EFECTO DE LA BIOFERTILIZACIÓN SOBRE EL CRECIMIENTO EN MACETA DE PLANTAS DE CAÑA DE AZÚCAR (*Saccharum officinarum*)

EFFECT OF BIOFERTILZATION ON THE GROWTH OF POTTED SUGARCANE PLANTS (*Saccharum officinarum*)

EFEITO DA BIOFERTILIZAÇÃO NO CRESCIMENTO DE PLANTAS EM VASOS açúcar de cana (*Saccharum officinarum*)

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ABSTRACT

The use of microorganisms as fertilizer has demonstrated beneficial effects on plant growth and is an alternative to chemical fertilizers. However, each microorganism has different beneficial effects. This study evaluated the effect of applying microorganism fertilizers, Azospirillum brasilense, Azotobacter chroccocum, and Trichoderma lignorum on the growth of potted sugarcane plants var. CC 934418. Plant growth was measured in terms of stem diameter, stem and root length, and the number of leaves and roots 15, 30, and 45 days after planting. Plant growth evidenced statistically significant differences among treatments. Microorganism

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fertilizers showed a positive effect on the growth of sugarcane plants, with Azospirillum brasilense and Trichoderma lignorum as the microorganisms that exercised the greatest effect on stem diameter, root systems, and plant foliation. Beneficial effects of Trichoderma lignorum on leaf growth were observed. This is a new scientific contribution since this species has not been reported as promoting plant growth.

RESUMEN

El uso de microorganismos como fertilizante, ha demostrado tener efectos benéficos sobre el crecimiento de plantas y son una alternativa al uso de fertilizantes químicos, sin embargo, cada microorganismo difiere en sus efectos benéficos. En este trabajo se evaluó el efecto de la aplicación de microorganismos fertilizantes, Azospirillum brasilense, Azotobacter chroccocum y Trichoderma lignorum sobre el crecimiento en maceta de plantas de caña de azúcar variedad CC 934418. El crecimiento de las plantas se midió en términos de diámetro del tallo, longitud de tallo y raíces, y número de hojas y raíces a los 15, 30 y 45 días de la siembra. El crecimiento de las plantas mostró diferencias estadísticamente significativas entre los tratamientos. Los microorganismos fertilizantes mostraron efecto positivo sobre el crecimiento de plantas de caña de azúcar, siendo Azospirillum brasilense y Trichoderma lignorum los microorganismos que ejercieron mayor efecto sobre el diámetro del tallo y los sistemas radical y foliar de la planta. Se observaron los efectos beneficiosos de Trichoderma lignorum sobre el crecimiento de la hoja. Este es un nuevo aporte científico, ya que esta especie no ha sido reportada como promotora de crecimiento vegetal.

RESUMO

O uso de microrganismos como fertilizante, tem sido demonstrado que têm efeitos benéficos no crescimento das plantas e são considerados uma alternativa ao uso de fertilizantes químicos, no entanto, cada microrganismo possui diferentes efeitos benéficos. Neste estudo foi avaliado o efeito da aplicação de microorganismos fertilizantes, Azospirillum brasilense, Azotobacter chroccocum e Trichoderma lignorum no crescimento de cana-de-açucar da variedade CC 934418 plantadas em vasos. O crescimento das plantas foi medido em termos do diâmetro do caule, comprimento de caule e da raiz e número de folhas e raízes nos dias 15, 30 e 45 após a semeadura. O crescimento da planta mostrou diferenças significativas entre os tratamentos. Os microrganismos fertilizantes mostraram efeito positivo sobre o crescimento das plantas de cana, os microrganismos Azospirillum brasilense e Trichoderma lignorum exerceram um efeito maior em diâmetro do caule, sistema radicular e folhas da planta. Os efeitos benéficos do Trichoderma em lignorum crescimento da folha foram observadas. Esta é uma nova contribuição científica vez que esta espécie não foi relatada como a promoção de crescimento vegetal.

PALABRAS CLAVE:

Caña de azúcar, crecimiento, azospirillum brasilense, azotobacter chroccocum, trichoderma lignorum.

