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EFFECT OF MYCORRHIZAE AND ACTINOMYCETES ON GROWTH AND BIOPROTECTION OF *Capsicum annuum* L. AGAINST *Phytophthora capsici*

Alfredo Reyes-Tena[1,2], Gabriel Rincón-Enríquez[1], Luis López-Pérez[2] and Evangelina Esmeralda Quiñones-Aguilar[1,*]

[1]Biotecnología Vegetal, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco. Av. Normalistas No. 800, Colinas de la Normal CP 44270, Guadalajara, Jalisco, México; [2]Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo. km 9.5 Carretera Morelia-Zinapecuaro, El Trebol CP 58880, Tarimbaro, Michoacán, México
[*]Corresponding author's e-mail: eqaguilar08@gmail.com, equinones@ciatej.mx

To evaluate the inoculation effect of Arbuscular Mycorrhizal Fungi (AMF) and actinomycetes on promoting plant growth and diminishing wilt in pepper plant (*Capsicum annuum* L.), a pot experiment was conducted under greenhouse conditions using a factorial design on random blocks. It was evaluated the native Cerro del Metate mycorrhizal consortium, and ABV39 and ABV02 actinomycetes were evaluated against the CH11 stain of *Phytophthora capsici*. Responses of the variables evaluated were plant height, stem diameter, number of leaves, total fresh and dry biomass, leaf area and radical volume, number of AMF spores, mycorrhizal colonization, roots infected with *P. capsici*, and severity of wilting utilizing an ordinal qualitative scale. The results showed that mycorrhizal plants and inoculated with ABV39 demonstrated the highest values for plant height, stem diameter, and number of leaves with 26.12 cm, 5.29 mm, and 35.12 leaves, respectively. Co-inoculation of AMF and both actinomycetes significantly promoted ($p \leq 0.001$) total fresh and dry biomass and leaf area of the plants without *P. capsici*. The disease severity of the AMF-inoculated plants diminished against plants without mycorrhization. However, when they were also co-inoculated with ABV39 or ABV02, disease severity was significantly reduced ($p \leq 0.05$), up to level 2 of 5 levels on a severity scale. These results show a synergetic effect with co-inoculation of AMF and actinomycetes in vegetal growth promotion and bioprotection against wilting caused by *P. capsici* in pepper plants.

Keywords: Arbuscular mycorrhizal fungi, biocontrol, microbial synergism, wilt, plant pathogen interaction, plant growth promoters, *Streptomyces*

INTRODUCTION

The major phytosanitary problem in the cultivation of pepper plants (*Capsicum annuum* L.) worldwide is the wilt caused by *Phytophthora capsici* Leonian (García-Rodríguez *et al*., 2010). This oomycete can cause losses of up to 80% in fruit production (Li *et al.*, 2007). Excessive application of biocides, mainly chemical, to combat this disease can cause damage to human health and the environment. In addition, this pathogen oomycete can generate resistance even to new fungi such as the pyrimorph (Pang *et al.*, 2013). Alternative handling of this disease comprises biological control using microorganisms that are antagonistic to the pathogenic agent (Segarra *et al.*, 2013). Arbuscular Mycorrhizal Fungi (AMF) are antagonistic to the plant pathogens of the plant's roots, this possible through different direct and indirect mechanisms of action (Abdel-Fattah *et al*., 2011; Jung *et al*., 2012). Mechanisms of direct action include competition for space and nutrients in the rhizosphere of plants creating networks of hyphae and roots blocking pathogen transmission. Al-Askar and Rashad (2010) showed that, in roots colonized by *Glomus mosseae*, *Glomus intraradices*, *Glomus clarum*, *Gigaspora gigantea*, and *Gigaspora margarita* in common bean roots, arbuscular mycorrhizal colonization significantly reduced infection by *Fusarium solani*. These fungi can change the patterns of root exudation, which indirectly promotes the establishment of beneficial microorganisms through qualitative and quantitative changes of their populations in the rhizosphere (Lioussane, 2010). Other mechanisms of indirect action of AMF in the bioprotection of plants against disease is Induced Systematic Resistance (ISR) (Haneef *et al.*, 2010), through which the plant enters a state of alert that maximizes the switching on of its defense mechanisms to counterattack the pathogens in any area of the vegetal tissue. In this regard, Ozgonen and Erkilic (2007) reported that mycorrhization with *Glomus etunicatum*, *Glomus fasciculatum*, and *G. margarita* reduced wilt severity in pepper plants; this was associated with the greater concentration of capsidiol found in mycorrhizal plants. This phytoalexin could be related with the delay of the development of necrosis by wilt (Sid-Ahmed *et al.*, 2000). Other mechanisms of action against plant pathogens are include structural functional compensation in the roots of diseased plants (Vierheilig *et al.*, 2008) and the maintenance of the redox equilibria during Oxidative Stress

