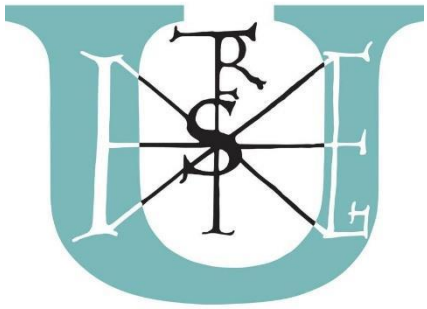

SZENT ISTVÁN UNIVERSITY

IDENTIFICATION AND IMPACT OF ARBUSCULAR MYCORRHIZAL FUNGI ON *ECLIPTA PROSTRATA* L.AND *CAPSICUM FRUTESCENS* L.

The Thesis of the Ph.D. dissertation

VO TRUNG AU

Gödöllő

2020

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1. BACKGROUND AND OBJECTIVES

Arbuscular mycorrhizal (AM) fungi, as common soil microbes, can colonize roots of most terrestrial plant species. These beneficial fungi have been reported to significantly contribute multiple benefits to host plants such as enhanced plant uptake of water and mineral nutrients, increased resistance/tolerance to environmental adversities. Therefore, this decreases input of inorganic fertilizers and pesticides in agriculture. AM fungi (AMF) help plants withstand mineral deficiencies, drought, and salinity (Katalin and Duc, 2019, Begum et al., 2019). Over the last decades, the application of AMF in agriculture has increased tremendously. The exploitation of AM symbiosis is one of the most effective practices to improve horticulture productivity (Birhane et al., 2012).

Chilli (*Capsicum frutescens* L.) belongs to the family Solanaceae with high Vitamin C, B, and capsaicin content. In Vietnam, this spice plant plays an important role in the daily diet of people and export. It provides high economic returns to farmers and thereby contribute to Gross domestic product (GDP) in Vietnam. Due to the fact that the introduction of non-native AMF could result in local soil disturbances, isolation of indigenous fungal symbionts is an important step in AM application into Chilli production in Vietnam.

Eclipta prostrata (L.), commonly known as a false daisy in English, is a popular plant in Asia. This plant has been utilized as folk medicine in China, Japan, India, Vietnam, and other tropical regions for the cure of respiratory disorders, including cough and asthma, infectious hepatitis, cardiovascular ailments, and hemorrhagic diseases (Yu et al., 2020). This medicinal plant has been used for the treatment of snakebite envenomation, HIV 1, diabetes type II, loose teeth, greying of hair, dizziness, and hemoptysis (Sun et al., 2010). Thanks to its various applications, wide attention has been paid by many researchers; however, there is a scarcity of information on AM application to the plant production, particularly alterations in phytochemical constituents induced by AMF and salt stress, which may cause substantial changes in its bioactive constituents and pharmacological activities. Therefore, the purpose of the present study was to explore the impact of AMF on Chilli and medicinal plants.

Objectives

Our aims were to

- Identify AMF species penetrating Chilli roots in inocula using tropical forest, agricultural, and grassland soils. Then DNA sequences and phylogenetic analyses of AMF were implemented.
- Investigate the impact of AM inoculation and growth substrate on biomass and content of polyphenols in *Eclipta prostrata*.
- Explore the effect of salt stress and AMF on plant performance and secondary compounds of *Eclipta prostrata.*
- Assess the impact of different AMF species on plant performance and secondary compounds of *Eclipta prostrata.*

2. MATERIALS AND METHODS

2.1. Target plants

Seeds of Chilli (*Capsicum frutescens* L.) hot varieties were collected at the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City (Vietnam). Cỏ mực *(Eclipta prostrata* L.*)* from Vietnam (original from Hong Dai Viet company in Vietnam) was used in our experiments.

2.2. Arbuscular mycorrhizal fungi inocula

Symbivit®, a commercial mycorrhizal product [a mixture of *Rhizophagus irregularis (G. intraradices), Funneliformis mosseae (G. mosseae), Claroideoglomus etunicatum (G. etunicatum), Claroideoglomus claroideum (G. claroideum), Rhizoglomus microaggregatum (G. microaggregatum*), and *Funneliformis geosporum (G. geosporum)*] (Symbiom Ltd., Lanskroun, Czech Republic; www.symbiom.cz) was utilized in the experiments including section 2.3.2; 2.3.3; and 2.3.4.

Three different AMF species originated from different mycorrhizal collections, *Septoglomus deserticola* (BEG 73), *Funneliformis mosseas* (SZIE), *Acaulospora lacunose* (BEG 78). Mycorrhizal inoculums were applied before transferring germinated seeds to pots of experiment 2.3.4.

Three types of soil were chosen as a source of AMF inoculum, following a land-use gradient. 1. soil from tropical Forest (FS) 2. Agriculture (AS) and 3. Grassland (GS) soils, was sampled from Vietnam. These soils were used to pots of experiment 2.3.1.

2.3. Plant growth and experiment design

2.3.1. Isolation and characterization of arbuscular mycorrhizal fungi potential for field application in a low input agriculture on Chilli (*Capsicum frutescens* L.) plant.

The experiment was carried out in plastic pots divided by a nylon mesh filter (40µm pore size) in two compartments. The upper part was filled with sand and peat with a ratio of 5:1, while the bottom part was filled with sand and peat with a ratio of 2:1 and was inoculated with 45 g of either forest or agriculture or grass soils separately (1.5% w/w) close to the interface with the upper compartment. Each treatment had ten biological replicates (10 pots/

treatment) and control (10 pots), resulting in 40 pots in the total. After 14-weeks of growth, agronomic variables, mycorrhizal colonization rate, counting spores, root DNA extraction, PCR cloning, and restriction fragment length polymorphism were performed.

