Effects of biological control agents on arbuscular mycorrhiza fungi *Rhizophagus clarus* in soybean rhizosphere

Leopoldo Sussumu Matsumoto[1], Igor Matheus Oliveira dos Santos[2], André Riedi Barazetti[2], Glenda Cavalari Simões[2], Tiago Nunes Farias[2] and Galdino Andrade[2,*]

[1]1Universidade Estadual do Norte do Paraná – Faculdades Luiz Meneghel (UENP-CLM), Departamento de Patologia Geral, CP 261, CEP: 86360-000, Bandeirantes, Paraná, Brazil. [2]Universidade Estadual de Londrina, CCB, Departamento de Microbiologia, Laboratório de Ecologia Microbiana, CP 10011, CEP: 86057-970, Londrina, Paraná, Brazil. *Corresponding author, E-mail: gal.andrade7@gmail.com

ABSTRACT

Microbial activity in the rhizosphere is essential for nutrient cycling, which can contribute to soil fertility and plant growth. This work aimed to evaluate the effects of two biological control agents (*Trichoderma* sp. and *Beauveria* sp.) on the functional groups of microorganisms in the soybean (*Glycine max*) rhizosphere and plant growth. The experiment was carried out in a greenhouse, and five replicates with one plant per pot (1000 mL), containing a mixture of soil: sand (4:1), were harvested and microbial communities evaluated at 7, 21, 45 and 60 days after soybean germination. The populations of heterotrophic bacteria (HBP), saprophytic fungi (SFP), fluorescent pseudomonads (PFP) and the functional groups of microorganisms related to carbon cycling [proteolytics (PP), amylolytics (AP) and cellulolytics (CP)], nitrogen cycling [dry weight of nodules (DWM)], and phosphorus cycling [AM fungi colonization (AM)] were estimated. A soil sample (1 g) was taken from the homogenized rhizosphere soil to estimate the culturable microbial community size. Samples were suspended in 9 mL of sterile saline (0.85%) and aliquots (50 μL) of ten-fold dilutions spread on the respective culture medium. Plates were incubated at 28 °C and CFU were counted. The results showed that biological controls agents such as *Trichoderma* sp. and *Beauveria* sp. presented different effects on microbial community and *Rhizophagus clarus* colonization. *Trichoderma* sp. had positive influence on plant growth and soil microbial community, except for AM fungi. However, *Beauveria* sp. showed no significant differences in all evaluations, including plant growth.

Key words: Rhizosphere, microbial communities, *Trichoderma* sp., *Beauveria* sp., biocontrol.

INTRODUCTION

Rhizosphere results from biological activity and physicochemical processes, which are influenced by plant roots. They supply exudates for microbial activity, producing some compounds that influence plant growth (Cely et al., 2016a). Accordingly, microorganism phenotypes could be grouped based on their functionality for each biogeochemical cycle (Andrade 2004). Microbial activity is essential for nutrient cycling (Kirk et al., 2004), and contributes to it in many aspects such as plant nutrition (Andrade 2004), plant health (Jung et al., 2012) and soil fertility (Cely et al., 2016b).

The dynamics of microbial interactions is regulated by synergistic and antagonistic processes that occur in many environments such as rhizoplane and rhizosphere (Andrade 2004). This process that regulates the interaction between microorganisms is involved in the success of microbial inoculants as well as biofertilization or biocontrol of root pathogens (Watts-Williams and Cavagnaro 2015). Nowadays, *Trichoderma* sp. and *Beauveria* sp. are important biological control agents. *Trichoderma* sp. produces antifungal compounds and hydrolytic enzymes (Mbarga et al., 2014) against *Rhizoctonia, Sclerotium, Phythium, Phytophthora* and *Fusarium. Beauveria* sp. are entomopathogenic fungi largely used in the biocontrol of many insect orders that attack cultures such as sugar cane, sunflower, banana, canola, among others.

Over the last years, the interest in biological control is increasing each year. This is supposedly due to the fact that biocontrol agents are not hazardous either for human and animal health, or for the environment (Bérdy 2005). Pesticides can cause negative impacts on the environment such as water contamination, soil microbial community, which can disturb nutrient cycles and probably plant health and growth (Aktar et al., 2009). However, some questions need to be answered. First of all, what is the cell density of the biocontrol agent found in natural environment where this microorganism lives? Second, what is the soil resilience capacity to receive millions of cells of one species many times a year? We have to think that living cells need nutrients to grow and multiply, and they certainly will compete with microbial communities, causing nutrient immobilization. Can this competition cause soil disturbance among functional groups of microorganisms?

