

**PROSPECTS OF ARBUSCULAR MYCORRHIZAL TECHNOLOGY FOR
GROWTH, MINERAL NUTRIENT ACQUISITION AND ESSENTIAL OILS OF
SELECTED *MENTHA* SPECIES**



By

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**DEPARTMENT OF BOTANY
UNIVERSITY OF PESHAWAR
PESHAWAR, PAKISTAN
2012**

DEDICATION

Sincerely dedicated to
my parents and teachers

UNIVERSITY OF PESHAWAR
PESHAWAR

**PROSPECTS OF ARBUSCULAR MYCORRHIZAL TECHNOLOGY FOR
GROWTH, MINERAL NUTRIENT ACQUISITION AND ESSENTIAL OILS OF
SELECTED *MENTHA* SPECIES**

A dissertation submitted in partial satisfaction

of the requirement for the degree of

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In

BOTANY

By

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This thesis, entitled “**Prospects of Arbuscular Mycorrhizal Technology for growth, mineral nutrient acquisition and essential oils of selected *Mentha* species**” submitted by Mrs. Tanvir Burni is hereby approved and recommended as partial fulfillment for the

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PUBLICATION OPTION

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ABSTRACT

The principal objective of the first phase of present study was to conduct survey and to generate baseline data regarding the mycorrhizal status and their morphological types in 15 selected medicinal plants of Lamiaceae from various places of Khyber Pakhtunkhwa (KP). The study revealed that all plants had mycorrhiza ranging from 55% in (*Ocimum americanum*) to 100% in (*Mentha arvensis* and *Salvia Spp*). AM morphological study revealed that Arum and Paris types were most frequent of all other types. These are first reports of its kind from KP.

AM fungi in the rhizospheric soils of two heavily infected *Mentha* species were isolated, quantified and identified. Possible correlations were established between their occurrence and various soil factors. The study reports diversity of AMF species in the rhizosphere of *Mentha* species (important medicinal hosts). Eleven AMF species including *Acaulospora melleae* Spain & Schenck, *Glomus fasciculatum* (Thaxter), *Glomus etunicatum* Becker & Gerdemann, *Glomus claroides* Schenck & Smith, *Glomus microcarpum* Tul & Tul, *Glomus australe* (Berkeley) Berch, *Glomus intradivus* Schenck & Smith, *Glomus aggregatum* Schenck & Smith, *Glomus constrictum* Trappe, *Glomus mosseae* Gerdemann & Trappe, *Gigaspora gigantea* Gerdemann & Trappe were isolated and identified from *Mentha longifolia* Huds. rhizosphere. Seven species including *Acaulospora leavis* Gerdemann & Trappe, *Glomus fasciculatum* (Thaxter), *Glomus invernatum* Hall, *Glomus aggregatum* Schenck & Smith, *Glomus mosseae*, Gerdemann & Trappe, *Glomus microcarpum* Tul & Tul and *Gigaspora albida* Schenck & Smith, were identified from *Mentha arvensis* L. Based on the morphological characteristics of spore, they were placed under three genera: *Acaulospora*, *Gigaspora* and *Glomus*. The present study highlighted the fact that this partnership is controlled by various edaphic factors and host species. Various indigenous AMF species in crude mixed cultures MC1 (11 Spp.) and MC2 (7 Spp.) were selected multiplied and used along with single exotic species *Glomus intradivus* (GI) in further experiments.

In the second phase different experiments were designed with an aim to find out some efficient and specific AM Fungal endophytes which could be used in future for

better performance, essential oil yield, biological mobilization of nutrients and conservation of selected *Mentha* species. Our results revealed that the native AMF inocula (MC1 and MC2) perform better than those, inoculated with monospecific *Glomus intraradicus* mycorrhizal plants performed better than the non mycorrhizal plants in terms of growth performance. As far as host species response is concerned *Mentha arvensis* performed better in most of the measured aspects as compared to *Mentha longifolia*. The findings also provided useful information on oil yield and its chromatographic profiles as affected by AMF inoculation. An increase in menthol and piperitone oxides (oxygenated monoterpenes) in response to various AMF inocula was noted.

MC1 (11 AMF Spp.) showed comparatively high potency towards developing symbiosis. Keeping this in view, it was selected for further experimentation. The results revealed that AM fungi effectively promoted the productivity of *Mentha* species and proved their role as bio-fertilizer. Inoculation with indigenous AMF species i.e. *Glomus*, *Acaulospora* and *Gigaspora* were found to be significantly effective in enhancing the herb yield in P-deficient soils as compared to non inoculated plants. These findings suggest that *Mentha* species may yield better and more effective with natural fertilizers using AM technology instead of relying on expensive fertilizers. It was noted that lower doses of P-fertilizers with AMF were more effective for host growth and favored good development of endophytes than luxury doses of phosphorus. Higher P doses declined the sporulation and colonization.

Present work revealed the effective performance of indigenous AMF fungi on growth, oil yield and biological mobilization of nutrients in *Mentha arvensis* in combination with natural alternatives (Rockphosphate and organic matter). Results suggested that selected native fungi (species of *Glomus*, *Acaulospora* and *Gigaspora*) were efficient to obtain and biologically mobilize nutrients from rock phosphate and organic matter and provide hosts for improved yield. Moreover both low and high doses of rock phosphate and organic matter exerted positive impacts on AMF efficacy, sporulation and colonization

The use of indigenous mixture of AMF might improve the chance of including any effective fungal isolate and also create a more suitable environment for the operation

of ecosystem processes which may prove to be better strategy for improving plant growth in nutrient deficient soil.

Use of bio-fertilizers like the present AMF for crop production is gaining momentum as they are eco-friendly as compared to chemical fertilizers. AMF should be managed to help in the reduction of synthetic fertilizer and other agrochemical inputs, thus enhancing the sustainability of the commercial cultivation of aromatic plants in low fertile soils.

TABLE OF CONTENTS		
S.No.	CONTENTS	PAGE No.
1.	CHAPTER 1: INTRODUCTION	1
	i. Mycorrhiza	1
	ii. Arbuscular mycorrhiza	2
	iii. Taxonomy of AM Fungi	2
	iv. Structure and Diversity of AM Morphologies	2
	v. AM Benefits to plant	3
	vi. Test plant	10
2.	CHAPTER 2: LITERATURE REVIEW	12
	i. Occurrence of mycorrhiza and host growth responses in medicinal plants	12
	ii. Effect of mycorrhizal fungi on secondary metabolites and essential oils of medicinal plants	16
	iii. Role of AM in nutrient acquisition	19
	iv. Effect of fertilizers on AM infection and spore	22
	v. Effect of fertilizer with AMF on growth	24
3.	CHAPTER 3: MATERIALS AND METHOD	27
	i. Survey	27
	ii. Plant and Period	27
	iii. Sampling	27
	iv. Estimation of AM root colonization	28
	v. Spore extraction and estimation of density	28
	vi. Soil characteristics	28
	vii. Statistical analysis	28
	viii. Experimental work	29
	ix. Biological material	29
	x. Mycorrhizal dependency	32
	xi. Extraction of essential oil	32
	xii. Gas Chromatography-Mass Spectrometry (GC-MS)	32

	xiii. Proximate Composition	33
	xiv. Mineral evaluation	36
	RESULTS AND DISCUSSION	38
4.	CHAPTER 4: Mycorrhizal status and diversity in mycorrhizal morphologies	38
5.	CHAPTER 5: Arbuscular mycorrhizal fungi (AMF) associated with the rhizosphere of <i>Mentha arvensis</i> and <i>Mentha longifolia</i>	46
	i.. Taxonomic distribution of AM fungi	47
	ii. Root colonization and spore density of AM fungi	48
	iii. Effect of chemical characteristics of soil on AMF spore	48
6.	CHAPTER 6: Effects of AM fungi on growth performance and nutrient acquisition	56
	i. Growth attributes	56
	ii. Mineral nutrient acquisition	64
	iii. Proximate composition	73
	iv. Root colonization & AMF spore density	83
	v. Essential oils yield	89
	vi. Chemical composition of essential oils	91
7.	CHAPTER 7: Effect of phosphorus fertilizers on growth of AM induced <i>Mentha</i> species	107
	i. Effect of indigenous AMF species on growth parameters	107
	ii. Mycorrhizal dependency (MD)	114
	iii. Macromineral acquisition	117
	iv. Micromineral acquisition	123
	v. Essential oils	127
	vii. Effect of fertilizers on AMF infections & the densities of AM spores	129

8.	CHAPTER 8: Combined effects of AMF species application, rock phosphates (RP) & organic matters (FYM) fertilizers on growth performance & chemical behaviours of <i>Mentha arvensis</i>	135
	i. Mycorrhizal dependency (MD)	136
	ii. Oil yield	137
	iii. Effect of AMF infections & spore densities	148
9.	CHAPTER 9: CONCLUSIONS & RECOMMENDATIONS	155-156
10.	CHAPTER 10: REFERENCES	157

LIST OF TABLES

Table No.	Table Title	Page No.
1.	Column oven programming.	33
2.	AM morphologies and mycorrhizal status of medicinal plants of Family Lamiaceae.	41
3.	Arbuscular mycorrhizal structural colonization (%) in some plants of Family Lamiaceae.	42
4.	The distribution of AMF species in various districts of KPK in the rhizospheric soils of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> .	51
5.	Total densities/100 gm. soil of AM spores from the rhizosphere of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> in various districts of KPK.	54
6.	Chemical characteristics of rhizospheric soil of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> from various locations of KPK and Co-efficient of correlation (r) of soils chemical characteristics with AM spore densities.	55
7.	Effects of AMF species on various growth parameter of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> .	60
8.	ANOVA values for different parameters.	61
9.	Effect of AM species on macro and micronutrients acquisition of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> .	67
10.	ANOVA for nutrients	68
11.	ANOVA of micronutrients	70
12.	Effect of AM species on Proximate composition (%) and oil yield (%) of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> .	77
13.	ANOVA for proximate analysis	78
14.	ANOVA for proximate analysis	79
15.	Root infection (%) and spore density/100gms soil of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> .	85
16.	Analysis of variance for infection	86
17.	ANOVA - oil yield	90
18.	Experiment 1. Effects of AMF inoculation on Chemical compounds (%) of essential oils of <i>Mentha longifolia</i> .	97
19.	Experiment 1. Chemical composition (%) of essential oils of <i>Mentha arvensis</i> .	99

20.	Effects of AMF species on growth parameters of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> .	110
21.	ANOVA for growth parameters.	111
22.	ANOVA for growth parameters.	112
23.	Effects of phosphorus fertilizers on Mycorrhizal Dependency (MD) % of <i>Mentha arvensis</i> and <i>Mentha longifolia</i> .	115
24.	Effect of AMF species and fertilizers on macronutrient acquisition of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> .	119
25.	ANOVA for macronutrients	120
26.	Effects of AMF species on micronutrient acquisition of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> .	124
27.	ANOVA for micronutrients	125
28.	Root infection (%) and spore density/100gms soil of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> .	131
29.	.Effects of various levels of fertilizers on AM infection Morphologies, external hyphae, internal hyphae, Arbuscules, vesicles, (%) in roots of mycorrhizal <i>Mentha arvensis</i> and <i>Mentha longifolia</i> .	132
30.	Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on dry weight (gms) and oil yield (%) of <i>Mentha arvensis</i> .	138
31.	ANOV for dry weight	139
32.	Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on macronutrient acquisition (%) of <i>Mentha arvensis</i> .	143
33.	ANOVA for macronutrients	144
34.	Effect of two levels of Rock Phosphate (RP) fertilizers with AM (M) and without AM (NM) on micronutrient acquisition of <i>Mentha arvensis</i> .	145
35.	ANOVA Micronutrients	146
36.	Effects of various levels of fertilizers on AM infection (%), spore density, external hyphae, internal hyphae, Arbuscules and vesicles (%) in roots of mycorrhizal <i>Mentha arvensis</i> .	150

LIST OF FIGURE

Figure No.	Figure title	Page No.
1.	a, b, c, Arum type mycorrhiza in the root cortex of <i>Mentha piperata</i> , <i>M. royleana</i> and <i>M. spicata</i> , respectively.	43
2.	Intermediate type mycorrhiza in the root cortex of <i>Mentha arvensis</i> , <i>Salvia lanata</i> and <i>S. nubicola</i> , respectively	44
3.	Paris type mycorrhiza in the root cortex of <i>Mentha longifolia</i> , <i>Rosmarinus officinalis</i> and <i>Ocimum basilicum</i> respectively.	45
4.	Spore of <i>Glomus etunicatum</i> , <i>Acaulospora melleae</i> , <i>Glomus fasciculatum</i> respectively Bar = 10um	52
5.	Spore of <i>Glomus mosseae</i> , <i>Glomus aggregatum</i> and <i>Glomus claroide</i> respectively. Bar = 10um	53
6.	Effect of AMF species on Shoot length, and Root length of <i>Mentha arvensis</i> .	62
7.	Effect of AMF species on Shoot length and Root length of <i>Mentha longifolia</i>	62
8.	Effect of AMF species on Fresh and Dry weight of <i>Mentha arvensis</i> .	63
9.	Effect of AMF species on Fresh and Dry weight of <i>Mentha longifolia</i> .	63
10.	Effect of AMF species on Macronutrients of <i>Mentha arvensis</i> .	71
11.	Effect of AMF species on Macronutrients of <i>Mentha longifolia</i> .	71
12.	Effect of AMF species on Micronutrients of <i>Mentha arvensis</i> .	72
13.	Effect of AMF species on Micronutrients of <i>Mentha longifolia</i> .	72
14.	Effect of AMF species on Ash contents of <i>Mentha arvensis</i> .	80
15.	Effect of AMF species on Fiber contents of <i>Mentha arvensis</i> (AM) and <i>Mentha longifolia</i> .	80
16.	Effect of AMF species on Fat contents of <i>Mentha arvensis</i> .	81
17.	Effect of AMF species on Protein contents of <i>Mentha arvensis</i> .	81
18.	Effect of AMF species on Carbohydrate contents of <i>Mentha arvensis</i> .	82

19.	Root infection (%) in <i>Mentha arvensis</i> .	87
20.	Root infection (%) in <i>Mentha longifolia</i> .	87
21.	Spore density/ 100 gm soil in <i>Mentha arvensis</i> .	88
22.	Spore density/ 100 gm soil in <i>Mentha longifolia</i> .	88
23.	Effect of AMF species on oil yield % of <i>Mentha arvensis</i> .	90
24.	Effect of AMF species on Oxygenated monoterpenes in <i>Mentha arvensis</i> .	101
25.	Effect of AMF species on Monoterpene hydrocarbons in <i>Mentha arvensis</i> and <i>Mentha longifolia</i> .	101
26.	Effect of AMF species on Sesquiterpene hydrocarbons in <i>Mentha arvensis</i> and <i>Mentha longifolia</i> .	102
27.	Effect of AMF species on Oxygenated sesquiterpene in <i>Mentha arvensis</i> and <i>Mentha longifolia</i> .	102
28.	Typical Gc-Ms chromatogram of essential oils from uninoculated (control plant) of <i>Mentha arvensis</i> .	103
29.	Typical Gc-Ms chromatogram of essential oils from GI inoculated plants of <i>Mentha arvensis</i> .	103
30.	Typical Gc-Ms chromatogram of essential oils from MC1 inoculated plants of <i>Mentha arvensis</i> .	104
31.	Typical Gc-Ms chromatogram of essential oils from MC2 inoculated plants of <i>Mentha arvensis</i> .	104
32.	Typical Gc-Ms chromatogram of essential oils from uninoculated (control plant) of <i>Mentha longifolia</i> .	105
33.	Typical Gc-Ms chromatogram of essential oils from GI inoculated plants of <i>Mentha longifolia</i> .	105
34.	Typical Gc-Ms chromatogram of essential oils from MC1 inoculated plants of <i>Mentha longifolia</i> .	106
35.	Typical Gc-Ms chromatogram of essential oils from MC2 inoculated plants of <i>Mentha longifolia</i> .	106
36.	Effect of AMF species on growth parameters of <i>Mentha arvensis</i> .	113

37.	Effect of AMF species on growth parameters of <i>Mentha longifolia</i> .	113
38.	Effect of phosphorus fertilizers on Mycorrhizal Dependency (MD) % of <i>Mentha arvensis</i> and <i>Mentha longifolia</i> .	116
39.	Effect of AMF species and fertilizers on macronutrient acquisition of <i>Mentha arvensis</i> .	122
40.	Effect of AMF species and fertilizers on macronutrient acquisition of <i>Mentha longifolia</i> .	122
41.	Effect of AMF species on micronutrient acquisition of <i>Mentha arvensis</i> .	126
42.	Effect of AMF species on micronutrient acquisition of <i>Mentha longifolia</i> .	126
43.	Effects of AMF species on oil yield of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> .	128
44.	Spore density/100gms soil of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> .	133
45.	Root infection in soil of <i>Mentha longifolia</i> and <i>Mentha arvensis</i> .	133
46.	a. Poorly develop arbuscules b. Terminal vesicles in roots of MP3 treated plants of <i>Mentha arvensis</i> .	134
47.	Poorly develop b. disintegrating arbuscules in roots of MP3 treated plants of <i>Mentha longifolia</i> .	134
48.	Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on dry weight (gms) of <i>Mentha arvensis</i> .	140
49.	Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on Mycorrhizal dependency (gms) of <i>Mentha arvensis</i> .	141
50.	Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on oil yield (%) of <i>Mentha arvensis</i> .	141
51.	Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on macronutrients of <i>Mentha arvensis</i> .	147
52.	Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on micronutrients of <i>Mentha arvensis</i> .	147

53.	Effects of various levels of fertilizers on spore density in <i>Mentha arvensis</i> .	151
54.	Effects of various levels of fertilizers on AM infection (%), external hyphae, internal hyphae, Arbuscules and vesicles (%) in roots of mycorrhizal <i>Mentha arvensis</i> .	151
55.	Heavy AM infection in RP1 treated plants of <i>Mentha arvensis</i> . Bar = 10µm	152
56.	Heavy AM infection RP3 treated plants of <i>Mentha arvensis</i> . Bar = 10µm	152
57.	Heavy AM infection in F1 treated plants of <i>Mentha arvensis</i> . Bar = 10µm	153
58.	Heavy AM infection in F2 treated plants of <i>Mentha arvensis</i> . Bar = 10µm	153

CHAPTER - 1

INTRODUCTION

i. Mycorrhiza

Fungi emerged in the proterozoic era that witnessed the suite of six major extinction events and other global climatic changes. Historically, fungi (including ancestral mycorrhizal fungi) were mining nutrients from primitive undeveloped soils (Bellgard & Williams, 2011). The concept of fungal root symbiosis has been a subject of extensive research. The occurrence of hyphae in the cell of various angiosperms, especially in members of the family Orchidaceae was described first by Reissek as early as 1847. However, it was the German Botanist Frank (1885) who coined the Greek term “Mycorrhizen” (fungus root). The “Mycorrhiza” was further differentiated into “Ectotrophisch” and “Endotrophisch” by Frank in 1887. Fossil records show that mycorrhizal associations are as old as terrestrial plants which mean that mycorrhizal associations have been around 370 million years ago in fossil of Rhynie chert. In 1900, Dangcard reported that VA mycorrhizae are widespread and observed arbuscule & vesicles and named the fungus as *Rhizophagus* (Nair, 2007).

The term mycorrhiza describes symbiotic association between fungi and plants. These associations are assumed to play an important role in the land colonization due to the ability of the associated symbiotic organisms in obtaining nutrients unavailable to non-mycorrhizal individuals (Smith & Read, 2008). In return the plants supply the fungal partner carbohydrates. Mycorrhizal associations are the outcome of 450 million years of co-evolution between plants and fungi, which has led to adaptation in both partners that consolidate their symbiotic development and function (Smith *et al.*, 2003). The ubiquitous occurrence of mycorrhizal associations in nature led to the formation of different symbiosis types.

Smith & Read (2008) classified mycorrhiza into seven types of equal rank. However, Imhof (2009) mentioned that the classification of mycorrhizas in seven types based on differences and similarities does not acknowledge the structural diversity of mycorrhizas. However, AM have turned out to be much more diverse in structural features than previously thought (Imhof, 2007; Dickson, 2004).

ii. Arbuscular Mycorrhiza (AM)

Traditionally the arbuscular mycorrhiza (AM) symbiosis is viewed as a classic mutualism interaction in which both partners benefits (Hodge, *et al.* 2010). Arbuscular mycorrhizal (AM) symbiosis is formed by approximately 80% of the vascular plant species in all terrestrial biomes (Smith *et al.*, 2010). In this obligate partnership, the fungal spores perceive the presence of the host plant through biochemical communication. Arbuscular mycorrhiza is established by forming arbuscules, which are highly branched tree like structures and are the sites of nutrient and carbon exchange between fungus and host plant (Miransari, 2010). Moreover, thick walled vesicles filled with lipids, inter and intracellular hyphae and very rarely hyphal coils are also parts of this association.

iii. Taxonomy of AM Fungi

A new monophyletic phylum, the Glomeromycota, for AM fungi has been established based on SSU and RNA gene sequencing (SchuBler *et al.*, 2001). The Glomeromycota is divided into four orders, eight families and ten genera. The genera which include most of the described of species are *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora*. According to latest taxonomy, AM fungi belong to the Glomeromycota phylum, including four families and twelve genera. To date more than 200 species of AM fungi have been described (Redecker & Phillip, 2006).

iv. Structure and diversity of AM Morphologies

There are two types of mycorrhiza according to the structures of the intraradical mycelium, the Arum-type and the Paris-type (Gallaud, 1905). In the Arum-type the fungal symbiont spreads in the root cortex via intercellular hyphae. Short side branches penetrates the cortex cells and produce arbuscules. The Arum-type is commonly described in fast growing root systems of crop plants. In the Paris-type the hyphae develop intracellular coils and spread directly from cell to cell within the cortex. Arbuscules grow from these coils. Co-occurrence of Arum-Paris-type morphologies of AM is found in cucumber and tomato (Kubato *et al.*, 2005).

v. AM benefits to plant

By forming an extended intricate hyphal network, AM fungi can efficiently absorb minerals nutrients from the soil and deliver them to their host plants in exchange for carbohydrates. They facilitate nutrients uptake, particularly with respect to immobile nutrients such as phosphorus and enhanced tolerance to drought, disease resistance, building up a macroporous structure of soil that allows penetration of water and air and prevent erosion, enhance photosynthesis and reduces stresses during – micro-propagation (Sharda & Rodrigues, 2009).

1. Macronutrients

a. Phosphorus uptake

Phosphorus is one of the essential mineral nutrients for plants. The preferred form of phosphorus taken up by the plants is Orthophosphate (Vance, 2003). Due to the fact that P is generally not very mobile in soil, a narrow depletion zone appears around P absorbing roots (Hinsinger *et al.*, 2005). The most well known positive effect of AM to the plants is the increased supply of mineral nutrients especially phosphorus (Strack *et al.*, 2003). 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 hyphal 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 improves productivity in low fertility soil and are particularly important for increasing ions such as PO_4^{3-} and immobile nutrient such as phosphorus (Jacobsen *et al.*, 1992). Plant roots 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 & Read, 2008). Phosphate is taken up by the extra radical mycelium and then transported within the fungus mycelium as polyphosphate (Ohtomo & 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 insoluble source of inorganic P in soil that are not available to the roots (Duponnois *et al.*, 2005).

b. Rock Phosphate uptake

The mechanism of P uptake by AM from insoluble form such as Rock phosphates is not yet clear. However, it is generally believed that AM fungi could have access to rock phosphate by altering pH or by the secretion of organic acids anions that may act as chelating agencies (Javaid, 2009). The mycorrhizal inoculation can thus help in effective utilization of rock phosphate by changing it into available form (Sabannavar & Lakshman, 2009).

c. Nitrogen uptake

Mycorrhizal fungi can also improve absorption of N from $\text{NH}_4\text{-N}$ mineral fertilizers, transporting it to the host plant. Its transport and absorption can also increase biomass production in soil (Liu *et al.*, 2002). AM fungi help in nitrogen fixation in legumes by enhancing phosphorus nutrition. (Javaid, 2009).

d. Carbon

The main fluxes in the AM symbiosis found to be of “C” from plant to fungus and of “P” and possibly “N” from fungus to plant opposite C-movement from fungus to plants only to occur in special cases where the plant has restricted “C” supply, mostly in achlorophyllous plants (Bidartondo and Bruns, 2002).

e. Other Macronutrients

Macronutrients such as magnesium (Mg), potassium (K) and calcium (Ca) (Liu *et al.*, 2002) also transported by AMF. Higher levels of K were noted in *Sorghum* colonized by *Glomus fasciculatum* (Sharif *et al.*, 2011). AM fungi increased Ca, P, K and Mg concentration of P and K from roots to above ground organs in *Pistacia* when inoculated with different species of *Glomus* (Miransari, 2010).

2. Micronutrients

Arbuscular fungi can also improve the uptake of several micro nutrients 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 sink for copper, cobalt and zinc (Cooper & Tinker, 1987).

Plants have varying mechanisms for mobilizing chelating and reducing ferric (Fe) in order to facilitate uptake (Treeby, 1992) indicated that AM fungi may facilitate the Fe uptake in acidic but not in alkaline soils. Rufyikiri *et al.* (2002) found that AM fungus could uptake and translocated Uranium towards the roots. At varying zinc levels mycorrhizal colonization increase zinc absorption and accumulation in the roots. This may help to explain the alleviation of zinc toxicity at high concentration.

Mycorrhiza were found to ameliorate the toxicity of trace metals in soybean and lentil plants growing in polluted soil (Jamal *et al.*, 2002). Jones & Leyval (1997) found that cadmium tolerant *Glomus mosseae* isolates AMF were responsible for uptake, transport and immobilization of cadmium. Copper was absorbed and accumulated by three AM fungal isolates, as observed in a study with *Glomus* spp. (Gonzalez-Chavez *et al.*, 2002). Literature available indicated resistance of AM fungi to aluminum (Al) (Cumming & Ning, 2003).

