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ORIGINAL RESEARCH ARTICLE

Influence of *Glucanoacetobacter Diazotrophicus* On The Rootcolonization Of *Glomus Fasciculatum* And Growth Of Sugarcane

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ABSTRACT

In the present study, thirteen locations were selected in the Cuddalore district of Tamil Nadu, to study the occurrence of AM fungi in sugarcane soils. The survey revealed the presence of AM fungi in the sugarcane soils. The sample collected from Vallampadugai recorded the highest root colonization percentage and spore number and Pichavaram recorded the least. Five different AM fungi were isolated from sugarcane rhizosphere soils collected from each of the above mentioned thirteen locations and they are identified as *Glomus mosseae*, *Glomus fasciculatum*, *Glomus versiforme*, *Acaulospora laevis* and *Gigaspora margarita*. Among the five different AM fungal isolates, *G. fasciculatum* recorded highest root colonization percentage, spore number, acid and alkaline phosphatase enzyme activities. The growth of sugarcane in sterilized and unsterilized soils was compared in pot culture conditions so as to determine relative mycorrhizal dependency (RMD) of this crop. The sugarcane grew better in unsterilized soil compared to sterilized soil. The RMD was found to be 31.80 per cent and sugarcane considered as moderately dependent plant for mycorrhiza. The effect of inoculation of five different AM fungi was estimated as MIE. Among the five AM fungi tested, *G. fasciculatum* recorded higher MIE than other AM cultures. The effect of different organic amendments, farm yard manure (FYM), groundnut cake (GNC), pressmud (PM) and neem cake (NC) on the growth parameters and mycorrhizal colonization of sugarcane var. CoSi 98071 was studied in pot culture. Among the different organic amendments, FYM recorded highest per cent root colonization, spore number, plant height and can girth on 180 DAP. The investigation conducted in the pot culture house of Department of Microbiology, clearly indicated that the AM fungi *G. fasciculatum* along with different organic amendments was found to influence the growth and development of sugarcane.

Key words: Sugarcane, AM fungi, *G. fasciculatum* and Organic amendments.

1. INTRODUCTION

Sugarcane, being the long duration crop producing huge quantum of crop biomass, demands for nutrient inputs in large quantities. It is estimated that on an average the sugarcane removes around 100:60:225 kg of N, P_2O_5 and K_2O respectively for the production of 100 tonnes of cane yield [1]. Unless adequate supply of these nutrients is ensured, the sugarcane yield tends to decline even on most fertile soils. However, the indiscriminate prescription of inorganic fertilizer alone in the long run deteriorates soil health resulting in drastic reduction of cane yield. Hence, there is a permanent need to preserve the soil fertility with balanced proportion of major, secondary and micronutrients for sustainable crop productivity. The approach of integrated nutrient management through biofertilizers with inorganic fertilizers in judicious proportions will go a long way to augment the important strategy of enhancing the soil fertility for increased crop productivity especially in a long duration crop like sugarcane.

Arbuscular mycorrhizae (AM fungi) are ubiquitous in nature and their occurrence is obvious in association with plants grown in

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cultivated soils, sand dunes, coal mines and aquatic environments [2], the environments like marine habitats [3], saline patches [4], mangrove vegetation [5], different types of soils [6], agroforestry trees in alfisol. They were known to enhance plant growth and biomass through better uptake of nutrients with water, resistance to drought and increased tolerance to invading plant pathogens [7].

The association between plants and mycorrhizal fungi is wide spread, occurring in 80% of the plant species. Arbuscular mycorrhizal (AM) association is the common and prevalent among the different types of mycorrhizae. AM fungi is the most abundant kind of mycorrhizae found in association with every taxonomic group of plants and the list of species not infected is probably far shorter than the infected ones, these fungal associations are beneficial to crop plants in many ways, including enhancing the nutrient availability especially phosphorus, enhance water uptake and induces resistant against diseases and increase the yield [8].

