

Storage Life of Arbuscular Mycorrhizal Fungal (AMF) Inoculum in Vermiculite Based Culture

S. Merina Prem Kumari*, B.Jeberlin Prabina

Department of Agricultural Microbiology, Agricultural College & Research Institute, Madurai, India

Abstract— Arbuscular Mycorrhizal fungi (AMF) is ubiquitous soil borne fungi which provide an intimate link between soil and nutrient absorbing organs of plants. AMF fungi optimize the uptake of phosphorus in plant which results in increase in yield. Production of AMF inoculum and the storage in a viable condition for a longer period of time is still a serious constraint. This study describes the survival capability and infectivity of AMF spores under different storage conditions. The vermiculite based AMF inoculum contained spores of AMF namely *Glomus* sp., *Gigaspora* sp., *Scutellospora* sp., *Entrophospora* sp., and *Acaulospora* sp., identified from the rhizosphere soil collected from the maize crop cultivated at Mellur block of Madurai District in Tamil Nadu, India and the mixed AMF colonized host root bits as propagules. The AMF inoculum spore count was 5-6 / 100g inoculum and the infectivity was 100% in the roots of maize plant at the time of packing of the inoculum for storage. During storage, at the end of 180 days the AMF spore count was reduced to 2-5 / 100g inoculum and the infectivity was reduced to 90-95%. But the AMF inoculum stored with 10% moisture content at 20°C - 30°C and under the light intensity of 1500 lux maintained the same initial spore count of 5-6 / 100g inoculum and infectivity of 99% for the storage period of 150 days (5 months). The results of the present study suggest that AMF propagules, spores and hyphae colonized root bits in the form of vermiculite based culture maintained with 10% moisture content at temperature range of 20°C - 30°C and under light condition of 1500 lux for 5 months can be expected to be viable for infectivity in crop plants.

Keywords— arbuscular mycorrhizal fungi, vermiculite, maize plant, storage, infectivity.

I. INTRODUCTION

The success of mycorrhizal fungi has been attributed to the role it plays in the mobilization of nutrients from the soil of all ecosystems (Bonfante and Perrotto, 2000). Mycorrhizal fungi provide inorganic nutrients mainly phosphorus and other complex compounds to the plant through the extensive network of their hyphae that forage for soil nutrients more effectively than plant roots (Van der Heijden *et al.*, 1998). There are different types of

mycorrhizal interaction which have been classified into ectomycorrhizae and endomycorrhizae based on the presence of various extra radical or intraradical hyphal structure (Bonfante and Perotto, 2000). Seven mycorrhizal types have been identified but the most common endomycorrhizae are arbuscular mycorrhizae (Brundrett *et al.*, 1996).

Arbuscular mycorrhizal (AM) fungi are obligate symbiotic fungi and endosymbionts of a variety of plants within the angiosperms, gymnosperms and pteridophytes (Strinberg and Rillig 2003; Smith and Read, 1997). Arbuscular mycorrhizal fungi are so named because they produce fine tree – like hyphal structure termed ‘arbuscules’ that occur within the root cortical cells of plants. They are responsible for the exchange of carbon needed for energy and nutrients after close contact is made with the host cell. Vesicles serve as carbon storage compartments for the fungi and rich in lipids.

Due to the obligate biotrophism, the production of inoculum is one of the obstacles for application of Arbuscular Mycorrhizal fungi (AM) to benefit crops of economic importance. The AMF inoculum should be produced in high density, and maintain the infectivity and effectivity for a long period of time. Although not much information regarding storage of AMF is available, the maintenance of the inoculum in the conditions under which it was produced is recommended.

It has also been mentioned that maintenance of AMF inoculum at low temperature stimulates germination and spore development. However, the ideal temperature for storage of AMF inoculum should be determined. Also there is lack of information on the shelf life of Arbuscular Mycorrhizal fungi. Hence the present study was undertaken to determine the appropriate storage condition for AMF inoculum as bio fertilizer that would facilitate long term storage and maintain infectivity after storage. With this aim, the objectives formulated for the research are: (i) to store the AMF inoculum under different temperature, moisture content and light/dark conditions, (ii) to evaluate the spore density and infectivity of AMF inoculum in the maize plant and finally (iii) to arrive at the exact temperature and moisture content for efficient storage of AMF inoculum.