(OS) promoted by the pathogen attack (Alejo *et al.*, 2008). On the other hand, actinomycetes are Gram-positive bacteria that are considered stimulators of plant growth, in that they can synthesize hormones and to solubilize and mineralize organic nutrients, which promote their assimilation by plants, rendering a nutritional benefit (Franco-Correa, 2008). These bacteria are very abundant in the soil, and many strains isolated from the rhizosphere of plants have exhibited an antimicrobial effect *in vitro* against fungal, bacterial, and oomycete pathogens of economic importance such as *Colletotrichum* spp. (Intra *et al.*, 2011), *Rhizoctonia solani* (Castillo, 2004), *Pseudomonas syringae* pv. *phaseolicola* (Rincón-Enríquez *et al.*, 2014), *Pythium aphanidermatum* (El-Tarabily, 2006), and *P. capsici* (Ezziyyani *et al.*, 2004). Actinomycetes can produce siderophores and enzymes that inhibit spore germination and the growth of certain fungi and oomycete pathogens. Xuan-Hoa *et al.* (2012) demonstrated that strain H7602 of *Streptomyces griseus* reduced, by 47.4%, the mortality of the root of pepper plants inoculated with *P. capsici*, and additionally increased fresh biomass as compared to the control. This latter bioprotection effect was related with chitinase activity and the β-1,3-glucanase of the actinomycete. Therefore, actinomycetes are important microbiological resources that could be applied in the biological control of plant diseases (Medina-Cuevas and Evangelista-Martínez, 2011). It is known that AMF promote the establishment of bacteria, furthering vegetal growth including actinomycetes that, at the same time, encourages an unfavorable environment for the establishment of soil-borne pathogens (Franco-Correa *et al.*, 2010). Based on the previously mentioned material, joint implementation of AMF and actinomycetes as biological control agents of plant diseases may comprise a promising alternative in the search for synergy in the control of pathogenic microorganisms. However, to date, to our knowledge, no studies of the combined use of these two groups of microorganisms in biological control have been reported, there being only are studies on the nutritional benefit of actinomycete-AMF interaction in *Trifolium repens* plants (Gómez-Vargas *et al.*, 2011). Hence, the objective of this research was to evaluate the promotion of vegetal growth and bioprotection against wilt created by *P. capsici* in pepper plants by means of the co-inoculation effect of AMF and actinomycetes.

MATERIALS AND METHODS

***Experimental conditions and plant material*:** The experiment was conducted during Spring-Summer 2014, in a plastic greenhouse of the Universidad Michoacana, Mexico (latitude, 19°45'95"N; longitude, 101°09'16"; altitude, 1,900 m) with natural light, temperature, and humidity conditions. Poblano pepper seeds of the "San Luis" variety were germinated on aluminum trays with sterilized sand (120°C/1.05464 kg cm^{-2}/3 h). The seeds were germinated on day 12 after planting. Then pepper seedlings were transplanted when they had two true leaves (30 days after showing) in a styrofoam pot with a 500-g substrate (mix of soil: sand, 1:1, v/v) sterile (120°C/15 psi/3 h) supplemented with 1% VermiCompost.

***Inoculation of microorganisms*:** A mycorrhizal consortium denominated Cerro del Metate (AMF) was employed. This consortium exhibited potential for reducing wilt severity in poblano pepper plants under greenhouse conditions (Reyes-Tena *et al.*, 2014a). The pepper plants were inoculated with 14.5 g of inoculum sand containing 80 spores from the AMF consortium. The inoculum content of the AMF spores was applied to the radical system of the pepper seedling at transplantation time. ABV39 actinomycetes (*Streptomyces* spp.) and the ABV02 strain (not identified) previously isolated from agave rhizospheres (Rincón-Enríquez *et al.*, 2014); these were inoculated 52 Days After Transplantation (DAT). These strains demonstrated *in vitro* antimicrobial activity against *P. capsici* (86% growth inhibition) (Reyes-Tena *et al.*, 2014b). The inoculum consisted of a Colony-Forming Unit (CFU) suspension obtained from pure cultures in solid medium ISP2 (Shirling and Gottlied, 1966). The plants were inoculated with 10^7 CFU g^{-1} of substrate, with an actinomycete alone or in combination. The inoculation was applied at the base of the pepper-plants' stems. The CH11 strain of *P. capsici* was inoculated 62 days after transplantation, and 1 mL of a suspension 10^4 zoospores mL^{-1} was prepared as per the method of Ristaino (1990), with slight modifications: these four were washed every 24 h of pieces of culture (with V8® juice agar) were carried out with sterile distilled water to induce greater sporangium formation. At the time of inoculation with the pathogen, the substrate where the plants were grown was saturated with sterile distilled water for 24 h to facilitate plant infection by the oomycete.

