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Arbuscular mycorrhizal fungi (AMF) product for enhancing plant growth promotion and plant protection in *Piper longum* L., *Zea mays* L. and *Coffea arabica* L.

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Abstract An investigation was determined the effectiveness of using Arbuscular Mycorrhizal Fungi (AMF) to enhance plant growth and protect host plants from fungal pathogen diseases. The study had also identified the best substrates for producing AMF spores for biofertilizer products. First, the dominant AMF species were isolated from soils where corn, pepper, and coffee were grown. They were identified as *Acaulospora longula* (Aca1) and *Gigaspora marganita* (Gig2, Gig3). These AMF spores were added to corn seeds, and after three days, fungal pathogens were introduced. The results showed that all AMF species promoted corn seed germination and plant growth. The best substrate for Aca1 to amplify its spores in host plant was MT5, consisting of soil, sand, and coconut fiber in the ratio of 1:1:1. Meanwhile, MT2, consisting of 2 parts soil, 1 part sand, and 1 part coconut fiber, was found to be the best substrate for Gig2 and Gig3; numbers of AMF spores were 487.5, 351.5, and 350.00 spores per 100 g substrate, respectively. These AMF spores's production were then tested for their effect on *Phytophthora capsici* Leonian, *Fusarium oxysporum* Schltdl, and *Rhizoctonia solani* Kuhn, causing root rot disease on *Piper longum* L., *Coffea arabica* L., and smut disease in *Zea mays* L. The results showed that AMF could protect plants from fungal pathogen diseases, reducing the number of infected host plants by 35 % after 3 months of inoculation in *P. longum*, 10 % in *C. arabica*, and 33.4 % in *Z. mays.*

Keywords: Biofertilizer, Corn seed germination, Plant pathogen

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Introduction

Arbuscular mycorrhizal fungi (AMF) are members of the sub-phylum Glomeromycota with almost 230 species actually described (Ahanger *et al*., 2014). The origin of AMF at least 400 million years ago indicates they are critical to growth and reproduction of both plant and fungus (Azcon-Aguilar and Barea, 1997). As a result of co-evolution, AMF are found in most habitats worldwide and in approximately 90 % of all plant species (Campos-Soriano *et al.*, 2012). These associations are "mutualistic" in that both host and fungus benefit. The plant receives inorganic nutrients and water from fungal hyphae foraging far beyond the root zone. The fungus, in turn, obtains a steady supply of carbon and energy directly from the plant with a minimum of competition from other soil microbes. Mycorrhizal symbioses benefit plant growth and plant protection (Jung *et al.*, 2015, Le *et al.*, 2018). Maize is one of the staple crops and pepper and coffee are industrial crops in Vietnam. They play an important in the economic development of agriculture in Vietnam, especially in the Tay Nguyen region. There have been few studies of the mycorrhizal fungi diversity in these crops (Mathur *et al.*, 2016, Pavithra and Yapa, 2018). However, their role in plant promotion and plant protection has not been studied extensively. In this study, AMF from maize was isolated from pepper and coffee field and investigated their diversity and analyzed their effects on plant growth promotion and plant protection.

Materials and methods

The research was taken from 2018 - 2022 in The Institute of Microbiology and Biotechnology- Vietnam National University; Plant protection Instutute and Institute of Natural Product Chemistry, VAST, Vietnam.

Sampling and medium preparation

AMF were isolated from 15 soil samples cultivated with maize (collected in Ha Noi, Viet Nam), 15 soil samples cultivated with pepper, and 15 soil samples cultivated with coffee (collected in Dak Lak, Vietnam). To collect soil samples, a 3 cm layer of surface soil around the tree roots was remove, and approximately 500 g of soil around each sample was taken to a depth of about 20 cm. The media for testing AMF using for corn growth promotion consisted of 5 solid media and a liquid MRS medium. The solid media were used for cultivated corn, the liquid medium was used for applying to the solid medium.

