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# Studies on Viable Inoculum Production in Selected *Glomeraceae* species

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## Abstract

Arbuscular mycorrhizal (AM) fungi are ubiquitous in natural ecosystems and form intimate symbiotic associations with the majority of terrestrial plant roots. It is well reported that AM fungi can promote the uptake of plant nutrients (especially P), alleviate drought stress, improve soil structure and protect plants against root pathogens. Inoculum production in AM fungi using soil as substrate is a natural and inexpensive method for the mass production of AM inocula. The objective of the present study was to document the suitability of appropriate substrates and hosts for mass production. Two experiments were performed. In the first experiment four AM species *i.e. Rhizophagus intraradices, Funneliformis mosseae, Rhizophagus clarus* and *Claroideoglomus etunicatum* were separately used to inoculate the host *Plectranthus scutellarioides* (L) R. Br. (coleus) with sand, soil, or both sand: soil (1:1) as substrates. The second experiment differed from the first only in host plant, where *Zea mays* L. and *Eleusine coracana* Gaertn. were used. Statistical analysis revealed significant differences in spore density for the various substrates. Sporulation in all the AM fungal isolates was greatest when sand alone was used and AM colonization was greatest in *Z. mays*.

***Key Words:*** Arbuscular mycorrhizal fungi; Substrate; Mass production; Colonization; Sporulation.

## Introduction

Mycorrhiza means fungus-roots association where in Glomeromycotan fungi intimately associate with plant roots forming a symbiotic relationship. In the association, the fungus receives sugars from the plant while facilitating plant uptake of nutrients (Schüßler *et al.,* 2007). It is estimated that more than 80% of all terrestrial plants form this type of association (Smith and Read, 1997). These organisms increase plant growth (Smith and Read, 1997), plant reproductive capacity (Lu and Koide, 1994), stress tolerance (Gupta and Kumar, 2000), and aid in management of plant health by repelling pests and pathogens (Gange and West, 1994). The primary benefit to the host plant is the enhanced uptake

of immobile soil nutrients particularly phosphorus (P) (Jakobsen, 1999). Greater host plant nitrogen accumulation is reported (Ibibijen *et al.,* 1996). Further the fungi are involved in nutrient cycling (Xavier and Germida, 2002). Factors such as soil pH, soil temperature, moisture and mineral and organic nutrient concentration play role in sporulation and spore germination of the fungi (Clark, 1997).

Techniques such as aeroponics, hydroponics and root organ culture have been regularly used for mass production of AM fungal spores. The hydroponic technique does not attain the large-scale inoculum production needed as roots are immersed constantly in common flowing solution (Sharma *et al.,* 2000). However, it produces clean, sheared AM fungal inocula and the risk of cross-contamination by other AM fungi is low (Ijdo *et al.,* 2011). Root organ culture (ROC) technique with transformed and non-transformed roots is expensive and, labour intensive (Sylvia and Jarstfer, 1992).

Arbuscular mycorrhizal fungi colonize its host plant forming different structures *viz.* hyphae, arbuscules and vesicles. Hyphae are the non-septate structures that are both intra-radical and extra-radical. Arbuscules are the highly branched haustoria-like structures and are the sites of active transport of nutrients mainly P. The vesicles are bulbous structures that store lipids and also function as chlamydospores. This study describes a standard technique for optimal mass production of AM inoculum in soil based substrates.

## Materials and Methods

Three different types of substrates *i.e*. sand, soil and sand and soil mixture (1:1) were used in the present study. Four AM fungal species belonging to genus *Glomus viz*., *R.intraradices* (isolate GUAMCC1#1d)*, F. mosseae* (isolate GUAMCC3#1a)*, C. etunicatum* (GUAMCC10#2a) and *R. clarus* (GUAMCC9#1a) were tested. Five grams of inoculum (consisting of spores and colonized root fragments) of each AM species were added per pot. Coleus was used as a host plant.

The pH was determined after 1:1 dilution with distilled water. The same solution was used to assess the electrical conductivity (EC) (Bower and Wilcox, 1965). Total nitrogen and available phosphorus were assessed by the Jackson (1971) method and exchangeable potassium was evaluated after extraction with ammonium acetate (Jackson, 1971). Soil organic matter was detected by using Walkley and Black's rapid titration method (Jackson, 1971).

**Experiment 1:** Pure cultures were prepared by inoculating the coleus plants separately with the four test fungal species in three different substrates *viz.,* sand, soil and mixture of sand and soil (1:1). In each case, three replicates were prepared (4 AM fungal species x 3 soil types x 3 replicates = 36 pots in total). Five grams inoculum was added per pot. The pots were maintained in the polyhouse at $28^0$C.

**Experiment 2:** Two host plants *viz., Zea mays* and *Eleusine coracana* were inoculated with each of the four AM species using only sand as a substrate (4 AM species x 2 plant species x 10 replicates = 120 in total). These were maintained in polyhouse and watered thrice a week. Hoagland's solution (minus P) was added after every 15 days. Root colonization was assessed using Phillips and Hayman, (1970) method.

AM spores were isolated by wet sieving and decanting (Gerdemann and Nicolson, 1963). Spores from each substrate were quantified using the method of Gaur and Adholeya (1994). Statistical analyses were carried by one-way ANOVA using randomized block design. The differences between the treatments were confirmed by using WASP (Web Based Agricultural package).