3. Salinity

AM symbiosis enhanced resistance of host plants to salinity stress. AM improvement of salt resistance has usually been associated with AM induced increases in P-acquisition and plant growth (Nasim, 2010). Arbuscular mycorrhizal fungi are able to biologically enhance plant growth and crop production in saline soils (Daei *et al.*, 2009). AM salt tolerant species can also alleviate salinity stress on plant growth by increasing of the concentrations of osmolytes, such as carbohydrates and electrolytes in plant roots as well as be improving respiration and transpiration (Feng *et al.*, 2002 and Daei *et al.*, 2009).

4. AM and drought stress

AM symbiosis is alleged to improve plant resistance to drought by inducing physiological drought tolerance, through increased dehydration avoidance and dehydration tolerance (Nasim, 2010). The AM fungi may induce increase in the level of soluble proteins which contribute to plant tolerance to stresses such as drought (Subramanian & Charest, 1998). Dell-Amico *et al.* (2002) proposed that enhanced water

uptake through improved hydraulic conductivity and better leaf conductance may provide protection against drought stress. Moreover, alteration of osmotic potential by AM is probably one of the most important reasons for the improved ability of the host plant to grow under water stress. Further, though the higher accumulation of organic products, e.g. betaine, proline and carbohydrates like mannitol, sucrose and ions including K and Cl (Ruiz-Lozano, 2003; Ruiz-Lozano *et al.*, 2006). Moreover, glomalin also improve soil moisture retention (Auge *et al.*, 2004b; Rilling, 2004).

5. AM as bio-control agent

The major benefits of AM mycorrhizal symbiosis includes an increased tolerance to root pathogens (Harries & Watson, 2004). This may be due to increased chitinase activity higher signification of the Xylem, changes in root exudation and improvement in nutrition (Kapoor & Mukerji, 2000). Mycorrhizal fungi also enhance the production of phenolics flavanoids in particular phytoalexins in the roots of the plants out and thereby act as bioprotectants against phytopathogens (Khade & Rodrigue, 2009). Moreover, the elevated Jasmonic acid levels also enhance the defense status of mycorrhizas plant (Cordier *et al.*, 1998). Furthermore, the potential of AM fungi to alleviate nematode induced plant stress has been frequently reported due to their ability to increase root growth and nutrient absorption, mechanically preventing nematode penetration and establishment, physiological and anatomical alterations in the host (Khade & Rodrigues, 2009).

6. Radionuclide pollutants

Radionuclides appear to be prevented from being translocated into the shoots of plants by mycorrhiza (Dighton, 2009). The protective effects of mycorrhizae was also shown in transformed carrot root in association with *Glomus mycorrhizae*, where uptake of radio-caesium was strongly correlated to fungi hyphal length. Fungal hyphae have a greater uptake capacity than in the host plant (de Boulois *et al.*, 2005). AM fungi, in association are able to absorb and retain heavy metals and radionuclide therefore it could be suggested that may be useful in the restoration and remediation of polluted environments (Dighton, 2009).

7. Restoration

Quilambo (2003) discussed the role of mycorrhizal in site restoration, suggesting that the influence of mycorrhizae is mainly to aid in soil stability and drought tolerance of colonizing plants. Moreover, mycorrhizae improve soil structure and assist the establishment of vegetation on poor soils in a restoration project (Dighton, 2009). The ability of fungi to bind soil particles is either by physical means or by the production of the sticky glycoprotein, glomalin, by some mycorrhizal fungi (Wright & Upadyhaya, 1998) that increases soil stability and prevent soil erosion.

8. Remediation of heavy metals

AM symbiosis with plants has been observed in soils containing heavy metals (Khan *et al.*, 2000). The AM symbiosis of enhanced phosphate uptake and decreased zinc and cadmium uptake compared with non-mycorrhizal treatment, indicating the great importance of even low levels of symbiosis under heavy metal stress (Hildebrandt *et al.*, 2007). Root AM colonization of plants under heavy metal stress results in expression of specific genes responsible for production of proteins that increase the tolerance of plants to stress (Rivera-Becerril *et al.*, 2005). Arbuscular mycorrhizal symbiosis can regulate the transcription of these genes (Lanfranco *et al.*, 2002; Hildebrandt *et al.*, 2007). It has been reported that AM hyphae produce glomalin (insoluble glycol-protein) called glomalin, binds to heavy metals (Miransari, 2010).

9. AM and herbivory

Herbivores and phytopathogens are known to directly or indirectly affect defence responses in roots and shoots of plants. Arbuscular Mycorrhizal fungi give some protection to the host plant by stimulating the production of secondary compound that would make the plant less palatable to herbivores (Toussaint, 2007). Available literature reports the reduction in survival of *Lepidopteran* larvae when fed with the leaves of AM plants compared to the leaves of non AM plants (Miransari 2010).

10. AM and soil aggregation

Glomalin is a glycoprotein produced by AM fungi which form a protective coat on fungal hyphae. This substance improve soil stabilization soil by forming (a protective polymer-like lattice on) the aggregate surface (Kristine, 2008). AMF play an important role in agro-ecosystems, including the involvement of the extra-radical mycellium in providing soil aggregation (Rilling, 2004). Rilling *et al.* (2002) described significant indirect effects of AM fungal hyphal length on water stable aggregates stabilization via the production of glomalin.

11. Mycorrhiza and Global Warming

AM produce a sticky glycoprotein called glomalin (Comis, 2002). From an ecological point of view one of the fascinating properties of Glomalin is that it contains 30 to 40 %. Carbon and, glomalin can comprise one third of all carbon in the soil and can persist for forty years. It has been estimated that up to a third of all the increase in Global CO₂ that has been generated since the industrial revolution can be attributed to carbon losses though poor agricultural practice. Because of glomalin high carbon contents, grass crops and natural grasslands are now being recognized as potentiality valuable for offsetting CO₂ emissions from industry and vehicle. Moreover, AMF are recognized as an important carbon sink can also store large amounts of carbon means that they can play an important role in dealing with global warming (Amaranthus *et al.*, 2009).

12. AM and weed management

Available data suggests that VAM can influence the nature of weed communities in mixed cultures systems in a number of ways, including altering the relative abundance of mycotrophic species. These effects may change the composition of weed communities. It is quite possible that interactions with VAM can enhance the beneficial effects of weeds on the functioning of agro-ecosystem (Miransari, 2010).

13. AM and micro-propagation

The mycorrhization technology has been used recently in many micropropagated crops in order to improve their survival rate and growth during post vitro stages. AM fungi also help to restore the biological and physical properties of soil. Moreover they are active in mobilizing minerals (Calvet *et al.*, 2001). More inoculation of micropropagated plants with AMF enhanced plant quality and plant growth (Estrada-luna *et al.*, 2000).

14. AM and biosynthesis of secondary plant metabolites in medicinal herbs

AM fungi colonize the plant roots during active plant growth and efficiently acquire nutrients for the plants, while the plant itself supplies the fungal symbiont with carbohydrates. Besides an improved biomass growth due to fungal colonization, a range of biological and chemical parameters in plants can be changed. Medicinal plants with high requirements on quality and quantity of herb drug is particular on the pharmacologically active compounds constitute a convenient partner for AM studies. The first investigations focused generally on the accumulation of secondary compounds in the aerial parts of AM treated culinary and aromatic herbs such as basil or fennel (Copetta *et al.*, 2006; Gupta *et al.*, 2002; Kapoor *et al.*, 2004; Toussaint *et al.*, 2007). The AM treated plants had better quantity of the essential oil. Sometimes also slight changes in the composition of the essential oil compounds were also found (Copetta *et al.*, 2006; Gupta *et al.*, 2002; Kapoor *et al.*, 2004). The alterations in the plants secondary metabolism are not only restricted to terpenes, the essential oil constituents, but also other chemical compound groups are affected. In a further study on basil, for example, an increased content of rosmarinic acid, a highly antioxidant phenolic compound, was detected in AM colonized plants (Toussaint *et al.*, 2008).

vi. TEST PLANT

GENUS *MENTHA*

Mentha belonging to the tribe Mentheae of subfamily Nepetoideae, family Lamiaceae. Genus *Mentha* mostly includes perennial and rarely annual species, known for being aromatic and pharmaceutically active. The plants have under and over ground

stolons and erect, square branched stem, with opposite pairs of oblong to lanceolate leaves, having serrate margins and downward insertion (Rose, 1981).

Species of *Mentha*

Around 25-30 species are found to occur in temperate areas of Eurasia, South Africa and Australia. The species are distributed in wide range of environments, but best suited to wet and moist soils, this tendency to spread makes it invasive (Brickell & Christopher, 2002). There are some problems in taxonomic classification of *Mentha* at species level, which arised form a very long history of cultivation, naturalization vegetative plasticity, hybridization and many different ploidy levels. Many specimens can only be named tentatively.

The most common species of genus *Mentha* found in Pakistan are *M. arvensis* L., *M. spicata* L., *M. longifolia*., *M. piperata* L. *M. royleana* Benth and *M. pulegium* L.

Cultivation

Mint species preferably grow well in cool, moist and partial shady places. however, they can also be grown in full sun. They are fast growing, extending along surfaces through a system of runners. (Purohitt & Viyas 2005).

Harvesting

No time is specified for the harvesting of mint leave, its can be done at anytime. Fresh mint leaves are used either immediately after harvest or stored for some days in plastic bags and refrigerated.

Dried mint leaves should be stored in an air tight container and placed in a dark, cool and dry place (Ortiz, 1992).

Chemical constituents and uses

Mentha contain volatile oil, composed of menthol, menthone, thymol, resin, gum and tannin. It contains eleven different oils including menthafuran 21 – 48%, elemol 24%, viridi floral 27%, B – caryophyllene and carophyllene oxide 15% each in different

species of *Mentha*. These are used as carminative, anti-septic, stimulant & very valuable in colics (Shinwari *et al.*, 2006).

Wild mint is a popular traditional medicine against various respiratory ailments but other uses have also been reported from various parts of world. Mostly the leaves are considered pharmaceutically important as they contain the major volatile oil content, which cure a number of diseases. Leaves are used in herbal tea that cures coughs, colds, stomachic cramps, asthmatic, Indigestion & Headaches. Externally, wild mint has been used to treat wounds and swollen glands. In his Book "traditional healing herbs" Robert, 1990 described various uses of *M. longifolia* and *M. aquatica*, which are delicious in salad and vegetable dishes. She also mentioned that *M. longifolia*, when rubbed onto the body and keeps mosquitoes away.

REVIEW OF LITERATURE

i. Occurrence of mycorrhiza and host growth responses in medicinal plants

Mycorrhizal investigations have advanced at an astonishing pace over the last two decades. Reviewing mycorrhizal occurrence in land plants and mapping the information onto the newly available plant phylogeny will furnish our knowledge on mycorrhizal abundance in plants, its biological importance and its impact on evolution of both plants and fungi.

Khaliq & Janardhanan (1997) found noteworthy variations in effectiveness of different VAM fungi on mints. VAM inoculation in general improved the shoot biomass production of all the six *Mentha* species. The results suggest the potential use of AM fungi for improving commercially cultivated mints.

Rai *et al.* (2001) demonstrated occurrence and positive growth responses of the medicinal plants *Spilanthes calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. A significant increase in growth and yield of both plant species was recorded relative to uninoculated controls. Shoot and root length, biomass, basal stem, leaf area, overall size, number of inflorescences and flowers and seed production were all enhanced in the presence of the fungus. Kapoor *et al.* (2002) found the positive effects of VAM fungal inoculation on growth of *Anethum graveolens* L. and *Trachyspermum ammi* (Lim). Al-Karaki (2002) studied the occurrence of AMF in garlic (*Allium sativum* L.). AMF colonization occurred at all samplings and the root colonization by AMF increased gradually with time in the AMF-inoculated treatments. Orłowska *et al.* (2002) reported the arbuscular mycorrhizal status of *Biscutella laevigata* and *Plantago lanceolata*. Mycorrhizal colonization was higher in *P. lanceolata* than *B. laevigata*. Gorski (2002) reported seventy six medicinal plants with vesicular arbuscular mycorrhizal association in a survey of the Azad Jammu and Kashmir, Pakistan. Great variations were found in the VAM infection percentage and the existent of hyphal infections. Herbaceous plants showed more infections than shrubby and woody plants.

The extent of root colonization by VA endophytes varied with the soil type and plant species.

Kapoor *et al.* (2003) inoculated VAM fungi (*Glomus macrocarpum* and *Glomus fasciculatum*) in *Foeniculum vulgare* and found that fungal inoculants, *G. fasciculatum* registered the highest growth. Dickson *et al.* (2003) investigated mycorrhizal colonization in *Linum usitatissimum* arbuscules occurred in pairs in adjacent cortical cells arising from a single, radial intercellular hyphae. These "paired" arbuscules often appeared to be at different developmental stages. Sumana & Bagyaraj (2003) studied influence of VAM fungi on growth response of Neem (*Azadirachta indica*). VAM inoculated seedlings had greater plant height and stem girth. The mycorrhizal root colonization and spore number in the root zone soil was also more in VAM in inoculated plants as compared non inoculated plant.

Muthukumar *et al.* (2006) found that roots of 79 medicinal and aromatic plants were heavily infected with mycorrhiza. The number of plants having Arum type mycorrhiza was greater than those having Paris-type. Panwar & Tarafdar (2006) evaluated the occurrence of three selected endangered medicinal plant species (*Leptadenia reitculata*, *Mitragyna parvifolia*, *Withania coagulans*) and arbuscular mycorrhizal fungal (AMF) associations with them. Five genera were identified in the rhizosphere of these selected plant species. A high diversity of AMF was observed which varied between different host plant species. *Glomus mosseae* and *Glomus rubiforme* were the most dominant species. Trindade *et al.* (2006) studied the arbuscular mycorrhizal (AM) associations in papaya (*Carica papaya*, L.) Papaya roots showed AM colonization, ranging from 6% to 83%. Spore numbers varied from 34 to 444/30g of soil. *Glomus etunicatum*, *Paraglomus occultum*, *Acaulospora scrobiculata* and *Gigaspora* sp. were the most common species.

Goo & He (2007) reported the heavy occurrence of VAM fungi in roots of *Atractylodes macrocephala*, *Datura stramonium*, *Gentiana scabra*, *Lycium chineses*, *Salvia miltiorrhiza* and many other medicinal plants. Liu *et al.* (2007) found the occurrence of AMF in licorice. The results showed that mycorrhizae were of the Arum-type and their colonization frequency and colonization intensity were found to be 80.0-84.6%, 49.4-60.0% respectively. Burni *et al.* (2007) reported that *Mentha longifolia*

showed heavy VAM infection (80%) as compared to *Nepeta cataria* (61.6%). In both the plants arbuscular infection was lower than vesicular infection.

Rapparini *et al.* (2008) found that *Artimisia annua* plants responded positively to AM inoculation. Mycorrhizal plants have more stem dry weight, shoot length and leaves than control plants.

Maicon *et al.* (2008) found the occurrence of VAM fungal infection in ginger (*Zingiber officinals*). Boureima *et al.* (2008) reported the occurrence of AMF in endangered medicinal plant species (*Leptadenia reiticulata*, *Mitragyna Curculigo orchioides*, *Withania coagulans*). Five genera were identified in the rhizosphere of these plant species. A high diversity of AMF was observed which varied between different host plant species. Among the five genera, *Glomus* occurred most frequently. Sharma *et al.* (2008) studied arbuscular mycorrhizal (AM) technology for the conservation of *Curculigo orchioides* an endangered medicinal herb. This study reports the effect of three arbuscular mycorrhizal (AM) fungal inocula on growth performance of 'in vitro' raised *C. orchioides* plantlets. The three AM fungal inocula consisted of two mono specific cultures of *Glomus geosporum* and *G. microcarpum* and one crude consortium of AM fungal spores isolated from rhizosphere soil of *C. orchioides* growing in natural habitat.

Karagiannidis *et al.* (2010) while conducting survey in three northern Greek mountain areas reported that all the investigated medicinal plants were found to be mycorrhizal. Uma *et al.* (2010) found the occurrence of VAM fungi in 38 ginger species (Zingiberaceae) and two spiral gingers (Costaceae). Radhika & Rodrigues, (2010) reported the occurrence and responsiveness of VAM fungal infection in thirty six medicinal plant species belonging to 25 families. Out of this 30 were reported as medicinal and 6 were not medicinal. Forty two AM fungal species belonging to five genera viz, *Glomus*, *Acaulospora*, *Gigaspora*, *Ambispora* and *Scutellospora* were recovered from the rhizospheric soil. *Glomus* was found to be the most dominant AM fungal species Chandra *et al.* (2010) found that mycorrhizal inoculation increased the dry matter yield of five medicinal plants *Abelmoschatus moshatus*, *Clitoria ternata*, *Plumbago zeylanica*, *Psoralea corylifolia* and *Withiana somnifera*. Kumar *et al.* (2010) studied the seasonal variation of arbuscular mycorrhizal fungal dynamics in the rhizospheric soil of five medicinal plants species (*Spilanthes acmella*, *Withiana*

somnifera, *Salvia officianalis*, *Mentha spicata* and *Melissa officinalis*). The study included vesicles and arbuscules formation in the root and AM fungal spore population in the rhizosphere of all five medicinal plants. Results showed a wide range of monthly changes throughout the year.

Ayoob *et al.* (2011) found *Catharanthus roseus* plant raised in the presence of AM fungi had increased growth in (shoot length, root length, leaf number, fresh weight and dry weight). The study suggested that *Catharanthus roseus* is dependent on the mycorrhizal fungi to a large extent for its growth. Burni *et al.* (2011) carried out investigations to identify, quantify and find out the AMF relationship with chemical characteristics of rhizospheric soils of *Mentha arvensis* and *M. longifolia* growing in some cultivated and natural sites of Khyber Pakhtunkhwa respectively. Seven AMF species with *Mentha arvensis* and eleven with *Mentha longifolia* were recorded. The dominant Genus was *Glomus* (11 species) followed by *Acaulospora* and *Gigaspora* (2 species each). The AMF spore densities in studied sites were ranged from 30 – 245 spores/10 gr. soil. AM colonization was ranged from 26.87 – 100.

Burni & Hussain (2011) investigated the mycorrhizal status of some selected medicinal plants of family Lamiaceae growing in Khyber Pakhtunkhwa. The mycorrhizal infections were present in all studied species. Typical Arum and Paris type mycorrhiza are recorded for the first time from the family Lamiaceae. The number of studied plants with Arum type was higher than Paris type while two plants were recorded with intermediate type. Variations in the percentage of general VAM infection were ranged from (55-100%). Yang *et al.* (2011) studied the root and rhizosphere soil samples of medicinal plant *Magnolia cylindrica* from China. Paris type AM was recognized. Seventeen species of AM fungi were isolated and identified from the rhizospheric soil samples. Thenmozi *et al.* (2011) studied the vesicular arbuscular mycorrhizal fungi colonization on some medicinal plants. A total number of ten medicinal plants species selected for the study. The predominant VAM spores observed in the soil sample includes *Glomus* and *Gigaspora* species. Among the rhizosphere soil examined, a maximum spore count of 80 spores/25 gram soil was reported in Brahmi (*Bacopa monneiri*) and a lowest of 15 spores/25 gram soil in Neela Amari (*Indigofera tinctora*).

ii. Effect of mycorrhizal fungi on secondary metabolites and essential oils of medicinal plants

Maier *et al.* (1999) found the accumulation of cyclohexenone derivatives by arbuscular mycorrhiza in tobacco roots. Abu-Zeyad *et al.* (1999) reported that AM fungi increased the growth and yield of the castanospermine in the leaves. Mathur & Vyas (2000) reported that mycorrhizal colonization increased accumulation of aminoacids, protein, chlorophyll and sugar contents as compared with non-mycorrhizal plants. Kapoor & Mukerji (2000) reported that mycorrhizal technology enhanced the growth, essential oil and yield of members of Labiateae and Umbelliferae. Akiyama & Hayashi (2002) analyzed Cucumber (*Cucumis sativus* L.) roots for secondary metabolites upon inoculation with the arbuscular mycorrhizal fungus, *Glomus caledonium*. It enhanced the accumulation of the triterpenes, of fennel seeds over non-mycorrhizal control. The essential oil characterization revealed that the level of anethol was significantly enhanced on mycorrhization.

Kapoor *et al.* (2002b) found that VAM inoculation increased the essential oil concentration in fruits of *Coriandrum sativum* by 43%. Although significant variation in effectiveness of the two fungal species was observed, the quality of essential oil was significantly enhanced with on mycorrhization. GC characterization of essential oil showed increased concentration of geraniol and linalool in plants inoculated with *Glomus macrocarpum* and *Glomus fasciculatum* respectively. Kapoor *et al.* (2002a) evaluated the effects of application of two arbuscular mycorrhizal (AM) fungi, *Glomus macrocarpum* and *G. fasciculatum*, on shoot biomass and concentration of essential oil in *Anethum graveolens* and *Trachyspermum ammi* fruits. On mycorrhization, the concentration of essential oil increased up to 90% in Dill and 72% in *Carum* over their respective controls. *Glomus macrocarpum* was more effective than *G. fasciculatum* in enhancing the oil concentration. The levels of limonene and carvone were enhanced in essential oil obtained from *G. macrocarpum*-inoculated dill plants, while *G. fasciculatum* inoculation resulted in a higher level of thymol in *Carum*. Gupta *et al.* (2002) found positive response of the VAM fungus *Glomus fasciculatum* on the essential oil yield related characters and nutrient acquisition in the crops of different cultivars of menthol mint (*Mentha arvensis*)

The VAM inoculation significantly increased the root colonization, plant height, fresh herbage and dry matter yield, oil content and oil yield as compared to non-inoculated cultivars.

Kapoor *et al.* (2003) found that two (AM) fungi; *Glomus macrocarpum* and *G. fasciculatum* significantly improved growth and essential oil concentration of *Foeniculum vulgare*, *Glomus fasciculatum* improved the highest growth and 78% increase in essential oil concentration.

Freitas *et al.* (2004) evaluated the effects of different species of arbuscular mycorrhizal fungi on the vegetative growth, production and composition of essential oil of *Mentha arvensis* grown in different phosphorus levels. The inoculated treatments provided increments of up to 89% in the essential oil and menthol contents in relation to treatment not inoculated. Khaosaad *et al.* (2006) determined the effects of root colonization by *Glomus mosseae* on the qualitative and quantitative pattern of essential oils (EO) in three oregano genotypes (*Origanum* sp.). As EO levels in P treated plants were not enhanced, it concluded that the EO increase observed in mycorrhizal oregano plants is not due to an improved P status in mycorrhizal plants, but depends directly on the AMF–oregano plant association.

Kapoor *et al.* (2007) found that inoculation by *Glomus macrocarpum* and *Glomus fasciculatum*, either alone or supplemented with P-fertilizer, significantly increased concentration of artemisinin in the fertilization. This suggests that the increase in artemisinin concentration may not be entirely attributed to enhanced P-nutrition and improved growth. Copetta *et al.* (2007) evaluated the effects of *Glomus mosseae*, *Gigaspora margarita* and *Gigaspora rosea* on shoot growth and essential oil production of *Ocimum basilicum*, *Gigaspora margarita* and *Gigaspora rosea* modified essential oil contents. Liu *et al.* (2007) reported that *Glomus mosseae* and *Glomus veriforme* enhanced the glycyrrhizin concentration in roots.

Chaudhary *et al.* (2008) compared the effectiveness of *Glomus macrocarpum* (GM) and *Glomus fasciculatum* (GF) on three accessions of *Artemisia annua*. The AM inoculation significantly increased concentration of essential oil and artemisinin in leaves as compared to non-inoculated plants. Khaosaad *et al.* (2008) stated that mycorrhization increased the levels of biochanin A in the root and the shoots and reduced the levels of

genistein in the shoot of red clover. The levels of the other isoflavones were not affected. Venkateswarlu *et al.* (2008) studied the effect of arbuscular mycorrhizal fungi (AMF) on the azadirachtin content in seed kernels of Neem trees. No significant differences were found in the tree height, girth 0at breast height and fruit yield but oil percentage, total triterpenoids and azadirachtin content in kernels increased significantly as a result of AM inoculation. Rapparini *et al.* (2008) found the stimulating effects of arbuscular mycorrhizal colonization on terpene emission and content of *Artemisia annua*.

Karagiannides *et al.* (2010) found the mycorrhizal and nutritional status and the essential oil content and composition of common medicinal and aromatic plants. A range of values for nutrient status and essential oil contents and composition was established.

Karagiannidis *et al.* (2011) found that mycorrhizal plants of oregano and mint plants had higher contents of essential oils and grew better than non mycorrhizal plants. Farkoosh *et al.* (2011) found the enhanced effect of mycorrhizal symbiosis and *Bacillus coagulance* on qualitative and quantitative traits of *Matricaria chamomilla* under different level of phosphorus. Result revealed that a significant effect of the interactive treatment in most studied properties and moreover enhancement in quality of yield was more than yield quantity. Dave & Tarafdar (2011) found the positive effect of vesicular arbuscular mycorrhizae on growth and saponin accumulation in *chlorophytum borvilianum*.

