Evaluation of the control ability of *Phytophthora* sp. to damage on chili plant (*Capsicum annuum* L.) using Arbuscular Mycorrhiza Fungi (AMF) product

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ABSTRACT

The Arbuscular Mycorrhizal Fungi (AMF) form a reciprocal symbiosis with approximately 80% of terrestrial plant species, including various agricultural crops. They provide essential nutrients to host plants, improving drought tolerance, salinity resistance, and disease resistance. This study aimed to evaluate the ability of AMF to control *Phytophthora* disease in chili plants grown in a net house. Eight treatments were applied, including two controls (without AMF), five with AMF supplementation at different formulation ratios (30%, 40%, 50%, 60%, and 70%), and one using a commercially available AMF product. The results showed that AMF enhanced plant growth and development while reducing the negative effects of *Phytophthora* sp. on chili plants. Compared to the control, the 70% AMF treatment exhibited the lowest disease incidence and severity indexes at 17, 24, 31, and 38 days. The corresponding disease index and disease rate during the observation period were 0.8%, 1.2%, 1.8%, and 2.3% for the index, and 4.0%, 5.4%, 8.9%, and 9.9% for the rate, respectively. Furthermore, 24 days after treatment, the 70% AMF treatment demonstrated a 70.1% disease prevention effect.

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1. Introduction

The area of land devoted to chili cultivation has increased in Vietnam due to the significant economic benefits of the industry. However, climate change has posed challenges to chili farming. Controlling pests and implementing effective cultivation and prevention strategies have become increasingly difficult tasks. Various pests and diseases can damage different parts of the chili plant, but *Phytophthora* spp. disease is among the most destructive.

Every stage of the plant's development is impacted by the disease, and widespread fungal spread can drastically reduce productivity. More importantly, the pathogen can persist in the soil and plant residues, posing a threat to subsequent crops. The disease causes blight in individual plants or groups, especially in water-saturated soil following irrigation or rainfall. Early symptoms include brown necrotic spots on the plant roots and crowns, followed by rapid disease progression and eventual plant death (Ozgonen & Erkilic, 2007).

Approximately 10,000 distinct fungal species are known to cause plant diseases; these fungi are frequently found in soil, air (as spores), and plant lesions (Agrios, 2005). The type of fungus that causes plant root disease varies depending on the host plant's nutrition, and the same is true for The Arbuscular Mycorrhizal Fungi (AMF) colonization in plant roots. Consequently, when AMF and pathogenic fungi are present in the rhizosphere of a host plant's roots, three entities are continuously interacting: the pathogenic fungus, the AMF, and the host plant. When *Phytophthora* sp. and AMF coexist, spatial competition may occur within the root cells, as they occupy different cortical cells of the host plant's roots (Azcón-Aguilar & Barea, 1997).

The trial results also showed that the percentages of healthy roots and shoot weights of mycorrhizal (*Glomus fasciculatus*) sweet orange seedlings inoculated with *Phytophthora parasitica* (20 or 50 chlamydospores per gram of soil) were higher than those of non-mycorrhizal seedlings at either inoculum density, as demonstrated by Davis & Menge (1981). Additionally, a study demonstrated that *Phytophthora* sp. and AMF compete within root cells. It was observed that the growth of *Phytophthora* sp. is restricted by AMF symbiosis, and the pathogen does not infect cells in the presence of AMF or in close proximity to the roots (Akhtar & Siddiqui, 2008).

Symbiosis of AMF in host plants reduces disease inoculation and confines injury locations within the host plant, thereby limiting the spread of the pathogen to other areas. Additional mechanisms by which AMF symbiosis decreases pathogen activity include the formation of wound barriers, clogging of air pores to allow only gas and water vapor to pass through, and improving the host plant's nutritional status to facilitate rapid root recovery after pathogen attack. AMF also act as a physical barrier against pathogens. The extracellular hyphae of AMF cover root tissues, thickening and strengthening them, making it more difficult for pathogens to attack (Akhtar & Siddiqui, 2008).

The AMF forms a reciprocal symbiosis with approximately 80% of terrestrial plant species, including various crops. AMF provide host plants with water and nutrients, primarily nitrogen, phosphorus, potassium, and trace elements, in exchange for photosynthetic products (Smith & Read, 2010). Additionally, AMF enhance drought tolerance, salinity resistance (Augé & Saxton, 2015), and disease resistance (Pozo & Azcón-Aguilar, 2007).

Moreover, AMF can compete with pathogens for space and nutrients both within the roots and in the soil surrounding the rhizosphere. In light of this, the application of biological measures to manage pests and diseases has emerged as an effective alternative to chemical treatments, offering comparable efficacy while being safe for humans and environmentally friendly.

2. Materials and Methods

2.1. Isolation and identification of *Phytophthora* sp. based on morphological characteristics

Collection of soil samples

Chili soil samples were collected in Hoc Mon, Ho Chi Minh City, in March 2022. The symptoms of *Phytophthora* sp. disease on chili plants include brown or black spots on the leaves and stem stubs, as well as rotted roots. Severely infected plants appear black, withered, and dead. Soil samples were collected from the vicinity of symptomatic plants at a depth of 5 to 15 cm. Five soil samples were taken from separate locations in each garden, thoroughly mixed, and combined into a final sample weighing 1,000 g per garden. After collection, the samples were preserved in zip bags labeled with the field name, sampling location, and accompanying code (VS, 1985).

***Isolation and identification of Phytophthora* sp.**

Indirect isolation of *Phytophthora* from soil samples was performed using the rose petal trap method. To begin, 100 g of soil was added to sterile distilled water in a ratio of 1 part soil to 3 parts water. The mixture was gently stirred, allowed to settle for 2 h, and then incubated in the dark at room temperature. Infected rose petals were observed after 1 - 3 days. Re-isolation was performed on water agar (WA) medium (20 g agar; 1,000 mL distilled water).

