

## Response of Three Vegetable Crops to VAM Fungal Inoculation in Nutrient Deficient Soils Amended with Organic Matter

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### Abstract

A mixed culture of indigenous endomycorrhizal fungi was multiplied in a nursery and tested for its ability to promote growth and yield of three agricultural crops. Onion, potato and garlic were successfully inoculated with VAM fungi in nutrient deficient soil amended with composted leaves of *Albizia lebek* (albizzia), *Populus deltoides* (poplar) and *Leucaena leucocephala* (leucaena). Colonization in onion reached approx. 85% of root length, followed by garlic and potato (65%). Inoculation response in terms of yield increase was maximum in onion (70%) whereas garlic and potato showed 30% and 48% increases respectively. Also, growth response to VAM fungus inoculation at the high level of organic amendment as well as production of infectious VAM population was demonstrated.

Keywords: Garlic, inoculum, onion, organic, potato, VAM

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## 1. Introduction

There is a marked change in practice and philosophy of agriculture, which is rapidly shifting from conventional to sustainable approaches. Environmental and economic concerns associated with increased use of chemical fertilizers and pesticides have stimulated interest in low-input agriculture as an alternative to conventional agriculture. This system involves the use of farm generated inputs or to exploit biological systems such as management of Vesicular-Arbuscular Mycorrhizal fungi (VAM) for improved efficiency of P use (Gavito and Varela, 1993; Douds et al., 1993).

VAM fungi are obligately symbiotic soil fungi that impart a variety of benefits on their hosts including growth and yield enhancement (Gianinazzi-Pearson and Gianinazzi, 1983; Menge, 1983). These demonstrated benefits indicate that VAM associations could be crucial for plant growth and economically viable yields under agricultural practice involving organic inputs and shunning chemical fertilizers. Organic matter may interact with VAM fungi to affect plant production (Liebhardt et al., 1989; Noyd et al., 1996). Although the relationship of organic matter and VAM development is unclear, VAM hyphae appear to proliferate more extensively in association with organic matter (Hepper and Warner, 1983; St. John et al., 1983).

The occurrence and stimulatory effect of mycorrhizal symbiosis on growth and nutrient uptake of onion (Powell et al., 1982; Vosatka, 1995; Nwaduwe and Chude, 1995) and potato (Bhattarai and Mishra, 1984; McArthur and Knowles, 1993a,b; Wang et al., 1993) are well documented. The effect of mycorrhiza upon plant growth and P uptake in garlic has also been studied (Shuja and Khan, 1977; Khan et al., 1980; Firdaus et al., 1988). The objectives of the current work was, firstly to compare the yield enhancement in potato, garlic and onion inoculated with a mixed population of VAM fungi at two levels of organic matter amendment, and secondly to examine the VAM fungal inoculum production in the amended soil.

## 2. Material and Methods

### *Experimental site and preparation of substrate*

The experimental site is located at Gwal Pahari in Haryana state, India (77°12'E and latitude 28°35'N) 255 m above the mean sea level and receives a mean annual rainfall of 500 mm. The soil (sandy loam Hyperthermic Typic Haplustalf) was collected from the field, sieved through a 3 mm mesh and amended with two levels of compost (Bharadwaj, 1995) made of degraded leaves of *Albizia lebek* (25%), *Populus deltoides* (50%) and *Leucaena*

*leucocephala* (25%) in the ratio of 1:1 and 2:1 (soil:compost). Each mixture was homogenized thoroughly and analyzed for P, organic C, N, pH and EC (Table 1).

