SUGARCANE BAGASSE: A NOVEL SUBSTRATE FOR MASS MULTIPLICATION OF *FUNNELIFORMIS MOSSEAE* WITH ONION AS HOST

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ABSTRACT

A pot experiment was conducted to test the influence of sugarcane bagasse (fibrous waste left over after sugarcane juice) as a substrate for the inoculum production of *Funneliformis mosseae* in terms of AM root colonization, spore number and AM colonization pattern using onion as host plant. Their effect on growth performance of onion was also recorded in terms of increase in plant height, above ground fresh and dry weight, root length, root fresh and dry weight. The experiment was a 3×4 factorial design employing three forms of bagasse (fresh, dry and compost) and their four different concentrations (without substrate, 25 g/pot, 50 g/pot and 100 g/pot). The results showed that the compost bagasse promoted higher AM root colonization and sporulation, followed by dry and fresh bagasse. Maximum AM spores, vesicles, arbuscules and 100 per cent colonized roots were detected in onion plants supplemented with 25 g compost bagasse. This treatment also influenced significant increase in plant growth. Although, increasing substrate concentration proved stimulatory to AM fungus as well as onion plant growth but highest concentration (100 g) proved inhibitory. Hence, compost bagasse can be exploited for the multiplication of *F. mosseae* by farmers as it is a cost effective method of production.

Keywords: *Allium cepa*, arbuscular mycorrhizal fungi, compost bagasse, *Funneliformis mosseae*, inoculum production

INTRODUCTION

The significant effects of Arbuscular mycorrhizal (AM) fungi on plant growth and nutrition has lead to an increase in the use of commercial AM fungal inoculum as biofertilizer (Schwartz et al., 2006). However, the obligate biotrophic nature of AM fungi has long been accepted as an obstacle for the large scale production of inoculum.

Several culture techniques are being used for the multiplication purpose but the multiplication of AM fungi in conjunction with suitable host plant roots under pot condition with appropriate substrate is the most widely used method (Ferguson and Woodhead, 1982).

The type of substrate used for AM fungal multiplication is an important factor for the propagation of AM fungal propagaules. Organic wastes are rich in nutrients and their positive influence on AM root colonization have been reported by many workers (Gaur and Adholeya, 2002; Gyndler et al., 2005; Perner et al., 2006; Kuppusamy and Kumutha, 2011; Douds et al., 2010; Tanwar et al., 2013). In Haryana, sugarcane is grown in districts like Yamuna Nagar, Kurukshetra, Ambala and Karnal which contribute 60% of sugarcane production. One tone of sugarcane produces approximately 33 kg of sugarcane bagasse, the residue left after pressing sugarcane stalks to extract juice. Out of which, 20-30% of bagasse used to meet the steam and power requirements and the remaining is wasted. This low cost substrate can be utilized for AM fungi inoculum production as such or after some treatment like drying and composting and thus could acts as suitable niche for the multiplication of AM fungi.

The physiology of the host plant also influence spore production (Simpson and Draft, 1990). Selection of appropriate nurse plant for the production of AM inoculum is needful as host plant plays an important role in AM fungal propagation. Plant tolerance to wide range of temperature, low levels of phosphorus and inherent resistant to diseases and insects relatively are important determinants for multiplication of AM fungi (Millner and Kitt, 1992). Onion (*Allium cepa* L.), a member of Liliaceae family is often colonized by AM fungi (Manjunath and Bagyaraj, 1984; Fusconi et al., 2005; Perner et al., 2007) and is recommended as suitable nurse plant for the multiplication of *Funneliformis mosseae* in organic waste amended medium (Kaushish et al., 2011). In this experiment also onion is used as nurse plant for multiplication of *F. mosseae* in the presence of sugarcane bagasse substrate in its three different forms (fresh, dry and compost) with an aim to see a) the possible role of different forms of bagasse in enhancing *F. mosseae* spore population and root colonization, and b) whether bagasse addition affects host plant growth improvement.

MATERIALS AND METHODS

Experimental design

The experiment was a 3×4 factorial in a completely randomized design employing three forms of bagasse substrates (fresh, dry and compost) and four different concentrations of each substrate used (without substrate, 25 g/pot, 50 g/pot and 100 g/pot) with onion as host plant. Each treatment was replicated five times giving a total of 60 pots.