***Experimental design, quantification of mycorrhizal colonization, and growth*:** An experiment was established in a randomized-block design where the factors assessed were 1) AMF with two levels: inoculated and without mycorrhizal, 2) Actinomycetes with four levels: ABV39, ABV02, ABV39-ABV02, and without an actinomycete, and 3) *P. capsici*: inoculated and non-inoculated the oomycete pathogen; each treatment (16) was repeated eight times and the experimental unit consisted of a plant. To evaluate the effect of mycorrhization in pepper-plant growth, at 86 DAT, plant-growth variables were measured, including plant height recorded from the stem base to the leaf apex, stem diameter determined with a digital caliper. At the stem base, and number of leaves recorded by direct counting. Total fresh biomass and dry biomass variables were also quantified utilizing a Mettler Toledo AT200 analytical scale, and radical volume by volume-displaced water; the leaf area was determined with an LI-3100 LI-COR planimeter. To estimate mycorrhizal colonization, the root thinning and staining

method of Phillips and Hayman (1970) was employed; 90 1-cm segments root stained for quantification of intraradical mycelium, vesicles, arbuscules, and spores were observed. The percentage of mycorrhizal colonization was determined by the method described by McGonigle *et al.* (1990). For measurement of spore density in the substrate, the spores were extracted with the wet sieving-and-decanting technique of Gerdemann and Nicholson (1963), in combination with the technique of flotation in sugar in gradient sucrose described by Walker *et al*. (1982). Spore count was carried out under a stereoscopic microscope with the aid of a manual counter. For determination of the colonization of *P. capsici* in pepper plants, this was conducted with roots that were disinfected with a solution of commercial chlorine 0.5%. Subsequently, the roots were cut into segments 1-cm long, which were placed in Petri dishes with medium (Potato Dextrose Agar [PDA]), with 20 root segments placed per crop box. The percentage of roots infected based on number of segments where the oomycete pathogen grew represented the total number of segments recorded after e days.

***Determination of the severity scale of P. capsici in pepper plants*:** An experiment under greenhouse conditions was established to register the wilt symptomatology of the poblano pepper-plant variety "San Luis", and an ordinal scale of disease severity was developed. Twenty-five pepper plants were used, in which 1 mL of a suspension of 10^4 zoospores per mL^{-1} of CH11 strain of *P. capsici* was inoculated, and there were 25 plant controls (without *P. capsici*). The experimental unit included a styrofoam pot with 500 g of a mixture of soil/sand ratio of 1:1 (v/v) sterile (120°C/1.05464 kg cm^{-2}/3 h) with pepper plants. From day 4 of inoculation with *P. capsici*, the symptomatology was recorded that presented on the plants' leaves, stems, and roots. To perform the previously mentioned technique, destructive sampling was carried out, in which two plants with *P. capsici* were removed, and two healthy plants were removed, to compare disease severity. The degree of wilt was recorded employing an ordinal severity scale. The scale consisted of six levels: (0) healthy plant; (1) slight wilt, small, perky curved leaves, stem necrosis in the basal area; (2) moderate wilt, curved sheets and up to 20% of detached leaves, stem necrosis of up to 20%; (3) strong wilt, curved sheets and up to 50% of detached leaves, and stem necrosis of up to 40% with a 30% loss of the root system, growth stops; (4) very strong wilt, very curved and leaves with >50% detached, stem necrosis of >50%, root system of >80%, and a 50% loss of radical volume, with root epidermis detaching with ease, and (5) severe wilting, plant death.

Statistical analysis: Data of the quantitative variables were analyzed using Analysis of Variance (ANOVA) and the Tukey multiple comparison test ($p \leq 0.05$). Disease severity-scale data were analyzed using the non-parametric Kruskal-Wallis test ($p \leq 0.05$), and the Dunn test ($p \leq 0.05$) was applied to determine statistical differences among treatments. Analysis of the correlation among variables of mycorrhizal colonization, roots infection by *P. capsici*, and total dry biomass was performed with the Pearson test ($p \leq 0.05$). Analyses were performed with the Statgraphics Centurion ver. XV.II statistical software package (Statgraphics, 2005).

RESULTS

The effect of A

***AMF and actinomycetes on pepper plant growth*:** It was found that application of AMF promoted plant growth ($p \leq 0.05$) in most the variables evaluated, except for radical volume (Table 1). In total dry biomass, mycorrhization promoted greater growth (3.02 g) against that recorded in non-mycorrhized plants (1.81 g). On the other hand, application of actinomycetes significantly promoted growth ($p \leq 0.05$), the ABV02 strain exhibited statistically superior values when compared with the control without actinomycete for the variables leaf number, radical volume, and fresh and

Table 1. The effect on plant growth of Arbuscular Mycorrhizal Fungi (AMF) and actinomycetes on poblano pepper plants "San Luis" variety under greenhouse conditions at 86 days after transplantation.

