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EFFICACY OF MICROBIAL BIOAGENTS FOR THE CONTROL OF COLLAR ROT DISEASE IN CHICKPEA

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Abstract

Study was conducted *In vivo* to evaluate the antagonistic effects of two microorganisms against collar rot disease of chickpea caused by the fungus *Sclerotium rolfsii*. The effect of microbial amendments viz., Vesicular arbuscular mycorrhizae (VAM) and root nodulating bacteria *Rhizobium* sp., on the collar rot disease in *Cicer* was studied individually and in combination when applied to the soil. The results depicted that 100% seedling mortality was recorded in the control treatment where only test fungus i.e., *S. rolfsii* was added to the soil. Maximum reduction in seedling mortality was obtained when VAM and *Rhizobium sp*. were applied in combination. Likewise, the two microorganisms when used alone significantly reduced the effect of disease on plant growth. Treatment with *Rhizobium* sp., had greater synergistic effect on plant height, shoot and root weight, nodules plant^{-1}, pods plant^{-1} and 100-seed weight of chickpea as compared to VAM. It was inferred that both the amendments used have effectively improved various yield parameters by controlling the disease and reducing seedling mortality.

Introduction

Chickpea (*Cicer arietinum* L.) is of great economic importance for the growers of the *Thal* region where 90 % area of this crop is planted. It is grown in Pakistan over an area of about one million hectares with annual production of 0.6 million tons and seed yield of 600 kg ha^{-1} (Anon., 2007). Collar rot of chickpea caused by *Sclerotium rolfsii* Sacc., is a widespread and devastating disease and a major constraint in most chickpea growing areas where it severely limits yield. The disease has been reported to cause seedling mortality in chickpea up to 90 % in favorable conditions (Gurha & Dubey, 1982). However, *S. rolfsii* was reported to be a destructive soil-borne pathogen causing diseases on many crop plants especially in tropics and subtropics (Mukherjee & Raghu, 1997). Besides sources of genetic resistance and chemical control, some bioamendments can help alleviate and overcome disease stresses in important crops. These may be used as a component of integrated control effort and in the development of strategies for productivity improvement through integrated supply of nutrients with the help of specialized soil microorganisms. In the past, workers have described the inhibition of growth and production of sclerotia in *S. rolfsii* by bacteria (Agrawal *et al*., 1977; Brathwaite & Cunningham, 1982) and mycorrhizal fungus (Krishna & Bagyaraj, 1983). The bacteria of the genus *Rhizobium/Bradyrhizobium* contribute to nutrition of legumes by fixing atmospheric nitrogen. Mycorrhizal plants increase the surface area of the root system for better absorption of nutrients from soil especially when the soils are deficient in phosphorus. Apart from the well-known effect of VAM-fungal associations on phosphate uptake, Zn, Cu, and NH_4 uptake is also enhanced in wheat, maize and potatoes (Jegan & Kumar, 2006). Similarly, the uptake of sulfur is enhanced by VAM-fungal infection in red clover and maize. Increased production of plant growth hormones such as cytokinins, gibberellins and chlorophyll contents of plants have been found to increase by VAM colonization (Allen, 1983; Jegan & Kumar, 2006).

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There are indications to show that VAM fungal associations with plant roots can help overcome water stress by stomata regulation in citrus. Growth in many legumes such as clover, groundnut, soybean, pigeon pea and chickpea is enhanced when a *Cicer* species of *Rhizobium* and a VAM fungus are co-inoculated. This additive effect was demonstrated to a greater degree in phosphorus deficient soils as shown with groundnut (Mahen *et al*., 1995). Our objective was to assess the potential antagonistic effect of two microbial bioagents individually and concomitantly on the viability of chickpea plants and their impact on plant growth and seed yield parameters. Ability of VAM to colonize chickpea rhizosphere was also investigated.

Materials and Methods

Two-kg capacity plastic pots were filled with steam sterilized soil. Soil was autoclaved once at 120 psi for 20 minutes to make it microbe-free. A representative soil sample was analyzed for important nutrients and suitability towards VAM and *Rhizobium* sp., application. VAM inoculum was obtained from the Department of Biological Sciences, Quaid-i-Azam University, Islamabad and that of *Rhizobium* from Biology and Biochemistry Section, NARC, Islamabad Different treatments were applied according to prescribed procedure. The pots were assigned the following treatments.