Soil characteristics

The experiment was carried out in a greenhouse at the Botany Department, Kurukshetra University, Kurukshetra, Haryana, India, from Mid November to February 2012. Light was provided by cool white fluorescent lamps (8000 lux) under a 16 hr photoperiod. The soil characteristics were: sand, 64.2%; silt, 21.81%; clay, 3.90%;

starting pH, 6.8; EC, 0.25 $dS \cdot m^{-1}$; total N, 0.042%; available P, 0.017% and organic carbon, 0.06%.

Selection and preparation of sugarcane bagasse substrate

Sugarcane bagasse was collected from local sugarcane juice vendors and was processed before use. The substrate was divided into three parts. One part was used as such called as fresh substrate, the other part was first sun dried for one day and then in the oven at 70°C for two consecutive days, grounded to make fine powder and used as dry substrate. The remaining part of the bagasse was packed in net bags and then buried under the soil for three months, for compost formation. All the substrates were autoclaved at 121ºC for 2 hr prior to use.

Selection of nurse plant

In the present study, onion (*Allium cepa* L.) was selected and used as host.

AM fungus isolation and production of starter inoculum

The AM fungal spores were isolated from the rhizospheric soil of onion by the wet sieving and decanting technique (Gerdemann and Nicolson, 1963) and identified using the key of Schenck and Perez (1990). *Funneliformis mosseae* (Nicol. and Gerd.) Walker and Schüßler (earlier known as *Glomus mosseae*) was found to be the dominant AM fungus. Starter inoculum of the AM fungus was raised using the funnel technique (Menge and Timmer, 1982) with maize for over a three month period.

Experimental setup

Soil from the experimental site was passed through a 2 mm sieve, added to a sand:soil (1:3) mix and autoclaved at 121ºC for 2 hr prior to use. Different concentrations (0, 25, 50 and 100 g) of each form of substrate (fresh, dry and compost) were added to earthen pots (25.4 × 25 cm), thoroughly mixed with sand:soil mixture to make final volume 2 kg. To this 200 g of AM inoculum (chopped AM colonized root pieces of maize, along with soil containing 350-420 AM spores per 100 g) raised from funnel technique was added. Onion seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite for 10 min, and subsequently washed with sterilized deionized water and placed in a shallow tray containing sterilized soil:sand (3:1) to germinate. At 20 days after emergence, single seedling was transplanted to each pot above the inoculum. Plants were watered daily to maintain the moisture at approximately 60% water holding capacity of the soil and 100 mL/pot Hoagland nutrient solution (Hoagland and Arnon, 1950) (without KH_2PO_4) was added to each plant at 15 day intervals.

Harvest and Analysis

Vegetative growth response was assessed after 90 days of planting, by uprooting the whole plant mechanically. Firstly plant height (cm) was recorded followed by washing of plants with running tap water and then plants were divided into roots and shoots. Roots and shoots were weighted separately 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 AM spore quantification was done using the procedure of Gerdemann and Nicolson (1963). The percentage mycorrhizal root colonization was determined using the method of Phillips and Hayman (1970) after clearing roots in 10% (w/v) KOH followed by staining

with 0.05% (w/v) trypan blue. The presence of various AM fungal structures i.e., mycelium, arbuscules and vesicles were recorded as nil (-), scanty (+), moderate (++) and abundant (+++).

Statistical analysis

The experimental data was subjected to analysis of variance and means were separated with least significant difference test using the Statistical Package for Social Sciences (ver. 11.5, Chicago, Ill.).

RESULTS

Effect of different forms of sugarcane bagasse on multiplication of *F. mosseae*

It is evident from the Table 1 that all the three forms of bagasse increased the per cent AM colonized roots and spore number compared to control (without substrate) but the degree varies. AM colonized roots were characterized by the presence of extramatrical hyphae, intraradical hyphae, arbuscules and vesicles. Among the various substrates tested, compost bagasse stimulated the production of maximum number of spores and per cent root colonization followed by dry and fresh bagasse respectively. Maximum spores and AM colonized roots were recorded in onion plants supplemented with 25 g compost bagasse. Abundant arbuscules and vesicles were seen in the onion roots supplemented with 25 g compost bagasse, followed by 50 g dry bagasse and 50 g fresh bagasse respectively. After 90 days of inoculation, vesicle formation was not detected in plants amended with 100 g fresh and dry substrate. The later not even influenced the formation of arbuscules.

