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Isolation and Application of Diazotrophic Bacteria as Microbial Inoculants for Sugarcane Crops for Drought Tolerance Nature

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ABSTRACT

Sugarcane, a critical commodity in India, necessitates technical advances in production efficiency to enhance the country's agricultural energy balance. This research included four replications and a total of 24 plots.In this experiment, analytical-grade chemicals and double-distilled water were used throughout the procedure. Each soil sample was tested for its texture, pH, EC, organic carbon content, and phosphorus availability, among other characteristics, before being analysed. Each site had 20-30 cores of 2.5 cm diameter x 15-20 cm length extracted from the sugarcane root rhizosphere soil, with the diameter and length varying from site to site. For the roots and the surrounding earth, we dug 15-20 cm deep into the ground. After being transferred to polythene bags and kept at 37°C overnight, the samples were used to isolate AM fungal spores. In order to statistically analyse the data obtained from diverse features, the RBD design was used.

Keywords – Gluconacetobacter Diazotrophicus and Herbaspirillumspp, Elicitors, Sugarcane, Xanthomonas Albilineans.

I. INTRODUCTION

Nowadays, inoculating plants with beneficial microorganisms is a popular practise in agriculture. It offers crops with a variety of advantages, including enhanced plant growth and disease prevention. The development and production of plant growth-promoting rhizobacteria (PGPR) may be influenced by root exudates from plants, which can have both immediate and long-term consequences. Apart from the above stated strains, PGPR may be found in Azospirillumbrasilense, Bacillus subtilis, and Enterobacter cloacae, Gluconacetobacterdiazotrophicus, Pantoeaagglomerans, and P. fluorescens, as well as Rhizobium leguminosarum, Sinorhizobiummeliloti, and P. fluorescens, and Pseu

Plant growth might be aided by PGPR in a variety of ways, including biochemical N2 fixation, phosphate solubilization, and the production of phytohormones. The production of antimicrobial compounds or the establishment of induced systemic resistance may also be exploited by PGPR to indirectly increase plant growth as an extra advantage, as previously mentioned (ISR). Sustainability in agriculture cannot be ignored

any longer, and microbial inoculants may prove to be a cost-effective method of maintaining crop output over the long run.[1-2]

II. INOCULANT CARRIERS

Since the beginning of the inoculant manufacturing sector, the industry has been focused on developing more efficient products at a lower cost that satisfy the needs and expectations of farmers everywhere. As an important aspect, microorganisms must be transported by a carrier that maintains cell viability for an extended period of time while also being easy to employ. The first commercially produced inoculant, "Nitragin" (Fig. 1), was made from gelatin, and later on, gelatin was utilized as a transporter for bacteria in nutritional media. In their stead came peat, which was less deadly but retained its status as the "gold" transporter until the late 1990s, when things began to change (Fig. 1)

Solid peat is composed of organic soil that has collected over a long period of time under certain environmental circumstances, resulting in the formation of solid peat. Peat is a common inoculant carrier due to its high quantity of organic matter, which feeds bacteria with essential nutrients via photosynthesis. Aside from that, the peaty matrix protects microorganisms from soil adversities and increases cell viability in conditions of limited water availability and high temperatures. Using adhesives for the peat seed inoculation method is vital because they aid in the bonding of the peaty matrix to the seeds.[4]

