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RESEARCH ARTICLE

Evaluating the Influence of Commercial Arbuscular Mycorrhizal Fungi (Greenical VAM^{TM}) on Sugarcane Growth and Yield

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Abstract

Objective: The present study aimed to assess the effects of Greenical VAM^{TM}, a commercial mycorrhizal fungi tablet comprised of *Rhizophagus fasciculatus* and *Rhizophagus intraradices*, on sugarcane growth and yield differences. **Methods:** Two sugarcane cultivars, Co 0238 and Co 86002, were exposed to two AMF inoculation levels, each replicated three times. Parameters included root colonization, soil properties, nutrient uptake, and sugarcane productivity. **Findings:** AMF significantly improved root colonization, soil properties, nutrient uptake, and overall sugarcane productivity in both cultivars. Co 0238 performed better, highlighting varietal differences. Our study emphasizes the potential of AMF like Greenical VAM^{TM} to enhance sugarcane development and yield under favorable conditions. AMF root colonization varied between 56% and 76% throughout the studied cultivars, with Co 0238 outperforming Co 86002 in terms of yield parameters. This improvement resulted in improved yield characteristics and overall sugarcane production. **Novelty:** This research pioneers the use of Greenical VAM^{TM} in sugarcane cultivation, with practical implications for higher yields. Each tablet boasts an impressive spore concentration of 50,000 spores per gram, surpassing existing formulations.

Keywords: Greenical VAM^{TM}; Mycorrhizae; Plant Growth; Root colonization; Sugarcane

1 Introduction

Sugarcane (*Saccharum spp.*) is a vital crop in tropical and subtropical regions, used for sugar production and ethanol production[(1)]. Specifically, in India, *Saccharum officinarum*, a subtropical species, is the main sugar-yielding crop, making it a crucial cash crop. Consequently, India emerges as the preeminent producer of sugarcane globally. This emphasizes the importance of the Indian sugar sector in contributing to the nation's economic growth by generating revenue and employment avenues. Sugarcane has a high demand for both water and phosphorus (P) to achieve optimal productivity, although it can maintain substantial yields with lower leaf nitrogen (N) concentrations. Agronomists predominantly employ mineral fertilizers, typically

characterized as NPK formulations, to augment crop yields [2]. This practice, though effective in enhancing yield, escalates production expenses and may have detrimental impacts on soil health. Notably, a majority of sugarcane cultivation occurs under rain-fed circumstances. During dry periods, low and inconsistent rainfall can lead to limited water supply and reduced nutrient availability, particularly for less mobile nutrients like phosphorus (P). When drought occurs during crucial growth stages like germination and early establishment, it can cause significant yield losses [3].When plants lack access to groundwater for several weeks, their physiological activity declines, leading to reduced plant biomass [4]. Recent advancements in management practices, encompassing enhanced tillage methods, refined fertilizer use, and strategic soil microbial community administration, aim to boost sugarcane yields [5,6].

Arbuscular mycorrhizal fungi (AMF) have gained attention as of late due to their potential to increase sugarcane yields. Nearly all land plants, including most crop species, can benefit from having AMF as a mutualistic partner in their root systems [7]. AMF, frequently categorized as bio-fertilizers, possess the capability to augment crop yields in an ecologically sustainable context [8]. This symbiotic association is markedly efficacious in facilitating the uptake of diffusion-limited nutrients, with phosphorus being especially salient [9]. It plays a pivotal role in augmenting water uptake and its subsequent conveyance to the host plant [10]. Given their integral function at the plant-soil nexus, these fungi emerge as vital components in managing soil health and optimizing agricultural productivity. Notably, sugarcane exhibits a favorable response to AMF, suggesting that effective stewardship of the AMF community may be instrumental in rendering sugarcane cultivation systems more ecologically sustainable [11,12]. Nonetheless, potential constraints on the utilization of AMF in soil have been proposed, stemming from observations that these fungi can exert detrimental effects on plant growth under conditions of elevated soil phosphorus levels [13].