The solid media are: MT1 (1 V_{soil} : 1 V_{sand} : 0.5 $V_{coconut\ fiber}$), MT2 (2 V_{soil} : 1 V_{sand} : 1 $V_{coconut\ fiber}$), MT3 (0 V_{soil} : 1 V_{sand} : 0 $V_{coconut\ fiber}$), MT4 (1

V_{soil} : 2 V_{sand} : 1 $V_{coconut\ fiber}$), MT5 (1 V_{soil} : 1 V_{sand} : 1 $V_{coconut\ fiber}$). The liquid medium is MRS, which consisted: 10 mL solution 1; 10 mL solution 2; 5 mL solution 3; 5 mL solution 4; 1 mL solution 5; and 10 g of sucrose per litter (Solution 1 consisted of: KNO_3 0.76 %, KCl: 0.65, $MgSO_4.7H_2O$: 7.39 % and KH_2PO_4: 0.041 %; Solution 2 contained: $Ca(NO_3)_2.4H_2O$ 3.59 %; Solution 3 included: Calcium panthotenate 0.009 %, Nicotinic acid (2000X): 0.01 %, Biotin: 0.00001 %, Thiamine hydrochloride: 0.01 %, Pyridoxine hydrochloride: 0.009 %, Cyanocobalamine: 0.004 % and stock in -20^oC; Solution 4 contained: NaFeEDTA 0.016 %; Solution 5 comprised of: 100 mL $MnSO_4.5H_2O$ 1.234 %, 100 mL of H_3BO_3 0.925 %, 5 mL of $CuSO_4.5H_2O$ 2.2 %, 1 mL of $Na_2MoO_4.2H_2O$ 0.12 %, and 1 mL of $(NH_4)_6Mo_7O_{24}.4H_2O$ 1.7 %).

Isolation of AMF

AMF was isolated by wet sieving method (Rillig and Mummey, 2006) and single spore isolation method. One hundred gram of soil samples was mixed into 500 mL of tap water and mixed, then allow heavier particles to settle for a few second. The soil solution was poured through 0.56 µm sieve to remove large pieces of soil particles, and wash the sieve with tap water to ensure that all small particles have passed through; re-suspend the liquid solution through the coarse sieve again, and allow remaining particles to settle for a few seconds. The suspension was pass through a series of 250 - 500 µm sieves until retaining the desired spores. The fungal spores were placed to a petri dish and examined under a dissecting microscope. Single spore was isolated under a stereo microscope and collected for further experiment.

Sterilization of AMF spores

Axenic culture: 15 mL spore suspension in a 50 mL sterilized centrifuge bottle was transferred to an equal amount of double strength surface sterilant (2 % chloramine). The spore suspension was shacked twice and allowed to stand for two minutes, then centrifuge for 3 minutes at 1750 rpm. It was resuspended in antibiotic solution of 1 % gentamycin sulfate, 2 % streptomycin sulfate (Selosse *et al.*, 2015), and stored at 4^oC until use. The fungal spores were surface sterilized with 0.05 % sodium hypochlorite for 5 minutes and resuspended the spores in sterile distilled water and store at 4 ^oC for further experiment.

For other purposes: Surface sterilizes them with 0.05 % sodium hypochlorite for 5 minutes using the same method as above. Resuspend the spores in sterile distilled water and store at 4 ^oC until use.

Morphological classification

Each type of spore was mounted in water and Melzer's reagent (Sharma *et al*., 2017) for identification (Olympus CX41, Japan). Single spore was observed before and after staining Melzer's reagent under microscope. The identification was based on spore colour, size, surface ornamentation and wall structure with references to the descriptions and pictures which provided by the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi.

DNA extraction, PCR amplification and sequencing

DNA extraction from single AMF spore was done by grinding with 0.1 g glass bead, then adding 250 ml of sufficient extraction buffer of 50 mM Tris-HCl (pH 8.0), 50 mM EDTA, 1 % betamecaptoethanol and 3 % sodium dodecylsulfate for 30 minutes. The extraction was performed by adding an equal volume of phenol/chloroform/isoamyl alcohol at the ration of 25:24:1 (PCI), mixed and centrifuged at 14000 rpm for 5 minutes at room temperature. The upper surface was transferred to a new tube and extracted with PCI, and followed by centrifugation as above before precipitation with 0.7 volume of isopropanol and 0.1 volume of 3M sodium acetate for 2 hours at -20 ^{o}C. The sample was then centrifuged at 4 ^{o}C for 15 min at 14000 rpm. The DNA pellet was rinsed with 70 % EtOH, air dried and re-suspended in 100 µl of distilled water.

