Bui CV *et al.* (2022)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 50, Issue 1, Article number 12609
DOI:10.15835/nbha50112609
Research Article

Effect of arbuscular mycorrhizal fungus on the growth and polyphenol production of medicinal plants: *Ehretia asperula* and *Solanum procumben*

Cuong V. BUI[1,2*], Quang D. LE[1,2], Anh T. K. VO[2], Lam D. TRAN[1,2*]

[1]*Graduate University of Science and Technology (GUST), Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam; ledangquang2011@gmail.com*
[2]*Institute for Tropical Technology (ITT), Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam; cbuivan@gmail.com (*corresponding author); vothikieuanh2013@gmail.com; tdlam@itt.vast.vn*

Abstract

The study was conducted to evaluate the influence of arbuscular mycorrhizal fungus (*Rhizophagus intradices*) on growth and polyphenol production of the two important and popular medicinal plants in Vietnam: *Ehretia asperula* Zoll. & Mor. and *Solanum procumbens* Lour. The results showed a significant effect of the fungus on the growth of these two species with the growth indices such as height, weight and P content that were all higher than those of non-AM plants; although the indices of AM symbiosis in the plant roots were not as high as other plants in previous studies. The effect of AM fungus on polyphenol production was different between the two species. In *E. asperula*, the effect of AM fungi on polyphenol production was not significant; whereas in *S. procumbens*, AM symbiosis significantly increased polyphenol production in plant biomass, especially in roots. The different growth times of the two species might cause the different effects of AM fungus on polyphenol production.

Keywords: arbuscular mycorrhizal fungi; medicinal plants; polyphenols; secondary metabolites

Introduction

Arbuscular mycorrhizal (AM) fungi are widely recognized as the oldest and most widespread plant symbionts which occur in the soil of most ecosystems (Schüssler *et al.*, 2001). After millions of years of evolving with plants, AM fungi still are essential associates of many plants and play an important role in absorbing nutrients, regulating development, and enhancing plant resistance and tolerance to environmental stresses (Redecker *et al.*, 2006; Rouphael *et al.*, 2015). If mycorrhization of roots is by any reason declined or not formed, plant productivity will be negatively affected. The hyphae of the fungi act as extend root system with several times larger than root only, which helps roots to reach deeper and wider in soils to seek water and food (Requena, 1997). When colonize to plant roots, AM will form special structures (intraradical hyphae, arbuscules, vesicules) inside root cells for nutrient exchange. Plants provide the fungi carbohydrates, and in turn, fungi support plants more water and minerals uptake from soils by their widespread hyphae (Smith and Read, 2010).

Received: 27 Dec 2021. Received in revised form: 16 Feb 2022. Accepted: 17 Feb 2022. Published online: 24 Feb 2022.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Besides the interest of AM fungi on plant growth, recent studies have focused on other valuable property of AM fungi, which is the enhancement of secondary metabolite production in medicinal plants. Several studies have shown that the colonization of AM fungi significantly increases secondary metabolite content in plant biomass (Karpool *et al.*, 2002; Zhao *et al.*, 2009; Jurkiewicz *et al.*, 2010; Nell *et al.*, 2010). Karpool *et al.* (2002a, b) reported a significant increase of essential oils in plants such as *Anethum graveolens*, *Trachyspermum ammi* and *Coriandrum sativum*, when those were colonized by AM fungi. Airam *et al.* (2009) observed the phenolic compounds in the roots of *Echinacea purpurea* increased if the plants were inoculated with AM fungus (*Glomus intradices*). Some other publications also reported the increase of secondary metabolites in different plants with AM fungi symbiosis (Zhao *et al.*, 2009; Jurkiewicz *et al.*, 2010; Nell *et al.*, 2010). The mechanism is supposed due to the response of plants to AM fungi penetration by generating secondary metabolites which function as signals for the contact between fungi and plants (Giovannetti *et al.*, 1994). On the other hand, the colonization of AM fungi also stimulates the immune system of plants to control the symbiosis of AM (Bednarek *et al.*, 2012), and the secondary metabolites contribute an important part of the plant immune system (Piasecka *et al.*, 2015). In the early stage of the symbiotic process, plants recognize AM fungi as parasites or pathogens (García-Garrido and Ocampo, 2002), which makes them activate the defense system to prevent the penetration of the fungi.

Vietnam is a tropical climate country with rich biodiversity. Many plant species have been cultivated and used as traditional medicinal sources. Some of them are endemic species with valuable medicinal substances. Two species of *Ehretia asperula* and *Solanum procumbens* are such valuable medicinal plants and grown popularly in the highlands of Vietnam. *E. asperula* is a perennial woody plant and *S. procumbens* is an herb. The source of medicinal substances obtained from these species has been studied and proven to have anti-cancer and anti-inflammatory properties (Nguyen *et al.*, 2017; Nguyet *et al.*, 2018; Kim *et al.*, 2019; Tran *et al.*, 2019). However, studies on the effects of microflora in general or AM fungi in particular on the growth and production of medicinal substances of these two species are very few. Therefore, we conducted a study on the effects of AM fungi on the growth as well as secondary metabolite production of these two popular medicinal plants in Vietnam.