2. MATERIALS AND METHODS

2.1. Survey of AM occurrence in sugarcane fields

The survey of AM (Arbuscular mycorrhizae) fungal occurrence was conducted from different locations of Cuddalore District of Tamilnadu, India, where sugarcane (*Saccharum officinarum*) is grown as a commercial crop. The locations selected for the present study were, Annamalai Nagar, Vilagam, Arasur, Cuddalore, Vallampadugai, Bhuvanagiri, Mutlur, Orathur, Palur, Parangipettai, Pichavaram, Puduchatram and Veeramudianatham.

2.2. Collection of samples from each location

The soil texture, soil pH, EC, organic carbon content, available phosphorus was estimated in each soil sample. In each location the sugarcane root rhizosphere soil samples (20-30 core of 2.5 cm dia × 15-20 cm length) were collected. The roots and surrounding soil were excavated to a depth of 15-20 cm. Samples were then transferred to polythene bags for the isolation of AM fungal spores. The percentage colonization was calculated by clearing and staining the roots [9] followed by the determination of per cent root colonization by Krishna and Dart [10].

2.3. Isolation and characterization of AM fungi

Different soil samples were examined for the presence of AM fungal spore by wet sieving (1000 – 45 μm) and decanting method described by Gerdemann and Nicolson [11]. These spores were cleaned of soil particles by sucrose density gradient centrifugation method and washed with distilled water [12]. This spores suspension were counted with stereozoom microscope (×47). During counting, morphologically similar spores were separated into groups, mounted and identified. The soil texture and the types of the spores in them were counted. Based on the taxonomic key of Gerdemann and Trappe [13] the spores of *Glomus fasciculatum, Glomus mosseae, Glomus versiforme, Aculospora laevis* and *Gigaspora margarita* were identified.

2.4. Estimation of AM fungal spores from soil

AM fungal spore population was estimated by wet sieving and decanting method of Gerdemann and Nicolson [14]. One hundred gram of rhizosphere soil sample was taken and mixed thoroughly in one litre of tap water to settle down the heavier particles for a few seconds. The suspension was decanted through a coarse soil-sieve (500-800μm sieve) to remove large pieces of organic matter. The liquid which passed through the sieve was collected separately and stirred to resuspend all particles. The suspension was decanted through a sieve fine enough to retain desired spores (38-250μm sieve). The material retained on the sieve was washed with a stream of water to ensure that all colloidal materials were passed through the sieve. The small amount of remaining debris were transferred to a shallow layer of water in a petridish and examined under a stereozoom microscope. The spore numbers from each soil sample were counted and expressed per 100 g of soil.

2.5. Screening AM fungal cultures for efficiency to colonize the roots of sugarcane

The isolated five different AM fungal cultures *viz*., *G. mosseae, G. fasciculatum, G. versiforme, A. laevis* and *G. margarita* were studied for their comparative efficiency to colonize the roots of sugarcane. The 20 kg capacity cement pots were filled with sand: soil mix (1:1) fumigated by using 2 per cent formaldehyde and grown with single budded sugarcane setts at two setts pot^{-1}. The pots were inoculated with different cultures of AM fungi (soil inoculation at 50 g pot^{-1}). The following observations were taken on 60^{th}, 90^{th} and 120^{th} days after planting (DAP). Relatively effective colonization in the sugarcane as evidenced by percent root infection [15]. Number of spores present in the 100g of rhizosphere soil of sugarcane [14]. The acid and alkaline phosphatase

enzyme activities of sugarcane [15].

