Mycorrhiza (1998) 7:307–312

ORIGINAL PAPER

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A comparison of AM fungi inoculants using *Capsicum* and *Polianthes* in marginal soil amended with organic matter

Accepted: 2 February 1998

Abstract Different types of nursery inocula formulations, namely mixed indigenous cultures and *Glomus intraradices* Schenck and Smith, were compared with commercially available inoculants of AM fungi in a pot experiment using two horticultural crops, *Capsicum* and *Polianthes*. Soil-based inocula and soil beads produced the highest response in both crops. *Glomus intraradices* resulted in the highest yield in both *Polianthes* (45% increase in spike length) and *Capsicum* (112% increase in fruit yield). Among the commercial inocula tested, only Mycorise enhanced spike length (33%) and fruit yield (11%) in the two hosts. Overall AM colonization was higher in *Polianthes* than in *Capsicum*. Sheared root inocula of *G. intraradices* resulted in high colonization (upto 68%) but the yield enhancement was lower than with soil-based formulations. The mixed indigenous culture produced the highest number of spores and propagules and commercial inocula the lowest.

Key words Arbuscular mycorrhiza · *Capsicum* · Formulations · Inocula · Organic matter · *Polianthes*

Introduction

AM fungi have been shown to enhance the growth of numerous plants of economic importance (Gianinazzi et al. 1989) but their widescale use in crop production is limited, in part due to the limited availability of AM inoculum in bulk quantities. Although some AM inoculants have been commercialized (Wood 1987; Sieverding 1991), inoculation has generally been investigated at the research level (Blal and Gianinazzi-Pearson 1989) or at a pre-commercial stage (Gianinazzi et al. 1990). Progress is being made, however, in practical aspects of mycorrhizal technology. Inocula are being produced and marketed for use in horticulture under various trade names (St.John and Evans 1990; Lovato et al. 1992). Precise information is required on the quality and growth-promoting potential of commercial inocula. Furthermore, it is important to determine their efficacy in a particular environment recommended for promoting the growth of a specific host plant. A standardized inoculum formulation would facilitate comparison and the study of the potential benefits of AM on plant productivity. Hall (1979) suggested infested soil pellets as method suitable for introducing AM fungi into soil and described a technique for the production of 10000 AM-infested soil pellets per day for use in large-scale field trials. In addition, spores, hyphae, vesicles and colonized roots sieved from pot cultures have been pelletized with alginates (Ganry et al. 1985; Strullu and Plenchette 1991). Sheared inocula have been used to establish effective symbiosis (Sylvia and Jarstfer 1992).

The objective of our study was to compare three types of AM formulation, namely nursery-produced soil-based inocula, their formulations into beads and sheared roots and the commercially available inoculants of AM (at their recommended doses) using *Capsicum* and *Polianthes* as host plants.

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Materials and methods

Preparation of pot mixture and AM inocula

The pot mixture was prepared by mixing two parts of the soil (sandy loam pH 8.2, NO_3N 124 mg/kg, available P 0.53 mg/kg, available K 124 mg/kg) from an experimental site at Gwal Pahari (Haryana, India) and one part of compost made from leaves of *Albizzia* and poplars (pH 7.2, available P 22 ppm, organic C 11.40%, N 0.75%). The compost-amended substrate had a pH of 7.3, available P 10 ppm, organic C 2.4% and N 0.35%. Earthernware pots filled with 7 kg of this substrate were used.

Preparation of inocula formulations

Soil-based inocula

Mixed indigenous culture (Mi), containing native populations of *Glomus, Gigaspora* and *Scutellospora* spp., and a pure culture of *Glomus intraradices* Schenck & Smith (DAOM 181602, Biosystematics Research Centre, Ottawa, Ontario, Canada) (Gi) were used as AM inocula after 1 year in earthenware pots with the above pot mixture and *S*orghum bicolor as the host plant in a greenhouse (30 ± 2 °C). At maturity in July, the above-ground plant material was the removed and the substrate allowed to dry in a greenhouse for 1 week at 25°C. The roots were finely chopped and the dried root/soil mixture was thoroughly mixed to obtain an homogeneous inoculum. Spores were isolated by wet sieving and decanting (Gerdemann and Nicolson 1963) and counted on filter paper (Gaur and Adholeya 1994). Percent root colonization was assessed as described by Biermann and Lindermann (1981) after staining the roots with acid fuchsin (Philips and Hayman 1970).