#### *Preparation of VAM fungus inocula*

Mixed indigenous culture, containing native populations of *Glomus*, *Gigaspora* and *Scutellospora* spp. (each constituting 60%, 30% and 10% respectively in the consortium), was used as VAM inocula after growing for one year in earthenware pots (7 kg capacity) with the above pot mixture (1:1 soil compost mixture) and *Sorghum bicolor* var. *sudanense* as the host plant in a greenhouse ( $30\pm 2^\circ\text{C}$ ). At maturity, the above ground plant material was removed and the substrate allowed to dry in a greenhouse for a week at  $25^\circ\text{C}$ . The roots were then chopped finely and the dried root/soil mixture was thoroughly mixed to obtain a homogenous inoculum. Spores were isolated by wet sieving and decanting (Gerdemann and Nicolson, 1963) and counted on filter paper (Gaur and Adholeya, 1994). The number of VAM infectious propagules (IP) in the inoculum was assessed (Gaur et al., 1998) and the value was found to be 25 IPs/g. Percent root colonization in the inocula was assessed as described by Biermann and Linderman (1981). The value of colonization percentage was 62.

#### *Experimental setup and design*

A field area of  $7.5 \times 7.5$  m was used in the experiment, with 12 treatments completely randomized within a  $3 \times 2 \times 2$  factorial structure. The treatments consisted of three host species cultivated at two fertility levels (compost levels), each inoculated or uninoculated with VAM fungi. Each treatment was replicated three times with ten plants per treatment. Land was prepared by repeated ploughing, hoeing, and planting. The compost amended soil mixtures were wetted with appropriate amount of water and used to form raised beds (30 cm wide  $\times$  224 cm long  $\times$  16 cm high) using 70 kg mixture for each bed. The beds were separated by a distance of 30 cm, so that a series of beds and channels were formed. A furrow (16 cm wide, 210 cm long and 8 cm deep) was made in each bed by removing the soil.

#### *Preparation and transplanting of onion seedlings*

Seedlings of onion were grown in nursery beds. Seeds of onion (*Allium cepa* L. cv Pusa red) were surface sterilized with 10%  $\text{H}_2\text{O}_2$  for five minutes and washed with distilled water. Separate nursery beds (3 m  $\times$  1 m) were made using the two soil mixtures. Surface sterilized seeds were sown in line at a

Table 1. Chemical analysis of unamended nursery soil, leaf compost and soil amended with two levels of compost Hf (1:1) and Lf (2:1) (soil:compost) at the time of initiation of the experiment.

	Substrate	pH (1:2.5H <sub>2</sub> O)	EC (dS/m)	Organic C (%)	Olsen P (ppm)	N (%)
1	Nursery soil	8.2	0.20	0.22	0.53	0.04
2	Compost	7.2	0.93	11.72	25	1.23
3	Amended soil (Hf)	7.7	0.73	2.92	9.40	0.62
4	Amended soil (Lf)	7.9	0.50	1.15	4.62	0.35

Values are the means of five samples. Hf = High fertility; Lf = Low fertility.

distance of 5–7 cm and 1 cm deep in soil. The beds were covered with dry grass until the germination was completed.

When the plants were 0.4–1 cm in diameter at the soil surface and 15–20 cm high, they were pulled out gently and transplanted to experimental beds. Each of the two types of treatment beds (compost levels) received plants from respective nursery beds. Ten seedlings were transplanted in each bed with 20 cm between the plants. Inoculum was applied in furrows at the rate of one kg per bed (25000 infectious propagules) before planting the seedlings. Seedlings were transplanted in the evening and irrigated immediately and every five to seven days thereafter. Also, 2–3 weeding and light hoeing were done at the early stages.

#### *Sowing of garlic*

Garlic (*Allium sativum* L.) was propagated by cloves. The cloves were separated, graded by weight (2 g), and surface sterilized as described above. Cloves were then planted in vermiculite, and sprouted for 5–6 days at 27°C. The sprouted cloves were planted directly in furrows in the experimental beds with 20 cm between plants. Inoculum application and other horticultural practices were the same as described for onion.

