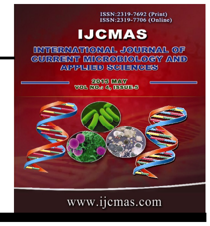

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 5 (2015) pp. 90-96**
http://www.ijcmas.com

Original Research Article

Significant Effect of Mycorrhization on some Physiological Parameters of *Salvia officinalis* Linn. Plant

Aditya Kumar[1*], Chhavi Mangla[2] and Ashok Aggarwal[3]

[1]Department of Botany, Dayanand Post Graduate College, Hisar-125001 Haryana, India
[2]Department of Botany, DAV College (Lahore), Ambala City-134003 Haryana, India
Mycology and Plant Pathology Laboratory
[3]Department of Botany, Kurukshetra University, Kurukshetra-136119 Haryana, India
**Corresponding author*

A B S T R A C T

Keywords

Trichoderma viride, *Glomus mosseae*, *Acaulospora laevis*, medicinal plants

The effect of two arbuscular mycorrhizal fungi (AMF) and *Trichoderma viride* on the physiological parameters of *Salvia officinalis* was studied in green house pot experiment. The arbuscular mycorrhizal (AM) plants had higher chlorophyll content (a, b and total) as compared with the non-AM plants. The *Salvia* plants inoculated with *Acaulospora laevis* plus *T. viride* had higher phosphorus content (0.844±0.02; 0.977±0.02) in shoot and root after 45 days. Likewise, ninety days after inoculation (DAI), maximum P content in shoot and root was observed in the plants treated with *A. laevis* plus *Glomus mosseae* (0.975±0.03) and *A. laevis* plus *T.viride* (1.223±0.04) respectively. Content of P was observed more in root than shoot. The results suggest that mycorrhizal symbiosis affects plant physiology thereby bioprospecting the possibility of mycorrhizal inoculation in order to improve the cultivation practices of medicinal plants.

Introduction

Salvia officinalis an evergreen sub-shrub enjoys the reputation of being a panacea because its wide range of medicinal effects. The plant has been reported to contain various types of secondary metabolites with anti-inflammation, antioxidant, antibacterial and mutagenic activities (Wang *et al.*, 1998; Baricevic and Bortol, 2000; Baricevic *et al.*, 2001). The extract of *Salvia* has been credited with medicinal use such as spasmolytic, antiseptic and astringent (Newall *et al.*, 1996). Modern day clinical trials have shown that its essential oil can improve memory and has shown promise in the treatment of Alzheimer's disease (Akhondzadeh and Abbasi, 2006).

Medicinal plants sector represents an important health and economic component of biodiversity. The rising demand for these species has exhausted its wild stock and necessitated large-scale cultivation. Hence, a scientific approach towards its cultivation and conservation with the help of AM technology could be a great option. A well developed arbuscular mycorrhizal system increases the sustainability of plant production. AM fungal symbiosis may be obtained by inoculation of plant with the desired efficient AM species. Pre-inoculation with AM fungi is an obvious management practice in crop established as transplants and may lead to increased yield not only in low P-soil but also in soil with moderate and even high phosphorus availability (Douds and Reider, 2003). Pre-inoculation with AM fungi has been demonstrated to increase higher biomass production of plants (Sorensen *et al.*, 2008).

The strains of *Trichoderma* induce metabolic changes in plants that increases resistance to a wide range of plant pathogenic micro-organisms and viruses (Harman *et al.,* 2004). The mobilization and further translocation of phosphorus from conidia of fungus *T. virens* into mycorrhizal *Pinus sylvestris* seedlings from organic matter has been well recognized (Zadworny *et al.,* 2008). The interactions between AM fungi and *T. viride* may be of crucial importance with in sustainable, low input agricultural cropping systems that rely on biological processes rather than agrochemicals to maintain soil fertility as well as plant health and growth (Artursson *et al.,* 2006; Kumar *et al.,* 2014).

Based on review of previous studies, the present work aims to examine the efficacy of AM inoculation and *T. viride* on different physiological parameters of *S. officinalis* after 45 and 90 days of inoculation.

Material and Methods

Plant material and growth conditions

Two months old seedlings of *S. officinalis* were procured from Dr. Y. S. Parmar University of Horticulture & Forestry, Nauni, Himachal Pradesh. Soil was collected from Botanical garden of Botany Department, Kurukshetra University, Kurukshetra and was sieved to remove the debris or large organic matter and then sterilized. Seedlings were grown in earthern pots (size 25×25 cm.) and in each pot, 10% inoculum of each AM fungi and *T. viride*, alone and in combination was added. The experimental pots were maintained in the polyhouse conditions where temperature (27-35ºC), humidity (80%) and light intensity (15000-19000 lux.) were maintained. Plants were watered regularly as and when required and nourished with Hoagland nutrient solution (without KH_2PO_4) after every 15 days during the course of experimentation. Three replicates were utilized for each treatment.