Soil-bead inocula

Homogenized soil inoculum of Mi and Gi was manually made into beads. Enough water was added to bind the soil and the mixture was then rolled by hand to form circular beads (0.50 cm diameter). A 300-g portion of moistened inoculum of both Mi and Gi was dried separately for testing the number of viable propagules. Prepared beads were spread on a slab (30 ± 2 °C) for 24 h to dry completely (0.2% moisture).

Sheared root inocula

Root samples were removed from freshly harvested, pots containing pure culture of *Gigaspora*. The root material was washed gently to remove adhering soil and blotted on filter paper before being cut into 1-cm lengths with scissors. Root samples were placed in 50 ml of water in the bowl of a food processor and sheared for 20 s. The sheared material was collected on a 5-μm mesh and air dried at 30 ± 2°C on a greenhouse bench. The dried, sheared root was then weighed and stored in a coldroom.

Flyash beads

Dried, sheared roots of Gi were was mixed in flyash. Gum acacia (Himedia RM682) was added (0.5 g per 100 gram of flyash mix) and beads made after addition of an appropriate amount of water. As before, a 300-g portion of the inoculum was dried in powder form for examination of the number of viable propagules. The beads were dried to 0.2% moisture in a greenhouse at 30 ± 2 C for 24 h.

Commercial inocula

Three commercial AM formulations were tested: Vaminoc (Microbiodiv, Agriculture Genetics Company, Royston, England), Dr. Kinkon (Idemitsu Kosan Co. Ltd., Tokyo, Japan) and Mycorise (Premier Tech., CP 3500, Quebec, Canada). The inocula were used at the recommended doses (Table 1). Vaminoc contained colonized sheared roots but spores were not observed, whereas Kinkon consisted of *Gigaspora margarita* Becker and Hall and *G. intraradices.* Mycorise consisted of spores of *G. intraradices.* Kinkon and Vaminoc were calcined montmorillonite based and Mycorise was perlite based.

Seed sterilization

Seeds of *Capsicum annuum* var. Pant-C-1 were surface disinfected with 10% H_2O_2 for 10 min, washed with sterile water and germinated on moist cotton layers at 30 °C in the dark for 48 h. Bulbs of *Polianthes tuberosa* var. Pearl were graded by weight (50 g) and surface sterilized with 10% H_2O_2 for 10 min before being repeatedly washed with sterile water.

Counting of propagules

Each formulation (except the commercial ones) was subjected to a bioassay to assess the number of propagules (IP) present. Inoculation potential was expressed as the number of propagules per 100 ml (IP 100 ml^{-1}) of substrate using a bioassay (Sharma et al. 1996) with *Zea mays*. The procedure was similar to that described by Plenchette et al. (1989) with some modifications. The inoculum (except the sheared root inoculum) was mixed with 20, 40, 60 and 80% of the sterilized soil and transferred to 5 × 9-cm plastic pots after homogenization. For sheared roots, 0.2, 0.5, 1.0 and 1.5 g of sheared roots was mixed homogeneously in 100 gm of sterilized soil and transferred to similar pots. Five replicates were prepared for each level of dilution. Eight germinated seeds of *Z. mays* were planted per pot and cultivated for 12 days in a greenhouse (30 ± 2 °C, RH 60%) before roots were washed, stained and processed for estimation of primary infection.

The number of primary entry points was counted on a whole root system under a stereoscopic microscope (× 40). Propagule density varied for each type of formulation and, therefore, the amount of each inoculum applied (Table 1) was adjusted to give 2000 IP per pot (except in the case of the commercial inoculas, where the recommended doses were used).

