

MUTUALISTIC AND ENDOPHYTIC MICROORGANISMS OF *ARTEMISIA ANNUA*: DESCRIPTION, ROLE AND USE

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Received: 2 November 2018; **Accepted:** 18 November 2018; **Published:** 30 December 2018

Abstract: *Artemisia annua* is an important medical plant that produces artemisinin used for its antimalarial, antibacterial and antifungal effects in modern medicine. The high demand and low artemisinin content in plants (0.01-2 %) has led to studies about alternative methods to increase yield. Biofertilizers (beneficial microbes and/or biological products that colonize roots, improve plant nutrition and growth) have been reported affecting secondary metabolism and the production of active ingredients of herbs. The purpose of this paper is to draw attention to the current status of the research on mutualistic and endophytic microorganism of *A. annua* that have the potential to increase the quality and quantity of the crude drugs, derived from the herb. Scientific papers in this field focus on the effects on inoculation with different microorganisms (arbuscular mycorrhizal fungi, endophytic bacteria and fungi) and the isolation of endophytes from *A. annua*. Bioinoculants can affect biomass, artemisinin and essential oil concentration, disease resistance, nutrient status, phosphatase activity, foliar glandular trichome density, leaf chlorophyll content, guaiacol peroxidase enzyme concentration, stomatal conductance and transpiration rate, and plant growth parameters (total weight, leaf yield, height, seed yield). The endophytes isolated from the plant are potential artemisinin content and plant stress resistance enhancers.

Keywords: *Artemisia annua*, artemisinin, mutualism, arbuscular mycorrhizal fungi, endophytic bacteria, endophytic fungi, plant stress resistance.

1. Introduction

The *Artemisia annua*, also known as sweet wormwood (Chinese: qing hao), is a plant belonging to the Asteraceae family native to temperate Asia, especially in China where it has been used for several centuries as therapy for cerebral fever (Chaudhary et al., 2008; Fairhurst and Wellems, 2015). It was naturalized in many countries including Argentina, Australia, Bulgaria, France, Hungary, Italy, Romania, Spain and the USA (Das, 2012; Bilia et al., 2014).

A. annua has become the focus of hundreds of papers since the early 1970s, after the discovery of its active component artemisinin (Wang et al., 2011; Calderón et al., 2013; Naeem et al., 2014). This complex structure, from the family of sesquiterpene trioxane lactone (Martínez et al., 2014) provides a wide spectrum of action against many diseases. Although artemisinin is no longer used as a drug itself, its derivatives are highly effective anti-malarials, which are

produced by chemical alteration of artemisinin (Hommel, 2008). According to WHO (2017), an estimated 216 million cases of malaria occurred worldwide with most cases being in the African region (90%). Parasite, predominantly *Plasmodium falciparum* resistance to artemisinin has been reported in five countries of the Greater Mekong subregion, therefore exploring all possible modes of action of artemisinin in order to develop new generation antimalarial drugs has become of great importance (Fairhurst and Dondorp, 2016).

Artemisinins (artemisinin and its chemical derivatives) have cytotoxic and inhibitory effect on various cancers, inflammatory diseases, viral (e.g. *Human cytomegalovirus*), protozoal (e.g. *Toxoplasma gondii*), helminthic (*Schistosom* sp., *Fasciola hepatica*) and fungal (e.g. *Cryptococcus neoformans*) infections (Ho et al., 2013). Hence, the interest in the isolation of artemisinin and the production of *A. annua* has increased worldwide (Sadiq et al., 2014). Unfortunately, its demand to production ratio is low. Artemisinin extraction from *A. annua* is highly influenced by the low artemisinin percentage in plants, which usually ranges from 0.01 to 2% dry weight (Liu et al., 2006; Keshavarzi et al., 2012), and the herb's dependence of temperature, humidity and soil types (White, 2008). Chemical synthesis is uneconomical, non-cost-effective and low yielding (Pandey and Pandey-Rai, 2015), however microbial genetic engineering is a potential alternative (Hommel, 2008). Progress has been made towards breaking the cost/yield barrier while it is yet unproven as commercially viable syntheses of artemisinin (Kung et al., 2018). Currently biosynthetic processes have proven to be the most efficient synthetic methods to produce artemisinin (Tang et al., 2018). Therefore, it is likely that *A. annua* will continue to be the main source of artemisinin (Hommel, 2008).

Various efforts have been carried out to increase the antimalarial compounds. These attempts can be divided into two categories that focus on improving the efficiency of artemisinin extraction (Briars and Paniwnyk, 2014) and the increment of antimalarial compounds in *A. annua* (Namuli et al., 2018). In order to increase the artemisinin concentration fertilizers (chemical, biological, organic and vermicompost), plant growth regulators (hormones), variation of growth conditions (light, water, macro and micronutrients) and the use of high-yielding clones or strains have been tried (Namuli et al., 2018). One of the issues encountered in medicinal plants cultivation is the unstable quality of the product. Biofertilizers, however, have been reported affecting secondary metabolism and the production of active ingredients of herbs (Zeng et al., 2013).

Microbial inoculants or biofertilizers are beneficial microbes and/or biological products that colonize roots, improve plant nutrition, growth, development and resistance to abiotic stresses (Monfil and Casas-Flores, 2014), thus they play an important role in sustaining productivity (Malhi et al., 2013). Bioinoculants can fix atmospheric nitrogen or enhance the solubility of soil nutrients which leads to their potential to increase the yield of crops (Namuli et al., 2018). However, their efficiency varies with nutrient type, source, soil type, climatic conditions, and species compatibility in the respective environment (Malhi et al., 2013; Berruti et al., 2016).

The aim of this article is to discuss the current status of the research on relationship between mutualistic or endophytic microorganisms and *A. annua* by reviewing a total of 37 papers in order to draw attention to, and arouse more interest in this research field. The publications were collected from two main research directions: *A. annua* inoculation with different microorganisms (arbuscular

micorrhizal fungi, endophytic bacteria and fungi) and their effect on plant growth and development; isolated endophytic microorganism from *A. annua* and their relationship with their host plant. Plants grown in poor-quality soil, under stressful conditions (arid conditions, low nutrient soil) or in intensely farmed land where topsoil is tripped-away can benefit from these organisms. They can positively influence mineral uptake (N, P, K, Ca, Mg, Zn, Cu, S), chlorophyll content in leaves, essential oil concentration, optimal growth of herbs, increase salt toxicity and water deficiency resistance in hosts and reduce susceptibility to root rot pathogens. The Global North becomes more suitable for plant growth due to global warming, while there is a decrease in agriculturally suitable land in the Global South and the Mediterranean area. Limits of land extensions and the increasing demand for agricultural products and medicinal plants raised attention to the usage of mutualistic and endophytic microorganisms (Zabel et al., 2017). Therefore, they could help meet the demand for artemisinin by increasing the artemisinin content, survival rate of herbs and enabling the production of plants in less favorable conditions, where malaria is present.

