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Article

Use of beneficial microorganisms in cape gooseberry crops

Wilmar Alexander Wilches-Ortiz[1,§]
María Margarita Ramírez-Gómez[1]
Diana Paola Serralde-Ordoñez[1]
Andrea María Peñaranda-Rolón[1]
Andrés Díaz-García[2]

1 Corporación Colombiana de Investigación Agropecuaria-Centro de Investigación Tibaitatá. Colombia

2 Corporación Colombiana de Investigación Agropecuaria-Centro de Investigación Sede Central. Vía Mosquera Bogotá km 14, Mosquera-Cundinamarca, Colombia. CP. 250047.

Autor para correspondencia: wwilches@agrosavia.co.

Abstract

Cape gooseberry (*Physalis peruviana* L.) is a tropical fruit of export interest in Colombia; one of the main causes of low productivity is associated with susceptibility to *Fusarium oxysporum* f. sp. *physali* (*Foph*), which causes vascular wilt. This study aimed to evaluate the use of arbuscular mycorrhizal fungi and plant growth-promoting bacteria individually and in mixture as a strategy for the tolerance of the crop to the disease. A validation trial of a cultivation cycle was established in 2022 and 2023 in Granada, Cundinamarca (Colombia) for three cape gooseberry materials (Dorada, Commercial, and Farmer) with three treatments; two correspond to inoculation with beneficial microorganisms with arbuscular mycorrhizal fungi (*Rhizoglomus irregulare* and *Acaulospora mellea*) (T1), arbuscular mycorrhizal fungi + plant growth-promoting bacteria (*Bacillus subtilis* Bs006) (T2), and a control without inoculation (T3). Fertilization of T1 and T2 was carried out at 50% and T3 at 100%. The severity of the disease, agronomic development, crop production, and fruit quality variables were evaluated. The results showed a relationship between the treatments of arbuscular mycorrhizal fungi with weight with and without a calyx, export fruits, and plant height. A relationship was also observed between those of arbuscular mycorrhizal fungi + plant growth-promoting bacteria with production, number of fruits, and °Brix. Finally, in the three materials, it was observed that, without inoculation, the progress of vascular wilt and fruits that do not meet export criteria were favored. It was concluded that inoculation with beneficial microorganisms exerts an antagonistic effect on vascular wilt disease caused by *Foph*.

Keywords:

Acaulospora mellea, Bacillus subtillis. Fusarium oxysporum f. sp. physali, Rhizoglomus irregulare.

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Introduction

Cape gooseberry (*Physalis peruviana* L.) is widely spread in the international market, mainly for its nutritional value (Etzbach *et al*., 2018). It has a high economic and social impact at the national level due to its importance for food security and its international demand. In 2021, cape gooseberry exports in Colombia increased by 16% and production increased by 7% (Agronet, 2022).

In Colombia, vascular wilt caused by *Fusarium oxysporum* f. sp. *physali* (*Foph*) is a phytosanitary limitation of cape gooseberry crops, causing losses of around 80% in production (Simbaqueba *et al*., 2018); additionally, its control is very difficult because chlamydospores remain viable for more than 15 years in soil and tolerate the effect of fungicides applied to it (García-Bastidas *et al*., 2019).

For the control of soil pathogens, the development of biological control measures with rhizospheric microorganisms is currently a promising alternative that leads to a decrease in the incidence and severity of the disease (Chávez-Ramírez *et al*., 2020). In this sense, Plant Growth-Promoting Bacteria (PGPB) and Arbuscular Mycorrhizal Fungi (AMF) trigger mechanisms associated with the suppression of plant diseases (El-Saadony *et al*., 2022).

Mohamed *et al*. (2019) highlighted the biocontrol activity of AMF and PGPB against phytopathogens in bean plants. The association of AMF and PGPB to reduce *Fusarium* wilt in cape gooseberry plants has been little explored. Therefore, the objective was to evaluate the biocontrol capacity of *F. oxysporum Foph* in the field with the application of AMF and in mixture with PGPB and its effect on crop productivity.

Materials and methods

Initial lot characterization

A cape gooseberry production lot with a history of vascular wilt disease was selected, in which an initial georeferenced grid-type sampling was carried out at equidistant points to determine the initial populations of *Fusarium* and AMF. The soil samples collected were taken for analysis at the Agricultural Microbiology Laboratory of the Colombian Corporation for Agricultural Research-AGROSAVIA (Tibaitata Research Center).

