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The effects of Vesicular Arbuscular Mycorrhizal (VAM) association on the nutritional values of *Mentha longifolia* L. at various levels of rock phosphate amendments

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Abstract

This study was aimed to evaluate the effect of VAM (Vesicular Arbuscular Mycorrhizae) on the nutritional analysis of *Mentha longefolia* L. Various levels of Rock Phosphate were applied in combinations with VAM or without VAM such as RP_0 (Without Phosphate) RP_1 (0.05 g), RP_2 (0.105 g) and RP_3 (0.157 g). Rhizospheric soil from the Agave plant having high spore number of different VAM fungi species i.e. *Glomus fasciculatum, G. mosseae* and *Acaulospora mellae* were applied. Proximate analysis showed an enhancement in crude protein, fat, ashes, moisture and crude fibers in mycorrhizal plants, however carbohydrate contents were reduced in mycorrhizal plants as compared to non-mycorrhizal plants. At various levels of Rock phosphate the overall performance of mycorrhizal groups was better than non- mycorrhizal groups. Mycorrhizal association facilitates the absorption of nutrients such as phosphate which is mostly non-mobile. This research work showed that VAM association is very crucial and important for growth of plants and increase fertility of soil as compared to non-mycorrhizal association.

Keywords: ***Mentha longefolia*** **L., Arbuscular Mycorrhizae, Phosphate rock, Rhizospheric soil.**

INTRODUCTION

Mycorrhizal associations are multifaceted interactions comprising diverse morphological, functional and evolutionary categories (Brundrett, 2002). The mycorrhizal symbioses represent a series of complex feedbacks between hosts and fungus that is governed by their physiology and nutrition. The outcomes of a mycorrhizal relationship depend on the balance between fungal demand for energy (in terms of carbon based compounds) and plant needs for nutrients (Miller *et al.* 2002). Most Mycorrhizae can be described as balanced mutuallistic associations in which the fungus and plant exchange, commodities required for the growth and survival of both partners. These occupy a separate isoclines from pathogenic, endo-Phytologistic or antagonistic associations in the continuum of plant fungus interaction (Brundrett, 2004). Morphological responses of the external mycelium of AM fungi in response to nutrient status of their environment have also been demonstrated (Leigh

et al. 2009), as have differing substrate colonization strategies among AM fungal genera (Cano and Bago, 2005). AM fungi colonize the root cortex of plants and develop an extrametrical hyphae network that can absorb nutrient from the soils (Plenchette *et al.* 2005). By forming an extended, intricate hyphal network, AM Fungi can efficiently absorb mineral nutrient from the soil and deliver them to their host plants in exchange for carbohydrates. They facilitate nutrient uptake, particularly with respect to immobile nutrients such as phosphorus and enhanced tolerance to drought, disease resistance, building up a macro-porous structure of soil that allows the penetration of water and air and prevent erosion, enhance photosynthesis and reduces stresses during micro-propagation (Sharda and Rodrigues, 2009).

The Arbuscular mycorrhizal association has been reported to improve the uptake of different mineral nutrients. However, phosphate has been in the focus because it can be a limiting factor for plant growth due to its immobility in the soil. The fine hyphae network is superior to the relatively thick roots and root hairs in accessing phosphate in the soil. On the other hand, high available Phosphate concentration often seems to induce a limitation of fungal colonization level by the plants (Redecker, 2005). Arbuscular mycorrhizal fungi have been shown to improve productivity in soils of low fertility and are particularly important for increasing ions such as Po_4^{3-} and immobile nutrient such as phosphorus (Jacobsen et al. 1992). Plant root alone may be incapable of taking up phosphate ions that are immobilized, i.e. in soil with a basic PH. The mycelium of AM fungi can however access to these P sources and make them available to the plants they colonized (Smith *et al.* 2006). Phosphate is taken up high affinity phosphate transporter in the extra-radical mycelium and then transported within the fungus mycelium as polyphosphate (Poly P) and once in the intra-radical hyphae the long chain is hydrolyzed facilitating transfer to the host plant (Ohtomo and Saito, 2005). Phosphorus taken up by AM fungi is considered to be from the same labile pool used by plant roots. However, there is increasing indication that mycorrhizal roots may be capable of using an insoluble source of inorganic P in soil that are not available to the roots (Duponnois *et al.* 2005). However, it is now clear that the external hyphae of AM fungi take up inorganic N as both NO_3^- and NH_4^+ (Jin *et al*. 2005; Govindarajulu *et al*. 2005) and organic N as amino acids (Hawkins *et al*. 2000) and transfer sometimes a large fraction of the plant. Besides phosphorus, arbuscular fungi can also improve the uptake of several micronutrients like Zinc (Zn), Copper (Cu), Iron (Fe) and Manganese (Mn) (Marschner, 1995). The AM fungi can take up these elements and store them so as to prevent their concentration to reach toxic levels. AM Fungi could act as a sink for Copper, Cobalt and zinc (Cooper and Tinker, 1987). The AM fungal association may induces increase in the level of soluble proteins which contribute to plant tolerance to stress such as drought (Ruiz-Lozano *et al*. 1995).

