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Arbuscular Mycorrhizal Fungi (AMF) effects on growth and nutritional performance of *Mentha arvensis* L. at various levels of rock phosphate amendments

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Present research work was performed to know the effects of AMF (Arbuscular Mycorrhizal Fungi) inoculation on biomass and growth performance of *Mentha arvensis* L. and different concentration of rock phosphate amendments were applied in association with AMF or without AMF such as RP_0 (without phosphate) RP_1 (0.05g), RP_2 (0.105g) and RP_3 (0.157g). Inoculum used was soil based and obtained from Agave plants with large number VAM spores i.e. *Glomus aggregatum*, *Glomus fasciculatum* and *Acaulospora mellea* were most prominent. The mycorrhizal plants showed improved growth significantly regarding root length, shoot length, number of leaves, fresh weight and dry weight as compared to non-mycorrhizal plants at low level of rock phosphate. In contradiction differences between mycorrhizal and non-mycorrhizal plants diminished at high levels. Similarly, nutritional analysis showed enhancement in crude fiber, protein and fat in mycorrhizal plants, however carbohydrate content was reduced in mycorrhizal plants. Mycorrhizal fungi enhanced the absorption of nutrients such as phosphate which is non-mobile and leads to enhanced plant growth. Overall mycorrhizal group shows better performance as compared to non-mycorrhizal group at various level of rock phosphate.

Keywords: *Mentha arvensis* L. Agave plant, Inoculum, AMF association, Rock Phosphate.

INTRODUCTION

Mycorrhiza word is derived from two Greak words 'Myco' means Fungus and 'Rhiza' means Roots of plants. Mycorrhizal association is a mutualistic balanced association between fungi and plants in which materials essential for survival and growth of both partners take place. These occupy a separate iso-cline from pathogenic, endophytic or antagonist associations in the betterment of fungus-plant interaction (Brundrett, 2004) and mycorrhizal plants shows better growth and biomass as compared to non-mycorrhizal plants (Fazal *et al.* 2020). On the basis of different fungal and plants symbionts there are many types of mycorrhizal associations, out of them (VAM) Vesicular Arbuscular Mycorrhizae is most crucial and wide spread and present in the roots of more than 80% of higher plants (Redecker, 2005) and viewed as a classic mutualism for the welfare of both the partners (Hodge *et al.* 2010). AM Fungal associations have great importance to plants due to their higher

capacity in increasing growth and yield through efficient nutrients uptake in infertile soils (Chen *et al.* 2005; Khalafallah and Abo-Ghalia, 2008). The potential of AM fungi as bio-fertilizers have been well accepted and documented in earlier studies (Kapoor *et al*. 2002). The most important and crucial role associated with the use of AM fungi include better nutrients absorption and improvement in growth and biomass of the plants (Kapoor *et al.* 2004). AM 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).

Phosphorus is very beneficial and important mineral nutrient for plants growth and the plant uptake it mostly in the form of ortho-phosphate (Vance, 2003). Due the fact that phosphate is generally not very mobile in soils, narrow depletion zones in the order of millimeters from around P- absorbing roots leading to low phosphate concentrations in soil can be limiting for plant growth performance (Hinsinger *et al.* 2005). Therefore, plants have developed mechanisms such as symbiotic relations with soil fungus in order to increase their access to soil phosphate. Probably the most important symbiotic association is the formation of mycorrhizae, a symbiotic association between plant roots and specific soil fungi (Khade & Rodrigues 2009). Mycorrhizal association is very crucial for all plants especially in medicinal plants and also presents in the member of Lamiaceae as in *Mentha arvensis* L. (Burni and Hussain 2013).

The term (AM) becomes more commonly used as compared to (VAM) because some fungi do not produce vesicles in the roots in these associations. However, there are problems with the use of Arbuscle alone to define AM (Brundrett, 2004). According to latest classification, AM fungi belong to phylum Glomeromycota phylum, with four families and 12 genera. Until now about 200 spps of VAM fungi have been described (Redecker and Philipp, 2006). The taxonomy and identification of AM fungi is still are based on the morphology of their spores but this criteria also leads to incorrect result. A new monophyletic phylum, Glomeromycota, for AM fungal associations had been established based on SSU rRNA gene sequencing techniques (SchuBler *et al*. 2001).