PCR amplification

The fungus-specific primers NS1 (5'-GTA GTCATA TGC TTG TCT C-3') and NS4 (5'-CTT CCG TCAATT CCT TTA AG-3') were used for amplification of 18S rRNA (Smith and Read, 2008). The second PCR (nested PCR) were carried out using AML1 (5'-ATC AAC TTTCGA TGG TAGGAT AGA-3') and AML2 (5'-GAACCC AAA CAC TTT GGT TTC C-3') (Spatafora *et al.*, 2016). Amplification of the DNA fragments were performed using the GeneAmp PCR System 9700 (Applied Biosystems) under the following thermal cycling program: eight mins of denaturation at 94 ℃, 35 thermal cycles of 30 s at 94 ℃, 45 s at 48 ℃, and 3 min at 72 ℃ were performed, followed by an extension step at 72 ℃ for 10 min. The PCR product were checked on 0.8 % agarose gel. Checking electrophoresis and purified using an AMPureKit (Agencourt Biosciences, Beverly, MA, USA). Sequencing reactions were performed by using the Big Dye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems) and the same PCR primers.

Phylogenetic Analysis

All the sequences were assembled and edited manually using BioEdit ver. 7.09 (Tom Hall, Ibis Biosciences, Carlsbad, CA, USA). The sequences were aligned with GenBank sequences retrieved from BLAST searches in the NCBI database (http://www.ncbi.nlm.nih.gov/) by using MEGA X (Kumar *et al.*, 2018). The phylogenetic analysis was performed based on their rDNA 18S regions. The maximum likelihood method and general time reversible model were used to infer the evolutionary history (Nei and Kumar, 2000). The bootstrap consensus tree inferred from 100 replicates was used to represent the evolutionary history of the analyzed taxa. The branches that corresponded to partitions reproduced in less than 50 % bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) is shown next to the branches. The initial tree(s) for the heuristic search were obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. A discrete gamma distribution was used to model the evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.6268)). The rate variation model allowed some sites to be evolutionarily invariable ([+*I*], 1.81 % sites). All positions with less than 95 % site coverage were eliminated, i.e., fewer than 5 % alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). There were a total of 434 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Nei and Kumar, 2000; Kumar *et al.*, 2018).

Testing AMF for maize seed germination with and without fungal pathogen

To test the activity of AMF on fungal pathogen by seeding maize seeds on a moist chamber (Petri dish), including 8 experiments (T1 - T8), 60 seeds/experiment (20 seeds per Petri dish, each experiment repeated three times). Each experiment corresponding to 1 - 2 species of fungal pathogens. The seed were sown and treated with AMF for 3 days. Then infected with fungal pathogens and monitored the experiments for 10 days for seed germination, calculate the maize seed germination ratio, the root length and the sprout length.

Eight treatments were designed as follow: T1 = Control (maize seed soaked with sterilized), T2 = maize seed treated with *Phytophthora capsici*, T3 = maize seed treated with *Acaulospora longula* (Aca1), T4 = maize seed treated with *Gigaspora marganita* (Gig2), T5 = maize seed treated with *Gigaspora marganita* (Gig3), T6 = maize seed treated with *Acaulospora longula* (Aca1) + *P. capsici*, T7 = maize seed treated with *Gigaspora*

marganita (Gig2) + *P. capsici*, T8 = maize seed treated with *Gigaspora marganita* (Gig3) + *P. capsici*.

Testing AMF for their spore amplification in corn

Maize seeds were surface-sterilized using 0.05 % NaOCl which put into prepared coconut fiber pots and socked in water for 30 min then sterilized at 120^{o} C for 20 min, the MRS medium was used for irrigating to the inoculum port every day. After 4 days of germination, each seeding pot was added 10 AMF spores, and without AMF served as control pot. Then, the seedlings were planted into inoculant chamber containing 500 g substrates (soil, sand and coconut fiber at difference ratios, MT1- MT5). The number of AMF, height and weight of corn, and AMF spore numbers were checked.

Effect of AMF on fungal pathogens of the host plant

Five grams of each AMF product (Gig2 and Gig3 inoculated in medium MT2, and Aca1 inoculated on medium MT5) prepared as described above were added to their respective host plant. Then the fungal pathogen was added into their host. After one week of AMF inoculation, the fungal pathogens were added to their host plants. Plant protection of AMF were checked after one, two- and three-months inoculation using parameters such as the ratio of plants infected by fungal pathogens, the length of the oldest shoot, the number of new shoots and other characteristics of the leaves and roots, such as color and the number of new roots.