The samples were sterilized with distilled water twice, washed with 70% ethanol for 30 sec, and rinsed again with distilled water. After sterilization, the samples were cut into small pieces and placed on filter paper to dry for 30 min. The dried samples were cultured on WA medium and incubated at 25°C in dark conditions. After 2 - 3 days on WA medium, the mycelium was transferred to carrot agar (CRA) medium (600 g carrots, 15 g $CaCO_3$, 15 g agar, 1,000 mL distilled water) and incubated at 25°C in dark conditions.

According to Ho (1990), Drenth & Sandall (2001), and Abad et al. (2023), morphological features are the basis for *Phytophthora* sp. identification. Key features include oospores, sporangium size and shape, chlamydospore characteristics, sporangiophore branching, and swollen mycelium.

Preparation of Phytophthora* sp. *solution

Phytophthora sp. was cultured at a concentration of 10^6 CFU/mL in LB medium, and chili plants were evenly watered with the fungal spore solution (50 mL of spore solution per pot) ten days after AMF supplementation.

2.2. Evaluation of AMF bioproducts for controlling *Phytophthora* sp.

Preparation of AMF product

The AMF products were created by mixing the biomass of two genera of AM fungi, *Glomus* sp. and *Acaulospora* sp., at a concentration of 10^2 IP/g (IP - inoculation potential).

Experimental design to evaluate the effect of AMF products on *Phytophthora* sp. diseases on chili plants

The experiment was conducted at the Research Institute for Biotechnology and Environment, Nong Lam University, Ho Chi Minh City, Vietnam. The experimental period

lasted six months, from May 2022 to November 2022. The experiment was carried out in a net house with an area of 50 m^2, where temperatures ranged from 35 - 40°C and humidity levels were between 50% - 65%

The scientific name of chili is *Capsicum frutescens*. Each chili pot contained 4 kg of steamed and sterilized soil and measured 25 cm × 20 cm × 20 cm. NPK fertilizer was applied following the 20 - 20 - 15 formula at a rate of 10 g per pot, with fertilization occurring every 10 days. After the chili plants were incubated in trays for 30 days, they were transplanted into pots, and the AMF product was applied one day later.

Eight randomized complete block design (RCBD) plots with one factor and three replications were used in the experiment. Each plot contained 30 pots of plants, totaling 240 pots. *Phytophthora* sp. was injected into NT1 (positive control) without the use of AMF. The NT2 (negative control) received neither AMF nor *Phytophthora* sp. In addition to being inoculated with *Phytophthora* sp., five plots (NT3, NT4, NT5, NT6, and NT7) were treated with AMF products at concentrations of 30%, 40%, 50%, 60%, and 70%, respectively. Commercial Mycorrhiza products with *Phytophthora* sp. were used in NT8 (Mycorrhiza). The NT8 (Mycorrhiza) was a product of the Institute of Soil and Agrochemistry, Ministry of Agriculture and Rural Development. Following treatment, the experimental monitoring period (days after treatment, DAT) was conducted at 17, 24, 31, and 38 days.

Methods for measuring indicators in experiments

- Plant height (cm): measure from the stem base to the top of the plant.
- Root length (cm): measure from the stem base to the longest root.
- Total number of roots (roots): count all roots emerging from the stem base.
- Root biomass (g): Record the fresh weight of the roots.
- Total number of spores (spore per 100 g soil): Following the method by Brundrett et al. (1996), spores were extracted from the soil using centrifugation in a 50% sucrose solution and moist sieving. Spores were counted under a microscope.
- Symbiotic AMF rate (%): root staining was performed using the method of Phillips & Hayman (1970). Indicators of AMF presence in roots include the branching structure of the mycelium, dusty appearance, and vesicles penetrating the root tissue. The AMF infection rate was calculated using the formula:

  Symbiosis Rate (%) = (Number of root segments containing AMF/Total number of detected root segments) × 100.
- Disease Rate (%): Calculated as

  Disease rate (%) = (Number of diseased roots/Total number of investigated roots) × 100 (VS, 2021).
- Disease Index (%): Calculated using the formula:

  Disease Index (%) = $\sum$ [(N1 × 1) + ... + (Nn × n)]/(N × K) × 100 (VS, 2021), where:
  - *N1* = Number of samples at harm level 1,
  - *Nn* = Number of samples at harm level *n*,
  - *n* = The nth harm level,
  - *N* = Total number of surveyed samples,
  - *K* = The highest harm level in the hierarchy.

Five disease levels, based on Belete et al. (2013), include:

- Grade 0: No decayed roots,
- Grade 1: 1 - 25% decayed roots,
- Grade 2: 26 - 50%,
- Grade 3: 51 - 75%,
- Grade 4: 76 - 100%.

- Control Effectiveness (%) (VS, 2022): Calculated as:

  Control Effectiveness (%) = (1 - Ta/Ca) × 100, where:

  - *Ta* = Disease index of the treatment plot at the time of the record following treatment,
  - *Ca* = Disease index of the control plot at the same time.

