(5.2)
F. oxysporum + G. intraradices + T. harzianum	29.43(5.5)	34.80(6.0)	37.53(6.2)	42.73(6.6)	36.1(6.0)
G. intraradices	23.66(5.0)	29.96(5.6)	31.40(5.7)	36.36(6.1)	31.18(5.7)
F. oxysporum	16.46(4.2)	0.0(1.0)	0.0(1.0)	0.0(1.0)	4.1(1.8)
T. harzianum	22.64(4.9)	27.30(5.3)	28.66(5.4)	33.50(5.9)	28.28(5.4)
Captan	18.00(4.2)	18.16(4.3)	20.00(4.6)	21.20(4.7)	19.34(4.5)
Control	16.76(4.2)	17.80(4.3)	19.50(4.5)	20.66(4.7)	18.68(4.4)
Mean	22.19(4.8)	22.72(4.6)	24.14(4.8)	26.63(5.0)	
CD at 5% level	Days = 0.04 Treatments= 0.06 Days × Treatments= 0.12				

** Figures in parenthesis are square root transformed values*

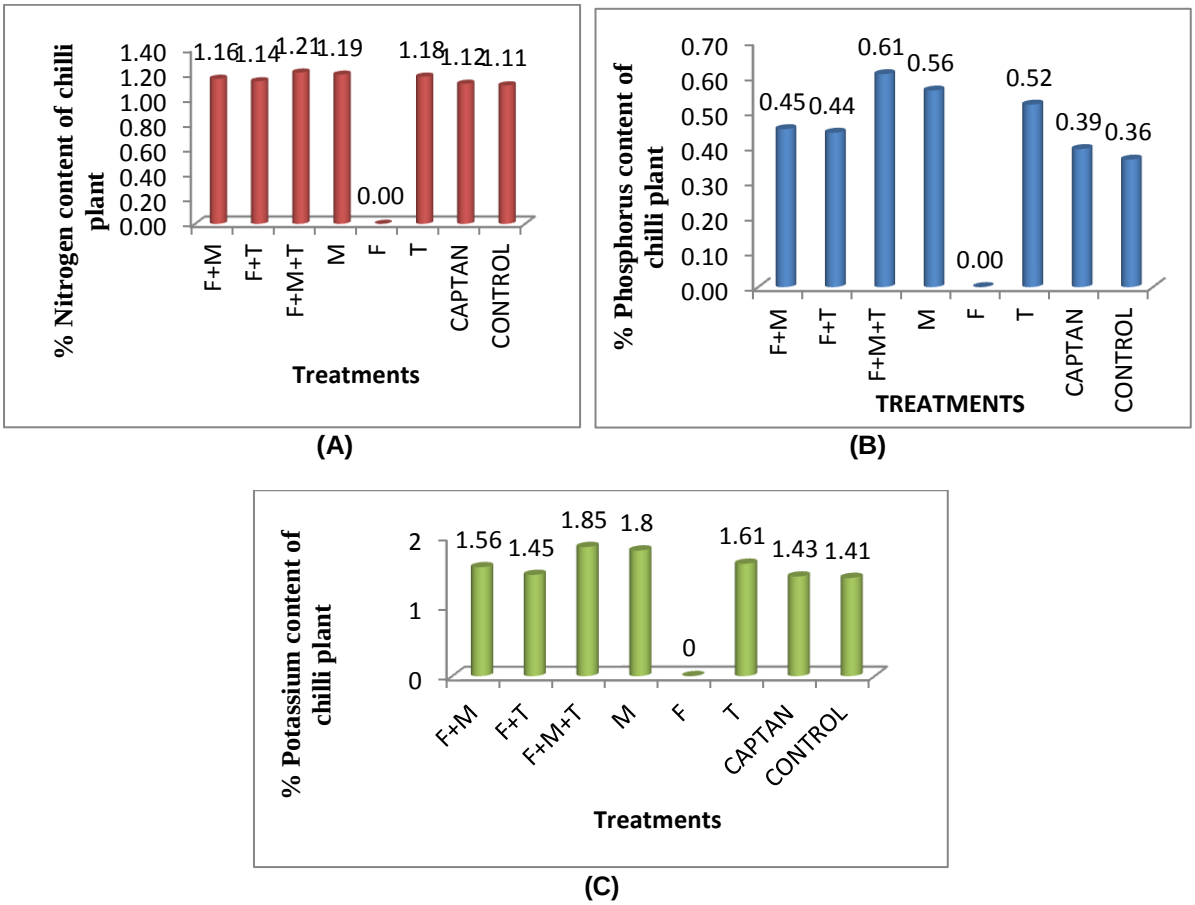


Plate 1. Effect of different treatments on the NPK (A, B and C resectively) of chilli shoot

nutrient uptake by plants, just as was demonstrated by Duan et al. [20] and Watts-Williams et al. [21], 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 [22]. However, Bodker et al. [23] 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* [24]. In certain studies, the disease inhibition by AMF was connected to their

ameliorative effects for plant nutrients especially for P-content [25,26]. Hassan Dar et al. [27] 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 [28-30].

5. CONCLUSION

This paper highlights the importance of arbuscular mycorrhiza and *T. harzianum.* Combination of *G. intraradices and T. harzianum* help in management of chilli wilt. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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