J. Biol. Chem. Chron. 2015, *1(1),* 37-40
ISSN (Print): 2454 – 7468
ISSN (Online): 2454 - 7476
www.eresearchco/jbcc/

The Influence of Mycorrhizal Interactions on some physiological parameters of *Spilanthes acmella* Murr. plant

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(Received 03 May, 2015; Accepted 30 June, 2015; Published 10 July, 2015)

ABSTRACT: Arbuscular mycorrhizal fungi usually enhance overall plant performance, yet their effects on some physiological parameters of *Spilanthes acmella* was evaluated in green house pot experiment. In the present investigation the presence of these bodies accelerate the rate of chlorophyll content and phosphorus after 45 and 90 days of inoculation. Results showed that maximum increase in chlorophyll content was recorded in dual combination of *A.laevis* plus *G.mosseae* (0.818±0.05; 2.074±0.07) after 45 and 90 days. Likewise, forty five days after inoculation, the maximum P-content in shoot and root was noticed in the treatment of *A.laevis* plus *T.viride* (0.999±0.03) and *G.mosseae* plus *T.viride* (0.551±0.02) respectively. After 90 days of inoculation, the highest increased P-content in shoot and root was observed in *A.laevis* plus *G.mosseae* (1.248±0.03) and *G.mosseae* plus *T.viride* (0.839±0.02) respectively. Results revealed that higher content of phosphorus was recorded in shoot than root.

Keywords: Arbuscular mycorrhizal fungi; *S.acmella; Glomus mosseae; Acaulospora laevis; Trichoderma viride;* medicinal plants.

INTRODUCTION: *S.acmella* is an indigenous herb, grown as an annual herb throughout the tropics. A number of constituents have been isolated from *S.acmella* like spilanthol isobutylamide and triterpenoids[1,2]. Different bioactive metabolites have also been reported from *S.acmella*[3]. It has a long history of use as a folklore remedy e.g. for toothache, rheumatism and fever[4]. In pharmaceuticals, the plant has found applications as an antitoothache, formulation for pain relief, swelling and gum infections and mouthwashes.

The microbial communities are responsible for development of soil structure through various biogeochemical cycles in order to maintain the soil health and quality[5]. 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. Biological control, the use of specific microorganisms that interfere with plant pathogens and pests, is a nature friendly ecological approach to overcome the problem caused by standard chemical methods of plant protection. The strains of *Trichoderma* induces metabolic changes in plants that increases resistance to a wide range of plant pathogenic microorganisms and viruses[6]. AM fungi and *Trichoderma* can interact synergistically to stimulate plant growth through a range of mechanism that includes improved nutrition acquisition and inhibition of fungal plant pathogens.

Natural products including medicinal plants have a great significance; therefore, it requires formulation of planning and strategies for their conservation and enhancement of their products. However, it is advisable to develop cheaper solution, as employing mycorrhizal inoculation, which has gained momentum in last couple of years. Hence, in the present study, analysis has been made to see the effect of AM fungi (*Glomus mosseae* and *Acaulospora laevis*) and *T. viride*, alone and in dual combination on different physiological parameters of *Spilanthes acmella* after 45 and 90 days of inoculation.

MATERIAL AND METHODS:

Experiment Conditions and Design: The experiment was conducted at green house at Kurukshetra University, Kurukshetra where all the conditions (27-35ºC temperature, 80% humidity, 15000-19000 lux. light intensity) were controlled.

Plant Material and AM Fungal Inoculums: Two months old seedlings of *S.acmella* were procured from Dr.Y.S. Parmar University of Horticulture & Forestry,

Nauni, Solan, Himachal Pradesh. The seedlings were maintained under poly-house conditions of Botany Department, Kurukshetra University, Kurukshetra. The strains of AM fungi i.e. *G.mosseae* and *A.laevis* were isolated from rhizospheric soil of selected medicinal plant and were mass produced. Different combinations of AM fungi and *T. viride* were utilized. *T.viride* was isolated from soil by Warcup's soil plate method and was mass cultured using wheat bran: saw dust medium.