Study factor	Plant height (cm)	Stem diameter (mm)	Number of leaves	Leaf area (cm^2)	Root volume (mL)	Biomass (g)	
						Dry	Fresh
Mycorrhizal							
With	23.80 a	5.01 a	33.44 a	78.29 a	2.48 a	3.02 a	12.37 a
Without	20.46 b	4.43 b	24.29 b	47.33 b	2.22 a	1.81 b	9.16 b
Actinomycetes							
Strain AB39	21.73 a	4.71 a	28.97 ab	64.23 ab	2.43 ab	2.55 ab	10.73 ab
Strain AB02	22.92 a	4.72 a	30.49 a	71.39 a	2.81 a	2.72 a	11.78 a
Strain AB39, AB02	22.18 a	4.81 a	29.28 ab	60.98 ab	2.18 ab	2.44 ab	11.10 ab
Without	21.68 a	4.65 a	26.75 b	54.64 b	1.97 b	1.95 b	9.47 b
P. capsici							
With	21.60 b	4.64 b	28.21 a	41.89 b	0.61 b	1.70 b	6.13 b
Without	22.68 a	4.81 a	29.52 a	83.73 a	4.08 a	3.13 a	15.41 a

Different letters in the same column by factor indicate significant differences among levels of study factors (Tukey, $p \leq 0.05$).

dry biomass. This shows that AMF and the ABV02 strain were capable of increasing pepper-plant growth. Finally, inoculation of the CH11 strain of *P. capsici* significantly diminished ($p \leq 0.05$) growth and plant biomass vs. healthy plants; this was reflected when a diminution of up to 94% presented with radical volume, 76% with lead area, 69% with dry biomass, and 78%, with fresh biomass.

The results of the combination of AMF and actinomycetes on growth variables in poblano pepper plants are presented in Table 2. In all variables, a statistically significant difference ($p<0.001$) was found between treatments. Generally, mycorrhizal plants (AMF) and without inoculation of the pathogen demonstrated greater growth in every evaluated variable; values recorded for the leaf area reflect this trend, where the mycorrhizal plants and without *P. capsici* exhibited values between 104 and 107.9 cm^2. The treatment in which the Cerro del Metate mycorrhizal consortium was inoculated (AMF) and actinomycete ABV39 in the absence of the pathogen (AMF, 39) recorded greatest height in plant growth, and greatest stem diameter, leaf number, leaf area, radical volume, and fresh and dry biomass, followed by the treatment with the inoculated AMF with actinomycete ABV02 without *P. capsici*. Both treatments were statistically superior ($p \leq 0.001$) to treatments in which the mycorrhizal consortium and the actinomycetes were not inoculated (PC and without AMF, ACTI, PC); these treatments recorded lowest values of fresh and dry biomass, and this variable significantly diminished ($p \leq 0.001$) in the treatments in which the plants were inoculated with *P. capsici*, because of the disease. However, treatments in which the mycorrhizal consortium and the actinomycetes were inoculated separately (AMF, 39, PC, and AMF, 02, PC) recorded a greater total fresh biomass (6.4 and 8.1 g, respectively) and total dry biomass (1.9 and 2.5 g, respectively), being statistically superior ($p \leq 0.05$) to the control, where no AMF and actinomycetes were inoculated (PC), which recorded total fresh and dry biomass of only 4.1 and 0.9 g, respectively. This suggests that mycorrhization and inoculation of actinomycetes in poblano pepper plants of the "San Luis" variety in the presence of the phytopathogen could aid in avoiding loss of the plant biomass. However, no significant interaction was found ($p = 0.65$) among AMF and actinomycete factors on total dry biomass, suggesting that growth promotion could solely be an effect of AMF inoculation.

Effect of AMF and actinomycetes on bioprotection against P. capsici in pepper plants: Twenty days after inoculation of *P. capsici*, all the pepper plants exhibited wilting symptoms (Fig. 1). However, when comparing disease symptomatology with the disease severity scale (Fig. 2), the non-parametric Kruskal-Wallis showed statistic difference between treatments (Statistical value = 31.33; $p \leq 0.001$), in which treatments (AMF, 02, PC) and (AMF, 39, PC) demonstrated a disease severity level of 2, according to the disease severity scale proposed (Fig. 3). In the treatment where only *P. capsici* was inoculated, the plants died due to the disease (level 5 on the scale).

Based on the results observed, co-inoculation with the Cerro del Metate consortium (AMF) and actinomycete ABV02 or ABV39 diminished wilt severity on poblano pepper plants to level 2 with respect to plants without co-inoculation with

Table 2. The effect of the Arbuscular Mycorrhizal Fungi (AMF) and actinomycetes combination on different parameters of plant growth in poblano pepper plants variety "San Luis" under greenhouse conditions at 86 days after transplantation.

