

## EFFECTS OF (VAM) VESICULAR ARBUSCULAR MYCORRHIZAE ON GROWTH PERFORMANCE OF *MENTHA LONGIFOLIA* L. AT VARIOUS LEVELS OF ROCK PHOSPHATE AMENDMENTS

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### خلاصہ

راک فاسفیٹ کے مختلف سطحوں پر مینٹھا لفولیا ایل. کی ترقی کی کارکردگی پر (VAM) ویکولر ایسکلر مکرورریزی کے اثرات کی تحقیقات کے لئے ایک کوشش کی گئی تھی۔ راک فاسفیٹ کے مختلف سطحوں کے ساتھ VAM کے ساتھ یا VAM کے بغیر (RP<sub>0</sub> فاسفیٹ کے بغیر (0.05) RP<sub>1</sub> (جی، 0.105) RP<sub>2</sub> اور RP<sub>3</sub> (0.157) کے ساتھ مل کر میں استعمال کیا گیا تھا۔ آداویز پلانٹ سے ریڑھ پروفک مٹی کی بڑی تعداد میں مختلف ویم فنگی، گلو مس فاسکلیٹیم، گلیو مس ماسی اور Acalospora ملاتے تھے۔ انو لوم استعمال کیا گیا تھا جس میں مٹی کی بنیاد پر انو لوم۔ مطالعہ نے واضح طور پر واضح اختلافات ظاہر کئے ہیں <0.05> مکرورریزی پلانٹ کی پر جاتیوں میں راک فاسفیٹ ترمیم کے مختلف سطحوں پر پلانٹ شوکی لمبائی، جڑ کی لمبائی، پتیوں کی تعداد، تازہ اور خشک وزن کے متعلق علاج اور غیر اہم فرقوں میں MRP<sub>3</sub> اور MRP<sub>2</sub> میں سب سے بہترین شوٹ کی لمبائی، تازہ وزن، خشک وزن اور پتیوں کا نمبر ریکارڈ کیا گیا تھا جبکہ ایم ایم بی 1 اور MRP<sub>3</sub> میں سب سے زیادہ جڑ کی لمبائی ریکارڈ کی گئی تھی۔ راک فاسفیٹ کے مختلف سطحوں پر اس کے مجموعی طور پر مکرورریزی کے مقابلے میں بہتر بنا یا گیا تھا

### Abstract

An effort was made to investigate the effects of VAM (Vesicular Arbuscular Mycorrhizae) on the growth performance of *Mentha longifolia* L. at various levels of rock phosphate. Various levels of rock phosphate were applied in combinations with VAM or without VAM such as RP<sub>0</sub> (Without Phosphate) RP<sub>1</sub> (0.05 g), RP<sub>2</sub> (0.105 g) and RP<sub>3</sub> (0.157 g). Rhizospheric soil from Agave plant with large number of spores of different VAM fungi, i.e. *Glomus fasciculatum*, *Glomus mosseae* and *Acaulos poramellae* were applied. Inoculum was used as soil based inoculum. The study revealed significant differences <0.05> among treatments and non-significant differences among replications regarding to the plant shoot length, length of roots, numbers of leaves, fresh and dry weight at different levels of the rock phosphate amendments in mycorrhizal plant species. The best shoot length, fresh weight, dry weight and leaves number in terms of the mean were recorded in MRP<sub>3</sub> and MRP<sub>2</sub> while the highest root length in terms of mean was recorded in MRP<sub>1</sub> and MRP<sub>3</sub>. At various levels of the rock phosphate the overall performance of mycorrhizal groups was better than non-mycorrhizal groups.

### Introduction

Vesicular Arbuscular mycorrhizae (VAM) is a mutualistic associations that occurs among majority of the plant species and influence a large number of key processes (Wang *et al.*, 2006; Rilling, 2009). There are many kinds of mycorrhizal associations, involving different fungal and plant symbionts, but the Arbuscular Mycorrhizae is the most wide spread (Redecker, 2005) and present in the roots of more than 80% of higher plants. Transport and absorption of minerals by VAM could increase the biomass production in the soil (Liu *et al.*, 2002). Mycorrhizal plants show higher Biomass, photosynthetic rate, contents of organic matter and responsive phosphate transporter gene in general than non-mycorrhizal plants (Derek *et al.*, 2005).