2.3.2 Impact of arbuscular mycorrhizal inoculation and growth substrate on growth, biomass, and polyphenols content in *Eclipta prostrata*

The proportions of sand-peat were prepared as the following ratios: 100:0; 80:20; 60:40; 40:60; 20:80 and 0:100 % (v/v). All the inoculated treatments (AMF+) were added with 15 grams/ 0.5kg substrate/pot of commercial product of arbuscular mycorrhizal fungi Symbivit® while the control plants were added 15 grams/ 0.5kg substrate/pot of autoclaved Symbivit. Each sand-peat ratio had ten biological replicates, resulting in 10 replicates x 6 ratios sand-peat x 2 treatments = 120 plants. After 7 weeks of growth, plant biomass and mycorrhizal colonization, polyphenol, total phenolic content were determined.

2.3.3. Impact of arbuscular mycorrhizal fungi on growth, biomass, and polyphenols content in *Eclipta prostrata* under salt stress

Three salinity levels (0, 100, and 200 mM NaCl) and two mycorrhizal treatments (non - mycorrhizal and mycorrhizal plants) were applied in this experiment. The 60:40 % (v/v) sand: peat proportion was used for cultivating *Eclipta prostrata* plants. AMF treatment was added with 90 grams/3kg substrate/pot of Symbivit product, the control was added 90 grams/3kg substrate/pot of autoclaved Symbivit product. Each treatment had ten biological replicates, resulting in 10 replicates x 3 salinity levels x 2 treatments = 60 plants. Shoot and root weight, height, root length, leaf area, chlorophyll fluorescence, mycorrhizal colonization rate, proline, and polyphenolics concentration were examined at 4 and 8 weeks of plant growth.

2.3.4. Impact of different arbuscular mycorrhizal inoculation on growth, biomass and total phenolic content in *Eclipta prostrata*

The experiment consisted of five groups with four different kinds of AMF species *Septoglomus deserticola* (BEG 73); *Funneliformis mosseas* (SZIE); *Acaulospora lacunose (*BEG 78); and the commercial product Symbivit, and control (without AMF). During 4 and 8 weeks, the number of leaves, height of plants were recorded. After 4 weeks and 8 weeks of growth, plants were

harvested and the biomass, root colonization, chlorophyll florescence, total phenolic content, proline, root length, and root biomass were measured.

2.4. Measurement of parameters

Assessment of mycorrhizal colonization was according to Trouvelot et al. (1986).

Spore counting in tropical forest soil (FS) agricultural (AS) and grassland (GS) soils and in the substrate of the pots after plant harvesting was examined following the description of Ianson and Allen (1986).

Assessment of plant growth rate and biomass, the **s**hoot height, branch length, branch number, leaf number were measured at the time as described above in each experiment. The fresh and dry weight of shoots and roots and leaf area were recorded. Whole shoots and roots were dried at 72h at 60^0C.

Leaf area was measured following the method of Glozer (2008).

Chlorophyll fluorescence, the maximum quantum efficiency of photosystem II (PSII) photochemistry (Fv/Fm), was determined according to the method of Oxborough and Baker (1997) and Nemenyi (1999).

Proline determination was measured according to Bates (1973).

Determination of total phenolic content was measured by Folin–Ciocalteu assay according to Lister (2001).

Determination of polyphenols by using high performance liquid chromatographic analysis as described in Vo et al. (2019)

Determination of antioxidant enzymatic activities, the protein concentration of all leaf extracts was estimated according to the method of Bradford (1976). Polyphenol oxidase (PPO, EC 1.10.3.1) activity was measured by modified Fehrmann and Dimond (1967) method. Peroxidase (POD, EC 1.11.1.7) activity was determined by Rathmell and Sequeira (1974) method. *Superoxide dismutase* (SOD, EC 1.15.1.1) activity was measured spectrophotometrically at 560 nm according to the method of Beyer and Fridovich (1987). *Catalase* (CAT, EC 1.11.1.6) activity was determined following the method of Aebi (1984).

2.5. PCR and cloning

Root DNA extraction analysis according to method of (Khan et al., 2007).

The AMF species composition inside the roots was analyzed by a PCR approach targeting a portion of the ribosomal Short Sub Unit (SSU). PCR was performed using the AMF specific primers AML1 (5'-ATCAACTTTCGATGGTAGGATAGA-3') and AML2 (5'-GAACCCAAA CACTTTGGTTTC C-3') by Lee et al. (2008).

2.6. Sequence editing and phylogenetic analysis

Sequence similarities were determined using the BLASTn sequence similarity search tool provided by GenBank. Only sequences belonging to Glomeromycota were selected for the subsequent analyses, and the others were discarded. Sequences editing was conducted manually using MEGA 4.0 (Tamura et al., 2007) and Chromas Lite 2.01.

2.7. Statistical analysis

Statistical analysis was carried out using SAS 9.1 (SAS Institute, Cary, NC) package for Windows. Means were compared by Tukey post-hoc test at $P < 0.05$. Principal component analysis (PCA) as a statistical procedure was used to investigate patterns in polyphenolic data, and to highlight similarities and dissimilarities in phenolic contents of *E. prostrata* with and AMF. Hierarchical cluster analysis (HCA) was performed to identify relative similarity among treatments, and the result was drawn as a dendrogram. The PCA and HCA were carried out by using an XLSTAT program.

3. RESULTS AND DISCUSSIONS

3.1. Isolation and characterization of promising arbuscular mycorrhizal fungal strains for field application in chilli plants in low input agriculture.

