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Growth promoting fungi increase the quality of *Coffea canephora* seedlings Pierre ex a. Froehner

Jucélia Rodrigues Alves[1], Flávio Neves Celestino[1], Alisson Gomes de Moraes[1], Ricardo Arizono dos Reis[1], Eduardo Domingos Grecco[2]

[1]Instituto Federal de Educação, Ciência e Tecnologia do Sudeste de Minas Gerais/IF Sudeste MG, Campus Manhuaçu, Manhuaçu, MG, Brasil
[2]Koppert Biological Systems Brasil, Domingos Martins, ES, Brasil
Contact authors: juceliarodriguesmtp@gmail.com; fncelestino@yahoo.com.br; alissongomes081@gmail.com; ricardo.arizono@gmail.com; egrecco@koppert.com.br
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ABSTRACT

Growth promoting fungi have shown an important role in the development of agricultural crops. Among these fungi, the genus *Trichoderma* stands out, and the entomopathogenic fungi of the genera *Beauveria* and *Metarhizium* have been gaining major importance. Thus, the aim of the present study was to evaluate the vegetative growth of conilon coffee seedlings inoculated with growth promoting fungi. For the production of seedlings, cuttings of clone A1 of conilon coffee were used, in standard substrate. The growth promoting fungi *Trichoderma harzianum*, *Beauveria bassiana* and *Metarhizium anisopliae* were used at a concentration of 1 x 10^7 conidia.mL^{-1}, inoculated via soil and leaf. Vegetative growth evaluations were performed at 180 days after cutting. The treatments corresponded to a 4 x 2 factorial arrangement, in a randomized block design, with three replications, comprising 24 plots. The data were submitted to analysis of variance and the means compared by the Scott-Knott grouping method ($p \leq 0.05$). The treatments did not promote gains in plant height, stem diameter, root length, root fresh mass, leaf area, shoot/root fresh and dry mass of conilon coffee seedlings. Growth promoting fungi *T. harzianum* and *B. bassiana* promoted a higher number of leaf pairs, higher fresh mass of aerial part and higher dry mass of aerial part. Additionally, *T. harzianum* promoted higher root dry mass in conilon coffee seedlings. Only for the number of leaf pairs, the inoculation via soil was higher than via leaf, while for the other parameters there was no difference regarding the inoculation method.

Key words: Conilon coffee; *Trichoderma harzianum*; *Beauveria bassiana*; *Metarhizium anisopliae*.

1 INTRODUCTION

Brazil is the largest producer and exporter of coffee, accounting for 31.9% of world exports (International Coffee Organization - ICO, 2021). The cultivated species are *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner. The states of Minas Gerais and Espírito Santo are the main producers and in the 2020 harvest they were responsible for 54.9 and 22.1% of the Brazilian production, respectively (Companhia Nacional de Abastecimento CONAB, 2021). In Brazil, more than 2 million hectares of coffee are cultivated, of which 12.8% are under development (CONAB, 2021). In this way, there is a constant demand for quality seedlings, for the planting of increasingly productive and sustainable crops.

Year after year, there has been a major need to reduce the use of pesticides in agricultural systems, looking for sustainability (Dara, 2019, Deguine et al., 2021). Under scenario that research on the use of plant growth-promoting fungi has been expanded, as they contribute to a better balance of agricultural systems, thus reducing the need for external inputs (Vega, 2018; Duong et al., 2020). Stenberg (2017) highlights the importance of better understanding the interactions within agricultural systems, so that integrated pest management (IPM) is actually applied. Studies of these interactions have been carried out, mainly, with fungi of the genera *Trichoderma* (Mello et al., 2020; Sood et al., 2020). However, recently, there are several reports of more complex interactions involving entomopathogenic fungi, mainly of the genus *Beauveria* and *Metarhizium* (Donga; Vega; Klingen, 2018; Jaber; Alananbeh, 2018; Mwamburi, 2021; Baron; Rigobelo, 2022).