Among the nutrients essential for plants Phosphorus is a very important one. Plants mostly up take phosphate in the form of Orthophosphate (Vance, 2003). As a fact Phosphorus is generally not mobile in the soil, so a narrow depletion zone about a millimeters form around phosphorus absorbing roots (Hinsinger *et al.*, 2005). The very positive performance of VAM for plants is the enhanced supply of mineral nutrients, especially phosphorus, which is mostly immobile one (Strack *et al.*, 2003). The mycellium of VAM fungi have access to these immobile phosphorus sources and transferred them to the colonized plants that are not available to the roots (Smith *et al.*, 2006; Duponnois *et al.*, 2005).

Now-a-days due to increase in the s rate of the phosphate containing fertilizers and with the low recovery by plants about (10-30%) from the applied characteristics, the developing countries trying to utilize the indigenous rock phosphate as a cheap alternative source. Utilization of rock phosphate is very useful for the

sustainability of the soil (Zapata and Axmann, 1995). Uses of phosphate solubilizing microorganisms such as arbuscular mycorrhizal fungal association is a very cheap and low energy cycle to enhance the agronomic effectiveness of rock phosphate by changing it into available form (Gyaneshwar *et al.*, 2002; Sabannavar and Lakshman, 2009).

In addition, Mycorrhizal association also enhances the up take of Nitrogen form  $\text{NH}_4\text{-N}$  mineral fertilizers passing it to the host plants. Extensive nitrogen uptake ability of VAM fungal association was demonstrated by using in-vitro model system by many workers such as (Jin *et al.*, 2005; Govindarajulu *et al.*, 2005; Howkins *et al.*, 2000). Likewise, the fungi obtain carbon from plants and transfer other mineral nutrients to the plants (Bidartondo *et al.*, 2002). Besides phosphorus, VAM fungi can also improve the uptake of several micro-nutrients like Zinc, Copper, Iron and Manganese (Marschner, 1995). The AM fungi absorb these mineral elements and store them and also prevent their concentration to reaching the toxic level (Cooper and Tinker, 1987).

### **Non-Nutritional Role of Vam Fungi**

The major advantage of VAM symbiosis is an increased tolerance to root pathogens and to environmental stresses (Harries and Watson, 2004). VAM fungi do not directly interact with harmful pathogens but have indirect effects, which may be physiological or physical changes through certain chemicals and morphological alteration in host plants. Mycorrhizal association also increased root length density and also change root morphology preparing the plants to discover more soil volume and to absorb more water as compared to non-mycorrhizal plants during drought conditions (Calvet *et al.*, 2001).

Arbuscular mycorrhizal fungi might be used to increase salt tolerance of plants utilizing such strains we would tend to increase the success of establishing mycorrhizae in crops growing on saline soil with improved production. Many workers such as (Muok and Ishii, 2006; Cho *et al.*, 2006) suggested that Arbuscular mycorrhizal fungi enhanced tolerance of different salts. Soil aggregation is very important for maintaining soil porosity, proper gases exchanges, facilitate biogeochemical cycle and water infiltration (Diaz-Zortica *et al.*, 2002). Mycorrhizal fungal association is also very essential for biological and physical properties of soil leading to growth and establishment of many crops, particularly plants of the intensive agricultural system, in which soil texture, soil structure, chemical composition and the micro-flora are mostly disturbed (Borkowska *et al.*, 2008).

### **Materials and Methods**

During the experiment clay soil and sand used were obtained from the ground of Botany Department, University of Peshawar. Soil sample was a chemical analysis of (NIFA) by different methods. The concentration of nitrogen in soil samples was determined by the Jeldhal method of Bremner and Mulvaney (1996). ABDTPA extractable P, Cu, Fe, Zn and Mn and soil PH by Richards (1954) organic matter of the soil by Nelson & Sommer (1982). The clay soil with PH of 7.9, electric conductivity  $0.676 \text{ ds/m}^2$ , Nitrogen, 0.033% and Phosphorus 0.001 ppm. The sand having PH, 8, electric conductivity  $0.325 \text{ ds/m}^2$ , Nitrogen, 0.056% and Phosphorus 0%. After the sieving, the clay soil was finely mixed with sand in a ratio of 2:1 resulting in sandy loam textured soil. A total of 32 pots with a diameter of 89cm and the length of 48cm were filled with 7 Kg of these nutrients lacking soil.

