Original Research Article

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

Aditya Kumar¹*, Chhavi Mangla² and Ashok Aggarwal³

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

*Corresponding author

**ABSTRACT**

The effect of two arbuscular mycorrhizal fungi (AMF) and *Trichoderma viride* on the physiological parameters of *Salvia officinalis* was studied in greenhouse 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.

**Keywords**

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

**Introduction**

*Salvia officinalis* an evergreen sub-shrub enjoys the reputation of being a panacea because of 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 spasmyloytic, 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 earthen 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 (27-35°C), 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$_2$PO$_4$) 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 Vanadomolybdo-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 total 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chlorophyll Content (mg./gm. fresh wt.)</th>
<th>% Phosphorus Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chl.a</td>
<td>Chl.b</td>
</tr>
<tr>
<td>Control</td>
<td>0.533±0.03&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.188±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Trichoderma viride</em></td>
<td>0.732±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.211±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Glomus mosseae</em></td>
<td>0.795±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.262±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Acaulospora laevis</em></td>
<td>0.645±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.199±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. laevis</em>+<em>G. mosseae</em></td>
<td>0.652±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.203±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>G. mosseae</em>+<em>T. viride</em></td>
<td>0.876±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.285±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. laevis</em>+<em>T. viride</em></td>
<td>0.760±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.252±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chlorophyll Content (mg./gm. fresh wt.)</th>
<th>% Phosphorus Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chl.a</td>
<td>Chl.b</td>
</tr>
<tr>
<td>Control</td>
<td>0.746±0.04&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.216±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Trichoderma viride</em></td>
<td>0.959±0.03&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.311±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Glomus mosseae</em></td>
<td>1.011±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.304±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Acaulospora laevis</em></td>
<td>0.814±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.244±0.04&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. laevis</em> + <em>G. mosseae</em></td>
<td>0.925±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.299±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>G. mosseae</em> + <em>T. viride</em></td>
<td>0.917±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.294±0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. laevis</em> + <em>T. viride</em></td>
<td>1.111±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.353±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* 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**


