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Effect of rhizobium, arbuscular mycorrhiza and rhizobacterial isolates on fenugreek (*Trigonella foenum-graecum*) growth and mycorrhiza population

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Abstract

Fenugreek (*Trigonella foenum-graecum* L.) belonging to the Leguminosae (Fabacecae family) is an important leguminous seed spice and well known aromatic and medicinal crop. Fenugreek seeds were treated as alone and in combination with *Rhizobium,* Arbuscular mycorrhizal (AM) fungi and rhizobacterial isolate previously isolated from rhizosphere and endorhizosphere of fenugreek plants. After six weeks plant growth parameters such as length, weight and number of nodules were recorded. Those roots receiving dual endophytes (*Rhizobium* + AM fungi) showed significantly higher values for plant height, as well as shoot weight but maximum root weight was recorded for seedling treated with all the three i.e. *Rhizobium* + AM fungi+ rhizobacterial isolate. Quantitatively maximum species of AM fungi (5 species) were isolated form rhizosphere of fenugreek plants receiving dual infection (*Rhizobium* + AM fungi) followed by plants colonized by *Rhizobium*, AM fungi and rhizobacterial isolates.

Key words: Fenugreek, rhizobium, mycrorhiza, nodules

Introduction

Fenugreek (*Trigonella foenum-graecum* L.**)** belonging to the Leguminosae (Fabacecae) family is an important leguminous spice and well known aromatic and medicinal herb. Fenugreek is of dual purpose-seed and leaf. It is commonly used to flavor liquors, bread, fish, salad, soups, cheese, curry and manufacture of pickles, perfumes, soap, cosmetics and cough syrup. It is a native of South-eastern Europe and Western Asia and widely cultivated in India which harbor great diversity of fenugreek.

In agricultural crop production systems, nitrogen is often the most limiting nutrient that dictates crop production. Despite its presence in large quantities in the atmosphere, plants cannot utilize nitrogen since it is inert. Legume – *Rhizobium* symbiosis is an important facet of symbiotic nitrogen fixation which is exploited to benefit agriculture and its sustainability. Arbuscular Mycorrhiza (AM) fungi are a symbiotic association between plant roots and fungi. The fungal partner is benefited by obtaining its carbon requirements from host's photosynthates and the plant in turn gains the much needed nutrients especially phosphorus, calcium, copper and zinc which would otherwise be inaccessible to the host. This uptake of nutrients is facilitated with the help of fine absorbing hyphae of the fungus. These fungi are associated with majority of agricultural crops. There are seven genera of these fungi that produce Arbuscular mycorrhizal symbiosis with plants. They are *Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora*, *Entrophospora*, *Archaeospora* and *Paraglomus*. Probably the most abundant fungi in agricultural soils are the arbuscular mycorrhizal (AM) fungi (Brahmaprakash and Sahu, 2012).

Low-input legume farming systems especially medicinal and seed spice crops like fenugreek often suffer nutrient deficits that limit plant performance. The symbiosis between plants and arbuscular mycorrhizal (AM) fungi efficiently promotes plant growth and nutrient uptake, especially in growth-limiting environments (Smith

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and Read, 2008). AM symbiosis seems to be particularly efficient for the acquisition of low-mobility nutrients such as phosphorus (P), magnesium, and zinc, although it traditionally has been considered irrelevant for plant nitrogen (N) nutrition. However, there is increasing evidence that AM symbiosis plays a significant role in plant N capture, especially under conditions of water and nutrient stress. These benefits mainly have been observed in pot studies; field studies have often produced contradictory results (Kaschuk *et al.,* 2010). The present work evaluated the effect of *Rhizobium,* AM fungi and rhizobacterial isolates through seed inoculation of fenugreek.

Materials and methods

Fenugreek plants as well as soil samples were collected from local farmers' fields for isolation of *Rhizobium* from root nodules and rhizobacteria from rhizosphere soils. The inoculums of rhizobia collected from 3-day old cultures of *Rhizobium* sp. isolated from fenugreek nodules, initially streaked on yeast extract mannitol agar (YEMA) medium and later raised in YEM broth was used at the rate of 1 ml per seed. Arbuscular Mycorrhizae spores were isolated from fenugreek fields through sieving and decanting techniques (Gerdemann and Nicolson, 1963) and thus isolated AM fungi spores were maintained on *Cenchrus ciliaris* L. as pot cultures and used as inoculum after root harvesting. The previously isolated microbial inoculants (Karel,2005) were used in the present investigation to study the effect of rhizobia, mycorrhizae and rhizobacteria on fenugreek growth and arbuscular mycorrhizal (AM) population.

Surface sterilized healthy seeds of fenugreek were selected and soaked overnight prior to sowing. Three replicates for each inoculation treatment were made, viz; (1) *Rhizobium* alone (2) AM alone (3) *Rhizobium* and AM (4) *Rhizobium,* AM and rhizobacterial isolate (5) control (no inoculation). Pot culture plants were maintained at a temperature range of 25-30 C and were irrigated with tap water as and when required. After six weeks, plants from each treatment were removed in such as way that their roots remained intact and then washed under slow water without damaging the plant and their root system. Plant growth parameters such as length, weight and number of nodules were recorded.

