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Full Length Research Paper

Interaction effects of *Glomus fasciculatum* and *Trichoderma viride* inoculations on groundnut plants inoculated with pathogen *Macrophomina phaseolina*

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An investigation was undertaken to evaluate interaction between arbuscular mycorrhizal (AM) fungus (*Glomus fasciculatum*) and *Trichoderma* species (*Trichoderma viride*) for their possible role in biological control ability in groundnut plant against soil-borne root pathogen *Macrophomina phaseolina* which is causal agent of root-rot. For this, groundnut seeds were treated with both AM fungi and *Trichoderma* singly or in combination of both in presence or absence of pathogen *M. phaseolina*. In the obtained results, the overall plant growth parameters such as shoot, root length, fresh, dry weight and leaf, pod numbers, disease severity, mycorrhizal dependency, physiological, bio-chemical activities and antioxidant enzyme activities were calculated after 30 and 60 days of sowing. The interaction result showed that the effect of pathogen was significantly reduced due to single or dual inoculation of either AM fungi or *Trichoderma* in terms of plant growth or disease severity. In defense related physiological, biochemical and antioxidant enzyme activity also showed marked increase due to single or dual inoculations of AM fungi or *Trichoderma* species. However, the dual inoculations of AM fungi/*Trichoderma* were more pronounced in increasing overall growth, reducing the disease severity caused by *M. phaseolina* and bringing about defense related physiological, bio-chemical, antioxidant enzyme activities in the presented pot culture investigation.

Key words: AM fungi, biological control, groundnut, *M. phaseolina, Trichoderma*.

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid. is a saprophytic fungi that infects about 500 plant species with wide range of host (Srivastava et al., 2001) by causing dry root rot/charcoal root-rot. *M. phaseolina* forms micro-sclerotina in soil which can survive adverse environmental conditions and may remain viable for more than 3 years (Kendig et al., 2000). There are various methodology availble today for the disease to be controlled by applying like chemicals such as fumigating by methyl bromide, chemical fungicides or soil drenching but in longer perspective it may prove costly economically and environmentally. So, biological control seems inevitable over harmful chemicals (Agrios, 2004). In that perspective for the biocontrol of plant pathogens, incorporation of rhizospheric micro-organisms have the potential (Ozgonen et al., 1999) besides providing promotion of growth in plants (Barea et al., 2005).

Among the beneficial bio-control agents, AM fungi have been constantly reported because of their role in improved plant nutrition and resistance to abiotic or biotic stress (Azcon-Aguilar and Barea, 1996; Caron, 1989; Linderman, 2000; Akkopru and Demir, 2005; Fritz et al., 2006). Also, the saprophytic fungi known as *Trichoderma* species in soil have been seen as promoter of plant growth by improving nutritional status or by providing defense from plant pathogens in host plants by employing several mechanisms such as mycoparasitism, antibiosis, competition and production of cell wall degrading enzymes (Harman, 2004; Shakeri and Foster, 2007; Mala et al., 2009). Therefore, AM fungi and *Trichoderma* species possess qualitities which could be suitable for effective biocontrol agents.

Hence, objectives of this research were to evaluate the effects of AM fungi and *Trichoderma* inoculations singly or in

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combination against pathogen *M. phaseolina* causing root-rot in groundnut plants.

MATERIALS AND METHODS

Plant Growth and Inoculation Conditions

The pathogen *M. phaseolina* was obtained from the Division of Agharkar Research Institute, Pune, India. *M. phaseolina* was mass multiplied on sorghum seeds which were soaked overnight in conical flask. From pure culture 7 mm mycelial disc was inoculated onto sorghum seeds and were incubated for 3 weeks and it served as pathogen inoculum. The AM fungus (*G. fasciculatum*) was isolated, identified and mass-multiplied on sorghum and guinea grass, from which 20 g of rhizospheric soil containing spores, colonized root pieces served as mycorrhizal inoculum. The *Trichoderma viride* (talc based) was obtained from the Agricultural College, Pune, India. Four seeds of local susceptible groundnut cultivar [Phule Pragati (JL-24)] were pre-treated with 10 g per kg with *T. viride* and mycorrhizal inoculum was placed below seeds for plantation on autoclaved soil. The pathogen inoculum (5 g) was placed in soil around roots of groundnut plants after 15 days of sowing. There were total 8 treatments as follows: 1. Control (uninoculated); 2. Control with *M. phaseolina* (C+Mp); 3. Control with *T. viride* (C+Tv); 4. Control with *T. viride* and *M. phaseolina* (C+Tv+Mp); 5. *G. fasciculatum* (Gf); 6. *G. fasciculatum* with *M. phaseolina* (Gf+Mp); 7. *G. fasciculatum* with *T. viride* (Gf+Tv); 8. *G. fasciculatum* with *T. viride* in presence of *M. phaseolina* (Gf+Tv+Mp).