KEYWORDS:

Saccharum, growth, azospirillum brasilense, azotobacter chroccocum, trichoderma lignorum.

PALAVRAS-CHAVE:

cana-de-açucar, crescimento, Azospirillum brasilense, Azotobacter chroccocum, Trichoderma lignorum.

INTRODUCTION

Biofertilization has been used as an alternative to chemical fertilizers to increase soil fertility and crop production in sustainable agriculture [1]. Use of microorganisms beneficial to agriculture started more than 60 y ago because of their capacity to convert unavailable and nutritionally important elements into available ones, and as an alternative to increase plant resistance to adverse environments [2,3]. Among the microorganisms that have had a beneficial effect on crop growth and yield, and found to be associated to plant rhizosphere, are species of the genera: *Azospirillium, Azotobacter, Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Rhizobium, Serratia,* and *Trichoderma* [4]. These microorganisms species benefit plant development owing to the fact that they increase nitrogen absorption, phytohormone synthesis, mineral solubilization, and iron chelates. Some can also induce resistance against pests and inhibit soil pathogens through the production of antimicrobial metabolites. Furthermore, they improve soil structure and reduce erosion [5, 3, 6, 7].

Diverse studies report that inoculation with only one beneficial microorganism generally increases plant growth and decreases pathogenic agents [8, 9]. Each microorganism has different beneficial effects, the inoculation effect with *Azospirillum* on plant growth, for example, is due to a secretion of substances promoting plant growth, mainly indoleacetic acid producing a positive effect on root development and water and mineral catchment [10, 11]. The beneficial effect of the *Trichoderma* spp. microorganism is that it establishes symbiotic rather than parasitic relationships with the plant increasing root growth and productivity, helping to overcome stress situations, and improving nutrient absorption [12]. Species of the *Trichoderma* genus are able to inhibit the growth of a variety of potentially pathogenic fungi, such as *Botrytis cinerea, Mucor piriformis, Rhizoctonia solani*, *Alternaria alternata, Fusarium udum,* and *Sclerotium cepivorum* [13, 14, 15, 16, 17]. However, the *lignorum* species is not reported as having biofertilizing potential. Bacteria of the *Rhizobiacea* genus establish symbiotic relationships with the roots of legumes and the effectiveness of this symbiosis will depend on the capacity of the legumes to form consistent nodules in their plant tissue and nitrogen fixation necessary for plant development under stress conditions. Furthermore, these bacteria stimulate phytohormone production, such as cytokinins, auxins, and gibberelins that are responsible for plant growth or elongation of the cell wall. In turn, the plant contributes all the necessary organic compounds for the growth of the microorganism [18, 19, 20]. In spite of the benefits that these microorganisms give to plants, few real applications are reported [1]. In the tomato (*Lycopersicon esculentum* Mill), inoculation with rhizobacteria exerted a positive effect on quality, specifically in size and texture [21]. Other authors have reported that they were able to increase growth and yield in apricots by using rhizosphere microorganisms [22], peanuts [23], and cacti [24], whereas Kızılkaya [25], found that strains of *Azotobacter chroococcum* exerted a positive effect on nitrogen yield and concentration in wheat.

Few studies have been found about biofertilizer use for sugarcane, in spite of the fact that asymbiotic nitrogen fixed by bacteria replaces 60% of the nitrogen needed by this cultivar (corresponding to kg N ha^{-1}) and it is harvested in more than 90 countries worldwide [26, 27, 28]. Mirza *et al*. [29], inoculated sugarcane seedlings micropropagated with strains of *Enterobacter sp* and achieved beneficial effects on growth. These authors indicated the potential of these strains as biofertilizers in sugarcane. Suman et al. [30], found substantial genetic diversity in some strains of *Acetobacter* for future research as microorganisms promoting plant growth, and suggested that strains of *Acetobacter* and *Azospirillum* can be used as the basic source for the development of sugarcane biofertilizers. Stamford et al. [31], evaluated the effectiveness of a biofertilizer on sugarcane yield and its effects on some chemical attributes of a soil with low P and K availability, and considered that biofertilizers are a potential source for use in sugarcane.

