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MASS MULTIPLICATION OF INDIGENOUS *ARBUSCULAR MYCORRHIZAE* FOR TEMPERATE FRUIT CROPS

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ABSTRACT

Arbuscular Mycorrhizal (AM) fungi hold considerable potential for use as inoculant and nowadays much attention is focused on mass production of AM fungal inoculum, since this is of paramount importance in improving plant production in agriculture and horticulture. However, effectivity of these fungi on crop growth relies on their production practices, typically the substrate selected for its mass multiplication. Therefore, as an initial step, this experiment was carried out to select a suitable substrate for the AMF inoculum production using methi and guinea grass as a host plant. For mass multiplication production of AM fungi, indigenous arbuscular mycorrhizal spores were isolated from different locations of Shimla district of Himachal Pradesh. The pot culture experiment was carried out under net house conditions and observations were noted after 3 and 6 months of multiplication. The maximum no. of spores (294.44 and 394.44 spores/50 g soil) was recorded at Dhochi site of Jubbal location, however, the minimum (155.56 and 222.22 spores/ 50g of soil) were noted Dhar site of same location. Similarly the maximum percentage of root colonization (10.66% and 13.61%) was recorded at Dhochi site of Jubbal location, whereas the minimum (5.17% and 7.17%) root colonization was noted at Dhar site of same location after 3 and 6 months of multiplication, respectively.

INTRODUCTION

Arbuscular Mycorrhiza (AM) has been described as "Universal symbiosis" in plant kingdom. About 80% of all terrestrial plant species form this type of symbiosis (Smith and Read, 1997) and 95% of the world's present species of vascular plants belong to families that are characteristically mycorrhizal. The widespread occurrence of AM fungi has been found in many fruit crops like apple, peach, strawberry, cherry, litchi, cashew, citrus, banana, guava, grapevine and mango (Hasan and Khan, 2005). These AM fungi improves plant growth through enhanced supply of essential nutrients and sustain soil health (Kirti *et al.*, 2016). Among them arbuscular mycorrhizal fungi are obligate symbionts which live in associations with the roots of approximately 80% of all plant species. Interest in AM fungi inocula propagation for agriculture is increasing due to their role in promotion of plant health, soil fertility, and soil aggregates stability in the developing economies (Abiala *et al.*, 2013 and Sharma *et al.*, 2016). The mycorrhizal symbiosis is a key stone to the productivity and diversity of natural plant ecosystems. The increasing use of chemical fertilizers for increasing crop productivity is now well known to adversely affect the quality of the soil (Gupta *et al.*, 2015 and Kirti *et al.*, 2016). Contrary to the chemical fertilizers, organic manures and bio inoculants particularly, AMF inoculum are less expensive and known to increase the productivity without harming the environment. Large scale multiplication of efficient fungi has been achieved by inoculating appropriate host plants such as clover, ray grass, sudan grass or maize that are grown in sterilized soil or any other rooting media such as clay, bark, pumice, and peat mixtures (Declerek *et al.*, 1996). One of the prime factors important for the effectivity of these fungi on crop is the substrate selected for its mass multiplication. For this reason, the majority of research groups try different substrates with nutrient solutions. But none has standardized a best substrate for AM inoculum production so that it can be adopted by companies. Therefore, we utilized maize as test plant in an experiment studying whether a) cheap sources of materials like inert material. It is therefore important to use arbuscular mycorrhizal (AM) fungi as biofertilizer. Since isolation and selection of AM species (effective for growth promotion) and raising of pure culture of these species is difficult, a suitable host is required to maintain the AM culture. So the present investigations focus mainly on mass multiplication of AM spores by guinea grass (*Panicum maximum*) as it form thick root mat and thrive well under mid hills and temperate climatic conditions.

MATERIALS AND METHODS

Pure culture preparation of AM fungi

Starter inoculum of selected AM fungus was raised by 'funnel technique' (Menge, 1984) using *Trigonella* spp. (methi) as a

host. AM fungal spores were picked up through auto pipette under stereoscopic binocular microscope and kept in 0.1 per cent sodium azide solution. These spores were mixed in upper layer of sterilized soil and sand mixture in the funnel. Thereafter seed of *Trigonella* spp. were surface sterilized with sodium hypochlorite. These seed were allowed to grow for 20-25 days in these funnel and the seedlings were watered with sterilized water twice a day as per requirement by using hand sprayer. The roots of methi plants were chopped and mixed with soil:sand mixture kept in polybags as a inoculum for mass multiplication.

