Pak. J. Phytopathol., Vol. 27 (02) 2015. 153-158

Official publication of Pakistan Phytopathological Society

Pakistan Journal of Phytopathology

ISSN: 1019-763X (Print), 2305-0284 (Online)

http://www.pakps.com

# INFLUENCE OF ROOT INOCULATIONS WITH VASICULAR ARBUSCULAR MYCORRHIZAE AND RHIZOMYX FOR THE MANAGEMENT OF ROOT ROT OF CHICKPEA

**Sundas Shakoor, Muhammad Inam-ul-Haq*, Shagufta Bibi, Raees Ahmed**

*Department of Plant Pathology, PMAS-University of Arid Agriculture Rawalpindi, Pakistan.*

**A B S T R A C T**

Chickpea is one of the most important crops grown worldwide including Pakistan. Root diseases are one of the most important limiting factors in chickpea production worldwide. In Pakistan chickpea crop is susceptible to various root pathogenic fungi like *Macrophomina phaseolina* causing dry root rot and *Rhizoctonia solani* causing wet root rot. Considerable evidence has been accumulated in recent years to support and identify the benefits associated with the use of vascular arbuscular mycorrhizae in crop protection. In the present study, efficacy of different treatments of Rhizomyx and VAM were checked against root rot of chickpea. It was observed that Rhizomyx and VAM produced significant results in controlling the root pathogenic fungi i.e. by minimizing the percent infection of chickpea root pathogenic fungi to a minimum level 0.5% and 0.10% while applying with *R. solani and M. phaseolina* respectively. *Glomus etunicatum*, *Glomus mosseae* and Rhizomyx inoculation alone and in combination significantly increased shoot fresh weight, plant length and number of pods in plants inoculated with *M. phaseolina* and *R. solani* over un-inoculated control, showed positive impact on the plant growth of chickpea, also give remarkable results in reduction of root rot severity index when applied alone and in combination with Rhizomyx. Endophytes colonize the roots of plants similar to that of root pathogenic fungi and biological control with endophytes offers an effective strategy for the management of root pathogenic fungi.

**Keywords**: Rhizomyx, vascular arbuscular mycorrhizae, *Macrophomina phaseolina, Rhizoctonia solani*.

## INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the world after bean (*Phaseolus vulgaris*) and peas (*Pisum sativum*), with Pakistan having chickpea production approximately 496 thousand tons on about 1.05 million hectares (Eco. Survey of Pakistan, 2011-12). The soil borne fungus *M. phaseolina* is endemic throughout the temperate and tropical regions of the world and has been isolated from over 400 plant species (Sikes *et al.,* 2009). Another root infecting fungus *Rhizoctonia solani* exists in the soil and attacks more than 2000 species of plants, mainly leguminous plants (Parmeter, 1970). Among the different types of mycorrhizae, arbuscular mycorrhiza is one of the most common and the most frequent endomycorrhiza all over the world. Arbuscular mycorrhizal (AM) fungi are non-pathogenic obligate symbionts (Iqbal *et al.,* 2005).

About 150 species belonging to the genera *Gigaspora* and *Scutellispora* (Gigasporaceae), *Glomus* and *Sclerocystis* (Glomaceae) and *Acaulospora* and *Entrophospora* (Acaulosporaceae) in the Zygomycete order Glomales of fungi are involved in Vesicular Arbuscular Mycorrhizal (VAM) fungi associations (Morton and Benny, 1990). Mycorrhizal fungi apparently can occupy a particular habitat for thousands of years with little genetic change (Trappe and Molina, 1986) and fossil evidence suggests that VAM associations have been present throughout most of the history of vascular plants (Pirozynski and Dalpé, 1989; Stubblefield and Taylor, 1988).

The present study is aimed at to compute the ability of the VAM fungi and Rhizomax to serve as a biological control agent against dry and wet root rot of chickpea which will help the plant pathologists in management of plant diseases.

* Corresponding Author:

Email: dr.inam@uaar.edu.pk

## MATERIALS AND METHODS

**Isolation of fungal pathogens:** Fungal pathogens i.e. *M. phaseolina* and *R. solani* were isolated from infected samples by placing on PDA and incubation was done at 22 ± 2 $^0$C for 5-7 days.

