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Rhizospheric effect of Endophytic Mycorrhiza and Trichoderma Viride on physiological parameters of *Mentha Spicata* linn.

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ABSTRACT: A pot experiment was employed to examine the impact of Arbuscular Mycorrhizal (AM) fungi (*Glomus mosseae* and *Acaulospora laevis*) along with *Trichoderma viride* on *Mentha spicata* after 45 and 90 days of inoculation. Soil microbes' effect on different physiological parameters was determined. The maximum stomatal conductance (morning and evening) was observed in the plants inoculated with *G.mosseae* plus *T.viride* after 45 and 90 days (102.3±0.45, 114.0±2.52; 134±2.00, 154.16±0.95). Likewise, higher phosphorus content in root and shoot was reported in inoculated plants over control. The total chlorophyll content was also found to increase in the plants treated with *A.laevis* (1.628±0.04) and *G.mosseae* (3.491±0.06) after 45 and 90 days respectively. The finding in the present work concludes that AM fungi along with *T.viride* influence the concentration of different physiological parameters.

Keywords: *M.spicata,* Arbuscular Mycorrhizal Fungi, *G.mosseae*, *A.laevis*, *T.viride.*

INTRODUCTION

The micro-organisms have posed influence in the social and economic structure of human civilization from time immortal. The mycorrhizal symbiosis represents a series of complex feedbacks between host and fungus that is governed by their physiology and nutrition [(1)]. These cause a series of dynamic biological and biochemical reactions such as organic matter decomposition, new material synthesis, rock weathering and element transformation in the soil and thus, affect the nutrient availability of plants [(2)].
One group of fungi that has been found to be active in rhizosphere of plant is the genus *Trichoderma*. Biological control, the use of specific micro-organisms 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* induce metabolic changes in plants that increase resistance to a wide range of plant pathogenic micro-organisms and viruses [(3)]. 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. These interactions 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 [(4)]. Such conditions justify the need to identify the best combination of AM fungal species and its bio inoculants in order to make better use of AM in plant improvement.
Hence, keeping the above facts in consideration, 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 *Mentha spicata* after 45 and 90 days of inoculation.

MATERIAL AND METHODS

2.1 Study Site: The current study was undertaken in poly house of Botany Department, Kurukshetra University, Kurukshetra, and Haryana, India during 2007-2009. The conditions like 27-35ºC temperature, 80% humidity and 15000-19000 lux. light intensity were maintained during the experiment.

2.2 Selection of AM endophytes: Two dominant strains of AM fungi i.e. *G.mosseae* and *A.laevis* were isolated from rhizospheric soil of selected medicinal plants and were used alone and in dual combination with *T.viride* to find out their potential on physiological parameters of *M.spicata*. Different combinations of AM fungi and *T. viride* were utilized.

2.3 Preparation of inoculum of AM Fungi and *T.viride*: Both AM fungi (*G.mosseae* and *A.laevis*) were mass produced by using different substrate and host. *T.viride* was isolated from soil by Warcup's soil plate method [(5)] and further mass cultured by using wheat bran: saw dust medium.

2.4 Preparation of Pots: Soil was collected from Botanical garden of Botany Department, Kurukshetra University, Kurukshetra and was sieved to remove the debris and large organic matter. One or two seedlings were grown in earthern pots (size 25×25 cm.) depending upon the nature of growth of plants. To each pot 10% inoculum of each AM fungi and *T.viride*, alone and in combination was added. Three replicates were utilized for each treatment.

2.5 Estimation of Chlorophyll Content: Chlorophyll content of all experimental plants was determined by the method of Arnon[(6)]. Pigments were calculated in terms of mg./gm. plant tissue on fresh weight basis.

2.6 Estimation of Phosphorus: 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 [(7)], which is based on the yellow colour of the unreduced Vanado-molybdo-phosphoric heteropoly complex.

2.7 Record of Data: The effect of different treatments was recorded at 45 and 90 days after planting (DAP). Among physiological parameters, chlorophyll (mg./gm.) and phosphorus (%) content were determined as discussed earlier. Stomatal conductance ($m.mol.^{-2}sec.^{-2}$) was also observed by using Porometer (AP4-Delta T devices, UK).

2.8 Statistical Analysis: Statistical 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. The present investigation was carried out in order to evaluate the potential of AM fungi and *T.viride* on physiological parameters of *M.spicata*. 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.

Table: I- Influence of Arbuscular Mycorrhizal Fungi and *T. viride* on growth performance of *M.spicata* after 45days.

