

Microbial bioinoculants on agronomic and physiological characteristics of chiltepín, *glabriusculum* variety

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ABSTRACT

Objective: This study aimed to evaluate the effects of *Bacillus subtilis* and arbuscular mycorrhizal fungi, applied individually and in combination under conventional and organic management, on agronomic, physiological, and microbiological variables of chiltepín cultivated under open-field conditions.

Design/methodology/approach: The experiment was conducted under open-field conditions with mulch, using a randomized complete block design with eight treatments and three replicates. The treatments consisted of individual and combined applications of *Rhizophagus irregularis* (BLACK RAY) and *Bacillus subtilis* (Bio Booster), applied via drench every 15 days at a dose of 5 mL per plant. Agronomic, physiological, and microbiological variables were assessed, including plant height, stem diameter, SPAD units, fresh and dry biomass, root length, percentage of mycorrhizal colonization, and the number of spores per 100 g of soil.

Results: The results revealed that the combination of arbuscular mycorrhizal fungi and *Bacillus subtilis* under conventional management promoted the greatest accumulation of total dry biomass, as well as the highest values of spore density and mycorrhizal colonization. Plant height and stem diameter did not exhibit significant differences among treatments. Overall, treatments involving microbial consortia outperformed individual applications, particularly when integrated with conventional fertilization.

Study limitations/implications: Chiltepín (*Capsicum annum* var. *glabriusculum*) is a wild species currently undergoing domestication, a process associated with high phenotypic and physiological variability. This condition may influence crop response to the application of microbial bioinoculants and to the management schemes evaluated, thereby limiting the uniformity of the observed responses and underscoring the importance of considering this biological characteristic when interpreting the results.

Findings/conclusions: The results indicate that the combined application of microbial bioinoculants enhances root system functioning and nutrient uptake efficiency in chiltepín, thereby demonstrating the potential of microbial consortia as a biological management strategy for this crop.

Keywords: *Capsicum annum* var. *glabriusculum*, bioinoculants, arbuscular mycorrhiza, *Bacillus subtilis*.



INTRODUCTION

Mexico harbors a remarkable diversity of chili peppers consumed fresh, dried, or pickled, encompassing a broad spectrum of flavors ranging from very sweet to extremely pungent. Among the latter, chiltepín (*Capsicum annuum* var. *glabriusculum*) stands out as the wild ancestor of cultivated chili pepper (*Capsicum annuum* L.) (Campos, 2019). Sinaloa is nationally recognized for its high agricultural productivity and its strategic role in the development of intensive cropping systems. In this context, chiltepín (*Capsicum annuum* L. var. *glabriusculum*) has begun to gain greater relevance in the state, particularly due to recent advances in its cultivation and domestication processes, which position Sinaloa as one of the leading states in this field (Gómez Anduro *et al.*, 2025). Nevertheless, the scientific knowledge currently available on this species in the state remains incipient, especially with regard to its natural distribution, ecological adaptation, and agronomic management under different production conditions. Although chiltepín is a species widely distributed in northwestern Mexico, most of the detailed ecological information comes from studies conducted in the state of Sonora. In that state, its presence has been documented in municipalities located in the south, central region, and eastern mountainous area, where it grows wild in riparian environments, ravines, and hillsides, associated with ecosystems such as desert and thorn scrub, tropical dry forest, and oak woodlands (Bañuelos *et al.*, 2008). This situation highlights the need to generate specific studies in Sinaloa that would allow a more precise characterization of local chiltepín populations and provide a scientific basis for their use and conservation in a context of increasing productive interest. Its occurrence in these arid and semi-arid environments reflects high physiological and adaptive plasticity, enabling it to withstand water and thermal stress conditions (Romero-Higareda *et al.*, 2023). Wild populations have been considerably affected by various factors, including low rainfall, frost, high temperatures, pests, diseases, as well as consumption by wild and domestic animals (SEMARNAT, 2010; Mc Caughey *et al.*, 2020). At present, producers have become more aware of the need to protect plants during fruiting and harvesting, from which they obtain a product of high economic value, with dried chiltepín reaching an average market price of 250 to 300 pesos per liter (Mc Caughey-Espinoza, 2020). Chiltepín is a plant genetic resource of high ecological, gastronomic, and economic value, whose domestication and agronomic management still present substantial knowledge gaps. Wild populations face threats such as habitat loss, unregulated collection, and environmental variability, making it necessary to generate sustainable production alternatives under controlled conditions (Pickersgill, 2007). Microbial bioinoculants, particularly *Bacillus subtilis* and arbuscular mycorrhizal fungi (AMF), have shown positive effects in multiple crops by improving nutrient uptake, stress tolerance, root development, photosynthetic efficiency, and the synthesis of secondary metabolites (Chatzistathis *et al.*, 2024). However, there is still limited information regarding their specific impact on chiltepín, especially in protected systems or under domestication processes. Evaluating the agronomic and physiological response of chiltepín to microbial inoculation will make it possible to develop sustainable management practices, reduce the use of chemical inputs, promote natural biostimulation, and contribute to the conservation and productive utilization of this native species. The results will provide a technical basis for technological production packages, productive scaling, and restoration or germplasm