MATERIALS AND METHODS

32 pots having 89 cm diameter and 48 cm length were filled with 7 kg of nutrient deficient sandy loam textured soil. Sand and clay soil was used in this research work was obtained from the ground of Botany department University of Peshawar. After sieving, clay soil was finely mixed with sand with the ratio of (2:1) resulting in sandy loam textured soil. Chemical analysis of the soil and sand samples was done at Nuclear Institute for Food and Agriculture (N.I.F.A) by different methods, Nitrogen concentration by K. Jeldhal method of Bremmer (1996). ABDTPA extractable P, Cu, Fe, Zn, and Mn and Soil PH by Richard (1954), electrical conductivity by Black, C.A (1965), soil organic matter by Nelson and Sommer (1982). The clay soil having PH (7.8), electrical conductivity (0.675 ds/m^2), Nitrogen (0.032%) and Phosphorus (0.2%). The sand shows PH of (8), Electrical conductivity of (0.325 ds/m^2), Nitrogen (0.056%) and Phosphorus (0%). After sieving, clay soil was finely mixed with sand in ratio of 2:1 resulting in sandy loam textured soil.

Inoculum applications:

In this research work the rhizospheric soil was obtained from the Agave plant having high number of different AM fungi species i.e. *Glomus fasciculatum, G. aggregatum* and *Acaulospora mellae.* Inoculum with fungal spores was used as soil based Inoculum and its preparation, placement and application were done by the methods provided by (Brundrett *et al*. 1996).

APPLICATION OF FERTILIZERS:

Following levels of Rock Phosphate were applied in combination with AM or without AM.

1-RP_0 No Phosphate added (Control)
2-RP_1 25% 0f the recommended dose
3- RP_2 50% of the recommended dose
4-RP_3 75% of the recommended dose

EXPERIMENTAL DESIGN, TREATMENTS AND REPLICATIONS:

1- M+ Mycorrhizae without Rock Phosphate (control).
2- M- Non-mycorrhizal without Rock Phosphate (control).
3- MRP_1+ Mycorrhizal + Rock phosphate level 1
4- MRP_{1-} Non-mycorrhizal + Rock Phosphate level 1
5-MRP_2+ Mycorrhizal + Rock phosphate level 2
6- MRP_2- Non mycorrhizal + Rock phosphate level 2
7- MRP_3+ Mycorrhizal + Rock phosphate level 3

8- MRP_3- Non-mycorrhizal + Rock phosphate level 3

EVALUATIONS

Following growth Parameters were determined.

1 - Root length 2- Shoot length 3- Number of leaves 4- Fresh weight 5- Dry weight.

STATISTICAL ANALYSIS:

Statistical analysis was done by calculating ANOVA (Analysis of variance) and LSD.

PROXIMATE ANALYSIS:

Dried powder of *Mentha arvensis* L. was analyzed at Agriculture University Peshawar KPK Pakistan for Ash and moisture contents, crude protein, Fat, fiber and carbohydrate on dry matter basis and final results are given in the table 6..

RESULTS

Shoot Length (cm):

Variance analysis revealed significant differences for replications and highly significant differences for treatments regarding the shoot length at various levels of rock phosphate in mycorrhizal and non-mycorrhizal plants (Appendix 1). It is evident from mean data that Mycorrhizal plants show better performance as compared to non-mycorrhizal plants at RP1 and RP2 Levels. While, at RP3 level non-mycorrhizal plants shows better performance than mycorrhizal one. Among controls mycorrhizal plants exhibited better performance in shoot length than non-mycorrhizal plants (Table 1, Fig 1).

Number of Leaves:

Analysis of variance exhibited non- significant differences for replications and significant differences for treatments in mycorrhizal and non-mycorrhizal plants (Appendix 2). Mean data indicated that at RP1 and RP2 show better results in mycorrhizal than non-mycorrhizal plants. While at RP3 level the differences were slight. Again mycorrhizal plants shows best performance in comparison to non-mycorrhizal one (Table 2, Fig 2).

Fresh Weight (g):

Analysis of variance cleared significant differences for replications and highly significant differences for treatments regarding the plant fresh weight in mycorrhyizal plants (Appendix 3). Mycorrhizal plants exhibited maximum growth at RP1 and RP2 as compared to non-mycorrhizal plants. However at RP3 level slight differences observed between mycorrhizal and non-mycorrhizal plants. Similarly mycorrhizal plants under control showed high fresh weight (table 3, Fig 3).