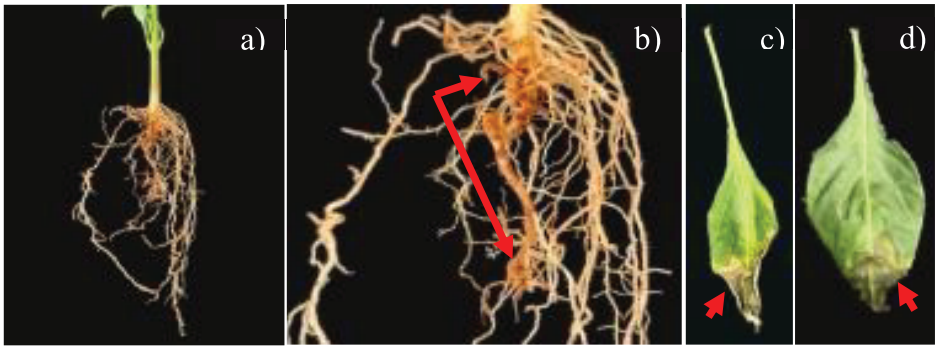
2.3. Data analysis

All experimental data evaluating the effect of AMF on chili plants were replicated three times. The data were processed to calculate the mean and standard deviation using Microsoft Excel 2019 software, and the average values were then analyzed using ANOVA with a significance level of 0.5% in SAS 9.1 software.

3. Results

3.1. Isolation and morphological identification of *Phytophthora* sp.

Phytophthora sp. was isolated and identified based on its morphological characteristics. In accordance with Koch's postulates, *Phytophthora* symptoms were reassessed on chili plants grown in net houses. The findings revealed that symptoms began to appear on the chili plants 40 days after inoculation (Figure 1).



Fiugre 1. The disease symptoms on chili plants 40 days after inoculation.
a-b) symptom on roots; c-d) symptom on leaf.

The pathogen was found to have survived in the soil sample based on the morphology of *Phytophthora* sp. on CRA media and the results of rose trapping (Figure 2, Figure 3). The mycelium of *Phytophthora* sp. consisted of indeterminate, colorless branches; colorless sporangia; and oval, ellipsoid, or ovoid shapes, with lengths ranging from 26 to 37.5 µm and widths between 15.6 and 20 µm. The length-to-width (L/W) ratio ranged from 1.7 to 1.9. The papillate sporangia are well-rounded, measuring 2.8 - 2.2 mm in length and 1.7 - 1.2 mm in width, although they may be absent. The chlamydospore has a thick wall, measuring 1.1 - 1.8 µm, with a diameter of 22 - 27 µm.

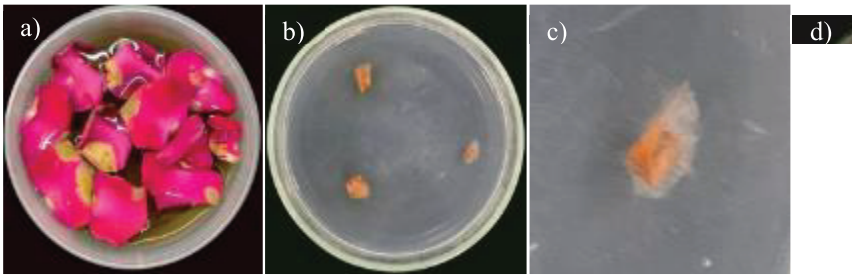


Figure 2. The isolation result of *Phytophthora* sp. by trapping rose. a) Trapping rose after 3 days; b-c), the isolation samples by trapping rose; d) Mycelium of *Phytophthora on* carrot agar (CRA) medium.

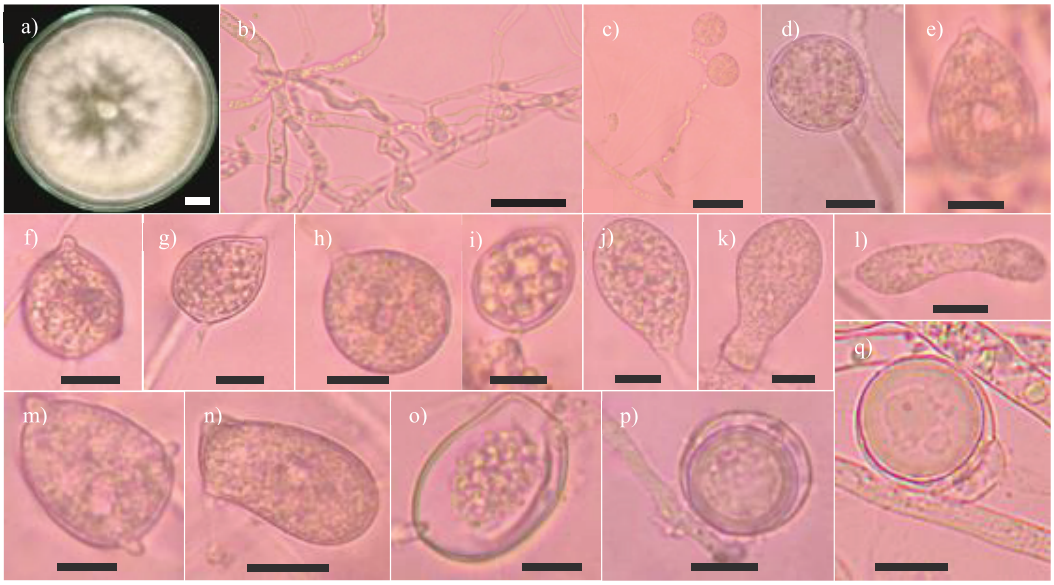


Figure 3. Morphology of P*hytophthora* sp. on carrot agar (CRA). a) colony on medium; b) swelling mycelium; c) Branches carrying pouch spores; d) chlamydospores; e-o) sporangium shape; p-q) oospores; (Bar = 10 µm).

According to Saltos et al. (2022), *P. capsici* causes damage to chili plants by impairing the water-conducting mechanisms of the roots and stems, resulting in light brown discoloration and withering of the leaf tips. Small, black lesions appear on the roots and stem stubs, initially spreading quickly before completely decomposing. The cortical tissue of the stem develops dry, dark brown, or black lesions.

