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Interaction Between Arbuscular Mycorrhizal and Antagonistic Rhizosphere Fungi in Peat Soil Enhancing Growth of *Coffea liberica* Seedlings

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ABSTRACT

This study aimed at determining the effect of Arbuscular Mycorrhizal Fungi (AMF) and Antagonistic Rhizosphere Fungi (ARF) on growth of *Coffea liberica* seedlings in peat soils. Eight AMF isolates (without AMF, *Glomus* sp.-1a, *Glomus* sp.-3c, *Acaulospora* sp.-1b, *Acaulospora* sp.-2d, *Glomus* sp.-1a + *Glomus* sp.-3c, *Acaulospora* sp.-1b + *Acaulospora* sp.-2d, and mixtures of *Glomus* sp.-1a + *Glomus* sp.-3c + *Acaulospora* sp.-1b + *Acaulospora* sp.-2d) were combined with five ARF types (without ARF, *Trichoderma* sp., *Aspergillus* sp., *Gliocladium* sp., and *Penicillium* sp.). Data were collected on the following variables: seedling height, leaf number, stem diameter, shoot and root dry weight, N and P uptake, and root infection by AMF. Results indicated that *Trichoderma sp.*, in combination with various types of AMF, was the best ARF in promoting *C. liberica* seedling growth and increasing N and P uptake. On the other hand, the mixture of *Glomus* sp.-1a + *Glomus* sp.-3c combined with various types of ARF was the best AMF in promoting seedling growth and increasing N and P uptake. It can be concluded that *Trichoderma* sp. and the mixture of *Glomus* sp-1a and *Glomus* sp-3c were best combination to be applied to promote the *C. liberica* seedlings grown in peat soil.

INTRODUCTION

Liberica coffee (*Coffea liberica*) is a coffee variety that thrives on the peat soil found in Jambi Province. It is widely grown in the Tanjung Jabung Barat Regency, which encompasses the districts of Pengabuan, Bram Itam, Senyerang, Kuala Betara, and Tungkal Hilir (Masyarakat Perlindungan Indikasi Geografis Kopi Liberika Tungkal Jambi, 2015). However, despite its adaptability to peat soil, Liberica coffee may not achieve optimal growth and production due to the limiting factors present in this type of soil, such as low chemical, physical, and biological fertility, as well as diseases caused by root fungi and leaf rust.

Efforts are needed to optimize the growth and production of Liberica coffee in peat soil, which is categorized as marginal with low chemical, physical, and biological fertility and is susceptible to diseases such as root fungi and leaf rust. One of these efforts is using beneficial rhizosphere microorganisms such as indigenous Arbuscular Mycorrhizal Fungi (AMF) and Antagonistic Rhizosphere Fungi (ARF). Previous reports indicate that AMF can improve water and nutrient uptake (Bhattacharjee & Sharma, 2012; Kartika et al., 2018; Treseder, 2013; Watts-Williams et al., 2014), increase plant tolerance against environmental stress (Ndiaye et al., 2011; Wu & Zou, 2010; Zhu et al., 2012) and heavy metal toxicity (Krishnamoorthy et al., 2015), enhance plant resistance to pests and diseases (Sylvia & Chellemi, 2001), control basal stem rot caused by *Ganoderma boninense* in oil palm (Rini, 2001), improve fruit nutritional quality of tomato (Hart et al., 2014) and increase the competitiveness of coffee plants against *Bidens pilosa* interference (França et al., 2016).

Antagonistic microorganisms present in the rooting zone have the potential to inhibit the spread of root infections caused by pathogens

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and can be highly effective as biological control agents. Each hostile fungus species is known to have the capacity to control various pathogenic fungi due to differences in their morphology and physiological characteristics. Studies have shown that *Trichoderma* sp. can control a range of fungal pathogens such as *Phytophthora palmivora, Rhizoctonia solani, Fusarium* spp., *Sclerotium rolfsii,* and *Pythium* spp. on crops such as peanut, tomato, cucumber, and durian (Ha, 2010). *Trichoderma* sp. is also as effective in controlling soil-borne fungal pathogens (Bastakoti et al., 2017), pistachio wilt disease (*Verticillium dahliae*) (Fotoohiyan et al., 2017), and cacao pod rot (*Phytophthora palmivora*) (Sriwati et al., 2019). Boughalleb-M'Hamdi et al. (2018) reported that *Aspergillus* sp. and *Trichoderma* spp. control *Macrophomina phaseolina*, *Fusarium solani,* and *Fusarium oxysporum* on melon and watermelon plants. Furthermore, Ruliyanti & Majid (2020) reported that applying *Gliocladium* sp. with vermicompost can control the pathogen *Fusarium oxysporum* and increase the growth and production of watermelon plants. Gómez-Muñoz et al. (2018) concluded a positive interaction between *Penicillium bilaii* and available phosphorus in increasing phosphorus uptake and maize plant growth.

