BIOFERTILIZATION WITH RHIZOBACTERIA AND A CONSORTIUM OF ARBUSCULAR MYCORRHIZAL FUNGI IN CITRUS ROOTSTOCKS

[BIOFERTILIZACIÓN CON RIZOBACTERIAS Y UN CONSORCIO DE HONGOS MICORRIZÓGENOS ARBUSCULARES EN PORTAINJERTOS DE CÍTRICOS]

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SUMMARY

The effect of individual and combined biofertilization with the plant growth promoting rhizobacteria (PGPR) strains FCA-8, FCA-56 and FCA-60 of *Pseudomonas putida* and arbuscular mycorrhizal fungi (AMF) on the growth of *Citrus volkameriana* and Rangpur lime rootstocks grafted with Tahiti lime was evaluated under nursery conditions. Plants were inoculated individually and combined with the three rhizobacterial strains and with AMF, and received 50% fertilization. These plants were compared with a control group that received nursery management with 100% fertilization. A 2 x 10 factorial split-plot experimental design with five replications per treatment was established. Variables measured included plant height, stem-base diameter, root length and volume, fresh and dry plant biomass, and bacterial and mycorrhizal colonization. Individual and combined biofertilization with PGPR and AMF promoted the growth of *C. volkameriana* and Rangpur lime grafted with Tahiti lime in the nursery, similar to the control with 100% fertilization. The three strains of *P. putida* and AMF applied individually or combined provided similar results to the control in the nutrient content of Tahiti lime leaves. The *P. putida* strains showed good capacity for colonization of the rhizosphere of *C. volkameriana* and Rangpur lime individually or combined with AMF.

Key words: *Pseudomonas putida*; plant growth; nutrition; mycorrhizal colonization.

RESUMEN

Se evaluó en vivero el efecto de la biofertilización individual y combinada con las cepas rizobacterianas FCA-8, FCA-56 y FCA-60 de *Pseudomonas putida* y hongos micorrizógenos arbusculares (HMA), sobre el crecimiento de los portainjertos *Citrus volkameriana* y lima Rangpur, e injertados con lima Persa. Las plantas se inocularon de forma individual y combinada con las tres cepas rizobacterianas y con HMA, y recibieron 50% de fertilización. Se compararon con el testigo que fue el manejo del vivero con 100% de fertilización. El diseño del experimento fue de parcelas divididas con cinco repeticiones. Se evaluaron las variables altura de planta, diámetro de la base del tallo, longitud y volumen de raíz, biomasa fresca y seca de planta, colonización bacteriana y micorrízica. La biofertilización individual y combinada con las tres cepas rizobacterianas de *P. putida* y los HMA estimularon el crecimiento de los portainjertos de *C. volkameriana* y lima Rangpur y del injerto lima Persa en vivero, siendo igual al testigo con fertilización completa. Las cepas de *P. putida* y los HMA aplicados en forma individual o combinados presentaron resultados similares al testigo en el contenido nutrimental de hojas de lima Persa. Las cepas de *P. putida* mostraron buena capacidad de colonización en la rizósfera de *C. volkameriana* y lima Rangpur en lo individual y combinadas con HMA.

Palabras clave: *Pseudomonas putida*; crecimiento vegetal; nutrición; colonización micorrízica.

INTRODUCTION

Citrus cultivation is of great importance in countries with subtropical and tropical climates due to the quantity of jobs that are generated during the production process, as well as by the income obtained from the industrialization and commercialization of fresh or processed fruit (Agustí, 2003; FAO, 2009). In Mexico the production of citrus is ranked 5th in global production and is carried out in 23 states (SIAP, 2009).