2. The effect of arbuscular mycorrhizal fungi (AMF) on *Artemisia annua* plants

Mycorrhiza is a mutualistic relationship between fungi and the roots of a plant, which are infected by the fungus. The rest of the fungal mycelium continues to grow in the soil and shares the absorbed nutrients and water with the plant host, while the fungus is provided with photosynthetic sugars by the host (Moore et al., 2011). Mycorrhizal networks have a significant effect on plant communities by leading to plant-to-plant transfers of nutrients or signals, thus increasing stress resistance (Heijden and Horton, 2009).

Mycorrhizas are present in more than 80% of angiosperms and gymnosperms (Bonfante and Anca, 2009; Barman et al., 2016). They have been classified into two types depending on the location of the fungal hyphae: ectotrophic (outside the root) and endotrophic (inside the root) (Bonfante, 2001). Endomycorrhizas can be divided in three groups: arbuscular (AM), ericoid and orchidaceous endomycorrhizas (Moore et al., 2011).

AMF, members of Glomeromycota, are not host specific, but depend on their host plant to complete the fungal life cycle (Balestrini et al., 2015; Berruti et al., 2016). AM colonies are usually formed by intracellular and intercellular hyphae, and intracellular arbuscules, while in soil AMF are distributed in large extraradical mycelium (Giovannetti and Sbrana, 1998; Moore et al., 2011). A wide range of soil microorganisms (nonbacterial microorganisms, mycorrhiza helper bacteria and plant growth promoting rhizobacteria) maintain a relationship with AMF (Miransari, 2011).

The effects of AMF on secondary metabolites of different host plants have been researched since the mid-twentieth century (Santander et al., 2017). AMF enhance the absorption of different soil nutrients including phosphorus, zinc, silicon, copper, magnesium, potassium, calcium, iron and nitrogen, thus alleviate salt stress (Evelin et al., 2009) and increase K^+/Na^+ ratio, growth and productivity of plants (Jeffries et al., 2003; Zeng et al., 2013; Garg and Bhandari, 2016). AM symbiosis can promote accumulation of terpenes, alkaloids, phenolic compounds and other substances in medicinal plants (Zeng et al., 2013). AMF hyphal length can reduce soil loss by preventing soil erosion (Mardhiah et al., 2016). Water transport in host plants can be influenced by AMF with hormonal changes, effective water absorption, direct water uptake through mycelium and osmotic regulation

(Santander et al., 2017). The aforementioned effects vary depending on the host plant species, soil type, nutrient status of the soil and the AMF species (Kim et al., 2017).

A. annua has been treated previously by AMF species from the genera *Acaulospora*, *Glomus* and *Rhizophagus*. Changes have been reported regarding biomass, plant growth, total leaf yield, artemisinin and essential oil content, nutrient status, phosphatase activity, foliar glandular trichome density, leaf chlorophyll content, GPOX enzyme concentration, stomatal conductance and transpiration rate (**Table 1**).

3. The effect of endophytic fungi on *Artemisia annua* plants

Endophytes are microorganisms including bacteria, archaea, fungi, and protists that inhabit the plant endosphere for at least parts of their life cycle where they can form different relationships (mutualistic, symbiotic, commensalistic, trophobiotic) (Ryan et al., 2008; Hardoim et al., 2015; Kandel et al., 2017; Singh et al., 2017). They can be classified into two groups: prokaryotic and eukaryotic endophytes (Hardoim et al., 2015). Another classification distinguishes endophytes that are unable to develop external structures and those that do generate external structures (nodules of nitrogen fixing bacteria) (Lacava and Azevedo, 2014).

Endophytic fungi-host interactions can be mutualistic, antagonistic and neutral. Their distribution is influenced by ecological and environmental conditions (temperature, humidity and levels of soil nutrition) (Jia et al., 2016). Endophyte-host association can be used to enhance the production of useful metabolites (Suryanarayanan et al., 2009). Three types of endophytic fungus-host relationship have been described: some endophytic fungi produce hormones to affect plant growth, some promote the accumulation of secondary metabolites such as medical components or drugs (Jia et al.,

2016), while others produce antimicrobial and antifungal substances (Torres and White, 2012). Chemicals produced by endophytic fungi can improve the resistance and yield of crops (Cavaglieri et al., 2004) and also can be toxic for insects (Hartley and Gange, 2009).

Associations between *A. annua* and endophytic fungi have been evaluated after treatment with species from the genera *Colletotrichum*, *Penicillium* and *Piriformospora*. Artemisinin concentration, growth of biomass, chlorophyll content, seed yield, secondary metabolites, adaptation to biotic and abiotic stresses have been influenced by the treatment (**Table 2**).

4. The effects of endophytic bacteria on *Artemisia annua* plants

The large variety of endophytic bacteria and the adaptation to different environments (tropic, temperate, aquatic, Antarctic, rainforests, xerophytic, deserts, mangrove swamps and also coastal forests, geothermal soils etc.) lead to a wide pharmaceutical and agricultural application (Singh et al., 2017).

Bacterial endophytes have shown to control plant pathogens, insects and nematodes (Mercado-Blanco and Lugtenberg, 2014) in order to reduce biotic and abiotic stresses for their host (Liarzi et al., 2016), they promote plant growth and increase plant yield by inhibiting growth promoting hormones, fixation of nitrogen, water uptake and phosphate solubilization (Taghavi et al., 2009; Griffin, 2014; Singh et al., 2017; Etminani and Harighi, 2018). Some endophytes are resistant to heavy metals or antimicrobials, and can degrade organic compounds (Sheng et al., 2008; Bian et al., 2011; Rajkumar et al., 2012), which makes them a possible option for phytoremediation (Ryan et al., 2008). Endophytes were successful in decreasing drought, heat and salt stress in host plants (Kandel et al., 2017).