Arbuscular Mycorrhizal Fungi and Antagonistic Rhizosphere Fungi synergistically increase growth, yield, and plant resistance against pathogens. Studies have shown that the application of AMF and ARF increases nutrient absorption and plant resistance to disease (Tsvetkov et al., 2014), promotes the growth of *Arachis hypogaea* (Yadav & Aggarwal, 2015), *Helianthus annuus* (Yadav et al., 2015), *Solanum lycopersicum* (Commatteo et al., 2019; Sohrabi et al., 2020), and apple seedlings (Zydlik et al., 2021), and increase productivity of Brassicaceae (Poveda et al., 2019). Furthermore, the application of AMF and ARF also increases the activity of defense enzyme and yield of *Capsicum annuum* (Duc et al., 2017), pigment, protein, and amino acids contents, as well as the activity of the phosphatase enzyme in *Allium cepa* (Metwally & Al-Amri, 2020; Metwally et al., 2021).

A previous study by Kartika et al. (2017) revealed that two genera of AMF (*Glomus* and *Acaulospora*) and four genera of ARF (*Aspergillus*, *Trichoderma*, *Gliocladium,* and *Penicillium*) were found in Liberica coffee rhizosphere in peat soil of Tanjung Jabung Barat Regency. Therefore, it is worthwhile to study the role of AMF and ARF in supporting the growth of Liberica coffee. This study aims to find the best interaction of AMF and ARF, which effectively promotes the seedling growth of *Coffea liberica* on peat soil.

MATERIALS AND METHODS

Study Area

This trial was conducted from May to December 2018 at Mekar Jaya Village, Betara District, West Tanjung Jabung Regency, Jambi. This area is located in geographic coordinates of 0.964334 °S and 103.383515 °E.

Experimental Design

This investigation employed a factorial completely randomized design. The first factor was the type of AMF (m0 = without AMF, m1 = *Glomus* sp.-1a, m2 = *Glomus* sp.-3c, m3 = *Acaulospora* sp.-1b, m4 = *Acaulospora* sp.-2d, m5 = mixture of *Glomus* sp.-1a + *Glomus* sp.-3c, m6 = mixture of *Acaulospora* sp.-1b + *Acaulospora* sp.-2d, m7 = mixture of *Glomus* sp.-1a + *Glomus* sp.-3c + *Acaulospora* sp.-1b + *Acaulospora* sp.-2d). The AMFs used were obtained from the rhizosphere of *Coffea liberica* grown in the study area.

The second factor was the type of ARF (f0 = without ARF, f1 = *Trichoderma* sp., f2 = *Aspergillus* sp., f3 = *Gliocladium* sp., f4 = *Penicillium* sp.). AMF and ARF were isolated from the rhizosphere of Liberica coffee grown in peat soil in Tanjung Jabung Barat Regency, Jambi Province. This made 40 combinations of AMF + ARF, repeated three times, resulting in 120 experimental units. Each unit consisted of 4 individual seedlings.

Media Preparation

Before sterilization, the growing media (peat soil) was wind-dried and sieved with a 10-mesh test sieve. The sterilization was done by heating the soil in a drum for about 8 hours. It was then put in black polyethylene bags (15 x 25 cm in size), each of 1.5 kg.

Seed Germination

Liberica seeds from the selected stock plant were sown on 5-cm thickness sand media on a seed bed. Seed germination occurred 30 days after sowing, and paranet protected seedlings from excessive sunlight and direct rainfall. Two-month-old healthy seedlings were transplanted into peat soil previously prepared in black polyethylene bags.

AMF and ARF Inoculation

The inoculum of AMF and ARF apply to the soil simultaneously as seedling transplantation.

Ten grams of each AMF and ARF inoculant were prepared for each seedling and thoroughly mixed with peat soil before use. Plant care was carried out daily, including watering, weeding, and pest and disease control.

Variables and Data Analysis

Data were collected on 7-month-old seedlings (5 months after transplantation). The observation was made on the height of seedlings, number of leaves, diameter of stems, dry weight shoots and roots, N and P uptakes, and root infection by AMF. Observation of AMF infections was done by root staining method (Kormanik & McGraw, 1982). Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) with α = 0.05 was employed in data analysis.

RESULTS AND DISCUSSION

Seedling Height

Combined with AMF, the highest seedling is achieved by applying *Trichoderma* sp. (Table 1). It shows a more compatible interaction between AMF and *Trichoderma* sp., compared to other ARF types (other than *Trichoderma* sp.). This interaction suggests that both fungi from the Liberica coffee rhizosphere can work synergistically in supporting the growth and development of seedlings. Similarly, Valentine et al. (2017) reported that a combination of AMF and *Trichoderma* sp. improved growth and seed production in rock melon (*Cucumis melo*). Besides, it also plays an essential role as an agent for soil biological control. Improving plant performance by the use of biological control can be caused by the role of plant pathogens. The same result is also reported by Krisdayani et al. (2020) on *Albizia chinensis*, Zydlik et al. (2021) on *Malus domestica,* and Sofian et al. (2022) on *Elaeis guineensis*, which show that the combination of FMA *Glomus* spp. and *Trichoderma* spp. can accelerate and increase the growth of seedlings.

