Journal of Experimental Sciences 2013, 4(1): 11-15
ISSN: 2218-1768
Available Online: http://jexpsciences.com/

journal of
experimental sciences

Effect of arbuscular mycorrhizal fungi on growth of groundnut and disease caused by *Macrophomina phaseolina*

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Abstract

Efficacy of arbuscular mycorrhizal fungi (AMF) was evaluated for the biological control of soil-borne plant pathogen *Macrophomina phaseolina* in groundnut plant. For this investigation pot culture technique was followed. Soil based mixture of AM fungi (*Glomus fasciculatum*) was inoculated onto the root of groundnut. In results the colonization by mycorrhizal fungi significantly resulted into decreased incidence of disease caused by *M. phaseolina*. The growth of groundnut showed marked increase due to mycorrhizal colonization viz. shoot and root length, fresh and dry weight, leaf, nodule and pod number. In presence of pathogen mycorrhizal dependency was significantly higher but degree of colonization went down. The content of chlorophyll was found to be increased significantly due to inoculation of AM fungi. The various bio-chemical and defense related enzyme activities were investigated and the results obtained showed significant increase in their activities due to pathogen as well as AM fungi inoculation. But, highest activities were recorded where both pathogen as well AM fungi were involved. Thus, inoculation of AM fungi showed great bio-control ability as well as growth promoter. Moreover, it showed their efficacy in inhibition of damaging effect caused by pathogen *M. phaseolina*.

Keywords: AM fungi, Biocontrol, Dependency, Plant growth, Root colonization.

INTRODUCTION

Macrophomina phaseolina Goid (Tassi.) is an important soil-borne pathogen which causes charcoal root-rot disease in 500 species throughout the world approximately Purkayastha et al [17]. In groundnut it causes severe charcoal root-rot disease. It has been often observed that once soil-borne pathogen is established in soil it becomes tedious to eradicate them even with harmful and costly chemical inputs. So, integrated biological management of soil-borne diseases has become a necessity for their eradication as well as for saving our ecosystem. In recent pasts arbuscular mycorrhizal fungi have shown encouraging results in this regards by many researchers. Several trials are being made with AM fungi continuously for biocontrol of several pathogens. So, for modernistic approach in today's agriculture practice in view of preserving our natural environment, for this suitable incorporation of AM fungi seems inevitable. Thus, in the present investigation role of AM fungi's bio control potential was evaluated with overall response of growth, defense related enzymes and disease incurred upon by pathogen *M. phaseolina* in groundnut plant.

Received:; Revised ; Accepted.

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Materials and methods

Biological material and fungal isolates

Groundnut seeds of susceptible variety [Phule Pragati JL-24] were obtained from Naik seeds, Maharashtra, Pune, India. Before trials in pot culture, seeds were surface sterilized with HgCl2 (0.02%) for 5mins and then washed with sterile distilled water.

The AM fungi *G. fasciculatum* (Thaxter Sensu Gerd.) Gerd. and Trappe was kindly provided by Dr. D. J. Bagyaraj. It was mass multiplied using different hosts such as *Sorghum vulgare* and *Panicum maximum* (Jacq.). It was maintained in pot cultures containing sterilized soil and sand. From these pots after three months, mycorrhizal inoculum (soil based) was placed at about 3-5 cm below each groundnut seeds under the soil surface before sowing. Mycorrhizal inoculation contained 20g of AM fungi inoculum of *G. fasciculatum* mixture containing spores, colonized root pieces and extrametrical mycelium in soil.

The pathogen *M. phaseolina* was kindly provided by Agharkar Research Institute, Maharashtra, Pune, India. For pathogen inoculum it was mass multiplied on sorghum grains. For that in sterile conical flasks of 500 ml capacity were filled with 100g water-soaked sorghum grains plugged with cotton. The bottles were then sterilized at a pressure of 15lbs for 20mins. With 5 mm mycelial disc from the active periphery of a 7-day-old pure culture of M *phaseolina* were inoculated on sterilized sorghum seeds in saline bottles and were incubated for one month at 28ºC ± 2ºC temperature for proper mycelial growth to them as pathogen inoculum. From this pathogen inoculum 5g was applied of groundnut seedling after fifteen days of groundnut plant's growth.