Table 1 Number of propagules in inoculum formulations of AM fungi applied

Formulation	Initial number of propagules per gram formulation	Formulation applied per pot (g)
Vaminoc	ND	1.0 (Recommended dose)
Kinkon	ND	1.0 (Recommended dose)
Mycorise	ND	1.0 (Recommended dose)
Soil-based inoculum (Mi)	20	100.0[1]
Soil-based inoculum (Gi)	18	111.0[1]
Soil beads (Mi)	15	133.3[1]
Soil beads (Gi)	14	142.9[1]
Flyash beads	50	40[1]
Sheared inocula	1000	2[1]
Control	ND	ND

[1] Total number of propagules per pot was 2000

Experimental set up and layout

Two hosts and 10 inoculum treatments were tested in a completely randomized design with 5 replications in a 10×2 factorial structure. The different formulations of AM represented 9 treatments with one noninoculated control. Two-day-old seedlings of *Capsicum* were transferred to earthernware pots filled with 7 kg of pot mix. Each pot received 4 seedlings, which were thinned to 1 plant per pot 10 days after germination. *Polianthes* bulbs were buried in pots covered with a 2-cm layer of the pot mix, one bulb per pot. Each inoculum formulation was applied in the form of a layer below and around the roots or bulbs. All pots were placed in a greenhouse (30 ± 2 °C). No fertilizer was applied during the the course of the experiment and watering was carried out once per week.

Measurements and analysis

Plants were harvested after 12 weeks. The fresh fruits (*Capsicum*) were weighed and the shoots cut apart. The length of each spike was measured in *Polianthes*. Shoots were severed just above the crown, weighed, rinsed in distilled water, dried at 70 °C for 48 h, weighed again and ground to pass through a 0.5-mm pore size screen and digested in an H_2SO_4–H_2O_2 mixture. The P and N contents were determined using the method of Jackson (1973).

Roots of plants were washed free of soil, cut into 1-cm segments, homogenized thoroughly and their total fresh weight determined. A subsample of root segments was taken for analysis of mycorrhizal colonization. Root samples were dried at 70 °C for 48 h to measure dry weight.

Percent AM colonization in roots was determined on 100 1-cm root segments per treatment. Roots were stained using the method of Philips and Hayman (1970). Root pieces were mounted between glass slides and examined at $\times 40$ magnification with a compound microscope (Leica, Gallen III). Colonization was assessed according to Biermann and Lindermann (1981) and expressed as the percent of root segment colonized for each root piece. Spores of AM fungi were extracted from 50-ml substrate samples using the technique of Gerdemann and Nicolson (1963). The spores retained on different sieves (0.25 mm, 0.15 mm, 75 μm, 38 μm, arranged in series) were collected in a beaker and recovered by sucrose density centrifugation. Only visually intact and lipid-filled spores were counted under a stereoscopic microscope (Gaur and Adholeya 1994). Sporocarps were gently crushed so that all the spores could be enumerated. A portion (300 g) of homogenized substrate from each pot was also used to determine of number of propagules (Sharma et al. 1996).

Statistical analysis

Treatment effects were determined by one-way analysis of variance (ANOVA) using a completely randomized design. The differences between treatments were confirmed by Duncan's Multiple Range Test (DMRT) using Costat Statistical software (Cohort, Berkeley, Calif.). A significance level of 95% was applied.

Results

Vegetative growth

Shoot matter of *Polianthes* did not increase significantly when inoculated with any of the AM formulations (Table 2). Inoculation with soil-based inocula or soil beads containing Mi or Gi produced the highest shoot dry weight in *Capsicum* (Table 3). Among the commercial inocula, Mycorise produced a significantly higher shoot dry weight than Kinkon, which was similar to that produced by sheared roots.