For *Foph*, pathogen detection was performed by growing dilutions of soil samples in Komada medium, and to quantify the AMF, the wet sieving and settling techniques were used (Gerdemann and Nicolson, 1963). This is in order to know the amount of spores per gram in the soil of the microorganisms prior to the establishment of the plant material and the initial populations of the pathogen.

Location, plant material, and microorganisms

After characterizing the lot, a field validation trial was established in Granada, Cundinamarca, one of the regions with the highest production of cape gooseberry in Colombia. The semi-commercial trial was randomly established in nine plots with experimental units of 72 plants each for two inoculation treatments (T1: AMF and T2: AMF + PGPB; 50%; chemical fertilization) and a control (T3: No inoculation; 100%; chemical fertilization) in three cape gooseberry materials corresponding to an ecotype of the region (farmer material; “Uchuvón”), a variety used commercially in various regions (commercial material) and the Dorada variety developed and released by AGROSAVIA (Núñez-Zarantes *et al*., 2016).

The AMF used were *Rhizoglomus irregulare* (Blaszkowski *et al*., 2008; Sieverding *et al*., 2014) and *Acaulospora mellea* (Schenck *et al*., 1984) belonging to the Germplasm Bank of the Collection of Microorganisms with Interest in Biofertilizers (CMIB) of AGROSAVIA (Figure 1). With respect to the PGPB strain, the bioproduct Natibac® was used, the active ingredient of which is *Bacillus subtilis* strain Bs006 (formerly *Bacillus amyloliquefaciens*) (Figure 1c), whose production and quality control were carried out at the facilities of the Agrosavia Bioproducts Pilot Plant.

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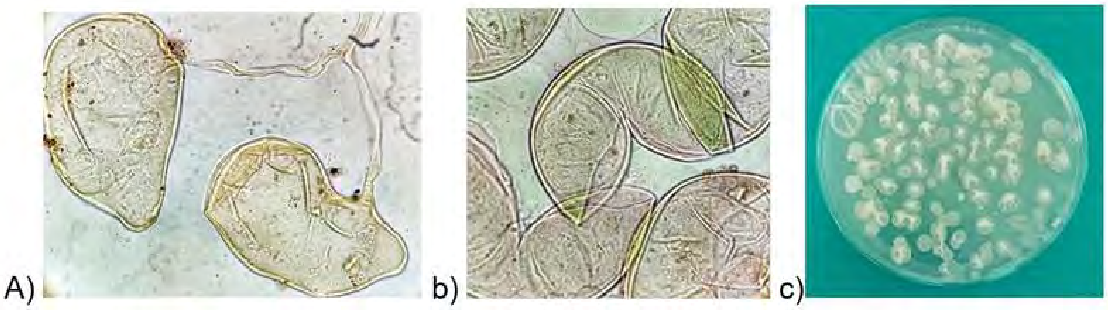
Figure 1. Beneficial microorganisms evaluated. Microscopy of AMF spores (100x) in polyvinyl alcohol-lactic acid-glycerol (PVLG) solution. a) *R. irregulare*; b) *A. mellea*; c) macroscopic image of *B. subtilis* on nutrient agar.



AMF were multiplied with onion (*Allium fistulosum* L.) hosts planted in 500 g pots in soil:sterile sand substrate (2:1 v/v). Quality control of the inoculum was carried out at five months by wet sieving and settling (Gerdemann and Nicolson, 1963) with a count of 144 spores g^{-1}.

The inoculation of AMF was carried out from 70 spores per plant in 72-cavity trays under mesh house conditions, which were then taken to the field. The form and frequency of application of Natibac® was made according to previously standardized conditions (Beltrán-Acosta *et al*., 2023). It was applied in the germination of cape gooseberry seedlings in mesh house conditions and in field transplantation with two boosters every 15 days in each of the stages (seedling and transplanting).

The crop management was carried out considering the biofertilizer potential of the microorganisms; therefore, for treatments 1 and 2, half the dose was applied and for the control treatment, the full recommended dose of chemical fertilization according to the soil analysis. The management of pests, diseases, weeds, and training were according to the producer's criteria in all treatments.