Modern agriculture is characterized by the excessive use of inorganic fertilizers and agrochemicals to manage and increase production and to control pests and diseases. This production system has generated serious environmental contamination problems, public health issues and reduced soil fertility (Leach and Mumford, 2008; Berg, 2009). Thus, it is necessary to implement sustainable production strategies to reduce the damage to human health and contribute to soil and water conservation, important components for the equilibrium and optimal operation of agroecosystems (Barea *et al.*, 2005; Rives *et al*., 2007; Ryan *et al*., 2008). Currently, agricultural biotechnology promotes alternatives for profitable and viable production such as biofertilization, which improves crop performance without damaging the environment or human health, and at the same time contributes to the improvement of soil fertility (Van Loon, 2007; Lambers *et al*., 2009). The growing interest in the biofertilization of agricultural production systems is based on the use of beneficial living microorganisms, such as plant growth-promoting rhizobacteria (PGPR) and the formation of arbuscular mycorrhizal fungi (AMF). These organisms are capable of stimulating the growth, development and health of the plant and improving soil fertility (Song *et al*., 2007).

Many genera of bacteria exist within the rhizobacteria, including *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter* and *Serratia*. The genus *Pseudomonas* includes free-living saprophytic microorganisms in the soil, decomposing organic matter, aquatic ecosystems and is associated with plants and animals (Berg, 2009). The most beneficial species in *Pseudomonas* are *P. putida* Trevisan and *P. fluorescens* Migula (Gravel *et al*., 2007), which fix atmospheric nitrogen, degrade aromatic compounds, promote the synthesis of plant growth regulators and other antagonistic metabolites such as siderophores, lithic enzymes (glucanases and chitinases) and antibiotics (Richardson *et al*., 2009; Ryan *et al*., 2009). These species have been extensively studied for their positive interactions on the growth, development and health of crops such as corn, wheat, tomato, lettuce, rice and citrus (Rives *et al*., 2007; Bhattacharjee *et al*., 2008; Santoyo *et al*., 2010).

Among the AMF are the genera *Gigaspora*, *Glomus*, *Acaulospora* and *Sclerocystis*, which establish symbiotic associations with more than 90% of plant species. This symbiosis influences the nutrition and growth of the plants, improves tolerance to water stress and resistance to soil pathogens, as well as promotes increased root volume and intervenes in the solubilization and absorption of phosphorous (Vogelsang *et al*., 2006; Bever *et al*., 2009). The potential of AMF has been evaluated in crops of agricultural importance such as corn, wheat, sugarcane, tomato, onion, coffee, pastures and citrus (Wang and Qiu, 2006; Lambers *et al*., 2009; Rigamonte *et al*., 2010).

The process of biofertilization using PGPR and AMF has viable potential for agricultural production plans that seek to improve plant growth because these microorganisms are positively synergistic (Bever *et al*., 2009; Lambers *et al*., 2009). Thus, the objective of the present study was to evaluate in a nursery the effect of individual and combined inoculation with three rhizobacterial strains and a consortium of AMF on the growth and nutrition of *Citrus volkameriana* and Rangpur lime grafted with Tahiti lime.

MATERIALS AND METHODS

Nursery location

The study was carried out in Martínez de la Torre, Veracruz, Mexico (20° 04' N and 97° 04' W, at an altitude of 151 m a.s.l.), in the San Manuel nursery which is certified for citrus production. Management of the plants used normal nursery operations with the difference that inoculation with rhizobacteria and/or mycorrhiza was according to the corresponding treatment.

Plants used

Considering the difference in growth that rootstocks conferred to scion, *C. volkameriana* Tan. & Pasq. and Rangpur lime (*Citrus* x *limonia* Osbeck) were selected; *C. volkameriana* is more vigorous than Rangpur lime (Curti-Díaz *et al*., 2000). The graft used was Tahiti lime (*Citrus latifolia* Tan.). When the rootstocks presented three pairs of true green leaves in the seedbed, they were transplanted to black polyethylene bags of 3.0 kg in capacity containing fertile river sediment (clay-loam), previously disinfected with Bunema® 55 GE (Buckman) at 20 mL L^{-1}. The graft with Tahiti lime was performed 120 days after the transplantation.

Propagation and maintenance of the inoculations

Rhizobacteria

The PGPR (*Pseudomonas putida*) used were supplied by the Universidad Veracruzana. The strains were labeled as FCA-8, FCA-56 and FCA-60, and were grown in the B-King (BK) culture medium [glycerol (IDQ®) 10 mL L^{-1}, peptone (BD-Bioxon®) 15 g L^{-1}, $MgSO_4$ (IDQ®) 1 mL L^{-1}, K_2HPO_4 (J.T. Baker®) 1.5 g L^{-1}]. Each strain was cultured in a total of 450 mL of BK culture medium, divided into three flasks with 150 mL in each; the media was sterilized for 15 min at 120 ºC (Garrity *et al*., 2005). Strain incubation was at 25 ºC for 96 h according to Díaz *et al*. (2001).

