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Research Article

Effect of Industrial Arbuscular Mycorrhizal Fungi (Ami Mycovita) on Sugarcane Production

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Abstract

This study assesses the effectiveness of Ami Mycovita, a commercial Vesicular Arbuscular Mycorrhizal (VAM) formulation containing *Funneliformis mosseae* on the growth of Sugarcane. In our study, we explored the effects of arbuscular mycorrhizal fungi inoculation on two sugarcane cultivars, Co 0238 and Co 86002, each replicated three times. Through comprehensive assessment of sugarcane productivity, root colonization, nutrient uptake and soil properties, we discovered significant improvements across all parameters following AMF application. Notably, Co 0238 exhibited superior performance compared to Co 86002, suggesting varietal disparities in response to AMF treatment. Our research highlights the promising potential of AMF, exemplified by Ami Mycovit, in enhancing sugarcane growth and yield. With root colonization of AMF ranging from 84% to 107% across the two cultivated varieties, Co 0238 demonstrated higher yield parameters, resulting in enhanced overall sugarcane production. The innovative use of Ami Mycovita in sugarcane farming not only has practical benefits for increasing crop production, but also has a remarkable spore concentration of 1000 spores per gram in its powdered form.

Keywords: Ami Mycovita; Arbuscular Mycorrhizal Fungi; *Funneliformis mosseae*; Sugarcane Growth

Abbreviation

VAM: Vesicular Arbuscular Mycorrhizal; NPK: Nitrogen Phosphorus Potassium; AMF: Arbuscular Mycorrhizal Fungi; CCS: Commercial Cane Sugar; FIA: Flow Injection Analysis; DW: Dry Weight; ANOVA: Analysis of Variance

Introduction

Sugarcane, classified under the *Saccharum spp.*, holds paramount importance in tropical and subtropical regions, serving as a primary crop for sugar and ethanol production [1]. Specifically, in India, *Saccharum officinarum*, a subtropical species, reigns as the predominant source of sugar, thereby positioning the country as a prominent global producer of sugarcane [2]. This underscores the pivotal role of India's sugar industry in driving economic growth through revenue generation and employment opportunities. Sugarcane exhibits a pronounced demand for both water and phosphorus (P) to achieve optimal productivity, while demonstrating the capability to sustain substantial yields with lower leaf nitrogen (N) concentrations. Traditional agricultural practices predominantly rely on mineral fertilizers, typically NPK formulations, to augment crop yields [3]. However, while effective, this approach incurs escalated production costs and potential deleterious impacts on soil health. Importantly, a significant portion of sugarcane cultivation takes place under rain-fed conditions. During periods of drought, characterized by inadequate and erratic rainfall, water scarcity ensues, resulting in compromised nutrient availability, particularly for less mobile nutrients such as phosphorus (P). Drought events occurring during critical growth stages, such as germination and early establishment, pose significant risks of yield reduction [4]. Prolonged periods of groundwater absence over several weeks

precipitate diminished physiological activity in plants, culminating in reduced biomass [5]. Recent advancements in agricultural management strategies, encompassing refined tillage methodologies, optimized fertilizer application, and targeted manipulation of soil microbial communities, aim to bolster sugarcane yields [6,7].

Arbuscular mycorrhizal fungi (AMF) have recently attracted considerable attention for their ability to enhance sugarcane yields. These fungi form mutually beneficial partnerships with most land plants, including many crops, residing within their root systems [8]. Often regarded as bio-fertilizers, AMF hold promise for boosting crop productivity in an environmentally sustainable manner [9]. This symbiotic relationship is particularly effective in facilitating the uptake of nutrients, especially phosphorus, which are limited by diffusion [10]. Moreover, AMF play a crucial role in improving water uptake and its subsequent delivery to the host plant [11]. Given their pivotal role in the plant-soil interface, these fungi are recognized as key players in soil health management and agricultural productivity enhancement. Notably, sugarcane shows a positive response to AMF, suggesting that careful management of AMF communities could promote the sustainability of sugarcane cultivation systems [12,13]. However, challenges in utilizing AMF in soil have been noted, as these fungi may negatively impact plant growth under conditions of high soil phosphorus levels [14].