For the agronomic evaluation, the variables of weight and number of fruits were measured in 10 random plants per plot. In addition, the total soluble solids (°Brix) were measured in these fruits with a refractometer. Finally, the total weight of fruits and the percentage of export quality of each plot were recorded.

Severity of vascular wilt caused by *Fusarium oxysporum* f. sp. *physali* (*Foph*)

The cape gooseberry vascular wilt severity scale (0 to 5) was used with joint records of the percentage of severity from 0 to 100%. The evaluation was carried out on the 72 plants per plot with readings of the percentage of leaf area and stems affected through visual perception from the lower to the upper third.

The severity data were used to determine the area under the disease progress curve (AUDPC), which is a useful quantitative summary of disease intensity over time. The method used was trapezoidal; the time variable (days after sowing) was discretized and the average intensity of the disease was calculated between each pair of time points (Madden *et al*., 2007).

Equation 1 expresses the calculation of the AUDPC, where the values obtained are standardized without units.

$$AUDPC=\sum_{i=1}^{Ni-1}\frac{\left(y_i+y_{i+1}\right)}{2}(t_{i+1}-t_i)$$

(Equation 1).

Where: N= number of disease measurements over time; t_i= sample time points in a sequence; y_i= associated measures of disease level.

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Statistical analysis

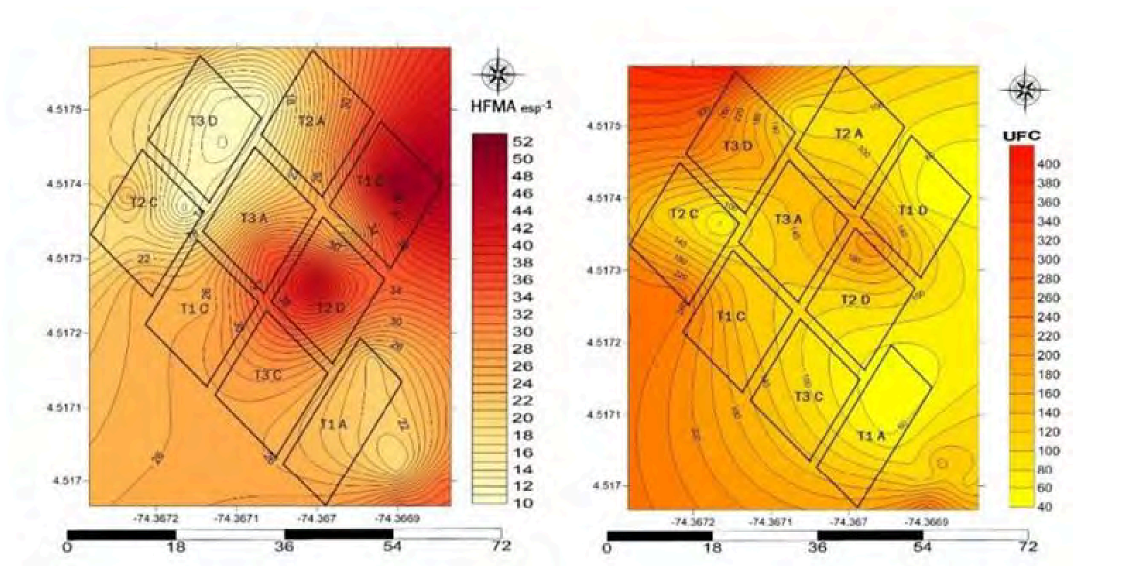
For agronomic, production and disease severity data, analysis of variance (α= 0.05), Student's t-tests and Tukey's multiple comparison ($p \leq 0.05$) were performed. An analysis of canonical correspondence between treatments and variables was included. The statistical program used was R® v.4.3.2 (R Core Team, 2020).

Results and discussion

Initial lot characterization

The data obtained in the laboratory from the soil samples was used to identify the initial distribution of the AMF and *Foph* populations in the lot. Figure 2 shows the maps that graphically represent the density of microorganisms in the soil by means of "IDW" (Inverse Distance Weighting) interpolation and illustrates the distribution of the plots randomly in the lot.