Root Length (cm):

Analysis of variance revealed significant differences for treatments and non-significant differences for replications regarding root length in mycorrhyizal and non-mycorrhizal plants (Appendix 4). Mycorrhizal plants showed better performance at RP1 and RP2 levels as compared to non-mycorrhizal plants. However at RP3 level low difference were present in mycorrhizal and non-mycorrhizal plants under control condition better performance was showed by mycorrhizal plants (table 4, Fig 4).

Dry weight (g):

Variance analysis indicated non-significant differences among the replications and treatments regarding the plant dry weight in mycorrhyizal and non-mycorrhizal plants (Appendix 5). At RP1 level the mycorrhizal plants showed maximum dry weight as compared to non-mycorrhizal plants. At RP2 and RP3 there were slight differences between mycorrhizal and non-mycorrhizal plants. Mycorrhizal plants under control condition showed maximum dry weight (table 5).

Proximate Analysis:

Dried powder of *Mentha arvensis* L. was analyzed for ash content, crude protein, crude fiber, fat, moisture contents and carbohydrate an dry matter basis and the results are given in (Table 6). Slight differences were observed among mycorrhizal and non-mycorrhizal plants regarding the ash contents. However mycorrhizal plants showed better result as compared to non-mycorrhizal plants.

Mycorrhizal plants have higher of crude protein as compared to non- mycorrhizal plants. Similar results were observed for crude fiber and fat contents, in which mycorrhizal plants exhibited better performance as compared to non-mycorrhizal plants. However, for moisture contents the non-mycorrhizal plants performed better, although the differences were slight.

For carbohydrate the non-mycorrhizal plants performed better as compared to mycorrhizal plants. However at RP2 level the differences were slight.

Appendix 1: Analysis of variance table for Shoot length of *Mentha arvensis* L.

Source	Degree of Freedom	Sum of Squares	Mean Square	F-value	Prob
Replications	3	237.50	79.167	3.28	0.0409
Treatments	7	845.55	120.793	5.01	0.0018
Error	21	506.18	24.104		
Total	31	1589.23			

Coefficient of variation: 21.48%Error Mean Square =24.10No. of observations to calculate a mean = 4
LSD value = 7.220 at alpha = 0.05

Appendix 2: Analysis of variance table for Number of leaves of *Mentha arvensis* L.

Source	Degree of Freedom	Sum of Squares	Mean Square	F-value	Prob
Replications	3	20.90	6.966	1.62	0.2143
Treatments	7	89.93	12.847	2.99	0.0242
Error	21	90.16	4.294		
Total	31	200.99			

Coefficient of variation: 17.66%, Error Mean Square = 4.294, LSD value = 3.047 at alpha = 0.05

Appendix 3: Analysis of variance table for Fresh weight of *Mentha arvensis* L.

Source	Degree of Freedom	Sum of Squares	Mean Square	F-value	Prob
Replications	3	6.63	2.211	3.45	0.0349
Treatments	7	41.89	5.984	9.35	0.0000
Error	21	13.44	0.640		
Total	31	61.96			

Coefficient of variation: 39.78% Error Mean Square =0.6400, LSD value = 1.176 at alpha = 0.05

Appendix 4: Analysis of variance table for Root length of *Mentha arvensis* L.

Source	Degree of Freedom	Sum of Squares	Mean Square	F-value	Prob
Replications	3	15.96	5.322	1.42	0.2643
Treatments	7	84.06	12.008	3.21	0.0179
Error	21	78.56	3.741		
Total	31	178.58			

Coefficient of variation: 28.92%Error Mean Square = 3.741LSD value = 2.844 at alpha = 0.05

Appendix 5: Analysis of variance table for Dry weight of *Mentha arvensis* L.

Source	Degree of Freedom	Sum of Squares	Mean Square	F-value	Prob
Replications	3	0.83	0.276	0.83	0.4930
Treatments	7	3.52	0.503	1.51	0.2181
Error	21	6.99	0.333		
Total	31	11.34			

Coefficient of variation: 97.96% LSD value = 0.849 at alpha = 0.05

Table1: Effect of Mycorrhiza and Rock Phosphate levels on Shoot length (cm) of *Mentha arvensis* L.

