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Mass multiplication of arbuscular mycorrhizal fungi associated with some leguminous plants: an ecofriendly approach

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Plant microbe interactions are interesting events that contribute to sustainable agriculture. The arbuscular mycorrhizal (AM) fungi enjoy a mutualistic association between the roots of most plant species and serve as the most common type of biofertilizer. However, production of inoculums is one of the hindrances in the large-scale production of AM fungi. In this context, a pot experiment was performed under polyhouse conditions, to evaluate the effect of chickpea husk as substrate with jowar (*Sorghum bicolor*), barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) as different host plant on mass multiplication of dominant AM fungi. The results revealed that AM fungal multiplication was significantly influenced by the presence of different concentrations of substrate and different type of the host plants used. Among the different hosts, sorghum showed prominent results pertaining to maximum inoculum production of *G. mosseae*. Spore numbers tend to increase with period of growth and increase in size of the host plants. Thus, the present study might be highly significant as it suggests an economical as well as eco-friendly species specific highly effective inoculum.

Keywords: *Acaulospora laevis*, AM fungi, Chickpea seed husk, *Glomus mosseae*, Mass multiplication

Intensification of agriculture is an inevitable compulsion to meet the increasing demand for food and fodder, and it has put an enormous burden on the natural ecosystem. Sustainable agriculture which encompasses soil and crop productivity by integration of agricultural management technology helps to enhance the farm profitability without compromising the environmental needs[1]. Researchers seek novel and effective technologies to improve crop productivity and profitability in a sustainable manner. Among the microbial communities, arbuscular mycorrhizal (AM) fungi are a mutualistic association between the roots of most plant species and fungi and serve as the most common type of biofertilizer used in agriculture system to increase plant production[2,3].

The arbuscular mycorrhizal (AM) fungi are not considered as host specific. However, some plants are more susceptible than others in relation to development of symbiosis[4]. Different practices such as use of waste substrates along with the traditional substrate (soil-sand mixture) are being tried for mass culture of AM fungi these days[5]. Therefore, the broad application of AM fungi has been limited by the difficulties in obtaining large quantities of pure inoculum and their commercial exploitation is still in its infancy[6].

AM fungi are maintained and mass produced in pot cultures on suitable host plants[7]. The host plant selected should be suitable to agro climate conditions of the area, having thick root system for sizeable sporulation and infection, annual in growth habit and adaptable to polyhouse conditions. The host plants also may stimulate selectively or limit sporulation of certain AM fungal species suggesting varied affinities between hosts and symbionts[8,9].

Quantitative and qualitative population of AMF depends upon several factors which include cultivation practices used for plant growth, environmental conditions, type of substrate and host plant. One of the most important considerations in the inoculum production is the choice of fungal isolates which are capable of growth promotion of target host plant[10,11]. Selection of suitable substrate for mass production of AM fungi is also important[12,13]. It is well known that organic wastes are rich in nutrients and have positive effect on AM root colonization[14-17].

Here, we attempted to prepare an economical and efficient medium, optimal concentration of substrate, and an appropriate host to give maximum spore production of the selected dominant fungi associated

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with most of the leguminous crops in minimum time. We opted chickpea seed husk for substrate because of its availability, nutritive value and low cost[18-20].

The chickpea husk was used as a substrate with different concentrations and jowar (*Sorghum bicolor*), barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) were used as hosts to see the influence of different host plant on mass multiplication of selected AM fungi.

Materials and Methods

Experimental design

The experiment was a 4×2×3 factorial in a completely randomized design employing three types of hosts (Barley, Wheat and Sorghum), two forms of substrates (Dry and Compost) of chickpea husk at four different concentrations (without substrate, 25, 50 and 100 g pot^{-1}) were used. Each treatment was replicated five times. The experiment was carried out in a greenhouse at the Botany Department, Kurukshetra University, Kurukshetra. Light was provided by cool white fluorescent lamps (8000 lux) under a 16 h photoperiod.

Soil characteristics

The soil characteristics were: sand-64.2%, silt-21.81%, clay-3.90%, starting pH- 6.8, EC-0.25dSm^{-1}, organic carbon-0.06%, total N-0.042%, available P-0.0018 kg/m^2, K-0.022 kg/m^2 and S-14.80 ppm.

