COMBINED EFFECT OF *Pseudomonas* sp. AND *Trichoderma aureoviride* ON LETTUCE GROWTH PROMOTION

EFEITO COMBINADO DE Pseudomonas sp. E Trichoderma aureoviride NA PROMOÇÃO DO CRESCIMENTO DE ALFACE

Argemiro Pereira MARTINS FILHO[1]; Erika V. de MEDEIROS[2]; Jesimiel Gomes BARBOSA[1]; Jorge Marcos Peniche BARBOSA[1]; Júlia KUKLINSKY-SOBRAL[2]; Cristina SOUZA-MOTTA[3]
1. Msc., Federal Rural University of Pernambuco- UFRPE, Unity Academic of Garanhuns, Garanhuns, State of Pernambuco, Brazil;. argemiro.ufra@gmail.com; 2. Ph.D Professor, Federal Rural University of Pernambuco- UFRPE, Unity Academic of Garanhuns, Garanhuns, State of Pernambuco, Brazil; 3. Ph.D Professor, Federal University of Pernambuco- UFPE, Micology Department, Recife, State of Pernambuco, Brazil.

ABSTRACT: Plant growth promotion by microorganisms may be a viable alternative to increase lettuce production through pathogens control and nutrients absorption increase. *Trichoderma* and *Pseudomonas* genus are examples of widely studied microorganisms with the capacity to promote plant growth. However, there are still gaps regarding the action of the combined effect of these two microorganisms. Therefore, the objective of this study was to evaluate the combined effect of *Pseudomonas* sp. UAGF14 and *Trichoderma aureoviride* URM5158 on the development of lettuce plants. The experimental design was completely randomized, with five treatments: CONT (control), CM (soil with organic fertilization), CMB (soil with organic fertilization and *Pseudomonas* sp.), CMF (soil with organic fertilization and *T. aureoviride*), and CMFB (soil with organic fertilization, *Pseudomonas* sp. and *T. aureoviride*), with ten repetitions. At 30, 40 and 60 days after sowing, the following parameters were analyzed: plant and canopy height and number of leaves. At 60 days after emergence, shoot dry matter, leaf area, root dry matter, root length and chlorophyll were analyzed. Catalase, peroxidase and polyphenol oxidase enzymatic activity were determined. The CMFB treatment had the highest means of lettuce growth promotion, confirming the synergistic effect of the combination of the two microorganism types, as it increased height, canopy, shoot and root dry matter, and chlorophyll levels compared to CONT, although did not differ from CM in some variables. Enzymatic activity was also influenced by the action of these microorganisms combined, evidencing by polyphenol oxidase increase. The CMFB or CM were efficient in promoting lettuce growth, showing positive response to the plant morphological and physiological characteristics. However, few responses were observed in lettuce plant growth in the first cycle evaluated after 60 days, compared CM and CMFB treatments, but both treatments showed superiority in lettuce plant growth submitted to CONT treatment. Therefore, further studies are needed to estimate the long-term effects of combined effect of *Pseudomonas* sp. UAGF14 and *T. aureoviride* URM5158 on crop productivity in field conditions.

KEYWORDS: *Lactuca sativa*. Plant growth promoters. Chlorophylla. Catalase. Polyphenol oxidase.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a widely grown crop and a vegetable popularly consumed throughout the world. Lettuce is harvested all over the world and makes up an important part of vegetables in the world's population diet, as it is a rich source of vitamins, minerals and fiber. It is the most popular vegetable among those whose leaves are eaten raw and still fresh (ZÁRATE et al., 2010). Lettuce crops are of great importance in the national and world scene.

The search for new technologies that are effective, low-cost and sustainable for lettuce management is growing (ZAIDI et al., 2015). In this regard, the use of plant growth promoters can be highly variable due to several limiting factors, including culture type, growth conditions and inoculum rate. Several mechanisms have been proposed to explain growth promotion, including pathogen control and increased nutrients absorption (STEWART; HILL, 2014).

Trichoderma is an example of a microorganism widely studied regarding its ability to promote plant growth. It has the capacity to parasitize pathogenic fungi through chitinase production (SILVA et al., 2016). In addition, it has the capacity to enhance plant defense against invading pathogens, even at locations that are far from the application point, being widely used in agriculture as commercial biofungicides. There is direct and indirect evidence of the involvement of

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Trichoderma antagonistic secondary metabolites against a considerable number of pathogenic bacteria, yeasts and fungi, as well as evidence of its beneficial effects on cultures. In addition, various *Trichoderma* strains are known to promote plant growth and abiotic stress tolerance (ZEILINGER et al., 2016).

