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Effects of AM Fungi on the Plant Growth and Root-Rot Disease of Chickpea

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Abstract: Influence of four species arbuscular mycorrhizal fungi, namely *Glomus intraradices*, *G. aggregatum*, *G. claroideum* and *Glomus* sp. were evaluated for the biocontrol of root-rot fungus *Macrophomina phaseolina* on chickpea (*Cicer areitinum* L. cv. Avarodhi) under glasshouse conditions. Application of these AM fungi cause an increase of plant growth, pod number, nodulation, chlorophyll and N, P, K contents in *M. phaseolina* inoculated plants and also reduced root-rot index. *Glomus intraradices* caused greatest increase in plant growth, pod number, nodulation, chlorophyll and N, P, K contents of fungus inoculated plants followed *by G. aggregatum*, *Glomus* sp. and *G. claroideum*. Percent of root colonization caused by *G. intrardices* followed by *G. aggregatum*, *Glomus* sp. and *G. claroideum* showed a descending order. Application of *G. intraradices* found to be the best for reducing root-rot index and improving plant growth parameters of chickpea.

Key words: Biocontrol · Chickpea · *Glomus* spp. · *Macrophomina phaseolina* · Root-rot

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop of India and a main source of dietary protein in the large vegetarian diet. This crop is often susceptible to root-rot fungus, *Macrophomina phaseolina* (Tassi) Goid. Because it has a very wide host range and infecting about 500 plant species [1]. This fungus is the major constraint in the successful cultivation of this important crop [2, 3].

Rhizosphere organisms provide an initial barrier against pathogens attacking the root [4] and microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents. Arbuscular mycorrhizal fungi colonize the roots of many crop plants [5, 6] and are great value in promoting the uptake of phosphorus, minor elements and water [7, 8] and also reduce the severity of several plant diseases [9-12].

In the present study an attempt was made to examine the effects of different AM fungi (*G. intraradices*, *G. aggregatum*, *G. claroideum* and *Glomus* sp.) on growth, pod number, nodulation, chlorophyll and N, P, K contents and on the root-rot disease of chickpea.

MATERIALS AND METHODS

Preparation and Sterilization of Soil Mixture: Sandy-loam soil (pH 7.2) collected from the field belonging to the Botany Department, Aligarh Muslim University, Aligarh, India was passed through a 9 mesh sieve. The soil was mixed with river sand and organic manure in a ratio of 3: 1: 1 (v/v) respectively and added to jute bags. A little water was poured into each bag to wet the soil before transferring to an autoclave for sterilization at 137.9 kPa for 20 min. Sterilized soil was allowed to cool at room temperature before filling the 15 cm diameter clay pots with 1 kg of the steam sterilized soil.

Growth and Maintenance of the Test Plants: The seeds of chickpea cv. Avarodhi were surface sterilized in 0.1% sodium hypochlorite for 2 min and then washed three times with distilled water. Five healthy seeds of similar seeds were sown in each pot and later thinned was done to maintain one seedling per pot. Two days after thinning, seedlings received the treatments as listed in table 1 and the uninoculated plants served as a control. The plants were kept on a glasshouse bench at 22±2°C and watered as needed.

AM Fungus Inoculum: Four species of AM fungi, *G. intraradices*, *G. aggregatum*, *G. claroideum* and *Glomus* sp. were used for the experiment. These AM spores were isolated from the soil samples collected from the Aligarh and adjoining areas. The inocula of AM fungi were maintained in the Department of Botany, Aligarh Muslim University, Aligarh on Rodes grass (*Chloris gayana*) Kunth grown in sandy-

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Table 1: Effect of four strains of AM fungi on the plant growth and root-rot index of chickpea. Value within each column followed by same letter is not significantly different followed by DMRT at p = 0.05