#### *Sowing of potato*

Certified potato tubers (*Solanum tuberosum* L. var. Kufri kisan.) were taken from 4°C (95% RH) storage, surface sterilized with 10% H<sub>2</sub>O<sub>2</sub> (5 minutes), and rinsed thoroughly with distilled water. Single-eye pieces (5 g) were cut from

the mid-region of the tuber and rinsed. After air drying for an hour, tuber pieces plants were planted in vermiculite and sprouted in the dark for 10 days at 27°C. Single tuber piece plants were transplanted to the mycorrhizal and non-mycorrhizal beds, keeping a distance of 20 cm between the plants. Inoculum placement and irrigation was the same as for onion.

#### *Harvest and analysis*

The crops were harvested at 16 weeks when about 70 per cent of the shoots were dry. Irrigation was stopped ten days before harvesting to stop growth. The onion and garlic bulbs and potato tubers were removed from each bed and weighed separately. Substrate from each of the beds was homogenized separately and kept in separate bags for further analysis.

Shoots of all plant species were severed just above the earth level, weighed, rinsed in distilled water, dried at 70°C for 48 hr, weighed, ground to pass a screen (0.5 mm pore size) and digested in H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> mixture. The P and N contents in the digest were determined using methods described by Jackson (1973). Roots were washed free of soil, cut into 1 cm segments, mixed thoroughly and weighed. A sub sample of root segments was taken for analysis of mycorrhizal colonization. Roots also were dried at 70°C for 48 hr before measuring dry weight.

Percent VAM fungus colonization in roots was determined on 100 root segments (1 cm each) per sample. Roots were stained using the method of Philips and Hayman (1970). Root pieces were mounted on glass slides and examined under 40× magnification with a compound microscope (Leica, Gallen III). The assessment of colonization was done according to the method of Biermann and Linderman (1981) in which colonization was expressed as the percent of each root segment length that was colonized. Spores of VAM fungi were extracted (Gerdemann and Nicolson, 1963) from 50 ml-homogenized substrate in three replicates for each treatment. The spores retained on different sieves were collected in a beaker and recovered by sucrose density centrifugation. Only visually intact spores were counted under a stereoscopic microscope (Gaur and Adholeya, 1994). The average number of spores in the 50 ml substrate soil was used to estimate the spores per 100 ml substrate at each of the treatments. Number of infectious propagules was determined as described by Gaur et al. (1998).

#### *Statistical analysis*

Treatment effects were determined by one-way analysis of variance (ANOVA) using a completely randomized design. The differences between

Table 2. Effects of inoculation with VAM fungi on various plant and fungal parameters in onion and garlic cultivated at two levels of fertility.

Host/ Treatments						
Fertility levels	VAM inoculation	Bulb diam. (cm)	Bulb fresh weight (g)	Colonization (%)	Shoot dry weight (g)	Root dry weight (g)
<b>Onion</b>						
Hf	Inoculated	7.8a	83.0a	84.6a	3.5a	0.8a
	Uninoculated	6.7b	74.0b	5.3d	2.3b	0.6b
Lf	Inoculated	6.5b	73.1b	73.2b	3.4a	0.4c
	Uninoculated	4.6c	42.2c	12.1c	1.0c	0.2d
LSD		0.3	2.9	1.9	0.1	0.0
<b>Garlic</b>						
Hf	Inoculated	4.8a	63.5a	56.2b	3.7a	1.1a
	Uninoculated	4.1b	53.3b	0.6d	3.4a	0.7b
Lf	Inoculated	3.8b	46.1c	65.4a	3.6a	0.6b
	Uninoculated	3.2c	35.0d	2.2c	2.0b	0.3c
LSD		0.3	2.8	1.0	0.4	0.1

Values of bulb diameter and bulb fresh weight are means of ten replicates and the rest are means of 3. Means in each column followed by the same letters are not significantly different at P 0.05 by Duncan's Multiple Range Test (DMRT). Hf = High fertility; Lf = Low fertility.

Table 3. Effects of inoculation with VAM fungi on various plant and fungal parameters in potato cultivated at two levels of fertility.