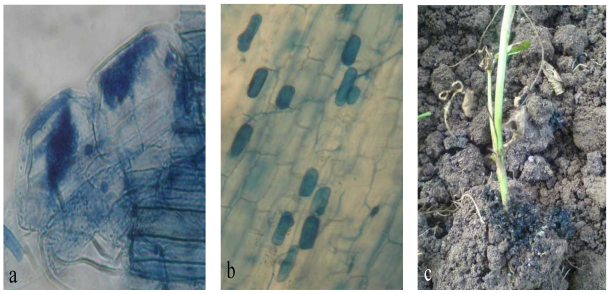

Fig. 1. Typical formation arbuscules: (a) and vesicles; (b) by AM fungi (*Glomus fasciculatum*) and groundnut roots showing charcoal root-rot (c) symptoms. in peanut roots

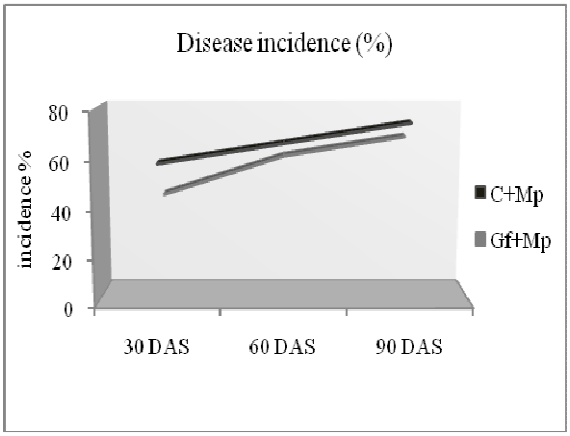

Fig. 2. Effect of *G. fasciculatum* inoculation on incidences of disease caused by pathogen *M. phaseolina* in groundnut plants

Data assessments

Disease incidences on groundnut plants were determined by observation of stem-rot incidence at the base using Kokalis's [8] formula. Randomly selected root samples were cleared in 10% KOH at 90°C for 1 hr were stained in 0.01% trypan blue according to Phillips and Hayman [15] for 10 mins. And colonization of roots by G. fasiculatum were estimated by Grid-line intersect method described by Giovannetti and Mosse [7]. Mycorrhizal dependency (MD) was determined by weighing dry weights of pathogenic and non-pathogenic mycorrhizal groundnut plants according to Plenchette [16].

After the growth of periods of 30, 60 and 90 days twelve plants from three pots from each treatment were carefully harvested. The groundnut plants were washed under with tap water and were determined for various morphological parameters. The length of shoot and root (cm), number of leafs, number of pods, number of nodules, fresh and dry weight (g) of all plant were measured.

Various physiological and bio-chemical parameters were assayed as follows: total chlorophyll according to Arnon [1], total proteins according to Lowry et al [9]; proline according to Bates et al [2]; peroxidase according to Putter [18]; total phenols according to Malick and Singh [12]; polyphenol oxidase total phenols as per Mahadevan and Shidhars [11]; superoxide dismutase (SOD) Beauchamp and Fridovich [3].

Statistical analysis

The data were analyzed by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). Duncan's multiple range test was applied as post hoc test at p= 0.05. Three replications were made, standard deviation (±) were values of mean. All the calculations were made by using a Statistical Package for Social Sciences (SPSS) for windows version 9.0 and Microsoft Excel 2007.

Results and Discussion

The groundnut plants showed significantly decreased incidences (Fig. 2) of stem-rot when inoculated AM fungi after various growth periods of 30, 60 and 90 days of sowing. The disease incidences were higher in non-mycorrhizal groundnut plants (58.33% after 30 days; 66.67% after 60 days; 75% after 90 days) as compared to mycorrhizal diseased groundnut plants where incidences were much lower (41.67% after 30 days; 58.33% after 60 days 66.67%) due to mycorrhizal fungi's colonization which indicated role of AM fungi in decreasing disease caused by pathogen *M. phaseolina*. Moreover, the mycorrhizal colonization was found to be decreased due to very presence of *M. phaseolina* as compared to their absence mycorrhizal groundnut plants. Already numerous studies have demonstrated significant role of AM fungi as biocontrol agents where it competes for host photosynthates with pathogens Smith and Read [20].