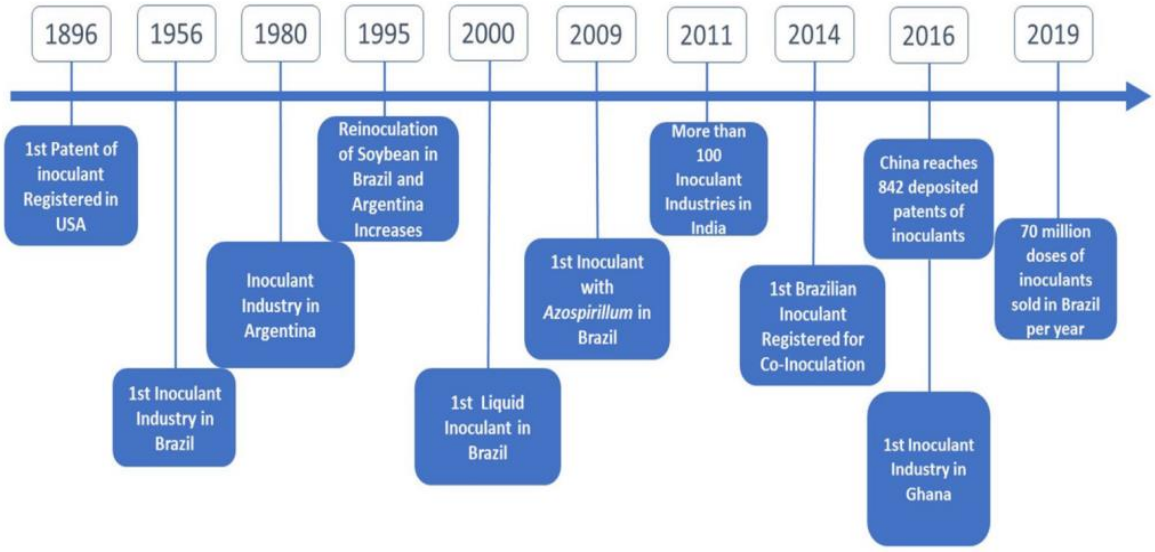

Figure1 : Inoculant development timeline, including some key milestones [4]

III. MATERIALS AND METHODS

AM fungal colonization in sugarcane roots

Using Phillips and Hayman's approach, the proportion of mycorrhizal colonisation in the root was found (1970). The roots of the field path plants were gently rinsed with tap water before being dried. The cytoplasm and nucleus of the host cell were cleaned prior to stain penetration by first immersing the washed roots in a 10% KOH solution. After that, it was autoclaved for roughly 20 minutes at 15 lb/sq.inch pressure. Hydrogen peroxide solution was used for 10 minutes to disinfect the root pieces after they had been digested. This process was repeated three or four times or until no brown colour was seen in the rinsed water. To ensure adequate

staining, the roots were acidified for three to four minutes with two percent hydrochloric acid Root pieces were dyed with 0.05 per cent tryphan blue in lactophenol and cooked for 10 minutes after the acid was drained out without washing with water. Stereozoom microscopy was used to study the ends of these roots. The percentage of AM fungal colonisation in each root segment was determined by analysing 50 root segments from each replication.

$$\text{Percent AM fungal colonization} \quad \frac{\text{Number of root bits with infection}}{\text{Total number of root bits examined}} \times 100$$

AM Fungal Spore Estimation from Soil

Gerdemann and Nicolson's wet sieving and decanting technique was used to determine the AM fungal spore population (1963). One litre of tap water was used to fully mix 100 grammes of field trail rhizosphere soil sample in order to settle down the heavier particles for a few seconds. To remove big bits of organic debris, the suspension was decanted through a coarse soil-sieve (500-800m sieve). Separately, all of the liquid that had gone through the sieve was collected and swirled to resuspend all of the particles. Filtering the suspension (38-250 microns sieve) was necessary to preserve the appropriate spores. In order to guarantee that all colloidal elements were removed from the sieve, a stream of water was used to wash the sieve. Petridish water and microscopes were used to analyse a little number of debris that was still floating in the air. Each soil sample's spore number was counted and given as a number of spores per 100 grammes of soil.

- ✓ **Leaf nitrogen**

Each treatment's third leaf from the top was gathered, dried, and powdered on a monthly basis for up to 10 months. The nitrogen content of these samples was then calculated using Humphries (1956)'s Kjeldhal technique and represented as mg per g of dry leaf weight in mg/g.

- ✓ **Package of Agronomic practices**

As described in the Crop Production Guide, the agronomic procedures were followed (1994).

IV. SOIL ANALYSIS

After collecting soil samples at two, four, six, and ten months, they were shade-dried, powdered and sieved through a 2 mm sieve from each plot. Listed below are the results of the testing done on the soil sample.

Nitrates in reserve the alkaline permanganate process.