3.1.1. Molecular analyses for identification of arbuscular mycorrhizal fungi in the roots

After 14 weeks, plants of each treatment were selected, and DNA were extracted from the roots. PCR amplification was performed with AML1 and AML2 primers specific for Glomeromycota. The amplification of DNA from RS, AS, and GS gave an expected product of approximately 800bp, as confirmed by gel electrophoresis. The bands purified from the gel were used to create a clone library. No band was detected for the CON (control) treatment.

3.1.2. Phylogenetic analyses

After editing, the sequences were analyzed by Blastn. Eleven sequences were found to be related to AMF sequences. Sequences corresponding to RP3 and RP4 were found to be related to plant DNA and Basidiomycota species, respectively. Non-AMF sequences were discarded before proceeding to phylogenic analyses.

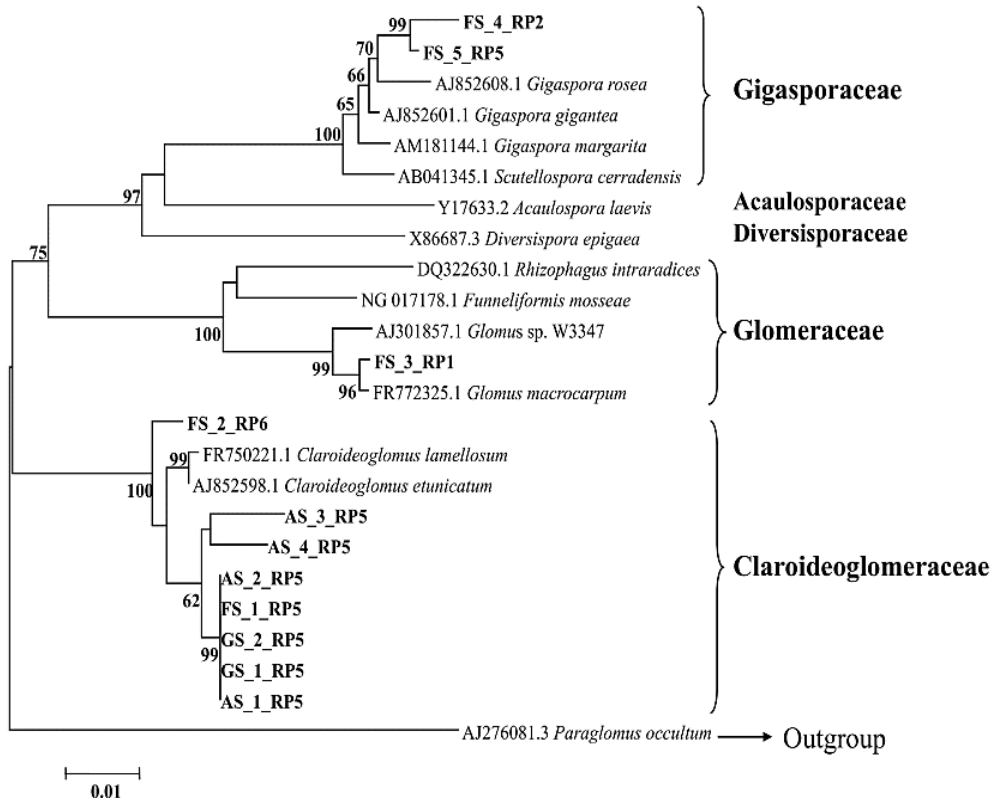

Figure 1: Neighbor-joining phylogenetic tree displaying the relationship between the AMF sequences recovered from Chilli roots and 21 reference sequences from GenBank representing some of the main Glomeromycota families. Sequences obtained in this work are in bold and labeled with the corresponding restriction pattern. *Paraglomus occultum* was used to root the tree. Phylogenetic groups are shown on the right side of the tree. Bootstrap values > 50 are shown near the nodes.

11 AMF sequences were used to build a phylogenetic tree (Fig.1). Eight of them, related to the restriction patterns RP5 and RP6, clustered together with

the reference sequences representing the Claroideoglomeraceae family. One sequence (RP2) clustered together with the reference sequence from *Gigaspora rosea*, while one (RP5) clustered basal with the group of reference sequences representing the family of Gigasporaceae. The sequence related to the restriction pattern 1 clustered with the sequence from *Glomus macrocarpum* in the family of Glomeraceae.

3.2. Impact of arbuscular mycorrhizal inoculation and growth substrate on biomass and polyphenols content in *Eclipta prostrata*

3.2.1. Effect of AMF inoculation and different nutrient supplies on measured plant parameters and phenolic concentration.

Fresh shoot biomass of inoculated plants was 131.89 g, 55% higher than that of non-inoculated plants (84.88 g). Dried shoot biomass of inoculated plants was (13.97 g), 67% greater than that of non-inoculated (8.38 g). Moreover, fresh and dried root biomass were 33.75 g and 4.68 g, respectively, which were 79% and 140% higher than those of non-inoculated plants (18.81 g and 1.95 g). Moreover, fresh and dried biomass of root and shoot were found a significant difference among 60:40 sand-peat-plant with others ($P<0.05$).

Different proportions of peat had significant effects on the concentration of total phenolic content (TP) of aerial parts, but mycorrhizal inoculation did not. Peat concentration higher than 40% (v/v) decreased the phenolic content of plants. *E. prostrata* growing in a 60/40% (v/v) sand and peat substrate ratio had the highest phenolic content in both treatments.

3.2.2. HPLC analysis of polyphenols from the aerial part of *E. prostrata*

In the HPLC analysis, the gradient elution applied was able to efficiently separate a wide range of phenolic compounds such as protocatechuic acid; 5-o-caffeoylquinic acid; dimethyl-wedelolactone; 4,5-dicaffeoylquinic acid; 3,5-dicaffeoylquinic acid; quercetin-3- arabinoside; luteolin; 4-o-caffeoylquinic acid; and wedelolactone, with dimethyl-wedelolactone and wedelolactone being abundant in all of the different samples examined.

