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Research Article

Beneficial microorganisms enhance the growth of basil (*Ocimum basilicum* L.) under greenhouse conditions

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Abstract

The integration of healthy management alternatives continues to be a challenge in the organic production of aromatic and medicinal plants, including of basil (*Ocimum basilicum* L.). The objective of this work was to evaluate the effects of three beneficial microorganisms (1) *Trichoderma harzianum* (TH), (2) *Bacillus subtilis* (BS), (3) *Glomus cubense* (GC) and their combinations on the growth of basil. A completely randomised design was used with a control and seven treatments with six repetitions. The control (1) was with no microorganism inoculation and the seven treatments were inoculations with the single or the combined microorganisms as follows: (2) TH, (3) BS, (4) GC, (5) TH+BS, (6) TH+GC, (7) BS+GC and (8) TH+BS+GC. Three harvests of fresh biomass were made and a number of growth variables were recorded: fresh and dry biomass, leaf area, number of commercial stems, stem length and thickness, Leaf length and width, relative chlorophyll concentration (SPAD readings) and the levels of N, P, K, Ca and Mg. Overall growth increased by 58% with TH+GC compared with the control and by 55% compared with the single inoculations (TH, BS and GC) and with the triple inoculation (TH+BS+GC). A growth increase of 51% was obtained with BS+GC compared with the control and of 38% compared with the other treatments. These results indicate co-inoculation of TH+GC or of BS+GC are useful alternative managements to increase greenhouse production of basil.

Keywords: arbuscular mycorrhizal fungi; aromatic plants; *Bacillus subtilis*; biofertilisers; *Glomus cubense*; *Trichoderma harzianum*

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Introduction

Because of their perceived nutraceutical (health related) and organoleptic (aroma, flavour) benefits, the use and consumption of medicinal and aromatic plants (MAP) has increased across the world. This increase has contributed to economic development in the agricultural sectors of many nations. The new focus has also delivered important environmental and social benefits (Kala, 2015; Salome-abarca *et al.*, 2015).

Furnell *et al.* (2019) reported that the three main MAP exporting countries are Mexico, Cameroon and South Africa, while the five main MAP importing countries (77% of total MAP consumption/utilisation) are France (26%), USA (16%), Japan (15%), Germany (11%) and Spain (7%). In Mexico, MAP comprise 8% of total production of organic plants. Of these, basil (*Ocimum basilicum* L.) is the main species produced for export with an average fresh biomass yield of 8.45 t ha^{-1} and a planted area of 417 ha, of which 208 ha are produced under organic systems, a further 5 ha is produced in greenhouses and the rest under conventional (i.e. non-organic) systems (CONAGUA, 2018; Chiquito-Contreras *et al.*, 2018). The main producing states in Mexico are Baja California (greenhouse), Morelos, Nayarit (conventional) and Baja California Sur (organic), the latter being the largest producer of basil (Sánchez-Verdugo and Avilés-Quevedo, 2012; Ojeda-Silvera *et al.*, 2015).

Plants of all species have developed a diversity of strategies to cope with the biotic and abiotic challenges faced in their natural habitats. A major one of these, relevant to most plant species, involves symbiotic relationships established with fungal and bacterial microorganisms. These microorganisms interact directly with the plant host, to increase its tolerance of these challenges and also to facilitate their access to belowground resources (water and minerals). In so doing, they result in a stimulation of the plant's physiological processes, and these are reflected in increases in yield. In a commercial environment, these symbioses often allow reduced rates of application of chemical fertilisers (López-Valenzuela *et al.*, 2019; Tamayo *et al.*, 2019).

The arbuscular mycorrhizal fungi (AMF) with their attributes of association with cultivated species, generate substantial improvements in plant growth, with positive effects on plant nutrition, on the uptake of water and nutrients, on soil stability, and systemic resistance to fungal diseases and other pests. These benefits create important and economically viable alternatives to more conventional methods of cultivation (Baum *et al.*, 2015; Esquivel-Quispe, 2020). It has been reported that *Trichoderma harzianum* is an opportunistic symbiont fungus of plants, able to produce elicitors that induce plant defences against pathogens and insects, solubilisation of soil-bound P, Mg, Fe, Mn and plant growth promoters (Sharma *et al.,* 2017; Hernández *et al.*, 2019).

Of the bacteria, it has been reported that *Bacillus subtilis* stimulates plant growth through the synthesis of plant growth regulators such as indole acetic acid, also enabling biological N fixation, solubilisation of P and production of siderophores, (Corrales *et al.,* 2017; Leal-Almanza *et al.*, 2018).