Progressive increase in the substrate concentration was not found to be stimulatory for *F. mosseae*. As increase in the concentration of dry bagasse from 25 to 50 g stimulated the formation of AM fungal propagules and after that 100 g bagasse showed inhibitory effects. In the same way least concentration of fresh and compost bagasse i.e., 25 g proved beneficial and increased concentration decreases mycorrhization. Out of fresh and dry bagasse, second most effective results were recorded in 50 g dry bagasse. A positive relationship was recorded between spore number and root colonization rate.

Effect of sugarcane bagasse and *F. mosseae* on growth performance of onion

Soil inoculation with bagasse significantly increased all the plant growth parameters (plant height, above ground fresh, dry weight, root length and root fresh, dry weight), besides increased per cent colonized roots and spore number, except for highest bagasse concentration (100 g). It is clear from the Table 2, significant increase in plant height was recorded in plants amendment with compost bagasse and 25 g compost bagasse resulted in maximum increase in plant height as compared to other treatments and control. At the same time, highest above ground fresh weight and maximum increase in root length was also recorded in the same treatment. Onion plants supplemented with 50 g dry bagasse increased the above ground fresh weight and root fresh and dry weight which was much superior over plants without bagasse.

DISCUSSION

The ability of AM fungus to spread and form hyphal network in the substrate is influenced by their physical properties of soil such as compaction and water retention rate (Gaur and Adoleya, 2000). Onion roots were successfully colonized by the *F. mosseae* hyphae in the presence of sugarcane bagasse but the degree varies with different substrates and their concentrations. *F. mosseae* responded more positively to the soil supplemented with compost bagasse than fresh and dry bagasse for its multiplication as well as for increasing most of the growth characteristics of onion and highlighted the synergistic interaction between all the three factors i.e., fungus, plant and substrate. As stated increasing fresh and dry bagasse concentration proved inhibitory to AM fungi which could be attributed to the high nutrient content of these materials or may be due to the presence of phytotoxic substances.

Sugarcane bagasse is a good source of cellulose, hemicellulose and lignin and earlier reports suggest reduction in root colonization by AM fungi in the presence of cellulose in the culturing media (Avio and Giovannetti, 1998; Gryndler et al., 2003). In the present investigation also, at higher substrate concentration, a slight reduction occurs in AM fungal multiplication and onion growth. This suggested the possible inhibitory effects of increased bagasse concentration to AM fungi. However, low addition level of bagasse proved beneficial for the AM fungi survival and proliferation as it increased root colonization as well as spore density. These results are in agreement with our previous finding (Tanwar et al., 2010), while using bagasse with sorghum and maize for the multiplication of *F. mosseae*.

There is an increase in the available water content of soil on addition of sugarcane bagasse (Kameyama et al., 2010) which could be an important factor instrumental for the multiplication of AM fungi. Composting is considered to be one of the most suitable ways of converting organic wastes into simpler products that are beneficial for plant growth (Stantiford, 1987) and considered appropriate for the AM fungi and host plants (Linderman and Davis, 2001). Microbial decomposition and mineralization of cellulose, hemicellulose, lignin and bagasse proteins increased soil nutrient content especially N, P, Ca and Mg required for plant growth (Meunchang et al., 2005). In the present investigation also soil amendment with compost bagasse proved more suitable for AM fungal multiplication and plant growth as compared to other two substrates.

Increased AM colonization of roots enhanced the population of AM spores as observed in the present study, since these two phenomenon are closely related to each other (Hayman, 1970). Increased sporulation might also be related to increased root biomass of the host plant due to the increase in the available nutrients in the soil on addition of bagasse or due to higher water holding capacity of sugarcane bagasse. Soil amendments with organic residues change the soil properties and also modify the plant and fungal responses to symbiosis. Similarly, Sousa et al., (2012) reported favourable response of goat manure incorporation on AM spore and glomalin production in the rhizosphere of cotton.

Onion plants used in the present study acts as a suitable host for increasing inoculum density due to its short life cycle, adequate root system, good colonization level and tolerance to low levels of soil phosphorus (IJdo et al., (2011). Enhanced root

colonization by *F. mosseae* due to the presence of abundant spores in the rhizosphere of onion has lead to increased root proliferation and surface area of roots which thereby absorb more nutrients from the soil and its accumulation in plants and hence plant growth. These results correspond with observations of Sharma and Adholeya (2000) and Bolandnazar (2009).