Especially, diseases caused by *P. capsici* can reduce productivity by up to 100% if they develop early in the season and under favorable conditions (Liu et al., 2014). This disease can persist for several years in the absence of a host due to resistant structures known as oospores (Gandariasbeitia et al., 2019). Temperatures between 25 and 28°C and high humidity (> 80%) promote fungal infection and disease propagation, leading to large epidemics (Granke et al., 2011) Humid soils are optimal for the primary inoculum (oospores) to infect susceptible plants (Gandariasbeitia et al., 2019). According to Mchau & Coffey (1995) and Shahnazi et al.

(2012), morphological characteristics form the basis for the identification and classification of many genera, such as *Fusarium* sp. and *Phytophthora* sp.

3.2. Effects of AMF product on the growth and development of chili

The effect of the AMF product on chili plant growth was investigated. Chili plant growth and development data (Figures 4 and 5) demonstrated that the AMF-treated plots outperformed the control plots in all measured parameters, including plant height, root length, number of roots, and biomass. Statistically significant differences were observed between the AMF-treated plots and the control, according to the results of the statistical analysis.

Figure 4A, the plant height target, demonstrated how plant height increased progressively throughout all recording stages. In comparison to the other plots, NT6 (60% AMF) and NT7 (70% AMF) displayed the best results (23.5 cm, 34.1 cm, 46.9 cm, and 62.1 cm) and (23.4 cm, 33.7 cm, 47.1 cm, and 63.0 cm), respectively. The lowest plant height values (18.3 cm, 24.4 cm, 30.2 cm, and 43.8 cm) were found in NT1.

At the follow-up phases, the root length target (Figure 4B) showed that root length results in the AMF-treated plots were better than those in the two control plots. NT6 (60% AMF) and NT7 (70% AMF) produced the best root length results at stages 17 and 24 DAT (26.5 cm, 25.6 cm, and 36.5 cm, 34.5 cm); NT3 (30% AMF) and NT6 (60% AMF) produced the best results at stage 31 DAT (47.4 cm and 46.3 cm); and NT7 (70% AMF) and NT8 (Mycorrhiza) produced the highest root lengths at stage 38 DAT, measuring 55.1 cm and 57.9 cm. Nevertheless, NT1 produced the lowest values (20.1 cm, 30.2 cm, 34.0 cm, and 40.8 cm).

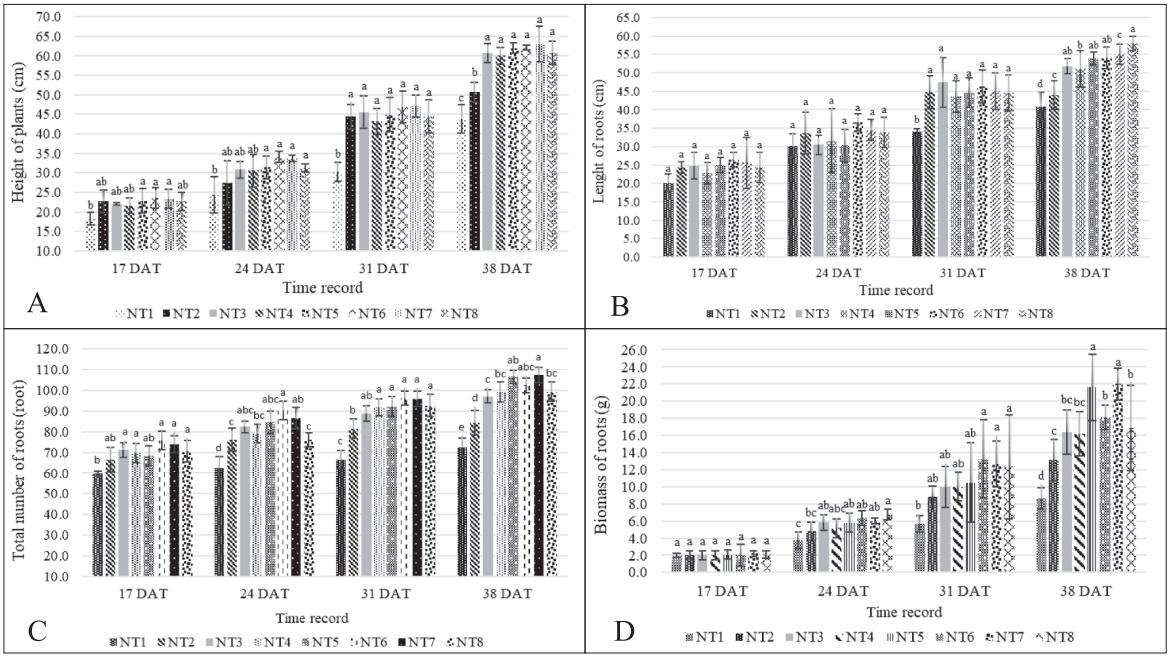


Figure 4. Growth target of chili plants. A: Height of plants; B: Length of roots; C: Total number of roots; D: Biomass of roots.

In Figure 4C, the total number of root targets is displayed. In comparison to the two control plots, the AMF treatment plots displayed a greater total number of roots, with statistically significant differences at the follow-up stages. The highest results were obtained by NT6 (60% AMF) and NT7 (70% AMF) at 17, 24, and 31 DAT (75.5 and 73.8 roots; 90.3 and 86.7 roots; 96.3 and 95.8 roots), while the best results were obtained by NT5 (50% AMF) and NT7 (70% AMF) at 38 DAT (106.5 roots and 107.3 roots). The NT1 achieved the lowest recorded values across all stages (59.8, 62.5, 66.7, and 72.5 roots).

