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Effect of Inoculation with Rhizobacteria and Arbuscular Mycorrhizal Fungi on Growth and Yield of *Capsicum chinense* Jacquin

M. Constantino [1], **R. Gómez-Álvarez** [*1], **J. D. Álvarez-Solís** [1], **V. Geissen** [1], **E. Huerta** [1] and **E. Barba** [1]

Abstract

We evaluated the effect of two rhizobacteria (*Azotobacter chroococcum* and *Azospirillum brasilense*) and a commercial product containing multiple strains of arbuscular mycorrhizal fungi (AMF) and an NPK fertiliser on the growth and yield of habanero chilli (*Capsicum chinense* Jacquin). All treatments were applied as single or combined inoculants, under nursery and field conditions, in a completely randomised design. The biofertilisers were applied to the roots by coating or dipping, with the inoculants in a solid or liquid support, respectively. At 30 days after inoculation, populations of 2.5×10^6 to 1.3×10^6 cfu g soil^{-1} of *A. brasilense* and 10.3×10^5 to 2.6×10^5 of *A. chroococcum* were detected in the rhizosphere of the crop. The prevalence of colonisation of plants inoculated with AMF ranged from 35 to 57%, with the greatest values recorded for the treatment involving single biofertilisation by root coating. In the nursery phase, single biofertilisation promoted a higher growth and nutrient content in the crop than combined biofertilisation. However, in the field phase the combined biofertilisation increased the nutrient content of the plant leaves, which was significantly greater than observed in the NPK treatment.

The highest yields were recorded for the treatments involving a single inoculation of *A. chroococcum* and for those with the multi-strain of AMF, with average values of 2.5 and 2.3 kg plant^{-1} respectively, compared with 1.0 kg plant^{-1} obtained with the treatment in which NPK fertiliser was applied.

Keywords: Biofertiliser, Azospirillum brasilense, Azotobacter chroococcum, PGPR, root coating

1 Introduction

Mexico has the greatest genetic diversity of chillies and peppers (*Capsicum* spp.), and occupies second place in terms of world production. However, yields are generally very low with a mean of 13.7 t ha^{-1}. In spite of this, *Capsicum* spp. represents one of the main horticultural exports of Mexico (FAOSTAT, 2005). In organic farming systems,

[1] El Colegio de la Frontera Sur, Unidad Villahermosa, Tabasco, Mexico
[*] corresponding author

the use of organic fertiliser and microbial inoculants or biofertiliser represents a sustainable alternative to high inputs of chemical fertilisers used in the conventional production systems (KENNEDY *et al.*, 2004).

Among the microorganisms that have been used as biofertilisers there is a group of bacteria known as plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), that have been recognized for their potential use in agriculture and horticulture (AZCON, 2000; LUCY *et al.*, 2004). The mechanisms by which PGPR promote plant growth are diverse, and often the beneficial effect is due to a combination of mechanisms (BASHAN *et al.*, 2004). Nitrogen fixation, the solubilisation of phosphorous in the rhizosphere and the production of phytohormones, enhance plant growth directly. In mycorrhizal associations, plants supply carbohydrates to fungi, while fungi improve plant nutrition by increasing the absorption and translocation of nutrients, principally P, as well as N, K, Cu, Zn and Mg (LINDERMAN, 1992).

The techniques that exist for the application of biofertilisers include seed coating (TERRY *et al.*, 2002), which reduces production costs by using lower volumes of inoculants (TAYLOR and HARMAN, 1990). In the case of crops that require transplantation, such as the habanero chilli, one inoculation technique consists in dipping the roots of seedlings in a mixture of solid biofertiliser and water immediately before transplantation (BASHAN, 1998).

In the present study we examined the effect of different methods of inoculation on the development of populations of *Azospirillum brasilense* and *Azotobacter chroococcum*, as well as micorrhizal colonisation in *Capsicum chinense* Jacquin. Additionally, we studied the effects of these treatments on growth and yield of *C. chinense* under tropical conditions.