Figure 2. Graphical representation of the density of the populations of arbuscular mycorrhizal fungi (AMF) and *Fusarium oxysporum* f. sp. *physali* (*Foph*) in the soil and distribution of the plots and treatments in the soil. T= treatment; A= Farmer material; C= Commercial material; D= Dorada variety.



The distribution of *Foph* in the lot is 40 to 400 colony-forming units (CFU) g^{-1} soil, which allowed the study to ensure the presence of the pathogen in the lot. For AMF, values of 10-40 g^{-1} soil spores were found, contents close to those reported in the study of these fungi in cape gooseberry crops in the same locality (Granada), which ranged from 5-35 spores g^{-1} soil (Ramírez-Gómez *et al*., 2019).

Severity of vascular wilt caused by *Fusarium oxysporum* f. sp. *physali* (*Foph*

When evaluating the development and progress of vascular wilt disease, a higher percentage of severity was observed in the control treatments, ratifying the values of the area under the disease progress curve, which present significant differences in the control treatments compared to inoculation, where it was lower for the mixture (AMF + PGPB), followed by the AMF alone.

Figures 3 and 4 show greater control of the disease with the mixture of AMF + PGPB compared to the control without inoculation, with statistical differences between the commercial and farmer materials in the AUDPC, which demonstrates the biocontrol potential of the disease by these microorganisms. This coincides with Moreno-Velandia *et al*. (2019) reports, where they demonstrated the biocontrol and plant growth-promoting activity of cape gooseberry with Bacillus velezensis against Fusarium wilt [Fusarium oxysporum f. sp. physali (Foph)].

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Figure 3. Severity of vascular wilt and area under the curve in the commercial material. AMF= *R. irregulare* y *A. mellea*; PGPB= *B. subtilis*; AUDPC= area under the disease progress curve; DDS= days after sowing. Means with different letters indicate significant differences between treatments (Tukey, *p*≤ 0.05).

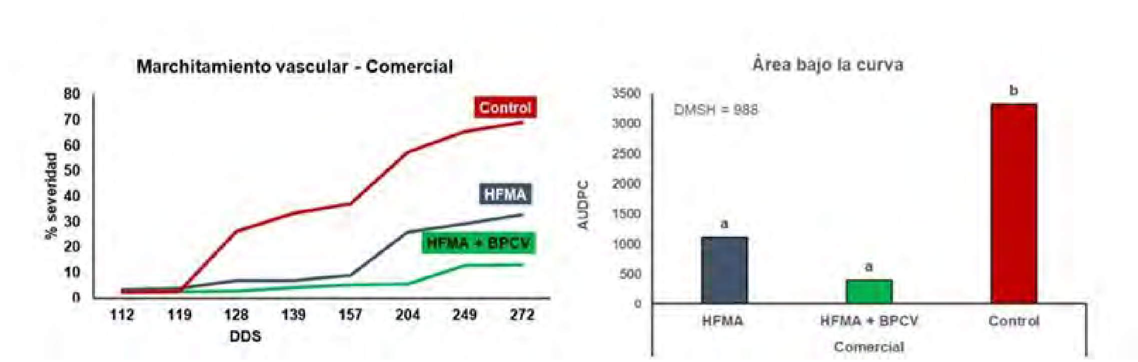
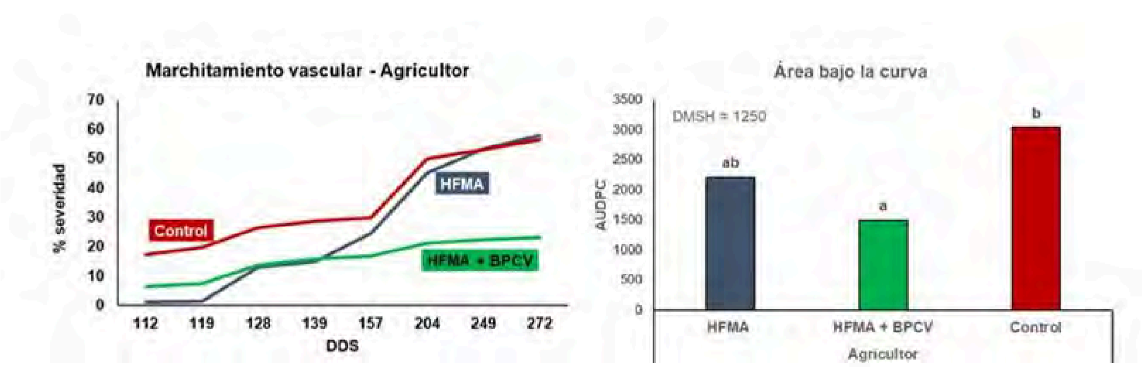