Results

Isolation and identification of AMF

From 15 soil samples collected from a corn-cultivated field in Luong Son, Hoa Binh province, 1564 AMF spore were isolated. They were divided into 5 genera: *Acaulospora, Dentiscutata, Gigaspora, Glomus* and *Rhizopagus*. *Acaulospora* was the most abundant genus, comprising of 27.19 % of the total spores. While 524 AMF spores were isolated from 5 soil samples collected from a coffee-cultivated field in Dak Lak province. They were distributed to: *Acaulospora, Dentiscutata, Gigaspora, Glomus* and *Rhizopagus*. *Gigaspora* was the most abundant genus, comprising 36.7 % of the total spores. And 669 AMF spores were isolated from 5 pepper-cultivated soil in Dak Lak which distributed among *Acaulospora, Dentiscutata, Gigaspora* and *Glomus*. *Gigaspora* was the most abundant genus in this case as well, comprising of 33.9 % of the total spores. The most abundant AMF isolated from corn (*Acaulospora*- Aca1), coffee

(*Gigaspora*- Gig2) and pepper (*Gigaspora*- Gig3) were selected for the next experiments. They were identified using 18S rDNA analysis with nested PCR and the NS1/NS4 and AML1/AML2 primer pairs. The results are presented in Figure 1 showed that Aca1 was nested in a single clade containing *Acaulospora longula* and *Acaulospora* sp. V3 with 57 % bootstrap value; Gig2 and Gig3 were nested in clade contained the *Gigaspora margariata* with 52 % bootstrap value (Figure 1). These results were once again made confirm the morphological identification for the conclusion name of the Aca1 should belonged to the *A. longula*, Gig2 and Gig3 should be *G. margariata*.

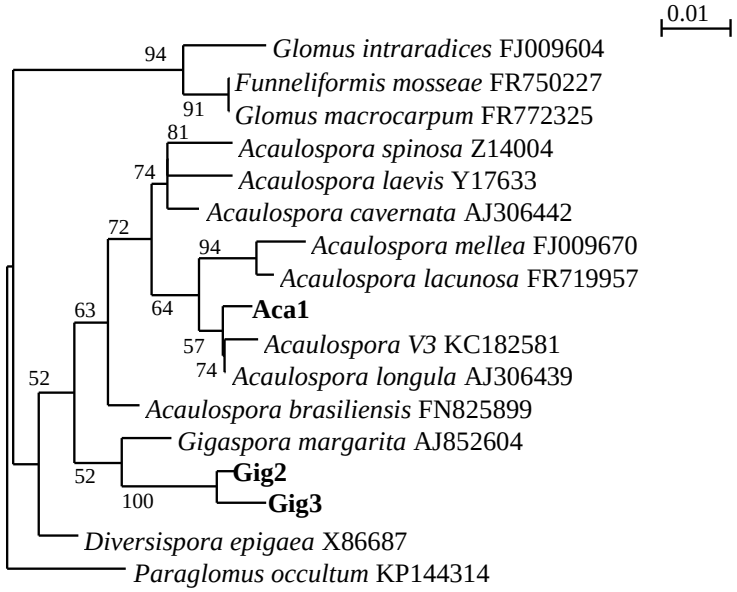

Figure 1. Phylogeny of the dominant AMF isolated from soil cultivated corn (Aca1), soil cultivated peppers (Gig2) and soil cultivated coffee (Gig3)

AMF enhance corn seed germination and their antifungal pathogen ability

The germination ratios of corn seed were tested with and without fungal pathogen infection. The results showed that in the none treated experiment, corn seed germinated rate was 90 ± 2 %; the root and sprout length were 11.2 mm and 10.5 mm, respectively. In in the AMF treated experiments, the germination ratios increase to 100 % in all 3 types of AMF; the root and sprout length increased to 17.3 - 18.6 mm and 12.1 - 19.1 mm, respectively.

In the experiment that plants were treated with *P. capsici*, the germination ratio of corn seed decreased to 26.65 %; and the root length, sprout length were 5.3 mm and 3.5 mm, respectively. While in the experiments that pathogen agents (*P. capsici*) were added after 3 days AMF

inoculation, the germination ratio of corn seed increased to 48.5 – 70 %, the root lengths were 6.2 - 7.2 mm and the sprout lengths were 4.7 - 6.5 mm, respectively (Table 1).