Treatment	Plant height (cm)	Stem diameter (mm)	Number of leaves	Leaf area (cm^2)	Root volume (mL)	Biomass (g)	
						Dry	Fresh
AMF,39, PC	21.9 abc	5.0 abc	31.1 a-d	59.8 cde	0.7 de	1.9 b-e	6.4 ef
AMF,39	26.1 a	5.3 a	35.1 a	107.9 a	5.1 a	4.1 a	18.4 a
AMF,02, PC	23.8 ab	5.0 abc	35.3 a	55.2 cde	0.7 de	2.5 a-d	8.1 de
AMF,02	26.4 a	5.1 ab	35.1 a	104.0 ab	4.7 ab	4.2 a	18.2 a
AMF,02, 39,PC	22.4 ab	5.0 abc	33.8 ab	49.7 def	0.3 e	2.5 a-d	7.2 de
AMF,02, 39	26.1 abc	5.1 ab	32.9 abc	91.3 ab	3.5 abc	3.4 ab	16.8 ab
AMF,PC	23.8 ab	4.8 abcd	30.9 a-d	50.8 de	0.3 e	2.2 a-d	6.9 ef
AMF,	22.6 abc	4.8 abcd	33.4 ab	106.2 a	4.5 ab	3.2 abc	17.0 ab
39,PC	18.3 c	4.0 e	25.5 cde	33.7 efg	0.9 de	1.1 ef	5.5 ef
39	20.6 bc	4.6 bcde	24.0 de	53.3 cde	3.0 bc	3.1 abc	12.4 bcd
02,PC	21.3 abc	4.8 cde	27.4 b-e	49.6 def	1.01 de	1.5 c-f	7.2 def
02	20.4 bc	4.3 de	24.1 de	75.7 bcd	4.87 a	2.6 a-d	13.6 abc
02,39,PC	20.0 bc	4.5 cde	20.6 e	19.8 fg	0.35 e	0.9 f	3.5 f
02,39	20.6 abc	4.7 abcd	29.5 a-d	82.0 abc	4.62 ab	2.9 a-d	16.6 ab
PC	20.1 bc	4.4 cde	20.9 e	15.4 g	0.67 de	0.9 f	4.1 f
Without AMF,ACTI,PC	20.3 bc	4.5 bcde	21.9 e	45.9 ef	2.37 cd	1.5 def	10.0 cde

Actinomycetes (ACTI): ABV39 strain (39); ABV02 strain (02); PC = CH11 strain of *P. capsici*. Different letters in the same column indicate significant differences between treatments, Tukey ($p \leq 0.05$).

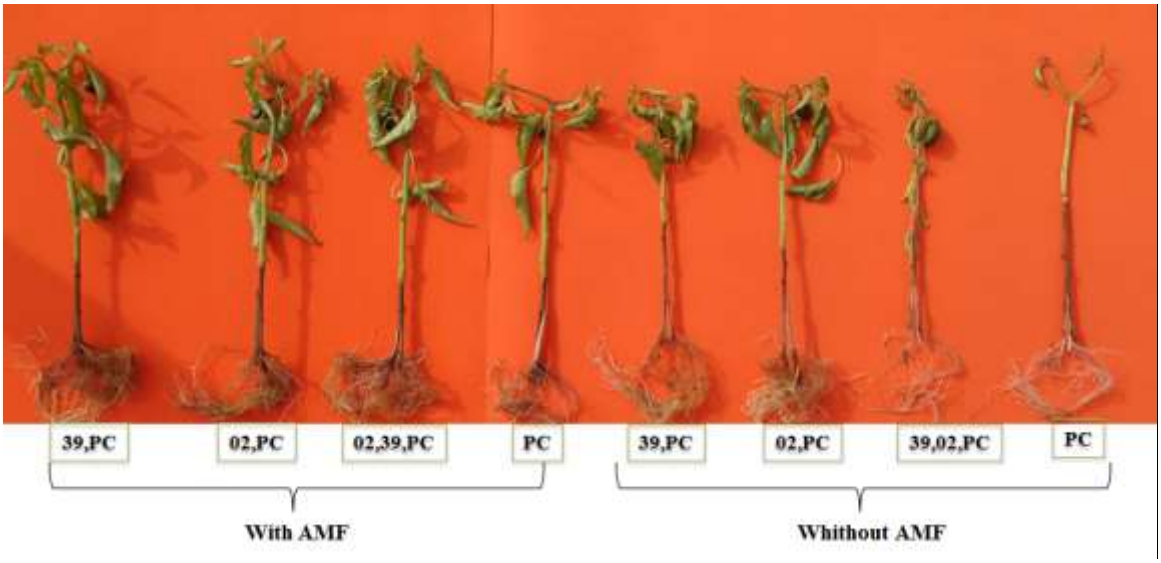

Figure 1. General aspect of the effect of inoculation of Arbuscular Mycorrhizal Fungi (AMF) and actinomycetes (02, 39) on the wilt severity of poblano pepper plants caused by *Phytophthora capsici* (PC). AMF: Cerro del Metate. Actinomycetes: ABV02 strain (02) and ABV39 strain (39).

