

SZENT ISTVÁN UNIVERSITY

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

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ABBREVIATIONS

- AM** Arbuscular mycorrhiza
- AMF** Arbuscular mycorrhizal fungi
- CAT** Catalase
- DAT** Days after transplanting
- DNA** Deoxyribonucleic acid
- E. prostrata** *Eclipta prostrata*
- EP** *Eclipta prostrata*
- ETI** Effector-triggered immunity
- HR** hypersensitive response
- ISR** Induced systemic resistance
- MAMPs** microorganism-associated molecular patterns
- MIR** Mycorrhiza-induced resistance
- PAL** Phenylalanine ammonia-lyase
- PCR** Polymerase chain reaction
- POD** Peroxidase
- PPO** Polyphenol Oxidase
- PSI** Photosystem I
- PSII** Photosystem II
- RH** Relative humidity
- ROS** Reactive oxygen species
- SAR** Systemic acquired resistance
- SOD** Superoxide dismutase
- VTC** Vacuolar transporter chaperone

1. INTRODUCTION

Most crops are cultivated with high input of mineral fertilizers and chemical pesticides, which has led to considerable damage to the ecosystem. The application of biological measures could provide an alternative pathway to reduce the ecological footprint, which benefits the consumers and reduces health risks. Sustainable agriculture can be achieved through the adaptation of crops to soil fertility properties and the reduction in the application of chemical fertilizers. Microbial biodiversity is found to be a crucial factor in controlling different biological processes in the soil, especially their relationship with the plant roots.

Another critical issue which the world faces is soil salinization. This soil problem is a growing environmental problem causing a severe menace to world food security. Application of fertilizers and irrigation in agriculture, together with overuse of groundwater resources and low rainfall, also contribute substantially to salinization ([Cantrell and Linderman, 2001](#), [Al-Karaki, 2006](#)). It is estimated that 50% of the total irrigated land in the world is negatively influenced by salt-affected soils ([Ruan et al., 2010](#)). More severely, ever-growing salinization of agricultural land is predicted to lead to a 30% and even up to 50% loss of agricultural areas in the next 25 years and by 2050, respectively and salt-affected soils have nearly reached to 34 million irrigated hectares ([FAO, 2012](#)), pose a global risk

Interestingly, under natural conditions, plants often interact with microbes, which directly mediates plant performance under various environmental conditions. Some plant-microbe associations lead to mitigation of stress-related damages and improvement of plant tolerance to environmental stresses ([Duc et al., 2018](#), [Katalin and Duc, 2019](#)). In addition, microorganisms play an important role in the rhizosphere, which has an impact on plant growth through their association in nutrient cycling and acquisition. In agriculture, microbes have an enormous essential application and are an integral component, including decomposing organic matter, drought tolerance, heat tolerance, resistance to insects, and resistance to plant diseases of the farming system. Arbuscular mycorrhizal fungi (AMF), as common soil microbes, can colonize the roots of most terrestrial plant species. These beneficial fungi have been reported to significantly contribute multiple benefits to its host plants such as enhanced plant uptake of water and mineral nutrients, increased resistance/tolerance to environmental adversities, therefore decreases input of inorganic fertilizers and pesticides in agriculture. AMF helps plants to 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. Hence, the exploitation of AM symbiosis is one of the most effective practices to improve crop 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 the people and export. It provides high economic returns to the farmer and thereby contribute to Gross domestic product (GDP) in Vietnam. Due to the fact that the introduction of non-native arbuscular mycorrhizal fungi could result in the local soil disturbance, isolation of indigenous arbuscular mycorrhizal fungi 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 arbuscular mycorrhizal fungi on two spice and medicinal plants.

Objectives

Our aims were:

To identify AMF species penetrating chilli (*Capsicum frutescens* L.) roots in inocula using tropical forest, agricultural, and grassland soils. Then DNA sequences and phylogenetic analyses of AMF were implemented.

To investigate the impact of AM inoculation and growth substrate on biomass and content of polyphenols in *Eclipta prostrata*.

To explore the effect of salt stress and AMF on plant performance and secondary compounds of *Eclipta prostrata*.

To assess the impact of different AMF species on plant performance and secondary compounds of *Eclipta prostrata*.

2. LITERATURE REVIEW

2.1. Arbuscular mycorrhizal fungi

2.1.1. Taxonomy of Arbuscular mycorrhizal fungi (AMF)

At present, AMF is categorized as a member of phylum Glomeromycota consisting of four orders (Glomerales, Archaeosporales, Paraglomerales, and Diversisporales), with 11 families, 25 genera, (Fig 1) (Redecker et al., 2013) and more than 270 species. Nonetheless, data based on next-generation sequencing of root samples (Kruger et al., 2012) and recent results (Chen et al., 2018) indicate that its number may be an order of magnitude higher.

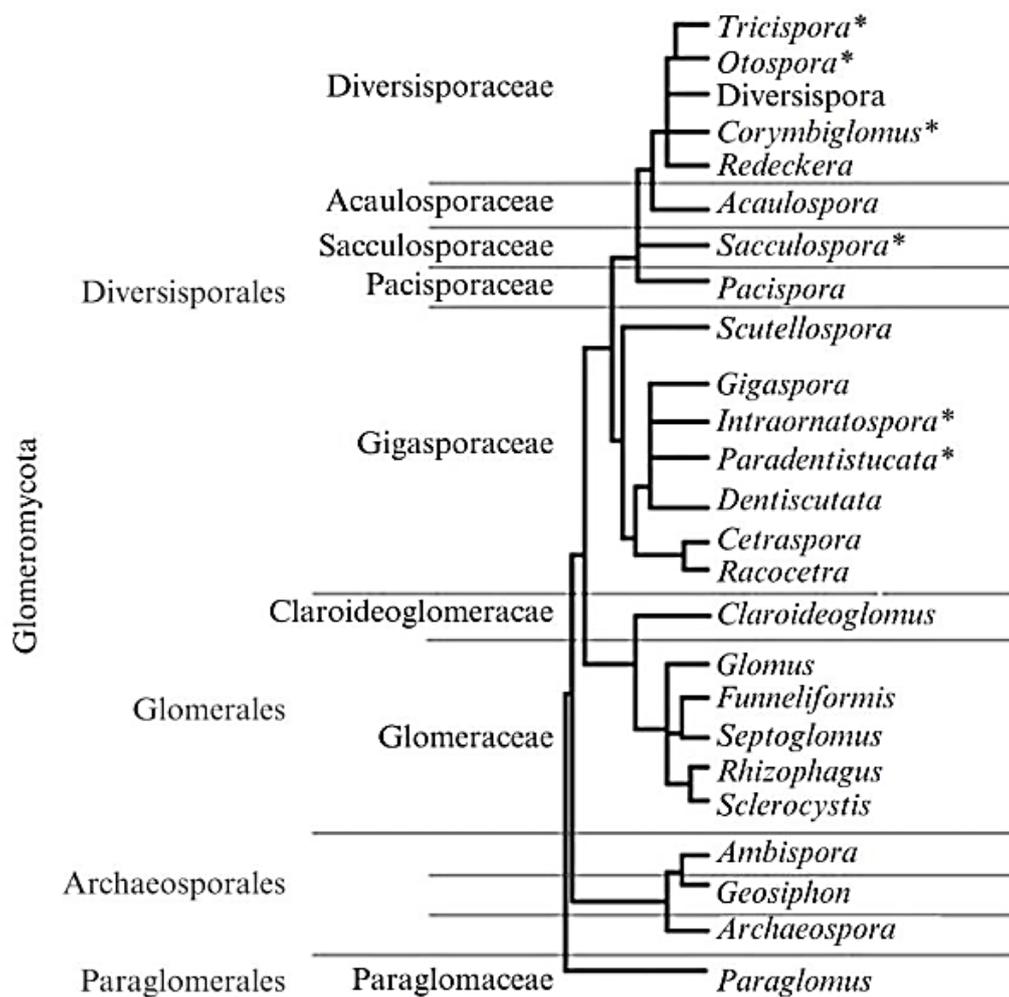


Figure 1: Taxonomy of AMF (Redecker et al., 2013, Kruger et al., 2012, Oehl et al., 2011)

*Insufficient evidence, but no formal action is taken.

2.1.2. Identification of arbuscular mycorrhizal fungi

The identification of AMF requires tools that provide criteria for defining and resolving biological groups at different taxonomic levels. AMF were identified by classical method based on spore morphology. The main criteria used for species delimitation are spore size, shape, color, basal structure, ornamentation, and wall or wall layer structure (Brundrett, 2009). Nevertheless, this could be misleading because the traditional method was influenced by the host plant in the experiment (Jansa et al., 2002), physiological parameters of the AMF, and environmental conditions (Walker, 1992). Several species form some spore morphs, and spore occurrence, as resting stages, does not show the active community of AMF (Hempel et al., 2007). In addition, in the case of samples from extraradical or intraradical mycelium, only molecular methods can be used for the identification of AMF species thanks to molecular markers (Senés-Guerrero and Schüßler, 2016). Indeed, molecular identification is the present methodology that has reclassified a variety of AMF species (Souza, 2015). rDNA internal transcribed spacer and DNA sequences of nuclear small subunit ribosomal RNA gene of mycorrhiza have been used (Gai et al., 2009, Binet et al., 2011). Also, the large ribosomal subunit (LSU) gene has been selected as a target for the Polymerase Chain Reaction (PCR) in numerous studies, since this gene consists of enough variation to identify AMF species (Johnson et al., 2015, Nielsen et al., 2016). A phylogenetic reference data for phytotaxonomic and systematics of mycorrhiza has been developed (Kruger et al., 2012), which has been exploited as DNA barcode for the fungal symbionts (Senés-Guerrero and Schüßler, 2016). This reference dataset is a crucial tool for AMF identification from environmental samples. Noticeably, various investigations have been implemented on AMF diversity in soils, using morpho-taxonomy (Marta, 2006, Villacrés and Boris, 2014) and more notably molecular tools (Hassan Sel et al., 2014, Ivan E. de la Providencia, 2015, Iffis et al., 2016), hence the accuracy of AMF species identification has improved.

2.1.3. Life cycle of arbuscular mycorrhizal fungi

AMF are obligate biotrophs because they rely on their host to complete their life cycle (Fig. 2). Multiple rounds of spore germination can occur in AM fungi. This exploratory hyphal development changes dramatically in the presence of plant-derived signals called strigolactones. They are phytohormones secreted from the plant roots and are able to stimulate hyphal branching, a key step in the root colonization process (Akiyama K., 2005). These hormones owe their name to the first natural strigolactones identified as a germination stimulant of the root parasitic plant *Striga lutea* (witchweed) (Cook, 1966). AM fungi respond to the plant exudates by producing mycorrhizal signal (myc factor). The myc factor activates symbiosis-associated genes in the host plant (Kaku et al., 2006).

When the AMF hyphae reach the root surface, hyphopodia are developed with a similar function to the appressorium of pathogen fungi (Bastmeyer et al., 2002). On the plant side, epidermal cells assemble a transcellular structure right below the fungal hyphopodium, a few hours before host cell penetration. A precise succession of processes leads to the formation of the so-called pre-penetration apparatus (PPA). This appears as a cytoplasmic column containing microtubules and microfilaments. Only after this transcellular tunnel has been formed, the fungus enters the cell, grows across it, and move towards the cortex, suggesting that the host plant has the major control over fungal penetration and development (Genre et al., 2005).

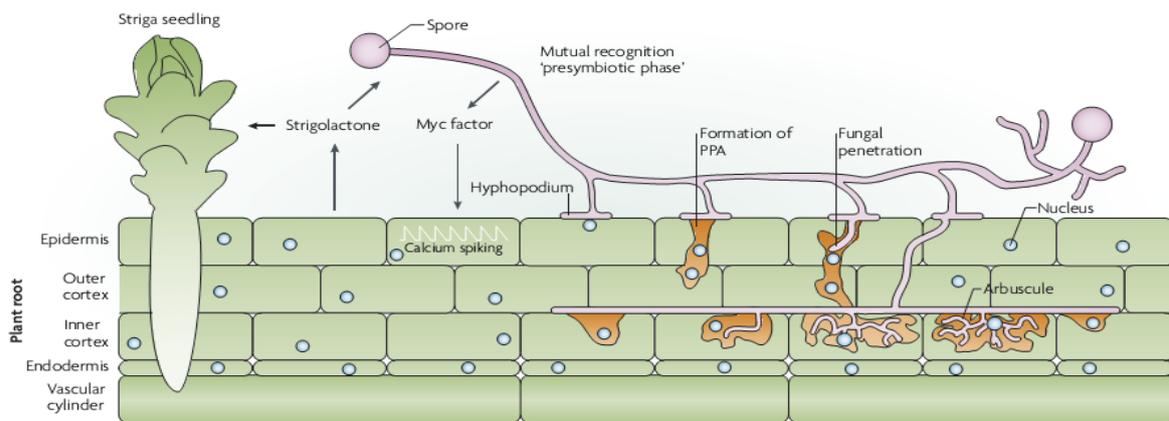


Figure 2: Arbuscular mycorrhizal fungi life cycle outside and inside the root (Parniske, 2008).

Beside intracellular arbuscula, the colonizing AM fungi sometimes produce vesicles in the apoplast, which are proposed to function as storage organs of the fungus. The life cycle ends with the synthesis of new spores typically outside of the plant root at the leading tip of individual fungi (Parniske, 2008).

Once inside, the apoplast fungal hyphae start to branch and grow laterally along the root axis. These hyphae induce the development of PPA-like structures in inner cortical cells (Genre et al., 2008), followed by penetration and formation of arbuscules. The fungal hyphae inside the cell branch repeatedly to produce the tree-shaped arbuscule structure surrounded by a new-formed plant-derived periarbuscular membrane (PAM). Nutrients are exchanged across the symbiotic interface between the fungus and the plant (periarbuscular matrix).

2.1.4. Benefits of arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi are the most common fungi in soils and account for 9–55% of the soil microbial biomass and 5–36% of the total soil biomass (Olson et al., 1999). These fungi are obligate root symbionts inhabiting almost all terrestrial ecosystems and play a vital role in agricultural ecosystems. Indeed, they can mutually associate with around 80% of vascular plants

and with approximately 90% of agricultural plants (Smith and Read, 2008). In this association, the host plant performance is enhanced through the uptake of mineral nutrient and water by the hyphal network of mycorrhizal partner (Smith and Read, 2008), whilst the fungus gains 10–20% of total photosynthates (Allen et al., 2003) and lipids (Bravo et al., 2017) from the host. Within root cells, arbuscular mycorrhizal fungi form typical tree-like structures, the arbuscules (from the Latin word *arbusculum*), or hyphal coils. Some also produce storage organs, termed vesicles (from this the former name Vesicular Arbuscular Mycorrhizae, VAM). The AM symbiosis represents an ancient symbiosis. Based on the fossil record and molecular data, arbuscular mycorrhizal fungi are believed to appear at least since the Ordovician (460 million years ago), which coincides with the event of the first land plants invading the terrestrial environment (Remy et al., 1994).

AM associations vary widely in structure, function, and benefits provided to the host plants. AMF hyphal network extends the rhizosphere and explores the surrounding soil deeply with a density of 100 meters of hyphae per cubic centimeter (Miller et al., 1995). This hyphal network, specialized for the uptake of macronutrients (Allen and Shachar-Hill, 2009) as well as micronutrients (Caris et al., 1998, George, 2000), provides a greater absorptive surface than root hairs, leading to better mineral nutrition for the plant. In return for supplying plants with nutrients and water, AM fungi obtain organic carbon in the form of carbohydrates from plants (Solaiman and Saito, 1997). It was estimated that about 20% of the photosynthesis products of land plants (approximately 5 billion tons of carbon per year) are addressed to AM fungi (Bago et al., 2000). In addition to a better mineral uptake, mycorrhizal fungi can enhance drought, saline stress tolerance, which are special features of AM to help host plants to grow under harsh environmental conditions (Jung et al., 2012, Birhane et al., 2012). Furthermore, AMF can increase plant resistance against pests and soil-borne pathogens (Pozo et al., 2009) and tolerance against toxic concentrations of heavy metals such as copper, zinc, lead (Huang et al., 2000, Ouziad et al., 2005, Gohre and Paszkowski, 2006).

In terms of soil properties, glomalin, a glycoprotein, produced abundantly by the fungal hyphae (Rillig et al., 2001) serve as a glue, binding nitrogen, carbon and other biological components of soil to clay, sand and the mineral components, hence enriching soil organic matter as well as soil carbon storage (Six et al., 2000). The glomalin content in soil links closely with water stability of aggregates (Wright and Upadhyaya, 1998). Thus, AMF can improve soil water relations and soil texture (Bethlenfalvay and Schuepp, 1994) markedly.

The application of AMF as biofertilizer in agriculture, horticulture, in site remediation, or landscape restoration had been highlighted. The fungi importantly contribute to maintenance and

enhancement of soil quality and fertility, regulation and functioning of plant communities and plant biodiversity, productivity in microcosms, particularly under conditions of phosphorus scarcity (van der Heijden et al., 1998, Smith and Read, 2008). Regarding environmental aspects, the utilization of AMF could lessen the input of inorganic fertilizers, in particular phosphorus fertilizers, and pesticides in agriculture thanks to AM-induced protection against a variety of detrimental environmental adversities and improvement of plant growth and productivity.

2.1.5. The role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen by target plants

Phosphorus (P) and nitrogen (N) elements play a significant role in plant metabolism, and their deficit can affect plant development. Nitrogen is one of the crucial elements in the nucleic acid structure, protein synthesis, and plays an essential role in the energy metabolism of the plant. Nitrogen exists in two forms in the soil: organic and inorganic. Plants use the organic form of nitrogen easier than the inorganic one. However, in the soil, inorganic nitrogen is more abundant than organic. Phosphorus plays a vital role in the cell membrane assembling, nucleic acid structure, protein synthesis regulation, and it is also the primary substrate in energy metabolism. Therefore, soil phosphorus availability is a parameter that strongly affects plant life, with significant consequences on crop yield. Total P concentration in the soil is high, but orthophosphate (Pi), the inorganic form used preferably by plants, is available in limited amounts because of its low solubility and slow diffusion rates. Consequently, a depletion zone is formed around actively absorbing roots.

To confront with Pi limitation, plants have evolved an array of adaptive strategies for improving Pi acquisition and use efficiency. One of these strategies applied by plants to overcome P deficiency is the symbiotic association with arbuscular mycorrhiza. The intensive hyphal network of AMF increases the supply of mineral nutrients to the host plant. Even though the main benefit of the mycorrhizal association is the P improvement for the host plant, AMF colonization often leads to improved uptake of nitrogen and other nutrients (Smith et al., 2011).

AM-associated plants possess two uptake pathways for P and N, a direct pathway by roots and a mycorrhizal pathway via the fungal symbiont (Fig. 3 and Fig. 4). The direct pathway is mediated by nutrient uptake transporters with high- or low affinity in the epidermis or root hairs. Particularly for P with low mobility in the soil, the uptake through the direct pathway is often restricted due to depletion zones around roots. In contrast, the mycorrhizal pathway begins with nutrient uptake by high-affinity nutrient transporters in the extraradical hyphae. Subsequently, the absorption is translocated to the intraradical hyphae in the root cortex, then transported to root cells thanks to

mycorrhiza-inducible nutrient transporters of the plant (Smith et al., 2011). However, a plant is concurrently colonized by AMF communities that can vary in their effectiveness with which their mycorrhizal pathway makes the contributions to the total uptake of nutrients in the host plant (Bücking and Kafle, 2015).

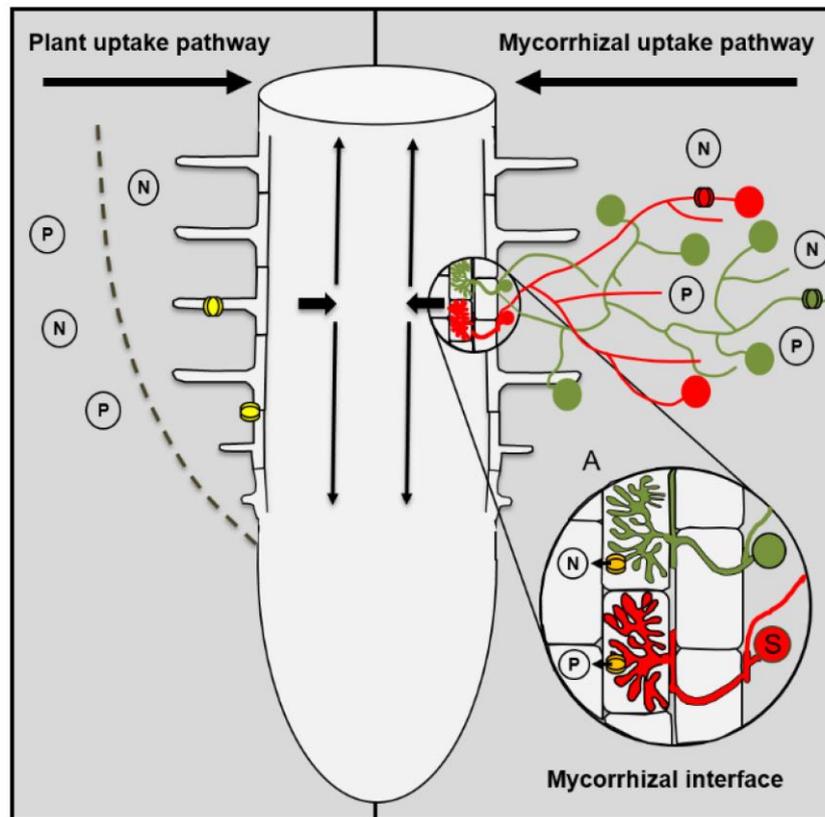


Figure 3. The direct and mycorrhizal pathway to acquire N and P in colonized plants (Bücking and Kafle, 2015). P and N can be taken up by transporters in epidermis or root hairs (yellow symbols) or through the mycorrhizal pathway where the nutrient uptake is implemented by fungal transporters in the extraradical hyphae (red or green symbols), the transport via the hyphae from the extraradical mycelium to the intraradical mycelium (see mycorrhizal interface), and the uptake from the mycorrhizal interface by AMF-inducible transporters of the host plant in the periarbuscular membrane (orange symbols). The green and red fungal structures represent the colonization of one host root by several AMF species which can vary in their effectiveness with which they are capable of taking up nutrients in the soil and transfer them to their host plant.

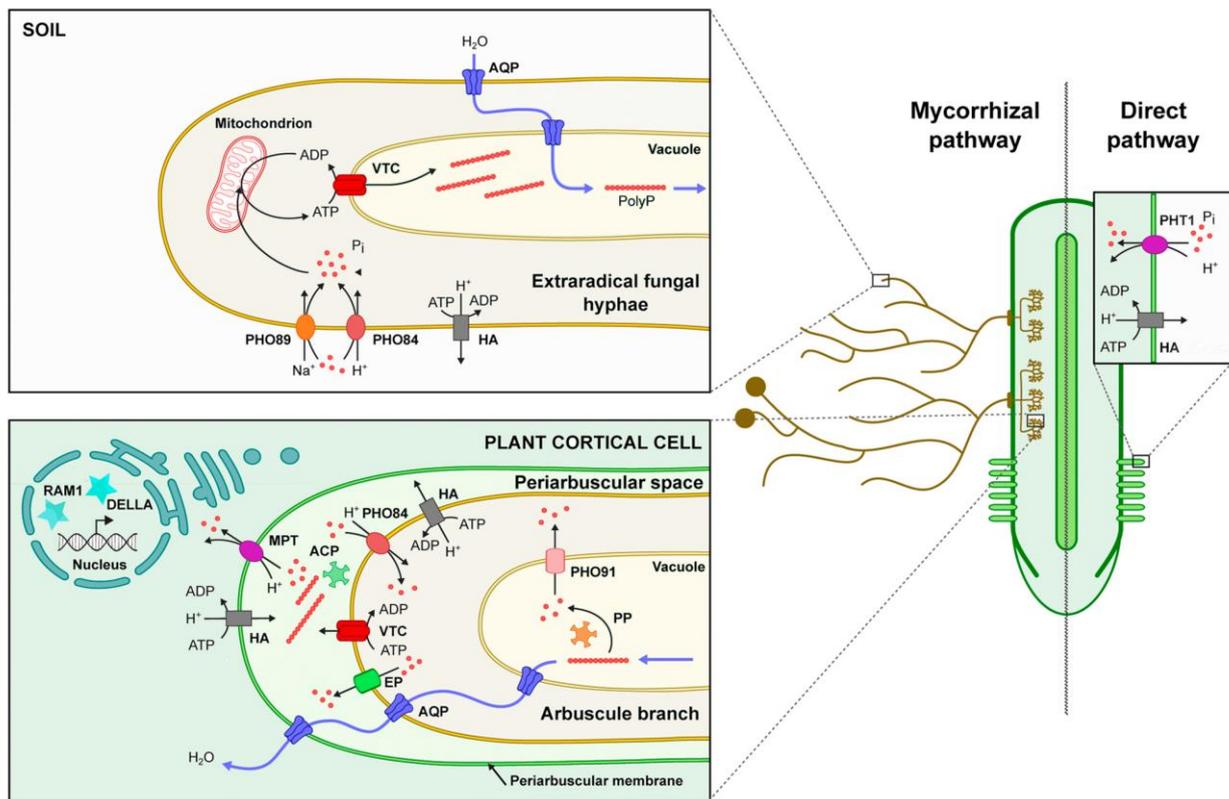


Figure 4. The Pi uptake pathways in colonized roots (Ferrol et al., 2019). Direct Pi uptake by roots involves the activities of a H⁺-ATPase (HA) and a Pi transporter of the Pht1 family. Mycorrhizal Pi acquisition begins with Pi uptake by the extraradical mycelium via a H⁺/Pi symporter (PHO84) promoted by a H⁺-ATPase (HA), or via a Na⁺/Pi symporter (PHO89). Once in the cytosol, Pi may be made into ATP in the mitochondria and then transformed into polyphosphate by the transporter chaperone complex (VTC) on the vacuole. Polyphosphate is transported to the intraradical hyphae by cytoplasmic streaming, promoted by the flow of water regulated by aquaporins (AQP). In the arbuscule, polyphosphate undergoes hydrolysis by a fungal polyphosphatase (PP), then Pi is transported to the cytosol via a Pi transporter (PHO91) in the vacuole. When Pi arrives in the fungal cytosol, it is either polymerized into polyphosphate via VTC complex on the plasma membrane or released into the periarbuscular space thanks to an unidentified efflux protein (EP). Next, the polyphosphate is hydrolyzed by acid phosphatase (ACP) originated from the host plant in the periarbuscular space. Last, Pi from the periarbuscular interface is taken up by plant cells thanks to mycorrhiza-inducible Pi transporters (MPT) on the periarbuscular membrane, which is transcriptionally modulated by the transcription factors DELLA and RAM1. Also, Pi transporters of the mycorrhizal fungi are expressed in the arbuscules in which they function as transeptors.