Table 1. The ability of AMF to promote maize seed germination and their ability to resistance fungal pathogens

Treatments	**Maize seed germination ratio (%)**	**Root length (mm)**	**Sprout length mm)**
T1 = None treated	$90^{a} \pm 2$	$11.2^{a} \pm 0.2$	$10.5^{a} \pm 0.2$
T2 = *Phytophthora* sp	$26.65^{b} \pm 3$	$5.3^{b} \pm 0.1$	$3.5^{b} \pm 0.3$
T3 = *A. longula* (Aca1)	$100^{c} \pm 0$	$17.3^{c} \pm 0.1$	$12.2^{a} \pm 0.2$
T4 = *G. gigantea* (Gig2)	$100^{c} \pm 0$	$18.1^{c} \pm 0.3$	$19.1^{c} \pm 0.2$
T5 = *G. marganita* (Gig3)	$100^{c} \pm 0$	$18.\ 6^{c} \pm 0.2$	$12.1^{a} \pm 0.02$
T6 = *A. longula* (Aca1) + *P. capsici*	$70^{d} \pm 2$	$7.2^{b} \pm 0.4$	$6.5^{d} \pm 0.3$
T7 = *G. gigantea* (Gig2) + *P. capsici*	$70^{d} \pm 1$	$7.1^{b} \pm 0.1$	$6.5^{d} \pm 0.6$
T8 = *G. marganita* (Gig3) + *P. capsici*	$48.5^{e} \pm 2$	$6.2^{b} \pm 0.3$	$4.7^{b} \pm 0.4$

The values with different superscript letters in a column are significantly different ($p < 0.05$).

AMF for corn growth promotion

In this study, *A. longula* Aca1 and *G. margariata* Gig2 and Gig3 were introduced into Zea mays to evaluate their impact on plant height, shoot dry weight, and the number of AMF spores. The control group, which did not receive any AMF injection, did not exhibit any AMF spores. However, the AMF experiments showed a significant increase in AMF spore production, ranging from 137.0 - 487, 127.0 - 351.5, and 136.67 - 350.0 spores/100g soil for Aca1, Gig2, and Gig3, respectively (P value < 0.05). The MT5, consisting of soil, sand, and coconut fiber in a 1:1:1 ratio, was the best mediums for Aca1 and MT2, containing soil, sand; and coconut fiber in a 2:1:1 ratio, was the best mediums for Gig2 & Gig3 to produce spores, with respective spore yielded of 485.5, 351.5, and 350.00 spores/100g soil (Table 2).

Moreover, the maize plants in the experimental groups demonstrated a significant improvement in height and shoot dry weight compared to the control group. The control group's plant height ranged from 34.0 - 38.4 mm, and shoot dry weight was 2.28-3.0 g/plant. Conversely, the experiments exhibited a height range of 44.3 - 52.0 mm, 44.5 - 50.0 mm, and 41.6 - 49.75 mm in Aca1, Gig2, and Gig3, respectively, and a weight range of 3.6 - 4.5 g, 3.64 - 4.5 g, and 3.2 - 4.0 g/plant in Aca1, Gig2, and Gig3, respectively (Table 2).

Table 2. Affection of Gig2, Gig3 and Aca1 on maize plant growth promotion

Indexes examined	Substrates				
	MT1	**MT2**	**MT3**	**MT4**	**MT5**
	Acaulospora longula Aca1				
P_{Aca1}(g)	3.78 ±0.13	4.5 ±0.21	3.6 ± 0.26	3.68 ± 0.10	3.78 0.45
H_{Aca1}(mm)	44.6 ±2.3	52.0 ±0.89	45.8 ± 2.39	44.3 ±1.2	44.4 ± 3.36
$Aca1_{spore/100g\ soil}$	137.0 ±20.5	219.75 ±18.39	191.0 ± 36.65	195.25 ± 50.76	487.5 ± 25.0
	Gigaspora marganita Gig2				
P_{Gig2}(g)	3.7 ±0.1	4.5 ±0.052	3.9 ± 0.412	3.64 ± 0.192	3.82 ± 0.068
H_{Gig2}(mm)	43.6 ±3.78	50 ±1.92	46.25 ± 2.74	46.13 ± 2.78	44.5 ± 3.11
$Gig2_{spore/100g\ soil}$	165.75 ±14.64	351.5 ±21.44	197.75 ± 17.75	127.0 ± 5.89	168.0 ± 25.75
	Gigaspora marganita Gig3				
P_{Gig3}(g)	3.36 ±0.77	4.0 ±0.04	3.2 ±0.33	3.38 ± 0.66	4.8 ± 0.82
H_{Gig3}(mm)	41.6 ±3.8	42.0 ±2.77	43.1 ± 4.92	43.13 ± 2.68	49.75 ± 3.63
$Gig3_{spore/100g\ soil}$	171.67 ± 14.74	350.00 ± 45.83	194.67 ± 3.06	136.67 ± 25.42	163.67 ± 37.17
Control					
$P_{Control}$(g)	2.28 ±0.8	3.0 ±0.8	2.3 ± 1.24	2.6 ±1.2	2.46 ± 1.2
$H_{Control}$(mm)	34.0 ±1.22	35.0 ±2.30	38.4 ± 2.61	37.0 ± 2.35	37.6 ± 2.88
$AMF_{spore/100g\ soil}$	0	0	0	0	0