Preparation of starter AM inoculum

Glomus mosseae and *Acaulospora laevis* were found to be the dominant AM fungal strains as described in our earlier research[21,22]. These spores were now multiplied with maize for a period of two months using the funnel technique[23]. For this, the selected isolated spores were first surface sterilized with 2% (w/v) Chlioamine-T for about 15 min followed by washing with sterilized deionized water. These spores were then checked for their viability using Thionin stain.

Ten healthy and viable spores were then used for the starter inoculum using maize as host. Sterilized funnel (250 mL) was filled with sterilized soil: sand (3:1) mixture, inoculated with the selected spores and five properly disinfected seeds of maize were sown in each funnel. After 60 days of growth, plant roots were analyzed for mycorrhizal colonization and rhizosphere soil was also tested for AM spore quantification. This inoculum was further used for the multiplication of AM fungi first under earthen funnels followed by bigger earthen pots. The inoculum obtained at this stage was further used for the present experiment.

Selection and preparation of substrate

Chickpea husk was collected from local flour mill and was processed before use. The substrate was divided into two parts. One part was used as such called as dry (grounded to make a fine powder). The remaining part of the substrates was packed in nylon net bags and then buried under the soil for three months for compost formation and homogenized before use to form a composite substrate.

Selection of host plant

Three different monocots belonging to family Poaceae i.e. wheat, barley and sorghum were selected as host plants and tested with substrate.

Experimental setup

Top soil (0-30 cm) from Botanical Garden was air dried, pulverized, passed through a 2 mm sieve. It is then mixed with sand: soil (1:3) and autoclaved at 121°C for 30 min for two consecutive days prior to use. Different concentrations of each substrate (0, 25, 50 & 100 g pot^{-1}) were added to earthen pots (25.4× 25 cm), thoroughly mixed with sand: soil mixture to make final volume of 2 kg. To this, 200 g of AM inoculum (chopped AM colonized root pieces of maize, along with soil containing 350-420 AM spores 100 g^{-1}) raised by the funnel technique was added.

For wheat, barley and sorghum, healthy seeds were surface sterilized with 0.5% sodium hypochlorite for 10 min, and subsequently washed with sterilized deionized water. Ten seeds were sown in each pot above the inoculum. After 15 days of growth, wheat, barley and Sorghum plants were thinned to five plants per pot. Plants were watered regularly and 100 mL pot^{-1} Hoagland's solution[24] (without KH_2PO_4) was added to each pot at 15 day intervals.

Harvest and analysis

Vegetative growth response was assessed 90 days after planting by manually uprooting the whole plant. Plant height (cm) and root length (cm) was recorded followed by washing of plants with running tap water. Roots and shoots were separated to determine their fresh weight (g), and then placed in an oven to dry at 70°C until a constant dry weight (g) was obtained.

The percentage mycorrhizal root colonization and AM spore quantification was done using the method of Philips and Hayman[25] and Gerdemann and Nicolson[26].

Statistical analysis

All results were analyzed using Analysis of Variance (ANOVA), followed by post hoc test through computer software SPSS 11.5 version. Means were ranked at $P \leq 0.05$ using Duncan's Multiple Range Test for comparison.

Results

Although all the host plants inoculated with *Glomus mosseae* and *Acaulospora laevis* produced fungal spores and colonized roots that were characterized by the presence of extrametrical hyphae, intraradical hyphae, arbuscules and vesicles, yet substantial differences were obtained with different hosts and concentration of substrate used.

Regarding *G. mosseae*, 50 g dry chickpea husk resulted in maximum plant height, shoot biomass, root biomass, root length as well as mycorrhizal colonization and AM spore number in comparison with control when barley was used as a host plant. Similarly, 100 g compost resulted in maximum plant height, AM spore number and maximum root colonization in comparison with control (Tables 1 & 2).

With wheat, maximum plant height, plant biomass, root length, AM spore number and root colonization

Table 1 — Inoculum production of *Glomus mosseae* using chickpea husk substrate (Dry and Compost) and Barley as a host