Figure 4.Severity of vascular wilt and area under the curve in the farmer material. AMF= *R. irregulare* y *A. mellea*; PGPB= *B. subtilis*; AUDPC= area under the disease progress curve; DDS= days after sowing. Means with different lett ers indicate significant differences between treatments (Tukey, *p*≤ 0.05).



The authors attribute an antagonistic potential against *Foph* to the test strain. This is complemented by the AMF evaluated (*R. irregulare* and *A. mellea*), which potentiated this antagonistic effect in conjunction with PGPB in the two cape gooseberry materials.

Figure 5 shows vascular wilt in the Dorada variety, where AMF stands out with lower percentages of severity compared to other treatments. This coincides with Ramírez-Gómez *et al*. (2016), where they found that cape gooseberry plants inoculated with a mixture of AMF had a lower incidence and severity of vascular wilt caused by *F. oxysporum* compared to the controls, which demonstrates good agronomic development and plant health in the crop.

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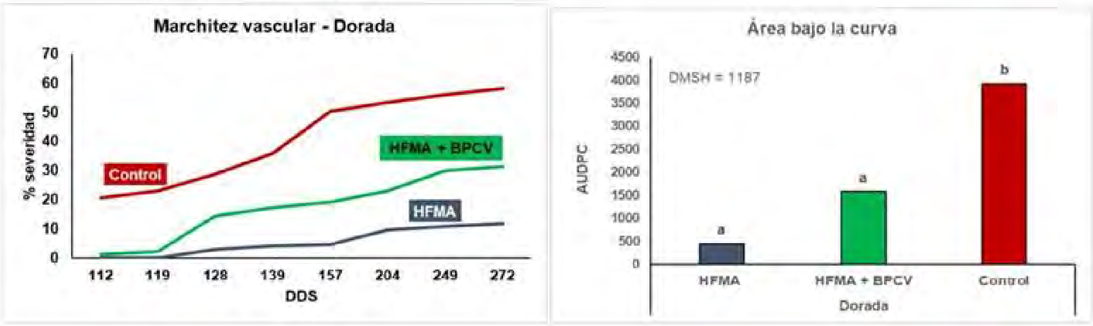
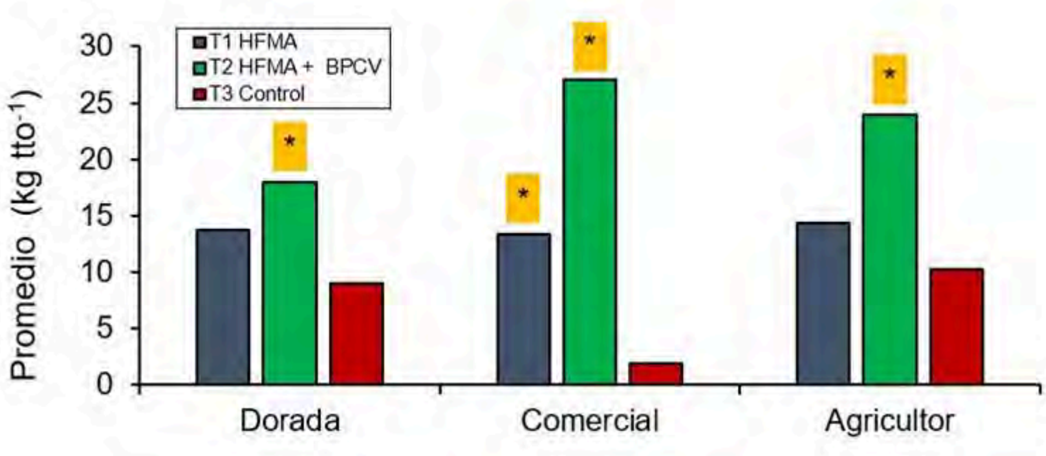
Figure 5. Severity of vascular wilt and area under the curve in the Dorada variety. AMF= *R. irregulare* y *A. mellea*; PGPB= *Bacillus subtilis*; AUDPC= area under the disease progress curve; DDS= days after sowing. Means with different letters indicate significant differences between treatments (Tukey, *p*≤ 0.05).