Phosphorus readily available Changes made to the molybdate blue ascorbic acid modification procedure

- **Preparation of plant samples**

Biomass output was recorded by taking plant samples every two months for P and leaf samples every six months for N analyses. In order to carry out the investigation, the following procedures were used:

- ✓ Nitrogen content Microkjeldahl method
- ✓ Phosphorus content Triple acid and digestion method

V. RESULTS AND DISCUSSION

Variations in root colonisation percentage and spore quantity 100g-1 soil were shown to be significant in this investigation. Soil samples from sugarcane rhizospheres were collected in Atarahi District at 20 different sites and then classified. Eight of the samples tested were classified as clay loam. Samples of clay, sandy clay, and

sandy loam were collected. The soil pH varied from 7.3 to 8.9, and the EC was 0.32 to 0.54 mmhos cm-1 in all samples. Each sample contained between 0.36 and 0.77 percent organic carbon, while the accessible phosphorus concentration varied from 11.18 to 21.10 kilogrammes per ha-1.

Table 1 : Sugarcane rhizosphere soil samples were collected and AM fungus isolated.

S. No.	Placeof the sample	Soil texture	pH	ECmmhos$_{cm}^{-1}$	Organiccarbon	Available phosphorus content (kgha^{-1})	Percent root colonization	AM sporepopulation 100 g^{-1} ofrhizospheresoil
1	Abdullahpur	Clayloam	8.1	0.43	0.51	17.48	48.0	98.0
2	Adampur	Clay	7.5	0.41	0.46	16.71	50.5	109.0
3	Ahirauli	Clayloam	7.7	0.47	0.60	16.69	51.0	100.5
4	Atarahi	Clayloam	8.0	0.50	0.63	11.18	40.5	80.0
5	Babarakha	Clay	7.5	0.41	0.59	18.59	66.0	126.0
6	Babupur	Clay	7.3	0.39	0.49	19.90	35.5	80.0
7	BadaouliNoniyan	Clayloam	7.4	0.36	0.44	20.04	40.0	84.4
8	Bahadurpur	Sandyclay	7.5	0.34	0.54	18.48	48.0	100.5
9	Bairagar	Clayloam	8.2	0.54	0.77	11.27	50.0	102.0
10	Atkadpur	Clayloam	8.0	0.46	0.70	13.24	50.0	100.5
11	Baghmurtza	Sandyclay	8.5	0.53	0.76	19.38	32.0	65.0
12	Bans Gopalpur	Sandyclay	7.4	0.32	0.47	19.10	39.2	80.5
13	Barrre Patti	Sandyloam	7.3	0.40	0.36	21.10	35.0	86.0
14	Bhakura	Sandyclay	8.44	0.50	0.71	12.00	29.8	67.0
15	Baboopur	Sandyclay	8.67	0.48	0.69	16.00	30.0	80.0
16	Alamgirpur	Clayloam	8.94	0.48	0.72	14.00	40.5	84.0
17	ChakPahalawan Tahir	Clayloam	8.64	048	0.62	18.00	40.0	80.0
18	Chaka Banki	Sandyclay	8.05	0.50	0.65	11.00	41.3	80.0
19	Chaktali	Sandyclay	8.86	0.50	0.61	13.00	34.9	67.0
20	Baijapur	Sandyclay	8.27	0.50	0.49	12.00	34.5	67.0

AM fungal colonisation and spore population have been unaffected by soil natural physico-chemical characteristics, such as pH, EC, and organic carbon content. AM fungal colonisation and spore production were negatively affected by the soil's phosphorus level. Root colonisation percentage and AM spore population in the soil were found to vary from 32.0 to 66.0 percent and 65.0 to 126.0 percent, respectively, for sugarcane roots.
The maximum root colonisation percentage and spore number 100g-1 were found in a sample taken from Babarakha (66.0 and 126.0). Root colonisation and spore count 100g-1 soil were lowest in the sample taken from Baghmurtza (32.0 and 65.0). G. mosseae, G. fasciculatum, G. versiforme, A. laevis, and G. margarita were among the species of G. sporulatum that Gerdemann and Trappe (1974) discovered using a stereozoom microscope on the isolated spores (Table 5.2).'

Mycorrhizal spores are influenced by soil type

More spores were found in clay loam soil (102 in 100 g-1 of soil) than in sandy clay (82.0), sandy loam (80.0) and clay (78.0) types. Fungi G. fasciculatum dominated all of the soil types, with G. mosseae and A. laevis in close second and third place respectively (Table 5.3 and Fig 5.1).