Treatments	R1	R2	R3	R4	Mean
M+	35.5	29.5	25.5	28.0	29.63 ^{a}
M-	16.0	21.0	16.0	11.0	16.00 ^{d}
MRP1+	36.0	36.0	30.0	19.0	30.25 ^{a}
MRP1-	25.5	18.0	29.5	22.0	23.75 ^{abc}
MRP2+	29.0	28.5	23.25	22.0	25.69 ^{ab}
MRP2-	26.25	19.0	12.5	10.0	16.94 ^{cd}
MRP3+	20.25	21.0	20.5	12.0	18.44 ^{cd}
MRP3-	23.0	21.0	14.5	30.0	22.13 ^{bcd}

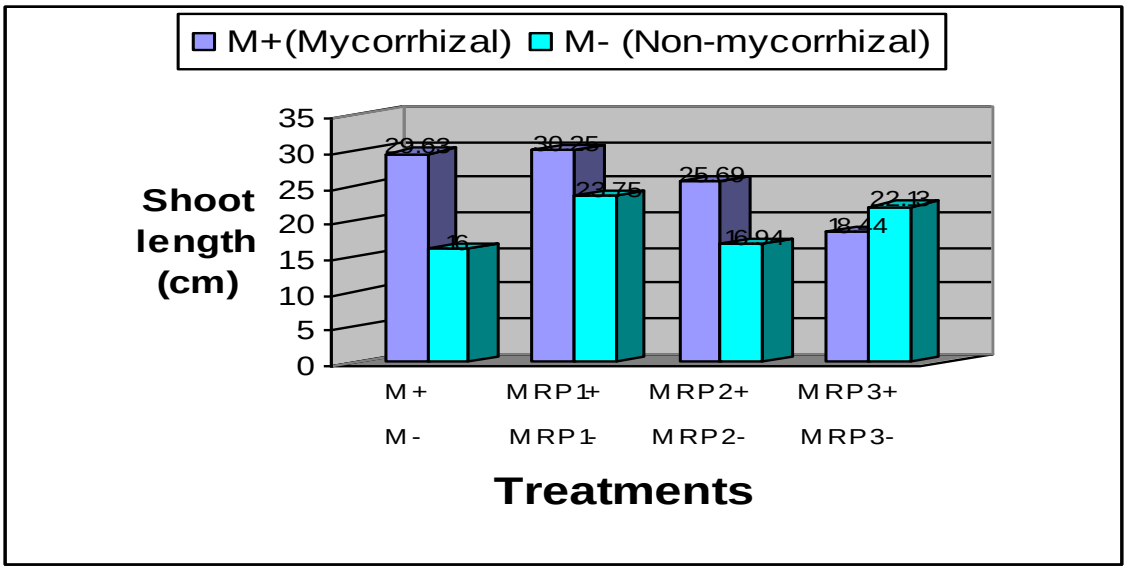

Figure 1

Table 2: Effect of Mycorrhiza and Rock Phosphate levels on Number of leaves of *Mentha arvensis* L.

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Treatments	R1	R2	R3	R4	Mean
M+	12.0	17.5	12.0	13.0	13.63 [a]
M-	10.0	11.5	9.5	9.0	10.00 [bc]
MRP1+	15.0	17.5	12.5	11.0	14.00 [a]
MRP1-	12.5	11.0	13.0	12.0	12.13 [ab]
MRP2+	12.5	11.5	12.0	12.0	12.00 [ab]
MRP2-	11.5	10.0	7.5	5.0	8.50 [c]
MRP3+	11.5	14.0	10.5	12.0	12.00 [ab]
MRP3-	11.5	10.0	9.0	16.0	11.63 [ab]