2 Materials and Methods

2.1 Microbiological and plant material

Pure strains of *A. chroococcum* and *A. brasilense* were used in the experiments. These were isolated by the Instituto de Investigaciones Fundamentales en Agricultura Tropical (INIFAT), La Habana, Cuba, and reproduced in laboratory cultures. The *MICORRIZA* (NOCON S.A. de C.V.) product was used for the mycorrhizal inoculants, which is a low cost and commercially available product in Tabasco State, Mexico. This contains multi-strain arbuscular mycorrhizal fungi at a concentration of 5×10^6 spores ml^{-1}. The habanero chilli seedlings (*Capsicum chinense* Jacquin) were obtained from seeds commercialized by SEMINIS S.A. de C.V., Mexico. Habanero chilli seedlings of 45 d old were used in the nursery and field experiments.

2.2 Characterization of the soil

The soil used in the experiments was a loamy eutric Fluvisol according to FAO / UNESCO's classification, with 26% clay, 36% silt and 38% sand, an apparent density of 1.3 g cm^{-3}, pH(H_2O) value of 6.7, organic matter content 1.6%, total N content of 0.1 g 100 g dry $soil^{-1}$, P(Olsen) content of 12.2 mg kg^{-1}, K content of 0.9 cmol kg^{-1},

Ca of 12.5 cmol kg^{-1}, Mg of 6.4 cmol kg^{-1} and a cation exchange capacity (CEC) of 24.3 cmol kg^{-1} (ammonium acetate 1N, pH 7). This type of soil is common in Tabasco State.

2.3 Preparation of biofertilisers

In the case of the liquid biofertilisers, *A. chroococcum* was cultured in liquid Ashby medium enriched with NH_4NO_3 (3 g l^{-1}) and yeast extract (0.1 g l^{-1}), whereas *A. brasilense* was grown in a nutrient broth (Bioxon). Both were incubated at 30°C with orbital agitation (150 rpm) for 60 hours in the case of *A. chroococcum* and 48 hours in the case of *A. brasilense*, to obtain a concentration of 1×10^9 colony forming units (cfu) ml^{-1}. The solid biofertilisers (*A. chroococcum*, *A. brasilense* and AMF) were prepared from the liquid biofertilisers using a substrate of dry sugarcane press mud with a particle diameter of 1 mm, sterilized at 1.2 atm for 1 hour. Every kilogram of substrate was inoculated with 500 ml of the liquid inoculants. The inoculated solid substrates were incubated at 30°C for 10 days. The concentration of *A. brasilense* and *A. chroococcum* in solid supports was 1×10^{10} cfu g^{-1} which was verified by the dilution plate count method. Agar Ashby medium was used for *A. chroococcum* and agar Congo red medium was used for *A. brasilense*.

2.4 Experimental design in the nursery

The nursery experiment was carried out in October 2005 in Tabasco, Mexico. Temperature ranged from 25 to 30°C during the experimental period. In this experiment we evaluated the effect of the different application methods (dipping and root coating in single or combined form) on rhizobacteria populations and micorrhizal colonisation as well as plant growth and leaf nutrient content. A completely randomised experiment was designed with 12 treatments and 5 repetitions (Table 1). Chilli plants were planted in seedling trays (150 individual cells). When plants had grown to the desired size they were individually inoculated with the treatments described in Table 1. Immediately after inoculation seedlings were individually transplanted to pots filled with 500 g of a substrate (soil: manure 1:1, previously sieved at 2 mm and sterilized twice at 1.5 atm for 30 min). The seedlings were watered daily with water to maintain the moisture at approximately 60% water holding capacity of the soil and maintained under nursery conditions during 30 days.

The single coating treatments (Ab_S, Ac_S, AMF_S) involved a 1:10 mixture of 10 g of biofertiliser and 90 ml of adherent (2 % w/v starch), whereas in the combined treatments (Ab_S+AMF_S, Ac_S+AMF_S) the mixture contained 10 g per inoculant and 180 ml of adherent. The root balls of seedlings were coated with the mixtures of the different treatments and allowed to dry in the shade for 10 min. In the treatments where non-combined biofertilisers were applied by dipping (Ab_L, Ac_L, AMF_L), the roots were submerged in their corresponding bacterial or AMF inoculants for five minutes. A 1:1 (v/v) mixture of each of the inoculants was previously prepared for the combined treatments applied by dipping roots (Ab_L+AML, Ac_L+AMF_L). Afterwards, the complete root ball of the seedling was submerged for 5 minutes in the inoculant preparation and