The integration of certain symbiotic microorganisms (*Glomus cubense*, *Trichoderma harzianum* and *Bacillus subtilis*) within organic management systems for basil cultivation, is expected to generate significant benefits over conventional methods. It should reduce disease and increase growth, resulting in increased production per unit area and reductions in the use of synthetic fertilisers. The objective of this work was to evaluate the effects of *Trichoderma harzianum, Bacillus subtilis*, *Glomus cubense* and their combinations on growth of basil under greenhouse conditions.

Materials and Methods

The study was carried out in a tunnel greenhouse with a whitish plastic covers above (30% shading) and anti-aphid mesh on the side walls, located at the Autonomous University of Morelos, Mexico (18 ° 58' 51'' N, 99 ° 13' 55'' E) at an altitude of 1.866 m. During the experiment, environmental data were recorded in the greenhouse with a data logger (Hobo® datalogger, model MX2301A, USA); the long-term average temperature was 22.4 °C and long-term average relative humidity was 53.0%.

Transplanting and application of beneficial microorganisms

Transplanting was carried out on March 1, 2020 into 20.3 cm plastic pots containing 2.8 kg of agricultural soil. Basil seedlings cv. 'Nufar' F1 produced in styrofoam trays with 200 cavities, from the company Fusión Mexicana Agropecuaria S.A. de C.V. located in Jojutla de Juárez, Morelos. The topsoil used in the experiment was obtained from a depth of 0-20 cm from the aforementioned company, it had a silty clay texture with the following chemical characteristics: pH 6.32; high CEC of 27.1 mEq 100 g^{-1}; normal contents of: organic matter 2.3%, EC 2.7 dS m^{-1}, total N 0.6 cmol kg^{-1}, available P (Bray-Kurtz) 5.1 ppm; high contents of K (7.4 cmol kg^{-1}), Ca (107.0 cmol kg^{-1}) and Na (14.7 cmol kg^{-1}); low contents of of Mg (9.0 cmol kg^{-1}) and normal Cu (0.03 cmol kg^{-1}), Mn (0.07 cmol kg^{-1}), Zn (0.04 cmol kg^{-1}) and B (0.39 cmol kg^{-1}). The soil was sterilised by solarisation (Katan and Gamliel, 2012), for this, it was covered with transparent plastic and exposed to sunlight for 30 days.

The inoculation of the basil seedlings with the species *T. harzianum* and *B. subtilis* obtained from the Technological University of the South of Morelos State (UTSEM), was carried out in a soil drench. Prior to inoculation these were grown on PDA culture media and nutrient agar at 25 ± 2 °C; the first inoculation was carried out at the time of transplanting and the second 15 days later, at a concentration of 1 x 10^5 spores per mL^{-1} and 1 x 10^5 bacteria per mL^{-1} prepared with sterile distilled water in a 1000 mL beaker. They were applied with a 1000 mL Batlle 730061UNID model spray that guaranteed the uniformity of the application to all plants. The species *G. cubense* with 70 spores per gram of inoculant and 50% radical colonisation, non-toxic and free of pathogens obtained through the State Council of Organic Fertilisers (CEFO) located in Oaxaca, Mexico, was prepared in a plastic container, in which the roots were immersed in a fluid paste of the mycorrhizal inoculant with a dose of 0.5 kg ha^{-1} of the product per 800 mL of distilled water at the time of transplanting (Fernández *et al.*, 2000).

Irrigation and mineral nutrition

Every two days 1 L of 50% Steiner's nutrient solution was supplied per pot. The solution was prepared from soluble commercial fertilisers: $Ca(NO_3)_2$, KNO_3, $MgSO_4$, K_2SO_4 and KH_2PO_4 (Steiner, 1984). The pH of the nutrient solution was adjusted between 5.5 and 5.7 with 95% sulfuric acid. As a source of micronutrients, ultrasol Micro Mix, SQM® was used at 20 g per 500 L of nutrient solution.

Experimental design and treatments

The three beneficial microorganisms were: *Trichoderma harzianum* (TH), *Bacillus subtilis* (BS) and *Glomus cubense* (GC). A completely randomised design was used with a control and seven treatments with six repetitions. The control (1) was with no microorganism inoculation and the seven treatments were inoculations with the single or the combined microorganisms as follows: (2) TH, (3) BS, (4) GC, (5) TH+BS, (6) TH+GC, (7) BS+GC and (8) TH+BS+GC (Table 1). The experimental unit was a pot containing one basil plant.