II. MATERIALS AND METHODS

Storage study

The vermiculite based AMF inoculum contained spores of *Glomus sp.*, *Gigaspora sp.*, *Scutellosporasp.*, *Entrophospora sp.*, and *Acaulosporasp.*, identified from the rhizosphere soil collected from the maize crop cultivated at Mellur block of Madurai District in Tamil Nadu, India and the mixed VAM colonized host root bits as propagules. The AMF inoculum consisting of a mixture of roots of maize, AMF hyphae and spores in vermiculite based culture was maintained in the mass production plot of the Department of Agricultural Microbiology, Agriculture College and Research Institute, Madurai and was used for the experiment.

The inoculum was stored in 150 gauge polythene covers and each cover contained 500g of vermiculite based AMF culture. The bags were sealed and the inoculum was stored at different moisture concentrations of 5%, 10% and 15% at various temperature of 10°C, 20°C and 30°C under both light (1500lux) and dark conditions for a storage period of six months. Four replications were maintained for each treatment.

Treatments

The treatments of the experiment are T₁ - Control (light-1500lux) ; T₂- Control(dark) ; T₃ - 10°C + 5% moisture content (MC) + dark ; T₄ - 10°C + 5% MC + light - 1500lux ; T₅ - 10°C + 10% MC + dark ; T₆- 10°C + 10% MC + light -1500lux ; T₇ - 10°C + 15% MC + dark ; T₈ - 10°C + 15% MC + light -1500lux ; T₉ - 20°C + 5% MC + dark ; T₁₀- 20°C+ 5% MC + light -1500lux ; T₁₁- 20°C+ 10% MC + dark ; T₁₂ - 20°C + 10% MC + light -1500lux ; T₁₃ - 20°C+15% MC + dark ; T₁₄ - 20°C+15% MC + light -1500lux ; T₁₅ - 30°C+ 5% MC + dark ; T₁₆ - 30°C + 5% MC + light -1500lux ; T₁₇ - 30°C+ 10% MC + dark ; T₁₈ - 30°C + 10% MC + light -1500lux ; T₁₉ - 30°C + 15% MC + dark ; T₂₀- 30°C + 15% MC + light -1500lux.

The control sample had the moisture content of 10%. 500g of vermiculite based AMF inoculum was stored in plastic covers in various storage conditions as described above. The samples were taken from plastic covers every month to observe the spore density and infectivity for a period of six months.

Spore density

The spore density was calculated by extraction of AMF spores from the stored inoculum by the Wet Sieving and Decantation technique developed by Gerdemann and Nicolson(1963). About 250g of soil was suspended in one litre of water in beaker and stirred thoroughly. Soil macro aggregates should be crushed with hand. After 10-30 seconds of settling down of soil particle the upper layer of soil suspension is passed through 180, 90 and 45 µm sieves arranged one below the other in the above said order. The procedure was repeated until the upper layer of

soil suspension is transparent. The sievings on the fine mesh is collected in a small beaker. Spores collected from soils are put in a small petridish and counted under a stereozoom microscope.

Infectivity test

The AMF inoculum stored in various conditions was tested for infectivity on roots of maize by analyzing the AMF infection percentage by Clearing and Staining method of Phillips and Hayman (1970). The 10g of AMF inoculum after storage was mixed with 90g of vermiculite and filled in plastic cups in which maize seeds were sown. After 10 days of growth, the maize seedlings were transferred to pots containing sterilized soil. The pots were maintained in a greenhouse for one month to develop AMF infection in the roots of maize. Later the maize rootswere collected and thoroughly washed in tap water, without disturbing the external mycelium. The roots were cut into one 1cm segments and immersed in FAA solution (Formaldehyde: Glacial acetic acid: Ethanol – 5:5:90) and kept for overnight. On the next day, FAA was decanted and root segments were washed free of FAA. The root segments were cleared in 10% KOH by autoclaving for 30 minutes at 15 lb pressure / sq inch. Then the root samples were rinsed in water for two to three times and acidified by soaking in 2% HCl for three minutes. The acid was poured off and the root segments were stained by immersing in 0.05% trypan blue in lactoglycerol (lactic acid : glycerol : distilled water - 4 : 2 : 2) and boiled for five minutes. The excess stain was poured off and the root segments were arranged on glass slides and examined under a microscope (10 x 10X) for AMF infection. The AMF infection percentage was calculated using the following formula.

$$\text{Percent root infection} = \frac{\text{Number of root segment infected}}{\text{Number of root segment examined}} \times 100$$

Statistical Analysis

All statistical analyses were performed with procedures of Agres version 3.01 for all data sets that were obtained over a period of time. This type of analysis was chosen under the premise that samples were estimated for spore count and infectivity by VAM colonization percentage after storage of AMF inoculum under different temperatures, moisture content and light/dark conditions.