## Results and Discussion

The results of soil analysis indicated that the pH of sand was lower than soil or sand: soil combination (Table 1). All AM species showed higher root colonization in sand at pH 6.9 than at pH 5.9 in soil. The analysis also revealed that sand was low in nutrient content compared to soil and sand: soil mix. This is consistent with Clark (1997) who reported that AM species performed best in limited nutrient conditions. The increase in root colonization in plants grown in sand may be attributed to the low levels of P where soil had a higher P content and recorded less root colonization and decreased sporulation (Fig. 1). Abbott and Robson (1991) reported that the addition of P to soil reduces AM colonization, suggesting that a higher concentration of P affects colonization levels and sporulation. In our study carbon (C) content of soil was greater than to the other two substrates, also possibly suggesting that high C content may be unsuitable for colonization and sporulation of AM fungi. The results suggest that sand may be a suitable substrate for the mass production of AM fungi.

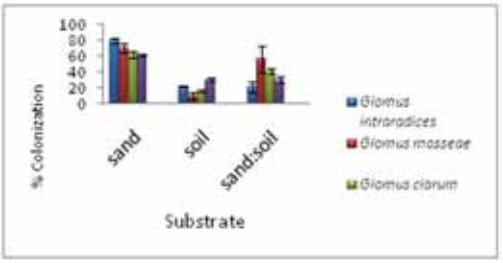

Fig. 1. Percentage colonization of *Coleus* roots.

Egerton-Warburton *et al*., (2007) demonstrated increased spore production in certain *Glomus* species (colonizers producing small spores) after N fertilization when associated with a $C_4$ host. Burrows and Pfleger (2002) demonstrated that AM fungal species producing large spores increase sporulation with increased plant diversity, while spore production of species producing small spores varied depending on the hosts species used. When the AM fungi are associated with diverse host plant species, e.g. in production fields or beds,

decrease in spore production may not occur. Moreover, the number of plant species and individuals, plant health, and developmental status could impact the performance of the associated AM fungi.

Table 1. Physico-chemical properties of substrates.

| Substrate | pH | EC (dS $m^{-1}$) | OC (%) | N (kg $Ha^{-1}$) | P (kg $Ha^{-1}$) | K (kg $Ha^{-1}$) |
|---|---|---|---|---|---|---|
| Sand | 6.97 | 0.077 | 0.20 | 75.26 | 3.64 | 67.20 |
| Soil | 5.90 | 0.705 | 1.60 | 664.83 | 12.93 | 310.24 |
| Sand: soil | 6.98 | 0.761 | 0.28 | 125.44 | 7.99 | 129.92 |

**Legend:** n = 3

Table 2. AM fungal spore density in substrates.

| | Spores $100g^{-1}$ substrate* | | | |
|---|---|---|---|---|
| | ***G. intraradices*** | ***G. mosseae*** | ***G. clarum*** | ***G. etunicatum*** |
| Sand | $350 \pm 24.97^{a}$ | $305 \pm 24.11^{b}$ | $248 \pm 37.07^{c}$ | $235 \pm 22.27^{c}$ |
| Soil | $150 \pm 8.14^{b}$ | $125 \pm 14.51^{d}$ | $132 \pm 27.80^{c}$ | $190 \pm 24.44^{a}$ |
| Sand: soil | $132 \pm 18.33^{c}$ | $200 \pm 22.27^{a}$ | $160 \pm 7.37^{b}$ | $151 \pm 5.16^{bc}$ |

*All values are mean of three readings (n=3). Means followed by different letters indicate that the treatments were significantly different. ($P \leq 0.05$).

When the host plant factor was evaluated, it was observed that *Z. mays* recorded higher root colonization than *E. coracana* (Fig. 2) which is in accordance with earlier observations (Patil *et al.,* 2013). A greater root colonization observed in *Z. mays* could be due to higher compatibility between the AM fungal isolate and plant (Kuppusamy and Kumutha, 2011). Although *Z. mays* and *E. coracana* have similar root systems, the extent of root colonization differed. Both species possess a root surface covered by two kinds of mucilage: a gelatinous material produced by the root cap, the other firmer and uniformly thickened, attached to the epidermal cells. In *E. coracana*, when the roots elongate, the mucilaginous mantle is detached, the cortical cells losing the site for AM. Thus, the endodermis remains

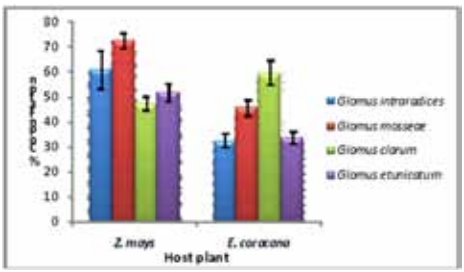


Fig. 2. Sand substrate Percentage AM colonization of plant roots.

as a root surface. In *Z. mays* this mantle is detached only with the epidermal and hypodermal cells (Mc Cully, 1987). These anatomical differences may influence AM fungal development.

In conclusion, the study revealed that both substrate and AM fungal species had a significant influence on AM root colonization. Substrate, host species, soil pH, P and organic matter are seen to influence the intra-radical development of the fungi. Sand may be the most effective, and cheapest, substrate for the mass production of AM fungi, with *Z. mays* as effective host plant. However, The INVAM website (http://invam.caf.wvu.edu) reports that spore numbers in some of the pot cultures decrease after successive propagation cycles. They suggested use of alternating the hosts when this problem occurs. The study warrants further research in this direction.

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