The level of the individual polyphenols was affected, to a high extent, by the proportions of peat and sand in the growing media. In the inoculated plants, such a tendency held true only for protocatechuic acid, 5-o-caffeoylquinic acid, quercetin-3-arabinoside, and 3,5-dicaffeoylquinic acid. In both inoculated and non-inoculated samples, peat proportions between 60% and

80% caused a drastic decrease in the content of all polyphenols detected in the extracts compared to others treatment except wedelolactone. Notably, a drastic decrease in the polyphenol content did not occur with 100% peat in both inoculated and control samples. With AMF inoculation, the concentration of luteolin was 45.74 mg/g at a 0/100% (v/v) sand and peat mixture, which was significantly higher than that determined in the other treatments ($P < 0.05$).

The average content of luteolin; 3,5-dicaffeoylquinic acid; wedelolactone; 4-o-caffeoylquinic acid; and protocatechuic acid was higher by 75%, 37%, 10%, 41%, and 67%, respectively, in mycorrhizal inoculated plants compared to their levels in the control ones. Whereas the content of 5-o-caffeoylquinic acid; dimethyl-wedelolactone; 4,5-dicaffeoylquinic acid; and quercetin-3-arabinoside was lower by 25%, 13%, 47%, and 31%, respectively. The highest level of protocatechuic acid (41.87 mg/g) was recorded in a 60/40% (v/v) sand and peat mixture by AMF+. In addition, the highest levels of wedelolactone, the major polyphenol, were found in plants grown in peat proportion between 0% and 40% in both inoculated and non- inoculated treatments.

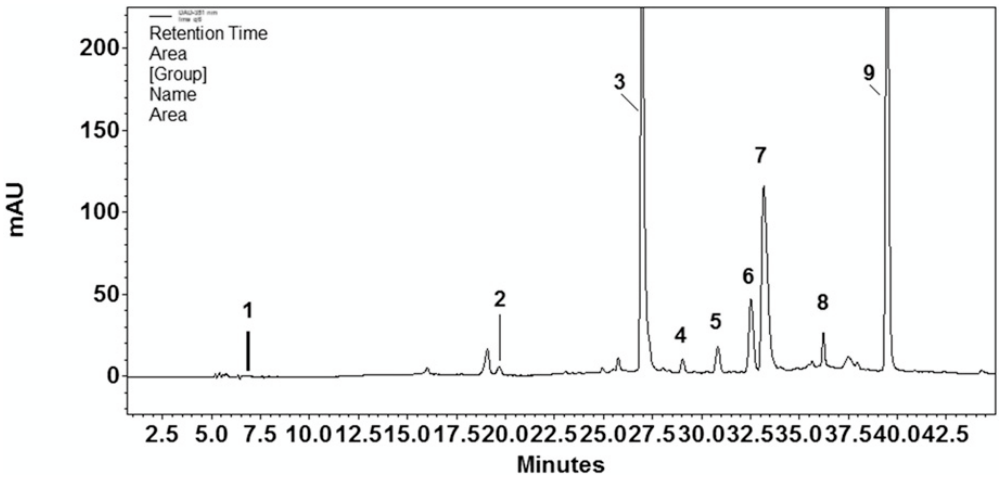

Figure 2. HPLC profile of effective polyphenols extracted from *E. prostrata* leaves. Different numbers represent polyphenols: 1: Protocatechuic acid; 2: 5-o-caffeoylquinic acid; 3: Dimethyl-wedelolactone; 4: 4-o-caffeoylquinic acid; 5: 3,5-dicaffeoylquinic acid; 6: 4,5-dicaffeoylquinic acid; 7: Quercetin-3-arabinoside; 8: Luteolin; 9: Wedelolactone.

3.3. Impact of salt stress and arbuscular mycorrhizal fungi on plant performance of *Eclipta prostrata*

3.3.1. Growth parameters

Table 1. Growth parameters of *Eclipta prostrata* plants not inoculated or inoculated with arbuscular mycorrhizal fungi under non-stress, moderate, and high saline conditions after four and eight weeks

Treatment		Root length (cm/plant)		Root weight (g/plant)		Shoot weight (g/plant)		Leaf number (leaf/plant)	
Stress conditions	Mycorrhizal inoculation	4 w	8 w	4 w	8 w	4 w	8 w	4 w	8 w
Non-stress	**AMF+**	19.7 ±3.5 a	25.5 ± 2.3 a	1.08 ± 0.38a	2.95 ± 0.72 a	6.35 ± 2.4 a	9.9 ± 2.6 a	18.9 ± 2.9 a	31.5 ± 10.1 a
	AMF-	11.8 ±1.4 ab	18.3 ± 1.5 ab	0.17 ± 0.1 ab	1.68 ± 0.56 b	1.43 ± 0.42 ab	4.4 ± 0.7 ab	10.0 ± 1.5 c	15.2 ± 1.5 bc
100 mM NaCl	**AMF+**	17.3 ±3.0 a	17.2 ± 2.3 b	0.78 ± 0.5 ab	0.41 ± 0.06 c	2.15 ± 1.4 ab	2.7 ± 0.3 b	15.8 ± 3.2 ab	21.8 ± 3.7 ab
	AMF-	12.5 ±3.3 ab	12.6 ± 0.6 b	0.53 ± 0.1 ab	0.15 ± 0.05 c	0.99 ± 0.33 ab	1.4 ± 0.1 bc	9.4 ± 0.4 c	11.1 ± 1.5 bc
200 mM NaCl	**AMF+**	8.8± 3.6 b	16.5 ± 3.9 b	0.11 ± 0.1 ab	0.19 ± 0.04 c	0.66 ± 0.4 b	1.6 ± 0.6 bc	12.8 ± 1.1 bc	11.7 ± 0.3 bc
	AMF-	7.7± 2.0 b	11.6 ± 2.1 b	0.03 ± 0.01 b	0.13 ± 0.02 c	0.44 ± 0.1 b	0.9 ± 0.4 c	9.4 ± 0.5 c	9.0 ± 1.7 c
Significant of Source of variation (NS = not significant, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001)									
Mycorrhizal inoculation (M)		**	***	*	**	**	***	***	***
Salt stress (S)		**	***	*	***	**	***	**	***
M*S		NS	NS	NS	**	NS	**	*	*