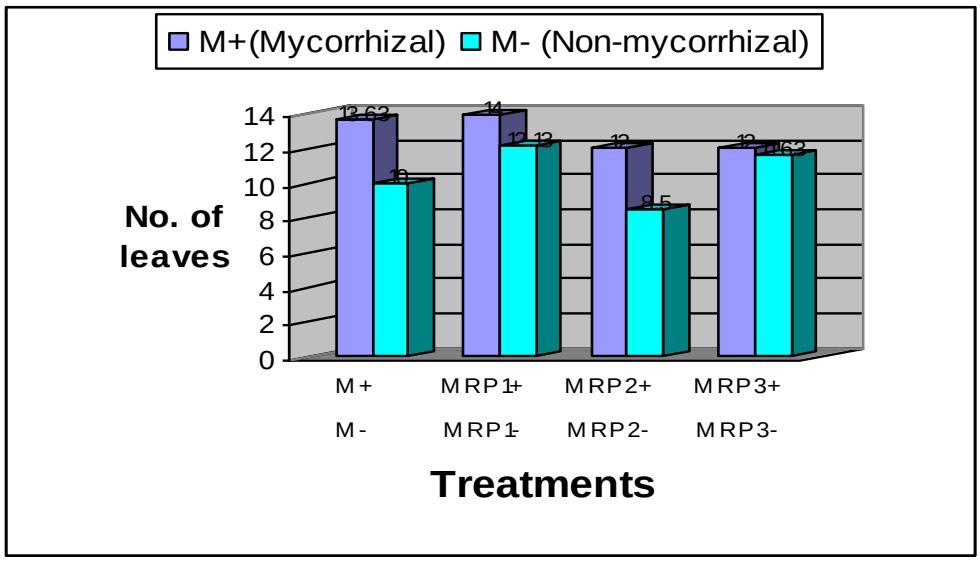

Fig 2

Table 3: Effect of Mycorrhiza and Rock Phosphate levels on Fresh weight (g) of *Mentha arvensis* L.

Treatments	R1	R2	R3	R4	Mean
M+	4.35	2.0	3.0	1.8	2.79 ^{bc}
M-	0.85	0.7	0.8	0.35	0.68 ^{de}
MRP1+	5.0	5.95	2.9	2.7	4.14 ^{a}
MRP1-	1.55	2.0	1.2	0.8	1.39 ^{de}
MRP2+	3.2	4.3	2.35	2.3	3.04 ^{ab}
MRP2-	0.95	1.15	0.25	0.15	0.63 ^{e}
MRP3+	2.85	2.0	1.65	0.85	1.84 ^{cd}
MRP3-	1.4	1.1	1.1	2.8	1.60 ^{de}

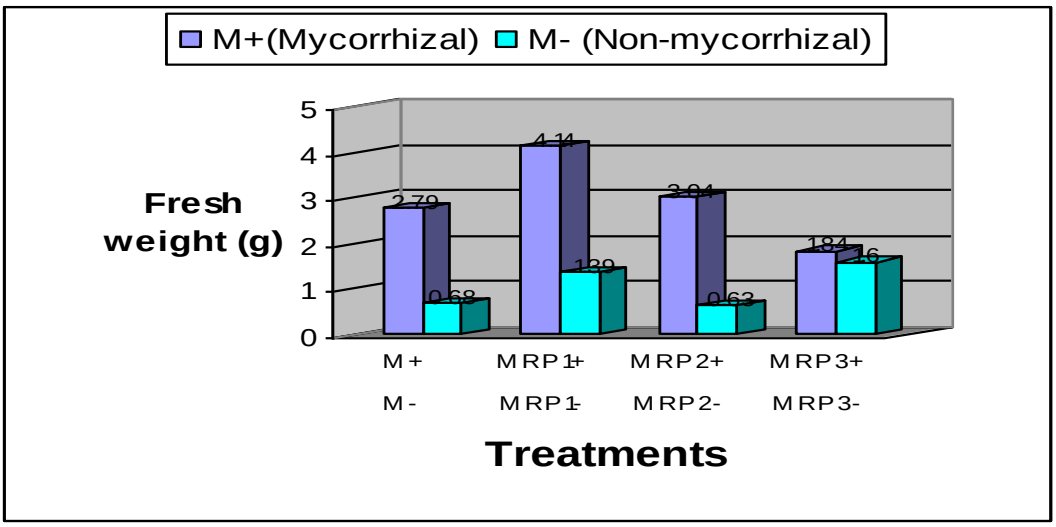

Fig 3

Table 4: Effect of Mycorrhiza and Rock Phosphate levels on Root length (cm) of *Mentha arvensis* L.

