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Influence of arbuscular mycorrhizal fungi and *Trichoderma viride* on growth performance of *Salvia officinalis* Linn.

Aditya Kumar*, Ashok Aggarwal and Sunita Kaushish

Department of Botany, Kurukshetra University, Kurukshetra - 136119 (Haryana), INDIA
*Corresponding author. E-mail: adityagohar@yahoo.com

Abstract : *Salvia officinalis* (Sage) is a popular kitchen herb, member of mint (Lamiaceae) family has been cultivated for its wide range of medicinal values. Arbuscular mycorrhizae (AM) are beneficial symbionts for plant growth and development and offer a viable replacement of high input agricultural technology employed for production of environmentally hazardous fertilizers. Therefore, the present study was focused to analyze the effect of two AM fungi (*Acalospora laevis* and *Glomus mosseae*) along with *Trichoderma viride*, alone and in combination, on different growth parameters of *S.officinalis* in a green house pot experiment with sterilized soil. AM inoculum and *T.viride* showed significant increase of different growth parameters after 45 and 90 days of inoculation. Among all treatments, dual combination of *A.laevis* plus *T.viride* was most effective in increasing shoot length, leaf area, root length, root weight, AM spore number and percent root colonization. Moreover, maximum increase in shoot biomass was found in plant treated with *T.viride*.

Keywords : *Salvia officinalis*, *Acaulospora laevis*, *Glomus mosseae*, *Trichoderma viride*, Growth response.

INTRODUCTION

Salvia officinalis Linn. is a evergreen sub- shrub and has been used traditionally for its anti inflammation, antidyspepsia, antioxidant, hypoglycemic properties and mutagenic activities (Wang, 1998; Baricevic and Bartol, 2000). Modern day clinical trials have shown that its essential oil can improve the memory and has shown promise in the treatment of Alzheimer's disease (Akhondzadeh and Abbasi, 2006).

Since the plant has a great significance due to its wide range of the therapeutic potential to treat a large number of ailments, it becomes necessary to enhance their biomass production and their quality in order to fulfill the need of society. Therefore, it requires formulation of planning and strategies for their conservation and enhancement of its products. However, the outputs are limited because of low level of fertilizer used and of the care taken to harvest. It is therefore advisable to develop cheaper solution, such as the mycorrhizal inoculation. The AM fungi are known to colonize a number of tropical plants including vegetables (Reddy *et al*., 2006).

Arbuscular mycorrhizal fungi are now a day well recognized as biofertilizers (Bohra *et al.*, 2007), biological control of root pathogens (Reddy *et al*., 2006), bioremediation (Li *et al*., 2006), increase in biomass of plant (Kumar *et al.*, 2008; Karthikeyan *et al*., 2008), nutrient content (Das *et al.*, 2007) and drought tolerance (Farahani *et al.*, 2008). Hence, in the present study, analysis has been made to see the effect of AM fungi (*A.laevis* and *G.mosseae*) and *T.viride*, alone and in dual combination, on different growth parameters of *S.officinalis* after 45 and 90 days of inoculation.

MATERIALS AND METHODS

Study Site: The study was undertaken in poly house of Botany Department, Kurukshetra University, Kurukshetra, India.

Sample collection and processing : Soil samples from the root zone of *S.officinalis* were collected to a depth of 5-30 cm. The soil samples were wet- sieved for spores using the technique of Gerdemann and Nicolson (1963) and quantification of AM spores was done by grid line intersect method (Adholeya and Gaur,1994). Root samples were rinsed with tap water and cut into 1 cm. pieces and performed with trypan blue according to rapid clearing and staining method of Philips and Haymann (1970). The percent AM root colonization was calculated by using the following formula:

$$\text{Percent AM root colonization} = \frac{\text{Total no. of root segments colonized}}{\text{Total no. of root segments examined}} \times 100$$

Mass multiplication and inoculation of AM spores and *T.viride* : Dominant AM spores i.e. *Acaulospora laevis* and *Glomus mosseae* isolated from rhizosphere of *S.officinalis*, were mass produced by Funnel technique

(Menge and Timmer, 1982) using maize as host. *T.viride* was isolated from the soil and then further mass produced in the medium of wheat bran, saw dust and distilled water prepared in the ratio of 3:1:4 (Sharma *et al.,* 2007).

Moreover, seedlings of *S.officinalis* were grown in earthen pots (Size 25×25) under poly house conditions in sterilized soil. To each pot 10% of inoculum of AM fungi and *T.viride* alone and in combination was added. The effects of different treatments were recorded after 45 and 90 days of inoculation on various growth parameters. Roots and shoots were harvested and weighted separately for their fresh weight and the oven dried to 70ºC for dry weight. Leaf area was studied using leaf area meter (Systronics 211) and stomatal conductance was observed by using porometer (AP4-Delta T devices,UK). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc test.