Harvests and variables measured

Basil is a perennial herbaceous plant and three harvests (cuts) of fresh stems were made. The first was on March 31, 2020 (30 days after transplanting), the second on April 14 and the third on April 29, all of the same year. For each harvest, the following variables were evaluated so the cumulative effects of the three harvests could be determined: the number of commercial stems; stem length from the base to apex (precision 0.01 cm ruler); stem thickness 1 cm above the base of the commercial stem cut (digital vernier, Traceable® Model 97152-16, USA); leaf length and width, measured below the first internode of the stem cut (digital vernier); relative chlorophyll concentration in mature, fully-expanded leaves (SPAD reading, Minolta® Model 502 Plus, Japan);

leaf area (all leaves were removed from the branches and stems and placed on a leaf area meter, (LI-COR® Model LI-3100C, USA); fresh and dry biomasses of each cut of commercial stems, separated into leaves and stems (digital scale OHAUS® Model Scout Pro SP401, USA, sensitivity 0.01 g). After the fresh weight had been obtained, organs were placed in brown paper bags and dried in a force ventilated oven (Luzeren® Model DHG9070A, China) at 60 °C for three days to constant weight. The macronutrient concentrations (N, P, K, Ca and Mg) were then determined in each organ. For N, the micro-Kjeldahl method was used (Alcántar and Sandoval, 1999), while for P, K, Ca and Mg, wet digestion was carried out with a mixture of perchloric and nitric acids 2:1 ratio (Alcántar and Sandoval, 1999). Macronutrient extraction was carried out from the dried biomass of each organ (leaves and stems) and the % concentrations (N, P, K, Ca and Mg) determined as:

Extraction of macronutrients (kg ha^{-1}) = [DM aerial part (g per plant) x concentration (%) of the element in the DM of the aerial part] x 10.

Table 1. Description of the treatments applied to basil plants (*Ocimum basilicum* L.)

Treatment	Concentration of inocula
Control	Without inoculation
TH	1 x 10^5 CFU mL^{-1}
BS	1 x 10^5 CFU mL^{-1}
GC	70 espores g^{-1} of soil
TH+BS	1 x 10^5 CFU mL^{-1} + 1 x 10^5 CFU mL^{-1}
TH+GC	1 x 10^5 CFU mL^{-1} + 70 espores g^{-1} of soil
BS+GC	1 x 10^5 CFU mL^{-1} + 70 espores g^{-1} of soil
TH+BS+GC	1 x 10^5 CFU mL^{-1} + 1 x 10^5 CFU mL^{-1} + 70 espores g^{-1} of soil

CFU = Colony formation unit. *TH = Trichoderma harzianum, BS = Bacillus subtilis, GC = Glomus cubense.*

Statistical analyses

These were carried out with the software IBM SPSS® Statistics for Windows vs 25 (IBM Corp, Armonk, New York, USA). The normality and homogeneity of variance were verified using the Levene and Kolmogorov-Smirnov test. Subsequently, the analysis of variance and the Tukey mean comparison test ($p \leq 0.05$) were carried out.

Results and Discussion

Fresh and dry biomasses

Significant differences ($p \leq 0.05$) were observed between the treatments in fresh and dry biomasses (Figure 1). The co-inoculation TH+GC increased fresh biomass by 67% compared with the control, the single inoculations (TH, BS and GC) and the three combined microorganisms (TH+BS+GC). Likewise, TH+GC increased dry biomass by 63% compared with the same treatments before mentioned. Similarly, the combined inoculation BS+GC increased fresh biomass by 45% and dry biomass by 55% compared with the control, the single inoculations (TH, BS and GC) and the three combined microorganisms (TH+BS+GC).

The results show that a direct interaction with these beneficial microorganisms in the rhizoplane of basil plants, has increased the physiological activity and other biotic and abiotic factors so as to increase plant biomass. The ascending biomass increments for TH+GC and BS+GC are likely due to some different specificity of the symbiotic activities of these microorganisms with basil, as new stems appear between harvests throughout the experimental period. This idea is also suggested in the report by Chiquito-Contreras *et al.* (2018) where mixed inoculations between mycorrhizae and bacteria of the *genus Stenotrophomonas rhizophila*, stimulated the processes involved in plant growth.

