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Inoculum Production of *Acaulospora laevis* using Fresh and decomposed Apple Pomace as Substrate

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Abstract

Because of the biotrophic nature of arbuscular mycorrhizal fungi, it is not been able to use on a commercial scale despite being aware of the potentiality of arbuscular mycorrhizal fungi in agriculture, forestry and horticulture research. For the commercial development of AM inoculants, a number of strategies have been followed time to time with their own merits and demerits. Three plant species viz. wheat, lemon grass and lily grass were examined for mass production of consortium of A. laevis, AM fungus present in the rhizosphere soil after adding different concentration of fresh and decomposed apple pomace as substrate. Out of the three test species, lemon-grass responded as the most suitable host showing highest colonization (89.7±0.50%; 75.0±1.58 spores with fresh and decomposed 86.6±1.90%; 72.2±1.92 spores substrate. It was also observed that plants having higher AM colonization showed higher AM spore production showing a positive correlation. They not only stimulated AM development, but also accelerated the root and shoot growth.

Keywords: Inoculum, *Acaulspora laevis*, AM fungi, apple pomace.

Introduction

The continuing increase in global population coupled with the limitations in the world's supply of natural resources and widespread degeneration of the environment presents a major challenge to the agricultural scientists today. Agriculture, the largest industry on earth, is exhausting the planet's biological support systems. Extensive use of chemicals in developing countries is often untenable because of cost, environmental and safety concerns. The cheap and non-destructive means of achieving high productivity aims at the establishment of a viable low input farming system. In order to implement such a plan, the judicious use of nature's own biofertilizers such as arbuscular mycorrhizal fungi (AMF) appears to be one of the suitable alternatives to this problem as it aids in the growth and multiplication of crop plants which prove to be the most effective alternative to chemical fertilizers for enhancing growth and biomass production Tiwari et al.[1]. Furthermore, modern agricultural practices based on fertilizer and pesticide application and involving mechanical soil preparation can also reduce mycorrhizal inoculum potential Ezawa et al [2]. In certain cases, the application of a mycorrhizal fungal inoculum development and the use of mycorrhizal fungi have grown considerably over the last three decades, with mycorrhizal fungi being seen as a natural means of improving plant production. Recently, particularly important break throughs have been made with regards to the growth of arbuscular mycorhizzal fungi Fortin et al[3]. These advances triggered a shift from the small-scale production of AM fungal inocula in pot cultures that started in the 1980s Dalpe and Monreal[4], towards the development of large-scale inoculum production and the production of a number of mycorrhizal fungal based products for use in agriculture, horticulture, forestry and the revegetation and restoration of disturbed or polluted sites. The large-scale use of mycorrhizal fungal inocula for plant production is still in it's infancy and high production costs, variable inoculum quality and official registration must all be overcome before a product can be marketed Dalpe and Monreal[4]. Currently, more than 20 companies are producing mycorrhizal inoculum world-wide Gianinazzi and Vosatka [5].

The large-scale application of mycorrhizal fungi is strongly dependent on the quality, availability and cost of inocula. The major challenge facing the development of a commercially viable AM fungal inoculum has been, and still is associated with the fact that AM fungi are strict biotrophs (i.e. they must be associated with a host plant to grow and complete their life-cycle) and that there is no means of producing AM fungi in axenic culture. Advances in inoculum production are needed to provide readily available and economically viable commercial mycorrhizal inocula. Intact natural terrestrial ecosystems are typically rich in mycorrhizal fungi, and soil samples collected from them usually exhibit a high mycorrhizal inoculum potential. In comparison to this, though inoculation by AM fungal spores significantly affects plant growth, the efficiency of this natural biofertilizer is restricted to a narrow spectrum of conditions. Confining all the factors into consideration, present study was undertaken to assess the mass production of *A. laevis* using different plant hosts with different concentrations of fresh and decomposed apple pomace.

Material and Methods

Mass production of AM fungi: Selection of appropriate host: For mass multiplication of AM fungi three host plant species viz. lemon grass, lily grass and wheat (figure-1) were tried for selection of suitable host for mass production of AM spores.