Trichoderma influence on plants goes beyond pathogens biocontrol and abiotic stresses amelioration, as it may also be associated with culture growth and biomass production stimulation. Some *Trichoderma* are able to provide nutrients and phytohormones, such as indole-acetic acid (IAA), which influences plant growth. Although *Trichoderma* fungus is more likely to stimulate growth by influencing the balance of hormones, such as IAA, gibberellic acid and ethylene, it also interferes in carbohydrate metabolism and photosynthesis (NAWROCKA, 2013; STEWART; HILL, 2014) helping plants tolerate better environmental stresses, such as salinity and drought. The fungal mycelium secretes different compounds that increase the root system branching capacity, improving nutrients and water absorption (LÓPEZ-BUCIO et al., 2015). Cerinolactone is a new metabolite has been isolated from *Trichoderma* and *Cerinum* cultures that activates plant defense mechanisms and regulates plant growth, suggesting that plant defense and development share common components in *Trichoderma* (HERMOSA et al., 2014).

On the other hand, plant growth-promoting bacteria, such as the *Pseudomonas* genus, improve horticultural crops biological and chemical characteristics (AHMAD et al., 2013), directly influencing on nutrient uptake and phytohormone levels increase, besides indirectly influencing on pathogens biocontrol and abiotic stresses tolerance (GLICK, 2012). Recent studies with *Pseudomonas putida* KT2440 demonstrated potential bioremediation of soils contaminated with organophosphates and pyrethroids (ZUO et al., 2015). *Pseudomonas* sp. is effective to decrease the disease in yellow chrysanthemums, although it does not affect viable phytoplasma presence in the new development of infected leaves (GAMALERO et al., 2010). In addition to antifungal activity, some *Pseudomonas* strains have the potential to synthesize indole-acetic acid and to solubilize phosphate (CORDERO et al., 2012).

Many studies showed *Trichoderma* spp. benefits related to plant diseases biological control (SILVA et al., 2016) and *Pseudomonas* benefits related to plant growth promotion. However, the combined effect of both microorganisms in the induction of systemic resistance in cultivated plants is still incipient. Alizadeh et al. (2013) studied the combination of *Trichoderma harzianum* Tr6 and *Pseudomonas* sp. PS14 as systemic resistance inducers in cucumber plants and *Arabidopsis thaliana*.

However, there is still a gap regarding the combined effect of these two microorganism types on lettuce crops growth and physiological responses. Therefore, the objective of this study was to evaluate the combined effect of *Pseudomonas* sp. UAGF14 and *Trichoderma aureoviride* URM5158 on lettuce growth.

MATERIAL AND METHODS

Pseudomonas and *Trichoderma* isolates obtainment and selection

Pseudomonas sp. UAGF14 was used with the following plant growth promotion characteristics: biological nitrogen fixation, phosphate solubilization, indole-acetic acid synthesis and exopolysaccharide production (SANTOS et al., 2016).

Trichoderma aureoviride URM 5158 was used in this study because, in a previous study, it was determined as the best chitinase producer and the best enzymatic activity producer for the ROS group in cassava plants (SILVA et al., 2016). This isolate was obtained at the URM culture collection (https://www.ufpe.br/micoteca/) and grown in potato dextrose Agar (PDA) medium, reactivated through three successive subcultures.

Evaluation of lettuce plant growh with *Pseudomonas sp.* and *Trichoderma aureoviride*

The bacteria *Pseudomonas* sp. UAGF 14 was inoculated into tryptone soya agar solid 10 % using the depletion technique, in order to obtain isolated colonies. From these colonies, 10 ml of liquid tryptone soy was picked and left under constant stirring for 24 hours. Then, 100 ml of the same medium was picked and left under constant agitation for 24 hours. Afterwards, the bacterial culture was diluted in Phosphate-buffered Saline (8 g L^{-1} NaCl, 0.2 g L^{-1} KCl; 1.44 g L^{-1} Na_2HPO_4; 0.24 g L^{-1} KH_2PO_4, pH 7.4), in order to reach the optical density (OD 600nm) of 0.095.

Trichoderma aureoviride URM 5158 was cultivated in 250-mL Erlenmeyer flasks containing 50 mL of PDA liquid. These plates were incubated at 26 °C ± 2 °C for 8 days.