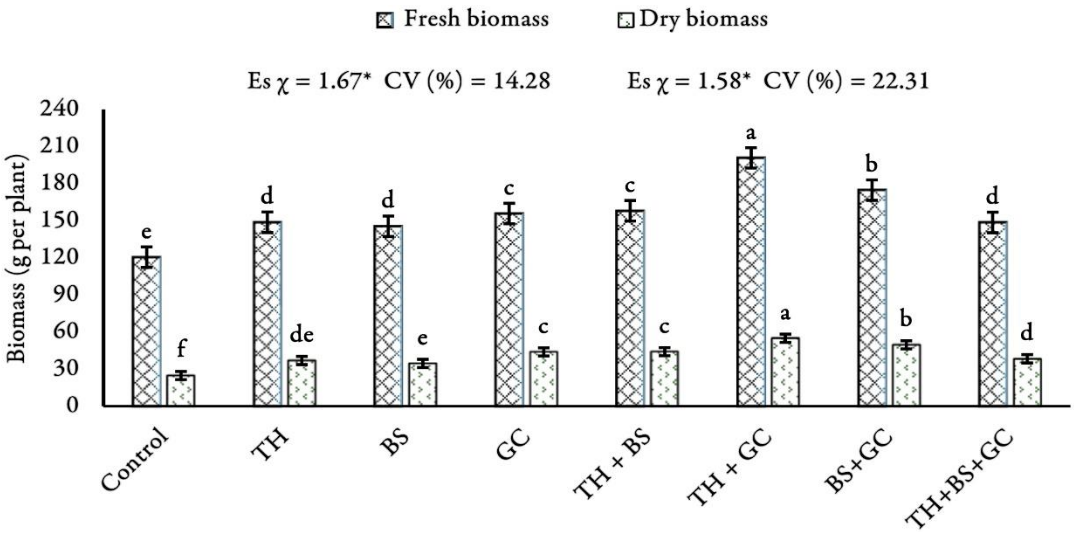

Figure 1. Fresh and dry basil biomasses for plants inoculated with beneficial microorganisms
TH = *Trichoderma harzianum;* BS = *Bacillus subtilis;* GC = *Glomus cubense*; Es χ = standard error of the mean; CV = Coefficient of variation. Bars indicate standard error. Different letters in the same column indicate significant differences according to Tukey's multiple range test (p≤0.05).

Álvarez *et al.* (2018) note that co-inoculated microbes encourage plant growth through synergism between the distinct benefits they each provide in terms of the metabolism of the plant. In contrast, Moncada *et al.* (2021) found a decrease in plant biomass of *Ocimum basilicum* L. plants grown in pots inoculated with *Bacillus* spp. compared with a 100% Steiner solution control. These authors hypothesised that the temperature of 15 °C that was recorded during the experiment could have delayed root growth and the activity of plant growth-promoting rhizobacteria (PGPR), which in turn limited the interaction of the *Bacillus* spp. with the plant host and any increase in the uptake of nutrients.

Meanwhile, Khalediyan *et al.* (2021) found a significant increase in fresh and dry weight of the aerial and root biomasses of *Ocimum basilicum* L. in plants co-inoculated with *G. mosseae* and *B. subtilis* compared to the control treatment and plants treated with mineral fertiliser. These authors attributed their results to the increase in the uptake of water and nutrients by the fungal and bacterial structures that also penetrate the plant cells and so stimulate the plant's physiological activity.

Riahi *et al.* (2020) reported that *Pseudomonas rhizophila* S211, *Halomonas desertis* G11 and *Oceanobacillus iheyensis* E9 significantly increased various biomass growth parameters of leaves and roots compared to the control in *Pelargonium graveolens* L'Hér plants. They also reported a 43.34% increase in biomass weight with the dual microorganism inoculation over the control. These effects were attributed to the production of siderophores, the solubilisation of minerals and the diffusion of substances that promote direct plant growth.

Hernández-Montiel *et al.* (2020) reported an increase of 19.29% in plant biomass in *Capsicum annuum* L. plants co-inoculated with rhizobacteria compared to the control. This positive effect was attributed to the ability of the bacterial inoculants to produce plant growth hormones which stimulate cell division and differentiation and thus an increase in biomass. The beneficial effects of *T. harzianum* combined with rhizobacteria have also been shown to increase plant survival and biomass production in *Arachis hypogaea L.* plants. However, their effects are less when applied separately (Neelipally *et al.*, 2020).

Leaf area

There were significant (p≤0.05) differences in leaf area between the evaluated treatments (Figure 2). TH, BS and TH+BS+GC did not show differences between them, but they did with the control. A similar effect was observed between GC and TH+BS. However, compared to the control, leaf area did show an upward trend of 55 and 45% with TH+GC and BS+GC, respectively. This could be due to synergisms between the microorganisms that stimulate the physiological systems of plants.