**Spores isolation and identification of VAM:** Spores of VAM were isolated from soil by wet-sieving and decanting techniques as described by Gerdeman and Nicolson, 1963 and Brundrett *et al*., 1996. Soil was suspended in water and then passed through sieves of different sizes. Spores suspension is then centrifuged with sugar solution and the spore layer was collected in a petri dish from just above the layer of sugar solution through syringe pipe. These spores were identified according to their morphological characteristics including shape, size, color, distinct wall layer, attached hyphae and surface orientation of spores as described by Schenck and Parez (1990).

**Evaluation of VAM and rhizomyx concentrations in pot experiment**

**Sterilization of soil mixture:** Sandy loam soil (pH 7.2) collected from the field of chickpea and was added to jute bags. Water was poured into each bag to wet the soil before transferring them to an autoclave for sterilization. Sterilized soil was allowed to cool to room temperature before filling 15-cm diameter clay pots with 1 kg of sterilized soil.

**Growth and maintenance of test plant:** The seeds of the chickpea were surface sterilized in 0.1 % sodium hypochlorite for 2 min. Five healthy seeds were sown in each pot and later thinning was done to maintain one seedling per pot. Two days after thinning seedling received the treatment and the un-inoculated plant were served as a control. The plants were kept on a glasshouse bench at 22±2 $^0$C and were watered as needed.

**Fungal inoculum:** The fungal inoculum was prepared by culturing the isolates on PDA at 25 $^0$C in the incubator. After 5-7 days of incubation, when fungal mycelium was obtained, it was maintained in the distilled water in the ratio of 1 mg per 10 ml of distilled water. Fungal inoculum was obtained by blending the fungal inoculum in distilled water for 5 min.

**AM inoculum:** The AM fungus, *G. etunicatum* and *G. mosseae*, was isolated from the soil of chickpea fields. The population of AM fungal inoculum was assessed by the most probable number method. Fifty grams of inoculum with soil was added around the seed to provide 500 infective propagules of *G. etunicatum* and *G. mosseae* per pot.

**Inoculation technique:** Inoculation of AM fungi alone and in combination with different concentrations of Rhizomyx was done in the soil around the root without damaging the roots. *M. phaseolina* and *R. solani*was also inoculated in the soil. The inoculum suspension of these microorganisms was poured around the roots and soil was replaced (Rangaswami, 1992). An equal volume of sterile water was added to control treatment.

**Experimental design:** The experiment was carried out in a completely randomized blocked design with three experimental variables:(a) control; (b) *Macrophomina phaseolina*; (c) *Rhizoctonia solani*. Each set was inoculated with the following treatments: (1) control; (2) Rhizomyx 2%; (3) Rhizomyx 4%; (4) Rhizomyx 6%; (5) *G. etunicatum*; (6) *G. mosseae*; (7) Rhizomyx 2% + *G. etunicatum*; (8) Rhizomyx 2% + *G. mosseae*; (9) Rhizomyx 2% + *G. mosseae* + *G. etunicatum*; (10) Rhizomyx 4% + *G. etunicatum*; (11) Rhizomyx 4% + *G. mosseae*; (12) Rhizomyx 4% + *G. mosseae* + *G. etunicatum*; (13) Rhizomyx 6% + *G. etunicatum*; (14) Rhizomyx 6% + *G. mosseae*; (15) Rhizomyx 6% + *G. mosseae* + *G. etunicatum*. Each treatment was replicated three times and the experiment was repeated twice.

**Evaluation of growth parameters:** The plants were harvested 90 days after inoculation. Data were recorded on Plant length, fresh shoot weight, number of pods and root-rot severity index. The root-rot index was determined by scoring on a scale ranging from 0 (no disease) to 5 (severe root rot) according to Johansen *et al*. (1994).

**Statistical analysis:** All data collected were analyzed statically using single factor analysis and least significant differences (LSD) were calculated at 5% level.

## RESULTS AND DISCUSSION

**Effect of VAM and rhizomyx on growth parameters:** Application of *G. mosseae* and *G. etunicatum* alone or in combination with Rhizomyx in different concentrations to plants without pathogens caused a significant increase in fresh shoot weight over the control without any antagonists or pathogens. The application of *G. mosseae* in combination with Rhizomyx 6% on plants without pathogens caused a greater increase in shoot fresh weight than *G. etunicatum* by 13.6% over the control, while *G. etunicatum* caused 7.6 % increases in fresh shoot weight. Combined inoculation of *G. mosseae* and *G. etunicatum* in the presence of Rhizomyx 6%

caused a greater increase (33.9%) in fresh shoot weight than that caused by Rhizomyx 2% (24.2 %) or by Rhizomyx 4% (19.9 %).