MATERIALS AND METHODS

Clay and sand soil was obtained from the ground of Botany Department, University of Peshawar. Chemical analysis of the soil and sand sample was done at N.I.F.A (Nuclear Institute for Food and Agriculture) by different methods, Nitrogen concentration of soil sample as determined by K. Jeldhal method of Bremmer and Mulvaney (1996). ABDTPA extractable P, Cu, Fe, Zn and Mn and soil PH by Richards (1954), soil organic matter by Nelson and Sommer (1982). Proximate analysis of samples was carried out at Agriculture University KPK Peshawar. The clay soil having PH, 7.8, electric conductivity 0.675 days/m^2, Nitrogen, 0.032% and Phosphorus 0.001 ppm. The sand had PH, 8, electric conductivity 0.325 days/m^2, Nitrogen, 0.056% and Phosphorus 0%. After sieving, clay soil was finely mixed with sand in a ratio of 2:1 resulting in sandy loam textured soil. 32 pots having 89 cm diameter and 48 cm length were filled with 7 Kg of this nutrient deficient sandy loam textured soil.

APPLICATION OF INOCULUM:

In the experimental work rhizospheric soil from *the Agave plant* having high spore number of different AM fungi species i.e. *Glomus fasciculatum, G. mosseae and Acaulospora mellae.* Inoculum was used as soil based inoculum. Mycorrhizal inoculums preparation, placement and application were done by the method given by Brundrett et al. (1996).

APPLICATION OF FERTILIZERS:

Following levels of Rock Phosphate were applied in combination with AM or without AM as shown in the table.

Table: Levels of rock phosphate were applied in combination with AM or without AM

S. No.	Soil	Detail
1	RP_0	(Without Phosphate)Control.
2	RP_1	(0.05 g)
3	RP_2	(0.105 g)
4	RP_3	(0.157 g)

Table 2: Experimental design, treatments and replications

S.No.	M+	Mycorrhizal without Rock phosphate (Control)
1.	M-	Non mycorrhizal without Rock phosphate (Control)
2.	MRP_1+	Mycorrhizal+ Rock Phosphate level 1
3.	MRP_1-	Non mycorrhizal+ Rock Phosphate level 1
4.	MRP_2+	Mycorrhizal+ Rock Phosphate level 2
5.	MRP_2-	Non mycorrhizal+ Rock Phosphate level 2
6.	MRP_3+	Mycorrhizal+ R ock Phosphate level 3
7.	MRP_3-	Non mycorrhizal+ Rock Phosphate 3

RESULTS AND DISCUSSION

Following the growth parameters were measured during experimentation.

Table 2: Parameters used for analysis in (%) at different level of rock phosphate amendments.

S.No.	Parameters	S.No.	Parameters
1.	Ashes and moisture	4.	Fibers
2.	Proteins	5.	Carbohydrates
3.	Fats/ Oils	--	--

Dried powder of *Mentha longifolia* L. were analyzed at Agriculture University Peshawar KPK Pakistan for ash and moisture contents, crude Protein, Fat, crude fiber and carbohydrate on dry matter basis and the results are given in table 1.

ASHES AND MOISTURE

The results of the Ashes and moisture contents of *Mentha longifolia* L. following 7 treatments are given in the table are shown in figure 1. Analysis of data revealed that mycorrhizal plants show higher contents of Ashes and moistures as compared to non-mycorrhizal plants. The highest Ashes contents were recorded in M+ (11.699) while the lowest in M- (8.980) and Moisture contents in MRP3+ (10.331) while the lowest in MRP2- (9.472). At various levels of Rock phosphate the overall performance of mycorrhizal was better than non- mycorrhizal.