The purpose of this paper is to evaluate the impact of two fungi used as biocontrol agents, *Trichoderma* sp. and *Beauveria* sp. on the functional groups of microorganisms in the soybean rhizosphere.

MATERIAL AND METHODS

Experimental Design

The experimental design (2 biocontrol agent x 4 harvest x 5 replicates), and respective non-inoculated control (n= 50), was arranged in a completely random design. Plant harvest and microbial community evaluations were carried out 7, 21, 45 and 60 days after soybean germination.

Results were evaluated by analysis of variance (ANOVA), the t test analysis were performed at $p \leq 0.05$ level of probability.

Biocontrol Agents

Trichoderma sp. (1.0 g kg^{-1} of commercial product Triconat PM®, 107 spore mL^{-1}) and *Beauveria* sp. (107 spore mL^{-1}) from our own collection were used.

Soil

The experiment was carried out in a greenhouse (28 ˚C/25 ˚C day/night, 80% humidity), and five replicates with one plant per pot. The oxisol soil (> 80% of clay) was used and contained: organic matter (g kg^{-1}) 21.5; pH (CaCl$_2$) 5.7; P (Mehlich) 39.3 mg dm^{-3}; K 0.52 cmol dm^{-3}; Ca 8.2 cmol dm-3; Mg 1.9 cmol dm^{-3}. The soil was mixed and homogenized with sand (4:1). The potting mixture was sterilized with steam flow (60 min day^{-1}), for three consecutive days, and distributed into 1000 mL pots.

Spore suspensions of the biocontrol agents (10 mL), *Trichoderma* sp. (107 spore mL^{-1}) or *Beauveria* sp. (107 spore mL^{-1}), were mixed in the soil before potting. Each pot received 10 mL of soil extract (500 g soil in 500 mL sterile water, filtered with filter paper No. 1), to recompose the natural microbial population of the soil, except for AM fungi.

AM fungi inoculum consisted of soil containing fungal spores and hyphae, as well as colonized root fragments obtained from pots with *Brachiaria decumbens* inoculated with R. *clarus.* Each pot received 2 g of AM fungi inoculum, one day before soybean was sowed.

Plant

Seeds of the soybean var. COODETEC 202 (early maturation, cycle of 118 days, acid soil tolerant) were surface-sterilized with hypochlorite solution 1% for 1 min. and washed three times with sterile distilled water. Three seeds were sowed in each pot, and three days after germination two seedlings were cut. After that, 10 mL of *Bradyrhizobium japonicum* CPAC 390 (109 mL^{-1}) was inoculated on the soil surface around the seedling.

Plants were harvested by cutting shoot on soil surface, and the shoot dry weight was recorded after being kept in oven at 70 ˚C for three days. Roots were washed, dried with soft paper and weighed. Dry shoots were grounded and the amount of Zn, Ca, Mg, Cu, Fe and Mn was estimated by multi-elementary analysis using mass emission spectrometry. The K was estimated by flame spectrometry and P by atomic emission spectrometry. The amount of nutrients accumulated was estimated by ratios between nutrient concentration and shoot dry weight.

Functional groups of microorganisms

The natural microbial population of the soil formed by heterotrophic bacteria (HBP), saprophyte fungi (SFP), fluorescent pseudomonas (PFP) and the functional groups of microorganisms related to carbon [proteolytics (PP), amylolytics (AP) and cellulolytics (CP)], nitrogen [dry weight of nodules (DWM)] and phosphorus [AM fungi colonization (AM)] biogeochemical cycles were estimated. A soil sample (1 g) was taken from the homogenized rhizosphere soil of each experimental unit to estimate the culturable microbial community size (Zuberer 1994). The samples were suspended in 9 mL of sterile 0.85% NaCl solution and maintained at 5 ˚C. Aliquots (50 µL) of ten-fold dilutions [10^{-6} (PP, AP, CP, HBP); 10^{-5} (SFP); 10^{-4} (PFP)] were spread on duplicate plates containing the respective culture medium (Chart 1).