The experiment was conducted in Regolithic Neosols collected in the native forest of a semiarid region in Pernambuco State, Brazil. This soil was considered sandy as physical and chemical attributes were shown.

The soil was dispensed into plastic pots with 4-L capacity. Pots received two previously disinfected (washed in a solution containing 5 ml of sodium hypochlorite diluted with 500 ml of autoclaved distilled water and washed in water) lettuce seeds (veronica cultivar) and daily irrigation.

Treatments that received the bacterium *Pseudomonas* sp. UAGF14: CMB (soil with organic fertilization and *Pseudomonas* sp. UAGF14) and CMFB (soil with organic fertilization, *Pseudomonas* sp. UAGF14 and *T. aureoviride* URM 5158) were inoculated with the bacteria by immersion in the inoculum for 30 minutes, and the material was agitated every 10 minutes. Treatments without bacteria - CONT (without manure, *Pseudomonas* sp. UAGF14 or *T. aureoviride* URM 5158), CM (organic fertilization) and CMF (soil with organic fertilization and *T. aureoviride* URM 5158) – were also submitted to the same procedure, being immersed in PBS buffer.

Inoculation with *T. aureoviride* URM 5158 was added to the soil. *Trichoderma* was selected according to the method by Abo-elyousr et al. (2014). A 100 ml dose of *Trichoderma* (1×10^6 conidia ml^{-1}) was added to the soil in CMF (soil with organic fertilization and *T. aureoviride* URM 5158) and CMFB treatments (soil with organic fertilization, *Pseudomonas* sp. UAGF14 and *T. aureoviride* URM 5158).

Experimental design and evaluated variables

The experimental design was completely randomized, with five treatments: CONT (control without organic fertilization, *Pseudomonas* sp. UAGF14 or *T. aureoviride* URM 5158), CM (organic fertilization), CMB (soil with organic fertilization and *Pseudomonas* sp. UAGF14), CMF (soil with organic fertilization and *T. aureoviride* URM 5158) and CMFB (soil with organic fertilization, *Pseudomonas* sp. UAGF14 and *T. aureoviride* URM 5158), with ten repetitions. The organic fertilization was made upon the recommendation of soil fertility analysis, with cattle manure.

At 60 days after emergence (DAE), the following variables were analyzed in lettuce plants: plant height; number of leaves; plant canopy; dry shoot matter; leaf area, through LI-3000 area meter; root length; root dry matter; and chlorophyll, through SPAD index model values (ClorofiLOG, *Falker Automação Agrícola*, Brazil). The SPAD value was obtained from three leaves.

Antioxidant enzymes extraction and estimation in lettuce plants was made with five leaves from each plant, which were homogenized at 4 °C to set the enzyme activities. The sample aforementioned was macerated in liquid N_2 and 4 mL of 50 mM potassium phosphate buffer (pH 7.0), in order to avoid phenol oxidative effects, and 0.05 g polyvinylpyrrolidone (PVP) was added to it. Concentrates were centrifuged in a refrigerated centrifuge (4 °C) at 10,000 x g for 10 min. Supernatants were stored in microtubes at -20 °C.

CAT activity (CAT, EC 1.11.1.6) was measured according to Havir and Mchale (1987). Peroxidase activity (POX, EC 1.11.1) was measured according to the method described by Urbanek et al. (1991), through the use of guaiacol and H_2O_2 as substrates. Polyphenol oxidase (PPO, EC 1.10.3.1) activity was determined through pyrogallol oxidation (KAR; MISHRA, 1976). All enzyme activities were expressed in units of U min^{-1} mg^{-1}.

Statistical analysis

Data were analyzed through one-way ANOVA in the SPSS (version 19) software. Means and standard errors were calculated in ten repetition (n= 10) values. Means were compared through Tukey's test; P values ≤ 0.05 were considered significant.

RESULTS AND DISCUSSION

Lettuce plants height, canopy and number of leaves matched with the linear regression (Fig. 1A, 1B E 1C), demonstrating interaction between days and the culture development. The height of lettuce plants that received CM did not differ between treatments with bacteria or fungi inoculated alone or associated, but all treatments showed difference compared to the control. However, even showed no statistical difference, the treatments with the use of both microorganisms presented the highest height at 60 DAE. The treatments CMF showed an increase in plant height of 131% and CMFB 206.2% in relation to the control.