Table 1. The average height (cm) of *C. liberica* seedlings grown in peat soil with different types of AMF + ARF (5-month-old)

Arbuscular Mycorrhizal Fungi (AMF)	Antagonistic Rhizosphere Fungi (ARF)				
	without ARF	*Trichoderma* sp.	*Aspergillus* sp.	*Gliocladium* sp.	*Penicillium* sp.
without AMF	11.17±0.50 $^{c}_{D}$	14.42±0.63 $^{a}_{D}$	12.50±0.25 $^{b}_{D}$	13.00 ± 0.75 $^{b}_{C}$	11.00±0.13 $^{c}_{D}$
Glomus sp-1a	14.83±1.25 $^{c}_{AB}$	16.08±0.13 $^{a}_{C}$	14.00±0.73 $^{b}_{C}$	14.83b±1.25 $^{c}_{B}$	13.83±0.75 $^{c}_{BC}$
Glomus sp-3c	13.67±0.50 $^{b}_{B}$	16.83±0.50 $^{a}_{C}$	12.83±0.00 $^{c}_{C}$	14.83±2.75 $^{b}_{B}$	14.00±0.25 $^{b}_{B}$
Acaulospora sp-1b	13.08±0.00 $^{bc}_{BC}$	16.25±1.63 $^{a}_{C}$	12.92±3.38 $^{c}_{C}$	11.17±1.25 $^{d}_{D}$	12.17±4.63 $^{cd}_{C}$
Acaulospora sp-2d	12.00±0.75 $^{d}_{CD}$	18.83±1.25 $^{a}_{B}$	14.33±1.75 $^{b}_{B}$	13.42±2.75 $^{bc}_{C}$	12.67±1.75$^{c}_{BC}$
Glomus sp-1a and 3c	13.17±1.25 $^{c}_{BC}$	21.83±1.00 $^{a}_{A}$	15.33±0.25 $^{c}_{A}$	16.83±0.38 $^{b}_{A}$	16.33±1.00 $^{bc}_{A}$
Acaulospora sp-1b and 2d	14.83±0.75 $^{b}_{A}$	21.33±0.25 $^{a}_{A}$	15.98±1.25 $^{ab}_{A}$	13.75±0.63 $^{b}_{BC}$	13.92±0.88 $^{b}_{B}$
Glomus sp-1a, sp-3c dan *Acaulospora sp-1b, sp-2d*	15.17±1.38 $^{b}_{A}$	16.17±0.25 $^{a}_{C}$	13.00±1.50 $^{bc}_{C}$	13.58±0.63 $^{b}_{C}$	12.33±0.75 $^{c}_{C}$

Remarks: Numbers followed by the same uppercase in the same columns and the same lowercase in the same rows indicate a non-significant difference according to DMNRT at α = 0.05

The highest seedling obtained at the combination of *Trichoderma* sp. + *Glomus* sp.-1a + *Glomus* sp.-3c and *Trichoderma* sp. + *Acaulospora* sp.-1b + *Acaulospora* sp.-2d. With *Aspergillus* sp., the highest seedling record in a combination of *Aspergillus* sp. + *Glomus* sp.-1a + *Acaulospora* sp.-2d. Applying *Gliocladium* sp. and *Penicillium* sp., the highest seedling growth is achieved in *Gliocladium* sp. + *Acaulospora* sp.-2d and *Penicillium* sp. + *Acaulospora* sp.-2d. Meanwhile, in the absence of ARF, applying *Glomus* sp.-1a, *Glomus* sp.-3c, and *Acaulospora* sp.-1b produced the highest seedlings (Table 1). These findings indicated that various types of ARF can interact with more than one type of AMF, where the combination of *Trichoderma* sp. + *Glomus* sp.-1a + *Glomus* sp.-3c and *Trichoderma* sp. + *Acaulospora* sp.-1b + *Acaulospora* sp.-2d showed the most significant seedling height. *Trichoderma* sp. can help create a healthy root environment so coffee seedlings can optimally absorb nutrients and water. According to Amiroh et al. (2020), *Trichoderma* sp. indirectly increases plant height by colonizing the root area and spreading to the root cortex so that the infection space for pathogens is reduced and plants can absorb water and nutrients. In addition, mycorrhizal hyphae also grew and spread out to increase the area of water and nutrient uptake. Thus, *Trichoderma* sp. and mycorrhizae cooperate synergistically in promoting plant growth.

Applying mycorrhizae also stimulates the formation of growth-stimulating hormones in plants, such as auxins and cytokinins, essential in cell division and elongation, increasing plant height. Haneefat et al. (2012) reported that soybeans treated with a combination of *Trichoderma harzianum* and *Glomus mosseae* grew better than those without treatment as the result of the accumulation of phytohormones, especially auxin and gibberellins. The ability of *Trichoderma* sp. to produce phytohormone auxin is determined by the presence of the main precursor L-tryptophan, which is created as plant exudate (Nafady et al., 2022).

Stem Diameter

The widest stem diameter is obtained using *Trichoderma* sp. with or without AMF. In the *Glomus* sp.-1a + *Glomus* sp.-3c + *Acaulospora* sp.-1b + *Acaulospora* sp.-2d, the widest diameter is achieved in combination with *Trichoderma* sp. and *Gliocladium* sp. (Table 2). The widest diameter is also found on the application of ARF along with *Glomus* sp.-1a + *Glomus* sp.-3c or *Glomus* sp.-3c without ARF (Table 2). This indicates that *Glomus* sp-1a or *Glomus* sp-3c can work synergistically with all types of ARF. *Trichoderma* sp. cooperates synergistically with all types of AMF in increasing the stem diameter of *C. liberica* seedlings. The research coincides with those of Idowu et al. (2016) findings on *Abelmoschus esculentus,* Dendang & Hani (2018) on *Calophyllum inophyllum*, and Krisdayani et al. (2020) on *Paraserianthes falcataria* seedlings.