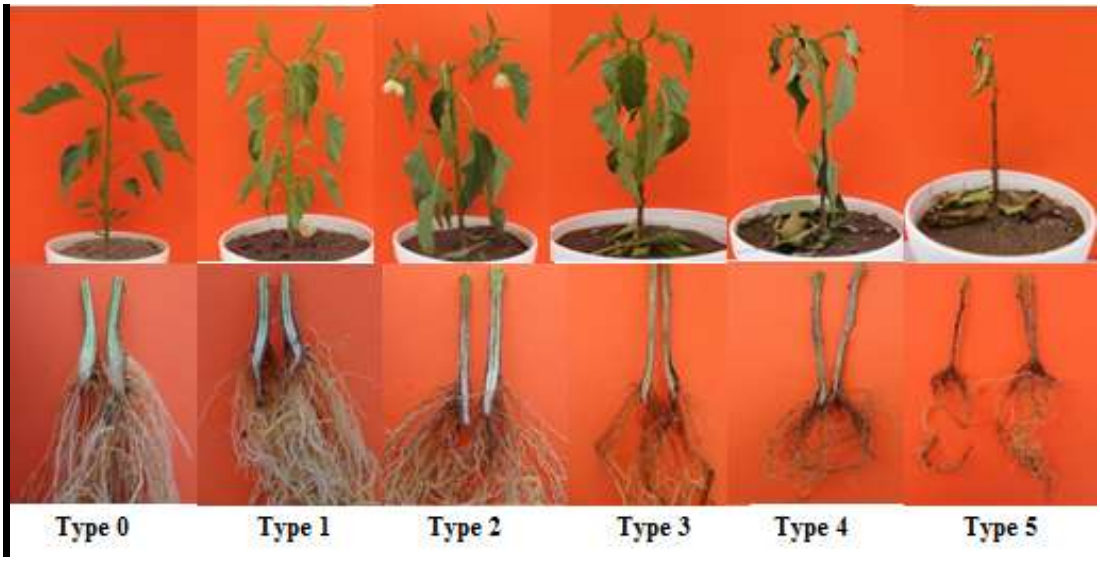

Figure 2. Morphological characteristics on plants and roots in the wilt severity scale of the poblano pepper variety "San Luis" induced by *Phytophthora capsica*.

microorganisms antagonistic to *P. capsici*. On the other hand, there was significant interaction (P=0.034) between AMF and actinomycetes and disease severity, suggesting a combined effect on wilt diminution. In the treatment in which the actinomycete strains were inoculated plus the phytopathogen without AMF (02, 39, PC), the plants presented a severity level of 4, which suggests that joint inoculation of these actinomycetes does not exert the bioprotection effect against wilt. This could indicate possible inhibition between both bacteria strains.

***Effect of mycorrhizal colonization on bioprotection against P. capsici on pepper*:** Percentage of mycorrhizal colonization, number of spores of AMF in the substrate, and percentage of infection in the roots evaluated by PC at the end of the experiment (86 DAT) are depicted in Table 3. In plants inoculated with AMF, high percentages of mycorrhizal colonization were recorded of up to 66%. In these treatments, mycorrhizal structures were observed in the root, mainly intraradical and extraradical mycelium, vesicles, and spores. On the other hand, a significant positive correlation ($p \leq 0.001$) was found between percentage of mycorrhizal colonization and total dry biomass (r=0.62); this confirms that inoculation

Table 3. Colonization, density of Arbuscular Mycorrhizal Fungi (AMF) spores, and *Phytophthora capsici* (PC) infection in the rhizosphere of poblano pepper plants variety "San Luis" under greenhouse conditions.

Treatment	Mycorrhizal colonization (%)	AMF spore density (spores in 100 g dry soil)	Root infection by *Phytophthora capsici* (%)
AMF,39,PC	60.0 a	190.0 abc	12.6 a
AMF,39	51.1 a	257.8 a	0.0 e
AMF,02,PC	58.9 a	187.8 abc	18.3 ab
AMF,02	46.7 a	243.3 a	0.0 e
AMF,02,39, PC	62.2 a	167.8 bc	26.6 abc
AMF,02,39	51.1 a	136.7 c	0.0 e
AMF,PC	66.7 a	217.8 ab	33.3 abc
AMF	52.2 a	125.6 c	0.0 e
39,PC	3.3 b	2.2 d	45.0 bcd
39	5.6 b	5.6 d	0.0 e
02,PC	1.1 b	3.3 d	48.3 cd
02	3.3 b	4.4 d	0.0 e
02,39,PC	1.1 b	7.8 d	51.6 cd
02,39	3.3 b	3.3 d	0.0 e
PC	3.3 b	4.4 d	70.0 d
Without AMF, ACTI, PC	2.2 b	1.1 d	0.0 e

Actinomycetes (ACTI): ABV39 strain (39); ABV02 strain (02). PC = CH11 strain of *Phytophthora capsici*. Different letters in the same column indicate significant differences between treatments (Tukey; $p \leq 0.05$).

with the mycorrhizal consortium promoted poblano pepper-plant growth under greenhouse conditions.