Selection of substrates: Two substrates i.e., fresh apple pomace and decomposed apple pomace collected from agroindustry Parwanoo (Himachal Pradesh) with traditional substrate i.e., sand: soil (1:3) in different ratios was selected to find out the most suitable substrate for the mass production of *A. laevis*.

Source of AM fungal spores: The rhizospheric soil samples of two plants under study i.e., *Bacopa monnieri/ Vitex negundo.* were examined to isolate the dominant AM fungi by using Gerdemann and Nicolson[6] technique, which was found to be the species of *Acaulospora.*

Selection of AM fungi: The efficient strain of AM fungus (*A. laevis*) was isolated from the rhizospheric soil sample of *Bacopa monnieri/ Vitex negundo*. The AMF propagules were obtained from the soil by 'Wet Sieving and Decanting Method' Gerdemann and Nicolson [6]. The starter inoculum of AM fungus (*A. laevis*) was raised by 'Funnel Technique' Menge and Timmer [7] using *Zea mays* as host. After 40 days, seedling roots were processed to study AM colonization[8], and soil samples were studied for spore quantification. AM fungal endophyte was identified by using the keys of Walker [9], Mortan and Benny[10].

Filling of pots: Pots were filled with sterilized soil: sand (1:3) and different concentration of substrate was added to make the final weight 1500 gm. Ten percent of the inoculum was added to the mixture in the upper part. The inoculum consisted of AM spores and AM colonized root pieces.

Surface disinfection of seeds and sowing of seedlings: Seeds of wheat were surface sterilized with 10% solution of Sodium hypochlorite for 1 - 2 min and then washed thoroughly with distilled water to remove sodium hypochlorite before sowing them. Ten seeds were selected and sown approximately 2 cm below soil in each pot. Ten days old seedlings of lemon grass and lily grass were procured from Chuharpur herbal park, Khizrabad, Yamunanagar which showed almost no mycorrhization.

Isolation of AM fungal spores: *A. laevis* spores were collected from the soil by wet sieving and decanting method[6] and enumerated according to Adholeya and Gaur [11].

Estimation of VAM root colonization: AM root colonization was estimated by Phillips and Hayman[8]. The total percentage of root colonization was determined by using the following formula:

$$\% \text{ Mycorrhizal root colonization} = \frac{\text{Total number of root segements colonized}}{\text{Total number of root segements examined}} \times 100$$

Multiplication and maintenance of AM fungi: The pure culture of isolated AM fungi was used for pot culture inoculations of lemon grass, lily grass and wheat *as* the hosts for their multiplication. Each treatment with different hosts and substrates was replicated five times. The plants were watered regularly and nourished by Hoagland nutrient solution (100 ml/pot) every 15 days up to 75 days.

Statistical analysis: The data was analysed with the help of two factor analysis of variance. The change in mycorrhizal spore count and percentage of root colonization was analysed both along hosts and along different substrates. Means were then ranked at $P \leq 0.05$ level of significance using Duncan's Multiple Range Test for comparison.

Results and Discussion

As evident from the results (table 1, 2) after 75 days of inoculation, AM spore population was found to be maximum at 600 gm. of fresh as well as decomposed apple pomace with all the three host plants i.e. wheat, lemon-grass and lily. Percentage mycorrhizal root colonization also showed the similar trend suggesting a positive correlation between spore population and root colonization. All the parameters were found to increase constantly from control to 200 gm. to 400 g. and 600 gm.

Best results of AM spore count and percentage root colonization were observed in lemon-grass (89.7 ± 0.50% ; 75.0 ± 1.58) with fresh apple pomace and (86.6± 1.90%; 72.2 ± 1.92) with decomposed apple pomace followed by lily (89.3 ± 0.61%; 37.6± 2.07) with decomposed apple pomace. With decomposed substrate, wheat plant showed better results than lily (72.6±2.81%) as regards the percentage root colonization.