Leaf Number

In the presence or absence of AMF, the most significant leaf number is obtained when *Trichoderma* sp. was applied (Table 3), which means that apart from being able to work alone, *Trichoderma* sp. can also work synergistically with all types of AMF. In addition, in applying *Glomus* sp-1a and the mixture of *Glomus* sp.-1a + *Glomus* sp.-3c, the highest growth rate is obtained with *Trichoderma* sp. and *Aspergillus* sp. This means that, in addition to *Trichoderma* sp. and *Aspergillus* sp. can work with those AMFs synergistically (Table 3). The results of this study follow previous investigations on the application of *Trichoderma* sp. and mycorrhiza, which are reported to increase plant growth (Iula et al., 2021; Metwally & Al-Amri, 2020; Metwally et al., 2021; Szczałba et al., 2019).

On the application of various ARF, the highest leaf number is obtained in combination with *Glomus* sp.-1a + *Glomus* sp.-3c, while in the absence of ARF, the highest leaf number is shown by *Glomus* sp.-3c (Table 3). *Glomus* sp. is a well-adapted AMF to a wide range of host plants and various environmental conditions. This species can work synergistically with different types of ARF (Asmarahman et al., 2018; Kartika et al., 2019; Rita et al., 2021; Sanana et al., 2022; Suryati, 2017; Tomo & Prasetya, 2021).

Table 2. The average stem diameter (mm) of *C. liberica* seedlings grown in peat soil with different types of AMF + ARF (5-month-old)

Arbuscular Mycorrhizal Fungi (AMF)	**Antagonistic Rhizosphere Fungi (ARF)**				
	without ARF	***Trichoderma* sp.**	***Aspergillus* sp.**	***Gliocladium* sp.**	***Penicillium* sp.**
without AMF	1.53±0.03 ^{b} F	1.85±0.03 ^{a} E	1.67±0.01 ^{ab} D	1.53±0.00 ^{b} C	1.62±0.01^{b} E
Glomus sp-1a	2.25±0.05 ^{b} CD	2.50±0.02 ^{a} BC	2.27±0.02 ^{b} AB	2.15±0.01 ^{b} AB	1.85±0.02 ^{c} D
Glomus sp-3c	2.53±0.23 ^{ab} A	2.63±0.00 ^{a} ABC	1.92±0.01d CD	2.15±0.25 ^{b} AB	2.35±0.30 ^{b} B
Acaulospora sp-1b	2.32±0.15 ^{ab} ABC	2.42±0.18 ^{a} C	2.18±0.32 bc B	2.07^{c}±0.47 ^{d} AB	1.90±0.01 ^{d} D
Acaulospora sp-2d	2.43±0.00 ^{bc} AB	2.70±0.00 ^{a} B	2.17±0.30 b BC	1.93±0.05 ^{c} B	1.97±0.18 ^{bc} CD
Glomus sp-1a and 3c	1.92±0.01 ^{c} E	2.80±0.08 ^{a} A	2.52±0.01 ab A	2.28±0.15^{c} A	2.65±0.20 ^{a} A
Acaulospora sp-1b and 2d	2.15±0.30 ^{b} CDE	2.68±0.13 ^{a} AB	1.90±0.15 c D	2.25±0.00 ^{c} A	2.20±0.35 ^{b} BC
Glomus sp-1a, sp-3c dan *Acaulospora sp-1b, sp-2d*	2.05±0.02 ^{ab} DE	2.15±0.01 ^{a} D	1.88±0.00 b D	2.12±0.01^{a} AB	1.88±0.00 ^{b} D

Remarks: Numbers followed by the same uppercase in the same columns and the same lowercase in the same rows indicate a non-significant difference according to DMNRT at α = 0.05

Table 3. The average leaf number of *C. liberica* seedlings grown in peat soil with different types of AMF + ARF (5-month-old)

Arbuscular Mycorrhizal Fungi (AMF)	**Antagonistic Rhizosphere Fungi (ARF)**				
	without ARF	***Trichoderma* sp.**	***Aspergillus* sp.**	***Gliocladium* sp.**	***Penicillium* sp.**
without AMF	8.00±0.00 ^{b} E	9.33±0.00 ^{a} E	9.00±0.00 ^{a} C	8.00±1.50 ^{b} D	8.00±1.00 ^{b} F
Glomus sp-1a	9.67±0.50 ^{b} C	10.67±0.50 ^{a} D	10.33±0.50 ^{a} B	10.33±0.00 ^{a} AB	8.67±0.50 ^{c} DE
Glomus sp-3c	11.00±0.00 ^{b} A	11.67±0.50 ^{a} BC	9.33±0.50 ^{d} C	10.00±0.50 ^{b} B	10.00±0.00 ^{c} B
Acaulospora sp-1b	10.67±0.00 ^{b} AB	11.33±1.00 ^{a} C	9.33±1.00 ^{d} C	10.00±0.00 ^{c} B	9.00±0.50 ^{d} CD
Acaulospora sp-2d	9.67±0.50 ^{c} C	11.67±0.50 ^{a} BC	10.00±0.50 ^{b} B	10.00±0.00 ^{c} B	8.33±0.50 ^{d} EF
Glomus sp-1a and 3c	9.00±0.00 ^{c} D	12.33±1.00 ^{a} A	11.33±0.50 ^{a} A	10.67±0.00 ^{b} A	10.67±0.50 ^{b} A
Acaulospora sp-1b and 2d	10.33±0.00 ^{b} B	12.00±0.50 ^{a} A	10.00±1.00 ^{b} B	9.33±1.00 ^{c} C	10.00±0.00 ^{b} B
Glomus sp-1a, sp-3c dan *Acaulospora sp-1b, sp-2d*	9.67±0.50 ^{b} C	10.67±0.50 ^{a} D	10.00±0.50 ^{b} B	10.00±0.00 ^{b} B	9.33±0.00 ^{c} C

