IJPSR (2015), Vol. 6, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 March, 2015; received in revised form, 06 June, 2015; accepted, 22 September, 2015; published 01 October, 2015

EFFECT OF AM FUNGI AND DIFFERENT SELENIUM LEVELS ON THE QUALITY OF *SALVIA MILTIORRHIZA* BGE

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Keywords:

AM fungi; *Salvia miltiorrhiza* Bge; Selenium; Medicinal ingredients

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ABSTRACT: The effects of AM fungi on Salvia miltiorrhiza under different selenium levels was researched using pot technology. The results showed that inoculation of AM fungi significantly increased the plant height, root length, each part weight, chlorophyll, protein and selenium content of Salvia miltiorrhiza under the same selenium level, in addition, MDA and proline contents decreased significantly. It can be seen that AM fungi could promote the growth of Salvia miltiorrhiza, enhance disease resistance and improve the uptake of nutrient elements. The contents of tanshinone IIA in inoculated plants were higher than non inoculated plants. While the selenium concentration was 40μg.ml^{-1}, the content of tanshinone IIA in inoculated plants was highest, that was 0.272%. The growth of Salvia miltiorrhiza, soluble sugar, MDA and the content of tanshinone IIA increased firstly and then decreased, but the contents of inoculated plants were higher than non inoculated plants, they reached the maximum at Se1(20μg.ml^{-1})~Se2(40 μg.ml^{-1}). From that proper concentration was cooperated with AM fungi on improving the growth and accumulating of medicinal components. Selenium content in Salvia miltiorrhiza Bge was positively correlated with selenium concentrations, but no obvious linear relationship, the distribution was more in the leaf than root. Inoculation of AM fungi on the Salvia miltiorrhiza had the best effect when the selenium concentration was 20~40 μg.ml^{-1}.

INTRODUCTION: *Salvia miltiorrhiza* Bge. belonged to the dicotyledonous labiatae family, and could remove blood stasis, induce menstruation and relieve menalgia, pure heart and dispel vexed, cool and carbuncle and so on [1]. As a kind of Chinese traditional medicine of removing blood stasis, the market demand of Salvia miltiorrhiza Bge was overmuch, but in recent years, Chinese herbal medicine's long-term extensive planting resulted in its production and medicinal ingredients decreased year by year, seriously degradation of germplasm resources. So it was important to make use of the modern biological technology to improve the growing conditions so as to improve the yield and quality of Salvia miltiorrhiza.

Arbuscular mycorrhizal (referred to as AM) fungi was a kind of beneficial microorganisms in soil, and could form a symbiotic relationship with the majority of terrestrial plants. A huge network of hyphae was formed in the surrounding of host plant root, thereby increased the root absorption of water and nutrients in soil, otherwise AM fungal hyphae would compound some substance to adjust some chemical reactions in plants through its own to promote the growth, stress resistance [2, 3] and improve the effective component of plants. He Xueli [4] researched AM fungi could promote the growth of Minqin Seriphidium, impove total N, total P, total

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(10).4174-80

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(10).4174-80

flavonoids contents and the resistance of plants under water stress; in addition, with the pot experiments, the content of two species of medicinal components total-coumarins and imperatorin had been increased in the root of Angelica dahurica inoculated with AM fungi [5]. Zhao Qinghua [6] researched the effect of AM fungi and nitrogen amount on the growth of tea, the results showed that AM fungi could improve the content of tea polyphenols, caffeine, amino acids and water extracts under the certain nitrogen level, which significantly improved the quality of tea. Liu Lili [7] confirmed that the 5 kinds of arbuscular mycorrhizal fungi had increased the selenium rich ability of Hongyang kiwifruit leaves, polysaccharide content and photosynthetic index.

Selenium was a necessary element in human and animals and suitable concentration could regulate some plants' growth [8]. Selenium deficiency easily cause antioxidant stress of human body, susceptible to diseases such as cancer [9]. While the intake of selenium in the human body directly or indirectly came from plants, so plants played an important role in the selenium ecological chain. What's more the plants could significantly enhance the resistance, improve its quality after selenium supplementation [10].