Response Variable Evaluated: Chlorophyll content of experimental plants was determined by the method of Arnon[7] and 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[8]. The effect of different treatments was recorded at 45 and 90 days after planting (DAP).

Statistical Analysis: Statistically interpretation of data was done 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. 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 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: Perusal of data depicted in Tables (I and II) showed the dependence of *S.acmella* on mycorrhizal symbiosis. Different physiological parameters significantly increased 45 and 90 days after inoculation.

Chlorophyll Content (Table 1, 2; Figure 1, 2): In the present investigation, when chlorophyll content was monitored, there were differences in content of chlorophyll a and chlorophyll b with different mycorrhizal inoculations. The highest increase in total chlorophyll content after 45 days of inoculation was observed in *A.laevis* plus *G.mosseae* (0.818±0.05) followed by *G.mosseae* plus *T.viride* (0.562±0.04) and *A.laevis* plus *T.viride* (0.519±0.05). After 90 days, the maximum increase in total chlorophyll content was also noticed in *A.laevis* plus *G.mosseae* (2.074±0.07) and minimum in control. The results are in conformity with the findings of Rao and Aseri[9] who recorded an increase in chlorophyll content and various metabolites by dual inoculation of AM fungi and rhizobacteria in *Emblica officinalis*. One of the important indicator of physiological activity is the rate of photosynthesis, which is related to the chlorophyll contents of the plants. Similarly, Haripriya and Sriramachadrasekharan[10] while worked on onion, reported an increase in chlorophyll content and related compounds due to inoculation of AM fungi (*G.mosseae*) whether used individually or in combination. The total chlorophyll content in *Mentha spicata* was also found to increase when plant was subjected to inoculate with AM fungi[11].

Table 1: Influence of AM Fungi and *T.viride* on different physiological parameters of *S.acmella* after 45days.

Treatments	Chlorophyll Content (mg./gm. fresh wt.)			% Phosphorus Content	
	Chl.a	Chl.b	Total Chl.	Shoot P	Root P
Control	*0.147±0.01^{d}	0.126±0.01^{d}	0.273±0.02^{e}	0.432±0.01^{f}	0.097±0.05^{f}
Trichoderma viride	0.181±0.01^{d}	0.215±0.02^{bc}	0.397±0.03^{d}	0.654±0.01^{d}	0.160±0.01^{d}
Glomus mosseae	0.256±0.03^{c}	0.235±0.02^{bc}	0.492±0.05^{bc}	0.561±0.02^{e}	0.456±0.03^{b}
Acaulospora laevis	0.237±0.02^{c}	0.194±0.01^{c}	0.431±0.04^{cd}	0.683±0.01^{d}	0.302±0.01^{d}
A.laevis + G.mosseae	0.482±0.03^{a}	0.336±0.02^{a}	0.818±0.05^{a}	0.762±0.02^{c}	0.311±0.01^{d}
G.mosseae + T.viride	0.318±0.01^{b}	0.244±0.02^{b}	0.562±0.04^{b}	0.902±0.02^{b}	0.551±0.02^{a}
A.laevis + T.viride	0.268±0.03^{b}	0.251±0.02^{b}	0.519±0.05^{b}	0.999±0.03^{a}	0.376±0.01^{c}

** 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: Influence of AM Fungi and *T.viride* on different physiological parameters of *S.acmella* after 90 days.

Treatments	Chlorophyll Content (mg./gm. fresh wt.)			% Phosphorus Content	
	Chl.a	Chl.b	Total Chl.	Shoot P	Root P
Control	*0.913±0.02^{d}	0.274±0.02^{a}	1.187±0.05^{c}	0.738±0.03	0.197±0.05
Trichoderma viride	1.074±0.03^{c}	0.359±0.02^{b}	1.433±0.06^{b}	1.117±0.02	0.274±0.03
Glomus mosseae	1.120±0.02^{bc}	0.372±0.02^{b}	1.489±0.04^{b}	0.963±0.04	0.775±0.03
Acaulospora laevis	1.084±0.04^{c}	0.354±0.02^{b}	1.438±0.07^{b}	0.912±0.02	0.618±0.05
A.laevis* + *G.mosseae	1.547±0.04^{a}	0.527±0.02^{a}	2.074±0.07^{a}	1.248±0.03	0.468±0.06
G.mosseae* + *T.viride	1.158±0.03^{b}	0.380±0.02^{b}	1.538±0.06^{b}	1.178±1.04	0.839±0.02
A.laevis* + *T.viride	1.159±0.03^{b}	0.361±0.02^{b}	1.507±0.03^{b}	1.049±0.04	0.455±0.03