Sugarcane fertilization consists in contributions of nutrients of the Nitrogen-Phosphorus-Potassium (NPK) type with the actual recommended dose of 250, 75, and 190 kg ha^{-1}, respectively, for each component. However, other authors recommend additional doses of NPK in clay soils (463, 261, and 226 kg/ha, respectively) whereas partially saline clay soils also require additional doses of NPK, 151, 121, and 35 kg ha^{-1}, respectively [32]. The indiscriminate use of chemical compounds for crop fertilization entails removal of nutrients and secondary micronutrients present in the soil, extracted from one harvest to another and without returning in an organic form. Due to this problem, it is necessary to apply sustainable agriculture through beneficial microorganisms or biofertilizers which not only compensate nutrient deficiencies, but rather increase crop productivity and plant nutrient assimilation efficiency [33]. Consequently, this study evaluated the effect of applying *Azospirillum brasilense, Azotobacter chroccocum,* and *Trichoderma lignorum* in sugarcane plant var. CC 934418 on stem diameter, stem and root length, and number of leaves and roots, and they were compared with sugarcane plants grown in a nutrient-rich substrate.

METHOD

Thirty-six certified sugarcane plants var. CC 934418 were obtained from Ingenio del Cauca Hacienda, Zanjón Rico, suerte 2, Miranda (Cauca, Colombia). Plants were potted in a nutrient-rich substrate, composition shown in Table 1.

Subsequently, 2 d after planting, 27 sugarcane plants were inoculated separately with 10 mL aqueous solutions of Azospirillum brasilense, Azotobacter chroccocum, and Trichoderma lignorum in concentrations of 2.5x107CFU mL-1. Strains were obtained from the microbial seed bank of the Biocontrol company and certified in the soil microbiology laboratory of the Universidad Nacional de Colombia, Bogotá campus, and in the Center for Microbiological Research of the Universidad de los Andes in Bogotá (CIMIC), Colombia.

After 15, 30, and 45 d of the inoculation, random samples were taken to analyze the inoculated plants for each microorganism and control. Subsequently, plants were washed in a water solution with 10% sodium hypochlorite, and measurements taken of stem diameter, stem and root length, and number of leaves and roots. Stem diameter was calculated with a Vernier caliper, stem and root length with a flexometer scaled 0 to 3 m, and the number of leaves and roots was counted manually. Experiments were carried out with three replicates and a completely random 4x3 factorial design as follows:

The inoculant type factor had four levels, Azospirillum brasilense, Azotobacter chroccocum, and Trichoderma lignorum, as well as a non-inoculated treatment used as the control.

Plant development time factor had three levels: development at 15, 30, and 45 d after inoculation.

The response variable was plant development 15, 30, and 45 d after inoculation with each microorganism measured for stem diameter, stem and root length, and number of leaves and roots.

Results were analyzed with ANOVA and multiple mean tests by the minimum significant difference method (MSD) adjusted at 95% with the statistical package SAS version 9.13.

RESULTS

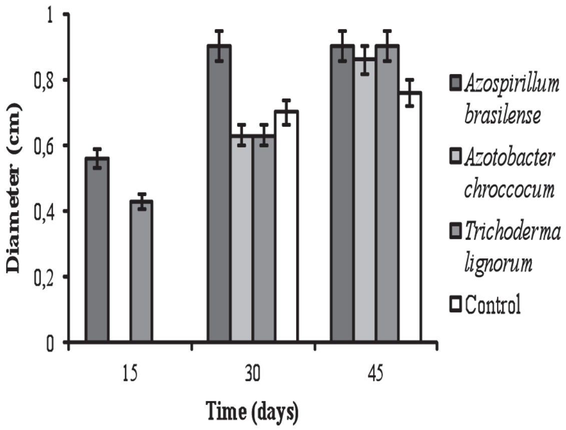
Stem diameter and length

Figure 1 shows the variation in stem diameter for inoculation with *Azospirillum brasilense, Azotobacter chroccocum,* and *Trichoderma lignorum* in sugarcane plants var. CC 934418. ANOVA demonstrated a highly significant difference among treatments. The MSD method did not show differences between the control and treatment with *Azotobacter chroccocum* in the three evaluated time periods. *Azospirilum brasilense* and *Trichoderma lignorum* displayed a beneficial effect on stem diameter from the first 15 d after planting. On day 30, *Azospirillum brasilense* significantly increased stem diameter whereas the *Azotobacter chrocooccum*