Table 1. The effect of AM fungi on *Artemisia annua* plants

AMF species	The effect of the treatment	References
<i>Acaulospora tuberculata</i>	-addition of mycorrhiza did not affect leaf yield, total biomass yield -inoculation enhanced the number of branches and the leaf artemisinin content (0.29%) -manure and mycorrhiza improved plant height, total dry weight, the number of branches	Rahman et al., 2014
<i>Glomus aggregatum</i>	-the treatment increased leaf yield and P content, total weight and height of the plants, and phosphatase activity in soil -no significant differences were found in leaf artemisinin content between treated (0.65%) and control plants (0.61%)	Awasthi et al., 2011
<i>Glomus macrocarpum</i>	-inoculation significantly increased the dry weight of shoot, production of herbage, nutrient status (P, Zn, Fe) of shoot, concentration of essential oil and artemisinin in leaves compared to control plants -inoculation resulted in highest artemisinin concentration -Mn concentration significantly decreased -Cu concentration was not influenced by the treatment	Chaudhary et al., 2007
	-the treatment significantly increased concentration of artemisinin -mycorrhizal plants had higher foliar glandular trichome density, accumulated more phosphorus in their shoots compared to control plants -Cu concentration was not influenced	Kapoor et al., 2007
	-inoculated plants had higher artemisinin content and biological yield -the addition of 40-80 mg/kg phosphorus fertilizer to the mycorrhizal colonization has increased mycorrhizal colonization and enhanced plant growth rate	Tan et al., 2013
	-the treatment enhanced leaf yield and total dry weight of the plants -significant increase in phosphatase activity and phosphorus concentration was observed -co-inoculation with <i>Bacillus subtilis</i> was effective in improving the height by 57.78%, total plant weight by 62.64%, leaf yield of plants by 66.6%, phosphatase activity and the artemisinin content (0.77 %) compared to control (0.61 %) or other tested bio-inoculants	Awasthi et al., 2011
<i>Glomus mosseae</i>	-mycorrhizal treatment improved the nitrogen, phosphorus and potassium uptake -mycorrhizal colonization led to changes in volatile components and increased the volatile oil content by 45.0% -stem, branch and leaf biomass was enhanced by 32.8%, 15.2% and 19.6% -inoculation increased the leaf chlorophyll content, net photosynthetic rate, stomatal conductance, transpiration rate, stem diameter and aboveground biomass	Huang et al., 2011
	-AM inoculation increased shoot elongation, total shoot length, the foliar mineral concentration of Mg -the emission of limonene and artemisia ketone was stimulated by the treatment -the total terpene content and emission was not affected by AM inoculation with or without bacteria -co-inoculation with bacteria led to higher stem dry weight and leaf mass per area compared to other treatments (AM only, P-supplemented non-mycorrhizal plants and control)	Rapparini et al., 2008

	-Na and K concentrations were lower in AM treatments compared to control plants -no differences in the rate of photosynthesis were observed	
<i>Glomus versiforme</i>	-mycorrhizal treatment improved the uptake of nitrogen, phosphorus and potassium -stem, branch and leaf biomass was enhanced by 26.5%, 10.1% and 14.9% -mycorrhizal colonization increased the leaf chlorophyll content, net photosynthetic rate, stomatal conductance, transpiration rate, stem diameter and aboveground biomass -the treatment changed the volatile oil components and increased volatile oil content in leaf by 25.0% -AMF treatment positively affected the uptake of plant nutrients and the density of glandular trichomes -a significant increase in biomass production and accumulation of artemisinin was observed	Huang et al., 2011
	-the treatment was not effective alone in enhancing growth and yield of plants -co-inoculation with <i>Stenotrophomonas</i> spp. improved its efficiency in increasing total dry weight and height -significant increase in phosphatase activity was observed -no significant differences were found in leaf artemisinin content between treated (0.68%) and control plants (0.61%) -combination with <i>Bacillus subtilis</i> led to significant increase in artemisinin content with 24.6%	Giri, 2017
<i>Rhizophagus fasciculatus</i> (Syn. <i>Glomus fasciculatum</i>)	-inoculation significantly increased the production of herbage, dry weight of shoot, nutrient status (P, Zn, Fe) of shoot, concentration of essential oil and artemisinin in leaves compared to control plants -Mn concentration significantly decreased -an increase in essential oil concentration of 66% was observed compared to non-inoculated plants -Cu concentration was not influenced by the treatment	Awasthi et al., 2011
	-the treatment significantly increased concentration of artemisinin -mycorrhizal plants had higher foliar glandular trichome density, accumulated more phosphorus in their shoots compared to control plants -inoculation in P-enriched soil significantly increased biomass, Fe and Zn concentrations in shoots, glandular trichome density, artemisinin concentration in leaves -Cu concentration was not influenced	Chaudhary et al., 2007
<i>Rhizophagus intraradices</i> (Syn. <i>R. irregularis</i> , <i>Glomus intraradices</i>)	-the treatment significantly improved guaiacol peroxidase enzyme concentration, fresh and dry biomass of leaves -the number of trichomes and artemisinin concentration was significantly increased by 40.7% and 17% compared to control -inoculation increased root biomass, had significantly greater inter-nodal length, more flavonoid content compared to control plants -the treatment did not significantly affect artemisinin content, the glandular trichome density and leaf biomass -inoculation resulted in increased endogenous jasmonic acid levels -the glandular trichome density and artemisinin concentration was enhanced -the treatment was effective in enhancing leaf yield, total dry weight, significantly improved K uptake and phosphatase activity -no significant differences were found in leaf artemisinin content between treated (0.69%) and control plants (0.61%)	Kapoor et al., 2007 Domokos et al., 2018 Fortin and Melchert, 2015 Mandal et al., 2015 Awasthi et al., 2011

Table 2. The effect of endophytic fungi on *Artemisia annua* plants

Endophytic fungi	The effect of the treatment	Reference
<i>Colletotrichum gloeosporioides</i>	-the treatment promoted the artemisinin production by 51.63% in <i>A. annua</i> hairy root cultures	Wang et al., 2006
<i>Colletotrichum</i> sp.	-when using elicitor extract from the fungus the artemisinin content increased in hairy roots by 64.29 % compared to control -peroxidase activity of hairy roots and Ca^{2+} accumulation in cortical cells was influenced -the elicitor extract from the fungus led to an increase in artemisinin content in hairy roots of <i>A. annua</i> (44% increase over the control)	Wang et al., 2002
<i>Penicillium oxalicum</i>	-inoculation of 30-day-old rooting plantlets with fungus enhanced the artemisinin concentration by 43.5% over control -the endophyte had positive effect over the growth of <i>in vitro</i> propagated <i>A. annua</i> plantlets and induced oxidative stress through the production of reactive oxygen species	Wang et al., 2001 Zheng et al., 2016
<i>Piriformospora indica</i>	-inoculation promoted the growth of on <i>in vitro</i> grown plantlets and increased the carotenoid, total soluble sugar, total soluble protein and flavonoids content -the artemisinin concentration was enhanced by 1.7-fold compared to control	Arora et al., 2017
	-the treatment significantly increased the plant biomass, total chlorophyll content in leaves, phosphorus and nitrogen content by 65.95% and 13.27%	Arora et al., 2016
	-the endophyte led to an increment in artemisinin content -dual inoculation with <i>Azotobacter chroococcum</i> enhanced plant height, total biomass and total leaf yield per plant by 63.51%, 52.61% and 79.70% compared to control	
	-root length, shoot length, stem size, root thickness, dry weight and fresh weight of <i>in vitro</i> raised plantlets increased due to the treatment -the chlorophyll and carotenoid content was also positively affected	Baishya et al., 2015
	-the treatment led to a 1.57 times increase in artemisinin production and promoted growth in hairy roots in liquid cultures	Ahlawat et al., 2014
	-the treatment significantly increased the average shoot number and biomass of <i>A. annua</i> cultures -the artemisinin content was enhanced by 60% compared to control	Sharma and Agrawal, 2013