Treatments		Shoot dry weight (g)	Increase (%)	No. of pods per plants	No. of nodules per root system	Percent Root colonization by AM fungi	Root rot index
Without							
M. phaseolina	Control	6.02d	-	25c	4ab	-	-
	G. intraradices	6.98a	15.95	34a	7a	68a	-
	G. aggregatum	6.76ab	12.29	32ab	6ab	66ab	-
	G. claroideum	6.46c	7.31	29b	5ab	61bc	-
	Glomus sp.	6.54bc	8.64	30b	4ab	63c	-
With							
M. phaseolina	Control	4.68h	-	16f	4ab	-	4
	G. intraradices	5.52e	17.95	24cd	6ab	56d	2
	G. aggregatum	5.30ef	13.25	23cde	5ab	53de	3
	G. claroideum	5.02g	7.26	20e	3b	46ef	3
	Glomus sp.	5.14fg	9.83	21de	4ab	50f	3

oam soil mixed with washed river sand and farm-yard manure in the ratio of 3:2:1 (v/v/v), respectively under glasshouse conditions. The population of AM fungal inoculum was assessed by the most probable number method [13]. Fifty grams of inoculum with soil was added around the seedling to provide 500 infective propagules of AM fungi per pot (1 g inoculum contains ten infective propagules). The crude inoculum consisted of soil, extra metrical spores and sporocarps, hyphal fragments and infected Rhodes grass fragments.

Fungal Inoculum: The strain of *Macrophomina phaseolina* used in this experiment was isolated from the chickpea field belonging to Anoopshar, Aligarh exhibiting root-rot symptoms. The culture of *M. phaseolina* isolated from chickpea field was maintained on potato dextrose agar (PDA). The fungal inoculum was prepared by culturing the isolates on Richard's medium [14] for 15 days in an incubator running at 25°C. After incubating the mat of fungal mycelium was washed in distilled water and was collected on blotting sheets to remove excess of water and nutrients. The inoculum was prepared by mixing 10 g fungal mycelium in 100 ml of distilled water and blending it for 30 s in a Waring blender. The 10 ml of this suspension containing 1 g fungus was used as inoculum.

Inoculation Technique: For the inoculation of AM fungi (*G. intraradices*, *G. aggregatum*, *G. claroideum* and *Glomus* sp.) and root-rot fungus *M. phaseolina* soil around the root was carefully removed without damaging the roots. The inoculum suspensions of these microorganisms were poured around the roots and the soil was replaced. An equal volume of sterile water was added to control treatments.

Experimental Design: The experiment was carried out in a completely randomized block design as listed in Table 1, comprising of five treatments (1) Control; (2) *G. intraradice*; (3) *G. aggregatum*; (4) *G. claroideum*; (5) *Glomus* sp. These five treatments were applied both in presence and absence of *M. phaseolina*. Each treatment was replicated five times.

Observations: The plants were harvested 90 days after inoculation. Data were recorded on plant length, dry shoot weight, number of pods, number of nodules, percentage of root colonization and root-rot index. Shoot were dried into hot air oven at 60 °C for 48 h and the weight of shoots recorded in g. Chlorophyll and N, P, K contents were estimated per gram of fresh leaf weight basis. Chlorophyll content was estimated by the method of Arnon [15] while the nitrogen content was estimated by the method of Lindner [16]. Phosphorus and potassium contents were estimated by the methods of Fiske and Subba Row [17] and flame photometer, respectively. Root rot index of plants inoculated with *M. phaseolina* were recorded by scoring the disease severity on 0-5 scale, where 0 = no disease and 5 = severe root-rot. The rotting was scored on the basis of percentage lesions in the root tissues where 0 = no root rot, 1 = up to 20% rotting, 2 = 21-40% rotting, 3 = 41-60% rotting, 4 = 61-80% rotting and 5 = more than 80% rotting [18]. The proportion of root colonized by AM fungi was determined using a grid intersecting method [19] after clearing the root with KOH in 0.05% trypan blue lactophenol.

Statistical Analysis: The dataset was analyzed statistically as a two factor experiment (*M. phaseolina* × AM fungi) by the method of Dospekhov

[20]. Least significant differences (L.S.D.) was calculated at p = 0.05. DMRT was employed to denote the differences between treatments.

