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ORIGINAL ARTICLE

Arbuscular Mycorrhizal Fungi Associated with Some Aromatic and Medicinal Plants

Yaghoub Raei, Weria Weisany*
Department of Plant Ecophysiology, Faculty of Agriculture, Tabriz University
Department of Plant Ecophysiology, Faculty of Agriculture, Tabriz University
Member of Young Researchers and Elite Club, Islamic Azad University, Sanandaj Branch, Iran
* Corresponding author: E-mail: Weria.Wisany@gmail.com

ABSTRACT
The importance of mycorrhiza is well documented by several researchers. Arbuscular mycorrhizal (AM) fungi have been widely used in agriculture to improve the cultivation of many crops such as medicinal plants. Medicinal plants have been used world-wide for thousands of years and are widely recognized as having high healing but minor toxic side effects. The scarcity and increasing of demand for medicinal plants and their products have promoted the development of artificial cultivation of medicinal plants. The improving the contents of secondary plant metabolites, antioxidant, photosynthesis and mineral nutrition through AM in medicinal plants is quite new, in the recent years some research work on that topic has been done. In this review, we have assembled and summarized the effects of AM symbioses on secondary metabolites antioxidant, photosynthesis and mineral nutrition of medicinal plants. We are convinced that the AM symbiosis will have benefit for the cultivation of medicinal plants and improve secondary metabolites, the rate of photosynthesis, antioxidants and nutrition uptake of medicinal plants.
***Keywords**: Arbuscular mycorrhizal, Medicinal plants, Photosynthesis, Secondary metabolites*

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INTRODUCTION

Due to beneficial effects of medicinal plants on health and wellness, these plants are used since ancient times around the world as medicine. In general, derived compounds from the secondary plant metabolism are responsible for the efficacy of medicinal plants. The term of mycorrhiza describes symbiotic associations between plants and fungi. These associations are assumed to play an important role in the land colonization by plants due to the ability of the symbiotic organisms in acquiring nutrients unavailable to non-mycorrhizal individuals [93,96].

The roots of many plant species live in symbiosis with certain soil fungi via establishing what are known as mycorrhiza. Mycorrhiza symbioses are essential for the sustainable management of agricultural ecosystems [49,12,96]. The name 'arbuscular' is derived from characteristic structures, the arbuscules (Figure 1) which occur within the cortical cells of many plant roots and also some mycothalli colonized by AM fungi. Together with storage vesicles located within or between the cells, these structures have been considered diagnostic for AM symbioses.

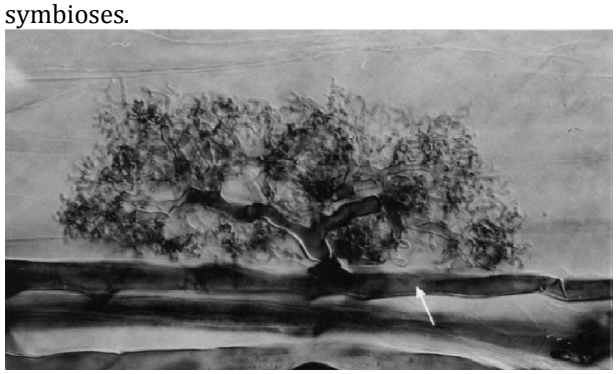

Figure 1. A mature *Arum*-type arbuscule of *Glomus mosseae* within a cortical cell of *Allium porrum* (leek). The arbuscule has grown from a well-developed intercellular hypha (arrow). From Brundrett et al. [17] with permission.

AM help plant species to uptake water and nutrients and make physiological changes to increase growth and productivity of host plants [39,15]. Although the increased allocation of biomass to leaves in AM plants is not universal, it has been accepted as a strong indication of a beneficial AM colonization. Research on the primary metabolism of the plant has also been addressed in studies investigating changes in photosynthetic rates and carbon assimilation and allocation in AM plants [115,116]. It is widely accepted that AM fungi improve phosphorous acquisition by the plant. Several studies have shown that AM also improve nitrogen acquisition [6,45,50,36], though this is more controversial. AM fungi play a key role in soil fertility and plant nutrition, enhancing the uptake and translocation of mineral nutrients—mainly P, N, S, K, Ca, Fe, Cu and Zn—from soil to host plants, by means of an extensive belowground hyphal network, which spreads from colonized roots into the soil environment [96,34].