Table 2 : Different AM fungal isolates from sugarcane rhizosphere soil samples were identified and characterised

S.No.	Characters	*Glomusmosseae*	*Glomusfasciculatum*	*Glomusversiforme*	*Acaulosporalaevis*	*Gigasporamargarita*
1	Sizeofspore	120µm	100–120µm	125–150µm	400µm	200–300µm
2	Sporeshape	Globose	Globosehypogeous	Globose	Globose	Ectocarpic
3	Colourofspore	Yellowtobrown	Yellowtoreddishbrown	Yellowtobrown	Outer wall –brownInnerwall–HyalineEllipsoid	White whenyoungandslightlyyellowish atmaturity
4	Sporocarp	Present	Present	Present	Present	Absent
5	Thicknessofsporewall	3–4µm	4–14µm	3–4µm	4–8µm	>20µm
6	Subtendinghypha e	Cylindricflared	Absent	Cylindricorflared	Notobservable	Bulbous(30–50µm)

Table 3 : species-level diversity in soil types and AM fungi

S.No.	Soil texture	Total AM fungal sporepopulationper100g of soilin each soil types	Types of AMfungi				
			Glomusmosseae	*Glomus fasciculata*	*Glomusvermiform*	*Acaulosporalaevis*	*Gigaspora margarita*
1	SandyClay	82.0	16.0	38.0	5.0	9.0	14.0
2	Sandyloam	80	16.0	34	6.0	10	14.0
3	Clayloam	102.0	22.0	48.0	7.0	11.0	14.0
4	Clay	78.0	16.0	38.0	5.0	9.0	10

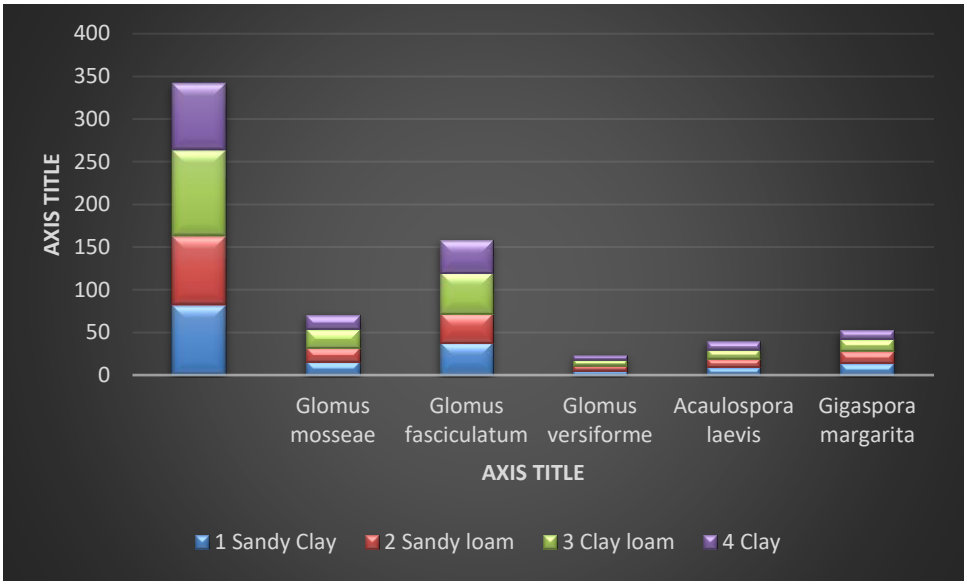

Fig. 3 : A species-level breakdown of soil types and AM fungus populations.

- **Sugarcane was used to test for the presence of five distinct AM fungus (CoC 24)**

Sugarcane var. CoC 24 was planted 60, 90, and 120 days following the emergence of five AM fungus isolates (G. mosseae, G. fasciculatum, G. versiforme, A. laevis, and Gi. margarita) in pot culture studies (DAP). Based on root colonisation %, spore quantity per 100 g, acid and alkaline enzyme activity, the most efficient AM fungal culture was chosen for further testing. The findings may be found in the following document: (Table 5.4).