Groundnut plants treated with mycorrhizal fungi showed better overall growth response (Tab. 2-4) in terms of length of shoot and root (cm), number of leafs, number of pods, fresh and dry weight (g) of all groundnut plants, whereas non-mycorrhizal showed normal growth response. Significantly lower response in growth parameters was observed in non-mycorrhizal pathogenic groundnut plants due to negative effect of pathogen *M. phaseolina* in comparison to mycorrhizal pathogenic ones where growth was recorded to be much better. The improved growth may be correlated to improved nutrition incurred upon by AM fungi Fritz [6].

The mycorrhizal colonization was observed in root samples of groundnut plants. The formation of structures such as arbuscules and vesicles were visible under the microscope. Mycorrhizal colonization was observed to be increasing with increasing growth periods of 30, 60 and 90 days. The colonization was 50%, 59.33% and 88% for mycorrhizal non-pathogenic groundnuts and 34%, 48.33% and 68% for mycorrhizal pathogenic groundnut plants (Tab. 1). Mycorrhizal dependency was significantly raised in pathogenic mycorrhizal treated groundnut plants due to presence of pathogen as compared to its absence. In mycorrhizal groundnut plants in presence of pathogen, the mycorrhizal dependency was significantly higher by 57.21%, 67.87%, 72.81% whereas it was not so higher in absence of pathogen (Tab. 1). Even the study of Declerck [5] showed increase in relative mycorrhizal dependency by mycorrhizal plants in presence of pathogen *Cylindrocladium spathiphylli* in comparison to its absence.

The marked growth of mycorrhizal plants showed significant increase in the content of photosynthetic pigments in groundnut plants in comparison to control ones. But lower level of these photosynthetic pigments was recorded in non-mycorrhizal

pathogenic ones as compared to AM fungi inoculated pathogenic groundnut plants which suggested that pathogens presence might have incurred in decreased formation of these photosynthetic pigments [Tab. 7].

The pronounced increase in the protein content has been suggested to induce fresh protein synthesis in host plants after infection or to the fungal proteins in mycorrhizal roots Mathur and Vyas [13]. Mohan et al [14] reported that the higher activity of polyphenol oxidase (PPO) in the diseased leaves of tomato might due to their participation in the oxidation of phenolic residues into cell wall polymers in the pathogen-infected cell. There was significant increase in shoot protein content and shoot polyphenol activity due to pathogen or mycorrhizal inoculation but more protein content and shoot polyphenol activity was recorded in mycorrhizal groundnut plants. The highest level of protein content and shoot polyphenol activity was recorded in groundnut plants where both pathogen and AM fungi were applied after their growth periods of 30, 60 and 90 days (Tab. 5).

In case when pathogen invades of host plant the first stage of defense mechanism in plants is the rapid accumulation of phenols at the infection site which restricts or may slow the growth of the pathogen because of its antioxidant and antimicrobial properties Lamba [9]. The proline are considered to provide disease resistance as they scavenge reactive oxygen species generated during pathogen attack and other kind of biological stresses Chen [4]. The main function of SOD is to scavenge the superoxide anion radicals, generated in various physiological processes, pathogen attack thereby preventing the oxidation of biological molecules from death Schinkel [19]. In the present experiment inoculations with AM fungi or pathogen resulted into significant increase in total phenol, proline content and superoxidase activity, increments were more significant due to mycorrhizal inoculation as compared to control non-mycorrhizal pathogenic ones but two fold increase in total phenol content, proline content and superoxidase activity was observed in mycorrhizal pathogenic ones after 30, 60 and 90 days after sowing. Interestingly, continuous increase was observed after various growth intervals showing increasing trend in total phenol content, proline content and superoxidase activity (Tab. 6-7).