Plates were incubated at 28 ˚C and CFU were counted after 3 days and 5 days, to allow for the development of slow-growing colonies. The CFU number was expressed per gram of dry soil. A colony was considered to have cellulolytic, amylolytic, and proteolytic activity if a degradation halo was present. The percentage of plants roots infected with AM fungi was estimated from stained samples (Philips and Hayman 1970) by the grid-line intersect method (Giovanetti and Mosse 1980) through microscopic examination. The total number and dry weight (60 ˚C 48 h^{-1}) of nodules of B. japonicum were also evaluated.

Chart 1. Culture media for enumerating functional group microorganism populations from C cycle (cellulolytic, amylolytic and proteolytic media) and culturable bacteria and fungi.

Cellulolytic (Wood, 1980)	5.0 g carboxymethyl cellulose, 1.0 g NO_3NH_4, 50 mL NaCl solution (0.85%), 950 mL soil extract (v:v), 15.0 g agar, pH = 7.0. Halo develop: Add 1M NaCl solution to the medium surface for 5 minutes; eliminate; add a 0.1% Red Congo solution for 30 minutes; wash with distilled water until halos around colonies are observed; count halo forming colonies.
Amylolytic (Pontecorvo et al., 1953)	10.0 g soluble starch, 10.0 g casein, 1.0 g glucose, 3.0 g Na2HPO4, 0.1 g $MgSO_4.7H_2O$, 15 g agar. 1000 mL distilled water. To observe the starch degradation halo, add an iodine solution to the surface of the medium on top of the formed colonies, remove the excess and count halo forming colonies.
Proteolytic (Wood, 1980 modified by Andrade in our Laboratory),	10.0 g Casein, 0.1 g yeast extract, 1.5 gKH2PO4, 0.5 g MgSO4.7H2O, 50 mL NaCl solution (0.85%), 15 g agar, 950 mL soil extract (v: v), pH= 6.8. Halo developed: Add 0.1N HCl solution for 2 min; eliminate; count halo forming colonies.
P1 media for fluorescent pseudomonads (Katoh and Itoh, 1983)	1.0 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.2 g KCl, 5.0 g $NaNO_3$, 1.0 g de sodium desoxicholate, 5.0 g betaine, 15 g agar, 1000 mL distilled water, pH 7.2.
Trypitic-soy agar MERCK® for culturable bacteria	40.0 g TSA per 1000 mL distilled water
Potato dextrose agar MERCK® for culturable fungi	39.0 g per 1000 mL distilled water

RESULTS AND DISCUSSION

Differences in plant growth were observed after 45 days of germination. Plants treated with Beauveria sp. inoculated with R. clarus had low growth when compared with other treatments.

After 60 days, plants treated with Trichoderma sp. inoculated or not with R. clarus, were bigger than other treatments, including the control (Figure 1A). No significant differences were observed after 45 days on root dry weight, but within 60 days, root growth increased on plants treated with Trichoderma sp. and Beauveria sp. when compared with control plant (Figure 1B).

Seeds of many plant species treated with Trichoderma sp. were protected against pathogens and increased plant growth. The stimulatory effects should be attributed to production of low weight compounds such as phytohormones, which are organic acids involved in nutrient solubilization increasing absorption of nutrients by plant (Li et al., 2015; Chagas et al., 2015).

The stimulatory effect observed on plant growth on soil treated with Trichoderma sp., was probably influenced by interaction between B. japonicum and Trichoderma sp. that provided more nutrients to the roots and protected them against pathogenic fungi (Rudresh et al., 2005).

Nodules dry weight decreased in plants treated with Trichoderma sp. plus R. clarus when compared with other treatments after 45 days. Nodules weight increased after 60 days in plants treated with Trichoderma sp. in presence or absence of AM fungi or treated with Beauveria sp. when compared with control samples (Figure 2A).

The presence of saprophytic fungi should influence microbial population including B. japonicum in the early stages of nodule formation on the rhizosphere. This results from the fact that they are involved in complex interactions such as antibiosis (Cook and Baker 1983), fungistasis (Pavlica et al., 1978) and mycoparasitism (Elad 1986).