** 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*

Phosphorus Content: It is envisaged from Tables (I,II) and Figures (I,II), the mycorrhizal inoculation significantly increased the phosphorus content in shoot and root over control after 45 and 90 days. Results revealed that higher content of phosphorus was recorded in shoot than root. Forty five DAI, the maximum P-content in shoot and root was noticed in the treatment of *A.laevis* plus *T.viride* (0.999±0.03) and *G.mosseae* plus *T.viride* (0.551±0.02) respectively. After 90 days of inoculation, the highest increased P-content in shoot and root was observed in *A.laevis* plus *G.mosseae* (1.248±0.03) and *G.mosseae* plus *T.viride* (0.839±0.02) respectively. In the AM inoculated seedlings, increased P uptake could be linked with significant AM fungal colonization of the root system and this increase in P uptake may also be due to an increase in the number of uptake sites per unit area of roots and a greater ability of these roots to exploit the soil nutrients[12]. AM inoculation significantly improves the absorption of phosphorus and other nutrients in plants by increasing the contact surface and explored soil volume and possibly facilitate the nutrient transport among plants[13]. Schweiger *et al.*[14] also reported that the colonization by the isolates of *Acaulospora laevis* resulted in the highest shoot P content. Significant effect of such type of symbiotic association on some physiological parameters of *Plantago lanceolata* has been well documented[15]. The AM fungi increase the root and leaf biomass as well as the P uptake in *Glycyrrhiza glabra* has been also studied[16].

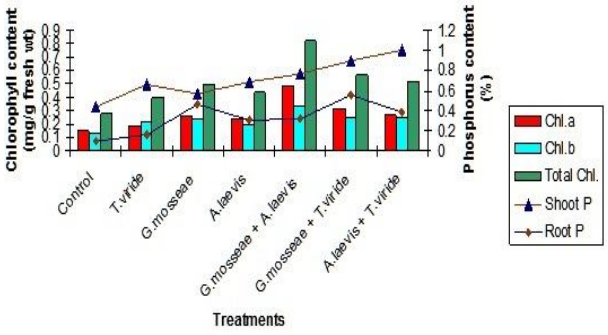

Figure 1: Efficacy of AM fungi and *T.viride* on chlorophyll and P content in *S.acmella* after 45 days.

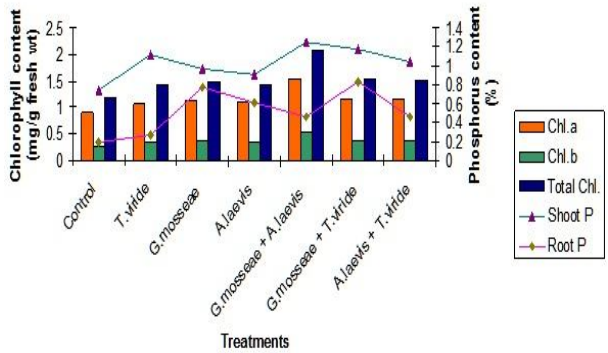

Figure 2: Efficacy of AM fungi and *T.viride* on chlorophyll and P content in *S.acmella* after 90 days.

CONCLUSION: This study demonstrates that arbuscular mycorrhizal fungi may have contrasting effects on physiological attributes of *S.acmella*. The AM fungi affect the level of phosphorus and chlorophyll content. Such conditions justify the need to identify the best combination of AM fungal species and can be applied to enhance the physiological parameters in medicinal plants.

ACKNOWLEDGEMENTS: The authors are thankful to Kurukshetra University, Kurukshetra for providing necessary facilities for the work undertaken.

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