Results and discussion

The effect of *Rhizobium* with or without AM fungi and /or rhizobacterial isolate inoculation was studied in pot culture experiment of fenugreek. The inoculated fenugreek seedlings were showing robust growth and remained healthy till six weeks of study period while the control pot seedling withered and remained stunted. Table 1 shows the plant growth parameters recorded after 6 weeks. The shoot weight of *Rhizobium* + AM fungi and rhizobacterial isolate treated plants were much higher than control (uninoculated) and similar pattern was observed with respect to root weight of fenugreek seedlings treated with these three microbial inoculants (*Rhizobium* + AM fungi + Rhizobacteria). The root system was much branched and better developed in mycorrhizal than non-mycorrhizal ones. Those roots receiving dual endophytes (*Rhizobium* + AM fungi) showed significantly higher values for plant height, as well

Table 1. Effect of microbial inoculation on plant growth parameters of fenugreek

Treatments	Plant Height (cm)	Shoot weight (g)	Root weight (g)
Rhizobium	25.33	17.96	9.70
AM fungi	25.52	9.92	10.25
Rhizobium + AM fungi	26.64	18.34	11.62
Rhizobium + AM fungi + rhizobacteria	25.60	18.08	11.94
Control	22.10	13.54	7.52
Mean	25.03	15.56	10.26

as shoot weight but maximum root weight was recorded for seedlings treated with all the three i.e. *Rhizobium* + AM fungi+ rhizobacterial isolate (Table 1). These observations on plant biometrics clearly indicated that dual infection resulted in best growth of fenugreek seedlings. This is in conformity with earlier work by Chaturvedi *et al.,* (1988) and Singh *et al.,* (1988).

Fenugreek seeds treated with *Rhizobium* and AM fungi had significantly more nodules than those treated with either *Rhizobium* or AM fungi alone; however it was at par with plants treated with *Rhizobium* + AM fungi + Rhizobacterial isolate (Fig.1). It has been well documented that inoculation of plants with AM fungi can stimulate nodulation and nitrogen fixation by legumes (Xie *et al*., 1995).

Low AM spore counts were recorded from the soils of uninoculated fenugreek plants as compared to those from inoculated ones. Six types of AM fungi spores were extracted and identified from the different pot culture soils, viz; *Glomus fasciculatum, G. heterosporum, G. occultum, G. pubescens, G. albidum and Scutellospora pellucida.* One AM fungus species, *G. fasciculatum* was recorded in all the treatments including control (Table 2). Quantitatively maximum species of AM fungi (5 species) were isolated from rhizosphere of fenugreek plants receiving dual infection (*Rhizobium* + AM fungi) followed by plants colonized by *Rhizobium*, AM fungi and rhizobacterial isolates (four species). Similarly, the highest per cent infection in root system as well the highest number of AM fungi spores per 15 g soil was recorded in dual culture treated fenugreek plants (Table 2).

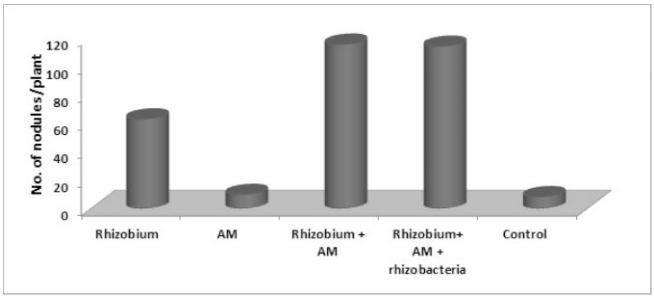

Fig.1: Influence of inoculation on root nodulation in fenugreek

AM fungi have been reported to increase growth of plants by enhancing nutrient uptake (Mathur and Vyas, 1995) through a reduction of the distance that nutrients must diffuse to plant roots by accelerating the rate of nutrient absorption and nutrient concentration at the absorbing surface and finally chemically modifying the availability of nutrients for uptake by plants through mycorrhizal hyphae. The present study on fenugreek inoculation with AM

Table 2. Influence of inoculation on root infection and AM spores in fenugreek

Treatments	AM infection in root system (%)	No. of AM spores 15 g^{-1} soil	Type of Spores
Rhizobium	29	16	*Glomus fasciculatum* *G. occultum*
AM fungi	66	28	*G. fasciculatum* *G. pubescens* *Scutellospora pellucida*
Rhizobium + AM	83	37	*G. fasciculatum* *G. albidum* *G. occultum* *G. pubescens* *S. pellucida*
Rhizobium + AM + rhizobacteria	81	35	*G. heterosporum* *G. fasciculatum* *G. occultum* *S. pellucida*
Control	16	10	*G. fasciculatum*
Mean	55	25.2	

fungi has also provided similar results as those obtained by several other researchers (Xavier and Germida, 2003). Earlier workers have revealed that AM fungi assist plants in accumulating higher concentration of phosphorus (Rengel 2002; Simard and Durall, 2004) which in turn have a positive effect on both nodulation and nitrogen fixation by *Rhizobium* inoculants.

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