III. RESULTS

Spore count and Infectivity of one month stored AMF inoculum

The T₁₈ treatment (30°C+10%MC+light) showed high spore count and infectivity in one month storage period followed by T₁₂ treatment (20°C+10%MC+light). T₁₁treatment (20°C+10%MC+dark) recorded a minimum

spore count and infectivity in one month of storage period. (Table.1; Fig.1&2)

Spore count and Infectivity of two months stored AMF inoculum

The T₁₈ treatment (30°C+10%MC+light) showed high spore count and infectivity in the second month of storage followed by T₁₀ treatment (20°C+5%MC+light). T₉ treatment (20°C+ 5%MC+dark) recorded a minimum spore count in two months storage period.(Table.1; Fig.1&2).

Spore count and Infectivity of three months stored AMF inoculum

The T₁₈ treatment (30°C+10%MC+light) showed high spore count and infectivity in the third month of storage followed by T₆ treatment (10°C+10%MC+light). T₁₉ treatment (30°C+ 15%MC+dark) recorded a minimum spore count and infectivity in the third month of storage. (Table.1; Fig.1&2).

Spore count and Infectivity of four months stored AMF inoculum

The T₁₈ treatment (30°C+10%MC+light) and T₁₂ (20°C+10%MC+light) treatments were on par and showed high spore count and infectivity in four months storage period followed by T₆ treatment (10°C+10%MC+light). T₁₉ treatment (30°C+15%MC+dark) recorded a minimum spore count and infectivity in four months storage period.(Table.1; Fig.1&2).

Spore count and Infectivity of five months stored AMF inoculum

The T₁₂ treatment (20°C+10%MC+light) showed high spore count and infectivity in the fifth month of storage followed by T₁₈ treatment (30°C+10%MC+light). T₅ treatment (10°C+10%MC+dark) recorded a minimum spore count in the fifth month of storage.(Table.1; Fig.1&2).

Spore count and Infectivity of six months stored AMF inoculum

The T₁₂ treatment (20°C+10%MC+light) and T₁₈ treatment (30°C+10%MC+light) were on par and showed high spore count and infectivity in the sixth month of storage followed by T₁₀ treatment (20°C+5%MC+light). T₁₉ treatment (30°C+15%MC+dark) recorded a minimum spore count and infectivity in six months of storage period. (Table.1; Fig.1&2).

IV. DISCUSSION

Arbuscular mycorrhizal fungi are soil fungi that colonize roots of the majority of crop plant, forming a mutualistic symbiosis. AMF infected root samples harvested from aeroponic culture the root inoculum when stored dry at 4°C for twenty three months recorded no significant reduction in propagules density (Sylvia and Jarstfer.,1991;Veresoglou *et al.*,2012).

Bahia grass and industrial sweet potato colonized by *Glomus deserticola*, *G.etunicatum* and *G. intraradices* were grown in aeroponic culture, retained their infectivity after cold storage at 4°C in either sterile water or moist vermiculite for at least four and nine months, respectively (Hung and Sylvia, 2000).

In this study, vermiculite substrate based AMF inoculum containing AMF colonized maize root bits and spores of *Glomus sp.*, *Gigaspora sp.*, *Scutellosporasp.*, *Entrophospora sp.*, and *Acaulosporasp.* is used as a propagule and stored in various storage conditions. The mycorrhizal parameters viz. spore density and AMF inoculum infectivity under storage at different temperatures, moisture content and light/dark condition were examined every month. During storage for up to five months the spore count and AMF inoculum infectivity was maintained and remained the same till 5 months of storage and after which it started to deteriorate. The various storage condition for AMF inoculum were compared and the statistical analysis clearly shows that the VAM inoculum stored under 30°C+10%MC+light and 20°C+10%MC+light recorded the highest spore count and AMF infectivity. The result agrees with few reports on VAM storage which indicate high viability of AMF spores at low temperature, low moisture content and under light condition.(Daft *et. al.*, 1987, Gerdeman and Trappe, 1994 ; Andre freire cruz,2004).Further investigation by Mugnier and Mosses indicated that spores of *G.mossae* retained their viability after cold storage at 10°C in a moist atmosphere. Later Daft and Spencer Thomas,1987 reported that spore infectivity was higher when the spores had been stored wet or in moisture condition. Further investigation is being carried out to find the AMF inoculum viability over years at various storage conditions.In the present investigation it is found that the storage conditions optimum for AMF inoculum storage is temperature range of 20°C to 30°C, moisture content of 10% and light intensity of 1500lux. It is concluded that AMF inoculum can be stored for five months under the above conditions, thereby maintaining the spore count of 5-6/100g of inoculum and infectivity of 98% to 100%.