2.6. Studies on the interaction of sugarcane with Arbuscular mycorrhizal fungi (pot experiment)

Sugarcane (CoSi 98071) was used to study its interaction with AM fungi in unsterile soil. The relative mycorrhizal dependency (RMD) was measured and Mycorrhizal Inoculation Effects (MIE) were measured for five AM fungi *viz*., *G. mosseae, G. fasciculatum, G. versiforme, A. laevis* and *G. margarita*. Cement pots (60 × 30 × 60 cm) were filled with 20 kg of clay loam soil with pH 7.2 and native mycorrhizal population of 30 spores per 100 g of soil. The Treatments are: T_1 - Sterilized soil, T_2 - Unsterilized soil, T_3 - Unsterilized soil + *G. mosseae*, T_4 - Unsterilized soil + *G. fasciculatum*, T_5 - Unsterilized soil + *G. versiforme*, T_6 - Unsterilized soil + *A. laevis* and T_7 - Unsterilized soil + *G. margarita*. Each treatment was replicated three times. For sterilized soil, the pots filled with soil were sterilized with 2% formaldehyde. The AM fungal inoculation was done by placing the inoculums (50 g inoculums with 150 infective propagules per g) 2 cm below the soil surface. The single budded sugarcane setts were planted as two setts per pot. Irrigation was given as required. Plants were harvested 150 DAP and dry weight of the plants were recorded after drying to a constant weight.

2.7. Mycorrhizal inoculation effects

Mycorrhizal inoculation effect (MIE), a measure of growth response of crop plants to mycorrhizal inoculation was measured for *G. mosseae, G. fasciculatum, G. versiforme, A. laevis* and *G. margarita* in sugarcane crop. MIE was calculated using formula proposed by Bagyaraj*et al.* [16].

2.8. Phosphatase activity of roots

The phosphatase activity was measured in roots. The enzyme phosphatase hydrolyzed para-nitrophenyl phosphate. The released p-nitrophenol was yellow in colour in alkaline medium and was measured at 725nm. The optimum pH for acid phosphatase was 4.5 and for alkaline phosphatase were 8.5 [17].

2.8.1. Enzyme extract

AM fungi inoculated 10 g of sugarcane roots were ground thoroughly with acid washed sand in a pre-chilled pestle and mortar in grinding medium containing 20 ml of 0.2 M acetate buffer (pH 4.5) for acid phosphatase or 0.2 M borate buffer (pH 8.5) for alkaline phosphatase. The homogenate was passed through four layers of cheese cloth and the filterate was centrifuged at 3000 rpm for five minutes. Supernatant was used as enzyme source.

2.8.2. Estimation of acid phosphatase

The substrate P-nitrophenyl phosphate of 1.0 g was dissolved in 100 ml distilled water. One ml of substrate was pipette out into a test tube and two ml of enzyme extract and five ml of 0.2 M acetate buffer (pH 4.5) were added. This was incubated for 24 hrs and one drop of 10 per cent TCA was added and centrifuged. From this, one ml of clear supernatant was taken in a test tube. To this supernatant, one ml of folinciocalteau reagent and 2 ml of 20 per cent sodium carbonate were added and boiled for one minute (at 100°C). Then the test tube was cooled and the volume was made upto 10 ml with distilled water. The colour intensity was read at 725 nm (red filter). Standard curve using P-nitrophenol was drawn and from this the activity was calculated.

2.8.3. Estimation of alkaline phosphatase

Alkaline phosphatase activity was measured by adopting the procedure described for acid phosphatase. Except that here the borate buffer (0.2 M pH 8.5) was used instead of acid buffer.

2.9. Influence of *Glucanoacetobacter diazotrophicus* on the root colonization of *Glomus fasciculatum*and growth of sugarcane

Cement pots of 20 kg capacity were filled with sterilized sand: soil (1:1) mixture *G. fasciculatum* soil based root inoculums at 50 g pot^{-1} was placed two cm below the soil surface as a thin uniform layer. Sugarcane setts (two budded) of var. CoSi 98071 were planted at two setts pot^{-1}. Before planting, the setts were treated with *G. diazotrophicus* culture suspension containing 1×10^7 CFU ml^{-1}. An absolute control was maintained without inoculation of *G. diazotrophicus* and *G. fasciculatum*. Five replications were maintained for each treatment. The following treatments were studied: T_1 -Absolute control; T_2 - *G. diazotrophicus* – 1×10^7 CFU ml^{-1}; T_3 –AM alone (*G. fasciculatum*) T_4 - *G. diazotrophicus* – 1×10^7 CFU m^{-1} + *G. fasciculatum*. The root colonization percentage, spore number and growth parameters were observed in each treatment on 120 DAP.