Generally, it is claimed that mycorrhizal fungi improve plant nutrient uptake thanks to fine exploration of the rhizosphere by the hyphae, which in return receive plant carbohydrates that are essential for completion of the fungal life cycle. This retains the concept of mutualism, i.e., an interaction of net benefit to both parties [104], and poses questions about the molecular mechanisms that allow nutritional exchange. Although mycorrhizal detection and investigations of their impacts on medicinal plants have rarely been conducted, they have been observed to be associated with medicinal and aromatic plants [40]. They promote the accumulation of effective ingredients of medicinal plants, which has become a hot area of research lately. Mandala (*Datura stramonium*) and Schizonepeta (*Schizonepeta tenuifolia*) were the first medicinal plants shown to be affected by AM fungi [112,113].

The symbiotic AM fungi can also induce changes in the accumulation of secondary metabolites, including phenolics in roots and aerial parts and also essential oil of host plants [26,90,120,24,107]. The recognition of the status of mycorrhizal association and its variation in medicinal and aromatic plants is, therefore, of particular concern to improve the quantity of pharmaceutical substances. During the establishment of the AM symbiosis, a range of chemical and biological parameters is affected in plants, including the pattern of secondary plant compounds. The accumulation of flavonoids [44,72,64], cyclohexanone derivatives and apocarotenoids [30,68,110,111], phytoalexins [103,120], phenolic compounds [90,37], triterpenoids [3], and glucosinolates [109] in plants colonized by AM fungi has been reported. There are several studies on the association of AM fungi with aromatic and medicinal plants, such as basil (*Ocimum basilicum*) [81,107,114], oregano (*Origanum onites*) [57], mint (*Mentha requienii*) [18,32], dill (*Anethum graveolens*) [53], fennel (*Foeniculum vulgare*) [55], coriander (*Coriandrum sativum*) [54,29], lavender (*Lavandula angustifolia*) [108], pelargonium (*Pelargonium peltatum*) [82] and sage (*Salvia officinalis*) [76].

In general, the mycorrhizal status of a great variety of medicinal plants has not so far been thoroughly investigated. However, there are some studies reporting on the colonization of medicinal plants by AM or ectomycorrhizal fungi. Lakshmipathy et al. [61] assessed the AM colonization intensity of five threatened medicinal plant species growing in southern India, and found varying colonization intensities depending on the plant species and the investigated site, and related to soil pH, P content and phosphatase enzyme activity. Cabello et al. [18] found that inoculation with *Glomus mosseae* increased root AM fungi colonization and biomass production in *Mentha piperita* plants. According to Gupta et al. [41] plants of *Mentha arvensis* inoculated with a *Glomus fasciculatum* isolate increased plant height, fresh and dry biomass, and the production of essential oils in comparison with non-mycorrhizal plants.

Here, we summarize the current researches progress on the relationship between AM and essential oil components, antioxidant, photosynthesis, plant growth and mineral nutrition of aromatic and medicinal plants. We consider that this research area is very important, and that the application of issuing knowledge can have great potential in improving the quality of medicinal materials.

Secondary metabolites

Many different secondary metabolites with different functions are produced during the growth and development of plants. It is known that secondary metabolite contents in root and shoot tissues of AM plants may increase over nonmycorrhizal plants [102]. Both, *Glomus macrocarpum* and *G. fasciculatum* significantly enhanced the concentration of the terpenoid artemisinin in *Artemisia annua* leaves [52]. Symbiosis between plants and AM fungi can promote the accumulation of several secondary metabolites in medicinal plants which play important roles in treating human diseases [53,54,55]. Mycorrhizal plants of *Castanospermum australe* contained higher amounts of the alkaloid castanospermine in their leaves in comparison with nonmycorrhizal controls with a differential effect of AM fungi [2]. In the available reports about these AM treated plants mainly the quantity of the essential oil was beneficially affected, sometimes also slight changes in the composition of the essential oil compounds were found [24,55,41]. In *Mentha arvensis* L. Gupta et al. [41] reported that mycorrhizal inoculation significantly increased oil content and yield compared to non-mycorrhizal plants. Freitas et al. [32] also observed that inoculation with AM fungi led to an increase of 89% in the essential oil and menthol contents of *M. arvensis* plants. In

Mentha piperita, Mucciarelli et al. [73] observed that colonization by a non-mycorrhizal fungus increased essential oil content and altered the oil composition.