Furthermore, it is important to note that AMF can potentially influence atmospheric carbon dioxide (CO_2) fixation by enhancing photosynthetic rates in host plants, leading to increased carbon sequestration and improved efficiency in transporting light assimilates from leaves to roots [15]. Additionally, numerous AMF species have been found to establish mutually beneficial relationships with sugarcane, as documented previously [16]. These symbiotic interactions highlight the significance of studying AMF in sugarcane cultivation, given their potential contributions to carbon cycling and plant development.

The main aims of this study were to investigate the impact of a commercial strain of Arbuscular Mycorrhizal Fungi (AMF) on sugarcane plants. A comparative analysis was carried out between the effects of commercial strains of Ami Mycovita™ and a control group in field conditions.

Materials and methods

Experimental design

The mycorrhizal inoculum Ami Mycovita™, containing *Funneliformis mosseae* (NCBI accession no. OM967381), was utilized as the Arbuscular Mycorrhizal Fungi (AMF) treatment. The study incorporated two sugarcane cultivars (Co 0238 and Co 085), with one cultivar treated with Ami Mycovita™ serving as the experimental group, while the other cultivar received no inoculation and served as the control. The experimental design employed was completely randomized and replicated three times for robustness and reliability of the results.

Field experiment

The field experiment was structured as a split-plot configuration within a randomized complete block design, with three replicates. The primary plot factor involved the selection of sugarcane cultivars (Co 0238 and Co 085), while the sub-plot element included the inoculation with Ami Mycovita and non-inoculation in the test and control groups, respectively. Each study area was divided into plots, each consisting of 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. Additionally, to establish a protective buffer zone around each plot, the same sugarcane variety was planted within a 2-meter perimeter.

The land preparation for sugarcane cultivation followed standard procedures, with an application rate of 100 g per acre of Ami Mycovita. The powder formulation of Ami Mycovita contains 1,000 spores per gram. Following this, soil samples were analyzed to determine their physicochemical properties. The soil was then shaped into rows to facilitate the planting process, and sugarcane setts were manually placed within these rows. Concurrently, Ami Mycovita was applied by spreading 100 g per acre, with the fungus injected into the soil. This inoculation process coincided with the day of sugarcane planting. In contrast, the control group did not receive any AMF inoculation.

Soil sampling and observation

Prior to soil sampling, meticulous removal of plant debris was undertaken from each plot, ensuring a clean sampling environment. Soil samples were collected from both center rows within

every plot. The soil was then sieved through a 2-millimeter mesh and subsequently dried in an oven. Comprehensive analysis of the soil's chemical properties, including key nutrients such as total phosphorous (P), nitrogen (N), potassium (K), and available phosphorous, was meticulously conducted.

Root samples were collected and carefully cleaned to remove soil particles before being preserved in 50% ethanol. The roots underwent a thorough washing under running water and were then sliced into 1 cm pieces. These root segments were subsequently cleared using 10% KOH solution heated to 90°C for 1 hour. Following this, they were stained with methyl blue lacto-glycerol. The extent of root colonization by arbuscular mycorrhizal fungi (AMF) was assessed by mounting stained root sections onto slides and examining them under a light microscope.

Arbuscular mycorrhizal fungi colonization in the root samples was evaluated. To extract the arbuscular mycorrhizal fungal spores from the rhizosphere soil, 5 grams of soil were subjected to flotation-centrifugation using 50% sucrose. The acquired spores were then examined and quantified under a microscope, with collection performed on filter paper designed with a grid pattern for precise enumeration.

Plant biomass and sugarcane productivity

Cane yields were determined by weighing harvested sugarcane stalks at the culmination of the 12-month growing cycle and converting this weight into tons per hectare, factoring in plant density. The evaluation of sugarcane quality involved analyzing the percentage of commercial cane sugar (CCS) and subsequently calculating sugar yields in tons per hectare. Shoot nutrient contents, including nitrogen (N), phosphorus (P), and potassium (K), were assessed by collecting, drying, and finely grinding individual plant samples comprising both leaves and stalks. Various analytical techniques were employed for nutritional analysis: flow injection analysis (FIA) for N, spectrophotometry for P, and flame photometry following wet digestion for K. Principal nutrient absorption was computed based on the dry weight (DW) of sugarcane yield, providing insights into nutrient utilization throughout the crop's development cycle.

Statistical analysis

In this study, data analysis comprised evaluating normality and homogeneity of variance prior to conducting a one-way analysis of variance (ANOVA), with the exception of the spore number of *Funneliformis mosseae*. The significance of the means was assessed using Tukey's honestly significant difference test (Tukey's HSD).