AM endophytes and *T.viride*

Two dominant species of AM fungi (*G. mosseae* and *A. laevis*) were isolated from rhizospheric soil of selected medicinal plant and were used alone and in dual combination with *T. viride*. The starter inoculum or pure culture of each selected AM fungus was raised by ‘‘Funnel Technique’’ of Menge and Timmer (1982) and further mass produced. *T. viride* was isolated from soil by Warcup’s soil plate method and further used for mass culturing using wheat bran: saw dust medium.

Estimation of physiological parameters

The content of Chlorophyll (a, b and total) in all experimental plants was measured by the method of Arnon (1949). The phosphorus content in shoot and root in the test plants was determined by Vanado-molybdo-phosphoric acid yellow colour method, in nitric acid system outlined by Jackson (1973). The effect of different treatments was recorded at 45 and 90 days after planting (DAP).

Statistical analysis

The data was statistically analyzed by using analysis of variance (ANOVA) followed by post hoc test through computer software SPSS 16.0 (SPSS Inc. Chicago, IL). Means were then ranked at P=0.05 level of significance using Duncan's Multiple Range Test for comparison.

Results and Discussion

Arbuscular mycorrhizal fungi are well known to enhance the nutritional status of several plants and thereby aid in increased growth and yield. The present investigation was carried out in order to evaluate the potential of AM fungi and *T. viride* on physiological parameters of *S. officinalis* plant. Results elucidated that the seedlings of all plants under investigation varied in their response to inoculation with AM fungi and *T. viride* in different combinations or treatments of inoculation and also showed the dependence of these plants on such types of inoculations.

Effect of inoculation on physiological parameters after 45 and 90 days (Table 1, 2)

Studied physiological parameters such as chlorophyll content and percent phosphorus content were analyzed after 45 and 90 days of inoculation. Both the parameters showed a varied degree of performance with different inoculants of AM fungi and *T. viride*, when used alone and in combination.

Chlorophyll content

AM symbiosis enhances the chlorophyll concentration of *S. officinalis* after 45 and 90 days of inoculation. As evident from the Tables (Iand II), dual combination of *G. mosseae* plus *T. viride* showed maximum increase in chlorophyll a (0.876±0.02), chlorophyll b (0.285±0.02) and total chlorophyll (1.161±0.04) after 45 days of inoculation. On the same lines, ninety DAI, maximum increment in chlorophyll a (1.111±0.02), chlorophyll b (0.353±0.04) and totol chlorophyll (1.451±0.07) was registered in the treatment of *A. laevis* plus *T. viride*.

Increase in total chlorophyll content in inoculated plants may be due to increased uptake of phosphorus which increases the photosynthetic activity of plants and ultimately the chlorophyll content. Similar findings were reported by Karthikeyan *et al.* (2009) and Colla *et al.* (2008). Chlorophyll a, chlorophyll b and total chlorophyll content of AM plants is always higher than that of non- AM plants (Rabie, 2005). Cantrell and Linderman (2001) observed significantly greener leaves (more chlorophyll) in mycorrhizal plants than those of non- mycorrhizal lettuce plants and non- AM onion to be stunted due to phosphorus deficiency.

Phosphorus content

In the present investigation, when P content was estimated in plants of *S. officinalis*, there was more increment in P content in all the inoculated seedlings over control. Content of P was observed more in roots than shoots. After 45 days of inoculation, maximum increase in shoot and root P content was found in *A. laevis* plus *T. viride* (0.844±0.02, 0.977±0.02) followed by *G. mosseae* plus *T. viride* (0.824±0.03, 0.882±0.02). Ninety DAI, maximum P content in shoot and root was observed in the plants treated with *A. laevis* plus *G. mosseae* (0.975±0.03) and *A. laevis* plus *T. viride* (1.223±0.04), respectively.