Inoculation with either *R. solani* or *M. phaseolina* alone or in combination significantly reduced fresh shoot weight over the un-inoculated control. Reduction in fresh shoot weight was greater when *R. solani* and *M. phaseolina* were applied together. Inoculation with *G. mosseae* and *G. etunicatum* alone or in combination with Rhizomyx 6% significantly increased fresh shoot weight of pathogen-inoculated plants. Again the application of *G. mosseae* caused a greater increase (21.4–22.6%) in fresh shoot weight of pathogen-inoculated plants than *G. etunicatum* (8.7–12.7%). Combined application of *G. mosseae* and *G. etunicatum* to pathogen-inoculated plants in combination with Rhizomyx 6% caused a greater increase (36.1–42.2%) in fresh shoot weight.

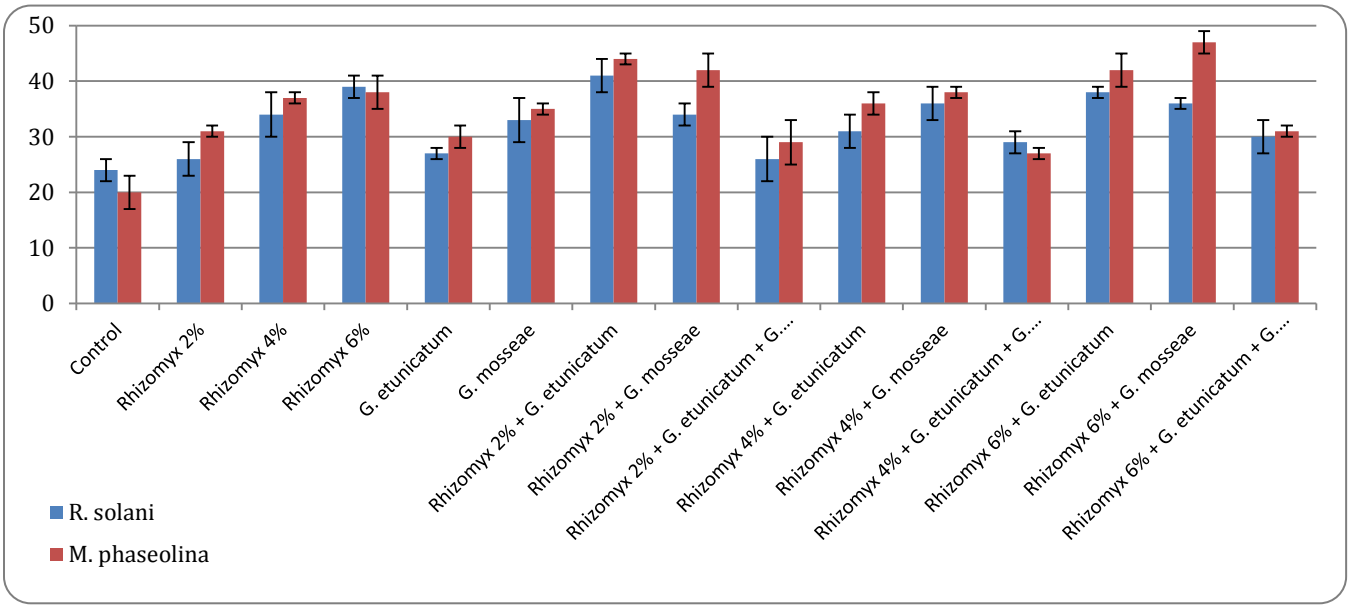


Figure 1. Plant Length (cm) after 90 days.