Root biomass

In *Polianthes*, root dry weight was significantly higher when inoculated with Mi or Gi in either soil-based or beads form than other formulations. Commercial inocula produced relatively low root biomass, with Kinkon the most effective. The lowest root dry weight with indigenous formulations was recorded when sheared inocula was used as such or in the form of flyash beads (Table 2). Inoculation with Mycorise resulted in the lowest root dry matter, which was statistically not different from that of sheared inocula and flyash beads. Dry root weight in *Capsicum* was not affected by inoculation with any formulation except soil-based Gi (Table 3).

P uptake

At harvest, a significant enhancement of P uptake was recorded in all treatments with *Polianthes* (except with the commercial inocula) and *Capsicum*. With both hosts, P uptake was highest in soil-based Mi. Shoot P did not differ significantly when Mi or Gi were applied in the form of soil-based inocula or beads except in *Capsicum* inoculated with Gi (Tables 2 and 3).

Yield

The different types of formulation differed with respect to increase in yield of the two host species. In *Polianthes*, an increase of 45% (29 cm spike length) was recorded after inoculation with soil-based Gi, 33% with Mycorise and 29% in the case of soil-based Mi (Fig. 1). Inoculation with soil beads containing Gi or Mi pro-

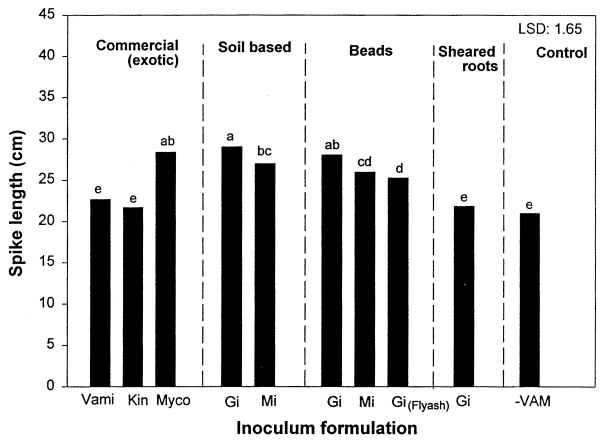

Fig. 1 Influence of inoculation with various AM formulations on spike length in *Polianthes*. Columns with the same letter are not significantly different ($P \leq 0.05$). (*Gi Glomus intraradices*, *Kin* KinKon, *Mi* mixed indigenous, *Myco* Mycorise, *Vam* Vaminoc)

Table 2 Effect of inoculation with various AM formulations on shoot P uptake, shoot/root dry weight, and percent colonization in *Polianthes tuberosa* after 12 weeks growth. Mean values in each column followed by the same letter do not differ significantly at $P \leq 0.05$. The values are followed by standard deviation between five replicates (*LSD* least significant difference)

AM Formulation	P (mg/g)	Shoot dry wt. (g)	Root dry wt. (g)	Percent colonization
Vaminoc	2.17 ± 0.16f	5.76 ± 0.13abc	3.86 ± 0.10de	55.4 ± 2.19cd
Kinkon	2.06 ± 0.1fg	5.54 ± 0.32bc	4.18 ± 0.46cd	57.8 ± 3.27c
Mycorise	2.08 ± 0.10fg	5.68 ± 0.30abc	3.66 ± 0.3e	42 ± 1.0e
Soil-based inoculum (Mi)	3.18 ± 0.2a	5.98 ± 0.15a	4.32 ± 0.3c	53.8 ± 2.16d
Soil-based inoculum (Gi)	2.89 ± 0.2bc	5.90 ± 0.33a	4.92 ± 0.2a	66 ± 4.74ab
Soil beads (Mi)	2.97 ± 0.1ab	5.96 ± 0.25a	4.46 ± 0.3bc	56.2 ± 2.78cd
Soil beads (Gi)	2.72 ± 0.4cd	5.82 ± 0.22ab	4.80 ± 0.5ab	67.0 ± 3.39ab
Flyash beads	2.53 ± 0.4de	5.94 ± 0.11a	3.86 ± 0.2de	64.4 ± 2.30b
Sheared root inocula	2.47 ± 0.1e	5.90 ± 0.19a	3.78 ± 0.1e	68.6 ± 2.89a
Control (-AM)	1.90 ± 0.1g	5.44 ± 0.20c	3.28 ± 0.2f	0.0
LSD	0.23	0.31	0.37	3.66