The substrate concentration also influenced the type of mycelium, vesicles and arbuscules. Mycelial growth was observed in all the concentrations except control. Very few vesicles were reported with 200 gm. substrate in all the host plants. In all the trap plants only vesicles were observed with 400 gm. substrate. In the lily host plant, arbuscules were observed with only 600 gm. of substrate suggesting thereby that in the current examination, application of different substrates influenced the formation of vesicles and arbuscules. Also, a positive correlation was observed between root colonization and spore production in all the treatments that could be attributed to the soil nature and the amount of substrate mixed thereby affecting the root infection, number of vesicles per root and ultimately the spore population. Enhancement in the formation of vesicles and arbuscules as an effect of application of substrate has been reported by Baby and Manibushanrao[12]. Muthukumar and Udaiyan[13] also reported an increment in AM spore number, when they used as compost at substrate. Stimulation of AM sporulation by organic substances like humic acid or chitin has also been described previously[14]. Jeffries and Barea [15]also found increased fungal hyphal growth and AM spore formation due to addition of organic matter. Likewise, increased mycorrhizal root colonization with organic substrate has been reported in

Sorghum bicolor 16. In the present study, application of substrate wastes significantly stimulated sporulation, promoting increase in number many folds. Spore density actually depends upon the concentration of substrates used as these wastes enhance the nutrient uptake thereby improving the root system, which favourably enhances the spore population in its surroundings.

Table-1
Effect of fresh apple pomace on mass production of *Acaulospora laevis* with one way and two way Anova

Substrate conc. (gm)	Sand : Soil (gm)	Host plant used	Fresh shoot weight (gm)	Dry shoot weight (gm)	Fresh root weight (gm)	Dry root weight (gm)	Percent mycorrhizal root colonization	Am spore count / 50 gm soil
Control	375:1125	Wheat	*10. 3 ± 0.53^{de}	2. 2 ± 0.45^{f}	12. 4 ± 0.51^{de}	8. 3 ± 0.43^{de}	37. 3 ± 3.65^{de}	13.4 ± 2.97^{ef}
200	325 : 975	Wheat	13. 3 ± 0.47^{cd}	4. 3 ± 0.43^{de}	13. 3 ± 0.47^{d}	9. 3 ± 0.52^{d}	39.0 ± 1.37^{d}	18.6 ± 2.61^{de}
400	275 :825	Wheat	16. 3 ± 0.47^{c}	4. 4 ± 0.27^{d}	30. 3 ± 0.37^{cd}	18.3 ± 0.43^{c}	59.1 ± 1.99^{bc}	23.2 ± 2.86^{cd}
600	225: 675	Wheat	16. 5 ± 0.40^{c}	4. 5 ± 0.45^{d}	54.7 ± 0.33^{c}	32.2 ± 0.43^{ab}	69.3 ± 1.49^{b}	39.2 ± 1.92^{ab}
Control	375:1125	Lemon grass	6. 7 ± 0.66^{e}	2. 4 ± 0.51^{e}	5 .0 ± 0.68^{f}	4.0 ± 0.68^{ef}	32.6 ± 1.49^{f}	12.0 ± 1.58^{f}
200	325 : 975	Lemon grass	13. 6 ± 0.49^{cd}	4. 3 ± 0.44^{de}	6. 3 ± 0.66^{ef}	2.4 ± 0.64^{f}	45.5 ± 2.49^{cd}	24.6 ± 3.85^{cd}
400	275 :825	Lemon grass	16. 4 ± 0.54^{c}	6. 4 ± 0.47^{c}	7. 4 ± 0.42^{e}	4. 5 ± 0.35^{e}	74.0 ± 2.24^{ab}	34.0 ± 2.92^{c}
600	225: 675	Lemon grass	20. 4 ± 0.89^{b}	7. 5 ± 0.43^{b}	13. 8 ± 0.43^{d}	7. 0 ± 0.47^{de}	89.7 ± 0.50^{a}	75.0 ± 1.58^{a}
Control	375:1125	Lily grass	11. 9 ± 0.38^{d}	5. 8 ± 0.55^{cd}	36. 7 ± 0.71^{bc}	10.6 ± 0.55^{cd}	35.5 ± 4.97^{e}	17.6 ± 1.14^{e}
200	325 : 975	Lily grass	18. 8 ± 0.62^{bc}	6. 9 ± 0.55^{b}	54.7 ± 0.48^{b}	19.5 ± 0.51^{bc}	46.3 ± 3.41^{c}	21.8 ± 2.49^{d}
400	275 :825	Lily grass	22.2 ± 0.74^{ab}	7.8 ± 0.19^{ab}	58.3 ± 0.72^{ab}	21.6 ± 0.93^{b}	73.1 ± 4.26^{b}	36.4 ± 2.07^{bc}
600	225: 675	Lily grass	29. 2 ± 0.38^{a}	8. 1 ± 0.28^{a}	76. 6 ± 0.58^{a}	33.5 ± 0.44^{a}	89.3 ± 0.61^{a}	37.6 ± 2.07^{b}
LSD ($P \leq 0.05$)			1.4224	0.5489	0.7692	0.7041	7.2386	3.1127
ANOVA (F 11,24			563.260	109.383	7761.039	1867.929	269.757	246.300
Host Conc.			623.095	313.175	94765.242	3526.094	93.397	203.199
Substrate Conc.			1548.632	184.970	3777.638	2020.951	487.367	572.008
Host × Substrate Conc.			94.738	21.189	504.566	560.103	33.997	107.791

