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Integrated Effects of Arbuscular Mycorrhizal Fungi and Vermicompost on Plant Growth, Nutrient Uptake, and Artemisinin Accumulation in *Artemisia annua* L.

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Abstract

Arbuscular mycorrhiza fungi (AMF) contribute to a positive role in secondary metabolism and production of active ingredients in aromatic and medicinal plants. This symbiotic association is particularly affected by the availability of phosphorus (P) in the soil. *Artemisia annua* L. is medicinal plant and used as anti-malarial drugs. Study was conducted on *Artemisia annua* L. using AMF, alone or combination with Vermicompost (VC) 0.05, 0.10 and 0.15 kg^{-1}. The effects of these fungi and their combinations with VC were determined in relation to the biomass, biochemical parameters, and essential oils content in *Artemisia annua* L. AMF treated plants with 0.1g kg^{-1} V.C. supply showed better growth, nutrients content and essential oils content compared to other treatment. These results suggest that AMF and V.C. combination enhancement plant growth, active constituent and essential oils content in plant. This combination may be substitute of inorganic fertilizer in cultivation of *Artemisia annua* L. Therefore, it is potentially representing an alternative way of promoting growth of this important medicinal herb, as natural ways of growing such crops are currently highly sought after in the herbal industry.

Keywords

Artemisia annua; Arbuscular Mycorrhizal Fungi; Essential Oil, Artemisinin, Vermicompost

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INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are an important group of soil microorganisms, classified in the phylum Glomeromycota (Schubler and Walker, 2010), AMF symbiosis is formed by approximately 80% of the vascular plant species in all terrestrial biomes (Smith et al 2010). Their beneficial effects on the nutrition and development of plants have been clearly shown since the extra-radical mycelium surrounding the plant roots (Gosling et al., 2006), not only extends the volume of the soil (Azcón et al., 2003) but also makes the absorption of the nutritive minerals more efficient (Schnepf et al., 2011). Inoculation with Arbuscular mycorrhizal fungi is often facilitating the acquisition of poorly accessible nutrients in plants (Cavagnaro, 2008) particularly phosphate (Smith et al., 2003) and thus promotes plant growth (Nell et al., 2010, Kumar et all 2017).

Vermicompost comprises the majority of nutrients in plant-available forms, such as nitrates, phosphates. It is characterized by a large surface area having many micro-sites for the microbial activity and

preservation of nutrients. It contains different microbial populations, plant growth regulator (cytokines and auxins). The most valued nutrients are exchangeable calcium and soluble potassium, which cause an increase in plant growth and secondary metabolite (Kumar et al 2017). The beneficial effects of vermicompost have been shown also in horticultural and agronomical crops.

Traditional systems of medicine are popular in developing countries, and up to 80% of the population relies on traditional medicines or folk remedies for primary health care needs. A great number of scientists and organizations turn their attention to traditional therapies in order to find and conserve important resources (Akerele, 1990). Medicinal herbs are widely sought worldwide for their natural properties.

The genus *Artemisia annua* comprises more than 150 species and is considered as one of the largest genera of the Asteraceae family (Evans, 1996). Several *Artemisia* species are used to treat malaria in various parts of the world. It is an annual herb which grows in several regions all over the world. The artemisinin of the plant is also used as perfumery (Bauer et al., 1997).

VAM fungi play an important role in ecological and productivity in plant (Fedderman et al., 2010). Mycorrhizal inoculation not only promoted the growth of medicinal plants but also improved the productivity and quantity of secondary metabolites. Many species belonging to the Asteraceae, including *Artemisia annua*, symbiosis with arbuscular mycorrhiza showed the better results (Wang and Qiu, 2006). In addition of AMF is increasing uptake of poorly available nutrients such as phosphorus and nitrogen (Smith and Read, 1997; Toussaint et al., 2004) or conferring protection against pathogens (Fillion et al., 1999).