Type of chickpea husk substrate	Conc. of each substrate (g pot^{-1})	Plant height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)
Dry	0	30.4±2.07$^{\dagger g}$	19.62±0.032^{f}	6.72±0.019^{f}	8.73±0.031^{f}	1.34±0.027^{h}	0.52±0.015^{h}
	25	46.6±3.20^{c}	24.22±0.011^{d}	7.30±0.024^{d}	16.2±0.033^{b}	3.21±0.023^{d}	1.20±0.015^{d}
	50	60.0±2.54^{a}	35.44±0.021^{b}	13.2±0.036^{b}	19.5±0.034^{a}	4.92±0.019^{a}	2.22±0.047^{a}
	100	37.4±1.94^{f}	22.43±0.027^{e}	6.33±0.046^{g}	9.27±0.025^{e}	2.24±0.041^{e}	0.70±0.020^{e}
Compost	0	27.8±1.48^{h}	22.21±0.016^{e}	7.29±0.016^{d}	10.0±0.026^{d}	2.07±0.025^{f}	0.57±0.015^{g}
	25	40.0±1.58^{e}	26.25±0.027^{c}	8.33±0.029^{c}	12.2±0.013^{c}	3.90±0.021^{c}	1.70±0.020^{b}
	50	45.6±2.40^{d}	39.36±0.031^{a}	14.2±0.040^{a}	10.3±0.020^{d}	4.29±0.016^{b}	1.33±0.027^{c}
	100	58.2±1.78^{b}	23.22±0.019^{de}	6.89±1.328^{e}	7.18±0.022^{g}	1.42±0.015^{g}	0.66±0.030^{f}
L.S.D ($P \leq 0.05$)		6.0274	3.534	0.5084	2.2793	0.3331	0.2304
ANOVA $F_{(7,15)}$	S type	115.492	163.905	51.895	94.195	91.958	49.384
	S conc.	548.43	239.432	432.76	765.22	523.54	286.55
	S type × S conc.	12.872	87.543	55.342	76.325	34.229	22.430

[Each value is a mean of five replicates, ±: standard deviation, S: Substrate. † indicates the level of significance at ($P \leq 0.05$) level. Means followed by same letter/s within a column are not significantly different over one another (Least significant different test, ($P \leq 0.05$)]

Table 2 — Inoculum production of *Glomus mosseae* using chickpea husk substrate (Dry and Compost) and Barley as a host

Type of chickpea husk substrate	Conc. of each substrate (g pot^{-1})	Pattern of Colonization			AM spore number/ 10 g of soil	% Root colonization
		Mycelium	Vesicles	Arbuscules		
Dry	0	+	–	+	52.6±2.30^{g}	24.2±1.30^{h}
	25	+	++	++	89.6±1.57^{c}	54.4±3.64^{c}
	50	+	+++	+++	97.8±1.48^{b}	84.2±1.92^{a}
	100	+	++	++	73.8±3.42^{e}	49.6±1.51^{f}
Compost	0	+	++	+	62.6±1.94^{f}	32.6±1.94^{g}
	25	+	+	+	91.2±2.38^{b}	53.4±1.14^{e}
	50	+	+	++	77.0±1.58^{d}	55.2±2.38^{d}
	100	+	+++	+++	102.4±2.07^{a}	82.4±1.81^{b}
L.S.D ($P \leq 0.05$)					17.2368	8.113
ANOVA $F^{(7,15)}$	S type				304.114	44.160
	S conc.				875.53	238.54
	S type × S conc.				14.331	45.331

[Each value is a mean of five replicates. ±: standard deviation, S: Substrate, AM: Arbuscular mycorrhizae,-: absent, +: scanty, ++: moderate, +++: abundant. † indicates the level of significance at ($P \leq 0.05$) level. Means followed by same letter/s within a column are not significantly different over one another (Least significant different test, ($P \leq 0.05$)]

was found to be highest with 50 g dry chickpea husk substrate as well as with 50 g compost (Tables 3 & 4).

Similarly, while using sorghum, 100 g dry chickpea husk substrate resulted in maximum plant height, plant biomass, root length as well as mycorrhization. In case of compost 50 g chickpea husk substrate resulted in maximum plant height as well as mycorrhization (i.e. AM spore number and percent root colonization (Tables 5 & 6). Overall, 50 and 100 g of both dry and compost substrate resulted in maximum mycorrhization, plant height and plant biomass.

Analysis of the effect of chickpea husk on *A. laevis* exhibited that all the three hosts tested influenced the root colonization and AM spore number. In case of Barley, using chickpea husk as substrate, 50 g of dry chickpea husk resulted in maximum plant height and 25 g of compost resulted in increase in plant height. In case of AM spore number and root colonization respectively, 25 g of dry and compost proved to be more efficient (Tables 7 & 8).

While using chickpea husk as substrate, wheat as a host 100 g of dry and compost resulted in maximum increment in plant height, 100 g dry substrate resulted in maximum AM spore number and root colonization and 50g compost resulted in maximum AM spore number and root colonization (Tables 9 & 10).