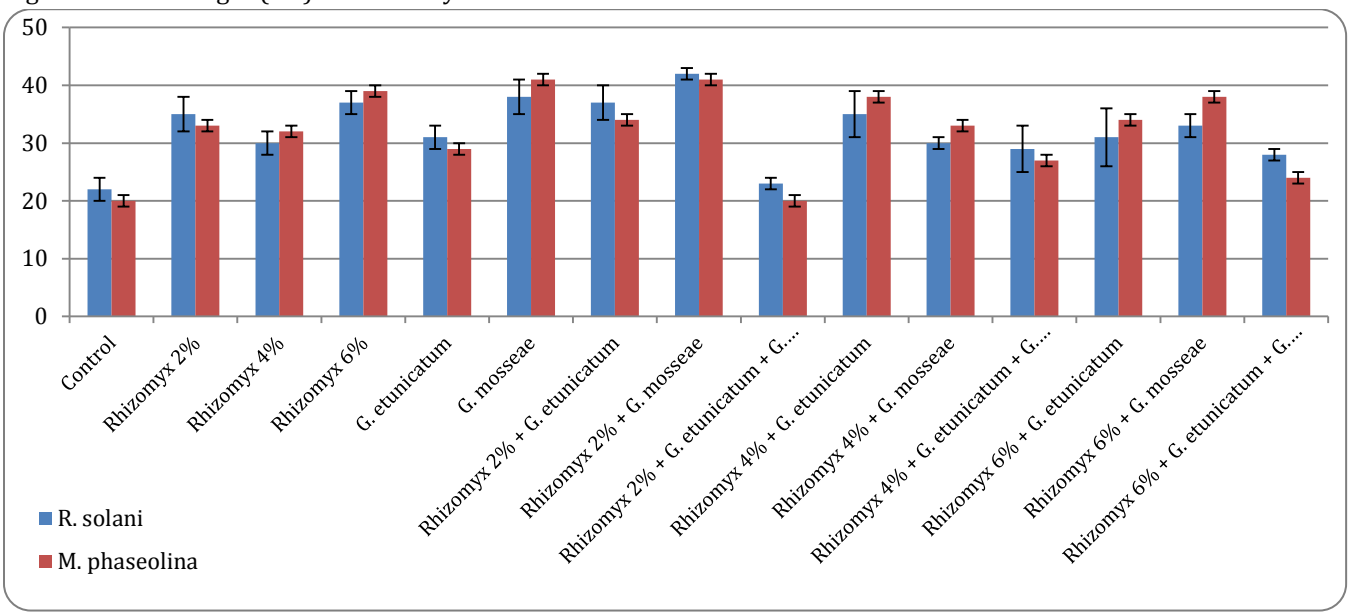


Figure 2. No. of pods after 90 days.

Application of *G. mosseae* and *G. etunicatum* alone with different concentrations of Rhizomyx significantly increased the number of pods per plants in both inoculated and un-inoculated plants over control. The number of pods per plants was reduced when plants were inoculated with either *R. solani* or *M. phaseolina*. Maximum increase in

number of pods was observed in case of Rhizomyx 2% applied in combination with *G. mosseae,* while in case of Rhizomyx application in combination with *G. mosseae* and *G. etunicatum* in different concentration, there was not significant increase in number of pods both in case of *R. solani* and *M. phaseolina.*