The AM colonization was highly inhibited by Trichoderma sp. during whole the experiment. After 25 days, R. clarus was stimulated by Beauveria sp,, although in 45 days no differences were observed in plants inoculated only
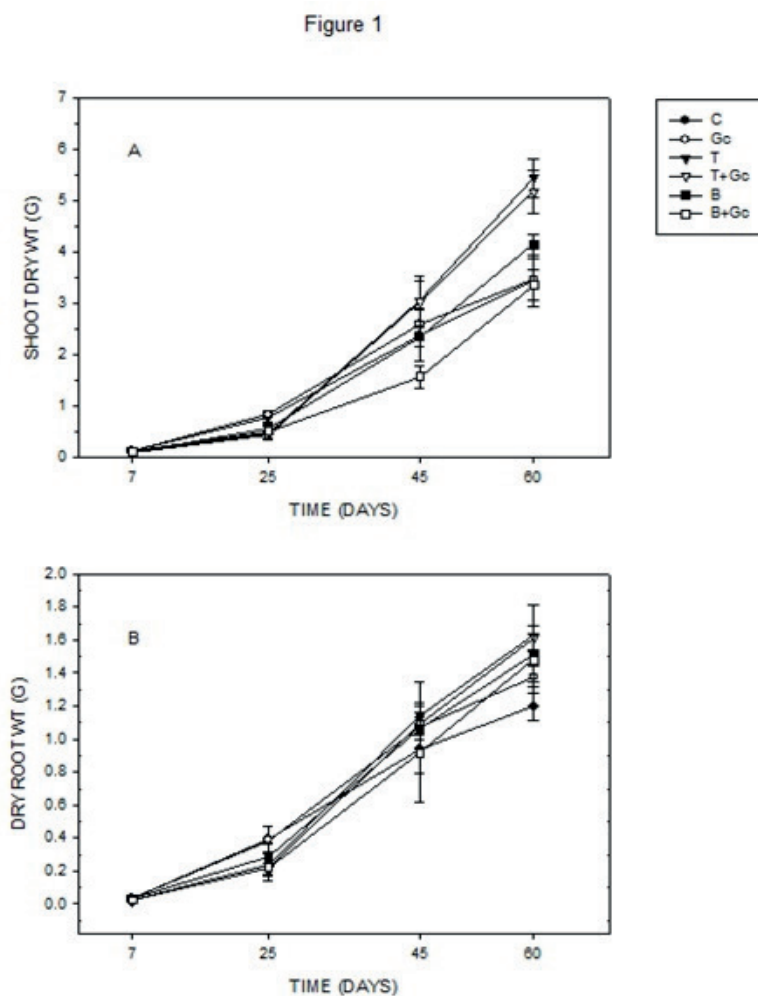
Figure 1

Figure 1. Effect of two biological control agents *Trichoderma* sp and *Beauveria* sp on plant growth (A) Shoot dry weigh; and (B) Dry root weight. The bar corresponds to standard error for each treatment in each time (p ≤0.05). The letters correspond to Control (C); *Glomus clarum* (Gc); *Trichoderma* sp (T); *Trichoderma + R. clarus* (T + Gc); *Beauveria* (B); *Beauveria + R. clarus* (B + Gc).

with *R. clarus* and *Beauveria* sp. plus *R. clarus*. However, after 60 days, colonization increased in plants treated with Beauveria sp. and *Trichoderma* sp. (Figure 2B).

In spite of the *Trichoderma* sp. increased plant growth, AM colonization was highly inhibited by *Trichoderma* sp. although stimulated by *Beauveria* sp. up to 25 days. The genus has different characteristics. First of all, *Trichoderma* sp. is myco-parasite and Baeauveria sp. is insect-parasite; the differences should influence the effect on AM colonization. In in vitro conditions where the influence of different *Trichoderma* sp. strains on AM fungi germination was compared. It was observed that T. *pseudokoningii* showed direct effect on spore germination, which suggested a direct interaction between AM fungi and Trichoderma sp. in the pre-symbiotic phase (Martinez et al., 2004). The same authors showed that AM colonization level in soybean depends on the Trichoderma sp. strain that was present. Some *Trichoderma* sp., which inhibits spore germination, could increase root colonization and plant growth. This means that the effect on AM spore germination is not correlated with low root colonization (Martinez et al., 2004). *Trichoderma* sp is not selective to parasite pathogenic fungi, and the effect on plant nutrition especially P supply decreased (Brimner and Boland 2003; Green et al., 1999). However, biocontrol agents may promote plant growth on the rhizosphere microcosm (Li et al., 2015; Chagas et al., 2015).