1. VAM+Test Plant (Mycorrhiza inoculation to healthy plants)
2. VAM+Test Plant+Pathogen (Mycorrhiza inoculation to diseased plants)
3. *Rhizobium*+Test Plant (*Rhizobium* inoculation to healthy plants)
4. *Rhizobium*+Test Plant+Pathogen (*Rhizobium* inoculation to diseased plants)
5. VAM+*Rhizobium*+Test Plant (Combined inoculation to healthy plants)
6. VAM+*Rhizobium*+Test Plant+Pathogen (Combined inoculation to diseased plants)

VAM inoculation was accomplished by placing a layer of inoculum 5 cm under the seed to ensure a good and early contact with the newly emerging roots as proposed by Jackson *et al*., (1972). Black color peat soil was impregnated with the bacterium *Rhizobium* sp., *Cicer* applied to seeds of chickpea var. C 44 in such a way that these were covered uniformly and received required number ($>10^8$/g soil) of bacterium. The uniformity of bacterium was ensured by plate counting with colony counter after making different dilutions of inoculum in water. For inoculating treatments 2, 4 and 6 with pathogen *S. rolfsii*, 4 wheat grains per plant having complete mycelial growth were placed in close contact with seven-day old chickpea seedlings in collar region. Seedling mortality due to *S. rolfsii* and the effect of VAM and *Rhizobium sp*., amendments on the survival of chickpea plants against the disease was recorded after 40 days. At later growth stages, the effect of the pathogen on various yield parameters in the presence and absence of amendments were recorded. These parameters include VAM colonization, nodules plant^{-1}, plant height (cm), pods plant^{-1} shoot dry weight (g), root dry weight (g) etc.

Results and Discussion

i. Effect on the mortality of seedlings due to *S. rolfsii*: The results in Table 1 indicate that in the absence of any bioamendment i.e., VAM and/or *Rhizobium*, 100% mortality of the seedlings of the chickpea cultivar was recorded due to test fungus *S. rolfsii*. Mortality rate was reduced to 42, 33 and 21%, respectively when chickpea seedlings in the presence of the fungus were inoculated with VAM, *Rhizobium* sp., and VAM+*Rhizobium* sp., Iqbal *et al*., (1990) while using VAM and *Aspergillus* sp. together recorded minimum infection of *Rhizoctonia solani*. Several other workers (Iqbal *et al*., 1988; Jain & Sethi, 1988 and Parasad, 1991) also reported VAM as an organism that alleviated the adverse effects of different plant pathogens. Mycorrhizal infection may alter the host plant metabolism through certain chemical, physiological and morphological inductions (Mosse, 1973). VAM inoculated plants have higher levels of arginine that is inhibitory to chlamydospores of disease causing pathogen (Baltruschat & Schonbeck, 1975). Insoluble polysacchrides accumulate in cell walls and lignin production is enhanced in mycorrhizal roots and these have more vascular bundles (Daft & Okusanya, 1973). In lignified and strengthened cell walls, the growth of pathogens is likely to be inhibited as was shown with *Fusarium oxysporum* and *Phoma terrestris* (Dehne *et al*., 1978). A stronger vascular system increases the flow of nutrients, imparts greater mechanical strength and thus diminishes the effects of pathogen to greater extent. It is speculated that mycorrhizal chickpea seedlings that survived in the presence of pathogen might have benefited from some or all of these modifications.

Application of *Rhizobium* sp., bacteria alone with the fungus has resulted in significant reduction in seedling mortality. Bhattacharyya & Mukerjee (1990); Ghaffar (1993); Ehtesham-ul-Haque & Ghaffar (1992) also recorded higher percentage of seedling viability with *Rhizobium* against the pathogen. It is thought that the bacterium may protect roots of legumes by producing a toxic metabolite called Rhizobiotoxine (Chakarborty & Purkayashta, 1984); by producing antibiotics (Malajczuk, 1983); by parasitizing the pathogen directly (Tu, 1978); by lyzing the pathogen (Malajczuk, 1984). Plants withstood fungus attack more effectively when the two amendments were used concomitantly. The explicit reason for this effect is the building up a more powerful defense by VAM and *Rhizobium* together against the fungus.