Evaluation of Treatments

The experiment was conducted in CRBD format with three replications. The evaluation of plant growth parameters such as shoot, root length, fresh, dry weight, leaf, pod numbers, physiological, biochemical and antioxidant activities were measured after 30 and 60 days of sowing as follows: 1. Acid and alkaline phosphatase (Lowry et al., 1954); 2. Total chlorophyll (Arnon, 1949); 3. Proline (Bates et al., 1973); 4. Protein (Lowry et al., 1951); 5. Total phenol (Malick and Singh, 1980); 6. Polyphenol, peroxidase (Putter, 1974) and 7. Superoxide dismutase (Beauchamp and Fridovich, 1971). The percent root colonization was determined by Giovannetti and Mosse, (1980) and mycorrhizal dependency was calculated according to Plenchette et al., (1983).

Statistical Analysis

The data were subjected to statistical scrutiny following one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± SD. Duncan's multiple range test was applied as post hoc test at $p=0.05$. All the calculations were made by using a Statistical Package for Social Sciences (SPSS) for windows version 9.0 and Microsoft Excel 2007 was used to analyze the data.

RESULTS AND DISCUSSION

Disease Severity

In the given Figure 1, the inoculations of AM fungi and *Trichoderma* in groundnut plants significantly reduced the disease severity percentage after 15 and 45 days of pathogen infection. The disease severity percentage after 45 days of pathogen infection in non-AM fungi but *Trichoderma* treated groundnut plants (C+Mp+Tv) was 81.48 % and 66.67 % in presence of pathogen in only AM fungi treatment (Gf+Mp). But combined inoculation of both *G. fasciculatum*/*T. viride* in presence of pathogen (Gf+Tv+Mp) was found to be most effective combination than single inoculation by either antagonists which reduced severity by 50 %. The associations with AM fungi and *Trichoderma* species have been best known to provide protection against pathogens by the mechanism of improved nutrition or competition for space and nutrients in host plants (Karagiannidis et al., 2002; Celar, 2003; Malik and Dawar, 2003).

Mycorrhizal Dependency

The results in Figure 2 showed that mycorrhizal dependency was higher in presence of pathogen (*M. phaseolina*) when inoculated with AM fungi or *Trichoderma*. The mycorrhizal dependency showed higher ranged between 58.93 - 83.78 % for diseased groundnut plants inoculated with AM fungi/*Trichoderma* singly or in combinations (Gf+Mp or Gf+Tv+Mp) as compared to single or dual inoculations of AM fungi/*Trichoderma* (Gf or Gf+Tv) in absence of pathogen in groundnut plants where it ranged between 38.46 - 44.37 %. The more pronounced effect of mycorrhizal dependency in presence of pathogen have been demonstrated by Declerck et al., (2002), where he showed that the presence of pathogen *Cylindrocladium spathiphylli* caused higher mycorrhizal dependency range in banana plants. However, the mycorrhizal dependency was lower in healthy groundnut plants in dual inoculation of AM fungi/*Trichoderma* (Gf+Tv) as compared to only AM fungi treatment (Gf) due to possible role shared by *Trichoderma* together with AM fungi in plants growth promotion. Already, both AM fungi and *Trichoderma* species have been acknowledged to provide growth by their role in phosphorous metabolism (Smith and Smith, 2012; Rudresh et al., 2005).