Bacteria population was stimulated by *Trichoderma* sp. after 60 days (Figure 3A). Fungi and proteolytic populations were also stimulated, although in 25 and 60 days (Figure 3B and 3C). Cellulolytic population was stimulated only by *Trichoderma* sp. after 60 days on plant growth (Figure 4A). *Trichoderma* sp. showed the same effect on amylolytic population that was observed on proteolytic (Figure 4B). Fluorescent pseudomonad was the only population stimulated by Trichoderma sp. at 45 days. The stimulatory effect was still observed in 60 days but in *Trichoderma* sp. minus AM (Figure 4C).

The stimulatory effect observed in the functional groups of microorganisms on the rhizosphere should be influenced by root exudates and lysate from AM fungi mycelia after feed by *Trichoderma* sp. Naseby et al. (2000) observed in cucumber roots that *Trichoderma* sp. population increase in terms of root length and bacteria population
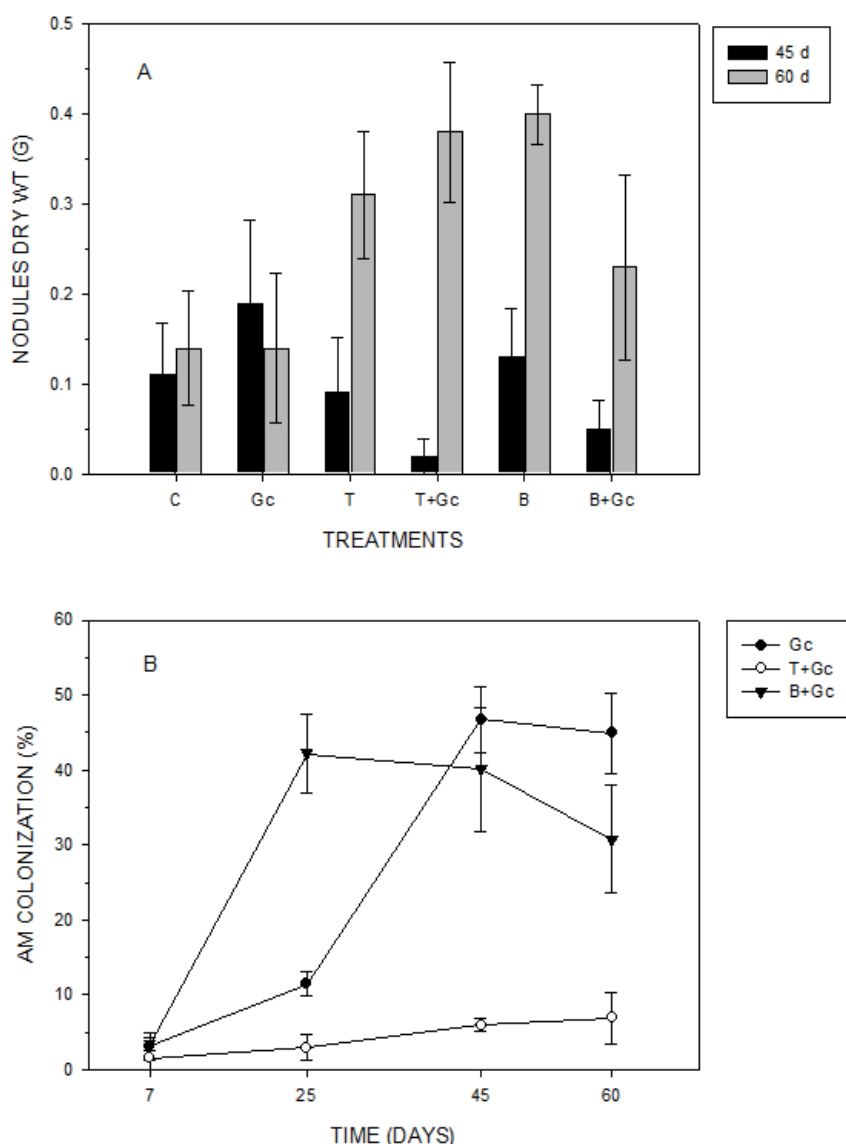
Figure 2

Figure 2. Effect of two biological control agents *Trichoderma* sp and *Beauveria* sp on (A) Nodules dry weight; (B) AM fungi colonization. The bar corresponds to standard error for each treatment in each time (p ≤ 0.05). The letters correspond to Control (C); *Glomus clarum* (Gc); *Trichoderma* sp (T); *Trichoderma + R. clarus* (T + Gc); *Beauveria* (B); *Beauveria + R. clarus* (B + Gc).

increase in terms of root length and bacteria population in presence of *Pythium*.