P: Shoot dry weight (g); H: plant height (cm), MT1 ($1V_{soil}$: 1 V_{sand} : $0.5V_{coconut\ fiber}$), MT2 ($2V_{soil}$: 1 V_{sand} : $1V_{coconut\ fiber}$), MT3 ($0V_{soil}$: 1 V_{sand} : $0V_{coconut\ fiber}$), MT4 ($1V_{soil}$: $2V_{sand}$: $1V_{coconut\ fiber}$), MT5 ($1V_{soil}$: 1 V_{sand} : $1V_{coconut\ fiber}$). The differences in each parameter shown in this table had statistically significant $P < 0.05$.

AMF for their host plant protection in vivo

Phytophthora capsici, *F. oxysporum* and *R. solani* were fungal pathogens infected to *P. longum, Z. mays* and *C. arabica; A. longula* Aca1, *G. marganita* Gig2 and *G. marganita* Gig3 were used for host plant protection. *Phytophthora capsici* showed significant ($p < 0.05$) pathogenic effect on *P. longum*, after 30 days of the inoculation, 40 % of the host strains was infected, the number increase to 50 % at the second months and keep the same rate after 3 months. *Rhizoctonia solani* have significant pathogen effect on *Z. mays* after 2 months inoculation 16.7 % host plant were effect by fungal pathogen. *Fusarium oxysporum* effect to *C. arabica* at the low rate, after 2 - 3 months of inoculation 10 - 15 % of the host strains was infected. In the experiment groups which (AMF + fungal pathogen) were inoculated in to their host, the rate of pathogen infection had been reduced dramatically: in the *P. longum*, this rate was reduce from 50 % to

15 % after 2 months inoculation; in the *Z. mays*, this rate was reduce from 46.7 % to 13.3 % after 3 months inoculation; in the *C. arabica* this rate was reduce from 15 % to 5 % after 3 months inoculation (Table 3).

Table 3. Effect of AMF on fungal pathogens of *Piper longum*, *Zea mays* and *Coffea arabica*

		Ratio of plant infected by fungal pathogen (%)		
Host	**Treatments (DAI)**	**30**	**60**	**90**
P. longum	Control	0.0^{a}	0.0^{a}	0.0^{a}
	P. capsica only	40.0^{c}	50.0^{c}	50.0^{c}
	AMF Gig2 only	0.0^{a}	0.0^{a}	0.0^{a}
	AMF Gig2 + *P. capsici*	15.0^{b}	15.0^{b}	15.0^{b}
C. arabica	Control	0.0^{a}	0.0^{a}	0.0^{a}
	F. oxysporum only	0.0^{a}	10.0^{b}	15.0^{c}
	AMF Gig3 only	0.0^{a}	0.0^{a}	0.0^{a}
	AMF Gig3 + *F. oxysporum*	0.0^{a}	0.0^{a}	5.0^{b}
Z. mays	Control	0.0^{a}	0.0^{a}	0.0^{a}
	R. solani only	0.0^{a}	16.7^{b}	46.7^{c}
	AMF Aca1 only	0.0^{a}	0.0^{a}	0.0^{a}
	AMF Aca1 + *R. solani*	0.0^{a}	6.7^{ab}	13.3^{b}

Note: Each value is the mean for four replicates. Values in column followed by the same letters are no significantly different ($p < 0.05$).
DAI: Days after inoculation.