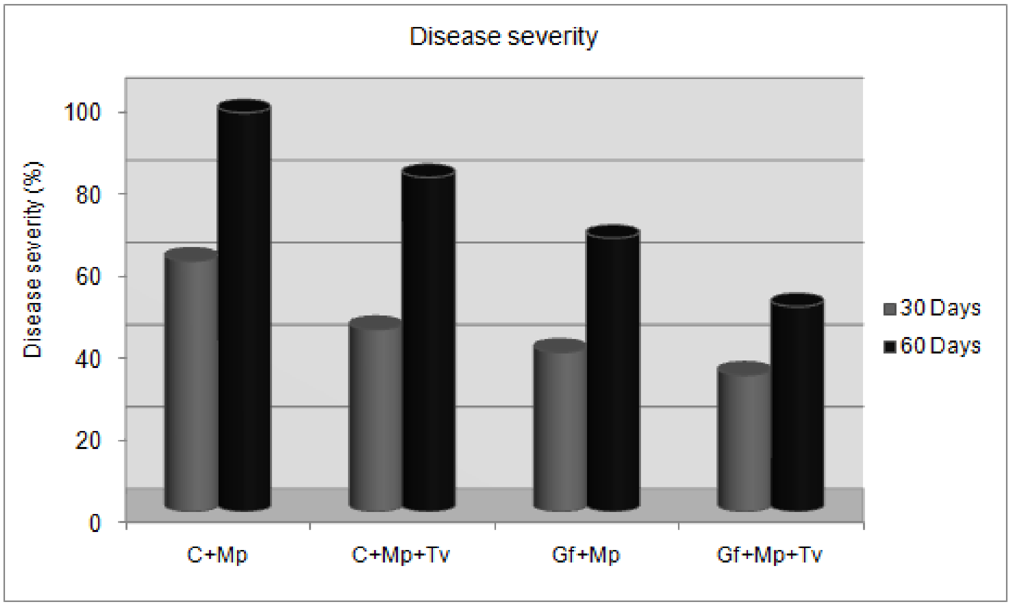

Figure 1. Effect of *G. fasciculatum* inoculation and *T. viride* on disease severity of *A. hypogaea* L. (JL-24):

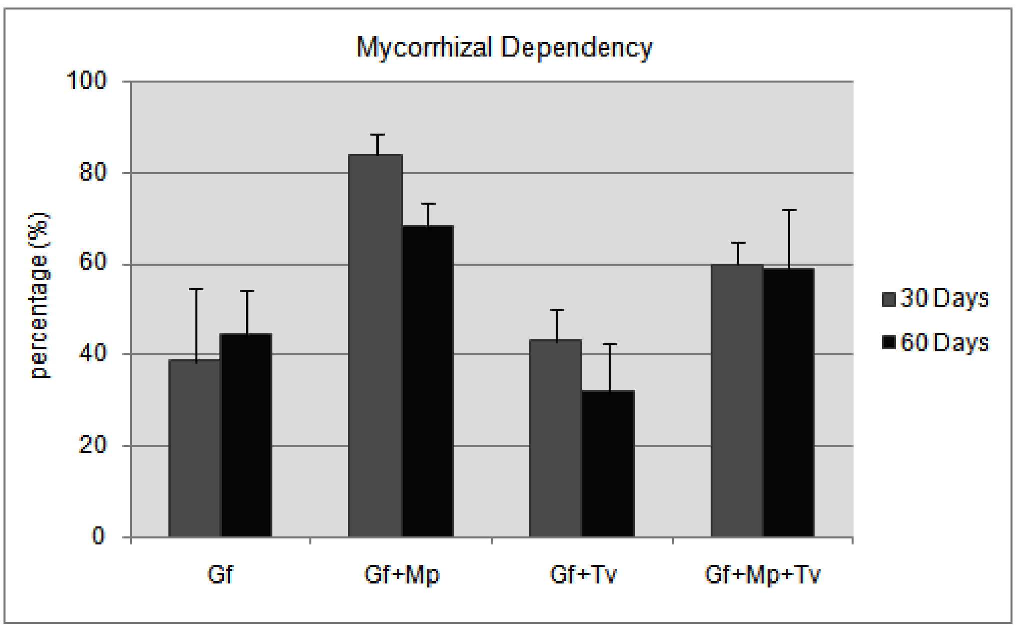

Figure 2. Effect of *G. fasciculatum* inoculation and *T. viride* on mycorrhizal dependency of *A. hypogaea* L. (JL-24):

Total Chlorophyll

The content of chlorophyll increased due to inoculation by AM fungi/*Trichoderma* which might mark overall well being for the growth of plants (Arfan et al., 2007). The total chlorophyll content was significantly higher by 35.77 % after 60 days of sowing in only mycorrhizal groundnut plants (Gf) as compared to any other treatment or non-mycorrhizal control ones followed by dual inoculations with both *G. fasciculatum* and *T. viride* without pathogen (Gf+Tv). In single inoculation of AM fungi in presence of pathogen (Gf+Mp) recorded increased total chlorophyll content in groundnut plants after 30 days of sowing by 50.87 % whereas 5.68 % increase was recorded in dual inoculations of AM fungi/*Trichoderma* treatment (Gf+Mp+Tv) which showed major role played by the AM fungi than *Trichoderma* species in increasing the chlorophyll content. The lowest chlorophyll content was observed in uninoculated control diseased ones (Figure 3).

