

# Evaluation of Different Medium for Producing on farm Arbuscular Mycorrhizal Inoculum

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## Abstract

Arbuscular mycorrhizal fungi (AMF) is a broad-spectrum non-specific organism known to colonize 85% of land plants hold considerable potential for use as inoculants. In the present perspective much attention is focussed on mass production of AMFinoculum, since this is of paramount importance in improving better uptake of nutrients, offers tolerance against a range of soil stresses, plant production and enhances the chances of plant survival. Given these benefits, utilization of the AMF symbiosis should be an important tool in sustainable agricultural systems. Producing AMF inoculum is a complex procedure involving selection of a host plant, finding the right mix or medium and the inoculum starter. Seven different substrates were tested for the production of AMF inoculum. Red earth in isolation or combination with solirite emerged as a suitable potential medium when compared to solirite for bulk production of pure, mature and infective AMF inoculum.

## Highlights

- AMF are naturally-occurring soil fungi that form a symbiosis with the roots of most crop plants benefitting in terms of increased nutrient uptake and enhanced biotic and abiotic resistance.
- Utilization of this symbiosis is a potentially essential part of sustainable agriculture.
- We developed an on farm method for farmers to produce inoculum of AMF which is effective, economical, and easy to use.
- Red earth in isolation or combination with solirite emerged as a suitable potential medium for production of AMF.

**Keywords:** Inoculum, Solirite, Red earth, Vermicompost

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Arbuscular mycorrhizal fungi (AMF) are one of the most widely distributed and ecologically important fungi with almost 400 million years of evolution history having a symbiotic associations with more than 80% of land plants (Douds and Siedel, 2012). Research by several workers have demonstrated that AMF render numerous benefits to plants, including increasing the absorption of macro and

micro nutrients, enhancing defence and resistance towards biotic and abiotic stress (Pal, 2011). AM fungi must be cultured in the presence of a live host plant roots to grow and reproduce ensuring culture purity. Commercial utilization of AMF has become difficult because of the obligate symbiotic nature and difficulty in culturing on laboratory media. Only the phase of the fungus inside the root can

absorb sugar and express certain metabolic pathways necessary for growth, such as the synthesis of fats. Therefore, the fungus has a very limited ability to grow asymbiotically. Failure by researchers to overcome these limitations has prohibited the growth of these organisms in pure culture on petri dishes or in fermenters for inoculum production (Pal *et al.*, 2013).

Several researches across the world proposed different methods of production of AMF inoculum as soil based culture as well as carrier based inoculums (Adholeya, 2003). Root organ culture and nutrient film technique provide scope for the production of soil less culture, but they are difficult, expensive and are impossible to achieve under existing available resources locally. As a carrier based inoculum AMF produced in a variety of ways utilizing laboratory, greenhouse, or field-based methods. Greenhouse methods include classical pot culture in which AM fungus spores are inoculated into greenhouse pots in which host plants are grown. Although inocula produced by these methods are commercially available but these methods involve huge expenditure starting from lab space, trained personnel for isolation of the AMF from the original media and/or mixing with a carrier substrate, packaging and transportation which is actually to be borne by the farmer.

On-farm production of inocula avoids many of these costs and could make this symbiosis, and the associated economic and environmental benefits, available to ultimate end user (Gaur *et al.*, 2000). In addition, these methods can produce inoculum containing the indigenous AM fungi already adapted to one's farm. It is therefore, consistent efforts have been made to establish an appropriate nursery medium for producing AMF inoculum. The goal of our research was to develop and standardise the medium for on-farm inoculum production system that generated a potent, effective, species rich inoculum that was inexpensive to produce. By avoiding the associated costs of commercially produced inoculum, on-farm production makes the economic and environmental benefits of AMF available to a larger number of farmers.

## Materials and Methods

### Inoculum source

The inoculum was a research product and contained *Glomus mossae* and *Glomus fasciculatum*. The product was obtained from T Stanes & Company Limited, Coimbatore, Tamil Nadu and consisted of fragments of colonised roots and spores in a clay support granule. This inoculum provided a uniform base

for the media experiment, eliminating problems due to AMF batch variation.

### Potting media

Eight different media in isolation or in combinations i.e., coarse texture sandy soil (T1), crushed red earth (T2), vermicompost (T3), solirite (T4), coarse texture sandy soil and solirite @ 1:3 ratio (T5), red earth and solirite @ 1:3 ratio (T6) and vermicompost and solirite @ 1:3 ratio (T7) were tested. A trench (0.5m x 0.5m x 0.3m) is formed and lined with black polythene sheet to be used as a plant growth tub. Thirty kg of the respective media/media mixture packed in the trench up to a height of 20 cm. Solirite (75% irish peatmoss and 25% horticulture grade expanded perlite and vermiculite) have been procured from Nirmal Enterprises, Lucknow.