2.1.6. Effects of arbuscular mycorrhizal fungi on plant tolerance against abiotic stresses

2.1.6.1. Nutrient uptake under abiotic stresses

AMF association is widely reported to significantly stimulate nutrient uptake of various macro-nutrients and micro-nutrients in plants, resulting in elevated photosynthate production and thus improved biomass accumulation (Chen et al., 2017, Mitra et al., 2019). AMF also effectively help host plants to acquire nutrients from the nutrient-poor soils (Kayama and Yamanaka, 2014). Under phosphorus-limited conditions, mycorrhizal colonization enhances phosphorus supply to the host roots. Indeed, AM symbiosis maintains N and P uptake, eventually sustaining plant development at lower and higher P levels under different irrigation regimes (Liu et al., 2014, Liu et al., 2018). Under drought stress, mycorrhizal inoculation substantially improved the contents of N, P, and Fe in *Pelargonium graveolens* L. (Amiri et al., 2016). Similarly, mycorrhizal *Pistachio* plants displayed high concentrations of P, K, Mn, and Zn under water scarcity (Bagheri et al., 2012). Furthermore, AMF application enhanced N and P levels in *Chrysanthemum morifolium* plants (Wang et al., 2018) and augmented seedling weight by heightening water content and intercellular CO₂, N, and P concentrations in *Leymus chinensis* plants (Jixiang et al., 2017). Under salinity stress, an improvement in levels of P, K, and Ca in *Euonymus japonica* plants due to AMF infection has been shown (Gomez-Bellot et al., 2015). It is believed that the fungal symbionts increase the uptake of most all nutrients while they lessen the absorption of Na and Cl in host plants, resulting in plant growth stimulation (Evelin et al., 2012). The interaction between AMF and salt stress significantly influences the contents of N and P, and the N:P ratio in shoots (Wang et al., 2018). In another report, native AMF inoculation significantly changed the N contents of crop plants (Turrini et al., 2018). Previous reports have illustrated that about 20–75% of the total N uptake of mycorrhizal plants could be transferred by the fungi to their host plants (Ahanger et al., 2014, Hameed et al., 2014, Hashem et al., 2018). Elevated N in AM plants evidently leads to higher leaf chlorophyll contents since N is one of the essential components of chlorophyll molecules (de Andrade et al., 2015). Other observations supporting the mycorrhiza-mediated improvement in N nutrition in host plants were described in several reports (Courty et al., 2014, Bücking and Kafle, 2015, Corrêa et al., 2015). AM colonization enhances N assimilation and C, N accumulation under ambient, and increased CO₂ levels (Zhu et al., 2016). In addition, AMF was demonstrated to enhance growth, accumulation of macro-nutrients and micro-nutrients, and their allocation in the olive plantlets cultivated under elevated Mn concentrations (Bati et al., 2015). The authors also showed that AMF was effective in limiting high accumulation of Fe, Mg, Mn, and Na in host roots.

2.1.6.2. Salinity

Soil salinization is a growing environmental problem causing a severe menace to world food security. Application of fertilizers and irrigation in agriculture, together with overuse of groundwater resources and low rainfall, also contribute substantially to salinization (Cantrell and Linderman, 2001, Al-Karaki, 2006). It is estimated that salinity and other soil problems such as soil degradation, erosion negatively influence 3.6 billion ha (about 69%) of world dryland agriculture (Riadh et al., 2010) whilst 50% of total irrigated land on the world is negatively influenced by salinity (Ruan et al., 2010). It is also calculated that the annual loss of US\$ 27.3 billion in world crop production is owing to land degradation as a result of salinity in irrigated areas (Qadir et al., 2014). More severely, ever-growing salinization of agricultural land is predicted to lead to a 30% and even up to 50% loss of agricultural areas in the next 25 years and by 2050, respectively and salt-affected soils have nearly reached 34 million irrigated hectares (FAO, 2012), posing a considerable global risk.

Salinity stress inhibits plant growth due to reducing the vegetative development and net assimilation rate leading to decreased crop productivity (Ahanger et al., 2017). It is known that salt stress featured by a high level of soil soluble salts, exerting ionic toxicity, lowered uptake of nitrate, phosphorus, potassium, and calcium and osmotic stress (Vahdati and Lotfi, 2013). The osmotic pressure of the alkaline soil solution is higher than that in plant cells, therefore, hindering plant uptake of minerals and water while available and large quantities of Na^+ , Cl^- ions can infiltrate the cells and instantly toxify membranes, disrupting cellular metabolic activities (Munns and Tester, 2008). Salinity also induces the excessive production of reactive oxygen species (Ahanger et al., 2018). Ionic toxicity, nutritional insufficiencies, and osmotic stress under saline stress altogether provoke metabolic imbalances and oxidative stress in plants.

Attempts are being made to find promising measures of obtaining improved crop productivity under salt stress conditions. One such measure is the judicious application of AMF for ameliorating the detrimental effects of saline on plants (Santander et al., 2019). Many studies have revealed the effectiveness of AMF to alleviate the salt-induced decrease in plant growth and yield under saline stress (Talaat and Shawky, 2014, Abdel Latef and Chaoxing, 2014). El-Nashar (2016) demonstrated that AMF increased leaf water potential, water use efficiency, and growth rate of *Antirrhinum majus* plants. More recently, Ait-El-Mokhtar et al. (2019) have described the beneficial influences of fungal symbiosis on plant physiology such as stomatal conductance, photosynthetic rate, and leaf water relations under salt stress conditions. AMF considerably mitigated the adverse effects on photosynthesis under saline stress (Sheng et al., 2011).

Mycorrhizal application remarkably enhanced chlorophyll content, photosynthetic rate, gas exchange traits, and water use efficiency of *Ocimum basilicum* plants subjected to salinity stress (Elhindi et al., 2017). *Allium sativum* plants colonized by AMF exhibited higher growth traits such as fresh and dry biomass, and leaf area index under salt stress (Borde et al., 2010). In addition, Wang et al. (2018) have shown substantial improvement in fresh and dry weights, and N content of roots and shoots thanks to the mycorrhizal application under moderate saline conditions. Plants associated with AMF showed heightened biosynthesis of salicylic acid, jasmonic acid, and some important inorganic nutrients. For example, contents of total N, P, K⁺, Ca²⁺, and Mg²⁺ were higher in the mycorrhizal *Cucumis sativus* plants in comparison to those nonmycorrhizal plants under salty conditions (Hashem et al., 2018). In lettuce, under salinity stress, mycorrhizal plants gained higher biomass production, elevated proline synthesis, augmented N uptake, and noticeable alterations in ionic relations, particularly decreased accumulation of Na⁺, than those in uninoculated plants (Santander et al., 2019).

2.1.7. Effects of arbuscular mycorrhizal fungi on secondary metabolism in medicinal plants

It is widely documented that the mutualistic association between AMF and medicinal plants can be applied for improved secondary metabolite production in plants. The secondary metabolites not only play an essential role in increasing the plant defense system but also can be utilized for healing human diseases. In the production of herb-based materials, it is important to adopt mycorrhizal technology to exploit the role of AM fungi in the cultivation of medicinal plants. In fact, there is a modification in the secondary metabolite accumulation owing to the occurrence of chemical and biological events during mycorrhization (Barrios, 2007, Cai et al., 2008). AMF can increase concentrations of secondary metabolites such as phenolic compounds, phytoalexins, flavonoids, cyclohexanone derivatives, apocarotenoids, triterpenoids, and glucosinolates in herbal, and medicinal plants (Singh et al., 2013, Smith and Read, 2008, Szakiel et al., 2011, Xiao et al., 2011, Zubek et al., 2013). The AM-induced improvements in both quantitative and qualitative triterpenoids in plants, for example, anethole, atracyllole, artemisinin, β -caryophyllene, thymol, stevioside, rebaudioside A, p-cymene, patchoulol, geraniol, glycyrrhizic acid, and valerenic acid in aromatic plants (Mandal et al., 2013, Lu et al., 2011, Arpana et al., 2008, Geneva et al., 2010, Jurkiewicz et al., 2010). Furthermore, many authors demonstrated that a rise in concentration of terpenoids (anethol, geraniol, forskolin, thymol) in medicinal and aromatic plants colonized by AMF is accountable for P availability (Bagheri et al., 2014, Zhang et al., 2010, Zhang et al., 2011) and terpenoid biosynthesis-related genes (Floß et al., 2008, Mandal et al., 2014, Mandal et al., 2015). Changes in the composition of essential oil (EO) contents in *Vetiver* roots in the presence of AMF and unidentified bacteria PGPR were observed (Adams et al., 2004). AM colonization

heightened the essential oil concentration (geraniol, linalool, menthone, menthol, carvone, and pulegone) in *Ocimum basilicum* (Copetta et al., 2006) and *Mentha arvensis* (Freitas et al., 2004). Alterations in accumulation and biosynthesis of alkaloids has been ascribed to AM association in numerous medicinal plants such as *Camptotheca acuminata* (Yu et al., 2010), *Catharanthus roseus* (Andrade et al., 2013), *Castanospermum australe* (Abu-Zeyad et al., 1999), *Phellodendron amurense* (Fan et al., 2006), *Datura stramonium* (Wei and Wang, 1989). Increased alkaloid production in medicinal plants is connected with the higher plant biomass in mycorrhizal plants, which are usually linked to the nutritional benefits of AM colonization (Liu et al., 2007). AMF directly modulates the expression of different genes related to the plant defenses, resulting in improved whole-plant fitness and its enhanced tolerance to biotic stresses (Jung et al., 2012, Zhou and Fan, 2007). In addition, mycorrhizal inoculation elevated alkaloid levels such as alliin, camptothecin, and ephedrine in many medicinal plants (Guo et al., 2010, Yu et al., 2010). Other researchers also reported enhanced production of some pharmacologically important monoterpene indole alkaloids (MIAs), such as ajmalicine, catharanthine, serpentine, vinblastine, vindoline, and vincristine in *Catharanthus roseus* and pyridine alkaloids (PAs) in *Nicotiana* species (El-Sayed and Verpoorte, 2007, Roepke et al., 2010). Regarding phenolics, it has been illustrated that AMF enhanced both quantity and quality of flavonoids and anthraquinone glycosides in medicinal plants (Francineyde et al., 2014, Bagheri et al., 2014, Oliveira et al., 2013). Jurkiewicz et al. (2010) observed an increase in chlorogenic and caffeic acid in *Arnica montana*. In other medicinal plants, AM application strengthened the production of phenolics in *Salvia spp.* (Yang et al., 2017), *Aloe vera* (Mamta et al., 2012), and *Viola tricolor* (Zubek et al., 2015). There were substantial increases in the level of caffeic and rosmarinic acids (Jugran et al., 2015, Zubek et al., 2015) and diobulbinone (Lu et al., 2015).

2.2. *Eclipta prostrata* L.

Eclipta prostrata L. (*E. prostrata*), commonly known as false daisy in English, is a popular plant in Asia. Although it is considered as a common weed by farmers, the whole plants have been utilized as folk medicine in China, Japan, India and other tropical regions in cure for 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, anti-HIV 1, diabetes type II, loose teeth, greying of hair, dizziness, and hemoptysis (Sun et al., 2010). In addition, its plant extracts have been reported to show a wide range of biological properties, consisting of anti-inflammatory (Kim et al., 2017), antitumor (Chung et al., 2017a), antihyperlipidemic (Zhao et al., 2015), antihyperglycemic (Rahman et al.,

2011), antioxidant activities (Chan et al., 2014), and mitigating the cognitive impairment induced by scopolamine (Jung et al., 2016).

2.2.1. Taxonomy, and distribution of *Eclipta prostrata*

The taxonomy of *E. prostrata* is as follows: division: Magnoliophyta; class: Magnoliopsida; order: Asterales; family: Asteraceae/Compositae; genus: *Eclipta*; and species: *E. prostrata* (Fig .5). The name *Eclipta* is originated from the Greek ekleipta, “to be deficient” because they lack the pappus on achenes (Fernald, 1950). The name *prostrata* comes from the Latin *prostratus*, referring to the prostrate growth habit (Radford, 1986).

E. prostrata is an indigenous plant of Asia but is at present broadly distributed in sub-tropical, tropical, and warm temperate regions on the globe (Liu et al., 2012). Indeed, it can be found in more than 87 countries. It is common in Asia, North America, and some countries in Africa, Europe, Central America, and the Caribbean (Anonymous, 2020). *E. prostrata* thrives well in moist soil, hilly regions, pH from 4-8, and a wide range of temperatures from 20-38°C (Chauhan and Johnson, 2008).



Figure 5. *E. prostrata* plants (A) and its flowers (B) and fruits (C) (Feng et al., 2019).

In Vietnam, *E. prostrata* is a common plant in the commercial medicinal market. It has been used as a folk medicine to treat bleeding internal and external, menorrhagia, hemorrhage, epistaxis, hemorrhoids, bloody defecation and urination, vomiting and coughing up blood, bleeding under the skin, measles, asthma, sore throat, burns, skin fungus, and furry tongue (Pham, 2000). In India, this plant is well known for hair rejuvenation, excellent appetizer, digestant, liver stimulant, anthelmintic, and analgesic. It is also used to cure loss of appetite, indigestion, anti-inflammation, liver, and spleen enlargements (Khare, 2010). In Korea, the plant is referred to as *Eclipta genus*.

It plays an important role in the pharmaceutical and food industries (Ko and Jeon, 2003). In Nepal, the plant juice, after mixing with an aromatic (essential oil), is used in the treatment of catarrhal inflammation and jaundice. The leaves are used to treat scorpion stings (Anonymous, 1998). In China, the plant is used for the treatment of teeth lost, dizziness, tinnitus, spitting blood, hematuria, and uterine bleeding (Sun et al., 2010).

2.2.2. Molecular characteristics of *Eclipta prostrata*

The pharmacology studies reported that *E. prostrata* plays an essential role in biological activities, including anti-cancer cytotoxic activity, anti-inflammatory, and antioxidant activities (Chung et al., 2017b). However, there is a rare genetic and genomic study for breeding and authentication of these plants. *E. prostrata* L., has a total of 22 alleles ($2n= 22$) (Pushpa et al., 2011). The genome size of *Eclipta* plant, which is about 4.27×10^9 bp and has been determined by DNA extracted from the fresh young leaves and used flow cytometry to calculate with *Pisum sativum* as standard (Ray et al., 2015). Genome size represents the genomic library and additional parameter for species-specific phenology (Arumugunathan et al., 1999, Lysak et al., 2000).

Also, the most important organelles in plants have gene sequences such as the chloroplast, mitochondria and nucleus. In that the chloroplast play a key role in active metabolic centers of photosynthesis, cellular reactions to signals, and response via retrograde signalling. Also, the biosynthesis of amino acid, nucleotides, fatty acids, phytohormones, vitamins and a plethora of metabolites, and the assimilation of sulphur and nitrogen also are implemented in these organisms. Besides, chloroplasts are the place where occurs the plant responses to heat, drought, salt, light, pH, and pathogens (Bobik and Burch-Smith, 2015, Daniell et al., 2016). Park et al. (2016) reported the complete chloroplast genome sequence of the *E. prostrata* L by de novo assembly method using whole-genome sequence data. The chloroplast genome of *Eclipta prostrata* was 151.757 kb in length, which was composed of a large single-copy sequence of 83.285 kb, a small single-copy region of 18.346 kb, and a pair of inverted repeat region of 25.063 kb (Fig. 6). And the genome harboured 80 protein-coding sequences, 30 tRNA genes, and 4 rRNA genes. Based on the chloroplast genome, the phylogeny tree analysis found *E. prostrata* L., close to *Helianthus annuus* (sunflower) plant with chloroplast protein-coding genes.

2.2.3. Phytochemical constituents of *Eclipta prostrata*

The phytochemical constituents of *E. prostrata*, determined by liquid chromatography (LC), ultraviolet (UV), mass spectrometry (MS), or nuclear magnetic resonance (NMR), provides a useful tool for rapid data collection, structure elucidation and precursor results (Wolfender et al., 2003). Previous research showed that *E. prostrata* contains different natural compounds such as flavonoids, alkaloids, alkynes, cardiac glycosides, triterpenoids saponins, phenolics, essentials oil,

thiophenes, coumestans, lipids, polyacetylene, and steroids (Zhao et al., 2001, Chang and Kim, 2009, Liu et al., 2012, Chung et al., 2017b).

In particular, *E. prostrata* has the highest content of tannin 11.86%, followed by saponin 1.7%, alkaloid 0.34%, and flavonoid 0.87% (Dhandapani and Sabna, 2008). Of these, flavonoids, thiophenes, coumestans, triterpenes, steroids are considered as primary constituents (Feng et al., 2019). Noticeably, environments, and other factors such as harvest time, storage time, and geographical sources may influence chemical components in *E. prostrata* (Chung et al., 2017b). It is rich in phenolic compounds, which are secondary metabolites synthesized by plants during normal development and in response to stress conditions such as infection, wound, and UV radiation (Canter et al., 2005).

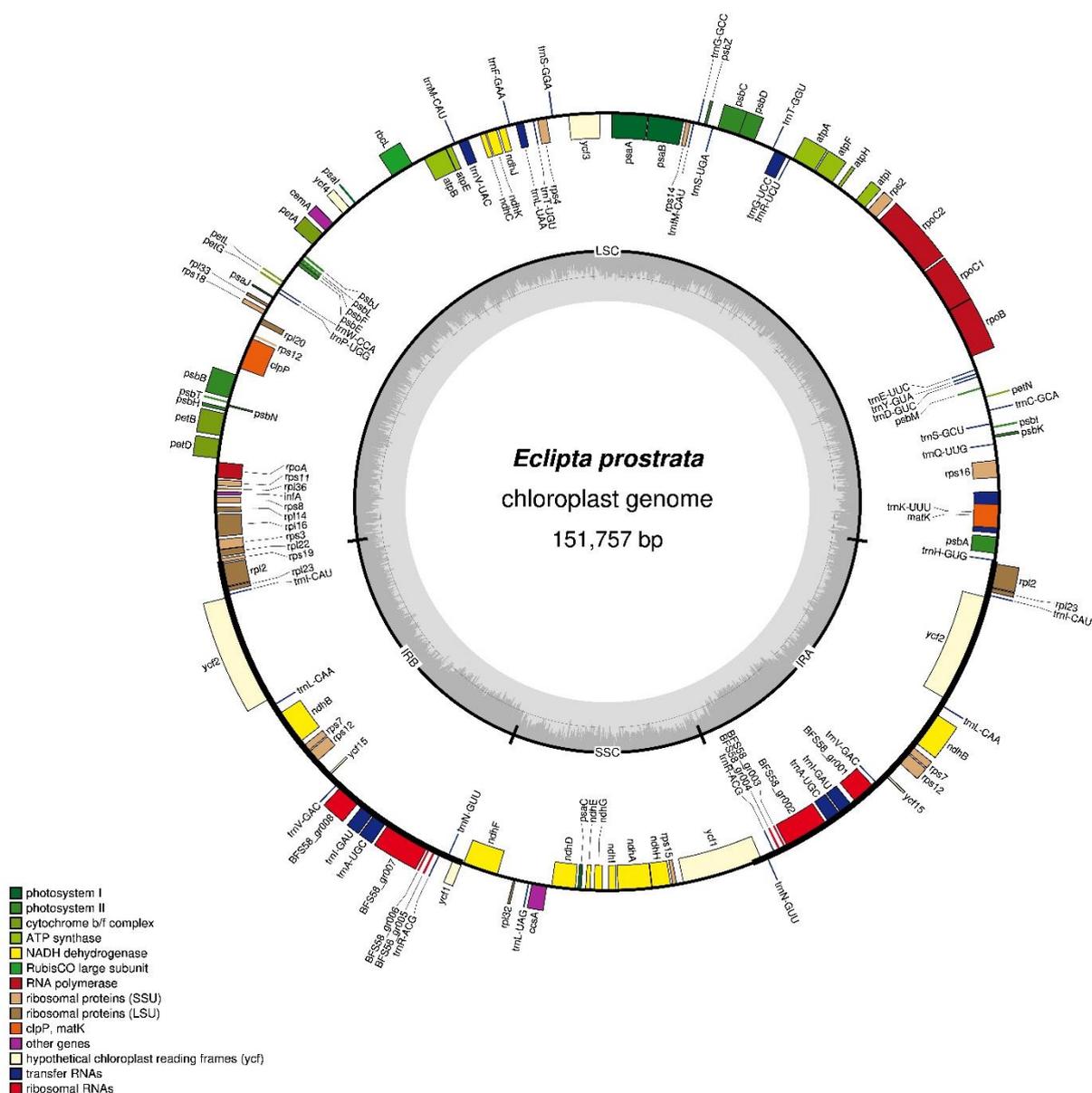


Figure 6: Chloroplast genome map of *E. prostrata* and inter-species polymorphic DNA markers. Chloroplast genome map of *E. prostrata* was generated using OGDRAW RefSeq: NC_030773.1 (<http://ogdraw.mpimp-golm.mpg.de/>).

Triterpenoids isolated from this plant displayed anti-proliferative (Lee et al., 2008b) and antimicrobial (Gopiesh et al., 2008) potentials. And the plant extracts were investigated for against of 28 strains of gram-negative and gram-positive bacteria (Cherdtrakulkiat et al., 2015). *E. prostrata* from China has a high content of saponins ranging from 426 to 13,056 milligrams per gram sample. The two main groups of chemical substances in *E. prostrata* are the flavonoids and triterpenoids. Saponins are especially abundant in triterpenoids (Han et al., 2015). Chemical components extracted from *E. prostrata* are shown in Table 1.

Table 1: Chemical components extracted from *E. prostrata* (reviewed by Feng et al. (2019))

Group	Compounds	Plant part
Flavonoids	Quercetin	Aerial
	Apigenin	Aerial
	Luteolin	Aerial
	Apigenin-7-O-glucoside	Leaves
	Buddleoside	Aerial
	Luteolin-7-O-glucoside	Aerial
	Diosmetin	Aerial
	7-O-methylorobol-4'-O- β -D-glucopyranoside	Aerial
	Pratensein	Aerial
	Pratensein-7-O- β -D-glucopyranoside	Aerial
	3'-O-methylorobol	Aerial
	Orobol	Whole plant
	Oroboside	Whole plant
	Orobol-5-O- β -D- glucopyranoside	Whole plant
	3'-O-methyl orobol-7-O- β -D-glucopyranoside	Whole plant
	Steroids	20-epi-3-dehydroxy-3-oxo-5,6-dihydro-4,5-dehydroverazine
Verazine		Leaves
Ecliptalbine		Leaves
20-epi-4 β -hydroxyverazine		Leaves
20-epi-25 β -hydroxyverazine		Leaves
20-epi-verazine		Leaves
4 β -hydroxyverazine		Leaves

	25 β -hydroxyverazine	Leaves
	Stigmasterol-3-O- β -D- glucoside	Whole plant
	Daucosterol	Whole plant
	Stigmasterol	Aerial
	3-O-(6-O-palmitoyl- β -D- glucopyranosyl)- stigmasterol	Whole plant
	β -sitosterol	Whole plant
Thiopenes	2-(penta-1,3-diynyl)-5-(3,4-dihydroxy-but-1-ynyl)-thiophene	Aerial
	5-(but-3-yne-1,2-diol)-5'-hydroxymethyl-2,2'-bithiophene	Aerial
	5'-isovaleryloxymethyl-5-(4-isovaleryloxy-but-1-ynyl)-2,2'-bithiophene	Aerial
	5-(3'',4''-dihydroxy-1''-butynyl)-2,2'-bithiophene	Aerial
	5-(3-butene-1-ynyl)-5'-ethoxymethyl-2,2'-bithiophene	Whole plant
	5-methanol-5'-(3-butene-1-ynyl)-2,2'-bithiophene	Whole plant
	5-aldehyde-5'-(3-butene-1-ynyl)-2,2'-dithiophene	Whole plant
	5-methoxymethyl-2,2':5',2''-terthiophene	Aerial
	5-ethoxymethyl-2,2':5',2''-terthiophene	Aerial
	α -terthienyl	Aerial
	α -formylterthienyl (syn. ecliptal)	Aerial
	α -terthienylmethanol	Aerial
	3'-methoxy-2,2':5',2''-terthiophene	Aerial
	2,2',5'',2''-terthiophene-5-carboxylic acid	Leaves
	5-hydroxymethyl-(2,2':5',2'')-terthienyl tiglate	Whole plant
	5-hydroxymethyl-(2,2':5',2'')-terthienyl agelate	Whole plant
Triterpenes	Echinocystic acid	Whole plant
	Ecliptasaponin A, B, C, D	Whole plant
	Eclalbasaponins I-XIII	Aerial/
	Oleanolic acid	Whole plant
	Eclalbatin	Whole plant
	β - amyrin	Aerial
	3,16,21- trihydroxy-olean-12-en-28-oic acid	Aerial
	3-oxo-16 α -hydroxy-olean-12-en-28-oic acid	Aerial

	β -amyrone	Aerial
	3 β ,16 β ,29-trihydroxyoleanane-12-ene-3-O- β -D-glucopyranoside	Aerial
	3,28-di-O- β -D-glucopyranosyl-3 β ,16 β -dihydroxy oleanane-12-ene-28-oleanic acid	Aerial
	Silphioside B, E	Aerial
	Echinocystic acid-28-O- β -D-glucopyranoside	Aerial
	Echinocystic acid-3-O-(6-O-acetyl)- β -D-glucopyranoside	Aerial
	3-O-(2-O-acetyl- β -D-glucopyranosyl) oleanolic acid-28-O-(β -D-gluco-pyranosyl) ester	Aerial
	3-O-(6-O-acetyl- β -D-glucopyranosyl) oleanolic acid-28-O-(β -D-gluco-pyranosyl) ester	Aerial
	3-O-(β -D-glucopyranosyl) oleanolic acid-28-O-(6-O-acetyl- β -D-glucopyranosyl) ester	Aerial
	3-O- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl oleanolic-18-ene acid-28-O- β -D-glucopyranoside	Aerial
	Ursolic acid	Whole plant
	α -amyrin	Whole plant
	28-O- β -D-glucopyranosyl betulinic acid 3 β -O- β -D-glucopyranoside	Aerial
Coumarins	Wedelolactone	Aerial
	Demethylwedelolactone	Aerial
	Dimethylewedelolactone-glucoside	Whole plant
	Isodemethylwedelolactone	Whole plant
	Coumestan	Whole plant

2.2.4. Pharmacological activities of compounds originated from *Eclipta prostrata*

There is an increasing trend of using natural herbs as ingredients to produce medicine around the world. According to the WHO, more than 75% of the world's population is using a natural herb to treat diseases. *E. prostrata* is an important medicinal herb that contains valuable natural compounds, especially tannin, wedelolactone, and flavonoids. Wedelolactone is found to possess potent hepatoprotective or antihepatotoxicity (Saritha et al., 2012), anti-tumor (Hsieh et al., 2015), anti-inflammatory (Yuan et al., 2013) effect, and neutralization of lethal and myotoxic activities of anti-snake venom (Mors et al., 1989). Phenolic compounds such as phenolic acids, tannins, and

flavonoids found in this plant have been shown to possess various biological activities, including anti-inflammatory, anti-carcinogenic and anti-atherosclerotic, which might be related to the antioxidant activity (Chung et al., 1998, Soobrattee et al., 2005). Different plant parts also have important functions in the treatment of diseases (Table 2).

Table 2: Pharmacological activities of *Eclipta prostrata*

Medicinal value	Part used, extracted contents	References
UVB protection	Aerial part	Chan et al. (2014)
Antidermatophic activity	Aerial part	Nagabhushan et al. (2013)
Antibacterial	Aerial part	Yu et al. (2020)
Antidiabetic principle	Whole plant	Rahman et al. (2011)
Malaria	Leaves part	Rajakumar and Abdul Rahuman (2011)
Anti-HIV-1 integrase	Aerial part	Tewtrakul et al. (2007)
Ovarian cancer cell	Aerial part	Kim et al. (2015)
Hepatotoxicity	Leaves part	Dheeba et al. (2012)
Lipid lowering	Whole plant or leaves part	Zhao et al. (2015), (Satheesh Naik et al., 2018)
Antivenom	Aerial part	Pithayanukul et al. (2004), Melo et al. (2010)
Inhibit cell migration <i>in vitro</i> and exhibits anti angiogenic <i>in vivo</i>	Aerial part	Kriengsak et al. (2008)
Immunomodulatory activity	Whole plant	Karthikumar et al. (2011)
Anti-proliferative in HBCs	Aerial part	Lee et al. (2008b)
Anti-inflammation	whole plant	Tewtrakul et al. (2011)
Osteoprotective	Triterpenoid	Deng et al. (2015)
Induces apoptosis	α - erthienylmethanol	Lee et al. (2015)
Against ticks and fluke in veterinary	Leaves part	Elango and Rahuman (2010)
against Japanese encephalitis vector, Culex tritaeniorhynchus	Leaves part	Elango and Rahuman (2010)

Against Fish nodavirus	Leaves part (Dasyscyphin C)	Krishnan et al. (2010)
Antimicrobial activity	Leaves (saponin)	Gopiesh et al. (2008)
Induces autophagic and apoptotic cell death in human ovarian cancer cells	Eclalbasaponin II	Cho et al. (2016)
Antisemitic activity	leaves	Elango et al. (2012)
Antitumor activity	Eclalbasaponin I	Liu et al. (2012)
Herbicidal Activity	2-phenyliminothiazolidine	Li et al. (2008)
Against Haemonchus contortus	leaves part	Kamaraj and Rahuman (2011)
Antioxidant	Leaves part	Rao (2009)
Anti-osteoporosis	Whole part	Zhao et al. (2019)
Improving spatial learning and memory deficits	Whole part	Xia et al. (2019)
Induction of anagen	Whole part	Lee et al. (2019)

3. MATERIALS AND METHODS

3.1. 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.