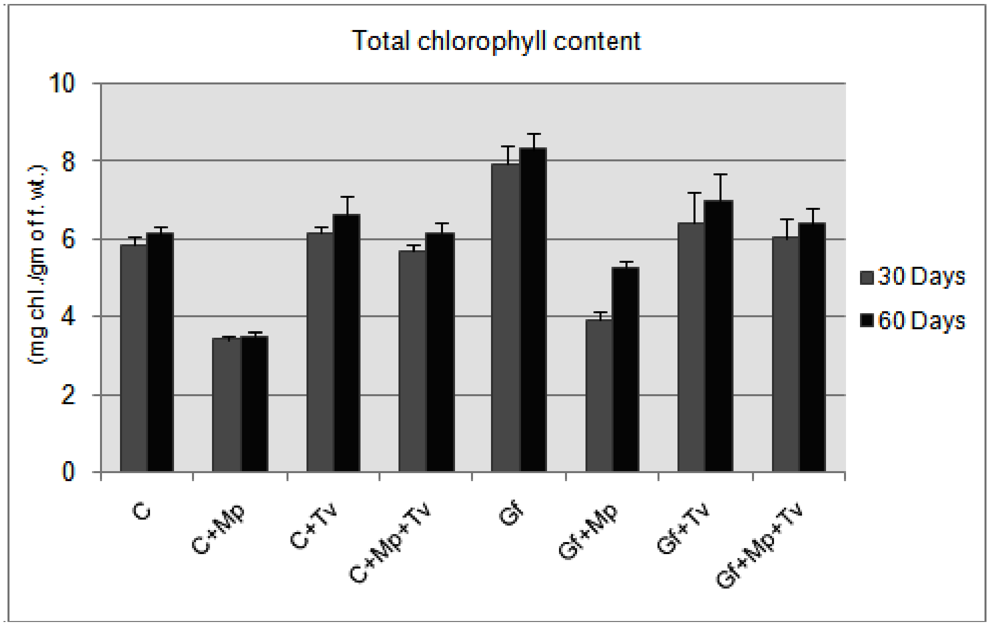

Figure 3. Effect of *G. fasciculatum* inoculation and *T. viride* on total chlorophyll content of *A. hypogaea* L. (JL-24):

Table 1. Effect of *G. fasciculatum* inoculation and *T. viride* on the shoot and root length of *A. hypogaea* L. (JL-24):

	Shoot length (cm)		**Root length (cm)**	
Treatments	**30 Days**	**60 Days**	**30 Days**	**60 Days**
C	20.67±1.25de	25.33±1.25b	27.67±1.25c	29.67±1.25c
C+Mp	17.33±3.40e	15.33±1.25d	18.00±0.82e	19.33±1.70e
C+Tv	23.67±0.47cd	25.33±1.25b	31.33±1.70ab	30.67±1.25c
C+Mp+Tv	18.00±1.63e	18.33±0.94c	23.33±1.25d	25.67±1.70d
Gf	28.67±1.25ab	30.67±1.25a	34.67±2.49a	36.00±2.16a
Gf+Mp	24.33±1.25cd	24.00±0.82b	25.33±1.70cd	31.33±1.25bc
Gf+Tv	29.00±1.63a	32.00±0.82a	32.67±1.25a	34.00±0.82ab
Gf+Mp+Tv	25.00±1.63bc	25.00±0.82b	28.67±1.25bc	30.00±0.82c

C: Control (Uninoculated); C+Mp: Control+*M. phaseolina*; C+Tv: Control+*T. viride*; C+Mp+Tv: Control+ *M. phaseolina* +T. *viride*; Gf: *G. fasciculatum*; Gf+Mp: *G. fasciculatum*+ *M. phaseolina*; Gf+Tv: *G. fasciculatum*+*T. viride*; Gf+Mp+Tv: *G. fasciculatum*+ *M. phaseolina* +*T. viride*. Means followed by the same subscript within column are not significantly different at p<0.05 (Duncan's multiple range test), n= three replicates.