* = Mean of five replicates, ±= Standard deviation, Mean value followed by differ alphabet/s within a colum do not differ significantly over one other at $P \leq 0.05$ lead by Duncan's Multiple Range Test .

Table-2
Effect of decompose apple pomace on mass production of *Acaulospora laevis* with one way and two way Anova

Substrate conc. (gm)	Sand : Soil (gm)	Host plant used	Fresh shoot weight (gm)	Dry shoot weight (gm)	Fresh root weight (gm)	Dry root weight (gm)	%root colonization	Am spore count / 50 gm soil
Control	375:1125	Wheat	*3.42 ± 0.33^{ef}	0.50 ± 0.16^{ef}	1.7 0 ± 0.07^{f}	0.36 ±0.13^{d}	41.0 ± 2.53^{cd}	25.4 ± 3.13^{e}
200	325 : 975	Wheat	4.46 ± 0.36^{e}	0.60 ± 0.30^{e}	4.22 ± 0.15^{e}	0.82 ± 0.08^{c}	62.5 ± 2.76^{b}	43.4 ± 1.95^{cd}
400	275 :825	Wheat	5.38 ± 0.31^{de}	1.38 ± 0.29^{de}	4.36 ± 0.25^{e}	1.10 ± 0.14^{c}	71.4 ± 2.41^{ab}	49.0 ± 0.71^{bc}
600	225: 675	Wheat	14.5 ± 0.38^{c}	2.34 ± 0.27^{cd}	7.24 ± 0.38^{d}	2.68 ± 0.13^{bc}	72.6 ± 2.81^{ab}	56.0 ± 3.00^{b}
Control	375:1125	Lemon grass	4.40 ± 0.46^{e}	1.40 ± 0.34^{d}	2.52 ± 0.44^{ef}	0.64 ± 0.27^{cd}	41.4 ± 2.05^{c}	31.8 ± 1.79^{d}
200	325 : 975	Lemon grass	7.40 ± 0.32^{d}	2.48 ± 0.37^{cd}	6.34 ± 0.34^{de}	1.34 ± 0.34^{c}	53.1± 2.86^{bc}	45.0 ± 2.23^{c}
400	275 :825	Lemon grass	11.5 ± 0.38^{cd}	4.54 ± 0.42^{c}	8.48 ± 0.41^{cd}	2.60 ± 0.35^{bc}	63.1 ± 3.32^{b}	71.0 ± 1.41^{a}
600	225: 675	Lemon grass	19.5 ± 0.27^{bc}	5.46 ± 0.34^{bc}	14.3 ± 0.33^{c}	11.4 ± 0.38^{b}	86.6± 1.90^{a}	72.2 ± 1.92^{a}
Control	375:1125	Lily grass	26.4 ± 0.27^{b}	7.16 ± 0.23^{b}	35.4 ± 0.30^{bc}	12.4 ± 0.42^{ab}	29.8± 2.08^{d}	25.2 ± 1.92^{ef}
200	325 : 975	Lily grass	34.3 ± 0.33^{ab}	9.54 ± 0.36^{ab}	39.4 ± 0.37^{b}	14.3 ± 0.38^{a}	42.1 ± 2.22^{c}	29.4 ± 1.94^{de}
400	275 :825	Lily grass	38.2 ± 0.34^{a}	10.4 ± 0.36^{a}	39.5 ± 0.30^{b}	14.3 ± 0.42^{a}	52.9 ± 2.68^{bc}	56.0 ± 1.58^{b}
600	225: 675	Lily grass	39.3 ± 0.30^{a}	10.7 ± 0.25^{a}	40.3 ± 0.23^{a}	14.5 ± 0.44^{a}	70.8 ± 1.21^{ab}	62.4 ± 1.67^{ab}
LSD ($P \leq 0.05$)			0.8742	0.7972	0.8152	0.808	6.3762	5.181
ANOVA (F 11,24			8100.952	762.379	13022.203	1954.886	220.724	340.976
Host Conc.			32290.717	2065.329	132792.5	8100.930	255.179	213.154
Substrate Conc.			3993.528	542.642	18086.398	4189.886	613.993	1749.806
Host × Substrate Conc.			280.489	34.752	3406.263	472.798	28.474	31.931