The interaction of plant-microbes in the rhizosphere microcosm is dynamic and mediated by low weight compounds produced by plant and microbial community (Andrade 2004; Matsumoto et al., 2005). Also, different volatile compounds are involved in the microbial community composition and its capacity to metabolize different carbon sources available in the rhizosphere (Marschner and Timonen 2005). A decrease of fluorescent pseudomonads should be associated with the change of root exudates and nutrients supply in the rhizosphere (Trabelsi and Mhamdi 2013).

The chemical shoot analysis was carried out after 45 and 60 days of plant growth. The manganese concentration in plants treated with Trichoderma sp. was four times bigger than other treatments, and the other nutrients analysed showed no differences (Table 1).

A high concentration of Mn found in plant tissue inoculated with *Trichoderma* sp. should be related with low level of AM colonization, as a result of some circumstances in which it may protect the plant against high uptake of Mn present in soil, decreasing Mn level in shoots (Nogueira et al., 2007).

Biological control agents such as *Trichoderma* sp. and *Beauveria* sp. showed different effects on microbial community and R. clarus colonization. The high levels of Mn did not affect plant health and plant growth when *Trichoderma* sp. decreases AM colonization. The fact that *Trichoderma* sp. decreased AM fungi is very important, because it is
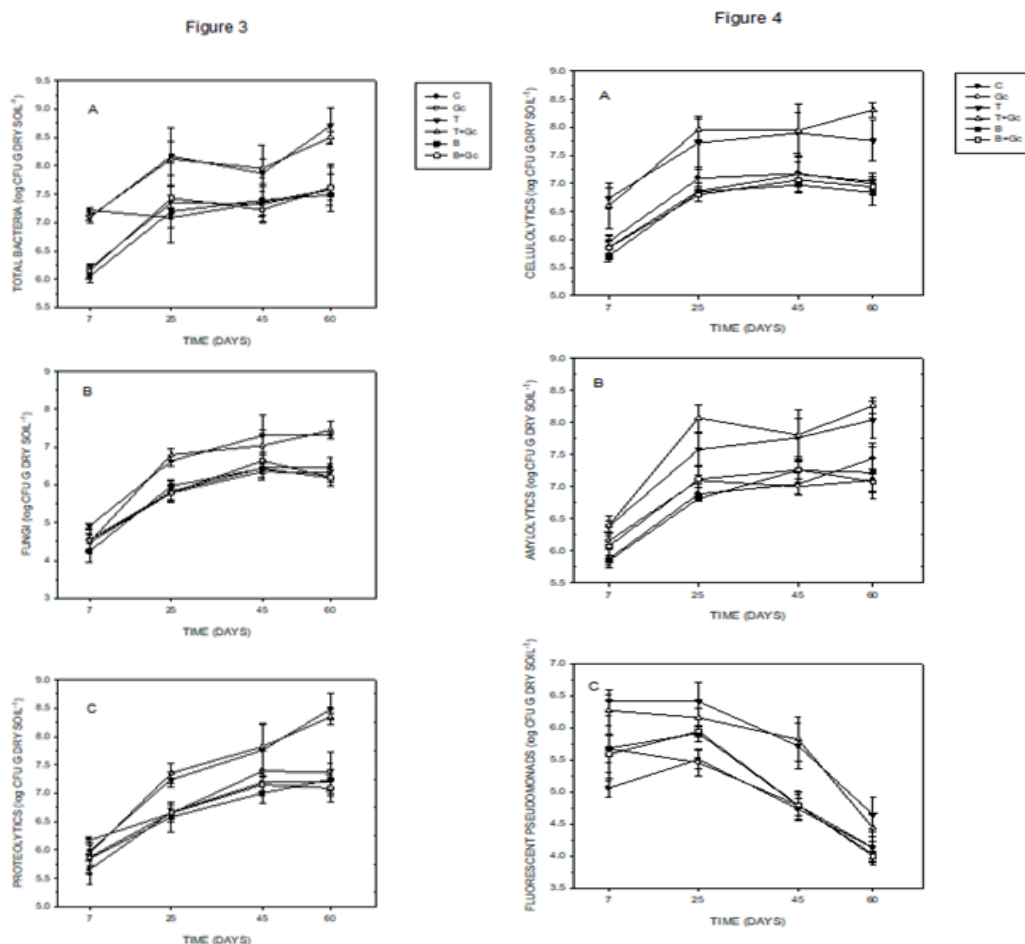
Figure 3. Effect of two biological control agents *Trichoderma* sp and *Beauveria* sp on populations of (A) Culturable bacteria; (B) Culturable fungi; (C) Proteolytic. The bar corresponds to standard error for each treatment in each time (p≤0.05). The letters correspond to Control (C); *Glomus clarum* (Gc); *Trichoderma* sp (T); *Trichoderma + R. clarus* (T + Gc); *Beauveria* (B); *Beauveria + R. clarus* (B + Gc).