Results and Discussion

Root colonization and spore density

Arbuscular mycorrhizal fungi root colonization was consistently observed following application. Statistical analysis revealed a significant treatment effect over three time periods, with plots that received inoculation (Ami Mycovita) showing significantly higher root colonization compared to non-inoculated plots (control). Notably, after 3 months, there were striking disparities between the test and control groups, with the control group exhibiting significantly reduced colonization. Although colonization levels converged after 12 months, inoculated plants still demonstrated considerably greater colonization than control plants in both varieties, as depicted in Figure 1. These findings parallel results observed in a study conducted on wheat cultivars, which showed a root colonization rate of 48% in the variety inoculated with AMF; however, it did not enhance nutrient uptake by the plant [17]. Additionally, in the present study, the selection of Ami Mycovita as the inoculant, along with its much higher spore count, likely played a pivotal role in enhancing root colonization.

The absence of *Funneliformis mosseae* spores was observed in the control group. Spore densities exhibited temporal variation, with peak densities occurring after 6 months. Interestingly, spore concentrations were lowest in the control group, which did not undergo any fertilization or inoculation. Figure 2 illustrates that *Funneliformis mosseae* spores predominantly contributed to the increased spore counts observed in the inoculated treatments. This phenomenon can be attributed to various factors such as environmental conditions, soil properties, plant species, and the number of spores inoculated, all of which can collectively influence the magnitude of spore production by AMF. It is plausible that the specific combination of these factors in our study favored a sustained increase in spore densities, resulting in outcomes superior to those observed in other experiments [18].

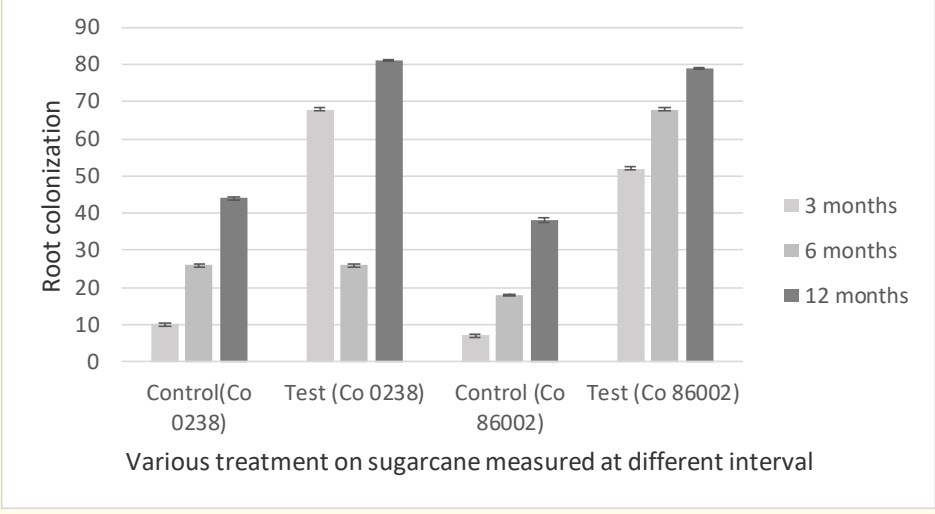

Figure 1: Root Colonization of Arbuscular mycorrhizal fungi in sugarcane.

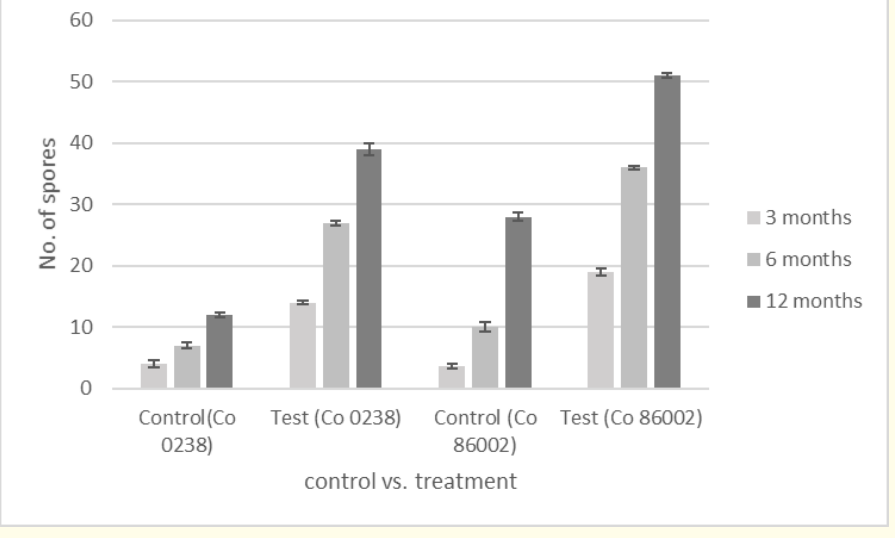

Figure 2: Spores counting.

