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REVIEW PAPER

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

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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 micorrhizal 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; Fairhust 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 derivates 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 (Fairhust and Dondorp, 2016).

Artemisinins (artemisinin and its chemical derivates) 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 grow 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 medical 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, silicium, 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
Acaulospora tuberculata	-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
Glomus aggregatum	-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
Glomus macrocarpum	-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
Glomus mosseae	-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 *Bacillus subtilis* 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
	-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
Glomus spp. (*G. mosseae*, *G. intraradices*, *G. viscosum*)	-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	
Glomus versiforme	-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%	Huang et al., 2011
Rhizophagus fasciculatus (*Syn. Glomus fasciculatum*)	-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	Giri, 2017
	-the treatment was not effective alone in enhancing growth and yield of plants -co-inoculation with *Stenotrophomonas* 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 *Bacillus subtilis* led to significant increase in artemisinin content with 24.6%	Awasthi et al., 2011
	-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	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 -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	Kapoor et al., 2007
Rhizophagus intraradices (*Syn. R. irregularis, Glomus intraradices*)	-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	Domokos et al., 2018
	-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	Fortin and Melchert, 2015
	-inoculation resulted in increased endogenous jasmonic acid levels -the glandular trichome density and artemisinin concentration was enhanced	Mandal et al., 2015
	-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%)	Awasthi et al., 2011

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

Endophytic fungi	**The effect of the treatment**	**Reference**
Colletotrichum gloeosporioides	-the treatment promoted the artemisinin production by 51.63% in *A. annua* hairy root cultures	Wang et al., 2006
Colletotrichum 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	Wang et al., 2002
	-the elicitor extract from the fungus led to an increase in artemisinin content in hairy roots of *A. annua* (44% increase over the control)	Wang et al., 2001
Penicillium oxalicum	-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 *in vitro* propagated *A. annua* plantlets and induced oxidative stress through the production of reactive oxygen species	Zheng et al., 2016
Piriformospora indica	-inoculation promoted the growth of on *in vitro* 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% -the endophyte led to an increment in artemisinin content -dual inoculation with *Azotobacter chroococcum* 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
	-root length, shoot length, stem size, root thickness, dry weight and fresh weight of *in vitro* 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 *A. annua* 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**
Agrobacterium rhizogenes	-the infected *A. annua* seedling tissues produced neoplastic roots characterized by high growth rate, genetic stability and producing higher levels of secondary metabolites	Soleimani et al., 2012
Azospirillum sp.	-the treatment increased protein content in plants compared to chemical fertilizers	Keshavarzi and Nik, 2011
Azotobacter chroococcum	-an increase in total soluble sugar, total soluble protein and flavonoids content and carotenoid accumulation was observed -the treatment promoted plantlet growth -the artemisinin content was increased by 1.3-fold compared to control	Arora et al., 2017
	-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% -dual inoculation with *Piriformospora indica* 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
Azotobacter sp.	-the treatment increased protein content in plants compared to chemical fertilizers	Keshavarzi and Nik, 2011
Bacillus sp.	-inoculation increased protein content in plants compared to chemical fertilizers	Keshavarzi and Nik, 2011
	- 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
Bacillus subtilis	-inoculation improved height, leaf yield and total dry weight of the plants -significantly increased the mycorrhizal colonization of *G. mossae* by 50.32 % and phosphorus concentration in plants -co-inoculation with *G. mossae* 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 -co-inoculation with *G. fasciculatum* increased the artemisinin content by 24.6%	Awasthi et al., 2011
Pseudomonas fluorescens	-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
Pseudomonas sp.	-the treatment increased protein content in plants compared to chemical fertilizers	Keshavarzi and Nik, 2011
Radiobacter 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

Stenotrophomonas 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 *G. fasciculatum* -co-inoculation with *G. mosseae* increased phosphorus concentration	Awasthi et al., 2011
Streptomyces 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**
Acremonium persicum	-the elicitor extract prepared from the fungi increased the potted plants biomass and artemisinin content	Hussain et al., 2017
Aspergillus spp.	-the 11 endophytic extracts had different inhibitory effects on microbial pathogens -*Aspergillus* spp. exhibited the strongest activity against *Escherichia coli*, *Staphylococcus aureus* and *Trichophyton rubrum*	Zhang et al., 2012
Aspergillus terreus	-the fungi was isolated from *A. annua*, but no effect was studied	Zhang et al., 2010
Cephalosporium sp.	-*Cephalosporium* sp. extract had the strongest antimicrobial activity against *Magnaporthe grisea*	Zhang et al., 2012
Cladosporium sp.	-the pure cultures of the fungi had the greatest antibacterial (against *Staphylococcus aureus*, *Streptococcus mutans*, *Salmonella typhi*, *Bacillus subtilis*) and antifungal activity (*Malassezia furfur, Candida albicans*) out of the seven endophytic fungi obtained	Purwantini et al., 2015
Cochliobolus lunatus	-the elicitor extract prepared from the fungi increased the plant biomass and artemisinin content	Hussain et al., 2017
Colletotrichum gloeosporioides	-an increase in the plant biomass and artemisinin content was observed	Hussain et al., 2017
Colletotrichum sp.	-the fungi can produce antimicrobial and plant growth regulatory metabolites -the extract has antibacterial (against *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina lutea* and *Pseudomonas* sp.) and antifungal activity (against *Candida albicans*, *Aspergillus niger*, *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia cerealis*, *Helminthosporium sativum, Phytophthora capisici*)	Lu et al., 2000
Curvularia pallescens	-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
Leptosphaeria 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 *A. annua*	Liu et al., 2002
Mucor sp.	-*Mucor* sp. extract showed the most pronounced effect on *Rhizoctonia cerealis*	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 (Franche 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.

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