

## RESEARCH ARTICLE

# A screening tool for Quality of Mycorrhizal bio fertilizer- Mycorrhiza Inoculum potential

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**ABSTRACT**

Mycorrhiza is a known bio-fertilizer since long and has been found to be mobilizing various nutrients, including phosphorus, iron and zinc in adequate quantities. Mycorrhizal Inoculum Potential or Infectivity Potential is an indicator of mycorrhizal activity in the plant and soil. It allows the quality/infectivity of inoculum to be evaluated and is used as a biological indicator. Mycorrhizal Inoculum Potential is the number of infectious fungal propagules in a sample which can be tested by bioassay using plant seedlings as host and measurement of mycorrhizal colonization after a defined period. The method used in this study is Mean Infection Percentage, an effective method for assessing Mycorrhiza Inoculum Potential. Five mycorrhizal bio-fertilizer samples were used to assess the quality and an in house developed mycorrhizal bio-fertilizer was used to compare the results. The quality of samples was decided based on its spore count, viability of Infective Propagules, Mycorrhizal Infectivity Potential and presence of other endophytes after 15 days. Samples: "A,B,C,D,E" were fertilizer samples from the market and sample "F" was in house developed bio-fertilizer. Spore count in sample A,B,C,D and E was found to be 10,000 /gm, 101/gm, 6 /gm, 2/gm and 50/gm respectively . Spores of *Glomus* genera were the main Mycorrhizal constituent of all the samples and their Mycorrhizal infectivity potential after 15 days was found to be 0%, 30%, 0%, 10%, and 0% respectively. As compared to the market samples, F sample which is an inhouse developed Mycorrhizal bio fertilizer had 3spores/gm and showed 50% Mycorrhizal Inoculum Potential. Other endophytes were observed in all the roots of host plant (*Zea mays* plant) inoculated with fertilizer samples. In conclusion, the in house, in vivo developed product was of good quality than other in-vitro developed bio-fertilizer samples.

**Keywords:** Mycorrhiza Inoculum Potential, Mycorrhiza bio-fertilizer, *Glomus*, Endophytes

**INTRODUCTION**

Field trials with Arbuscular mycorrhizas are still beset with the issues of huge scale production of AM Fungi, its storage and application to the crop

An alternate approach is to control Indigenous Arbuscular mycorrhizal (AM) fungi by cultural practices or by the utilization of soil amendments that increase the effectiveness of the natural inocula. (Daft, 1992). Several bioassays are developed to indirectly count the quantity (or relative amount) of infectious fungal propagules during a sample use. (www.INVAM). Inoculum of Vesicular-Arbuscular Mycorrhizal fungi consists of various kinds of infective propagules: spores, vesicles (members of Glomineae only), hyphal fragments, and hyphae from mycorrhizal root fragments. whereas some analysis has indicated that hyphae and roots are most infective, this conclusion can't be generalized to all or any isolates of a species or perhaps to all or any species. (Abbott and Robson, 1981) The advantages and edges of adopting mycorrhizae in agriculture, permits us to raise, visualize the scope of this development at the crop level and , intern, the impact of its long- term adoption on the standard of life in improvement in nutrition, tolerance to water stress, proof against low temperature, transformation of root design, diversity of microbes in soil development, resistance against pathogens, enhanced synthesis of primary or secondary metabolites and improvement within the quality and amount of agricultural product. (Abbott and Robson, 1991b). Development and production of AM fungal inocula and bio-fertilizer is a laborious and cost consuming process because of obligate and bio tropic nature of AM fungi, hence to standardize the production process of AM fungi inocula and bio-fertilizer is of very much importance. Numerous plant species may be utilized in infectivity assays, since most have some dependence on the mycorrhizal association and can become inhabited. The foremost dependent plant species of grasses are most popular to optimize.



**Fig. 1: method for developing in vivo mycorrhiza or setting up MIP**

The main aim of this study was to find out and compare the performance of market fertilizer samples with inhouse developed bio- fertilizer sample

## MATERIALS AND METHODS

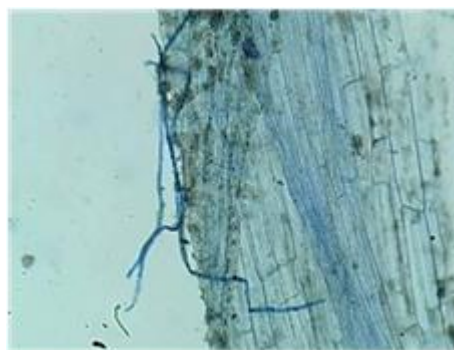
1. Host plant used for checking mycorrhiza infection is *Zea mays*.
2. The red laterite soil and pot used for MIP testing was sterilized.
3. Sterilization technique used for soil is autoclaving soil at 1.5Kg/cm<sup>2</sup> at 128°C for 30 minutes.
4. Pot was sterilized using alcohol swab.
5. Experiment was set up in the pot as shown in figure 1
6. Amount of mycorrhizal bio-fertilizer used for Mycorrhizal inoculums potential is 5 gm of each sample in each pot.
7. The plant was uprooted 15 days after germination and roots were checked for mycorrhizal infection by Modified Philips and Hayman,1970