The plant canopy of lettuce showed the same pattern of plant height in which all the treatments that received CM were superior to the control, for example, the treatment with inoculation of both microorganisms (CMFB) showed a significant difference in relation to the control, with increase of 194.5% in canopy plants. For the number of leaves, the CMFB treatment showed a significant difference in relation to the control, showing an increase of 287.3% at the end of the crop cycle, demonstrating interaction between plant growth promoter microorganisms.

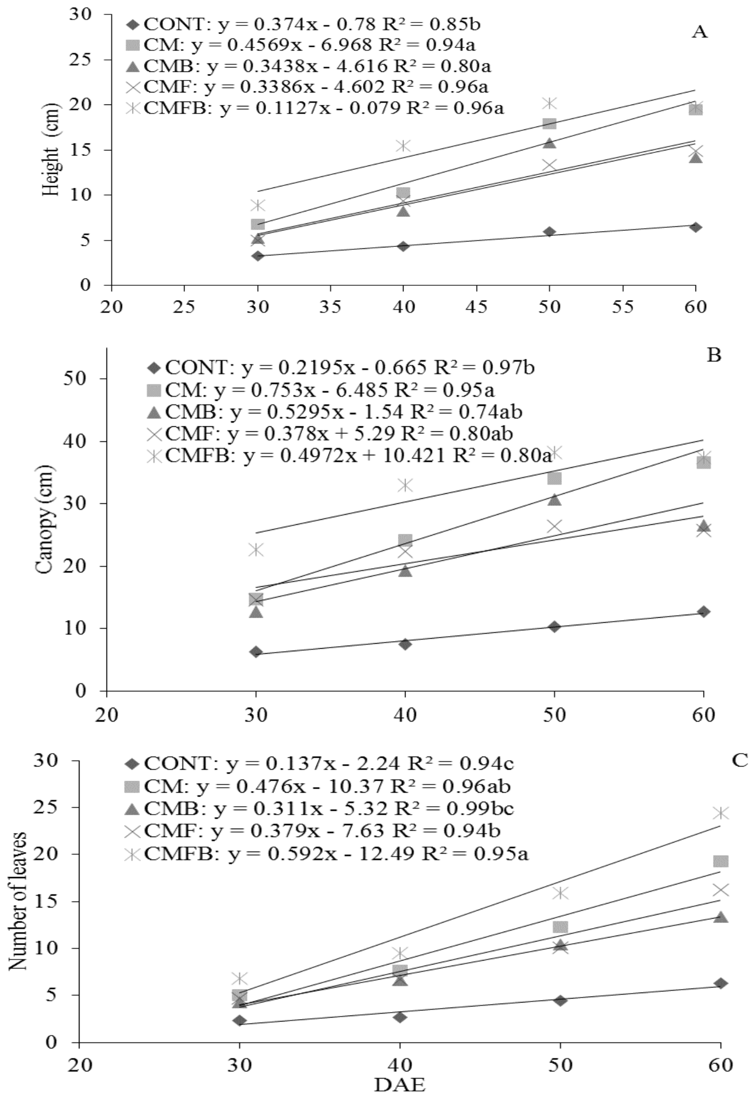

Figure 1. A) Height, B) Canopy and C) Number of leaves of lettuce plants after inoculation with *Pseudomonas* sp. UAGF14 and *Trichoderma aureoviride* URM 5158. CONT= control, CM= cattle manure, CMB= cattle manure + bacteria (*Pseudomonas* sp.*)*, CMF= cattle manure + fungi (*T. aureoviride*), CMFB= cattle manure + fungi + bacteria.

Probably, these results are related to the secondary metabolites released by the bacteria in the environment, and a variety of ecological processes may be involved due to the presence of plant growth promoting substances, such as phytohormones, siderophores and antibiotics, among others (COELHO et al., 2008). The results are similar to those found by Zuffo et al. (2016) when analyzing lettuce cultivars growth in a Yellow Dystrophic Latosol in interaction with organic residues (rice hull and bovine manure), who obtained 26 leaves at 40 days after emergence. Here, the treatment that received only organic residues did not differ from the treatments that received CM associated with microorganisms, showing that only the manure is able to help the plant to develop. Likewise, Cecílio Filho et al. (2008) evaluated the yield and commercial characteristics of the lettuce crop in an Eutrophic Red Latosol in protected environment and in a different growing season, obtaining means of 25 leaves during the crop cycle. However, Sousa et al. (2014), using different biofertilizers in the lettuce crop, obtained an average of 21.51 leaves. Clay soils have the ability to adsorb organic waste, providing organic matter accumulation and increasing microorganism action, such as fungi and bacteria, providing stability for soil aggregates and working as an environment that is conducive to plant development.