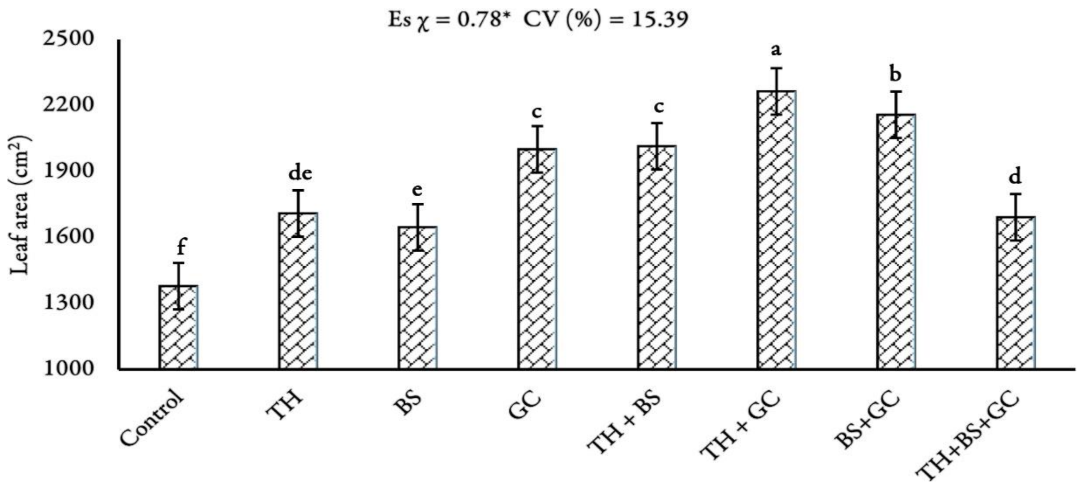

Figure 2. Basil leaf area inoculated with beneficial microorganisms
TH = Trichoderma harzianum; BS = Bacillus subtilis; GC = Glomus cubense; Es χ = standard error of the mean; CV = Coefficient of variation. Bars indicate standard error. Different letters in the same column indicate significant differences according to Tukey's multiple range test ($p \leq 0.05$).

These results suggest that the effects of the plant-microbe interaction may have been to stimulate the uptake of water, essential nutrients and the production of metabolites such as enzymes, plant growth-promoting compounds, organic acids etc that promote the leaf area of the plants. Bhat *et al.* (2020) suggests the increase in leaf area in plants inoculated with microbes is likely the production of a wide range of beneficial strategies and mechanisms in the rhizosphere that make nutrients more available to plants. Rivera *et al.* (2020) also found that the leaf area of *Pennisetum purpureum* and *Nicotiana tabacum* L. increased gradually due to benefits from associated mycorrhizal inoculants under a range of edaphic and environmental conditions.

Pan *et al.* (2020) found positive effects on aerial biomass accumulation in *Elaeagnus angustifolia L.* with applications of AMF and PGPR. Simple inoculation of *Glomus. mosseaese* increased the aboveground biomass by 64% compared with the control. They too reported a similar response with the addition of *Bacillus amyloliquefaciens* and attribute the results to the specificity of the plant to the AMF strain, rather than to the richness or microbial diversity in the plant's rhizosphere.

The contrasting responses between our inoculation treatments, could be because beneficial microorganisms do not always have the same responses in different host plant species due to their different functional interactions and edaphic environments. However, when correctly applied their symbiotic interactions with the host stimulate its growth (Tian *et al.*, 2020).

Growth variables

The statistical analysis showed significant differences ($p \leq 0.05$) among the treatments in the various parameters we measured (Table 2). There was a gradual trend in all variables with the co-inoculation of TH+GC followed by with BS+GC. In contrast, GC and TH+BS showed similar responses in all growth variables. Likewise, the application of TH+BS+GC and the single inoculations TH and BS were not significantly different. The sequential harvests during the experimental period and the relationships with TH+GC and BS+GC increased the number and commercial quality of the stems harvested. It is assumed that the plants took up water and soil minerals via the associated microorganism structures, with the microorganism better able to obtain carbon compounds from the host while also enhancing the development of the host's aerial biomass (Arango *et al.*, 2013). Abdollahi Arpanahi *et al.* (2020) found the morphological parameters were significant in the plants treated with PGPR compared to the controls. In contrast, an inappropriate selection of rhizobacteria in a culture can inhibit host plant growth, due to the deficient or excessive concentration of hormones, as well as to incompatibility between microorganism and host plant (Anguiano-Cabello *et al.*, 2019; Rivera *et al.*, 2020).