Cheng Jialing applicated with suitable concentration of Na_2SeO_3 on the mulberry leaves, so that the content of selenium, amino acid, soluble protein, soluble polysaccharides and flavonoids were increased, the nutrition and health value were improved. While as a kind of selenium rich plant, *Salvia miltiorrhiza* supplemented with exogenous selenium could not only improve the yield and content of selenium, but also the selenium compounds and medicinal components *in vivo* may have the synergistic effect in the treatment of many diseases [11].

This experiment studied the effect of different content of selenium and AM fungi on the growth and medicinal components content in Salvia miltiorrhiza, in order to have a better development and utilization of AM fungal germplasm resources, improve the yield and quality of Salvia miltiorrhiza, promote the accumulation of selenium and medicinal ingredients, and play a better pharmacological action of Salvia miltiorrhiza.

MATERIALS AND METHODS:

Experiment materials:

Salvia miltiorrhiza seeds purchased from the Hebei Baoding Anguo medicine market in the march of 2013. Microbial inoculants obtained from rhizosphere soil containing spores and mycelium of ryegrass inoculated with AM fungi, each 10g dried soil containing 50 spores. Sodium selenite solution was sprayed on the foliar.

The tested soil was farmland cinnamon soil in Hebei Baoding, the collecting depth was about 0~20 cm, over 2 mm sieve, according to the mass ratio of soil: sand = 2: 1 mixed stand-by application. pH = 8.15, alkaline hydrolysis N: 58.137μg·g^{-1}, available phosphorus:15.96μg·g^{-1}, organic matter:12.97mg·g^{-1}, available potassium: 105.362μg·g^{-1}. Total selenium: 0.186mg·kg^{-1}, effective selenium: 18.767μg·kg^{-1}. Pot container was hard plastic basin with a hole in bottom, specification: 21.5 cm×16 cm×20.5 cm.

Experimental design:

Culture medium was non sterilized soil. The experiment set AM fungi and content of selenium two factors, while selenium amount set 4 levels, Na_2SeO_3 concentrations were 0, 20, 40, 80μg·ml^{-1}, the two treatments of AM fungi inoculation and non inoculation were set on the same selenium level, each treatment was replicated 4 times, that was total 32 basins.

Seeding on 2013-04-10, three kilogram soil for each pot, the fertilizers: 0.963g $CO(NH_2)_2$, 0.576g KH_2PO_4 and 0.636g K_2SO_4. Each inoculation treatment add inoculants 50g, applicated for two layers, while the control sample add equal amount of sterilizing inoculants. Daily management was implemented in Hebei University glass greenhouse, made a reasonable control of moisture and temperature, made sure each pot 3 trees after emergence, regular watering Ripper, selenium was sprayed on 2013-06-20. Configuring mass concentrations were 0, 20, 40, 80μg·ml^{-1} Na_2SeO_3, a selenium treatment was conducted every 20 days, 3 times in a row, and harvested on 2013-11-10.

Experimental methods:

The height of plant and length of main root were measured by tape after harvested. Dry mass of over ground and underground were measured by scale. The chlorophyll was extracted by acetone overnight, and then determined by UV spectrophotometer. The content of soluble protein was determined by Kaumas Coomassie brilliant blue G-250 staining method. The content of soluble sugar was determined by anthrone colorimetric method; Selenium was first made [12] into complex with o-diaminobenzene, and then determined by UV spectrophotometer. Tanshinone II_A was extracted by toluene and determined by HPLC [1].

RESULTS AND ANALYSIS:

The effect of AM fungi and selenium content on the growth of salvia miltiorrhiza. It can be seen from **Table 1**, on the same selenium level, the height, root shoot ratio, and dry mass over ground and underground of plants inoculated with AM fungi were significantly increased, indicated that AM fungi could significantly increased the growth of Salvia miltiorrhiza's each part comparing with back plants. AM fungi and selenium concentration did not play a leading role on the growth of root, this may be the main factor of root length had no significant changes.