### Inoculation

Five hundred grams of AMF mother culture was mixed into the top 2-5 cm of medium in each trench. Each trench was wetted with water to maintain adequate moisture for proper seed germination. Each trench was planted with 5% sodium hypochlorite surface sterilized seed of sorghum (*cv* CSU-15). Additionally, to prevent the spread of pathogens, the host plant should be from a different family than the inoculated crop. Due to the fact that the inoculum system targets vegetable producers, sorghum, a member of the grass family, is an ideal general host. During sowing 1 g urea, 1 g super phosphate and 0.5 g muriate of potash was applied in each trench. Ten grams of urea was applied twice on 4 and 6 weeks after sowing.

### Harvest

Stock plants are grown for 8 weeks. Quality test on AMF colonization in root samples is carried out on 4th and 6th week. Sample roots were stained to confirm that they had been colonized. The aerial parts of the plants were removed and the pots dried for another weeks. Once the pots were dried, the roots were removed and the media collected. The inoculum is obtained by cutting all the roots of stock plants. There are three types of infectious propagules of AM fungi: spores, pieces of colonized roots, and viable hyphae. All three are produced in the on-farm system. The inoculum produced consists of a mixture of potting media, spores, pieces of hyphae and infected root pieces. Thus within 6 weeks 30 kg of AMF inoculum could be produced from 0.25 sq meter area.

### Determination of moisture , available nutrients and spore count

For determination of moisture content, 5 gm of prepared inoculum was taken on a dry petri-dish. It was heated in an oven for about 5 hours at 65°C, constant weighing was done. Cooling is done in a desicator and weigh. Percentage loss in weight was estimated as moisture content of the inoculum. Organic carbon was estimated following the principle of redox titration using 1N  $K_2Cr_2O_7$ , standard 0.5 N ferrous ammonium sulphate and diphenylamine indicator. Estimation of nitrogen, phosphorus and potassium in inoculum was performed by kjeldahl's, klett summerson and flame photometer method (Singh and Rakshit, 2013). Total viable propagules of AMF was measured by wet sieving and decanting technique after Gerdemann, and Nicolson (1963).

### Statistics and experimental design

Trenches were arranged in a completely randomized block design in the farmer's field. The treatments were replicated thrice. The data obtained were analyzed by a one factorial analysis of variance, with substrate as experimental factor using AGRES software as per described by Panse and Sukhatme (1985). When the Fischer's values were significant, mean values were compared by Fisher's least square difference test ( $P < 0.05$ ).

### Results

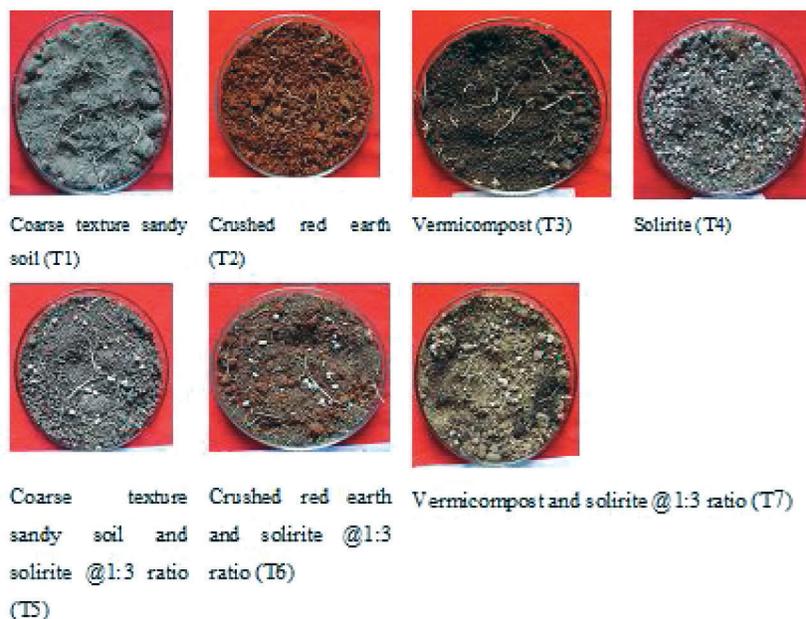


Fig.1: AMF inoculums produced from different medium

The inoculum produced are provided as granular substrates made from mixed materials such as solirite, vermicompost, coarse sand and red earth in which segments of colonized roots, spores, and filamentous networks are distributed (Figure 1). Using the on-farm system, sorghum typically have 70-80% of their root length colonized by mycorrhizal fungi. The host plant's roots is chopped up in order to take advantage of the mycorrhizal vesicles inside that contain energy reserves. Therefore, even small root pieces contain AM fungi and can be mixed into the medium to increase the number of infectious propagules. We have found that the best way to harvest the spores and viable hyphae part of the inoculum is to cut off dead leaves, remove the root ball from the trenches, and shake off the medium into a large bin. The root system can then be cut into pieces with scissors and mixed into the inoculum. From the above study it is relevant that mix of AMF species are well adapted to in vivo propagation and these characteristics make them excellent candidates for commercial inoculums production.