Table 1. Chemical composition of the commercial substrate used in sugarcane plants var. CC 934418

Total nitrogen (N)	2,0 %	Ashes	48%
Total phosphorus (P2O5)	1,5 %	Maximum Humidity	20%
Soluble potassium (K2O)	1,5 %	pH	7,14
Calcium (CaO)	2,0 %	Density	0,47 g/cm3
Magnesium (MgO)	1,5 %	Cation exchange capacity	38,2 meq/100g
Oxidable organic carbon	17%	Water retention capacity	147,25 %
Carbon-Nitrogen relationship	8,6	Electrical conductivity	10,12 ds/m

Figure 1. Variation in stem diameter because of inoculation with *Azospirillum brasilense, Azotobacter chroccocum,* and *Trichoderma lignorum* in sugarcane plants var. CC 934418



and *Trichoderma lignorum* treatments did not present statistical differences. By day 45, plants inoculated with *Azospirillum brasilense* and *Trichoderma lignorum* displayed a greater diameter (0.9 mm).

Diverse studies have reported the beneficial effects of biofertilizers on plant growth. Singh and Datta [34], obtained superior growth and grain yield in plants treated with Anabaena variabilis. Plant growth is affected by the presence of microorganism pathogens [35, 36] which become resistant to agrochemicals. The use of microorganism fertilizers decreases resistance against these pathogens and benefits their growth [35, 36]. This is explained by the production of antibiotics, siderophores, and antagonist substances which acts as biological control [37]. The beneficial effect on stem diameter was observed in this study, although the production of the aforementioned substances was not quantified. Given the agrobiotechnological potential found in this study, identification and quantification of the named substances could be considered as the objective of a future study.

Figure 2. Variation in stem length because of inoculation with *Azospirillum brasilense, Azotobacter chroccocum,* and *Trichoderma lignorum* in sugarcane plants var. CC 934418

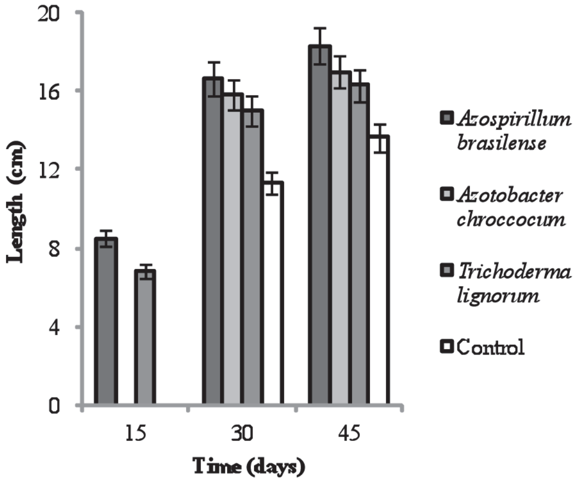


Figure 2 shows the variation in stem length for inoculation with *Azospirilum brasilense, Azotobacter chrococcum,* and *Trichoderma lignorum* in sugarcane plants var. CC 934418. ANOVA demonstrated a highly significant difference among treatments. The MSD test showed differences among all treatments in the three evaluated time periods (15, 30, and 45 d). *Azospirilum brasilense* and *Trichoderma lignorum* displayed a beneficial effect on stem length from the first 15 d after planting. *Azotobacter chrococcum* showed an effect on stem length starting from day 30.