* =Mean of five replicates, ±= Standard deviation, Mean value followed by differ alphabet/s within a colum do not differ significantly over one other at $P \leq 0.05$ lead by Duncan's Multiple Range Test .

Present results are in accordance with Tanwar et al [17] who found an increase in AM spore population, % root colonization, fresh and dry root and shoot weight as an effect of sugarcane bagasse and ash. AM fungal colonization depends upon the type of host as well. Because, AM fungal spores multiply only in association with plant roots, which act as suitable ecological niche for germination of spores[1,8]. Chaturvedi et al[19] also evaluated influence of host on AM fungal community using a monocot (*Zea mays*) as a trap crop in soils collected from different localities. In the present study, both lemon-grass and lily-grass proved to be suitable hosts along with wheat for mass multiplication of *A. laevis,* as these grew fast with extensive root system while providing favourable conditions for higher root colonization and sporulation. AM fungi respond to host exudates with extensive hyphal growth and branching Giovanneti et al[20]. The healthy plant growth and more root system development in the current analysis are also confirmed by the documentation of Scheloske et al[21] who reported high degree of mycorrhizal colonization and more dry weight in mycorrhizal roots compared to non-mycorrhizal control. Moreover, AM fungi enhanced plant growth at all the concentrations of applied substrates as compared to control ones (without substrates). Similar were the observations made by Sharma et al [22], Tanwar et al[17] and Chauhan et al [23]suggesting thereby that without mycorrhizal fungi, plants showed poor growth.

Wheat

Lemon grass

Lily grass

Figure-1

Mass Production with three hosts wheat, lemon grass, lily grass

Conclusion

Under certain conditions, the mycorrhizal inoculum potential of a soil is reduced and artificial inoculation can help enhance plant survival, establishment and growth. Presently, the benefits of using mycorrhizal fungi can be clearly measured when plants are grown under suboptimal conditions. However, this is not always a meaningful benefit to growers, and the mass screening of mycorrhizal fungal strains should be done to find strains capable of giving a more rapid and consistent benefit in terms of plant growth. In this period of enhanced environment awareness, there is an increasing demand for more environmentally friendly and sustainable plant production technologies. This will hopefully lead to quicker, but equally efficient technologies to market products more rapidly and, in turn, pave the way for a healthier environment.

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