Materials and Methods

Plants

The seeds of *Ehretia asperula* Zoll. & Mor. and *Solanum procumbens* Lour. were collected from Hoa Binh province, Vietnam. The seeds were dried and stored at room temperature.

AM fungus

Rhizophagus intradices (DAOM 181602) was cultivated in carrot (*Daucus carota* L.) root organ cultures on Petri dishes as described by Bui and Franken (2018).

Soil preparation

Soil was loam soil: 30-40% sand, 10% humus, 50-60% alluvial soils, no nutrients addition to facilitate the colonization of AM fungi. To eliminate the available AM propagules, the soil was pasteurization in 3 circles (each circle: heating to 80 °C for 24 hours, then cooling down to room temperature for 24 hours). After the pasteurization, the original microbial system of the soil (but not AM propagules) was added by 100 ml soil extract per 1 kg pasteurized soil. The soil extract was obtained by adding 150 ml distilled water to the 100 g soil and shaking for 1 hour, then filtering through paper (Whatman, Schleicher and Schuell, Germany) to get soil extract solution.

Seedling preparation and culture

The seeds were surface sterilized by ethanol 70% for 5 minutes, then by NaClO 3% for 3 minutes. The sterilized seeds were placed on water agar plates and incubated in dark for 10 days to germinate. The seedlings then were transferred to pots containing 2 kg pasteurized soils. Two treatments (AM plants and non-AM plants) were set up. The AM plants were added by AM spores from root organ culture to seedling roots (50 ± 5 spores per one seedling), and the controls (non-AM plants) were added by distilled water instead of AM spores. Plants were daily irrigated (100 ml/day).

Plant biomass measurement

After the growth period (*E. asperula*: 6 months and *S. procumbens*: 4 months), the plants were harvested for analysis. Harvested plants were divided into root and shoot parts. The fresh and dry weight of root and shoot were measured and recorded by a weight balancer. The leaf number and total area were also measured. A mobile app., Petiole (http://petioleapp.com/), was used to calculate leaf area. The dried roots were used for mycorrhization analysis, and P uptake, as well as natural products, was also measured.

AM assessment and spore count

The roots were firstly cleared by KOH 10% then stained with trypan blue 0.05%. The AM colonization was calculated according to Trouvelot *et al.* (1986) as described below:

- Frequency of AM colonization in the root system:

F% = (No. of AM fragments/total No.)*100

- Intensity of the AM colonization in the root system:

M% = (95n5+70n4+30n3+5n2+n1)/(No. total), where n5 = number of fragments rated 5; n4 = number of fragments 4 etc.

- Arbuscule abundance in the root system:

A% = a*(M/100)

AM spores were isolated by wet sieving together with a centrifuge in sucrose solution (30% w/v), according to Turnau et al. (2005). The spores were counted and collected under a binocular microscope.

P uptake in biomass

P accumulation in shoot and root was analyzed using an ICP-OES system. In brief, the dried shoots and roots were grounded to powder (2 mm in size). Around 200 mg of grounded shoots and roots were digested with 5 ml 65% HNO_3 and 3 ml 30% H_2O_2 in a microwave extraction for 15 minutes. The digested solution was made up to a volume of 50 mL with double distilled water. The P concentration was analysed by an ICP-OES system after filtration (Bui and Franken, 2018).

Polyphenol analysis

The analysis of polyphenol content in plant shoots and roots is based on the gallic acid equivalence (GAE) method with Folin-Ciocalteu's reagent (FCR) according to ISO 14502-1. Briefly, each sample was extracted by adding 70% methanol at 70 °C and vortex. The extract was centrifuged at 3500 rpm for 10 min. The supernatant was decanted in a tube (two times). The extracts were pooled with 70% methanol and diluted 100 times with distilled water. The diluted extract was mixed with Folin-Ciocalteu's reagent and sodium carbonate solution in water. The total polyphenol content was expressed as Gallic acid equivalents (GAE) in g/100 g material and converted to the percentage of polyphenol in the tested biomass in this study. The concentration of polyphenols in samples was derived from a standard curve of Gallic acid ranging from 10 to 50 µg/mL (absorbance at 765 nm, $R^2 = 0.9985$).

Statistical analysis

The statistical analysis was carried out with the software Statistica (version 12). The variables were tested for normality of distribution (Kolmogorov–Smirnov test). The variables which were not normally distributed or given in percentage values were transformed by logarithm or arcsine, respectively. Two-way analysis of variance (ANOVA) and T-test were used for data analysis. Tukey HSD was performed at $p \leq 0.05$ in case of significant interaction between factors after ANOVA, and the growth parameters between AM plants and non-AM plants were compared by T-test at $p \leq 0.05$. All data are shown as mean values with standard deviations (SD).

Results

The parameters were analyzed (after 6 and 4 months of *E. asperula* and *S. procumbens*, respectively) including mycorrhization, plant growth, P uptake and polyphenol production.

Mycorrhization

To confirm the penetration of AM fungi to plants, the roots were stained to observe the specific structure of AM fungi and quantify the extent of colonization. Due to the natural black pigment of the *E. asperula* plant, the staining process was modified by adding NaClO 3% for whitening before being treated with trypan blue stain. The appearance of AM spores was also taken into account.