Selection of host

Guinea grass (*Panicum maximum*) was selected as a suitable host as it form thick roots and thrive well under mid hills and temperate climatic conditions for mass production of AM spores.

Selection of substrates and filling of pots

Sterilized mixture of soil, sand and FYM in the ratio of 1:1:1 was filled in plastic pots having about 3 kg capacity. The substrates were sterilized in an autoclave at 15 lbs for half an hour, for three consecutive days for the complete destruction of all kinds of microbes or their reproductive structure.

Inoculation of pots with AMF inoculum

Twenty five percent of the inoculum (200 g) was added to the soil, sand and FYM mixture in the upper part. The inoculum consists of about 150 AM spores and AM colonized root pieces.

Sowing seeds of *Panicum maximum* and maintenance of pots

Seeds of guinea grass were surface sterilized with 0.01% solution of sodium hypochlorite for 1-2 min and then washed thoroughly with distilled water to remove sodium hypochlorite before sowing them. The pure culture of isolated AM fungi was used for pot culture inoculations and guinea grass used as the hosts for their multiplication. Each treatment was replicated three times. Plants were grown in net-house conditions. The plants were watered regularly up to 6 months. Root and soil samples were taken every 30 days for examination. Spores were collected from the guinea grass soil by wet sieving and decanting method (Gerdemann and Nicolson, 1963). The AM colonization was assessed by taking tertiary roots. The root system was carefully removed and washed gently with tap water. Tertiary roots were stained according to the method described by Phillips and Hayman (Phillips and Hayman, 1970). The per cent colonization was assessed in accordance with intersect method (Giovannetti and Mosse, 1980) and will be calculated as:

$$\text{Per cent root colonization} = \frac{\text{Number of mycorrhizal infected root segments observed}}{\text{Total number of root segments examined}} \times 100$$

Statistical analysis

The data recorded for various parameters under laboratory conditions were statistically analyzed as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Mass multiplication of indigenous arbuscular mycorrhizal fungi with guinea grass

The multiplications of AM spores were carried out under laboratory conditions in funnels using sterilized soil and sand mixture in the ratio of 1:1 for 20-25 days. On the basis of germination and growth of *Trigonella* spp. (methi) plants out of 35 indigenous inoculum only 24 inoculum were selected for mass multiplication (Fig.1). The selected indigenous

Table 1: Spore counts (no. of spore/50g of soil) of indigenous AM fungi during mass multiplication with guinea grass (*Panicum maximum*) under net house conditions

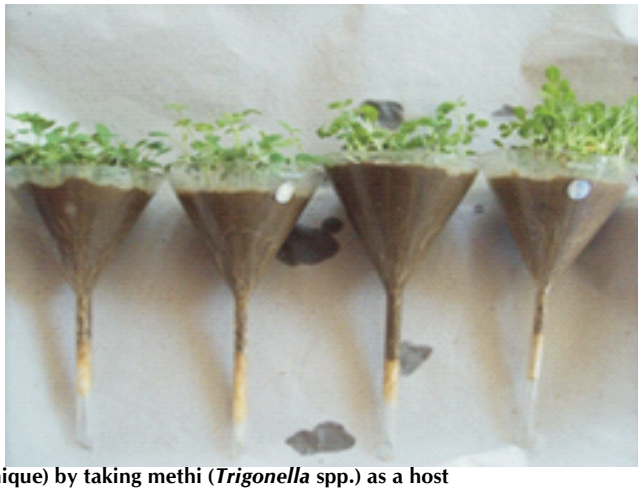
Location	Amf- Spore Population (Per 50 G Of Soil)								
	After-3months					After-6 Months			
		Site 1	Site 2	Site 3	Mean	Site 1	Site 2	Site 3	Mean
Jubbal	Dhochi	216.67	383.33	283.33	294.44	333.33	416.67	400.00	383.33(*30.18)
	Dhar	133.33	166.67	166.67	155.56	200.00	216.67	250.00	222.22(*42.85)
Kotgarh	Kandiyali	186.67	210.00	233.33	210.00	266.67	266.67	233.33	255.56(*12.17)
	Thanedhar	270.00	233.33	200.00	234.44	316.67	283.33	300.00	300.00(*27.96)
$Cd_{0.05}$	Location	17.03			8.11				
	Sites × Location	29.50			14.04				

Values in (*) showed per cent increase after 6 months over 3 months

Table 2: Root colonization (%) of guinea grass (*Panicum maximum*) by indigenous AM fungal inoculums under net house conditions