Mycorrhiza

The AMF were supplied by the Universidad Veracruzana. This consortium is comprised of infective propagules of *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler comb. nov., *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler comb. nov., *Glomus aggregatum* N.C. Schenck & G.S. Sm., *Glomus* sp., *Gigaspora albida* N.C. Schenck & G.S. Sm., *Acaulospora morrowiae* Spain & N.C. Schenck and *Acaulospora scrobiculata* Trappe. The propagation of the consortium was carried out in a sterilized mixture 2:1 (v/v) of soil and river sand (Zulueta *et al*., 2010).

Biofertilization of *Citrus volkameriana* and Rangpur lime

Rootstock inoculation was carried out at the moment of transplantation from the seedbed to the polyethylene bags. The PGPR were prepared to a concentration of 10^9 cells mL^{-1} of BK culture media, adjusted with sterile physiological saline solution using a digital spectrophotometer (Thermo-Spectronic, Genesys 20®) and according to the McFarland scale (Rives *et al*., 2009). The inoculation with the PGPR was carried out by immersing the roots in 500 mL of bacterial suspension for 20 min. The inoculation of AMF consisted of completely impregnating the roots with 10 g of inoculum per plant.

In addition to the inoculation with the rhizobacteria and the AMF, the plants were fertilized at 30 days after transplantation with the formula 18-46-00 (N-P-K). The control was fertilized according to nursery management protocol, which was the application of 5 g per plant, and in those treatments with biofertilization 2.5 g per plant was applied.

Variables evaluated

Variable measurement was carried out equally between the rootstocks and grafts. For *C. volkameriana* and Rangpur lime, the variables measured were: plant height (cm), stem base diameter (mm), root length (cm) and volume (cm^3), dry and fresh plant biomass (g), bacterial colonization (CFU g^{-1} of roots) according to Bashan *et al*. (1996), as well as the percentage of total mycorrhizal colonization (hyphae, arbuscules and vesicles) using the technique of clearing and staining roots (Phillips and Hayman, 1970) and observing them under an optical compound microscope (Nikon®) (Biermann and Linderman, 1981).

Variables evaluated for the Tahiti lime graft included: height (cm), graft base diameter (mm), leaf area (cm^2), dry and fresh plant biomass (g), and leaf content of N, P, K, Ca, Mg, Fe, Cu, Zn and Mn (Maldonado *et al*., 2008). For nutrient determinations a compound sample from five plants per treatment was used, which did not include replications. All variables were measured when the plants reached 12 months of age, a time defined by the technical-productive system of the nursery.

Experimental design and statistical analysis

A split-plot factorial design was used in which the plots represented by *C. volkameriana* and Rangpur lime were divided into 10 sub-parcels (10 treatments) having five replications, with plant as the experimental unit. The treatments evaluated were: 1) Control, 2) FCA-8, 3) FCA-56, 4) FCA-60, 5) AMF-1, 6) Mix (mixture of the three rhizobacterial strains), 7) Mix + AMF, 8) FCA-8 + AMF, 9) FCA-56 + AMF and 10) FCA-60 + AMF. The control was according to the normal management of the nursery. Analyses of variance and comparison of means (Tukey test; $P \leq 0.05$) were used to analyze the data in SAS for Windows (Rebolledo, 2002).