ii. Effect on colonization of chickpea roots by VAM: Data revealed that mycorrhizal colonization in the control treatment dropped from 56 to 30% with the addition of fungus. Co-inoculation of VAM and *Rhizobium* sp., enhanced mycorrhizal infection to 82% but addition of fungus to this treatment significantly lowered colonization by VAM to 70% (Table 2). Results are in conformity with those of Ramraj & Sharmugam (1990) where combined inoculation of *Rhizobium* and *G. etunicatum* increased mycorrhizal colonization. Jain & Sethi (1988) reported that *Meloidogyne incognita* or *Heterodera cajani* reduced VAM infection and spore production considerably. It was noted in this study that impact of pathogen in reducing mycorrhizal colonization was less severe when the plants were inoculated with VAM and *Rhizobium* sp., simultaneously.

iii. Effect on growth and yield parameters: The nodulation activity expressed as number of nodules plant^{-1}was negatively affected in the presence of fungus and dropped from 35 to 19 nodules plant^{-1} compared with *Rhizobium* treatment. Chickpea plants produced more nodules per plant (58) when seeds were inoculated with *Rhizobium* and mycorrhizae together (Table 3) but addition of fungus to this treatment reduced the nodules (43) significantly. Earlier work on this aspect supports our findings. Patil (1985) found that infection of *S. rolfsii* caused reduction in the development of effective nodules per plant. The dry weight and total nitrogen content of nodules from fungus-infected plants were also low. Bhattacharyya & Mukerjee (1990) also noted reduced nodulation by *S. rolfsii* in groundnuts.

Table 1. Effect of different treatments on the seedling mortality due to *S. rolfsii*.

Treatments	**% Mortality**
Seedlings + Fungus	100.00 d
VAM + Seedlings + Fungus	41.66 c
Rhizobium + Seedlings + Fungus	33.34 b
VAM + *Rhizobium* + Seedlings + Fungus	20.84 a
LSD (p=0.05)	**8.28**

Table 2. Percent infection of chickpea roots by VAM as affected by different treatments.

Treatments	**% Infection**
VAM	55.9 b
VAM + Fungus	29.9 a
VAM + *Rhizobium*	81.7 d
VAM + *Rhizobium* + Fungus	69.5 c
LSD (p=0.05)	**3.94**

Table 3. Growth and yield parameters of chickpea as affected by different treatments.

Treatments	**Nodules plant^{-1}**	**Shoot weight (g)**	**Root weight (g)**	**Plant height (cm)**	**Pods plant $^{-1}$**	**100-seed weight (g)**
VAM	-	2.13 b	0.19 b	21.0 c	8.2 b	16.5 b
VAM+Fungus	-	1.25 a	0.11 a	14.8 a	4.9 a	11.3 a
Rhizobium	34.9 b	3.30 d	0.32 d	25.0 d	13.0 d	23.0 d
Rhizobium+Fungus	18.5 a	2.62 c	0.22 c	18.2 b	10.2 c	19.0 c
VAM+*Rhizobium*	57.7 d	5.40 f	0.81 f	35.7 f	19.9 f	28.1 f
VAM+*Rhizobium*+Fungus	43.1 c	4.20 e	0.70 e	29.1 e	15.5 e	25.0 e
LSD (p=0.05)	**7.29**	**0.62**	**0.05**	**2.88**	**2.60**	**2.68**

Inoculation of chickpea with microbial amendments of VAM and *Rhizobium sp*., individually and in combination improved plant height, root and shoot dry weight, pods plant^{-1} and 100-seed weight effectively. These plant traits were adversely affected when the fungus was incorporated in these treatments, however, its impact was reduced in the presence of these bio-amendments (Table 3). The synergistic effect of these microbes has been discussed in detail previously. Singh (1990) reported an equal increase in shoot length due to VAM and *Rhizobium* inoculation. Similar results have been reported by Ehteshamul-Haque *et al*., (1994) where they found that combined use of *B. japonicum* and *Tolaromyces flavus* produced chickpea plants with greater height and shoot weight. Mukhtar & Khan (1989) found that *S. rolfsii was* responsible for restricted growth of chickpea plants. Bhandal *et al*., (1989) recorded the increased shoot biomass for *Rhizobium* inoculated pea plants. The findings of the study are also in agreement with those of Ramraj & Sharmugam (1990) who reported that dual inoculation with *G. etunicatum* and *Rhizobium* increased biomass to a greater extent than when either was inoculated alone.

Conclusions

Chickpea seedlings mortality due to collar rot was reduced remarkably as a result of VAM and *Rhizobium* sp., treatments applied individually and simultaneously. Chickpea growth and yield parameters were significantly improved with the use of bioamendments (VAM and *Rhizobium*) both in the presence and absence of *S. rolfsii*. *Rhizobium* had a greater synergistic effect than VAM on the growth and yield parameters of chickpea.

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