Treatments	R1	R2	R3	R4	Mean
M+	7.25	10.5	7.5	11	9.063 ^{a}
M-	5.75	5.75	7.75	3.0	5.563 ^{b}
MRP1+	7.0	6.5	7.0	5.5	6.500 ^{ab}
MRP1-	4.0	4.25	5.5	4	4.439 ^{b}
MRP2+	7.0	14.75	8.5	6.0	9.063 ^{a}
MRP2-	3.75	7.75	4.25	4.0	4.938 ^{b}
MRP3+	9.0	6.75	7.75	5.5	7.250 ^{ab}
MRP3-	6.5	6.25	5.0	8.5	6.688 ^{ab}

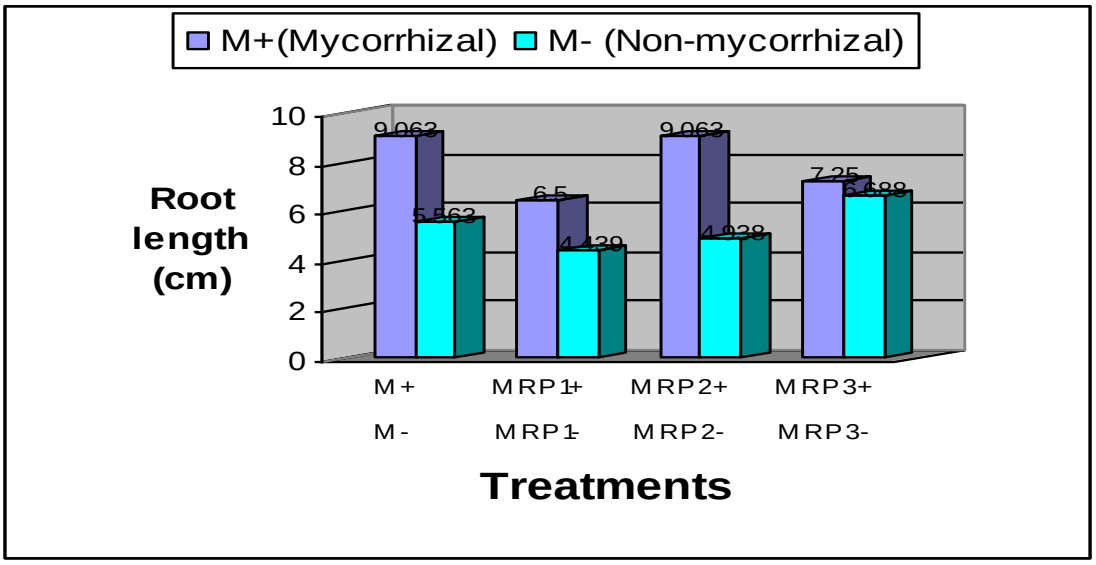

Fig 4

Table 5: Effect of Mycorrhiza and Rock Phosphate levels on dry weight (g) of *Mentha arvensis* L.

Treatments	R1	R2	R3	R4	Mean
M+	2.85	0.35	0.85	0.6	1.162
M-	0.25	0.35	0.2	0.1	0.225
MRP1+	1.15	1.9	0.9	0.1	1.012
MRP1-	0.4	0.2	0.55	0.4	0.387
MRP2+	0.6	1.4	0.5	0.2	0.675
MRP2-	0.4	0.2	0.1	0.1	0.20
MRP3+	0.5	0.6	0.3	0.2	0.40
MRP3-	0.35	0.35	0.3	1.6	0.65

Table 6: Proximate Analysis of *Mentha arvensis* L.

Treatments	Percent on dry matter basis					
	Ash	Crude protein	Crude fiber	Fat	Moisture	Carbohydrate
M+	11.706	16.153	25.887	2.288	8.774	35.192
M-	11.475	14.685	19.780	1.896	9.055	43.109
MRP1+	11.487	16.452	26.460	6.493	8.783	30.325
MRP1-	11.403	16.153	20.579	1.794	8.983	41.088
MRP2+	11.551	14.370	24.375	1.497	9.182	39.025
MRP2-	11.538	13.741	23.338	1.196	9.282	40.905
MRP3+	11.661	15.209	22.316	4.486	9.191	37.137
MRP3-	11.258	13.541	17.691	3.493	9.851	44.166

Plate 1: Effect of Mycorrhiza and RP0 (Control) levels on growth of *Mentha arvensis* L.