RESULTS

Applications of all the arbuscular mycorrhizal fungi (*G. intraradices, G. aggregatum, G. claroideum* and *Glomus* sp.) to plant without pathogenic fungus caused a significant increase shoot dry weight over uninoculated ones (Table 1). Application of *G. intraradices* to plants without fungus caused a greater increase shoot dry weight than caused by any of the AM fungi tested. *G. intraradices* caused an increase in shoot dry weight (15.95%) over the uninoculated ones while increase in shoot dry weight caused by *Glomus aggregatum* (12.29%), *Glomus* sp. (8.64%) and *G. claroideum* (7.31%) (Table1).

Inoculation of fungus *M. phaseolina* caused a significant reduction in shoot dry weight over the uninoculated ones (Table 1). Meanwhile, inoculation of all the AM fungi significantly increased the shoot dry weight of pathogenic fungus inoculated plants. Inoculation of *G. intraradices* caused a greater increase in shoot dry weight (17.95%) of the *M. phaseolina* inoculated plants followed by *G. aggregatum* (13.25%), *Glomus* sp. (9.83%) and *G. claroideum* (7.26%) (Table 1).

The numbers of pods per plants were significantly reduced in plants inoculated with *M. phaseolina*. Applications of *G. intraradices, G. aggregatum, G. claroideum* and *Glomus* sp. to plants caused a significant increase the number of pods per plants both *M. phaseolina* inoculated and uninoculated ones (Table 1). Nodulation was very poor in all plants either inoculated with AM fungi or with *M. phaseolina* (Table 1). Percentage root colonization was high in plants inoculated with *G. intraradices* (68%) followed by *G. aggregatum* (66%), *Glomus* sp. (63%) and *G. claroideum* (61%). In presence of pathogenic fungus root colonization caused by AM fungi was found to be reduced (56-46%) (Table1). The root rot severity index was 4 when *M. phaseolina* was inoculated alone and severity index was dropped to 2 when *M. phaseolina* inoculated plants were treated with *G. intraradices*. In other treatments the index was reduced to 3 (Table 1).