Table 4. Effect of AMF and fungal pathogens in their host plants in growth promotion

Host	**Treatments**	**Height of plants (cm)**	**Number of leaves produced**
P. longum		18.5^{b}	2.2^{b}
	P. capsica only	17.1^{a}	0.5^{a}
	AMF Aca1 only	23.9^{d}	3.4^{d}
	AMF Aca1 + *P. capsici*	21.8^{c}	2.7^{c}
C. arabica	Control	25.7^{c}	8.3^{b}
	F. oxysporum only	24.1^{a}	7.2^{a}
	AMF Gig2 only	26.9^{d}	9.0^{c}
	AMF Gig2 + *F. oxysporum*	25.2^{b}	8.1^{b}
Z. mays	Control	85.0^{b}	8.1^{b}
	R. solani only	76.3^{a}	7.7^{a}
	AMF Gig3 only	102.7^{d}	8.8^{c}
	AMF Gig3 + *R. solani*	94.3^{c}	8.3^{b}

Note: Each value is the mean for four replicates. Values in column followed by the same letters are no significantly different ($p < 0.05$).

Effect of AMF and fungal pathogens on their host plants have not only observed on the ratio of plants infected fungal pathogen infected but also their growth rates. The results showed that *P. capsici*, *F. oxysporum*, and *R. solani* significantly ($p<0.05$) respectively hindered the growth of *P. longum*, *C. arabica* and *Z. mays*, leading to reduced growth rates as evidenced by the decreased number of leaves (0.5, 7.2, and 7.7 leaves, respectively) and plant's height (17.1, 24.1, and 76.3 cm, respectively) when compared to the control experiment (2.2, 8.3, and 8.1 leaves and 18.5, 25.7, and 85.0 cm, respectively). *A. longula* (Aca1) and *G. marganita*

(Gig2, Gig3) had a significantly ($p<0.05$) greater effect on the number of leaves and height of plants, resulting in an increase to 3.4, 9.0, and 8.8 leaves and 23.9, 26.9, and 102.7 cm, respectively. When compared to the treatment of pathogens only, the AMF control of the pathogen's effect on P. *longum*, *C. arabica*, and *Z. mays*, *A. longula* (Aca1) + *P. capsici*, *G. marganita* (Gig2) + *F. oxysporum*, and *G. marganita* Gig3 + *R. solani* exhibited significant control of the pathogen ($p<0.05$), resulting in an increase of 2.2, 0.9, and 0.6 leaves and a plants's height of 4.7, 1.1, and 18 cm, respectively (Figure 2, Table 4).