RESULTS AND DISCUSSION

Shoot length: AM inoculation significantly increased the shoot length of *S.officinalis* after 45 and 90 days. Increase in shoot length was maximum in dual inoculation of *A.laevis* plus *T.viride* (Table-1).This shows the efficacy of AM fungus and *T.viride* on growth of *S.officinalis.* Artursson *et al.* (2006) reported the synergestic effect of dual inoculation of AM fungi and *Trichoderma* on plant growth through a mechanism that includes nutrient absorption and inhibition of plant pathogens. Significant increment in height after 45 and 90 days of inoculation could be attributed to the AM colonization as it known to enhance plant growth by nutrient uptake and secreting plant growth hormones. The increase in height could be due to the greater rate of photosynthesis (Allen *et al*., 1981). Kungiu *et al.* (2008) reported the progressive increase in shoot length of *Senna spectabilis* with AM inoculation.

Shoot biomass: It is envisaged from Table-1, the plants inoculated with *T.viride* showed highest biomass. This may be due to increased uptake of inorganic nutrients via increased absorbing surface by inoculated plants and can be due to maximum branched shoots of this plant as compared to other treatments. The efficacy of *Trichoderma* for plant growth promotion may be due to higher nutrient uptake particularly N,P,K,Fe,Zn and Cu in several crops including sugarcane (Shukla *et al*., 2008; Howell, 2003). *Trichoderma* are known to produce a number of antibiotics such as trichodermin, trichodermol, polyketides, peptaiboils, sesquterpenes and steroids. They are frequently associated with both biocontrol activity and promotion of plant and root growth (Chet *et al*., 2006, Harman *et al.,* 2004). The stimulated growth with *Trichoderma* inoculation has also been reported on tomato (Ozbay *et al*., 2004) and on *Nicotiana benthamiana* (Charon *et al*., 2007).

Root length: Table 1 indicates that the maximum increase in root length was found in dual inoculation of *A.laevis* plus *T.viride*. Perrin(1990) found that mycorrhizal inoculation protects the roots from soil pathogens and tends to increase growth of roots. The maximum increase in root length might be due to mycelial network of AM fungi which extends deeper to invade nutrient depletion zone. Kumar *et al.* (2008) while working on *Spilanthes acmella,* reported the maximum increase in root length when inoculated with AM fungi and *Trichoderma viride*. Significant increase in root length with AM fungi have also been reported by Reddy *et al.* (2006) and Akhtar and Siddique (2007).

Leaf area: AM inoculation significantly increased the leaf area of all treated plant as compared to control plant (Table-1). It was found that after 45 days of inoculation the leaf area was found maximum in the plant treated with *T.viride* and 90 days after inoculation (DAI) the maximum increase in leaf area was recorded in the treatment of *A.laevis* plus *T.viride*. Higher leaf area in AM inoculated plant might be as a result of enhanced nitrogen acquisition (Tobar *et al*., 1994b) by host plant through the external hyphal transport of NO_3 (Tobar *et al.,* 1994a) or nitrogen assimilating enzymes(Cliquet and Stewart, 1993). The increase of leaf area with AM inoculation would be beneficial by maintaining a higher photosynthetic rate (Auge *et al.,* 1987; Panwar, 1993). A positive effect of leaf area to AM inoculation has been also reported in Sesame (Boureima *et al*., 2007) and Sorghum (Ebel *et al*., 1994).

Root biomass: The result (Table-1) show that the amount of root biomass has been found to be increased progressively irrespective of treatment over control. The increase in root biomass (fresh and dry) was observed maximum with of *T.viride* after 45 days. After 90 days the increase in fresh root biomass was found maximum with *A.laevis* plus *T.viride* but the dry root weight was found maximum with *G.mosseae* plus *T.viride*. The increase in fresh root weight in inoculated plants could be either with increased mycorrhizal colonization or due to the formation of external mycelial network around the roots by AM fungi. The results are in agreement with the findings of earlier investigators (Karthikeyan *et al.,* 2008; Gupta and Janardhanan, 1991) who observed improved root biomass production in different plants due to AM fungi inoculation.

AM Spore count and Mycorrhizal colonization: The status of spore population and degree of mycorrhizal infection was also studied under different treatments and result depicted in Table-1 showed that the mycorrhizal spore count was found maximum in plants inoculated with *A.laevis* plus *T.viride* and highest AM root colonization

Table-1. Influence of Arbuscular mycorrhizal fungi and *T. viride* on growth performance of *S. officinalis* after 45days and 90 days.