RESULTS AND DISCUSSION

Growth of *Citrus volkameriana* and Rangpur lime

The analysis of variance showed significant differences in root volume ($P = 0.0063$), fresh plant biomass ($P = 0.0008$) and dry plant biomass ($P = 0.0073$). Dry plant biomass in *C. volkameriana* between Mix and AMF was significantly different. For Rangpur lime, significant differences in plant height were revealed for Mix and FCA-8 in relation to the control (Table 1). According to the results obtained on the growth of *C. volkameriana* and Rangpur lime (Table 1), there are various studies that show responses to inoculations with AMF and PGPR on the growth, development, health and performance of different plant species (Barea *et al*., 2005; Berg, 2009; Santoyo *et al*., 2010). Rives *et al*. (2009) mention that the use of rhizobacteria significantly favors the growth and development of plants. They found that 10 local strains of *P. putida* in rice promoted growth and development, with significant differences in dry and fresh biomass and root length. These results were not detected in the present study, possibly because the control under nursery management included a doubling of chemical fertilizer compared to the other treatments, as well as being influenced by the substrate. Similar values to the control were found indicating that the biofertilization treatments substituted 50% of the missing chemical fertilization. The results obtained provide continued incentive to test potential native *Pseudomonas* rhizobacteria to promote growth in plants from varied agricultural interests. As well, they indicate the importance to test these microorganisms in the field.

Table 1. Effects of biofertilization with rhizobacteria and arbuscular mycorrhizal fungi on the growth of *Citrus volkameriana* and Rangpur lime.

Treatments	Plant height (cm)	Stem diameter (mm)	Root length (cm)	Root volume (cm^3)	Fresh plant biomass (g)	Dry plant biomass (g)
C. volkameriana						
Control	34.50 a	9.43 a	25.74 a	35.60 a	42.52 a	15.61ab[1]
FCA-8	34.04 a	9.21 a	29.12 a	36.00 a	54.95 a	16.99 ab
FCA-56	35.26 a	9.41 a	28.28 a	35.60 a	55.18 a	18.40 ab
FCA-60	34.46 a	9.55 a	24.30 a	38.80 a	54.97 a	19.12 ab
AMF	35.46 a	8.62 a	26.26 a	34.00 a	49.66 a	14.16 b
Mix	34.30 a	9.46 a	26.10 a	42.00 a	56.33 a	19.63 a
Mix + AMF	35.08 a	9.35 a	27.92 a	36.60 a	49.17 a	16.94 ab
FCA-8 + AMF	34.78 a	9.45 a	25.34 a	37.20 a	49.29 a	17.79 ab
FCA-56 + AMF	33.96 a	8.94 a	29.66	36.60 a	47.17 a	18.66 ab
FCA-60 + AMF	34.82 a	9.36 a	26.40	35.60 a	49.38 a	17.25 ab
Rangpur lime						
Control	32.22 b[1]	8.79 a	23.40 a	29.20 a	42.11 a	15.22 a
FCA-8	36.58 a	9.71 a	29.12 a	33.20 a	48.73 a	17.94 a
FCA-56	35.70 ab	9.47 a	25.70 a	33.00 a	47.17 a	17.40 a
FCA-60	35.36 ab	9.59 a	23.82 a	27.40 a	42.45 a	16.45 a
AMF	35.92 ab	9.49 a	24.10 a	32.40 a	45.41 a	17.10 a
Mix	36.26 a	9.18 a	25.72 a	29.00 a	43.29 a	15.90 a
Mix + AMF	35.34 ab	9.28 a	23.36 a	30.60 a	45.02 a	15.87 a
FCA-8 + AMF	34.62 ab	9.50 a	24.76 a	26.00 a	41.48 a	15.39 a
FCA-56 + AMF	35.74 ab	8.94 a	25.66 a	30.00 a	43.17 a	15.76 a
FCA-60 + AMF	34.68 ab	9.45 a	25.80 a	30.80 a	45.94 a	15.79 a

Mix = mixture of three rhizobacterial strains.
[1]Values with the same letter in the same column are not significantly different (Tukey, $P \leq 0.05$).

Growth of Tahiti lime promoted by biofertilization

The analysis of variance of the split-plots showed significant differences for the general model ($P = 0.0004$) and for the interaction of rootstock by treatment ($P = 0.0316$). The Tukey test separated the means of the five variables studied between the two rootstocks (Table 2).

Table 2. Means of the growth-related variables for Tahiti lime grafted to *Citrus volkameriana* and Rangpur lime and biofertilized with rhizobacteria and arbuscular mycorrhizal fungi.