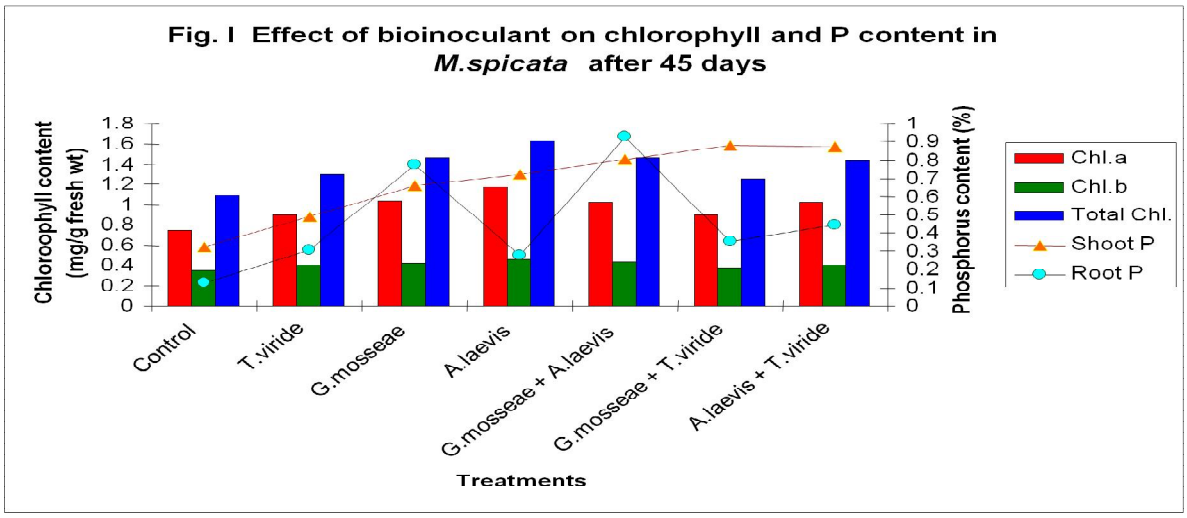
Treatments	Chlorophyll Content (mg./gm. fresh wt.)			% Phosphorus Content		Stomatal Conductance (mmol^{-2}s^{-2})			
	Chl.a	Chl.b	Total Chl.	Shoot P	Root P	Morning		Evening	
Control	*0.746±0.02^{d}	0.346±0.03^{c}	1.092±0.05^{d}	0.320±0.01	0.131±0.01	**L**	30.56±1.10^{g}	**L**	32.03±0.15^{g}
						U	9.31±0.23^{f}	**U**	10.36±0.83^{e}
Trichoderma viride	0.897±0.02^{c}	0.408±0.04^{abc}	1.305±0.07^{c}	0.488±0.04	0.311±0.01	**L**	64.8±0.9^{e}	**L**	69.33±1.05^{e}
						U	24.4±0.36^{d}	**U**	25.93±0.50^{c}
Glomus mosseae	1.048±0.04^{b}	0.417±0.05^{abc}	1.466±0.10^{b}	0.660±0.03	0.773±0.08	**L**	55.66±0.25^{f}	**L**	61.46±1.11^{f}
						U	14.66±0.25^{e}	**U**	15.73±0.60^{d}
Acaulospora laevis	1.173±0.02^{a}	0.455±0.02^{a}	1.628±0.04^{a}	0.719±0.03	0.280±0.03	**L**	69.23±0.75^{d}	**L**	78.73±0.65^{d}
						U	26.26±0.51^{c}	**U**	29.46±0.20^{b}
A.laevis* + *G.mosseae	1.024±0.03^{b}	0.438±0.02^{ab}	1.459±0.05^{b}	0.808±0.03	0.923±0.03	**L**	91.66±1.05^{b}	**L**	96.76±0.45^{b}
						U	29.16±0.70^{b}	**U**	29.83±0.55^{b}
G.mosseae* + *T.viride	0.889±0.02^{c}	0.374±0.02^{bc}	1.263±0.05^{c}	0.879±0.03	0.351±0.03	**L**	102.3±0.45^{a}	**L**	114.0±2.52^{a}
						U	37.56±0.32^{a}	**U**	38.13±1.25^{a}
A.laevis* + *T.viride	1.028±.003^{b}	0.412±0.03^{abc}	1.440±0.06^{b}	0.871±0.03	0.440±0.03	**L**	78.3±0.6^{c}	**L**	84.9±1.40^{c}
						U	25.1±0.26^{d}	**U**	28.33±1.20^{b}