Karagiannides *et al.* (2012) reported that *Glomus lamellosum* on root colonization, growth, essential oil and nutrient acquisition of *Santolina chamaecyparissus*, *Salvia officinalis*, *Lavandula angustifolia*, *Geranium dissectum*, and *Origanum dictamnus* significantly improved the growth, essential oil production and nutrient contents compared. It is concluded that the use of *Glomus lamellosum* may allow plant growth in low fertility soils, reduce fertilizer inputs and increase aromatic plant production of essential oils, while they indicate that it may be possible to use mycorrhizae to affect the quality of the essential oil produced.

iii. Role of AM in nutrient acquisition

Cigar *et al.* (2000) found that mycorrhizal inoculation on cucumber plant growth caused higher uptake of P, Zn and Mn. Liu *et al.* (2000) found that the effects of *G. intraradices* on Zn, Cu, Mn, and Fe uptake varied with micronutrient and levels added to soil. Saito (2000) observed that the most common benefit hosts plants receive from arbuscular mycorrhizal fungi is enhanced phosphorus nutrition. George (2000) observed that the colonization of plant roots by arbuscular mycorrhizal (AM) fungi greatly affect the plant uptake of mineral nutrients. Davies *et al.* (2000) demonstrated that under reduced phosphorus (P) conditions, AMF enhanced plant vegetative and reproductive growth. Growth of AMF plants at low P was comparable with non AMF plants at moderate availability. Al-Karaki (2000) determined that AMF-inoculated garlic (*Allium sativum* L.) had less P use efficiency than uninoculated plants.

Fusconi *et al.* (2001) concluded that AM fungi significantly improved plant growth in soils with low phosphorus (P) availability. Inoue *et al.* (2001) investigated that there was no significant interaction ($P > 0.05$) between phosphorus fertilizer and AMF inoculation on nitrogen phosphorus and potassium uptake. Alloush & Clark (2001) found that nutrients commonly limiting in acidic soil (e.g., calcium, magnesium and potassium) was greatly enhanced in AMF compared to non Mycorrhizal (non AMF) plants. Rao & Tak (2001) found that total uptake of many nutrients but not of Potassium and Sodium, was significantly enhanced upon AM fungal inoculation. Nelson & Achar (2001) described stimulation of growth and nutrient uptake by VAM fungi in *Brassica oleracea* var. *capitata*.

Gyaneshwar *et al.* (2002) described role of soil microorganisms in improving P nutrition of plants. Hartwig *et al.* (2002) found that AMF colonization neither improved the N nor P nutrition status, but led to an improved total P uptake. da Silveira *et al.* (2002) found the positive effects of six species of AMF on growth of avocado (*Persea* sp.) plants. Fisher & Jayachandran (2002) described that AMF significantly increased the dry weight and total P content of two endangered plant species from South Florida.

Kapoor *et al.* (2003) studied AM inoculation of plant along with phosphorus fertilization significantly enhanced growth and P-uptake of plants. Nowak (2004)

investigated that in geranium N, P and K concentrations were higher in the mycorrhizal plants compared to the non-mycorrhizal. Kerur & Lakshman (2004) examined effects of phosphate fertilizer and VAM fungi on two floricultural plants showed enhanced nutrients in shoots, when compared to non-inoculated plants. Zandavalli *et al.* (2004) found that the leaf concentrations of P, K, Na, and Cu were higher while those of Ca, Mg, Fe, Mn, and B were lower AM inoculated plants.

Redecker (2005) reported that AM improve the uptake of different mineral nutrients. Sailo & Bagyaraj (2005) stated that *Coleus* plants raised in presence of most of the AM fungi had increased P contents over those grown in the absence of soil inoculation with AM fungi. Duponnois *et al.* (2005) reported that plants inoculated with AM fungi utilized more soluble phosphorus from soil mineral phosphate than non-inoculated plants. Giri *et al.* (2005) found that concentration of mineral nutrients was significantly higher in AM inoculated seedlings than in non-inoculated in *Cassia siamea* plant.

Caglar & Akgun (2006) studied effects of vesicular-arbuscular mycorrhizal (VAM) fungi on the seedling growth of three *Pistacia* species and studied that mycorrhizal inoculations increased the leaf N, but not P and K contents. Koske & Gemma (2006) studied the effects of arbuscular mycorrhizae on growth of *Osteomeles anthyllidifolia* and *Psidium cattleianum* and found that leaf-tissue P of both species was significantly higher in inoculated plants.

Ouahmane *et al.* (2007) investigated that *C. atlantica* plants inoculated with native AM fungi mobilized P from KRP more efficiently than those mycorrhized with *G. intraradices*. Bennett & Bever (2007) observed that mycorrhizal fungi can increase plant growth through uptake of essential nutrients. Tawaraya *et al.* (2007) studied that effect of arbuscular mycorrhizal colonization on nitrogen and phosphorus uptake and growth of *Aloe vera* and reported that AM colonization increased the nutrient uptake and growth.

Pagano *et al.* (2008) reported that inoculated plants showed greater height and diameter growth and dry matter and nutrient concentration in plants of *A. peregrine*. Manoharan *et al.* (2008) studied that VAM mycelium in soil results in greater efficiency of nutrient absorption particularly for slowly diffusing mineral ions, especially phosphorous. Nwoke *et al.* (2008) studied that the shoot dry matter yield and P

accumulation correlated significantly with the root length of cowpea and the average diameter of sorghum roots. Porras-Soriano *et al.* (2009) reported that AM fungi increased growth, nutrient uptake in Olive trees under nursery conditions. Aher (2009) demonstrated that *Arachis hypogea* inoculated with *Glomus fasciculatum* showed improved growth and nutrient uptake than the uninoculated control plants. Omomowo *et al.* (2009) reported that inoculation of AM fungi with rhizobia significantly increased N, P, K, Ca, Mg, Na, Fe in seeds of cowpea over that of the uninoculated control. Kafkas & Ortas (2009) determined the effects of arbuscular mycorrhizae for enhancing *Pistacia* species seedling growth by improving phosphorus and zinc uptake.

Cwala *et al.* (2010) found that mycorrhizal plants of tomato performed better than the other plants in terms of their P, K, Ca, Mg and Mn nutrition. Alizadeh *et al.*, (2010) reported that mycorrhiza significantly increased the absorption of nitrogen, phosphorous and potassium in shoot and root. Ratti *et al.* (2010) observed the enhancement of macro (P, S) and micro (Cu, Mn) nutrients in *Catharanthus roseus* after inoculation with AM fungi. Baird *et al.* (2010) recorded that no impact of AMF colonization on P concentration and uptake by organically grown field pea and lentil.

Yaseen *et al.* (2011) found that in AM inoculated Cowpea (*Vigna unguiculata*) varieties out performed than non-inoculated plants in terms of nutrient uptake. Batnal & Lakshman (2011) found the positive symbiotic response of *Phyllanthus emblica* vars. Wild and chakaiya to different AM fungi in terms of plant height, biomass, stem diameter, number of leaves and P uptake. Karagiannidis *et al.* (2011) found the positive effect of three Greek fungi on growth and, nutrient acquisition in oregano and mint. They found the enhanced uptake of both macro and micronutrients in AM inoculated plants.

iv. Effect of Fertilizers on AM infection and Spore

Joner (2000) found the additions of NPK led to reduced colonization. While moderate quantities of FYM have less adverse effects on AM than equivalent amounts of nutrients in NPK fertilizers.

Linderman & Davis (2001) concluded that inoculating plants roots with mycorrhizal fungi in combination with composted organic amendment extract was

beneficial. Muthukumar & Udaiyan (2002) found that spore populations and colonization of cowpea roots by AM fungi were higher in soils amended with organic manure than in non-amended soils. Cowpea growth and yield were positively related to spore populations of *Glomus aggregatum*, *Glomus geosporum* and *Scutellospora calospora* but were negatively correlated to spore populations of *Acaulospor scrobiculata* and *Glomus sinuosum*. Martin *et al.* (2002) found the application of dry olive residue decreased the percentage of AM colonization of plants except those were inoculated with *Glomus mosseae* or *Glomus deserticola*.

Gaur & Adholeya (2002) studied five fodder crops, *Zeamays*, *Medicago sativa*, *Trifolium alexandrium*, *Avena sativa* and *Sorghum vulgare* inoculated with indigenous arbuscular mycorrhizal fungi amended with organic matter. All these test plants showed differences in percentage of AM colonization. This study clearly indicates the potential of using indigenous AM inoculations in fodder crops grown in marginal soils.

Gryndler *et al.* (2002) found in organic manure treatments the length of AM mycelium and spore formation increased than with mineral fertilization. Cavender *et al.* (2003) found that vermicompost stimulates mycorrhizal colonization of root of *Sorghum bicolor*.

Linderman and Davis (2004) found that the effect of organic and inorganic fertilizer on arbuscular mycorrhiza formed by *Glomus intraradices*. Results showed that organic fertilizers favours AM establishment and function more than inorganic fertilizers Tanu *et al.* (2004) observed effect of different organic manures/composts on the herbage and essential oil yield of *Cymbopogon winterianus* and their influence on the native AM population in a marginal alfisol. The type and dose of the various organic amendments significantly influenced the indigenous AMF infectious propagules in soil. Highest number of AMF propagules were recorded in the leaf compost amended pots. Ryan *et al.* (2004) found that at high P-concentration the effect of mycorrhiza is either nil or negative and dependency on AMF was negatively correlated with soil phosphorus contents. Garampalli *et al.* (2005) found the flyash amendment affect significantly the intensity of VAM fungal colonization inside the plant roots and also suppressed the formation of VAM fungal structures (Vesicles and arbuscules) completely at higher concentration.

Aryal *et al.* (2006) found the arbuscular mycorrhizal infection rates and frequency of arbuscule formation were always high in the organic fertilized plants than in the chemically fertilized plants and moreover the inoculation increased AM infectivity in the organic fertilized plants but not in the chemically fertilized plants. Panwar & Tarafdar, (2006) also found negative correlation between AMF spores and soil phosphorus. Gryndler *et al.* (2001) reported organic and mineral fertilization respectively increases and decrease the development of external mycelium of AMF in long term field experiment. Mineral fertilization reduced the growth of AMF of hyphal length whereas organic manuring alone increased the growth of AMF.

Javaid & Riaz (2008) found green leaf manures adversely affected the AM colonization of maize and were significantly correlated with different parameters of arbuscular & vesicular colonization.

Assaf *et al.* (2009) found AM fungal spore population & colonization levels substantially enhanced by the application of olive pomace wastes.

Galvan *et al.* (2009) found onion roots from organic fields had higher fractional colonization levels than those from conventional fields. This suggests that farming systems as such did not influence AMF diversity, but rather specific environmental conditions or agricultural practices.

Sharif *et al.* (2011) showed that low availability of soil phosphorus increases VAM colonization.

v. Effect of fertilizer with AMF on Growth

Caravaca *et al.* (2002) found composted residue greatly increased macronutrient content, soil microbial activity and enzyme activities and decreased soil bulk density. The addition of composted residue to soil with mycorrhizal inoculation had increased the plant height of *Pistacia lentiscus* seedlings by 106% with respect to control. Gaur & Adholeya (2002) studied five fodder crops, *Zeamays*, *Medicago sativa*, *Trifolium alexandrinum*, *Avena sativa* and *Surghum vulgare*, inoculated with arbuscular mycorrhizal (AM) fungi amended with organic matter under field conditions. Shoot and root dry weights and total uptake of P and N of all the test plants were significantly increased by AM inoculation. Osorio *et al.* (2002) found plant growth and mycorrhizal

activity were severely constrained in the un-amended soil which had low organic matter content, low pH and low availability of phosphorus, boron, calcium, magnesium and potassium. Plants grew significantly better in the organic matter amended soil.

Aryal *et al.* (2003) found better positive effects of AMF inoculations in organic plants than in chemically treated plants suggesting higher dependency of organic plants on these symbionts for better growth and development. Cavender *et al.* (2003) found the addition of vermicompost to substrates increased N, P and K levels. Vermicompost stimulated mycorrhizal colonization of roots. Shoot and root dry weights were increased by vermicompost in the absence of AMF. Inoculation with AMF reduced dry weights.

Tanu *et al.* (2004) evaluated four organic amendments leaf compost (LC), vegetable compost (VC), poultry manure (PM), and Sewage-sludge (SSL), for their effects on the herbage yield, essential oil content and inoculum potential of native arbuscular mycorrhizal fungi on the three varieties of *Cymbopogon vinturians*. Alguacil *et al.* (2004) found that mycorrhizal inoculation with AM fungi was the most effective treatment for enhancement of shoot biomass, particularly with *G. mosseae*. The addition of composted sewage-sludge alone was sufficient to restore soil structural stability but was not effective with respect to improving the performance of *Retama Sphaerocarpa* plants.

Caravaca *et al.* (2004) found mycorrhizal inoculation treatments stimulated significantly the production of shoot biomass, to a higher extent than the addition of the amendment alone to the soil. Linderman & Davis (2004) found that release of nutrients from organic fertilizers, as a result of microbial activity, favours AM establishment and function more than most inorganic fertilizers unless P levels of the latter are low.

Hameeda *et al.* (2007) studied the effect of composts or vermicomposts on sorghum growth. Both Rice straw compost (RSC) and Rice straw vermicompost (RSVC) were applied at different concentrations with and without Arbuscular Mycorrhizae (AM). The study showed that the application of microbial inoculants along with higher concentrations of composts may not be synergistic for plant growth.

Perner *et al.* (2007) studied the effect of arbuscular mycorrhizal colonization and two levels of compost supply on nutrient uptake and flowering of *Pelargonium* plants. Plants grown on a peat based substrate with two rates of compost addition and with and

without arbuscular mycorrhizal (AM). Thus concluded that addition of compost in combination with mycorrhizal inoculation can improve nutrient status and flower development of plant.

Das *et al.* (2008) conducted an experiment to study the effect of bio-fertilizers on the biomass yield and N P K content in Stevia (*Stevia rebaudiana*). The result showed that yield and N P K content in Stevia plant increased initially and thereafter the same amount decrease with the combined treatment of bio-fertilizer rather than individual treatment. Javaid & Riaz (2008) found out effect of green leaf manure of *Azadirachta indica* A. juss., *Eucalyptus citriodora*, *Chenopodium album* L. and *Parthenium hysterophonis* L. on crop growth and arbuscular mycorrhizal (AM) colonization in maize. All applied green leaf manures adversely affected the plant growth. Shoot biomasses were significantly correlated with different parameters of arbuscular and vesicular colonization.

Amaya *et al.* (2009) suggested that AMF increased nutrient acquisition, from an organic fertilizer source by enhancing acid phosphates and alkaline phosphatase activity this facilitating P acquisition, increasing photosynthesis, and improving plant growth. Darzi *et al.* (2009) found maximum vegetative growth of AMF inoculated fennel plants at 100% rock phosphate level as compared to non inoculated plants. Sabnnavar & Lakshman (2009) reported the enhanced growth and nutrient uptake as compared to control plants at high rockphosphate levels (100%) in varieties of *Sesamum*.

Bahadur *et al.* (2009) carried out a field experiment in lettuce (*Lactuca sativa* L.) to study the effect of organic compared with inorganic inputs on physiology, growth, yield and quality. The integrated use of organic manures and bio-fertilizers exhibited significantly higher head length and diameter. Head weight and yield under combined use of organic manures and biofertilizers were noticed. Assaf *et al.* (2009) studied the impact of olive pomace wastes and fungicide treatment on indigenous arbuscular mycorrhizal fungi associated with chickpea (*Cicer arietinum* L.) under field conditions. It was concluded that organic matter increased growth and yield of chickpea plants in the field.

Objectives of study

The principal objective of the present study was to investigate the mycorrhizal status of selected plants of Lamiaceae and the role of Arbuscular Mycorrhizal technology in their growth performance of some selected species of *Mentha* species under various soil amendments. To achieve this objective the proposed study is aimed.

- To find out the mycorrhizal status and associated AM (Arbuscular mycorrhizal) fungi with selected medicinal plants of family Lamiaceae.
- To evaluate the role of AM technology on growth, mineral nutrient acquisition and essential oils of selected *Mentha* species of under inorganic and organic fertilizer amendments.
- To screen out the possible effects of fertilizer amendments on below ground parameters i.e. Root infection and AMF Flora.
- To find out some efficient and specific AM endophytes which could be used in future for better performance yields, biological mobilization of nutrients and conservation of medicinal plants.

MATERIALS AND METHODS

The present research work consisted of survey followed by the experimental work.

i. SURVEY

It was carried out to find out the mycorrhizal status and the morphology types of AM infection in the roots of selected medicinal plants of Lamiaceae from various sites of Khyber Pakhtunkhwa (KPK). AM fungi in the rhizospheric soils of two heavily infected *Mentha* species were also isolated, quantified and identified; possible correlation were established between their occurrence and various soil factors .

ii. Plants and Period

The present investigations was carried on 15 selected medicinal plants of family Lamiaceae including *Mentha longifolia* L., *Rasmarinus officinals* L., *Ocimum basilicum* L., *Mentha piperata* L., *Mentha royleana* Benth., *Mentha arvensis* L., *Mentha spicata* L., *Ocimum americanum* L., *Origanum vulgare* L., *Ocimum majorana* L., *Coleus forskohlii* L., *Coleus aromaticus* Benth., *Lallementia rovelena* Benth., *Salvia lanata*, and *Salvia nubicola* Wall ex. sweet. Plants were collected growing wild or cultivated in various sites of Peshawar, Haripur, Havelian and Manshera from February 2007 to May 2008.

iii. Sampling

The roots and rhizospheric soil samples were collected at 10-30cm depths in five replicates per plant from various sites of Peshawar, Haripur, Havelian and Manshera following the method of Jalaluddin & Anwar (1991) and Panwar and Tarafdar (2006). Before sampling, the soil from the upper layer scapped off to remove foreign particles and litter. All the collected soil samples from the rhizosphere of a plant were homogenizes replication wise before sieving (< 2mm mesh size) to remove stones, plant debris and coarse roots. Plants were rooted out carefully, avoiding damage to secondary and tertiary rootlets, which are the site of endophyte development. Samples were stored in polythene bags at 5⁰C for further studies.

iv. Estimation of AM root colonization

Roots were separated from soil, washed in tap water and finely cut into 1 cm pieces followed by clearing with 10%KOH and stained with acid fuchsin solution (Phillips & Hayman, 1970). Assessment of fungal infection was conducted on each sample by glass slide method (Giovannetti & Mosse, 1980). Fifty root segments were studied in each sample, total root infections (%). External hyphal (%), Internal hyphal (%) vesicular and arbuscular (%) infection were expressed as percent of total root segments. The stained roots were examined under compound microscope (10X and 45X). Microphotographs of best representative slides were taken.

v. Spore extraction and estimation of density

Spores of AMF were extracted from the field and successive pot culture soils by the wet sieving and decanting technique of Gerdemann & Nicolson (1963). Total number of mycorrhizal fungal spores in the soil samples were estimated by the method of Gaur & Adholeya (1994). Spore densities were expressed as the number of spores per 100g of soil. The isolated spores were picked up with needle under and were mounted in polyvinyl lacto glycerol (PVLG). All the spores (including broken ones) were examined using compound microscope. Taxonomic identification of spores up to species level was based on spore size, spore colour, wall layers and hyphal attachments using the identification manual of Standard monographs (Schenck & Perez 1990, Redecker & Phillips, 2006)

vi. Soil characteristics

Soil pH was determined by pH meter. AB-DTPA extractable P, K and micronutrients were determined after Soltanpour & Schwab, (1977). Total Nitrogen concentration was done by Kjeldhal method (Bremer & Mulvancy, 1982), Lime contents by (Black, 1965), Soil organic matter by (Nelson & Sommers, 1982).

vii. Statistical analysis

The data on the quantitative distribution of AM spores and experimental results were statistically analysed by using analysis of variance (ANOVA). Relationship

between AM spores and soil characteristics were found by computing coefficient of correlation (r) by the formula.

$$r = \frac{SSxy}{\sqrt{SSx.SSy}}$$

Standard error ($\pm$) was also measured for various parameters.

viii. Experimental Work

ix. Biological material

Soil and plant materials

Air dry subsoil (100cm depth) was collected from the University of Peshawar having the pH 7.7 ± 0.23 , organic matter (%) 0.34 ± 0.21 , available nitrogen (%) 0.017 ± 0.01 , phosphorus (%) 5.25 ± 0.16 , texture silt, loam sand 54.2%, silt (38.0%), clay (17.8%). Healthy suckers of *Mentha* species were collected from Herb Garden Qarshi industry Haripur and PCSIR Labs. Peshawar. Washed with water cut into 6cm pieces (2 nodes in each piece), surface sterilize with 50% ethanol for 30-45 seconds and washed thoroughly with sterilized water for 4-5 minutes (Khaliq & Janadhanan, 1997).

AMF INOCULA

Three types of AMF inocula were used in various experiments with variable species compositions isolated from various sites as follows.

1. **Gi.** Mono specific commercial inoculum of AMF species *Glomus intraradices* (Gi) (**Myce-Pro** obtained from Germany provided by Department of Soil and Environmental Sciences, KPK Agricultural University Peshawar).
2. **MC1.** Multi indeginous AMF inoculum having eleven species (*A. melleae* Spain & Schenck, *Glomus fasciculatum* (Thaxter), *G. etunicatum* Becker & Gerdemann, *G. claroides* Schenck & Smith, *G. microcarpum* Tul & Tul *G. australe* (Berkeley) Berch, *G. intaradicus* Shenck & Smith, *G. aggregatum* Schenck & Smith *G. constrictum* Trappe and *G. mosseae*, Gerdeman & Trappe and *Gigaspora gigantea* Gerdemann & Trappe) were collected from the rhizosphere soil of *Mentha longifolia*.

3. **MC2** Multi indigenous AMF inoculum having seven species MC2 (*A. leavis* Gerdemann & Trappe, *G. microcarpum* Tul & Tul, *G. fasciculatum* (Thaxter), *G. mosseae*. Gerdemann & Trappe *G. aggregatum* Schenck & Smith, *Glomus inveranum* Hall and *Gigaspora. albida* Schenck & Smith) collected from the rhizospheric soil of *Mentha arvensis*.

Inoculation

Pot culture were established by following the method of Bouamri *et al.* (2006). Soil cultures of various AMF species were maintained on *Mentha arvensis* by the method Silviera *et al.*, 2006 for multiplication of AMF. A furrow (10m long and 3cm deep) was made in the centre of each pot (89 cm diameter) and the 150 gm of mixed rhizobase and soil base inoculum was placed in furrow in each pot. One piece sterilized stolon was placed in each furrow by following the method of Khaliq & Janardhanan (1997).

Experimental site

The study was conducted at the experimental garden of Department of Botany, University of Peshawar. It was lies between 33°-42 to 34° North latitude and 71°42 to 71° 42 East longitude. Climate of Peshawar is semiarid with very hot summers and mild winters. Winter in Peshawar, starts in mid-November and ends in late March. Summer months are May to September. The mean maximum temperature in summer surpasses 40°C (104°F) during the hottest month and the mean minimum temperature is 25°C (77°F). The mean minimum temperature during winter is 4°C (39°F) and maximum is 18.35°C (65.03°F).

Experiment 1

This experiment was conducted to see the effects of indeginous AMF species on growth performance, nutrient acquisition, proximate composition, essential oil yield and chemical profile of *Mentha arvensis* and *Mentha longifolia*.

Various indeginous AMF species in crude mixed cultures MC1 (11 Sps.) and MC2 (7 Sps.) were multiplied and used along with single exotic species *Glomus*

intraradicus (GI) as treatments with non inoculated (control). Experimental design was RCBD with 5 replications and four treatments. Statistical data were evaluated by analysis of Variance (Two Factor Anova). Probabilities of significance among treatments and LSD values ($P < 0.05$) were used to compare means through MSTSATC package.

Experiment 2

The second experiment was run to find out the effects of AMF species with application of P fertilizer on growth performance, MD values, oil yield, infection and spore density on *Mentha arvensis* and *Mentha longifolia*.

The pot experiment was aimed to see out the effect and rates of inorganic P fertilizers with and without AMF (MC1) inoculation on growth performance, nutrient acquisition, oil yield, MD (Mycorrhizal Dependency), on AMF infection in roots and spore density in rhizospheric soils of test species. Experiment design was RCBD with 8 treatments (5 replications). Four levels of water soluble phosphate in the form of superphosphate (Recommended dose 90kg/hac) of fertilizers were maintained i.e. Po control (non phosphorus added), P1 (25% of fertilizer), P2 (50 %), P3 (100%). with mycorrhiza (M) and without mycorrhiza (NM). Trail was conducted in 2010.

Experiment 3

The third experiment was performed to investigate the effects of AMF species with various levels of Rock phosphate and organic fertilizer on growth performance, MD values, oil yield, infection and spore density in *Mentha arvensis*.

The pot experiment was conducted to find out the effects and rates of rock phosphate and organic fertilizers (FYM) with and without AMF (MC1) inoculation on growth performance, nutrient acquisition, oil yield, MD (Mycorrhizal Dependency), on AMF infection in roots and spore density in rhizospheric soils of MA. Experiment design was RCBD 8 treatments with 5 replications. Four levels of Rock phosphate (RP 34%P) obtained from Hazara mines (Recommended dose 90kg/hac for each) of fertilizers were maintained i.e., RP0 control (non phosphorus added), RP1 (50% of fertilizer) RP2 (100 %), F1 (50% FYM) F2 (100%) with mycorrhiza (M) and without mycorrhiza (NM). Trial was conducted in 2011.