Using sorghum as host and chickpea husk as a substrate, 50 g of dry substrate resulted in maximum

Table 3 — Inoculum production of *Glomus mosseae* using chickpea husk substrate (Dry and Compost) and wheat as a host

Type of chickpea husk substrate	Conc. of each substrate (g pot^{-1})	Plant height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)
	0	25.6±2.88$^{\dagger h}$	3.424±0.003^{h}	1.23±0.003^{h}	9.40±0.08^{g}	1.23±0.027^{h}	0.92±0.002^{a}
Dry	25	30.8±1.64^{g}	6.825±0.002^{c}	2.32±0.003^{d}	13.8±1.30^{e}	2.33±0.027^{d}	0.10±0.001^{f}
	50	44.0±2.54^{e}	7.327±0.023^{b}	3.42±0.019^{b}	19.2±1.78^{b}	2.96±0.024^{a}	0.20±0.003^{c}
	100	38.8±0.83^{f}	5.001±0.001^{f}	1.99±0.002^{f}	13.0±1.41^{d}	1.55±0.027^{g}	0.10±0.002^{e}
	0	47.0±3.00^{d}	5.433±0.002^{e}	2.02±0.002^{e}	13.0±1.22^{d}	1.63±0.027^{f}	0.10±0.003^{e}
Compost	25	50.4±3.78^{c}	5.929±0.014^{d}	2.79±0.002^{c}	14.2±0.18^{c}	2.46±0.028^{c}	0.13±0.002^{d}
	50	58.0±0.70^{a}	8.326±0.026^{a}	3.79±0.001^{a}	20.3±0.02^{a}	2.62±0.021^{b}	0.22±0.003^{b}
	100	52.0±1.87^{b}	4.332±0.003^{g}	1.52±0.001^{g}	12.2±1.64^{f}	1.93±0.027^{e}	0.13±0.001^{d}
L.S.D (P≤0.05)		3.3561	0.7083	0.3983	1.1982	0.4182	0.2318
ANOVA $F_{(7,15)}$	S type	99.349	6.081	37.836	6.218	2.231	11.132
	S conc.	27.963	75.043	171.822	102.034	102.034	229.678
	S type × S conc.	53.276	100.737	47.164	27.563	27.563	56.443

[Each value is a mean of five replicates, ±: standard deviation, S: Substrate. † indicates the level of significance at (P≤0.05) level. Means followed by same letter/s within a column are not significantly different over one another (Least significant different test, (P≤0.05)]

Table 4 — Inoculum production of *Glomus mosseae* using chickpea husk substrate (Dry and Compost) and wheat as a host

Type of chickpea husk substrate	Conc. of each substrate (g pot^{-1})	Pattern of Colonization			AM spore number/ 10 g of oil	% Root colonization
		Mycelium	Vesicles	Arbuscules		
	0	+	–	+	30.8±2.77$^{\dagger g}$	17.0±2.12^{g}
Dry	25	+	++	++	202.4±1.81^{d}	51.8±2.04^{f}
	50	+	+++	+++	213.4±3.13^{c}	73.4±3.13^{c}
	100	+	+	++	93.4±3.13^{e}	43.0±3.46^{d}
	0	+	+	++	43.0±3.46^{f}	24.4±3.91^{e}
Compost	25	+	++	++	263.4±3.13^{b}	83.2±2.77^{b}
	50	+	++	++	303.4±3.13^{a}	93.2±2.77^{a}
	100	+	+	+	213.4±3.13^{c}	73.6±3.04^{c}
L.S.D (P≤0.05)					29.7372	7.4757
ANOVA $F_{(7,15)}$	S type				21.553	47.819
	S conc.				274.950	517.979
	S type × S conc.				21.239	14.833

[Each value is a mean of five replicates, ±: standard deviation, S: Substrate, AM: Arbuscular mycorrhizae,-: absent, +: scanty, ++: moderate, +++: abundant. † indicates the level of significance at (P≤0.05) level. Means followed by same letter/s within a column are not significantly different over one another (Least significant different test, (P≤0.05)]

Table 5 — Inoculum production of *Glomus mosseae* using chickpea husk substrate (Dry and Compost) and Sorghum as a host