Host	Treatments		Shoot dry weight per plant	Root dry weight per plant	Colonization (%)
	Fertility levels	VAM inoculation			
<b>Potato</b>	Hf	Inoculated	12.53a	0.41a	65.68a
		Uninoculated	8.53c	0.21b	2.40b
	Lf	Inoculated	10.7b	0.17c	67.99a
		Uninoculated	6.4d	0.13d	3.28b
LSD		0.31	0.13	1.76	

Values are means of three replicates. Means followed by same letters are not significant at P=0.05 by Duncan's Multiple Range Test (DMRT). Hf = High fertility; Lf = Low fertility.

treatments were confirmed by Duncan's Multiple Range Test (DMRT) using Costat Statistical Software (Cohort, PO Box 1149, Berkeley, CA 94701, USA). A significance level of 95% was applied.

### 3. Results

#### *Plant growth*

Biomass of inoculated plants was greater than uninoculated plants for all the species tested. At low fertility, dry shoot weight of inoculated plants was greater than their respective control plants: 233% for onion, 80% in garlic, and 67% in potato (Tables 2 and 3). At high fertility, increases of 52, 9 and 46% (over controls) were recorded in dry shoot of onion, garlic and potato, respectively. Dry shoot weights of inoculated onion and garlic plants cultivated at higher fertility did not differ significantly from those at low fertility. In contrast, the value of dry shoot weight in uninoculated controls was high at higher fertility as compared to the uninoculated controls at low fertility. Dry root weights of inoculated plants also were higher than those of uninoculated plants in all the hosts tested. Root weights of both inoculated and uninoculated plants increased with increase in fertility.

Inoculation with VAM fungi significantly increased bulb diameter and bulb weight of both onion and garlic (Table 2). Significant ( $P < 0.05$ ) yield increase for inoculated over uninoculated plants was apparent, particularly at low fertility. At low fertility, inoculation with VAM fungi produced 71, 31 and 48% increase in yield over uninoculated plants in onion, garlic and potato, respectively, as compared to 12, 19 and 10% increase in yields of the respective plants at high fertility (Fig. 1).

#### *Shoot mineral concentration*

The P concentration of shoots was significantly ( $P < 0.05$ ) higher in inoculated than uninoculated plants, but the effect of inoculation on P uptake varied with fertility. Compared to uninoculated plants, increase in P uptake by VAM inoculation was higher at low than at high fertility. N concentration was also high in shoots of inoculated plants as compared to uninoculated plants (Table 4).

#### *Root colonization*

Percentage root colonization of the three hosts by VAM fungi responded differently at the two fertility levels. In onion, percent colonization increased

Table 4. Influence of VAM inoculation on nutrient uptake by shoots of potato, garlic and onion cultivated in soils at two levels of fertility.

Host	Treatments		P (mg/g)	N (%)
	Fertility levels	VAM inoculation		
Potato	Hf	Inoculated	2.02a	3.64a
		Uninoculated	1.86b	3.05b
	Lf	Inoculated	1.19c	2.07c
		Uninoculated	1.02d	1.60d
	LSD		0.01	0.16
Garlic	HF	Inoculated	1.38a	0.84a
		Uninoculated	1.18b	0.27b
	Lf	Inoculated	1.20b	0.15c
		Uninoculated	0.90c	0.07d
	LSD		0.12	0.02
Onion	Hf	Inoculated	1.49a	1.68a
		Uninoculated	1.15c	1.39b
	Lf	Inoculated	1.35b	1.40b
		Uninoculated	0.80d	1.17c
	LSD		0.02	0.04

Values are means of three replicates. Means in each column followed by the same letters are not significant at  $P=0.05$  by Duncan's Multiple Range Test (DMRT). Hf = High fertility; Lf = Low fertility.

by 15% with increase in fertility but it decreased by 16% in garlic (Table 2). In contrast, no significant difference was observed in potato between the colonization levels in roots of plants cultivated at the two fertility levels (Table 3). Overall highest root colonization was recorded in onion followed by potato and garlic (Tables 2 and 3).