Growth Parameters

The overall growth parameters in terms of shoot, root length, fresh, dry weight and leaf, pod numbers were observed to be higher in case of mycorrhiza/*Trichoderma* treatments. The overall growth parameters were observed to be lower in groundnut plants due to presence of pathogen. However, the combined inoculations of AM fungi/*Trichoderma* (Gf+Tv+Mp) showed better results in presence of pathogen. The growth parameters were observed to highest in groundnut plants where both *G. fasciculatum*/*T. viride* (Gf+Tv) were inoculated followed by single inoculations of either AM fungi or *Trichoderma* or control ones (Table 1, 2, 3). But, AM fungi seemed to have performed better than *Trichoderma* in terms of promotion in groundnut plants. The marked growth promotion shown by AM associations can be correlated for their ability to increase nutritive value to host plants, especially P which is required for all organisms and it is also well known aspect of AM fungi (Smith and Smith, 2012). Also, Rudresh et al., (2005) showed the positive attributes of phosphorous metabolism in plants-growth by

Table 2. Effect of *G. fasciculatum* inoculation and *T. viride* on the fresh and dry weight of *A. hypogaea* L. (JL-24):

	Fresh weight (g)		Dry weight (g)	
Treatments	**30 Days**	**60 Days**	**30 Days**	**60 Days**
C	5.87±0.55d	6.87±0.66e	3.34±0.22b	3.42±0.09cd
C+Mp	1.30±0.14f	2.46±0.36f	0.79±0.18d	1.61±0.06e
C+Tv	6.34±0.78d	7.34±0.18de	3.50±0.34b	4.23±0.09bc
C+Mp+Tv	3.53±0.37e	4.26±0.39f	2.05±0.35c	2.12±0.25de
Gf	11.00±1.29ab	12.34±2.13ab	5.68±0.97a	6.32±1.04a
Gf+Mp	8.90±0.63c	9.53±0.38cd	4.99±0.66a	5.16±0.84ab
Gf+Tv	12.48±1.28a	12.82±1.74a	6.18±0.30a	6.34±0.91a
Gf+Mp+Tv	9.44±1.37bc	10.11±0.92bc	5.15±0.94a	5.45±0.93ab

C: Control (Uninoculated); C+Mp: Control+*M. phaseolina*; C+Tv: Control+*T. viride*; C+Mp+Tv: Control+ *M. phaseolina* +T. *viride*; Gf: *G. fasciculatum*; Gf+Mp: *G. fasciculatum*+ *M. phaseolina*; Gf+Tv: *G. fasciculatum*+*T. viride*; Gf+Mp+Tv: *G. fasciculatum*+ *M. phaseolina* +*T. viride*. Means followed by the same subscript within column are not significantly different at p<0.05 (Duncan's multiple range test), n= three replicates.

Table 3. Effect of *G. fasciculatum* inoculation and *T. viride* on the leaf and nodule number of *A. hypogaea* L. (JL-24):

	Leaf number (no.)		Nodule number (no.)	
Treatments	**30 Days**	**60 Days**	**30 Days**	**60 Days**
C	49.33±4.19e	63.67±3.40d	6.67±1.25cd	5.00±0.82e
C+Mp	22.33±3.09f	22.00±2.16f	1.00±0.82d	1.00±0.82e
C+Tv	65.00±4.08d	85.00±4.08c	8.33±1.25bcd	14.33±3.09cd
C+Mp+Tv	44.33±3.30e	40.67±2.49e	5.33±0.47cd	5.33±0.47e
Gf	94.67±3.68b	124.67±6.85a	15.33±6.94b	30.67±6.02b
Gf+Mp	76.67±3.40c	83.33±3.40c	11.00±2.9bc	12.33±2.05d
Gf+Tv	120.00±8.1a	123.33±2.3a	23.33±4.0a	25.67±2.49b
Gf+Mp+Tv	91.00±2.94b	104.67±4.5b	8.00±3.27bcd	19.00±2.94c

C: Control (Uninoculated); C+Mp: Control+*M. phaseolina*; C+Tv: Control+*T. viride*; C+Mp+Tv: Control+ *M. phaseolina* +T. *viride*; Gf: *G. fasciculatum*; Gf+Mp: *G. fasciculatum*+ *M. phaseolina*; Gf+Tv: *G. fasciculatum*+*T. viride*; Gf+Mp+Tv: *G. fasciculatum*+ *M. phaseolina* +*T. viride*. Means followed by the same subscript within column are not significantly different at p<0.05 (Duncan's multiple range test), n= three replicates.