The use of fungi of the genus *Trichoderma*, which are important biological control agents, can reduce the presence of several plant pathogens present in the soil (Mello et al., 2020). These fungi use different mechanisms to control plant pathogens that include mycoparasitism, competition for space and nutrients, antibiotic secretion and enzymatic cell wall degradation (Mello et al., 2020; Baron; Rigobelo, 2022). In addition to protection against plant pathogens, several mechanisms for promoting vegetative growth were obtained with *Trichoderma* spp., including improved root system development and auxin production, siderophore production, increased drought tolerance, defense protein expressions within the plant, phosphate solubilization, and increased resistance to salinization (Saravanakumar; Arasu; Kathiresan, 2013; Contreras-Cornejo et al., 2014, Sood et al., 2020).

Entomopathogenic fungi, already recognized for their high potential in the biological control of pests, have demonstrated complex relationships with plants as endophytes, that is, they colonize the internal tissues of plants for some time or throughout their life cycle, without causing visible signs of infection (Vega, 2018; Celestino et al., 2020). In this context, these fungi are able to act as antagonists of plant pathogens through by using a diverse range of mechanisms, such as the production of metabolites (antibiotics, volatile compounds and enzymes), competition for resources (space, nutrients and water) and mycoparasitism, induction of systemic plant resistance and increases in plant growth, resulting in reduced plant pathogen activity (Vega, 2018; Quesada-Moraga, 2020; Baron; Rigobelo, 2022). Based on the above, the objective of the present study was to evaluate the vegetative growth of conilon coffee seedlings inoculated with growth promoting fungi.

2 MATERIAL AND METHODS

2.1 Experimental site

The experiment was carried out in a nursery covered with a shading screen (50% reduction in light intensity), at the Manhuaçu *Campus* of the Federal Institute of Education, Science and Technology of the Southeast of Minas Gerais (IF Sudeste MG), in Manhuaçu, Minas Gerais (20°14'45.11"S; 42° 9'1.49"W), at an elevation of 800 m.

2.2 Experimental procedure

Conilon coffee cuttings of the clone A1 were obtained in the Capixaba Institute for Research, Technical Assistance and Rural Extension (Incaper), Cachoeiro de Itapemirim, Espírito Santo (20°45'9.10"S; 41°17'25.55"O), at an elevation of 82 m. Black perforated polyethylene bags measuring 9 x 20 cm were used, and a standard substrate consisting of soil, manure, single superphosphate and potassium chloride (Ribeiro; Guimarães; Alvarez, 1999). The substrate was disinfested by solarization according to Ghini (2004). After preparation of the polyethylene bags, the vegetative propagation of cuttings of clone A1 was carried out. The cuttings were prepared according to the procedure of Verdin Filho et al. (2014). A horizontal cut of 1 cm was made above the insertion of the plagiotropic branches, and another straight horizontal cut of 3 to 4 cm was made below the insertion of the leaves. Further, 2/3 of the leaf blade of each leaf was cut with the objective to reduce evapotranspiration, thus avoiding excessive water loss. Irrigation and fertilization were carried out according to the recommendations for the production of coffee seedlings (Sakiyama et al., 2015).

The growth promoting fungi tested were commercial formulations of *Trichoderma harzianum* (Rifai) (Ascomycota: Hypocreales), *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) and *Metarhizium anisopliae* (Metsch.) Sorokin (Ascomycota: Hypocreales), as Trichodermil® SC (Isolate: Esalq 1306; Active ingredient: 2 x 10^9 viable conidia mL^{-1}; Koppert Brasil, Piracicaba, SP), Boveril® WP (Isolate: ESALQ PL63; Active ingredient: 1 x 10^8 viable conidia g^{-1}; Koppert Brasil, Piracicaba, SP) and Metarril® WP (Isolate: ESALQ E9; Active ingredient: 1.39 x 10^8 viable conidia g^{-1}; Koppert Brasil, Piracicaba, SP), respectively. Two inoculation methods were used to test these growth promoting fungi, via soil and leaf application. For each commercial formulation, a suspension was prepared at 1 x 10^7 conidia mL^{-1} and added Tween® 80 PS at 0.05% (v v^{-1}). Conilon coffee cuttings were disinfested in sodium hypochlorite (NaClO) at 0.5% (v v^{-1}) for five minutes. Then, they were rinsed and washed in sterile distilled water for three times. For the soil inoculation, 10 mL of the conidia suspension were applied to the soil surface in each bag (Posada et al., 2007). Further, the top of the bags was covered with aluminum foil (Posada et al., 2007). The leaf inoculation was carried out with a manual sprayer by applying, 5 mL $plant^{-1}$. In the check treatments, water was applied with addition of Tween® 80 PS at 0.05% (v v^{-1}) under the same conditions as previously described.