Addition of microorganisms associated with organic residues has innumerable benefits to the soil, such as microbial biomass activity increase, which is directly linked to nutrient availability through phosphate solubilization and nitrogen mineralization, essential elements for vegetable development. The number of leaves found in lettuce plants in the present study corroborate with that of Sottero et al. (2006), who obtained a mean of 23 leaves in the treatment with *Pseudomonas* isolates when studying rhizospheric colonization and lettuce growth promotion.

Lettuce height obtained with *Pseudomonas* sp. UAGF14 and *T. aureoviride* URM 5158 at 40 DAE and 60 DAE in this study are superior to that verified by Rekha et al. (2007), who studied the effect of *Pseudomonas* sp. associated with humic acid on lettuce plants growth. According to the authors, vigorous initial development was observed, with remarkable growth after 14 days of inoculation and stabilization between 20 and 40 days after inoculation, where an average of 12 cm of shoot height was obtained. However, Kozusny (2014) studied rhizospheric colonization and growth promotion by *Bradyrhizobium* strains in lettuce seedlings and obtained a mean of 7.9. In the study aforementioned, lettuce development has demonstrated a response to the combined action of microorganisms. This effect is related to manure organic residue in conjunction with growth promoting microorganisms through solubilization of inorganic phosphate to organic phosphate by soil bacteria and fungi action, in which *Pseudomonas* so. UAGF14 and *T. aureviride* URM 5158 (GRAVEL et al, 2007) species are highlighted. Phosphate solubilizing microorganisms, such as *Pseudomonas* spp., were reported in lettuce growth promotion, increasing phosphate absorption (CHABOT et al., 1996). Gatiboni et al (2008) studied microbial biomass phosphorus and acid phosphatases activity and observed that phosphate solubilization makes phosphorus available to the plants, stimulating the growth and the formation of the root system.

All treatments increased lettuce leaf area, root length, shoot dry matter and root dry matter compared to the control (Fig. 2). However, in some variables, plants submitted to CM treatment did not differ statistically from the treatment that received both microorganisms, although the latter showed higher means than the other treatments. For example, plants submitted to CMFB showed increases of 430.5% and 759.7% for leaf area and dry matter, respectively, compared to the CONT. The results are possibly related to the ability of the *Trichoderma* genus fungus to improve nutrient absorption, induce resistance and promote plant growth (HARMAN, 2011). Hoyos- carvajal et al. (2009) reported that growth stimulation is evidenced by biomass, yield, stress resistance and nutrient absorption increase. Harvest and yield increases associated with *Trichoderma* presence was observed in a wide range of horticultural species, such as cucumber, eggplant, peas, pepper, radish, tomato, carrot and lettuce (GRAVEL et al., 2007).

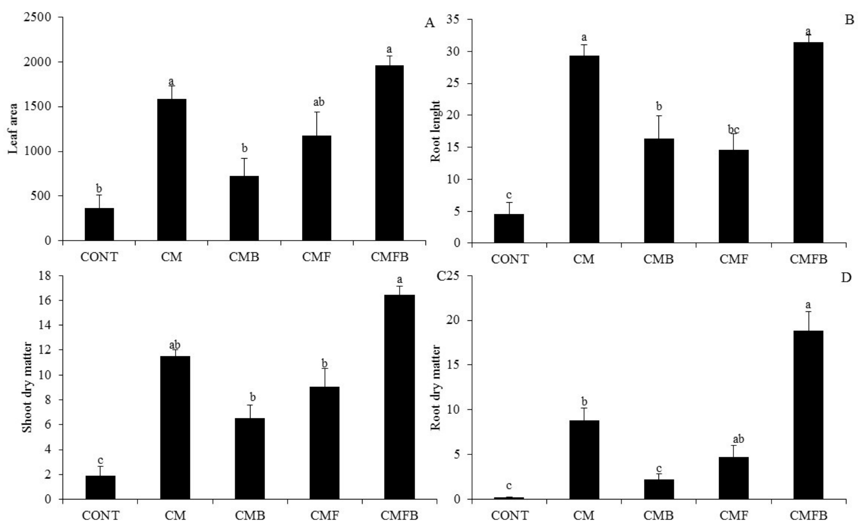

Figure 2. A) Leaf area, B) root length, C) shoot dry matter and D) root dry matter. CONT= control, CM= cattle manure, CMB= cattle manure + bacteria (*Pseudomonas* sp. UAGF14*)*, CMF= cattle manure + fungi (*Trichoderma aureoviride* URM 5158), CMFB= cattle manure + fungi + bacteria. Different lowercase letters indicate significant differences ($P < 0.05$) by ANOVA, followed by Tukey's test.