### Spore count and Spore isolation

by Gerdeman and Nicolson's method, 1963 (wet sieving and decanting)

### Soil based inoculum

This is the most commonly used inoculant technique. Soil inocula are produced using traditional pot culture technique by multiplying AM inoculant in the soil mixture. The success of good soil inoculum production depends on selection of good host plant, efficient AM strain and a suitable substrate in which AM Fungus can be mass multiplied. (Bagyaraj et al., 2002) (Singh et al., 2016).



**Fig.2: Mycorrhizal hyphae point**

**Preparation of in vivo mycorrhizal bio-fertilizer**

3 sets of Mycorrhizal pots were taken each containing 5kg of substrate soil: sand in the ratio of 3:1 and 50gm of coco peat was added in each pot. 5 gms of *Funneliformis mosseae* containing 50 spores were inoculated at the time of germination of seeds. The plants were watered every alternate day. The plants were uprooted 90 days after sowing and analyzed for Spore density. (Shweta et.al,2016) 50 spores in 5kg of soil was added. After 90days spore density was found to be 60 spores per 20gm of soil, which is much higher compared to the results obtained by (Mala et al., 2010) i.e 769/50 g at the 6<sup>th</sup> month after inoculation. Here the time period was also less and spore density was high within 3 months only.

**RESULTS AND DISCUSSION**

The Bio fertilizer samples used A,B,C,D and E in the proposed study were containing Mycorrhizal propagules which were in vitro produced. These samples were subjected to MIP studies to assess the quality. Sample F was in vivo developed in house product with soil as carrier material. In vivo mycorrhizal inoculation treatments had high infectivity potentials, whereas the equivalent in vitro treatments were less effective at colonizing the plant's root system. Although all types of propagules of AM fungi (spores, root fragments, and hyphae) are able to initiate AM symbiosis. (Mosse, 1988) the production system of AM fungi itself can have implications in experimental results, thus requiring thorough investigation of mechanisms involved as well as monitoring of responses in cropping systems. (Calvet

2013) . Spore count in sample A,B,C,D and E was found to be 10000/gm, 101/gm, 6 /gm, 10 spores/5gm and 50/gm respectively. 3spores /gm was present in in vivo developed inhouse product sample F. As per (FCO 1985), requirement, a fertilizer sample should have 60 spores/g. Per that rule only sample A and B are passing the requirement but are not found to be effective in the field. However we suspect more number of spores in the bio-fertilizer samples as the spores must have changed their morphology when other ingredients are added in fertilizer sample. Changes in morphology of spores were not studied. This could be one of the reason for less spore count.

As per the procedure given in FCO, 1985, after 15 days of growth plant is uprooted and checked for Mycorrhizal Inoculum Potential i.e. whether mycorrhiza is showing any entry point in plant root or not which is termed as infectivity potential. Mycorrhizal infectivity potential was 0%, 30%, 0%, 10%, and 0% respectively. As compared to the market samples, F sample which is a inhouse developed Mycorrhizal biofertilizer shows 50% Mycorrhizal Inoculum Potential. Root colonization achieved by in vivo root fragments when used as inoculums was much higher despite the uncertain density of propagules within the plant roots. A previous study done by (Plenchette et al., 1996) says Loss of infectivity along generations of inocula produced in vitro, both for spores and mycorrhizal root pieces, might reduce the infectivity and the effectiveness of the subcultures. This could be one of the reason for 0 % or no infection seen in bio-fertilizers sample A,B,C,D and E inoculated maize plants.

**Table 1: Results after 15 days**

Samples	A	B	C	D	E	F
Total spore count	10000/gm	101/gm	6 /gm	10 spores/5gm	50/gm	3/gm
Type of spore present	<i>Glomus intraradices</i>	<i>Accaulospora and Glomus</i>	<i>Glomus intraradices</i>	<i>Glomus etunicatum</i>	<i>Glomus intraradices/ Glomus Fasciculatum</i>	<i>Glomus mosseae</i>
Infectivity potential	0%	30%	0%	10%	0%	50%
Presence of other endophytes	present	present	Present	present	Present	Absent

Dominant mycorrhizal type found in all types of bio-fertilizer samples was *Glomus*. A research conducted by Krishnamoorthy, 2015 says that T-RFLP and DGGE analysis confirmed the dominance of *Funneliformis mosseae* and *Rhizophagus intraradices* spores in major type of fertilizers and natural environments which was conducted to find out abundant mycorrhizal species.