Location	Amf- Root Colonization (%)								
	After - 3 Months					After - 6 Months			
		Site 1	Site 2	Site 3	Mean	Site 1	Site 2	Site 3	Mean
Jubbal	Dhochi	11.83	9.97	10.17	10.66	13.83	14.17	12.83	13.61(*27.67)
	Dhar	4.83	5.50	5.17	5.17	6.50	7.17	7.83	7.17(*38.68)
Kotgarh	Kandiyali	6.83	7.50	6.78	6.78	9.17	9.50	6.83	8.50(*25.36)
	Thanedhar	8.07	9.80	9.00	8.96	9.17	10.50	10.50	10.06(*12.27)
$Cd_{0.05}$	Location	0.42				0.37			
	Sites × Location	0.73				0.65			

Values in (*) showed per cent increase after 6 month over 3month

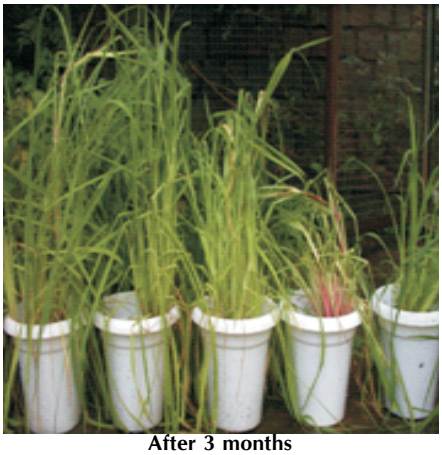


General view: Inoculated sterilized soil and sand mixture (Funnel technique) by taking methi (*Trigonella* spp.) as a host

Control ←

→Inoculated

Under laboratory conditions



After 3 months

After 6 months

At the time of harvesting

Under net house conditions

Figure 1: Mass Multiplication Of Amf Inoculum

inoculum spore culture from laboratory conditions were taken for mass multiplication under net house conditions in plastic pots having Soil: Sand: FYM mixture in the ratio of 1:1:1 by taking guinea grass (*Panicum maximum*) as a living host plant.

Spore counts (no. of spore/50g of soil) of indigenous AM fungi during mass multiplication with guinea grass (*Panicum maximum*) under net house conditions

The Soil:Sand:FYM mixture collected from pots under net house conditions after 3 and 6 months of mass multiplication with guinea grass (*Panicum maximum*) depicted presence of spore number and percentage of root colonization (Table 1).

spore counts after 3-months of mass multiplication. AMF spore

populations varied from 155.56 to 294.44 spores per 50 g of soil. The maximum (294.44 spores/50 g soil) numbers of spore were noted at Dhochi site of Jubbal location. However, the minimum (155.56 spores per 50g of soil) were recorded at Dhar site of Jubbal location. The similar trend of spore multiplication was noted after 6 months of growth of guinea grass. Spores population varied from 222.22 to 383.33 spores per 50 g of soil. The maximum (383.33 spores/50 g soil) numbers of spore were noted at Dhochi site of Jubbal location and the minimum (222.22 spores per 50g of soil) were noted at Dhar site of same location.

Our results are in conformation with earlier works carried out by Bharat (2011) Sharma (2016) who reported that AMF inoculum soil contained 300-400 spores/g of soil.

Root colonization (%) of guinea grass (*Panicum maximum*) by indigenous AM fungal inoculums under net house conditions

There was significant variation in root colonization of guinea grass after 3 months of growth under natural condition by different indigenous inoculums of AM fungi. The root colonization varied from 5.17 to 10.66 per cent as (Table-14). The maximum (10.66%) root colonization were noted at Dhochi site of Jubbal location and the minimum (5.17%) root colonization were noted at Dhar site of same location. The similar trend of root colonization by indigenous inoculums was noted after 6 months, with colonization percentage variation from 7.17 to 13.61 per cent. The maximum (13.61%) root colonization were noted at Dhochi site of Jubbal location and the minimum (7.17%) root colonization were noted at Dhar site of same location. Our results are in coloboration of similar work done by Sharma (2016) who had reported root colonization in guinea grass ranging from 16.67% to 46.67% after six months under net house conditions. Dohroo *et al* (2013) observed a great variation in root colonization by AM fungi with different crops. Further, Oehal and Sieverding (2004) reported that abundance of arbuscular mycorrhizal spores is a good indicator of root colonization and productivity of the crop. Baath and Hayman (1984) studied the effect of plant density on AMF root colonization and reported that low root colonization may be attributed to high plant density. The AMF inoculum prepared by growing guinea grass was applied @ 200g/pot by ensuring 5-6 spores/g of inoculum. Results also envisaged that either host plants favour the association of particular AM species or AM fungi may show some preference for the host. Therefore, from the present investigation it was concluded that *Panicum maximum* can be used as a host for mass propagation of pure cultures of AMF.