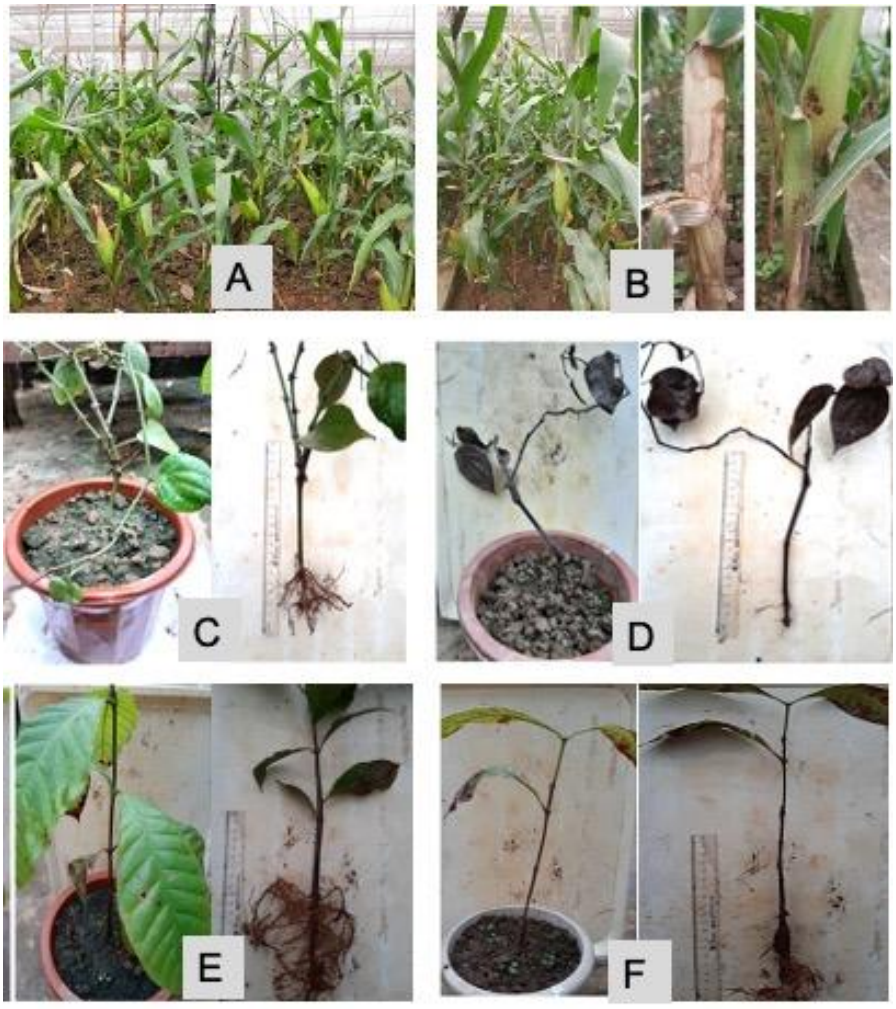

Figure 2. In vivo experiments of plant protection and plant growth promotion of AMF after 3 months inoculation. A. *Zea mays* infected by Aca1 and *Rhizoctonia solani.* B. *Zea mays* infected with *Rhizoctonia solani.* C. Pepper infected by Gig2 and *Phytophthora capsici.* D. Pepper infected by *Phytophthora capsici.* E. Coffee infected by Gig3 and *Fusarium oxysporum.* F. Coffee infected by *Fusarium oxysporum*

Discussion

There were numerous studies conducted in Vietnam have identified AMF in crops such as corn, coffee and pepper, primarily through morphological observations with *Acaulospora, Glomus* and *Gigaspora* are the dominant genera in *Zea mays* (Le *et al.*, 2018), *Coffea arabica* (Le *et al.*, 2021), and *Piper nigrum* (Le *et al.*, 2021). In this study, the dominant AMFs were confirmed based on morphology observation and utilization of 18S rDNA analysis, making the finding more credible. Furthermore, the results of this study had consisted with other previous studies in confirming that AMF could enhance the growth of various plants, including *Piper longum* (Gogoi and Singh, 2011), *Zea mays* Zhao *et al.* (2015), Mathur *et al.* (2016), *Glycine max* L. (Pavithra and Yapa, 2018) and some plants in the mining associated clay (Song *et al.*, 2020). Besides that, the results of AMF propagation of our research are comparatively better than those observed in certain previous studies. For example, in the study conducted by Selvakumar *et al.* (2018), the inoculation of AMF spores into *Sorghum bicolor* L. plants for a duration of 240 days resulted in a spore count ranging from a mere 6.4 to 112.3 spores/100g of soil. In contrast, our study attained a significantly higher spore count of 350 - 487.5 spores/100g of substrate.

The group of fungal pathogens causes the largest damage to agriculture among all other plant disease groups. Among them, *Fusarium, Phytophthora*, and *Rhizoctonia* are three of the most dangerous pathogenic species, reducing the productivity and quality of their host plants (Sarah *et al.*, 2017). They cause wilting, leaf blight, and other diseases in many plant species, such as coffee (Ploetz, 2006; Alemu, 2012; Al-Areqi *et al.*, 2015), pepper (Pérez-Hernández *et al.*, 2014, Cerkauskas, 2017; Jibat and Alo, 2021; Tena *et al.*, 2022), and maize (Singh *et al.*, 2018, 2020). This claim was validated in a study where *F. oxysporum, P. capsici,* and *R. solani* were examined on coffee, pepper, and maize, and found to significantly suppress the growth of the crops, as measured by the ratio of plant infected by fungal were higher compare with the control, while the number of leaves produced and leaf length were lower than in the control group. And when using AMF as a protective agent, it could reduce the rate of fungal infection as well as make the increasing of the number of leaves and leaf length of their host plant. These results were similar with the previous studies, such as in Al-Areqi *et al.* (2015) reported that the inoculation of the coffee plants with AMF after their inoculation with *Fusarium solani* had increase the length of plant (3.33 cm) compare to the control.

Overall, the findings of this study is not only validated the role of AMF in enhancing seedling establishment, plant growth, and plant protection, but also indicated that maize serves as a highly effective host for AMF, resulting in rapid and efficient spore production compared to other host plants. Moreover, soil, sand, and coconut fiber with different ratios

were identified as suitable substrates for AMF propagation. These findings hold great potential for the development of AMF-based biofertilizers and the adoption of sustainable agricultural practices.

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