Other endophytes were not identified during spore isolation from sample but when roots were checked for Mycorrhizal Infection Potential, endophytes were observed in the roots. They grow much faster than mycorrhiza. The size of the mature chlamydospores varies between (14) 16 in length and (9) 10 and 17(20) mm in width (Kost et al,2013)

## CONCLUSION

In conclusion, Infection unit originates from entry points and represent the initial phase of fungal growth within the root cortex which may lead to general colonization of roots. (Cox G et.al 1974), Mycorrhiza Inoculum Potential is a useful tool for deciding quality of mycorrhizal bio-fertilizer. The in house in- vivo developed bio -fertilizer was found to be much effective compared to the in -vitro developed biofertilizers which had more spore count .Thus the Mycorrhiza Inoculum Potential test is a useful tool to find out quality and efficiency of AM fungal bio-fertilizers.

The above study and our results suggests that infective propagules need not be only spores, they may be infected root pieces with vesicles and arbuscules.

## REFERENCES

- Abbot LK and Robson AD (1991) Factors influencing the occurrence of arbuscular mycorrhizae, Agri. Ecosyst. Environ,35:121- 150
- Cinta Calvet et al. (2013) Plant Growth Stimulation and Root Colonization Potential of In Vivo versus In Vitro Arbuscular Mycorrhizal Inocula, HORTSCIENCE 48(7):897-90
- Cox G and Sanders P (1974) Ultrastructure of the host fungus interface in a Vesicular arbuscular mycorrhiza. New Phytol 73: 901-912
- Daft MJ (1992) Use of mycorrhizas in agriculture: Problems and prospects. In: Read, D.J.; Lewis, D.H.; Fitter, A.H.; Alexander, I.J. (Eds.). Mycorrhizas in ecosystems. Wallingford: CAB International, 1992. P.198-201
- Dhananjaya Pratap Singh, Harikesh Bahadur Singh, Ratna Prabha (2016) Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 2: Functional Applications, ISBN-978-81-322-2642-0 DOI 10.1007/978-81-322-2644-4
- FCO, Fertilizer Control Order 1985, p206-2011
- Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc., 46:235- 244.
- Gerhard Kost et al,2013, Morphology and Ultrastructure of Piriformosporaindica, sebaiales and their biotechnological applications, p397, ISBN-978-3- 642-33801-4
- Abbott L and Robson A (1981) Aust. J. Agric. Res. 32:631-639.
- Mala WJ, Kumari IS, Sumanasena HA, Nanayakkara CM (2010) Effective Spore Density of *Glomus mosseae*, Arbuscular Mycorrhiza (AM), for Inoculation of Rooted Cuttings of Black Pepper (*Piper nigrum* Linn.) Trop. Agri. Res., 21(2): 189-197.
- Mosse B (1988) Some studies relating to 'independent' growth of vesicular-arbuscular endophytes. Can. J. Bot. 66:2533-2540
- O'halloran PI, Miller MH, Arnold G (1986) Absorption of P by corn (*Zea mays* L.) as influenced by soil disturbance. Canadian Journal of Soil Science, Ottawa, 66:287-302
- Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of colonization. Trans Br Mycol Soc 55:158-160
- Plenchette C, Declerck S, Diop TA and Strullu DG (1996) Infectivity of monoxenic subcultures of the arbuscular mycorrhizal fungus *Glomus versiforme* associated with Ri-T-DNA transformed carrot root. Appl. Microbiol. Biotechnol. 46:545-548.
- Ramasamy Krishnamoorthy et al. (2015) Arbuscular Mycorrhizal Fungi Community Structure, Abundance and Species Richness Changes in Soil by Different Levels of Heavy Metal and Metalloid Concentration, PLoS ONE 10(6): e0128784. doi: 10.1371/journal.pone.0128784
- Jain Shweta B and Sunita Chahar (2016) Effect of AM Fungi *Funneliformis mosseae* on the Growth of *Ocimum sanctum*. *Int.J.Curr.Microbiol.App.Sci.* 5(4): 64-67. doi:http://dx.doi.org/10.20546/ijcmas.2016.504.010
- Smith SE and NA walker (1981) A quantitative study of mycorrhizal infection in *Trifolium*: Separate determination of the rates of infection and of mycelial growth, New. Phytol 89; 225-240

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