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Evaluation of various substrates for mycorrhizal inoculum production

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ABSTRACT

In the present study, six media were standardized to investigate their suitability for on-farm arbuscular mycorrhizal fungi (AMF) production by examining their physical condition and nutrient status which could be a major determinant of AMF propagule in the final product. The experiment was conducted with Sorghum as a host plant which was inoculated with *Funneliformis mosseae* and *Rhizoglomus intraradices*. Solirite alone recorded 4.66–24.66% higher root colonization than other medium while for spore density in per g of finished product, solirite and red soil mixture (106.33) was found superior followed by solirite and biochar mixture (94.33) and solirite alone (83), respectively, and lowest in soil medium (63 to 74). Moreover, spore density and root colonization were significantly and positively correlated ($R^2 = 0.583$, $P<0.05$) with each other. Solirite alone or mixed with other media improved water holding capacity and porosity with optimum nutrient availability which culminated in better host plant growth and ultimately higher number of AMF propagules.

Keywords: Arbuscular mycorrhizal fungi, Micronutrients, Porosity, Root colonization, WHC

Arbuscular mycorrhizal fungi (AMF) as a symbiotic partner of most of the land plants are imperative for growth and development. AMF provides several benefits to the plants such as improves nutrient and water availability, alleviates abiotic stress, disease, and gives pest resistance (Kumar *et al.* 2016). In order to get these benefits, viable AMF propagules in the soil must be optimum which can be achieved either by adopting ideal farm management practices or by external application of commercially available AMF inoculum (Jansa *et al.* 2006, Berruti *et al.* 2015). In general, external application of AMF inoculum is an easy and rapid strategy but the quality and cost of the commercially available product always remains questionable. To reduce the cost of commercially available AMF inocula, on-farm production of AM biofertilizer could be economical and viable alternative for the farmers. In this technique, producer can use locally available resources along with indigenous AMF species. Generally, AMF propagules in finally prepared product largely depends on the physio-chemical characteristics of the substrate. Among these parameters, the roles of substrate P level and the size of the particle are already well established (Gaur and Adholeya 2000, Maitra *et al.* 2021). However, limited literature is available on carbon, nitrogen and micronutrient status of the substrate along with their physical condition and their influence on quality of AMF inoculum. The objective of this work was to find out the effect of carbon, nitrogen and micronutrient status, physical condition of media on AMF spore density and root colonization in finished product to maximize the production of high quality inoculum.

MATERIALS AND METHODS

Experimental set up: An experiment was conducted during August-September 2015 in the nethouse of the Department of Soil Science and Agricultural Chemistry of Institute of Agricultural Sciences, BHU, Varanasi. Total six different media i.e., Alluvial soil, crushed red earth, solirite (low P content.), Alluvial soil with solirite (SA) @1:3 ratio, red earth with solirite (SR) @1:3 ratio and biochar with solirite (SB) @1:3 ratio were used for on-farm AMF inoculum production. The physico-chemical analysis of rice husk biochar collected from a rice mill of village Kollana, Mirzapur, UP, India showed bulk density 0.40 Mg/m^3, porosity 72%, water holding capacity (WHC) 218%, *p*H 9.8 N 0.10%, P 0.20 and K 0.15%.

10 g of mycorrhizal consortium containing *Funneliformis mosseae* and *Rhizoglomus intraradices* mycorrhizal spp. were mixed in top of 2–5 cm of the medium. Each medium was planted with sterilized seed of the host plant (sorghum) and fertilized with 0.79 g urea and 0.48 g diammonium phosphate (*DAP*) and 0.38 g muriate of potash (MOP) per

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pot. After 8 weeks, the aerial parts of plants were removed and pots were dried for another week.

Sample collection and analysis: The data on growth attributes of sorghum were recorded 8 weeks after sowing and root and shoot biomass was collected and dried in oven at 105°C for 24 h and dry weight was measured for calculating root to shoot ratio. After cutting the aerial part, collected medium was stored in sufficient amount at 4°C for various physio-chemical and mycorrhizal analyses. Spores of AMF were quantified in per g of finished product, collected from each bag via wet sieving and centrifugation (Gerdemann and Nicolson 1963). Roots were collected from the sample bags, rinsed, cleared and stained using trypan blue and AMF colonization was quantified using the gridline intersect method (Newman 1966). Total root length (cm) was estimated by line interception method (Tennant 1975).

Total concentration of chlorophyll was determined using DMSO (dimethylsulfoxide) method (Borghezan *et al.* 2003) while a vailable N, P and K in plant samples were estimated by the methods suggested by Subbiah and Asija (1956), Olsen (1954) and Black (1965), respectively. For micronutrient analysis, samples were digested with di-acid mixture of HNO_3:$HClO_4$ (9:1 v/v) and the analysis was performed using atomic absorption spectrophotometer. For different physio-chemical analysis, various substrate used in pot experiment were sieved and analyzed using a standard protocol. Different methods were used including bulk and particle density with pycnometer bottle in laboratory using disturbed soil sample (Black 1965); Water holding capacity (WHC) by methods of Keen box (Piper 1966); Soil porosity as:

$$(BD/PD)*100$$

where BD, bulk density; PD, particle density (Mg/m^3).