Table 3. The effect of endophytic bacteria on *Artemisia annua* plants

Endophytic bacteria	The effect of the treatment	References
<i>Agrobacterium rhizogenes</i>	-the infected <i>A. annua</i> seedling tissues produced neoplastic roots characterized by high growth rate, genetic stability and producing higher levels of secondary metabolites	Soleimani et al., 2012
<i>Azospirillum</i> sp.	-the treatment increased protein content in plants compared to chemical fertilizers	Keshavarzi and Nik, 2011
<i>Azotobacter chroococcum</i>	-an increase in total soluble sugar, total soluble protein and flavonoids content and carotenoid accumulation was observed	Arora et al., 2017
	-the treatment promoted plantlet growth	
	-the artemisinin content was increased by 1.3-fold compared to control	
	-the treatment increased plant biomass, total chlorophyll content by 51.97%, phosphorus content by 31.90%, nitrogen content by 29.20% and artemisinin content by 70%	
<i>Azotobacter</i> sp.	-dual inoculation with <i>Piriformospora indica</i> enhanced plant height, total biomass and total leaf yield per plant by 63.51%, 52.61% and 79.70% compared to control	Arora et al., 2016
	-the treatment increased protein content in plants compared to chemical fertilizers	
<i>Bacillus</i> sp.	-inoculation increased protein content in plants compared to chemical fertilizers	Keshavarzi and Nik, 2011
	-inoculation increased protein content in plants compared to chemical fertilizers	Keshavarzi and Nik, 2011
<i>Bacillus subtilis</i>	-AM fungi in association with different bacteria increased stem dry weight and leaf mass per area	Rapparini et al., 2008
	-the total terpene content and emission, and the rate of photosynthesis was not affected	
	-inoculation improved height, leaf yield and total dry weight of the plants	
	-significantly increased the mycorrhizal colonization of <i>G. mossae</i> by 50.32 % and phosphorus concentration in plants	
	-co-inoculation with <i>G. mossae</i> was highly effective by improving the height by 57.78%, total plant weight by 62.64%, leaf yield of plants by 66.60%, phosphatase activity, and the artemisinin content by 26.2 % compared to control	
<i>Pseudomonas fluorescens</i>	-co-inoculation with <i>G. fasciculatum</i> increased the artemisinin content by 24.6%	Awasthi et al., 2011
	-AM fungi in association with different bacteria increased stem dry weight and leaf mass per area	
<i>Pseudomonas</i> sp.	-the total terpene content and emission, and the rate of photosynthesis was not affected	Rapparini et al., 2008
	-the treatment increased protein content in plants compared to chemical fertilizers	Keshavarzi and Nik, 2011
<i>Radiobacter</i> spp.	-AM fungi in association with different bacteria increased stem dry weight and leaf mass per area	Rapparini et al., 2008
	-the total terpene content and emission, and the rate of photosynthesis was not affected	

<i>Stenotrophomonas</i> spp.	-inoculation improved the height, leaf yield and total weight of the plants -the leaf artemisinin content was 0.73%, compared to 0.61% in control -the treatment improved the population of <i>G. fasciculatum</i> -co-inoculation with <i>G. mosseae</i> increased phosphorus concentration	Awasthi et al., 2011
<i>Streptomyces</i> spp.	-AM fungi in association with different bacteria increased stem dry weight and leaf mass per area -the total terpene content and emission, and the rate of photosynthesis was not affected	Rapparini et al., 2008

Table 4. Isolated fungi from *Artemisia annua* and their importance

Isolated fungi	The effect of the fungi	Reference
<i>Acremonium persicum</i>	-the elicitor extract prepared from the fungi increased the potted plants biomass and artemisinin content	Hussain et al., 2017
<i>Aspergillus</i> spp.	-the 11 endophytic extracts had different inhibitory effects on microbial pathogens - <i>Aspergillus</i> spp. exhibited the strongest activity against <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> and <i>Trichophyton rubrum</i>	Zhang et al., 2012
<i>Aspergillus terreus</i>	-the fungi was isolated from <i>A. annua</i> , but no effect was studied	Zhang et al., 2010
<i>Cephalosporium</i> sp.	- <i>Cephalosporium</i> sp. extract had the strongest antimicrobial activity against <i>Magnaporthe grisea</i>	Zhang et al., 2012
<i>Cladosporium</i> sp.	-the pure cultures of the fungi had the greatest antibacterial (against <i>Staphylococcus aureus</i> , <i>Streptococcus mutans</i> , <i>Salmonella typhi</i> , <i>Bacillus subtilis</i>) and antifungal activity (<i>Malassezia furfur</i> , <i>Candida albicans</i>) out of the seven endophytic fungi obtained	Purwantini et al., 2015
<i>Cochliobolus lunatus</i>	-the elicitor extract prepared from the fungi increased the plant biomass and artemisinin content	Hussain et al., 2017
<i>Colletotrichum gloeosporioides</i>	-an increase in the plant biomass and artemisinin content was observed	Hussain et al., 2017
<i>Colletotrichum</i> sp.	-the fungi can produce antimicrobial and plant growth regulatory metabolites -the extract has antibacterial (against <i>Bacillus subtilis</i> , <i>Staphylococcus aureus</i> , <i>Sarcina lutea</i> and <i>Pseudomonas</i> sp.) and antifungal activity (against <i>Candida albicans</i> , <i>Aspergillus niger</i> , <i>Gaeumannomyces graminis</i> var. <i>tritici</i> , <i>Rhizoctonia cerealis</i> , <i>Helminthosporium sativum</i> , <i>Phytophthora capsici</i>)	Lu et al., 2000
<i>Curvularia pallescens</i>	-the elicitor extract prepared from the fungi increased the potted plants biomass and artemisinin content and induced maximum growth (amongst the tested endophytic fungi)	Hussain et al., 2017
<i>Leptosphaeria</i> sp.	-leptosphaerone, a new bioactive and/or chemically new compound that may contain great medicinal or agricultural potential, was isolated from the studied endophyte of <i>A. annua</i>	Liu et al., 2002
<i>Mucor</i> sp.	- <i>Mucor</i> sp. extract showed the most pronounced effect on <i>Rhizoctonia cerealis</i>	Zhang et al., 2012

Studies have been conducted on the relationship between *A. annua* and prokaryotic endophytes from the phylum Firmicutes (genus *Bacillus*), Actinobacteria (genera *Stenotrophomonas* and *Streptomyces*) and Proteobacteria (genera *Agrobacterium*, *Azopirillum*, *Azotobacter*, *Pseudomonas* and *Radiobacter*). The majority of these studied bacterial endophytes are nitrogen-fixing (NF) bacteria. Plants are unable to directly assimilate molecular nitrogen, which is a limiting nutrient in most environments. However, biological nitrogen fixation developed only by prokaryotic cells enables this process (Franché et al., 2009).

The treatment lead to increased protein, chlorophyll and artemisinin content, and enhanced phosphatase activity and plant growth parameters (total weight, leaf yield, height) (Table 3).