3.2. Arbuscular mycorrhizal fungi inocula

Symbivit®, a commercial mycorrhizal product [a mixture of *Rhizophagus irregularis* (*G. intraradices*), *Funneliformis mosseae* (*G. mosseae*), *Claroideoglossum etunicatum* (*G. etunicatum*), *Claroideoglossum claroideum* (*G. claroideum*), *Rhizoglossum microaggregatum* (*G. microaggregatum*), and *Funneliformis geosporum* (*G. geosporum*)] (Symbiom Ltd., Lanskroun, Czech Republic; www.symbiom.cz) was utilized in the experiments including section 3.3.2; 3.3.3; and 3.3.4.

Moreover, an inocula, including three different AMF species, were propagated separately in open-pot cultures with *Zea mays* and *Medicago truncatula* as host plants for 9 months. The basics of AMF samples originated from different national mycorrhizal collections, *Septoglossum deserticola* - (BEG 73), *Funneliformis mosseae* (SZIE), *Acaulospora lacunose* (BEG 78). Mycorrhizal inocula of high quality consisted of sand, peat, mycelia, infected root fragments, and quantitative ≥ 10 spores/ gram. Mycorrhizal inoculums were applied before transferring germinated seeds to pots of experiment 3.3.4.

Three types of soil were chosen as a source of AMF inoculum, following a land-use gradient. 1. soil from tropical forest (FS) was sampled at Nam Cat Tien (11°23'15.0" N; 107°28'05.0" E) in Dong Nai province, Vietnam. The soil was described as sandy and clay-rich. The dominant resident plant was *Tetrameles nudiflora*, a large deciduous tree species widespread in Southeast Asia. 2. Agricultural (AS) and 3. Grassland (GS) soils, both sandy, were sampled at the district of Ho Chi Minh city (10°53'17.2"N; 106°40'03.1"E), Vietnam, from a field cultivated with cassava (*Manihot esculenta*) and from an adjoining meadow, respectively. These soils were used to pots of experiment 3.3.1.

3.3. Plant growth and experiment design

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

This experiment was carried out from September to November, 2015. Five soil cores per soil type including forest, agriculture and grass soils at a depth of 30 cm from the top land cores from the same soil type were ground and mixed together homogeneously. In order to evaluate the contribution of the AMF consortia to the plant growth, the tests were carried out in plastic pots 18cm x 18cm x 14cm in size divided by a nylon mesh filter (40µm pore size) in two compartments (Appendix Fig. 28). Peat, mixed with sand, was used for its ability to retain moisture and as a source of mineral nutrients at low concentration. The mixture of sand-peat was autoclaved at 121°C for two hours, and its pH was adjusted to 6.5. 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. The bottom compartment was inoculated with 45 grams of either forest or agriculture or grassland soils separately (1.5% w/w) close to the interface with the upper compartment. Because of the size of its pores, the nylon mesh filter represented a physical barrier for the roots to spread into the bottom compartment where peat was present at higher concentrations while AMF hyphae could have access to both compartments (Smith and Read, 2008). Five pots were prepared for each soil type, and five pots without soil inoculation were added to the trials as control (CON). The plants were grown in a climatic chamber EKOCHL 1500 (24/28°C, 60% RH, 16h light/8 dark) and watered three times per week for fourteen weeks. Each treatment had ten biological replicates (10 pots/treatment) and control (10 pots), resulting 40 pots in the total. Seeds were surface sterilized with 80% alcohol and pre-germinated in Petri dishes under moist conditions. When two cotyledons appeared, after about three days, seedlings were transplanted into pots (three seedlings per pot). After transplanting, seedlings were watered with Long Ashton nutrient solution for 7 days and subsequently with distilled water. Pots were kept in a climatic chamber EKOCHL 1500 (18/24°C, 60% RH, 16h light). After 14-weeks of growth, agronomic variables and mycorrhizal colonization rate were analyzed, the counting spores, root DNA extraction, PCR cloning, and restriction fragment length polymorphism were performed.

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

This experiment was carried out from February to June, 2016. The *Eclipta prostrata* seeds were sterilized with 1% NaOCl then washed with sterilized water several times before germination on the filter paper in Petri-dishes at 26°C for 3 days. Pre-germinated seeds were sown in plastic pot 10 x 6 x 14cm in size containing 0.5kg of sterile mixture of sand and peat mixed evenly. The

proportions of sand-peat were prepared as the following ratios: 100:0; 80:20; 60:40; 40:60; 20:80 and 0:100. The increase of peat is an increase in nutrients in the mix. All the inoculated plants (AMF+ plants) were added with 15 grams of commercial product of arbuscular mycorrhizal fungi Symbivit® [a mixture of *Rhizophagus irregularis* (*G. intraradices*), *Funneliformis mosseae* (*G. mosseae*), *Claroideoglobus etunicatum* (*G. etunicatum*), *Claroideoglobus claroideum* (*G. claroideum*), *Rhizoglobus microaggregatum* (*G. microaggregatum*), and *Funneliformis geosporum* (*G. geosporum*)] while the control plants were added 15 grams of autoclaved Symbivit. These plants were grown in a climatic chamber EKOCHL 1500 (24/28°C, 60% RH, 16h light/8 dark) and watered three times per week for seven weeks. Each sand-peat ratio had ten biological replicates, resulting in a total 10 x 6 ratio sand-peat x 2 treatment = 120 plants.

When plants completed a one-week growth stage, branch length, branch number, leaf number, shoot height were recorded. After 7 weeks of growth, plant biomass and mycorrhizal colonization, polyphenol, total phenolic content were determined. Fully expanded leaves (excluding petioles) and root samples were immediately frozen in liquid nitrogen and stored at -80°C until further analysis.

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

The experiment was implemented between September, 2016 to January, 2017. A pot experiment was conducted at Szent István University using a randomized complete block design with three salinity levels (0, 100, and 200 mM NaCl) and two inoculant status (non - mycorrhizal and mycorrhizal plants) for six treatments total. The *Eclipta prostrata* seeds were sterilized with 1% NaOCl then washed with sterilized water several times before germination on the filter paper in petri- dishes at 26°C for 3 days. Pre-germinated seeds were sown in each plastic pots 18cm x 18cm x14cm in size containing 3kg of sterile sand and peat and evenly mixed. The 60:40 % (v/v) sand: peat proportion was used for cultivating *Eclipta prostrata* in the study of sand/peat medium and AMF impact on the growth of *E. prostrata*. AMF treatment was added with 90 grams of Symbivit product, the control was added 90 grams of autoclaved Symbivit product. Each treatment had ten biological replicates, resulting in a total of 10 x 3 salinity levels x 2 treatment = 60 plants. The plants were grown in a climatic chamber EKOCHL 1500 (24/28°C, 60% RH, 16h light/8 dark) and watered three times per week for eight weeks. 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. Fully expanded leaves (excluding petioles) and root samples were immediately frozen in liquid nitrogen and stored at -80°C until further analysis.

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

The experiment was carried out from May to September, 2017. The *Eclipta prostrata* seeds were sterilised with NaOCl 1% then washed with sterilized water several times before germination on the filter paper in petri- dishes at 26°C for 3 days. Subsequently, the germinated seeds of *Eclipta prostrata* were placed in 0.5-liter plastic pots 10 x 6 x 14cm in size filled with an autoclaved mixture of sand and peat (60:40 % (v/v)). 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 commercial products named Symbivit [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*)], and control treatment without AMF. 15 grams of mycorrhizal fungus inoculums were used per each plant (≥ 10 spores/1 mg sand), the control was added 15 grams of autoclaved mycorrhizal fungus inoculums. The plants were grown in a climatic chamber EKOCHL 1500 (24/28°C, 60% RH, 16h light/8 dark) and watered three times per week for eight weeks.

During 4 and 8 weeks, the number of leaves, height of plants were recorded. After 4 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 recorded. Fully expanded leaves (excluding petioles) and root samples were immediately frozen in liquid nitrogen and stored at -80°C until further analysis.

3.4. Assessment of mycorrhizal colonization

Fifteen root fragments (1 cm long) from each plant were collected and stained with Trypan Blue (Kormanik and McGraw, 1982, Trouvelot et al., 1986) to evaluate the level of mycorrhization.

Staining procedure:

- 1) clearing of the roots with 5% potassium hydroxide (KOH) for 30 minutes to remove the protoplasm from the cells.
- 2) washing three times with tap water
- 3) acidification with 1% hydrochloride acid (HCl) for 30 minutes
- 4) staining in 0.05% Trypan Blue (TB) solution overnight at room temperature
- 5) storing in glycerin/water/lactic acid (1:1:1) detaining solution
- 6) root pieces mounted on a microscope slide with a drop of the detaining solution

Once prepared, the slides were observed under a stereomicroscope at 100X magnification.

3.5. Spore counting in soil

Spore abundance was measured in the soils used for inoculation including tropical forest soil (FS) agricultural (AS) and grassland (GS) soils and in the substrate of the pots after plant harvesting. AMF spores were isolated in 3 replications from 35 g of air-dried soil/pot-substrate by wet sieving through 200 and 30 μm sieves, followed by sucrose gradient centrifugation (Janson and Allen, 1986). After centrifugation, spores were transferred into Petri dishes and counted under a stereomicroscope at 100X magnification. Spore abundance was expressed as the number of AMF spores per gram of soil.

3.6. Assessment of plant growth rate and biomass

3.6.1. Treatment of media and arbuscular mycorrhizal fungi effect on the growth, biomass, and polyphenolic content in *Eclipta prostrata*

Shoot height, branch length, branch number, leaf number were recorded at one week after transplanting the seedling, and repeated during the seven weeks of growth period. After seven weeks of growth, plants were harvested without damaging the root system. The fresh and dry weight of shoots and roots and leaf area were recorded. Whole shoots and roots were dried at 72h at 60°C.

3.6.2. Treatment of salt stress and effect of different AMF species on the growth and secondary metabolism, and enzymatic activity of *Eclipta prostrata*

Shoot height, branch length, branch number, leaf number were recorded at one week after transplanting the seedling, and at four- and eight-weeks growth period. Plants were harvested after four weeks and eight-weeks growth. The fresh biomass of shoots and roots, root length, and leaf area were recorded. Whole shoots and roots were dried at 72h at 60°C.

3.7. Measurement of leaf area

Leaf area was measured following the method of Glozer (2008). Two leaves at node 4 branches of an individual plant in each treatment with 3 biological replicates were randomly selected. Leaf were scanned by using scan machine and calculated average leaf area by using Image J software.

3.8. Chlorophyll a fluorescence determination

Chlorophyll a fluorescence, the maximum quantum efficiency of photosystem II (PSII) photochemistry (F_v/F_m), was determined after 30 min of dark-adaption by using a Walz-PAM 2500 (Germany) fluorometer according to the method of Oxborough and Baker (1997) and

Nemenyi (1999). Four fully developed leaves from the shoot apex of a single plant in five biological replicates in each treatment were measured.

3.9. Proline determination

Proline content test was used to determine the proline content in the fresh leaves. After various treatments in plant, proline content were quantified by the acid ninhydrin procedure of Bates (1973). 0.5 gram of fresh plant material was homogenized in 10ml of 3% aqueous sulfosalicylic acid and homogenate was filtered through Whatman # 1 filter paper. Then, a 1:1:1 solution of proline, ninhydrin acid, and glacial acetic acid was added and incubated at 100°C for 1 hour. The reaction was arrested in an ice bath, the chromophore was extracted with 4 ml toluene, and its absorbance at 520 nm was determined in a BioMate spectrophotometer (Thermo Spectronic). Proline concentration was determined from the standard curve and calculated on a fresh weight basis as follow:

$[(\mu\text{g proline} / \text{ml} * \text{ml tolulene} / 115.5 \mu\text{g}) / \mu\text{mole}] / [(g \text{ sample}) / 5] = \mu\text{moles proline/g of fresh weight material.}$

3.10. Determination of total phenolic content

Total phenolics (TP) concentration was measured by Folin–Ciocalteu assay Lister (2001). The analyses of total polyphenols were completed according to Folin-Denis method by spectrophotometry at 760 nm using catechin as standard and Folin-Ciocalteu's phenol reagent (A.O.A.C., 1990). 20 ml 60% ethanol was added to approximate 2 g of plant material, mixed well, and then filtered. Folin-Denis reagent (0.5 ml) was added to 1 ml filtered sample, and the content of the tube was mixed thoroughly. After 3 min, 1 ml of saturated Na₂CO₃ was added. The mixture was completed to 10 ml with distilled water, and it was allowed to stand for 30 min at room temperature. The absorbance was determined at 760 nm using gallic acid as standard. Total polyphenol contents are given as mg/100 g of fresh weight. Fiber content was measured by a digestion treatment following the protocol of A.O.A.C. 985.29. The standard graph was prepared with gallic acid as reference compound ($y = 0.0025x - 0.0408$, $R^2 = 0.9925$).

$Total \text{ phenolic content } (\% \text{ w/w}) = GAE \times V \times D \times 10^{-6} \times 100 / W$, *GAE* - Gallic acid equivalent ($\mu\text{g/ml}$), *V* - Total volume of sample (ml), *D* - Dilution factor, *W* - Sample weight (g).

3.11. Determination of polyphenols by using high performance liquid chromatographic analysis

To extract polyphenols, each aerial part of *E. prostrata* fresh plant material (0.5 g, well-blended) was crushed in a crucible mortar in the presence of quartz sand. With the gradual addition of 20

ml of a mixture of 45% MeOH + 45% EtOH + 10% water, the crushed sample was transferred to an Erlenmeyer flask and subjected to an ultra-sonication force using an ultrasonic water bath device for 4 min. Then the sample was shaken by a mechanical (GLF3005) shaker for 15 min. The mixture was kept overnight at 4⁰C, then filtered through a PTFE filter paper. It was further cleaned-up by passing through a 0.22 mm PTFE HPLC syringe filter before injection on to the HPLC column for the analysis of polyphenols. We used Nucleosil C18 HPLC column, 100, Protect-1 (Macherey-Nagel, Duren, Germany), 3 mm particle size, 150 · 4.6 column to separate phenolic compounds using a gradient elution of 1% formic acid in water (A) and acetonitrile (B), with a flow rate of 0.6 mL·min⁻¹. Gradient elution started with 2% B; it changed in 10 min to 13% B, then in 5 min to 25% B, and then in 15 min to 40% B, and finally, in 7 min it turned to 2% B. The peaks were identified by comparing their retention times and spectral characteristics with available standards such as catechin, quercetin-3-glucoside, luteolin-glucoside, luteolin, wedelolactone, and dimethyl-wedelolactone (Sigma-Aldrich Ltd., Hungary). For quantification of phenolic compounds, the area of each peak was integrated at the maximum absorption wavelength, and the concentrations were calculated by relating the areas of peaks to those of the available external standards (Merken and Beecher, 2000). In the case of no standard materials available, the polyphenol compounds were tentatively identified based on their retention recognized on chromatogram and spectral properties (Fang et al., 2015). The standard materials were singly injected as external standards and chromatographed with the samples as well.

3.12. Determination of antioxidant enzymatic activities

0.5 g of frozen (-80°C) leaf material of each sample from treatments was homogenized in liquid nitrogen with 3 ml of 50 mM Tris-HCl buffer (pH 7.8) containing 7.5% (w/v) polyvinylpyrrolidone K25 and 1 mM Na₂EDTA, and centrifuged at 10,000 x g for 20 minutes at 4⁰ C. The supernatants were used for measuring peroxidase, polyphenol oxidase, superoxide dismutase, and catalase 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. The 2.2 ml of reaction mixture made up of 0.1 M sodium phosphate buffer (pH 6.0), 1 mM Na₂EDTA, 20 mM catechol with 200 µl of the crude leaf extract was used to assay the enzyme activity at 400 nm in 10 minutes. Changes of absorbance per protein concentration per unit time were estimated.

Peroxidase (POD, EC 1.11.1.7) activity was determined by Rathmell and Sequeira (1974) method. Briefly, 10 µl plant extract was added to 2.2 ml of reaction mixture consisting of 0.1 M sodium phosphate buffer (pH 6.0), 100 µl of 50 mM Guaiacol, 100 µl of 12 mM H₂O₂. The absorbance

was recorded at 436 nm in 5 minutes. The enzyme activity was calculated by the changes in absorbance per mg protein per minute.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured spectrophotometrically at 560 nm according to the method of [Beyer and Fridovich \(1987\)](#). Shortly, 20 µl of the crude extract and 20 µl of 1 mM riboflavin were added to 2 ml of reaction mixture composed of 50 mM phosphate buffer (pH 7.8) consisting of 2 mM EDTA, 0.025% Triton X-100, 55 µM Nitroblue tetrazolium (NBT), and 9.9 mM L-methionine. One unit of SOD activity (U) was defined as the required enzyme volume to result in 50% inhibition of the reduction of NBT as recorded at 560 nm.

Catalase (CAT, EC 1.11.1.6) activity was determined following the method of [Aebi \(1984\)](#). The 3-ml reaction mixture consisting of 2 ml of leaf extract diluted (x200) in the buffer of 50 mM potassium phosphate (pH 7.0) and 10 mM of hydrogen peroxide. The absorbance decrease at 240 nm of the reaction was recorded as deposition level of H₂O₂. The enzyme activity was expressed as the changes in absorbance per protein concentration per unit time.

3.13. Root DNA extraction

Molecular analyses were performed (according to the plant variables measured) for each treatment. Three root fragments (1 cm long) per plant were used for DNA extraction. Root fragments were homogenized directly in 100µl of extraction buffer (3% w/v CTAB, 100 mM Tris-HCl pH 8, 2 M NaCl, 25 mM EDTA pH 8, 4% b-mercaptoethanol v/v, and 5% PVP w/v) and incubated at 70°C for 30 min. After incubation, the mixture was cooled at room temperature before adding an equal volume of chloroform: isoamyl alcohol (24:1). After 10 min shaking at room temperature, the mixture was centrifuged at 12000 rpm for 10 min at 25°C. The aqueous phase was transferred to a fresh tube, and the DNA was precipitated by adding 0.6 volumes of ice-cold isopropanol and stored at -20°C for 30 min. Precipitated DNA was centrifuged at 12000 rpm for 10 min at 4°C. The supernatant was decanted carefully, and pellet washed with 80% ethanol. The pellet was dried at 37°C for 12 min and dissolved in 40µl of 1X TE ([Khan et al., 2007](#)).

3.14. PCR and cloning

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'-ATCAACTTTTCGATGGTAGGATAGA-3') and AML2 (5'-GAACCCAAA CACTTTGGTTTC C-3') by [Lee et al. \(2008a\)](#).

Amplifications were carried out using the Phusion High-Fidelity DNA Polymerase (Thermo Scientific) with the following thermal profile: initial denaturation at 98°C for 1 min, followed by

35 cycles at 98°C for 10 sec (denaturation), 64°C for 15 sec (annealing), 72°C for 24 sec (extension), followed by a final extension at 72°C for 5 min.

PCR products were analyzed by gel electrophoresis. Bands at the expected size of 800 bp were cut out, and DNA was extracted with the Illustra GFX™ PCR DNA and Gel band purification kit (GE Healthcare Life Sciences) according to the manufacturer's instructions.

Purified DNA fragments were cloned into CloneJET™ PCR Cloning Kit (Fermentas) and transformed into *Escherichia coli* DH5α according to the manufacturer's instructions.

Transformants were checked by PCR for the presence and size of the insert.

Positive clones were tested for restriction fragment length polymorphism (RFLP) by digestion with *Hinf I* (Promega) and analyzed by gel electrophoresis (2.5% agarose in tris-borate-EDTA; TBE). Representatives for each restriction profile found were selected. Plasmids were extracted with the Wizard® Plus SV Minipreps DNA Purification System Kit (Promega) and sent for sequencing to Biomi Ltd. (Agricultural Biotechnological Center, Gödöllő).

3.15. 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.

Sequences were aligned by MUSCLE with those identified by the BLAST search and with reference sequences of the major groups of Glomeromycota.

Phylogenetic tree inference, using the neighbor-joining method, was computed with MEGA 4.0 software assessing Kimura-2p model as distance method and 1000 replicates of non-parametric bootstrapping.

3.16. Restriction fragment length polymorphism analysis

Positive clones were analyzed for restriction fragment length polymorphism (RFLP) by digestion with *Hinf I* (Promega) and electrophoretic run on 2.5% TBE agarose gel. Representative clones were selected for each restriction profile found. Plasmids were extracted with the Wizard® Plus SV Minipreps DNA Purification System Kit (Promega) and sent for sequencing to Biomi Ltd (Agricultural Biotechnological Center, Gödöllő).

3.17. 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. Sequence editing was conducted manually using MEGA 4.0 (Tamura et al., 2007) and Chromas Lite 2.01. Sequences were aligned by MUSCLE with reference sequences identified with blastn and sequences representing the major taxonomic groups of Glomeromycota. Phylogenetic tree inference, using the neighbor-joining method, was computed with MEGA 4.0 software assessing Kimura-2p model as distance method and 1000 replicates of non-parametric bootstrapping.

3.18. Statistical analysis

Statistical analysis was carried out using the 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 without arbuscular mycorrhizal fungi (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.

4. RESULTS AND DISCUSSIONS

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

4.1.1. Assessment of root colonization by arbuscular mycorrhizal fungi

Percentages of mycorrhizal frequency (F) were calculated by the MycoCalc program using the scores of mycorrhizal colonization and arbuscular abundance. FS, AS, and GS treatments were found significantly different ($p < 0.05$) when compared with the control treatment (CON) (Fig. 7). The highest score was found in the roots of chilli inoculated with forest soil (FS), significantly different from AS and GS soil ($P < 0.05$). The frequency increased from 0% in the control treatment to 56% (AS), 63% (GS), and 81.78% in the FS treatment.

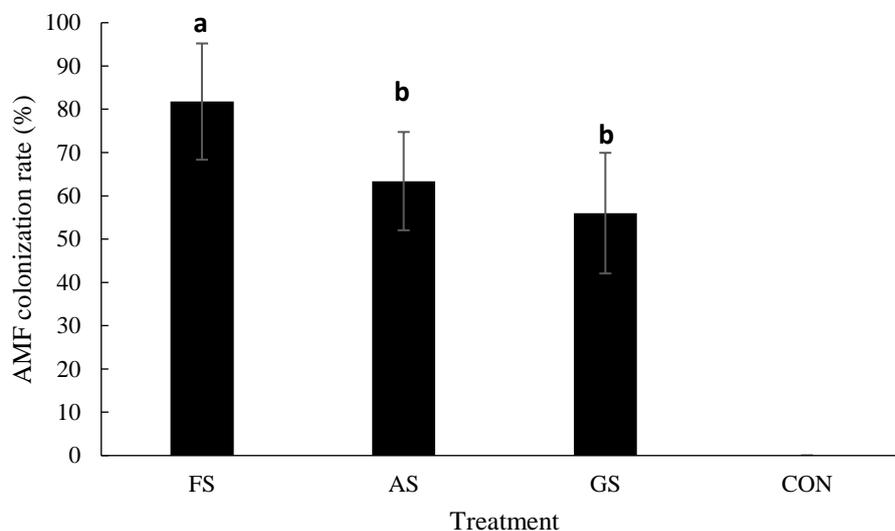


Figure 7: Percentage of mycorrhizal frequency (F). Bars on the top of each column represent the standard deviation ($n=5$). Tropical forest soil (FS); Agriculture soil (AS) and Grassland soil (GS) and control (CON). Different letters indicate a significant difference according to the Tukey posthoc test ($P < 0.05$).

4.1.2. Amount of AMF spores in the soils

Before substrate inoculation, spore abundance was measured in the three different soils chosen as a source of AMF inoculum. The number of spores (average of three independent replications) observed per gram of soil was 4.34 ± 1 in the tropical forest soil (FS), 6.26 ± 1 in the agricultural soil (AS), and 8.57 ± 2 in the grassland soil (GS).

After plant harvesting, the sand-peat substrate was collected, and the spore abundance measured. In the control pots, no spores were detected. Similar spore abundance was found among the different treatments (1.2 spores/g). There are no significant differences between the three sets of pots inoculated with different soils ($P > 0.05$).

4.1.3. Plant growth parameters and shoot, root biomass

The plant growth after two weeks started to show a perceptible change among the treatments. At the moment of the plant harvesting, after 14 weeks, the average shoot length of FS plants was the highest (14.32 cm) followed by GS (11.57 cm), AS (11.01 cm), and CON (5.18 cm) plants respectively (Table 3). Post hoc test showed that the CON plants were significantly different ($p < 0.05$) from the inoculated plants belonging to the FS, AS and GS treatments. According to the test, the FS plants were significantly higher than AS and GS plants. No significant differences were observed between AS and GS treatments (Table 3).

Table 3. Growth parameters in chilli plants with different soil inoculants from Vietnam. Standard deviation is provided with the values. Different letters in each parameter indicate a significant difference, according to the Tukey test ($P < 0.05$). CON: control plants; FS: plants inoculated with forest soil; AS: plants inoculated with agricultural soil; GS: plants inoculated with grassland soil.

Treatment	Shoot height (cm/plant)	Root length (cm/plant)	Dry shoot weight (mg/plant)	Dry root weight (mg/plant)	Leaf number (leaf/plant)
CON	5.18±1.9c	9.88±0.09a	20±7c	10±3.5c	3.26±1.2b
FS	14.32±1.15a	13.43±0.02a	74±13a	26±3.3a	5.71±1.29a
AS	11.01±3.09b	12.70±0.02a	47±14b	20±2.2b	5.04±1.22ab
GS	11.57±2.69b	12.44±0.02a	45±12b	18±1.9b	4.83±1.39ab

Different letters denote significant differences according to Tukey's post hoc test ($P < 0.05$) among treatment after 14 weeks.

The average leaf number recorded was highest in the FS treatment, with an average of six leaves/plant followed by AS and GS treatments with five leaves/plant in both treatments. The lowest average leaf number was observed in the CON treatment with three leaves/plant (Table 3). There is a significant difference between FS treatment compared with the CON treatment ($p < 0.05$) and no significant difference between AS, GS with CON. The assessment of root length after 14 weeks showed a positive effect of the substrate inoculation on the root growth. The average root length of FS plants was the highest (13.43 cm) followed by AS (12.70 cm), GS (12.44 cm), and CON (9.88 cm) plants, respectively (Table 3). Bonferroni test showed no

significant difference among the different types of inoculation ($p>0.05$).

The average dry shoot and root weight were measured after 14 weeks confirmed the trend seen on the shoot length measurements. The weight of inoculated plants was significantly higher compared to the control plants. FS plants had a significantly higher shoot and root dry biomass (74 and 26 mg, respectively) than the plants belonging to the AS and GS treatments (Table 3). No significant differences were observed between AS and GS treatments.

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

After 14 weeks, the 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.