Table 3 Effect of inoculation with various AM formulations on shoot P uptake, shoot/root dry weight and percent colonization in *Capsicum annuum* after 12 weeks growth. Mean values in each column followed by the same letter do not differ significantly at $P \leq 0.05$. The values are followed by standard deviation between five replicates. (*LSD* least significant difference)

AM Formulation	P (mg/g)	Shoot dry wt. (g)	Root dry wt. (g)	Percent colonization
Vaminoc	1.84 ± 0.05d	4.13 ± 0.69bc	1.44 ± 0.19b	32.32 ± 0.38f
Kinkon	1.64 ± 0.05e	3.81 ± 0.75c	1.52 ± 0.19b	32.00 ± 0.07f
Mycorise	1.9 ± 0.12d	4.82 ± 0.36b	1.42 ± 0.11b	45.21 ± 0.43c
Soil-based inoculum (Mi)	2.64 ± 0.15a	5.64 ± 0.51a	1.5 ± 0.16b	47.88 ± 0.41d
Soil-based inoculum (Gi)	2.22 ± 0.13c	5.64 ± 0.54a	1.88 ± 0.08a	69.05 ± 0.10a
Soil beads (Mi)	2.54 ± 0.05ab	5.63 ± 0.55a	1.48 ± 0.18b	47.97 ± 0.81d
Soil beads (Gi)	2.4 ± 0.1b	5.59 ± 0.68a	1.36 ± 0.05b	57.85 ± 0.38bc
Flyash beads	2.2 ± 0.18c	4.59 ± 0.41b	1.36 ± 0.05b	58.77 ± 0.04b
Sheared inocula	1.98 ± 0.13d	4.17 ± 0.29bc	1.38 ± 0.19b	56.80 ± 3.18c
Control (-AM)	1.40 ± 1.2f	2.73 ± 0.52d	1.42 ± 0.21b	0.78 ± 0.24g
LSD	0.15	0.70	0.19	1.37

duced results similar to those with soil-based inocula. Sheared roots encapsulated in flyash beads produced a spike length (25 cm) significantly higher than that with sheared root alone (22 cm). Vaminoc and Kinkon produced relatively low spike lengths.

Similar responses to the various formulations were observed with *Capsicum*, though the overall increase in yield was higher in *Capsicum* (112%) than in *Polianthes* (45%). This increase was observed after inoculation with soil-based Gi, in contrast to 70%, 27%, 22% and 11% with Mi, flyash beads, sheared roots and Mycorise, respectively (Fig. 2).

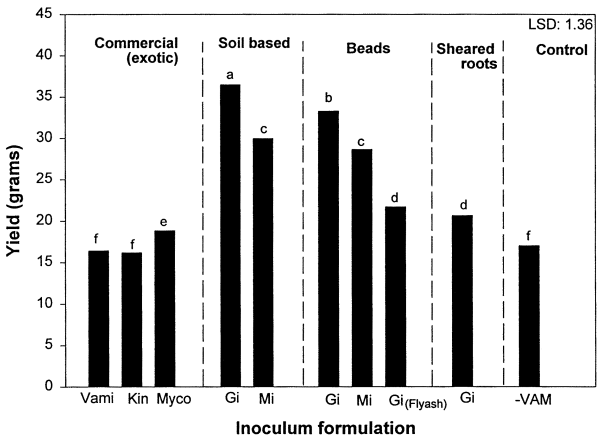

Fig. 2 Influence of inoculation with various AM formulations on yield of *Capsicum*. Columns with the same letter are not significantly different ($P \leq 0.05$). Abbreviations as in Fig. 1