4 w, 8 w, four weeks and eight weeks of growth, respectively.
AMF, arbuscular mycorrhizal fungi; For each parameter, the means ± standard deviation is presented (n = 3). Different letters in each parameter indicate significant differences according to the Tukey test (P<0.05) among treatments.

Salt stresses, particularly at 200 mM NaCl, considerably decreased all growth parameters tested at four and eight weeks. There were no significant differences in root length, root and shoot weight, and plant height between colonized and uncolonized plants under both salt levels during plant growth. (P>0.05). Root weight was remarkably higher in inoculated plants versus uninoculated plants under non-stress conditions at eight weeks (Table 1).

Mycorrhizal inoculation markedly increased leaf number, stem diameter, and leaf area of non-stress plants at both times of measurement. Intriguingly, AM colonization remained no change in leaf number of host plants (versus non-stressed AM plants) subjected to moderate salt stress (100 mM NaCl) at four weeks while the stress significantly lessened leaf number in non-AM plants (versus non-stressed non-AM plants). However, high salinity (200 mM NaCl) considerably decreased the colonization percentage to 29.56% after 4 weeks. Interestingly, we did not measure any substantial differences in mycorrhizal colonization rates after eight weeks of growth. Their rates were 51.89%, 47.44%, and 43% in colonized plants under non-stress, moderate, and high salt stress, respectively.

3.3.2. Polyphenol compounds

In the HPLC analysis, the gradient elution applied was able to efficiently separate a wide range of phenolic compounds was examined. The level of the individual polyphenols was affected, to a great extent, by salt stress, particularly at 200 mM NaCl when plants reached eight weeks of aged. After four weeks of plant growth, mycorrhizal treatment remarkably increased 4-o-caffeoylquinic acid (by 166.6% as compared with the counterpart of non-AM plants), 3,5 – dicaffeoylquinic acid (404.8%) and ferulicquinic acid (2901%) under non-stress conditions. Nevertheless, caffeic acid and dimethyl wedelolactone in non-stress mycorrhizal plants were under the detection limit at four weeks. Similarly, at eight weeks, mycorrhizal colonization had a significant positive influence on 3,4-dicaffeoyl-quinic acid (increased by 146.8%, in comparison to the counterparts of uncolonized plants), luteolin-glucoside (by 804.5%), luteolin (detectable) while a considerable negative effect on ferulic acid (decreased by 48.6% in relation to non-AM plants), dimethyl wedelolactone (by 39.6%) in host plants under non-stress conditions.

Under moderate salt stress, the concentration of wedelolactone, 4-o-caffeoylquinic acid, and ferulic acid were substantially lower in inoculated four-week plants as compared to those of uninoculated plants. Dimethyl wedelolactone was under the detection limit in AM plants under such stress, but their ferulicquinic acid was detectable. Intriguingly, we found no significant differences in all phenolic compounds between eight-week AM and non-AM plants exposed to moderate salinity (100 mM NaCl).

When exposure to high salt stress, fungal colonization substantially influenced the level of 3,5 – dicaffeoylquinic acid, 4,5- dicaffeoylquinic acid, detectable ferulicquinic acid, and detectable luteolin-glucoside at four weeks but negatively affected ferulic acid content. Noticeably, the concentration of most phenolic compounds was markedly heightened in mycorrhizal plants, except that of dimethyl wedelolactone, quercetin-arabinoside, luteolin at eight weeks. The highest levels of wedelolactone and 4,5-dicaffeoyl-quinic acid were found in non-stress mycorrhizal plants, whereas ferulicquinic acid was under the detection limit at eight weeks.

After four weeks of plant growth, there was a considerable effect of mycorrhizal inoculation (M) on contents of caffeic acid ($P<0.01$), 3,5–dicaffeoylquinic acid ($P<0.01$), luteolin-glucoside ($P<0.001$), luteolin-7-o-glucoside ($P<0.05$), ferulic acid ($P<0.001$), dimethyl wedelolactone ($P<0.001$), ferulicquinic acid ($P<0.001$), 4,5-dicaffeoylquinic acid ($P<0.001$) and quercetin-arabinoside ($P<0.05$). Saline had a substantial impact on the levels of all polyphenol compounds tested (at least $P<0.05$), except dimethyl wedelolactone and 5-o-caffeoylquinic acid. Interactions between two main effects on caffeic acid ($P<0.05$), 3,5 – dicaffeoylquinic acid ($P<0.001$), ferulic acid ($P<0.01$), ferulicquinic acid ($P<0.001$), 4,5-dicaffeoylquinic acid ($P<0.05$) were found. When plants reached eight weeks of age, mycorrhizal colonization significantly influenced the concentrations of all polyphenol compounds (at least $P<0.05$), excluding ferulic acid and dimethyl wedelolactone. Similarly, salinity caused sharp changes on all polyphenols (with at least $P<0.01$), except 4,5-dicaffeoylquinic acid. Interactions between two main effects on almost all polyphenols were observed (at least $P<0.05$).