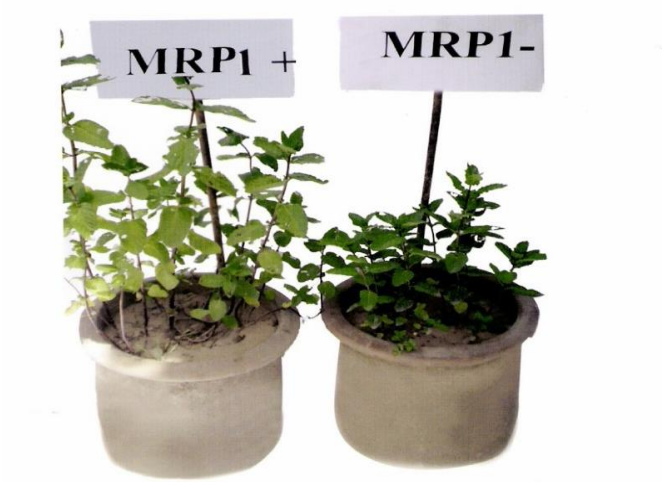

Plate 2: Effect of Mycorrhiza and RP1 levels on growth of *Mentha arvensis* L.

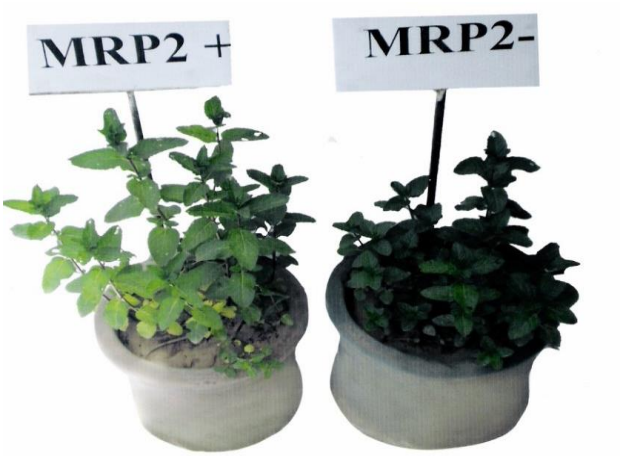

Plate 3: Effect of Mycorrhiza and RP2 levels on growth of *Mentha arvensis* L.

Plate 4: Effect of Mycorrhiza and RP3 levels on growth of *Mentha arvensis* L.

DISCUSSION

The major physiological basis for symbiotic mutualism association is bi-directional nutrients transfer (Smith and Smith 1997). Arbuscular mycorrhizal (AM) fungi is crucial and vital components of nearly all terrestrial ecosystems, forming mutually beneficial (mutualistic) symbioses with the roots of around 80% of vascular plants and often increasing phosphate (P) uptake and growth. In an AM plant, P (as orthophosphate) can be absorbed both directly at the soil-root interface through root epidermis and root hairs and via the "Mycorrhizal" pathway via external AM hyphae in soil. AM hyphae have the ability to grow beyond the nutrients depletion zone and deliver P to the root is thought to be the main basis for their positive effects on P uptake and plant growth (Smith and Read, 2008). Mycorrhizal association is very beneficial for nutrients uptake such as phosphorous, nitrogen, sulphur, zinc, iron, and many more leading to increase root length, shoot length, number of leaves, fresh weight and dry weight (Fazal *et al.* 2018) also enhanced and improve crude protein, crude fiber, fat and moisture contents but carbohydrate contents were reduced due to fungal utilization in return (Fazal *et al*. 2020).

Enhanced growth of mycorrhizal plants as compared to non-mycorrhizal plants have been explored by many other workers such as (Al-Karaki, 2002; Estaun *et al*. 2003; Paradi et al. 2003; Zandavalli *et al.* 2004; Li *et al*. 2005; Ouahmane *et al.* 2007; Ennett and Bever, 2007; Meghvans *et al.* 2008; Dianda, 2009; Kafkas and Ortas, 2009; Selvaraj *et al.* 2009; Yaseen *et al.* 2011; Alsamowal *et al.* 2013 and Burni *et al.* 2013).