However, it was reported that *Glomus mosseae* directly increases the essential oil content in shoots of *Origanum* sp., (Khaosaad et al., 2006) as well as sweet basil (Copetta et al., 2006). Arbuscular mycorrhiza increases artemisinin accumulation in *Artemisia annua* by higher expression of key biosynthesis genes via enhanced jasmonic acid levels.

The objective of this study was therefore to determine the influence of AMF inoculation and vermicompost fertigation on the growth, biomolecules, nutritional and essential oils content in *Artemisia annua*.

MATERIAL AND METHODS

The pot experiment was generated as comparisons between the following 8 treatments, as independent treatments arranged in a complete randomized design with three replicates

The experiment was done in Department of Life Sciences KVSCOS, SVSSU. Garden soil was fumigated applying 0.1% commercial formaldehyde. The fumigated soil was covered with a black polythene sheet and left for a week. Thereafter, the polythene was removed to dissipate the fumigate. After a week soil was transfer into pots for mass multiplication. In order to assess the effect of AMF fungi and vermicompost fertilizer on growth, Biomolecule, nutritional and essential oils content of *Artemisia annua* plant. Inoculation treatments (non-inoculated test control and seedlings inoculated with AMF) and three doses of VC (0.05, 0.1, and 0.15 kg kg−1 soil) with three replicates. Seeds were sown directly into pots. The pots contained 5 kg of sand and soil mix (50:50) with or without (control). AMF used from the previous culture which isolated from Garden soil of SVSU. About 35 days old seedlings were transplanted in row on first week of December. 500-gram soil inoculums (90-100 endomycorrhizal spore /10-gram soil along with 200 mg chopped AM colonized sorghum root (infection level 80%) was placed in furrow. Each pot before planting was observing up to seedling formation and maturation.

Biomass yield

Root and shoot fresh and dry biomass were measured and expressed in grams. This material was dried in the oven at 80ºC for 72 hours, after which the dry biomass of root and shoot were recorded.

Estimation of mycorrhizal root colonization

Mycorrhizal root colonization was determined after clearing roots with KOH and staining with trypan blue (Phillips and Hayman 1970). Root segments (1 cm length) were observed under light microscope, six microscope slides per replicate. Percent root colonization was assessed and scored following the method of Biermann and Linderman (1981). Frequency of arbuscule and vesicle was occurrence determined using a gridline intersection method (Giovannetti and Mosse 1980). A minimum of 100 root segments were used for this enumeration, and the colonization by AMF was calculated using the following formula.

$$\text{Per cent colonization (\%)} = \frac{\text{Total number of root segments colonized}}{\text{Total number of root segments studied}} \times 100$$

Quantification of mycorrhiza. The stained roots were cuts into segment of 1 cm length. 50 such segment was mounted in lactophenol and pressed lightly with thumb to prepare to squash. This squash preparation observed under a compound microscope to study the characteristic of AM fungi in the roots.

Photosynthetic pigment concentration (Hiscox and Israelstan, 1979)

Fresh leaves of plant were cut into small pieces, 0.1 g of tissue was taken in a test tube containing 7 ml Dimetthyl sulphoxide (DMSO) and incubated at 65°C for 1 hour. To this aliquot, 3 ml DMSO was added to make total volume 10 ml. Optical density was taken at 730 nm 510nm, 645nm and 663nmusing UV Spectrophotometer. Chlorophyll a, b and carotenoids were calculated by formulae given by Arnon (1949).

Estimation of Net photosynthetic rate, stomatal conductance and internal CO_2

Net photosynthetic rate (PN), stomatal conductance and internal CO_2 were measured on sunny days at 1100 hours using fully expanded leaves of *A. annua* with the help of an IRGA (Infra-Red Gas Analyzer, LI-COR 6400 Portable Photosynthesis System), Before recording the measurement, the IRGA was calibrated and zero was adjusted approximately every 30 min during the measurement period. Each leaf was enclosed in gas exchange chamber for 60 s (Gago et al 2016).