Agronomic practices

Hoeing, weeding and irrigation were done after Purohit & Viyas (2005). *Mentha* suckers were sown on 25 February 2010 in 8 kg capacity earthen pots. A basal dose of urea 100mg/kg and KCl 20mg/kg along with other fertilizers (KH₂PO₄/Rock phosphate /organic fertilizers) were added to pots. A light irrigation was given after planting and subsequent irrigation was done whenever required and kept the soil moist. Pots were maintained under natural light and temperature. Harvesting was done on 20 June 2010 after 120 days of sowing. The harvesting was done in bright sunlight after 10 A. M. in the morning. Plants were up rooted, measurements (shoot length, root length, fresh weight, dry weight were taken).

x. Mycorrhizal dependency (MD)

Mycorrhizal dependency (MD) value of *Mentha* species was calculated by the formula given by Plenchette *et al.* (1983).

$$\text{Formula} = \frac{\text{Dry weight of mycorrhizal plants} - \text{Dry weight of non mycorrhizal plants}}{\text{Dry weight of mycorrhizal plants}}$$

xi. Extraction of essential oil

A modified Clevenger type apparatus was used for the extraction of essential oils from the leaves through hydro-steam distillation. The leaves were thoroughly washed, cut into small pieces, placed in distillation flask and subjected to hydro- steam distillation for 4 hours. The steam and vaporized oils were condensed into liquid by a vertical condenser and stored in measuring tube. Being immiscible and lighter than water, the volatile oil separated out as an upper layer. The oil was then separated from water and collected in small bottles, dried with anhydrous sodium sulphate, sealed, labeled and stored in light resistant vials at 4–6°C for further use (Essien *et al.* 2008).

xii. Gas Chromatography - Mass Spectrometry (GC-MS)

The chemical composition of essential oils from the leaves of *Mentha arvensis* and *Mentha longifolia* were determined by GC-MS Model QP 2010 plus ((Shimadzu)

operating in EI mode at 70 eV equipped with a split - split less injector (Split ratio, 1:50). Helium was used as carrier gas with flow rate of 1 ml/min. A capillary column (Length: 30 m, id: 0.25 mm, thickness: 0.25 µm, DB-5MS Agilent technologies, USA) treated with 95 % dimethyl and 5 % diphenyl polysilphenylene. Dichloromethane HPLC grade was used as reagent. The following standard conditions were maintained during the operation of MS-GC analysis.

Inject temperature: 240 °C

Ion source temperature (EI): 240 °C

Interface temperature: 240 °C

Pressure: 80 K Pa

GC program time: 46.67 minutes total

Solvent cut time: 2.5 minutes

MS start time: 3 minutes

MS end time: 46 minutes

Acquisition time: scan

M/Z: 40-500

The process was programmed as per temperature condition below (Table 1).

Table 1. Column oven programming.

Rate (°C/min)	Temperature (°C)	Hold (minutes)
-	40	0
3	90	0
10	240	15

xiii. Proximate Composition

Moisture, ash, crude fats and crude protein contents were determined by standard methods of AOAC (2000).

Determination of Crude Fat (%)

Crude fat was determined by ether extract method using Soxhtec (labconco) apparatus. One g moisture free sub sample was wrapped in filter paper, placed in thimble and then introduced in the extraction tube. Pre-weighed, clean and dry receiving beaker

was filled 1/ 3rd with petroleum ether and fitted into the apparatus. Extraction was done up to complete drying of solvent.

The percent of crude fat was determined as follow.

$$\% \text{ Crude Fat} = \frac{(\text{Weight of beaker} + \text{Ether extract}) - (\text{Weight of Beaker}) \times 100}{\text{Weight of Sample}}$$

Determination of Ash (%)

A clean crucible was heated in a muffle furnace at 660⁰C for an hour, then cooled it in desiccators and weighed it as (W₁). One g sample was placed in the crucible (W₂). The sample was charred over the burner with the help a blowpipe. The crucible was then placed in a muffle furnace at 550⁰C for 6-8 hours. After the complete ignition the furnace was turnoff. The crucible was cooled and weighed (W₃). Percent ash was calculated as follows.

$$\% \text{ Ash} = \frac{\text{Weight of Ash (W}_3 - \text{W}_1) \times 100}{\text{Weight of sample}}$$

Determination of Crude fiber (%)

Crude fiber was determined by acid and alkali digestion method. The weighed samples were first digested with acid and then with alkali. One gm sample was taken in a clear beaker and 100 ml of 2.5 % HCl was added to it. The mixture was boiled with stirring for about half an hour. It was drained into the beaker. The fiber residue was again digested in 2.5 % Na OH in a similar manner like that of acid digestion. The residue so collected was transferred to a dried crucible to remove the moisture. Then the weight of the dry crucible was noted. The crucible was then kept in furnace for red dull heat till the formation of white and grey ash. The crucible was cooled in desiccator and weighed again. The loss in weight of the dry residue upon ignition was taken as the amount of crude fiber. Percent crude fiber was calculated as follows.

$$\% \text{ Crude Fiber} = \frac{(\text{W}_1 - \text{W}_2 \times 100) \times F}{\text{Wt. of Sample}}$$

Where

F = Value of crude fat

W₁ = Weight of dry crucible

W₂ = Weight of crucible after heating

Determination of crude protein (%)

One g sample was taken in a digestion flask and 8 g of digestion mixture K_2SO_4 : $CuSO_4$ (8:1) and 12 ml of conc. H_2SO_4 was added. Some piece of pumice stone was also added to avoid bumping of solution. The flask was swirled in order to mix the contents thoroughly. It was then placed on heater under fume hood to start digestion and continued till the mixture becomes clear. After digestion, the heater was turned off and flask was cooled. The digest was then transferred to 100ml flask and volume was made up to the mark by the addition of distilled water. Distillation of the digest was performed in markam still distillation apparatus. 10 ml of digest was taken with the help of pipette and was introduced in the distillation tube through funnel. Then 10 ml of NaOH was gradually added through the same way. Distillation was continued for at least 10 minutes and NH_3 produced was collected as NH_4OH in a conical flask containing 20ml of 4% boric acid solution with few drops of modified methyl red indicator. The tip of condenser was washed in the flask with distilled water. During distillation pink color changed to yellowish due to NH_4OH . The distillate was then titrated against standard 0.05HCl till the appearance of pink color. A blank was also run following the steps as mentioned above. % crude protein was calculated as follows.

$$i. \quad \% \text{ Crude Protein} = 6.25 \times \%N$$

$$ii. \quad \% N = \frac{(S - B) \times N \times 0.014 \times D \times 100}{Wt.of.sample \times V}$$

Where

S = Sample titration reading

B = Blank titration reading

N = Normality of HCl

D = Dilution of the sample after digestion

V = Volume taken for distillation

0.014 = Miliequivalent Wt of Nitrogen

Determination of nitrogen free extract

Nitrogen free extract represents the digestible carbohydrate and value can be obtained by subtracting the sum of the percentage of moisture, crude fiber, crude protein, crude fat and ash from 100.

$$\text{NFE} = 100 - (\% \text{ Moisture} + \text{crude fat} + \text{crude protein} + \text{fiber} + \text{ash})$$

Determination of carbohydrate

Carbohydrate was computed by subtracting the weights of protein, fats, crude fibers, ash, and moisture contents from 100.

xiv. Mineral Evaluation

Wet Digestion

One gram dried crude powdered plant sample was taken in a beaker and 10 ml concentrated HNO_3 (Nitric acid) was added. It was allowed to stand overnight. After 24 hours 4ml perchloric acid was added to each sample and kept for 25 minutes. The sample was heated till it evaporated to a small volume. The samples were cooled and then transferred to a 50ml volumetric flask and diluted with 100ml solution and filtered. The stock solutions were marked with names and coded numbers and were analyzed through atomic absorption spectrometer for quantitative detection of the following elements.

Preparation of stock/ standard solutions (100ppm)

10 ml of 1000 ppm standard stock solutions of each element was taken in 100 ml volumetric flask and was made to the volume with double distilled water.

Standard solutions (2.5, 5, 10 ppm)

Working standard solutions (2.5, 5, 10 ppm) were prepared by taking 2.5, 5, and 10 ppm of 100 ppm stock solution in a series of 100ml volumetric flask and volume was made up to the mark with double distilled water.

Procedure

The instrument was set according to the instrumental conditions required for respective elements. The respective cathode lamp was turned on and allowed to warm up for 10 minutes and the air acetylene flame was ignited. The instrument was calibrated and standardized with working standard of 2.5, 5, and 10ppm of the standard stock solution of respective minerals (Parkin Elmer). Working standards were run as unknown to verify the standardization. The sample stock solution was aspirated into the flame and concentration of respective element was calculated in ppm (AOAC 2000).

RESULTS AND DISCUSSION

The principal objective of the first phase of present study was to conduct survey and to generate baseline data regarding the mycorrhizal status and its morphology types in some selected medicinal plants of Lamiaceae from various sites of Khyber Pakhtunkhwa (KPK). AM fungi in the rhizospheric soils of two heavily infected *Mentha* species were also isolated, quantified and identified; possible correlation were established between their occurrence and various soil factors. Various indigenous AMF species in crude mixed cultures MC1 (11 Sp.) and MC2 (7 Sp) were selected multiplied and used along with single exotic species *Glomus intraradici* (Gi) in further experimentation. In the second phase different experiments were designed with an aim to find out some efficient and specific AM Fungal endophytes which could be used in future for better performance, essential oil yields, biological mobilization of nutrients and conservation of selected *Mentha* species with highest AM infections. Results of present research work are discussed below.

i. Mycorrhizal status and diversity in mycorrhizal morphologies

The present survey was undertaken to examine the mycorrhizal status, colonization and morphology of AM in the roots of 15 selected medicinal plants of Family Lamiaceae growing wild or cultivated in various sites (Manshera, Haripur, Havelian and Peshawar) of Khyber Pakhtunkhwa.

All the studied medicinal plants were mycorrhizal (Table 2), which ranged from 55 (*Ocimum americanum*) to 100%. (*Mentha arvensis* and *salvia* Spp). Our results agreed with those of Khaliq & Janardhanan (1997), who reported AM colonization levels in different species of *Mentha*, ranging from 32% (*Mentha viridis*) to 85.5% (*Mentha arvensis*). On the contrary, Muthukumar *et al.* (2006) found no infection in *Mentha arvensis*. Our results also agree with those of Karagiannidis (2010) and Fuchs & Haselwandter (2004) who also reported the occurrence of mycorrhiza in some other members of family Lamiaceae.

During present studies AM morphology was also recorded in the investigated species of Lamiaceae for the first time from KPK (Table 2). The Arum-type mycorrhiza was characterized by the presence of intracellular arbuscules, intercellular hyphae and vesicles and were observed in *M. piperata*, *M. royleana*, *M. spicata* (Table 3 & Figs.1-ac). While intracellular hyphal coils, intracellular vesicles and arbuscular were characteristics of Paris-type seen in prevalent in *Mentha longifolia*, *Rosmarinus officinalis*, *Ocimum basilicum*, (Figs. 3-ac). While *Mentha arvensis*, *Salvia lanata*, *S. nubicola* could be placed under intermediate category (Figs. 2-ac). Muthukumar & Parkash (2009) also reported the occurrence of Arum type in *Ocimum basilicum* and *O. americanum*. While Arum and intermediate types was recorded by Dickson (2004). Furthermore, Zubeck & Blaszkowski (2009) observed that *Mentha citrate*, *Origanum majoroma*, *Salvia officinalis* and *Thymus vulgaris* were colonized by AMF (67 – 100%), which were mostly of Arum type.

It is reported that morphological type is controlled by the host plant and results suggests that plant genotype strongly influences AM morphology. It may also happen that different morphological types are found in the same genus (Muthukumar & Parkash, 2009). Moreover, the co-occurrence of both Arm and Paris types in families like Arecaceae, Poaceae, Euphorbiaceae, Fabaceae, Rubiaceae, Solanaceae and Lamiaceae has also been reported (Smith & Smith, 1997). Muthukumar *et al.* (2006) reported Arum-type in *Leucas aspera* and *Ocimum tenuiflorum*. The physiological and functional disparity between Arum and Paris types is still not clear but it has been reported that the development of Arum-type is faster than that of Paris type (Brundrett & Kendrick, 1990; Cavagnaro *et al.*, 2001). Anatomical characters of host roots might influence the AM morphology (Brundrett & Kendrick, 1988, 1990).

Recently, Kubato *et al.* (2005) stated that the morphology of AM type depends on the interaction between both the host plant and fungal species. It is essential to examine a wider range of plants growing in different habitats to understand such morphological variations of AM. Cavagnaro *et al.* (2001) found that both the AM morphological types in *Lycopersicon esculentum* (wild type tomato) depended on the fungal species. Yamato (2004) also stated that AM morphology type was determined by both the plant and fungal

species. Moreover, environmental factors such as temperature, light intensity and soil moisture may also affect AM morphology (Cavagnaro *et al.*, 2001).

In the present investigation different percentages of various morphological parts (external hyphae, internal hyphae, arbuscules and vesicles) were also recorded (Table 3). Variations in the morphology of vesicles were observed in roots of studied plants. These variations might be due to infection by multiple endophytes (Burni *et al.*, 1993, 2008). Variations in the infection levels may also be due to the reasons that host response can differ with fungal species and seasonal development (Bethylenfalvay & Ames 1987). Moreover, environmental factors have strong and sometimes unpredictable effects on infection as well as functioning of the AM endophytes. Host genome also affect the establishment and morphology of AM infection (Lackie *et al.*, 1987).

Conclusion

The present investigation suggests the possibility of AMF applications in herbal industry in order to improve herb production and quality. Considering the impact of mycorrhizal fungi It seems to be crucial that in the case of species dependence on an AMF for their performance (e.g. growth, secondary metabolite production) more attention should be paid on soil monitoring and mycorrhizal development during the process of cultivation (Zubek & Blaszkowski, 2009). The present results might enable us to select plant species that are highly colonized by AMF and to find out the mycorrhizal dependency and the influence of AMF on the production of secondary metabolites.

Table 2. AM morphologies and mycorrhizal status of medicinal plants of Family Lamiaceae.

S.No	Species	Collection sites	Morphology Types	General infection (%)
1.	<i>Mentha arvensis</i> L.	Peshawar	Intermediate	100 ± 0.78
2.	<i>M. longifolia</i> L.	Peshawar	Paris	80 ± 0.56
3.	<i>M. piperata</i> L.	Peshawar	Arum	70 ± 0.30
4.	<i>M. royleana</i> Benth	Manshera	Arum	76 ± 0.64
5.	<i>M. spicata</i> L.	Haripur	Arum	60 ± 0.65
6.	<i>Rosmarinus officinalis</i> L.	Peshawar	Paris	66 ± 0.50
7.	<i>Ocimum basilicum</i> L.	Peshawar	Paris	60 ± 0.80
8.	<i>O. americanum</i> L.	Peshawar	Arum	55 ± 6.30
9.	<i>Origanum vulgare</i> L.	Manshera	Arum	78 ± 0.38
10.	<i>O. majorana</i> L.	Manshera	Arum	50 ± 4.45
11.	<i>Coleus forskohlii</i> L.	Peshawar	Arum	70 ± 0.6
12.	<i>Coleus aromaticus</i> Benth	Manshera	Arum	60 ± 0.79
13.	<i>Lallementia rovelena</i> Benth.	Peshawar	Arum	60 ± 1.85
14.	<i>Salvia lanata</i> Roxburgii	Galliat	Intermediate	95 ± 1.25
15.	<i>S. nubicola</i> Wall ex. Sweet	Manshera	Intermediate	100 ± 2.40

Mean $\pm$ Standard error

Table 3. Arbuscular mycorrhizal structural colonization (%) in some plants of Family Lamiaceae.

S.#.	Species	External Mycelium	Internal Mycelium	Arbuscules	Vesicle
1.	<i>Mentha arvensis</i> L.	9.1 $\pm$ 0.54	19.8 $\pm$ 0.25	49.9 $\pm$ 1.24	21.2 $\pm$ 2.24
2.	<i>M. longifolia</i> L.	9.5 $\pm$ 0.46	38.8 $\pm$ 0.44	18.5 $\pm$ 0.48	13.2 $\pm$ 0.50
3.	<i>M. piperata</i> L.	18.3 $\pm$ 0.25	12.2 $\pm$ 0.15	17.4 $\pm$ 0.38	22.1 $\pm$ 0.19
4.	<i>M. royleana</i> Benth.	10.4 $\pm$ 0.54	29.6 $\pm$ 0.55	28.2 $\pm$ 0.60	7.8 $\pm$ 0.60
5.	<i>M. spicata</i> L.	14.3 $\pm$ 0.57	10.7 $\pm$ 0.45	20.5 $\pm$ 0.55	14.5 $\pm$ 0.64
6.	<i>Rosmarinus officinalis</i> L.	13.2 $\pm$ 0.63	22.5 $\pm$ 0.52	23.5 $\pm$ 0.61	6.8 $\pm$ 0.58
7.	<i>Ocimum basilicum</i> L.	11.2 $\pm$ 0.48	19.8 $\pm$ 0.39	17.2 $\pm$ 0.47	11.80 $\pm$ 0.36
8.	<i>O. americanum</i> L.	8.7 $\pm$ 0.76	14.6 $\pm$ 0.60	12.2 $\pm$ 0.72	19.50 $\pm$ 0.70
9.	<i>Origanum vulgare</i> L.	18.3 $\pm$ 0.25	16.5 $\pm$ 0.19	32.8 $\pm$ 0.16	10.40 $\pm$ 0.20
10.	<i>O. majorana</i> L.	8.9 $\pm$ 0.35	14.7 $\pm$ 0.39	20.3 $\pm$ 0.30	6.10 $\pm$ 0.29
11.	<i>Coleus forskohlii</i> L.	14.8 $\pm$ 4.45	10.2 $\pm$ 2.11	30.6 $\pm$ 0.99	14.4 $\pm$ 0.90
12.	<i>Coleus aromaticus</i> Benth	10.3 $\pm$ 0.51	18.5 $\pm$ 0.41	15.3 $\pm$ 0.39	15.9 $\pm$ 2.25
13.	<i>Lallementia royleana</i> Benth.	8.6 $\pm$ 0.65	19.4 $\pm$ 0.61	13.7 $\pm$ 0.71	18.3 $\pm$ 0.48
14.	<i>Salvia lanata</i> Roxburgii	14.6 $\pm$ 0.98	40.6 $\pm$ 0.76	20.5 $\pm$ 0.56	19.3 $\pm$ 0.57
15.	<i>S. nubicola</i> Wall ex. Sweet	6.7 $\pm$ 1.22	12.2 $\pm$ 1.98	13.6 $\pm$ 0.80	17.5 $\pm$ 0.46

Mean: $\pm$ Standard error

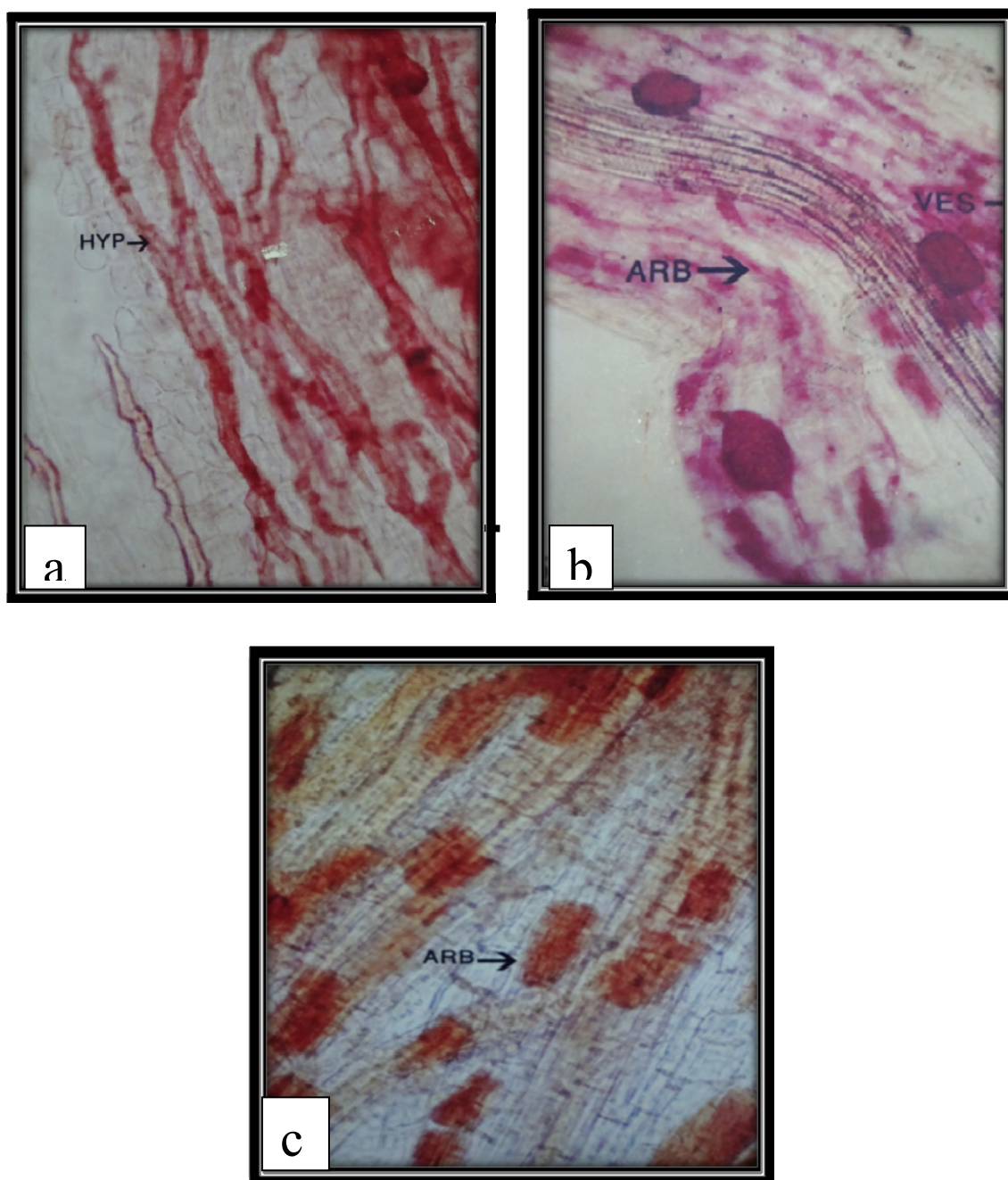


Fig 1. a,b,c. Arum type mycorrhiza in the root cortex of *Mentha piperata*, *M. royleana* and *M. spicata*, respectively. ARB: Arbuscules: VES: Vesicles: HYP: Hyphae. M

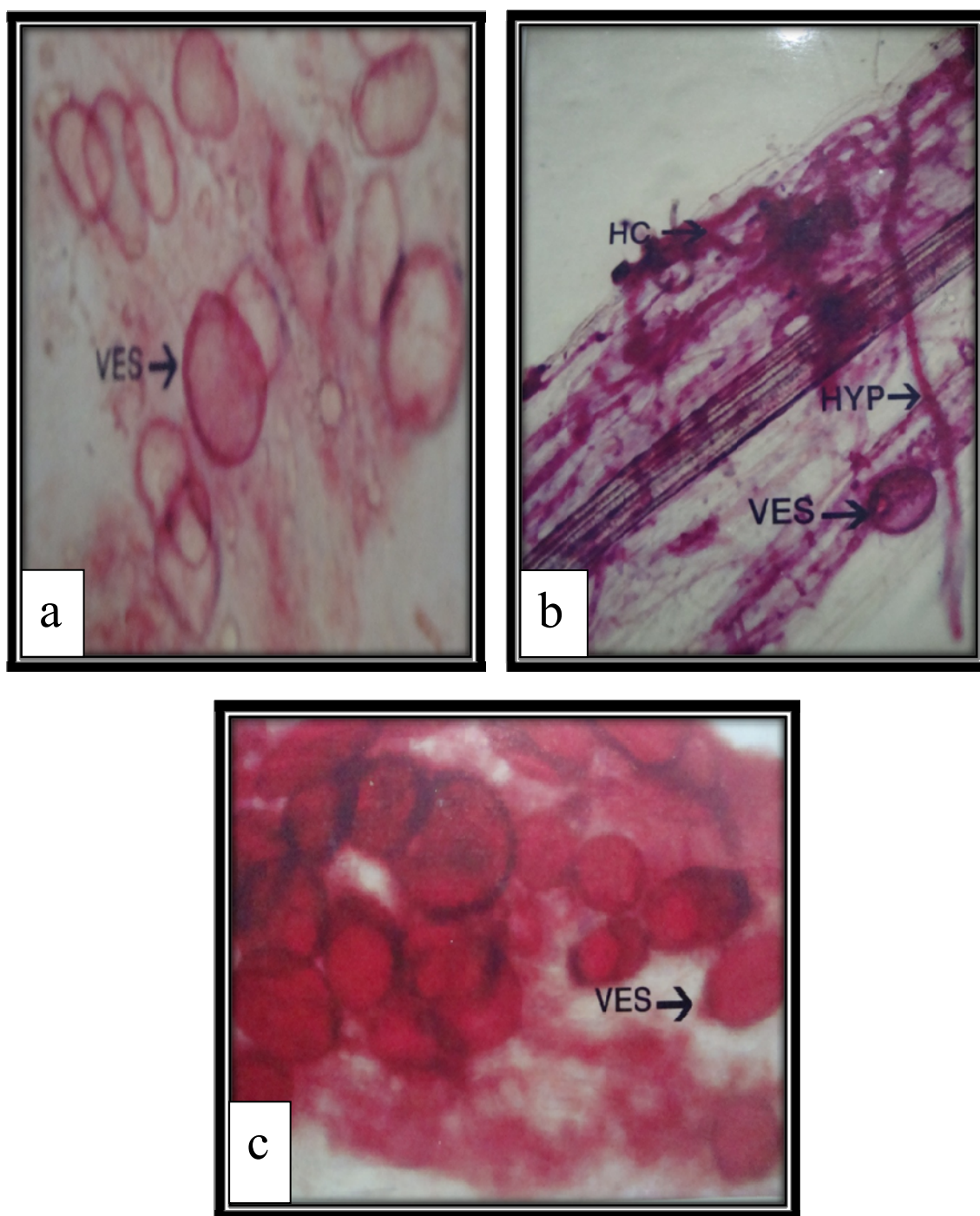


Fig. 2 a,b,c. Intermediate type mycorrhiza in the root cortex of *Mentha arvensis*. *Salvia lanata* and *S. nubicola*, respectively. VES: Vesicles; HYP: Hyphae; HC: Hyphal coils.