2.3 Assessments of the vegetative growth

Vegetative growth assessments were performed 180 days after inoculation. The plant height (cm) and the stem diameter (mm) were measured. The stem diameter was measured at the substrate level with the aid of a digital caliper. The number of leaf pairs of the plants in each plot was counted and the average number of true leaves per plant was calculated. The leaf area was also determined, based on the length and width of a leaf of each pair of true leaves measured. The leaf area of each pair of leaves was obtained by the product width x length x 0.667 x 2 (pair of leaves), as proposed by Barros, Maestri and Braga Filho (1973). The leaf area of the seedling was obtained by the sum of the areas of the leaf pairs of each plant. The average leaf area was obtained based on the values of the leaf areas of the seedlings in the plot, with the results expressed in cm^2. The plants were taken to the laboratory where the roots were washed with tap water using a 0.5 mm diameter sieve. After this procedure, the fresh mass (g) (root and shoot) was determined by using an analytical precision scale of 0.001 g. The root length (cm) was measured with the aid of a millimeter ruler. The determinations of the dry masses (g) (root and shoot) were performed after the samples were passed through a forced air circulation oven at 70 °C for 72 hours. The ratios of shoot fresh mass/root fresh mass and dry mass shoot/root dry mass were obtained.

2.4 Statistical analysis

Each experimental plot consisted of 16 plants arranged in four rows of four plants, with the four central plants consisting of the useful experimental plot. Treatments corresponded to a 4 x 2 factorial arrangement (Growth promoting fungi: *T. harzianum, B. bassiana, M. anisopliae* and control; Inoculation methods: via soil and leaf), in a randomized block design with three replicates. From the interactions were obtained 8 treatments, totaling 24 plots. The data of the vegetative growth were subjected to analysis of variance and the means compared by the Scott-Knott grouping method, at 5% probability.

3 RESULTS

Plant height, stem diameter, root length, root fresh mass of the conilon coffee seedlings showed no significant interaction between the factors, growth promoting fungi (*T. harzianum*, *B. bassiana* and *M. anisopliae*) and inoculation method (soil and leaf) (Figure 1A, 1B, 1E, 1F, 1G and 1H, Figure 2A and 2B). Furthermore, when each factor was analyzed separately, no differences were observed between treatments for the vegetative growth variables (Figure 1A, 1B, 1E, 1F, 1G and 1H, Figure 2A and 2B).

The number of leaf pairs of conilon coffee seedlings showed no interaction between factors, fungi growth promoters (*T. harzianum*, *B. bassiana* and *M. anisopliae*) and inoculation method (soil and leaf) (Figure 1C and 1D). Analyzing just the factor fungi growth promoters, it was observed that *T. harzianum and B. bassiana* promoted higher number of leaf pairs in the conilon coffee seedlings (Figure 1C). When considering only the inoculation method, there was a higher number of leaf pairs in the plants inoculated via soil as compared to the inoculation via leaf (Figure 1D).

The fresh mass of the aerial part of the conilon coffee seedlings showed no interaction between the factors, growth promoting fungi (*T. harzianum*, *B. bassiana* and *M. anisopliae*) and inoculation method (soil and leaf) (Figure 2C and 2D). However, when considering only the factor fungi growth promoters, the fungi *T. harzianum* and *B. bassiana* promoted higher fresh shoot mass for the conilon coffee seedlings (Figure 2C). In contrast, the inoculation method (soil and leaf) did not differ for this vegetative growth parameter (Figure 2D).