The degree of influence of different AM fungi on the same medicinal plant or of the same AM fungus on different medicinal plants can vary. Jurkiewicz et al. [51] reported that inoculate composed of different AM fungi differed not only in their effectiveness in establishing symbiosis and promoting growth of *Arnica montana*, but also in the degree to which they increased plant contents in phenolic acids and sesquiterpene lactones. AM symbiosis significantly increased the contents of essential oils in dill, carum, and coriander, and *G. fasciculatum* appeared to be more effective than *G. macrocarpum* [53,54]. Rasouli-Sadaghianil et al. [85] also reported that *G. fasciculatum* may have a higher symbiotic potential in increasing essential oil contents in basil. In studies on *Ocimum basilicum* [24] and *Mentha arvens* [32], it was shown that AM fungal root colonization increases the essential oil contents and in *O. basilicum* alterations of the essential oil composition has been reported [24]. The first investigations focused generally on the accumulation of secondary compounds in the aerial parts of AM treated culinary and aromatic herbs such as basil or fennel [24,107,54,41]. In the available reports about these AM treated plants mainly the quantity of the essential oil was beneficially affected, sometimes also slight changes in the composition of the essential oil compounds were found [24,55,61]. As reviewed by Toussaint et al. [107], the reasons for the AM effects on the secondary metabolites are still unclear, even though speculations attribute this effect to the improved phosphorus status or the changed hormonal balance of the plants.

Until recently, very little attention has been paid on the accumulation of secondary compounds in the aerial parts of mycorrhizal plants. Khaosaad et al. [57] observed changes of essential oil concentration (but not composition) following mycorrhizal inoculation of oregano genotypes (Figure 2). Copetta et al. [24] found the increased abundance of glandular hairs, and essential oil yield in mycorrhizal inoculated *O. basilicum*. In studies on *Coriandrum, Anethum and Foeniculum vulgare*, it was shown that AM fungi root colonization enhances the essential oil quality by altering essential oil components [53,54,55]. Khaosaad et al. [57] also found that essential oil concentration significantly was increased in two oregano (*Origanum* sp.) genotypes associated with *G. mosseae*, but the levels of essential oil in plants treated with P are not changed, which indicates that the increase in essential oil concentration in mycorrhizal oregano plants is not due to an improved P nutrition, but directly depends on association with the AM fungus.

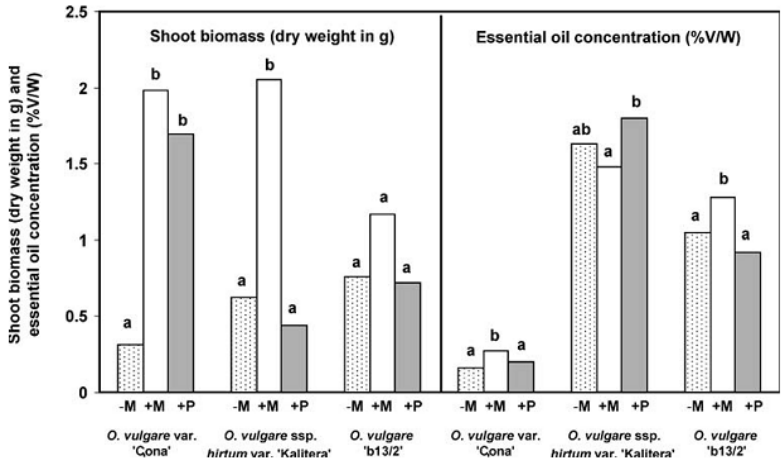

Figure 2. Shoot biomass and content of essential oil in leaves of mycorrhizal (+M) and nonmycorrhizal (−M) oregano genotypes and of nonmycorrhizal oregano genotypes supplied with a P solution (+P). Within each genotype bars with the same letter are not significantly different [57].