The target for root biomass (Figure 4D) demonstrated that the root biomass in the plots increased steadily over the course of each stage, with the AMF treatment plots outperforming the two control plots at 24, 31, and 38 DAT. Results showed that NT8 (Mycorrhiza) produced the best results at 24 DAT (6.8 g); NT7 (70% AMF) produced the best results at 31 DAT (12.7 g); and NT7 produced the best results at 38 DAT (22.0 g). Furthermore, NT1 produced the lowest root biomass readings (3.8, 5.6, and 8.7 g).

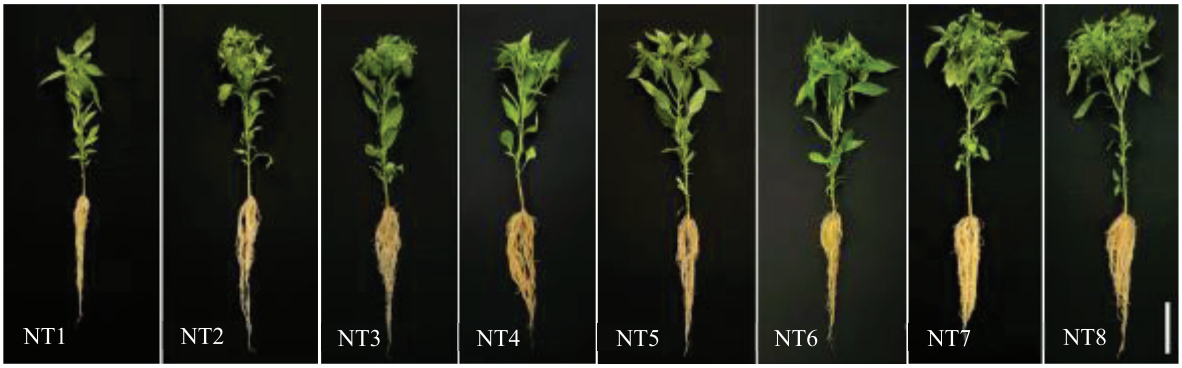


Figure 5. Growth target of chili plants (Bar = 20 cm).

In this experiment, monitoring indicators were collected independently for each plant (destruction indicators), although all plants were subjected to the same experimental conditions (AMF strain, irrigation water, fertilizer, care, etc.). Each plant exhibited different growth patterns, particularly in terms of root development below ground. Additionally, when inoculating AMF into each experimental pot, the symbiosis between the AMF strain and the roots of each plant varies, even though the strain is the same. This variation leads to different levels of root system growth. The experimental data represent the averages for each individual plant, so differences in data collection may arise between plants within the same treatment or between different treatments.

These studies have demonstrated that the addition of AMF products to plant soil influences the growth and development of chili plants, enhancing root biomass and promoting the production of leaves, flowers, fruits, height, plant length, and the number of roots. In the Thai provinces of Ubon Ratchathani and Sisaket, organic chili farms were evaluated for their AMF variety, based on the work of Boonlue et al. (2012). According to the findings, 14 species of AMF were identified, classified into 4 genera: *Acaulospora* sp. (4 species), *Entrophospora* sp. (1 species), *Glomus* sp. (7 species), and *Scutellospora* sp. (2 species). The genus *Glomus* sp. was found to be the most prevalent, followed by *Acaulospora* sp. The total number of AMF spores was consistent across all survey stations.

Additionally, the experimental results demonstrated that, compared to chili plants without AMF treatment, chili plants treated with AMF exhibited higher growth, blooming, and fruiting, as well as increased phosphorus (P) absorption capacity. The results indicated that mycorrhizal inoculation enhances the root absorption area (Anditya et al., 2021). Plant height, branch count, stem diameter, header width, and leaf area index all increased following mycorrhizal inoculation and liquid organic fertilizer treatment. Furthermore, the yield components productivity, fruit weight, fruit length, and fruit number were enhanced. Treatment with 10 g AMF per plant and 15 mL LIF per liter produced the best productivity (11.16 tons/ha), 33% higher than the untreated plants.

3.3. Effect of AMF product on disease rates and disease index on chili roots

The data showed that, during the monitoring period, both the disease index and the disease rate on chili roots were recorded (Table 1). When comparing the AMF-treated plots to the control, statistically significant changes in both the disease rate and disease index were observed. At all recording periods, the disease rate for variety NT7 (70% AMF) was lower than that of NT1 (9.5%, 14.5%, 24.0%, 28.2%) at 4.0%, 5.4%, 8.7%, and 9.9%, respectively. Similarly, the disease index increased gradually at each stage for NT7 (0.8%, 1.2%, 1.8%, and 2.3%) compared to NT1 (1.9%, 3.8%, 5.9%, and 7.1%), with values of 1.1%, 2.6%, 4.1%, and 4.8%, respectively.

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Table 1. Disease rate and disease index on chili plants (%)

Plots	Disease rate (%)				Disease index (%)			
	17 DAT[1]	24 DAT	31 DAT	38 DAT	17 DAT	24 DAT	31 DAT	38 DAT
NT1 (PC)[2]	9,5^{a}	14,5^{a}	24,0^{a}	28,2^{a}	1,9^{a}	3,8^{a}	5,9^{a}	7,1^{a}
NT2 (NC)[3]	0,0^{c}	0,0^{d}	0,0^{c}	0,0^{c}	0,0^{c}	0,0^{d}	0,0^{c}	0,0^{c}
NT3 (30% AMF)	5,7^{b}	9,3^{b}	11,7^{b}	14,4^{b}	1,1^{b}	1,9^{b}	2,6^{b}	3,2^{b}
NT4 (40% AMF)	5,2^{b}	6,8^{c}	10,6^{b}	14,9^{b}	1,0^{b}	1,4^{bc}	2,6^{b}	3,0^{b}
NT5 (50% AMF)	5,4^{b}	6,0^{c}	10,5^{b}	14,7^{b}	1,1^{b}	1,4^{bc}	2,5^{b}	2,9^{b}
NT6 (60% AMF)	4,4^{b}	6,3^{c}	9,7^{b}	13,0^{b}	0,9^{b}	1,4^{bc}	2,1^{b}	2,6^{b}
NT7 (70% AMF)	4,0^{b}	5,4^{c}	8,7^{b}	9,9^{b}	0,8^{b}	1,2^{c}	1,8^{b}	2,3^{b}
NT8 (Mycorrhiza)	4,7	6,1^{c}	11,7^{b}	12,4^{b}	0,9^{b}	1,6^{bc}	2,3^{b}	3,0^{b}
P	**	**	**	**	**	**	**	**