### **Application of inoculums**

In the experiment rhizospheric soil of *Agave* plant with high spore number of many VAM fungal species, i.e. *Glomus fasciculatum*, *Glomus mosseae* and *Acaulosporamellae*. Inoculum used was soil based and its preparation, placement and application were carried out by using the method of Brundrett *et al.*, 1996.

### **Evaluation:**

Following growth parameters were measured.

- i) Shoot length ii) Root length
- ii) Fresh weight iv) Dry weight
- iii) Number of leaves

### **Statistical Analysis**

ANOVA was carried out for all dependent variable data (root length, shoot length, fresh weight, dry weight and number of leaves) to check whether the F test was significant and means were compared by the Adhoc Turkey test.

## Results

### 1. Shoot length “height” (cm)

Results of Mean, ANOVA and LSD test of shoot length of *Mentha longifolia* L. following 7 treatments are given in table, plates and appendices 1. Analysis of variance revealed significant differences among treatments and non-significant differences among replications regarding to the plant shoot length at different levels of rock phosphate in mycorrhizal and non-mycorrhizal plants. The highest shoot length in term of mean was recorded in MRP3, and MRP2 while lowest in M+. While height of MRP1 was in between above two extreme. The overall performance of mycorrhizal plants was better than non-mycorrhizal plants.

### 2. Root length in (cm)

The results of the Means, ANOVA and LSD test of root length of *Mentha longifolia* L. following 7 treatments are given in table, figure and appendices 2. Analysis of variance revealed non-significant differences among replications and treatments regarding to the root length at various levels of Rock phosphate. The highest root length in term of mean was recorded in MRP1 and MRP3 and lowest in M+. The root length of MRP2 was in between above, the two extreme. At different levels of rock phosphate plants with VAM association showed better root length than non-mycorrhizal.

### 3. Fresh weight in “g”

The results of Means, ANOVA and LSD test of fresh weight of *Menthalongifolia* L. following 7 treatments are given table, figure and appendices 3. Analysis of variance showed significant differences among treatments and non-significant difference among replications regarding to the plant fresh weight at various levels of Rock phosphate. The highest fresh weight in term of mean was recorded in MRP3, and MRP2 and lowest in M+. While the fresh weight of MRP1 was in between above two extreme. Overall mycorrhizal plants showed better fresh weight than non-mycorrhiza.

### 4. Dry weight in “g”

The results of Means, ANOVA and LSD test regarding to dry weight of *Menthalongifolia* L. following 7 treatments are given in the table, figure and appendices4. Analysis of variance exhibited non-significant differences among treatments and replications regarding to the plant dry weight at various levels of Rock phosphate. The highest dry weight in term of mean was recorded in MRP3, and MRP2 and lowest in M+. While the fresh weight of MRP1 was in between above two extreme. Overall the performance of Mycorrhizal was better than non-mycorrhizal at various levels of Rock phosphate

### 5. Number of leaves

The results of means, ANOVA and LSD test regarding to the number of leaves of *Menthalongifolia*L. following 7 treatments are given in table, figures and appendices 5. Analysis of variance revealed significant differences among treatments and non-significant difference among replications regarding to the number of leaves at various levels of Rock phosphate. The highest number of leaves in term of mean was recorded in MRP3, and MRP2 and lowest in M+, While the number of leaves of MRP1 was in between above two extreme. Regarding to the number of leaves the performance of VAM plants was better than non-mycorrhizal one.