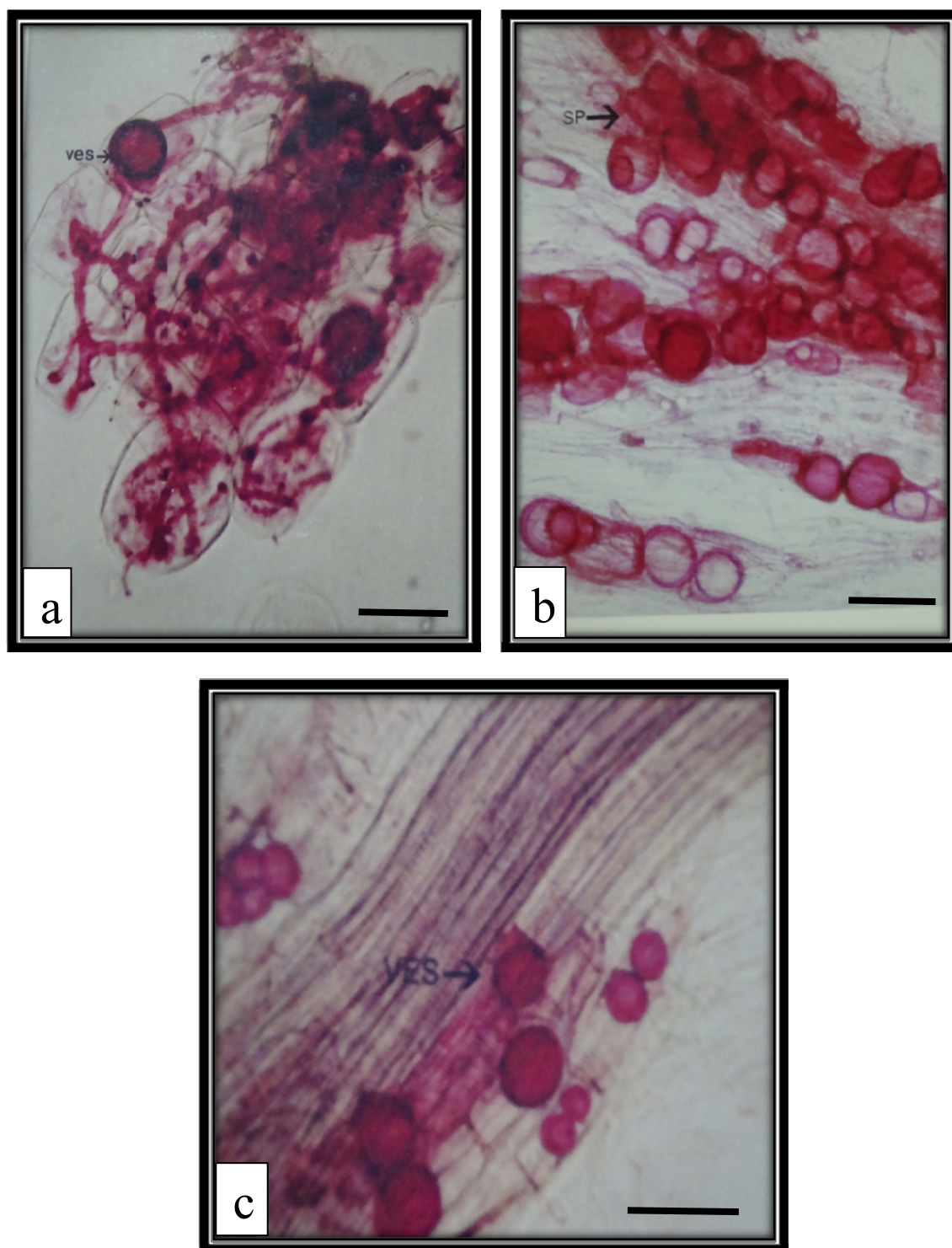


Fig. 3 a,b,c. Paris type mycorrhiza in the root cortex of *Mentha longifolia*, *Rosmarinus officinalis* and *Ocimum basilicum*, respectively. SP: Spores: ARB: Arbuscules: VES: Vesicles: Bar =10 μ m

CHAPTER - 5

ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ASSOCIATED WITH THE RHIZOSPHERE OF *MENTHA ARVENSIS* L. AND *MENTHA LONGIFOLIA* HUDS.

VAM fungi are ubiquitous in occurrence and some workers have reported their presence from various locations in Pakistan (Jalal-ud-din *et al.*, 2008). However, there is less information about their occurrence and distribution in the soils of KPK. (Burni & Ilahi, 2004; Zainab & Burni, 2005; Sharif *et al.*, 2005; Nasruallah *et al.*, 2010). Khyber Pakhtunkhwa Province of Pakistan is very rich in medicinal and aromatic flora but only few reports are available regarding their mycotrophic status and associated AMF flora (Burni *et al.*, 2007, 2011).

Knowledge about the presence and diversity of AMF in a specific area is an essential step for utilizing these fungi in any application (Wang *et al.*, 2006). The identification of indigenous AMF is a fundamental requirement to understand biodiversity and essential for monitoring changes in natural, managed or disturbed ecosystems (Khanum, 2007).

Keeping in view the importance of these micro-organisms present work was undertaken to investigate two selected species of *Mentha* growing in KPK. Eleven AMF species (*A. melleae* Spain & Schenck, *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker & Koske, *Glomus etunicatum* Becker and Gerdemann, *Glomus claroides* Schenck & Smith, *Glomus microcarpum* Tul & Tul, *G. australe* (Berkeley) Berch, *Glomus intaradicus* Schenck & Smith, *Glomus aggregatum* Schenck & Smith, *Glomus constrictum* Trappe, *Glomus mosseae*. Gerdeman & Trappe, *Gigaspora gigantea* Gerdemann and Trappe. *Glomus microcarpum* Tul & Tul) were recovered from *Mentha longifolia* rhizosphere (Table 4) and seven species (*Acaulospora leavis* Gerdemen & Trappe, *Glomus fasciculatum* (Thatex) Gerdemann & Trappe emend. Walker & Koske, *Glomus inveranum* Hall, *Glomus aggregatum* Schenck & Smith, *Gigaspora albida* Schenck & Smith, *Glomus mosseae*, Gerdemann & Trappe.) from *Mentha arvensis* (Table 4). Based on morphological characteristics of spore, they were placed under three genera *Acaculospora*, *Gigaspora* and *Glomus*.

i. Taxonomic distribution of AM Fungi

Fourteen AMF species were identified in studied sites of KPK (Table 4; Figs. 4, 5). *Glomus* species were common and made up for more than 75% of total isolates followed by *Acaulospora* and *Gigaspora*. The present findings confirm the ubiquitous nature of AMF spores. Our findings corroborate with the findings of Morton (1988) that genus *Glomus* is a predominantly distributed genus in the soil all over the world. The dominance of *Glomus* in the present study agrees with the findings of Panwar & Tarafdar (2006), Pande & Tarafdar, (2004), Burni & Illahi (2004), Mridha & Dhar (2007), Sharma *et al.*, (2009), Burni *et al.*, (2004). Further, Wang & Shi (2006) also reported 122 AM species in the rhizosphere of different plants in various environment of China. Zang *et al.* (2003) and Tao *et al.* (2004) reported the dominance of *Glomus*. The dominance of *Glomus* species in different locations might be due to wide tolerance to the changing habitat conditions. They can survive in acidic and alkaline soils (Pande & Tarafdar, 2004).

Glomus fasciculatum was the most common species in all the investigated sites. This agree with the findings of Sharif *et al.* (2005), who found *Glomus fasciculatum* to be the most predominant species in the soils of KPK. Moreover, Nasim *et al.* (1999) also observed the spores of *Glomus fasciculatum* associated with grasses from different locations of Pakistan. Jalal-ud-din & Anwar (1991) also recorded *Glomus fasciculatum* in wheat fields of Sindh and Punjab. *Glomus aggregatum* was also common species and present study agree with Jalaluddin & Anwar (1991), who found this species in the wheat fields of Sindh.

Arbuscular mycorrhizal fungi have a broad ecological range that play important role in ecosystem diversity. The population and distribution depends upon various abiotic and biotic factors (Mohammad *et al.*, 2003). Changes in total spore population in different ecosystems have been reported by various workers (Louis & Lim, 1987; Saif, 1986; Mridha & Dhar, 2007).

ii. Root colonization and spore density of AM fungi

Roots of all investigated plants exhibited AM association (Table 4). Both the species of *Mentha* tested positive for AM fungal colonization.

Root colonization was characterized by the presence of external hyphae, internal hyphae, arbuscules and vesicles. Arbuscular infection was common in *Mentha longifolia* whereas vesicular infections were more prevalent in *Mentha arvensis*. The results of spore densities means (Table 5) revealed that the four investigated sites were different with respect to the total number of densities of AMF spores. Among them, maximum spore densities recorded in Manshera (245 ± 2.06) and minimum in Havellian (50 ± 0.26).

The total AMF spore densities in different soils of *Mentha* species ranged 50 - 245 spores / 100g of soil. Our results are in line with Hindumathi *et al.* (2011) who also found a wide variations in spore density in the rhizospheric soils of legumes. Kumar *et al.* (2010) reported the arbuscular fungal dynamics in the rhizospheric soils of five medicinal plants. They reported that AMF spore density ranged from 19.33 to 68.66/10 gm soil of *Mentha spicata*. Radhika & Rodrigues (2010) recorded spore density of 85spores/100gm in the rhizospheric soil of *Mentha* & other species. The quantitative distribution of AMF spore of various studied sites showed high variation. Sharif *et al.* (2005) and Nasrullah *et al.* (2010) also stated that AMF spore vary considerably from place to place according to physical and chemical nature of the soil. Our results are strengthened by the findings of Hindumathi *et al.* (2011), who also indicated variations in the quantitative distribution due to pH, texture and mineral nutrients of soil.

iii. Effect of chemical characteristics of soil on AMF spores

The relationship between AMF spore densities and chemical characteristics of soils for both *Mentha* species was determined statistically by computing the co-efficient of correlation (r). The soil samples were analyzed for chemical characteristics. The results (Table 6) show that soil pH ranged between 7.00-8.08. Kamal & Parsad (1995) reported that alkaline pH (8.6) favours AMF spore population. A positive correlation between soil pH and AMF spores was found in the present case. The findings are in line

with the findings of Panwar & Tarafdar (2006). In this respect the present result revealed that phosphorus ranged between 2.25-5.99 ppm in the investigated sites. There was a negative correlation between soil phosphorus and AMF spores. Some other workers (Janaki & Manoharachary, 1994; Korthari & Singh, 1996; Panwar & Tarafdar, 2006) also found negative correlation between AMF spores and soil phosphorus. Sharif *et al.* (2011) showed that low availability of soil phosphorus increases VAM colonization. The present results negate the findings of Chandrasekara *et al.* (2005), who did not find any significant relationship with available phosphate.

Moreover, present results showed positive correlation between soil nitrogen (N) and AMF spores, and this agrees with the findings of Panwar & Tarafdar (2006). There was a positive correlation between K and AMF spores. However our results are negated by Hindumathi *et al.* (2011) who reported negative correlation between AMF spores and potassium.

Wu *et al.* (2006) evaluated the influence of organic matter on AMF with variable results indicating different responses on plants and fungi. Present data regarding the Pearson correlation/ co-efficient revealed positive values between AMF and organic matter. Our results are supported by Burni & Ilahi (2004) and Hindumathi *et al.* (2011) who also reported that soil with high organic matter contents were also rich in AMF spores density. This may be attributed to the fact that organic matter increases the water holding capacity of soil that might enhance the sporulation of AMF.

In the present investigation the extent of root colonization varied in *Mentha* species which might be attributed to the differential preference of the AM fungi to various species (Khade & Rodrigues, 2009). Many other researchers also reported that root colonization of AMF is genetically controlled (Sensory *et al.*, 2007). Karangiannidis *et al.* (1997) also reported significant differences between four grapevine rootstocks regarding their abilities to form AM association. Recently, Burni *et al.* (2011) also stated variations in AM root colonization in various members of Lamiaceae. Different edapho-climatic factors like soil type, nutritional status of soil, soil pH, organic matter, soil moisture, rain fall and temperature etc might be responsible for variations in root colonization and spore population (Hindumathi *et al.*, 2011).

CONCLUSION

The study concludes the positive association of AMF in two species of *Mentha* in various districts of KPK. It also highlighted the fact, that this partnership is controlled by various edaphic factors, and host varieties. The present results revealed the rich diversity of AMF associated with this pharmaceutically medicinal herb offers the feasibility of using these fungi as biofertilizers to enhance their productivity.

Table 4. The distribution of AMF species in various districts of KPK in the rhizospheric soils of *Mentha longifolia* and *Mentha arvensis*.

S. No.	AM FUNGI	DISTRICTS							
		Peshawar 359 (m)		Haripur 610 (m)		Havellian 203 (m)		Mansehra 975.36 (m)	
		ML	MA	ML	MA	ML	MA	ML	MA
1.	<i>Acaulospora.melleae</i> Spain & Schenck	+	-	-	-	+	-	-	-
2.	<i>Acaulsporaleavis</i> Gerdemen & Trappe	-	+	-	-	-	-	-	+
3.	<i>Glomus fasciculatum</i> (Thatex) Gerdemann & Trappe emend. Walker & Koske	+	+	+	+	+	+	+	+
4.	<i>Glomus etunicatum</i> Becker and Gerdemann	-	-	+	-	-	-	+	-
5.	<i>Glomus claroides</i> Schenck & Smith	-	-	+	-	-	-	+	-
6.	<i>Glomus australe</i> (Berkeley) Berch	-	-	-	-	+	-	+	-
7.	<i>Glomusintaradicus</i> Schenck & Smith	-	-	+	-	+	-	+	-
8.	<i>Glomus aggregatum</i> Schenck & Smith	+	+	+	+	+	+	+	+
9.	<i>Glomus constrictum</i> Trappe.	-	-	+	-	+	-	+	-
10.	<i>Glomusmosseae</i> , Gerdemann & Trappe	+	+	+	+	+	+	+	+
11.	<i>Gigaspora gigantea</i> Gerdemann and Trappe	-	-	-	-	+	-	-	-
12.	<i>Glomus microcarpum</i> Tul & Tul	+	+	+	+	+	+	+	+
13.	<i>Glomus inveranum</i> Hall	-	+	-	+	-	+	-	+
14.	<i>Gigasporaalbida</i> Schenck & Smith	-	-	-	-	-	+	-	-

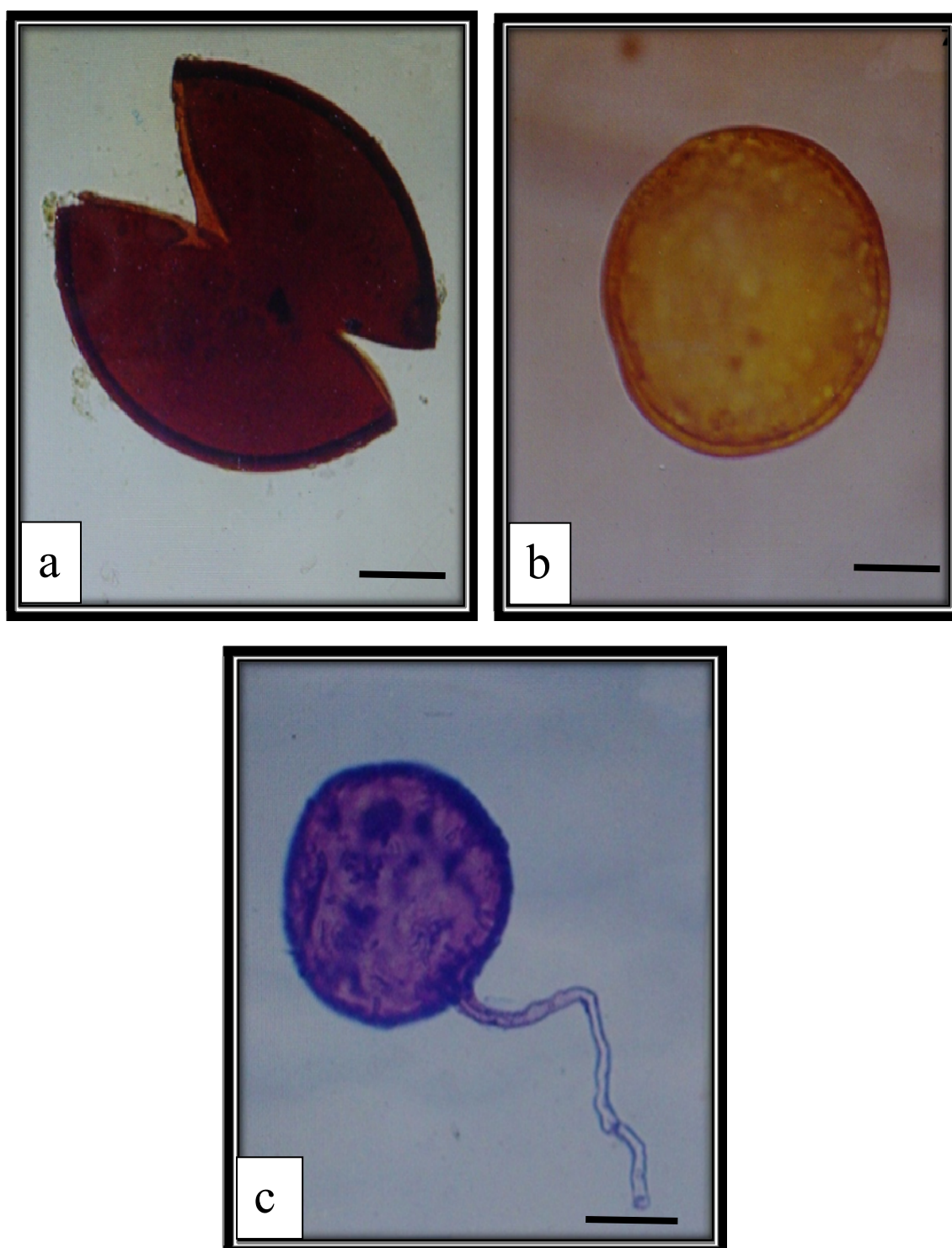


Fig. 4. a,b,c. Spore of *Glomus etunicatum*, *Acaulospora melleae* and *Glomus fasciculatum*, respectively. Bar = 10μm

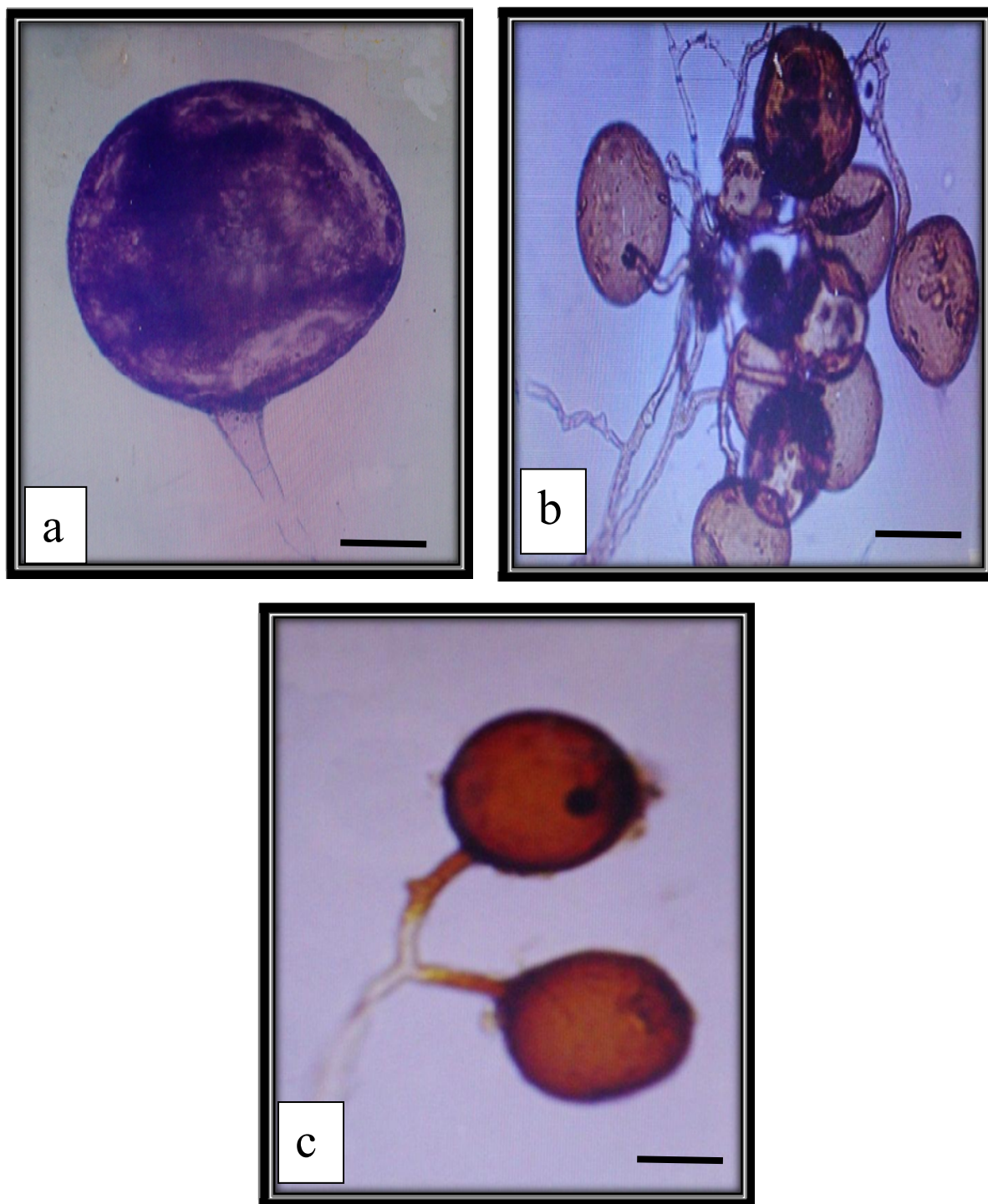


Fig. 5 a,b,c. Spore of *Glomus mosseae*, *Glomus aggregatum* and *Glomus claroide*, respectively. Bar = 10 μ m

Table 5. Total densities/100 gm. soil of AM spores from the rhizosphere of *Mentha longifolia* and *Mentha arvensis* in various districts of KPK.

		Districts			
		Peshawar	Haripur	Havelian	Mansehra
<i>Mentha longifolia</i>	AM colonization	40.50 $\pm$ 2.5	80.68 $\pm$ 1.45	70.25 $\pm$ 1.36	100 $\pm$ 2.02
	Spore density	190 $\pm$ 1.89	134 $\pm$ 1.29	150 $\pm$ 0.9	245 $\pm$ 2.06
<i>Mentha arvensis</i>	AM colonization	26.87 $\pm$ 0.99	66.90 $\pm$ 1.88	30.34 $\pm$ 1.12	90.7 $\pm$ 1.12
	Spore density	138 $\pm$ 0.88	187 $\pm$ 1.07	50 $\pm$ 0.56	152 $\pm$ 3.08

Standard error $\pm$

Table 6. Chemical characteristics of rhizospheric soil of *Mentha longifolia* and *Mentha arvensis* from various locations of KPK and Co-efficient of correlation (r) of soils chemical characteristics with AM spore densities.

Soil character	Species	Districts				r-Values
		Peshawar	Haripur	Havelian	Mansehra	
pH	ML	8.00±0.01	7.51±0.02	7.85±0.03	8.08±0.01	+0.9
	MA	7.25±0.02	7.05±1.10	7.00±0.8	7.80±0.02	±0.7
OM (%)	ML	0.95±0.05	1.03±0.04	0.78±0.01	1.77±0.03	+0.9
	MA	2.50±0.02	4.52±0.07	3.90±0.03	3.04±0.01	±0.1
Lime (%)	ML	10±0.02	0.70±0.01	11.4±0.21	1.50±0.01	-0.6
	MA	11.3±0.05	11.7±0.03	8.75±0.01	2.50±0.04	±0.2
N (ppm)	ML	0.41±0.02	0.27±0.02	0.34±0.07	0.35±0.01	+0.8
	MA	0.25±0.02	0.23±0.03	0.38±0.04	0.25±0.01	±0.9
P (ppm)	ML	3.99±0.02	2.25±0.02	5.89±0.03	2.60±0.02	-0.8
	MA	2.76±0.01	5.99±0.07	6.1±20.01	3.17±0.05	-0.5
K (ppm)	ML	124±0.05	109.2±0.04	156.2±0.01	186.9±0.05	+0.07
	MA	300.2±0.02	223.8±0.04	180±0.01	312.8±0.06	+0.2
Zn (ppm)	ML	0.61±0.07	0.99±0.01	0.14±0.02	0.52±0.06	-0.13
	MA	0.14±0.06	0.19±0.04	0.88±0.02	0.23±0.01	-0.8
Fe (ppm)	ML	15.07±0.08	12.89±0.01	13.00±0.01	16.84±0.01	-0.14
	MA	16.06±0.03	14.46±0.01	11.16±0.02	17.82±0.01	-0.9
Cu (ppm)	ML	1.40±0.01	2.90±0.01	2.60±0.01	1.65±0.03	-0.1
	MA	2.70±0.04	2.65±0.01	2.00±0.03	1.30±0.07	-0.3
Mn (ppm)	ML	13.60±0.01	9.45±0.02	10.40±0.01	19.25±0.01	-0.9
	MA	16.80±0.02	10.75±0.03	12.20±0.04	17.45±0.01	-0.3

Mean ± Standard error

EFFECTS OF AM FUNGI ON GROWTH PERFORMANCE AND NUTRIENT ACQUISITION

Arbuscular mycorrhizae is the most widespread plant symbiosis that often improves plant productivity (Fedderman *et al.*, 2010). Recently, mycorrhizal inoculation not only promoted the growth of medicinal plants but also improved the productivity and quantity of chemicals. Hence, there is an upcoming demand for research in improving the quality and quantity of drugs produced from native medicinal plants in relatively less time with application of AM fungi (Karthikeyan *et al.*, 2009). Moreover AMF are not host specific and show wide host range, but their affinity to the host is always preferential (Rogers *et al.*, 1994), thus suggesting the need for selecting efficient AMF for a particular host.