From 60th to 120th DAP, the sugarcane root colonisation of AM fungus grew more and more rapidly. Plants inoculated with G. fasciculatum had a higher percentage of root colonisation, the spore number $100g^{-1}$ of rhizosphere soil, the acid and alkaline phosphatase enzyme activities than plants inoculated with G. versiforme, A. laevis or Gi. margarita. Sugarcane inoculations with G. fasciculatum (78.20, 180.00) had the maximum root colonisation and spore number, followed by G. mosseae (61.20, 175.00), G. versiforme (46.20, 165.60), A. laevis (57.00, 170.00), and Gi. margarita (52.60, 164.30) on the 120th day after planting (DAP).

Table 4 : Concentrations of carbon sources that G. diazotrophicus isolates from fungal spores can grow on in semisolid LGI medium

S.No.	Isolates	Sucroseconcentration(%)							Glucoseconcentration(%)						
		Control	2.5	5	10	15	20	25	Control	2.5	5	10	15	20	25
1	GdAVS[1]	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
2	GdVVS[1]	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
3	GdAVS[2]	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
4	GdCVS	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
5	GdVSVS	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
6	GdBVS	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
7	GdMVS[1]	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
8	GdOVS	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
9	GdPVS[1]	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
10	GdPVS[2]	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+

11	GdPVS^3	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
12	GdPVS^4	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
13	GdVVS^2	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
14	GdMVS^2	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
15	GdAVS^3	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
16	GdKVS^1	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
17	GdTVS	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
18	GdSVS	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
19	GdKVS^2	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
20	GdMVS^3	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+
21	PAL5	-	+	++	+++	++	++	+	-	+	++	+++	++	++	+

+ – Light pellicle formation; ++ – Thick pellicle formation; +++ – Thick and deep pellicle formation; - – No growth 1234 – Isolate differentiation for the designation PVS; GdVSVS – Isolate from Babarakha superior isolate

- **Growth in LGI broth**

G. diazotrophicus cultures grew exponentially in LGI broth between 168 and 180 hours. G. diazotrophicusGdVSVS had the highest OD value (1.58 hours) of the 21 cultures tested, followed by G. diazotrophicusGdTVS (1.57 hours) and G. diazotrophicus PAL5 (1.58 hours) (1.56). The G. diazotrophicus isolate GdPVS4 was the sluggish grower, with an OD value of 1.37 at 180 hours of cultivation (Table 5.16).

- **Growth in acetic LGI broth**

In acetic LGI broth, the growth of all 20 isolates was greater than in LGI broth. One of the G. diazotrophicus strains, GdVSVS, achieved the highest OD value of 1.63, followed by GdPAL5 (1.60) and four G. diazotrophicus at 180 hours (Table 5.17 and Fig. 5.2).

VI. ANALYZING THE EFFICACY OF G. DIAZOTROPHICUS ISOLATES

For the purpose of screening isolates for their ability to fix nitrogen and dissolve phosphate

All 20 G. diazotrophicus isolates and the reference strain PAL5 showed significant nitrogenase activity. As shown in Fig 5.3, the highest concentration of C2H4/hr/mg cell protein was found in G. diazotrophicusGdVSVS, followed by G. diazotrophicus GdKVS2. Beyond nitrogenase activity, all the strains possessed phosphate solubilizing capacities of at least 0.58 g PO4/0.5 mg insoluble P/mg sucrose used..... There were 20 isolates of G. diazotrophicus. The one with the highest phosphate solubilizing ability (0.78 g PO4/0.5 mg insoluble P/mg sucrose) was GdVSVS. G. diazotrophicus, GdKVS2 and PAL-5 followed (Table 5.18 and 5.19).