Additionally, it's worth noting that Arbuscular Mycorrhizal Fungi (AMF) can potentially impact atmospheric carbon dioxide (CO_2) fixation. This impact arises from their ability to enhance the photosynthetic rates in host plants, which in turn increases carbon sequestration and improves the efficiency with which the plant transports light assimilates from its leaves to its roots [14]. Furthermore, it is worth noting that numerous AMF species establish mutually beneficial relationships with sugarcane, as previously documented [15]. These symbiotic relationships underscore the potential significance of studying AMF in sugarcane cultivation due to their possible contributions to carbon cycling and plant development.

The primary objectives of this research were to analyze the effects of a commercial strain of Arbuscular Mycorrhizal Fungi (AMF) on sugarcane plants. A comparative study was conducted for commercial strains of Greenical VAM^{TM} in the field in conjunction with a control.

2 Methodology

2.1 Experimental design

Mycorrhizal inoculum Greenical VAM^{TM} containing *Rhizophagus fasciculatus* and *Rhizophagus intraradices* (NCBI accession no. OR563927 and OR563925) was incorporated as AMF. The research included two sugarcane cultivars (Co 0238 and Co 86002), with the one treated with Greenical VAM^{TM} serving as the test and the one without any inoculation serving as the control. The design was completely randomized and replicated three times.

2.2 Field experiment

The field experiment was conducted in the year 2021-2022, in the growing season of September to April, in the Bardoli Village of Gujarat, India (21.103569°N, 73.190867°E). The soil characteristics of the field are mentioned in Table 1 . It followed a split-plot configuration inside a randomized complete block design, and there were three replicates. The primary plot factor involved the selection of sugarcane cultivars (Co 0238 and Co 86002), while the sub-plot element included Greenical VAM^{TM} inoculation and non-inoculation in the test and control, respectively. Each study area had been organized into plots, each comprising four rows measuring 15 meters in length and 6 meters in width. These rows were spaced 1.8 meters apart, with a 20-centimeter gap between individual sugarcane plants. To establish a protective buffer zone around each plot, the same sugarcane variety was also planted within a 2-meter perimeter.

The land preparation for sugarcane cultivation adhered to standard procedures, with the application rate of 5 g per acre. A tablet formulation of Greenical VAM^{TM} has 50,000 spores per gram. Subsequently, soil samples underwent analysis to determine their physicochemical properties. The soil was shaped into rows to facilitate the planting process, and sugarcane setts were manually placed within these rows. Concurrently, by spreading 5 g of Greenical VAM^{TM} per acre, the fungus was injected into the soil. This inoculation coincided with the day of sugarcane planting. In contrast, the control group received no AMF inoculation.

Table 1. Soil characteristics of the experimental area

Parameters	Quantity
pH	6.93
EC	0.63 dS/m
Organic Carbon	0.68%
Available N	216.23kg/ha
Available P	57 kg/ha
Available K	432 kg/ha
Available S	6.70 ppm

2.3 Soil Sampling and Observation

Plant debris was removed meticulously before soil samples were taken via both center rows within every plot. These samples of the plant's rhizosphere, taken from depths of 0 to 30 cm, were taken at three different periods: three, six, and twelve months post-planting. Following collection, the soil was sieved through a 2-millimeter mesh and subsequently dried in an oven. The soil's chemical properties, encompassing vital nutrients like total phosphorous (P), nitrogen (N), potassium (K), and available P, were then meticulously analyzed.

Root samples were obtained, and dirt was meticulously removed before being preserved in 50% ethanol. The roots were washed in running water, then sliced into 1 cm pieces before being cleared using 10% KOH over 90°C for 1 hour. Subsequently, they were stained with methyl blue lacto-glycerol. The proportion of root colonization by arbuscular mycorrhizal fungi (AMF) was determined by mounting stained root sections onto slides while examining them beneath a light microscope.

Arbuscular mycorrhizal fungi colonization in the root samples was assessed. To extract the Arbuscular mycorrhizal fungal spores from rhizosphere soil, 5 grams of soil were subjected to flotation-centrifugation using 50% sucrose. Microscopical examination and quantification of the acquired spores required collection on filter paper patterned like a grid.