bank strategies. The aim of this study was to evaluate the effect of *Bacillus subtilis* and arbuscular mycorrhizal fungi, applied individually and in combination under conventional and organic management, on agronomic, physiological, and microbiological variables of chiltepín grown under open-field conditions.

MATERIALS AND METHODS

Experimental site

The study was conducted during the 2025-2026 autumn-winter growing cycle under open-field conditions at the experimental field of the Universidad Tecnológica de Culiacán, located in Culiacán, Sinaloa, Mexico.

Experimental area and land preparation

The experiment was established on a total area of 90 m², distributed in two 45-m-long mulched rows. Prior to crop establishment, the soil was prepared through conventional plowing, harrowing, and leveling in order to obtain suitable conditions for transplanting.

Plant material and crop establishment

Chiltepín pepper plants at 40 days after sowing (DAS) were used, produced in germination trays. Transplanting was carried out manually under open-field conditions. Planting density was 2.0 m between rows and 0.40 m between plants.

Experimental design and treatments

The experiment was established under a randomized complete block design with eight treatments and three replicates (four plants per replicate), for a total of 96 plants. The treatments consisted of drench applications performed every 15 days using BLACK RAY [*Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler, 2010], applied at a dose of 5 mL per plant, and Bio Booster, a biological control agent formulated from *Bacillus subtilis*, also applied at a dose of 5 mL per plant. Their characteristics, dose, application method, and frequency are described in Table 1. Applications were made directly to the soil in the root zone, beginning at 40 DAS.

Table 1. Description of the treatments evaluated.

Treatments	Description	Application time	Dose
1	AMF + ORG	Transplant	5 mL/plant
2	<i>B. subtilis</i> + ORG	Transplant	5 mL/plant
3	<i>B. subtilis</i> + AMF + ORG	Transplant	5 + 5 mL/plant
4	AMF + CONV	Transplant	5 mL/plant
5	<i>B. subtilis</i> + CONV	Transplant	5 mL/plant
6	<i>B. subtilis</i> + AMF + CONV	Transplant	5 + 5 mL/plant
7	CONV CONTROL	Transplant	—
8	ORGANIC CONTROL	Transplant	—

AMF=arbuscular mycorrhizal fungi; ORG=organic management; CONV=conventional management.

Irrigation and agronomic management

Irrigation and fertilization were performed using a drip system, with a frequency of three irrigations per week and an approximate duration of one hour per irrigation, adjusted according to climatic conditions and soil moisture. Agronomic management was uniform across all treatments, following the technical recommendations for chiltepín pepper cultivation in the region.

Variables evaluated

During the development of the experiment, growth and physiological response variables were evaluated, including plant height (cm), stem diameter (mm), SPAD units, biomass, percentage of mycorrhizal colonization, and number of AMF spores in 100 g of soil. Evaluations were carried out every 15 days, beginning at 15 days after transplanting (DAT) and continuing until 80 days after transplanting.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA), and a mean comparison test was performed at a significance level of $p \leq 0.05$ using SAS statistical software version 9.4. During the development of the field experiment, several activities were carried out to evaluate crop growth and physiological status (Figure 1). First, treatments were applied via drench directly to the root zone in order to ensure homogeneous distribution of the solutions and promote their interaction with the root system. Subsequently, stem diameter and plant length were measured as indicators of vegetative growth, and relative chlorophyll content was assessed using a portable SPAD meter to estimate the nutritional status and photosynthetic activity of the plants.

Plant height

Plant height was recorded from the base of the stem to the apex of the plant using a measuring tape. Measurements were taken every two weeks, always at the same time of day and following the same criterion. This parameter made it possible to monitor longitudinal growth dynamics and the physiological response to the treatments (Hurtado *et al.*, 2017).