In general, the product ion of phenolic compounds and terpenoids, the components of essential oils, is considered as a defensive response to fungal colonization. Given the fungicide properties of several constituents of essential oils, and the increased production of these metabolites in mycorrhizal plants, it has been suggested that they could be synthesized as a defensive response to AM fungi presence [24].

Antioxidant enzyme

AM symbiosis influences the primary metabolism of host plants, it induces important changes in enzymatic activities of superoxide dismutase and catalase [91,70]. Ectomycorrhizal symbiosis between the mycelia and the roots of some plants could have the important effects in the levels of antioxidants of both partners: fungal mycelium and plant roots. In the early steps of mycorrhizal associations an oxidative burst might occur through the rapid production of high amounts of ROS (reactive oxygen

species) in response to external stimulation [62,10]. Therefore, the production and/or activity of antioxidants, including enzymes (e.g. superoxi dedismutase, catalase and peroxidase) [10,74] or phenolic compounds, might be increased in plant roots and/or mycelia [75,86]. A "GmarCuZnSOD" [63] and likely other anti-oxidative enzymes could be activated, in order to overcome the ROS-inactivating system related to the host defense, providing the first contact between the host roots and AM fungus, that differentiates into the appressorium and colonize the root cortex. Previous our studies showed that inoculation of chickpea by AM significantly increased antioxidant enzyme activity. The most POD and PPO activities were recorded for inoculated plants with *G. etunicatum* and *G. versiform* species (Figure 3), and the most APX activity was observed in plants inoculated with *G. intraradices* [100,101]. In a further study on basil, for example, an increased content of rosmarinic acid, a highly antioxidant phenolic compound, was detected in AM colonized plants [24]. Higher levels of SOD activities are observed the colonized roots of lettuce with *G. mosseae* or *G. deserticola*, compared to non-mycorrhizal controls, when plants are subjected to drought stress conditions [91].

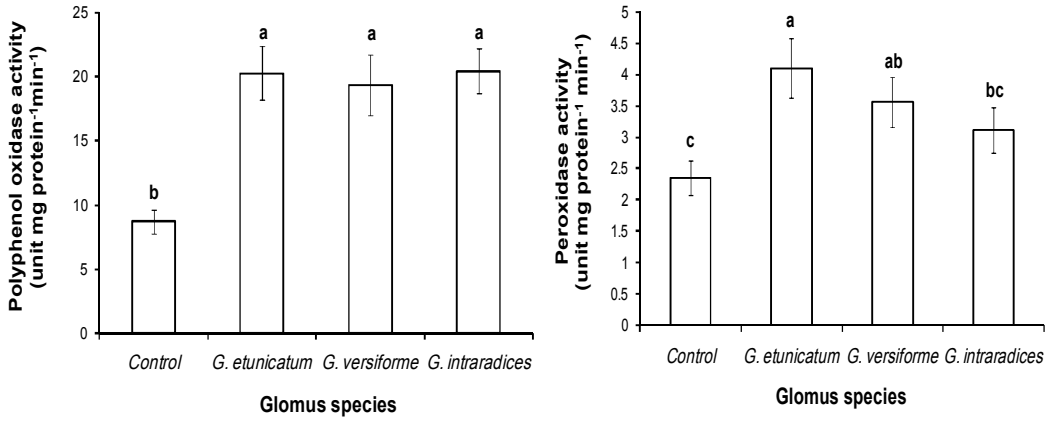

Figure 3. Polyphenol oxidase (PPO) and peroxides (POD) enzymes activities in non-inoculated (control) and inoculated chickpea by *Glomus* species (*G. versiforme*, *G. intraradices* and *G. etunicatum*). The results are means (n=3) ± SE. Means with the same letter for each stage are not significantly different (Duncan's Multiple Range test; $P \leq 0.05$).

Photosynthesis

Most of the studies suggested that AM fungi symbiosis helps in increasing the rate of photosynthesis, storage of photosynthates and export at the same time [9]. AM fungi associations have been shown to improve photosynthetic efficiency by improving P nutrition in plants [71], owing to an effect of P status on CO_2 assimilatory reactions. The mechanisms by which AM fungi trigger changes in the concentration of phytochemicals in plant tissues are not yet well understood [107].