Total organic carbon (TOC) and nitrogen (TN) content of different media were analysed using CHNS analyser. Available Zn, Mn, Cu and Fe were determined by DTPA extractable method (Lindsay and Norvell 1978).

Statistical analysis: Experimental units were arranged according to a completely randomized design (CRD). Analysis of variance (ANOVA) was used to test the statistical difference between different variables. The mean significant differences were compared by Tukey's HSD test at $P<0.05$. The Pearson correlation analysis among parameters was performed using the R-square (R version 3.5.1).

RESULTS AND DISCUSSION

Physical property of media: To ascertain the physical condition of different medium, their bulk density, particle density, water holding capacity and porosity were estimated (Table 1). Particle density was found significantly lower in SB (1.54 Mg/m^3) followed by SR (1.86 Mg/m^3) and SA (1.86 Mg/m^3) while soil based medium reported higher values with maximum in red soil (2.67 Mg/m^3). Bulk density also followed similar trend as noticed in particle density and varied from 0.50 to 1.57 Mg/m^3. Porosity of the media was found 7.39, 26.60, 30.54, 31.54 and 38.92% higher in SB compared to solirite mix, SA, SR, alluvial and red soil, respectively. Solirite mix and its combination with other media improved their water holding capacity significantly. Solirite alone showed maximum WHC (359.33%) followed by SB (271.33%) and SR (177.35%).

Solirite alone or in mixture improves physical property by increasing porosity, WHC, and reducing bulk and particle density which resulted in greater AMF propagules (Gaur and Adholeya 2000, Caravaca *et al.* 2002). Red soil has poorest physical property due to the presence of cementing agent such as iron and Al- oxide leading to higher compaction while its combination with solirite enhances AMF propagules significantly which could be ascribed to lower P content in red soil (Gosling *et al*. 2006) and optimum aeration and water retention of subsequent mixture of solirite and red soil.

Nutrient status of media: Nutrient status was determined by estimating TOC, TN, C:N ratio and micronutrient content of various growth media. TOC in different treatment varied from 7.89 to 16.98 g/kg with significantly higher content in soil based medium compared to SR and solirite alone (Table 1). SA had 1.24, 1.69 and 1.90 times higher TOC than SB, SR and solirite alone. Similar to TOC, treatment containing solirite mix with other medium except SA had significantly reduced TN compared to soil based medium (Table 1). Micronutrient (Zn, Cu, Fe and Mn) analysis of media showed that solirite mixed subsrate contained higher amount of microntutrients than soil based medium (Table 1).

Table 1 Physical properties of different medium used for on-farm AMF production

Treatment	Particle density	Bulk density	WHC (%)	Porosity	TOC (g/kg)	TN (g/kg)	Micronutrient content (ppm)			
							Zn	Cu	Fe	Mn
Alluvial soil	2.47b	1.33b	42d	46.33bc	14.58ab	0.64a	3.33	5.93	17	15.31
Red soil	2.67a	1.57a	33d	41.33c	15.42ab	0.59ab	2.80	5.00	37	28.53
Solirite mix	2.04c	0.75c	359a	62.67a	8.80c	0.37c	17.32	46.68	360	36.60
SA	1.86d	0.93b	169c	49.67b	16.98a	0.64a	20.00	60.00	446	72.67
SR	1.86d	0.98b	177c	47.00bc	10.78c	0.38c	17.90	43.74	348	58.62
SB	1.54e	0.50d	271b	67.67a	14.11b	0.62ab	21.85	62.23	482	92.67

SA, Alluvial Soil with solirite; SR, red earth with solirite; SB, biochar with solirite. Data (mean) followed by similar latter are not significant different at $p < 0.05$ level of significance according to Tukey HSD.