Therefore, present experiment was designed to test the symbiotic efficiency of various AMF species isolated from different sites on host growth responses of two species of *Mentha* i.e., *Mentha arvensis* and *Mentha longifolia*, respectively were evaluated, in terms of growth parameters, nutrient acquisition, proximate composition essential oil yield, composition, root colonization and spore density. Present study also aimed to select symbiotic efficient strains for *Mentha* species in order to harness the maximum benefit from the AMF in herb industry.

i. Growth attributes

The mean data regarding various growth parameters was analysed by ANOVA and LSD of both test species treated with three AMF inocula as given in (Tables 7, 8). It was seen that AM fungi significantly ($p < 0.000$) promoted the growth of both the species of *Mentha*. However, maximum enhancement of plant height, root length, fresh weight, dry weight, root colonization and spores population was observed in the case of *Mentha arvensis* than *Mentha longifolia*.

Shoot length

The results showed (Table 7; Figs. 6, 7) the mycorrhizal plants attained significantly better height as compared to respective control plants. Similar results have been observed by Gupta *et al.* (2002) in three cultivars of menthol mint (*Mentha arvensis*). It has been suggested that VAM inoculated plants had greater mitotic activity of stem cells, resulting in taller plants (Tarafdar & Marschner, 1995). This may be because of fact that extra-radical mycelium (being smaller in diameter than roots) penetrates better beyond the depletion zone for better acquisition of nutrients (Mengel & Kirkby, 2001). The increased plant height of AM inoculated plants was attributed to enhanced acquisition of mineral nutrients with low mobility, such as P, Zn, Cu and Fe (Chandanie *et al.*, 2009; Al-Karaki, 2000).

It is evident from mean data (Table 7; Figs.7, 8) that AMF inoculum MC1 (mixture of 11 species) promoted the shoot length (43.53cm) of *Mentha arvensis* and *Mentha longifolia* (40.94cm) followed by MC2 (mixture of 7 species) in *Mentha arvensis* (40.48cm) and *Mentha longifolia* (34.74 cm). Significantly least values (35.96 cm and 30.70cm) were encountered in GI (single species, *Glomus intraradicus*) treated plants followed by their corresponding control plants (19.96cm and 20.58cm). Similar findings were reported by Sharma *et al.* (2009), who found low performance of test species *Curculigo orchiodes* inoculated with *G. intraradicus*. Likewise, Rowe *et al.* (2007) also stated that plants colonized by a commercial inoculum of *G. intraradicus* responded negatively than native or locally adapted field inocula. Furthermore Kliromonas (2003) who explained that in nature plants exert control over AMF populations to select the best mutualists. Moreover, they also observed that mycorrhizal effectiveness is higher when plants are associated with home fungi.

Root Length

The root length (Table 7; Figs. 7, 8) in *Mentha arvensis* and *Mentha longifolia* rated mycorrhizal plants out performed significantly than non mycorrhizal. Similar results were also exhibited by AMF inoculation in *Spilanthes somnifera* and *Withania somnifera*. The present results are also in accordance with other workers (Motosugi *et al.*,

2002; Jayachandran & Shetty, 2003; Turkmen *et al.*, 2008). These findings are also in conformity with Gogoi & Singh (2011) who while working on the comparative efficiency of twenty three AMF species on growth attributes of *Piper longum* found significantly positive effects of three *Glomus* species on root growth. The consistent results obtained in the present case might be due to that mycorrhizal inoculation changed the root morphology, increases the lateral root number and length explained by Kumar *et al.* (2007). Moreover, mycorrhiza also reduces soil compaction that facilitates root development (Miransari, *et al.* 2007).

The comparative performance of various AMF species showed that MC1 responded significantly better in *Mentha longifolia* and *Mentha arvensis* (12.16 cm and 13.600 cm) followed by MC2 (11.82 cm and 11.94 cm) thereafter by GI (11.40 cm and 9.600 cm) and least in control (7.360 cm and 9.110 cm). The present findings showing different response of *Mentha* species to three mycorrhizal fungal inocula confirm previous reports on variable response of a plant species to inoculation with different native AMF species with different number and type (Kitajima, *et al.* 2003; Van-der Heijden *et al.* 1998). It is also known that the natural AMF inocula collected from various sites can define competitive outcomes among plant species and modify the degree of benefit conferred by AMF (Moora *et al.*, 2004).

Fresh weight

The results (Tables 7, 8; Figs. 8, 9) revealed that the mycorrhizal plants registered significantly ($p < 0.000$) high fresh weight in response to AM inoculation. The maximum fresh weight was recorded in MC1 (18.72gms) of *Mentha longifolia*, closely followed by (18.13 gms) in the same treatment. Next higher values were recorded in MC2, i.e. 17.11gms and 15.82gms in *Mentha arvensis* and *Mentha longifolia* respectively. GI treated *Mentha arvensis* plants exhibited significantly high value (15.82gms) as compared to *Mentha longifolia* (13.34gms) followed by control. The present results are favoured by the findings of Freitas *et al.* (2004), who noticed better fresh weight in inoculated *Mentha arvensis* plants. Our results also agreed with Khaliq & Janardhanan (1997) who found the positive and variable influence of various AMF inocula on fresh weight of *Mentha arvensis* that ranged from 71% to 192.5%. The effect of mycorrhiza on

fresh weight could be related to P nutrition (Torelli *et al.*, 2000). However, increased growth of mycorrhizal plants may also be linked to overcoming two types of inhibitions of photosynthetic activity. First, by drawing upon the starch reserves in the leaves of the host plant, the VAMF reduces the starch-sink inhibition in leaves. Secondly, increasing the concentration of inorganic P in the chloroplast reduces the ADP/ATP limitation of photosynthesis (Harris & Paul, 1987).

Dry weight

The perusal of data (Table 7, 8; Figs. 8, 9) pertaining to effect of three AMF inocula on dry weight of *Mentha arvensis* showed non significant differences. However differences exist between mean values and enhancement followed the general trend i.e., MC1>MC2>Gi followed by control plants of both species. Generally mycorrhizal plants outperformed the non-mycorrhizal. In terms of dry weight production there was a tendency for MA to give a more positive response to inoculation as compared to control. Similar trend was also revealed by Karagiannidis *et al.* (2011) who found that mint plants colonized with AMF exhibited from 2 to 3 fold higher dry weight compared to non mycorrhizal plants. Moreover, our findings are also by work of Karagiannidis *et al.* (2012) who noticed significantly higher dry weight in response to *G. etunicatum* inoculation in three *Mentha* species.

Present study suggested that the host species exhibited differential growth responses to AMF. A number of factors might affect the growth response of host plants to AMF colonization, including the genotypes of host plants (Sensory *et al.*, 2007). Different levels of responsiveness among different genotypes in the same crop species have been demonstrated in previous studies (Declerck *et al.*, 1995; Linderman & Davis, 2004).

Table 7. Effects of AMF species on various growth parameter of *Mentha longifolia* and *Mentha arvensis*.

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indeginous. AMF (MC1 and MC2). Treatments were tested with two way ANOVA.

Species	Treatments	Shoot length (cm)	Root length (cm)	Fresh weight (gm.)	Dry weight (gm.)
<i>Mentha longifolia</i>	C	20.58 ^e	9.110c	12.20 ^e	4.384d
	GI	30.70 ^d	9.600c	13.34 ^e	5.460b
	MC1	40.94 ^b	13.600a	18.72 ^a	6.672a
	MC2	34.74 ^c	11.94ab	15.90 ^{cd}	5.678b
<i>Mentha arvensis</i>	C	19.96 ^e	7.360c	15.54 ^d	4.598d
	Gi	35.96 ^c	11.40ab	15.82 ^{cd}	5.230c
	MC1	43.53 ^a	12.16a	18.13 ^{ab}	6.770a
	MC2	40.48 ^b	11.82ab	17.11 ^{bc}	5.980b
LSD at 5%		2.435	1.477	1.455	0.4786
P VALUE	T	0.0000	NS	0.0001	NS
	S	0.0000	0.0000	0.0000	0.0000
	(T × S)	0.0026	0.0794	0.0037	NS

Each value is a mean of five replicates. Values followed by different letters are significantly different ($p < 0.05$). P (Probability), NS (Non significant), LSD (Least Significant Test), T (Treatment), S (species).

Table 8. ANOVA values for different parameters.

Source	Degree of Freedom	Sum of Squares	Mean Square	F value	Prob.
a. Shoot length					
Replications	4	8.337	2.084	0.5902	
Treatments (T)	1	105.041	105.041	29.7433	0.0000
Varieties (S)	3	2681.247	893.749	253.0736	0.0000
T×S	3	64.279	21.426	6.0670	0.0026
Error	28	98.884	3.532		
Total	39	2957.788			
Coefficient of variation: 5.63 %					
b. Root length					
Replications	4	4.385	1.096	0.4219	
Treatments (T)	1	1.421	1.421	0.5470	
Varieties (S)	3	121.443	40.481	15.5803	0.0000
T×S	3	19.541	6.514	2.5069	0.0794
Error	28	72.750	2.598		
Total	39	219.540			
Coefficient of variation: 14.82 %					
c. Fresh weight					
Replications	4	16.983	4.246	3.3647	0.0228
Treatments (T)	1	25.075	25.075	19.8721	0.0001
Varieties (S)	3	124.492	41.497	32.8872	0.0000
T×S	3	21.380	7.127	5.6481	0.0037
Error	28	35.331	1.262		
Total	39				
Coefficient of variation: 7.08 %					
d. Dry weight					
Replications	4	2.468	0.617	2.2586	0.0880
Treatments (T)	1	0.092	0.092	0.3374	
Varieties (S)	3	26.039	8.680	31.7733	0.0000
T×S	3	0.407	0.136	0.4961	
Error	28	7.649	0.273		
Total	39	36.655			
Coefficient of variation: 9.34 %					

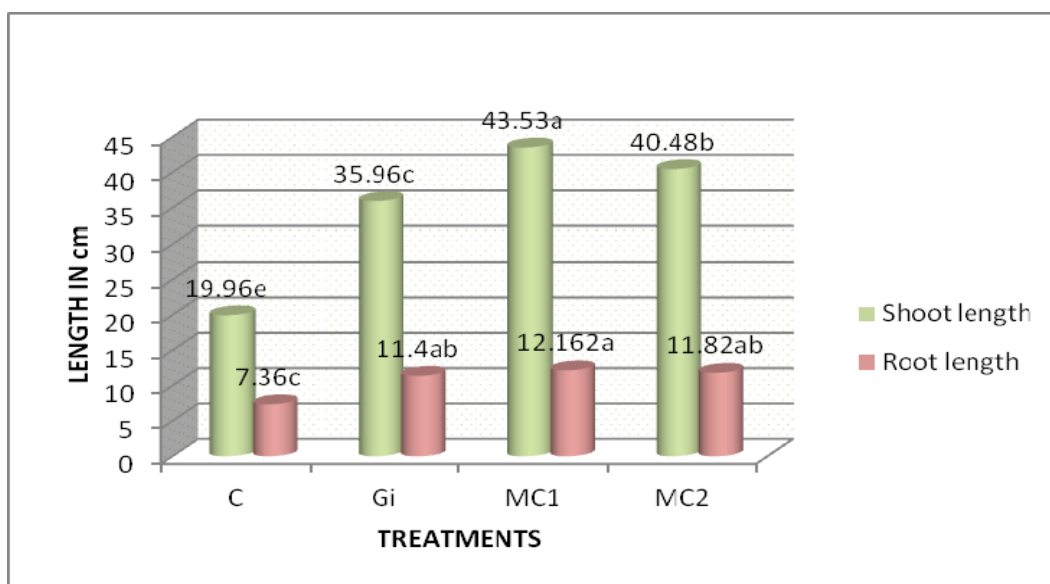


Fig. 6. Effect of AMF species on shoot length and root length of *Mentha arvensis*. Plants were grown non inoculated (C) or inoculated with Gi (*Glomus intraradicus*) Indigenous. AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$).

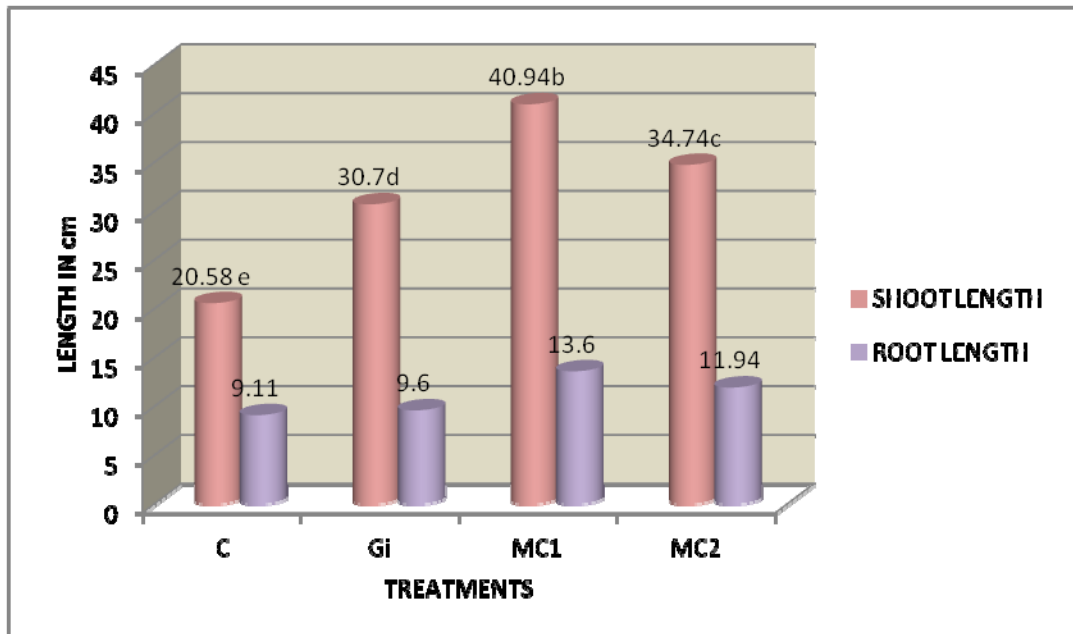


Fig. 7. Effect of AMF species on shoot length and root length of *Mentha longifolia*. Plants were grown non inoculated (C) or inoculated with Gi (*Glomus intraradicus*) Indigenous. AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$).

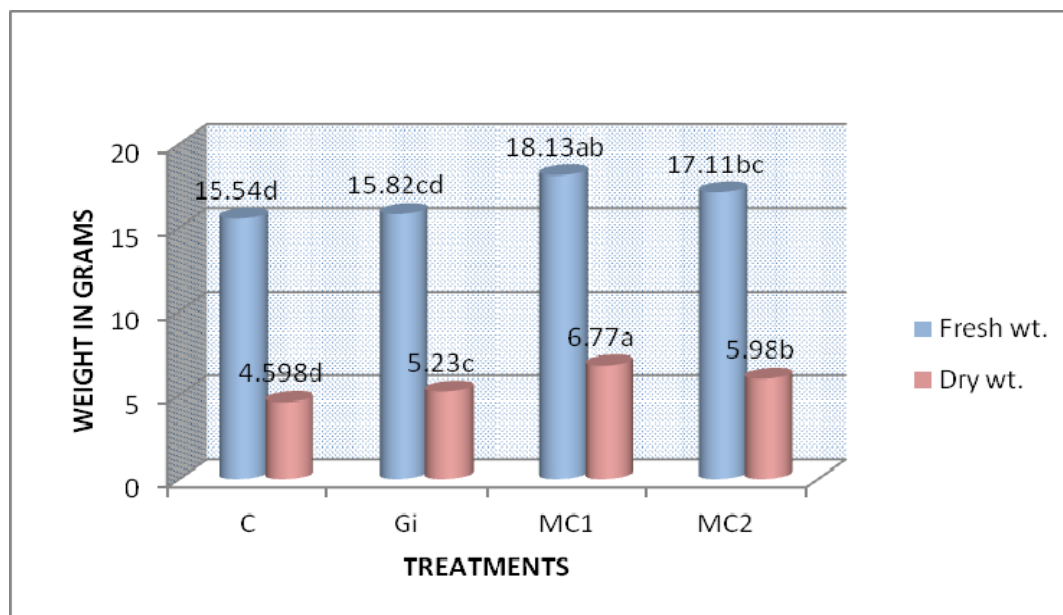


Fig. 8. Effect of AMF species on fresh and dry weight of *Mentha arvensis*.

Plants were grown non inoculated (C) or inoculated with Gi (*Glomus intraradicus*) Indigenous AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$).

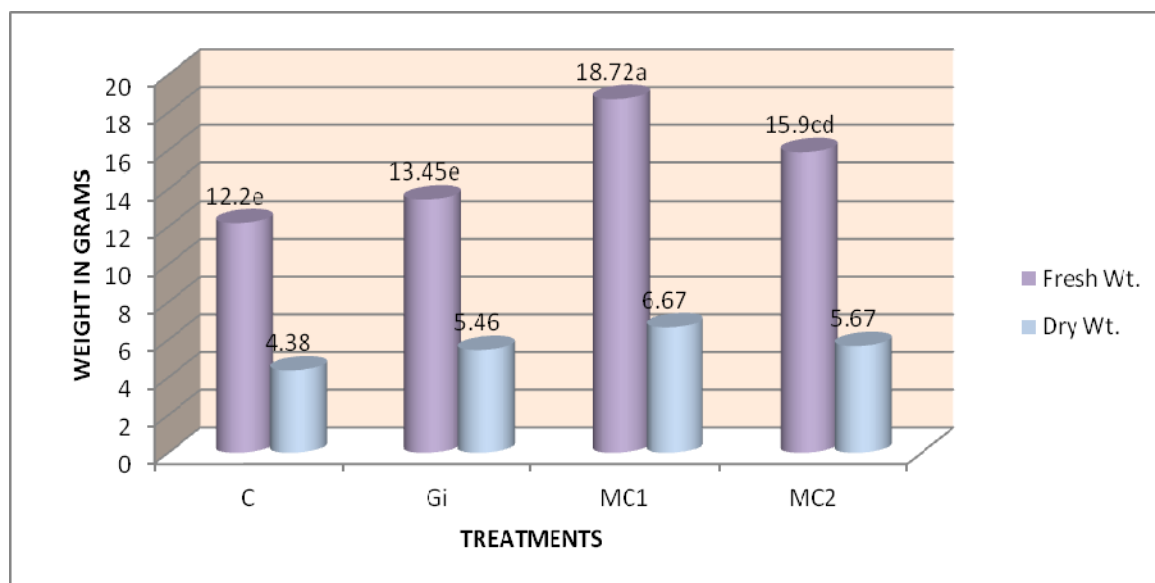


Fig. 9. Effect of AMF species on fresh and dry weight of *Mentha longifolia*.

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indigenous AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$).

ii. Mineral Nutrient acquisition

Arbuscular mycorrhizal (AM) fungi are symbiotic fungi that enhance host plant uptake of relatively immobile nutrients, particular phosphorus and several micronutrients (Javaid, 2009). Mycorrhizal root system increases the absorptive area of 10-1000 times, therefore, improves the ability to utilize the soil resources. AM fungi can capture and transfer all of the 15 major and minor nutrients necessary for plant growth (Lester, 2009). In the present investigations analysis of tissues of mycorrhizal and non mycorrhizal *Mentha* species was carried out by standard methods. The results of Means, LSD test and ANOVA for (*Mentha arvensis* and *Mentha longifolia*) are given below (Tables 9, 10; Figs. 10, 11).

Macro mineral acquisition

There was significant increase of P uptake in *Mentha arvensis* (1.14%) in plants treated with MC1 while plants inoculated with MC2 and GI showed same level (0.8%). Mycorrhizal plants of *Mentha longifolia* showed similar P uptake in MC1 and MC2 (0.8%) while it was low in Gi (0.7%) treatment. Similar findings have been reported by Karagiannidis *et al.* (2011) as they found that non mycorrhizal oregano and mints plants had significantly low uptake of macro and micronutrients as compared to mycorrhizal plants. Various AM fungi such as *G. lamellosum* and *G. etunicatum* isolates had varied effects in the levels of nutrient elements in Oregano and mint plants.

Arbuscular mycorrhizal association has been reported to improve the uptake of different mineral nutrients with phosphate in the focus due to its role as limiting factor because of its immobility in the soil. The AM fungi form fine hyphal network which is superior to the relatively thick roots and root hairs in accessing phosphate in the soil (Redecker, 2005). Further on, as explained by Sylvia *et al.* (1993) the fungal hyphae have smaller diameter than roots and access smaller soil pores thus effectively increasing the surface area for nutrient acquisition especially P (Henrike *et al.*, 2007) by making P available to root system that might improve of plant growth (Rejali *et al.*, 2008). Nitrogen contents (Table 9, 10) significantly in all the mycorrhizal treated plants of both species of *Mentha*. In *Mentha longifolia* maximum value was recorded in MC1 (2.70%), followed

by MC2 (2.2%) and least in GI treated plants (0.9%) as compared to their respective controls (0.2%). Similar trend was also noticed in *Mentha arvensis*. Mader *et al.* (2000) also stated that mycorrhizal colonization improve plant N nutrition and the results agree with that and also with Gogoi & Singh (2011), who similarly reported that AMF increased growth and N contents. Likewise, Karagiannidis *et al.* (2011) found that nitrogen contents ranged from 2.49% to 2.84% in mycorrhizal plants was significantly higher than control (0.71- 0.88%) in mints. The present findings are also supported by other workers (Caglar & Akgun, 2006; Tawaraya *et al.* 2007; Meding & Zasoki, 2008).

The uptake of potassium was significant as revealed by ANOVA (Tables 9, 10). The differences were significant ($P < 0.000$) between treatments. The general trend was $MC1 > MC2 > Gi > Control$ as was also observed for other nutrients (N & P). However, the differences in response between the two species was non significant as the recorded values were statistically at par with each other, i.e., maximum being in MC1 (2.540 and 2.660) followed by MC2 (1.900 and 1.860) and GI (1.400 and 1.460) in *Mentha longifolia* and *Mentha arvensis*, respectively. Generally mycorrhizal treatments enhanced the uptake of K. Similar observations were also documented by various researchers in different plants *Cassia saimea* (Giri *et al.*, 2005) and cowpea (Omomowo *et al.*, 2009; Yaseen *et al.*, 2011). Mycorrhizal inoculation also enhanced K uptake in various varieties of tobacco (Janouskova *et al.*, 2007).

Micro mineral acquisition

For micro nutrients including Fe, Zn and Cu (Tables 9, 11; Figs. 12, 13) revealed that mycorrhizal plants had significantly higher concentrations than non-inoculated. Similarly, other findings (Cardoso & Kuyper, 2006; Meding & Zasoski, 2008) reported increased uptake of Cu and Zn by mycorrhizal plants. Arbuscular fungi improved the uptake of several micro nutrients like zinc, copper, iron and manganese (Yaseen *et al.* 2011). Moreover, mycorrhizal fungi improve the uptake of some water insoluble nutrients from soil that are normally inaccessible to plants (Kumar *et al.*, 2007) by their enzymatic activities (phosphatases, proteases, chelating compound) or by the alteration of physical and chemical properties in the soil (Abdelhafez & Abdel-Monsief, 2006).

However, Karagiannidis *et al.* (2012) observed that *Mentha* species had similar concentrations of N, B, Zn, Cu and Mn in inoculated and control plants. However improved uptake was found in other nutrients. This uptake selectivity reflects a well regulated nutrient homeostasis in mycorrhizal plants (Ning & Cumming, 2001).

The effect of various AMF species on the uptake of micro minerals among treatments followed the general trend with slight deviations. Maximum values were recorded for MC1 and minimum in GI in both test species. The results showed that the native mycorrhizal fungi in mix culture out performed as compared to single foreign strain. Posta *et al.*, (1994) stated that native mix AMF inoculation strongly modified functional abilities of the soil microflora especially its capacity to metabolize carboxylic acids. It is well demonstrated that root mycorrhizal colonization changes the number of aerobic bacteria in the rhizosphere. It is stated that soil around the external mycelium constitutes another specific microbial compartment called Hyphospheres, which is influenced by native fungal exudations supporting the bacterial activities (Andrade *et al.*, 1998; Mansfeld-Giese *et al.*, 2002; Marshner & Timonen, 2005). Such micro-organisms affect mycorrhizal functions such as nutrient and water uptake carried out by external hyphae of AM fungi (Ouahmane *et al.*, 2007).

Evidence suggest that diversity not the species composition of a mycorrhizal community affects function where nutrient influx into host increases with increased fungal species richness, irrespective of the actual species composition (Sharma *et al.*, 2009).

Table 9. Effect of AM species on macro and micronutrients acquisition of *Mentha longifolia* and *Mentha arvensis*. Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indigenous AMF (MC1 and MC2). Treatments were tested with two way ANOVA.