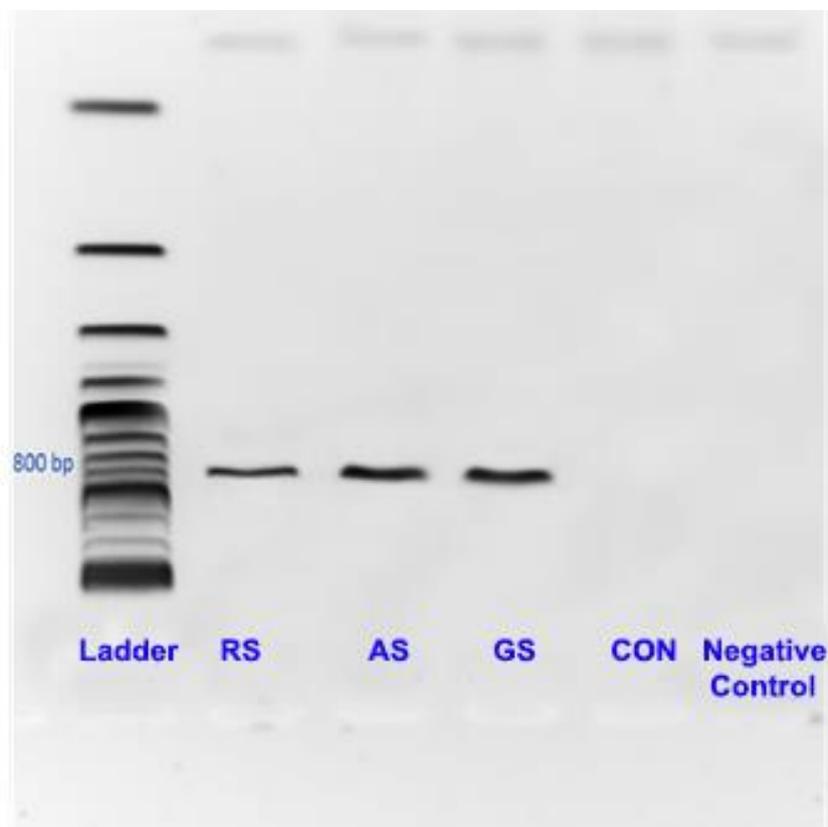


Figure 8: Gel electrophoresis of PCR products amplified from root DNA using AML 1 and AML 2 primers. Gene Ruler 100 bp Plus DNA Ladder (Thermofisher scientific company) was used to assess the band sizes. Forest soil (FS); Agriculture soil (AS) and Grassland soil (GS) and Control soil (CON).

The amplification from RS, AS, and GS DNA gave an expected product of approximately 800bp, as showed in the gel electrophoresis picture (Fig. 8). The bands purified from the gel were used to create a clone library. No band was detected for the CON treatment.

After transformation, a colony screening was performed to obtain at least 20 positive clones (containing the fragment with the expected size) per treatment. The PCR products from the positive clones were digested with *HinfI* and run on a high-resolution agarose gel (2.5%). In total, six restriction profiles were detected (Figure 9).

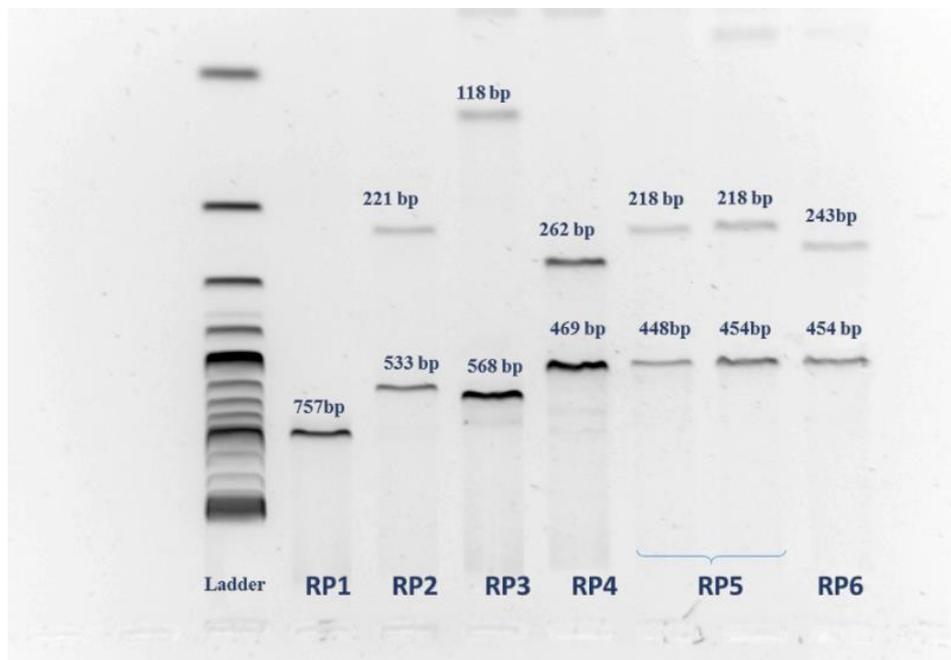


Figure 9: Gel electrophoresis showing the restriction patterns detected, named from RP1 to RP6. The size of the fragments was obtained by *in silico* digestion after sequencing. Two different restriction patterns, not visible on gel electrophoresis, are present for the RP5.

The restriction profile 5 (RP5) was the most abundant, with 52 clones on 60 clones analyzed (Table 13). All the six restriction patterns were represented among the clones analyzed for the FS treatment. In the AS and GS clones, only two restriction patterns were found. Fourteen clones, representing the restriction patterns detected, were sent for sequencing.

Table 13. Number of restriction profiles of 60 colonies for inoculation treatments

	RP1	RP2	RP3	RP4	RP5-6	RP7
FS	2	1	1	1	14	1
AS			1		19	
GS		1			19	

4.1.5. 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 phylogenetic analyses. 11 AMF sequences were used to build a phylogenetic tree (Fig.10). 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.

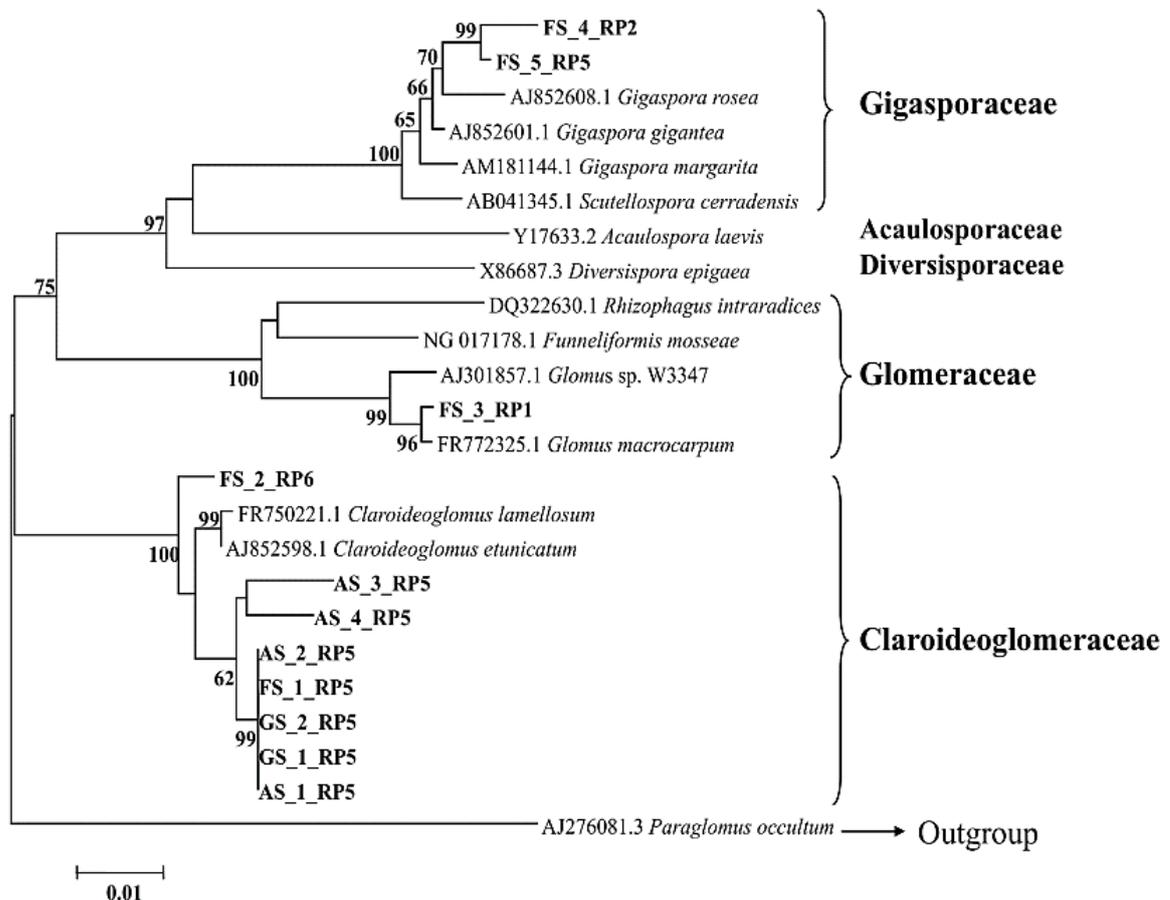


Figure 10: Neighbor-joining phylogenetic tree displaying the relationship between the AMF sequences recovered from chilli roots and 21 reference sequences from GenBank representing some of the main Glomeromycota families. Sequences obtained in this work are in bold and labeled with the corresponding 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.

4.1.6. Discussion

All chilli seeds could germinate and grow in the artificial culture system set up to conduct the experiment. However, the rate of plant growth was slower than natural plant growth because of the extreme deficiency of nutrients (plants were watered without any nutrient solution, only with distilled water). Moreover, AMF spores were found in the top and bottom layer of the pots, and the roots showed a certain degree of mycorrhization, indicating that the experiment was successful

in the achievement of the AMF mycelium development across the mesh membrane (pore size 40µm). During 14 weeks, differences in plant growth between the treatments with inoculation and control treatment were observed. This result demonstrated that plants in FS, AS, and GS treatments could receive better nutrition through the AMF mycelium also developed in the compartment rich in peat content, where plant roots could not have access.

The percentage of mycorrhizal frequency was the highest (82%) in FS, followed by 60% of AS and 56% of GS. Our results showed that the effects on plant growth were directly correlated with the percentage of AMF mycorrhization. In fact, even if the spore abundance measured in the soil used for the inoculation was the lowest among the three soils used, at the end of the experiment the results showed that FS was the best treatment for the plant growth, with the highest average of shoot biomass (0.45 g), shoot height (14.32 cm) and leaf number (6 leaves per plant). No significant differences were observed between AS and GS plants, characterized by a similar rate of mycorrhization.

Generally, a strong mycorrhization is associated with reduced development of the root apparatus of the plant because the root wall supplied nutrients by the AMF mycelium does not need to invest resources in the root's development (Bonfante and Perotto, 1995). In the present study, this was not observed, probably due to the extreme deficit of nutrients in the pot system.

Specific primer AML1 and AML2 were used to verify the presence and identify the AMF taxa in plant roots (Lee et al., 2008a). All of the treatments inoculated gave a positive PCR product, and as expected, no amplification was detected in the control plant roots. Overall, in the restriction analyses, seven restriction patterns were observed. All of restriction patterns were detected in the FS clones, while two restriction patterns were found in the other treatments. After sequencing, two restriction patterns (only three of sixty clones analyzed) were found associated with no-AMF sequences, demonstrating the high specificity of the primers used. The phylogenetic analyses showed that there was a good correlation between the restriction patterns observed and the phylotypes identified. One restriction pattern was associated with two different phylotypes. In silico restriction confirmed that the two phylotypes were associated with two almost identical restriction patterns.

In total, the sequences were distributed in three families. Most of them associated with two restriction patterns, clustered in the family of *Claroideoglomeraceae*, two sequences clustered in the family of *Gigasporaceae* but a different taxonomic position, and one was strictly associated with the *Glomus macrocarpum* species in the family of *Glomeraceae*.

Considering the single treatments, in the FS treatment (inoculated with tropical forest soil) the higher number of taxa was found, followed by GS and AS treatment. These results concur with the data reported by Öpik et al. (2006), where the number of AM fungal taxa per host plant species differed between habitat types: a significantly higher richness was found in tropical forests (18.2 fungal taxa per plant species), followed by grasslands (8.3), temperate forests (5.6) and habitats under anthropogenic influence (arable fields and polluted sites, 5.2).

The plants in FS treatment showed the highest values of growth parameters in chilli plant. According to the molecular data, it is not possible to recognize specific taxa associated with the best results of plant parameters measured. In fact, sequences in all the treatments mostly related to the *Claroideoglomeraceae*. Furthermore, several authors reported that the communities of spores

in the soil and fungi colonizing roots were not necessarily identical (Clapp et al., 1995, Balestrini et al., 2010); neither there was a direct relationship between sporulation and root colonization levels (Dodd et al., 2000).

Even though there is no obvious specificity between a host plant and colonizing AM fungal species, it was observed by several authors that there could be preferential associations (Torrecillas et al., 2012). In the present study, it could be supposed that the host plant species influenced the AMF assemblage inside the roots in order to privilege the colonization by members of the *Claroideoglomeraceae* family. It has to be mentioned that a sequence found in FS treatment was clustered in the *Gigasporaceae* even if the restriction profile was looking identical in the gel electrophoresis, to the one associated with the *Claroideoglomeraceae* sequences.

Consequently, it was not possible to recognize the effective abundance of such taxa inside the roots of FS plants. In addition, the molecular analyses were limited to a low number of clones (20 per treatment), and the molecular target used as well as the type of restriction analyses (with only one enzyme) could not allow a deep resolution regarding the analyses of AMF taxa. It was demonstrated that some functional traits could be different among strictly related species and strains belonging to the same species (Takács et al., 2006). From this point of view, it could be hypothesized that the highest root colonization, as well as the growth effects observed in the FS plants, could be explained by species or strains different from those present in the GS and AS plants even if belonging to the same AMF family.

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

4.2.1. Mycorrhizal inoculation rate

The result showed that mycorrhizal plant had successful root colonization, while no infection was observed in non-inoculant plants. The highest percentage of root colonization ($76.23\% \pm 15.6$) was found in the presence of a substrate containing a sand/peat ratio of 60/40% (v/v), followed by a higher sand proportion at the same rate with peat 80:20; 100:0; 40:60; 20:80; and 0:100 % (v/v) (Fig. 11). There were no significant differences in root colonization at higher than 40% (v/v) peat ratios.

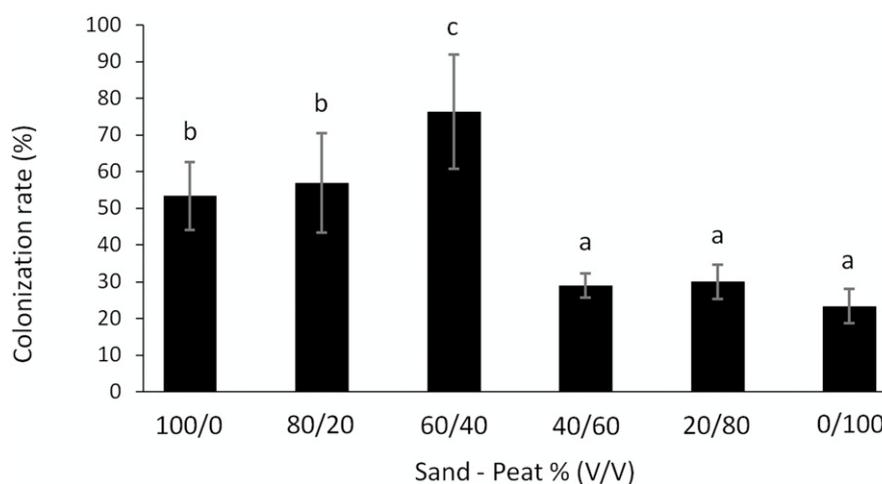


Figure 11. Mycorrhizal colonization rate (%) of *E. prostrata* after 7 weeks of growth at different rates of sand and peat as a growth substrate. Different letters indicate a significant difference according to the Tukey posthoc test ($P < 0.05$).

4.2.2. Effect of AMF inoculation and different nutrient supplies on measured plant parameters

After seven weeks of growth, inoculation with the commercial product Symbivit significantly increased the growth of *E. prostrata* compared to the control plant (Table 4).

The highest values were found by all measured growth parameters (shoot height, branch number, branch length, and leaf number) in case of both treatments at 60/40 % (v/v) sand-peat mixture ratio. Plant performance indicates that this substrate composition is the best for enhancing the growth of both inoculated and non-inoculated plants. The highest difference between mycorrhizal and non-mycorrhizal plants was found in sand substrate representing the lowest nutrient supply.

The branching of the target medicinal plant started in the second week and terminated after six weeks of growth. The total average of the branch number and branch length of inoculated plants

were higher by 31% and 32% than observed in the control plants, respectively, after seven weeks of growth. (Table 4).

Table 4 The average growth parameter of *Eclipta prostrata* (mean \pm SD, n=10) in seven weeks of inoculated (AMF+) and control not inoculated (AMF-) plants. AMF, arbuscular mycorrhizal fungi.

Sand/Peat % (V/V)		Branch length (cm/plant)	Branch number (branch/plant)	Leaf number (leaf/plant)	Shoot height (cm/plant)
100/0	AMF+	7.975 \pm 0.651 e	4.60 \pm 1.37 e	23.50 \pm 3.01 f	11.913 \pm 1.225 d
	AMF-	0.150 \pm 0.038 f	0.40 \pm 0.21 f	11.33 \pm 1.38 f	7.050 \pm 1.417 e
80/20	AMF+	19.513 \pm 4.079 ab	17.38 \pm 2.00 bcd	130.75 \pm 16.03 bc	23.363 \pm 3.915 ab
	AMF-	16.688 \pm 3.012 bcd	16.00 \pm 4.24 cde	113.50 \pm 20.28 cde	19.125 \pm 4.330 bcd
60/40	AMF+	22.750 \pm 3.383 ab	25.00 \pm 3.46 a	178.57 \pm 23.49 a	25.500 \pm 3.063 ab
	AMF-	19.938 \pm 2.673 ab	21.88 \pm 2.85 ab	157.71 \pm 17.84 ab	22.600 \pm 3.090 ab
40/60	AMF+	19.300 \pm 0.602 abc	12.57 \pm 2.92 de	100.25 \pm 16.33 de	21.688 \pm 1.263 abc
	AMF-	154.75 \pm 23.52 d	11.43 \pm 1.76 e	97.00 \pm 14.22 de	17.750 \pm 1.913 d
20/80	AMF+	19.543 \pm 2.176 ab	19.57 \pm 6.65 abc	121.71 \pm 33.26 cde	20.714 \pm 3.329 ab
	AMF-	15.100 \pm 1.057 d	11.14 \pm 2.03 e	83.43 \pm 12.53 e	17.100 \pm 1.056 d
0/100	AMF+	20.625 \pm 0.851 a	17.43 \pm 3.92 bcd	105.60 \pm 8.31 cde	22.838 \pm 2.206 a
	AMF-	15.538 \pm 2.359 cd	12.78 \pm 2.44 de	101.65 \pm 9.38 cde	18.750 \pm 3.212 cd
Significant of Source of variation (ns = not significant, *P \leq 0.05, **P \leq 0.01, ***P \leq 0.001)					
Growing media (GM)		***	***	***	***
Mycorrhizae(M)		***	***	***	***
M*GM		*	*	NS	NS

Different letters in each parameter indicate a significant difference according to the Tukey test (P<0.05). NS non-significant difference

The leaf area of *E. prostrata* was largest at AMF+ 60:40 sand-peat with 21.65 cm² leaf⁻¹ and lowest in control 100:0 sand-peat 2.06 cm² leaf⁻¹. The difference was found between 100:0 sand-peat (8.09 cm² leaf⁻¹) and control 100:0 sand-peat (2.06 cm² leaf⁻¹). The average number of leaves of mycorrhizal plants (AMF+) grown on substrates with different sand/peat ratios was slightly higher (17%) than in the control one (Table 5).

A measurement of fresh biomass of shoot in inoculated plants was 131.89 g, 55% higher than non-inoculated 84.88 g in the fresh biomass. The dried biomass of shoots in the inoculated plants was (13.97 g), 67% greater than non-inoculated (8.38 g). Moreover, the total fresh root biomass was 33.75 g, 79% higher than non-inoculated 18.81 g, and 4.68 g, higher 140% than non-inoculated

plants 1.95 g in the total dried biomass (Table 5). Moreover, fresh and dried biomass of root and shoot were found a significant difference among 60:40 sand-peat-plant with others ($P < 0.05$) (Table 5).

Table 5. Change in the biomass of shoot, roots, and leaf area of *Eclipta prostrata* (mean \pm SD, n = 5) the different treatments after 7 weeks of growth.

Treatment ^z		Shoot ^{yx}		Root ^{yx}		Leaf area (cm ² /plant) ^{zy}
Sand/ Peat (%) (v/v)		Fresh (g/plant)	Dry (g/plant)	Fresh (g/plant)	Dry (g/plant)	
AMF+	100/0	1.99 \pm 0.47 f	0.13 \pm 0.027 ef	0.25 \pm 0.09 d	0.06 \pm 0.02 d	8.09 \pm 0.32 e
AMF-	100/0	0.39 \pm 0.09 g	0.03 \pm 0.022 f	0.18 \pm 0.49 d	0.01 \pm 0.005 d	2.06 \pm 0.30 f
AMF+	80/20	19.38 \pm 3.82 de	2.34 \pm 0.73 bc	9.79 \pm 1.21 ab	1.41 \pm 0.16 b	18.04 \pm 1.71a bc
AMF-	80/20	14.37 \pm 3.84 e	1.91 \pm 0.47 bcd	6.61 \pm 0.59c	0.72 \pm 0.15 c	13.74 \pm 1.00 d
AMF+	60/40	38.85 \pm 3.92 a	3.93 \pm 0.46 a	11.68 \pm 0.43 a	1.75 \pm 0.15 a	21.65 \pm 3.05 a
AMF-	60/40	27.43 \pm 3.97 bc	2.74 \pm 0.53 b	8.95 \pm 1.78 b	0.92 \pm 0.17 c	15.13 \pm 2.28 cd
AMF+	40/60	14.83 \pm 1.94 e	1.48 \pm 0.27 cd	1.52 \pm 0.25 d	0.14 \pm 0.03 d	18.90 \pm 0.82 abc
AMF-	40/60	14.41 \pm 3.87 e	1.27 \pm 0.49 cd	1.32 \pm 0.57 d	0.11 \pm 0.025 d	16.59 \pm 3.75 bcd
AMF+	20/80	33.41 \pm 4.91 ab	3.89 \pm 0.92 a	5.73 \pm 0.54 c	0.70 \pm 0.15 c	19.49 \pm 1.35 ab
AMF-	20/80	12.58 \pm 2.97 e	1.15 \pm 0.43 de	0.96 \pm 0.17 d	0.08 \pm 0.01 d	18.85 \pm 2.79 abc
AMF+	0/100	23.43 \pm 4.72 cd	2.10 \pm 0.43 bcd	4.78 \pm 0.67 c	0.62 \pm 0.12 c	18.46 \pm 2.39 abc
AMF-	0/100	15.70 \pm 1.55 e	1.28 \pm 0.31 cd	0.79 \pm 0.36 d	0.11 \pm 0.04 d	16.91 \pm 2.02 bcd
Significant of Source of variation (ns = not significant, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$)						
Growing media (GM)		***	***	***	***	***
Mycorrhizae(M)		***	***	***	***	***
M*GM		***	***	***	***	***

^zAMF+ represents the mycorrhizal; AMF- represents the nonmycorrhizal plants.

^yDifferent letters in each parameter indicate significant difference according to the Tukey test ($P < 0.05$) among sand and peat ration.

^x***Significant at $P < 0.001$.

4.2.3. Proline concentration and chlorophyll fluorescence parameters of *E. prostrata*

The plants grown under different proportions of sand and peat varied in their Fv/Fm (Fig. 12A). The highest quantum efficiency of photosystem II was recorded in inoculated treatment 60/40% (v/v) sand/peat mix; however, no significant difference could be detected between inoculated and non-inoculated treatment. Both the mycorrhizal fungi and growth substrate have a significant influence on proline concentration of the leaves. Increasing the proportion of peat in substrate resulted in a small-scale increase in proline concentration, reaching the highest level of both treatments in 100% peat (Fig. 12B).

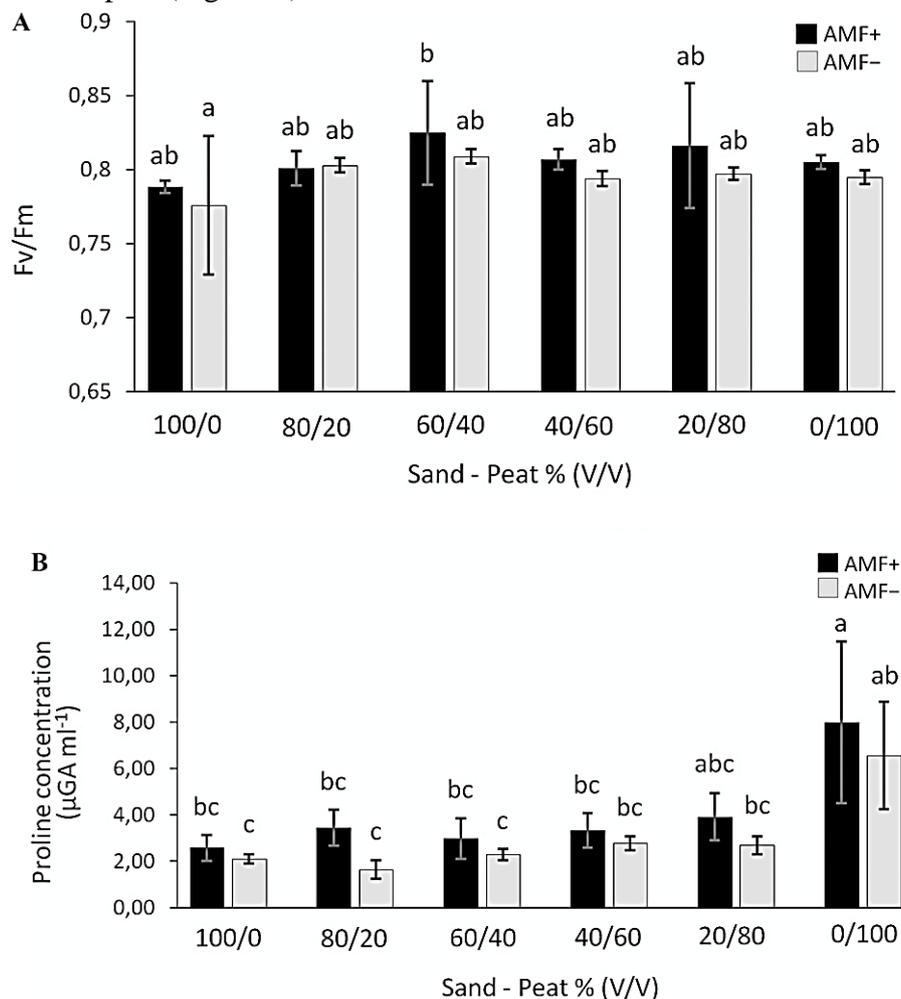


Figure 12. Fv/Fm quantum efficiency of photosystem II. (A), Proline concentration (B) in the leaves of *E. prostrata* after seven weeks of growth, using different rates of sand and peat as the growth substrate. Black and grey columns refer to AMF treated (AMF+) and Control (AMF-) plants, respectively. Different letters indicate a significant difference, according to the Tukey posthoc test ($P < 0.05$).

4.2.4. Total phenolic concentration from aerial part of *E. prostrata*

Different proportions of peat had significant effects on the concentration of total phenolic content (TP) of aerial parts, but mycorrhizal inoculation did not (Fig. 13). 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.