TABLE 1: EFFECTS OF AM FUNGI ON THE GROWTH OF *SALVIA MILTIORRHIZA* UNDER DIFFERENT SE LEVELS

Na_2SeO_3concentration ($\mu g \cdot ml^{-1}$)	Inoculation	Plant height (cm)	Root length (cm)	Root shoot ration(%)	Dry mass(g)	
					Overground	Underground
0	AM	23.420±1.730A	21.067±0.902A	0.74±0.013B*	2.834±0.222A*	2.243±0.024B
	CK	19.830±1.956a	19.167±2.082a	0.639±0.007b	1.389±0.069a	2.093±0.443a*
20	AM	21.750±1.410A*	21.500±0.500A	0.758±0.009A*	3.258±0.484A*	3.204±0.481A*
	CK	19.250±1.521a	18.667±0.764a	0.657±0.011a	1.835±0.344a	1.522±0.246ab
40	AM	22.500±0.870A*	21.667±0.764A	0.773±0.007A*	2.866±0.281A*	2.870±0.279A*
	CK	18.670±1.253a	19.667±1.756a	0.658±0.008a	1.978±0.369a	1.533±0.170ab
80	AM	15.670±0.121B*	21.333±0.764A*	0.762±0.007A*	2.651±0.237A	2.164±0.160B*
	CK	20.170±1.900a*	17.167±1.258a	0.645±0.007ab	1.938±0.434a	1.343±0.242b

Note: The date is "mean±standard deviation" Under different selenium levels, different capital letters mean significant difference among inoculated plants, and lowercases in the same column mean significant difference among non-inoculated plants. The sign * means significant difference between inoculated and non-inoculated plants under same selenium level. All the differences are at 5% level. The same below.

On different selenium levels, the height of inoculated plants were basically the same on Se_0~Se_2 levels, but it significantly decreased on Se_3, illustrated that high selenium level inhibited the growth of Salvia miltiorrhiza. While plants without inoculation did not change significantly. The root length of inoculated and non inoculated plants had no significantly change with the increased of selenium, but on the level of Se_2 valued maximum. Selenium could improve the root shoot ratio of inoculated and non inoculated plants, but the concentration of Se had no obvious effects. The dry mass over ground of inoculated and non inoculated plants had no obvious differences between Se_0 to Se_3, which was consistent with the change of plant height, but increased firstly with the change of selenium concentration and then decreased, the inoculated reached maximum at Se_1, while non inoculated at Se_2, illustrated that AM fungi enhanced the promoting effect of selenium on the growth of Salvia miltiorrhiza. The dry mass underground of inoculated plant increased firstly and then decreased with selenium concentration increased, and there was significant difference between Se_2, Se_1, and Se_0, Se_3, reached at maximum on Se_1 level, while dry mass underground of the non inoculated plants significantly reduced after added selenium. From this we can see that AM fungi could significantly promote the growth of Salvia miltiorrhiza, the effect of selenium on the growth of Salvia miltiorrhiza is not apparent, but AM and selenium together had the synergy effect, they can significantly promote plant growth, plant height, root length, quality of each part. But it could also inhibit the growth of Salvia miltiorrhiza Bge when the concentration of selenium was higher. So the best selenium concentration is Se_1~ Se_2.

The effect of AM fungi and selenium on the physiological indexes of salvia miltiorrhiza. It can be seen from **Table 2**, on the same selenium level, the content of chlorophyll and protein in the leaf of Salvia miltiorrhiza seedlings inoculated with AM fungi were significantly increased, showed that the inoculated plants grew well, and had stronger photosynthesis and higher survival ability. The mass fraction of proline and malonaldehyde were lower, illustrated that AM fungi could increase the resistance of plants. The content of soluble sugar was lower than non inoculated strains, so AM fungi could not improve the content of soluble sugar.

On the different selenium levels, the mass fraction of chlorophyll decreased gradually with the increasing of selenium concentration, and had no significant decrease for inoculated plants, but it was opposite for non inoculated ones, indicated that selenium affect the synthesis of chlorophyll and AM fungi could reduce this effect. There was no significant difference between the content of protein for inoculated and non inoculated plants with the increase of selenium concentration. The content of soluble sugar for inoculated plants increased firstly and then decreased with the adding of selenium, and reached the highest at Se_1, but reached the highest at Se_2 for non strains. The molal concentration of Malonaldehyde was highest at Se_2 for inoculated ones, but at Se_1 for non inoculated plants, and had significant differences with different selenium concentrations. In addition the mass fraction of proline significantly decreased for all plants with the increase of selenium, illustrated that selenium could increase the resistance of plants to the environment.