2.4 Plant Biomass and Sugarcane Productivity

Cane yields were calculated by weighing harvested sugarcane stalks at the end of the 12-month growing cycle and converting that weight into tons per hectare after accounting for plant density. The assessment of sugarcane quality involved the analysis of the commercial cane sugar (CCS) percentage and the subsequent calculation of sugar yields in tons per hectare. Shoot nutrient contents, such as nitrogen (N), phosphorus (P), and potassium (K), were analyzed by collecting, drying, and finely grinding individual plant samples consisting of leaves as well as stalks. A nutritional analysis employed various methods: flow injection analysis (FIA) for N, spectrophotometry for P, and flame photometry following wet digestion for K. Principal nutrient absorption was computed based on sugarcane yield dry weight (DW), revealing nutrient usage throughout the crop's development cycle.

2.5 Statistical analysis

In this study, data analysis involved assessing normality and variance homogeneity before applying a one-way analysis of variance (ANOVA), excluding the spore number of *Rhizophagus fasciculatus* and *Rhizophagus intraradices*. The significance of the means was determined using Tukey's honestly significant difference test (Tukey's HSD) in Graphpad Prism version 10.0.

3 Results and Discussion

3.1 Root Colonization and Spore Density

Arbuscular mycorrhizal fungi root colonization was consistently present in an application. Statistical analysis revealed a significant treatment effect over three time periods, with plots that received inoculation (Greenical VAM^{TM}) displaying significantly higher root colonization compared to non-inoculated plots (control). Root colonization after 3 months revealed striking disparities between the test and control categories, with the control category exhibiting much reduced colonization. Colonization levels converged after 12 months, although inoculated plants still showed considerably greater colonization than control in both varieties, as shown in Figure 1 . Similar results were observed in the study conducted on wheat cultivars, displaying a root colonization rate of 48% in the variety inoculated with AMF[16]. However, it did not improve the nutrient uptake by the plant. Furthermore, in the present study, the choice of Greenical VAM^{TM} as the inoculant and a much higher

spore count may have played a crucial role in enhancing root colonization.

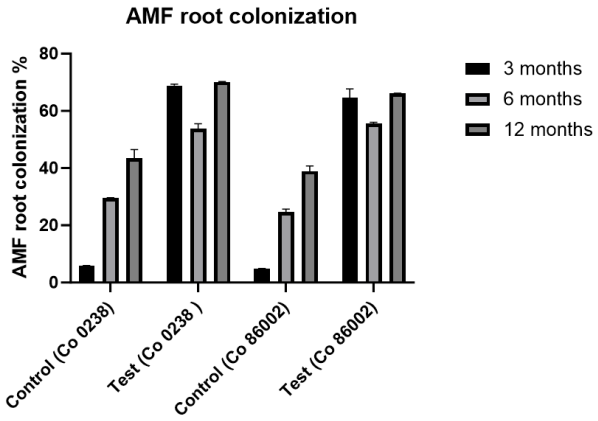

Fig 1. Root colonization by AMF at the three different sampling intervals

The absence of *Rhizophagus fasciculatus* and *Rhizophagus intraradices* spores was noted in control. Spore densities displayed temporal variation, with the peak densities occurring after 6 months. The spore concentrations were lowest in the control group, which did not undergo any fertilization or inoculation. It is clear from Figure 2 that *Rhizophagus fasciculatus* and *Rhizophagus intraradices* spores are mostly responsible for the increased spore counts seen in the infected treatments. It is attributed to the particular environmental conditions, soil properties, plant species, and number of spores inoculated, which can all together influence the magnitude of spore production by AMF. It is possible that the specific combination of these factors in our study favored a sustained increase in spore densities, leading to results superior to those observed in other experiments [17].