Table 1: Experimental treatments

Treatments	*Description*	*Support*	*Application method*
Ab_S	*A. brasilense*	solid	root coating
Ab_L	*A. brasilense*	liquid	root dipping
Ac_S	*A. chroococcum*	solid	root coating
Ac_L	*A. chroococcum*	liquid	root dipping
AMF_S	Arbuscular mycorrhizal fungi	solid	root coating
AMF_L	Arbuscular mycorrhizal fungi	liquid	root dipping
Ab_S+AMF_S	*A. brasilense*+Arbuscular mycorrh. fungi	solid	root coating
Ab_L + AMF_L	*A. brasilense*+Arbuscular mycorrh. fungi	liquid	root dipping
Ac_S + AMF_S	*A. chroococcum*+Arbuscular mycorrh. fungi	solid	root coating
Ac_L + AMF_L	*A. chroococcum*+Arbuscular mycorrh. fungi	liquid	root dipping
Control	—	—	—
Chemical Fertiliser	N:P:K (25:10:30 g plant^{-1})	—	soil application

allowed to dry in the shade for 10 min. For the NPK treatment a dose of 25:10:30 g plant^{-1} was applied directly to the substrate, adding all the P and K but only half of the N. The other half of N was added at the moment of transplantation in the field. Samples of 10 g of rhizospheric soil per plant were collected 30 days after inoculation to determine the rhizobacterial population. Soil samples were suspended in 90 ml sterile distilled water and shaken for 30 min at 150 rpm. Immediately after shaking, each suspension was serially diluted by pipetting 1 ml aliquots into 9 ml sterile water, to obtain a final dilution of 10-5 fold. A 0.1 ml volume of each dilution of the series was plated on Petri dishes with agar Congo red medium for the *A. brasilense* treatment, whereas Burk's N-free medium was used for *A. chroococcum* treatments. Three replicate dishes were prepared for each dilution. Agar plates were incubated at 30°C for 48-96 hours. After incubation, the number of colony forming units (cfu) g^{-1} of soil was determined by the pour plate method. Rhizobacteria were identified considering cellular and colony morphology, and by Gram staining (Holt, 2000, Bergey's Manual). Mycorrhizal colonization of roots in terms of percent infection was measured according to the method of Phillips and Hayman (1970).

2.5 Experimental design in the field

A group of 60 chilli plants were prepared in the same way as described for the nursery experiment. Chilli plants were transplanted to a biointensive tropical organic garden (soil characteristics as described in the nursery experiments). The plants were planted at a distance of 0.8 m between each other in a completely randomised design with 12 treatments and 5 repetitions (n= 5 plants). Plant growth and nutrient content were evaluated in leaves collected at 80 days after planting i.e. 110 after inoculation. The plants were harvested at 8 months after sowing and the yield was determined according to the fresh weight of the chilli fruits.

2.6 Evaluation of the growth and nutrient content of chilli plants

In the nursery experiment, the plant height (cm), number of leaves, fresh biomass and nutrient content were evaluated 30 days after inoculation or fertilisation. For nutrient analyses, the N content was determined by semi-micro Kjeldahl procedure (Bremmer, 1965). Total P and K content were determined by $HNO_3 - HClO_4$ treatment and were measured by vanadomolybdate spectrophotometry and flame atomic-absorption spectrometry, respectively. In the field phase, the height (cm), number of branches and stem diameter at the base of the plant (cm), were evaluated 110 days after sowing. The leaves samples were collected from each plant during flowering to determinate the total nutrient content (N, P and K) using methods mentioned above. The fruits were harvested after 200 days.

2.7 Statistical analysis

Results were then subjected to a one way ANOVA, and means were compared by Duncan's multiple range Test ($p<0.05$). In the case of non normaly distribution data, an angular transformation (for the mycorrhizal colonisation data) and a logarithmic transformation were applied (for the rhizobacterial population). All analyses were performed using STATISTICA version 6.0 Software.