3. RESULTS

3.1. Survey of AM fungal occurrence in sugarcane fields of Cuddalore District of Tamilnadu

The AM fungal colonization and spore load in the rhizosphere soil of sugarcane collected from Annamalai Nagar, Vilagam, Arasur, Cuddalore

and Vallampadugai, Cuddalore District, Tamilnadu, India were presented in (**Table 1**).

3.2. Influence of soil types on mycorrhizal spore population

Among the four different soil types, clay loam soil, recorded the highest spore population (100 in 100 g^{-1} of soil) followed by clay soil, sandy clay (75.0 in 100g^{-1} of soil) and sandy loam (68.0 in 100g^{-1} of soil) types. Among the five different AM fungal isolates *G. fasciculatum* was the predominant species in all the soil types followed by *G. mosseae*, *G. margarita*, *A. laevis* and *G. versiforme* (**Table 2 & 3**).

3.3. Screening of five different AM fungi in sugarcane (CoSi 98071)

Pot culture experiments were carried out to study the effect of the five isolates of AM fungi *viz*., *G. mosseae*, *G. fasciculatum*, *G. versiforme*, *A. laevis* and *G. margarita* on the root colonization of sugarcane var. CoSi 98071 on 60, 90 and 120 days after planting (DAP). Based on the percentage of root colonization, spore number 100 g^{-1} of rhizosphere soil, acid phosphatase and alkaline phosphatase activity, the efficient AM fungus was selected. The results are presented in (**Table 4**). The sugarcane root colonization by AM fungi increased with the age of crop from 60th DAP to 120thDAP. The per cent root colonization, spore number 100g^{-1} of rhizosphere soil, the acid and alkaline phosphatase enzyme activities were highest in *G. fasciculatum* inoculated plants compared with *G. mosseae*, *G. versiforme*, *A. laevis* and *G. margarita* inoculated plants.

The highest per cent root colonization and spore number of sugarcane were recorded in *G. fasciculatum* inoculation (76.28, 181.48) followed by *G. mosseae* (62.23, 176.20), *G. versiforme* (47.24, 164.48), *A. laevis* (56.22, 170.93) and *G. margarita* (52.21, 166.75). The highest acid and alkaline phosphatase activities were recorded in *G. fasciculatum* inoculated roots (28.78, 27.38 µg 24 hrs^{-1} 10 g^{-1} of root) followed by *G. mosseae* (28.58 and 26.60) and others between 25.50 to 28.78 and 22.25 to 27.38 respectively for acid and alkaline phosphatase activity. In general, the acid phosphatase activity was more, compared to alkaline phosphatase activity. The values obtained in *G. mosseae*, *G. margarita* and *G. versiforme* were on par.

3.4. Studies on the interaction of sugarcane (CoSi 98071) and AM fungi

In general, the sugarcane var. CoSi 98071 grew better in unsterilized soil compared to sterilized soil. The relative mycorrhizal dependency (RMD) for sugarcane var. CoSi 98071 was 31.80 per cent. Based on this, sugarcane was considered moderately dependent on Arbuscular mycorrhiza. The effect of inoculations of five different AM fungi *G. mosseae*, *G. fasciculatum*, *G. versiforme*, *A. laevis* and *G. margarita* was compared with uninoculated unsterilized soil on shoot dry weight of sugarcane var. CoSi 98071 was studied in a pot culture experiment. Based on shoot dry weight at 150 DAP, the mycorrhizal inoculation effect (MIE) of the five different AM fungal cultures was recorded. The data are presented in (**Table 5**).