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REFERENCES

Abiala, M. A., Popoola O. O., Olawuyi O.J., Oyelude, J.O., Akanmu A.O., Killani, A.S., Osonubi, O. and Odebode, A. C. 2013. Harnessing the potentials of vesicular arbuscular mycorrhizal (VAM) fungi to plant growth: a review. *International J. Pure and Applied Science and Technology*. **14(2)**: 61-79.

Baath, E. and Hayman, D. S. 1984. Effect of soil volume and plant density on mycorrhizal infection and growth response. *Plant Soil*. **77:** 373-36.

Bharat, N. K. 2011. Effect of commonly used fungicides on arbuscular-mycorrhizal association with apple roots. *World J. Science and Technology*. **1:** 61-64.

Declerek, S., Strulla, D. G. and Plenchette, C. 1996. *Mycological Research*. **10:** 1237-1242.

Dohroo, A., Sharma, D. R. and Dohroo, N. P. 2013. Occurrence of arbuscular mycorrhizae in rhizospheric soils of different crops and agroclimatic zones of H.P. *Indian J. Agriculture Research*. **47(4):** 373-376.

Gerdemann, J. W. and Nicolson, T. H. 1963. Spores of mycorrhizal *Endogoene* species extracted from soil by wet sieving and decanting. *Transcation of British Mycological Society*. **46:** 235-244.

Giovannetti, M. and Mosse, D. 1980. An evaluation of techniques for measuring VAM infection in roots. *New Phytologist*. **84:** 489-500.

Gomez, K., A. and Gomez, A. A. 1984. *Statistical procedure for agricultural research*. IInd *ed.* John Wiley and Sons, New York, pp. 357-427.

Gupta, S., Kaushal, R., Kaundal, K., Chauhan, A. and Spehia, R., S. 2015. Efficacy of indigenous plant growth promoting rhizobacteria on capsicum yield and soil health. *Research on Crops*. **16:** 123-132.

Hasan, K. and Khan, M. N. 2005. Status of arbuscular mycorrhiza in mango in six districts of Uttar Pradesh. *Mycorrhiza News*. **16(4):** 16-18.

Kirti, S., Sharma, R., Dipta, B., Pawar, R., Kaushal, R. and Bharat, N. K. 2016. Status of arbuscular mycorrhizae associated with rhizosphere of sweet cherry (*Prunus avium*) in high hills temperate zone of Himachal Pradesh. *Indian Phytopathology*. **69(3):** 286-289.

Kirti, S., Dipta, B., Bhardwaj, S., Pawar, R. and Kaushal, R. 2016. Screening and characterization of plant growth promoting rhizobacteria associated with cherry (*Prunus avium* L.).*The Bioscan* **11(4):** 2111-2115.

Kuppusamy, S. and Kumutha, K. 2012. Standardization of the spore density of AM fungal inoculum for effective colonization. *International J. Agriculture Science*. **4(2):** 176-181.

Menge, J. A. 1984. Inoculum production in VA mycorrhiza. *Soil Science Society*. **42:** 926-930.

Oehal, F. and Sieverding, E. 2004. *Paciospora*, a new vasicular arbuscular mycorrhizal fungus genus in the *Glomeromycetes*. *J. Applied Botany*. **78:** 72-82.

Phillips, J., M. and Hayman, D. S. 1970. Improved procedure for clearing roots and staining parasitic and VA-mycorrhizal fungi for rapid assessment of infection.*Transcation of British Mycological Society*. **55:** 158-161.

Rana, S., Sharma, R. and Kaur, M. 2015. Biochemical characterization of fluorescent *Pseudomonas* species isolated from normal and replantsites of apple orchards. *International J. Farm Sciences*. **5(3):** 165-170.

Sharma, R. 2016. Studies on the influence of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria on nutrient uptake and biomass production in cherry (*Prunus avium* L.). Dr.Y S Parmar University.of Horticulture and Forestry, Nauni-Solan (H.P.)

Sharma, R., Kirti, S., Dipta, B., Kaushal, R., and Bharat, N. K. 2016. Biodiversity of arbuscular mycorrhizal fungi in sweet cherry (*Prunus avium* L.) of Kullu district of Himachal Pradesh. *The Bioscan*. **11(3):** 1555-1558.

Smith, S. E. and Read, D. J. 1997. Mycorrhizal symbiosis. 2nd edition. Academic Press, Inc San Diego Calofornia. ISBN 0-12-652840-462