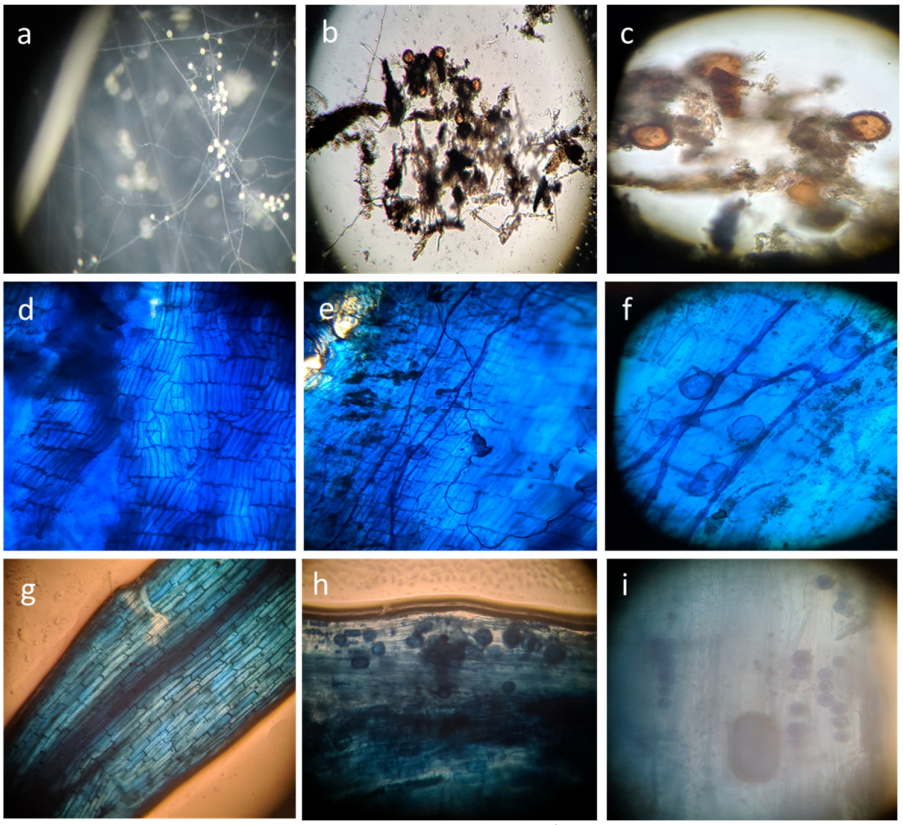

Figure 1. Spores in soils and AM colonization in plant roots. a) *R. intradices* spores in Petri dish before being transferred to seedlings. b-c) spore clusters found in soils (× 100 and × 400). d) the roots of *E. asperula* plant without AM. e-f) the root of *E. asperula* plants with AM hyphae and vesicules (× 100 and × 400). g) the roots of *S. procumbens* plants without AM. h-i) the roots of *S. procumbens* plants with AM hyphae and vesicules (× 100 and × 400)

E. asperula plants: the AM frequency (F%) of AM fungi in the roots was around 54%, which means half of the root system was colonized by AM fungi. The AM intensity (M%) was not as high as frequency, at around 33.4%, while the arbuscule abundant (A%) was 14.9% (Figure 1 and Table 1). After treating by NaClO, the structure of AM fungi in the roots was clearer with hyphae and vesicules. Arbuscules were more difficult to observe because of the dark and firm roots. Spore in soils rarely appeared as a single spore but a cluster surrounded by hyphae (Figure 1bc). Around 15 spores per 10 grams of soil were counted. AM fungi colonization or spores were not detected in non-AMplants (Figure 1d and Table 1).

S. procumbens plants: the frequency (F%) of AM fungi colonization was higher than that of the *E. asperula* plants (72.19%), but the intensity and arbuscule abundant tended to decrease. The AM spore number in soil was also less than that in soil of *E. asperula* plants (Table 1).

Table 1. The growth and colonization parameters of the two plant species with (M+) and without (M-) AM fungus

Species/parameters			M+	M-	T-test
E. asperula	Fresh weight (g)	Shoot	77.7 ± 18.6	72 ±2.7	ns (n = 3)
		Root	114.1 ±3.6	82.6 ± 20.5	ns
	Dry weight (g)	Shoot	35.8 ± 3.9	29.2 ± 0.9	*
		Root	35.6 ± 4.8	26.1 ± 1.1	*
	Leaf-	Number	12.3 ± 1.5	16.6 ± 3.0	ns
		Total area (cm^2)	547.4 ± 8.6	515.6 ± 16.1	*
	Mycorrhization	Frequency (F%)	54 ± 5.2	-	
		Intensity (M%)	33.4 ± 6.4	-	
		Arbuscule (A%)	14.9 ± 6.6	-	
	Spore number (per 10 g soil)		16.00 ± 1.73	-	
S. procumbens	Fresh weight (g)	Shoot	50.95 ± 8.91	39.03 ± 3.49	* (n = 4)
		Root	54.37 ± 9.81	48.80 ± 6.23	ns
	Dry weight (g)	Shoot	22.87 ± 3.31	14.95 ± 2.29	*
		Root	17.31 ± 0.93	15.28 ± 0.99	*
	Leaf-	Number	16.5 ± 3.41	12.0 ± 2.16	ns
		Total area (cm^2)	106.9 ± 11.75	103.0 ± 17.66	ns
	Mycorrhization	Frequency (F%)	72.19 ± 8.72	-	
		Intensity (M%)	30.71 ± 8.48	-	
		Arbuscule (A%)	12.66 ± 4.23	-	
	Spore number (per 10 g soil)		14.66 ± 2.08	-	