Note: in the same value group, values with the same accompanying characters are not statistically significant.
[1]DAT: day after treatment.[2] PC: positive control. [3]NC: negative control. AMF: Arbuscular Mycorrhizal Fungi.

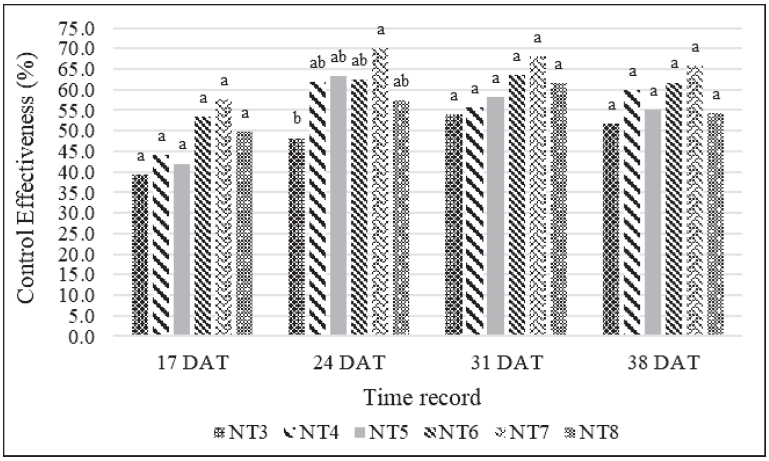


Figure 6. The control effectiveness of *Phytophthora* sp. disease on chili roots.

According to Figure 6, the control efficiency of AMF for *Phytophthora* sp. increased at DAT 17 and 24 but gradually decreased at DAT 31 and 38. Furthermore, the difference in control effect between NT3 (39.4%, 48.2%, 54.0%, 51.7%) and NT7 (57.6%, 70.1%, 68.0%, 65.9%) was 18.2%, 21.9%, 14.0%, and 14.2%, respectively. Thus, NT7 (70% AMF) showed the highest control efficiency, while NT3 (30% AMF) had the lowest at all stages. Specifically, the NT7 plot had the best result for controlling *Phytophthora* sp., with an effect of 70.1% at 24 DAT. Additionally, chili plants in NT1 showed disease symptoms at 38 DAT, whereas the AMF-treated plants exhibited no symptoms at the same time (Figure 7).

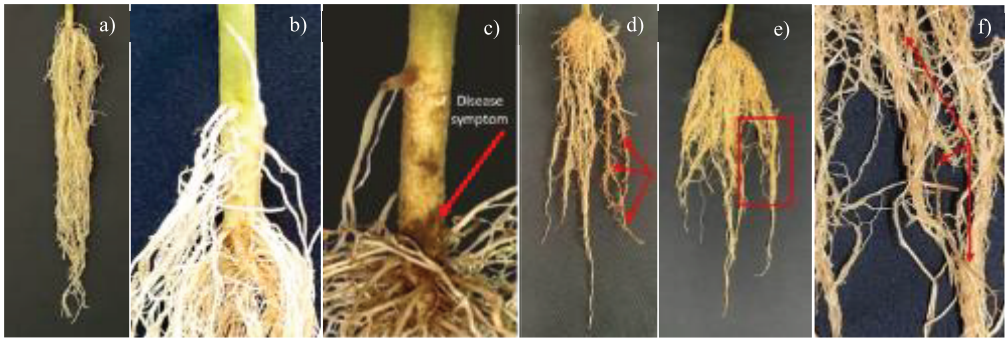


Figure 7. Root system of chili plants. a-b) the root system is not diseased; c-f) *Phytophthora*-disease roots (c,d: roots without Arbuscular Mycorrhizal Fungi (AMF) product; e,f: plant roots with AMF product).

The study by Ozgonen & Erkilic (2007) on chili plants revealed the effect of AMF species (*Glomus mosseae*, *Glomus etunicatum*, *Glomus fasciculatum*, and *Gigaspora margarita*) in controlling Phytophthora blight disease caused by *Phytophthora capsici* under four-week inoculation conditions in greenhouse settings. In these conditions, *G. fasciculatum* significantly boosted yield by 22%. Under pot, greenhouse, and field conditions, *G. mosseae* reduced *P. capsici* disease severity by 91.7%, 43.0%, and 57.2%, respectively. In summary, AM fungi, particularly *G. mosseae*, improved plant growth and reduced Phytophthora blight in chili plants.

AMF-infected roots were 39% and 30%, respectively, substantially fewer than those of plants without AMF, according to a study on tomato plants by Vigo & Hooker (2000) conducted 7 and 16 days after inoculation with the *Phytophthora parasitica* strain. The study also found that AMF had no effect on the spread of the disease or the necrotic regions of infected roots. Plants without AMF exhibited 61% more severe infections 26 days after inoculation with the *Phytophthora parasitica* strain compared to plants with AMF (31%). It was concluded that the effect on the number of infected foci is one mechanism by which AMF achieves biological control of this pathogen in tomatoes.