PROTEINS

The Proteins contents of *Mentha longifolia* L. following 7 treatments are given in the table 3 and figure 2. Analysis of data revealed that Mycorrhizal plants show higher contents of Proteins as compared to non-mycorrhizal plants. The highest Protein content was recorded in M+ (15.808) while the lowest in MRP2- (8.802) and differences were very less among MRP1 and MRP3. At various levels of Rock phosphate the overall performance of mycorrhizal was better than non- mycorrhizal.

FATS / LIPIDS

The results of fat contents of *Mentha longifolia* L. following 7 treatments are given in the table. and figure 3. Analysis of data revealed that mycorrhizal plants show higher contents of fats as compared to non-mycorrhizal plants. The high lipid contents were recorded in M+ (5.49) while the lowest in MRP1- and MRP3- (1.59) each and MRP1- and MRP1+ show very close similarity. At various levels of Rock phosphate the overall performance of mycorrhizal was better than non-mycorrhizal.

FIBERS

The results of fiber contents of *Mentha longifolia* L. following 7 treatments are given in the table 3 and figure 4. Analysis of data revealed that mycorrhizal plants show higher contents of fiber as compared to non-mycorrhizal plants. The highest fiber contents were recorded in M+ (30.70) followed by MRP1+ (26.8) while the lowest in M- (17.54). At various levels of Rock phosphate the overall performance of mycorrhizal was better than non- mycorrhizal.

CARBOHYDRATE

The results of Carbohydrate contents of *Mentha longifolia* L. following 7 treatments are given 3 and figure 5 in the table. Analysis of data revealed that Non- mycorrhizal plants show higher contents of carbohydrate as compared to mycorrhizal plants. The high carbohydrate contents were recorded in M- (49.745) followed by Mrp2- (48.024) while lowest in M+ (26.05). At various levels of Rock phosphate the overall performance of non-mycorrhizal was better than mycorrhizal.

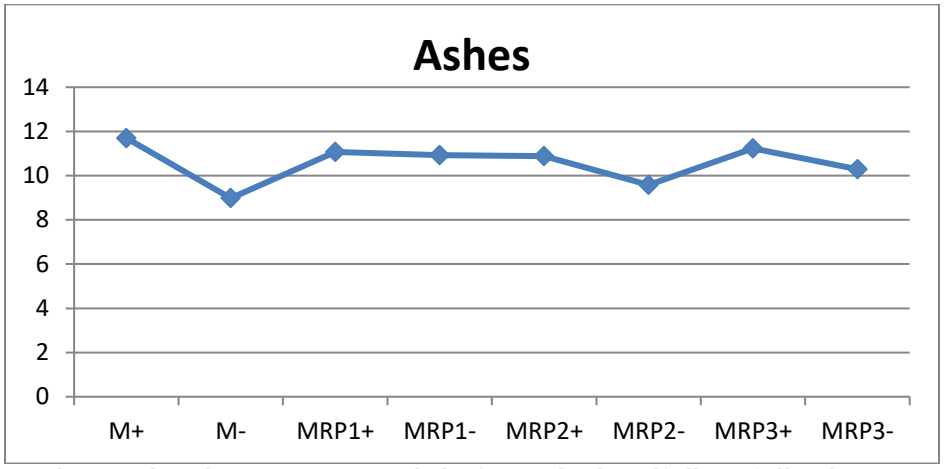

Figure 1: Ashes and moisture contents (%) of *mentha longifolia* L. following 7 treatments.

