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Full Length Article

Efficiency of *Glomus fasciculatum* and *Trichoderma viride* in bio-control of soil-borne pathogen (*Macrophomina phaseolina*) on different groundnut cultivars

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ABSTRACT

The present work was carried out on Groundnut for evaluating arbuscular mycorrhizal fungi along with *Trichoderma* as biological control agent against soil-borne pathogen *Macrophomina phaseolina.* In pot culture experiment groundnut seeds were inoculated with mycorrhizal inoculum and pre-treated with talc based *Trichoderma viride* singly or in combination before inoculation of pathogen *M. phaseolina*. After particular periods the emergence of disease incidence and severity were found to be lower in diseased groundnut plant where both antagonists were inoculated when compared with single inoculation of either antagonist or non-mycorrhizal control ones. The biochemical and antioxidant activities were observed to be increased due to inoculation of mycorrhizal fungi and *Trichoderma*. Thus, espousal of integrated management by AM fungi along with *Trichoderma* species seems to be economic and promiscuous way to improve to control *M. phaseolina* in low-input agricultural practice.

Keywords: AM fungi, biocontrol, *G. fasciculatum*, *M. phaseolina* and *T. viride*.

INTRODUCTION

To cope with ever increasing population and food demand, there is an urgent need to optimize production of food with canvassing to control plant disease without further degrading the climate. Even though today numerous different strategies have been employed to prevent plant diseases, evidence has showed that harnessing indigenous or introduced soil microbial inoculants influences plant health and productivity (Wu *et al.,* 2013). The pathogen *Macrophomina phaseolina* (Tassi) Goid., needs to be controlled as it causes charcoal-rot disease on more than 500 plant species throughout the world (Purkayastha *et al.,* 2006).

In that context, the arbuscular mycorrhizal fungi (AMF) symbiosis is a well known terrestrial symbiotic association formed between fungi and roots of vascular plants (Douds and Siedel, 2012). AMF is particularly relevant due to implications for fitness of plant (Verbruggen *et al.,* 2013) by their ability to protect against plant pathogens by several mechanisms (Doley, 2012, Arabi *et al.,* 2013), thereby leading to productivity in host plants. Another fungus such as *Trichoderma* species which occurs worldwide and generally associated with soil around plant roots and debris has also been identified as potential biological agents to control plant diseases (Schuster and Schmoll, 2010). Therefore, several crops could get benefits from indigenous or introduced microbes (Lakshman and Kadam, 2011).

Hence, in the present experiment we carried out with single and combined inoculation of AMF along with *Trichoderma* spp. to evaluate its efficacy against pathogen *M. phaseolina* in different *Arachis hypogaea* L. cultivars.

MATERIALS AND METHODS

Plant Material

Seeds of local susceptible [Phule Pragati (JL-24)] as well as resistant groundnut cultivars (Western-51) were obtained from Naik seeds, Maharashtra, Pune, India, for the pot culture experiment.

AM fungal inoculum and *Trichoderma* strain

AM spores were isolated (Gerdemann and Nicolson, 1963) and identified (Schenck and Prez, 1987). For preparing AM fungal inoculum, AMF was maintained and multiplied in pots with hosts *Sorghum vulgare* and *Panicum maximum* which consisted of 20 g of rhizospheric soil containing spores and colonized root pieces whichlater on wereinoculated at about 3-5 cm below each groundnut seeds before sowing in pot.Talc based *T. viride* was obtained from Department of Plant Pathology, Agricultural College of Pune, Maharashtra, India.

Pathogen strain and mass inoculum

The strain of *M. phaseolina* was obtained from through the Division of Mycology and Plant Pathology, ARI, Pune, Maharashtra, India. Mass multiplication of *M. phaseolina* was carried out on Sorghum grains (250 g) soaked overnight in sterile water taken in 500 ml capacity saline bottles tightly plugged with cotton. After sterilizing the seeds,5 mm mycelial disc from a week old pure culture of *M. phaseolina* was inoculated in bottles and were incubated for a month at 28°C. This grain culture (5 g) served as pathogen inoculum.