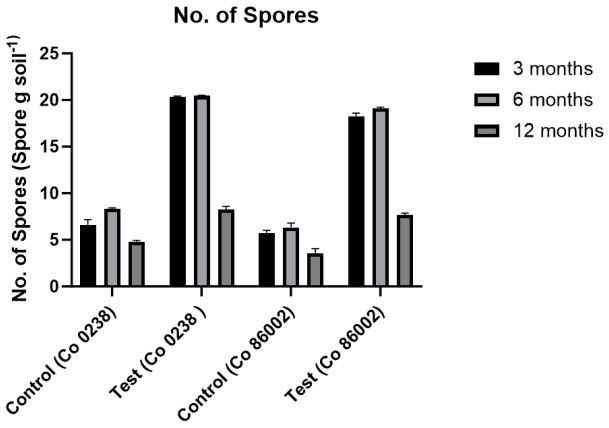

Fig 2. Number of AMF spores at the three different sampling intervals

3.2 Soil Properties

Rhizosphere nutrient concentrations peaked at 6 months post-planting, declining afterward. Table 2 when compared to the test and control groups, the control group's total rhizosphere N was considerably lower after 3 months. Rhizosphere nitrogen was at its maximum at 6 months, although Greenical VAM^{TM} inoculation enhanced it after 3 months.

Upon 3 months of AMF treatment, total P levels in the rhizosphere reached a maximum, which steadily decreased until 12 months (Table 1). Available P was consistently higher in mycorrhizal treatments compared to non-inoculated ones after 3 months. Whereas, Greenical VAM^{TM} also had a notable effect on total K level, with the highest observed increase occurring three months after treatment. These results are correlated with the study conducted on maize sorghum, where AMF inoculation

Table 2. Impact ofGreenical VAM^{TM} on rhizosphere chemical properties of sugarcane. Values represent mean ± SD (n = 3)

	3 months			6 months			12 months		
	Total N (mg Kg^{-1})	Total P (mg Kg^{-1})	Total K (mg Kg^{-1})	Total N (mg Kg^{-1})	Total P (mg Kg^{-1})	Total K (mg Kg^{-1})	Total N (mg Kg^{-1})	Total P (mg Kg^{-1})	Total K (mg Kg^{-1})
Control	197±09	75±12	400±46	187±9	72±15	319±11	130±23	52±10	320±41
Test	284±23	115±42	489±68	400±43	200±92	488±57	220±31	59±44	373±56

considerably increased soil nutrient availability[18].

3.3 Effect of Greenical VAMTM on Plant growth and nutrient uptake

Mycorrhizal treatment resulted in a significant increase in shoot dry weight compared to the control. The Greenical VAM^{TM} treatment applied to Co 0238 resulted in the greatest dry weight of the stalks (Figure 3). AMF colonization was also significantly correlated with shoot dry weight after 3 months.

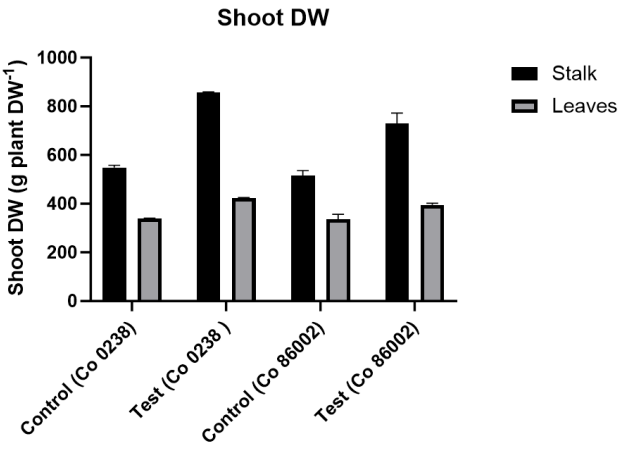

Fig 3. Impact of Greenical VAM^{TM} on Stalk and Leaf after 12 months

Greenical VAM^{TM} inoculation significantly enhanced plant productivity, as indicated in Figure 4 . Both sugarcane varieties, when subjected to the experimental conditions, demonstrated a marked improvement in yield performance for both cane and sugar extraction. Notably, the percentage increase in yields was more pronounced in the Co 86002 variety compared to Co 0238, especially in sugar extraction. However, in absolute terms, Co 0238 had marginally higher yields in both categories under the influence of Greenical VAM^{TM}.