Production

According to the results of the Student's t-test and with the *p*-value obtained, with a significance level of 5%, it was concluded that the sample means differed statistically from the control for the inoculation treatments, which is corroborated in the graph with differences in the production obtained between the AMF + PGPB and control treatments without inoculation in each variety. AMF treatment and the control show significant differences in the commercial material (Figure 6).

Figure 6. Production of cape gooseberry fruits per plot in three materials evaluated in the field. AMF= *R. irregulare* y *A. mellea*; PGPB= *B. subtilis*. Means with * indicate significant differences compared to the control treatment in each material (t-Student, *p*≤ 0.05).



These results are consistent with others in nightshades, such as Desai *et al*. (2020), where, in *Solanum lycopersicum* L. and *Capsicum annuum* L. plants inoculated with *Bacillus sonorensis* and *Funneliformis mosseae*, they revealed healthy phytosanitary status and vigorous growth compared to those not inoculated. Beltrán-Acosta *et al*. (2023) found that inoculation with *B. subtilis* increased stem length by 58% and aerial dry weight of cape gooseberry seedlings by 119% compared to control.

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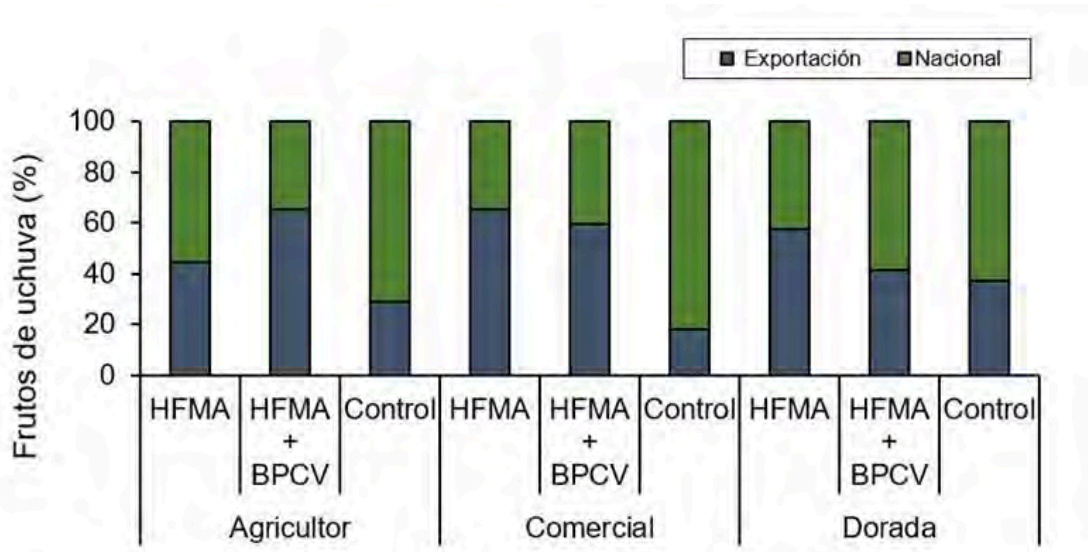
Guana *et al*. (2011) evaluated the inoculation with AMF in cape gooseberry, identifying the potential of *A. mellea* in root development in the nursery stage and greater biomass production. In production, they found a 45% increase with *A. mellea* compared to the control treatment and an effect of more than 60% due to the inoculation of *A. mellea* and *R. irregulare* (formerly *Glomus intraradices*) with a lower percentage of fruit cracking.

The present study assessed the quality of the fruit obtained based on the marketing destination, which is defined by the demands of the international market, where the inoculation treatments reflected a higher percentage of export fruits compared to the control for the materials under study (Figure 7).

Figure 7.Marketing of cape gooseberry fruits in the different materials evaluated in the field. AMF= *R. irregulare*; *A. mellea*; PGPB= *B. subtilis*.



Correlations between factors and response variables

Figure 8 shows the behavior and interaction of the three cape gooseberry materials under study with the different treatments. Through the canonical correspondence analysis (CCA), with 71.5% of the total variance, the positive correlations between variables and treatments were confirmed, where the high affinity between AMF and the variables of weight with and without calyx, export fruits and plant height stands out, with a greater representation in agricultural and commercial materials.