Remarks: Numbers followed by the same uppercase in the same columns and the same lowercase in the same rows indicate a non-significant difference according to DMNRT at α = 0.05

Shoot Dry Weight

The heaviest shoot dry weight is obtained using *Trichoderma* sp., either with or without the AMF combination, followed by *Penicillium* sp. in combination with *Acaulospora* sp.-2d (Table 4). This indicates that *Trichoderma* sp. can work synergistically with all AMF species in increasing shoot dry weight, while *Penicillium* sp. could only synergize well with *Acaulospora* sp.-2d. *Trichoderma* sp. in the rhizosphere established an association, thus improving nutrient uptake and growth (Shoresh et al., 2010). It colonized within roots and improved nutrient uptake, resulting in better plant growth and development and enhanced plant resistance to abiotic stresses. Applying *T. harzianum* increased the concentration of trace and essential elements in the shoots and roots of cucumber and tomato seedlings (Azarmi et al., 2011). This is due to the production of phytohormones, siderophores, and phosphate-solubilizing enzymes (Doni et al., 2014). Phytohormones such as cytokinins, indole-3-acetic acid, and gibberellins (Tjamos et al., 2010) stimulate root growth and increase plant roots' porous surface. According to Khan et al. (2017), *Trichoderma* sp. increases growth and yield primarily through its ability to degrade complex organic in soil into simpler compounds available and easily absorbed by plants. Kour & Kaur (2022) reported that *Trichoderma* sp. acts as an agent of biocontrol and stimulates tolerance to abiotic stresses, resulting in better plant growth and yield.

In the application of ARF, the heaviest shoot dry weight is obtained with *Acaulospora* sp.-2d (Table 4), indicating that *Acaulospora* sp.-2d can cooperate with all types of ARF. A previous study shows that *Acaulospora* sp. is a type of AMF that increased the growth of *Canavalia ensiformis* (Akib et al., 2018) and *Hevea brassiliensis* seedlings (Margarettha, 2014). According to Sharma et al. (2017), *Trichoderma* sp. and AMF interact synergistically through a signal transduction mechanism by releasing biomolecular compounds that act as a messenger and are accepted by AMF as an interacting receptor.

Table 4. The average shoot dry weight (g) of *C. liberica* seedlings grown in peat soil with different types of AMF + ARF (5-month-old)

Arbuscular Mycorrhizal Fungi (AMF)	Antagonistic Rhizosphere Fungi (ARF)				
	without ARF	*Trichoderma* sp.	*Aspergillus* sp.	*Gliocladium* sp.	*Penicillium* sp.
without AMF	0.80 ± 0.01^{c} E	1.72 ± 0.02^{a} D	0.81 ± 0.00^{b} E	0.96 ± 0.00^{b} D	0.88 ± 0.00^{bc} E
Glomus sp-1a	1.63 ± 0.01^{bc} B	1.93 ± 0.00^{a} BC	1.48 ± 0.01^{c} B	1.49 ± 0.01^{c} C	1.74 ± 0.00^{b} B
Glomus sp-3c	1.83 ± 0.01^{a} BC	1.91 ± 0.01^{a} BCD	1.23 ± 0.01^{c} C	1.80 ± 0.01^{ab} AB	1.66 ± 0.01^{b} B
Acaulospora sp-1b	1.65 ± 0.01^{a} A	1.75 ± 0.01^{a} CD	1.74 ± 0.00^{a} A	1.36 ± 0.01^{b} C	1.36 ± 0.01^{b} CD
Acaulospora sp-2d	2.04 ± 0.01^{b} E	2.26 ± 0.01^{a} A	1.75 ± 0.01^{c} A	1.92 ± 0.14^{b} A	2.22 ± 0.39^{a} A
Glomus sp-1a and 3c	1.63 ± 0.59^{b} C	1.82 ± 0.10^{a} BCD	0.88 ± 0.50^{d} D	1.33 ± 0.07^{c} C	1.20 ± 0.54^{c} D
Acaulospora sp-1b and 2d	1.83 ± 0.55^{b} B	2.00 ± 0.05^{a} B	1.45 ± 0.38^{c} BC	1.95 ± 0.07^{ab} A	1.58 ± 0.49^{c} BC
Glomus sp-1a, sp-3c dan *Acaulospora sp-1b, sp-2d*	1.36 ± 0.13^{d} D	1.89 ± 0.11^{a} BCD	1.53 ± 0.15^{c} B	1.72 ± 0.16^{b} B	1.26 ± 0.08^{d} D

Remarks: Numbers followed by the same uppercase in the same columns and the same lowercase in the same rows indicate a non-significant difference according to DMNRT at $\alpha = 0.05$