Plant growth

E. asperula plants: the growth of plants in different treatments was similar in the first three months. From the 4th month, the differences in height, leaf area emerged, which showed the advantage of the AM plants compared to those of non-AM plants. At harvesting after 6 months, the obvious higher root biomass of the AM plants than non-AM plants was observed (Figure 2). Despite the "no significant" difference of the fresh weight, the dry weight data showed the AM plants had significantly higher in both shoot and roots than those of non-AM plants (Table 1). Leaves of non-AM plants were higher in number but significantly lower in area.

S. procumbens plants: the difference in height between the plants with and without AM fungi was obvious after one-month growth. After four months, AM plants showed significantly higher growth than non-AM plants, which was indicated by dried biomass and height (Figure 2). However, the number and total area of leaves between the two treatments did not show a significant difference (Table 1).

Figure 2. Plant biomasses after the growth period of A) *E. asperula* plants and B) *S. procumbens* plants. M+ and M- indicate for plants with and without AM, respectively

P uptake

One of the main effects of AM fungi on plant growth is that AM fungi enhance P uptake from the soil for plant needs. This characteristic of AM fungi has been reported in several studies (review see Smith and Read, 2008). For this reason, P uptake was analyzed in the plant biomasses of this study.

E. asperula plants: the results demonstrated that the P concentration in the shoots was higher than in the roots, ranging from 1.04 to 1.2 g/kg dry weight. Generally, P concentrations in plant biomass of the two treatments were not significantly different; although P concentrations in AM plants were higher than plants non-AM plants. In contrast, P content in roots of AM plants showed significantly higher than those of non-AM plants. The higher dry weight of AM-plants led to a more P content in both shoots and roots compared to non-AM plants (Figure 3).

S. procumbens plants: the P concentration in AM plants was especially high in the roots compared to the shoots, as was the P content. In non-AM plants, the P concentration in roots and shoots was similar, there was no significant difference. Regarding P content in biomass, AM plants had a higher P content in both roots and shoots than those of non-AM plants (Figure 4).

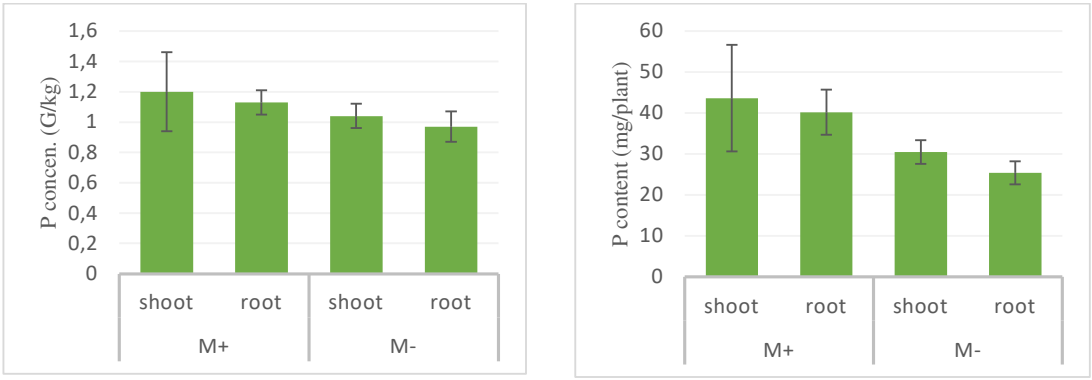

Figure 3. The P concentration and P content in root and shoot biomasses of *E. asperula* plants. Two-way ANOVA analysis showed no significant difference of P concentration and content in M+ and M- plants