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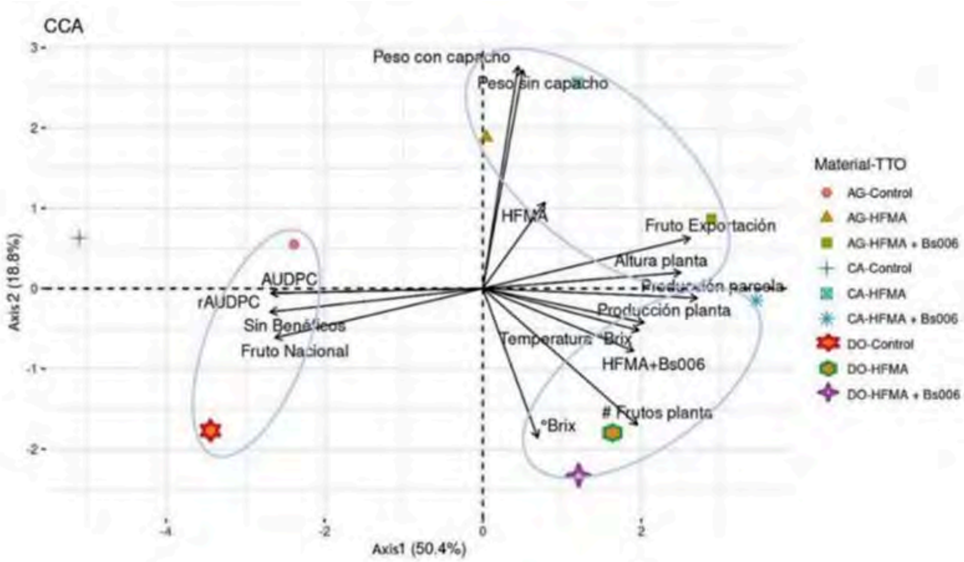
Figure 8.Canonical correspondence analysis of beneficial microorganisms with production, quality, and tolerance to F. oxysporum f. sp. physali in three cape gooseberry materials. AG= Agricultor; CA= Comercial; DO: Dorada; control= no inoculation; AMF= *R. irregulare*, *A. mellea*; Bs006= *B. subtilis*; AUDPC= Area under the disease progress curve; rAUDPC= relative of AUDPC.



There was also a high relationship between AMF+PGPB with production, number of fruits, and °Brix. In the three materials without inoculation, there is a correlation with the variables of AUDPC and its relative, as well as with the fruits of lower quality (national), variables that tend to be inversely proportional to AMF inoculation.

These results are consistent with other studies, such as Wilches-Ortiz *et al*. (2022), where they report the contribution of AMF *R. irregulare* and *A. mellea*, directly related to the quality of panela in sugarcane. In other nightshades, such as *S. lycopersicum*, studies by Schubert *et al*. (2020) showed that *R. irregulare* contributes to the improvement of quality and °Brix in tomato fruits. In the present study, this potentiated relationship was reflected in the mixture of AMF with PGPB, which contributed to the production of cape gooseberry fruits and their °Brix.

Conclusions

The inoculation of the assessed beneficial microorganisms exerts an antagonistic effect on the vascular wilt disease caused by *Fusarium oxysporum* f. sp. *physali* (*Foph*); this effect is potentiated with the two functional groups: arbuscular mycorrhizal fungi and plant growth-promoting bacteria (AMF + PGPB).

AMF lead to a higher export fruit production and plant height of cape gooseberry, whereas the mixture of AMF and PGPB was related to higher fruit production and fruit quality expressed in degrees Brix.

It was shown that the lack of inoculation of both AMF alone and mixed with PCBP favored vascular wilt caused by *Foph*, as well as a decrease in production and quality of the fruit, without meeting export standards. The present study establishes itself as a basis for future research on the biocontrol effect of these microorganisms with potential for transfer to other crops.

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knowledge for innovation that reduces the low competitiveness of cape gooseberry derived from the Covid-19 emergency through the reduction of vascular wilt in Ubaté and Granada, Cundinamarca. Agreement 2049. BPIN 2020000100700

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