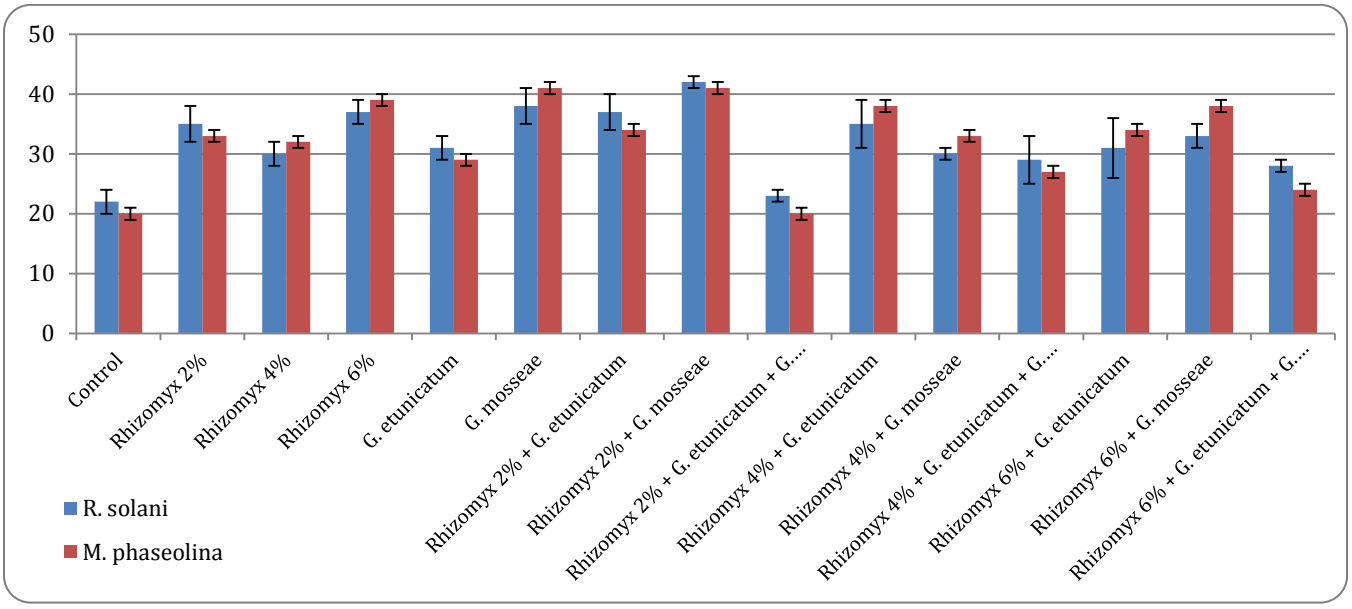


Figure 2. No. of pods after 90 days.

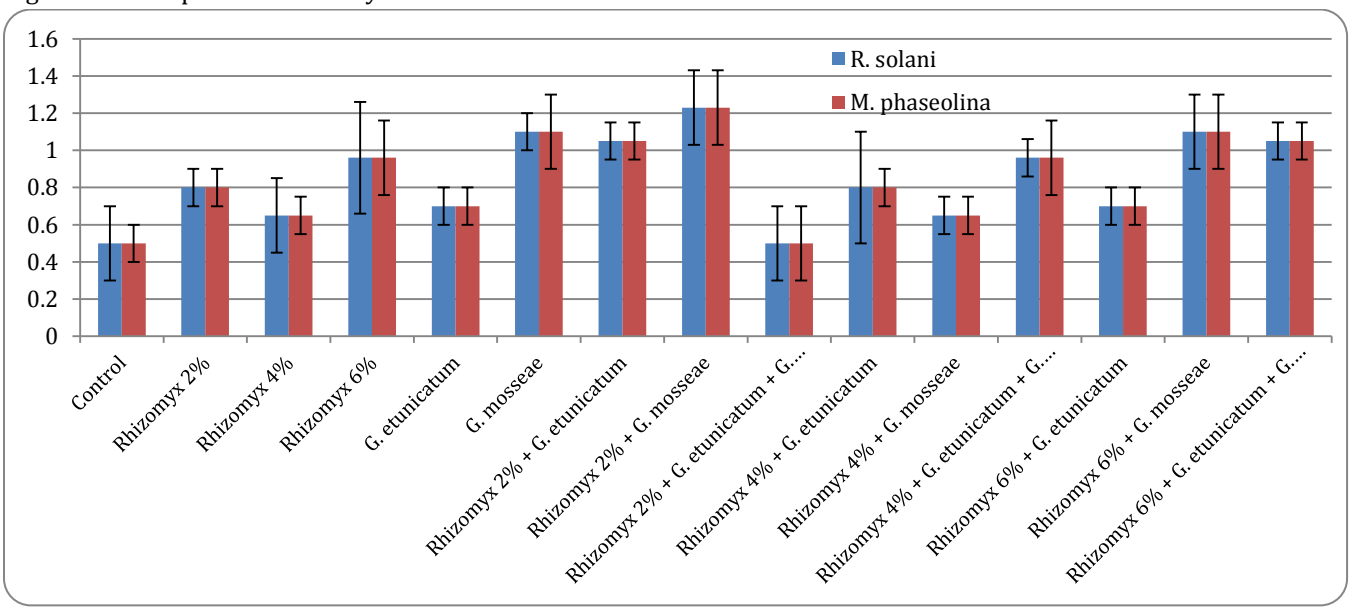


Figure 3. Fresh Shoot Weight (g) after 90 days.

**Root-Rot Severity Index**

Root-rot severity was 03 according to the root rot severity scale, when *M. phaseolina* was applied and 05 when *R. solani* was applied without AM fungi. Severity was reduced to plants inoculated with *R. solani* or *M. phaseolina* when treated with *G. mosseae* and *G. etunicatum*. Severity was two when pathogen inoculated plants were treated with *G. mosseae* and *G. etunicatum* in combination with Rhizomyx 2% or 4%. Maximum reduction in root rot severity index was observed when Rhizomyx 6% was applied in combination with *G. mosseae*. While in case of treatment applied Rhizomyx in combination with both *G. mosseae* and *G. etunicatum* at the same time, root rot severity was not reduced, may be due to the inhibitory effect of two VAM fungi applied (Figure 4).