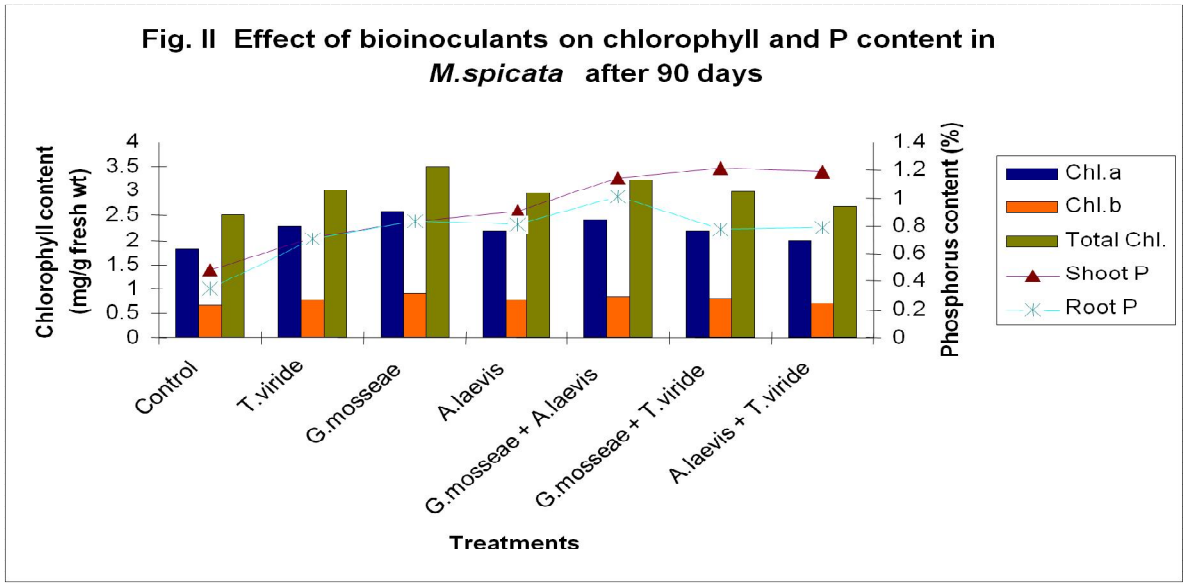
** Each value is an average of three replicates*
Means values followed by different alphabet/s are significant over one another by Duncan's Multiple Range Test at P= 0.05.
± Standard Deviation
U Upper surface of leaf
L Lower surface of leaf

Table: II- Influence of Arbuscular Mycorrhizal Fungi and *T. viride* on growth performance of *M.spicata* after 90 days

Treatments	Chlorophyll Content (mg./gm. fresh wt.)			% Phosphorus Content		Stomatal Conductance (mmol^{-2}s^{-2})			
	Chl.a	Chl.b	Total Chl.	Shoot P	Root P	Morning		Evening	
Control	*1.834±0.05^{f}	0.673±0.02^{e}	2.508±0.07^{e}	0.489±0.04^{d}	0.348±0.02^{d}	**L**	37.33±0.32^{g}	**L**	40.05±0.26^{g}
						U	14.6±0.26^{f}	**U**	19.2±0.90^{f}
Trichoderma viride	2.279±0.03^{c}	0.745±0.03^{cd}	3.024±0.07^{c}	0.721±0.03^{c}	0.709±0.03^{c}	**L**	85.43±0.35^{e}	**L**	97.23±1.0^{e}
						U	28.36±0.30^{d}	**U**	32.06±0.30^{d}
Glomus mosseae	2.584±0.02^{a}	0.907±0.03^{a}	3.491±0.06^{a}	0.834±0.05^{b}	0.832±0.03^{b}	**L**	72.2±0.40^{f}	**L**	78.9±0.45^{f}
						U	21.5±0.52^{e}	**U**	24.06±0.20^{e}
Acaulospora laevis	2.194±0.01^{d}	0.760±0.03^{bc}	2.955±0.05^{c}	0.906±0.03^{b}	0.810±0.02^{b}	**L**	91.8±0.45^{d}	**L**	106.23±2.05^{d}
						U	27.76±0.35^{d}	**U**	35.13±1.00^{c}
A.laevis* + *G.mosseae	2.423±0.05^{b}	0.824±0.05^{b}	3.247±0.09^{b}	1.150±0.06^{a}	1.002±0.02^{a}	**L**	109.33±1.05^{b}	**L**	125.43±0.70^{b}
						U	38.33±0.68^{b}	**U**	45.8±0.45^{b}
G.mosseae* + *T.viride	2.168±0.02^{d}	0.800±0.03^{bc}	2.969±0.06^{c}	1.219±0.04^{a}	0.776±0.09^{bc}	**L**	134.36±2.00^{a}	**L**	154.16±0.95^{a}
						U	45.43±0.32^{a}	**U**	59.66±1.18^{a}
A.laevis* + *T.viride	1.981±0.08^{e}	0.688±0.04^{de}	2.669±0.03^{d}	1.192±0.05^{a}	0.783±0.07^{bc}	**L**	97.96±0.25^{c}	**L**	115.13±0.66^{c}
						U	31.43±0.25^{c}	**U**	33.13±0.86^{d}