T. harzianum. Earlier, in a similar type of interaction presented in results (Table 1, 2, 3) have been demonstrated between AM fungi incorporated along with *Trichoderma* species in increasing total plant biomass of plants (Calvet et al., 1993; Srinath et al., 2003).

Changes in Acid and Alkaline Phosphatase Activity

The acid phosphatase activity significantly increased after 30 and 60 days of sowing by 158.65 % in Gf; 53.45 % in Gf+Tv respectively. In single or dual inoculation of *G. fasciculatum*/*T. viride* treatment with pathogen showed increased acid phosphatase activity after 30 days of sowing by 109.31 % in Gf+Mp and 18.75 % in Gf+Mp+Tv.

The activities of alkaline phosphatase increased after 30 and 60 days of sowing by 159.33 % in Gf and 53.38 % in Gf+Tv. The alkaline phosphatase activity was higher when inoculated with *G. fasciculatum* or *T. viride* singly or in combination with or without pathogen after 30 days of sowing by 116.52 % for Gf+Mp and 63.83 % for Gf+Mp+Tv (Tab. 4). The increased phosphatase activity has been linked to their activity by mycorrhizal colonization in host plants (Allen et al., 1995). Moreover, restriction in phosphate acquisition occurs during stress conditions (Barrett-Lennard et al., 1982) that is why present investigation showed reduced phosphatase activity in presence of pathogen *M. phaseolina*. Also, the increased phosphate amount could account for lowering of pathogen effects.

Changes in Proline and Protein Content

The root proline content showed increase after 60 days of sowing which was higher by 170.66 % in healthy AM fungi

Table 4. Effect of *G. fasciculatum* inoculation and *T. viride* on the acid and alkaline phosphatase of *A. hypogaea* L. (JL-24):

	Acid phosphatase (moles of p-nitrophenol released g^{-1} of fresh weight)		Alkaline phosphatase (moles of p-nitrophenol released g^{-1} of fresh weight)	
Treatments	**30 Days**	**60 Days**	**30 Days**	**60 Days**
C	0.099±0.006d	0.120±0.008c	0.112±0.008cd	0.179±0.019cd
C+Mp	0.089±0.005d	0.104±0.007c	0.092±0.008d	0.159±0.019d
C+Tv	0.186±0.027c	0.221±0.007b	0.192±0.0081b	0.214±0.014c
C+Mp+Tv	0.189±0.025c	0.255±0.013b	0.133±0.005c	0.197±0.012cd
Gf	0.256±0.011a	0.339±0.012a	0.290±0.024a	0.296±0.013ab
Gf+Mp	0.187±0.016c	0.257±0.024b	0.200±0.013b	0.272±0.024b
Gf+Tv	0.216±0.011bc	0.339±0.032a	0.274±0.009a	0.328±0.016a
Gf+Mp+Tv	0.224±0.005ab	0.235±0.008b	0.217±0.019b	0.274±0.030b

C: Control (Uninoculated); C+Mp: Control+*M. phaseolina*; C+Tv: Control+*T. viride*; C+Mp+Tv: Control+ *M. phaseolina* +*T. viride*; Gf: *G. fasciculatum*; Gf+Mp: *G. fasciculatum*+ *M. phaseolina*; Gf+Tv: *G. fasciculatum*+*T. viride*; Gf+Mp+Tv: *G. fasciculatum*+ *M. phaseolina* +*T. viride*. Means followed by the same subscript within column are not significantly different at $p<0.05$ (Duncan's multiple range test), n= three replicates.