The dry mass of root and aerial part of the conilon coffee seedlings showed no interaction between the factors, growth promoting fungi (*T. harzianum*, *B. bassiana* and *M. anisopliae*) and inoculation method (soil and leaf) (Figure 2E, 2F, 2G, 2H). However, when considering only the factor growth promoting fungi, it was observed that the fungus *T. harzianum* promoted higher dry mass of root and aerial part for the conilon coffee seedlings. On the other hand, the dry mass of the aerial part of the seedlings treated with the fungus *B. bassiana* did not differ from this (Figure 2E and 2G). Nevertheless, the inoculation method (soil and leaf) did not differ for these growth parameters (Figure 2F and 2H).

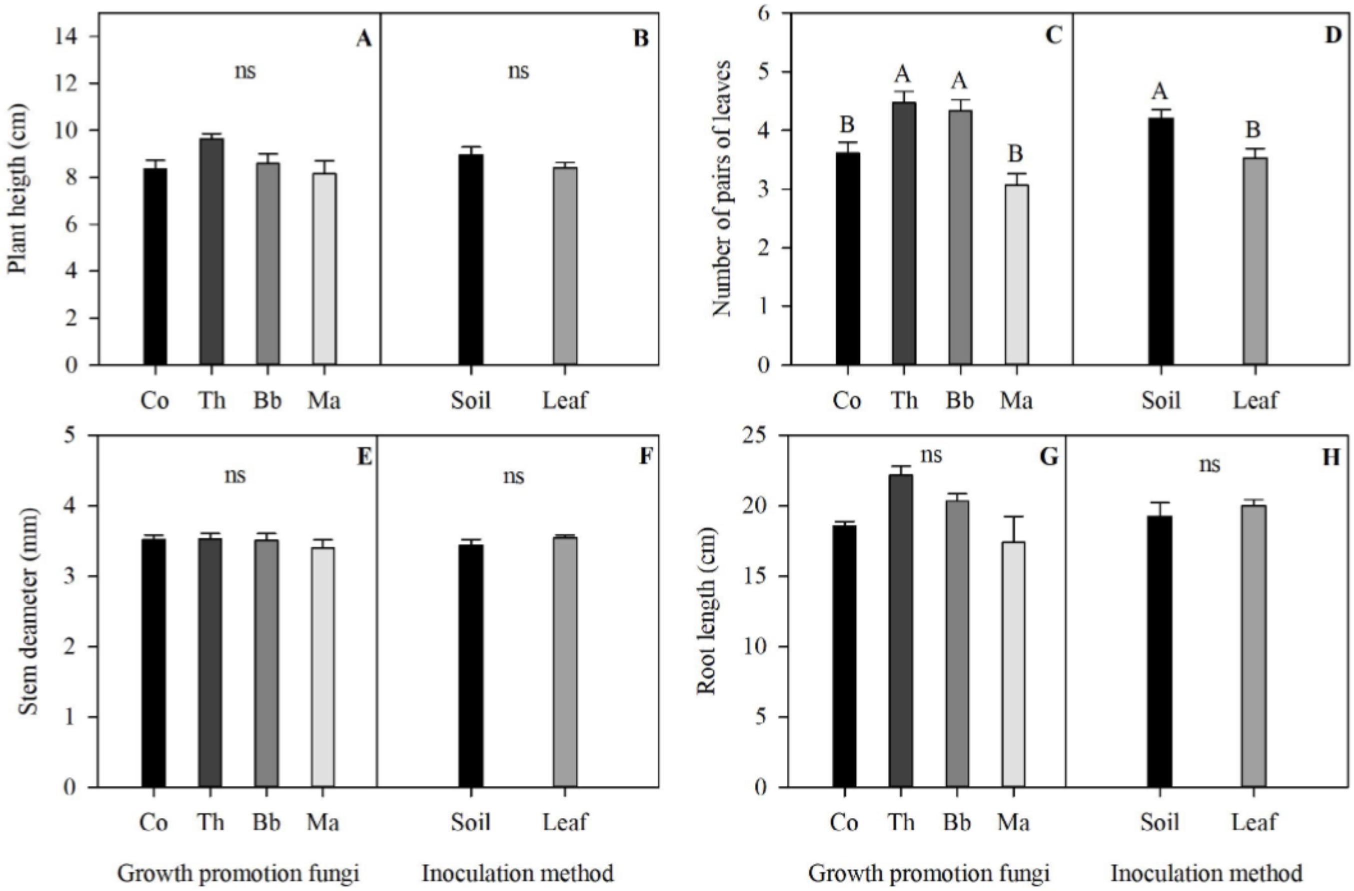

Figure 1: Plant height, number of leaf pairs, stem diameter and root length of the conilon coffee seedlings, inoculated via soil or leaf with the growth promoting fungi *Trichoderma harzianum* (Th), *Beauveria bassiana* (Bb) and *Metarhizium anisopliae* (Ma), and the control (Co). A,B,E,F,G,H Not significant (ns). C Bar (± EP) under different capital letters differ by the Scott-Knott grouping method ($p \leq 0.05$). D Bar (± EP) under different capital letters differ by the F test ($p \leq 0.05$).