But the content of chlorophyll, soluble sugar and malondialdehyde in fresh leaves of salvia miltiorrhiza reduced significantly on the condition of high selenium, showed that the higher selenium affect the health of Salvia miltiorrhiza. Taken together, AM fungi and selenium played a synergistic effect on the growth and increasing resistance of plants, so the optimal cultural conditions for Salvia Miltiorrhiza was inoculated with AM fungi and sprayed selenium when the concentration was 20~40μg·ml^{-1}.

TABLE 2: EFFECTS OF AM FUNGI ON THE PHYSIOLOGICAL INDEX OF *SALVIA MILTIORRHIZA* UNDER DIFFERENT SE LEVELS

Na_2SeO_3 concentration (μg·ml^{-1})	Inoculation	Chlorophyll (mg.g^{-1})	Protein (mg.g^{-1})	Soluble sugar (mg.g^{-1})	Malonaldehyde (mmol.g^{-1})	Proline (μg.g^{-1})
0	AM	1.765±0.368A*	4.461±0.276A*	5.261±0.559B	3.042±0.021D	11.185±2.901A
	CK	1.423±0.053a	4.054±0.121a	6.097±0.116bc*	3.052±0.065c*	15.845±0.310a*
20	AM	1.758±0.074A*	4.373±0.069A*	6.090±0.075A	3.336±0.049C	9.670±0.152A
	CK	1.315±0.144ab	4.067±0.0514a	6.325±0.188b	3.658±0.138a*	14.545±0.627b*
40	AM	1.690±0.076A*	4.554±0.026A*	5.056±0.127BC	3.583±0.064A	9.200±0.054B
	CK	1.244±0.156ab	3.982±0.126a	7.069±0.050a*	3.122±0.085c*	13.910±0.884b*
80	AM	1.603±0.065A*	4.598±0.079A*	4.572±0.046C	3.449±0.070B*	8.640±0.203C
	CK	1.186±0.019b	4.105±0.126a	5.881±0.107c*	3.307±0.049b	9.730±0.573c*

The selenium content of Salvia miltiorrhiza:

It can be seen from **Fig. 1**, the selenium concentration in each part of Salvia miltiorrhiza increased significantly, and selenium in the stem and leaf was more than root for most Salvia miltiorrhiza, that was opposite only on the level of Se_1 with inoculated plants and Se_0 with all plants. Whant's more selenium of high dosage group was higher than that of low dose group, and there was a positive correlation between selenium concentration in different part of Salvia miltiorrhiza and conentration of Na_2SeO_3, but they had no significant linear relationship. The mass fraction of selenium in each part of Salvia miltiorrhiza was significantly improved for inoculated ones, but on the level of Se_3, selenium in the inoculated strains was lower than non inoculated strains, that was probably due to that higher concentrations of selenium reduced the promotion of AM fungi to absorb and utilize selenium.

The content of tanshinone:

The root of Salvia miltiorrhiza inoculated and non inoculated with AM fungi were extracted after selenium treatment, and then the standard and samples of tanshinone II_A were analyzed by HPLC according to Chinese Pharmacopoeia, and meet

the relevant requirements of system suitability test, obtained the mass fraction of tanshinone II_A on each level as shown in **Fig.2**. It can be seen from the figure, the concentration of tanshinone II_A in inoculated strains were higher than that of non inoculated plants on the same selenium level, and increased significantly on Se_0, Se_2, and Se_3 levels. The mass fraction of tanshinone II_A was increased firstly and then decreased on different selenium levels, and reached the peak at Se_2.What's more there was significant differences between each level especially for Se_2 with others. From this we could see that selenium could promote the accumulation of tanshinone II_A, but higher selenium concentration will also play the bad role. The HPLC of tanshinone II_A extraction for inoculated plants on the level of Se_2 was analyzed, obtained that the area of peak was 1589868. The mass fraction of tanshinone II_A was 0.272% after calculated that was in line with the provisions of Chinese Pharmacopoeia (more than 0.2%).