Table 5. Effect of *G. fasciculatum* inoculation and *T. viride* on the root protein and proline content of *A. hypogaea* L. (JL-24):

	Protein (μ^{-1} g^{-1} fresh weight)		Proline (mg g^{-1} of fresh weight)	
Treatments	**30 Days**	**60 Days**	**30 Days**	**60 Days**
C	0.096±0.004e	0.107±0.003f	0.604±0.056d	0.734±0.030f
C+Mp	0.116±0.016de	0.142±0.007de	0.865±0.143c	1.020±0.096e
C+Tv	0.127±0.011d	0.139±0.011e	0.857±0.045c	0.912±0.053ef
C+Mp+Tv	0.132±0.007cd	0.161±0.010d	1.085±0.121c	1.370±0.154d
Gf	0.153±0.019bc	0.184±0.008c	1.460±0.137b	1.988±0.174c
Gf+Mp	0.174±0.006b	0.205±0.012b	1.598±0.048ab	2.305±0.031b
Gf+Tv	0.175±0.008b	0.191±0.005bc	1.587±0.143ab	2.214±0.049b
Gf+Mp+Tv	0.227±0.004a	0.240±0.013a	1.724±0.076a	2.614±0.054a

C: Control (Uninoculated); C+Mp: Control+*M. phaseolina*; C+Tv: Control+*T. viride*; C+Mp+Tv: Control+ *M. phaseolina* +*T. viride*; Gf: *G. fasciculatum*; Gf+Mp: *G. fasciculatum*+ *M. phaseolina*; Gf+Tv: *G. fasciculatum*+*T. viride*; Gf+Mp+Tv: *G. fasciculatum*+ *M. phaseolina* +*T. viride*. Means followed by the same subscript within column are not significantly different at $p<0.05$ (Duncan's multiple range test), n= three replicates.

treatments (Gf) followed by 142.76 % for AM fungi/*Trichoderma* treatments (Gf+Tv), 126.04 % in diseased AM fungi treatments (Gf+Mp) and 90.81 % in combined inoculations of AM fungi and *Trichoderma* (Gf+Mp+Tv). The root protein content was higher after 30 days of sowing by 60.21 % in Gf, 50.00 % in Gf+Mp, 38.62 % in Gf+Tv and 71.68 % in Gf+Mp+Tv (Tab. 5). The marked increases in content of proline are known for their ability to scavenge reactive oxygen species (ROS) produced during biotic stresses (Chen and Dickman, 2005). The increased protein content may be related to plant protection by AM fungi and *Trichoderma* species against plant pathogens (Gianinazzi-Pearson et al., 1994; Harman, et al., 2004).

Changes in Total Phenol and Polyphenol Activity

The total phenol recorded to be highest after 60 days of sowing in Gf+Mp by 93.57 % followed by 71.91 % for Gf, 60.83 % for Gf+Tv and 49.73 % for Gf+Mp+Tv. The root polyphenol activities were observed to be higher when inoculated either by *G. fasciculatum*/*T. viride* singly or in dual combination with or without pathogen *M. phaseolina* as compared to non inoculated controls ones after growth period of 30 days and 60 days of sowing (51.02 % for Gf, 39.06 % in Gf+Mp, 25.86 % in Gf+Tv, 20.51 % in Gf+Mp+Tv) (Tab. 6). The content of total phenols and polyphenol activity were highest in diseased groundnut plants treated with both AM fungi/*Trichoderma* (Gf+Tv+Mp).

Table 6. Effect of *G. fasciculatum* inoculation and *T. viride* on the root total phenol and polyphenol oxidase activity of *A. hypogaea* L. (JL-24):

	Total phenol (mg g^{-1} fresh weight)		Polyphenol oxidase (min^{-1} g^{-1} fresh weight)	
Treatments	30 Days	60 Days	30 Days	60 Days
C	0.057±0.005f	0.120±0.009e	0.408±0.063e	0.442±0.038c
C+Mp	0.071±0.004e	0.123±0.013e	0.533±0.088cde	0.650±0.109ab
C+Tv	0.070±0.003e	0.119±0.012e	0.483±0.052de	0.542±0.063bc
C+Mp+Tv	0.088±0.004d	0.182±0.014d	0.650±0.156abc	0.708±0.113a
Gf	0.089±0.004cd	0.214±0.011bc	0.617±0.052bcd	0.633±0.080ab
Gf+Mp	0.109±0.007b	0.238±0.007b	0.742±0.076ab	0.775±0.090a
Gf+Tv	0.098±0.004c	0.191±0.0010cd	0.608±0.052bcd	0.692±0.076ab
Gf+Mp+Tv	0.121±0.005a	0.272±0.022a	0.783±0.088a	0.792±0.101a

Table 7. Effect of *G. fasciculatum* inoculation and *T. viride* on the shoot superoxide dismutase and root peroxidase acitivity of *A. hypogaea* L. (JL-24):