Pseudomonas genus rhizobacteria of the fluorescent group can promote plant growth in different cultures, such as lettuce (COELHO et al., 2008; BASHAN et al., 2014). These benefits are due to the production of phytohormones, such as IAA, which can be synthesized by these microorganisms, as well as hydrocyanic acid (HCN), a volatile compound that inhibits phytopathogen development. In addition, phosphate solubilization and biological nitrogen fixation also collaborate with plant growth (GUIMARÃES et al., 2012; CIPRIANO, 2013; AHEMAD; KIBRET, 2014). Coelho et al. (2008) found that the lettuce rhizosphere has the highest number of *Pseudomonas* sp. bacteria. The presence of these bacteria helps leaf development, increasing the lettuce crop photosynthetic rate. Higher shoot dry matter accumulation is due to the higher leaf area, since the value of this variable is directly associated with that of the plant photosynthetic area. Plants with higher photosynthetic area, consequently, will have higher photoassimilate production, resulting in growth and development (ZUFFO et al., 2016).

Leaf area, root length, shoot dry matter and root dry matter showed synergistic interaction between growth promoting microorganisms (CMFB), obtaining a significant difference compared to other treatments in the present study, except with CM in some variables. Lower results than those of this study were found by Pantano et al. (2015), when studying the development of lettuce cultivars, obtaining a mean leaf area of 1692 m^2 and 14.52 g of shoot dry matter. Lower scores were also found by Zuffo et al. (2016), which obtained means of 7 g of shoot dry matter and 2225 cm^2 of leaf area, a value similar to that found in this study. Lettuce plants that received the combination of fungus and bacteria (CMFB) showed higher root length and root dry matter in relation to CONT, with an increase of 583% and 8773%, respectively, but was statistically similar to the CM treatment (Figure 2). Sottero et al., 2006, found similar results in the inoculation of *Pseudomonas* spp. of fluorescent group in the lettuce crop, showing a significant root dry matter increase in the seedlings in which they were inoculated in relation to the control.

The chlorophyll a and chlorophyll b found in lettuce plants submitted to all treatments were statistically superior to the control. However, plants submitted to CMFB treatment showed an increase of 137, 142, and 138% in chlorophyll a, chlorophyll b and chlorophyll a + b values, respectively (Fig. 3) compared to the control. Suzuki et al. (2014) found similar results in their experiment with hydroponic lettuce in the two cultivation situations evaluated (in nutrient deficiency presence and absence), and treatments with plant growth promoting bacteria (BPCP) inoculation increased chlorophyll content.

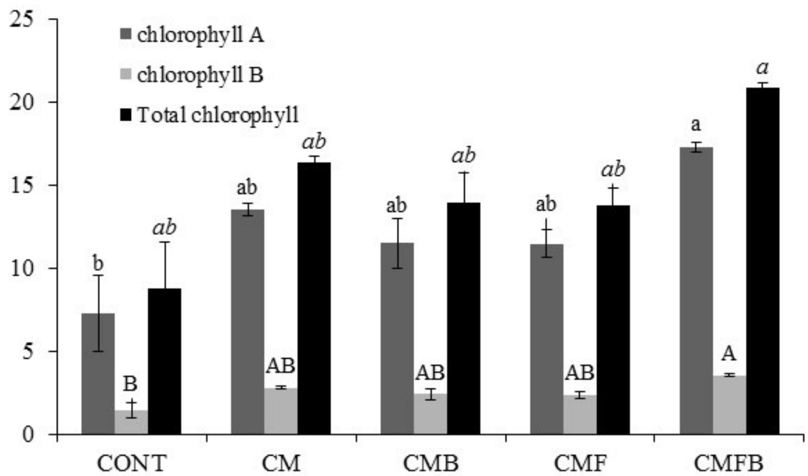

Figure3. Chlorophyll A, B and total in lettuce plant leaves after inoculation with *Pseudomonas* sp. UAGF14 and *Trichoderma aureoviride* URM 5158. CONT= control, CM= cattle manure, CMB= cattle manure + bacteria (*Pseudomonas* sp. UAGF14), CMF= cattle manure + fungi (*Trichoderma aureoviride* URM 5158), CMFB= cattle manure + fungi + bacteria. Different lowercase letters indicate significant differences ($P < 0.05$) in Chlorophyll A; capital letters indicate significant differences in Chlorophyll B, and italic case letters indicate significant differences in total chlorophyll by ANOVA, followed by Tukey's test.