AM colonization and spore production

In general, percent colonization was higher with *Polianthes* as host plant than with *Capsicum*. Colonization of *Polianthes* roots by commercial inocula did not exceed 60%, whereas Mi and Gi produced 54% and 66% colonization, respectively. Percent colonization did not differ significantly between beads and soil-based inocula. Sheared roots produced relatively higher colonization when used as roots alone than when encapsulated in flyash (Table 2). In *Capsicum*, the highest colonization was recorded after inoculation with soil-based Gi, followed by sheared roots and Mi. Percent colonization was significantly lower with soil beads of Mi, whereas soil beads of Gi produced results similar to those obtained with soil-based inocula. Among the commercial inocula, Mycorise produced the highest colonization of *Capsicum* roots, followed by Vaminoc and Kinkon (Table 3).

Overall, the production of propagules was higher with *Polianthes* than with *Capsicum*. With *Polianthes*, Mi produced the highest IP, followed by the beads, soil-based Gi, flyash beads containing sheared roots and sheared roots alone (Fig. 3). In general, the commercial inocula produced the lowest IP values. The formulation of soil-based inocula into beads did not significantly affect IP (Fig. 3). A similar trend was noted in *Capsicum*. The highest IP was recorded with Mi, followed by Gi, sheared roots, flyash containing sheared roots, Vaminoc, Kinkon and Mycorise (Fig. 4).

Spore production varied with the host used. With *Polianthes*, except for Mi, the number of spores recorded was less than the IP of the respective formulation. Mi produced the highest number of spores followed by Gi and sheared root alone. Among the commercial inocula, Kinkon produced the highest number of spores, followed by Vaminoc and Mycorise (Fig. 3). Overall spore production was lower than IP in *Capsicum*. Spore production was highest after inoculation with soil-based Mi, followed by Gi, sheared root inocula and flyash beads with sheared inocula. Among the commercial inocula, Kinkon produced the highest number of spores, followed by Vaminoc and Mycorise (Fig. 4).

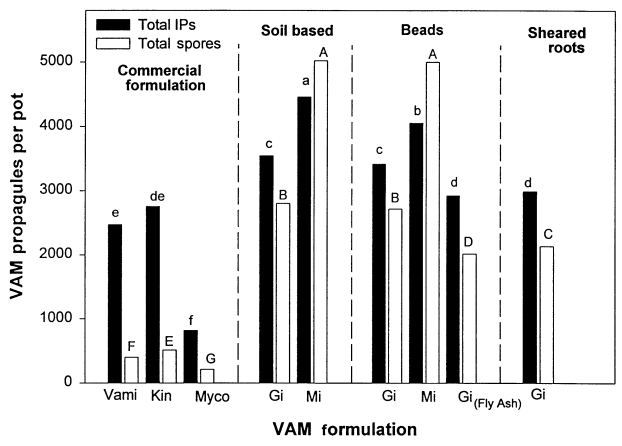

Fig. 3 Buildup of the AM population in *Polianthes* inoculated with various AM formulations. Columns with the same letter are not significantly different ($P \leq 0.05$). Abbreviations as in Fig. 1

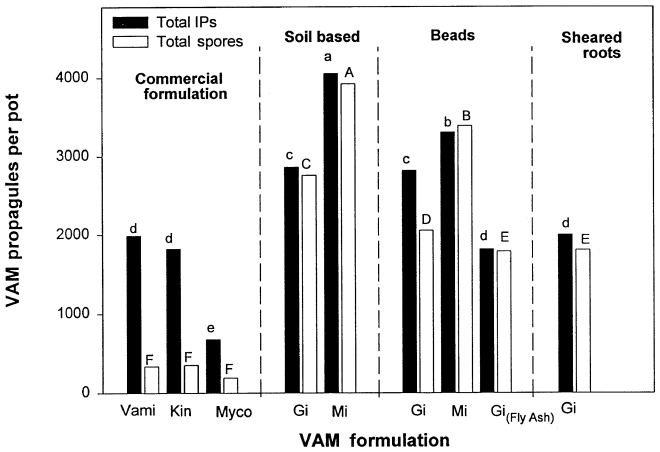

Fig. 4 Buildup of the AM population in *Capsicum* inoculated with various AM formulations. Columns with the same letter are not significantly different ($P \leq 0.05$). Abbreviations as in Fig. 1