Soil properties

The concentrations of nutrients in the rhizosphere reached their peak at 6 months post-planting, gradually declining thereafter. Table 1 indicates that, compared to the test group, the control group exhibited significantly lower total rhizosphere nitrogen levels after 3 months. Rhizosphere nitrogen levels peaked at 6 months, with Ami Mycovita inoculation enhancing nitrogen levels after 3 months.

After 3 months of AMF treatment, total phosphorus (P) levels in the rhizosphere peaked, gradually decreasing until 12 months (Table 1). Available phosphorus (P) consistently remained higher in mycorrhizal treatments compared to non-inoculated ones after 3 months. Additionally, Ami Mycovita also significantly influenced total potassium (K) levels, with the highest observed increase occurring three months after treatment. These findings align with a

	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	171 ± 44	67 ± 35	339 ± 11	169 ± 13	66 ± 17	297 ± 41	119 ± 17	62 ± 04	227 ± 35
Test	291 ± 56	181 ± 22	491 ± 05	386 ± 57	213 ± 86	447 ± 61	249 ± 45	147 ± 48	310 ± 47

Table 1: Impact of Ami Mycovita on rhizosphere chemical properties of sugarcane. Values represent mean ± SD (n = 3).

study conducted on maize sorghum, where AMF inoculation substantially increased soil nutrient availability [19].

Effect of Ami Mycovita on plant growth and nutrient uptake

The mycorrhizal treatment led to a significant increase in shoot dry weight compared to the control group. Particularly, the application of Ami Mycovita treatment to Co 238 resulted in the highest dry weight of the stalks (Figure 3). Additionally, AMF colonization exhibited a significant correlation with shoot dry weight after 3 months.

Ami Mycovita inoculation significantly enhanced plant productivity, as illustrated in Figure 4. Both sugarcane varieties, when subjected to the experimental conditions, showed a noticeable improvement in yield performance for both cane and sugar extraction. Notably, the percentage increase in yields was more pronounced in the Co 085 variety compared to Co 238, particularly in sugar extraction. However, in absolute terms, Co 238 exhibited marginally higher yields in both categories under the influence of Ami Mycovita.

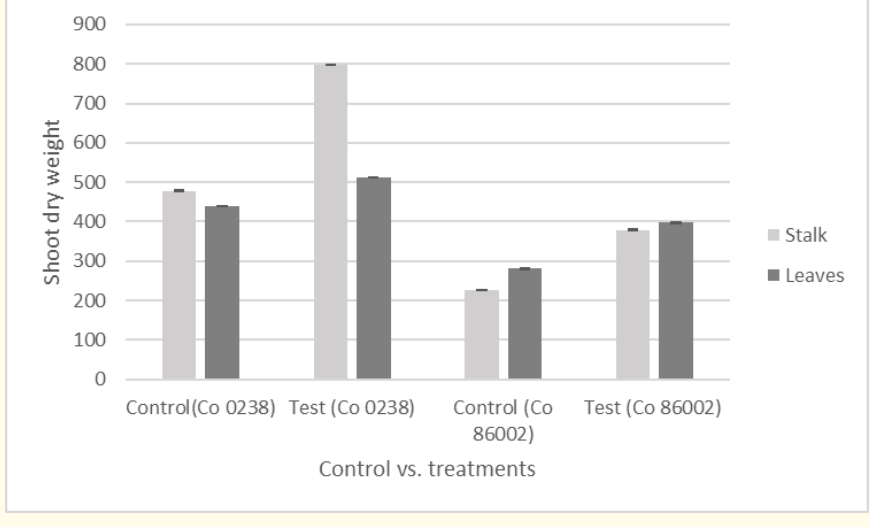

Figure 3: Effect of Ami Mycovita on Shoot dry weight in sugarcane.