3 Results and Discussion

3.1 Rhizobacteria and mycorrhizal colonisation

The populations of both rhizobacteria decreased after inoculation during the period of the experiments. According to Barea and Azcón-Aguilar (1982), the decrease in the population of bacteria after inoculation may be related to difficulties in adapting to their new environment. However, the root exudates play a significant role in the growth of microorganisms (Narula *et al.*, 2005). This exudation is reduced after 60 d past planting and much of the plant's energy reserves are channeled towards fruit/seed formation, thus causing an exponential decline in the survival of introduced bacteria. The population of *A. brasiliense* found in the rhizosphere of habanero chilli ranged from 1.3×10^6 to 2.4×10^6 cfu g^{-1} soil and was significantly greater (p¡0.05) than that determined for *A. chroococcum* with values of 6.8×10^5 to 1.0×10^6 cfu g^{-1} soil (Table 2). Root coating and dipping, either single or combined inoculation did not significantly affect the population of either rhizobacteria species.

Soil microorganisms influence AM fungal development and the establishment of symbiosis but no clear pattern of response has been found. Negative impacts upon the AM fungi include a reduction in spore germination and hyphal length in the extrametrical stage, decreased root colonisation and a decline in the metabolic activity of the internal mycelium (Wyss *et al.*, 1992). According to our results, the highest incidence of mycorrhizal colonisation (57.3%) was observed in the treatment AMF_S involving single biofertilisation by root coating (Table 2). The combined inoculation of rhizobacteria and AMF resulted in a lower colonisation in comparison with the treatments with AMF as the single inoculants (Table 2). These results differ from those of Fitter and Garbaye (1994) who reported that rhizobacteria increased the capacity of AMF to colonise

the roots of plants. However, according to GIANINAZZI-PEARSON (1982), free-living bacteria such *Azotobacter* and *Azospirillum* spp. can increase microbial populations in the rhizosphere of mycorrhizal plants.

Table 2: Population of rhizobacteria and mycorrhizal colonisation in *Capsicum chinense* Jacquin 30 days after inoculation in the different treatments under nursery conditions.

Treatments	*Rhizobacterial population (cfu g soil^{-1})*	*Mycorrhizal colonisation (%)*
Ab_S	1.3×10^6 ^{a}	nd
Ab_L	2.5×10^6 ^{a}	nd
Ac_S	7.0×10^5 ^{bc}	nd
Ac_L	6.8×10^5 ^{bc}	nd
AMF_S	nd	57.3 ^{a}
AMF_L	nd	47.3 ^{b}
Ab_S + AMF_S	1.5×10^6 ^{a}	42.0 ^{bc}
Ab_L + AMF_L	1.5×10^6 ^{a}	41.0 ^{cd}
Ac_S + AMF_S	10.3×10^5 ^{b}	35.3 ^{de}
Ac_L + AMF_L	2.6×10^5 ^{c}	37.0 ^{cde}
Control	nd	nd
NPK	nd	nd

Means followed by different letters are significantly different based on Duncan's multiple range test ($p < 0.05$), $a > b > c$. nd= not determined. cfu= colony forming units. Treatment codes are given in Table 1.

3.2 Effect of the treatments on the growth and nutrition of chilli plants: nursery experiment

The treatment Ab_S resulted in a significantly positive effect on the height of the plants (Table 3), in comparison with Ac_S, Ab_L+AMF_L, Ac_S+AMF_S, Ac_L+AMF_L and the Control. No significant differences occurred among the other treatments. The highest fresh biomass and number of leaves were also observed in the Ab_S treatment, and this was significantly different from Ac_S, Ac_L, Ab_S+AMF_S, Ab_L+AMF_L, Ac_S+AMF_S, Ac_L+AMF_L and the Control ($p<0.05$). No synergistic effects that might favour the growth of the crop were observed in the treatments involving combinations of biofertilisers (Table 3). Several studies have reported increases in growth and development for crops such as tomato, coriander, pepper and lettuce after inoculation with *Azotobacter* and *Azospirillum* (TERRY *et al.*, 2002; BASHAN *et al.*, 2004). However, the roots of pepper normally form symbiotic associations with AMF (MENA-VIOLANTE *et al.*, 2006), and the potential for AMF to increase plant growth under conditions of low soil P content has been well documented (LINDERMAN, 1992). In contrast, some Glomus isolates have been shown to stimulate plant growth independent of plant P nutrition or when P is not limiting (DAVIES JR. *et al.*, 1993).