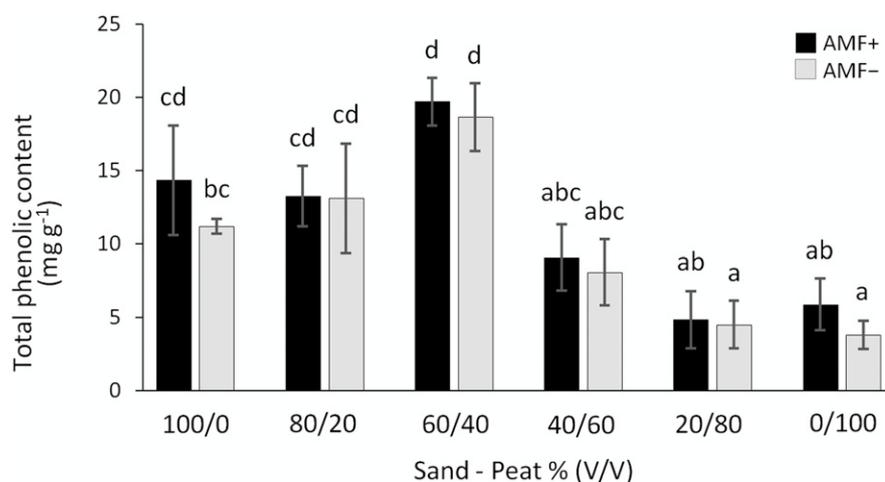


Figure 13. Total phenolic content (TP) in leaves of *E. prostrata* after 7 weeks of growth at different rates of sand and peat as the growth substrate. Black and grey columns refer to AMF treated (AMF+) and Control (AMF-) plants, respectively. Different letters indicate a significant difference according to the Tukey post-hoc test ($P < 0.05$).

4.2.5. 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 (Tables 6 and 7, Fig. 14), 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 (Table 6 and 7). 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 ($P < 0.05$) (Table 7).

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+ (Tables 6 and 7). 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.

Table 6. Change in the concentration of polyphenol compounds (mean \pm SD, n = 3) under different treatments.

Sand/Peat % (V/V)	Inoculation ^z	Protocatechuic acid(μ g/g) ^{yx}	5-o-caffeoylquinic acid (μ g/g) ^{yx}	Dimethyl Wedelolactone (μ g/g) ^{yx}	4-o-caffeoyl quinic acid (μ g/g) ^{yx}
100/0	AMF+	3.59 \pm 1.61 c	65.09 \pm 18.32 bc	989.00 \pm 246.84 a	67.95 \pm 35.92 b
	AMF-	4.43 \pm 0.4ac	173.34 \pm 53.47 a	826.09 \pm 330.35 ab	69.85 \pm 38.47 b
80/20	AMF+	19.20 \pm 4.4 b	57.15 \pm 26.21 c	776.55 \pm 146.96 abc	70.73 \pm 20.29 b
	AMF-	23.57 \pm 5.9 b	136.02 \pm 32.7 ab	1255.23 \pm 124.6 a	253.49 \pm 3.78 a
60/40	AMF+	41.87 \pm 9.7 a	169.89 \pm 36.16 a	1001.86 \pm 187.47 a	55.53 \pm 39.32 bc
	AMF-	5.46 \pm 1.34 c	56.09 \pm 29.22 ac	1066.79 \pm 265.17 a	50.39 \pm 16.58 bc
40/60	AMF+	4.11 \pm 2.2 c	9.26 \pm 1.29 c	214.55 \pm 63.36 c	UDL
	AMF-	4.63 \pm 2.69 c	13.44 \pm 6.51 c	401.05 \pm 53.13 bc	UDL
20/80	AMF+	1.88 \pm 0.47 c	16.22 \pm 2.2 c	231.42 \pm 110.9 c	15.49 \pm 11.45 bc
	AMF-	3.76 \pm 1.22 c	12.34 \pm 1.08 c	260.81 \pm 68.38 bc	UDL
0/100	AMF+	3.19 \pm 1.78 c	8.11 \pm 0.24 c	711.73 \pm 95.18 abc	UDL
	AMF-	2.33 \pm 0.39 c	45.4 \pm 8.81 c	722.81 \pm 283.03 abc	25.47 \pm 12.4 bc
Significant of Source of variation (ns = not significant, *P \leq 0.05, **P \leq 0.01, ***P \leq 0.001)					
Growing media (GM)		***	***	***	***
Mycorrhizae (M)		***	*	ns	***
M*GM		***	***	ns	***

^zAMF+ represents the mycorrhizal; AMF– represents the nonmycorrhizal plants.

^yDifferent letters in each parameter indicate significant difference according to the Tukey test (P < 0.05) among sand and peat ration.

^xNS = contrast is nonsignificant, *significant at P < 0.05, ***significant at P < 0.001. UDL = under detection limit.

Principal component analysis (PCA) was applied to assess the data on phenolic content in *E. prostrata* plants determined by HPLC. As demonstrated in Figure 5A and Principal component 1 (Factor 1) explains up to 55.58% of the total variance and is characterized mainly by protocatechuic acid; 5-o-caffeoylquinic acid; demethyl-wedelolactone;4,5-dicaffeoylquinic acid; quercetin-3- arabinoside; wedelolactone; and 3,5-dicaffeoylquinic acid. Principal component 2(Factor 2), explaining 12.52%, is contributed mainly by luteolin-glucoside. The PCA scatter plot showed 68.10% of the total variability in the phenolic data set. Through PCA and AHC, it is

important to note that the C treatment cluster group has significant differences in polyphenolic contents compared to other treatments (Fig. 15 and 16 A, B).

Table 7. Change in the concentration of polyphenol compounds (mean \pm SD, n = 3) under different treatments.

Sand/Peat % (v/v)	Inoculation ^z	Quercetine-3-arabinoside (μ g/g) ^{yx}	Luteolin (μ g/g) ^{yx}	3,5-dicaffeoyl-quinic acid (μ g/g) ^{yx}	Wedelolactone (μ g/g) ^{yx}	4,5-dicaffeoyl-quinic acid (μ g/g) ^{yx}
100/0	AMF+	146.62 \pm 30.52 bc	39.74 \pm 4.58 ab	99.97 \pm 17.89 a	3263.99 \pm 950.64 a	27.04 \pm 17.8 ab
	AMF-	74.52 \pm 28.52 cd	21.45 \pm 6.26 bc	87.69 \pm 15.38 ab	2376.25 \pm 604.10 abc	10.23 \pm 4.55 bc
80/20	AMF+	161.66 \pm 18.09 b	22.52 \pm 11.8 2 abc	62.63 \pm 10.48 bc	1362.73 \pm 79.05 cde	17.57 \pm 10 abc
	AMF-	253.90 \pm 49.28 a	23.08 \pm 4.84 abc	43.85 \pm 8.79 cd	1953.80 \pm 407.92 bcd	29.50 \pm 8.18 ab
60/40	AMF+	169.17 \pm 24.25 b	22.58 \pm 7.54 abc	62.90 \pm 10.61 bc	3063.97 \pm 524.18 ab	38.03 \pm 12.06 a
	AMF-	155.27 \pm 27.54 b	12.35 \pm 0.3 c	43.70 \pm 6.17 cd	2588.27 \pm 399.36 ab	15.13 \pm 5.4 bc
40/60	AMF+	1.51 \pm 0.38 d	1.39 \pm 0.58 c	7.18 \pm 3.91 ef	1010.37 \pm 141.22 de	1.90 \pm 0.08 c
	AMF-	UDL	12.73 \pm 4.85 c	4.91 \pm 2.79 ef	957.39 \pm 129.32 de	2.28 \pm 0.67 c
20/80	AMF+	UDL	14.10 \pm 3.73 c	4.45 \pm 2.11 f	757.71 \pm 113.33 de	2.39 \pm 0.71 c
	AMF-	133.21 \pm 47.5 bc	5.03 \pm 3.04 c	ND	659.31 \pm 86.17 e	2.39 \pm 0.9 c
0/100	AMF+	21.89 \pm 3.53 d	45.74 \pm 18.9 4 a	37.37 \pm 17.33 cde	1188.25 \pm 90.34 cde	8.09 \pm 1.75 bc
	AMF-	112.23 \pm 23.61 bc	8.63 \pm 2 c	19.81 \pm 11.61 def	1165.19 \pm 99.3 cde	7.87 \pm 2.34 bc
Significant of Source of variation (ns = not significant, *P \leq 0.05, **P \leq 0.01, ***P \leq 0.001)						
Growing media (GM)		***	***	***	***	***
Mycorrhizae (M)		***	***	***	ns	ns
M*GM		***	***	ns	ns	**

^zAMF+ represents the mycorrhizal; AMF- represents the nonmycorrhizal plants.

^yDifferent letters in each parameter indicate significant difference according to the Tukey test (P < 0.05) among sand and peat ration.

^xNS = contrast is nonsignificant, ***significant at P < 0.001. UDL = under detection limit.

4.2.6. Discussion

Several studies are reporting the advantages, prospects, and feasibility of AMF introducing to plant production (Birhane et al., 2012, Gianinazzi et al., 2010, Ryan and Graham, 2002). Moreover, AM symbiosis has been found to increase the active bio-compounds of some medicinal plants (Zeng et al., 2013); but data regarding *E. prostrata* has not been published until now. The increased

demand for sustainable cultivation of *E. prostrata*, together with its high concentration of secondary metabolites used in human medicine and plant protection, draws attention to AMF.

Our results indicate that AMF inoculation beneficially affected the growth parameters and some secondary metabolites of *E. prostrata* (Tables 4, 5, 6, 7). In most case, AM symbiosis significantly increased the fresh and dry weights of shoots and roots compared with the control, nonmycorrhizal plants (Table 4). The highest dry weight of shoots was found in the presence of mycorrhizal fungi both at 60/40% (v/v) and 20/80% (v/v) sand/peat proportion. In nonmycorrhizal treatment, plant shoot and root growth showed maximum values only in 60/40% (v/v) sand/peat mixture. The beneficial effect of peat on growth of plants could be attributed to the increased aerobic conditions and better than water-holding capacity in sand, in addition to the influence on the spread and colonization of AMF (Linderman and Davis, 2003).

Moreover, higher phosphorus and other nutrients in peat than in sand could explain the increased growth compared with plants growing in sand. Our results indicated that the greatest extent of mycorrhizal colonization found at 60/40% (v/v) sand and peat ration is correlated with the positive effects of AMF on plant growth, thus this finding is agreeing with study of Stevens et al. (2011). Higher than 40% peat concentration had a negative effect on root colonization by AMF, similar to the work of Ma et al. (2007) and Marschner et al. (2006); but an opposite result was also reported by Vosátka (1995). On the other hand, some researchers showed that the effect of AMF symbiosis does not correlate with colonization level (Toussaint, 2007) and was most effective when root colonization scored from 20% to 30% (Feldmann et al., 2009).

Besides shoot and root weight, the leaf area is also important from the agronomical point of view and is influenced by various nutrient supplies (Taranet et al., 2017) or/and mycorrhizal inoculation (Liang et al., 2018). AMF had positive influence on leaf area of *E. prostrata*, a finding that is similar to the results of Birhane et al. (2012), who reported an enhanced leaf area, biomass, and stomatal conductance in *Boswellia papyrifera* plant inoculated with AMF. In addition to the beneficial effects of AMF on nutrient uptake, mycorrhizal symbioses often give a balance to different stress conditions (Estrada et al., 2013), driving electron transport from an excited PSII reaction center. However, in our work, no differences were found in chlorophyll fluorescence (Fv/Fm) between mycorrhizal and nonmycorrhizal plants; similar to the work of Parádi et al. (2003). Significantly, higher proline content of both treatments (AMF+, AMF-) was measured in 100% peat substrate. Proline plays an important role in plants: it protects the plants from various stresses and also helps plants to recover from stress more rapidly (Hossain et al., 2014).

The increased weight of roots has particular importance not only in increasing the absorption surface but also in enhancing the secondary metabolites such as phenolic content from *Echinacea purpurea* (Araim et al., 2009) and stigmaterol, ecliptal, and heptacosanol from *E. prostrata* (Chung et al., 2017b). Plants protect themselves from different biotic and abiotic stress factors by synthesizing phenolic compounds, which act as a screen inside the epidermal cell layer, and by activation of the antioxidant systems at both the cell and whole organism level (Bhattacharya et al., 2010, Lattanzio et al., 2006, Sirvent and Gibson, 2002). The importance of flavonoids in ultraviolet protection has also been proved using mutant ultraviolet-hypersensitive phenotypes of *Arabidopsis* (Ryan et al., 2001). Preformed antibiotic compounds such as phenolic and polyphenolic compounds are ubiquitous in plants and play an important role in nonhost resistance to pathogens (Lattanzio et al., 2006). Furthermore, Stoms (1982) showed that the polyphenolic

compounds have a crucial role in regulating the growth and development of plants. The phenolic compounds have antioxidant properties also, which can reduce the peroxidation of membrane lipids by decreasing their fluidity in consequence, limiting the diffusion of free radicals that have been proved using *Allium sativum* L. (Bozin et al., 2008). Moreover, there is another valuable property of polyphenolic compounds: having the ability and capacity to chelate heavy metal ions, as demonstrated in a study with *Nymphaea* sp. (Lavid et al., 2001).

The polyphenol profiles of *Eclipta prostrata* measured in aerial parts showed the same tendency to the previous finding wedelolactone, and dimethyl-wedelolactone were the main components (Fang et al., 2015, Mendes et al., 2014, Murali et al., 2002). Additionally, Fang et al. (2014) determined luteolin-glucoside and 4,5-dicaffeoylquinic acid as polyphenols in the extract of *E. prostrata*.

Dimethyl-wedelolactone (DWL) is an important polyphenolic component in the profile of *E. prostrata* due to its antihepatoma toxic properties. Using 60/40% sand/peat as a growth substrate, we found about ten times higher concentration of DWL than reported by Murali et al. (2002). Some minor poly-phenols (such as 5-o-caffeoylquinic acid, quercetin-3-arabinoside, 4-o-caffeoylquinic acid, and protocatechuic) are detected for the first time in the extract of *E. prostrata* samples. Protocatechuic acid plays an essential role in the tolerance of rice during anaerobic flooding germination, promotion of shoot elongation, and the increase in chlorophyll b (Khanh et al., 2018). Our results indicate that growth medium, together with mycorrhizal inoculation, induced changes in tested secondary metabolites of *E. prostrata*. These findings confirm some previous work, where qualitative changes due to AMF were recognized in alkaloid, terpene, flavonoid, and phenolic acids in some medicinal plants (Zeng et al., 2013), but not in *E. prostrata*. To the best of our knowledge, our study is the first report on how AMF symbiosis to influence plant performance and active ingredients of *E. prostrata*. The mechanism by which AMF triggered the quality and quantity of secondary metabolites products in medicinal plants can be multidirectional and not well understood (Toussaint et al., 2007). On the one side, AMF symbiosis can significantly increase the contents of some secondary metabolites of medicinal plants due to increased nutrient availability to plants (Chandra et al., 2010). Enhanced N-uptake by AMF is well documented (Johansen et al., 1996), showing an increased synthesis of amino acids and specific metabolites in medicinal plants. Our results showed higher phenolic content (Fig. 13), and an increase of main polyphenols (such as wedelolactone; luteolin; 4,5-dicaffeoylquinic acid; and quercetin) of mycorrhizae inoculated plants compared with the controls (Tables 6 and 7). These findings agree with other studies where the target plants *Cynara cardunculus* and *Ocimum basilicum* increased the phenolic contents (Zeng et al., 2013). The increased concentration in total phenols can be explained by the influence pathways producing fatty acids, amino acids, and apocarotenoids in the cycle of tricarboxylic acids, which results in sub-products used in the integration of phenolic compounds (Lohse et al., 2005).

Moreover, Kapoor et al. (2002) showed that increased absorption of mineral nutrients (especially P and N), together with changed phytohormone level in the mycorrhizal plant (Toussaint et al., 2007), enhanced plant terpenoids and phenolic acids. Another reason could be that AMF can induce defense related compounds (including the production of phenolic compounds) in plants (Volpin et al., 1994). However, AMF did not always increase the phenolic content in *Ocimum basilicum* and *Salvia officinalis* (Lee and Scagel, 2009), and the different genotype of host plants also influenced it.

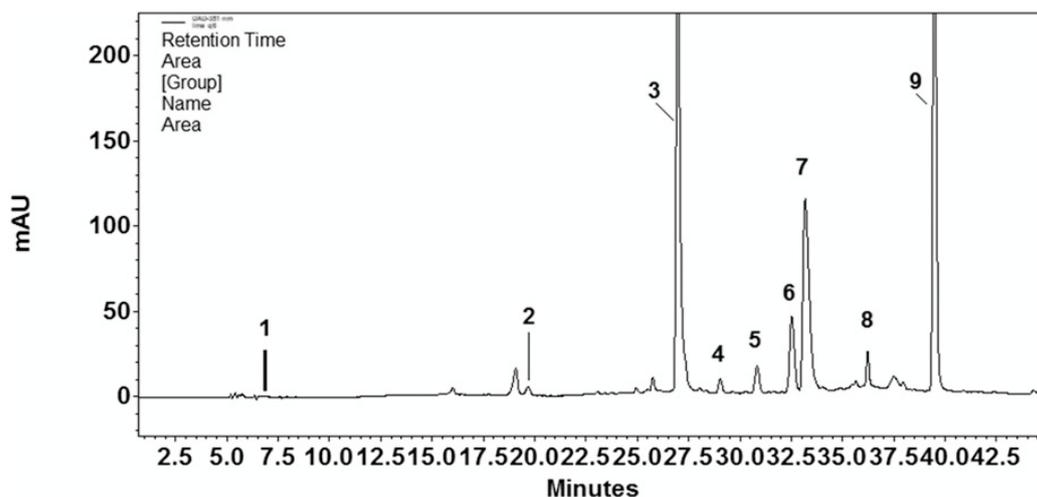


Figure 14. HPLC profile of effective polyphenols extracted from *E. prostrata* leaves. Different numbers represent polyphenols: 1: Protocatechuic acid; 2: 5-o-caffeoylquinic acid; 3: Dimethyl-wedelolactone; 4: 4-o-caffeoylquinic acid; 5: 3,5-dicaffeoylquinic acid; 6: 4,5-dicaffeoylquinic acid; 7: Quercetin-3- arabinoside; 8: Luteolin; 9: Wedelolactone.

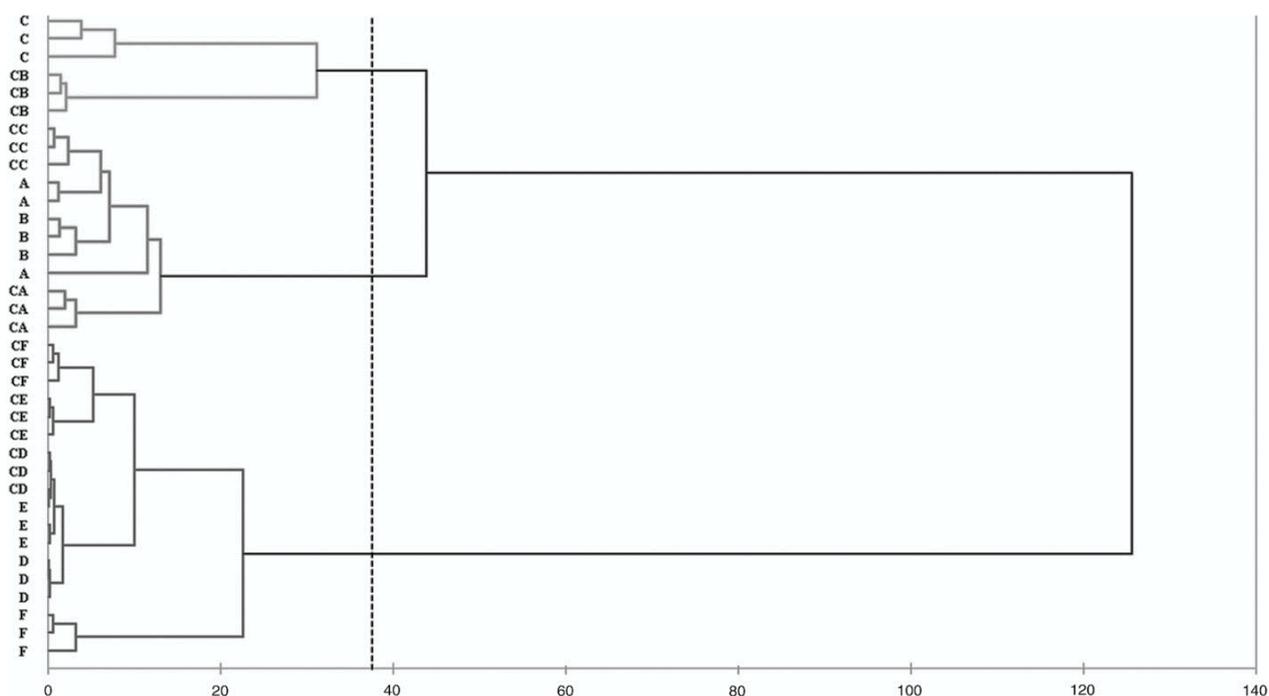


Figure 15. Dendrogram for 12 treatments with and without AMF obtained from the hierarchical cluster analysis. AMF+: mycorrhizal plants, AMF–: nonmycorrhizal plants; A: AMF+ 100/0 sand/peat % (v/v); B: AMF+ 80/20 sand/peat % (v/v); C: AMF+ 60/40 sand/peat % (v/v); D: AMF+ 40/60 sand/peat % (v/v); E: AMF+20/80 sand/peat % (v/v); F: AMF+ 0/100 sand/peat % (v/v); CA: 100/0 sand/peat % (v/v); CB: 80/20 sand/peat % (v/v); CC: 60/40 sand/peat % (v/v); CD:40/60 sand/peat % (v/v); CE: 20/80 sand/peat % (v/v); CF: 0/100 sand/peat % (v/v).

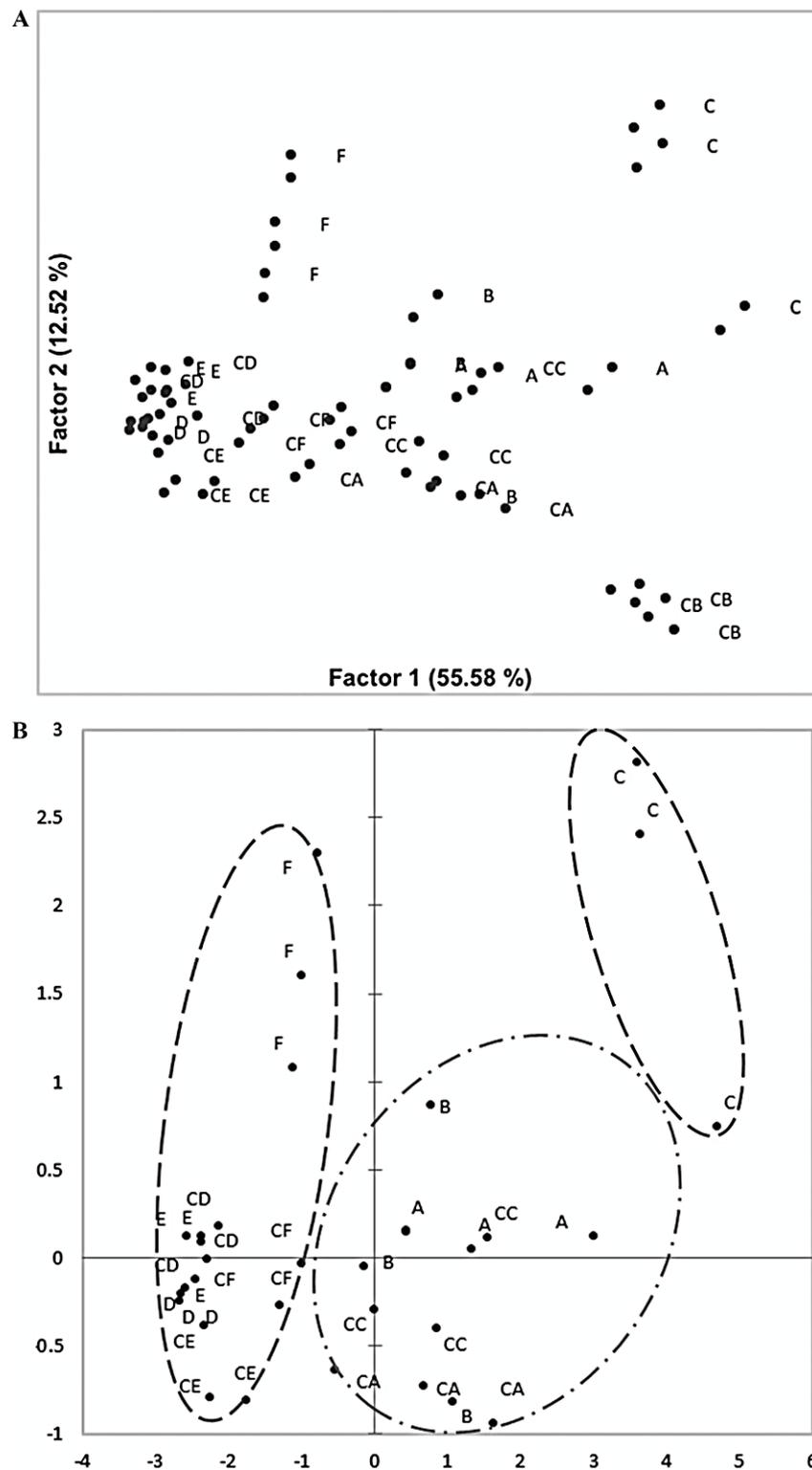


Figure. 16. Principal component analysis of polyphenolic data of 12 different treatments. A scatter plot (PC1 versus PC2). (A), A zoomed in scatterplot on the cluster containing treatments (B). A: AMF+ 100/0 sand/peat % (v/v); B: AMF+ 80/20 sand/peat % (v/v); C: AMF+ 60/40 sand/peat % (v/v); D: AMF+ 40/60 sand/peat % (v/v); E: AMF+ 20/80 sand/peat % (v/v); F: AMF+ 0/100 sand/peat % (v/v); CA: 100/0 sand/peat % (v/v); CB: 80/20 sand/peat % (v/v); CC: 60/40 sand/peat % (v/v); CD: 40/60 sand/peat % (v/v); CE: 20/80 sand/peat % (v/v); CF: 0/100 sand/peat % (v/v).

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

4.3.1. Root colonization rate

Non-AM plants had no mycorrhizal colonization during plant growth. After four weeks of growth, the mycorrhizal colonization rate of AM plants reached 54% under non-stress conditions, while the rate was 58.44% in those treated with 100 mM NaCl (Fig. 17A). No significant differences could be found between inoculated plants under non-stress conditions and salt stress at 100 mM NaCl.