Type of chickpea husk substrate	Conc. of each substrate (g pot^{-1})	Plant height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Root length (cm)	Fresh root weight (g)	Dry root weight (m)
Dry	0	62.8±1.92^{f}	19.33±0.016^{f}	5.61±0.020^{g}	9.02±0.019^{f}	3.03±0.015^{h}	1.09±0.016^{h}
	25	81.2±1.92^{c}	22.54±0.038^{e}	6.33±0.017^{e}	16.3±0.025^{c}	5.25±0.027^{e}	2.34±0.023^{e}
	50	89.2±1.92^{b}	24.26±0.022^{d}	7.84±0.015^{d}	19.3±0.026^{b}	8.32±0.018^{d}	3.43±0.027^{d}
	100	92.2±2.28^{a}	28.33±0.014^{b}	8.02±0.024^{b}	22.3±0.027^{a}	8.89±0.034^{b}	3.62±0.013^{c}
Compost	0	53.0±1.58^{g}	20.53±0.024^{g}	5.82±0.014^{f}	10.3±0.018^{d}	3.52±0.018^{g}	1.24±0.023^{g}
	25	62.8±1.92^{f}	19.32±0.022^{f}	5.33±0.016^{h}	9.40±0.020^{e}	3.94±0.021^{f}	1.74±0.031^{f}
	50	78.8±0.83^{d}	25.44±0.019^{c}	7.92±0.018^{c}	19.4±0.027^{b}	8.40±0.027^{c}	4.46±0.024^{a}
	100	66.4±2.07^{e}	29.36±0.029^{a}	8.27±0.018^{a}	22.7±0.021^{a}	9.04±0.023^{a}	3.71±0.021^{b}
L.S.D (P≤0.05)		5.8648	11.741	0.4958	1.5307	0.7636	0.06167
ANOVA $F_{(7,15)}$	S type	63.901	107.756	355.109	140.810	112.618	70.497
	S conc.	142.664	887.142	166.664	195.288	533.047	412.155
	S type × S conc.	36.835	87.833	29.997	91.758	163.994	52.306

[Each value is a mean of five replicates, ±: standard deviation, S: Substrate. † indicates the level of significance at (P ≤0.05) level. Means followed by same letter/s within a column are not significantly different over one another (Least significant different test, (P ≤0.05)]

Table 6 — Inoculum production of *Glomus mosseae* using chickpea husk substrate (Dry and Compost) and Sorghum as a host

Type of chickpea husk substrate	Conc. of each substrate (g pot^{-1})	Pattern of Colonization			AM spore number/10 g of soil	% Root colonization
		Mycelium	Vesicles	Arbuscules		
Dry	0	+	–	–	96.4±2.07^{h}	55.0±2.54^{g}
	25	+	++	+	126.6±3.20^{f}	74.0±2.28^{e}
	50	+	++	+	287.2±1.78^{c}	83.6±1.01^{cd}
	100	+	+++	+++	298.8±1.30^{b}	97.0±1.41^{b}
Compost	0	+	+	–	105.6±1.51^{g}	57.2±1.72^{f}
	25	+	++	++	175.4±3.04^{e}	78.2±2.56^{d}
	50	+	++	++	383.8±3.40^{a}	85.6±2.72^{c}
	100	+	+++	+++	202.0±1.87^{d}	99.8±1.60^{a}
L.S.D (P≤0.05)					23.7372	7.4757
ANOVA $F_{(7,15)}$	S type				21.553	47.819
	S conc.				274.950	517.979
	S type × S conc.				21.239	14.833

[Each value is a mean of five replicates, ±: standard deviation, S: Substrate, AM: Arbuscular mycorrhizae,-: absent, +: scanty, ++: moderate, +++: abundant. † indicates the level of significance at (P ≤0.05) level. Means followed by same letter/s within a column are not significantly different over one another (Least significant different test, (P ≤0.05)]

Table 7 — Inoculum production of *Acaulospora laevis* using chickpea husk substrate (Dry and Compost) and Barley as a host

Type of chickpea husk substrate	Conc. of each substrate (g pot^{-1})	Plant height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)
Dry	0	22.6±2.15	8.356±0.021	2.37±0.017	5.442±0.017	2.32±0.014	0.98±0.002
	25	33.8±1.93	14.38±0.024	7.45±0.027	12.33±0.011	4.34±0.023	1.36±0.028
	50	41.4±1.20	11.47±0.016	5.33±0.018	10.30±0.018	3.28±0.008	1.97±0.014
	100	32.2±1.72	10.39±0.018	5.12±0.032	09.89±0.130	3.10±0.013	1.81±0.018
Compost	0	28.0±1.41	7.352±0.017	2.04±0.017	05.01±0.014	2.01±0.010	0.80±0.002
	25	53.2±1.60	13.44±0.022	7.20±0.019	12.03±0.027	4.01±0.013	1.04±0.018
	50	42.4±1.62	10.10±0.020	4.90±0.027	09.92±0.022	3.01±0.015	1.43±0.014
	100	35.6±2.15	9.398±0.022	5.01±0.014	09.45±0.028	2.92±0.018	1.20±0.028
L.S.D (P≤0.05)		3.94425	1.4156	0.2801	3.0467	0.4892	0.342
ANOVA $F_{(7,15)}$	S type	125.514	177.336	101.490	16.851	114.595	80.635
	S conc.	723.867	448.930	129.849	128.433	630.607	188.051
	S type × S conc.	25.268	135.310	45.960	28.795	20.817	27.982