Several cultures showed chlorophyll content increase under BPCPs inoculation, with a significant increase in chlorophyll a content in cowpea (SANTOS et al., 2016). In tomato plants, these bacteria increased chlorophyll a, b and total contents and promoted seedling shoot growth of *Santa Clara* and *Cereja* cultivars (SZILAGYI-ZECCHIN, et al., 2015).

Chlorophyll increase is closely related to nutrients availability, such as N and P (MALAVOLTA, et al., 1997; NASCIMENTO et al., 2014). These green pigments are fundamental for photosynthesis and are commercially important for improving food quality (STREIT et al., 2005).

Antioxidant enzyme activities in lettuce plants varied among the treatments, except for the CAT activity that showed no statistical differences (Fig. 4). CMB and CMFB promoted a significant polyphenol oxidase (PPO) activity increase in relation to the control. The PPO enzyme acts under the phenolic compounds and is bound to the cell membranes, being activated only when released by membrane damage (TAVEIRA et al., 2012). PPO, besides occurring in plant tissues, can also be found in microorganisms, mainly in fungi and bacteria (ALVARENGA, 2011). This enzyme increase may be related to the microorganism that produces this activity in its structures. Similar results were observed by Awad et al. (2015), while analyzing trans-resveratrol and glycine betaine that affect the quality, antioxidant capacity, antioxidant compounds and enzymatic activity of *Vitis* sp.

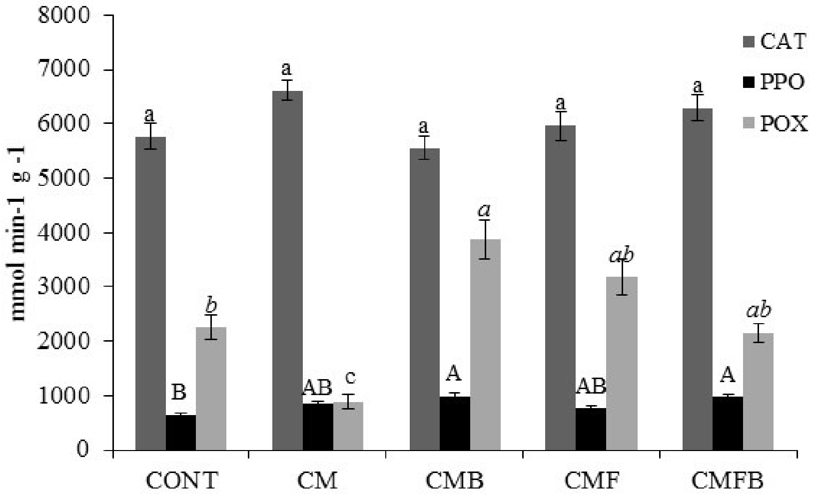

Figure 4. Enzimatic activities in lettuce plants inoculated with *Pseudomonas* sp. UAGF14 and *Trichoderma aureoviride* URM 5158. Catalase (CAT), polyphenol oxidase (PPO) and Peroxidase (POX). CONT= control, CM= cattle manure, CMB= cattle manure + bacteria (*Pseudomonas* sp. UAGF14), CMF= cattle manure + fungi (*Trichoderma aureoviride* URM 5158), CMFB= cattle manure + fungi + bacteria. Different lowercase letters indicate significant differences ($P < 0.05$) in CAT activity; capital letters indicate significant differences in PPO activity and italic case letters indicate significant differences in in POX activity by ANOVA, followed by Tukey's test.

The CMB treatment induced a significant increase of 71.93% in POX activity compared to the control. Inoculation of seeds with *Pseudomonas* bacteria increased nutrients availability, especially phosphorus, because soil-inoculated bacteria can increase POX enzyme activity, helping in the adaptation of plants under phosphorus deficiency. There is increasing evidence that the *Pseudomonas* genus can confer tolerance on plants through antioxidant enzymes activation for various abiotic stresses, such as the toxicity of some nutrients. However, interaction of nutrient with the bacterium increases antioxidant enzyme activity (ISRAR et al., 2016), as occurred in this study. Similar results were observed by Borkowski et al. (2015), while analyzing the oxidative stress in bacteria (*Pseudomonas* sp.) exposed to silicon carbide nanostructures.