Root Dry Weight

The heaviest root dry weight is obtained on the application of *Trichoderma* sp. with or without AMF, followed by *Penicillium* sp. in combination with *Acaulospora* sp.-2d and *Gliocladium* sp. in combination with *Acaulospora* sp.-1b + *Acaulospora* sp.-2d (Table 5). Meanwhile, on medium supplemented with various types of ARF or without ARF, the highest root dry weight is obtained in combination with *Acaulospora* sp.-2d. (Table 5). The healthy growth of Liberica coffee seedlings by AMF application is presumably due to increased nitrogen and phosphorus uptakes made possible through arbuscular mycorrhizal association. The association increases root growth and improves root interception and mineral absorption. The capacity of AMF mycelia for water and mineral absorption is higher than plant roots, thus improving water and mineral absorption in plants. It is reported by Nzanza et al. (2012), Colla et al. (2014) and Valentine et al. (2017) that the combination of mycorrhizae and *Trichoderma* sp. stimulated healthy root growth. Mycorrhizal infection might also increase root length by the formation of mycorrhizal hyphae.

Contreras-Cornejo et al. (2020) claim that the indole-3-acetic acid produced by *Trichoderma* sp. stimulated root growth and changed root architecture, increasing root mass and area for microbial colonization and improving nutrient uptake. Nafady et al. (2022) prove that *Trichoderma* sp. and mycorrhizae also increase plant growth and nutrient uptake, stimulate plant resistance, and reduce nematode populations and penetration rates. In addition, *T. harzianum,* as a biocontrol agent, could produce plant growth-promoting substances and hydrolytic enzymes.

N Uptake

Either with or without combination with AMF, the application of *Trichoderma* sp. results in the highest N uptake (Table 6). On the other hand, with the application of ARF, the most increased N uptake is recorded on applying a mixture of *Glomus* sp.-1a + *Glomus* sp.-3c, either in combination with *Trichoderma* sp. Meanwhile, with *Aspergillus* sp. or *Penicillium* sp., the highest N uptake is recorded with *Acaulospora* sp.-2d. In applying *Gliocladium* sp., the most increased N uptake is shown by its combination with the mixture of *Acaulospora* sp.-1b + *Acaulospora* sp.-2d (Table 6).

Table 5. The average root dry weight (g) of *C. liberica* seedlings grown in peat soil with different types of AMF + ARF (5-month-old)

Arbuscular Mycorrhizal Fungi (AMF)	Antagonistic Rhizosphere Fungi (ARF)				
	without ARF	*Trichoderma* sp.	*Aspergillus* sp.	*Gliocladium* sp.	*Penicillium* sp.
without AMF	0.30±0.01 ^{c} C	0.67±0.01 ^{a} C	0.33±0.01 ^{c} E	0.65±0.01 ^{a} E	0.53±0.00 ^{b} F
Glomus sp-1a	0.59±0.01 ^{bc} B	0.79±0.01 ^{a} B	0.57±0.01 ^{c} D	0.67±0.01 ^{b} CD	0.55±0.01 ^{c} EF
Glomus sp-3c	0.75±0.01 ^{c} A	0.97±0.02 ^{a} A	0.48±0.01 ^{d} BC	0.69±0.00 ^{c} BC	0.86±0.01 ^{b} B
Acaulospora sp-1b	0.71±0.01 ^{b} A	0.94±0.00 ^{a} A	0.67±0.00 ^{cd} A	0.59±0.01 ^{d} B	0.79±0.01 ^{b} BC
Acaulospora sp-2d	0.73±0.12 ^{c} A	1.00±0.08 ^{a} A	0.86±0.07 ^{b} D	0.83±0.10 ^{b} B	0.99±0.20 ^{a} A
Glomus sp-1a and 3c	0.69±0.04 ^{b} A	0.99±0.01 ^{a} A	0.48±0.03 ^{c} D	0.70±0.21 ^{b} B	0.48±0.10 ^{b} F
Acaulospora sp-1b and 2d	0.39±0.16 ^{d} C	0.97±0.10 ^{a} A	0.55±0.27 ^{c} D	0.93±0.21 ^{a} A	0.70±0.33 ^{b} CD
Glomus sp-1a, sp-3c dan *Acaulospora sp-1b, sp-2d*	0.57±0.20 ^{bc} B	0.78±0.02 ^{a} BC	0.72±0.13 ^{a} B	0.52±0.05 c E	0.63±0.20 ^{b} DE

Remarks: Numbers followed by the same uppercase in the same columns and the same lowercase in the same rows indicate a non-significant difference according to DMNRT at α = 0.05

The research results show that different types of AMF applied to peat soil worked synergistically with *Trichoderma* sp. This is following the work by Bhuvaneswari et al. (2014) on *Capsicum annuum,* Dehariya et al. (2015) on pigeon pea, Ban et al. (2018) and Domínguez et al. (2016) on *L. esculentum* and *Phaseolus vulgaris*, and Halifu et al. (2019) on *Pinus sylvestris. Trichoderma* sp. produces decomposing enzymes that will decompose organic materials. The decomposition process releases nutrients, primarily N and P, bound in complex compounds. Thus, *Trichoderma* sp. increased enzyme activity and nutrient content in soil rhizosphere and promoted nutrient transfer from soil to roots. This is because *Trichoderma* sp. can colonize the interior of roots (Kleifeld & Chet, 1992). *Trichoderma* sp. influenced plant development by the production of hormones (Windham et al., 1986), solubilization of insoluble nutrients (Altomare et al., 1999), increasing less-available minerals uptake and translocation (Baker, 1989), and production of plant hormone analogous (Cutler et al., 1989).