Discussion

This investigation compared various available AM fungi inoculum formulations for affects on productivity of *Polianthes* and *Capsicum*. Certain commercial formulations resulted in higher colonization, IP production and crop yield than others. All the commercial inocula colonized roots of *Capsicum* and *Polianthes* but only Mycorise resulted in large increases in yield. Similar yield enhancement after inoculation with commercial inocula has been reported in various crops. For example, Nutri-Link containing an isolate of *G. intraradices* was used with success for inoculating lettuce and tomato (Datnoff et al. 1990, 1991). The present study confirms variation of response between host species and shows that AM fungi which perform well under one set of environmental conditions with one host may not do so well with another host under different conditions (Carling and Brown 1980). The substrates of the commercial inocula used in this study varied (sterile perlite or clay granules) but this did not seem to effect the results.

Both host plants showed higher growth and yield when inoculated with Gi than with Mi, though colonization by Gi was less than by Mi. This supports the concept that intensity of infection is a relative parameter (Abbott and Robson 1978). In fact, a high level of infection may cause a drain of photosynthates (Clapperton and Reid 1992), which is not compensated for by improved mineral nutrition of host plant. Thus, Mi containing a mixed population of *Gigaspora, Scutellospora and Glomus* may affect shoot parameters of the host by influencing the energy sink represented by formation of endomycorrhiza. The optimal level of infection depends on the efficiency and turnover of various factors, such as photosynthesis rates.

Infected root pieces have been used as AM inocula for effectively colonizing different host plants (Manjunath and Bagyaraj 1981; Biermann and Lindermann 1983; Sylvia and Jarstfer 1992). Roots colonized by *Glomus* species can serve as inocula because of the presence of intraradical vesicles (Biermann and Lindermann 1983) and, for a few species, spores, e.g. *G. intraradices* (Grahm and Lindermann 1986). When used as an inoculum, fragments of roots containing intraradical vesicles produced rapid colonization and response in host plants (Powell 1976; Warner and Mosse 1980). Regrowth of hyphae from root species colonized by AM fungi has also been observed by many workers (Stahl 1949; Tolle 1958) and this is stimulated by root exudation (Tolle 1958).

The two inocula used in this study (Mi and Gi) produced different levels of root colonization, spore numbers and other propagules. The differences between the inocula may be related to the different patterns of sporulation of the species within them. Native isolates have been shown to produce greater colonization of root length than exotic isolates (Sylvia et al. 1993), and Sainz and Arines (1988) reported that native AM fungi were more effective than introduced fungi in improving plant growth and P uptake. Inoculation of *Capsicum* with soil-based inocula or sheared roots of Gi led to the same level of colonization but distinctly different effects on plant yield. This suggests that a one time assessment of colonization may not be related to potential crop yield and that more harvests over a longer time period are needed to examine colonization.

In the present investigation the major isolate identified (*G. intraradices*) was found highly suitable for promoting colonization and plant yield of *Capsicum* and *Polianthes*. This information can be used in further studies to integrate AM fungi into a potentially active role in major horticultural crops. However, further experimentation is necessary to carefully define optimal doses, efficacy under different soil conditions and host specificity.

Acknowledgements The present study was supported by the Department of Biotechnology, Government of India. Thanks are due to the Director, Tata Energy Research Institute for providing infrastructural support and the Council of Scientific and Industrial Research for granting a fellowship to Dr. Atimanav Gaur. We are also grateful to Dr David Douds (Agricultural Research Service, USDA, Wyndmoor, Philadelphia) for providing the *Glomus intraradices* inoculum. We thank Ms Anupama Rattan and Mr. M.P. Sharma for support during the experiments and for valuable suggestions. The wordprocessing of the manuscript by Bijesh Kamath is greatfully acknowledged.

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