Experimental design

Four seeds of groundnut were sown in plastic pots in the green house and were watered. After 15 days, pathogen inoculum (5 g) wasinoculated per pot at soil around the basal region of groundnut plants. There was no addition of pesticides or fertilizers.Complete randomized block design(CRBD) was followed, with eight treatments in triplicates as follows: (1) Controls (2) Control+*M. phaseolina* (Mp) (3) Control+*T. viride* (Tv) (4) Control+Mp+Tv (5) Gf (*G. fasciculatum*) (6) Gf+Mp (7) Gf+Tv (8) Gf+Mp+Tv. The sample plants were harvested after 30, 60 and days after sowing (DAS) for various observation of parameters.

Determination of disease incidence and severity

Disease incidence was observed as per Kokalis (1992). Disease severity was evaluated using rating scale of 1-5 as per by Shokes *et al.,* 1996.

Determination of percent root colonization

The root samples were immersed in 10% KOH at 90°C for 1 hour and stained in 0.01 % trypanblue for 10 minutes (Phillips and Hayman, 1970). The percent root colonization by G*lomus fasciculatum* were determined by Grid-line intersects method (Giovannetti and Mosse, 1980).

Assay of biochemical activities

Biochemical activities assayed were total proteins (Lowry *et al.,* 1951), proline (Bates *et al.,* 1973), total phenols (Malick and Singh, 1980), and antioxidant enzymes assayed were peroxidase (PER) (Putter, 1974), polyphenol oxidase (Mahadevan and Shirdhar, 1982).

Statistical analysis

Data were subjected to mean value of three replicates. The one way statistical analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was followed. The values are mean ± SD. Duncan's multiple range test was applied as post hoc test at p=0.05. The calculations were made by using a Statistical Package for Social Sciences (SPSS Inc. 1999) for windows version and Microsoft Excel 2007 to analyze the data.

RESULTS AND DISCUSSION

Percent root colonization

Percent root colonization was highest in only mycorrhiza treated groundnut plants as compared to pathogen's or *Trichoderma's* presence in both the cultivars. However, resistant cultivar W-51 showed lower colonization percentage as compared to susceptible variety (JL-24) which probably requires more protection against *M. phaseolina* after 30, 60 and 90 DAS (Fig. 1).

Disease incidence

In both cultivars the incidences of disease significantly got reduced when inoculated with AMF or *Trichoderma* singly but dual inoculation led to marked decrease in disease incidence (Fig. 2; 3).

Changes in protein, proline and total phenol content

Variability in biochemical activity in groundnut cultivars (JL-24 and W-51) was observed after the growth period of 30, 60 and 90 DAS. The shoot protein and proline content significantly increased after inoculation with *G. fasciculatum*/*T. viride* singly or in combination in both groundnut cultivars. But in presence of pathogen shoot protein and proline content were highest. The protein content increased significantly which may be induced by mycorrhiza (Shaul *et al.,* 1999). Moreover, there are reports of protein specific plant-pathogen interaction (Carvalho *et al.,* 2006).

Table 1: Protein content in groundnut cultivars inoculated with *G. fasciculatum* and *T. viride* after 30, 60 and 90 days after AM inoculation.