Nutrient analysis (Allen 1989)

Phosphorus The method was used to estimate the total phosphorus in digested material. A 5 mL aliquot was taken into a 10 ml test tube and 1 ml molybdic acid (2.5%) was added carefully, followed by addition of 0.4 ml 1-amino-2-naphthol-4 sulphonic acid. When the colour turned blue, the volume was made up to 10 ml with DDW. The solution was shaken for 5 minutes and was then transferred into a spectrophotometric tube. The OD of the solution was read on the spectrophotometer at 620 nm.

Potassium, Cupper, Manganese, and Zinc. Procedure amount of K, Cu, Mn, and Zn in sample solution was estimated using atomic absorption spectrophotometer. Calibration curve was prepared from standards, which was used to determine (K, Cu, Mn, and Zn) ppm in sample solution.

Macronutrient and Micronutrient was calculated by using the following formula:

$$\textbf{Macro. or micronutrients (µg g-1)} = \frac{\textbf{Macro. or micronutrients}(\textbf{ppm}) * - \textbf{Sol. volume } (\textbf{ml})}{\textbf{Sample weight } (\textbf{g})}$$

Essential oils estimation

Essential oils of *Artemisia annua* was extracted by hydrodistilation with a modified Clevenger trap (Simon and Quinn 1988). The distillation equipment consists of a boiler, distillation still condenser and receiver. 100-gram herbage was crushed in electric grinder with distilled water. The pulverized mass loaded into the still for distillation. The condenser cooled the hot vapours received from the still. The jet of vapours consisting of steam and essential oils were cooled in the condenser tubes which condensate flowed out into the receiver. The oil was drawn off and the volume of oil was noted. The percentage of essential oil content measured in volume/100 g fresh weight basis.

Statistical analyses

Treatment differences between treatments were determined using Duncan's multiple range tests at a significance level of 95%.

Table 1. Physicochemical properties of experimental soil.

Texture	Organic carbon %	pH (1:2.5)	Electrical conductivity (dS m−1) (1:2.5)	N	P	P	Ca	Na	Zn	Mn	Fe	Cu
Sandy clay loam	1.31	7.50±0.2	0.46	97.2	32.6	8.99	0.46	7.05	7.15	1.12	6.95	0.83

RESULTS

Effect of AMF and Vermicompost on biomass yield:

Artemisia annua plants responded positively to both of vermicompost and AMF inoculation showed increased shoot and root biomass. The results presented in Table 2 showed that all the parameters of plant biomass, considered in the present study were significantly increased in arbuscular mycorrhiza (AM) with vermicompost 0.1gram/kg soil treated plants as compared to other treatments (Table 2)

Table 2. Effect of AMF and Vermicompost fertilizer on fresh and dry weight of root and shoot per plant of *Artemisia annua* L.

Growth condition	Shoot fresh weight (in gram)	Shoot dry weight (in gram)	Root fresh weight (in gram)	Root dry weight (in gram)
AMF	54.33±1.35	6.55±0.17	7.99±0.15	3.86±0.12
0.05 VC	66.05±1.72	8.33±0.22	6.33±0.22	4.31±0.12
0.10 VC	74.01±1.88	9.11±0.24	6.74±0.21	5.01±0.15
0.15VC	75.01±1.88	9.11±0.24	6.74±0.21	5.01±0.15
AMF + 0.05 VC	93.1±2.98	9.89±0.26	12.3±0.39	3.47±0.15
AMF + 0.10 VC	98.99±3.12	10.6±0.33	10.91±0.36	4.22±0.13
AMF + 0.15VC	94.1±2.98	9.61±0.26	10.3±0.39	3.42±0.15
Control	18.87±0.52	5.01±0.14	4.01±0.13	3.54±0.15

Photosynthetic pigments concentration:

The estimated levels of chlorophyll pigments show that in the presence of vermicompost, the content of chlorophyll a and b is slightly higher in mycorrhizal plants than in non-vermicompost and control plants (Figure 1). Carotenoids content in *Artemisia annua* plants co-inoculated with AMF and vermicompost had highest carotenoids concentration than the plants given in other treatments (Figure 1).