The possible explanation may be the presence of extra-radical mycelium in the soil. Its primary function is the absorption of resources and nutrients from the soil. The increased efficiency of mycorrhizal roots versus non-mycorrhizal roots is caused by the active uptake and transport of nutrient by mycorrhizae (Quilambo, 2003). As the fungi hyphae have very smaller diameter than plants roots and therefore access smaller soil pores which effectively increase the volume of exploitation (Givovannetti, 2001). The mycorrhizal colonization may also induce the formation of lateral roots or increase root branching (Cisternesi *et al.* 1988). According to Clapperton and Reid (1992) higher root/shoot ratios indicate highly developed and extensive root system with efficient nutrient absorption. Therefore mycorrhizal plants can absorb more P at lower concentration in the soil solution than non-mycorrhizal plants (Plenchette and morel, 1996). The acquisition of other nutrients such as Cu, Mg and Fe in significantly higher concentration also occurs in mycorrhizal plants in comparison to non-mycorrhizal plants (Sharma et al. 2008). Moreover, mycorrhizal fungi secrete enzymes phosphatases, proteases, chealating compound to capture nutrient from soil organic and inorganic material that are not normally accessible to plants (Kumar *et al.* 2007). More efficient absorption of nutrients and water in inoculated plants is the possible justification for larger number of leaves, which facilitate better development of the aerial part of the plant in relation to the non-inoculated plants (Silveira *et al.* 2006). Results from this study are in consistent

with the fact that mycorrhizal inoculation changed the root morphology, increases the lateral root number and length. AM inoculated plants developed more economical root system, which is more efficient in absorption of nutrient and water. Mycorrhizal inoculation stimulates rooting and growth (Kumar *et al.* 2007) and also reduces soil compaction which results in root development (Miransari, 2007). The activity of Phytohormones like Cytokinin and Indole acetic acid is significantly higher in plants inoculated with AM. Higher hormones production results in better growth and development of plant (Allen *et al.* 1991).

Proximate Analysis:

Crude protein:

As evident from the result that crude protein was high in mycorrhizal plants as compared to non-mycorrhizal plants. According to (Fazal *et al.* 2020 and Ratti *et al*. 2010) *Glomus mosseae* inoculated plants of *Catharanthus roseus* exhibited higher level of protein (8.27 mg/g) than non-mycorrhizal (6.04) and other mycorrhizal inoculated plants. *Glomus mosseae* induced more leaf protein than *G. aggregatum* and *G. fasciculatum*. Similar results were also observed by (Young et al. 1972).

Carbohydrate:

Data of results showed that non-mycorrhizal plants have high carbohydrate level as compared to mycorrhizal plants. It is mostly accepted fact that the AM fungi are obligate symbionts and the carbohydrates are transferred from autotroph to heterotroph (Lewis, 1975 and Fazal et al. 2020). This transfer may cause reduction in carbohydrate level of the host. However our results are not in agreement with those of (Wu et al. 2010) who showed that the sole AMF inoculation markedly increased leaf sucrose content and leaf and root glucose content, compared to the non-AMF + P treatments.

Fat:

Our research studies showed high amount of fat contents in mycorrhizal plant as compared to non-mycorrhizal plants. Similar results were also found by (Cooper and Losel, 1978 and Fazal *et al*. 2020). According to them infected roots contained more total lipid than uninfected roots.

Ashes and moisture

The results of the Ashes and moisture contents of *Mentha arvensis* L. cleared that mycorrhizal plants shows higher contents as compared to non-mycorrhizal plants. Similar results were also found by (Fazal *et al.* 2020).

Fiber

Fiber contents of *Mentha arvensis* L. showed that mycorrhizal plants showed higher contents as compared to non-mycorrhizal plants. Our results was matching with that of (Fazal *et al.* 2020), accordingly AMF plants contained more total fiber than uninfected root.

CONCLUSION

From this research work it is cleared that mycorrhizal plats performed vey well and this association is very important for the growth og Mentha *arvensis* L. at various level of phosphate amendments. Mostly phosphate is immobile in soil. VAM association enhance its uptake leading to overall increase in growth performance such as root length, shoot length, number of leaves, fresh weight and dry weight. Proximate analysis showed that protein, lipids, fibers, ashes and moisture contents of mycorrhizal plants were better than non mycorrhizal plants 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.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

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

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