SPAD unit determinations

SPAD values were recorded every 15 days using a SPAD-502 meter. Three readings were taken per plant on the most fully expanded and well-illuminated leaf, at the distal portion of the adaxial surface, midway between the leaf margin and the midrib. Measurements were obtained by clamping the sensor onto the leaf, and all readings were performed at the same time of day (8:00 to 10:00 solar time) (de Souza *et al.*, 2019).

The SPAD value serves as a rapid indicator of nutritional status, particularly nitrogen content, as well as photosynthetic efficiency.

Stem diameter

Stem diameter was assessed using a high-precision digital caliper. Measurements were performed every two weeks. To ensure sampling uniformity, stem diameter was recorded



Figure 1. The image illustrates the activities involved in crop establishment (a, b), inoculation (c), and data collection, including SPAD readings (d), plant height (e), and stem diameter (f).

2 cm above the stem base, avoiding areas with bifurcations or abnormal thickening. Before each measurement session, the caliper was zero-calibrated to ensure data accuracy. Readings were then taken directly in the field, taking care not to exert excessive pressure on the plant tissue.

Root length

After careful extraction of the root system and washing of the roots to remove substrate residues, the total length of the main root was measured using a millimeter ruler.

Biomass

Plant growth was evaluated by determining fresh biomass (FW) and dry biomass (DW) of both the aerial part and the root system. Plants were separated into shoots and roots, after which the following variables were recorded: FW (fresh biomass), corresponding to the weight measured immediately after harvest; and DW (dry biomass), corresponding to the weight obtained after oven-drying at 65-70 °C until constant mass. This measurement allowed the estimation of plant matter accumulation and the effect of the treatments on overall growth.

Mycorrhizal colonization

The percentage of mycorrhizal colonization was determined using the method proposed by McGonigle *et al.* (1990), which provides an objective measure of root colonization by

arbuscular mycorrhizal fungi. Root fragments were placed vertically on microscope slides (three slides per plant with 100 fragments), and a drop of acidified glycerol was added. Observations were performed under an Olympus CX21 compound microscope (Olympus America Inc., Center Valley, PA, USA).

Spore counting

Spore extraction was carried out using the wet sieving, decanting, and sucrose-gradient centrifugation method, modified from Gerdemann and Nicolson (1963) and Daniels and Skipper (1972). Spores were counted under a stereoscopic microscope using representative subsamples of 100 g of soil. This count indicates fungal multiplication or persistence (Figure 2).

Evaluation at 80 days

All destructive parameters, including biomass, root length, colonization, and number of spores, were recorded at 80 days after transplanting, when the plants had reached an intermediate stage of development. This evaluation made it possible to integrate physiological, agronomic, and microbiological variables in order to characterize the effects of the treatments.