Watanarojanaporn et al. (2011) report that an experiment was carried out to compare the capacity of two AMF species in suppressing root rot on citrus plants caused by *Phytophthora* sp. Furthermore, by facilitating the plant's increased uptake of nutrients, particularly phosphorus (P), AMF enhanced its ability to protect the root system. The findings of this study were generally consistent with those of other researchers. The results demonstrated that, following 38 days of AMF inoculation in a greenhouse setting, the treatment with AMF products reduced the disease rate by 53%.

3.4. Effect of AMF product on total number of spores and symbiotic rate of AMF in rhizosphere soil and root of chili plants

At every stage, there were more AMF spores overall and a higher rate of symbiosis between the roots and rhizosphere soil of chili plants (Figure 8). All of the plots treated with the AMF product showed a statistically significant difference, with outcomes that increased steadily across all phases. In comparison to the other treatments (Figure 8A), NT 7 (70% AMF) and NT 8 (Mycorrhiza) produced the most AMF spores overall, with no statistically significant difference between the two treatments at any of the recording stages. In contrast, NT 3 (30% AMF) produced the fewest spores per 100 g of soil.

The symbiosis ratio (Figure 8B) revealed that for NT 6 (60% AMF) and NT 7 (70% AMF) at DAT 17, 24, and 31, the symbiosis rate was highest (32.7% and 31.0%; 38.0% and 36.3%; 41.7% and 43.0%). At DAT 38, NT 7 and NT 8 (Mycorrhiza) exhibited the highest rates (52.0% and 55.0%). Regarding the total number of AMF spores, the lowest symbiosis rates (23.3%, 29.0%, 31.0%, and 32.0%) were observed in NT 3 (30% AMF).

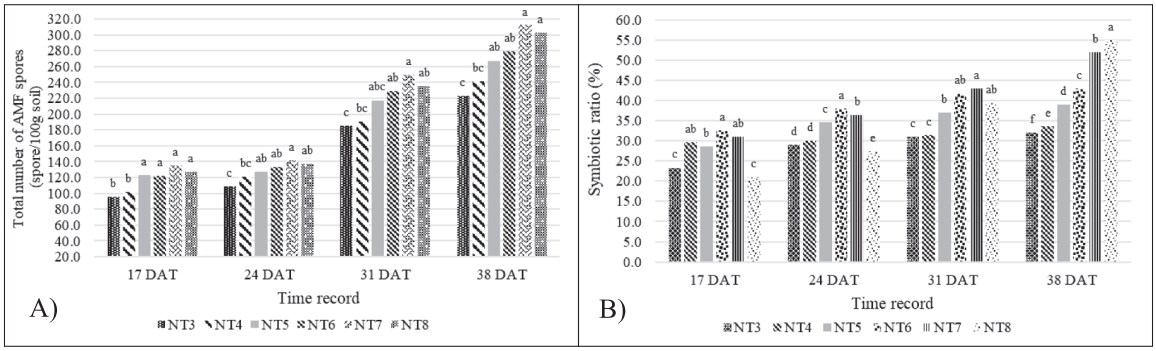


Figure 8. Total number of spores and symbiosis rate of Arbuscular Mycorrhizal Fungi (AMF) in rhizosphere soil and roots of chili plants.

According to Ozgonen & Erkilic's (2007) study on chili plants, four weeks after transplanting, the percentage of pepper roots colonized by AM fungi ranged from 61.3% to 68.1%. The colonization percentages for plants inoculated with *Glomus fasciculatum* and *Glomus mosseae* were 68.1% and 65.7%, respectively. Research has demonstrated that mycorrhizal fungi impact the growth of chili plants, specifically root length and plant height. Additionally, the number of symbiotic mycorrhizal fungal spores, which include various AM fungal strains such as *Acaulospora* sp., *Gigaspora* sp., *Glomus mosseae*, and *Scutellospora* sp., was 51 spores per 50 grams of soil (Aulia et al., 2021).

3.5. Following treatment with AMF products, AMF fungal species were detected in chili roots and rhizosphere soil

According to the identification results, *Glomus* sp. and *Acaulospora* sp. genera were found in the rhizosphere soil of chili plants. As shown by the genus *Glomus* sp. (Figure 9), spores can be found singly or in clusters, with straight spore stalks attached to the mycelium. The spores are spherical or almost spherical, with smooth surfaces, and are pale yellow, orange, brown, or reddish-brown in color. In the genus *Acaulospora* sp. (Figure 10), the spores are oval or spherical and grow singly. The spore wall consists of two to three layers, with one thin outer layer that is colorless. Large oil droplets are visible inside the spores, which have a rough surface with tiny cavities. The spores are pale yellow, yellow-orange, or orange in color.

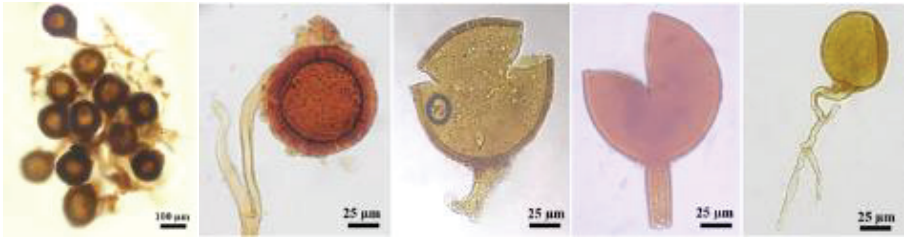


Figure 9. Phenotypes of spores of the genus *Glomus* sp. at the 40X.