#### *VAM fungus multiplication*

Highest multiplication of VAM fungal propagules in terms of number of spores and infectious propagules was observed when onion was the host, followed by garlic and potato (Fig. 2). Increase in fertility level resulted in significant decrease in number of spores when onion and potato were used. In contrast, cultivation of onion produced more of VAM infectious propagules at high fertility though the situation was reverse in potato. The change in



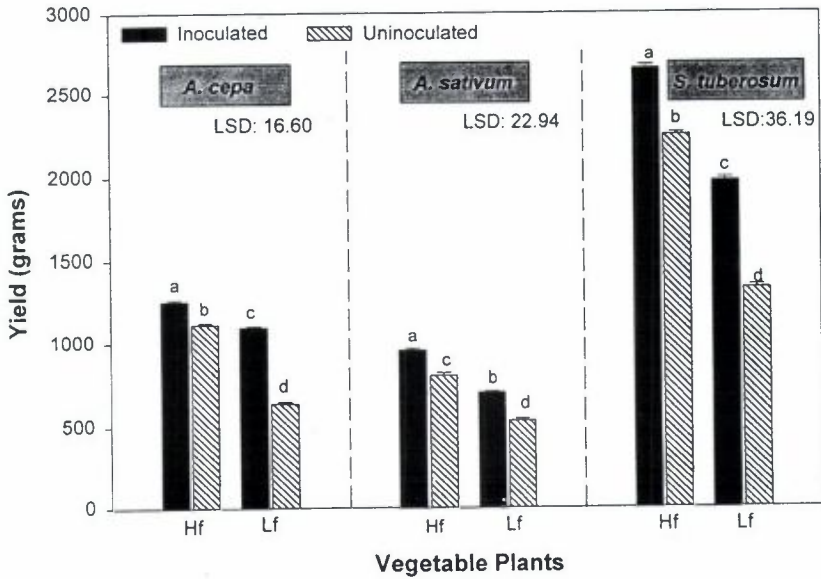


Figure 1. Influence of various vegetable crops on VAM fungus inoculum production. Columns with same letters are not significantly different at P 0.05. Hf = High fertility; Lf = Low fertility.

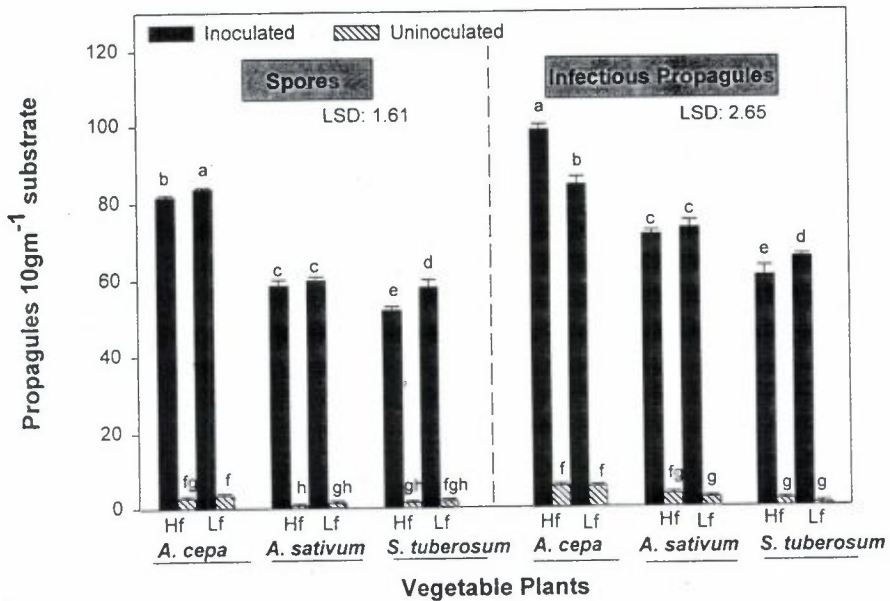


Figure 2. Influence of VAM fungus inoculation on yield of various vegetable crops. Columns with same letters are not significantly different at P 0.05. Hf = High fertility; Lf = Low fertility.

fertility level did not significantly effect the production of VAM fungal propagules with garlic as the host. The increase in infectious propagules at harvest (as compared to the initial population, 25,000), was 27, 20 and 17-fold respectively in onion, garlic and potato higher fertility. At low fertility, the respective increase in infectious propagules was 24, 20 and 18-fold.