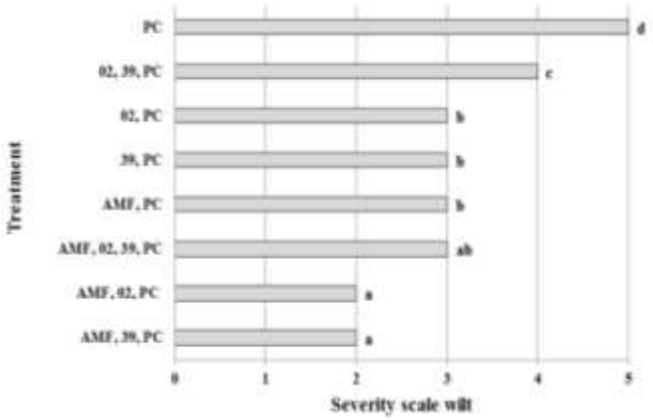

Figure 3. Bioprotection of Arbuscular Mycorrhizal Fungi (AMF) and actinomycetes against wilt of poblano pepper plant caused by *Phytophthora capsici* (PC) under greenhouse conditions at the end of the experiment. AMF: Cerro del Metate. Actinomycetes: ABV02 strain (02) and ABV39 strain (39). CH11 strain of *Phytophthora capsici* (PC). Different letters indicate significant differences between treatments, Dunn ($p \leq 0.05$).

With regard to density spores in the substrate, the treatments were statistically different ($p \leq 0.001$); treatments with mycorrhizal plants demonstrated a greater number of spores vs. non-mycorrhizal plants. In the treatment in which AMF was inoculated with PC without actinomycetes, an average increase of 92.2 spores was observed vs. the same treatment, but without PC (AMF), which might indicate stimulation by rhizosphere competence on the presence of PC and the absence of actinomycetes. Percentage of infection on the roots by *P. capsici* presented different statistics ($p \leq 0.001$) among treatments in which the plant pathogen was inoculated (Table 3). Lower rates of root infection by *P. capsici* were found in treatments with AMF inoculation, with treatments (AMF, 39, PC) and (AMF, 02, PC) exhibiting a minor infection of the phytopathogen. This suggests an antagonistic effect by competition for infection spots in the root between AMF and PC. This effect was confirmed by finding a significant negative correlation ($p \leq 0.001$) when comparing the percentage of mycorrhizal colonization with the variables: percentage of infection on roots with *P. capsici* (r= -0.7304) and the disease severity level (r= -0.506).

DISCUSSION

***Plant growth promotion by AMF and actinomycetes*:** Promoting plant growth and biomass by AMF on poblano pepper plants, reflected in the increase of the evaluated variables, agrees with what was reported by Alonso-Contreras *et al.* (2013), who found significant increases in stem diameter, leaf area, and dry biomass by the effect of the inoculation of a mycorrhizal consortium of pepper plants. Other studies have reported that AMF can promote growth and biomass of different plants of commercial interest, such as *Zea mays* (Celebi *et al.*, 2010), *Lycopersicum esculentum*

(Tahat *et al.*, 2008), and *Carica papaya* L. (Quiñones-Aguilar *et al.*, 2014). On the other hand, the recorded growth in terms of most the evaluated variables by treatments in which the mycorrhizal consortium was inoculated and actinomycete ABV39 or ABV02 without PC could represent a possible positive synergetic effect in the promotion of plant growth. In this respect, the results are like those studies conducted by Gómez-Vargas *et al.* (2011), in which the authors found that a strain of phosphate-mineralizing *Streptomyces* spp. (MCR26) acted synergistically with an AMF (*Glomus* spp.). These microorganisms increased the foliar uptake of phosphorus levels in *Trifolium repens* (white clover) plants, which stimulated the production of biomass and mycorrhizal colonization compared with the inoculation of each individual organism. According to Ramasamy *et al*. (2011), plant growth- promoting rhizobacteria, such as actinomycetes, could positively influence the development of mycorrhizal symbiosis through different mechanisms of action. In this regard, Carpenter-Boggs *et al*. (1995) found 19 actinomycetes capable of stimulating *in vitro* germination of *G. margarita* by producing volatile compounds that could benefit the plant in terms of greater growth.