	Protein content (protein in μ^{-1} g^{-1} fresh weight)					
Cultivar	**JL-24**			**W-51**		
Treatments	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
C	0.074±0.006e	0.069±0.006f	0.077±0.008f	0.092±0.005f	0.088±0.004f	0.106±0.004e
C+Mp	0.125±0.003d	0.117±0.003e	0.119±0.011e	0.139±0.007e	0.132±0.006e	0.164±0.005d
C+Tv	0.116±0.010d	0.107±0.007e	0.117±0.006e	0.130±0.011e	0.125±0.005e	0.161±0.006d
C+Mp+Tv	0.153±0.008c	0.140±0.003d	0.143±0.008d	0.169±0.005d	0.165±0.010d	0.193±0.014c
Gf	0.178±0.007b	0.173±0.005c	0.189±0.015c	0.200±0.011c	0.188±0.014c	0.207±0.011c
Gf+Mp	0.201±0.009a	0.196±0.004b	0.217±0.006ab	0.226±0.005b	0.219±0.007b	0.231±0.015b
Gf+Tv	0.178±0.004b	0.164±0.005c	0.199±0.009bc	0.218±0.012bc	0.198±0.009c	0.229±0.012b
Gf+Mp+Tv	0.211±0.012a	0.225±0.008a	0.231±0.008a	0.249±0.014a	0.228±0.003b	0.271±0.006a

Table 2: Proline content in groundnut cultivars inoculated with *G. fasciculatum* and *T. viride* after 30, 60 and 90 days after AM inoculation.

	Proline content (mg g^{-1} fresh weight)					
Cultivar	**JL-24**			**W-51**		
Treatments	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
C	0.527±0.052d	0.0080±0.0013c	0.0108±0.0014e	0.0145±0.0013e	0.733±0.052e	1.036±0.067e
C+Mp	1.105±0.181bc	0.0100±0.0015c	0.0135±0.0022de	0.0188±0.0013d	1.153±0.143d	1.367±0.083d
C+Tv	0.929±0.145c	0.0123±0.0036c	0.0149±0.0007d	0.0196±0.0008d	1.000±0.124d	1.313±0.068d
C+Mp+Tv	1.056±0.106c	0.0114±0.0014c	0.0162±0.0002cd	0.0204±0.0006d	1.417±0.141c	1.888±0.089c
Gf	1.309±0.034ab	0.0173±0.0012b	0.0184±0.0021c	0.0248±0.0016c	1.690±0.150b	2.299±0.066b
Gf+Mp	1.358±0.026a	0.0171±0.0013b	0.0239±0.0020b	0.0279±0.0014ab	2.007±0.042a	2.485±0.105ab
Gf+Tv	1.303±0.038ab	0.0188±0.0023ab	0.0244±0.0015b	0.0262±0.0010bc	1.973±0.157a	2.387±0.196b
Gf+Mp+Tv	1.435±0.037a	0.0220±0.0018a	0.0284±0.0011a	0.0294±0.0011a	2.041±0.117a	2.693±0.159a

Table 3: Total phenol content in groundnut cultivars inoculated with *G. fasciculatum* and *T. viride* after 30, 60 and 90 days after AM inoculation.

	Total phenol content (mg g^{-1} fresh weight)					
Cultivar	**JL-24**			**W-51**		
Treatments	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
C	0.062±0.005e	0.161±0.0040f	0.175±0.009f	0.193±0.014f	0.069±0.006e	0.100±0.007f
C+Mp	0.129±0.006d	0.193±0.003de	0.208±0.009e	0.228±0.012ef	0.147±0.011d	0.188±0.006d
C+Tv	0.128±0.005d	0.190±0.006e	0.216±0.009e	0.246±0.021e	0.137±0.004d	0.150±0.009e
C+Mp+Tv	0.136±0.003cd	0.204±0.003d	0.228±0.011e	0.264±0.020e	0.174±0.007c	0.206±0.013d
Gf	0.152±0.012bc	0.263±0.005c	0.275±0.013d	0.325±0.017d	0.198±0.018b	0.242±0.011c
Gf+Mp	0.157±0.003b	0.289±0.004b	0.327±0.013c	0.364±0.020cd	0.269±0.009a	0.325±0.009b
Gf+Tv	0.151±0.007bc	0.273±0.010c	0.369±0.012b	0.395±0.026b	0.215±0.011b	0.314±0.011b
Gf+Mp+Tv	0.179±0.018a	0.335±0.004a	0.411±0.007a	0.455±0.019a	0.273±0.013a	0.389±0.011a

Table 4: Shoot peroxidase activity in groundnut cultivars inoculated with *G. fasciculatum* and *T. viride* after 30, 60 and 90 days after AM inoculation.