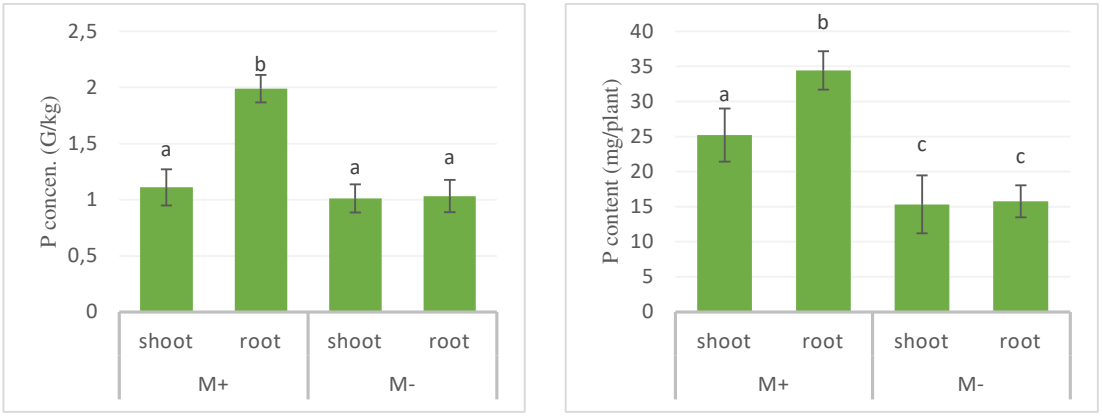

Figure 4. The P concentration and P content in root and shoot biomass of *S. procumbens* plants
The different letters indicate significant differences according to posthoc Tukey HSD test

Polyphenol accumulation

In addition to assess the effect of AM fungus on plant growth, we also evaluated the effect of AM fungus on the production of polyphenols in plant biomass. Polyphenols are a large and important group of bioactive compounds which commonly found in plant species as secondary metabolites. For this reason, polyphenols were chosen to test in this study.

Generally, the results showed that the polyphenols accumulated more in the roots than in the shoots of both *E. asperula* and *S. procumbens* plants (from 2 to 4 times higher, respectively). The polyphenol concentration of *E. asperula* with AM fungus was lower than those of non-AM plants; however, the contents were still higher especially in the roots, because of larger biomass (Figure 5). In contrast with *E. asperula*, the polyphenol concentration in *S. procumbens* plants with AM fungus was higher than that of non-AM plants, and accumulated mainly in roots. This led to significantly higher polyphenol content in root and shoot biomasses of AM plants than non-AM plants (Figure 6).

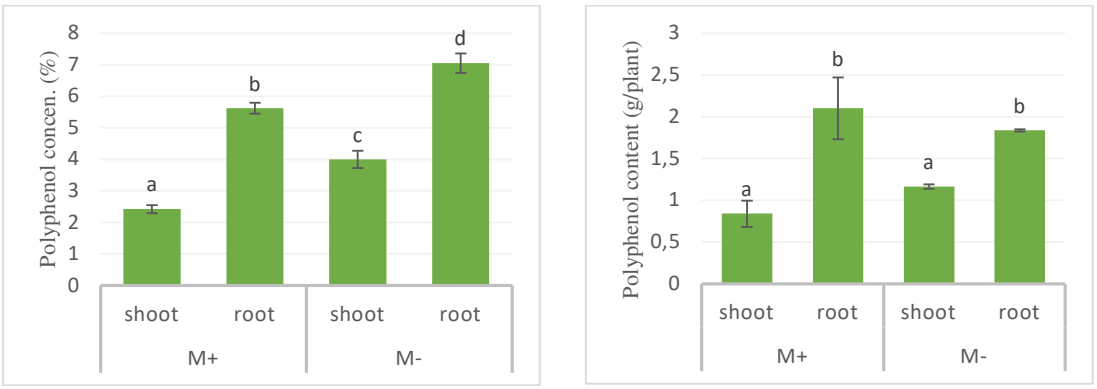

Figure 5. The polyphenol concentration and content in root and shoot biomass of *E. asperula* plants
The different letters indicate significant differences according to posthoc Tukey HSD test

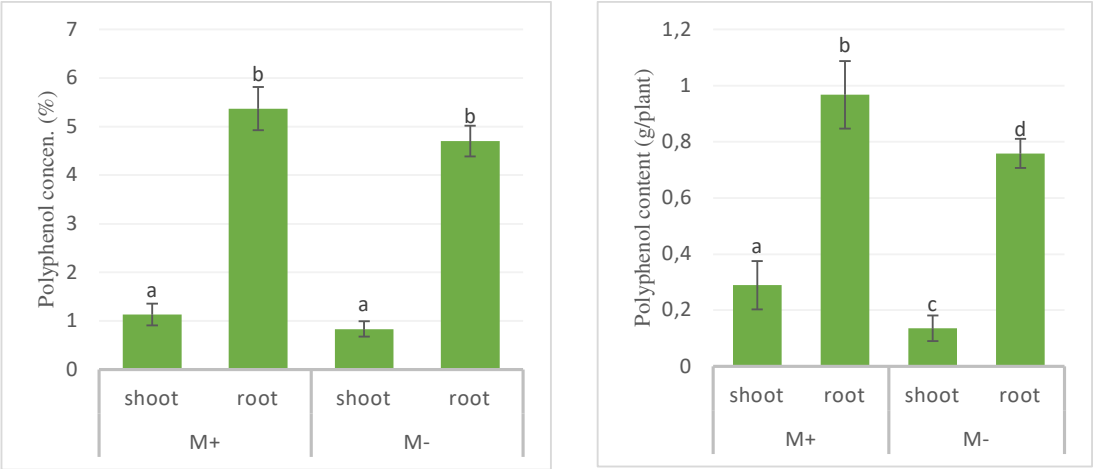

Figure 6. The polyphenol concentration and content in root and shoot biomass of *S. procumbens* plants
The different letters indicate significant differences according to posthoc Tukey HSD test.