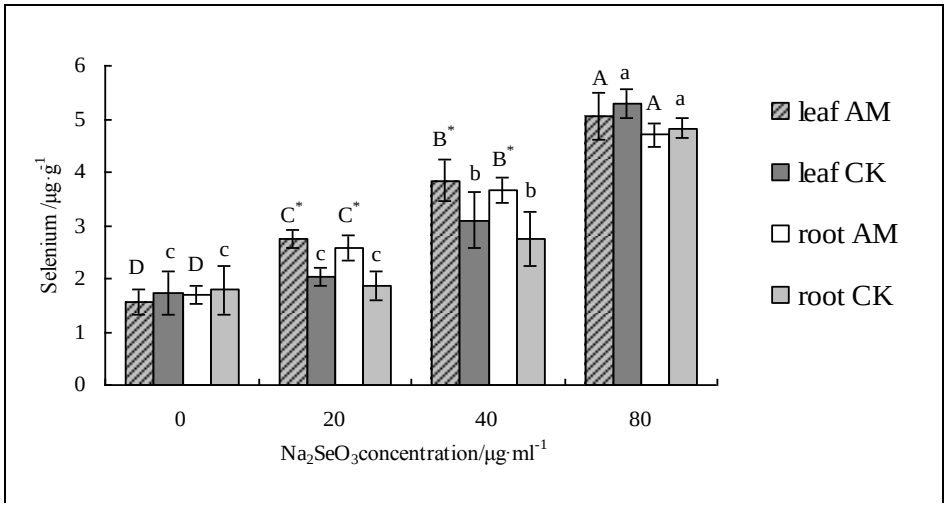

FIG.1: THE CONTENT OF SE IN *SALVIA MILTIORRHIZA* EACH PART WITH AM FUNGI UNDER DIFFERENT SELENIUM LEVELS

Note: Under different selenium levels, different capital letters mean significant difference among inoculated plants, and lowercases mean significant difference among non-inoculated plants. The sign * means significant difference between inoculated and non-inoculated plants under same selenium level. All the differences are at 5% level. The same below.

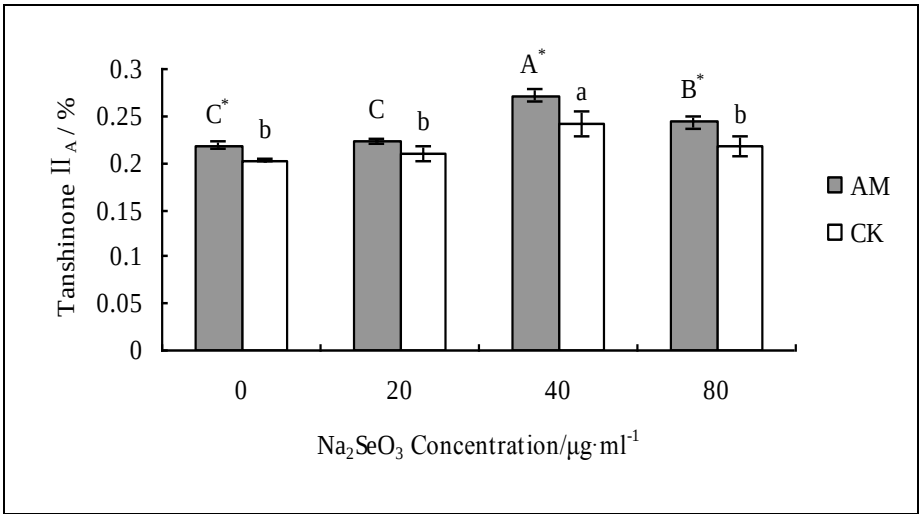

FIG. 2: THE CONTENT OF TANSHINONE II_A IN *SALVIA MILTIORRHIZA* WITH AM FUNGI UNDER DIFFERENT SELENIUM LEVELS

DISCUSSION: AM fungi could promote the growth of Salvia miltiorrhiza, improve the absorption of nutrients and accumulation of medicinal components, and strength the resistance of plant. Jinghua Yellow [13] reported that AM fungi could improve the growth of Artemisia annua, promote the absorption of N, P, K elements and accumulation of medicinal components.