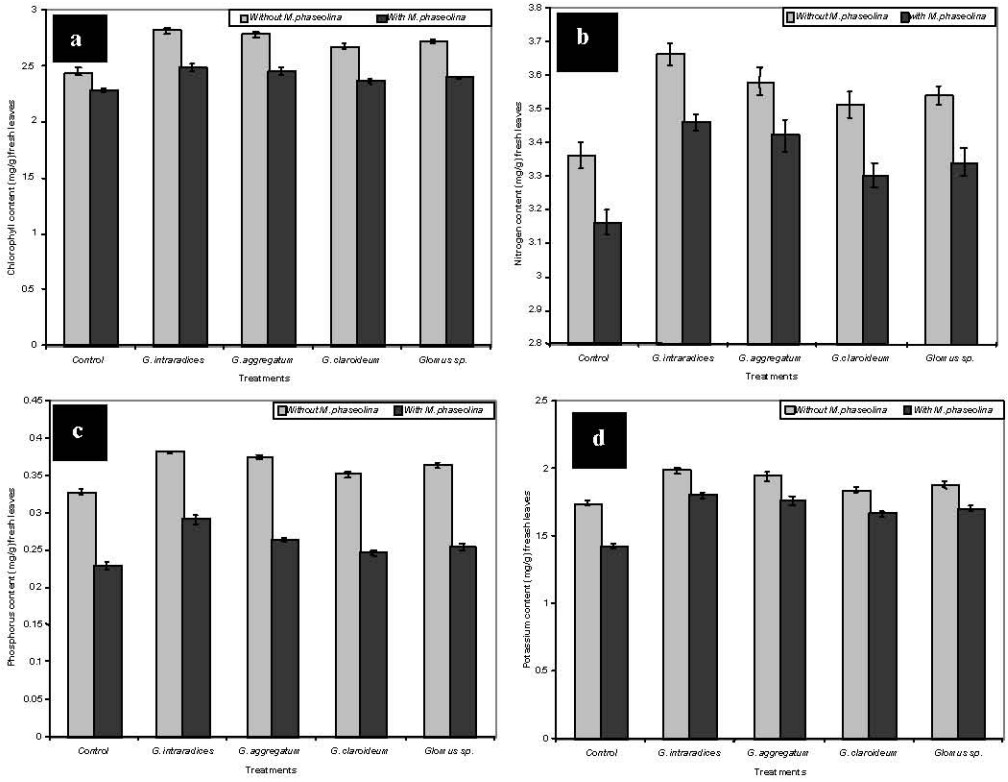

Fig. 1: Effects of four strains of AM fungi on the a) chlorophyll b) nitogen c) phosphorus d) potassium in chickpea plants inoculated with *Macrophomina phaseolina* or in uninoculated control. Bars indicate standard error

Applications of *G. intraradices, G. aggregatum, G. claroideum* and *Glomus* sp. to plant without fungus significantly increased the chlorophyll, nitrogen, phosphorus and potassium content over uninoculated ones (Fig.1a-d). The application of *G. intraradices* to plants without *M. phaseolina* caused a greater increase in chlorophyll, nitrogen, phosphorus and potassium contents than cause by other AM fungi tested (Fig.1a-d). *Macrophomina phaseolina* significantly reduced chlorophyll, nitrogen, phosphorus and potassium content over the uninoculated ones. Application of all the AM fungi *G. intraradices, G. aggregatum, G. claroideum* and *Glomus* sp. significantly increased the chlorophyll, nitrogen, phosphorus and potassium content of *M. phaseolina* inoculated plants (Fig. 1a-d).

DISCUSSION

The present study presumed that AM fungi improved the growth of *M. phaseolina* inoculated plants. It is obvious from the earlier reports that AM fungi had lower incidence of Fusarium wilt in alfalfa [21] while *G. fasciculatum* reduced the Fusarium wilt of pigeon pea [12]. It was obvious from the reports that presence of AM fungi reduced the root-rot severity in peas [22]. Reduced damaged by pathogens in mycorrhizal plants may be due to physiological and biochemical changes in the host or to an increase in the flow of nutrients which gives mechanical strength [23]. The root-rot severity of plants inoculated with *M. phaseolina* was also reduced by *G. intraradices* [2, 3]. The reduction in root-rot of pea caused by *Aphanomyces euteiches* was observed [24] and also application of *G. intraradices* reduced the Fusarium wilt up to 17% on tomato. In addition to changes in nutrient uptake in the root system, a mycorrhizosphere effect and activation of plant defense mechanisms are thought to be responsible for disease inhibition by AM fungi [25, 26]. The obvious contribution to reduction of root disease is to increase nutrient uptake, particularly P and other mineral, because AM symbiosis results in more vigorous growth of plants and the plants it may be more resistant or tolerant to pathogen attacks [26]. Similarly the effect of AM fungi or Phosphorus addition to reduce root rot of bananas caused by *C. spathiphyylii* was also observed [27]. It has been hypothesized that direct competition between root pathogens require host nutrient for the reproduction and development and the direct competition between the AM fungi may be the cause of their inhibition [28].

Greater tolerance is also attributable to increased root growth and phosphate status of the plant [29]. In addition to P, AM fungi can enhance the uptake of Ca, Cu, Mn, S and Zn [30, 31]. Host susceptibility to pathogens or tolerance the diseases process can be influenced by the nutritional status of the host and the fertility status of the soil [32]. The root colonized by mycorrhizal fungi also exhibited greater chitosanase, chitinase and β-1,3 glucanses activity [22, 33, 34]. These enzymes can be inhibitory to certain fungal pathogens [36]. This may be a reason AM fungi caused better reduction of root-rot disease caused by *M. phaseolina*. Among all the AM fungi tested *G. intraradices* was found best in improving plant growth, chlorophyll, nitrogen, phosphorus and potassium content and also reducing root-rot indices than other AM fungi used. The results emphasize the need of further investigation on the mechanisms by which AM fungi induce resistance in their hosts and to better define the environmental conditions enhancing diseases resistance.

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