3.3.3. Antioxidant enzyme activities

At four weeks of growth, mycorrhizal plants gained the highest POD activity under moderate salt stress, while the activity of this enzyme was lowest in nonmycorrhizal plants subjected to moderate saline conditions (Fig. 3A). No significant differences could be found in other treatments. Interestingly, no significant differences could be observed among all treatments under non-stress and salt stress conditions at eight weeks of plant growth (Fig. 3B).

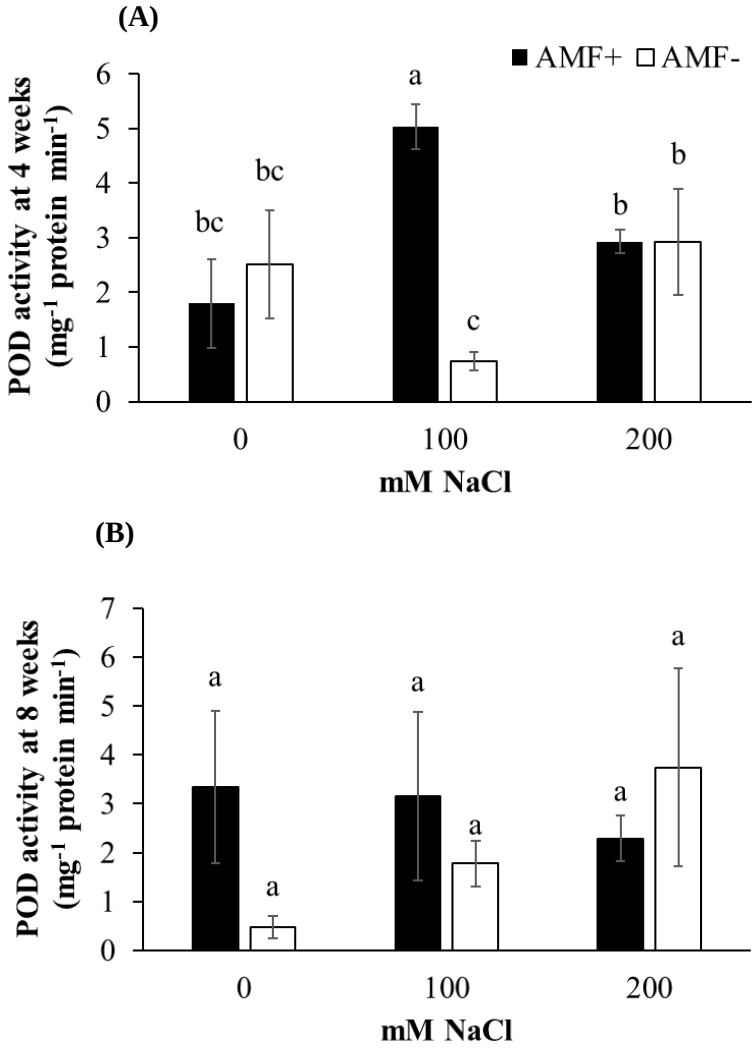

Figure 3. Peroxidase (POD) activity in leaves of *Eclipta prostrata* plants not inoculated or inoculated with arbuscular mycorrhizal mycorrhiza under non-stress, moderate, and high saline conditions at four weeks (A) and eight weeks (B) of growth. AMF+, AMF-, with and without arbuscular mycorrhizal fungi, respectively. Each bar shows mean ± standard deviation (n = 3). Different letters indicate significant difference among treatments according to the Tukey test ($P<0.05$).

Under non-stress conditions, mycorrhizal application significantly dropped CAT activity in plants at four weeks of growth (Fig. 4A). Moderate salt stress-induced a substantially higher level of this enzyme activity in colonized plants

but significantly decreased it in nonmycorrhizal plants as compared to corresponding ones.