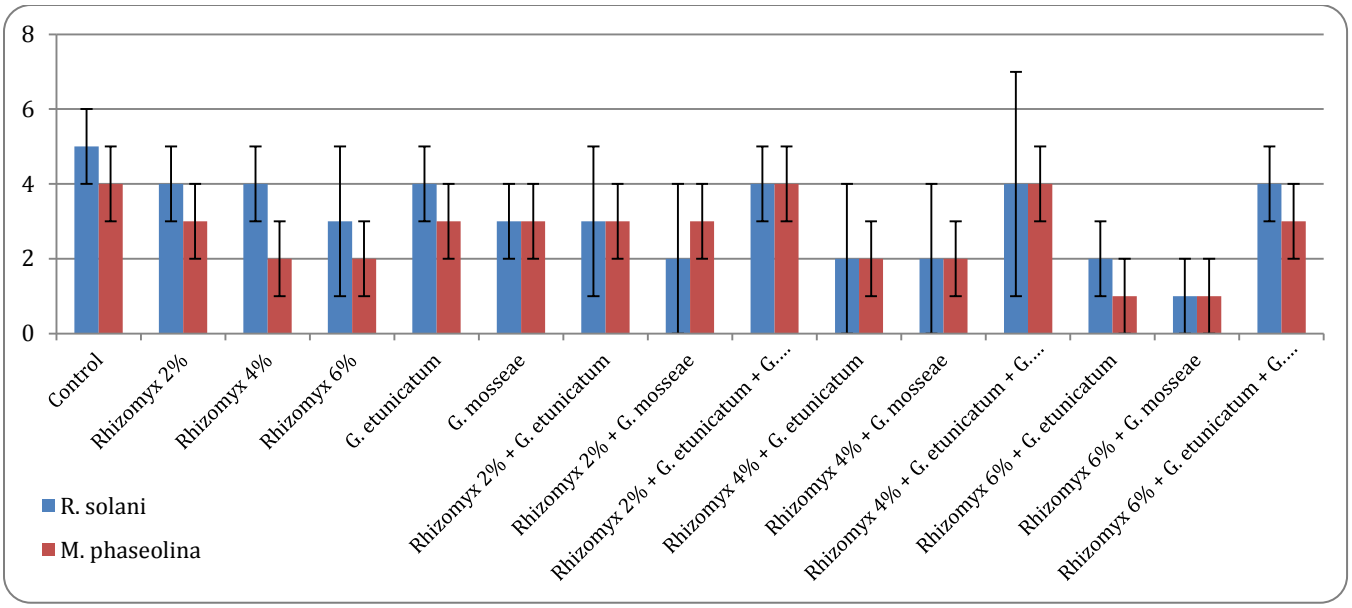


Figure 4. Root rot severity index after 90 days.

*G. intraradices*, *P. alcaligenes* and *B. pumilus* were used individually and concomitantly to control the root-rot disease complex of chickpea. PGPR isolates that promote and stimulate colonization by the AM fungus are called mycorrhiza helper bacteria (Artursson *et al.,* 2006), and they also stimulate the germination of AM spore and mycelial development (Nasim *et al.,* 2008). Combined application of AM fungus and PGPR caused a greater increase in root colonization than did the individual application. Moreover, combined applications of *G. intraradices* and the PGPR isolates inhibit pathogens more (Redden and Berger, 2007) than did the individual applications. Concomitant establishment also improves plant rooting and enhances plant growth and nutrition (Azcon-Aguilar and Barea, 1996), which resulted in a greater increase in plant growth.

The present study demonstrated that AM fungi and plant-growth-promoting rhizobacteria can coexist without adversely affecting each other. In fact, suitable combinations of these biocontrol agents can increase plant growth and resistance to pathogens.