Table 2. Growth variables evaluated in basil inoculated with beneficial microorganisms

Treatment	Number of commercial stems	Stem length (cm)	Stem thickness (mm)	Leaf length (mm)	Leaf width (mm)
Control	10.25 e	21.55 e	5.05 f	90.34 e	51.26 e
TH	12.50 d	23.45 d	5.77 d	94.09 d	53.99 d
BS	12.25 d	23.01 d	5.53 e	93.31 d	54.14 d
GC	14.00 c	24.44 c	6.18 c	97.55 c	57.89 c
TH+BS	15.00 c	24.63 c	6.31 c	98.72 c	58.54 c
TH+GC	19.00 a	27.15 a	7.24 a	106.17 a	64.93 a
BS+GC	16.75 b	26.02 b	6.84 b	102.65 b	61.46 b
TH+BS+GC	12.25 d	23.25 d	5.68 de	94.21 d	54.23 d
Es χ	0.50*	0.27*	0.09*	0.79*	0.68*
CV (%)	19.71	7.13	11.36	5.24	7.70

TH = Trichoderma harzianum; BS = Bacillus subtilis; GC = Glomus cubense; Es χ = standard error of the mean; CV = Coefficient of variation. Different letters in the same column indicate significant differences according to Tukey's multiple range test ($p \leq 0.05$).

Abdel-Rahman *et al.* (2011) found a growth stimulation of three varieties of *Ocimum basilicum L.* treated with *B. subtilis* and AMF. Likewise, they indicate that mycorrhizal colonisation was superior with respect to PGPR and they also reported that a duality of microorganisms provided a greater response compared to individual ones. In this context, Mohamed *et al.* (2019) in a trial carried out under greenhouse conditions, demonstrated the effect of single or combined inoculations of mycorrhizae, *Bacillus subtilis* and *Pseudomonas fluorescens* with *Phaesolus vulgaris L.* They report that the combined treatments with microorganisms were more effective than the individual treatments for increasing growth. Other studies indicate that the use of *Trichoderma* contributes to the balance of hormones such as indole acetic acid, gibberellic acid, ethylene, which is reflected in the quality and safety aspects for the commercialisation of these species (Stewart and Hill, 2014; Peccatti *et al.*, 2019).

Makarov *et al.* (2020) indicate that the growth of plants inoculated with microorganisms gradually increases compared with non-inoculated controls, regardless of the soil medium in which they are established due to increases in the activity of microorganism exoenzymes that stimulate plant growth. Similarly, Singh *et al.* (2021) report that plants have evolved in close association with the beneficial microorganisms that are involved in the biosynthetic pathway of plant metabolism. Moreover, they have numerous secondary functions in plant survival and growth. The physiology and biochemistry of inoculated crops in different ecosystems are reflected in the photosynthetic pigmentation of the leaves (carotenoids and chlorophyll), antioxidant potential, root volume, greater efficiency in the uptake of nutrients due to appropriate use of endophytic fungi and rhizobacteria (Malik *et al.*, 2021).

Relative chlorophyll concentration

There were significant ($p \leq 0.05$) differences in the SPAD readings in our basil plants inoculated with TH, GC or BS (Figure 3). Co-inoculation with TH+GC and BS+GC increased chlorophyll concentration by 23 and 19%, respectively, compared to the control. These responses can be attributed to the synergy between the inoculants and the plants, by promoting the photosynthetic activity with a coefficient of variation of 6.17%. Sánchez *et al.* (2018) and Ajeng *et al.* (2020) indicate that the greater intensity of green pigmentation is due, among other factors, to the greater uptake of N, P and K that are all involved in the photosynthetic machinery.

The combined use of these organisms increases the uptake of nutrients from the soil and thus the leaf chlorophyll content, regardless of the chemical and physical characteristics of the soil.

On the other hand, the intensity of the greenness of the leaves may be related to the mechanisms involved in the species of fungi and bacteria in the elicitation process that interacts in the plant biochemical pathways to produce secondary metabolites in large quantities. In this way, the also raise the quality of the crop in terms of growth, aroma, flavour and colour (Aguirre-Becerra *et al.*, 2021).