5. Isolated endophytes from *Artemisia annua* and their importance

Prokaryotic endophytes isolated from *A. annua* belong to the genera *Nocardia*, *Nonomuraea*, *Pseudonocardia*, *Rhodococcus* and *Streptomyces*: *Nocardia artemisiae* sp. nov. (Zhao et al., 2011a), *Nonomuraea endophytica* sp. nov. (Li et al., 2011), *Pseudonocardia bannaensis* sp. nov. (Zhao et al., 2011b), *Pseudonocardia* sp. (Li et al., 2012), *Pseudonocardia xishanensis* sp. nov. (Zhao et al., 2012a), *Rhodococcus artemisiae* sp. nov. (Zhao et al., 2012b) and *Streptomyces endophyticus* (Li et al., 2013). Their impact on *A. annua* has not been the main goal of these studies. However, the results suggest that certain endophytic actinobacteria can stimulate plant defense responses thus affecting the artemisinin content.

The isolated endophytic fungi are from the genera *Acremonium*, *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Cochliobolus*, *Colletotrichum*, *Curvularia*, *Leptosphaeria* and

Mucor. The antimicrobial and antifungal activities of the fungal endophytes have been studied (Table 4).

Conclusions

The shortage of wild medicinal plant resources and the increasing demand for herbal material increased the interest in alternative methods to enhance productivity. In this review, it has been pointed out that overall the inoculation with mutualistic and endophytic microorganisms leads to positive outcomes (enhancing plant growth, biomass, artemisinin and essential oil concentration, disease resistance), which makes them a potential alternative to existing yield increasing methods.

Some of the AM fungi and endophytes obviously favor the plant growth and development, influencing the quality and quantity of the crude drugs derived from *A. annua*. Co-inoculation of AM fungi and bacterial endophytes had the most impact on host plants, followed by single inoculation with AM fungi.

No publications about cultivation of *Artemisia annua* in open field conditions by inoculation with mutualistic or endophytic microorganisms were found. In the future the initiation of competition experiments with AMF and other soil inhabiting microorganisms would be of high interest.

AMF inoculation increased guaiacol peroxidase enzyme (GPOX) activity in *A. annua* plants. Further studies would be necessary to elucidate the mechanism of tolerance to water deficiency in case of inoculated *A. annua* plants.

Endophytes isolated from the plant could be used in medicine and in agriculture for their antimicrobial effect. These endophytes are potential artemisinin content and plant stress resistance enhancer.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial

or financial relationships that could be construed as a potential conflict of interest.

References

1. Ahlawat S, Saxena P, Alam P, Wajid S, Abdin M Z (2014) Modulation of artemisinin biosynthesis by elicitors, inhibitor, and precursor in hairy root cultures of *Artemisia annua* L. *Journal of Plant Interactions*, 9:1,811-824, doi: 10.1080/17429145.2014.949885
2. Arora M, Saxena P, Abdin M Z, Varma A (2017) Interaction between *Piriformospora indica* and *Azotobacter chroococcum* governs better plant physiological and biochemical parameters in *Artemisia annua* L. plants grown under in vitro conditions. *Symbiosis*, Volume 75, Issue 2, pp 103–112. doi: 10.1007/s13199-017-0519-y
3. Arora M, Saxena P, Choudhary D K, Abdin M Z, Varma A (2016) Dual symbiosis between *Piriformospora indica* and *Azotobacter chroococcum* enhances the artemisinin content in *Artemisia annua* L. *World J Microbiol Biotechnol* (2016) 32:19. doi: 10.1007/s11274-015-1972-5
4. Awasthi A L, Bharti N, Nair P, Singh R, Shukla K A, Gupta M M, et al., (2011). Synergistic effect of *Glomus mosseae* and nitrogen fixing *Bacillus subtilis* strain Daz26 on artemisinin content in *Artemisia annua*. *Appl. Soil Ecol.* 49:125–130. doi: 10.1016/j.apsoil.2011.06.005
5. Baishya D, Deka P, Kalita M C (2015) In vitro co-cultivation of *Piriformospora indica* filtrate for improve biomass productivity in *Artemisia annua* (L.). *Symbiosis*, Volume 66, Issue 1, pp 37–46. doi: 10.1007/s13199-015-0331-5
6. Balestrini R, Lumini E, Borriello R, Bianciotto C (2015) Plant-soil biota interactions. *Soil Microbiology, Ecology and Biochemistry*, ed Paul E A, London, Academic Press, Elsevier, pp 311-338. doi: 10.1016/b978-0-12-415955-6.00011-6
7. Barman J, Samanta B, Saha B, Datta S (2016) Mycorrhiza: the oldest association between plant and fungi. *Siraj Datta*, Dec 14, 2016
8. Berruti A, Lumini E, Balestrini R, Bianciotto V (2016) Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. *Front. Microbiol.*, 6:1559. doi: 10.3389/fmicb.2015.01559
9. Bian G, Zhang Y, Qin S, Xing K, Xie H, Jiang J (2011) Isolation and biodiversity of heavy metal tolerant endophytic bacteria from halotolerant plant species located in coastal shoal of Nantong. *Wei Sheng Wu Xue Bao*, 51(11):1538-47.
10. Bilia A R, Santomauro F, Sacco C, Bergonzi M C, Donato R (2014) Essential oil of *Artemisia annua* L.: an extraordinary component with numerous antimicrobial properties. *Complementary and Alternative Medicine* 2014(1):159819. doi: 10.1155/2014/159819
11. Bonfante P (2001) At the interface between mycorrhizal fungi and plants: the structural organization of cell wall, plasma membrane and cytoskeleton. In: Hock B. (eds) *Fungal Associations. The Mycota (A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research)*, vol 9. Springer, Berlin, Heidelberg, pp 45-61. doi: 10.1007/978-3-662-07334-6_4
12. Bonfante P, Anca I A (2009) Plants, mycorrhizal fungi, and bacteria: a network