VAM fungi when applied to the pot experiment; it reduced the disease incidence of root pathogenic fungi. The excellent result was obtained by T7 (*G. aggregatum*). This treatment reduced the incidence of root pathogenic fungi to a significantly minimum level. According to the observations, it was concluded that different species of VAM produced good results as compared to the single treatment of Rhizomyx when applied alone. In T7 (*G. aggregatum*) reduced the incidence of root pathogenic fungi to a minimum level of 13.33 and 12.66 percent of *M. phaseolina* and *R. solani*. Percent disease infection caused by *M. phaseolina* and *R. solani* in chickpea plant after inoculation with VAM was calculated by using the using the formula of percent infection. It has previously been reported that application of mixture of isolates inhibits pathogen growth more efficiently than single isolate (Fritz *et al*., 2006; Pozo and Azco´n-Aguilar, 2007). The reason why application of single isolate does not control disease in better way might be related to insufficient root colonization. Therefore, these mechanisms by applying a mixture of the isolates lead to more effective or at least more reliable biocontrol of root pathogenic fungi of chickpea. Root colonization by arbuscular mycorrhizal fungi has been frequently reported to reduce root infection by various root borne pathogens (Azcon-Aguilar and Barea, 1996; Smith and Read, 1997.

**REFERENCES**

Artursson, V., D. F. Roger, and J. K. Jansson. 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environmental microbiology, 8 (1): 1-10.

Azcon-Aguilar, C. and J. M. Barea. 1996. Arbuscular mycorrhizas and biological control of soil borne plant pathogens-an overview of mechanisms involved. Mycorrhiza, 6: 457-464.

Economic Survey of Pakistan. 2012. Pakistan Bureau of Statistics. Govt. of Pakistan, Islamabad.

Fritz, M., I. Jakobsen, M. F. Lyngkjaer, H. Thordal-Christensen, and J. Pons-Kühnemann. 2006. Arbuscular mycorrhiza reduces susceptibility of tomato to *Alternaria solani*. Mycorrhiza, 16: 413-419.

Iqbal, S. M., I. U. Haq, A. Bukhari, A. Ghafoor and A. M. Haqqani. 2005. Screening of chickpea genotypes for resistance against *Fusarium* wilt. Mycopath., 3(2):1-5.

Johansen, C., L. Krishnamurthy, N. P. Saxena, and S. C. Sethi. 1994. Genotypic variation in moisture response of chickpea grown under line-source sprinklers in a semi-arid tropical environment. Field Crops Research, 37(2): 103-112.

Morton, J. B. and G. L. Benny. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders Glomineae and Gigasporineae and two new families Acaulosporaceae and Gigasporaceae with an emendation of Glomaceae. Mycotaxon, 37: 471- 491.

Nasim, G., A. Ali, A. Munawar and R. Bajwa. 2008. Seasonal dynamics of AM fungi in sugarcane in relation to red rot disease.

Parmeter, J. R. 1970. *Rhizoctonia solani,* Biology and Pathology. Univ. of California Press, Berkeley Los Angeles and London. p. 255.

Pirozynski, K. A. and Y. Dalpé. 1989. Geological history of the Glomaceae with particular reference to mycorrhizal symbiosis. Symbiosis, 7:1-36.

Pozo, M. J. and C. Azco´n-Aguilar. 2007. Unraveling mycorrhiza-induced resistance. Current Opinion in Plant Biology, 10: 393–398.

Rangaswami, G. 1992. Diseases of Crop Plants in India, Prentice Hall of India Pvt. Ltd., New Delhi, p.520.

Redden, B. and J. Berger. 2007. History and origin of chickpea. In: Chickpea Breeding and Management. S.S. Yadav, B. Redden, W. Chen, B. Sharma (eds.). CAB International, pp.1-13.

Schenck, N.C. and Y. Perez. 1990. Manual for the identification of VA mycorrhizal fungi (Vol. 286). Gainesville: Synergistic Publications.

Sikes, B. A., K. Cottenie and J. N. Klironomos. 2009. Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. J. Ecol., 97: 1274–1280.

Smith, S. E. and D. J. Read. 1997. Mycorrhizal symbiosis, Academic, London.

Stubblefield, S. P. and T. N. Taylor. 1988. Recent advances in paleomycology. New Phytol., 108: 3-25.

Trappe, J. M. and R. Molina. 1986. Taxonomy and genetics of mycorrhizal fungi: their interactions and relevance. In: Physiology and Genetical Aspects of Mycorrhizae (Ed. By V. Gianinazzi-Pearson and S. Gianinazzi). pp. 133-146.