There are very few studies on the synergism between AMF and rhizobacteria with respect to the promotion of plant growth in pepper plants; however, Kim *et al.* (2010) reported that the inoculation of a consortium of three AMF and two strains of *Methylobacterium oryzae* significantly increased fresh biomass in red pepper plants (*C. annuum* L.), which agrees with what was observed in this investigation. Although synergetic interaction between AMF and rhizobacteria in promoting plant growth has been documented (Miransari, 2011), the mechanisms underlying these associations are not yet completely clear (Artursson *et al.*, 2006); however, these microorganisms are an important biotechnical alternative in plant nutrition within sustainable agriculture (Gamalero *et al.*, 2004).

***Bioprotection by AMF and actinomycetes*:** Treatments with co-inoculation of AMF and actinomycetes demonstrated diminishing on wilt diminution, this suggesting a possible synergetic relationship between both microorganisms in bioprotection against *P. capsici*. The capacity of AMF to control soil disease would be strongly related with their capacity to promote the establishment of rhizobacteria antagonistic to plant pathogens (Lioussanne, 2010), as in the particular case of actinomycetes in which some of the species of the genus *Streptomyces* bacteria are even considered mycorrhizal assistants (lves-Rigamonte *et al.*, 2010). On the other hand, it is possible that bioprotection and biomass growth of biomass in plants inoculated with the Cerro del Metate mycorrhizal consortium is possibly due to an improvement in nutrition by means of the mycorrhization, as mentioned by Gómez-Dorantes *et al*. (2008), where previously mycorrhized tomato plants (*Solanum lycopersicum*) possessed reduced susceptibility to *P. capsici*. This effect was attributed to nutritional improvement and the competence of carbon composites among the microorganisms. In this regard, the negative correlation between percentage of mycorrhizal colonization and percentage of infection of *P. capsici* roots found in this study suggests a possible mechanism of competence for spots of infection inside pepper-plant roots. Mycorrhization could also have induced the systematic resistance in plants inoculated with the AMF consortium. Ozgonen and Erkilic (2007) mentioned that induction of systematic resistance in mycorrhizal pepper plants was mainly reflected in the increased concentrations of jasmonic acid, ethylene, and capsidiol. Another possible explanation is the accumulation of phenolic compounds and proteins related with the pathogenesis in mycorrhized plants (Ozgonen *et al.*, 2009). Another indirect mechanism that might be implicated in reduction of severity by means of the mycorrhization effects comprise modifications in radical architecture and maintenance of redox balance in response to biotic stress (Azcón-Aguilar and Barea, 1996).

About the bioprotection promoted by actinomycetes against *P. capsici*, Ezziyyani *et al.* (2004) demonstrated that *Streptomyces rochei* inhibited up to 81% of *P. capsici* radical growth in 4 days, while on day 7, the actinomycete was able to parasitize, promoting the production of mycelial degradative enzymes of the plant pathogen oomycete. Ezziyyani *et al.* (2007) purified, from *S. rochei*, the compound 1 propane, 1-4 chlorophenyl with antagonistic capacity against this phytopathogen. There are very few studies on the application of actinomycetes in the biocontrol of diseases under plant conditions; however, recently, Goudjal *et al.* (2014) found that application of a consortium of six actinomycetes coated in tomato seeds reduced, by up to 86.6%, the of incidence on the disease known as damping-off caused by *Rhizoctonia solani* in seedbeds with infested substrate. Additionally, this application significantly increased biomass, root length, and seedlings. Xuan-Hoa *et al.* (2012), while evaluating the effect of an elaborated extract based on *S. griseus* (strain H7602) vs. *P. capsici* on potted pepper plants at a greenhouse, found that the extract reduced up to 47.4% of root mortality increased fresh radical biomass by 56.4%, with the mechanism of action mainly attributing to enzymatic activity in the substrate (chitinase and β-1,3 glucanase) of this strain. These reports indicate a clear bioprotective effect and promotion of the actinomycete growth. Based on the results of the present study, a synergetic effect was observed in the promotion and growth of bioprotection against *P. capsici* by the effect of co-inoculation of AMF and actinomycetes; both microorganisms could be benefited by the direct and indirect mechanisms of action such as the promotion of mycorrhization, the solubilization of organic nutrients, or by antibiosis and competition against the plant pathogen.

***Conclusions*:** The results of this study showed that

mycorrhizal and actinomycete co-inoculation in poblano pepper plants variety "San Luis" increased plant growth and plant biomass without *P. capsici*. In plants inoculated with the plant pathogen, mycorrhization and co-inoculation of actinomycete ABV02 or ABV39 strains reduced wilt severity compared with plants into which no antagonistic microorganisms were inoculated. Probably one of the main mechanisms of bioprotection by the mycorrhizal consortium is against wilt, due to competition for space in the mycorrhizosphere and root, finding significant negative correlations between mycorrhizal colonization and percentage of root infection by *P. capsici* or disease severity.

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