Species	Treatments	P %	N %	K %	Mg %	Fe ppm	Cu ppm	Zn ppm
<i>Mentha longifolia</i>	C	0.1980 ^d	0.2740 ^h	0.580 ^d	0.1600 ^c	55.10 ^f	6.240 ^f	43.60 ^e
	Gi	0.8200 ^b	0.9080 ^g	1.400 ^c	0.2400 ^{fg}	60.48 ^e	6.800 ^{ef}	48.74 ^c
	MC1	1.140 ^a	2.700 ^b	2.540 ^a	0.8600 ^a	75.66 ^a	8.700 ^d	55.76 ^a
	MC2	0.8400 ^b	2.202 ^c	1.900 ^b	0.6800 ^{bc}	66.62 ^d	7.780 ^{de}	50.12 ^b
<i>Mentha arvensis</i>	C	0.2600 ^d	1.156 ^f	0.558 ^d	0.3600 ^{ef}	51.48 ^g	7.480 ^e	29.62 ^f
	Gi	0.7000 ^c	1.472 ^e	1.460 ^c	0.5000 ^{de}	65.80 ^d	11.40 ^c	47.58 ^{cd}
	MC1	0.8800 ^b	2.940 ^a	2.660 ^a	0.7800 ^{ab}	73.60 ^b	17.26 ^a	55.40 ^a
	MC2	0.8000 ^b	2.022 ^d	1.860 ^b	0.6000 ^{cd}	68.54 ^c	13.44 ^b	47.54 ^d
LSD at 5%		0.09979	0.1152	1.107	0.1469	0.8526	1.054	1.190
P VALUE	S	0.0010	0.0000	NS	0.0476	0.0721	0.0000	0.0000
	T	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	(T × S)	0.0006	0.0000	NS	0.0020	0.0000	0.0000	0.0000

Each value is a mean of five replicates. Values followed by different letters are significantly different ($p < 0.000$) P (Probability), NS (Non significant), LSD (Least Significant Test), T (Treatment), S (species).

Total 39 22.184

Coefficient of Variation: 7.30%

K		Degrees of	Sum of	Mean	F	
Value	Source	Freedom	Squares	Square	Value	Prob
ANOVA-Mg						
2	Var.(V)	1	0.056	0.056	4.2453	0.0476
4	Treat.(T)	3	1.945	0.648	48.9245	0.0000
6	AXB	3	0.245	0.082	6.1572	0.0020
-7	Error	32	0.424	0.013		

	Total	39	2.670			

Coefficient of Variation: 22.03%

Table 11. ANOVA OF MICRONUTRIENTS

K		Degrees of	Sum of	Mean	F	
Value	Source	Freedom	Squares	Square	Value	Prob
ANOVA - Fe						
2	Var.(V)	1	1.517	1.517	3.4601	0.0721
4	Treat.(T)	3	2395.115	798.372	1820.8842	0.0000
6	AXB	3	121.772	40.591	92.5768	0.0000
-7	Error	32	14.030	0.438		
	Total	39	2532.435			

Coefficient of Variation: 1.02%

K		Degrees of	Sum of	Mean	F	
Value	Source	Freedom	Squares	Square	Value	Prob
ANOVA - Cu						
2	Var.(V)	1	251.502	251.502	3739.8122	0.0000
4	Treat.(T)	3	198.715	66.238	984.9559	0.0000
6	AXB	3	68.515	22.838	339.6024	0.0000
-7	Error	32	2.152	0.067		
	Total	39	520.884			

Coefficient of Variation: 2.62%

K		Degrees of	Sum of	Mean	F	
Value	Source	Freedom	Squares	Square	Value	Prob
ANOVA - Zn						
2	Var.(V)	1	204.304	204.304	239.6527	0.0000
4	Treat.(T)	3	1859.149	619.716	726.9399	0.0000
6	AXB	3	304.626	101.542	119.1108	0.0000
-7	Error	32	27.280	0.853		
	Total	39	2395.359			

Coefficient of Variation: 1.95%

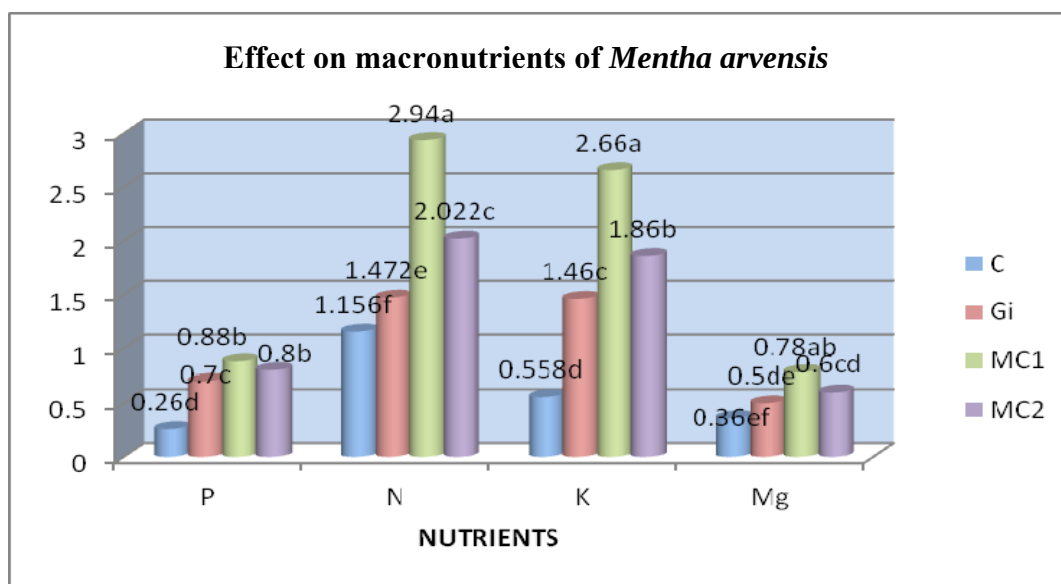


Fig. 10. Effect of AMF species on macronutrients of *Mentha arvensis*.

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indeginous. AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$)

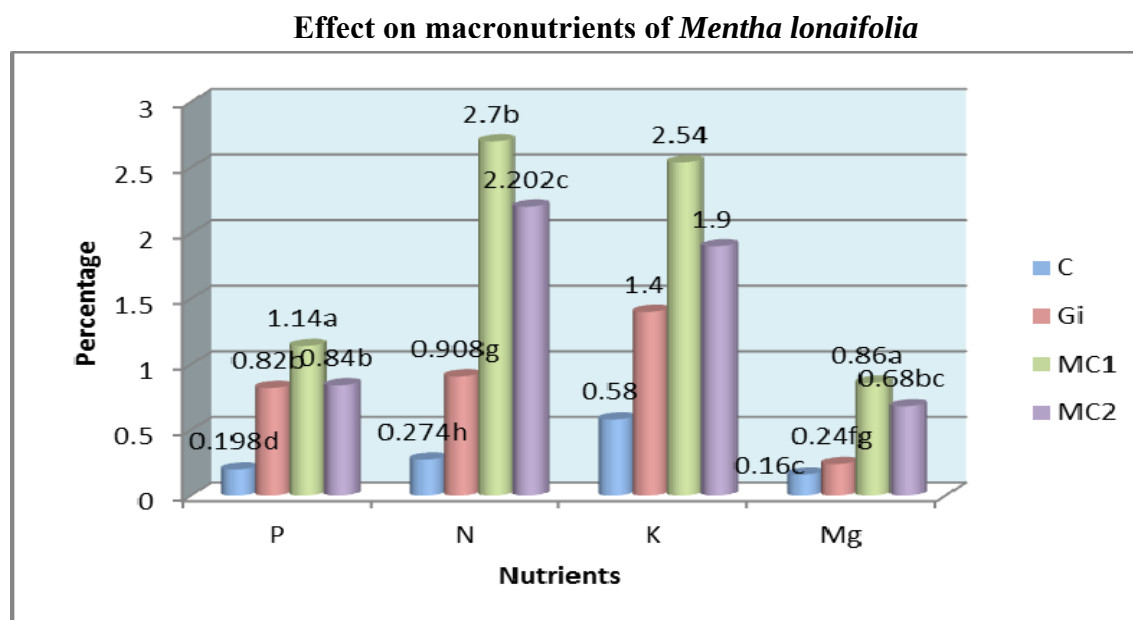


Fig. 11. Effect of AMF species on macronutrients of *Mentha longifolia*.

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indeginous. AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$).

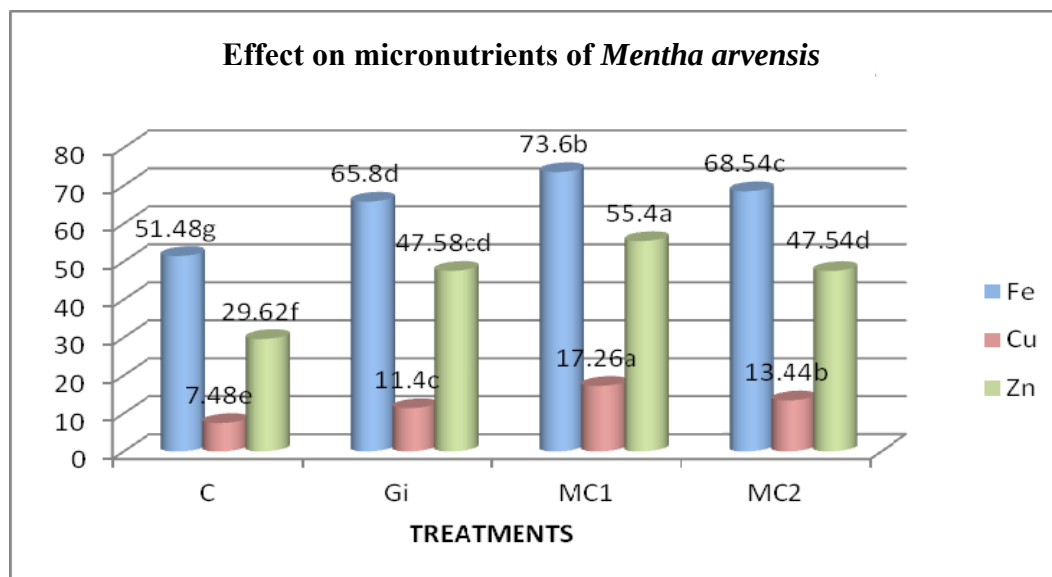


Fig. 12. Effect of AMF species on micronutrients of *Mentha arvensis*.

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indeginous. AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$)

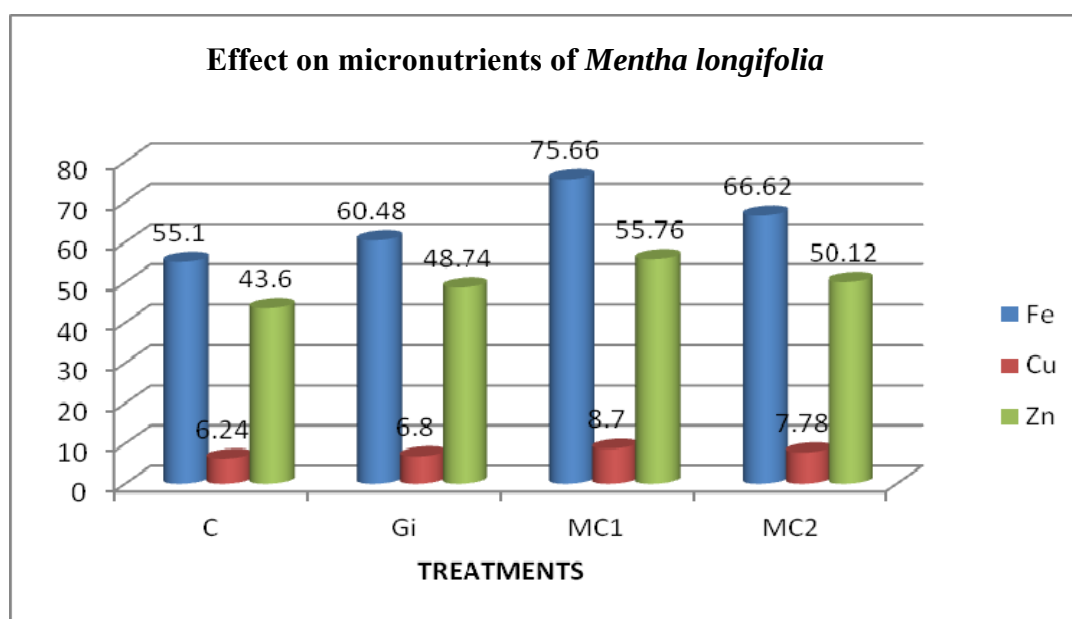


Fig. 13. Effect of AMF species on micronutrients of *Mentha longifolia*.

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indeginous. AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$).

iii. PROXIMATE COMPOSITION

The estimation of protein contents, carbohydrate, lipids, moisture and ash percentage are necessary to ensure the requirements of food regulators (Omomowo *et al.*, 2009). The present research work therefore also investigated the effect of AMF inoculation on proximate composition of test species.

1. Total Ash

The Ash values varied from 9.534% to 11.846% in treated plants of ML and MA. Maximum values were recorded in MC1 (11.846 & 11.240%) in both the species which were statistically at par with each other. (Fig. 14) Least values were noticed in GI treated plants having statistically similar values in the test species. Generally, mycorrhizal plants had higher ash contents compared to their corresponding control plants. Similar results were also held positively by Mehrvarz & Chaichi, (2008), who observed that mycorrhizal inoculated plants of Barely (*Hordeum vulgare* L.) exhibited higher level of total ash (8.05%) than non-mycorrhizal (7.84%). Elsheikh & Muhamedzein, (1998) also reported that AM inoculation increased ash content of groundnut seeds.

2. Crude Fiber

Chemically fibers consist of non starch polysaccharides. The crude fibre is made up of all organic compounds of the plant cell membranes and supporting structures remained after the removal of crude proteins, fats and nitrogen free extract. Data regarding the mean values, LSD and ANOVA regarding fibre contents of test species in various mycorrhizal treatments are given in (Tables 12, 13; Fig. 15). It showed that non-mycorrhizal plants had significantly higher crude fibers as compared to mycorrhizal plant. There were significant differences between species and treatments ($p < 0.000$). The fibre contents varied from 17.74% in non-inoculated to maximum in MC1 (25.36 %) followed by MC2 (22.48%) and thereafter in GI (18.08%) in *Mentha arvensis*. However, some deviation from general trend was also noted in ML as highest contents

were recorded in MC1 followed by GI (23.17) than control (19.15%). Our results are in agreement with those of Manoharan *et al.* (2008). On the contrary, Adewole & Ilesanmi (2011), stated that the control treatment (non-mycorrhizal) gave maximum values (27.33%) of crude fiber as compared to the mycorrhizal treatments.

3. Crude Fat

Looking at the overall percentage of fat contents (Tables 12, 13) showed significant differences ($P < 0.000$) among various treatments of test species. Fat contents ranged from 1.576% in non-inoculated ML to 6.454% in MC1 treated plants of MA. However, fat values recorded for ML showed reverse trend, i.e., maximum 2.492 % in GI followed by MC1 (2.070%) than MC2 (1.634%) (Fig. 16). Similar results were also reported by Cooper & Losel, (1978) as they stated that infected roots contained more total lipids than uninfected roots. Likewise, Omomowo *et al.* (2009) also observed that inoculation with *Glomus mosseae* caused higher fat contents of cowpea than uninoculated plants.

4. Crude Protein

Physiological functions in living organism depends on proteins as they are catalyst and enzymes. They carry all the chemical changes involved in plant growth. The result (Table 12; Fig. 17) showed that crude proteins enhanced in mycorrhizal plants compared to non-mycorrhizal plants. ML inoculated with MC1 had higher concentration of protein (17.04%), followed by MC2 (14.36%) and GI (13.02%) while, MA also followed the same trend maximum protein contents being (16.37%) in MC1 followed by MC2 (13.48%) and least was recorded in GI (12.19%). Ratti *et al.* (2010) stated that *Glomus mosseae* inoculated plants of *Catharanthus roseus* exhibited higher level of protein (8.27mg/g) than non-mycorrhizal (6.04). *Glomus mosseae* induced more leaf protein than *G. aggregatum* and *G. fasciculatum*. Similar results were also observed by Manoharan *et al.* (2008) the leaf protein contents of all mycorrhizal inoculated seedlings showed a greater increase than the non-mycorrhizal seedlings. Subramanian & Charest

(1998) and Wu *et al.* (2006) also obtained in the sense that the crude protein contents were higher in AM than non-mycorrhizal plants.

Earlier studies also showed similar trend for medicinal plants when subjected to AM inoculation that depends on the mycorrhizal fungal species (Rajeskumar *et al.*, 2008; Ndiaye *et al.*, 2009).

5. Carbohydrate

Carbohydrates are initially synthesized in plants from a complex series of reactions linking photosynthesis and provide energy through metabolism pathways and cycles. The carbohydrate content and LSD, ANOVA (Table 12, 14; Fig. 18) indicated that mycorrhizal plants significantly better performed ($P < 0.000$) in MC1, MC2 and G1 as compared to control in both the test species. Maximum concentration (37.42 - 42.07%) were recorded in mycorrhiza *Mentha longifolia* as compared to *Mentha arvensis* (30.00 – 38.00%). The findings agree with those of Wu *et al.*, (2010), who concluded that the AMF inoculation markedly increased leaf sucrose content and leaf and root glucose content, compared to the non-AMF treatments. Similarly, the present results are in accordance with those of Khalafallah & Abou-Ghalia (2008) who reported that mycorrhizal plants had higher carbohydrates contents than non-mycorrhizal wheat plant.

However, the results are contradictory to those of Manoharan *et al.* (2008) who reported decreased soluble sugars contents in mycorrhizal seedlings in the leaves of *Cassia siamea*, *Samanea saman*, *Erythrina variegata*, *Delonix regia* and *Sterculia foetida* than non-mycorrhizal seedlings. They explained that the decrease in soluble sugars might be due to the fact that carbohydrate are transferred from host to the fungal partner.

These findings revealed that AMF multiple culture (MC1 and MC2) performed better in mono species (G1) cultures in terms of nutrient acquisition proximate profile. These findings also coincide with Ratti *et al.* (2010) who stated all *Glomus* species, except *G. intraradicus*, enhanced all morphological and physiological parameters of test species. AMF are multifunctional and differ with respect to function, AMF with much species diversity give more advantage to host plant. Because more species means more functions fulfilled and more opportunities for beneficial relationship (Jakobsen *et al.*, 2005). Above mentioned facts may justify the results of present investigation. Similarly

Zubek *et al.* (2011) reported the enhanced growth performance in *Hypericum perforatum* inoculated with AMF mix. This could be due to improved mineral nutrition or to AMF-induced changes in the phytohormonal balance. During present investigations we found the superior symbiotic efficiency of mixed culture of native AMF (species of *Glomus Acaulospora* and *Gigaspora*) as compared to exotic strain of *Glomus intraradicus*. Conversely Dupponnois *et al.*, (2005) found *G. intraradicus* the most effective in promoting the host growth.

Table 12. Effect of AM species on Proximate composition (%) and oil yield (%) of *Mentha longifolia* and *Mentha arvensis*.

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*). Indigenous AMF (MC1 and MC2). Treatments were tested with two way ANOVA.

Percent on dry matter basis						
Varieties	Treatments	Ash (%)	Fiber (%)	Fats (%)	Proteins (%)	Carbohydrates (%)
<i>Mentha longifolia</i>	C	11.096a	23.17 ^{bc}	1.576 ^f	12.26 ^f	35.06 ^e
	Gi	9.534b	23.45 ^b	2.492 ^d	15.08 ^c	37.42 ^d
	MC1	11.846a	25.36 ^a	2.070 ^e	17.04 ^a	42.07 ^a
	MC2	11.510a	19.15 ^d	1.634 ^f	14.36 ^d	40.17 ^b
<i>Mentha arvensis</i>	C	10.380b	17.74 ^e	3.338 ^c	13.22 ^e	29.13 ^g
	Gi	9.656c	18.08 ^e	2.396 ^{de}	14.20 ^d	30.00 ^f
	MC1	11.240a	25.35 ^a	6.454 ^a	16.38 ^b	38.00 ^c
	MC2	11.350a	22.48 ^c	4.394 ^b	13.48 ^e	37.06 ^d
LSD at 5%		0.659	0.8209	0.4012	0.4094	0.4889
P VALUE	S	0.1471	0.0000	0.0000	0.0010	0.0000
	T	0.0000	0.0000	0.0000	0.0000	0.0000
	(T × S)	NS	0.0000	0.0000	0.0000	0.0000

Each value is a mean of five replicates. Values followed by different letters are significantly different ($p < 0.05$) P (Probability), NS (Non significant), LSD (Least Significant Test), T (Treatment), S (species)

Table 14. ANOVA FOR PROXIMATE ANALYSIS

K		Degrees of	Sum of	Mean	F	
Value	Source	Freedom	Squares	Square	Value	Prob
ANOVA - PROTEINS						
2	Var. (V)	1	1.329	1.329	13.1798	0.0010
4	Treat. (T)	3	83.210	27.737	275.1491	0.0000
6	AXB	3	5.942	1.981	19.6472	0.0000
-7	Error	32	3.226	0.101		
Total						
		39	93.706			

Coefficient of Variation: 2.19%

K		Degrees of	Sum of	Mean	F	
Value	Source	Freedom	Squares	Square	Value	Prob
ANOVA - CARBOHYDRATES						
2	Var. (V)	1	263.169	263.169	1826.9921	0.0000
4	Treat. (T)	3	435.397	145.132	1007.5499	0.0000
6	AXB	3	27.611	9.204	63.8941	0.0000
-7	Error	32	4.609	0.144		
Total						
		39	730.787			

Coefficient of Variation: 1.05%

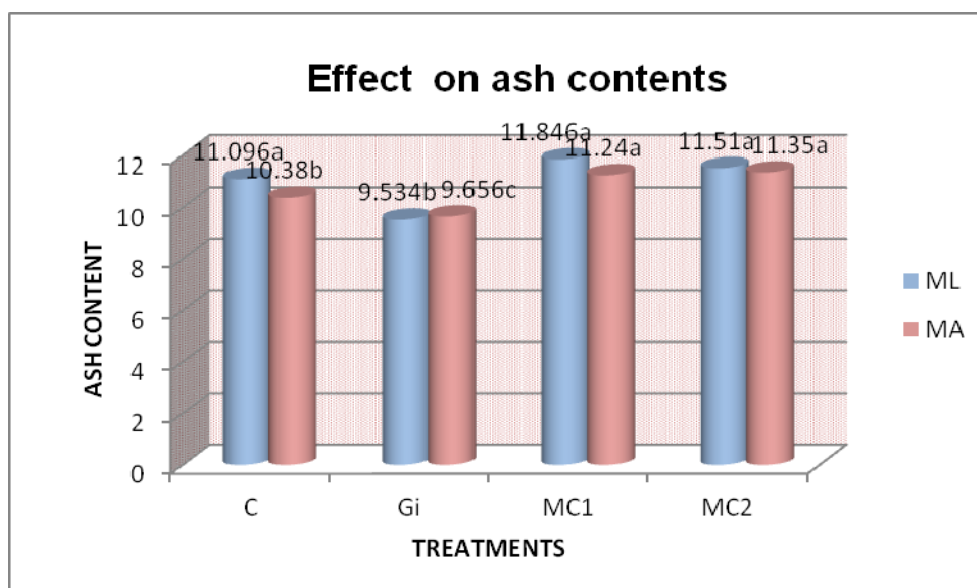


Fig. 14. Effect of AMF species on ash contents of *Mentha arvensis* (MA) and *Mentha longifolia*. (ML).

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indeginous. AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$)

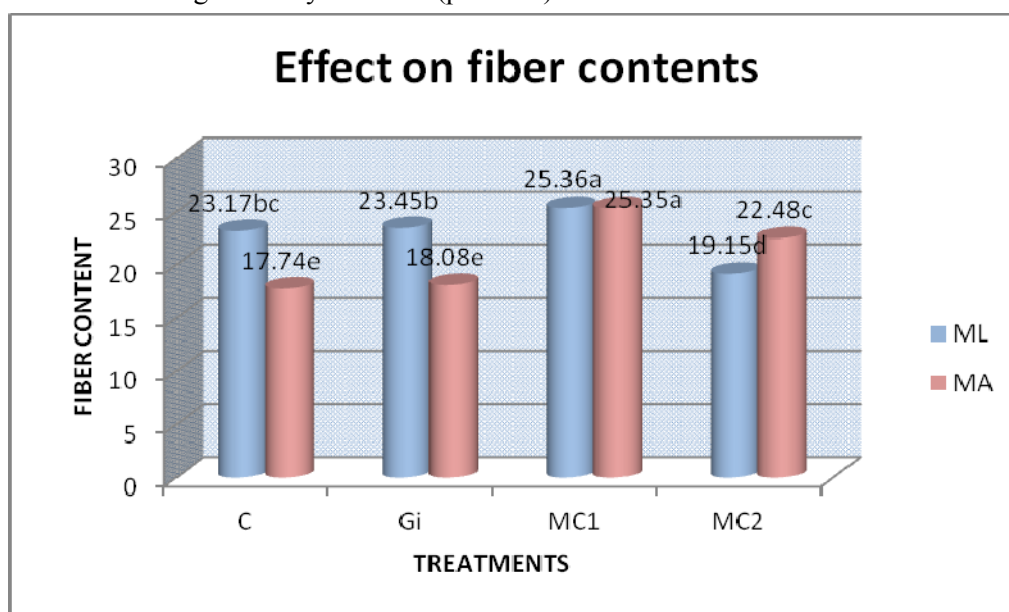


Fig. 15. Effect of AMF species on fiber contents of *Mentha arvensis* (MA) and *Mentha longifolia*. (ML).