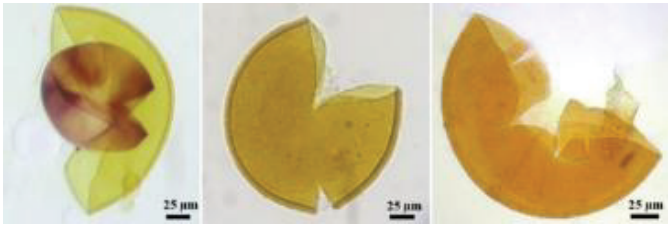


Figure 10. Phenotypes of spores of the genus *Acaulospora* sp. at the 40X.

The symbiotic AMF structures in the tissue of chili roots (Figure 11) showed that mycelium, vesicles, and arbuscular formations (stained with trypan blue) were present in every plot that received AMF treatment. In the intercellular spaces, the mycelium developed along the root cells in two directions (Figure 11c). Trypan blue, a dark dye, was present in the vesicle structures, which were oval in some cases and rectangular in others (Figure 11d). The arbuscular structure, which arises from the branching mycelium, was distributed within the root cells (Figure 11c, d). A ring-coiled filamentous structure was also observed in the root cells, according to the observation results (Figure 11e). The AMF fungi invaded the roots of chili plants, forming endohyphae and arbuscules.

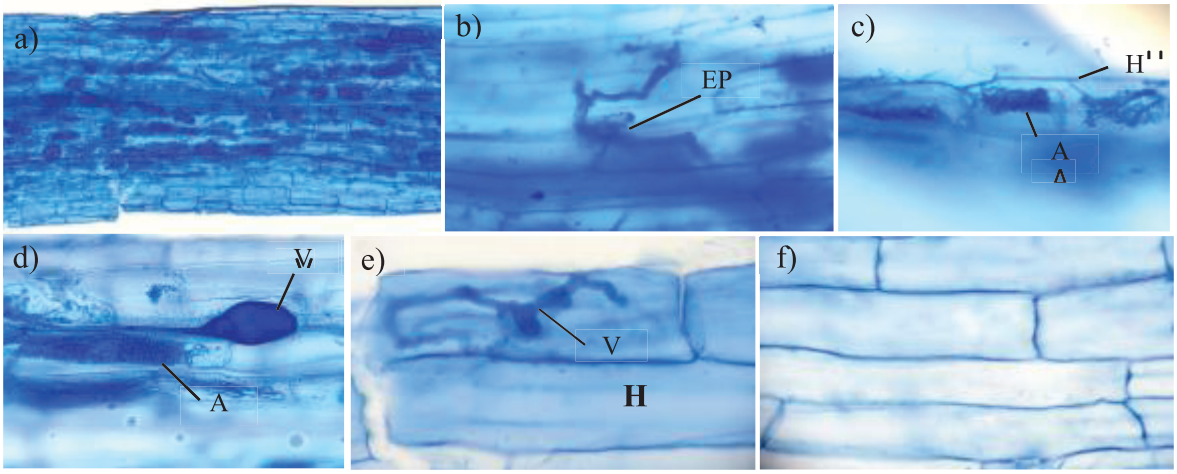
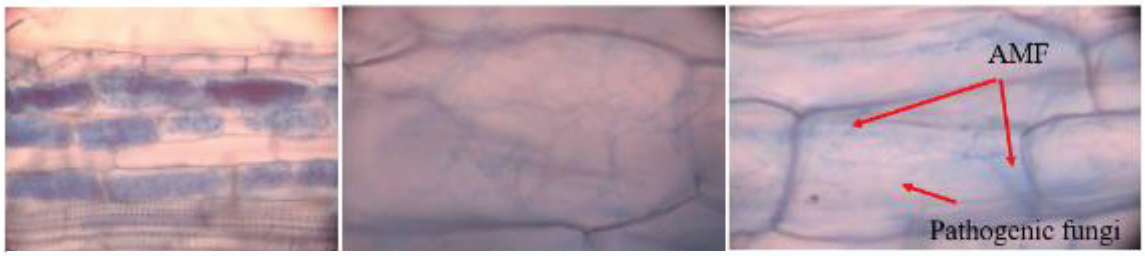


Figure 11. Symbiosis of Arbuscular Mycorrhizal Fungi (AMF) in chili roots and at 10X and 40X. a) AMF is present in roots at 10X; b) The point of infection of AMF; c - e) Symbiotic structure in the roots; f) Root cells do not have symbiosis; A (Arbuscule), H (Hyphae), V (Vesicle).



Chili root tissue with a symbiotic AMF | Chili root tissue has a fungal pathogen | Root tissue with parasitic fungal diseases and symbiotic AMF

Figure 12. Characteristics of chili root tissue in the presence of parasitic diseases and symbiotic Arbuscular Mycorrhizal Fungi (AMF).

Within 38 days of AMF product treatment, the source of fungal diseases caused by *Phytophthora* sp. on the roots of the chili plants was isolated (Figure 12). To contain the disease and prevent its spread, AM fungi developed structures that inhibited the growth of *Phytophthora* fungi. By forming wound barriers and obstructing the stomata small openings in the plant that allow only gases and water vapor to pass through AM fungi's invasion of the host plant's cells limits the pathogen's ability to spread. This action localizes the damage to the host plant, thereby reducing the overall spread of the disease.

4. Conclusions

The experiment assessing the efficacy of AMF treatment in controlling *Phytophthora* sp. on chili plants grown in net houses revealed that the AMF-treated plots outperformed the control plots in stimulating plant growth, as evidenced by increases in plant height, root length, root number, and biomass 31 days after treatment. In the AMF-treated plots, where *Phytophthora* sp. was controlled, both the disease rates and disease index were lower than in the untreated plots (positive control). Among the AMF plots, NT7 (70% AMF) yielded the lowest results. However,

by 24 DAT (days after treatment), NT7 (70% AMF) showed the highest control efficiency for *Phytophthora* sp. disease at 70.1%.

Conflict of interest

The authors have no conflict of interest to declare.

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