Graft host	Stem height (cm)	Stem diameter (mm)	Leaf area (cm^2)	Fresh plant biomass (g)	Dry plant biomass (g)
C. volkameriana	53.76 a[1]	7.40 a	988.03 a	48.46 a	16.02 a
Rangpur lime	49.46 b	6.65 b	890.88 b	40.94 b	13.85 b

[1]Means with the same letter in the same column are not significantly different (Tukey, $P \leq 0.05$).

Evaluations of treatment effects on Tahiti lime in each one of the rootstocks showed significant differences in *C. volkameriana* for dry plant biomass ($P = 0.0184$). Means comparison tests showed that strain FCA-60 worked best in the production of dry matter in comparison to AMF and in FCA-56 + AMF treatments (Table 3). Tahiti lime grafted onto Rangpur lime did not show significant differences for any of the variables studied (Table 3).

Rhizobacteria and AMF can improve the growth and development of the citrus evaluated, similar to the findings of Alarcón *et al*. (2003), because even with half of the fertilization they provided similar results to the control. Santillana (2006) found similar results for beans and corn, and showed differences with the control for three native strains of *Pseudomonas* sp. in terms of aboveground dry plant biomass and dry root biomass.

Table 3. Effects of biofertilization with rhizobacteria and arbuscular mycorrhizal fungi on the growth of Tahiti lime grafts on two rootstocks.

Treatments	Stem height (cm)	Stem diameter (mm)	Leaf area (cm^2)	Fresh plant biomass (g)	Dry plant biomass (g)
Citrus volkameriana					
Control	49.56 a	6.97 a	935.38 a	46.47 a	15.97 ab[1]
FCA-8	55.64 a	7.29 a	989.38 a	46.38 a	15.67 ab
FCA-56	53.24 a	7.16 a	883.48 a	43.79 a	14.96 ab
FCA-60	61.92 a	7.93 a	1213.38 a	61.15 a	20.92 a
AMF	49.28 a	6.76 a	921.58 a	43.61 a	13.33 b
Mix	57.34 a	7.98 a	1111.34 a	52.93 a	17.72 ab
Mix + AMF	47.72 a	7.36 a	1024.83 a	52.83 a	16.06 ab
FCA-8 + AMF	51.60 a	7.74 a	955.94 a	46.86 a	15.97 ab
FCA-56 + AMF	57.66 a	7.30 a	910.43 a	44.60 a	14.44 b
FCA-60 + AMF	53.68 a	7.48 a	934.54 a	45.98 a	15.21 ab
Rangpur lime					
Control	50.02 a	6.48 a	803.57 a	37.52 a	13.35 a
FCA-8	53.80 a	6.57 a	923.49 a	40.14 a	13.52 a
FCA-56	48.72 a	6.97 a	911.28 a	43.66 a	15.13 a
FCA-60	44.12 a	6.70 a	923.70 a	42.25 a	14.81 a
AMF	52.62 a	6.76 a	952.23 a	45.13 a	15.34 a
Mix	49.48 a	6.77 a	812.82 a	40.92 a	12.84 a
Mix + AMF	52.36 a	6.51 a	930.58 a	40.92 a	13.43 a
FCA-8 + AMF	46.76 a	6.52 a	859.49 a	38.87 a	13.40 a
FCA-56 + AMF	47.32 a	6.60 a	881.30 a	39.37 a	12.94 a
FCA-60 + AMF	49.40 a	6.63 a	910.20 a	40.56 a	13.76 a

Mix = mix of three rhizobacterial strains.
[1]Means with the same letter are not significantly different (Tukey, $P \leq 0.05$).

Even though the effects from the combined biofertilization of PGPR with AMF were not detected in this study, diverse reports have indicated the importance of using inoculations where both are associated. This is so because the individual qualities that each type of microorganism confers to the plant strengthens the plants' phenological development when both types are combined and interact in the rhizosphere to provide a positive synergism that improves growth, development, yield and health of the plants (Bever *et al*., 2009; Lambers *et al*., 2009; Richardson *et al*., 2009).