- of interactions. Annual Review of Microbiology, 63:363-383. doi: 10.1146/annurev.micro.091208.073504
13. Briars R, Paniwnyk L (2014) Potential methods to improve the efficiency of artemisinin extraction from *Artemisia annua*. In: Aftab T., Ferreira J., Khan M., Naeem M. (eds) *Artemisia annua - Pharmacology and Biotechnology*. Springer, Berlin, Heidelberg, pp 124–137. doi: 10.1007/978-3-642-41027-7_8
14. Calderón F, Wilson D M, Gamo J (2013) Antimalarial drug discovery: Recent progress and future directions. Progress in Medicinal Chemistry 52:97–151. doi: 10.1016/B978-0-444-62652-3.00003-X
15. Cavaglieri L., Passone A., Etcheverry M. (2004). Correlation between screening procedures to select root endophytes for biological control of *Fusarium verticillioides* in *Zea mays* L. Biol. Control. 31, 259–267. doi: 10.1016/j.biocontrol.2004.07.006
16. Chaudhary L V, Kapoor R, Bhatnagar K A (2008) Effectiveness of two arbuscular mycorrhizal fungi on concentrations of essential oil and artemisinin in three accessions of *Artemisia annua* L. Appl. Soil Ecol. 40, 174–181. doi: 10.1016/j.apsoil.2008.04.003
17. Das S (2012) *Artemisia annua* (qinghao): a pharmacological review. International journal of pharmaceutical sciences and research: 4573–4577. doi: 10.13040/IJPSR.0975-8232.3(12).4573-77
18. Domokos E, Jakab-Farkas L, Darkó B, Bíró-Janka B, Mara Gy, Albert Cs, Balog A (2018) Increase in *Artemisia annua* plant biomass content and guaiacol peroxidase activity using the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. Front Plant Sci. 2018; 9: 478. doi: 10.3389/fpls.2018.00478
19. Etminani F, Harighi B (2018) Isolation and identification of endophytic bacteria with plant growth promoting activity and biocontrol potential from wild pistachio trees. Plant Pathol. J. 34(3):208-217. doi: 10.5423/PPJ.OA.07.2017.0158
20. Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Annals of Botany, 104: 1263–1280. doi:10.1093/aob/mcp251
21. Fairhurst R M, Dondorp A M (2016) Artemisinin-Resistant *Plasmodium falciparum* Malaria. Microbiol Spectr. 4(3). doi: 10.1128/microbiolspec.EI10-0013-2016.
22. Fairhurst R M, Wellems T E (2015) Malaria (*Plasmodium* species). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (Eighth Edition):3070-3090. doi: 10.1016/B978-1-4557-4801-3.00276-9
23. Fortin S, Melchert V (2015) Effect of mycorrhizae on *Artemisia annua*. Worcester Polytechnic Institute.
24. Franche C, Lindström K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59. doi: 10.1007/s11104-008-9833-8
25. Garg N, Bhangari P (2016) Silicon nutrition and mycorrhizal inoculations improve growth, nutrient status, K⁺ /Na⁺ ratio and yield of *Cicer arietinum* L. genotypes under salinity stress. Plant Growth Regulation 78(3):371-387. doi: 10.1007/s10725-015-0099-x
26. Giovannetti M, Sbrana C (1998) Meeting a non-host: the behaviour of AM fungi. Mycorrhiza 8:123-130. doi: 10.1007/s005720050224
27. Giri B (2017) Mycorrhizal fungus *Rhizophagus fasciculatus* promotes artemisinin accumulation in *Artemisia annua*. In Paper Presented at the Tropentag,

- Future Agriculture: Socio-Ecological Transitions and Bio-Cultural Shifts, Bonn.
28. Griffin M R (2014) Biocontrol and bioremediation: two areas of endophytic research which hold great promise. *Advances in endophytic research*. Verma V C, Gange A C, Springer India, 2014, pp 257-282. doi: 10.1007/978-81-322-1575-2_14
29. Hardoim P R, van Overbeek L S, Berg G, Pirttilä A M, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol Mol Biol Rev*. 79(3):293-320. doi: 10.1128/MMBR.00050-14
30. Hartley S E, Gange A C (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. *Annu. Rev. Entomol*. 54, 323-342. doi: 10.1146/annurev.ento.54.110807.090614
31. Heijden M G A, Horton T R (2009) Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology*. 97, 1139–1150. doi: 10.1111/j.1365-2745.2009.01570.x
32. Ho W E, Peha H Y, Chan T K, Wong F (2013) Artemisinins: Pharmacological actions beyond anti-malarial. *Pharmacology & Therapeutics* 142:126–139. doi: 10.1016/j.pharmthera.2013.12.001
33. Hommel M (2008) The future of artemisinins: natural, synthetic or recombinant? *Journal of Biology* 2008, 7:38. doi: 10.1186/jbiol101
34. Huang J H, Tan J F, Jie H K, Zeng R S (2011) Effects of inoculating arbuscular mycorrhizal fungi on *Artemisia annua* growth and its officinal components. *Yingyong Shengtai Xuebao*, 2011, Vol. 22 Issue 6, p1443-1449.
35. Hussain M A, Mahajan V, Rather I A, Awasthi P, Chouhan R, Dutt P, Sharma Y P, Bedi Y S, Gandhi S G (2017) Isolation and identification of growth promoting endophytic fungi from *Artemisia annua* L. and its effects on artemisinin content. *Trends Phytochem. Res*. 1(4): 207-214.
36. Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea J M (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol Fertil Soils*, 37:1–16. doi: 10.1007/s00374-002-0546-5
37. Jia M, Chen L, Xin H L, Zheng C J, Rahman K, Han T, Qin L P (2016) A Friendly Relationship between Endophytic Fungi and Medicinal Plants: A Systematic Review. *Front Microbiol*. 2016; 7: 906. doi: 10.3389/fmicb.2016.00906
38. Kandel S L, Firrincieli A, Joubert P M, Okubara P A, Lestion N D, McGeorge K M, Doty S L (2017) An in vitro study of bio-control and plant growth promotion potential of Salicaceae endophytes. *Front Microbiol*, 13;8:386. doi: 10.3389/fmicb.2017.00386
39. Kapoor R, Chaudhary V, Bhatnagar AK (2007) Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in *Artemisia annua* L. *Mycorrhiza* 17:581–587. doi: 10.1007/s00572-007-0135-4
40. Keshavarzi M H B, Mousavi-Nik S M, Abdin M Z (2012). The effect of biological and chemical fertilizers on chlorophyll and artemisinin content in *Artemisia annua* L. In The 1st international and the 4th national congress on recycling of organic waste in agriculture. pp. 1–8. doi:
41. Keshavarzi M H B, Nik A M M (2011) The effect of biological and chemical fertilizers on protein content in *Artemisia annua* L. Leaves. *Annals of Biological Research*, 2011, 2 (5) :596-601