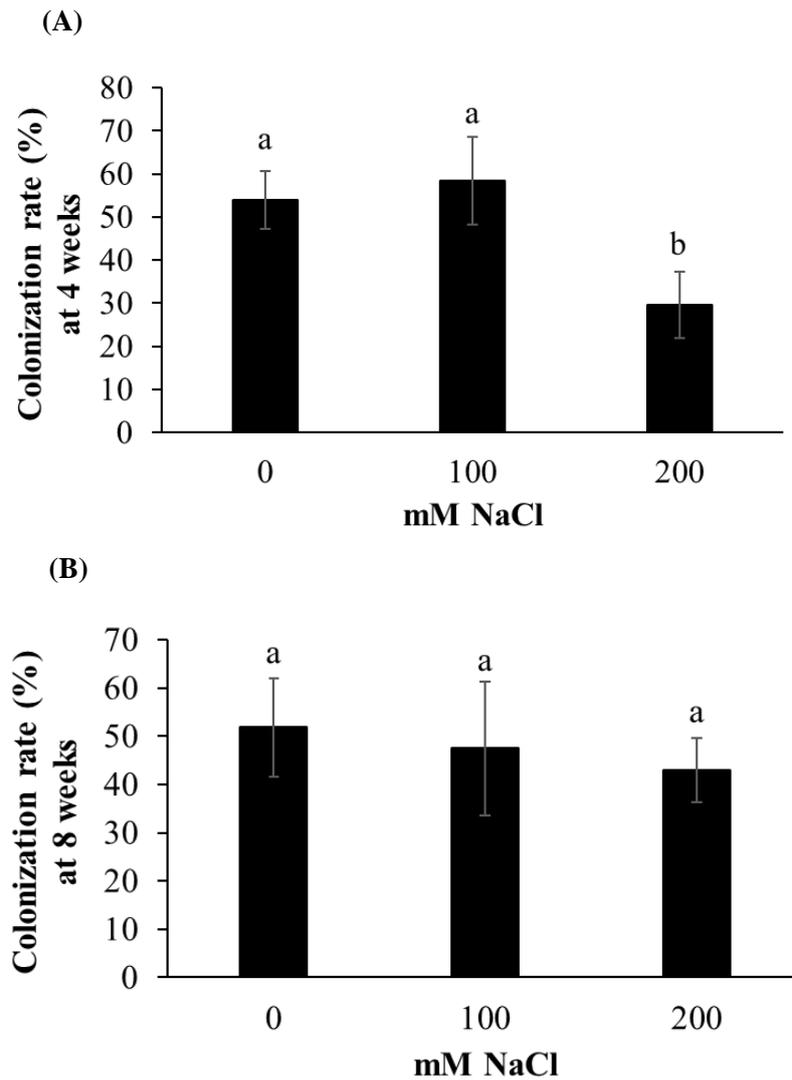


Figure 17. Mycorrhiza colonization rate (%) of *Eclipta prostrata* plants under non-stress, moderate, and high saline conditions at four weeks (A) and eight weeks (B) after growth. Each bar shows the mean \pm standard deviation ($n = 3$). Different letters indicate significant difference among treatments according to the Tukey test ($P < 0.05$).

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 (Fig. 17B). Their rates were 51.89%, 47.44%, and 43% in colonized plants under non-stress, moderate, and high salt stress, respectively.

4.3.2. Growth parameters

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. Root weight was remarkably higher in inoculated plants versus uninoculated plants under non-stress conditions at eight weeks.

Table 8. 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)		Plant height (cm/plant)		Stem diameter (mm/plant)		Leaf area (cm ² /plant)	
		4 w	8 w	4 w	8 w	4 w	8 w	4 w	8 w	4 w	8 w	4 w	8 w	4 w	8 w
Stress conditions	Mycorrhizal inoculation														
Non-stress	AMF+	19.7 ± 3.5 a	25.5 ± 2.3 a	1.08 ± 0.38 a	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	13.7 ± 6.0 a	25.5 ± 6.5 a	3.4 ± 0.7 a	4.31 ± 0.8 a	15.85 ± 2.6 a	23.81 ± 3.8 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	11.3 ± 6.0 ab	24.6 ± 6.6 a	2.0 ± 0.3 bc	2.93 ± 0.2 b	8.73 ± 0.7 b	11.93 ± 2.8 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	7.9 ± 2.1 abc	13.3 ± 2.2 b	2.4 ± 0.1 b	2.45 ± 0.1 b	8.37 ± 0.6 bc	14.38 ± 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	6.9 ± 2.2 abc	10.9 ± 3.0 bc	1.9 ± 0.1 bc	2.13 ± 0.1 b	5.24 ± 0.8 cd	7.63 ± 1.0 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	5.6 ± 0.7 bc	10.1 ± 2.1 bc	1.8 ± 0.3 bc	2.36 ± 0.4 b	6.60 ± 0.5 bcd	9.15 ± 1.8 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	3.8 ± 0.5 c	5.0 ± 0.6 c	1.4 ± 0.2 c	2.06 ± 0.4 b	3.28 ± 0.6 d	7.28 ± 1.5 c
Significant of Source of variation (NS = not significant, * P < 0.05, ** P < 0.01, *** P < 0.001)															
Mycorrhizal inoculation (M)		**	***	*	**	**	***	***	***	NS	*	***	**	***	***
Salt stress (S)		**	***	*	***	**	***	**	***	***	***	***	***	***	***
M*S		NS	NS	NS	**	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.

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) (Table 8).

4.3.3. Proline concentration

Salinity remarkably increased proline concentrations in both mycorrhizal and nonmycorrhizal plants at our first measurement (four weeks of growth). Nonetheless, there are no significant differences between inoculated and uninoculated plants under different conditions. Similarly, after eight weeks of growth, the proline level in plants under salt stresses was substantially higher than under non-stress. Interestingly, mycorrhizal treatment considerably strengthened the proline level (up to 24.57 $\mu\text{GA ml}^{-1}$) in plants treated with 100 mM NaCl.

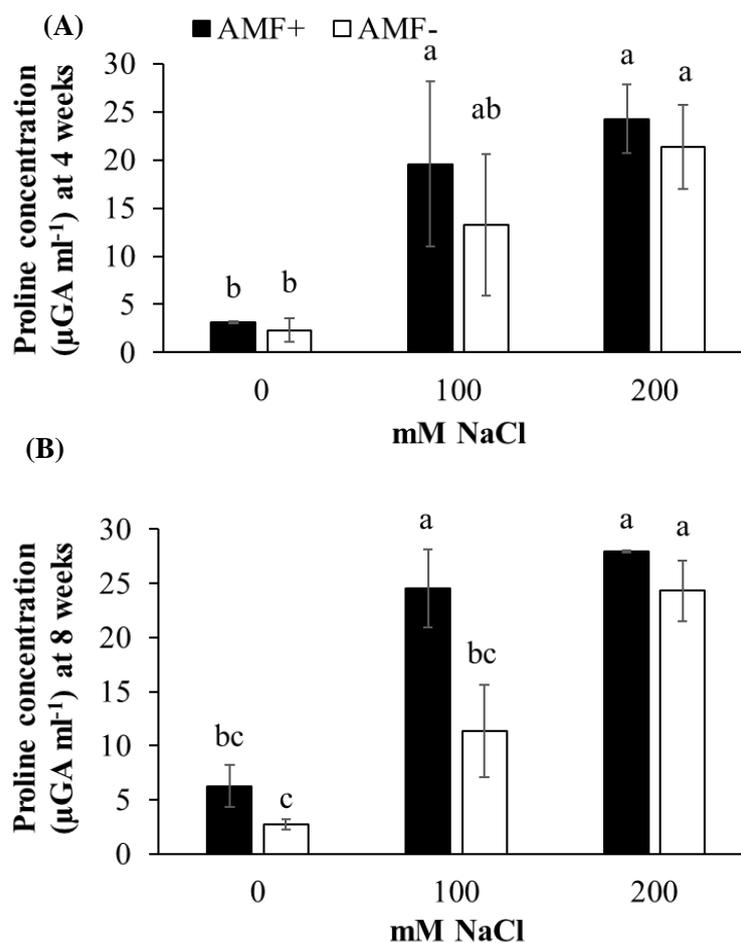


Figure 18. Proline concentration in leaves of *Eclipta prostrata* plants not inoculated or inoculated with arbuscular mycorrhiza under non-stress, moderate, and high saline conditions at four weeks (A) and eight weeks (B) of growth. AMF+, AMF-, with and without arbuscular mycorrhizal fungi, respectively. Each bar shows the mean \pm standard deviation ($n = 3$). Different letters indicate significant difference among treatments according to the Tukey test ($P < 0.05$).

As shown in Table 9, there were significant effects of mycorrhizal inoculation (M) and salt stress (S) on proline concentration measured at eight weeks ($P < 0.001$) and Fv/Fm at four weeks ($P < 0.001$ and $P < 0.05$, respectively) while only interaction between two main effects on proline at eight weeks was observed ($P < 0.001$). In addition, mycorrhizal treatment substantially influenced on

Fv/Fm at eight weeks ($P < 0.001$), POD activity at four weeks ($P < 0.01$). Salinity remarkably affected proline at four weeks ($P < 0.001$), PPO activity at four weeks ($P < 0.001$) and eight weeks ($P < 0.05$), SOD activity at four weeks ($P < 0.05$), CAT activity at four weeks ($P < 0.001$) and eight weeks ($P < 0.05$).

4.3.4. Chlorophyll fluorescence

Although salt stresses slightly increased the maximal photochemical efficiency of photosystem II (F_v/F_m) in plants, no significant differences between mycorrhizal and nonmycorrhizal plants were found at four and eight weeks of growth. However, mycorrhizal colonization considerably elevated F_v/F_m in plants under non-stress conditions after eight weeks of growth. (Fig.19).

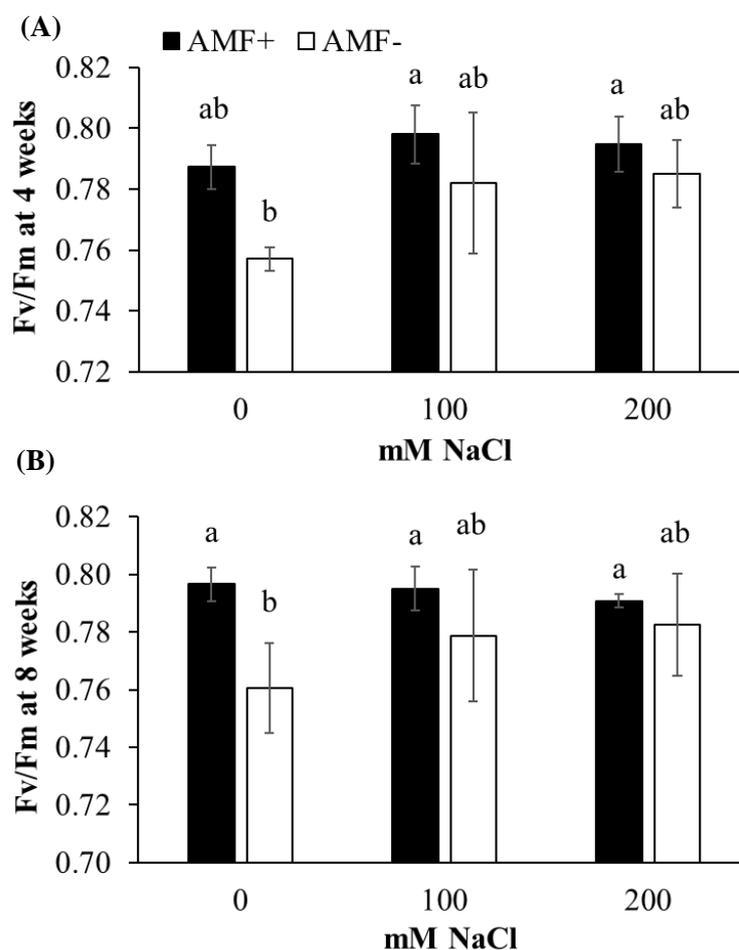


Figure 19. Maximal photochemical efficiency of photosystem II (F_v/F_m) in leaves of *Eclipta prostrata* plants not inoculated or inoculated with arbuscular mycorrhizal fungi under non-stress, moderate, and high saline conditions at four weeks (A) and eight weeks (B) after growth. AMF+, AMF-, indicate treatments with and without arbuscular mycorrhizal fungi, respectively. Each bar shows the mean \pm standard deviation ($n = 3$). Different letters indicate significant difference among treatments according to the Tukey test ($P < 0.05$).

Table 9. Effects of arbuscular mycorrhizal inoculation (M), salt stress (S), and their interaction between M and S on different parameters.

Parameter	Mycorrhizal inoculation (M)	Salt stress (S)	M*S
Proline at 4 weeks	NS	***	NS
Proline at 8 weeks	***	***	***
F _v /F _m at 4 weeks	***	*	NS
F _v /F _m at 8 weeks	***	NS	NS
PPO at 4 weeks	NS	***	NS
PPO at 8 weeks	NS	*	NS
POD at 4 weeks	**	NS	NS
POD at 8 weeks	NS	NS	NS
SOD at 4 weeks	NS	*	NS
SOD at 8 weeks	NS	NS	NS
CAT at 4 weeks	NS	***	NS
CAT at 8 weeks	NS	*	NS

NS = not significant; *, ** and *** mean significant difference at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively, according to Tukey's post hoc test.

4.3.5. Polyphenol compounds

In the HPLC analysis, the gradient elution applied was able to efficiently separate a wide range of phenolic compounds such as wedelolactone; 4-o-caffeoylquinic acid; caffeic acid; 3,4-dicaffeoylquinic acid; 3,5-dicaffeoylquinic acid; luteolin-glucoside; luteolin-7-o-glucoside; ferulic acid; dimethyl-wedelolactone; ferulicquinic acid; 4,5-dicaffeoyl-quinic acid; quercetin-arabioside; luteolin; 5-o-caffeoylquinic acid (Table 10 and 11) in plants at four and eight weeks under different growth conditions, with 4,5-dicaffeoyl-quinic acid and wedelolactone being abundant in all of the different samples 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-arabioside, 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-arabioside ($P<0.05$). 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 $P<0.05$).

Table 10. Change in the content of polyphenol compounds in *Eclipta prostrata* plants inoculated or not inoculated with arbuscular mycorrhizal fungi under non-stress, moderate, and high saline conditions after four weeks.

	Stress conditions										
	Non-stress		100 mM NaCl		200 mM NaCl						
Inoculation Compounds	AMF+	AMF-	AMF+	AMF-	AMF+	AMF-	Significant of Source of variation (NS = not significant, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001)	Mycor-rhizae (M)	Salt stress (S)	M*S	
Wedelolactone (µg/g)	1388.79 ± 430.42 ab	676.62 ± 328.85 bc	465.37 ± 55.53 c	1620.31 ± 239.16 a	324.24 ± 22.42 c	228.80 ± 91.18 c			NS	***	NS
4-o-caffeoylquinic acid (µg/g)	100.98 ± 10.93 b	37.87 ± 1.00 c	71.71 ± 24.91 bc	165.42 ± 44.35 a	81.81 ± 12.96 bc	73.24 ± 25.41 bc			NS	**	NS
Caffeic acid (µg/g)	UDL	3.49 ± 1.49 b	6.32 ± 2.30 ab	9.47 ± 2.49 a	5.65 ± 0.58 ab	5.17 ± 0.33 b			**	***	*
3,4-dicaffeoyl-quinic acid (µg/g)	64.44 ± 3.77 a	54.81 ± 9.89 ab	44.21 ± 9.64 ab	62.28 ± 26.41 ab	31.93 ± 18.93 ab	20.27 ± 2.37 b			NS	**	NS
3,5 – dicaffeoyl-quinic acid (µg/g)	130.54 ± 36.16 b	25.86 ± 11.10 c	189.67 ± 68.05 ab	243.65 ± 28.40 a	154.97 ± 21.42 ab	3.99 ± 1.99 c			**	***	***
Luteolin-glucoside (µg/g)	13.97 ± 6.25 b	6.98 ± 2.99 b	29.59 ± 6.45 a	18.93 ± 5.98 ab	17.29 ± 1.52 ab	UDL			***	***	NS
Luteolin-7-o-glucoside (µg/g)	251.45 ± 54.88 ab	292.97 ± 21.92 a	104.30 ± 27.55 c	224.88 ± 98.73 abc	90.79 ± 31.89 c	113.60 ± 13.32 bc			*	***	NS
Ferulic acid (µg/g)	108.7 ± 30.4 bc	74.24 ± 26.41 c	57.79 ± 6.02 c	251.78 ± 81.64 ab	84.45 ± 23.96 c	311.6 ± 76.54 a			***	*	**
Dimethyl wedelactone (µg/g)	UDL	151.47 ± 66.77 ab	UDL	220.72 ± 81.21 a	74.16 ± 18.44 b	63.11 ± 14.80 b			***	NS	NS
Ferulicquinic acid (µg/g)	400.96 ± 77.36 a	13.36 ± 6.93 c	58.13 ± 23.36 b	UDL	38.90 ± 3.60 b	UDL			***	***	***
4,5-dicaffeoyl-quinic acid (µg/g)	1122.38 ± 283.27 ab	81.24 ± 4.88 c	1364.81 ± 479.98 a	1248.44 ± 315.98 a	774.85 ± 129.05 ab	43.19 ± 21.14 d			***	***	*
Quercetin-arabioside (µg/g)	296.59 ± 49.11 a	196.64 ± 92.93 ab	251.45 ± 32.78 ab	231.69 ± 43.35 ab	185.90 ± 17.44 ab	134.69 ± 11.96 b			*	*	NS
Luteolin (µg/g)	10.96 ± 4.34 b	5.65 ± 1.14 b	35.57 ± 13.2a	21.92 ± 12.08 ab	12.30 ± 1.52 ab	19.27 ± 8.36 ab			NS	**	NS
5-o-caffeoylquinic acid (µg/g)	9.31 ± 3.19 ab	5.48 ± 1.49 b	8.30 ± 2.29 ab	9.97 ± 1.00 ab	6.98 ± 1.00 ab	14.80 ± 5.55 a			NS	NS	NS

AMF+, AMF- represent mycorrhizal and nonmycorrhizal plants, respectively.

For each parameter, the means ± standard deviation is shown (n = 3)

Different letters in each parameter indicate significant differences according to the Tukey test (P<0.05) among all treatments under different growth conditions. UDL, under detection limit.

Table 11. Change in the content of polyphenol compounds ($\mu\text{g/g}$) in *Eclipta prostrata* plants inoculated or not inoculated with arbuscular mycorrhizal fungi under non-stress, moderate, and high saline conditions after eight weeks.

	Stress conditions									
	Non-stress		100 mM NaCl		200 mM NaCl					
Inoculation Compounds	AMF+	AMF-	AMF+	AMF-	AMF+	AMF-	Significant of Source of variation (NS = not significant, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$)	Mycorr-hizae (M)	Salt stress (S)	M*S
Wedelolactone ($\mu\text{g/g}$)	1526.64 \pm 207.47a	1318.92 \pm 157.92ab	617.17 \pm 238.89c	359.07 \pm 78.55c	1103.13 \pm 292.97b	268.58 \pm 18.73c		***	***	**
4-o-caffeoylquinic acid ($\mu\text{g/g}$)	114.60 \pm 43.81bc	168.53 \pm 52.21b	128.55 \pm 37.11bc	51.21 \pm 16.97c	348.28 \pm 9.467a	30.26 \pm 8.47c		***	**	***
caffeic acid ($\mu\text{g/g}$)	35.54 \pm 5.84a	31.81 \pm 6.18a	13.75 \pm 3.85b	5.65 \pm 1.14b	29.40 \pm 5.48a	3.32 \pm 1.14b		***	***	**
3,4-dicaffeoyl-quinic acid ($\mu\text{g/g}$)	314.80 \pm 44.24a	127.55 \pm 30.18bc	47.86 \pm 6.86cd	21.57 \pm 1.40d	144.48 \pm 41.71b	20.62 \pm 5.01d		***	***	**
3,5-dicaffeoyl-quinic acid ($\mu\text{g/g}$)	417.10 \pm 111.42ab	452.92 \pm 103.65ab	232.85 \pm 66.67bc	72.15 \pm 35.95c	505.72 \pm 81.16a	29.26 \pm 13.44c		***	***	***
Luteolin-glucoside ($\mu\text{g/g}$)	30.12 \pm 9.55ab	3.33 \pm 1.15c	29.56 \pm 6.33b	16.96 \pm 3.42bc	52.32 \pm 12.46a	20.45 \pm 7.21bc		***	**	NS
Luteolin-7-o-glucoside ($\mu\text{g/g}$)	422.52 \pm 139.11a	418.59 \pm 70.51a	187.34 \pm 31.67bc	171.58 \pm 50.82bc	362.73 \pm 67.76ab	109.12 \pm 52.31c		*	***	*
Ferulic acid ($\mu\text{g/g}$)	174.39 \pm 63.01b	339.36 \pm 92.48a	118.58 \pm 59.06b	69.50 \pm 3.89b	337.81 \pm 43.84a	52.32 \pm 7.47b		NS	**	***
Dimethyl wedelac-tone ($\mu\text{g/g}$)	575.99 \pm 209.10b	954.68 \pm 200.01a	112.60 \pm 32.73c	80.81 \pm 10.48c	341.30 \pm 155.95bc	53.86 \pm 5.18c		NS	***	**
Ferulicquinic acid ($\mu\text{g/g}$)	UDL	UDL	UDL	UDL	UDL	UDL				
4,5-dicaffeoyl-quinic acid ($\mu\text{g/g}$)	2401.23 \pm 652.94a	1839.46 \pm 506.78ab	1529.16 \pm 520.47ab	913.79 \pm 142.71ab	2389.61 \pm 1087.18a	424.42 \pm 54.75b		*	NS	NS
Quercetin-arabioside ($\mu\text{g/g}$)	760.90 \pm 261.47a	430.28 \pm 121.43ab	151.80 \pm 57.84b	119.39 \pm 48.33b	367.21 \pm 26.40b	71.78 \pm 17.83b		**	***	NS
Luteolin ($\mu\text{g/g}$)	44.55 \pm 13.12a	UDL	9.63 \pm 7.20bc	6.58 \pm 2.10c	20.26 \pm 3.47b	7.08 \pm 2.98bc		***	**	***
5-o-caffeoylquinic acid ($\mu\text{g/g}$)	46.84 \pm 20.22a	40.95 \pm 10.05a	22.17 \pm 6.88ab	10.64 \pm 2.06b	47.33 \pm 1.49a	8.32 \pm 2.49b	***	***	**	

AMF+, AMF- represent mycorrhizal and nonmycorrhizal plants, respectively.

For each parameter, the means \pm standard deviation is shown (n = 3)

Different letters in each parameter indicate significant differences according to the Tukey test ($P < 0.05$) among all treatments under different growth conditions. UDL, under detection limit

4.3.6. Antioxidant enzyme activities

No significant differences in PPO activity between mycorrhizal and nonmycorrhizal plants under non-stress conditions, and at both salinity stress levels were seen at four and eight weeks of growth (Fig. 20). Nonetheless, high salt stress substantially decreased the enzyme activity in four-week plants.

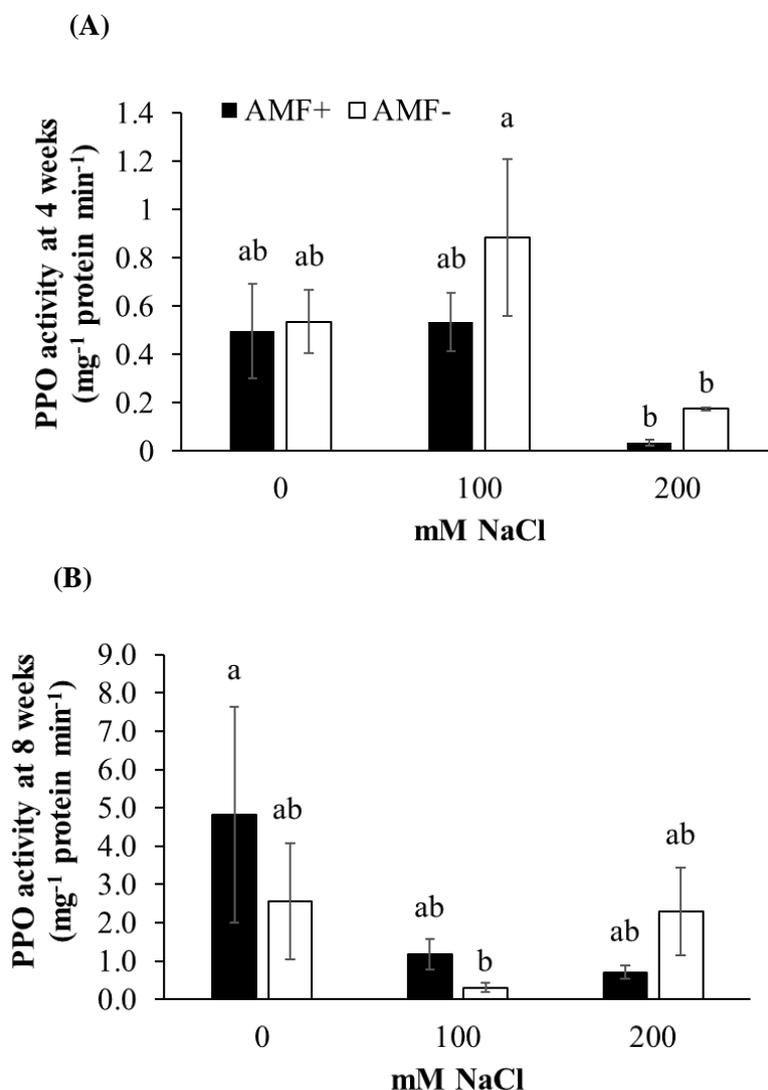


Figure 20. Polyphenol oxidase (PPO) activity in leaves of *Eclipta prostrata* plants not inoculated or inoculated with arbuscular mycorrhiza under non-stress, moderate, and high saline conditions at four weeks (A) and eight weeks (B) of growth. AMF+, AMF-, with and without arbuscular mycorrhizal fungi, respectively. Each bar shows the mean \pm standard deviation ($n = 3$). Different letters indicate significant difference among treatments according to the Tukey test ($P < 0.05$).

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. 21A). 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. 21B). In terms of SOD activity, no significant differences could be detected in mycorrhizal and non-mycorrhizal plants under non-stress and both salt levels at four and eight weeks after plant growth (Fig. 22).

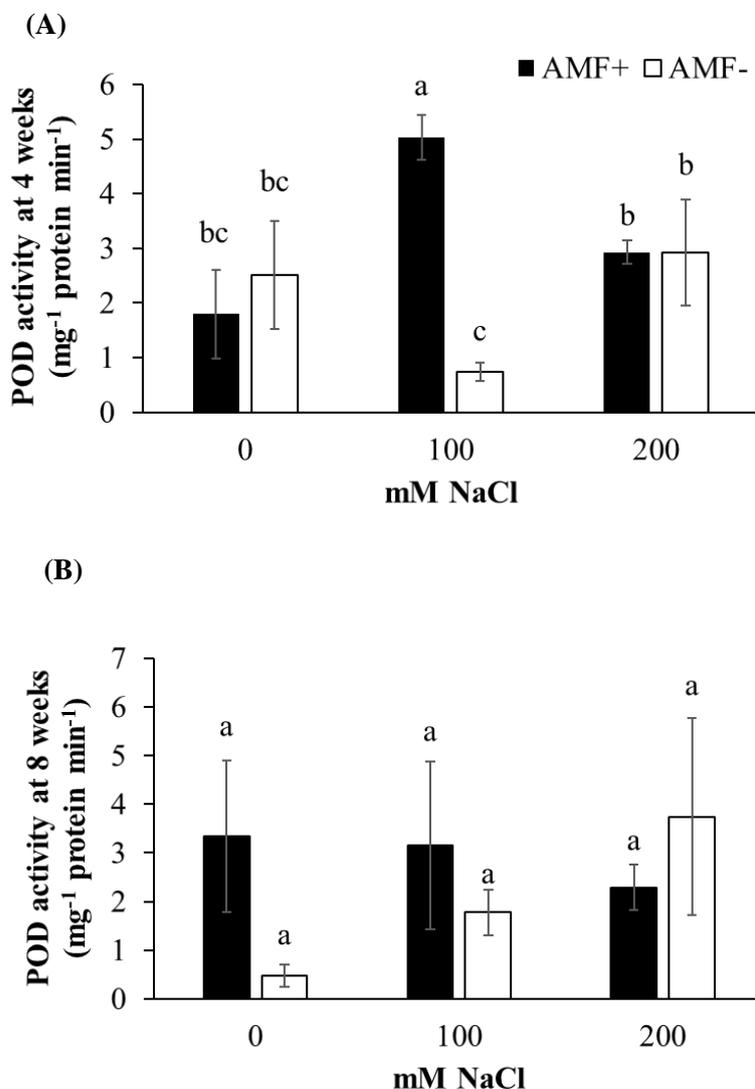


Figure 21. Peroxidase (POD) activity in leaves of *Eclipta prostrata* plants not inoculated or inoculated with arbuscular mycorrhizal mycorrhiza under non-stress, moderate, and high saline conditions at four weeks (A) and eight weeks (B) of growth. AMF+, AMF-, with and without arbuscular mycorrhizal fungi, respectively. Each bar shows mean \pm standard deviation ($n = 3$). Different letters indicate significant difference among treatments according to the Tukey test ($P < 0.05$).