Nutrient content in Tahiti lime leaves

Table 4 shows the macro- and microelement contents in Tahiti lime leaves in the different treatments. In Tahiti lime grafted on *C. volkameriana*, the highest values for N (3.10%) and P (0.24%) were with AMF; however, the difference with that of smaller value (FCA-60) was only 0.33% and 0.04%, respectively. In the combined biofertilization using strain FCA-56 with AMF, the content of K was 0.19% greater than the control. In the microelement assessments, no tendency for any of the treatments with individual or combined biofertilization was observed. According to the variability of responses in Table 4, the treatments did not show a clear effect on nutrient content; bioinoculation permits plants to grow similar to the control where 100% fertilization was applied.

For the graft on Rangpur lime the results were very similar to those on *C. volkameriana* where no significant tendency was observed for any of the treatments. A very low value for Cu was observed for the treatment with the mixture of the three bacterial strains, while a high value for Mn was observed in the control and in treatment FCA-8 (Table 4). These values were likely due to external factors and not by treatment effects; however, to determine this it will be necessary to use known quantities of nutrients and to provide them to the plants in a sterile substrate.

Due to the capacities of the PGPR and AMF to fix atmospheric nitrogen, solubilize phosphorous, and to produce plant growth regulators and other metabolites that favor plant vigor and health, these microorganisms have been extensively used to improve crop nutrition and contribute to reducing fertilizer use, because their activity influences the plant rhizosphere and improves absorption of macro- and microelements (Ryan *et al*., 2009; Rigamonte *et al*., 2010).

Table 4. Influence of biofertilization with rhizobacteria and arbuscular mycorrhizal fungi on the nutrient composition Tahiti[1] lime leaves from two rootstocks.

Treatments	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
	----------%----------					-------mg kg^{-1}----------			
Citrus volkameriana									
Control	3.06	0.19	1.65	3.10	0.28	175	28	19	97
FCA-8	3.08	0.19	1.47	4.56	0.30	200	18	22	102
FCA-56	2.86	0.20	1.68	3.31	0.25	212	45	24	55
FCA-60	2.77	0.20	1.74	3.21	0.27	159	28	22	44
AMF	3.10	0.24	1.71	3.06	0.29	205	30	23	48
Mix	2.83	0.21	1.78	4.89	0.30	210	32	21	43
Mix + AMF	2.95	0.21	1.59	3.18	0.25	188	15	19	44
FCA-8 + AMF	2.97	0.21	1.71	3.40	0.29	231	23	23	66
FCA-56 + AMF	2.95	0.23	1.84	4.22	0.30	331	35	17	53
FCA-60 + AMF	3.07	0.21	1.78	3.11	0.26	205	24	21	59
Rangpur lime									
Control	2.93	0.19	1.65	2.15	0.33	132	30	21	70
FCA-8	2.94	0.18	1.96	2.61	0.25	120	30	16	43
FCA-56	3.11	0.19	1.95	2.86	0.29	143	37	19	42
FCA-60	2.99	0.20	1.90	3.77	0.38	140	33	22	38
AMF	3.04	0.22	1.69	3.15	0.32	123	31	21	38
Mix	2.81	0.22	1.83	3.00	0.38	140	12	21	38
Mix + AMF	2.99	0.23	1.51	2.86	0.32	200	21	23	43
FCA-8 + AMF	2.97	0.23	1.81	2.88	0.22	186	40	23	43
FCA-56 + AMF	2.77	0.21	1.47	4.40	0.30	234	27	21	43
FCA-60 + AMF	2.83	0.22	1.86	3.34	0.26	170	21	18	44

Mix = mix of three rhizobacterial strains.
[1]Values obtained from one compound sample from five plants per treatment.

The growing interest in beneficial microorganisms in general, and particularly in the rhizobacteria of the genera *Pseudomonas*, *Bacillus*, *Azospirillum*, and *Azotobacter* for incorporation into systems of agricultural production, is due primarily to their ease of application. They can be inoculated on seeds, foliage, roots or directly in the ground, places where, once established in the rhizosphere of the plants, they can multiply in response to root exudates, leading to enhanced plant growth and/or the biological control of plant diseases. Such qualities are the result of aggressive root system colonization (Castro-Sowinski *et al*., 2007; Fernández-Herrera *et al.*, 2007; Santoyo *et al*., 2010).