Marwani et al. (2013) reported that mycorrhizae can increase the absorption of N, P, K, Ca, and Mg elements. Nitrogen is one of the most essential elements in forming chlorophyll. Mycorrhizae can increase nitrogen absorption due to the presence of the nitrate-reductase enzyme so that mycorrhizae can absorb nitrogen in the form of nitrate (Susilo, 2018). Kartika et al. (2018) also found that *Jatropha curcas* treated with mycorrhiza have significantly higher levels of N, P, and K than those without mycorrhiza. The application of mycorrhiza also saved the use of P fertilizer by 50%.

P Uptake

Applying *Trichoderma* sp. with or without AMF results in the most significant P uptake (Table 7). Further, the highest rate of P uptake is obtained when a mixture of *Glomus* sp.-1a + *Glomus* sp.-3c is combined with *Trichoderma* sp. However, *Acaulospora* sp.-2d resulted in the most significant P uptake when applied solely or combined with *Aspergillus* sp. or *Gliocladium* sp. (Table 7).

Table 6. Nitrogen uptake (mg per plant) of *C. liberica* seedlings grown in peat soil with different types of AMF + ARF (5-month-old)

Arbuscular Mycorrhizal Fungi (AMF)	**Antagonistic Rhizosphere Fungi (ARF)**				
	without ARF	***Trichoderma* sp.**	***Aspergillus* sp.**	***Gliocladium* sp.**	***Penicillium* sp.**
without AMF	15.72±0.00 ^{cd} F	47.53±0.00 ^{a} E	20.02±0.02 ^{c} E	30.03±0.41 ^{b} D	24.04±0.05 ^{c} E
Glomus sp-1a	36.08±0.13 ^{c} CD	58.97±0.03 ^{a} D	40,49±0.35 ^{by} B	42.76±0.19 ^{b} C	36.44±0.04 ^{c} C
Glomus sp-3c	38.9±0.11 ^{c} BCD	58.65±0.06 ^{a} D	33.35±0.09 ^{d} C	42.39±0.07 ^{c} C	44.19±0.16 ^{b} B
Acaulospora sp-1b	35.62±0.06 ^{bc} D	59.10±0.03 ^{a} D	39.81±0.23 ^{b} B	33.04±0.26 ^{c} D	33.42±0.05 ^{c} D
Acaulospora sp-2d	43.22±0.43 ^{d} AB	63.81±0.30 ^{a} CD	50.50±0.23 ^{c} A	48.25±0.78 ^{c} B	59.33±0.90 ^{b} A
Glomus sp-1a and 3c	44.48±1.06 ^{b} A	73.96±0.33 ^{a} A	27.82±1.09 ^{d} D	42.12±0.59 ^{b} C	32.68±1.14 ^{c} D
Acaulospora sp-1b and 2d	39.77±1.32 ^{c} ABC	69.71±0.42 ^{a} AB	40.77±1.39 ^{c} B	57.24±056 ^{b} A	41.22±1.57 ^{c} BC
Glomus sp-1a, sp-3c dan *Acaulospora sp-1b, sp-2d*	29.26±0.05 ^{c} C	66.37±1.17 ^{a} BC	44.B02±0.60 b	41.58±0.12 ^{b} C	32.01±0.29 ^{c} D

Remarks: Numbers followed by the same uppercase in the same columns and the same lowercase in the same rows indicate a non-significant difference according to DMNRT at α = 0.05

Trichoderma and AMF work synergistically in increasing nutrient uptake to increase the growth of Liberica coffee seedlings. Al-Asbahi (2012) reported that the symbiosis of *Trichoderma harzianum* Rifai KRL-AG2 and AMF in wheat results in the release of volatile biomolecules by the *T. harzianum* which indirectly strengthens the association between AMF and wheat roots, and the presence of a wheat protein that is homologous to arbuscular mycorrhizal proteins that play a role in AMF-plant interactions.

Furthermore, Dwiastuti et al. (2015) suggested that *Trichoderma* sp. have more opportunity to compete for a place to live and food sources, quickly penetrate the cell wall, and enter the cell to take nutrients and produce antibiotics that kill pathogenic fungal cells. Therefore, *Trichoderma* sp. helps create a healthy root area so plants can optimally absorb nutrients and water. Basri (2018) claimed that the water and nutrient uptake area increased as mycorrhizal hyphae network expanded. The finer size of hyphae compared to root hairs allowed them to infiltrate soil micropores to absorb water in shallow soil water content. Water uptake by mycorrhizal-associated plants also carries dissolved nutrients such as N, K, and S by mass flow. In addition, high P uptake is also due to the fact that fungal hyphae secreted phosphatase enzymes that released P from specific bonds, making it available to plants.