	Peroxidase activity (min^{-1} mg^{-1} protein)					
Cultivar	**JL-24**			**W-51**		
Treatments	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
C	0.00313±0.00051f	0.00501±0.00051d	0.00773±0.00078e	0.00877±0.00135d	0.00397±0.00078e	0.00543±0.00030f
C+Mp	0.00710±0.00106de	0.00773±0.00078bc	0.01044±0.00164cde	0.01274±0.00106bc	0.00752±0.00089cd	0.00752±0.00089def
C+Tv	0.00564±0.00051e	0.00564±0.00051cd	0.00856±0.00078de	0.00940±0.00102d	0.00585±0.00106de	0.00689±0.00051ef
C+Mp+Tv	0.00794±0.00106cd	0.00815±0.00153b	0.01128±0.00135bcd	0.01316±0.00153bc	0.00856±0.00194bc	0.00940±0.00102bcd
Gf	0.00773±0.00059d	0.00689±0.00051bcd	0.01003±0.00102cde	0.01211±0.00078c	0.00898±0.00129bc	0.00898±0.00164cde
Gf+Mp	0.00940±0.00051bc	0.00877±0.00102b	0.01337±0.00129ab	0.01483±0.00156ab	0.01044±0.00106b	0.01128±0.00135b
Gf+Tv	0.01003±0.00051b	0.00835±0.00078b	0.01253±0.00135bc	0.01399±0.00030bc	0.01065±0.00051b	0.01065±0.00051bc
Gf+Mp+Tv	0.01190±0.00051a	0.01274±0.00156a	0.01587±0.00129a	0.01713±0.00106a	0.01420±0.00078a	0.01483±0.00059a

Table 5: Shoot polyphenol oxidase activity in groundnut cultivars inoculated with *G. fasciculatum* and *T. viride* after 30, 60 and 90 days after AM inoculation.

	Polyphenol oxidase activity (min^{-1} g^{-1} fresh weight)					
Cultivar	**JL-24**			**W-51**		
Treatments	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
C	0.425±0.066e	0.475±0.100c	0.508±0.058d	0.608±0.029d	0.442±0.063e	0.467±0.080d
C+Mp	0.625±0.075c	0.850±0.066a	0.950±0.066bc	1.075±0.050bc	0.683±0.080cd	0.717±0.076c
C+Tv	0.483±0.038de	0.683±0.101b	0.792±0.138c	0.883±0.153c	0.550±0.075de	0.575±0.075d
C+Mp+Tv	0.708±0.052bc	0.908±0.063a	0.967±0.076bc	1.117±0.063b	0.733±0.113bc	0.767±0.101bc
Gf	0.583±0.038cd	0.842±0.06i3a	0.900±0.066bc	1.042±0.063bc	0.625±0.050cd	0.708±0.076c
Gf+Mp	0.775±0.115ab	0.925±0.025a	1.075±0.130ab	1.242±0.163ab	0.833±0.063b	0.892±0.052b
Gf+Tv	0.642±0.063c	0.892±0.138a	0.958±0.153bc	1.083±0.166bc	0.750±0.050bc	0.842±0.029bc
Gf+Mp+Tv	0.858±0.088a	1.000±0.075a	1.167±0.104a	1.350±0.115a	0.983±0.088a	1.100±0.090a

C: Control; 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*. Different alphabets in same line indicates significance difference at p=0.05 as per Duncan's multiple range test, each value represents means ± SE of three replicates.