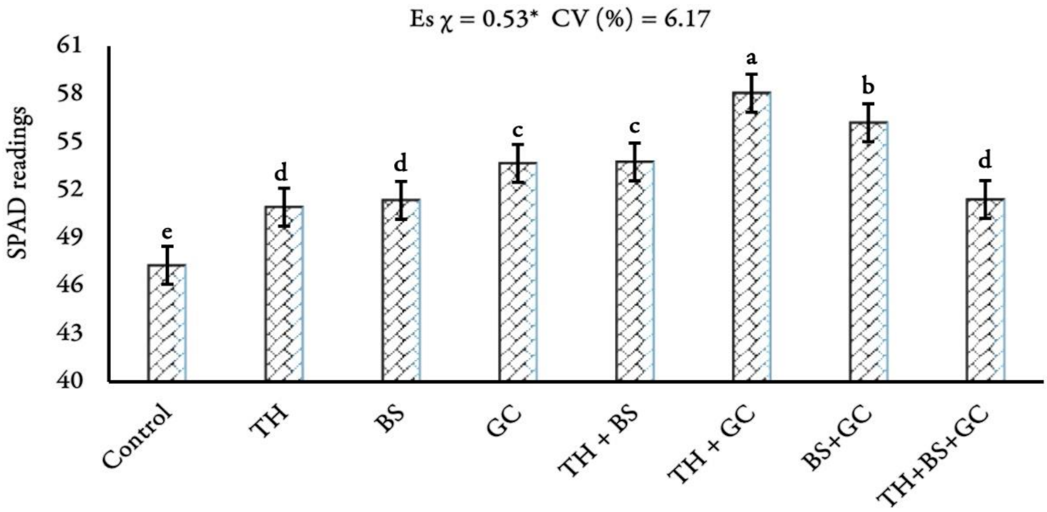

Figure 3. SPAD readings for leaves of basil plants inoculated with beneficial microorganisms
TH = Trichoderma harzianum; BS = Bacillus subtilis; GC = Glomus cubense; Es χ = standard error of the mean; CV = Coefficient of variation. Bars indicate standard error. Different letters in the same column indicate significant differences according to Tukey's multiple range test ($p \leq 0.05$).

Favourable trends have been reported in chlorophyll content of basil plants inoculated with AMF consortia and combined with PGPR, due to the direct or indirect mechanisms in which microbes produce antibiotics, metabolites, phytohormones and organic compounds that stimulate nutrient uptake and plant growth (Chiquito-Contreras *et al.*, 2018; Anguiano-Cabello *et al.*, 2019). Likewise, Emmanuel and Babalola (2020) and Suchitra *et al.* (2020) mention that mycorrhizal fungi increase mineral uptake and provide protection from abiotic stresses in plants to changes in photosynthetic products. Similarly, the microbe effects are greater when combined with PGPR suggesting that, by intervening in the physiology of plants, control the levels of plant hormones or indirectly reduce the inhibitory effects of pathogens.

Bordoloi and Shukla (2020) reported that the chlorophyll concentration in *Piper mullesua* plants grown under greenhouse conditions varied significantly between isolates of arbuscular mycorrhizal fungi. Likewise, they report that there was a higher trend ($p > 0.001$) in mycorrhizal treatments compared to non-inoculated controls in sterile soil. In turn, Gómez-Bellot *et al.* (2020) in *Viburnum tinus* L. plants found leaf chlorophyll concentration as well as the stomatal conductance were increased with inoculation of a microbial complex of AMF, compared to simple applications of mycorrhizae. These authors associated these effects with the specificity of the microbial complex in the rhizosphere under different irrigation conditions.

In consideration of the benefits of *T. harzianum* in combination with PGPR, Singh *et al.* (2020) showed in greenhouse-grown *Ocimum sanctum L.* that the combined effect of these improved the uptake of soil nutrients and the photosynthetic efficiency of the leaves to generate increased fresh biomass weight (83.78%), compared to uninoculated control plants. Neelipally *et al.* (2020) demonstrated that *Arachis hypogaea* L. plants inoculated with *T. harzianum* + *Bradyrhizobium spp* in the greenhouse generated higher dry biomass and chlorophyll concentration ($p < 0.0001$) compared to simple applications and the control treatment. However, they found that the chlorophyll index and the accumulation of N in plant tissues was higher with the simple inoculation of *Bradyrhizobium* with respect to *T. harzianum*, due to the interaction effects between endophytic fungi and rhizobacteria, as well as the particular functions of each in the growth of plants.

Macronutrient extraction

Compared to the control, the uptake of macronutrients increased 80% in basil plants treated with beneficial microorganisms (Table 3), with significant differences ($p \leq 0.05$) due to inoculation with TH+GC and BS+GC which showed increases of 82 and 71%, respectively. A similar effect was also observed between GC and TH+BS for all macronutrients. The inoculations with TH, BS, and with TH+BS+GC behaved similarly. The response found in this variable coincided with those described previously, in relation to the fact that the basil plants established a direct relationship between the mixed applications of the inoculants TH+BS and TH+GC unlike the single inoculations with TH, BS and GC and with the triple inoculation TH+BS+GC.