[Each value is a mean of five replicates, ±: standard deviation, S: Substrate. † indicates the level of significance at (P ≤0.05) level. Means followed by same letter/s within a column are not significantly different over one another (Least significant different test, (P ≤0.05)]

Table 8 — Inoculum production of A. laevis using chickpea husk substrate (Dry and Compost) and Barley as a host

Type of chickpea husk substrate	Conc. of each substrate (g pot^{-1})	Pattern of Colonization			AM spore number/ 10 g of soil	% Root colonization
		Mycelium	Vesicles	Arbuscules		
Dry	0	+	–	+	71.8±2.13^{h}	33.8±4.40^{h}
	25	+	++	++	222.4±2.05^{a}	92.6±1.74^{a}
	50	+	+++	+++	209.6±1.85^{b}	84.8±1.32^{c}
	100	+	++	++	194.0±3.34^{d}	72.4±1.62^{e}
Compost	0	+	++	+	79.4±2.15^{g}	32.0±3.63^{g}
	25	+	+	+	200.8±2.48^{c}	86.4±1.49^{b}
	50	+	+	++	173.6±2.05 ^{e}	83.4±2.41^{d}
	100	+	+++	+++	142.0±1.89^{f}	69.0±2.28^{f}
L.S.D ($P \leq 0.05$)					20.054	4.8852
ANOVA $F_{(7,15)}$	S type				8.017	148.808
	S conc.				1137.199	566.480
	S type × S conc.				26.122	180.542

[Each value is a mean of five replicates, ±: standard deviation, S: Substrate, AM: Arbuscular mycorrhizae,-: absent, +: scanty, ++: moderate, +++: abundant. † indicates the level of significance at ($P \leq 0.05$) level. Means followed by same letter/s within a column are not significantly different over one another (Least significant different test, ($P \leq 0.05$)]

Table 9 — Inoculum production of *Acaulospora laevis* using chickpea husk substrate (Dry and Compost) and wheat as a host

Type of chickpea husk substrate	Conc. of each substrate (g pot^{-1})	Plant height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)
Dry	0	16.8±1.78^{f†}	1.325±0.002^{f}	0.98±0.002^{e}	05.4±2.07^{g}	0.98±0.001^{g}	0.24±0.002^{e}
	25	22.4±2.30^{e}	2.431±0.002^{e}	1.02±0.002^{d}	11.4±1.34^{d}	1.23±0.001^{f}	0.72±0.002^{d}
	50	32.4±1.81^{d}	3.416±0.002^{d}	1.54±0.018^{c}	12.8±1.30^{c}	1.53±0.002^{e}	0.84±0.004^{c}
	100	39.0±2.00^{a}	4.280±0.003^{a}	1.92±0.003^{a}	15.6±1.51^{b}	1.83±0.001^{d}	0.99±0.002^{b}
Compost	0	22.4±1.81^{e}	4.125±0.002^{b}	1.90±0.003^{a}	18.4±1.34^{a}	2.12±0.003^{a}	1.02±0.003^{a}
	25	35.6±2.40^{c}	3.902±0.002^{c}	1.58±0.001^{b}	15.0±1.87^{b}	2.10±0.002^{b}	0.99±0.004^{b}
	50	28.6±1.34^{b}	1.131±0.002^{g}	0.98±0.002^{e}	09.4±1.67^{e}	1.92±0.002^{c}	0.82±0.002^{c}
	100	40.2±2.28^{a}	1.005±0.002^{h}	0.72±0.002^{f}	06.4±1.94^{f}	0.84±0.002^{h}	0.12±0.003^{f}
L.S.D ($P \leq 0.05$)		7.829	12.382	15.624	18.235	7.2982	5.252
ANOVA $F_{(7,15)}$	S type	240.977	534.782	143.009	31.879	540.328	190.725
	S conc.	95.290	188.405	59.109	268.770	108.122	64.325
	S type × S conc.	17.580	31.973	32.516	24.127	25.320	32.865