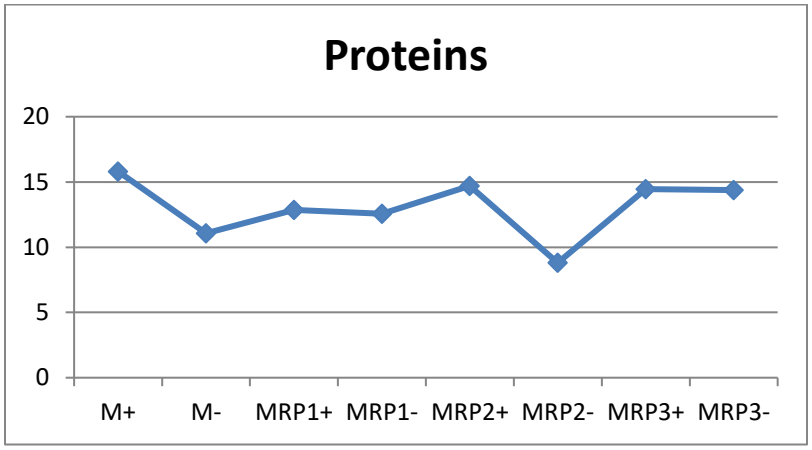

Figure 2: proteins contents (%) of *mentha longifolia* L. following 7 treatments

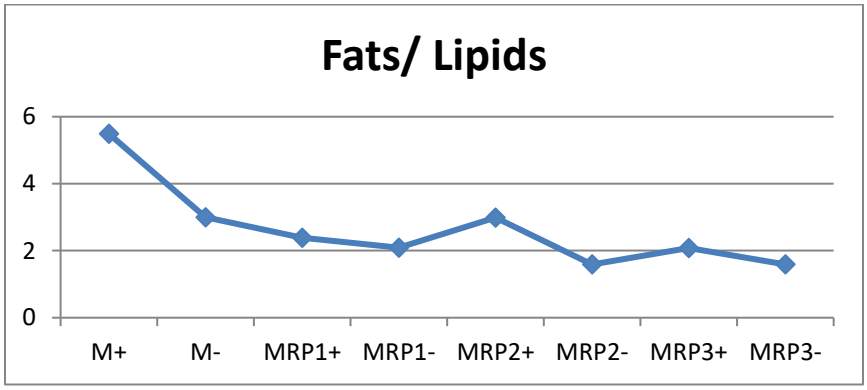

Figure 3: Fat contents (%) of *mentha longifolia* L. following 7 treatments.

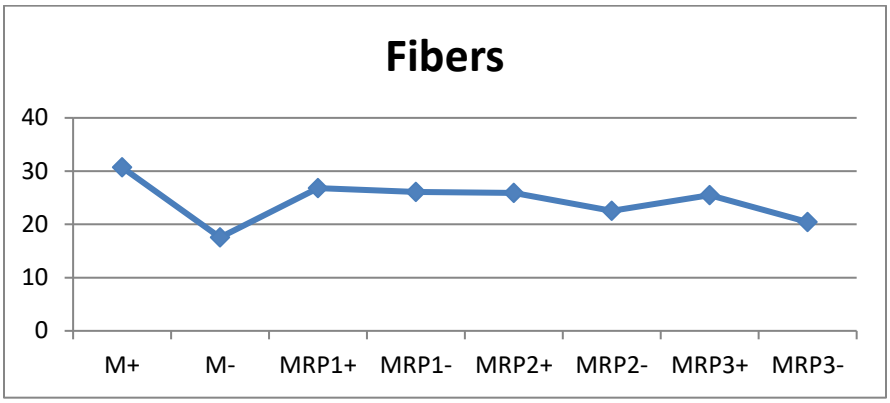

Figure 4: Fiber contents (%) of *mentha longifolia* L. following 7 treatments

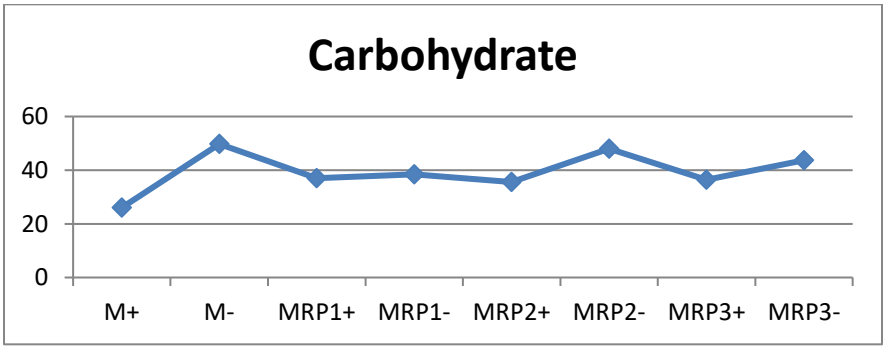

Figure 5: Carbohydrate contents (%) of *mentha longifolia* L. following 7 treatments

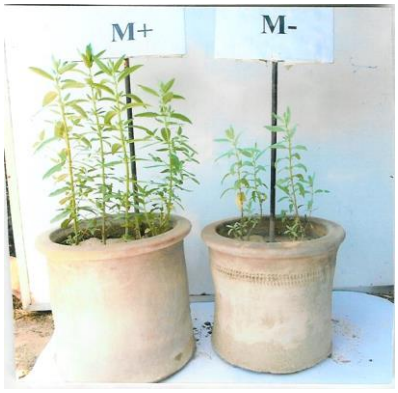

Plate. 1 Effect of Mycorrhiza and RPO (control) on growth of *Mentha longifolia* L.