Discussion

Arbuscular mycorrhizal fungi contribute to plant growth by enhancing nutrient and mineral uptake via their hyphal network spreading widely in soils, and this was proved by many reports which were conducted in different plants (review see Smith and Read, 2010). Besides enhancing nutrient uptake, AM fungi are able to produce phyto-hormone and to compete with pathogens, improving plant immunity to cope with abiotic and biotic stresses from the environment (Porcel *et al.*, 2006; Aroca *et al.*, 2007; Hildebrandt *et al.*, 2007). The positive effects of AM fungus in this experiment were clearly observed by the higher biomass of the plants inoculated with AM. Increasing root biomass is one of the main effects of AM fungi on plant growth (Li *et al.*, 2011; Galindo *et al.*, 2013). This increasing helps plants to uptake more nutrients from soils, and importantly, to create more contact between plant roots and fungal hyphae for exchanging water, minerals and foods (Smith and Smith, 2011). Results of P uptake can partly mirror for the advantage of nutrient exchange between plants inoculated with fungi. In *E. asperula*, the P concentration of AM plants tended to be higher than of non-AM plants, but the higher was not significant. The P content, however, was significant higher due to the bigger biomass weight, especially in the root system. Interestingly, the advantage of AM colonization on plant growth was observed only after three months of culture. This implies that the penetration of AM fungus to the root system as well as to form structures inside root cells took a longer time than other reported plants (Smith and Read, 2010). This effect on *S. procumbens*, however, was observed much earlier, within a month of growth. The height of the AM plants started to exceed non-AM plants after a month and showed significance at harvest time. There was even a sign of slow-growing after 3 months in non-AM plants in comparison with AM plants. The colonization parameters of both plant species showed an average level in comparison with other agricultural plants (Smith *et al.*, 2011; Bui and Franken, 2018). The frequency of colonization demonstrated the extent of root system performed connection with AM fungi; and this parameter in *S. procumbens* was higher than in *E. asperula*, but the intensity was a bit lower, meaning the AM structures (like hyphae, arbuscules, vesicules) was not so dense in roots. Despite the average level of colonization, the effect of the AM fungus on the growth of both plant species was clearly observed.

Previous studies have shown that the interaction between AM fungi and plants depends on the secondary metabolites such as the polyphenol group which acts as signaling factors for the primary contact (Giovannetti *et al.*, 1994; Buee *et al.*, 2000; Nagahashi and Douds, 2000). Quercetin and quercitrin (flavonoids) are polyphenols that are secreted by plant roots to induce AM fungi propagules into contact with the roots and penetrate them to form symbiosis (Tian *et al.*, 2021). The symbiosis of AM fungi affects the

production of secondary metabolites in general or polyphenols in particular. Several studies on medicinal plants showed an increase in concentrations of such compounds if the AM colonize plant root (Rosa-Mera *et al.*, 2011; Zubeck *et al.*, 2012). Because besides the function as signaling factors, secondary metabolites also play a role as anti-pathogen in the immune system of plants that fight against infection (Bednarek *et al.*, 2012; Piasecka *et al.*, 2015). In the early stage of contact with AM fungi, plants recognize them as pathogen or parasites, which makes the plants activate the defense system to resist the penetration, and releasing secondary metabolites is one of the activities for defending responses from plants (García-Garrido and Ocampo, 2002).

However, in *E. asperula* plants, polyphenol concentrations were lower in plants with AM than those without AM fungus, although the total content in plant biomass was not significantly different due to the higher weight of AM plants. This scenario is quite common when comparing the concentration and content of a substance in plants with and without AM colonization. This is called the "dilution effect" which implies that the growth of AM plants is usually faster and higher than that of non-AM plants, resulting in lower concentration but the total amount remains unchanged or even higher (Jarell and Beverly, 1981; Khan *et al.*, 2000; Davis, 2009). Nevertheless, the results demonstrated that the effect of AM fungus on polyphenol production in *E. asperula* plants was not obvious in this experiment. Most likely, the *E. asperula* is a perennial woody plant and the polyphenol accumulation might not yet enter the main stage, so the influence of AM fungus at this experimental time was not significant. Normally, after two-year growth, the bioactive substances accumulating in *E. asperula* biomasses are enough to harvest and use (Nguyet *et al.*, 2018; Kim *et al.*, 2019). Maybe, it is still early to see an obvious effect of the AM fungus on the polyphenol production in *E. asperula* plants.

In contrast, the AM colonization in *S. procumbens* plants tended to increase the concentration of polyphenols in both root and shoot biomasses, and the polyphenol content was significantly higher than that of non-AM plants. In this case, the "dilution effect" found in *E. asperula* was unlikely to occur and the effect of AM on the polyphenol production was significant. This can also be explained that *S. procumbens* is an herb with a shorter life cycle than *E. asperula*. The plants can be harvested with their highest bioactive substance accumulation after six-month growth (Tran *et al.*, 2019). So, the four-month growing period in this experiment is enough for the plants to be mature and accumulate secondary metabolites, and the effect of AM fungi was obviously observed.