Table.1 Effect of Arbuscular Mycorrhizal Fungi and *T. viride* on growth performance of *S. officinalis* after 45 days

Treatments	**Chlorophyll Content (mg./gm. fresh wt.)**			**% Phosphorus Content**	
	Chl.a	**Chl.b**	**Total Chl.**	**Shoot P**	**Root P**
Control	*0.533±0.03[e]	0.188±0.01[b]	0.721±0.05[e]	0.310±0.02[e]	0.406±0.02[d]
Trichoderma viride	0.732±0.03[d]	0.211±0.01[b]	0.943±0.04[c]	0.641±0.03[c]	0.811±0.06[bc]
Glomus mosseae	0.795±0.03[b]	0.262±0.03[a]	1.057±0.07[b]	0.561±0.02[d]	0.872±0.07[b]
Acaulospora laevis	0.645±0.02[d]	0.199±0.01[b]	0.844±0.03[d]	0.634±0.05[c]	0.778±0.03[c]
A.laevis+ G.mosseae	0.652±0.02[d]	0.203±0.01[b]	0.855±0.04[d]	0.730±0.03[b]	0.787±0.03[c]
G.mosseae+ T.viride	0.876±0.02[a]	0.285±0.02[a]	1.161±0.04[a]	0.824±0.03[a]	0.882±0.02[b]
A.laevis+ T.viride	0.760±0.02[c]	0.252±0.02[a]	1.012±0.04[bc]	0.844±0.02[a]	0.977±0.02[a]

* Each value is an average of three replicates

Means values followed by different alphabet/s are significant over one another by Duncan's Multiple Range Test at P= 0.05.

± Standard Deviation

Table.2 Effect of Arbuscular Mycorrhizal Fungi and *T. viride* on growth performance of *S. officinalis* after 90 days

Treatments	Chlorophyll Content (mg./gm. fresh wt.)			% Phosphorus Content	
	Chl.a	**Chl.b**	**Total Chl.**	**Shoot P**	**Root P**
Control	*0.746±0.04[e]	0.216±0.01[c]	0.963±0.06[c]	0.525±0.04[e]	0.677±0.04[e]
Trichoderma viride	0.959±0.03[bc]	0.311±0.03[ab]	1.270±0.06[b]	0.726±0.04[c]	1.063±0.04[bc]
Glomus mosseae	1.011±0.03[b]	0.304±0.03[ab]	1.315±0.06[c]	0.617±0.01[d]	0.952±0.05[d]
Acaulospora laevis	0.814±0.02[d]	0.244±0.04[bc]	1.058±0.07[c]	0.636±0.03[d]	1.020±0.02[c]
A.laevis* + *G.mosseae	0.925±0.02[c]	0.299±0.03[ab]	1.224±0.06[b]	0.975±0.03[a]	1.009±0.02[c]
G.mosseae* + *T.viride	0.917±0.02[c]	0.294±0.02[ab]	1.212±0.05[b]	0.871±0.03[b]	1.099±0.03[b]
A.laevis* + *T.viride	1.111±0.02[a]	0.353±0.04[a]	1.451±0.07[a]	0.931±0.03[a]	1.223±0.04[a]

* Each value is an average of three replicates

Means values followed by different alphabet/s are significant over one another by Duncan’s Multiple Range Test at P= 0.05.

± Standard Deviation

Such higher P content in AMF inoculated plants is attributed to higher influx of P into the plant system through AM fungi which explores the soil volume beyond P depletion zone. As suggested by Tinker (1975), increase in P efficiency by mycorrhizal plants could arise due to following factors: (i) morphological changes in the plant (ii) provision of additional or more efficient absorbing surface in the fungal hyphae with subsequent transfer to the host and (iii) ability of the mycorrhizal root or hyphae to utilize source of P not available to non mycorrhizal roots. Further, results obtained from present study also corroborated with findings of Khare and Rodrigues (2009) and Arpana *et al.* (2008) who reported an increased P content in mycorrhizal *Carica papaya* and *Pogostemon cablin* plants over non mycorrhizal plants when these plants were subjected to inoculate with different AM fungal strains.

Beneficial AMF are one of the important cornerstones of sustainable agricultural systems. The results of the present study clearly brought out the beneficial effect of inoculation with *G. mosseae*, *A. laevis* and *T. viride*, alone and in different combinations on various physiological parameters *S. officinalis*. As mycorrhizal fungi are beneficial for plant establishment, this study provides a good scope for commercially utilizing the efficient strains of AM fungi to exploit them for their beneficial effects with other beneficial rhizospheric microflora in establishment of seedlings, increase in productivity and reduce the fertilizer application required for obtaining economic production of these plants under field conditions.

Acknowledgements

The authors are thankful to Kurukshetra University, Kurukshetra for providing financial support and extending essential facilities to carry out this research work.

References

Akhondzadeh, S., Abbasi, S.H. 2006. Herbal medicine in the treatment of Alzheimer disease. *Pam. J. Alzheimers Disother Deman.*, 21: 113–118.