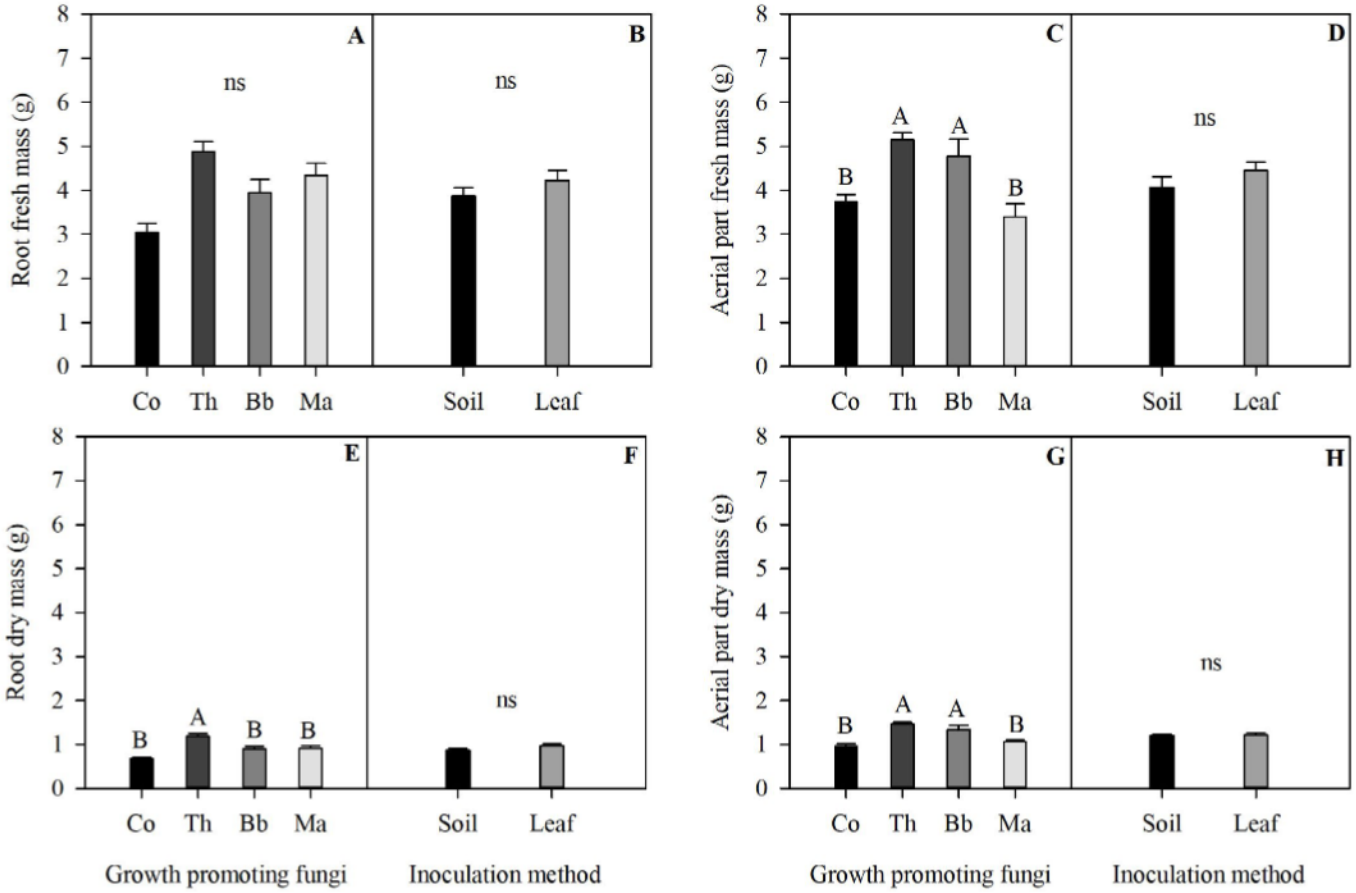

Figure 2: Fresh root and shoot matter and dry root and shoot matter of conilon coffee seedlings, inoculated via soil or leaf with the growth promoting fungi *Trichoderma harzianum* (Th), *Beauveria bassiana* (Bb) and *Metarhizium anisopliae* (Ma), and the control (Co). B,D,F,HNot significant (ns). A,C,E,GBar (± EP) under different capital letters differ by the Scott-Knott grouping method ($p \leq 0.05$).

Leaf area, fresh and dry mass ratio of shoot/root of the conilon coffee seedlings, showed no significant interaction between the factors, growth promoting fungi (*T. harzianum*, *B. bassiana* and *M. anisopliae*) and inoculation method (soil and leaf) (Figure 3). In addition, when each factor was analyzed separately, no differences were observed between the treatments for the aforementioned vegetative growth variables (Figure 3).

4 DISCUSSION

Regarding plant height, the results obtained corroborate with those found for clonal eucalyptus seedlings inoculated with *T. harzianum* and *Trichoderma virens* (Miller, Giddens, Foster) (Ascomycota: Hypocreales), via substrate and leaf, at 1 x 10^8 conidia mL^{-1}, as these fungi also did not change plant height. However, they promoted an increase in the dyrm diameter of eucalyptus seedlings from mini-cuttings, and an increase ranging from 40 up to 110% in the number of leaf pairs (Azevedo et al., 2017). Similar results were also obtained for cambara seedlings (*Gochnatia polymorpha*) inoculated with *T. harzianum* by incorporation of the formulated (1 x 10^8 conidia g^{-1}) to the substrate at a ratio of 4g kg^{-1} (Machado et al., 2015).