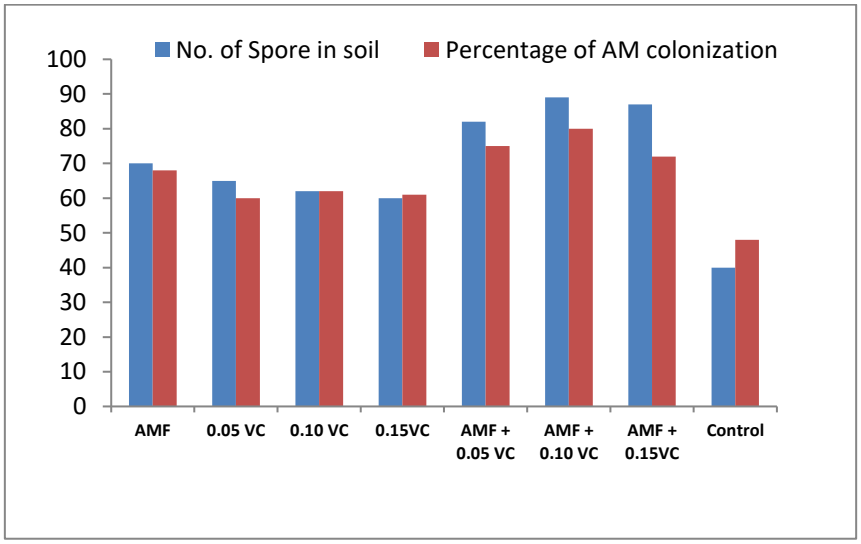

Figure 1. Effect of AMF and Vermicompost fertilizer on Number of Spore and AM colonization.

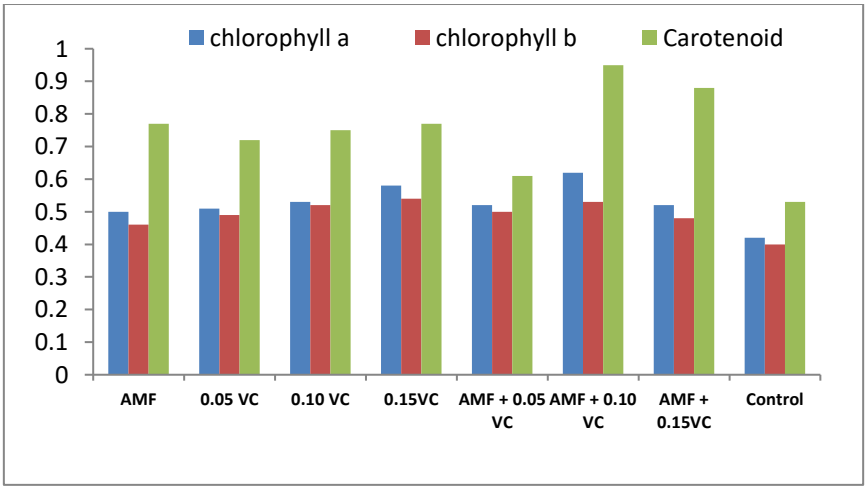

Figure 2. Effect of AMF and Vermicompost fertilizer on photosynthetic pigment.

Table 3. Effect of AMF and Vermicompost fertilizer on growth parameters of *Artemisia annua* L.

Treatments	Net photosynthetic rate (µ mol CO_2 m-2s-1)	stomatal conductance (mol m-2s-1)	Internal CO_2 (µ mol m-2s-1)
AMF	15.53±0.51	0.30±0.002	360±9.15
0.05 VC	16.12±0.42	0.33±0.002	356±8.80
0.10 VC	16.58±0.47	0.38±0.002	392±11.44
0.15VC	16.48±0.58	0.37±0.002	381±13.54
AMF + 0.05 VC	16.76±0.40	0.36±0.002	385±10.53
AMF + 0.10 VC	17.36±0.52	0.39±0.001	387±13.07
AMF + 0.15VC	17.22±0.59	0.38±0.002	370±9.62
Control	13.97±0.43	0.28±0.002	321±12.33

Table 4. Effect of AMF and Vermicompost fertilizer on macro and micro nutrient concentration of *Artemisia annua* L.