Table 3: Growth and nutrient content (Total N, P, K) in plant tissue of *Capsicum chinense* Jacquin at 30 days post-inoculation in the nursery experiment.

	Growth parameters			*Nutrient content (%)*		
Treatments	Number Leaves	Height (cm)	Fresh weight (g)	N_{Total}	P	K
Ab_S	28.0 ^{a}	27.72 ^{a}	12.55 ^{a}	4.32 ^{a}	0.53 ^{b}	1.43 ^{e}
Ab_L	19.2 ^{cde}	26.38 ^{ab}	10.81 ^{ab}	4.18 ^{ab}	0.58 ^{a}	1.44 ^{e}
Ac_S	26.2 ^{ab}	22.96 ^{bcd}	8.41 ^{bcd}	3.78 ^{ef}	0.44 ^{d}	1.56 ^{de}
Ac_L	18.8 ^{cde}	25.08 ^{abcd}	9.25 ^{bcd}	3.75 ^{ef}	0.41 ^{e}	1.85 ^{c}
AMF_S	19.2 ^{cde}	24.96 ^{abcd}	10.01 ^{abcd}	4.07 ^{bc}	0.56 ^{a}	1.61 ^{d}
AMF_L	20.8 ^{cd}	26.08 ^{abc}	10.57 ^{abc}	4.00 ^{cd}	0.50 ^{c}	1.61 ^{d}
$Ab_S + AMF_S$	26.0 ^{b}	24.26 ^{abcd}	8.83 ^{bcd}	3.68 ^{f}	0.41 ^{e}	1.80 ^{c}
$Ab_L + AMF_L$	19.8 ^{cde}	21.20 ^{d}	7.19 ^{d}	3.73 ^{ef}	0.38 ^{f}	2.16 ^{b}
$Ac_S + AMF_S$	27.0 ^{ab}	21.86 ^{cd}	9.09 ^{bcd}	3.88 ^{de}	0.37 ^{f}	2.15 ^{b}
$Ac_L + AMF_L$	20.8 ^{cd}	21.56 ^{d}	7.70 ^{cd}	3.78 ^{ef}	0.43 ^{cd}	1.92 ^{c}
Control	18.0 ^{e}	20.96 ^{d}	7.59 ^{cd}	3.61 ^{f}	0.37 ^{d}	1.45 ^{e}
NPK	18.8 ^{cde}	24.94 ^{abcd}	10.58 ^{abc}	4.13 ^{bc}	0.52 ^{bc}	2.44 ^{a}

Means followed by different letters are significantly different based on Duncan's multiple range test ($p < 0.05$), $a > b > c$. Treatment codes are given in Table 1.

Previous studies have reported that the combined application of PGPR and AMF increased growth and development in inoculated plants (Bashan *et al.*, 2004). In our study, the greatest response in *C. chinense* was obtained when the biofertilisers were applied as single inoculants. The incipient effect on the growth of the plants with the combination of rhizobacteria and mycorrhiza may be related to the competition of each symbionts for carbonated compounds, a situation in which the host plant must both satisfy its physiological requirements and provide energy to the symbiont microorganisms (Azcon, 2000).

Significantly higher N content in plant tissue of *C. chinense* was observed in the treatments Ab_S (4.3%) and Ab_L (4.2%). The P content of plant tissue was significantly higher in treatments Ab_L (0.58%) and AMF_S (0.56%), than the others. The NPK treatment resulted in the highest K content (2.44%), (Table 3). The increase in the nutrient content of plants inoculated with PGPR is mainly due to the changes produced in the morphology of the roots by the phyto-hormones that are synthesized by many rhizobacteria and that result in an increase in root surface area (Bashan *et al.*, 2004). However, PGPR may also enhance mineral uptake, not only as a consequence of the increase in root surface area but also by stimulating proton efflux activity (Bashan, 1990). (Murty and Ladha, 1988) showed that *Azospirillum* inoculation increased P and NH_4^+ uptake by rice plants, although, whether this was a result of increased nutrient mobilization, or a secondary effect of improved root growth, was not demonstrated. Additionally, the roots that are colonized by mycorrhizal fungi use the extraradical mycelium to explore a greater volume of soil, and translocate nutrients from the soil to the plant more efficiently, resulting in improved plant nutrition (Linderman, 1992).