Likewise, a tendency for increased N and K uptake was observed (expressed as kg ha^{-1}) compared with the other macronutrients. Similarly, P was the element most strongly taken up by the plants. This could be associated with the participation of N, P and K in photosynthesis, respiration, photosynthate translocation, protein synthesis and activation of key enzymes for various biochemical functions in plants (Delgado-Ospina *et al.*, 2012); In addition to the fact that microorganisms also exert effects on the uptake of macronutrients, which coincides with that reported by Bordoloi and Shukla (2020) who argue that microorganisms also participate in the supply and uptake of nutrients by plants.

Table 3. Uptake of macronutrients by basil plants inoculated with beneficial microorganisms

Treatment	N	P	K	Ca	Mg
	kg ha^{-1}				
Control	1.66 e	1.66 e	5.15 e	0.56 e	0.54 e
TH	3.44 d	3.44 d	10.62 d	1.17 d	1.07 c
BS	3.03 d	3.03 d	9.35 d	1.03 d	0.80 d
GC	6.80 c	6.80 c	20.48 c	2.26 c	1.09 c
TH+BS	6.48 c	6.48 c	20.98 c	2.31 c	1.15 c
TH+GC	10.43 a	10.43 a	32.17 a	3.54 a	1.96 a
BS+GC	8.10 b	8.10 b	24.97 b	2.73 b	1.54 b
TH+BS+GC	3.07 d	3.07 d	9.48 d	1.04 d	0.79 d
Es χ	0.15*	0.15*	0.48*	0.04*	0.03*

TH = Trichoderma harzianum; BS = Bacillus subtilis; GC = Glomus cubense; Es χ = standard error of the mean.
Different letters in the same column indicate significant differences according to Tukey's multiple range test ($p \leq 0.05$).

Arango *et al.* (2012) reported a significant increase in the level of macronutrients in mycorrhizal plants of *Menta piperita* L. grown in a greenhouse, with a direct relationship between fresh and dry matter and leaf area at 60 days after transplantation. Studies conducted by Delgado-Ospina *et al.* (2012), indicate that the uptake of nutrients can vary according to the species and the phenological phases of the crop. They reported K as the element with the highest foliar extraction requirement and P and Mg with lower demands in *Lippia organoides* H.B.K plants grown in a greenhouse.

As has also been reported, the use of *T. harzianum* as a disease control fungus and growth promoter in aromatic and medicinal plants mixed with other beneficial microorganisms or inorganic fertilisers increase the uptake of essential soil nutrients, and plant growth and yield (Quiroga *et al.*, 2015). On the other hand, Sun *et al.* (2020) reported a significant increase in the content of N, P, K, Ca and Mg in tea plants (*Camellia sinensis* (L.) O. Kuntze inoculated with *Glomus etunicatum* under shaded conditions. At the same time, they indicate that the results could be due to the involvement of mycorrhizae in the induction of plant resistance to stress, increased root biomass and uptake of nutrients and the hormone content of the root, actively regulating the transport pathway and synthesis.

Ortas *et al.* (2021) suggest mycorrhizal colonisation regulates the expressions of chloroplast genes in leaves and improves plant water status. They also confirm that essential soil nutrients are taken up by extra-radical hyphae and the differences in their acquisition, as well as in transpiration and stomatal conductance are related to the mycorrhizal efficiency of different species or the combination with other microorganisms, which is related with the results obtained in the present study.

Conclusions

Compared with the control, a 58% increase in growth of basil was obtained with inoculation with the combination *Trichoderma harzianum* + *Glomus cubense*, and a 55% increase in growth with respect with single inoculations of *Bacillus subtilis*, *Glomus cubense*, *Trichoderma harzianum* and with the triple inoculation of these microorganisms. Secondly, an increase of 51% in the growth of this fine herb was obtained with the co-inoculation of *Bacillus subtilis* + *Glomus cubense* with respect to the control and 38% with the other treatments, respectively. These plant growth responses are reflected in increases in both fresh and dry biomasses, leaf area, various morphological variables, the concentration of chlorophyll and the amounts of the main macronutrients. In contrast, the effects found with the joint application of the three inoculants and simple applications were lower in all the variables evaluated. These results suggest that the combined inoculation of beneficial microorganisms *Trichoderma harzianum* + *Glomus cubense* or *Bacillus subtilis* + *Glomus cubense*, is a useful and more sustainable alternative management for cultivation of basil under greenhouse conditions.

Authors' Contributions

Conceptualisation: PJL; JACG, YTA; execution of the investigation: YTA; drafting-original draft: YTA, PJL, JCHG; Methodology, review and supervision: PJL, IAT; advice and review: DGS, JOPG, VLM, MCRB, OBF.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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