[Each value is a mean of five replicates, ±: standard deviation, S: Substrate. † indicates the level of significance at ($P \leq 0.05$) level. Means followed by same letter/s within a column are not significantly different over one another (Least significant different test, ($P \leq 0.05$)]

Table 10 — Inoculum production of *Acaulospora laevis* using chickpea husk substrate (Dry and Compost) and wheat as a host

Type of chickpea husk substrate	Conc. of each substrate (g pot^{-1})	Pattern of Colonization			AM spore number/ 10 g of soil	% Root colonization
		Mycelium	Vesicles	Arbuscules		
Dry	0	+	–	+	23.8±1.30^{g}	21.0±2.00^{f}
	25	+	++	++	151.4±1.34^{d}	46.6±3.20^{e}
	50	+	+++	+++	85.6±2.60^{f}	53.4±3.13^{de}
	100	+	+	++	173.4±3.13^{b}	72.4±1.81^{c}
Compost	0	+	+	++	31.0±2.54^{e}	18.6±2.70^{g}
	25	+	++	++	163.4±3.50^{c}	57.6±1.94^{d}
	50	+	++	++	194.6±3.64^{a}	91.2±2.38^{a}
	100	+	+	+	154.4±3.84^{d}	82.4±1.81^{b}
L.S.D ($P \leq 0.05$)					9.382	10.591
ANOVA $F_{(7,15)}$	S type				545.54	185.725
	S conc.				118.122	59.817
	S type × S conc.				30.430	28.654

[Each value is a mean of five replicates, ±: standard deviation, S: Substrate, AM: Arbuscular mycorrhizae,-: absent, +: scanty, ++: moderate, +++: abundant. † indicates the level of significance at ($P \leq 0.05$) level. Means followed by same letter/s within a column are not significantly different over one another (Least significant different test, ($P \leq 0.05$)]

Table 11 — Inoculum production of *Acaulospora laevis* using chickpea husk substrate (Dry and Compost) and Sorghum as a host

Type of chickpea husk substrate	Conc. of each substrate (g pot^{-1})	Plant height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)
Dry	0	77.0±2.60^{f†}	10.32±0.021^{f}	2.32±0.017^{g}	5.35±0.025^{e}	2.33±0.024^{f}	0.93±0.001^{f}
	25	83.8±1.16^{d}	14.39±0.017^{d}	5.43±0.012^{d}	10.3±0.028^{c}	5.30±0.018^{d}	2.34±0.003^{d}
	50	103.0±1.41^{b}	16.38±0.024^{a}	6.82±0.008^{a}	14.3±0.028^{a}	6.12±0.017^{b}	2.73±0.027^{b}
	100	73.0±2.28^{e}	8.332±0.024^{g}	3.42±0.016^{e}	5.00±0.014^{f}	2.00±0.014^{g}	0.90±0.002^{g}
Compost	0	81.6±1.01^{g}	12.39±0.020^{e}	3.45±0.027^{e}	8.34±0.017^{d}	2.72±0.016^{e}	0.97±0.002^{e}
	25	112.4±1.62^{a}	15.31±0.020^{b}	5.81±0.010^{c}	11.3±0.024^{b}	5.41±0.021^{c}	2.54±0.017^{c}
	50	96.6±1.01^{c}	14.99±0.010^{c}	6.15±0.020^{b}	14.4±0.024^{a}	6.25±0.024^{a}	2.92±0.018^{a}
	100	74.4±2.05^{e}	08.31±0.021^{g}	3.32±0.017^{f}	4.92±0.016^{g}	1.96±0.018^{h}	0.90±0.002^{g}
L.S.D ($P \leq 0.05$)		6.380	0.3295	1.0725	1.5019	0.3295	0.2982
ANOVA $F_{(7,15)}$	S type	96.360	47.735	40.325	830.120	41.586	23.892
	S conc.	125.819	132.929	191.917	182.915	73.116	42.260
	S type × S conc.	47.049	59.329	42.235	45.432	22.925	39.429

[Each value is a mean of five replicates, ±: standard deviation, S: Substrate. † indicates the level of significance at ($P \leq 0.05$) level. Means followed by same letter/s within a column are not significantly different over one another (Least significant different test, ($P \leq 0.05$)]