Fidelibus[14] showed that AM fungi could promote the growth of various lemon and development of root. In this experiment, inoculated with AM fungi could significantly increased the growth of Salvia miltiorrhiza. While the height and dry mass of inoculated plants were better than the non inoculated plants, from that we could see Salvia miltiorrhiza inoculated with AM fungi had a well growing, so the mass fraction of chlorophyll and proteinin fresh leaves was higher. Compared with non inoculated plants, the resistance to environment was stronger, so the content of soluble sugar, proline and Malonaldehyde in young leaves of Salvia miltiorrhiza decreased significantly. In addition, the root grew more stronger after AM fungi formed symbiosis with Salvia miltiorrhiza, so the root shoot ratio and dry mass underground were higher than that of non inoculated plants. While the strong roots could absorb more nutrients in the soil, and then promote the growth and accumulation of medicinal components. What's more, the mass fraction of selenium in each part of inoculated strains was higher than that of non inoculated ones, at the same time, the suitable amount of selenium could also promote the accumulation of tanshinone II_A.

Selenium was a trace amount element whose physiological function was complex, which could adjust the dynamic life activities of plants and animals, and had a closely relationship with plant health [15]. While the Chinese herbal medicine enriched with selenium was regarded as selenium drug, not only could improve the human selenium deficiency, but also enhance the efficacy of Chinese herbal medicine themselves. Qiang Liu [16] analyzed forms of selenium systematically in the selenium enriched Salvia miltiorrhiza, obtained that the supplementation of selenium could increase the content of Cu, Ni, Mn in Salvia miltiorrhiza. Wenbo Zhang [11] researched the secondary metabolites in selenium enriched plants, showed that Se accumulated cultivation could improve the content of tanshinone $_A$ and salvianolic acid B, and enhance antioxidant activity of the two *in vitro*.

In this experiment the growth of Salvia miltiorrhiza increased firstly and then decreased with the addition of selenium, showed that higher concentrate selenium inhibited the growth, while suitable concentration (Se_1~Se_2) promoted. But selenium could not improve the content of chlorophyll and protein, which may be related to the differences between species or the combination of selenium and protein *in vivo* [17]. The distribution law of selenium is stems and leaves＞roots, which are determined by mass fraction of selenium in roots, stems and leaves of Salvia seedlings. First of all, selenium is enriched in the organs which are near the source of selenium, probably due to the use of spraying selenium on leaves.

In this experiment, the inoculation of AM fungi can improve the absorption of selenium of Salvia seedlings, but when the mass concentration of selenium is higher, the inoculated strains and the non-inoculated strains of selenium content were both higher. Perhaps when the concentration of selenium is higher, AM fungi is not the main factor that influences the absorption of selenium on Salvia, so there are non-inoculated strains with higher selenium content. When the mass concentration of selenium is 20~40μg.ml^{-1}, inoculation AM fungi can greatly promote the absorption of selenium in Salvia. AM fungi and roots formed a huge hyphae network after symbiosis that makes Salvia grow better, promote the absorption of selenium in plants, and their synergy can improve the absorption of nutrients in soils in Salvia and Enrichment of effective medicinal components, enhance the resistance of plants.

CONCLUSION: According to the experimental results, AM fungi and selenium have a significant interaction effect for Salvia, the optimal conditions for planting in the experiment turn out to be that the AM fungi is inoculated, and the mass concentration of selenium is about 20~40 μg.ml^{-1}, it has important significance for improving the quality of Salvia, ensure that Danshen medicinal value, and to realize the modernization of planting traditional Chinese medicine.

ACKNOWLEDGEMENTS: The authors thank to college of pharmaceutical sciences, Hebei University for providing all the facilities to carry out this work.

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How to cite this article:
Jianheng LI, Yajun QIAO, Junxiang Qi, Hanqiu WU and Xiaoyu YANG: Effect of Am Fungi and Different Selenium Levels on the Quality of Salvia Miltiorrhiza Bge. Int J Pharm Sci Res 2015; 6(10): 4174-80.doi: 10.13040/IJPSR.0975-8232.6(10).4174-80.