Table 2 Effect of different growth medium on plant growth, nutrient content and mycorrhizal propagules

Treatment	*pH* (cm)	PDW (g/plant)	RDW (g/plant)	RL (cm)	R:S ratio	Chl (mg/g)	SD	RC (%)	Macronutrient (%)			Micronutrient (ppm)			
									N	P	K	Zn	Cu	Fe	Mn
Alluvial	45.08cd	5.83ab	1.13cd	2109de	0.20bc	1.50cd	74ac	68ac	0.44c	0.12b	1.11b	23.68bc	6.08b	70.42c	13.05a
Red soil	40.97d	4.49b	0.76d	1633e	0.18c	1.26d	63a	65a	0.40c	0.11b	1.15b	16.12c	7.94ab	69.01c	15.39a
Solirite	54.26a	7.63a	2.43a	3767b	0.33ab	2.06bc	83abc	89b	0.61ab	0.14ab	1.32ab	32.75ab	11.71a	89.99ab	15.09a
SA	47.69bc	5.39ab	1.56bc	2600cd	0.29abc	1.84c	82abc	75abc	0.51bc	0.12ab	1.24ab	27.61b	9.99ab	77.05bc	12.03a
SR	51.70ab	5.67ab	1.79b	3333bc	0.32abc	2.14ab	106b	85bc	0.62a	0.15a	1.41a	31.25b	11.31a	101.30a	14.49a
SB	53.31a	7.45a	2.72a	4667a	0.38a	2.52a	94bc	78abc	0.56ab	0.13ab	1.28ab	41.68a	11.23a	91.50ab	14.01a

Data (mean) followed by similar latter are not significant different at $p < 0.05$ level of significance according to Tukey HSD.

Symbiotic association of AMF with host plant is largely dependent on relative availability of C, nitrogen (N) and phosphorus (P) and their lower concentration in substrate was considered ideal for the AMF mass production (Johnson *et al.* 2013). In addition, we have also estimated Zn, Cu, Fe and Mn content of medium and found that all micronutrients except Mn were significantly and positively correlated with AMF propagules. Our results conform with Datta and Kulkarni (2012) who also reported closer and positive association of Cu, Zn and Fe contents with root colonization and spore density. However, lower germination of spores and mycelial growth in the study of Moreira and Siqueira (2002) might be due to higher Mn and Fe concentration in substrate and also could be the reason to explain their contrasting result compared to our findings.

Mycorrhizal property: AMF inoculum was developed using different solirite mix and soil based medium. Spore density and root colonization were estimated in different medium to determine their potential or suitability for inoculum production (Table 2). Root colonizaion varied from 68 to 89.33% and was significantly higher in solirite based medium. Solirite mix exhibited an increment in root colonization to an extent of 5.51, 14.53, 19.64, 31.37 and 38.14% over SR, SB, SA, alluvial and red soil, respectively. Compared to root colonization, spore density in per g of inoculum was reported highest under SR (106.33) followed by SB (94.33) and lowest under alluvial (74) and red soil (63).

Growth attributes and nutrient uptake by host plant: AMF reportedly enhance the growth of host plant under diverse soil and climatic conditions. All the growth attributes except plant dry weight increased significantly in solirite mixed treatments (Table 2). Plant height ranged from 40.97 to 53.31 cm and was significantly higher in Solirite, SB and SR medium. Plant dry weight reported non-significant difference while root dry weight showed significant increment in solirite and SB medium i.e. 35.50–219.74 and 11.93–257.89%, respectively, than other medium. Total root length was found significantly higher in SB and showed 19.27, 28.56, 44.29, 54.81 and 64.99% increment than solirite, SR, SA, alluvial and red soil, respectively. R:S ratio was influenced by solirite presence and reduced significantly in red (0.18) and alluvial soil (0.20). Chlorophyll content followed the trend similar to plant height showing variation from 1.26 to 2.52 mg/g fresh weight of leaf with significantly higher value in SB (2.52) and SR (2.14). In present study, NPK content in sorghum exhibited almost similar trend and ranged from 0.40 to 0.62, 0.11 to 0.15 and 1.11 to 1.41%, respectively (Table 2). Among micronutrients, zinc was observed significantly higher in SB (41.68 ppm) and solirite (32.75 ppm) while Fe and Cu content were found significantly higher in solirite based medium than soil based medium and ranged from 69.00–101.30 and 6.08–11.71 ppm, respectively. The Mn content ranged from 12.03 to 15.39% but with non-statistical difference.

AMF on-farm production, largely dependent on suitable host plant with shorter life cycle and adequate