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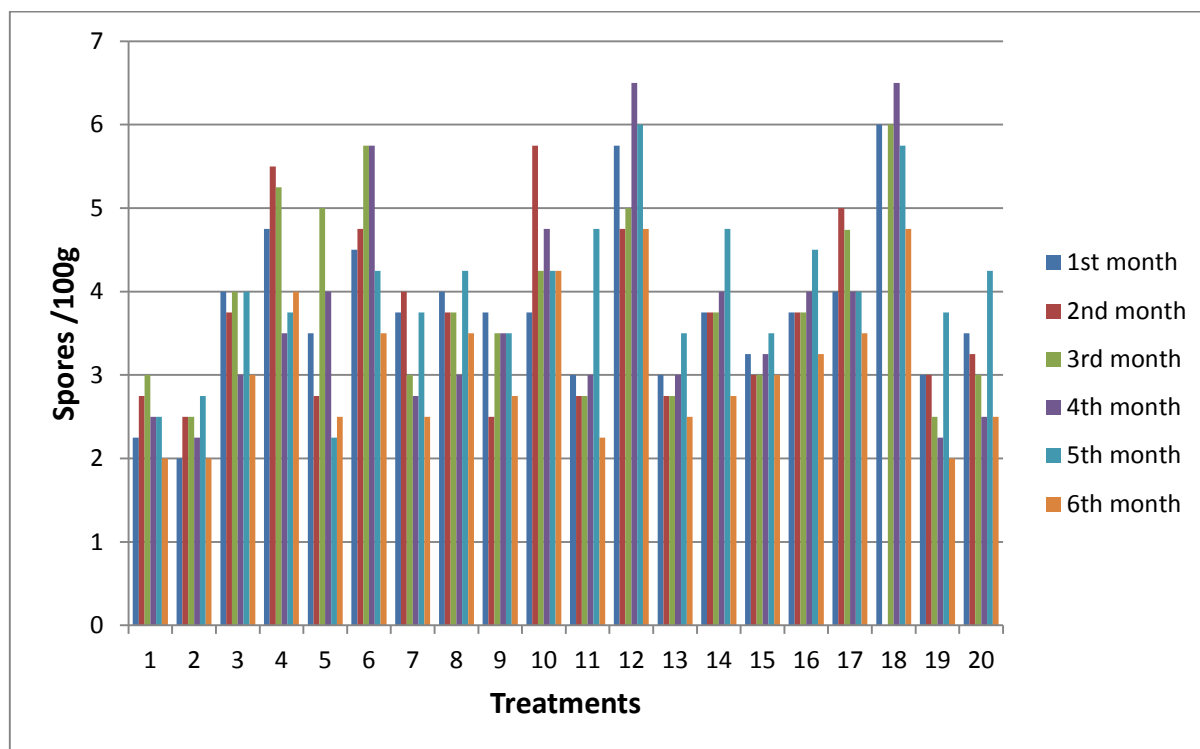