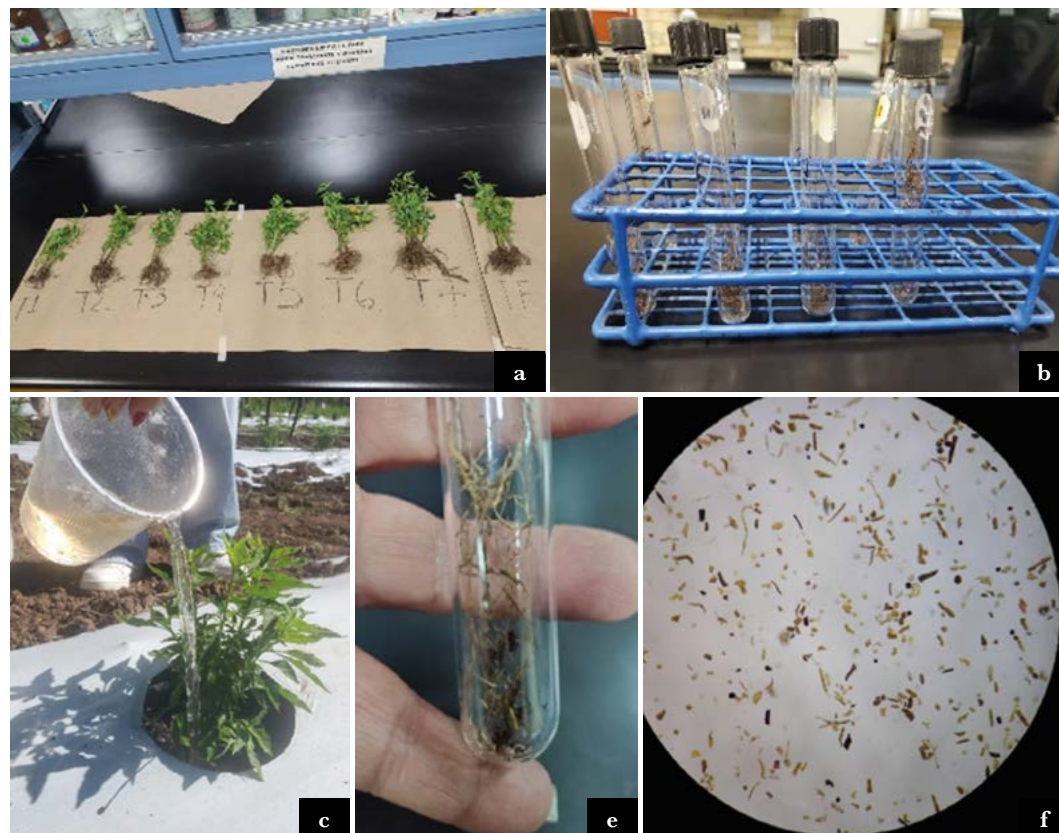


Figure 2. Sampled plants (a), sample preparation (b, c), root cleaning for the removal of soil residues, and spore counting of arbuscular mycorrhizal fungi (d, e).

RESULTS AND DISCUSSION

The results of the principal component analysis revealed that the agronomic and physiological variables evaluated in chiltepín were closely associated with the type of agricultural management and the application of microbial bioinoculants. The high proportion of variability explained by the first two principal components (88.22%) indicates that principal component analysis was an appropriate tool for identifying patterns of association between treatments and response variables, as shown in Figure 3, in agreement with previous multivariate studies conducted in complex agricultural systems (Hair *et al.*, 2019).

The first principal component (PC1), which accounted for the largest proportion of total variation, was associated with variables indicative of vegetative growth and biomass accumulation, such as plant height and the dry weight of different plant organs. The placement of treatments involving *Bacillus subtilis* and arbuscular mycorrhizal fungi under conventional management in the positive quadrant of this component suggests a favorable crop response. This behavior is consistent with what has been described for plant growth-promoting bacteria, which can stimulate plant development through the synthesis of growth regulators, improved nutrient availability, and enhanced phosphorus and nitrogen uptake efficiency (Vessey, 2003; Backer *et al.*, 2018). The positive association between biomass-related variables and treatments involving arbuscular mycorrhizal fungi supports the role of these symbionts in improving plant nutritional status. Several authors have documented that mycorrhizal colonization increases the efficiency of uptake of poorly mobile nutrients, which translates into greater shoot and root growth (Smith & Read, 2008). In this regard, Rouphael *et al.* (2015) indicate that arbuscular mycorrhizal fungi (AMF) act as biostimulants by promoting both root architecture and canopy development. The second principal component (PC2) was mainly related to root system variables, such

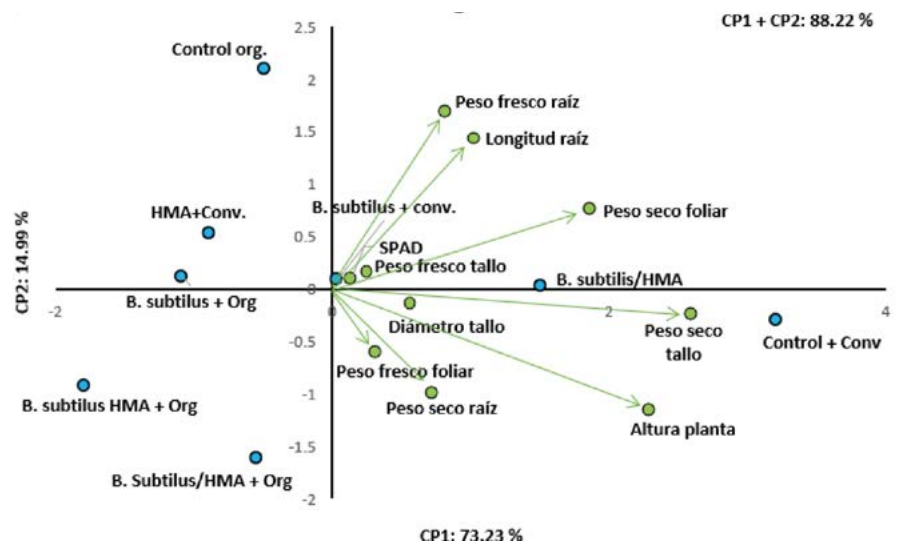


Figure 3. Cartesian plane showing the dispersion of treatments and the evaluated variables corresponding to the agronomic and physiological characteristics of chiltepín treated with *Bacillus subtilis* and arbuscular mycorrhizal fungi under conventional and organic management in open-field conditions.