Quality of AMF biofertilizer is one of the most important factors resulting in their success or failure and acceptance or rejection by end-user, the farmers. Specifications as per FCO have been mentioned in the table 1. The results showed that, although all trenches produced spores, there were substantial differences in spore formation and root growth between media (Table 1). The least spores were

**Table 1:** Specification of AM biofertilizers produced from different medium

Parameters /Inoculum	Base	Viable cell count (% pass through 250 micron IS sieve, 60 BSS)	Moisture content (%) maximum	pH	Total viable propagules/gm offinished product, minimum	Efficiency character (infection points in test roots/gm of mycorrhizal inoculum used)	NPK (%)	Organic matter (%C)
AM biofertiliser as per FCO	Fine Powder/ granules/ root biomass mixed with growing substrate	90	8-12	6-7.5	100	80	-	-
Coarse texture sandy soil	Fine Powder/ root biomass mixed with growing substrate	55	6	7.8	55	ND	1.1	0.37
Crushed red earth	Fine Powder/ granules root biomass mixed with growing substrate	60	7.5	6.4	85	ND	1.3	0.42
Vermicompost	Granules/ root biomass mixed with growing substrate	70	10.6	7.8	65	ND	1.9	9.8
Solirite	Fine Powder/ granules /root biomass mixed with growing substrate	90	13	7.5	105	ND	1.5	>20
Coarse texture sandy soil:Solirite (1:3 )	Fine Powder/ granules /root biomass mixed with growing substrate	62	8	7.7	64	ND	1.2	>20
Crushed Red earth: Solirite(1:3 )	Fine Powder/ granules /root biomass mixed with growing substrate	74	9	7.3	95	ND	1.4	>20
Vermicompost : Solirite(1:3 )	Fine Powder/ granules /root biomass mixed with growing substrate	84	10	7.6	87	ND	1.7	>20
SEm+	-	7.5	4.1	0.2	11.2	-	0.2	-
LSD(0.05)	-							



obtained from coarse textured sandy soil, but these were significantly different ( $P>0.05$ ) from rest of the treatments.

The most spores were obtained with solirite, which was followed by crushed red earth and solirite combination, crushed red earth, vermicompost and solirite combination. Solirite in isolation or combination with other substrates resulted greater root mass and multiplication of the AMF propagule because of its bulk density value is similar to that of soil ( $1.1 \text{ Mg m}^{-3}$ ) and particle size also effected the result. Greater spore formation in media of this particle size may be attributed to optimal aeration, drainage and oxygen supply. The decrease in spore formation of the larger particle size may be explained by relatively less water retention and a propensity for dehydration, leading to fluctuations in soil moisture on fungal hyphae and consequently lower spore production. Root mass was greatest in vermicompost media. It is likely that the greater available nutrients in these trenches directly boosted root growth, whereas in those trenches augmented with NPK substantially fewer nutrients were available. This suggests that the nutrient status of the media is of secondary importance to density and particle size for AMF spore production.

As a nutrient dense medium, vermicompost supplies all the nutrients needed for sorghum growth as well as a broad array of microbes that benefit soil health and suppress plant disease. However, due to compost's high P concentration, it must be diluted with a nutrient poor substrate. Another benefit of this dilution is the resulting light weight medium that can be easily recovered and utilized. An important consideration in AM fungus production is the level of available P in the media in which the plant hosts are grown. Plants growing in high P situations limit colonization of their roots by AM fungi. Maximal production of inoculum in this system requires the proper dilution of the nutrient rich vermicompost with a nutrient poor substrate such as perlite or vermiculite. This is because colonization of roots by AM fungi, and hence growth of the fungus, is inhibited by high nutrient levels, notably of available P. Further, diversified medium with different nutrient levels and their dilution with solirite responded differently which is manifested through the observations obtained (Table 1).

### Summary and conclusions

In summary, the present results have identified red earth in isolation or combination with solirite emerged as a suitable potential medium when compared to solirite for bulk

production of pure, mature and infective AMF inoculum. The red earth media is cheap, easily obtained with a bulk density  $1.3 \text{ Mg m}^{-3}$  and produces approximately 85-95 spores/g of finished inoculum. Although inoculums produced by the locally available soil contain less number of spore compared to solirite but it had the added benefit of containing a diverse group of locally-adapted mycorrhizal fungi that could be used to boost a farm's native populations. Additionally, some research suggests that the indigenous AM fungi are more effective in promoting plant growth in their local soil than introduced species. To obtain a locally adapted and taxonomically diverse inoculum, field soil can be mixed into the dilute compost mix as a source of native AM fungi (Douds et al. 2006). Of particular interest was the observation that medium density had a greater effect on spore production than particle size and root mass. Carrier based inoculum remains the preferred propagation technique, as it provides a convenient and relatively economic method to produce mycorrhizal inoculum on a large scale

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