Treatment	Change in height (cm)	Leaf area (sq cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	AM spore number /10 g o soil	AM root colonization (%)	Stomatal conductivity ($mmol^{-2}s^{-2}$)	
After 45 Days											
Control	*8.23±0.15	2.06±0.60	9.56±0.32	2.28±0.01	0.44±0.02	0.51±0.06	0.16±0.02	22.33±4.72	22.47±0.61	L	15.7±0.81
										U	4.1±0.25
Trichoderma viride	14.03±0.20	5.30±0.50	12.60±0.10	4.98±0.01	1.50±0.05	1.89±0.02	0.53±0.02	34.33±1.52	28.86±1.26	L	58.3±0.79
										U	13.3±0.40
Glomus mosseae	17.1±0.26	2.96±0.20	12.06±0.15	2.55±0.10	0.92±0.08	1.06±0.02	0.23±0.05	30.0±2.0	39.76±1.50	L	92.66±0.61
										U	21.9±0.20
Acaulospora laevis	16.56±0.20	3.76±0.15	11.66±0.35	2.85±0.05	1.19±0.07	1.08±0.01	0.36±0.02	44.66±2.51	46.23±0.90	L	78.2±0.20
										U	25.3±0.37
A.laevis+ G.mosseae	15.56±0.15	4.23±0.70	12.93±0.15	2.66±0.18	0.78±0.05	0.92±0.04	0.31±0.02	52.33±1.52	54.51±0.60	L	54.03±0.15
										U	23.1±0.25
G.mosseae+ T.viride	21.86±0.37	4.20±0.10	14.46±0.20	3.88±0.11	1.41±0.02	1.54±0.03	0.39±0.02	62.33±1.52	69.1±0.96	L	104.9±0.15
										U	26.66±0.20
A.laevis+ T.viride	23.46±0.45	4.30±0.70	17.03±0.41	4.89±1.03	1.48±0.02	1.62±0.02	0.49±0.01	78.0±3.0	60.2±1.90	L	96.1±0.35
										U	21.0±0.20
After 90 Days											
Control	*14.1±0.26	2.50±0.20	11.3±0.2	4.54±0.81	1.33±0.03	0.62±0.09	0.21±0.01	46.0±2.0	30.0±1.75	L	24.33±0.40
										U	7.34±0.12
Trichoderma viride	15.96±0.30	5.33±1.30	13.53±0.20	12.74±1.0	3.91±1.06	2.62±0.11	0.63±0.02	52.66±1.52	37.96±1.41	L	72.8±0.1
										U	27.4±0.15
Glomus mosseae	18.4±0.26	4.1±0.43	14.36±0.15	7.84±0.11	1.45±0.05	1.25±0.09	0.35±0.01	65.0±1.0	42.66±0.25	L	118.03±0.30
										U	39.7±0.25
Acaulospora laevis	29.65±0.35	4.63±0.30	14.83±0.25	8.50±0.59	2.89±0.02	1.22±0.01	0.44±0.02	57.0±1.0	51.93±0.25	L	107.4±0.25
										U	36.9±0.20
A.laevis+ G.mosseae	22.7±0.26	4.33±0.64	18.4±0.26	4.56±0.55	2.52±0.01	1.13±0.04	0.34±0.01	79.66±1.52	57.02±1.52	L	102.6±0.26
										U	35.1±0.32
G.mosseae+ T.viride	31.8±1.85	4.63±0.83	18.93±0.35	8.56±0.77	2.60±0.02	2.10±0.01	0.71±0.02	92.0±2.0	74.5±2.09	L	131.6±0.40
										U	45.06±0.25
A.laevis+ T.viride	35.3±1.21	5.6±0.25	19.93±0.45	6.36±0.13	2.25±0.06	3.02±0.07	0.67±0.03	101.66±5.03	82.3±3.21	L	128.06±0.35
										U	39.3±0.45

According to one way analysis of variance (ANOVA) of data, the mean difference is significant at 0.05 levels., * Each value is an average of three replicates ± Standard deviation, L-Lower surface, U-Upper surface

was found in the dual combination of *G.mosseae* plus *T.viride* after 45 days and in *A.laevis* plus *T.viride* after 90 days respectively. Griffee and Metha (2000) and Earanna *et al.* (2001) reported a positive response in respect to spore count and root colonization while working on *Catharanthus roseus* and *Coleus aromaticus* respectively. The increase in AMF spore population could be due to enhanced spread of AM fungal hyphae as reported by Muthukumar and Udaiyan (2002) and Koomen *et al.* (1987). The plant under different treatments also showed the AM association, which confirms the host dependency on endomycorrhizae. Similar was the assessment of Arshi and Roy (2008) in *Gliricidia sepium*.

Stomatal conductance: Mycorrhizal colonization of roots also influences the stomatal behaviour of leaves. Stomatal conductance increased when inoculated with different combination of AM fungi and *T.viride*. Result depicted in Table 1 showed that the dual interaction of *G.mosseae* plus *T.viride* significantly increased the stomatal conductance in *S.officinalis*. The progressive increase in stomatal conductance in AM inoculation might be due to higher photosynthetic rate and number of stomata on lower surface of leaf. Higher rate of stomatal conductance in mycorrhizal plants have also been reported by Auge (2001). Fidelbus *et al*. (2001) also reported positive role of AM fungi on citrus seedlings when exposed to severe soil drying by lowering the rate of leaf conductivity.

The tremendous advances in research on mycorrhizal physiology and ecology over the past 40 years have led to a greater understanding of the multiple roles of AMF in the ecosystem. Therefore, in the present investigation, the efficacy of two strains of AM fungi along with *T.viride* was analyzed on different growth parameters of *S.officinalis*. As mycorrhizal symbiosis is a highly evolved mutualistic relationship for plant establishment, so this study provides a great future for utilizing the efficient strains of mycorrhizal fungi to exploit them for the beneficial effects in establishment of seedlings, increase in productivity and reduce the fertilizer application required for obtaining economic production of *S.officinalis* plant under field conditions.

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