Figure 4. Effect of two biological control agents *Trichoderma* sp and *Beauveria* sp on populations of (A) Cellulolytic; (B) Amylolytic; (C) Fluorescent pseudomonads. The bar corresponds to standard error for each treatment in each time (p≤0.05). The letters correspond to Control (C); *Glomus clarum* (Gc); *Trichoderma* sp (T); *Trichoderma + R. clarus* (T + Gc); *Beauveria* (B); *Beauveria + R. clarus* (B + Gc).

Table 1. The nutrient concentration of chemical shoot analysis (N, P, K, Mg, Cu, Fe, Mn and Zn) after 45 and 60 days of soybean inoculated with *R. clarus*.

Treatments	Day	N	P	K	Mg	Cu	Fe	Mn	Zn
		kg^{-1} dry shoot				mg kg^{-1} dry shoot			
Control	45	17.50	3.15	13.98	5.94	14.76	1144.80	194.22	120.06
	60	14.00	3.27	6.99	4.66	7.80	354.00	229.80	102.72
Trichoderma	45	22.40	2.45	13.98	5.64	10.80	529.80	880.56	114.48
	60	16.10	1.81	6.99	5.09	6.90	382.80	888.06	156.54
Beauveria	45	18.20	3.14	12.24	5.38	8.64	288.00	175.14	87.00
	60	14.70	3.00	5.24	5.13	6.60	350.40	191.46	96.30

widely used in organic farms, and it is crucial to know how they interact with the functional groups of microorganisms in soil microcosms.

ACKNOWLEDGMENTS

REFERENCES

Aktar MDW, Sengupta D and Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary Toxicology 2: 1-12.

Andrade G (2004) Role of functional groups of microorganisms on the rhizosphere microcosm dynamics. In: Varma A, Abbott L, Werner D and Hampp R (eds.) Plant Surface Microbiology. Springer-Verlag, Berlin, p. 51-69.

Bérdy J (2005) Bioactive microbial metabolites: a personal view. The Journal of Antibiotics 58: 1-26.

Brimner TA and Boland GJ (2003) A review of the non-target effects of fungi used to biologically control plant diseases. Agriculture, Ecosystems and Environment 100: 3-16.

Cely MVT, de Oliveira AG, Freitas VF, Luca MB, Barazetti AR, Santos IMO, Gionco B, Garcia GV, Prete CEC and Andrade G (2016a) Inoculant of arbuscular mycorrhizal fungi (Rhizophagus clarus) increase yield of soybean and cotton under field conditions. Frontiers in Microbiology 7: 720.

Cely MVT, Siviero MA, Emiliano J, Spago FR, Freitas VF, Barazetti AR, Goya ET, Lamberti GS, dos Santos IMO, de Oliveira AG and Andrade G (2016b) Inoculant of Schizolobium parahyba with mycorrhizal fungi and plant growth-promoting rizobacteria increases wood yield under field conditions. Frontiers in Plant Science 7: 1708.

Chagas LFB, Chagas Junior AF, Carvalho MR, Miller LO and Colonia BSO (2015) Evaluation of the phosphate solubilization potential of Trichoderma strains (Trichoplus JCO) and effects on rice biomass. Journal of Science and Plant nutrition 15: 794-804.