* 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
U Upper surface of leaf
L Lower surface of leaf





3.1 Stomatal Conductance: Stomatal conductance of all inoculated plants increased after 45 and 90 days over control. Maximum increase in stomatal conductance (morning and evening) was found in the plants inoculated with *G.mosseae* plus *T.viride* (102.3±0.45, 114.0±2.52; 134.36±2.00, 154.16±0.95) on lower surface of leaves. More stomatal conductance was registered in evening time. One possibility for higher mycorrhizal stomatal conductance is more efficient extraction of soil moisture by mycorrhizal root system in dry soil. Hyphae of mycorrhizal fungi *G.clairodeum* can apparently make significant contribution to water uptake by cowpea roots [8]. Others have suggested that extraradical hyphae or increased root branching may allow mycorrhizal roots to more fully explore a particular soil volume extending soil water depletion zones and giving a mycorrhizal root system more access to available water[9&10]. Increased stomatal conductance or gas exchange rates in AM plants have been well recorded under amply watered and drought conditions [11&12]. Thakur [13] also reported a significant increase in stomatal conductance (19.35% more) in AM treated plants in *Phaseolus radiatus* over untreated ones.

3.2 Chlorophyll Content: Significant improvement in the total chlorophyll content of plants treated with AM fungi was observed as compared to control (Table I, II). A similar trend was observed for chlorophyll a and b. Fortyfive DAI, maximum amount of chlorophyll a was observed in the plants treated with

A.laevis (1.173±0.02) followed by *G.mosseae* (1.048±0.04), while after 90 days of inoculation, it was recorded higher in *G.mosseae* (2.584±0.02) followed by *A.laevis* plus *G.mosseae* (2.423±0.05). Similarly, maximum amount of chlorophyll b was registered in the plants treated with *A.laevis* (0.455±0.02) and *G.mosseae* (0.907±0.03) after 45 and 90 days respectively. However, total chlorophyll was also found maximum in *A.laevis* (1.628±0.04) and *G.mosseae* (3.491±0.06) after 45 and 90 days of inoculation. The increased amount of chlorophyll content in leaves indicates the photosynthetic efficiency. Mycorrhizal seedlings show a greater increase in the rate of photosynthesis than their controls which may be due to increase in the content of total chlorophyll [14&15]. Mycorrhizal plants have higher total chlorophyll and caretenoids than the non mycorrhizal plants [16&17]. The increase in Chlorophyll a, Chlorophyll b and total chlorophyll contents observed in the present study is in concurrence with the reported findings of Manoharan[18], who have observed that mycorrhizal tree seedlings have higher chlorophyll concentration.

3.3 Phosphorus Content: Regarding the phosphorus content in *M.spicata* seedlings, all the inoculation treatments had higher P- content compared to uninoculated seedlings. After 45 days, maximum shoot P-content was observed in the plants treated with *G.mosseae* plus *T.viride* (0.879±0.03) followed by *A.laevis* plus *T.viride* (0.871±0.03). Similarly, maximum root P-content was observed in the treatment of *A.laevis* plus *G.mosseae* (0.923±0.03) followed by *G.mosseae* (0.773±0.08). Higher concentration of phosphorus was reported in root than shoot. Ninety DAI, maximum P- content in shoot was recorded in the dual combination of *G.mosseae* plus *T.viride* (1.219±0.04) followed by *A.laevis* plus *T.viride* (1.192±0.05) and maximum root P- content was found in *A.laevis* plus *G.mosseae* (1.002±0.02) followed by *G.mosseae* (0.832±0.3) treatment. Here, shoot P-content was reported more than root P- content. Variation in results in plant responses to P-uptake acknowledge to the high functional diversity among AM fungal species [19]. Certain combinations of host and fungus are more or less compatible than others and species or strains of AM fungi can vary in their capacity to take up P from soil and transfer it to host plant [20&21]. AM fungi allow the root system to exploit a greater volume of soil P by (i) extending away from the root zone (ii) exploring smaller soil pores not reached by the root hairs and (iii) effective acquisition of organic phosphate by production of extracellular acid phosphatases [22]. Hyphae of mycorrhiza splash solvent acid of phosphorus (e.g. Malic acid) that causes increasing absorbption of phosphorus [23]. *Trichoderma* sp. is known to solublize unavailable form of P and convert them to an available form thus enabling better uptake by AM fungi [24].

CONCLUSION

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 of *M.spicata*. The research in the field of arbuscular mycorrhizal (AM) symbiosis has taken a giant leap in the past two decades, as demonstrated by the large amount of literature being published every year. Much of the research has documented the effect of AM fungi as well as *Trichoderma* on plant growth and development.

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