	Superoxide dismutase (unit g^{-1} fresh weight)		Peroxidase (min^{-1} mg^{-1} protein)	
Treatments	30 Days	60 Days	30 Days	60 Days
C	1.280±0.204e	3.192±0.064e	0.00355±0.00078e	0.00397±0.00078e
C+Mp	1.680±0.098e	3.464±0.329e	0.00773±0.00078cd	0.00961±0.00078cd
C+Tv	1.560±0.098e	4.248±0.443d	0.00564±0.00153d	0.00815±0.00051d
C+Mp+Tv	2.320±0.247d	4.672±0.123d	0.00731±0.00106cd	0.01003±0.00051c
Gf	2.680±0.396d	5.200±0.169c	0.00710±0.00078cd	0.00940±0.00051cd
Gf+Mp	3.360±0.098c	6.552±0.225b	0.00835±0.00078bc	0.01253±0.00051b
Gf+Tv	2.720±0.150d	6.308±0.193b	0.01003±0.00051ab	0.01232±0.00129b
Gf+Mp+Tv	4.000±0.247b	6.840±0.230b	0.01107±0.00078a	0.01483±0.00078a

C: Control (Uninoculated); C+Mp: Control+*M. phaseolina*; C+Tv: Control+*T. viride*; C+Mp+Tv: Control+ *M. phaseolina* +T. *viride*; Gf: *G. fasciculatum*; Gf+Mp: *G. fasciculatum+ M. phaseolina*; Gf+Tv: *G. fasciculatum+T. viride*; Gf+Mp+Tv: *G. fasciculatum+ M. phaseolina +T. viride*. Means followed by the same subscript within column are not significantly different at $p<0.05$ (Duncan's multiple range test), n= three replicates.

Moreover, the phenolics are long known to have antimicrobial properties and are involved in rapid accumulation at infection sites, also oxidized phenols are known to be more toxic than non-oxidized form (Mohammadi and Kazemi, 2002; Hilal et al., 2006). Also, increased accumulation of phenols reflects increased lignifications, which indicates possible bio-protection in plants (Cordier et al., 1998).

Changes in Antioxidant Enzyme Activity

Generally an organism protects themselves from harmful oxidative stresses by synthesis of antioxidant enzymes. The present investigation revealed that the root peroxidase (PER) and shoot superoxide dismutase (SOD) activity increased due to mycorrhiza/*Trichoderma* inoculations. In diseased mycorrhizal plants (Gf+Mp), the peroxidase enzyme activity increased by 30.43 % after 45 days of pathogen infection but in dual inoculations of AM fungi/*Trichoderma* (Gf+Tv+Mp), the increase was observed to be 51.42 % after 15 days of pathogen infection and it showed 51.28 % increase after 60 days of sowing in dual inoculation of AM fungi/*Trichoderma* in healthy groundnut plants (Gf+Tv) as compared to their respective control ones. The SOD enzyme activity in shoot of groundnut plants was found to be highest after 60 days of sowing in diseased mycorrhizal ground (89.15 % for Gf+Mp). But, when both mycorrhiza/*Trichoderma* was inoculated in presence of pathogen (Gf+Tv+Mp), it showed 46.40 % increase. Whereas, single or dual inoculations of mycorrhiza/*Trichoderma* in healthy plants showed increase by 62.91 % in Gf and 48.49 % in Gf+Tv as compared to control ones. However, the PER and SOD activity was highest in dual inoculation of antagonists in diseased groundnut plants (Gf+Tv+Mp). The AM specific defense related enzymes such as peroxidase and superoxide dismutase might have expressed during colonization process and they are considered to provide lignifications which might have led to reduction in disease of groundnut plants (Lambais, 2000; Pozo et al., 2002). Moreover, SOD enzymes are known for scavenging free-radicals thereby helps in prevention of lipid peroxidation or oxidative damage in cell (Mittler, 2002). Hence, from the results it may be concluded that treatment with both *G. fasciculatum* and *T. viride* individually as antagonists were effective for management of root-rot fungus *M. phaseolina* on groundnut plants. But, the combined treatments of *G. fasciculatum*/*T. viride* were found to be far better combination in reducing the pathogen *M. phaseolina*. Also, the combined inoculation could induce protein, proline, total phenol, polyphenol oxidase and antioxidant enzyme synthesis which might have played key role in the defense system of groundnut plants.

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