All the five AM fungal cultures inoculated in sugarcane var. CoSi 98071 significantly increased the shoot dry weight over uninoculated in unsterile soil. The mean mycorrhizal inoculation effects (MIE) of *G. mosseae*, *G. fasciculatum*, *G. versiforme*, *A. laevis* and *G. margarita* were 18.8, 20.5, 14.8, 17.7 and 16.4 respectively. The inoculation of *G. fasciculatum* recorded the highest MIE among the five AM cultures tested and selected for further studies. Based on the root colonization percentage, spore number, acid and alkaline phosphatase activities, RMD and MIE *G. fasciculatum* was found to be efficient and selected for further studies.

3.5. Survival of *G. fasciculatum*in different storage temperatures

The number of infective propagules (IP) found in sand: soil mix stored at different temperature is given in (**Table 6**). The initial number of infective propagules present in sand: soil mix was 6.3 IPg^{-1}. Survival was 13.90 per cent at 4°C, 11.00 per cent at 25°C and 8.30 per cent at 40°C after 12 months of storage. The population of AM fungi steadily decreased in 12 months of storage period. Storage for six months at 4°C retained the viability of inoculums at 55.13 per cent compared to 35.42 and 32.30 per cent at 25°C and 40°C respectively. The decrease in survival was more after six months of storage in all the three temperatures.

3.6. Effect of different organic amendments on the growth parameters and mycorrhizal colonization (*G. fasciculatum*) of sugarcane (CoSi 98071) in pot culture

In general, all organic amendments showed positive influence on the proliferation of AM fungi (**Table 7**). Among different organic amendments farm yard manure (FYM) significantly stimulated the highest per cent root

colonization and spore number (56.94 and 157.81) followed by pressmud (52.21 and 147.15), coconut cake (48.20 and 144.82) and neem cake (48.1 and 143.13) on 180 DAP.

The corresponding trend was observed in plant parameters viz., plant height, girth of the cane (Table 7). The significant increase in plant height (137.32 cm) and cane girth (10.87) were recorded in FYM amended treatment followed by pressmud, coconut cake and neem cake on 180 DAP. The results obtained in press mud and neem cake were on par.

Table 1: Survey of AM fungal occurrence in sugarcane fields of Cuddalore District of Tamilnadu

Place of the sample	Soil texture	pH	EC m mhos cm^{-1}	Organic carbon	Available P ($kg\ ha^{-1}$)	Percent root colonization	AM spore population 100 g^{-1} of rhizosphere soil
Annamalainagar	Clay loam	8.1	0.43	0.51	17.48	48.0	98.0
Vilagam	Clay	7.5	0.41	0.46	16.71	50.5	109.0
Arasur	Clay loam	7.7	0.47	0.60	16.69	51.0	100.5
Cuddalore	Clay loam	8.0	0.50	0.63	11.18	40.5	80.0
Vallampadugai	Clay	7.5	0.41	0.59	18.59	62.8	128.0
Bhuvanagiri	Clay	7.3	0.39	0.49	19.90	35.5	80.0
Mutlur	Clay loam	7.4	0.36	0.44	20.04	40.0	84.4
Orathur	Sandy clay	7.5	0.34	0.54	18.48	48.0	100.5
Palur	Clay loam	8.2	0.54	0.77	11.27	50.0	102.0
Parangipettai	Clay loam	8.0	0.46	0.70	13.24	50.0	100.5
Pichavaram	Sandy clay	8.5	0.53	0.76	19.38	34.8	67.0
Puduchatram	Sandy clay	7.4	0.32	0.47	19.10	39.2	80.5
Veeramudianatham	Sandy loam	7.3	0.40	0.36	21.10	35.0	68.0

Table 2: Characterization of different AM fungal isolates from sugarcane rhizosphere soil