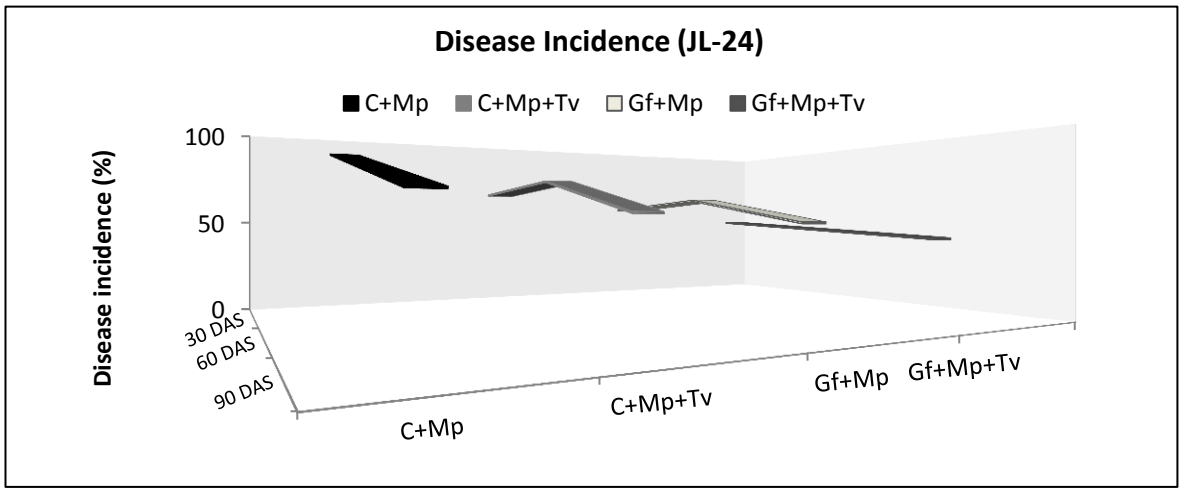

Figure 1: Effect of mycorrhiza/*Trichoderma* treatment on disease incidence in groundnut (JL-24)

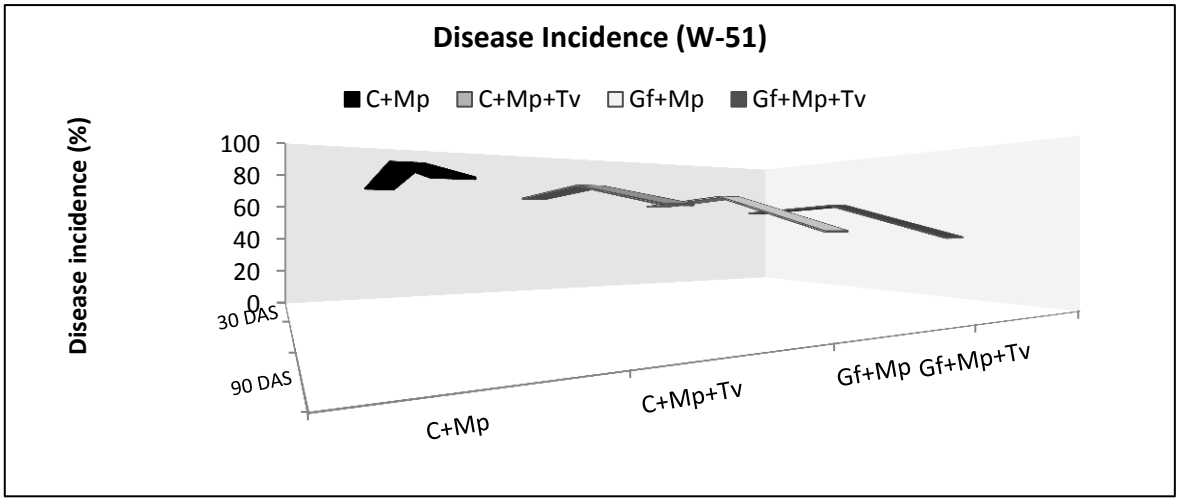

Figure 2: Effect of mycorrhiza/*Trichoderma* treatment on disease incidence in groundnut (W-51)