42. Kim S J, Eo J K, Lee E H, Park H, Eom A E (2017) Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. *Mycobiology*, 45(1): 20–24. doi: 10.5941/MYCO.2017.45.1.20
43. Kung S H, Lund S, Murarka A, McPhee D, Paddon C J (2018) Approaches and Recent Developments for the Commercial Production of Semi-synthetic Artemisinin. *Front Plant Sci.* 31;9:87. doi: 10.3389/fpls.2018.00087
44. Lacava P T, Azevedo J L (2014) Biological control of insect-pest and diseases by endophytes. *Advances in endophytic research*. Verma V C, Gange A C, Springer India, 2014, pp 231-256. doi: 10.1007/978-81-322-1575-2_13
45. Li J, Zhao G Z, Huang H Y, Zhu W Y, Lee J C, Zu L H, Kim C J, Li W J (2011) *Nonomuraea endophytica* sp. nov., an endophytic actinomycete isolated from *Artemisia annua* L. *International Journal of Systematic and Evolutionary Microbiology*, 61, 757–761. doi: 10.1099/ij.s.0.022558-0
46. Li J, Zhao G Z, Zhu W Y, Huang H Y, Xu L H, Zhang S, Li W J (2013) *Streptomyces endophyticus* sp. nov., an endophytic actinomycete isolated from *Artemisia annua* L. *International Journal of Systematic and Evolutionary Microbiology* 63, 224–229. doi: 10.1099/ij.s.0.035725-0
47. Li J, Zhao G-Z, Varma A, Qin S, Xiong Z, et al., (2012) An Endophytic *Pseudonocardia* Species Induces the Production of Artemisinin in *Artemisia annua*. *PLoS ONE* 7(12): e51410. doi:10.1371/journal.pone.0051410
48. Liarzi O, Bucki P, Miyara S B, Ezra D (2016) Bioactive volatiles from an endophytic *Daldinia cf. concentrica* isolate affect the viability of the plant parasitic nematode *Meloidogyne javanica*. *PLoS ONE* 11(12): e0168437. doi:10.1371/journal.pone.0168437
49. Liu C, Zhao Y, Wang Y (2006) Artemisinin: current state and perspectives for biotechnological production of an antimalarial drug. *Appl Microbiol Biotechnol*, 72:11–20. doi: 10.1007/s00253-006-0452-0
50. Liu J Y, Liu C H, Zou W X, Tian X, Tan R X (2002) Leptosphaerone, a metabolite with a novel skeleton from *Leptosphaeria* sp. IV403, an endophytic fungus in *Artemisia annua*. *Helvetica*, Volume 85, Issue9, pp 2664-2667. doi: 10.1002/1522-2675(200209)85:9<2664::AID-HLCA2664>3.0.CO;2-R
51. Lu H, Zou W X, Meng J C, Hu J, Tan R X (2000) New bioactive metabolites produced by *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*. *Plant Science* 151:67–73. doi: 10.1016/S0168-9452(99)00199-5
52. Malhi S S, Sahota T S, Gill K S (2013) Potential of Management Practices and Amendments for Preventing Nutrient Deficiencies in Field Crops under Organic Cropping Systems. *Agricultural Sustainability* pp 77–101. doi: 10.1016/B978-0-12-404560-6.00005-8
53. Mandal S, Upadhyay S, Wajid S, Ram M, Jain D C, Singh V P, Abdin M Z, Kapoor R (2015) Arbuscular mycorrhiza increase artemisinin accumulation in *Artemisia annua* by higher expression of key biosynthesis genes via enhanced jasmonic acid levels. *Mycorrhiza*. 25(5):345-57. doi: 10.1007/s00572-014-0614-3
54. Mardhiah U, Caruso T, Gurnell A, Rillig M (2016) Arbuscular mycorrhizal fungal hyphae reduce soil erosion by surface water flow in a greenhouse experiment. *Appl Soil Ecol* 99:137–140. doi: 10.1016/j.apsoil.2015.11.027
55. Martínez M J A, Olmo L M B, Ticona L A, Benito P B (2014) Pharmacological Potentials of Artemisinin and Related

- Sesquiterpene Lactones: Recent Advances and Trends, In: Aftab T., Ferreira J., Khan M., Naeem M. (eds) *Artemisia annua - Pharmacology and Biotechnology*. Springer, Berlin, Heidelberg, pp 75-93. doi: 10.1007/978-3-642-41027-7_5
56. Mercado-Blanco J, Lugtenberg B J J (2014) Biotechnological applications of bacterial endophytes. *Current Biotechnology*, 3(1):60-75. doi: 10.2174/22115501113026660038
57. Miransari M (2011) Interactions between arbuscular mycorrhizal fungi and soil bacteria. *Appl Microbiol Biotechnol*, 89:917–930. doi:10.1007/s00253-010-3004-6
58. Monfil V O, Casas-Flores S (2014) Molecular mechanisms of biocontrol in *Trichoderma* spp. and their applications in agriculture. *Biotechnology and Biology of Trichoderma*, pp 429–453. doi: 10.1016/B978-0-444-59576-8.00032-1
59. Moore D, Robson G, Trinci A (2011) 21th century guidebook of fungi. Cambridge University Press, United States of America, New York
60. Naeem M, Idrees M, Singh M, Kahn M M A, Moinuddin (2014) *Artemisia annua*: a miraculous herb to cure malaria. In: Aftab T., Ferreira J., Khan M., Naeem M. (eds) *Artemisia annua - Pharmacology and Biotechnology*. Springer, Berlin, Heidelberg, pp 27–49. doi: 10.1007/978-3-642-41027-7_3
61. Namuli A, Bazira J, Casim T U, Engeu P O (2018) A review of various efforts to increase artemisinin and other antimalarial compounds in *Artemisia Annua* L plant. *Cogent Biology* 4: 1513312. doi: 10.1080/23312025.2018.1513312
62. Pandley N, Pandley-Ray S (2015) Updates on artemisinin: an insight to mode of actions and strategies for enhanced global production. *Protoplasma*, 253(1):15-30. doi: 10.1007/s00709-015-0805-6
63. Purwantini I, Wahyono, Mustofa, Asmah R (2015) Isolation of endophytic fungi from *Artemisia annua*, L and identification of their antimicrobial compound using bioautography method. *Int J Pharm Pharm Sci*, Vol 7, Issue 12, 95-99.
64. Rahman W, Widyatmoko D, Lelono A A (2014) The effect of NPK fertilizer, manure, vesicular arbuscular mycorrhiza (VAM) on the growth, biomass and artemisinin content of *Artemisia annua* L. *Jurnal Biologi Indonesia* 10(2): 285-296. doi: 10.14203/jbi.v10i2.2107
65. Rajkumar M, Sandhya S, Prasad M N, Feritas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. *Biotechnol Adv.*, 30(6):1562-74. doi: 10.1016/j.biotechadv.2012.04.011
66. Rapparini F, Llusia J, Penuelas J (2008) Effect of arbuscular mycorrhizal (AM) colonization on terpene emission and content of *Artemisia annua* L. *Plant Biol* 10:108–122. doi: 10.1055/s-2007-964963
67. Ryan R P, Germaine K, Franks A, Ryan D J, Dowling D N (2008) Bacterial endophytes: recent developments and applications. *FEMS Microbiology Letters*, volume 278, issue 1, pp 1-9. doi: 10.1111/j.1574-6968.2007.00918.x
68. Sadiq A, Hayat M O, Ashraf M (2014) Ethnopharmacology of *Artemisia annua* L.: A Review. In: Aftab T., Ferreira J., Khan M., Naeem M. (eds) *Artemisia annua - Pharmacology and Biotechnology*. Springer, Berlin, Heidelberg, pp 9–25. doi: 10.1007/978-3-642-41027-7_2
69. Santander C, Aroca R, Ruiz-Lozano J M, Olave J, Cartes P, Borie F, Cornejo P (2017) Arbuscular mycorrhiza effects on plant performance under osmotic stress.