Increased photosynthesis may be mediated directly via increased availability of inorganic phosphate (Pi) in the leaves [94] or by increased specific leaf area [31]; it may sometimes be associated with increased hydration of the leaves [98]. As plant species differ in the sensitivity of their photosynthetic mechanisms to P deficiency [27], this would be a possible basis for differences in response to mycorrhizal infection. Many experiments on mycorrhizal effects on plant growth have shown that the rate of photosynthesis is higher in mycorrhizal than non-mycorrhizal plants [4, 60,65,99]. Zubek et al. [123] reported that AM fungi species specificity in the stimulation of *Hypericum perforatum* L. photosynthetic activity and the production of secondary metabolites. Inoculation with the AM fungi resulted in higher photosynthetic performance values in comparison to all the other treatments. This could be the result of improved plant phosphorus and/or nitrogen nutrition due to symbiosis. A similar tendency was found in the studies conducted by Zubek et al. [124] concerning the response of *Inula ensifoli*a L. to AM fungi. In other plant species such as *Citrus aurantium*, AM fungi enhanced photosynthetic activity was correlated with increased tissue P and chlorophyll content, and ribulose-1,5-bisphosphate carboxylase/oxygenase activity, RuBPCO [77]. The C fixed by the plant during vegetative growth is allocated above-ground to photosynthetic tissue and below-ground to nutrient-absorbing roots and mycori'hizas. In AM fungi the destinations in roots and extraradical hyphae of photosynthetically incorporated ^{14}C have been traced. Ho and Trappe [47] performed the earliest experiments which demonstrated that, over a period of weeks, small amounts of labelled photosynthate appeared in extra radical hyphae and spores of AM fungi.

Plant Growth and Mineral Nutrition

The effects of mycorrhizal infection on the growth and nutrition of plants have recently been reviewed [1,33,42,46,95]. The Conducted studies with artemisia (*Artemisia annua*), an important other medicinal

herb, showed contradictory effects on biomass and pharmacologically active compounds upon AM fungi inoculation in two independent studies. In one study was observed no significant effect on biomass and the total essential oil content but on some single compound levels [84]. While in the second study both the shoot biomass and the essential oil content were increased. Mycorrhization significantly increased the shoot biomass in *O. vulgare* var. Cona and *O. vulgare* ssp. *hirtum* var [57] (Figure 2). It is well known that AM fungi can increase the uptake of micronutrients and other mineral nutrients with low mobility including the aforementioned Fe [20], Zn [59] and Cu [66]. In greenhouse grown lettuces with optimal irrigation, mycorrhizal symbiosis improved the levels of Cu and Fe [13], but the effect was dependent on lettuce cultivar and source of the applied P for plant growth [14]. The fungal hyphal network is ideally positioned to efficiently take up nutrients and water from the soil, but only a few fungal transporters that are involved in this process, including those that transport phosphate [43,69], ammonium [67] and zinc [35], have been cloned.

One underlying mechanism to increase the rate of uptake of P is the high efficiency of which mycorrhizal roots exploit the soil profile, with hyphae extending beyond the depletion zone surrounding the absorbing root and its root hairs [21,79,80,106]. This fits with what is known of the factors that determine rates of nutrient supply to and uptake by roots. For non-mobile nutrients such as P (and to a lesser extent K^+ and NH^+_4), root growth, root radius, development of root hairs, and initial concentration in the soil solution are more important determinants of the rate of uptake than are the kinetic properties of the uptake systems of the root [21,79,19]. Mycorrhizal modification of the nutrient uptake properties of roots depends upon (a) development of extramatrical hyphae in soil, (b) hyphal absorption of phosphate, (c) translocation of P through hyphae over considerable distances, and (d) transfer of P from the fungus to the root cells. There are clear evidences that confirm all these processes take place. Mycorrhizal fungi have considerable ability to translocate nutrients, although there is little comparative data for different host-fungus combinations. Elements include P, Zn, S, Ca, and N [6,22,23]; and the distances over which translocation can take place exceed the radius of any depletion zone likely to develop around an actively absorbing root [87,88].