Arnon, D.T. 1949. Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris. Plant Physiol.*, 24: 1–5.

Arpana, J., Bagyaraj, D.J., Parkasa Rao, E.V.S., Parameswaran, T.N., Abdul Rahiman, B. 2008. Symbiotic response of Patchouli (*Pogostemon cablin* (Blanco) Benth. to different arbuscular mycorrhizal fungi. *Adv. Environ. Biol*., 2(1): 20–24.

Artursson, V., Finlay, R.D., Jansson, J.K. 2006. Interactions between AMF and bacteria and their potential for stimulating plant growth. *Env. Microbiol*., 8(1): 1–10.

Baricevic D., Sosa S., Della Loggia R., Tubaro A., Simonovska B., Krasna A., Zupancic A. 2001. Topical anti-inflammatory activity of *Salvia officinalis*L. leaves: the relevance of ursolic acid. *J. Ethnopharmacol*., 75: 125–132.

Baricevic, D., Bartol, T. 2000. The biological/ pharmacological activity of the *Salvia* genus. In: S.E. Kintzios (Ed.), SAGE- The genus *Salvia* spp. Harwood Academic Publishers, Amsterdam, The Netherlands. Pp. 143–184.

Cantrell, C., Linderman, R.G. 2001. Preinoculation of lettuce and onion with VA mycorrhizal fungi reduce deleterious effects of soil salinity. *Plant soil*, 233: 269–281.

Colla, G., Rouphael, Y., Cardarelli, M., Tullio, M., Rivera, C.M., Rea, E. 2008. Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. *Biol. Fert. Soils*, 44: 501–509.

Douds, D.D., Reider, C. 2003. Inoculation with mycorrhizal fungi increases the yield of green peppers in a high P soil. *Biol. Agric. Hortic*., 21: 91–102.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M. 2004. *Trichoderma* spp.: Oppurtunistic avirulent plant symbionts. *Nature Microbiol. Rev*., 2: 43–56.

Jackson, M.L. 1973. Soil chemical analysis, Prentice Hall Pvt. Ltd., New Delhi, India. Pp. 239–241.

Karthikeyan, B., Joe, M.M., Jaleel, C.A. 2009. Response of some medicinal plants to vesicular arbuscular mycorrhizal inoculations. *J. Sci. Res*., 1(1): 381–386.

Khare, S.W., Rodrigues, B.F. 2009. Studies on effects of arbuscular mycorrhizal (AM) fungi on mineral nutrition of *Carica papaya* L. *Not. Bot. Hort. Agrobot. Cluj*., 37(1): 183–186.

Kumar, A., Mangla, C., Aggarwal, A., Srivastava, V. 2014. Rhizospheric effect of endophytic mycorrhiza and *Trichoderma viride* on physiological parameters of *Mentha Spicata* linn. *Asian J. Adv. Basic Sci.*, 2(1): 99–104

Menge, J.A., Timmer, L.M. 1982. Procedure for inoculation of plants with VAM in the laboratory, greenhouse and field. In: N. C. Schenck (Ed.), Methods and principles of mycorrhizal research. A.P.S. Press, St. Paul, Minnesota. Pp. 59–68.

Newall, C.A., Anderson, I.A., Philipson, J.D. 1996. Herbal medicines: A guide for healthcare professionals. The Pharmaceutical Press, London, 231 Pp.

Rabie, G.H. 2005. Influence of arbuscular mycorrhizal fungi and kinetin on the response of mungbean plants to irrigation with seawater. *Mycorrhiza*, 15: 225–230.

Sorensen, J.N., Larsen, J., Jakobsen, I. 2008. Preinoculation with arbuscular mycorrhizal fungi increases early nutrient concentration and growth of field- grown leeks under high productivity conditions. *Plant Soil*, 307: 135–147.

Tinker, P.B. 1975. Soil chemistry of phosphorus and mycorrhizal effects on plant growth. In: F.E., Sanders, F.E., B. Mosse, and P.B. Tinker (Eds.), Endomycorrhizas. Academic Press, London. Pp. 353–371.

Wang, M., Li, J., Rangarajan, M., Shao, Y., La Voie, E.J., Huang, T.C., Ho, C.T. 1998. Antioxidative phenolic compounds from sage (*Salvia officinalis*). *J. Agr. Food Chem*., 46: 4869–4873.

Zadworny, M., Gorski, Z., Koczorowska, E., Werner, A. 2008. Conidia of *Trichoderma virens* as a phosphorus source for mycorrhizal *Pinus sylvestris* seedlings. *Mycorrhiza*, 19: 61–66.