Characters	*Glomus mosseae*	*Glomus fasciculatum*	*Glomus versiforme*	*Acaulospora laevis*	*Gigaspora margarita*
Size of spore	120 µm	100 – 120 µm	125 – 150 µm	400 µm	200 – 300 µm
Spore shape	Globose	Globose hypogeous	Globose	Globose	Ectocarpic
Colour of spore	Yellow to brown	Yellow to reddish	Yellow to brown	Outer wall – brown Inner	White when young and
Sporocarp	Present	Present	Present	Present	Absent
Thickness of spore wall	3 – 4 µm	4 – 14 µm	3 – 4 µm	4 – 8 µm	> 20 µm
Subtending hyphae	Cylindric flared	Absent	Cylindric or flared	Not observable	Bulbous (30 – 50 µm)

Table 3: Influence of soil types on spore population of AM fungi

Soil texture	Total AM fungal spore population per 100 g of soil in each soil types	Types of AM fungi				
		Glomus mosseae	*Glomus fasciculatum*	*Glomus versiforme*	*Acaulospora laevis*	*Gigaspora margarita*
Sandy Clay	75.0	15.0	35.0	5.0	8.0	12.0
Sandy loam	68.0	15.0	28.0	5.0	8.0	12.0
Clay loam	100.0	20.0	50.0	6.0	9.0	15.0
Clay	75.0	15.0	35.0	5.0	8.0	12.0

Table 4: Screening of different AM fungal species in Sugarcane var CoSi 98071 under pot culture study

AM fungal inoculation	% root colonization			Spore number /100g of rhizosphere soil			Acid phosphatase activity (µg/ 24 hrs^{-1} 10 g^{-1} of root)			Alkaline activity (µg/ 24 hrs^{-1}. 10g^{-1} of root)		
	Sampling period in days			Sampling period in days			Sampling period in days			Sampling period in days		
	60	90	120	60	90	120	60	90	120	60	90	120
Glomus mosseae	40.25	55.43	62.23	143.23	165.98	176.20	25.58	26.63	28.58	24.34	25.62	26.60
Glomus fasciculatum	52.23	62.30	76.28	152.20	169.45	181.48	26.50	27.78	28.78	25.73	27.01	27.38
Glomus versiforme	25.30	37.25	47.24	126.75	157.01	164.48	22.45	24.25	25.50	21.22	21.90	22.25
Acaulospora laevis	36.57	49.33	56.22	138.53	162.95	170.93	24.38	25.45	27.28	23.01	24.14	24.48
Gigaspora margarita	29.28	42.40	52.21	131.48	157.98	166.75	23.55	25.03	26.28	21.60	22.70	22.67
SE	2.59	2.12	2.02	2.27	0.52	1.14	0.31	0.17	0.33	0.45	0.47	0.61
CD (p = 0.05)	7.24	6.03	5.75	6.49	1.50	3.28	0.89	0.51	0.95	1.31	1.35	1.76

Table 5: Studies on the interaction between sugarcane (CoSi 98071) and AM fungi

S. No	Treatments	Plant dry weight on 150 DAP (g/plant)
1	Sterilized soil	7.57
2	Unsterilized soil	10.98

3	Unsterilized soil + *G. mosseae*	13.51
4	Unsterilized soil + *G. fasciculatum*	13.80
5	Unsterilized soil + *G. versiforme*	12.89
6	Unsterilized soil + *A. laevis*	13.34
7	Unsterilized soil + *Gi. Margarita*	13.13
SE		0.03
CD (p = 0.05)		0.09

Table 6: **Survival of AM fungi *G. fasciculatum* soil based root inoculums at different storage temperatures**

Months	Sand: soil (1:1) mix (I.P g^{-1})		
	4°C	25°C	40°C
0	6.3 (100.0)	6.3 (100.0)	6.3 (100.0)
2	6.3 (100.0)	5.9 (93.3)	5.9 (93.3)
4	5.7 (90.16)	5.5 (86.94)	5.3 (83.7)
6	3.4 (55.13)	2.3 (35.42)	2.3 (32.30)
8	2.0 (32.2)	1.5 (22.54)	1.5 (22.54)
10	1.2 (17.71)	0.83 (13.20)	0.56 (8.4)
12	0.90 (13.90)	0.80 (11.00)	0.53 (8.30)
SE	0.04	0.04	0.06
CD (p = 0.05)	0.12	0.13	0.17