Antioxidant enzyme activities provides resistance to plants against biotic and abiotic stresses. Microorganisms have been used to provide tolerance to plants against various stresses, induced through antioxidant enzymes. Growth promoting activity has been identified as responsible for casting metal chelating substances, such as siderophore chelating iron, in the rhizosphere. Thus, the plant is influenced to absorb several metals, activating its defense mechanism against stress through antioxidant enzymes activation (GURURANI et al., 2013). That is why the results of this study showed catalase increase using fungus/bacterial co-inoculation, since they can activate the defense mechanism of this enzyme. Similar results were observed by Islam et al. (2014), who analyzed the influence of *Pseudomonas aeruginosa* on oxidative stress tolerance in wheat under Zn.

CONCLUSION

The results indicate beneficial effects of cattle manure and the combined use of *Pseudomonas* sp. UAGF14 and *Trichoderma aureoviride* URM5158 on lettuce plants growth promotion, confirming the synergistic effect of the combination of the two microorganism types, as it increased height, canopy, shoot and root dry matter, and chlorophyll a, b and total levels, besides increasing enzymes expression related to ROS group resistance induction, mainly to PPO. However, few responses were observed in lettuce plant growth with these in the first cycle evaluated after 60 days when compared CM and CMFB treatments, but both treatments showed superiority compared to the control. Therefore, further studies are needed to estimate the long-term effects of combined effect of *Pseudomonas* sp. UAGF14 and *T. aureoviride* URM5158 on crop productivity in field conditions.

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RESUMO: A promoção do crescimento das plantas por micro-organismos pode ser uma alternativa viável para aumentar a produção de alface através de controle de patógenos e aumento da absorção de nutrientes. O gênero fúngico *Trichoderma* e o gênero bacteriano *Pseudomonas* são exemplos de micro-organismos amplamente estudados com capacidade para promover o crescimento da planta. No entanto, ainda existem lacunas quanto à ação do efeito combinado desses dois micro-organismos. Portanto, o objetivo deste estudo foi avaliar o efeito combinado de *Pseudomonas* UAGF14 e *Trichoderma aureoviride* URM5158 sobre o desenvolvimento de plantas de alface. O delineamento experimental foi completamente casualizados, com cinco tratamentos: CONT (controle, sem fertilização orgânica), CM (solo com fertilização orgânica), CMB (solo com fertilização orgânica e *Pseudomonas* sp.), CMF (solo com fertilização orgânica e *T. aureoviride*) e CMFB (solo com fertilização orgânica, *Pseudomonas* sp. e *T. aureoviride*), com dez repetições. Aos 30, 40 e 60 dias após a semeadura, foram analisados os seguintes parâmetros: altura da planta e dossel e número de folhas. Aos 60 dias após a emergência, a matéria seca da parte aérea, a área foliar, a massa seca das raízes, o comprimento radicular e a clorofila foram analisados. Catalase, peroxidase e atividade enzimática da polifenol oxidase foram determinadas. O CMFB apresentou o maior crescimento de alface, confirmando o efeito benéfico da combinação dos dois tipos de micro-organismos com a planta, na medida em que aumentou a altura, o dossel, a matéria seca da parte aérea e da raiz, e os níveis de clorofila em relação ao CONT, embora não tenha diferido do CM em algumas variáveis. As atividades enzimáticas também foram influenciadas pela ação desses micro-organismos combinados, evidenciada pelo aumento de polifenol oxidase. O CMFB ou CM foram eficientes na promoção do crescimento da alface, mostrando respostas positivas às características morfológicas e fisiológicas. Entretanto, poucas respostas foram observadas no crescimento da alface no primeiro ciclo da planta avaliado depois de 60 dias, comparando os tratamentos CM e CMFB, mas ambos tratamentos mostraram superioridade em relação ao crescimento das plantas de alface submetidas ao tratamento controle. Por isso, são necessários futuros estudos para estimar à longo prazo o efeito combinado de *Pseudomonas* sp. UAGF14 e *Trichoderma aureoviride* URM5158 na produção de cultura em condições de campo.

PALAVRAS-CHAVE: *Lactuca sativa*. Promotores de crescimento de plantas. Clorofila a. Catalase. Polyfenol oxidase.

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