For fresh mass, the results of this study corroborate with those obtained for passion fruit seedlings of cuttings inoculated with *T. harzianum* and *Trichoderma asperellum* Samuels, Lieckf & Nirenber (Ascomycota: Hypocreales). In addition, the symbioses also promoted higher total dry mass of plants (Santos; Melo; Peixoto, 2010). Similarly, chickpea plants inoculated with *T. harzianum,* at 4.09 x 10^8 conidia mL^{-1}, presented higher aerial and root dry mass (Kapri; Tewari, 2010). For rice cultivation, four isolates of *Trichoderma* spp. obtained in the Amazon rainforest, inoculated via seed and leaf, at 6 x 10^8 conidia mL^{-1}, promoted higher aerial and root dry masses (Silva et al., 2012). Higher dry biomass was also observed in cotton plants inoculated with *B. bassiana*, at 1 x 10^6conidia.mL^{-1} (Lopez; Sword, 2015).

Despite *M. anisopliae* not having positively changed the growth of conilon coffee seedlings, in other crops, such as corn, soybeans, broad beans, common beans and wheat, species of this genus promoted increases in the vegetative growth (Khan et al., 2012; Liao et al., 2014; Behie; Bidochka, 2014; Jaber; Enkerli, 2016). In this way, it is clear the need to expand the studies with species of this genus with coffee tree, as in the previously mentioned crops, other species were used such as

M. robertsii, M. acridom and *M. flavoviride* (Khan et al., 2012; Behie; Bidochka, 2014; Liao et al., 2014; Jaber; Enkerli, 2016).