as root length and fresh root weight, which allowed the differentiation of treatments with a greater emphasis on belowground growth. This pattern has previously been reported in studies in which the interaction between rhizospheric and mycorrhizal microorganisms promotes root system expansion and greater soil exploration (Barea *et al.*, 2005). Conversely, the dispersion of treatments under organic management across different quadrants of the biplot suggests that the response to bioinoculants was modulated by the particular conditions of this production system. In this regard, Hartmann *et al.* (2015) indicate that the native soil microbial community, together with nutrient availability and organic matter content, can significantly influence the effectiveness of inoculated microorganisms. Taken together, the results confirm that the combined application of *B. subtilis* and arbuscular mycorrhizal fungi promotes growth and biomass accumulation in chiltepín, particularly under conventional management, while the agricultural management system plays a pivotal role in the magnitude and expression of these effects.

Spores in 100 g of soil

The presence of arbuscular mycorrhizal fungi (AMF) had a remarkable effect on spore production in the soil and was strongly influenced by the type of management applied. Treatments 4 (26.33 spores), 1 (29.00 spores), and 3 (32.33 spores) showed significant increases relative to the controls, falling within the same statistical group and reaching values five to ten times higher than those of the untreated controls. However, Treatment 6, applied under conventional management, exhibited the highest sporulation, with 55.67 spores, differing statistically from the remaining treatments and consolidating its position as the most efficient treatment (Figure 3). These results clearly indicate that conventional management enhanced spore production, whereas under organic management the response was more moderate. The observed response reflects the dependence of the soil-plant system on symbiotic associations. Although soil fertility was not directly evaluated, the results suggest that treatments under conventional management favored greater sporulation, probably due to the immediate availability of nutrients, in contrast to organic management, where nutrient release is slower and more gradual (Smith & Read, 2008; Diagne *et al.*, 2020). Taken together, the results indicate that arbuscular mycorrhizae were the main factor increasing sporulation and that the type of management was decisive for the expression of this response. Treatment 6, under conventional management, stood out as the most effective, demonstrating that the combination of AMF with conventional fertilization practices can strengthen symbiotic activity and enhance chiltepín productivity (Zhao *et al.*, 2023). Regarding photosynthetic efficiency, measured by SPAD, no significant differences were observed among treatments, which is consistent with previous reports indicating that the SPAD response to biofertilizers is limited when the crop does not experience nitrogen or iron constraints (Bulgari *et al.*, 2019).

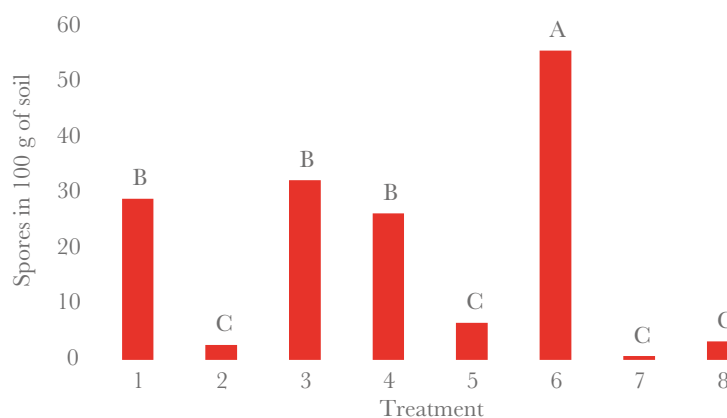
Stem diameter

The data show that, under the conditions evaluated, the bioinoculants did not generate statistically significant differences in stem diameter. Nevertheless, the results suggest that the type of management applied may influence the response, since treatments under

conventional management tended to exhibit slightly greater stem diameters than those under organic management. This indicates that the interaction between arbuscular mycorrhizae and agronomic management may modulate the expression of structural plant growth. The literature supports that soil context and phosphorus availability strongly influence the effect of AMF, whereas the response to other bioinoculants may depend on their interaction with fertilization and the type of management employed (Anguiano-Cabello *et al.*, 2019; Qu *et al.*, 2021; Zeng *et al.*, 2024).