Cook RJ and Baker KF (1983) The nature and practice of biological control of plants pathogens. American Phytopathological Society, Saint Paul, 539p.

Elad Y (1986) Mechanisms of interaction between rhizosphere microorganisms and soilborne plant pathogens. In: Jensen V, Kjoller A and Sorensen LH (eds.) Microbial communities in soil. Elsevier, London, pp. 49-61.

Giovanetti M and Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular micorrhizal infections in roots. New Phytologist 84: 489-500.

Green H, Larse J, Olsson PA, Jensen DF and Jakobsen I (1999) Suppression of the biocontrol agent Trichoderma harzianum by mycelium of arbuscular mycorrhizal fungus Glomus intraradices in root-free soil. Applied and Environmental Microbiology 65: 428-1434.

Jung SC, Martinez-Medina A, Lopez-Raez JA and Pozo MG (2012) Mycorrhiza-induced resistance and priming of plant defenses. Journal of Chemical Ecology 38: 651-664.

Kirk JL, Beaudette LA, Hart M, Moutoglis P, Klironomos JN, Lee H and Trevors JT (2004) Methods of studying soil microbial diversity. Journal of Microbiology Methods 58: 169-188.

Li R-X, Cai F, Pang G, Sehn Q-R, Li R and Chen W (2015) Solubilisation of phosphate and micronutrientes by Trichoderma harzianum and its relationship with the promotion of tomato plant growth. PloS ONE 10: e0130081.

Marschner P and Timonen S (2005) Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere. Applied Soil Ecology 28: 23-36.

Martinez A, Obertello M, Pardo A, Ocampo JA and Godeas A (2004) Interactions between Trichoderma pseudokoningii strains and the arbuscular mycorrhizal fungi Glomus mosseae and Gigaspora rosea. Mycorrhiza 14: 79-84.

Matsumoto LS, Martines AM, Avanzi MA, Albino UB, Brasil CB, Saridakis DP, Rampazo LGL, Zangaro W and Andrade G (2005) Interaction among functional groups in the cycling of carbon, nitrogen and phosphorus in the rhizosphere of three successional species of tropical woody trees. Applied Soil Ecology 28: 57-65.

Mbarga JB, Begoude BAD, Ambang Z, Meboma M, Kuate J, Schiffers B, Ewbank W, Dedieu L and ten Hoopen GM (2014) A new oil-based formulation of Trichoderma asperellum for the biological control of cacao black pod disease caused by Phytophthora megakarya, Biological Control 77: 15-22.

Naseby DC, Pascual JA and Lynch JM (2000) Effect of biocontrol strains of Trichoderma on plant growth, Pythium ultimum populations, soil microbial communities and soil enzyme activities. Journal of Applied Microbiology 88: 161-169.

Nogueira MA, Nehls U, Hampp R, Poralla K and Cardoso EJBN (2007) Mycorrhiza and soil bacteria influence extractable iron and manganese in soil and uptake by soybean. Plant and Soil 298: 273–284.

Pavlica DA, Hora TS, Bradshaw JJ, Skogerboe RK and Baker R (1978) Volatiles from soil influencing activities of soil fungi. Phytopathology 68: 758-765.

Philips DA and Hayman DS 1970 Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55: 158-161.

Rudresh DL, Shivaprakash MK and Prasad RD (2005) Effect of combined application of Rhizobium, phosphate solubilizing bacterium and Trichoderma spp. on growth, nutrient uptake and yield of chickpea (Cicer aritenium L.). Applied Soil Ecology 28: 139-146.

Trabelsi D and Mhamdi R (2013) Microbial inoculants and their impact on soil microbial communities: a review. BioMed Research International 2013: 863240.

Watts-Williams SJ and Cavagnaro TR (2015) Using mycorrhiza-defective mutant genotypes of non-legume plant species to study the formation and functioning of arbuscular mycorrhiza: a review. Mycorrhiza 25: 587-597.

Zuberer DA (1994) Recovery and enumeration of viable bacteria. In: Weaver RW, Augle JS and Bottomley PS (eds) Methods of Soil Analysis, part 2. Microbiological and Biochemical Properties. Books series 5. Soil Science Society of America, Madison, WI, p. 120-157.