Fig.1: Spore count in AMF inoculums stored for various duration

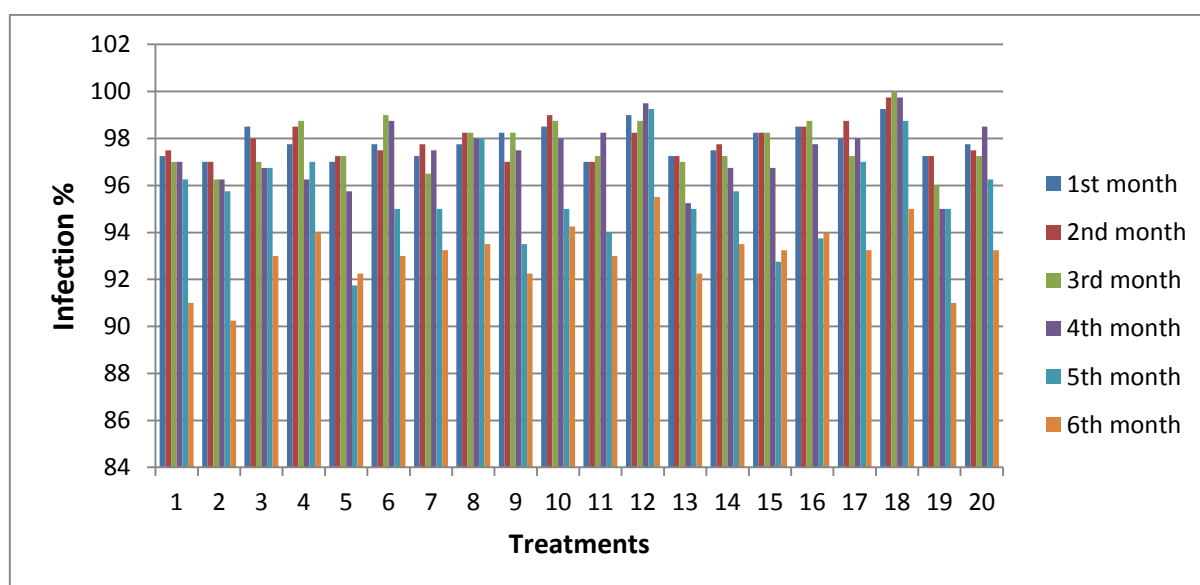


Fig.2: Infectivity of AMF inoculums stored for various duration

Table.1: Spore count and Infectivity of AMF inoculums stored for various duration

Treatment	Treatment details	1 month		2 months		3 months		4 months		5 months		6 months	
		Spores /100g	Infection %	Spores /100g	Infection %	Spores /100g	Infection %	Spores /100g	Infection %	Spores /100g	Infection %	Spores /100g	Infection %
T1	Control (light 1500lux)	2.25	97.25	2.75	97.50	3.00	97.00	2.50	97.00	2.50	96.25	2.00	91.00
T2	Control-Dark	2.00	97.00	2.50	97.00	2.50	96.25	2.25	96.25	2.75	95.75	2.00	90.25
T3	10°C + 5% MC + dark	4.00	98.50	3.75	98.00	4.00	97.00	3.00	96.75	4.00	96.75	3.00	93.00
T4	10°C + 5% MC + light	4.75	97.75	5.50	98.50	5.25	98.75	3.50	96.25	3.75	97.00	4.00	94.00
T5	10°C + 10% MC + dark	3.50	97.00	2.75	97.25	5.00	97.25	4.00	95.75	2.25	91.75	2.50	92.25
T6	10°C + 10% MC + light	4.50	97.75	4.75	97.50	5.75	99.00	5.75	98.75	4.25	95.00	3.50	93.00
T7	10°C + 15% MC + dark	3.75	97.25	4.00	97.75	3.00	96.50	2.75	97.50	3.75	95.00	2.50	93.25
T8	10°C + 15% MC + light	4.00	97.75	3.75	98.25	3.75	98.25	3.00	98.00	4.25	98.00	3.50	93.50
T9	20°C + 5% MC + dark	3.75	98.25	2.50	97.00	3.50	98.25	3.50	97.50	3.50	93.50	2.75	92.25
T10	20°C + 5% MC + light	3.75	98.50	5.75	99.00	4.25	98.75	4.75	98.00	4.25	95.00	4.25	94.25
T11	20°C + 10% MC + dark	3.00	97.00	2.75	97.00	2.75	97.25	3.00	98.25	4.75	94.00	2.25	93.00
T12	20°C + 10% MC + light	5.75	99.00	5.00	98.25	5.00	98.75	6.50	99.50	6.00	99.25	4.75	95.50
T13	20°C + 15% MC + dark	3.00	97.25	2.75	97.25	2.75	97.00	3.00	95.25	3.50	95.00	2.50	92.25
T14	20°C + 15% MC + light	3.75	97.50	3.75	97.75	3.75	97.25	4.00	96.75	4.75	95.75	2.75	93.50
T15	30°C + 5% MC + dark	3.25	98.25	3.00	98.25	3.00	98.25	3.25	96.75	3.50	92.75	3.00	93.25
T16	30°C + 5% MC + light	3.75	98.50	3.75	98.50	3.75	98.75	4.00	97.75	4.50	93.75	3.25	94.00
T17	30°C + 10% MC + dark	4.00	98.00	5.00	98.75	4.74	97.25	4.00	98.00	4.00	97.00	3.50	93.25
T18	30°C + 10% MC + light	6.00	99.25	6.50	99.75	6.00	100.0	6.50	99.75	5.75	99.00	4.75	95.00
T19	30°C + 15% MC + dark	3.00	97.25	3.00	97.25	2.50	96.00	2.25	95.00	3.75	95.00	2.00	91.00
T20	30°C + 15% MC + light	3.50	97.75	3.25	97.50	3.00	97.25	2.50	98.50	4.25	96.25	2.50	93.25
SE d		0.381	0.652	0.402	0.744	0.456	0.984	0.557	0.970	0.484	0.853	0.512	0.707
CD(.05)		0.763	1.304	0.871	1.489	0.912	1.469	1.014	1.322	0.970	1.206	1.024	1.414