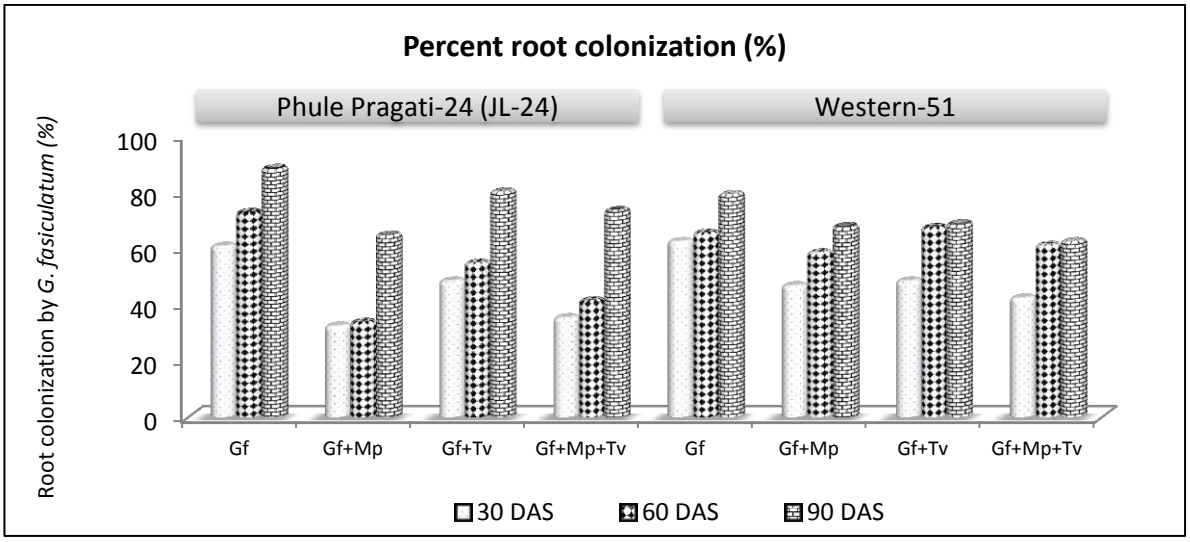

Figure 3: Percent root colonization

Gf: *G. fasciculatum*; Gf+Mp: *G. fasciculatum+M. phaseolina*; Gf+Tv: *G. fasciculatum+T. viride*; Gf+Mp+Tv: *G. fasciculatum+M. phaseolina+T. viride*. Bars represents standard errors of the means, n=3.

The increased proline content might have induced biotic factors (Grote and Claussen, 2001). The overall protein and proline content was higher in resistant cultivar (W-51) (Tab. 1; 2).

The total phenol content ofboth groundnut cultivars (JL-24, W-51) in presence of pathogen was highest in dual mycorrhiza/*Trichoderma* treatment. But, W-51 showed higher overall content of total phenols than JL-24 (Tab. 3). The total phenol increase may be attributed to high phenol levels which are known to be anti-microbial and found to be higher in resistant cultivars (Singh *et al.,* 2010).

Changes in antioxidant enzyme activity

The PER activity in shoot of both groundnut cultivars varied with treatments.The activities were highest in groundnut inoculated with both antagonists in presence of pathogen followed by single inoculations by either antagonists or control ones. But, comparatively higher activities were observed in case of W-51 (Tab. 4). The PPO activity in shoot of JL-24 was highest in *G. fasciculatum*/*T. viride* with pathogen as compared to non-mycorrhizal/*Trichoderma* controls ones in both cultivars. However, pathogen's presence increased the level of PPO in control ones without any antagonists (Tab. 5). There are reports of increased antioxidant activities in response to infection in host plant which provides defenses from reactive oxygen species (Anand and Mohan, 2014, Maksimov *et al.,* 2014).

Hence, the efficient biocontrol characteristics such as improved growth, anatomical changes in roots, competition and mycoparasitism of AMF as well as *Trichoderma* spp. can be exploited as biological fungicides in near future for the sustainable agricultural system (Wu *et al.,* 2013; Topoloveć-Pintaric *et al.,* 2013).

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