#### 4. Discussion

This study clearly demonstrated the effectiveness of VAM in increasing crop yield and high rate of VAM fungus multiplication in compost-amended substrate under field condition.

Organic amendment supported both high crop yield and VAM fungus population in the present study. The percent increase in yield for inoculated plants over their controls was greater at low than high fertility. In addition, a high level of VAM fungus population was recorded at both the fertility levels. Other works (Noyd et al., 1996) also have demonstrated enhanced plant cover and biomass, mycorrhizal infectivity and spore population by addition of composted yard waste. Limonard and Ruissen (1989) reported 3–4 fold increase in colonization of roots of potato and wheat due to change from conventional high input to a low input organic farming system. Douds et al. (1993) found that soil under a LISA system (Low Input Sustainable Agriculture) involving animal and green manure had greater capacity to produce VAM fungus colonization. The high level of organic amendment (high fertility) led to a decrease in VAM fungal population. The results of others have suggested that high level of organic matter may be detrimental to the VAM symbiosis (Aziz and Habte, 1988). Moreover, a negative correlation between higher soil-available P and mycorrhizal growth response, as reported earlier (Sylvia and Neal, 1990), could have resulted in this decrease.

The extent of positive growth response in our experiment is in agreement with the results reported by others. Furlan and Bernier-Cardou (1989) reported a 41% increase by VAM fungus inoculation in onion. Snellgrove and Stribley (1986) found a positive effect of pre-inoculation in the production of bulbs with diameter higher than 20 mm. In contrast, Sylvia and Neal (1990) did not find any increase of growth of plants by VAM inoculation in a pot trial. Dry shoot biomass of mycorrhizal plants of onion or garlic did not differ significantly at the two fertility levels. However there was an increase in bulb with the increase in fertility. This may indicate that the mycorrhizal plants allocate more biomass to bulbs where nutrient requirement is greater. Inoculation of crops with VAM fungi in this study was done by introducing enough inoculum directly with the soil to establish vigorous infection, thus ensuring substantial infection when the plants' demand for phosphate was maximum.

These crops were inoculated using single strains of VAM fungi in earlier demonstrations (Powell et al., 1982; Rai, 1990; McArthur and Knowles, 1993 a,b; Vosatka 1995). Instead, we have used a mixed consortium of *Gigaspora*, *Glomus* and *Scutellospora*, that has been multiplied and well acclimatised to the conditions at the experimental site. Inoculation with multiple VAM fungi is often associated with more consistent benefits to plant growth over a range of environmental conditions than associations with a single mycosymbiont (Daft and Hogarth, 1983; Koomen et al., 1987). Also, diversity in the VAM fungal population, capable of responding to environmental stress, has been proposed to account for the high productivity of some soils (Ellis et al., 1992). An increase in the overall hyphal density of VAM mycelium around roots may also result from multiple VAM-fungal infections compared with single species. In contrast, there is evidence for competition with the presence of more than one VAM fungus in host rhizospheres (Bethlenfalvay et al., 1982; Wilson, 1984).

Thus, mycorrhizal inoculation has potential to enhance the production of bulbs and tubers, particularly in nutrient deficient soils using biologically-based farming systems, which has an impact on both production and conservation in sustainable agriculture. In addition, a build up of the mycorrhizal inoculum during the cropping of these hosts has been demonstrated in soil amended with organic matter. This could be useful in decreasing inoculum costs in future years.

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