Determination of rhizobacterial populations and mycorrhizal colonization

Populations of rhizobacteria obtained from the individual and combined biofertilizations differed significantly among treatments (P = 0.0001). In both rootstocks, the significant differences were between the control and the AMF which were similar to each other (Figure 1) in comparison with all other treatments which were statistically similar.

The rhizobacterial populations obtained from the treatments that combined them with the AMF indicate that no antagonistic relation between the microorganisms existed. Indeed, both are capable of being complemented and to be more effective when interacting in the plant rhizosphere, interaction that can be selective and depend on the number and type of bacterial and fungal species (Terry and Leyva, 2006; Rigamonte *et al*., 2010).

The unidentified bacterial populations quantified in the control and AMF treatments were produced through repopulation from environmental contamination provided by the cultural practices performed during the production process in the nursery. This contamination indicates that the effect of the soil disinfectant (Bunema® 55 GE) used in the nursery was only temporary, permitting subsequent induced and natural microorganismal recolonization.

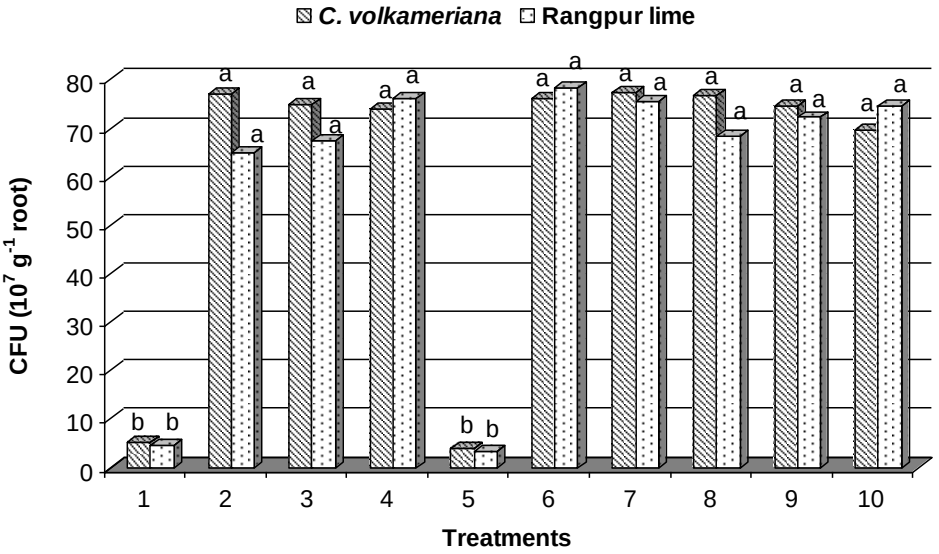

Figure 1. Abundance of PGPR in the rhizosphere of *Citrus volkameriana* and Rangpur lime: 1) Control, 2) FCA-8, 3) FCA-56, 4) FCA-60, 5) AMF, 6) Mix (mix of the three rhizobacterial strains), 7) Mix + AMF, 8) FCA-8 + AMF, 9) FCA-56 + AMF and 10) FCA-60 + AMF.
[a,b]Values with the same letter are not significantly different (Tukey, $P \leq 0.05$).

The benefit of the AMF in the plants is influenced by its capacity to colonize the root system and to establish a symbiotic relationship with its host. Given the treatments evaluated, the results of the analysis of variance showed significant differences between rootstocks ($P = 0.0013$) and among treatments ($P = 0.0001$). In *C. volkameriana*, the greatest mycorrhizal colonization (97.67%) was for individual inoculation with AMF. In contrast, for Rangpur lime 63.80% was recorded for Mix (mixture of the three rhizobacterial strains) with AMF. In the controls and treatments with individual rhizobacterial inoculation and Mix, no mycorrhizal colonization was found (Figure 2).