**Table.1. Effect of VAM Fungi and Rock Phosphate on Shoot length (cm) of *Mentha longifolia* L.**

TREATMENTS	Replicates				MEANS± SE
	R1	R2	R3	R4	
M+	34.5	27.5	19.5	42.5	<b>31 ± 0.3</b>
M-	32.5	23	26	33.5	<b>28.75± 0.2</b>
MRP1+	41	49	38	35	<b>40.75± 0.3</b>
MRP1-	36	25	39.5	37	<b>34.375 ± 0.2</b>
MRP2+	43	31.5	27.5	27	<b>32.25± 0.3</b>
MRP2-	29.5	22.5	23	24.5	<b>24.875± 0.2</b>
MRP3+	41.5	32	33.5	26	<b>33.25± 0.3</b>
MRP3-	26	28.5	15.5	19.5	<b>22.375 ± 0.2</b>

**Table.2. Effects of VAM Fungi and Rock Phosphate on Root length (cm) of *Mentha longifolia* L.**

TREATMENTS	Replicates				MEANS± SE
	R1	R2	R3	R4	
M+	6	7.5	7.15	7	<b>6.91± 0.3</b>
M-	6	5	8.5	8	<b>6.87± 0.2</b>
MRP1+	7.5	8	10.25	8	<b>8.43± 0.3</b>
MRP1-	7	6	7	6.75	<b>6.68± 0.2</b>
MRP2+	5.25	8.25	8	8.75	<b>7.56± 0.3</b>
MRP2-	7	7.5	6.5	7	<b>7± 0.2</b>
MRP3+	11.5	7.5	6.25	6.5	<b>7.93± 0.3</b>
MRP3-	6.25	8	4.55	7.25	<b>6.51± 0.2</b>

**Table.3. Effect of VAM Fungi and Rock Phosphate on Fresh weight (g) of *Mentha longifolia* L.**

TREATMENTS	Replicates				MEANS± SE
	R1	R2	R3	R4	
M+	2.55	1.9	1.15	5.05	<b>2.663 ± 0.3</b>
M-	3	1.11	1.6	3	<b>2.170 ± 0.2</b>
MRP1+	3.4	5.1	2.8	4.75	<b>4.012 ± 0.3</b>
MRP1-	3.25	1.8	4	3.95	<b>3.25± 0.2</b>
MRP2+	5.15	3.55	2.4	2.45	<b>3.38± 0.3</b>
MRP2-	2.35	1.3	2.3	1.3	<b>1.812 ± 0.2</b>
MRP3+	5.1	4.15	2.35	1.3	<b>3.457 ± 0.3</b>
MRP3-	2.15	1.25	0.95	0.65	<b>1.25± 0.2</b>

**Table.4 Effect of VAM Fungi and Rock Phosphate on Dry weight (g) of *Mentha longifolia* L.**

TREATMENTS	Replicates				MEANS± SE
	R1	R2	R3	R4	
M+	0.55	0.55	0.35	1.35	<b>0.7± 0.2</b>
M-	1.1	0.15	0.55	0.9	<b>0.67± 0.3</b>
MRP1+	0.75	1.3	0.75	1.55	<b>1.08± 0.2</b>
MRP1-	0.65	0.5	1.2	1.05	<b>0.85± 0.3</b>
MRP2+	1.4	0.9	0.65	0.55	<b>0.87± 0.2</b>
MRP2-	0.55	0.25	0.5	0.25	<b>0.38± 0.3</b>
MRP3+	2.75	2.1	0.5	0.35	<b>1.42± 0.3</b>
MRP3-	0.6	0.4	0.3	0.25	<b>0.38± 0.2</b>

**Table.5. Effect of VAM Fungi and Rock Phosphate on number of leaves of *Mentha longifolia* L.**

TREATMENTS	Replicates				MEANS± SE
	R1	R2	R3	R4	
M+	51	27	16	62	<b>39± 0.2</b>
M-	42	28	37	47	<b>38.5± 0.2</b>
MRP1+	62	87	46	57	<b>63± 0.3</b>
MRP1-	51	42	56	48	<b>49.25 ± 0.3</b>
MRP2+	67	51	52	47	<b>54.25± 0.2</b>
MRP2-	50	39	26	41	<b>39± 0.2</b>
MRP3+	61	41	58	48	<b>52± 0.3</b>
MRP3-	50	35	20	15	<b>30± 0.2</b>