An-Dong et al [7] reported that AM inoculation greatly increased P concentration in the leaves and flowers of *Lonicera confusa*. The uptake of N, P, and K were significantly also enhanced by AM inoculation in *Lonicera confusa* seedlings (Figure 4). The increasing in plant biomass by AM inoculation has been reported for other perennial medicinal plants like palmarosa (*Cymbopogon martinii*) and kalmegh (*Andrographis paniculata*) [39,8]. AM fungi can accelerate decomposition and directly acquire nitrogen from organic material [48]. A fungal amino-acid transporter [92] and an ammonium transporter that might be involved in nitrogen uptake by extraradical hyphae have been cloned [67]. Long-distance transport to the plant probably mainly proceeds through arginine [36,25]. A fungal pathway of uptake, translocation, and transfer of nitrogen to roots may also contribute with higher inflows of N into mycorrhizal roots [5]. A study has confirmed both to increase P-mediated in N_2 fixation and to enhance N uptake from soil by mycorrhizal *Hedysarum coronarium* [11].

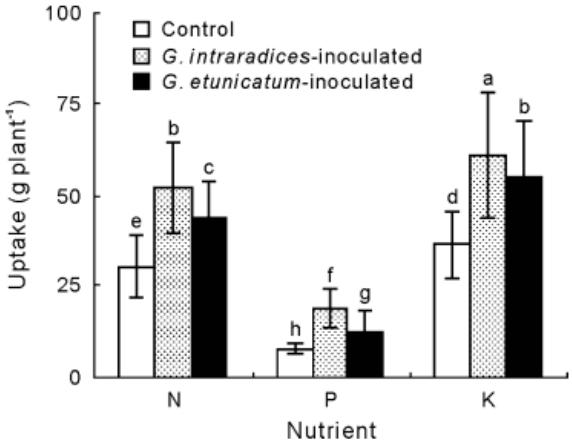

Figure 4. Nutrient uptake by the *Lonicera confusa* plants uninoculated (control) or inoculated with *Glomus etunicatum* and *Glomus intraradices* 5 years after transplanting. Values are means with standard deviations shown by vertical bars. Same letter above the bars indicates no significant differences at P< 0.05 [48].

Improved P nutrition, as well as directive fungal effects, may be implicated in the enhancement of uptake macronutrients such as K and S [83,89], and micronutrients such as Cu and Zn by plants [22,58,105].

Many studies have indicated that AM contributes to plant growth via assimilation of immobile soil nutrients (P and Zn) [56]. Probably this resulted from a greater absorption surface area providing by extensive fungal hyphae. An increased exploitation of the soil volume is especially important for the uptake of less mobile nutrients like P, Zn and Cu [97]. Zolfaghari et al [122] have suggested that AM fungi inoculation significantly increased plant height, fresh and dry matter yield, oil content and oil yield compared to non-inoculated basil plants. Essential oil content in the inoculated plants with *G. mosseae* and *G. fasciculatum* was significantly higher than other treatments.

CONCLUSION

The symbiosis of plant roots with AM fungi is known to be one of the most ancient and widespread plant strategies to enhance nutrient acquisition which copes with the environmental stress [16]. There have been several previous evaluations and summaries about the advantages, prospects, and feasibility of introducing AM fungi into the process of cultivation of medicinal plants [38,121,119,118]. Until recently very little attention has been paid on the accumulation of secondary compounds, antioxidant, photosynthesis and mineral nutrition in the aerial parts of mycorrhizal plants. In this paper, we have tried to provide a comprehensive review of information available about research on AM and secondary metabolites of medicinal plants, in order to set a basis for future work. Copetta et al. [24] have suggested that significantly increased levels of essential oil in *O. basilicum* colonized by three different AM fungi result from significantly larger numbers of peltate glandular trichomes on the basal and central leaf zones [24]. In several studies, AM fungi and dark septate endophytes (DSE) have been found to enhance plant growth, photosynthetic activity, phosphorus content, act antagonistically towards soil borne fungal pathogens, and modify the concentration of plant metabolites [77,117]. mycorrhizal plants can have higher photosynthetic rates compared to non-mycorrhizal plants. Consequently, the uptake of N, P, and K was also enhanced significantly by AM inoculation [28]. In conclusion, in order to further knowledge about the organic production of herbal materials, much effort is needed in research on the role of AM fungi and the AM symbiosis and the use of mycorrhizal technology in the cultivation of medicinal plants.

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