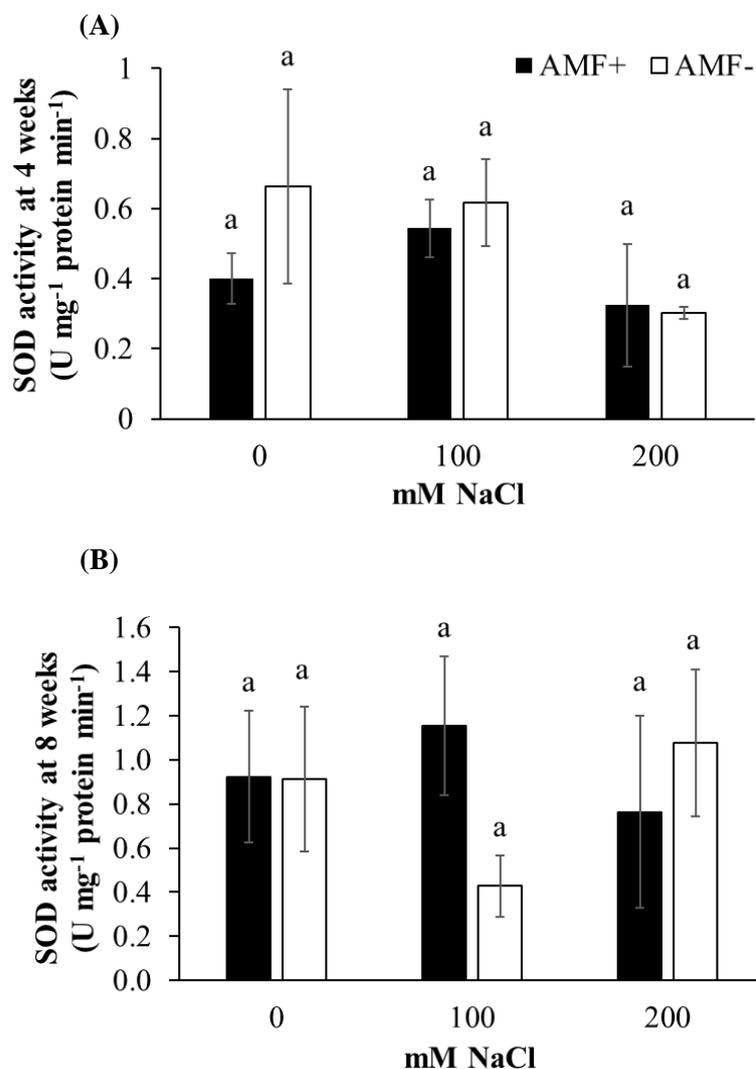


Figure 22. Superoxide dismutase (SOD) activity in leaves of *Eclipta prostrata* plants not inoculated or inoculated with arbuscular mycorrhizal mycorrhiza under non-stress, moderate, and high saline conditions at four weeks (A) and eight weeks (B) of growth. AMF+, AMF-, with and without arbuscular mycorrhizal fungi, respectively. Each bar shows the mean \pm 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. 23A). 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. 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. 23B).

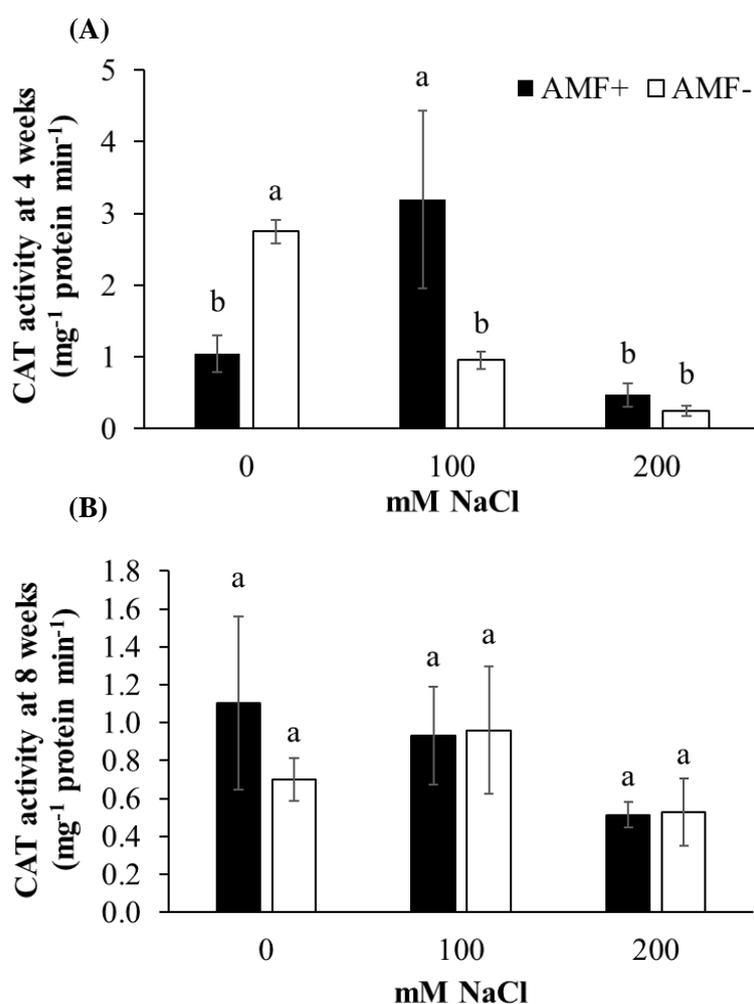


Figure 23. Catalase (CAT) activity in leaves of *Eclipta prostrata* plants not inoculated or inoculated with arbuscular mycorrhizal mycorrhiza under non-stress, moderate, and high saline conditions at four weeks (A) and eight weeks (B) after growth. AMF+, AMF-, with and without arbuscular mycorrhizal fungi, respectively. Each bar shows the mean \pm standard deviation ($n = 3$). Different letters indicate significant difference among treatments according to the Tukey test ($P < 0.05$).

4.3.7. Discussion

Although AMF were reported to improve plant performance under salinity in many plant species (Santander et al., 2019, Wang et al., 2019b, Wang et al., 2019a, Abdel Motaleb et al., 2020), this is the first report on positive influences of AMF on *Eclipta prostrata* medicinal plant under salt stresses. In the present study, mycorrhizal colonization rate was markedly decreased after four weeks of plant growth due to the high saline level (200 mM NaCl), but this decrease did not occur at the later stage of plant growth (at eight weeks). This may be owing to the direct inhibitory effect of NaCl on the AMF at the beginning stage of plant growth, then AMF may adapt to the higher

level of salt. Previous reports illustrated that salinity decreased AM colonization rate though it depended on cultivars and AMF isolates (Santander et al., 2020, Wang et al., 2019b).

The inhibitory impacts of salt stress on the growth parameters were recognized in our study, which is in line with earlier studies (Santander et al., 2020, Wang et al., 2019b). The reasons could be the fact that salinity is believed to inhibit plant growth as a consequence of nutrient imbalance, cytotoxicity owing to excessive uptake of Na⁺ and Cl⁻ ions, osmotic stress, and oxidative stress (Isayenkov and Maathuis, 2019). AM inoculation has been reported to enhance the growth characteristics of several plants exposed to saline conditions, such as in bean (Abdel Motaleb et al., 2020), soybean (Hashem et al., 2019), lettuce (Santander et al., 2019), sweet sorghum (Wang et al., 2019a). These results supporting beneficial effects of the AM application on growth parameters of host plants under salinity are inconsistent with our observation. However, its beneficial influences on several growth parameters were seen under non-stress conditions. This may be due to the specific interaction between fungal and plant partners, as reported in earlier studies (Duc, 2017, Duc et al., 2018).

Osmolytes play an important role in plant protection from ultrastructure damage caused by salinity. Proline, one of the crucial osmolytes, plays a crucial role in osmoregulation in plants subjected to salt stress (Ahmad et al., 2012) and serves as an efficient scavenger of reactive oxygen species (ROS) (Hayat et al., 2012). Our results showed that mycorrhizal plants significantly induced a higher proline level than non-AM plants under saline at 100 mM NaCl, which is a confirmation of the results of Santander et al. (2019) and Kong et al. (2019).

As crucial parts of the photosynthetic apparatus, photosystems I (PS I) and II (PS II) are susceptible to saline conditions. Salinity can demolish the reaction center of PS II, disturb electron transport from PS II to PS I, and eventually result in a drop in photosynthesis (Wang et al., 2019b). Therefore, damage to PS II under salt stresses was measured at four and eight weeks of growth in our study. We found that the maximal photochemical efficiency of PS II (Fv/Fm) was not affected by salinity and AMF, indicating that salinity did not impair the photosynthetic system under our experimental conditions. The results were not in line with other work (Wang et al., 2019b), illustrating that the symbiotic fungi could improve the photosynthetic capacity of host plants subjected to salt stress. The reasons may be accountable for differences in growth conditions, stress treatments, stress duration, plant and AMF species.

Plants accumulate phenolics as a defensive mechanism to deal with various abiotic stresses (Cheynier et al., 2013). Phenolic compounds possess antioxidant properties thanks to the capacity of scavenging reactive oxygen species (ROS), donating electrons, hydrogen atoms, or chelate

metal cations (Sharma et al., 2014). Our results showed that wedelolactone and 4,5-dicaffeoylquinic acid were the major components of phenolic compounds in *E. prostrata* plants during growth stages under non-stress and saline conditions. In our previous study, wedelolactone and dimethyl wedelolactone were proved as two main constituents of phenolics in *E. prostrata* plants grown in substrates with different proportions of sand and peat (Vo et al., 2019). The difference in the main constituent of polyphenols (4,5-dicaffeoylquinic acid in the present experiment versus dimethyl wedelolactone in the previous experiment (Vo et al., 2019) may be attributable mainly to different growth conditions we applied. The findings show that mycorrhizal colonization caused changes to tested secondary metabolites of *E. prostrata* plants under both levels of salinity at the early stage of plant growth. Noticeably, at the later growth stage (eight weeks after growth), AMF significantly enhanced most phenolic components (ten of thirteen polyphenols examined) in host plants under high salt stress (200 mM NaCl), whereas there were no substantial changes in the contents of polyphenols between AM and non-AM in plants subjected to moderate salt stress. Discrepant observations on phenolic compounds accumulation in plants under stress were reported. Our results concur with Zhang et al. (2013), who demonstrated significant increases in levels of phenolic compounds in inoculated plants. These increases might be due to the beneficial effects of AMF inoculation on relative water content and nutritional status in host plants. In addition, mycorrhization also exerts transcriptional and metabolic alterations in host plants (Salvioli et al., 2012, Jung et al., 2012), which may lead to changes in phenolic profiles. In contrast, Santander et al. (2019) pointed out considerably lower phenolics in leaves of two lettuce cultivars colonized by AMF under salt stresses. To our best knowledge, our work is the first study on effects of the fungal symbiosis on active ingredients of *E. prostrata* plants under saline stresses.

It is well known that plants can activate effective antioxidant systems where PPO, SOD, POD, CAT are important enzymes to protect themselves against oxidative stress. In the plant cell, SOD, the first line of defense against ROS (Kang et al., 2012), catalyzes the dismutation of O_2^- to H_2O_2 . Subsequently, CAT and other antioxidative enzymes detoxicate H_2O_2 to O_2 and H_2O (Apel and Hirt, 2004). POD catalyzes H_2O_2 reduction by diverse molecules such as phenolic compounds and directly participate in ROS removal (Kravic et al., 2013). In the present work, POD and CAT activity were highly induced in mycorrhizal plants exposed to moderate salinity (at 100 mM NaCl) at four weeks of plant growth while mycorrhizal application did not change the activity of other enzymes at both times of measurement under salt stresses. This may suggest that POD and CAT were two major antioxidative enzymes in mycorrhizal *E. prostrata* plants only at the early plant growth stage to alleviate the oxidative stress caused by saline conditions. Interestingly, the defense

enzymes induced by AMF varied with plant age (Mayer et al., 2019), which is in accordance with our observation. Similarly, strengthened POD and CAT activity were found in other plants inoculated with AMF under salinity conditions (Wang et al., 2019a, Santander et al., 2019). This implies that mycorrhizal application could mitigate the damage of ROS, protect the *E. prostrata* plants against the oxidative stress induced by salinity.

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

4.4.1. Root colonization rate

No AM colonization was observed in control plants (uninoculated plants). The colonization rate of plants inoculated with *Acaulospora lacunose* (Al) (25%) was significantly lower than plants inoculated with other AMF species and mixture of AMF (from 48.33% to 55%) at four weeks of growth, whereas no significant differences in this rate among eight-week plants inoculated with different single AMF species and mixture of AMF were found (Fig. 24).

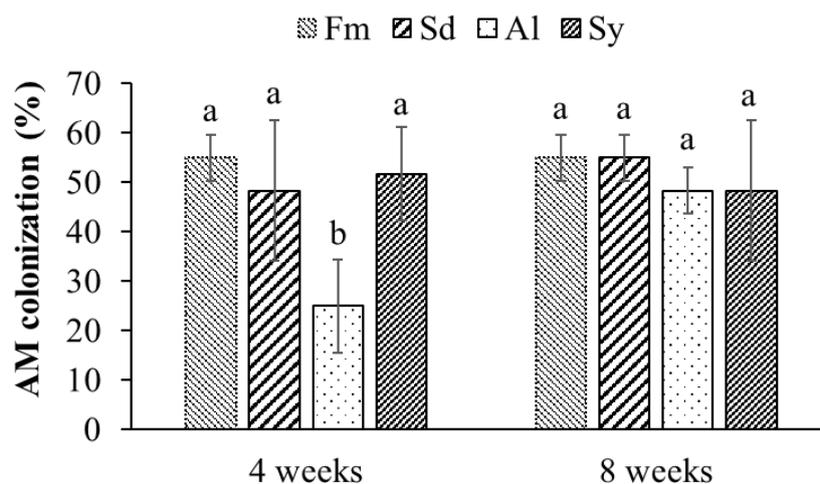


Figure 24. Mycorrhizal colonization rate (%) of *Eclipta prostrata* plants not inoculated or inoculated with *Funneliformis mosseae* (Fm), *Septoglomus deserticola* (Sd), *Acaulospora lacunose* (Al), symbivit (Sy) including six AMF isolates at four weeks (A) and eight weeks (B) of growth. Each bar shows the mean \pm standard deviation ($n = 3$). Different letters indicate significant difference among treatments according to the Tukey test ($P < 0.05$)

4.4.2. Growth parameters

There were no significant differences in root weight, leaf number among all treatments at four and eight weeks of growth (Table 12). Application of Sd and Al substantially induced longer root length than other fungal inoculants and control plants at eight weeks after growth. Inoculation with Symbivit (Sy) substantially increased shoot weight in relation to the corresponding control plants

at eight weeks of growth. In contrast, plants colonized by *Septoglomus deserticola* (Sd) had a considerably lower shoot weight than non-mycorrhizal plants during plant growth. Interestingly, mycorrhizal application significantly decreased plant height, as compared with uncolonized plants at four and eight weeks of growth, except Sy treatment and Fm treatment (at eight weeks after growth). Regarding leaf area, no beneficial effects of AMF inoculations were observed in host plants.

Table 12. Growth parameters of *Eclipta prostrata* plants inoculated with different arbuscular mycorrhizal fungi or not inoculated after four and eight weeks.

Treatment		Ct	Fm	Sd	Al	Sy
Root length (cm/plant)	4 w	19± 4b	17.17±2.02b	27.67±4.04a	25 ± 6.56ab	15.36±2.56b
	8 w	19 ± 3.35b	19 ± 2.53b	25 ± 2.76a	25.6 ± 4.46a	21.17±2.47ab
Root weight (g/plant)	4 w	1.49 ± 1.05a	1.67 ± 0.19a	1.69 ± 0.40a	1.83 ± 0.76a	1.53 ± 0.03a
	8 w	1.81 ± 0.13a	2.38 ± 0.62a	2.02 ± 0.18a	2.33 ± 0.16a	2.37 ± 0.30a
Shoot weight (g/plant)	4 w	4.44±1.12ab	3.26 ± 0.30bc	2.09 ± 0.17c	3.07±0.90bc	4.95 ± 0.30a
	8 w	6.01 ± 0.91b	6.05 ± 1.16b	3.15 ± 0.55c	4.98±1.20bc	10.58±1.86a
Leaf number (leaf/plant)	4 w	14.89 ± 4.8a	12.89 ± 5.1a	13.78±4.29a	17 ± 6.68a	12.89±2.47a
	8 w	20 ± 5.53a	20.86 ± 5.8a	18.86±4.14a	22.85±11.36a	21.43±4.86a
Plant height (cm/plant)	4 w	15.38±3.07a	9.44± 2.28b	8.51± 1.78b	10.72±4.36b	11.64±1.57ab
	8 w	40.88±10.05ab	46 ± 5.62ab	34 ± 7.57b	38.71±3.60b	52.71±7.41a
Leaf area (cm ² /plant)	4 w	15.66±2.73ab	15.33±1.95ab	11.12±1.04b	15.04±1.52ab	16.32±1.52a
	8 w	17.48±0.91ab	17.741±5.43b	12.80±2.62b	17.18±1.22b	24.36±1.79a

4 w, 8 w, four weeks and eight weeks, respectively, after plant growth

For each parameter the means ± standard deviation is shown (n = 5)

Different letters in each column indicate significant difference according to the Tukey test (P<0.05) among treatments - *Funneliformis mosseae* (Fm), *Septoglomus deserticola* (Sd), *Acaulospora lacunose* (Al), Symbivit (Sy) including six AMF isolates at four weeks and eight weeks after growth.

4.4.3. Proline concentration

Although inoculation with varying species of AMF induced slightly higher proline concentration in plants as compared with control plants, particularly at four weeks of plant growth, there were no significant differences among all treatments during plant growth (Fig. 25).

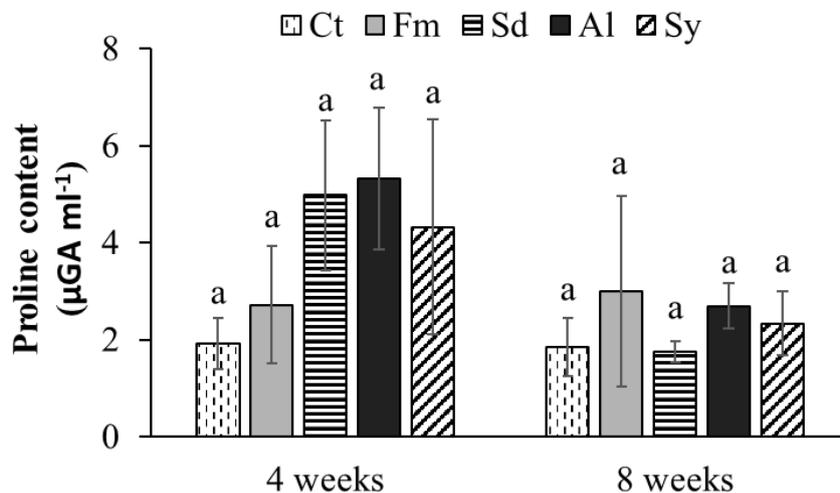


Figure 25. Proline content of *Eclipta prostrata* plants not inoculated or inoculated with *Funneliformis mosseae* (Fm), *Septoglomus deserticola* (Sd), *Acaulospora lacunose* (Al), symbivit (Sy) including six AMF isolates at four weeks (A) and eight weeks (B) after growth. Each bar shows the mean \pm standard deviation ($n = 3$). Different letters indicate significant difference among treatments according to the Tukey test ($P < 0.05$)

4.4.4. Chlorophyll fluorescence

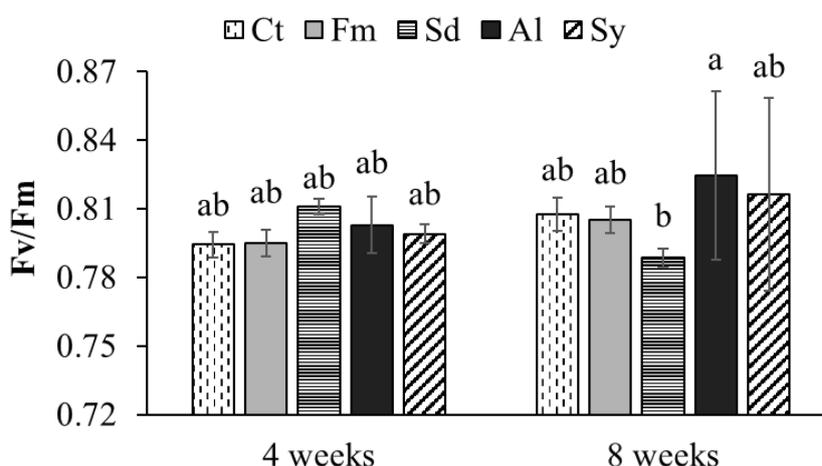


Figure 26. Maximal photochemical efficiency of photosystem II (Fv/Fm) of *Eclipta prostrata* plants not inoculated or inoculated with *Funneliformis mosseae* (Fm), *Septoglomus deserticola* (Sd), *Acaulospora lacunose* (Al), symbivit (Sy) including six AMF isolates at four weeks (A) and

eight weeks (B) after growth. Each bar shows the mean \pm standard deviation ($n = 6$). Different letters indicate significant difference among treatments according to the Tukey test ($P < 0.05$).

No significant differences in Fv/Fm quantum efficiency of photosystem II were found among all treatments at four weeks of growth (Fig. 26). When plants reached eight weeks old, Fv/Fm of plants inoculated by *Acaulospora lacunose* (Al) was remarkably higher than that of plants treated with Sd. Besides this, we did not find any significant differences in Fv/Fm, among other treatments, including control plants.

4.4.5. Total phenolic concentration

As Figure 27 shows, there were no significant differences in total phenolic content between control plants and mycorrhizal plants at four weeks of growth. However, the total phenolic level in the treatment of Symbivit (Sy) was remarkably lower than that in plants inoculated with *Funneliformis mosseae* (Fm) and *Septogloium deserticola* (Sd). At eight weeks of growth, *Septogloium 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 AM and non-AM plants were observed.

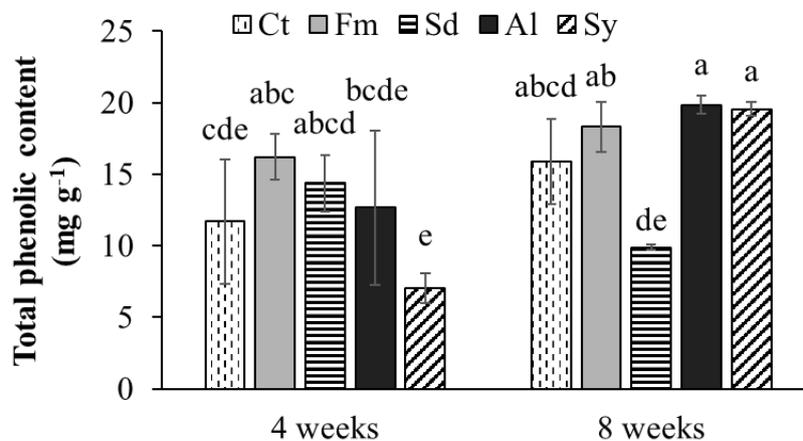


Figure 27. Total phenolic content of *Eclipta prostrata* plants not inoculated or inoculated with *Funneliformis mosseae* (Fm), *Septogloium deserticola* (Sd), *Acaulospora lacunose* (Al), symbivit (Sy) including six AMF isolates at four weeks (A) and eight weeks (B) after growth. Each bar shows the mean \pm standard deviation ($n = 4$). Different letters indicate significant difference among treatments according to the Tukey test ($P < 0.05$)

4.4.6. Discussion

In this study, the plant growth characteristics and physiological responses of *Eclipta prostrata* medicinal plants inoculated with different single AMF species and the mixture of six AMF isolates were investigated to find out differences in mycorrhizal effects resulted from AM applications on the medicinal host plants. The results showed that there were no significant differences in AM colonization among colonized plants in different AM treatments at eight weeks of plant growth, which was inconsistent with earlier reports (Fall et al., 2015, Duc and Posta, 2018). The authors supported that the different colonization rate of AMF species or isolates reflected various AM colonization strategies (Duc and Posta, 2018), or AMF strains had a particular selectivity to their host plants (Chen et al., 2014).

It is believed that AM colonization improves the growth of host plants. In the present work, we found that the influence of AMF species on growth characteristics in host plants was different (Table 12). Sd and Al remarkably improved their host root length while decreased shoot weight in relation to the corresponding control plants. Interestingly, the application of AMF mixture (Sy) considerably increased shoot weight but no other growth parameters, as compared to those of control plants. This may highlight the specificity of interaction between AMF and plants. Despite the low host specificity of AMF, each mycorrhiza species widely varies in the responsiveness to the host plant (Katalin and Duc, 2019). Indeed, AMF species differ in the capability of spreading mycelia, the viability, structure, and possibility of anastomosis (Abbott and Robson, 1985, Giovannetti et al., 2001).

Proline has a significant role in osmoregulation and functions as an effective scavenger of reactive oxygen species (Hayat et al., 2012). Enhanced salt stress tolerance with a higher proline concentration in mycorrhizal plants has been shown in our salinity study (in section 4.3.2) and other reports (Santander et al., 2019, Kong et al., 2019). Under non-stress conditions, we found no significant differences in proline content between non-mycorrhizal plants and mycorrhizal plants inoculated by different AMF species, which is in line with previous findings (Vo et al., 2019, Sinha and Raghuwanshi, 2016). Similarly, there were no substantial differences in Fv/Fm value the quantum efficiency of photosystem II between control plants and plants inoculated with different AMF species in our present results. AM colonization failed to positively influence Fv/Fm value in their host plants in this study, which concurs with the observations in our previous study (Vo et al., 2019). This may indicate that proline concentration and Fv/Fm may be enhanced in mycorrhizal plants only under environmental adversities.

Phenols are essential ingredients present in most plants due to their redox properties (Soobrattee et al., 2005). They play a crucial role in the formation of various biomolecules that protect plants against stresses (Sarma et al., 2002). These compounds are demonstrated to indirectly induce lignification in plants that serve as a barrier to pathogen attack, water loss, and enhance plant growth (Saxena et al., 2015). Our results show that there were no significant differences in total phenolic concentration between control plants and plants inoculated with diverse AMF species during plant growth. This observation is in accordance with earlier reports on mycorrhizal *E. prostrata* plants (Sinha and Raghuwanshi, 2016). Nevertheless, our findings also indicate that Sy remarkably induced lower total phenols than Fm and Sd at four weeks of growth. Also, the content of total phenols in plants treated with Sd was considerably lower than that in plants colonized by other AM treatments. Thus, the influence of each AMF species or AMF mixture on total phenolic content might be distinct in their host plants.

4.5. 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-arabioside, 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*.

6. SUMMARY

A variety of previous reports show that AMF are able to enhance plant performance and resistance/tolerance to various stresses. Our study supported that AM inoculation brings benefits to the chilli plant (*Capsicum frutescens* L.), and the medicinal plant *E. prostrata* with novel results through four different experiments in controlled environment.

Our first experiment was performed to examine the effect of inoculation of AMF strains by using forest soil (FS), agricultural soil (AS), and grassland soil (GS) in Vietnam as inoculants on plant growth of chilli (*Capsicum frutescens* L. Our results demonstrated that inoculation with different types of soils in Vietnam substantially promoted plant growth, in which FS treatment stimulated highest growth parameters of chilli plants. The traditional method used for trap-culture design with the mesh 30µm pore size was applied to limit the expansion of root *Capsicum frutescens* L downstream to rich nutrients at the bottom layer (sand: peat (2:1)). We isolated and analyzed the phylogeny of 11 AMF strains from different types of soils in Vietnam.