Table 7: Effect of different organic amendments on the mycorrhizal colonization (*G. fasciculatum*) and growth of sugarcane (CoSi 98071) in pot culture

S. No	Organic amendments	Percent root Colonization			Spore number/100 g soil			Height of the cane (cm)			Girth of the cane (cm)		
		Sampling period in days			Sampling period in days			Sampling period in days			Sampling period in days		
		60	120	180	60	120	180	60	120	180	60	120	180
1	Control	12.21	31.06	40.19	115.19	125.85	137.80	42.10	89.30	109.74	4.56	7.25	9.74
2	Farm yard manure	15.28	58.89	58.94	142.14	149.29	157.81	69.44	110.51	137.32	6.86	9.52	10.87
3	Groundnut cake	15.00	36.38	48.20	127.82	135.84	144.82	51.43	96.54	125.31	5.73	7.84	10.07
4	Neem cake	15.00	36.18	48.11	127.47	135.23	143.13	50.04	96.35	123.93	5.54	7.86	10.00
5	Press mud	15.18	48.18	52.21	128.02	138.17	147.15	55.26	99.36	127.64	5.96	8.02	10.22
SE		1.30	1.43	1.46	1.64	1.10	1.05	1.53	1.18	0.91	0.08	0.17	0.06
CD (p = 0.05)		4.00	4.07	4.12	4.71	3.15	2.97	4.34	3.37	2.54	0.21	0.48	0.15

4. DISCUSSION

AM fungi is the most abundant kind of mycorrhizae found in association with every taxonomic group of plants and the list of species not infected is probably far shorter than the infected ones and in the current status these fungal and bacterial associations are beneficial to crop plants in many ways, including enhancing the nutrient availability especially nitrogen and phosphorus, enhance water uptake and induces resistant against diseases and increased the yield [18]. The results of the present survey in thirteen different locations in Cuddalore District of Tamil Nadu State of India, where sugarcane is grown as a commercial crop revealed the ubiquitous nature of AM fungi in sugarcane rhizosphere soil and the occurrence of AM fungi in soils has been reported in various kinds of environments [19]. The rhizosphere soil and root samples collected from wheat and lentil at 11 sites across four zones of Saskatchewan in Canada were analyzed for spore number, level of AM fungal colonization and AM fungal species. The number of spores ranged from 78 to 272 g^{-1} soil. The level of colonization varied from site to site and the difference were more pronounced in wheat than lentil [20]. The root colonization percentage in sweet potato ranged from 30 to 70 per cent [21] and in cotton ranged from 40 - 80 [22].

Interestingly in the present study, the root colonization percentage and spore number of AM fungi in the rhizosphere samples and sugarcane in the range of 34.8 to 62.8 and 67.0 to 128.0 100 g^{-1} of soil respectively. The thirteen locations comprised of four different soil types i.e., sandy clay, sandy loam, clay loam and clay. Although

there was no apparent relationship between soil characteristics (pH, EC and organic carbon) and AM fungal root colonization and spore number was noticed. The available phosphorus inversely correlated with AM fungal root colonization and spore number. These results are in accordance with the findings of Miranda and Harris [23] in leek and Chandrasekara*et al*. [24] in sunflower.

In the present study, the four different AM fungi were isolated from sugarcane rhizosphere soil and characterized as *Glomus mosseae, Glomus fasciculatum, Glomus versiforme, Acaulospora laevis* and *Gigaspora margarita*. In the present investigation, in all the soil type were qualitatively and quantitatively enumerated. *G. fasciculatum* was predominant in all the soil types and the highest spore population was observed in clay loam soil [25].