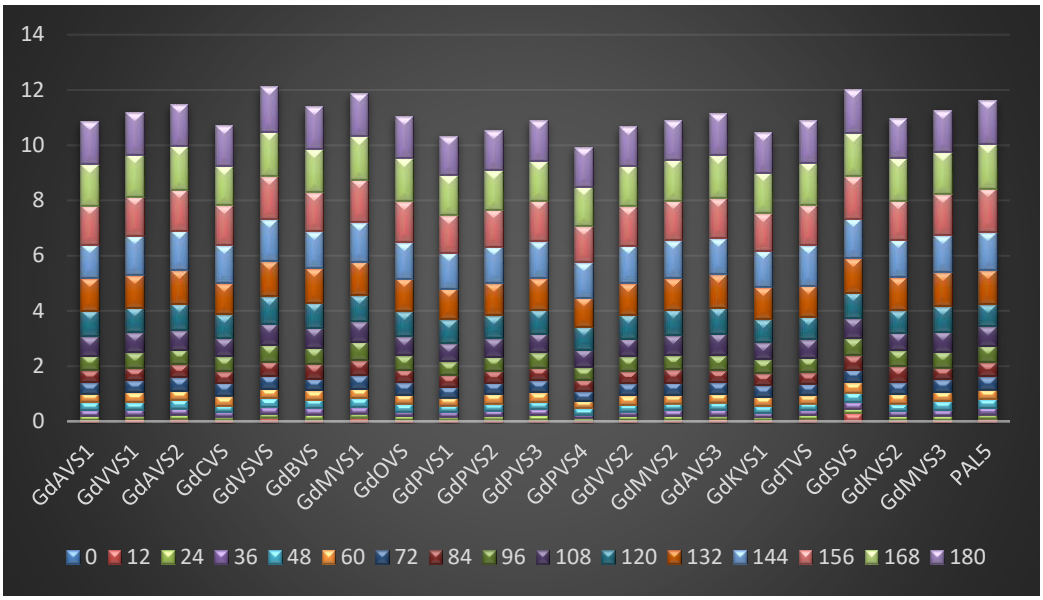

Fig. 4 Ampicillin-resistant G. diazotrophicus spores in acetic LGI broth

VII. CONCLUSION

Mycorrhizae (the relationship between plant roots and fungus) is found in over 80 percent of plant species. Among the many mycorrhizal fungi, AM fungi are the most common and the list of species that are not infected is likely to be much shorter than the infected ones. These fungal associations are beneficial to crop plants in many ways, including increasing nutrient availability, increasing water uptake and inducing resistance to disease and increasing crop yield. It seems that Gluconacetobacterdiazotrophicus plays a significant role in the plant's nitrogen supply via biological nitrogen fixation. G. diazotrophicus is also known for its ability to solubilize phosphorus, produce plant growth hormone indole acetic acid (IAA), and control red rot disease. It was found that G. diazotrophicus and AM fungal inoculation increased sugarcane growth and development by fixing nitrogen in various sugarcane parts (roots stems and leaves) along with producing growth promoting hormones and by solubilizing, mobilising, and protecting sugarcane plants from stress as well as pathogens.

- Samples of sugarcane rhizosphere soil were collected and analysed for physiochemical parameters, and AM fungal spores were successfully recovered, described, and then utilised to isolate G.diazotrophicus from all 20 distinct sugarcane rhizosphere soil samples (endophyte). Sugarcane rhizosphere soil samples were found to be infected with AM fungus, according to the study.
- Five different AM fungal isolates were isolated, characterised and finally identified as Glomus mosseae, Glomus fasciculatum. Glomus versiforme. Acaulosporalaevis. and Gigaspora margarita. Heterogenous soil types were observed in all the 20 different locations, namely sandy clay, sandy loam, clay and clay loam.
- Sugarcane grew better in unsterilized soil compared to sterilised soil in screening trials.
- G. fasciculatum reported as having the greatest root colonisation percentage (78.20), spore number (180.00), acid and alkaline phosphatase enzyme activity respectively (28.00, 26.30 g 24 h-1 of root). The RMD was determined to be 31.80%. Mycorrhizae-dependent sugarcane was classified somewhat reliant, and G. fasciculatum had the greatest MIE in mycorrhizae infection efficiency (MIE) (20.30).

- A pot culture experiment was used to determine the number of infectious propagules in the environment. After six months of storage at all temperatures, 50% of the population had died. After a year in storage, survival rates were 13% at 4°C, 11% at 25°C, and 8% at 40°C.
- It was shown that mycorrhizal root cultures of sorghum may be affected by IAA concentrations as low as 20 ppm and as high as 80 ppm. Root length, biomass, and colonisation percentage were all maximum at 60 ppm IAA after 30 days (16.60 cm, 1.30 g, and 46.60%).
- Co-inoculation of G. diazotrophicus and G. fasciculatum saved at least 50% of N and P, according to the findings of this research.
- An inoculation of AM fungus spores with G. diazotrophicus will need more research before it can be used as an endophyte.

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