Azospirillum brasilense demonstrated the greatest benefit on sugarcane plant stem length, which could be a consequence of phytohormone production that stimulating nutrient absorption and subsequent plant development. Stem length of Prunus cerasifera L. clone Mr.S 2/5 plants inoculated with Azospirillum brasilense Sp245 was significantly higher than non-inoculated plants [38]. Donoso et al. [39], reported significant differences in the height and biomass of Pinus Radiata when Trichoderma harzianum was used jointly with a nutrient-rich substrate (compost). Trichoderma spp. is considered to be a plant disease control agent and it is found on the market as a biofertilizer [12, 40]. Shukla et al. [41], suggest that the beneficial effect exerted on sugarcane yield for Trichoderma sp. is due to the soil condition as regards carbon and nitrogen requirements.

Root length and number of roots

Figures 3 and 4 show the respective variations in root length and number of roots with inoculation with Azospirillum brasilense, Azotobacter chrococcum, and Trichoderma lignorum in sugarcane plants var. CC 934418. ANOVA demonstrated significant differences among treatments for root length. The MSD method showed differences between the control plants and those treated as biofertilizers. Root length varied between 4.66 and 30.66 cm. No differences in root length were noticed

Figura 3. Variation in root length because of inoculation with *Azospirillum brasilense, Azotobacter chroccocum,* and *Trichoderma lignorum* in sugarcane plants var. CC 934418.

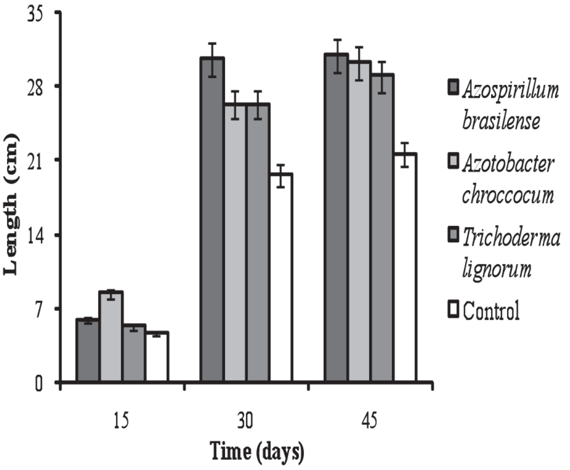
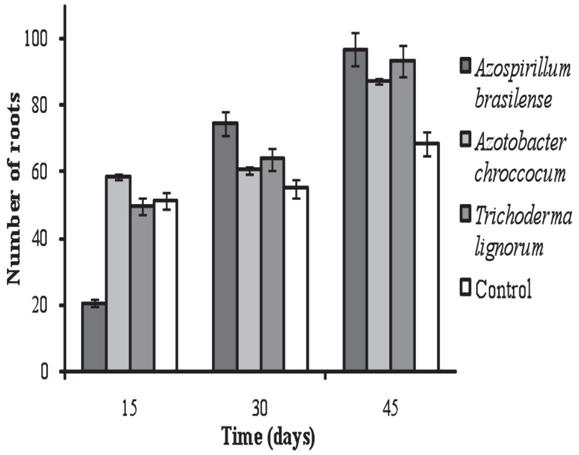


Figure 4. Variation in number of roots because of inoculation with *Azospirillum brasilense, Azotobacter chroccocum,* and *Trichoderma lignorum* in sugarcane plants var. CC 934418



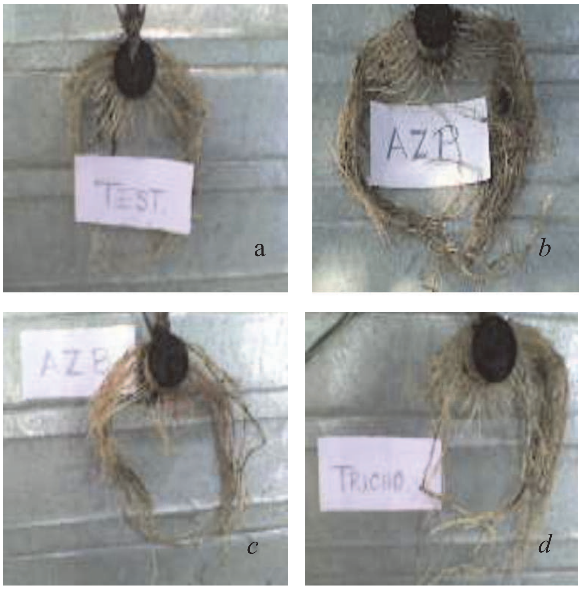
after 30 d in plants inoculated with Azotobacter chroccocum and Trichoderma lignorum, whereas there were no differences among plants sown with Azospirillum brasilense, Azotobacter chroccocum, and Trichoderma lignorum after 45 d. These results can be compared with those reported by Mena-Violante and Olalde-Portugal [21], who found significant increases in root length of tomato plants inoculated with Bacillus subtilis BEB-ISbs (BS13) in relation to control treatments. The increase in the root system length leads to a higher water and nutrient absorption. Moutia et al. [42], achieved an increase of 15% in plant growth (shoot height) and 75% in root dry mass in the sugar cane variety M 1176/77 inoculated with Azospirillum sp.