- Mycorrhiza, 27(7):639-657. doi: 10.1007/s00572-017-0784-x
70. Sharma G, Agrawal V (2013) Marked enhancement in the artemisinin content and biomass productivity in *Artemisia annua* L. shoots co-cultivated with *Piriformospora indica*. *World J Microbiol Biotechnol.* 29:1133–1138. doi: 10.1007/s11274-013-1263-y
71. Sheng X F, Xia J J, Jiang C Y, He L Y, Qian M (2008) Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. *Environ. Pollut.*, 156:1164–1170. doi: 10.1016/j.envpol.2008.04.007
72. Singh D G, Kumar A, Lata S, Johri R M (2017) Effect of arbuscular mycorrhizal fungi on plant growth, essential oil and Artemisinin content of *Artemisia annua* L. *International Journal of Pharmacology and Biological Sciences*, 11(1): 33-44.
73. Soleimani T, Keyhanfar M, Piri K, Hasanloo T (2012) Morphological evaluation of hairy roots induced in *Artemisia annua* L and investigating elicitation effects on the hairy roots biomass production. *International Journal of Agriculture: Research and Review*, vol. 2, pp 1005-1013
74. Suryanarayanan T S, Thirunavukkarasu N, Govindarajulu M B, Sasse F, Jansen R, Murali T S (2009) Fungal endophytes and bioprospecting. *Fungal Biology Reviews* 23:9-19. doi: 10.1016/j.fbr.2009.07.001
75. Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N, Barac T, Vangronsveld J, Van Der Lelie D (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. *Appl. Environ. Microbiol.* 75:748–757. doi: 10.1128/AEM.02239-08
76. Tan W D, Shen M J, Qiu H J, Zeng F L, Huang J H, Huang R S, Luo W G, Liu Y X (2013) Effects of different phosphorus treatments on Arbuscular mycorrhizal formation, growth and artemisinin content of *Artemisia annua*. *Journal of Southern Agriculture*, 2013 Vol.44 No.8 pp.1303-1307
77. Tang X, Demiray M, Wirth T, Allemann R K (2018) Concise synthesis of artemisinin from a farnesyl diphosphate analogue. *Bioorg Med Chem.* 26(7): 1314–1319. doi: 10.1016/j.bmc.2017.03.068
78. Torres M, White J F (2012) Endophytic fungi. *Encyclopedia of Science & Technology*, McGraw-Hill Education, pp 241-269. doi: 10.13140/2.1.3835.2169
79. Varma A, Bakshi M, Lou B, Hartmann A, Ouelmueller R (2012) *Piriformospora indica*: a novel plant growth-promoting mycorrhizal fungus. *Agric Res.* 1(2):117–131. doi: 10.1007/s40003-012-0019-5
80. Wang B, Sui J, Yu Z, Zhu L (2011) Screening the hemostatic active fraction of *Artemisia annua* L. In-vitro. *Iran J Pharm Res* 10:57–62.
81. Wang J W, Xia Z H, Tan R X (2002) Elicitation on artemisinin biosynthesis in *Artemisia annua* hairy roots by the oligosaccharide extract from the endophytic *Colletotrichum* sp. B501. *Acta Botanica Sinica* 44(10):1233-1238
82. Wang J W, Zhang Z, Tan R X (2001) Stimulation of artemisinin production in *Artemisia annua* hairy roots by the elicitor from the endophytic *Colletotrichum* sp. *Biotechnology Letters*, Volume 23, Issue 11, pp 857–860. doi: 10.1023/A:1010535001943
83. Wang J W, Zheng L P, Tan R X (2006) The preparation of an elicitor from a fungal endophyte to enhance artemisinin production in hairy root cultures of

- Artemisia annua L. Sheng Wu Gong Cheng Xue Bao, 22(5):829-34.
84. White NJ (2008) Qinghaosu (Artemisinin) The price of success. Science 320:331–334. doi:10.1126/science.1155165
85. WHO (2017) World Malaria Report. Luxemburg: Marketing and Dissemination, World Health Organization
86. Zabel F, Putzenlechner B, Mauser W (2014) Global agricultural land resources – a high resolution suitability evaluation and its perspectives until 2100 under climate change conditions, PLoS ONE 9(9): e107522. doi: 10.1371/journal.pone.0107522
87. Zeng Y, Guo L P, Chen B D, Hao Z P, Wang J W, Huang L Q, Yang G, Cui X M et al., (2013) Arbuscular mycorrhizal symbiosis and active ingredients of medicinal plants: current research status and prospectives. Mycorrhiza, 23(4):253–65. doi: 10.1007/s00572-013-0484-0
88. Zhang H W, Zhang J, Hu S, Zhang Z J, Zhu C J, Ng S W, Tan R X (2010) Ardeemins and cytochalasins from Aspergillus terreus residing in Artemisia annua. Planta Med 2010; 76:1616–1621. doi: 0.1055/s-0030-1249781
89. Zhang H, Bai X, Wu B (2012) Evaluation of antimicrobial activities of extracts of endophytic fungi from Artemisia annua. Bangladesh J Pharmacol 7: 120-123. doi: 10.3329/bjp.v7i2.10951
90. Zhao G Z, Li J, Zhu W J, Klenk H P, Xu L H, Li W J (2011) (a) Nocardia artemisiae sp. nov., an endophytic actinobacterium isolated from a surface-sterilized stem of Artemisia annua L. International Journal of Systematic and Evolutionary Microbiology, 61, 2933–2937. doi: 10.1099/ijs.0.029306-0
91. Zhao G Z, Li J, Zhu W J, Li X P, Tian S Z, Zhao L X, Xu L H, Li W J (2011) (b) Pseudonocardia bannaensis sp. nov., a novel actinomycete isolated from the surface-sterilized roots of Artemisia annua L. Antonie van Leeuwenhoek 100:35–42. doi: 10.1007/s10482-011-9562-5
92. Zhao G Z, Li J, Zhu W J, Tian S Z, Zhao L X, Yang L L, Xu L H, Li W J (2012) (a) Rhodococcus artemisiae sp. nov., an endophytic actinobacterium isolated from the pharmaceutical plant Artemisia annua L. International Journal of Systematic and Evolutionary Microbiology, 62, 900–905. doi: 10.1099/ijs.0.031930-0
93. Zhao G Z, Li J, Zhu W J, Wei D Q, Zhang J L, Xu L H, Li W J (2012) (b) Pseudonocardia xishanensis sp. nov., an endophytic actinomycete isolated from the roots of Artemisia annua L. International Journal of Systematic and Evolutionary Microbiology 62, 2395–2399. doi: 10.1099/ijs.0.037028-0
94. Zheng L P, Tian H, Yuan F Y, Wang W J (2016) The influence of endophytic Penicillium oxalicum B4 on growth and artemisinin biosynthesis of in vitro propagated plantlets of Artemisia annua L. Plant Growth Regulation, Volume 80, Issue 1, pp 93–102. doi: 10.1007/s10725-016-0162-2