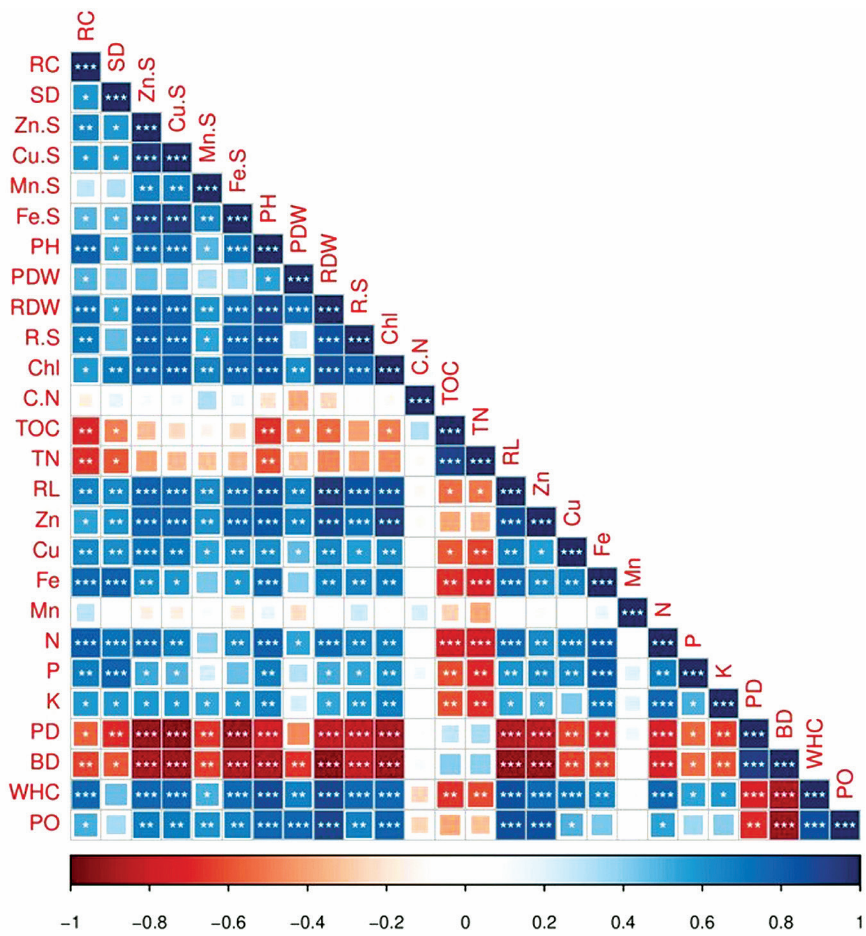

Fig 1 Correlation matrix of different variables with mycorrhizal propagules; blue colour correspond to (+) positive interaction, red color corresponds to (−) negative interaction, white corresponds to neutral interaction among variables. RC, Root colonization percentage; SD, spore density, Zn.S, Cu.S, Mn.S and Fe.S = Zinc, copper, iron and manganese content in various medium; PDW, plant dry weight; RDW, root dry weight; R:S, root to shoot ratio; Chl, chlorophyll; RL, root length; PD, particle density; BD, bulk density; WHC, water holding capacity; PO, porosity; PH, plant height.

root development, influences proliferation and sporulation of AMF. In our study, shoot and root development was influenced greatly in solirite mix with biochar and other medium by improving organic matter (Sonika *et al.* 2013) and physical condition of growth substrate (Singh and Sainju 1998) which resulted in larger root biomass and R:S ratio. Higher plant growth attributed to greater photosynthates accumulation which on subsequent allocation to rhizosphere results in greater AMF development (Řezáčová *et al.* 2018). In exchange of plant C, AM fungi improves the plant nutrient acquisition from soil or growth medium (Chen *et al.* 2017). In our study, AMF root colonization and spore density were strongly correlated to N, P, K, Zn, Cu and Fe content in plant tissue.

Correlation among variables: In present study, root colonization showed a significantly negative linear relationship with TOC (R^2 = -0.692, P< 0.01), TN (R^2 = -0.677, P< 0.01), PD (R^2 = -0.554, P<0.05) and BD (R^2 = -0.630, P<0.01) while medium Zn (R^2 = 0.646, P<0.01), Cu (R^2 = 0.580, P<0.05), Fe (R^2 = 0.486, P<0.05), *p*H (R^2 = 0.774, P<0.01), WHC (R^2 = 0.752, P<0.01) and porosity (R^2 = 0.503, P<0.05) exhibited significantly positive correlation (Fig 1). Spore density also showed almost similar association with described variables except WHC and porosity. In addition to this, spore density was positively correlated (R^2 = 0.583, P<0.05) with the root colonization. Other than medium property, plant growth attributes and its nutrient content such as root dry weight, root length, Zn, Cu, Fe, N, P, K were associated positively and significantly to AMF variables while plant dry weight (R^2 = 0.491, P<0.05) and R:S ratio (R^2 = 0.683, P< 0.01) were significantly associated with root colonization only.

In conclusion, medium like solirite which is lower in total carbon, nitrogen with good physical condition and micronutrient content ensured greater root growth and mass multiplication of AM propagules. Solirite mix with red soil and biochar emerged as best medium for on-farm AM production and produced higher AMF spore count and root colonization. Sponge-like matrix of biochar responsible for slow release of moisture results in greater moisture holding capacity which in turns plays a pivotal role for greater AM survivability. Red earth and alluvial soil were observed to have significantly lower spore count in isolation than in combination with solirite medium because of higher compaction, poor drainage and aeration, lower root proliferation and root growth. This indicates that the physical status of the media is equally important to their nutrient management for greater AMF inoculum production.

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