Root Infection

There is no interaction between AMF and ARF in the root infection variable (Table 8). In the AMF group, the highest root infection is achieved by the mixture of *Glomus* sp.-1a + *Glomus* sp.-3c (93.67%). However, this did not significantly differ from *Acaulospora* sp.-2d (93.33%), *Glomus* sp.-3c (92.67%), *Acaulospora* sp.-1b + *Acaulospora* sp.-2d (92.00%), or *Glomus* sp.-1a + *Glomus* sp.-3c + *Acaulospora* sp.-1b + *Acaulospora* sp.-2d (92%). Whereas in the ARF group, the highest root infection rate is on the application of *Trichoderma* sp. (84.38%), though it does not significantly differ from *Aspergillus* sp. (81.04%) or ARF-free treatment (80.21%). Chandanie et al. (2009) found that *Glomus mosseae* on root growth of *Cucumis sativus* does not depend on ARF. However, *G. mosseae* colonization may be increased by the presence of *Trichoderma* sp.

Table 7. Phosphorus absorption (mg per plant) of *C. liberica* seedlings grown in peat soil with different types of AMF + ARF (5-month-old)

Arbuscular Mycorrhizal Fungi (AMF)	Antagonistic Rhizosphere Fungi (ARF)				
	without ARF	*Trichoderma* sp.	*Aspergillus* sp.	*Gliocladium* sp.	*Penicillium* sp.
without AMF	2.90±0.02 $^{c}_{E}$	7.86±0.02 $^{a}_{D}$	3.01±0.04 $^{c}_{E}$	4.81±0.00 $^{b}_{C}$	4.25±0.00 $^{b}_{D}$
Glomus sp-1a	5.96±0.03 $^{cd}_{C}$	9.34±0.04 $^{a}_{BC}$	5.64±0.02 $^{d}_{B}$	6.60±0.01 $^{bc}_{B}$	7.17±0.00 $^{b}_{A}$
Glomus sp-3c	7.49±0.00 $^{b}_{B}$	9.24±0.01 $^{a}_{C}$	4.67±0.00 $^{c}_{CD}$	7.50±0.00 $^{b}_{A}$	7.11±0.02 $^{b}_{A}$
Acaulospora sp-1b	6.74±0.02 $^{b}_{BC}$	8.28±0.00 $^{a}_{D}$	6.82±0.06 $^{b}_{A}$	5.09±0.00 $^{d}_{C}$	5.86±0.02 $^{c}_{B}$
Acaulospora sp-2d	8.22±0.03 $^{b}_{A}$	9.23±0.09 $^{a}_{C}$	7.54±0.02 $^{c}_{A}$	7.78±0.02 $^{bc}_{A}$	6.67±0.06 $^{d}_{AB}$
Glomus sp-1a and 3c	7.57±0.23 $^{b}_{AB}$	13.86±0.06 $^{a}_{A}$	4.17±0.14 $^{e}_{D}$	6.01±0.10 $^{c}_{B}$	5.09±0.18 $^{d}_{B}$
Acaulospora sp-1b and 2d	6.54±0.21 $^{c}_{C}$	10.93±0.13 $^{a}_{AB}$	5.13±0.08 $^{d}_{BC}$	7.66±0.08 $^{b}_{A}$	6.85±0.25 $^{c}_{A}$
Glomus sp-1a, sp-3c dan *Acaulospora sp-1b, sp-2d*	4.93±0.03 $^{c}_{D}$	9.43±0.02 $^{a}_{BC}$	5.82±0.06 $^{b}_{B}$	6.44±0.01 $^{b}_{B}$	4.98±0.03 $^{c}_{C}$

Remarks: Numbers followed by the same uppercase in the same columns and the same lowercase in the same rows indicate a non-significant difference according to DMNRT at α = 0.05

Table 8. The percentage of root infection on *C. liberica* seedlings grown in peat soil with different types of AMF + ARF (5-month-old)

Treatments	No ARF	*Trichoderma* sp.	*Aspergillus* sp.	*Gliocladium* sp.	*Penicillium* sp.
No AMF	1.67±0.00	0.00±0.00	1.67±2.50	3.33±2.50	1.67±0.00
Glomus sp.-1a	91.67±0.00	93.33±0.00	91.67±2.50	90.00±0.00	91.67±0.00
Glomus sp.-3c	91.67±0.00	96.67±0.00	93.33±2.50	91.67±2.50	90.00±0.00
Acaulospora sp.-1b	90.00±0.00	95.00±0.00	91.67±0.00	91.67±2.50	90.00±0.00
Acaulospora sp.-2d	91.67±2.50	100.00±0.00	93.33±2.50	90.00±2.50	91.67±0.00
Glomus sp.-1a + sp.-3c	93.33±2.50	98.33±2.50	93.33±2.50	91.67±2.50	91.67±2.50
Acaulospora sp.-1b + sp.-2d	91.67±2.50	95.00±0.00	91.67±2.50	90.00±0.00	91.67±0.00
Glomus sp.-1a + sp.-3c + *Acaulospora* sp.-1b + sp.-2d	90.00±0.00	96.67±0.00	91.67±2.50	91.67±2.50	90.00±0.00

CONCLUSION

Trichoderma sp. and the mixture of *Glomus* sp.-1a + *Glomus* sp.-3c is the best combination of Antagonistic Rhizosphere Fungi and Arbuscular Mycorrhizal Fungi to be applied to promote the growth of *Coffea liberica* seedlings in peat soil.

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