The relationship between growth promoting fungi and host plants presents high complexity, as they depend on several extrinsic factors such as interaction with other microorganisms, climatic conditions, nutrient availability, soil temperature and humidity, among others (Machado et al., 2015; Jaber; Enkerli, 2016). Such complexity contributes to variation in the response of the host plants (vegetative growth) due to interactions with growth promoting fungi (Machado et al., 2015; Jaber; Enkerli, 2016). In this regard, it is possible that, in conditions of biotic and abiotic stress, higher increases in the vegetative growth of the conilon coffee tree are observed.

The fungi *Trichoderma* spp. have the ability to solubilize phosphates, turning them available to plants (Kapri; Tewari, 2010). The inoculation of *T. asperellum* significantly reduced the use of phosphorus fertilization in onion (*Allium cepa*) cultivation (Ortega-Garcia et al., 2015). This mechanism is of paramount importance given the low availability of this nutrient, particularly in tropical soils. In addition, fungi of this genus can produce indole-3-acetic acid (IAA), a plant hormone of the auxin group, which promotes several positive effects on the development of shoots and roots, such as tropism, cell division

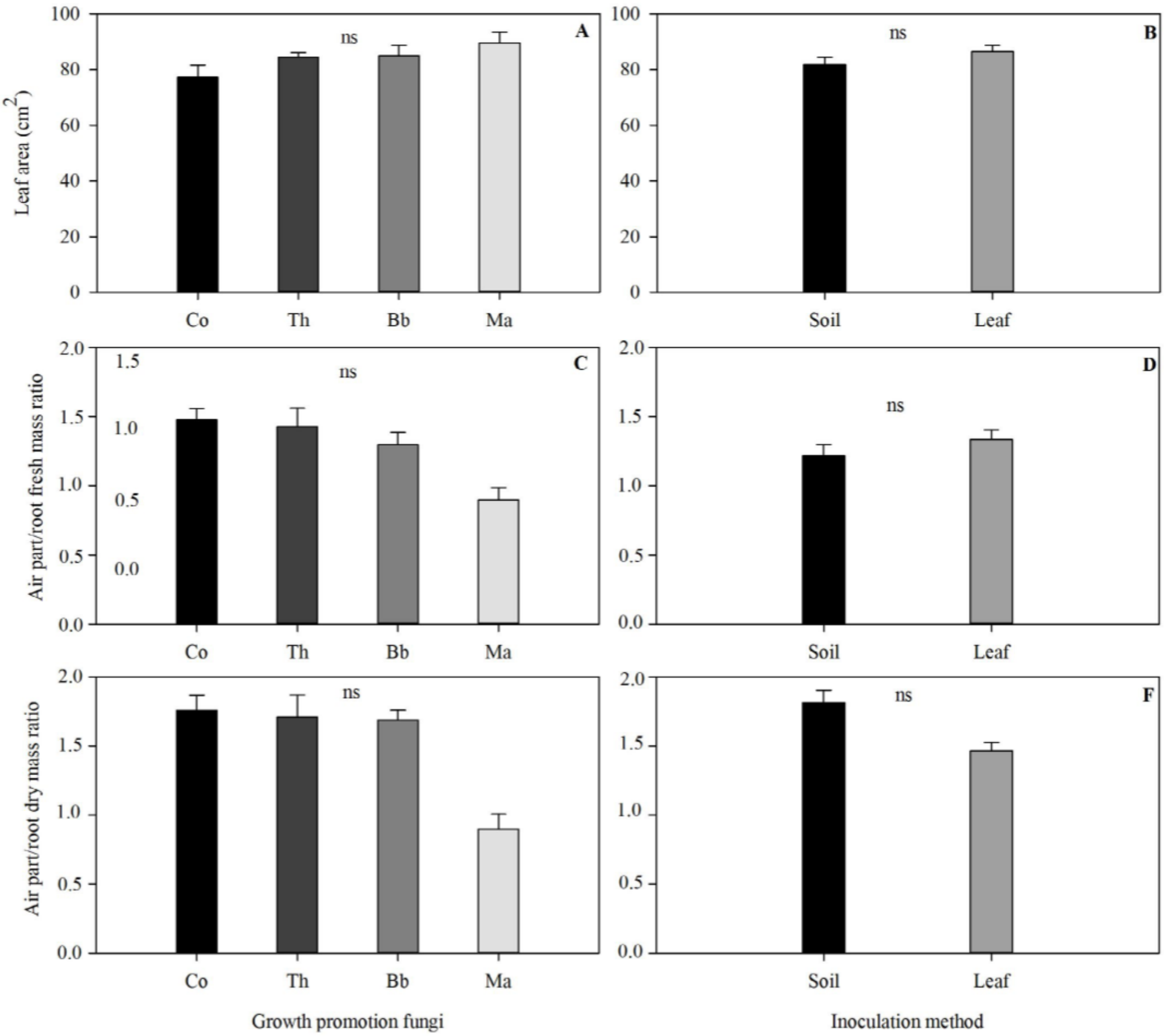

Figure 3: Leaf area, fresh mass and dry mass ratio of shoot/root of conilon coffee seedlings, inoculated via soil or leaf with growth promoting fungi *Trichoderma harzianum* (Th), *Beauveria bassiana* (Bb) and *Metarhizium anisopliae* (Ma), and the control (Co). [A,B,C,D,E,F]Not significant (ns).

and elongation responses, vascular tissue differentiation and initiation of the root formation process (Jaroszuk-Ściseł; Kurek; Trytek, 2014). Such mechanisms may possibly be involved in the promotion of vegetative growth provided by *T. harzianum* to the seedlings of the conilon coffee tree.

In addition to the species of the genus *Trichoderma*, the entomopathogenic fungi of the genera *Beauveria* and *Metarhizium* are able to improve the absorption of nutrients by the plants (Behie; Zelisko; Bidochka, 2012; Behie; Bidochka, 2014). Five species of *Metarhizium* (*M. robertsii*, *M. guizhouyces*, *M. brunneum*, *M. flavoviridae* and *M. acridum*) and *B. bassiana* were able to kill larvae of *Gallery mellonella*, colonize endophytically and carry out the transfer of N from the insect to *Glycine max* (soybean) (Fabaceae), *Phaseolus vulgaris* (common bean) (Fabaceae), *Triticum aestivum* (wheat) (Poaceae) and *Panicum virgatum* (Poaceae) (Behie; Bidochka, 2014). Therefore, the fungus *B. bassiana* may have employed this mechanism to promote higher vegetative growth of the conilon coffee plants.