Treatments	Phosphorus			Potassium			Cupper			Manganese			Zinc			Iron		
	Shoot	Root	Fruit	Shoot	Root	Fruit	Shoot	Root	Fruit	Shoot	Root	Fruit	Shoot	Root	Fruit	Shoot	Root	Fruit
AMF	0.32±0.01	0.32±0.01	0.34±0.01	1.45±0.04	1.71±0.06	1.46±0.04	18.58±0.51	16.42±0.53	16.39±0.49	116.21±4.11	115.33±4.98	106.23±3.21	28.58±1.21	26.77	21.08±0.92	98.58±2.95	94.42±2.83	93.42±3.83
0.05 VC	0.33±0.01	0.33±0.01	0.31±0.01	1.31±0.03	1.33±0.04	1.04±0.03	18.53±0.49	17.51±0.49	16.85±0.50	118.10±3.22	117.10±4.11	111.10±3.27	28.51±1.10	26.77	22.41±0.82	98.53±3.04	92.51±2.47	90.57±2.17
0.10 VC	0.35±0.01	0.34±0.01	0.32±0.01	1.23±0.03	1.09±0.05	0.93±0.02	16.70±0.63	15.11±0.47	15.86±0.47	121.21±3.12	127.21±5.12	112.24±2.12	26.73±1.18	24.33	18.85±0.71	92.7±2.78	89.11±2.44	87.21±3.44
0.15VC	0.35±0.01	0.34±0.01	0.34±0.01	1.82±0.05	1.67±0.06	1.67±0.05	17.71±0.55	16.81±0.61	16.22±0.48	125.45±4.05	123.45±4.05	115.45±5.15	27.71±1.02	25.33	19.71±0.45	89.71±2.87	88.81±3.77	85.81±3.11
AMF + 0.05 VC	0.35±0.01	0.35±0.01	0.35±0.01	1.5±0.06	1.31±0.04	1.54±0.07	17.63±0.63	16.89±0.42	16.89±0.51	127.54±3.05	129.53±5.65	117.54±3.05	27.65±1.10	26.5	21.65±0.62	82.63±3.11	96.89±4.07	92.89±3.07
AMF + 0.10 VC	0.36±0.01	0.35±0.01	0.31±0.01	0.92±0.03	0.89±0.03	0.88±0.04	14.41±0.45	14.01±0.53	14.55±0.43	140.88±3.02	137.88±5.02	130.80±3.72	24.41±0.91	23.83	17.41±0.81	81.41±2.44	87.01±3.21	83.41±3.55
AMF + 0.15VC	0.36±0.01	0.35±0.01	0.35±0.01	1.5±0.06	1.31±0.04	1.54±0.07	17.63±0.63	16.89±0.42	16.89±0.51	127.54±3.05	129.53±5.65	117.54±3.05	27.65±1.10	26.5	21.65±0.62	82.63±3.11	96.89±4.07	92.89±3.07
Control	0.30±0.01	0.29±0.01	0.31±0.01	0.92±0.03	0.89±0.03	0.88±0.04	14.41±0.45	14.01±0.53	14.55±0.43	140.88±3.02	137.88±5.02	130.80±3.72	24.41±0.91	23.83	17.41±0.81	81.41±2.44	87.01±3.21	83.41±3.55

Mycorrhizal parameters

The number of spores in soil was significantly higher in vermicompost and AMF inoculated plants in comparison to other treatments. The number of spores was minimum in control plants. (Figure 2).

Biochemical parameters: Phosphorus and other nutrients content was measured at flowering stage. *Artemisia annua* plants increasing pattern was observed in Phosphorus and other nutrient content with vermicompost and mycorrhizal treatments. But minimum was in control plant (Table 3).

Estimation of Net photosynthetic rate, stomatal conductance and internal CO_2

Net photosynthetic rate (PN), stomatal conductance and internal CO_2 observed maximum at mycorrhizal and vermicompost treatment but minimum at control (Table 4).