Upon inoculation with Ami Mycovita, both sugarcane varieties, Co 238 and Co 085, exhibited a significant increase in nutrient content. The notable elevation, particularly in nitrogen and phosphorus levels, suggests the effectiveness of the experimental conditions in enhancing nutrient uptake or retention in plant tissues. The consistent percentage increase across the two varieties implies that THE applied test conditions have a broad effect on the nutrient profile, irrespective of the variety (Figure 5). Several prior studies [20-22] have also underscored the beneficial impact of AMF inoculation on sugarcane production. Importantly, our study introduces a novel dimension by being the first to report the effects of increased spore density within a commercial Ami Mycovita

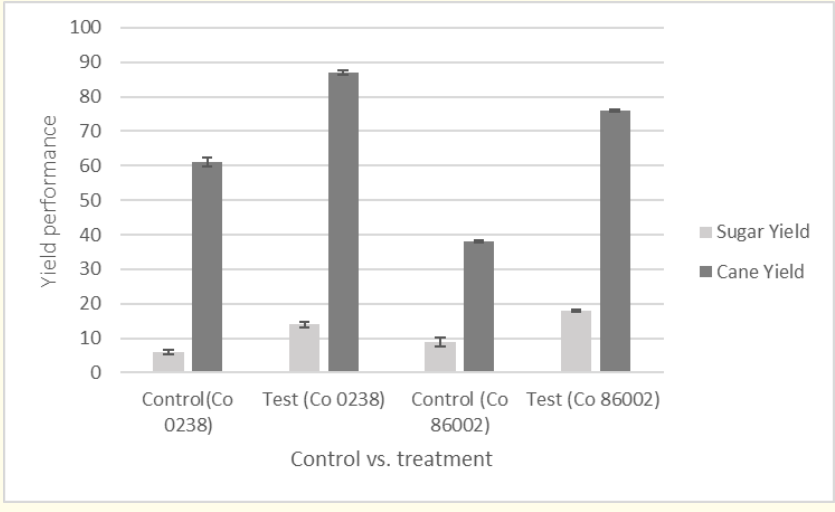

Figure 4: Effect of Ami Mycovita on yield.

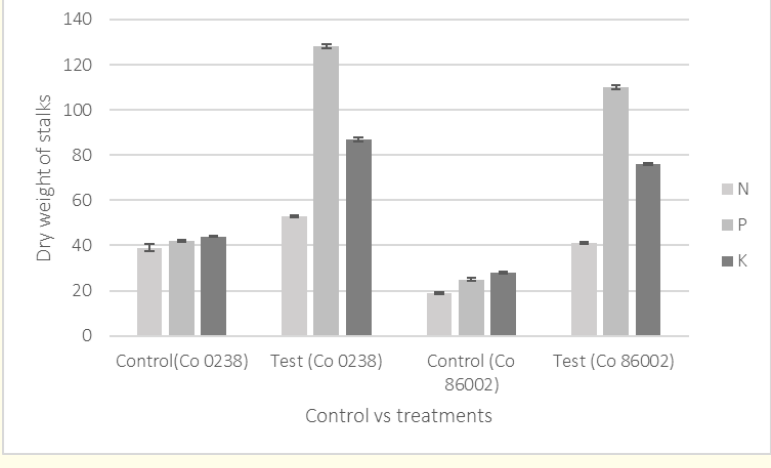

Figure 5: Effect of Ami Mycovita on Nutrient uptake in sugarcane.

formulation containing AMF. This higher spore density not only resulted in 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 underscores the potential benefits of optimizing AMF formulations to enhance both crop productivity and soil health, ultimately promoting sustainability and economic viability in sugarcane farming practices.

Conclusion

Ami Mycovita represents a novel and pioneering advancement in agriculture. Its distinctive powder formulation, boasting an exceptional spore concentration of 1000 spores per gram of Vesicular Arbuscular Mycorrhizae (VAM), has demonstrated remarkable potential in enhancing sugarcane growth, yield, soil health, and nitrogen management. This innovative approach suggests applicability across various plant species and ecological conditions, offering the promise of improved agricultural yields and plant health.

Future studies should further explore Ami Mycovita's versatility across different crops and environments, validating its potential as a significant resource in modern agriculture. Additionally, practical recommendations should be developed for farmers and growers to effectively integrate this innovative formulation into their cultivation practices, ensuring that its benefits are fully realized.

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