On day 30, was observed the greatest root length in plants inoculated with Azospirillum brasilense (30.66 cm). This explained by the high production of indoleacetic acid that produce plant growth [43]. Mehnaz and Lazarovits [44], confirmed this theory, when they examined the capacity of Azospirillum lipoferum N7 as a plant-growth promoting agent in corn var. 39D82. Results show that inoculation with biofertilizer microorganisms increases the length of the sugarcane plant root system, indicating a greater growth of absorbent hairs and therefore, greater nutrient catchment [12, 10, 11].

ANOVA demonstrated highly significant differences among treatments for the number of roots. The MSD test did not show any differences between Azotobacter chrocooccum and Trichoderma lignorum. On day 15, the control did not present significant differences in relation to the treatment inoculated with Trichoderma lignorum, whereas when Azospirillum brasilense was used, a smaller effect was observed on the studied variable (20 roots) compared to Azotobacter chrocooccum (58 roots), Trichoderma lignorum (50 roots), and the control (51 roots). However, on day 30 and 45 of the experiment, the number of biofertilized sugarcane plant roots increased considerably in relation to the control treatment. Ribaudo et al. [45], evaluated parameters associated with the growth of tomato seeds inoculated with Azospirillum brasilense FT326 where increases were found in the length of the main root hair, root surface, and root and shoot fresh weight. These authors propose ethylene as an intermediary in the signal path stimulating plant growth.

The control produced 68 roots by day 45 while plants inoculated with Azospirillum brasilense, Azotobacter chrocooccum, and Trichoderma lignorum generated 97, 87, and 93 roots, respectively. Treatment with Azospirillum brasilense had the highest number of roots during the last days of the experiment (Figure 5), followed by Trichoderma lignorum. These results can be compared with those reported by Pereyra et al. [46], who found beneficial effects on the root system of wheat plants inoculated with Azospirillim brasilense. On the other hand, sugar cane has the necessary roots to ensure its maximum growth in optimal soil conditions, which is benefitted by inoculation with

Figure 5. Root development in sugarcane plants var. CC 934418 with 45 d of growth. 5a: Control, 5b: Inoculation with Azospirillum brasilense, 5c: Inoculation with Azotobacter chrococcum. 5d: Inoculation with Trichoderma lignorum



Azospirillum strains stimulating root growth. Furthermore, it plays an important role when the plant faces drought conditions [42].

In other studies, inoculation of seaweed with distinct varieties of Azotobacter significantly increased the root biomass mean up to 98.2 % in relation to the control treatment [47]. These results agree with those reported by Harman et al. [12], who informed that Trichoderma lignorum strains are able to colonize roots and increase their growth.