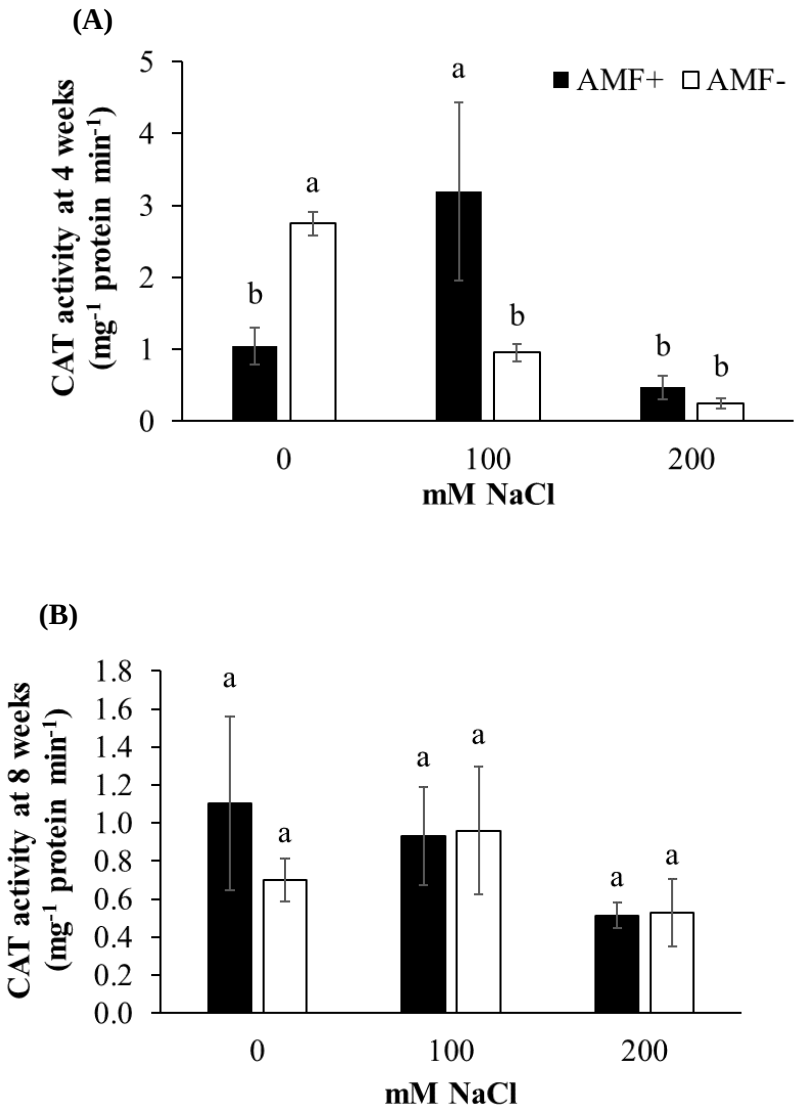

Figure 4. Catalase (CAT) activity in leaves of *Eclipta prostrata* plants not inoculated or inoculated with arbuscular mycorrhizal mycorrhiza under non-stress, moderate, and high saline conditions at four weeks (A) and eight weeks (B) after growth. AMF+, AMF-, with and without arbuscular mycorrhizal fungi, respectively. Each bar shows the mean ± standard deviation (n = 3). Different letters indicate significant difference among treatments according to the Tukey test ($P<0.05$).

When plants were exposed to high salt concentration, no changes in CAT activity were recorded in mycorrhizal plants, while CAT activity was markedly decreased in uncolonized plants in comparison to the corresponding ones under non-stress conditions. No significant differences in CAT activity in both mycorrhizal and non-mycorrhizal plants under all conditions were found at eight weeks of growth (Fig. 4B).

3.4. Impact of different arbuscular mycorrhizal fungi species on plant performance of *Eclipta prostrata*

3.4.1. Growth parameters

There were no significant differences in root weight, leaf number among all treatments at four and eight weeks of growth ($P>0.05$). Mycorrhizal application significantly decreased plant height, in *Septoglomus deserticola* (Sd) 34 cm plant^{-1} and in *Acaulospora lacunose* (Al) 38.71 cm plant^{-1}, 17% and 5% lower than non-inoculated plants (Ct) 40.88 cm plant^{-1} ,respectively, except Symbivit (Sy) plants was 52.71 cm plant^{-1}and *Funneliformis mosseae* (Fm) plants 46 cm plant^{-1} , 29% and 13% higher than non-inoculated (Ct) 40.88 cm plant^{-1} at eight weeks of growth, respectively. Regarding leaf area, no beneficial effects of AMF inoculations were observed in host plants ($P>0.05$). Interestingly, a measurement of shoot weight in Symbivit (Sy) plants was 10.58 g, 76% higher than non-inoculated (Ct) 6.01 g at eight weeks of growth, respectively. In contrast, shoot weight in plants colonized by *Septoglomus deserticola* (Sd) was 3.15 g and *Acaulospora lacunose* (Al) 4.98 g, 47% and 17% lower than non-mycorrhizal (Ct) plant 6.01 g after eight weeks of growth, respectively.

3.4.2. Total phenolic concentration

There were no significant differences in total phenolic content between control plants and mycorrhizal plants at four weeks of growth ($P>0.05$) (Fig.5). However, the total phenolic level in the treatment of Symbivit (Sy) was remarkably lower than that in plants inoculated with *Funneliformis mosseae* (Fm) and *Septoglomus deserticola* (Sd). At eight weeks of growth, *Septoglomus deserticola* (Sd) caused a significant decrease in total phenolic concentration in plants, as compared with plants colonized by other fungal symbionts (Fm, Al, Sy) at eight weeks of growth. Nevertheless, no significant differences in total phenolic levels between inoculated and non-inoculated plants were observed.

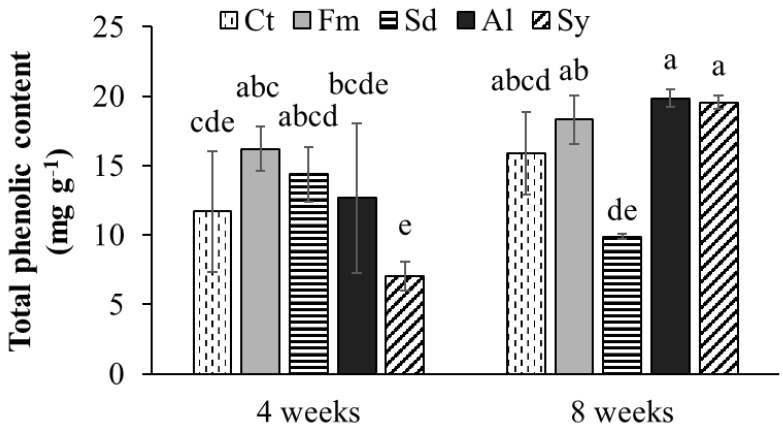

Figure 5. Total phenolic content of *Eclipta prostrata* plants not inoculated or inoculated with *Funneliformis mosseae* (Fm), *Septoglomus deserticola* (Sd), *Acaulospora lacunose* (Al), symbivit (Sy) including six AMF isolates at four weeks (A) and eight weeks (B) after growth. Each bar shows the mean ± standard deviation (n = 4). Different letters indicate significant difference among treatments according to the Tukey test (P<0.05)

4. Novel scientific results

1. Isolation, sequencing, and phylogenetic analyses of 11 AMF strains present in soil samples collected from tropical forest, agricultural and grassland soils in Vietnam such as 8 AMF strains of them belonging to the Claroideoglomeraceae family, 1 AMF strain belonging to Glomeraceae and 2 AMF strains belonging to Gigasporaceae family.