Table 12 — Inoculum production of *Acaulospora laevis* using chickpea husk substrate (Dry and Compost) and Sorghum as a host

Type of chickpea husk substrate	Conc. of each substrate (g pot^{-1})	Pattern of Colonization			AM spore number/ 10 g of soil	% Root colonization
		Mycelium	Vesicles	Arbuscules		
Dry	0	+	–	–	63.4±2.80^{f}	29.0±1.78^{g}
	25	+	++	++	200.8±2.77^{d}	75.8±2.31^{c}
	50	+	+++	++	250.0±2.28^{a}	82.8±1.72^{a}
	100	+	++	+	48.6±2.15^{g}	25.8±1.72^{h}
Compost	0	+	++	++	80.4±2.87^{e}	38.4±2.05^{e}
	25	+	++	++	213.4±2.80^{c}	78.2±2.56^{b}
	50	+	+++	++	226.0±2.60^{b}	72.8±2.31^{d}
	100	+	++	++	47.6±2.41^{g}	31.8±2.13^{f}
L.S.D ($P \leq 0.05$)					6.5243	9.3295
ANOVA $F_{(7,15)}$	S type				73.116	20.280
	S conc.				41.586	45.272
	S type × S conc.				22.913	39.998

Each value is a mean of five replicates, ±: standard deviation, S: Substrate, AM: Arbuscular mycorrhizae,-: absent, +: scanty, ++: moderate, +++: abundant. † indicates the level of significance at ($P \leq 0.05$) level. Means followed by same letter/s within a column are not significantly different over one another (Least significant different test, ($P \leq 0.05$).

plant height, AM spore number and root colonization while in compost 25 g of substrate resulted in maximum increment in plant height, AM spore number and root colonization (Tables 11 & 12). The inoculum production of *A. laevis* also varied considerably with different hosts and different concentrations of the substrates.

Discussion

Mass production of the dominant AM fungi depends upon the type of host as well as the duration of infection of these symbiotic organisms. It is the host type, which is more important for AM fungal colonization and subsequent spore production AM spore production[27,28].

Variation in the capacity of host plant for mass multiplication of AM fungi might be due to the specific variation in host plant root type, its anatomy and morphology, nutrient and endogenous hormone level characteristics and the environmental interaction[29,30].

Among all the host plants, sorghum showed prominent results pertaining to maximum inoculum production of *G. mosseae*. In contrast, wheat was found to be most compatible host for mass multiplication of AM fungi followed by Barley. Kormanik *et al.*[31] found increased sporulation with Sorghum having finer roots in comparison to maize. Moreover, the suitability of these species could be due to the production of a wide variety of water soluble and volatile organic compounds that may serve as

stimulant, attractants, nutrient sources, and even as genetic regulatory signals for AM fungi for better colonization[32].

Barley used in the present study also acts as a suitable host for increasing AM inoculum density due to its short life cycle, adequate root system, good colonization level and tolerance to low levels of soil phosphorus[6]. It may be due to concentration of substrate used i.e. chickpea husk, as barley has a larger root system, a positive effect on root colonization was observed.

Addition of organic substrate in the soil can efficiently increased plant growth besides promoting mycorrhizal multiplication. It has been reported by various workers[33-35]. Members of Graminae (Poaceae) possess rapidly developing fibrous root systems making them ideal trap plants.

Spore numbers tend to increase with age of the crop. Chaurasia & Khare[36] while mass producing AM fungi with four different host plants reported a gradual increase in root colonization and spore number with period of growth and increase in size of the plants.

AM fungal colonization and subsequent spore formation and production depend upon the type of host as well as the duration of infection of these symbiotic organisms[37]. Generally, with increase in the growth period after infection, root colonization of host also increases. However, this increase in colonization and period do not have greater bearing on spore production. It is the host type, which is more important for spore production[38]. Therefore, the relationship between colonization and rate of colonization with growth period do not vary greatly in different hosts. As in the present investigation, addition of substrates enhanced the mycorrhizal colonization and spore population with different trap plants.

Muthukumar & Udaiyan[39] also reported an enhancement in the AM spore population when they used compost as a substrate. The positive effect of organic matter on AM growth could be an effect of higher humidity since organic matter has a beneficial effect on soil structure and water holding capacity.

Assessment of the effects of substrate and hosts on the response of mycorrhizae is cost-effective and valuable tools for production of inoculum at a large scale. It might be useful to develop package practices for agricultural or horticulture crops.

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