Table 1. AM colonization and mycorrhizal dependency in groundnut after 30, 60 and 90 days of sowing.

	AM colonization (%)			Mycorrhizal dependency (%)		
Treatments	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
C	0.00	0.00	0.00	0.00	0.00	0.00
C+Mp	0.00	0.00	0.00	0.00	0.00	0.00
Gf	50.00±4.08	59.33±3.68	88.00±6.38	49.55±48.82	37.13±48.82	44.57±48.82
Gf+Mp	34.00±3.74	48.33±2.87	68.00±7.26	57.21±43.89	67.87±43.89	72.81±43.89

C: control, C+Mp: control inoculated with *M. phaseolina*, Gf: *G. fasciculatum* inoculated, Gf+Mp: *G. fasciculatum* and *M. phaseolina* inoculated, data were means of three replicates, error bars (±) represents mean value, DAS=Days after sowing.

Table 2. Shoot and root length (cm) in groundnut after 30, 60 and 90 days of sowing.

	Shoot Length (in cm)			Root Length (in cm)		
Treatments	**30 DAS**	**60 DAS**	**90 DAS**	**30 DAS**	**60 DAS**	**90 DAS**
C	18.00±0.82b	22.67±1.25b	29.00±0.82b	26.33±1.25c	26.00±1.63b	27.33±1.70b
C+Mp	14.67±1.70c	11.67±1.25c	14.33±2.05c	22.00±0.82d	14.00±0.82c	16.67±1.25c
Gf	26.67±1.25a	29.67±1.70a	39.00±2.16a	34.67±1.70a	33.33±2.49a	41.67±4.78a
Gf+Mp	28.67±1.70a	28.67±2.87a	31.00±1.63b	29.33±1.25b	28.33±0.94b	31.00±1.63b

Table 3. Fresh and dry weight (g) in groundnut after 30, 60 and 90 days of sowing.

	Fresh weight (in gm)			Dry weight (in gm)		
Treatments	**30 DAS**	**60 DAS**	**90 DAS**	**30 DAS**	**60 DAS**	**90 DAS**
C	3.92±0.76b	4.92±0.66c	6.49±0.47c	2.04±0.44bc	3.24±0.46b	3.81±0.50c
C+Mp	2.98±0.61b	3.26±0.50d	3.32±0.51d	1.25±0.49c	1.48±0.29c	1.48±0.21d
Gf	8.97±0.59a	9.29±0.80a	13.57±0.38a	4.05±0.52a	5.15±0.53a	6.87±0.42a
Gf+Mp	4.31±1.00b	7.45±0.29b	8.63±0.77b	2.91±0.44b	4.62±0.09a	5.44±0.34b

Table 4. Leaf and pod number (no.) in groundnut after 30, 60 and 90 days of sowing.

	Leaf numbers (in no.)			Pod numbers (in no.)		
Treatments	**30 DAS**	**60 DAS**	**90 DAS**	**30 Days**	**60 Days**	**90 Days**
C	54.00±7.12b	61.67±1.25c	86.33±6.02b	1.67±0.47b	1.33±0.47b	4.67±0.94a
C+Mp	29.00±4.55c	48.00±4.97d	60.67±4.64c	0.33±0.47c	0.67±0.47b	2.00±0.82b
Gf	82.33±7.41a	91.33±5.56a	112.33±4.78a	3.33±0.47a	4.33±0.47a	6.33±1.25a
Gf+Mp	75.00±11.05a	79.33±3.68b	92.33±9.46b	2.33±0.47ab	3.67±0.47a	5.00±0.82a

C: control, C+Mp: control inoculated with *M. phaseolina*, Gf: *G. fasciculatum* inoculated, Gf+Mp: *G. fasciculatum* and *M. phaseolina* inoculated, Duncan's multiple range tests was applied as post hoc test at p= 0.05, means followed by a common letter were not significantly different by DMRT. Data were means of three replicates, error bars (±) represents mean value, DAS=Days after sowing.