2. Varying peat and sand rates, representing different nutrient supplies, had significant impacts on both mycorrhizal colonization and growth responses. The growth substrate with 60/40% (v/v) sand and peat ratio is the best for large-scale cultivation of *E. prostrata*, moreover supporting the highest total phenolic content.

3. Through high-performance liquid chromatography (HPLC) analysis, nine individual phenolic components were analyzed, including wedelolactone and

dimethyl-wedelolactone at the highest concentration. Some of the identified compounds, such as 5-o-caffeoylquinic acid, quercetin-3-arabinoside, 4-o-caffeoylquinic acid, and protocatechuic acid, have not been reported previously in *E. prostrata* cultivars. Also, wedelolactone and dimethyl wedelolactone being abundant in all of the different samples examined Using hierarchical cluster analysis, multiple groups are represented, suggesting the role of mycorrhizal inoculation, growth substrate, and their interactive effect on secondary metabolites of *E. prostrata*.

4. AM inoculation enhanced *Eclipta prostrata* (L.) plant growth under non-stress conditions, but this enhancement was not apparent under salt stresses. AM treatment strengthened plant tolerance to moderate salinity by increasing proline level (at eight weeks after growth), activities of main antioxidant enzymes POD and CAT (at four weeks after growth) in *Eclipta prostrata* plants.

5. HPLC analysis of phenolic compounds illustrated that the AM symbiosis induced significant changes in phenolic profiles 4,5-dicaffeoyl-quinic acid and wedelolactone being abundant in all of the different samples examined of *E. prostrata* under both levels of salt stress at the early stage of plant growth (after four weeks). Most phenolic compounds were enhanced in AM plants under severe salinity, thus substantially influenced phytochemistry constituents of this medicinal plant.

6. The beneficial impacts of various AMF treatments (three single AMF species and a mixture of six AMF species) on plant growth characteristics were different. The positive effects of *Septoglomus deserticola* (Sd) and *Acaulospora lacunose* (Al) on root length at eight weeks of growth while the beneficial influence of Symbivit (Sy) on shoot weight at eight weeks of growth was observed. In the total phenolic content, plants inoculated by either

Funneliformis mosseae (Fm) or Sd had a considerably higher level of total phenolics than plants colonized by Sy at four weeks of growth. At eight weeks of growth, the total phenolic content of Fm, Al, and Sy treatment was substantially higher than that of Sd application. Therefore, these highlighted the specificity of interaction between AMF species and the medicinal plant *E. prostrata*.

5. CONCLUSIONS

In our first study in *Capsicum frutescens*, an experimental system to measure the AMF inoculum potential in different soils was set up. The results demonstrated that soils with different land-use could host AMF communities able to stimulate plant growth differently. After that, we isolated and sequenced 11 AMF strains from tropical forest, agricultural and grassland soils in Vietnam. This experimental system applied on a large scale in the aim of commercial inocula development could be suitable to test a very high number of AMF strains at the same time, avoiding the time-costing step of strain isolation and propagation. Furthermore, the use of AMF consortia instead of a single isolate would allow reproducing partially those conditions of competition that occur in a field when a commercial inoculum has to coexist with the indigenous AMF population.

The next study presents for the first time how arbuscular mycorrhizal fungi and different sand and peat proportions influence the growth rate and the polyphenol profile of the medicinal plant *E. prostrata*. Our results showed that a 60/40% (v/v) sand and peat ration seemed to be the best and is thus recommended for large-scale cultivation of *E. prostrata*, moreover supporting the highest total phenolic content of plants. The AMF inoculation successfully affected the growth, biomass, and polyphenol components of *E. prostrata*. Through an HPLC analytical method, polyphenol compositions were successfully assessed in *E. prostrata*, and nine individual phenolic components were quantified. Further research will be carried out under different types of abiotic and biotic stress conditions, focusing on single or mix arbuscular mycorrhizal fungi in an open field experiment. A better understanding of the phenolic composition of *E. prostrata* and factors influencing it helps to identify new industrial applications of this medicinal

plant (together with arbuscular mycorrhizal fungi), and moreover, help to develop new strategies for the prevention and treatment of different diseases.

We also investigated the beneficial effects of AMF on the medicinal plant *E. prostrata* under moderate and severe salt stress during plant growth. The findings indicate that AM inoculation enhanced the host plant growth under non-stress conditions, but this enhancement was not apparent under salt stresses. However, AM treatment strengthened plant tolerance to moderate salinity by increasing proline level (at eight weeks after growth), activities of main antioxidant enzymes POD and CAT at four weeks after growth in host plants. HPLC analysis of phenolic compounds illustrated that the AM symbiosis induced significant changes in phenolic profiles of *E. prostrata* under both levels of salt stress at the early stage of plant growth (at four weeks after germination). Noticeably, most phenolic compositions were enhanced in AM plants under severe salinity, thus substantially influenced phytochemicals of this medicinal plant.

In our last experiment, we investigated the effects of different single AMF species and mixed AMF inoculant on plant performance of *E. prostrata* during the plant growth. The results demonstrated that the beneficial impacts of AMF on plant growth characteristics and total phenolic content in plants were distinct among different AMF treatments, therefore highlighting the specificity of interaction between AMF species and the medicinal plant *E. prostrata.*

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