Essential oils concentration: The concentration of essential oil of *Artemisia annua* plants increased significantly with mycorrhization and Vermicompost treatment (Table 4).

Table shows the concentration of essential oil maximum in AMF with 0.10gm Vermicompost treatments and minimum oil concentration was in control plant.

DISCUSSION

The present investigation demonstrated that the combined application of arbuscular mycorrhizal fungi (AMF) and vermicompost significantly enhanced growth, physiological, and biochemical parameters of *Artemisia annua*. The synergistic effect of these bio-inoculants was evident in improved biomass production, photosynthetic pigments, nutrient acquisition, and essential oil accumulation.

The increased shoot and root biomass observed in AMF and vermicompost-treated plants, particularly at 0.10 g/kg soil, may be attributed to improved nutrient availability and enhanced root architecture. AMF establish a symbiotic relationship with plant roots, facilitating better uptake of phosphorus and water through their extensive hyphal network, while vermicompost improves soil fertility and microbial activity. Similar findings were reported by Smith and Read (2008), who described the positive role of AMF in improving plant growth through nutrient mobilization. Likewise, Atiyeh et al. (2002) observed significant biomass enhancement in plants supplemented with vermicompost due to improved soil physicochemical properties.

Photosynthetic pigment analysis revealed higher chlorophyll a, chlorophyll b, and carotenoid contents in co-inoculated plants compared to control plants. The increased chlorophyll concentration indicates improved photosynthetic machinery and greater light-harvesting efficiency. This may result from increased nitrogen, magnesium, and phosphorus availability, which are essential for chlorophyll synthesis. Kumar et al 2017 reported similar enhancement in chlorophyll content in medicinal plants inoculated with AMF, suggesting improved nutrient assimilation. The elevated carotenoid content may also contribute to better antioxidant protection and photoprotection under stress conditions (Kumar and Johri 2017).

The higher spore density observed in AMF and vermicompost-treated soil indicates that vermicompost creates a favorable rhizospheric environment for fungal multiplication and colonization. Organic matter from vermicompost likely supports microbial interactions that stimulate AMF sporulation. This agrees with findings by Cavender et al. (2003), who showed that organic amendments improve mycorrhizal colonization and spore production.

The enhanced phosphorus and other nutrient contents in treated plants further support the role of AMF and vermicompost in nutrient acquisition. Phosphorus is one of the most limiting nutrients for plant growth and secondary metabolite synthesis. AMF improve phosphorus uptake by increasing the absorptive surface area of roots, while vermicompost provides readily available macro- and micronutrients. Similar results were reported by Azcón et al. (2003), where mycorrhizal plants showed increased nutrient accumulation and improved plant metabolism.

Gas exchange parameters such as net photosynthetic rate (PN), stomatal conductance, and internal CO_2 concentration were significantly higher in AMF and vermicompost-treated plants. This indicates improved carbon assimilation and stomatal regulation, likely resulting from enhanced nutrient uptake and better water status. Improved photosynthetic efficiency has also been linked to mycorrhizal associations in medicinal plants by Wu and Xia (2006).

Essential oil content, an important secondary metabolite in *Artemisia annua*, was significantly increased by the combined application of AMF and vermicompost. The maximum essential oil concentration recorded under AMF + 0.10 g vermicompost treatment suggests that improved nutrient status and photosynthetic efficiency positively influenced secondary metabolite biosynthesis. Previous studies by Copetta et al. (2006) also reported enhanced essential oil yield in medicinal plants under mycorrhizal inoculation, indicating the role of AMF in stimulating terpenoid biosynthesis.

Overall, the present findings suggest that AMF and vermicompost act synergistically to improve plant growth, nutrient uptake, photosynthetic performance, and essential oil production in *Artemisia annua*. This integrated biofertilizer strategy may provide an eco-friendly and sustainable alternative to chemical fertilizers for large-scale cultivation of medicinal plants.

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