Percentage of mycorrhizal colonization

Analysis using a generalized linear model (GLM) showed that the evaluated treatments had a highly significant effect on the percentage of mycorrhizal colonization ($p < 0.0001$), explaining 98% of the total observed variation. The mean comparison test distinguished three clearly defined statistical groups. The group with the lowest colonization consisted of the control treatments and those inoculated exclusively with bacteria. A second group, with intermediate values, corresponded to treatments that included arbuscular mycorrhizal fungi and microbial mixtures. Finally, the Mixed + Conventional treatment stood out by recording the highest percentage of colonization. These results confirm that inoculation with arbuscular mycorrhizal fungi consistently promotes root colonization compared with treatments lacking mycorrhizae. Likewise, the greater colonization observed in treatments with microbial mixtures suggests that the interaction among different microorganisms may generate synergistic effects (Figure 4), facilitating the establishment and functioning of mycorrhizal symbiosis, particularly when the soil has adequate nutritional support (Smith & Smith, 2011; Rouphael & Colla, 2020). In this regard, the combination of mycorrhizae with other beneficial microorganisms under conventional fertilization schemes appears to optimize the plant-soil interaction, promoting more efficient and sustained colonization. These findings underscore the relevance of integrating microbial consortia as a viable strategy to improve root system functionality and nutrient use in chiltepín cultivation. Spore density in 100 g of soil showed statistically significant differences among the eight treatments evaluated ($p \leq 0.05$). Treatment 6 recorded the highest number of spores

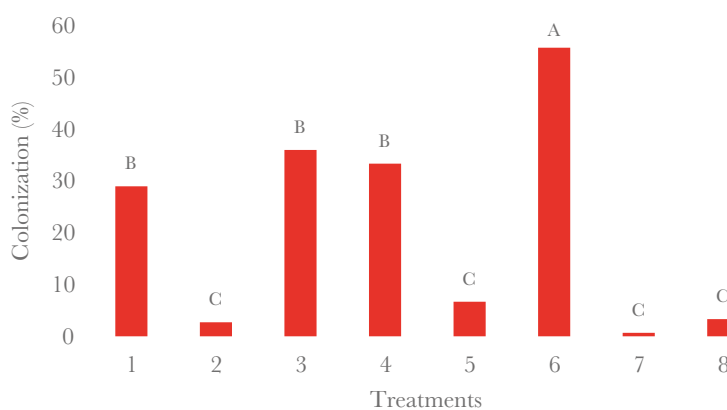


Means with a common letter are not significantly different ($p > 0.05$)

Figure 4. Spores in 100 g of soil.

(approximately 55), forming an independent statistical group A (Figure 3). Treatments 1, 3, and 4 showed intermediate values (approximately 25-32 spores) and were grouped in category B, with no differences among them but values lower than those of Treatment 6. In contrast, Treatments 2, 5, 7, and 8 exhibited the lowest spore densities (<10 spores), constituting group C and being significantly lower than groups A and B. These results demonstrate a differential response in soil spore abundance depending on the treatments applied.

The percentage of mycorrhizal colonization showed statistically significant differences among treatments ($p \leq 0.05$) (Figure 5). Treatment 6 exhibited the highest value ($\approx 55\%$), being independently classified in group A. Treatments 1, 3, and 4 recorded intermediate levels ($\approx 28-36\%$) and were grouped in category B, with no significant differences among them, although their values were lower than that of Treatment 6. In contrast, Treatments 2, 5, 7, and 8 showed the lowest percentages (<10%), constituting group C and differing significantly from groups A and B. These results demonstrate a differential effect of the treatments on mycorrhizal colonization.



Means with a common letter are not significantly different ($p > 0.05$).

Figure 5. Percentage of mycorrhizal colonization.

CONCLUSIONS

The application of microbial bioinoculants promoted root system development and biomass accumulation in chiltepín pepper, particularly when arbuscular mycorrhizal fungi were combined with *Bacillus subtilis*. The type of agricultural management significantly modulated the effects of the inoculants. Under conventional management, the combined treatments showed the highest mycorrhizal colonization and spore production, whereas under organic management the response was more moderate, although still positive, demonstrating that the effectiveness of these microorganisms depends on the conditions provided by the management system applied.

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