Upon inoculation with Greenical VAM^{TM} both sugarcane varieties, Co 0238 and Co 86002, showcased a marked elevation in nutrient content. The pronounced increase, especially in nitrogen and phosphorus, could be indicative of the effectiveness of the test conditions in enhancing nutrient uptake or retention in the plant tissues. The consistent percentage increase across the two varieties suggests that the applied test conditions have a broad effect on the nutrient profile, regardless of the variety (Figure 5). Numerous prior studies[19–21]have also highlighted the beneficial impact of AMF inoculation on sugarcane production. Significantly, our study introduces a novel dimension. It is the first to report the effects of an increased spore density within a commercial Greenical VAM^{TM} formulation containing AMF. This higher spore density not only led to increased biomass and enhanced soil microbial activity but also improved soil biological quality. Consequently, this translated into a substantial increase in sugarcane yield. This innovative aspect of our research emphasizes the potential benefits of optimizing AMF formulations to enhance both crop productivity and soil health, ultimately advancing sustainability and economic viability in sugarcane farming practices.

Hence, the study presents clear and significant results, demonstrating the significant impact of AMF (Greenical VAM^{TM}) on sugarcane growth and yield, along with a noticeable difference between the two sugarcane cultivars, Co 0238 and Co 86002. However, the findings was limited to the specific conditions and cultivars used in this study, and their applicability to other regions or sugarcane varieties could be addressed.

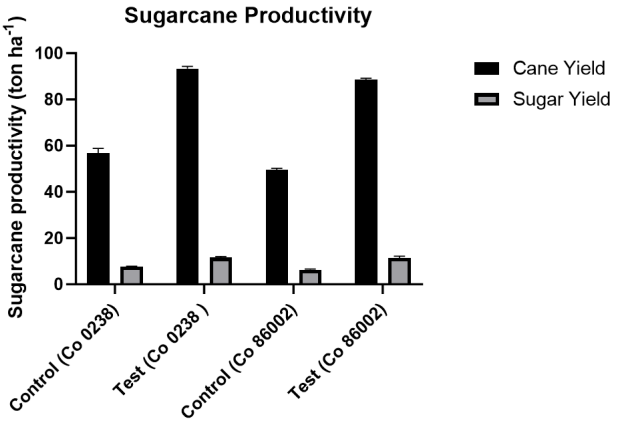

Fig 4. Impact of Greenical VAM^{TM} on sugarcane productivity

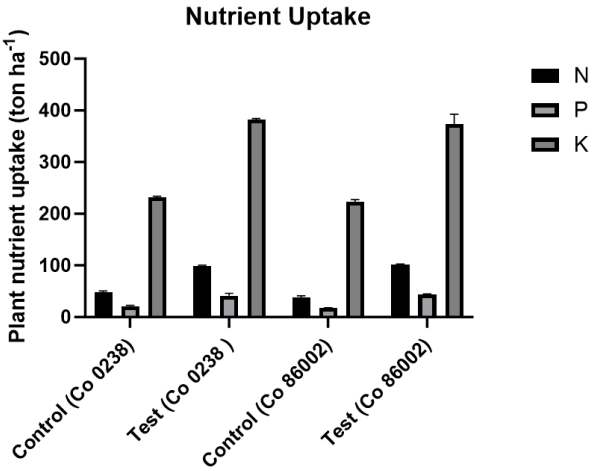

Fig 5. Impact of Greenical VAM^{TM} on plant nutrient uptake

4 Conclusion

Greenical VAMTM, a unique tablet formulation of Vesicular Arbuscular Mycorrhizae (VAM), significantly boosts sugarcane growth and yield by improving soil health, nitrogen management, and root colonization with its high spore concentration of 50,000 spores per gram. These promising results suggest potential applications to various plant species, offering a versatile method for enhancing crop yield and plant health across different environments. Further research could explore its adaptability and long-term effects, considering ecological variability and potential environmental impacts due to high spore concentrations.

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