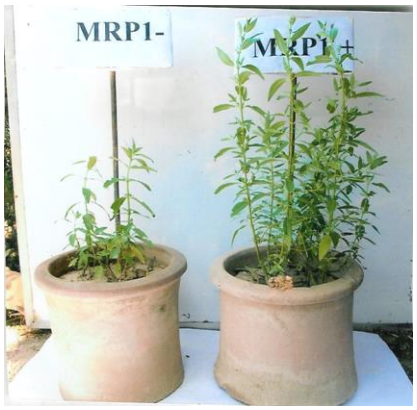

Plate. 2 Effect of Mycorrhiza and RP1 on growth of *Mentha longifolia* L.

Plate. 3 Effect of Mycorrhiza and RP2 on the growth of *Mentha longifolia* L.

Plate. 4 Effect of Mycorrhiza and RP3 on growth of *Mentha longifolia* L.

DISCUSSION

In the present day, an increase in the price of phosphate fertilizers coupled with low recovery (10–30%) of Phosphorus from applying characteristic, the developing tropical countries are attempting to utilize their indigenous reactive ground phosphate rock as a cheap alternative source. The use of Rock Phosphate has been proposed for sustainability purposes (Zapata and Axmann, 1995). The use of phosphate solubilizing microorganisms, including arbuscular mycorrhizal (AM) Fungi has been proposed as a low-cost and low energy mechanism to help increase the agronomic effectiveness of rock phosphate fertilizers (Gyaneshwar *et al*. 2002). The mycorrhizal inoculation can thus help in effective utilization of rock phosphate by changing it into the available form which is later taken up by the plant for their best growth and development (Sabannavar and Lakshman, 2009). As AMF association is very crucial for plant growth, productivity, concentration of secondary metabolites and nutrient uptake.

So the present research work was undertaken to find the effect of VAM inoculation on *Mentha longifolia* L. The result shows that slight differences were observed among mycorrhizal and non-mycorrhizal plants regarding the ash and moisture contents. Mycorrhizal plants enhanced the amount of crude proteins, fat, fibers as compared to non-mycorrhizal plants, however carbohydrate contents of non-mycorrhizal plant was higher as compared to mycorrhizal plants. Mycorrhizal fungi increased tremendously the productivity, growth, nutrient uptake and environmental stresses. Lot of work had been done on the role of AM fungi in medicinal plants showing mycorrhizal plants better performance as compared to non-mycorrhizal plants such as (Jackobsen *et al*. 2000; Abdel-Hafez and Abdel-Monsief 2006; Duponnois *et al*. 2005; Mosse *et al*. 1977; Derek *et al.* 2005; Quilambo, 2000; Inouce *et al*. 2001; Nowk, 2004; Techapinyawat *et al*., 2003 ; Kanno *et al.* 2006; Kerur and Lakshman, 2006; Rapparini *et al*. 2008; Aher *et al*. 2009; Cigar et al. 2000; Gupta *et al.* 2002; Rydlova *et al*. 2010; Ndiaye *et al*. 2009; Rapparini *et al*. 2008; Caravaca *et al*. 2003; Azcon *et al.* 2003; Rausch *et al*. 2001; Harrison *et al*. 2002; Paszkowski *et al*. 2002; Parras-Sariano *et al*. 2009; Jeffries *et al.* 2003; Bucher *et al*. 2009). Mycorrhizal plants show higher Biomass, photosynthetic rate, carbohydrate contents, mycorrhizal colonization and responsive Phosphate transporter gene in general than non-mycorrhizal plants (Derek *et al.* 2005).

CONCLUSION

From this research work it is cleared that mycorrhizal association is very crucial for growth and biomass of Mentha longifolia at various level of rock phosphate amendments. As rock phosphate is mostly immobile in soil. VAM association enhance its uptake leading to overall increase in protein, lipids, fibers, ashes and moisture contents but carbohydrate content reduced because fungi in this association consumed it in return of nutrients.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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the research work.

AUTHOR CONTRIBUTIONS

ZF performed the research work under the supervision of TB. LB facilitated during thesis writing and research work. KS and AH Helped in data analysis, Review the manuscript and tabulation of data, while AM facilitated in nutritional analysis. All authors read and approved the final version.

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