3.3 Effect of the treatments on the growth and nutrition of chilli plant: field experiment

Under field conditions the plant response to biofertiliser inoculation may be influenced by factors such as soil parameters, climatic conditions and other microbial interactions (Lucy *et al.*, 2004). Moreover, in unsterilized field soil conditions competition may occur between introduced and native microbial populations. In this study, the maximum plant growth values in the field occurred when the biofertilisers were applied singly (Table 4). The treatments that significantly stimulated the height of the plants were AMF by root coating or dipping (AMF_S and AMF_L) and *A. chroococcum* by root coating (Ac_S). Additionally, treatments Ac_S and AMF_L resulted in stem diameter values that were significantly high than observed in other treatments ($p < 0.05$). The number of branches was significantly greater in the AMFs treatment than the Control ($p < 0.05$) but did not differ significantly with respect to the other treatments.

Table 4: Growth and nutrient content (Total N, P, K) in plant tissue of *Capsicum chinense* Jacquin at 30 days post-inoculation in the nursery experiment.

Treatments	*Growth parameters*			*Nutrient content (%)*		
	Height (cm)	*Stem diameter (cm)*	*Number of branches*	N_{Total}	P	K
Ab_S	29.40 ^{bcd}	0.47 ^{f}	6.4 ^{a}	3.50 ^{e}	0.50 ^{a}	2.42 ^{a}
Ab_L	33.60 ^{bc}	1.27 ^{bc}	6.6 ^{a}	3.65 ^{d}	0.32 ^{f}	1.77 ^{c}
Ac_S	42.20 ^{a}	1.50 ^{ab}	7.6 ^{a}	2.79 ^{h}	0.36 ^{e}	1.12 ^{f}
Ac_L	34.20 ^{b}	1.20 ^{bcd}	7.8 ^{a}	3.24 ^{f}	0.33 ^{f}	1.39 ^{de}
AM_S	45.80 ^{a}	1.27 ^{bc}	8.0 ^{a}	3.00 ^{g}	0.21 ^{h}	1.34 ^{ef}
AM_L	42.80 ^{a}	1.73 ^{a}	7.4 ^{a}	3.04 ^{g}	0.35 ^{ef}	1.84 ^{c}
Ab_S + AM_S	30.20 ^{bcd}	0.90 ^{de}	7.0 ^{a}	4.35 ^{a}	0.40 ^{d}	1.62 ^{cd}
Ab_L + AM_L	27.00 ^{cd}	0.70 ^{ef}	5.4 ^{ab}	4.02 ^{c}	0.49 ^{ab}	2.11 ^{b}
Ac_S + AM_S	26.80 ^{cd}	0.70 ^{ef}	5.2 ^{ab}	4.17 ^{b}	0.47 ^{b}	2.23 ^{ab}
Ac_L + AM_L	25.80 ^{d}	1.10 ^{cd}	7.8 ^{a}	4.38 ^{a}	0.43 ^{c}	1.73 ^{c}
Control	25.20 ^{d}	0.43 ^{f}	3.3 ^{b}	2.80 ^{h}	0.23 ^{gh}	1.26 ^{ef}
NPK	27.80 ^{bcd}	1.03 ^{cde}	6.8 ^{a}	2.94 ^{g}	0.25 ^{g}	1.43 ^{de}

Means followed by different letters are significantly different based on Duncan's multiple range test ($p < 0.05$), $a > b > c$. nd= not determined. cfu= colony forming units. Treatment codes are given in Table 1.