SE = Standard error

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

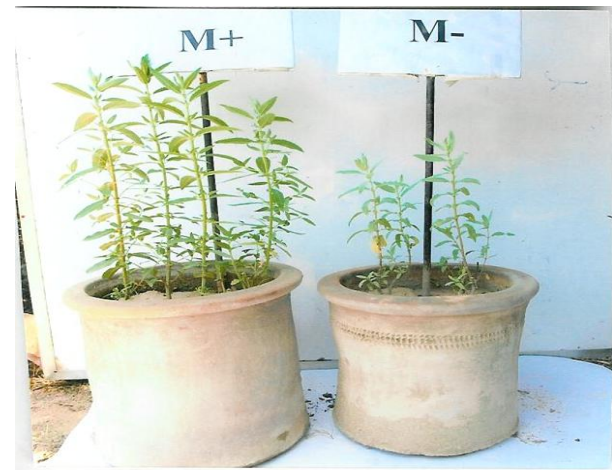


Plate.2 Effect of VAM and RP1 on growth of *Mentha longifolia* L.

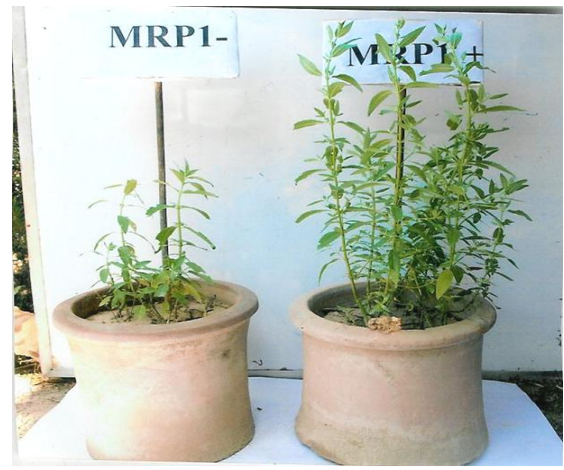
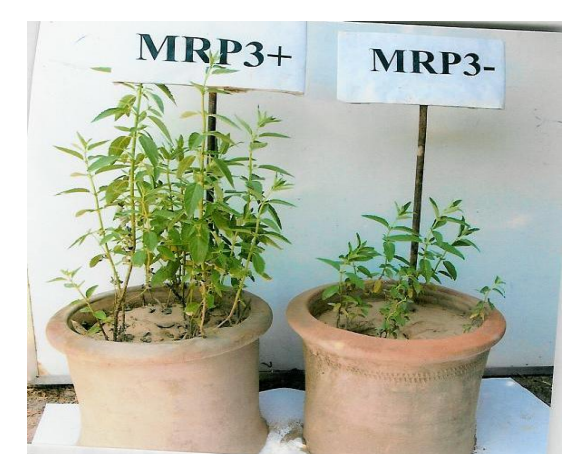


Plate.3 Effect of VAM and RP2 on growth of *Mentha longifolia* L.



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



**Appendices:** Analysis of variance effect of AM fungi and rock phosphate with different variables.

Variable.1 Effect of AM Fungi and Rock Phosphate on shoot length of *Mentha longifolia* L.

**Analysis of variance table**

Source	Degree of. the freedom	Sum of the .squire	Mean-squire	F.Value	Probi
Replications	3	254.46	84.820	2.31	0.1052
Treatments	7	920.12	131.445	3.59	0.0107
Error	21	769.60	36.648		
Total	31				

Grand Mean = 30.953 Grand Sum = 950. 500 Total Count = 32  
Coefficient of Variation= 19.56%

Variable. 2(c) Effect of AM Fungi and Rock Phosphate on root length of *Mentha longifolia* L

**Analysis of variance table**

Source	Degree of .freedom	Sum of squire	Mean squire	F.Value	Prob
Replications	3	0.49	0.162	0.07	0.9737
Treatments	7	12.63	1.804	0.81	0.5861
Error	21	46.56	2.217		
Total	31	59.67			

Grand Mean= 7. 241 Grand Sum = 231.700 Total Count = 32  
Coefficient of Variation= 20.56