Conclusions

In conclusion, the role of the AM fungus on the positive growth of the two medicinal plant species was clearly observed, although the time to see the effects was different. The AM plants were higher with bigger biomasses than those of non-AM plants after the culture period. Regarding the ability of the fungus to enhance polyphenol production, the effect was different between the two species. Forming symbiosis with the AM fungus decreased polyphenol concentration in both shoots and roots of *E. asperula*. Whereas in *S. procumbens*, the colonization of the fungus increased polyphenol concentration and content in all parts of the plants.

Authors' Contributions

CVB: conceptualization, writing – original draft, data curation, investigation; QDL: validation, investigation, methodology; ATKV: greenhouse trial, formal analysis, data curation; LDT: review and editing, project supervision. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research is funded by Graduate University of Science and Technology (GUST) under grant number GUST.STS.ĐT2019-HH02. We also would like to thank to Institute for Tropical Technology (ITT), Vietnam Academy of Science and Technology (VAST), for providing research facilities.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Araim G, Saleem A, Arnason JT, Charest C (2009). Root colonization by an arbuscular mycorrhizal (AM) fungus increases growth and secondary metabolism of purple coneflower, *Echinacea purpurea* (L.) Moench. Journal of Agricultural and Food Chemistry 57(6):2255-2258. *https://doi.org/10.1021/jf803173x*

Aroca R, Porcel R, Ruiz-Lozano JM (2007). How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? New Phytologist 173(4):808-816. *https://doi.org/10.1111/j.1469-8137.2006.01961.x*

Bednarek P (2012). Chemical warfare or modulators of defence responses–the function of secondary metabolites in plant immunity. Current Opinion in Plant Biology 15(4):407-414. *https://doi.org/10.1016/j.pbi.2012.03.002*

Buee M, Rossignol M, Jauneau A, Ranjeva R, Bécard G (2000). The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Molecular Plant-Microbe Interactions 13(6):693-698. *https://doi.org/10.1094/MPMI.2000.13.6.693*

Bui VC, Franken P (2018). Acclimatization of *Rhizophagus irregularis* enhances Zn tolerance of the fungus and the mycorrhizal plant partner. Frontiers in Microbiology 9:3156. *https://doi.org/10.3389/fmicb.2018.03156*

Davis DR (2009). Declining fruit and vegetable nutrient composition: what is the evidence? HortScience 44(1):15-19. *https://doi.org/10.21273/HORTSCI.44.1.15*

De la Rosa-Mera CJ, Ferrera-Cerrato R, Alarcón A, de Jesús Sánchez-Colín M, Muñoz-Muñiz OD (2011). Arbuscular mycorrhizal fungi and potassium bicarbonate enhance the foliar content of the vinblastine alkaloid in *Catharanthus roseus*. Plant and Soil 349(1):367-376. *https://doi.org/10.1007/s11104-011-0883-y*

Galindo-Castañeda T, Romero HM (2013). Mycorrhization in oil palm (*Elaeis guineensis* and *E. oleifera* x *E. guineensis*) in the pre-nursery stage. Agronomía Colombiana 31(1):95-102.

García-Garrido JM, Ocampo JA (2002). Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. Journal of experimental Botany 53(373):1377-1386. *https://doi.org/10.1093/jexbot/53.373.1377*

Giovannetti M, Sbrana C, Logi C (1994). Early processes involved in host recognition by arbuscular mycorrhizal fungi. New Phytologist 127(4):703-709.

Hildebrandt U, Regvar M, Bothe H (2007). Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68(1):139-146. *https://doi.org/10.1111/j.1469-8137.1994.tb02973.x*

Jurkiewicz A, Ryszka P, Anielska T, Waligórski P, Białońska D, Góralska K, ... Turnau K (2010). Optimization of culture conditions of Arnica montana L.: effects of mycorrhizal fungi and competing plants. Mycorrhiza 20(5):293-306. *https://doi.org/10.1007/s00572-009-0280-z*

Kapoor R, Giri B, Mukerji KG (2002a). Glomus macrocarpum: a potential bioinoculant to improve essential oil quality and concentration in dill (*Anethum graveolens* L.) and carum (*Trachyspermum ammi* (Linn.) Sprague). World Journal of Microbiology and Biotechnology 18(5):459-463. *https://doi.org/10.1023/A:1015522100497*

Kapoor R, Giri B, Mukerji KG (2002b). Mycorrhization of coriander (*Coriandrum sativum* L) to enhance the concentration and quality of essential oil. Journal of the Science of Food and Agriculture 82(4):339-342. *https://doi.org/10.1002/jsfa.1039*

Khan AG, Kuek C, Chaudhry T, Khoo CS, Hayes WJ (2000). Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41(1-2):197-207. *https://doi.org/10.1016/S0045-6535(99)00412-9*

Kim DD, Nguyet VT, Anh HX, Trang NTT, Chuyen NH, Ha TTH, Dat NT (2019). Cytotoxic phenolic constituents from the leaves of *Ehretia asperula*. Bangladesh Journal of Pharmacology 14(4):196-197.