AM fungal colonization in the roots of sugarcane may be due to fungal preference by the host and due to the factors influencing the mycotrophy of sugarcane. Mallosha *et al*. [26] worked on the selection of efficient AM fungi for tomato. Tomato seedlings inoculated with *Glomus leptotichum* recorded higher root colonization percentage, growth and yield parameters than *G. intraradices*. The results of the present investigation under pot culture revealed that among the five AM fungal species tested, *G. fasciculatum* was found to the most effective AM fungus for colonizing the roots of sugarcane (CoSi 98071) [27]. The root colonization percentage was 76.28 the number of spores were 181.48 100 g^{-1} of soil and the acid and alkaline phosphatase activities were 28.78 and 27.38 µg of phenol released 24 h^{-1} 10 g^{-1} of fresh root. Though a particular AM fungi can colonize many host plants, it has a preferred host which exhibits maximum symbiotic response when colonized by that particular AM fungal species [28].

In the present study, the infective propagules percentage decreased to 13.90, 11.00 and 8.30 per cent during the 12 months of storage of the *G. fasciculatum* inoculums at 4°C, 25°C and 40°C respectively. At six months, 4°C storage retained the viability of the inoculums at 55.13 per cent compared to 35.4 and 32.3 per cent at 25°C and 40°C respectively. These results are in accordance with the findings of Harinikumar and Bagyaraj [29] that the *G. fasciculatum* infective propagule numbers greatly decreased upto 10 months of storage reaching around 11 per cent. After 10 months, survival slowly decreased to zero per cent in 40 to 46 months. *G. epigaeum* sporocarps apparently stored very well fresh and individual spores of this species could be stored in water saturated bentonite clay at 5°C.

Species and strains of AM fungi have been shown to differ in enhancing nutrient uptake and plant growth [25]. Variations in the extent and effect of AM fungal colonization have also been linked to the genotype of the host plant [29]. The recent isolation of mycoplant mutants and the discovery that AM fungal colonization was a heritable trait suggest the possibilities of tailoring plant-fungus combinations for maximum efficiency.

Plant differs greatly in their need for an response to mycorrhizal infection. Researchers defined relative mycorrhizal dependency (RMD) as the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility. Plenchette *et al*. [30] proposed another formula to calculate relative mycorrhizal dependency (RMD) of crop plants under field conditions. They compared plants in fumigated and non-fumigated soil. In the present study, the relative mycorrhizal dependency of the crop was 31.80 per cent. The sugarcane is a moderately dependent crop to AM fungi. In the present pot culture study, sugarcane var. CoSi 98071 was highly responsive to *G. fasciculatum* inoculation with 20.15 per cent mycorrhizal inoculation effect (MIE) compared to *G. mosseae* (18.8%). *A. laevis* (17.7%), *G. margarita* (16.4%) and *G. versiforme* (14.9%). This clearly established the sugarcane CoSi 98071 and *G. fasciculatum* interaction increased the growth (MIE). Among the different AM fungi, the genus *Glomus* was more effective AM fungal symbiosis to sweet potato than *Acaulospora* or *Scutellospora* [31].

Organic amendments influence proliferation of AM fungi [32]. VA-mycorrhizal inoculation and addition of organic manure increased plant growth and increased shoot dry weight, N and P uptake of wheat cvcyiza 157 plants [33]. Organic amendments with narrow C:N ratio and moderate N, P and C like Farmyard manure (FYM) have shown a more pronounced effect on AM fungal proliferation. The highest root colonization percentage (100, 98) and spore number (307, 274.50 g^{-1} of soil) were observed in inoculation of *G. fasciculatum* in conjunction with FYM for wheat genotypes DWR-39 and DWR-187, than biogas spent slurry, groundnut cake, glyricidia,

jowar straw and rice bran [37]. Interestingly in the present study, the highest root colonization percentage (58.94).

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