Variable. 3 (b). Effect of AM Fungi and Rock Phosphate on a fresh weight of *Mentha longifolia* L

**Analysis of variance table**

Source	Degree of freedom	Sum of square	Mean square	F.Value	Prob
Replications	3	5.92	1.974	1.41	0.2683
Treatments	7	23.48	3.354	2.39	0.0576
Error	21	29.45	1.402		
Total	31	58.85			

Grand Mean= 2.714 Grand Sum = 86.840 Total Count= 32

Coefficient of Variation= 43.64

Variable. 4 (b). Effect of AM Fungi and Rock Phosphate on dry weight *Menthalongifolia*L.

**Analysis of variance table**

Source:	Degree of freedom	Sum of square	Mean square	F.Value	Probi
Replications	3	00.81	00.269	0.95	0.4333
Treatments	7	3.39	0.484	1.72	0.1592
Error	21	5.92	0.282		
Total	31	10.12			

Grand Mean= 0.798 Grand Sum= 25.550 Total Count= 32

Coefficient of Variation= 66.51

Variable. 5 Effect of AM Fungi and Rock Phosphate on number of leaves of *Mentha longifolia* L

**Analysis of variance table**

Source:	Degree; of freedom	Sum -square	Mean-Square	F.Value	Probi
Replications	3	987.75	329.250	2.24	0.1136
Treatments	7	3251.00	464.429	3.16	0.0192
Error	21	3088.75	147.083		
Total	31	7327.50			

Grand Mean= 45.625 Grand Sum= 1460.000 Total Count = 32

Coefficient of Variation= 26.58%

## Discussion

The results of Means, ANOVA and LSD test of *Mentha longifolia* L. showed that mycorrhizal plants shows better growth rate regarding to root length, shoot length, fresh weight, dry weight and number of leaves than non-mycorrhizal one at various levels of rock phosphate. The possible explanation may be that mycorrhizal association significantly enhanced the uptake of N and P then control one (Rehman *et al.*, 2006). The fungal hyphae covered large areas of soil and uptake P which are out of nutrient depletion zone that largely build up about the root surface at lesser carbon cost leading to increased root growth in inoculated plants and also root length than control one (Azcon *et al.*, 2003).Mycorrhizal inoculation increases transpiration and photosynthetic rate and chlorophyll concentration in plant than control one. It was hypothesized that AM inoculation enhance the growth and secondary metabolism. Overall VAM colonization drastically increases the biomass of the shoot and root and also increase concentration of Protein and most of the Phenol contents in the roots (Araim *et al.*, 2009).

Lot of works had been done on the role of AM fungal association which enhanced tremendously the productivity, growth, nutrient uptakes and environmental stresses of plants in medicinal plants such as (Jackobsen *et al.*, 2000; Duponnois *et al.*, 2005; Derek *et al.*, 2005; 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;Caravaca *et al.*, 2005;Azcon *et al.*, 2003;Harrison *et al.*, 2002; Paszkowski *et al.*, 2007; Parras-Sariano *et al.*, 2009; Jeffries *et al.*, 2003; Bucher *et al.*, 2009).

Mycorrhizal association can enhance in successful use of the rock phosphate by varying it into obtainable form, and uptake by the plants for enhanced growth and development (Sabannavar and Lakshman, 2009). Mycorrhizal association and plant growth is related because of high uptake of P, Zn and Mn (Cigar *et al.*, 2000). AM inoculation also enhanced plant biomass and total uptake of the P, N and many other nutrients through the activities of dehydrogenase, phosphatase and nitrogen as enzymes (Rao and Tak, 2001). VAM associations increased P and N uptake of the plants by entrapping Phosphate, Nitrate and Ammonium in the soil(Khan *et al.*, 2008).VAM fungi enhanced uptake of nutrients such as K, N, P, Ca and Mg contents and also increases the number of leaves (Satter *et al.*, 2006).As AMF association is very crucial for plant growth, productivity, secondary metabolites and nutrient uptake. So the present research work was carried out to find the outcome of VAM immunization on *Mentha longifolia* L.

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