The second experiment in the controlled environment was carried out to explore the impact of arbuscular mycorrhizal inoculation and growth substrate on biomass and content of polyphenols in *Eclipta prostrata*. Mycorrhizal inoculation alters some secondary metabolites of *E. prostrata*, showing significant differences in polyphenol contents between the treatments. Moreover, varying peat and sand rates, representing different nutrient supplies, had substantial impacts on both mycorrhizal colonization and growth responses. Our results highlight that 60/40% (v/v) sand and peat ration is the best for large-scale cultivation of *E. prostrata*, moreover supporting the highest total phenolic content. 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. Using hierarchical cluster analysis, multiple groups are represented, suggesting the role of mycorrhizal inoculation, growth substrate, and their interaction on secondary metabolites of *E. prostrata*. 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 develop new strategies for the prevention and treatment of different diseases.

The next experiment was conducted to investigate the impacts of AMF on the medicinal plant *E. prostrata* under moderate (100 mM NaCl) and severe salt (200 mM NaCl) stress during plant growth. The results show that the AM application improved the host plant growth under non-stress

conditions. Nonetheless, this enhancement was not apparent under saline conditions. AM inoculation also increased plant tolerance to moderate salinity by enhancing proline level (at eight weeks after growth), antioxidant activities of POD and CAT at four weeks, and CAT at eight weeks after growth in colonized plants. HPLC analysis of phenolic constituents demonstrated that the AM colonization significantly altered phenolic profiles of *E. prostrata* under both levels of salt stress at the early stage of plant growth. Importantly, most phenolic compounds were elevated in inoculated plants under severe salinity, thus remarkably modified phytochemistry constituents of this medicinal plant.

Finally, we found out the effects of three single AMF species (*Funneliformis mosseae*, *Septoglomus deserticola*, *Acaulospora lacunose*) and mixed six AMF species [a mixture of *Rhizophagus irregularis* (*G. intraradices*), *Funneliformis mosseae* (*G. mosseae*), *Claroideoglobus etunicatum* (*G. etunicatum*), *Claroideoglobus claroideum* (*G. claroideum*), *Rhizoglobus microaggregatum* (*G. microaggregatum*), and *Funneliformis geosporum* (*G. geosporum*)] inoculants on plant performance of *E. prostrata* during the plant growth. The findings indicated that the beneficial influences of AMF on plant growth characteristics and total phenolic content in plants were distinct among different AMF treatments, thus showing the specific interaction between different AMF species and the medicinal plant *E. prostrata*.

6. ÖSSZEFOGLALÁS

Számos tanulmány számol be arról, hogy az AM gombák javítják a növények teljesítményét és rezisztenciáját/toleranciáját a különféle stresszekkel szemben. Kutatásunk új eredményei négy különböző, kontrollált környezetben végzett kísérletből származnak és alátámasztják ezt a megállapítást: az AM-oltás pozitívan hat a chili paprika (*Capsicum frutescens* L.) és az *E. prostrata* növényekre.

Első kísérletünk az eltérő területekről - Vietnámból származó erdészeti talaj (FS), mezőgazdasági talaj (AS) és gyeptalaj (GS) oltóanyagkénti használatával - származó AM gomba törzsekkel történő oltás növekedésre gyakorolt hatásait vizsgálta, *Capsicum frutescens* L. növényeken. Eredményeink azt mutatták, hogy a különféle vietnámi talajokkal történő oltás jelentősen elősegítette a növény növekedését, kiemelve az FS kezelést, amely a legnagyobb mértékű változást eredményezte a chili paprika növekedési paramétereiben. A hagyományos csapda kultúra módszerét használtuk, ahol a tenyészedényeket két részre osztottuk egy 30 µm-es pórus átmérőjű hálóval. Mindezzel meggátoltuk a *Capsicum frutescens* L. gyökereinek a lefelé irányuló, tápanyagban gazdag alsóbb rétegekbe (homok : tőzeg (2:1)) történő terjedését. A domináns fajok egy részét izoláltuk és elemeztük 11 különböző típusú talajból származó vietnámi AM gomba törzs filogenetikai helyét is.

A második kísérletünkben az arbuskuláris mikorrhiza oltás és a szubsztrátum *Eclipta prostrata* biomasszájára és polifenol tartalmára gyakorolt hatását vizsgáltuk. A mikorrhiza oltás megváltoztatta az *E. prostrata* néhány másodlagos metabolitjának szintjét, szignifikáns különbségeket eredményezve a polifenol-tartalomban a kezeléseik között. Ezenkívül a különböző tőzeg- és homokarányok, amelyek a különböző tápanyag-ellátottságot képviselik, jelentős hatást gyakoroltak mind a mikorrhiza kolonizációra, mind a növekedési válaszokra. Az eredményeink azt mutatták, hogy a homok és tőzeg 60/40 % (v/v) aránya a legjobb az *E. prostrata* tömeges termesztésére, ezenfelül ez az arány támogatja a legnagyobb totál fenol tartalmat. Nagy teljesítményű folyadékromatográfiás (HPLC) elemzéssel kilenc egyedi fenol-komponenst is elemeztünk a növényből, melyek közül a wedelolakton és a dimetil-wedelolakton volt jelen a legmagasabb koncentrációban. Az azonosított komponensek közül néhányat, mint az 5-o-koffeokininsavat, a kvercetin-3-arabinozidot, a 4-o-koffeokininsavat és a proto-catechuinsavat elsőként mutattunk ki *E. prostrata* növényekben. A hierarchikus klaszteranalízis során több csoport is reprezentált, jelezve a mikorrhiza oltás, a szubsztrátum és a köztük fellépő interakció szerepét az *E. prostrata* másodlagos metabolitjainak változásában. Az *E. prostrata* fenol tartalmú anyagai összetételének megismerése és az azt befolyásoló tényezők jobb megértése elősegítik a gyógynövény (és az arbuskuláris mikorrhiza gombák) új ipari alkalmazásának felismerését, és új stratégiák kidolgozását a különféle betegségek megelőzésére és kezelésére.

Harmadik kísérletünk célja az volt, hogy megvizsgáljuk az AM gomba hatását az *E. prostrata* gyógynövény növekedésére mérsékelt (100 mM NaCl) és súlyos (200 mM NaCl) só stressz mellett. Eredményeink megerősítették, hogy az AM alkalmazása javítja a gazdanövény növekedését stressz mentes körülmények között. Ez a pozitív hatás azonban só stresszes körülmények között nem jelentkezett szignifikánsan. Az AM-oltás a növényi toleranciát is növeli mérsékelt só stressz mellett, azáltal, hogy megemeli a prolin szintjét (a növények nyolc hetes korában), a POD és a CAT antioxidáns aktivitását a négy hetes, a CAT antioxidáns aktivitását pedig nyolc hetes korban a kolonizált növényekben. A fenol tartalom HPLC analízise kimutatta, hogy az AM-kolonizáció szignifikánsan megváltoztatta az *E. prostrata* fenol profilját mindkét só stressz szint mellett a növény-növekedés korai szakaszában. Fontos kiemelni, hogy a legtöbb

fenolos vegyület szintje megnövekedett az oltott növényekben erős sóstressz hatására, és ezzel egyidejűleg jelentősen megváltozott a gyógynövény fitokémiai összetétele.

Végül három AMF-fajt (*Funneliformis mosseae*, *Septoglomus deserticola*, *Acaulospora lacunose*), és egy hat AM gomba-fajta keverékét tartalmazó [*Rhizophagus irregularis* (*G. intraradices*), *Funneliformis mosseae* (*G. mosseae*), *Claroideoglomus etunicicus* (*G. etunicatum*), *Claroideoglomus claroideum* (*G. claroideum*), *Rhizoglomus microaggregatum* (*G. microaggregatum*) és *Funneliformis geosporum* (*G. geosporum*)] oltóanyag *E. prostrata* növényi teljesítményére gyakorolt hatását vizsgáltuk a növény növekedése során. Az AM gomba növényi növekedésre és a növények totál fenol tartalmára gyakorolt jótékony hatása különböző mértékű volt, az egyedi AM gomba kezelések eltérő eredményeket hoztak. Mindezen eredmények rávilágítanak a különböző AM gomba fajok és az *E. prostrata* gyógynövény közötti specifikus kölcsönhatásokra, melyek a gyakorlati alkalmazásukkor figyelembe kell venni.

7. APPENDICES

7.1. REFERENCES

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7.2. SUPPLEMENTAL FIGURES AND TABLES

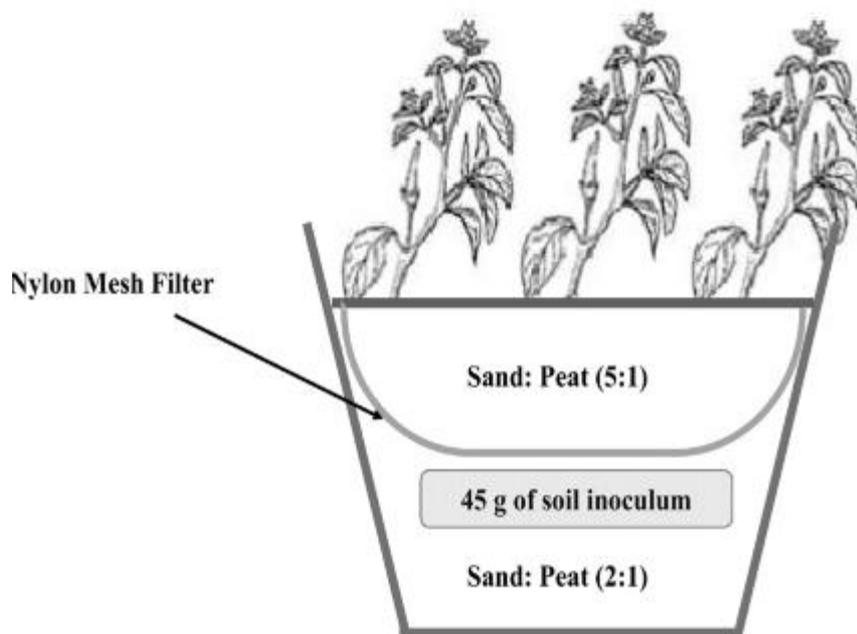


Figure 28. Schematic representation of the bi-compartment pot culture system adopted in the experiment.

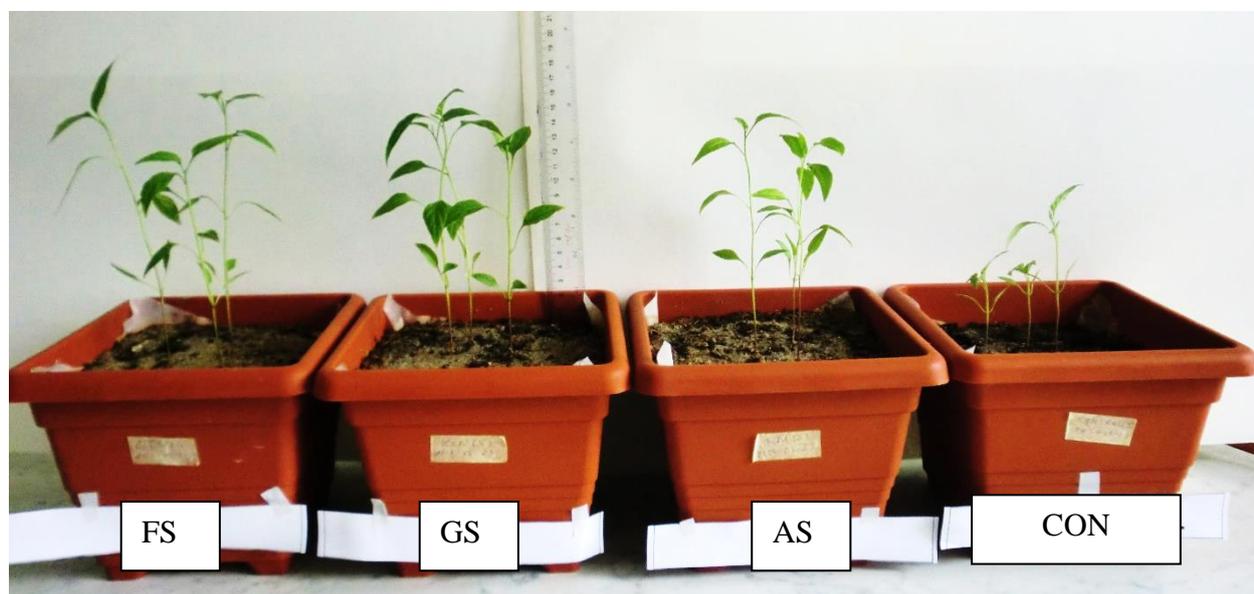


Figure 29. Comparison between inoculated and non-inoculated treatments CON (one pot per treatment) of chilli (*Capsicum frutescens*) after 14 weeks of growth. Tropical forest (FS) Agriculture (AS) and Grassland (GS) soils. (Photo by Vo, 2015).

Table 14. The main chemical characteristics of sand and peat substrate.

	pH	Phosphorus (mg/kg)	Potassium (mg/kg)	Carbonate %	Nitrogen %	Dry matter content (m/m%)
Peat	6.45±0.06	1610 ± 14	3320 ± 24	0.94±0.018	42.93±0.347	15.7 ± 0.1
Sand	7.23±0.03	62.16 ± 0.78	2485 ± 11	0.01±0.0015	0.38±0.081	79.4 ± 0.3

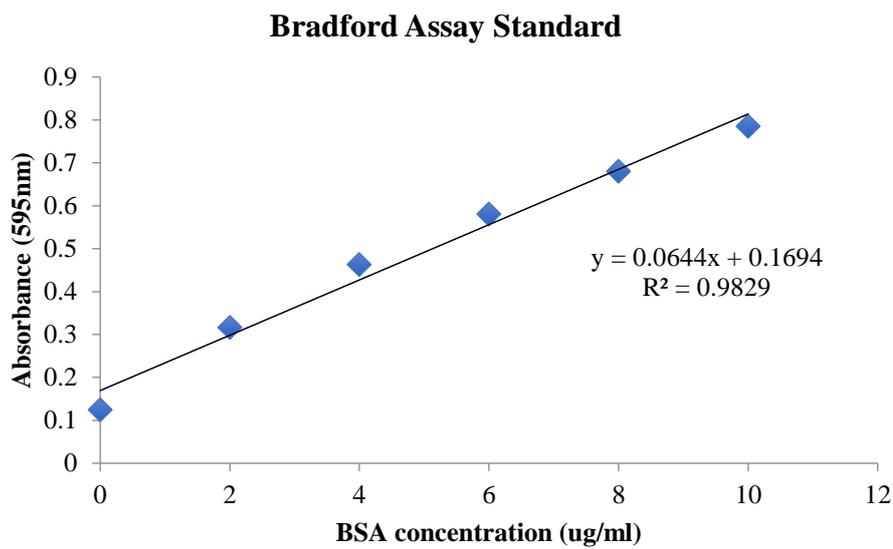


Figure 30. Bovine serum albumin standard curve for Bradford assay



Figure 31. Eight-weeks *Eclipta prostrata* plants with and without arbuscular mycorrhizal fungi (Symbivit) were grown in a growth chamber. Abbreviations: AM1 (Amf + 100/0 sand-peat % (v/v)), AM2 (Amf + 80/20% (v/v) sand-peat), AM3 (Amf + 60/40 sand-peat % (v/v)), AM4 (Amf + 40/60 sand-peat % (v/v)), AM5 (Amf + 20/80 sand-peat % (v/v)), AM6 (Amf + 0/100 sand-peat % (v/v)). NONAM1 (100/0 sand-peat % (v/v)), NONAM2 (80/20 sand-peat % (v/v)), NONAM3 (60/40 sand-peat % (v/v)), NONAM4 (40/60 sand-peat % (v/v)), NONAM5 (20/80 sand-peat % (v/v)), NONAM6 (0/100 sand-peat % (v/v)). (Photo by Vo, 2016)

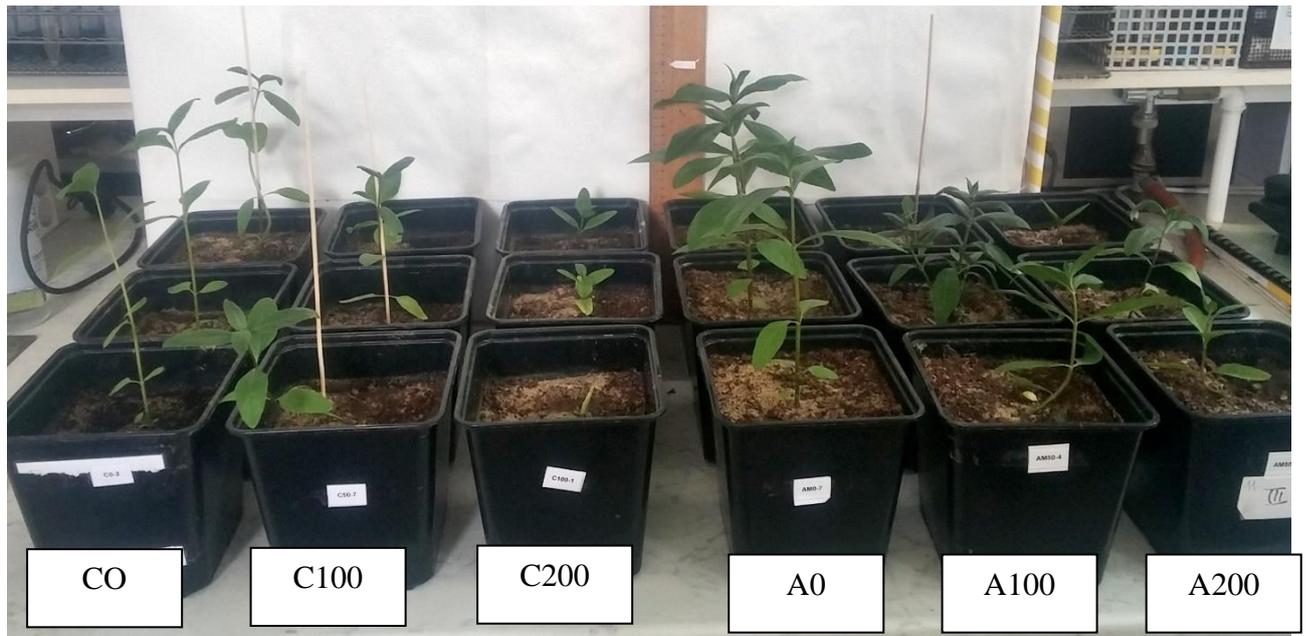


Figure 32a. Four-weeks *Eclipta prostrata* plants with arbuscular mycorrhizal fungi (Symbivit) under salt stress condition were grown in a growth chamber. A0, A100, A200 stand for 0 mM, 100 mM and 200 mM NaCl concentration with Symbivit (arbuscular mycorrhizal fungi) and C0, C100, C200 are control without Symbivit (arbuscular mycorrhizal fungi). (Photo by Vo, 2017).

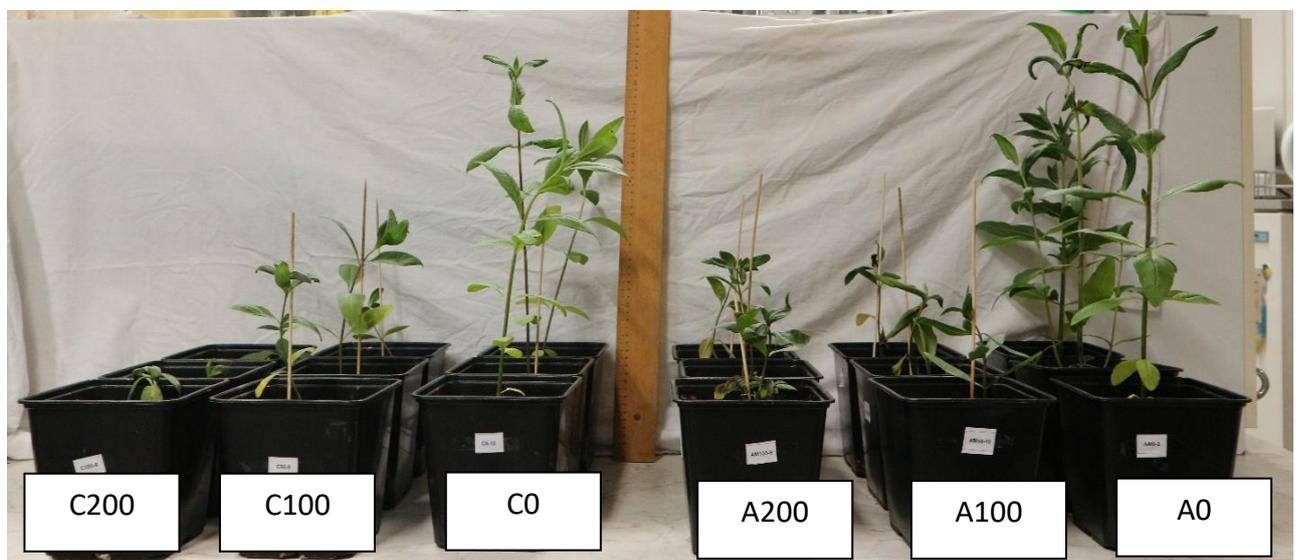


Figure 32b. Eight-weeks *Eclipta prostrata* plants with arbuscular mycorrhizal fungi (Symbivit) under salt stress condition were grown in a growth chamber. A0, A100, A200 stand for 0 mM, 100 mM and 200 mM NaCl concentration with Symbivit (arbuscular mycorrhizal fungi) and C0, C100, C200 are control without Symbivit (arbuscular mycorrhizal fungi). (Photo by Vo, 2017).

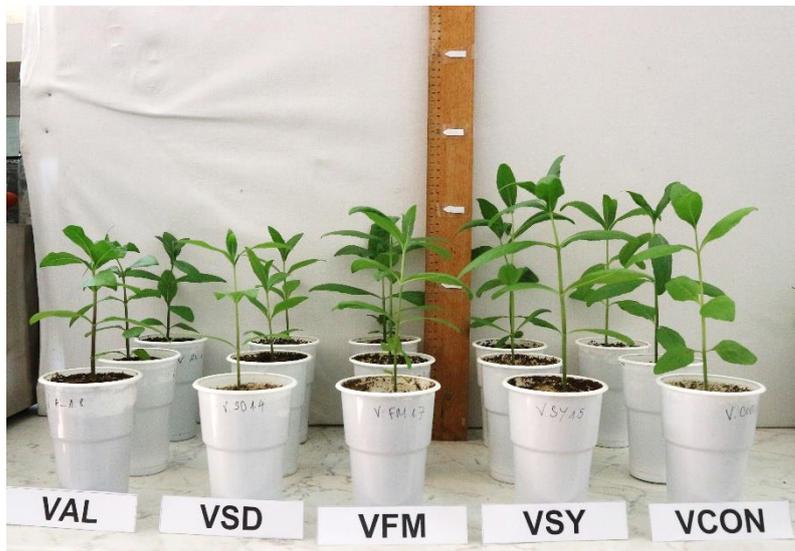


Figure 33a. Four -weeks *Eclipta prostrata* plants without (CON) or with arbuscular mycorrhizal fungi (Symbivit) under salt stress condition were grown in a growth chamber. *Funneliformis mosseae* (Fm), *Septogloium deserticola* (Sd), *Acaulospora lacunose* (A1), symbivit (Sy). V: Vietnam. (Photo by Vo, 2017).



Figure 33b. Eight -weeks *Eclipta prostrata* plants without (CON) or with arbuscular mycorrhizal fungi (Symbivit) under salt stress condition were grown in a growth chamber. *Funneliformis mosseae* (Fm), *Septogloium deserticola* (Sd), *Acaulospora lacunose* (A1), symbivit (Sy), V: Vietnam. (Photo by Vo, 2017).

8. LIST OF PUBLICATIONS

Peer -reviewed scientific articles

Au Trung VO, Imane HADDID, Hussein DAOOD, Zoltan MAYER and Katalin POSTA (2019). Impact of Arbuscular Mycorrhizal Inoculation and Growth Substrate on Biomass and Content of Polyphenols in *Eclipta prostrata*, HortScience 54(11), 1976-1983. DOI: <https://doi.org/10.21273/HORTSCI14227-19> IF 0.906 Q2.

MAYER Zoltan, JUHASZ Akos, **VO Trung Au**, and POSTA Katalin (2019). Impact of arbuscular mycorrhizal fungi on some defense enzyme activities at an early stage of maize (*Zea mays* L) under different abiotic stress. Applied ecology and environmental research. 17 (3): 6241-6253. DOI: http://dx.doi.org/10.15666/aeer/1703_62416253. IF: 0.689 Q3.

Hong Duc NGUYEN, **Au Trung VO**, Katalin POSTA.(2017). Impacts of arbuscular mycorrhizal fungi on plant growth and yield of three pepper genotypes. Journal of Agricultural and environmental sciences, Volume 4, Number 1. DOI: 10.18380/SZIE.COLUM.2017.4.1.suppl.

Au Trung VO, Franco MAGURNO, Katalin POSTA (2015). Evaluation of different Vietnamese soils as potential source of Arbuscular mycorrhizal fungal inoculum in *Capsicum frutescens*. Journal of Agricultural and environmental sciences, Vol 2, No 2. Page 48-58. DOI: 10.18380/SZIE.COLUM.2015.2.2.49.

Conferences

Trung Au VO, Franco MARGUNO, Katalin POSTA. (2016). Effects of mycorrhizal fungi on growth of Vietnam – chili pepper plant in low input nutrient condition. Nature conservation investigations in NATURA 2000 sites, in „Sustainable development in the Carpathian basin III”, Szent Istvan University, Gödöllő.

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Imane HADDIDI, **Au Trung VO**, Katalin POSTA. (2019). Changing phenolic and proline content of *Eclipta prostrata* under impact of arbuscular mycorrhizal fungi and different sand/peat media. Abstract Book –18th Alps-Adria Scientific Workshop. Cattolica, Italy 1st - 6th April. doi:10.34116/NTI.2019.AA.23.

RÁCZ Viktória, **Trung Au VO**, MAYER Zoltán, JUHÁSZ Ákos, POSTA Katalin. (2018). Effects of false daisy (*Eclipta prostrata* L.) extract on some plant pathogen microorganisms. 60th Georgikon Scientific Conference, Hungary, 130-131.

VO Trung Au, NGUYEN Hong Duc, POSTA Katalin. (2017). How do arbuscular mycorrhizal fungi affect the growth of *Eclipta prostrata* under different nutrient supplies? Poster. Asia Mycological Congress, Ho Chi Minh City, Vietnam, 10-13 Oct (2017).

VO Trung Au, NGUYEN Hong Duc, POSTA Katalin. (2017). Impact of arbuscular mycorrhizal inoculation and nutrient supply on the growth of medicinal plant, *Eclipta prostrata* L.

Presentation. 16th Alps – Adria Workshop – Synergism in science. Opatija, Croatia, 3 – 8th Apr (2017).

Other scientific articles with another topic published during PhD program

DAM Sao Mai, **VO Trung Au**, NGO Ke Suong, (2015). Consumer Willingness to Use for Roasted Coffee: A Vietnamese Case Study, *Journal of Food and Nutrition Sciences*. Special Issue: Food Processing and Food Quality. Vol. 3, No. 1. Page 152-156.

Sao Mai DAM, **Trung Au VO**. (2018). Optimizing polysaccharide extraction from the spore of Vietnamese lingzhi (*Ganoderma lucidum*) via enzymatic method. *ISHS Acta Horticulture 1213: III Asia Pacific Symposium on Postharvest Research, Education and Extension: APS2014*.

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Vo Trung Au