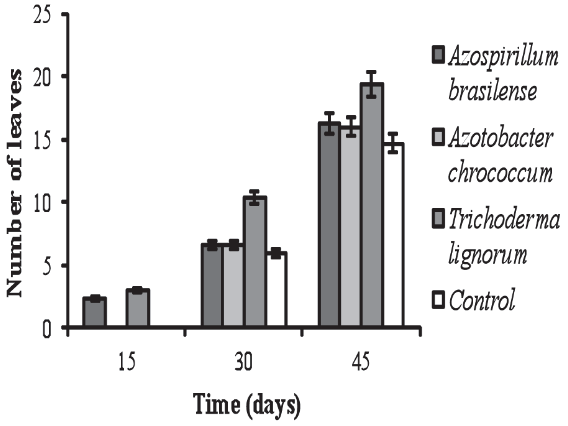
Results of root number and length obtained in our study can be compared with those achieved by Baset Mia et al. [48], who showed that inoculation with rhizobacteria (Azospirillum brasilense Sp7 and Bacillus sphaericus UPMB10) and nitrogen supplements stimulated root length and number in banana plants (Musa spp. cv. ‘Berangan’, type AA) with a 43% root increase in inoculated plants. The authors also compared these results with those obtained when bacterial strains were used without any nitrogen supplement, obtaining significant results. Gamal-Eldin and Elbanna [49], in studies with purple, nonsulfur photosynthetic bacteria (Rhodobacter capsulatus), achieved beneficial effects in terms of high rice plant yield (Oryza sativa).

Number of leaves

Figure 6 shows the variation in the number of leaves for inoculation with Azospirillum brasilense, Azotobacter chrococcum, and Trichoderma lignorum in sugarcane plants var. CC 934418. The MSD test revealed significant differences among treatments. Inoculation with Trichoderma lignorum showed marked differences with other treatmemnts in the evaluated time periods. This may be due to T. lignorum which is capable of producing compounds such as peptides, proteins, and low-weight molecular compounds that have an antibiotic activity against microorganism pathogens, thus promoting plant growth [50]. Yadav et al. [51], achieved increases in sugar cane nutrient availability and yield in Trichoderma viride applications, trash mulch, and trash burn.

These results can be compared with those obtained by Ravikumar et al. [47], who found that inoculation with Azotobacter chroococcum stimulated an increase in leaf area in Rhizhopora seedling species. This effect is explained by the capacity of the microorganism to fix atmospheric nitrogen and make it available to the plant. Furthermore, this microorganism in its culture medium secretes hormones such as auxins, gibberelins, and cytokinins which stimulate plant growth [52]. Other authors evaluated the effect of fertilizers, such as urea and Azotobacter chroococcum strains on blackberry growth (Morus spp.) and its leaf quality under saline conditions. They found higher means in the number of primary branches, plant height, and leaf yield in plants supplied with a biological fertilizer. Greater pigment content was also obtained in the last treatment [53]. It is

Figure 6. Variation in number of leaves because of inoculation with Azospirillum brasilense, Azotobacter chroccocum, and Trichoderma lignorum in sugarcane plants var. CC 934418



important to mention that foliar application is more effective than soil application because atmospheric nitrogen fixation is sufficient in the phylloplane [54]. Sudhakar et al. [55], also evaluated Azotobacter, Azospirillum, and Beijerinckia as foliar biofertilizers in blackberry plants (Morus spp.) with the Azotobacter treatment showing the best leaf yield. The application of these nitrogen-fixing bacteria improved leaf quality in relation to its protein content. Other authors found positive responses in the leaf area of Punica granatum L plants when Azospirillum brasilense, Azotobacter chroococcum, Glomus fasciculatum, and Glomus mosseae were used as biofertilizers, and where A. brasilense was the microorganism that produced the highest number of leaves [56]. Furthermore, Azotobacter has been reported as producing substances that stimulate seed germination, plant flowering, vigor, yield, and resistance to disease. It has been declared a nitrogen-fixing microorganism which increases soil fertility and productivity of different crops [57]. González et al. [58], reported significant changes in fresh weight, adaxial leaf thickness, and root width for in vitro experiments with pineapple inoculated with Azotobacter chroococcum.

CONCLUSIONS

Biofertilizers are a good alternative to using chemical fertilizers in sugar cane crops variety CC 934418. Applying Azospirillum brasilense, Azotobacter chroccocum, and Trichoderma lignorum in sugar cane plants, variety CC 934418, stimulates plant growth. Azospirillum brasilense significantly increased stem diameter, stem length, root number and length. Similarly, beneficial effects of Trichoderma lignorum on leaf growth were observed. This is a new scientific contribution since this species has not been reported as promoting plant growth.

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