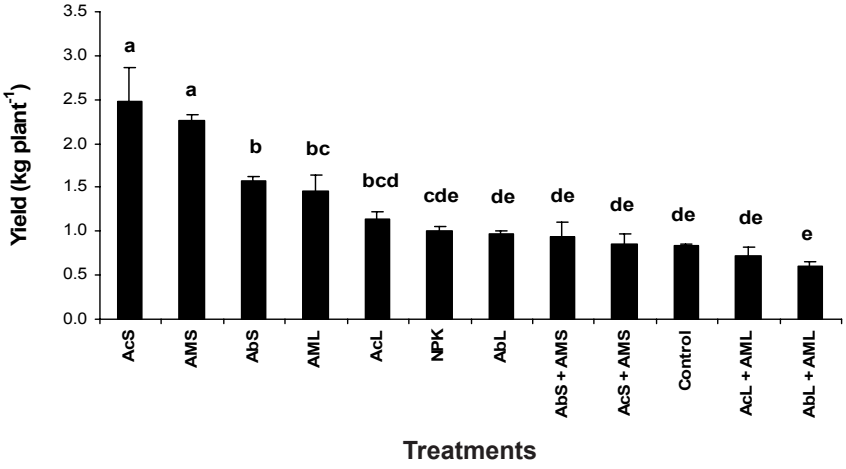
The combined inoculation with rhizobacteria and AMF seem to be the most effective treatment to improve N uptake. The leaf analysis of plants (Table 4) showed a greater N content with treatments Ac_L+AMF_L and Abs+AMFs with 4.3% for both treatments, and this was significantly greater than observed in the other treatments ($p < 0.05$). Generally, *Azotobacter* does not form intimate metabolic associations with the host plant and they therefore have a limited supply of carbon to provide energy for nitrogen fixation (Hubbell and Gaskins, 1984). Spores of mycorrhizal fungi seem to provide *Azotobacter* with an operational base in the vicinity of roots and a supply of carbon that increases the efficiency of both mycorrhizae and *Azotobacter*. In the case of P and

K, the Ab_S treatment resulted in the highest content (0.5% and 2.4%, respectively) and this was significantly greater than observed in treatments Ab_L, Ac_S, Ac_L, AMF_S, AMF_L, Ab_S+AMF_S, Ac_L+AMF_L, Control and NPK ($p < 0.05$).

3.4 Effect of the treatments on the yield of chilli plants

The use of PGPR to increase crop yield has been limited due the variability and inconsistency of results between laboratory, greenhouse and field studies. The application of biofertilisers had a positive effect on the yield of chilli fruits. The maximum values were recorded for treatments Ac_S with 2.5 kg plant^{-1} (25 t ha^{-1}) and AMF_S with 2.3 kg plant^{-1} (23 t ha^{-1}), compared with 1.0 kg plant^{-1} (10 t ha^{-1}) that was obtained with the NPK treatment and 0.8 kg plant^{-1} (8 t ha^{-1}) obtained in the Control treatment (Figure 1). Treatments that used combinations of biofertilisers resulted in significantly lower yields than those that were applied as single inoculants. It is also important to point out that the yield obtained was significantly greater with the treatments applied in solid supports compared with those applied in liquid supports.

Figure 1: Effect of the different treatments on the yield of *Capsicum chinense* Jacquin. Means followed by different letters are significantly different based on Duncan's multirange Test ($p < 0.05$), a > b > c. Errors bars = standard error of mean (n= 5).



In this study, *Azotobacter* in solid support (Ac_S) resulted in increases in plant height, the stem diameter and the yield but not in the nutrition of habanero chilli. These results suggest that plant responses to these bacteria could be associated with plant growth hormones, rather than the results of nitrogen fixation and phosphate solubilisation. Dobbelaere *et al.* (2002) also found that *A. brasilense* and *A. irakense* strains stimulated overall plant growth, including root development and increased yield of spring wheat and maize. However, neither rhizobacteria species affected the N content of

plants or grains. The effect of AMF on *Capsicum annuum* L. plants has been studied, in some detail with records of positive effects on growth, development, yield and some parameters of fruit quality such as size, colour and pigment content (Aguilera-Gómez *et al.*, 1999; Mena-Violante *et al.*, 2006).

4 Conclusion

The application of biofertilisers to *C. chinense* plants had a positive effect on the growth and nutrition of the crop with respect to the control and the NPK fertiliser treatment. The application of a root coating technique did not modify the concentration of the population of rhizobacteria or mycorrhizal fungi in the rhizosphere of *C. chinense*, but, this technique resulted in greater yields than the application of biofertilisers by root immersion. Biofertilisation with *Azotobacter chroococcum* and arbuscular mycorrhizal fungi applied as single inoculants in solid supports and by root coating provided the highest crop yields that even exceeded that of the NPK treatment. It is possible therefore, to obtain good yields with the application of biofertilisers under tropical conditions similar to those established in this study.

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