Table 5. Shoot protein content and PPO activity in groundnut after 30, 60 and 90 days of sowing.

	Shoot protein content mg/gm of fresh weight			Shoot PPO activity ∆ O.D/min/gm fresh weight		
Treatments	**30 DAS**	**60 DAS**	**90 DAS**	**30 DAS**	**60 DAS**	**90 DAS**
C	0.072±0.007c	0.097±0.004d	0.119±0.0009d	0.233±0.011b	0.291±0.031b	0.325±0.040c
C+Mp	0.146±0.011a	0.150±0.010b	0.250±0.0007b	0.458±0.047a	0.491±0.023a	0.583±0.051ab
Gf	0.100±0.005b	0.131±0.006c	0.227±0.0094c	0.450±0.040a	0.483±0.031a	0.500±0.061b
Gf+Mp	0.147±0.007a	0.177±0.004a	0.313±0.0016a	0.475±0.020a	0.550±0.040a	0.683±0.023a

Table 6. Shoot total phenol and proline content in groundnut after 30, 60 and 90 days of sowing.

	Shoot total phenol content mg/gm of fr. wt.			Shoot proline content µ mole/gm		
Treatments	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
C	0.159±0.007c	0.176±0.010c	0.246±0.012d	0.0155±0.0013c	0.0200±0.0012b	0.0151±0.0009c
C+Mp	0.271±0.012b	0.307±0.010b	0.427±0.014c	0.0202±0.0022b	0.0222±0.0017b	0.0208±0.0011b
Gf	0.385±0.018a	0.412±0.010a	0.466±0.013b	0.0288±0.0010a	0.0315±0.0011a	0.0312±0.0013a
Gf+Mp	0.380±0.015a	0.413±0.006a	0.499±0.016a	0.0321±0.0011a	0.0306±0.0012a	0.0315±0.0009a

Table 7. Shoot SOD activity and total chlorophyll content in groundnut after 30, 60 and 90 days of sowing.

	Shoot SOD activity unit/g fresh wt./hr.			Total Chlorophyll (mg chl./gm of fr. wt.)		
Treatments	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
C	1.720±0.204b	2.080±0.371b	2.680±0.150c	5.26±0.54	7.95±0.54	14.59±0.63
C+Mp	4.280±0.150a	4.720±0.599a	5.560±0.247b	3.59±0.52	6.62±0.64	10.62±0.71
Gf	4.800±0.353a	4.920±0.098a	6.680±0.408a	14.22±0.65	25.25±0.51	18.20±0.63
Gf+Mp	4.840±0.408a	5.560±0.408a	7.280±0.503a	10.46±0.45	11.45±0.52	15.29±0.64

C: control, C+Mp: control inoculated with *M. phaseolina*, Gf: *G. fasciculatum* inoculated, Gf+Mp: *G. fasciculatum* and *M. phaseolina* inoculated, Duncan's multiple range tests was applied as post hoc test at p= 0.05, means followed by a common letter were not significantly different by DMRT. Data were means of three replicates, error bars (±) represents mean value, DAS=Days after sowing.

Conclusion

The groundnut plants of susceptible variety (Phule Pragati-JL 24) were subjected to inoculation by AM fungi (*G. fasciculatum*) and pathogen (*M. phaseolina*). Several parameters which indicates overall decrease in disease caused by the pathogen was studied.

The study showed remarkable results when groundnut plants were inoculated with AM fungi. The study confirmed that mycorrhizal plant grows greatly in comparison to healthy non-mycorrhizal groundnut plants. Moreover, in pathogenic groundnut plants, harmful effects of pathogen can be lowered with the help of mycorrhizal inoculations.

Acknowledgment

First author is thankful to the authorities of University Grants Commission, New Delhi, for sanctioning fellowship under RGNF (No. F. 14-2(SC)/2009(SA-III). Authors are thankful to Prof. S. S. Deokule, Head, Department of Botany, University of Pune, Pune, India for providing all necessary assistance that were required for carrying out this research work.

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