Li H, Ye Z, Chan W, Chen X, Wu F, Wu S, Wong MH (2011). Can arbuscular mycorrhizal fungi improve grain yield, As uptake and tolerance of rice grown under aerobic conditions? Environmental Pollution 159(10):2537-2545. *https://doi.org/10.1016/j.envpol.2011.06.017*

Nagahashi G, Douds DD Jr. (2000). Partial separation of root exudate components and their effects upon the growth of germinated spores of AM fungi. Mycological Research 104(12):1453-1464. *https://doi.org/10.1017/S0953756200002860*

Nell M, Wawrosch C, Steinkellner S, Vierheilig H, Kopp B, Lössl A, Franz C, Novak J, Zitterl-Eglseer K (2010). Root solonization by symbiotic arbuscular mycorrhizal fungi increases sesquiterpenic acid concentrations in *Valeriana officinalis* L. Planta Medica 76:393-398. *https://doi.org/10.1055/s-0029-1186180*

Nguyen TL, Pham TH, Huynh TTH (2017). Evaluating the systematic position of *Ehretia asperula* Zoll. & Moritzi based on ITS1, matK and trnL-trnF DNA sequences. Vietnam Journal of Science, Technology and Engineering 59(4):61-65. *https://doi.org/10.31276/VJSTE.59(4).61*

Nguyet VT, Dat NT, Ha TTH, Chuyen NH, Hang NT, Kim DD (2018). Evaluating cytotoxic effect of the extracted compounds from Ehretia asperula Zoll. & Mor. stem on several cancer cell lines. Academia Journal of Biology 40(2):145-152.

Piasecka A, Jedrzejczak-Rey N, Bednarek P (2015). Secondary metabolites in plant innate immunity: conserved function of divergent chemicals. New Phytologist 206(3):948-964. *https://doi.org/10.1111/nph.13325*

Porcel R, Aroca R, Azcon R, Ruiz-Lozano JM (2006). PIP aquaporin gene expression in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants in relation to drought stress tolerance. Plant Molecular Biology 60(3):389-404. *https://doi.org/10.1007/s11103-005-4210-y*

Redecker D, Raab P (2006). Phylogeny of the glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. Mycologia 98(6):885-895. *https://doi.org/10.1080/15572536.2006.11832618*

Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M, ... Colla G (2015). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Scientia Horticulturae 196:91-108. *https://doi.org/10.1016/j.scienta.2015.09.002*

Schüßler A, Kluge M (2001). Geosiphon pyriforme, an endocytosymbiosis between fungus and cyanobacteria, and its meaning as a model system for arbuscular mycorrhizal research. Fungal associations, Springer 151-161. *https://doi.org/10.1007/978-3-662-07334-6_9*

Smith FA, Smith SE (2011). What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants? Plant and Soil 348(1):63-79. *https://doi.org/10.1007/s11104-011-0865-0*

Smith SE, Jakobsen I, Grønlund M, Smith FA (2011). Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiology 156(3):1050-1057. *https://doi.org/10.1104/pp.111.174581*

Smith SE, Read DJ (2010). Mycorrhizal symbiosis. Academic press.

Tian B, Pei Y, Huang W, Ding J, Siemann E (2021). Increasing flavonoid concentrations in root exudates enhance associations between arbuscular mycorrhizal fungi and an invasive plant. The ISME Journal 1-12. *https://doi.org/10.1038/s41396-021-00894-1*

Toussaint JP, Smith F, Smith S (2007). Arbuscular mycorrhizal fungi can induce the production of phytochemicals in sweet basil irrespective of phosphorus nutrition. Mycorrhiza 17(4):291-297. *https://doi.org/10.1007/s00572-006-0104-3*

Tran TTT, Dang HP, Nguyen TN (2019). Chemical constituents from methanolic extract of *Solanum procumbens* Lour (Solanaceae). Vietnam Journal of Science, Technology and Engineering 61(3):9-11. *https://doi.org/10.31276/VJSTE.61(3).09-11*

Trouvelot A, Kough J, Gianinazzi-Pearson V (1986). Estimation of vesicular arbuscular mycorrhizal infection levels. Research for methods having a functional significance. Physiological and genetical aspects of mycorrhizae. Aspects physiologiques et genetiques des mycorhizes: proceedings of the 1st European Symposium on Mycorrhizae, Dijon, 1-5 July 1985, Paris: Institut National de le Recherche Agronomique, c1986.

Turnau K, Jurkiewicz A, Lingua G, Barea J, Gianinazzi-Pearson V (2005). Role of arbuscular mycorrhiza and associated microorganisms in phytoremediation of heavy metal-polluted sites. Trace elements in the environment. Biogeochemistry, biotechnology, and bioremediation. CRC Taylor & Francis, Boca Raton, London, New York, pp 235-252.

Zhao J, Deng H, He X (2009). Effects of AM fungi on the quality of trueborn Angelica dahurica from Hebei province. Acta Agriculturae Boreali-Sinica 24:299-302.

Zubek, S., Mielcarek S, Turnau K (2012). Hypericin and pseudohypericin concentrations of a valuable medicinal plant *Hypericum perforatum* L. are enhanced by arbuscular mycorrhizal fungi. Mycorrhiza 22(2):149-156. *https://doi.org/10.1007/s00572-011-0391-1*