Other mechanisms that may be involved in the increased vegetative growth of the conilon coffee plants promoted by *T. harzianum* and *B. bassiana* are the systemic activation of resistance to plant pathogens and the production of antibiotics and secondary metabolites. Cucumber roots inoculated with *T. harzianum* showed higher expression of peroxidase and chitinase activities, improving the plant's resistance to the attack of plant pathogens (Yedidia; Benhamou; Chet, 1999). Harzianic acid, a secondary metabolite, derived from *T. harzianum*, showed antibiotic activity against *Pythium irregulare*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani* in *in vitro* test (Manganiello et al., 2018). Endophytic blastospores of *B. bassiana* provide high resistance against the plant pathogen *Botrytis cinerea* (Sui et al., 2022). Thus, studies with growth-promoting fungi applied to coffee farming should be expanded, as they may have a fundamental role for the sustainability of this agricultural system.

5 CONCLUSIONS

The treatments with the growth promoting fungi *T. harzianum, B. bassiana* and *M. anisopliae* did not promote gains in plant height, stem diameter, root length, root fresh mass, leaf area, shoot/root fresh and dry mass of conilon coffee seedlings.

Growth promoting fungi *T. harzianum* and *B. bassiana* promoted higher number of leaf pairs, higher shoot fresh mass and higher shoot dry mass.

The fungus *T. harzianum* promoted higher root dry mass in conilon coffee seedlings. Only for the parameter number of leaf pairs, inoculation via soil was more efficient than via leaf, while for the other parameters there was no difference in the method of inoculation.

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7 AUTHORS' CONTRIBUTION

FMB wrote the manuscript and performed the experiment; TH supervised the experiment and co-work the manuscript, and RFS review and approved the final version of the work, MAC conducted all statistical analyses; Performed the experiment; RFS review and approved the final version of the work.

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