A positive interaction was observed between the host plant, *F. mosseae* and sugarcane bagasse and depending upon the availability of substrates, either compost or dry bagasse which can be utilized for the inoculum production of *F. mosseae*. This is a simple and inexpensive method of multiplication of AM fungi where the colonized onion roots along with the rhizospheric soil containing *F. mosseae* spores and hyphae can be used as source of crude inoculum.

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mosseae with onion as host

Type of sugarcane substrate	Conc. of each substrate (g pot^{-1})	AM spore number /10 g of soil	AM root colonization (%)	Pattern of colonization		
				Mycelium	Vesicles	Arbuscules
Fresh	0	85.80±9.42e†	59.52±7.13c	+	+	+
	25	223.0±12.80c	74.00±4.95b	++	+	++
	50	132.6±14.33d	75.32±6.40b	++	++	++
	100	98.40±17.39e	50.09±5.00d	+	-	+
Dry	0	76.40±8.73ef	55.73±10.75	+	+	+
	25	163.2±18.84cd	61.08±9.45c	++	+	+
	50	325.0±12.51b	98.00±4.47a	+++	++	++
	100	53.20±10.92f	36.70±5.24e	+	-	-
Compost	0	73.00±7.58ef	50.40±7.23d	+	+	+
	25	428.6±18.19a	100.0±0a	+++	+++	+++
	50	236.2±15.9c	76.40±4.16b	+++	+	++
	100	108.6±9.71de	66.24±5.01c	++	+	+
L.S.D (*P*≤0.05)		17.2368	8.113			
ANOVA (F2,48)	S type	304.114	44.160			
(F3,48)	S conc.	875.33	238.54			
(F6,48)	S type × S conc.	14.331	45.331			

Each value is a mean of five replicates, ±: standard deviation, S: Substrate, AM: Arbuscular mycorrhiza, -: 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 difference test, *P*≤0.05).

response of onion

Type of sugarcane substrate	Conc. of each substrate (g pot^{-1})	Plant height (cm)	Above ground weight (g)		Root length (cm)	Root weight (g)	
			Fresh	Dry		Fresh	Dry
Fresh	0	33.95±5.35e†	10.10±0.72de	1.69±0.36d	17.28±1.28e	1.45±0.30de	0.68±0.13d
	25	62.90±6.32c	23.66±0.60d	3.03±0.27b	28.64±2.43b	2.67±0.30bc	1.05±0.15bc
	50	66.66±7.43c	30.03±1.12c	2.86±0.29b	29.90±0.93b	2.22±0.44c	0.99±0.19c
	100	27.54±4.34f	20.28±0.85d	1.07±0.30e	20.16±1.59c	0.95±0.15e	0.34±0.07f
Dry	0	30.72±2.51e	6.700±0.91e	1.46±0.56d	11.44±0.79f	1.07±0.13e	0.67±0.07
	25	60.18±2.90c	12.11±1.21	2.30±0.25c	12.10±0.44f	1.94±0.19d	0.93±0.19c
	50	75.58±5.03b	45.32±1.75ab	4.80±0.23a	19.72±1.44c	4.71±0.39a	2.09±0.36a
	100	29.20±2.21e	4.371±0.51f	1.07±0.15e	9.761±0.60	0.98±0.14e	0.46±0.10e
Compost	0	38.16±4.89d	9.381±0.71de	1.04±0.13e	16.10±1.70d	0.85±0.11f	0.56±0.15d
	25	84.70±3.40a	49.02±6.27a	4.32±0.77a	32.48±2.72a	3.15±0.36b	2.02±0.17a
	50	80.18±4.07a	31.04±4.16c	2.97±0.17b	28.02±2.32b	2.27±0.24c	1.42±0.23b
	100	83.68±5.47a	37.59±5.17b	3.50±0.37ab	24.22±2.94bc	2.15±0.12c	1.19±0.17bc
L.S.D (P≤0.05)		6.0274	3.534	0.5084	2.2793	0.3331	0.2304
ANOVA (F2,48)	S type	115.492	163.905	51.895	94.195	91.958	49.384
(F3,48)	S conc.	548.43	239.432	432.76	765.22	523.54	286.55
(F6,48)	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≤0.05 level. Means followed by same letter/s within a column are not significantly different over one another (Least significant difference test, P≤0.05).