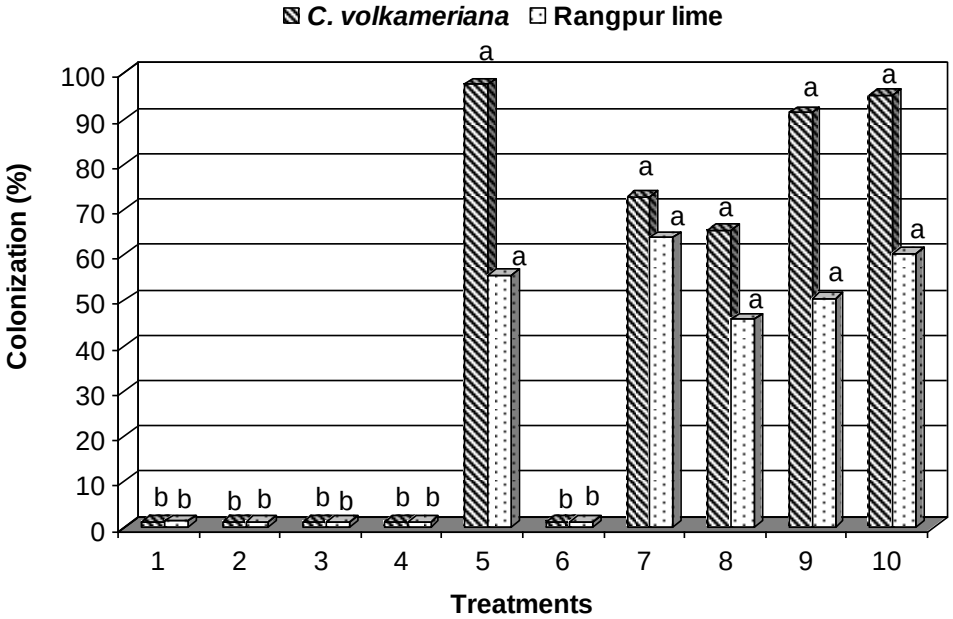

Figure 2. Rhizospheric colonization by the mycorrhizal consortium AMF in *Citrus volkameriana* and Rangpur lime: 1) Control, 2) FCA-8, 3) FCA-56, 4) FCA-60, 5) Consortium AMF, 6) Mix (mix of the three rhizobacterial strains), 7) Mix + AMF, 8) FCA-8 + AMF, 9) FCA-56 + AMF and 10) FCA-60 + AMF.
[a,b]Values with the same letter are not significantly different (Tukey, $P \leq 0.05$).

It is important to note the difference in mycorrhizal colonization percentage between the rootstocks *C. volkameriana* and Rangpur lime. This difference is related to the competition that rhizobacteria have for space and root exudates; however, coexistence of both microorganisms in the root systems of the plants was possible. Berg (2009) mentions that between communities of bacterial and AMF there is a positive synergism, where, from time to time, one of the two dominates the colonization of the rhizosphere due to its invasive capacity and effectivity for rapid establishment of the symbiotic relationship (Lambers *et al*., 2009; Rigamonte *et al*., 2010).

Citrus volkameriana showed greatest symbiotic capacity with the AMF. According to Tapia-Goné *et al*. (2010), this capacity depends on the infective ability and effectiveness of the fungus. The infective process refers to the capacity of the fungus to penetrate and invade the root system intensely and to explore the soil, as well as its ability to persist in the production system. The effectiveness of the fungus is demonstrated by improved development of the host indirectly or directly. The indirect benefit is protection against soil pathogens and stress, as well as increasing soil aggregation and stability, qualities that promote hyphal development. The direct benefit is the improved absorption of nutrients as phosphorous, zinc and copper. Besides the abilities of the fungus, environmental factors and the plant species play important roles in the efficient establishment of the symbiosis (Berg, 2009; Bever *et al*., 2009).

CONCLUSION

Individual and combined biofertilization with PGPR and the AMF positively stimulated the growth of the citrus graft hosts *C. volkameriana* and Rangpur lime, as well as the Tahiti lime graft in the nursery, providing results equal to 100% chemical fertilization. The *P. putida* strains and the mycorrhizal consortium applied in individual or combined form contributed to the increased nutrient content of Tahiti lime leaves, in particular strain FCA-56 and consortium AMF, for the contents of N and P assimilated by the plants. The *P. putida* strains showed good capacity at colonizing the rhizosphere of *C. volkameriana* and Rangpur lime individually and combined with consortium AMF. Better mycorrhizal symbiosis with *C. volkameriana* was shown individually and associated with strains FCA-56 and FCA-60.

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