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indeginous. AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$)

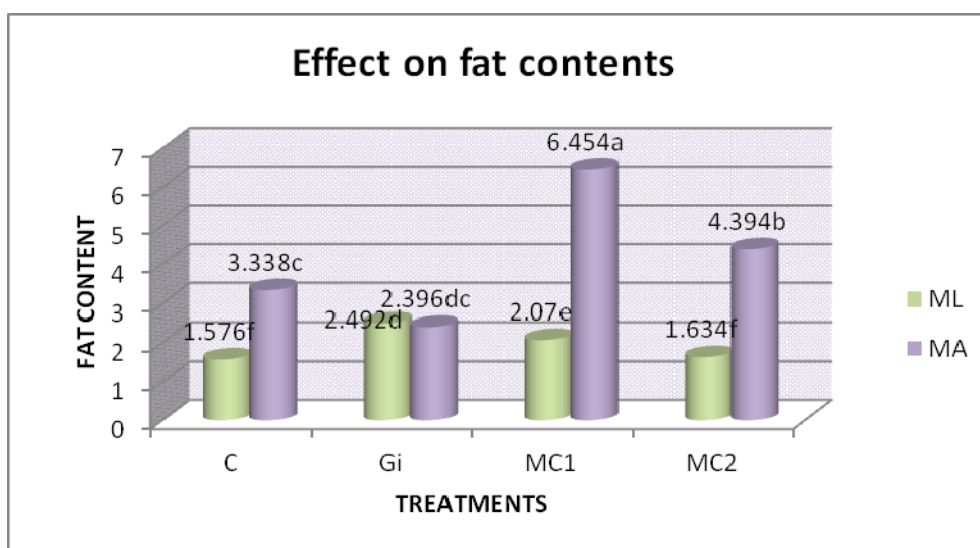


Fig. 16. Effect of AMF species on fat contents of *Mentha arvensis* (MA) and *Mentha longifolia*. (ML).

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indigenous. AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$)

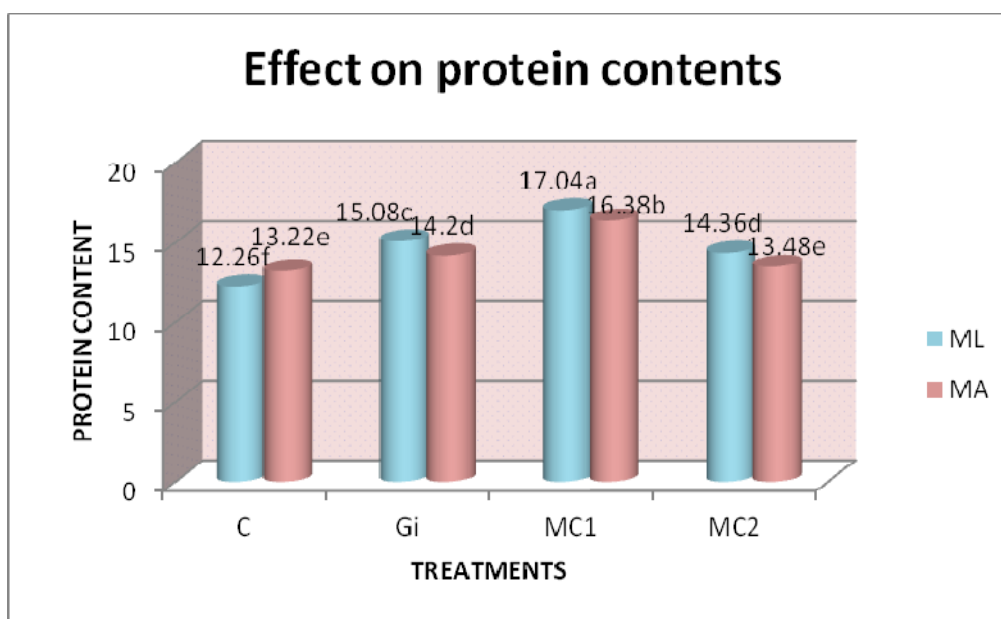


Fig. 17. Effect of AMF species on protein contents of *Mentha arvensis* (MA) and *Mentha longifolia*. (ML).

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indigenous. AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$)

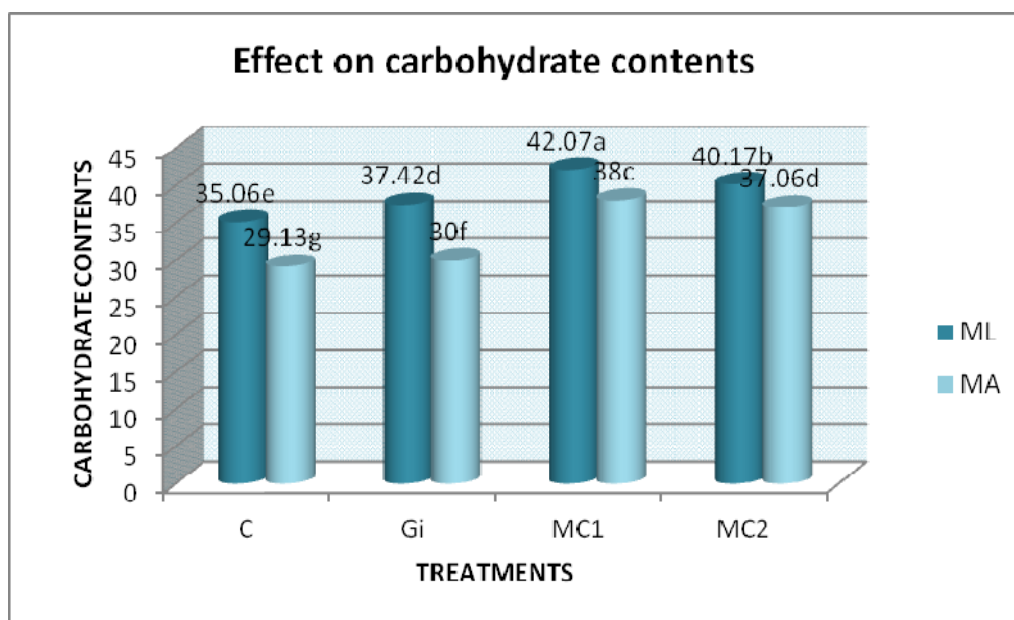


Fig. 18. Effect of AMF species on carbohydrate contents of *Mentha arvensis* (MA) and *Mentha longifolia*. (ML).

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indeginous. AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$)

iv. ROOT COLONIZATION AND AMF SPORE DENSITY

The mean and ANOVA data on percent colonization and spore count are given in (Tables 15, 16). Mycorrhizal plants of *Mentha arvensis* and *Mentha longifolia* treated with MC1 showed maximum colonization 80.00% and 84.96% respectively (Figs. 19, 20). While significantly lowest colonization levels 38.76% and 34.4% were observed in both plants inoculated with Gi. Similar trend of root colonization was noticed in *Mentha longifolia* maximum being in MC1 (84.96%) and minimum in Gi (34.7%). Both investigated *Mentha* species, responded positively to VAM inoculation. However, the symbionts showed certain degree of host selectivity. This is evident from the colonization frequency of the plants by the VAM fungi employed in present studies. *Mentha arvensis* was extensively colonized by MC1, consequently the association enhanced biomass production as compared to the other two inocula of AM fungi. Therefore, the degree of selective effectiveness is evident among the three VAM fungi inocula used for inoculating *Mentha* species. The results are supported by Khaliq & Janardhanan, (1997), who observed significant variations in effectiveness of *Glomus* species. AM species on mints. Moreover, they suggested that variation in the degree of the symbionts effectiveness should be considered before large scale inoculation programme.

Karagiannidis *et al.* (2010) found root colonization in various *Mentha* species that ranged from 8 to 100%. However, Karagiannidis *et al.* (2011) found non significant differences between treatments in root colonization. The effect of AM fungi on the plants depends strongly on the extent of colonization (Copetta *et al.*, 2006). Moreover, studies on three genotypes of oregano showed that essential oils in mycorrhizal plants do not rely on available P in the soil, but on the association with mycorrhizae Khaosaad *et al.* (2006). The present study showed variations in root colonization in investigated species of *Mentha*. Karagiannidis *et al.* (2011) stated that there might be differences in the responsiveness to mycorrhization among varieties of the same plant.

It was also observed that the spores density in rhizospheric soil of both species followed the same pattern (Figs. 21, 22). Significantly high spore count (380/100gm soil) was noticed in MC1 followed by MC2 and least 130/100gm encountered in Gi in *Mentha arvensis*. Moreover, there was positive correlation of AMF spore population with root colonization. On the other hand Khaliq & Janardhanan (1997) did not find any correlation between spore density and root colonization as they found maximum spore count in *G. aggregatum* inoculated pots (600/100 gm soil) while the root colonization was only 61%.

Table 15. Root infection (%) and spore density/100gms soil of *Mentha longifolia* and *Mentha arvensis*.

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indigenous AMF (MC1 and MC2). Treatments were tested with two way ANOVA.

Species	Treatments	Infection (%)	Spore density /100g. soil
<i>Mentha longifolia</i>	GI	34.70 ^f	150.8 ^e
	MC1	84.96 ^a	357.0 ^b
	MC2	58.04 ^d	180.2 ^c
<i>Mentha arvensis</i>	Gi	38.76 ^e	130.0 ^f
	MC1	80.00 ^b	380.0 ^a
	MC2	65.00 ^c	170.0 ^d
LSD at 5%		1.278	8.624
P VALUE	T	0.0000	0.2800
	S	0.0000	0.0000
	(T × S)	0.0000	0.0000

Each value is a mean of five replicates. Values followed by different letters are significantly different ($p < 0.05$). P (Probability) NS (Non significant). LSD Least Significant Difference Test. T. Treatment S. species.

Table 16. ANALYSIS OF VARIANCE FOR INFECTION

K		Degrees of	Sum of	Mean	F	
Value	Source	Freedom	Squares	Square	Value	Prob
ANOVA-INFECTION						
2	Var.(V)	1	30.603	30.603	31.9114	0.0000
4	Treat.(T)	2	10489.761	5244.880	5469.1128	0.0000
6	AXB	2	193.214	96.607	100.7372	0.0000
-7	Error	24	23.016	0.959		
	Total	29	10736.593			

Coefficient of Variation: 1.63%

K		Degrees of	Sum of	Mean	F	
Value	Source	Freedom	Squares	Square	Value	Prob
ANOVA-SPORES						
2	Var.(V)	1	53.333	53.333	1.2218	0.2800
4	Treat.(T)	2	302124.200	51062.100	3460.7583	0.0000
6	AXB	2	2610.867	1305.433	29.9068	0.0000
-7	Error	24	1047.600	43.650		
	Total	29	305836.000			

Coefficient of Variation: 2.90%

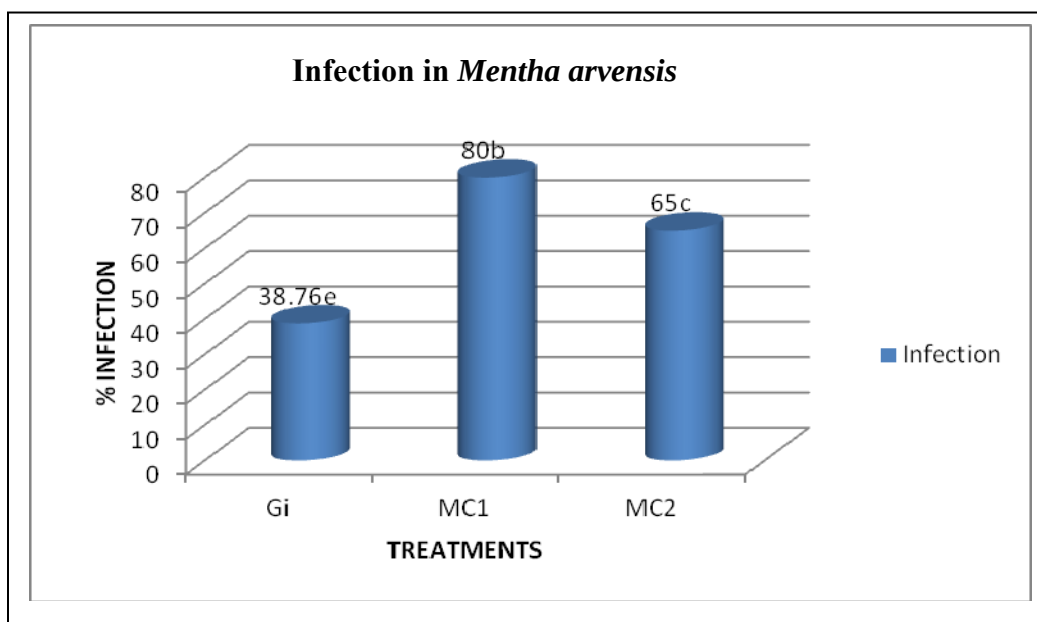


Fig. 19. Root infection (%) in *Mentha arvensis*.

Plants were grown or inoculated with GI (*Glomus intraradicus*) Indigenous AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$).

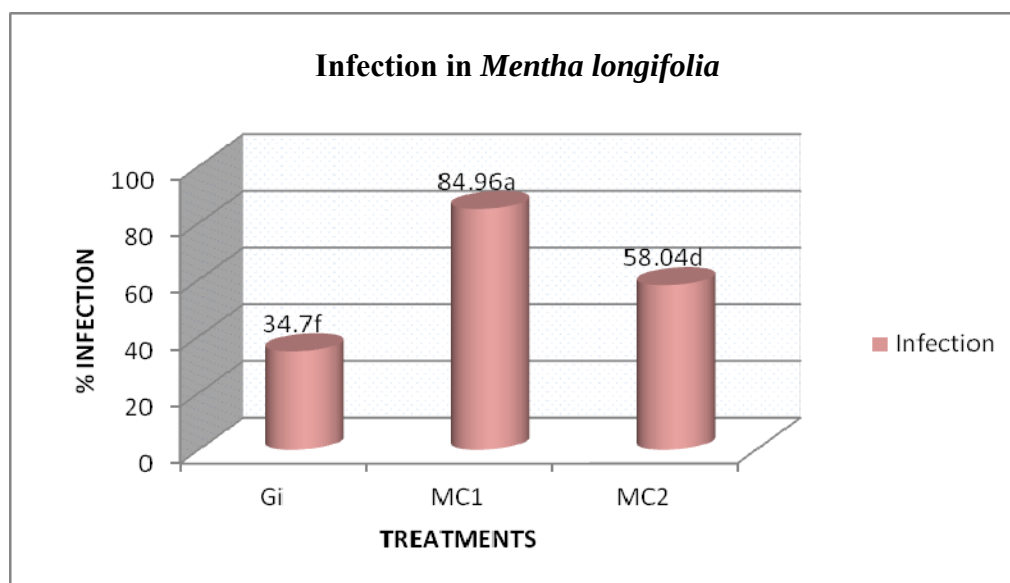


Fig. 20. Root infection (%) in *Mentha longifolia*.

Plants were grown or inoculated with GI (*Glomus intraradicus*) Indigenous AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$).

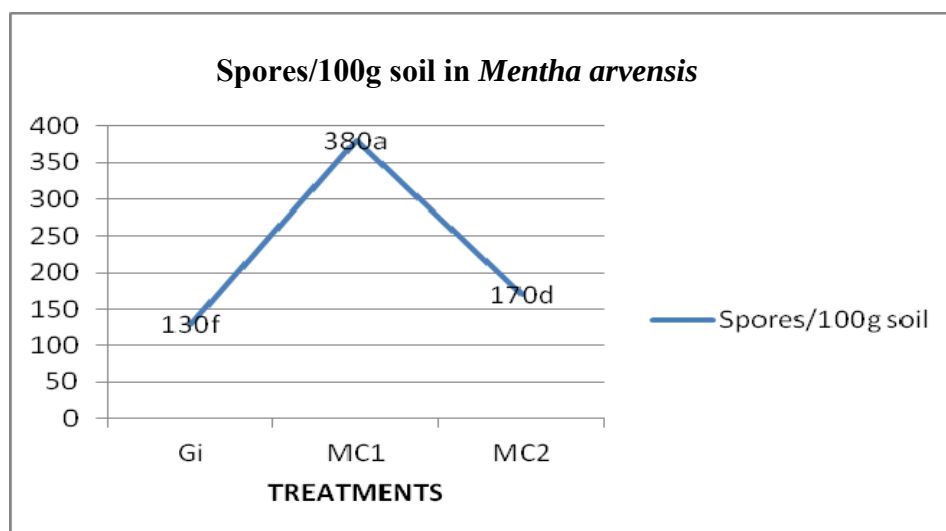


Fig. 21. Spore density /100gm soil in *Mentha arvensis*.

Plants were inoculated with GI (*Glomus intraradicus*) Indigenous AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$).

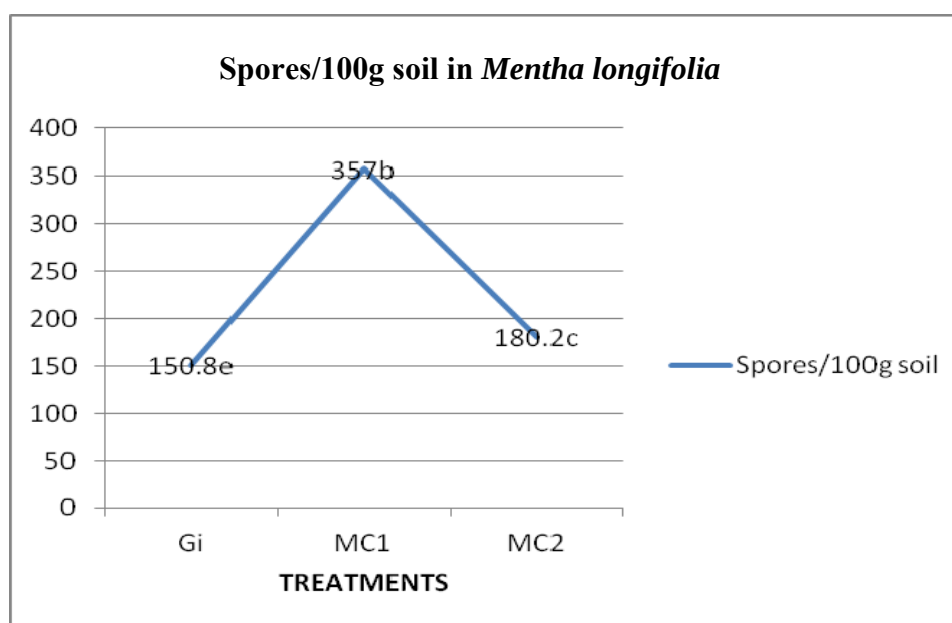


Fig. 22. Spore density /100gm soil in *Mentha longifolia*.

Plants were inoculated with Gi (*Glomus intraradicus*) Indigenous AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$).

v. ESSENTIAL OILS YIELD

Essential oils are volatile, lipophilic mixtures of secondary compounds, mostly containing monoterpenes, sesquiterpenes and phenylpropanoids. (Khaosaad *et al.*, 2007). The production of essential oils of medicinal plants may be influenced by plant physiology, cultivar, soil environment and nutrition. Even minor quantitative and qualitative enrichment of their essential oils may have significant impact on their value (Karagiannidis *et al.*, 2010). There is a growing trend research that indicates that AM influences essential oil production quantitatively and qualitatively (Copetta *et al.*, 2006; Frietas *et al.*, 2004; Khaosaad *et al.*, 2006) and this may also rely on the symbiotic fungus and plant accession. (Chaudhary *et al.*, 2008; Kapoor *et al.*, 2004). The collected data in (Table 17; Fig. 23) indicated a better performance of mycorrhizal fungi regarding oil yield as compared to non inoculated control. *Mentha arvensis* inoculated with mixed culture of 11 indigenous species (MC1) was found to contain significantly ($p < 0.000$) higher amount (2.0%) of essential oil, as compared to un inoculated control plants (1.00%). In *Mentha longifolia* oil yield was same (1.99%) in the same treatment. Studies on *Mentha* species shows that AM fungi improved the quality and quantity of essential oils that depended upon plant variety (Gupta *et al.*, 2002; Frietas *et al.*, 2004). Moreover, observations of Karagiannidis *et al.*, (2010) also favour our findings, who reported enhanced from 1.5 to 3.6% essential oil yield in different species of mycorrhizal *Mentha species* from different locations of Northern Greece.

The present results are also parallel with those of Karagiannidis *et al.* (2012), who noticed significantly enhanced essential oil production in mycorrhizal plants that ranged from 24% - 56.95% in medicinal plants of Lamiaceae. Moreover, the increased essential oil in *Salvia officinalis* by *Glomus intraradices* was reported by Geneva *et al.* (2010). Similar to the present finding Venkateshwar *et al.*, (2002) also reported that AM inoculated *Geranium* had significantly higher (2.1%) essential oil yield.

Table 17. ANOVA - OIL YIELD

K	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
ANOVA-OIL YIELD						
2	Var. (V)	1	1.384	1.384	88.6012	0.0000
4	Treat. (T)	3	34.996	11.665	746.8771	0.0000
6	AXB	3	0.215	0.072	4.5855	0.0088
-7	Error	32	0.500	0.016		

	Total	39	37.094			

Coefficient of Variation: 6.95%

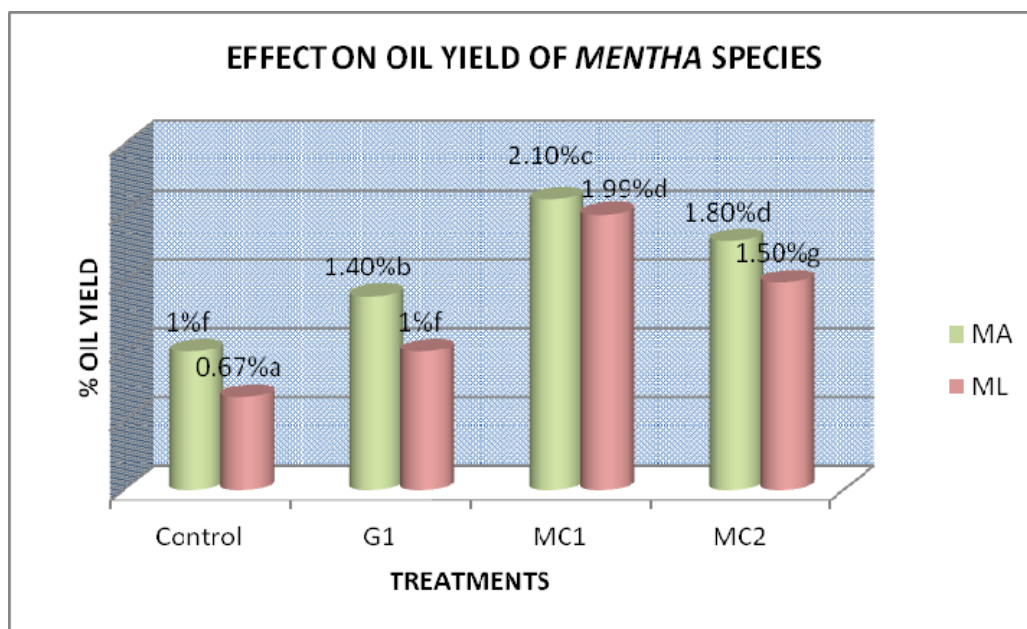


Fig. 23. Effect of AMF species on Oil yield % of *Mentha arvensis* and *Mentha longifolia*.

Plants were grown non inoculated (C) or inoculated with GI (*Glomus intraradicus*) Indigenous. AMF (MC1 and MC2). Values followed by different letters are significantly different ($p < 0.05$).

vi. CHEMICAL COMPOSITION OF ESSENTIAL OILS

Among plant secondary metabolites terpenoides are the most abundant and diverse class of natural compounds. The diversity of terpenoides is probably a reflection of their diverse biological activities in nature, which has made them a broadly used resources for traditional and modern human exploitation. Being essential components, essential oils are important for fragrance and flavour industries (Caputi & Aprea, 2011). The essential oils from the leaves of *Mentha longifolia* and *Mentha arvensis* inoculated with various AMF species were obtained through hydro-distillation and further analyzed for chemical composition through GC-MS. Identified chemicals, their respective chemical group, percent concentration retention time are given (Tables 18, 19). Typical GC-MS chromatograms of essential oils from *Mentha arvensis* and *Mentha longifolia* under various treatments are given in (Figs. 28 through 35).

On the whole 21 to 29 chemical compounds were identified in the essential oils of *Mentha arvensis* and *Mentha longifolia* respectively under various mycorrhizal treatments. These all identified chemical compounds can be placed in four chemical groups, i.e., 1. monoterpene. 2. hydrocarbons. 3. oxygenated hydrocarbons, sesquiterpenes hydrocarbons and 4. oxygenated sesquiterpenes.

1. Oxygenated Monoterpenes

The essential oils of AM treated *Mentha arvensis* and *Mentha longifolia* mainly consisted of Oxygenated monoterpenes, that ranged between 98.1 - 99.59% and 84.48 - 97.93%, respectively (Fig. 24). Menthol and piperitenone oxide were the principle oxygenated monoterpenes recovered in *Mentha arvensis* and *Mentha longifolia*. These findings are supported by the work of Singh *et al.* (2005), who also found high level of menthol in *Mentha arvensis*. Gullace *et al.* (2007) reported piperitenone oxide cis-piperitone epoxide pulegone, as the main component of essential oils from *Mentha longifolia* growing in Turkey. Vijoem *et al.* (2006) reported piperitenone oxide (15-66%) as the main compounds identified in the essential oils of *Mentha longifolia*. The present findings agree with Kapoor *et al.* (2002b) who also studied the effect of association of *Glomus macrocarpum* and *G. fasciculatum* on the concentration and composition of

essential oil in coriander (*Coriandrum sativum*) species. Mycorrhization was significantly enhanced the quality of essential oil. GC characterization of essential oil showed increased concentration of geraniol and linalool in plants inoculated with *G. macrocarpum* and *G. fasciculatum*, respectively. Moreover, Gupta *et al.* (2002) also found that VAM inoculation significantly increased the menthol contents in different cultivars of *Mentha*. Moreover, Kapoor *et al.* (2002a) noticed enhanced oxygenated monoterpenes (limonene and carvone) and thymol in essential oil obtained from AM-inoculated dill and carum plants, respectively. Frietas *et al.* (2004) also observed that inoculation with AMF increased the essential oil and menthol contents to 89% in mints. Contrarily, no menthol was detected in small quantities to less than 0.2% in mycorrhizal mints (Karagiannidis *et al.*, 2010). It may be attributed to changes in the synthetic pathways and the role of essential oils in plant physiology.

2. Monoterpene Hydrocarbons

The total monoterpene hydrocarbons (Fig. 25) in *Mentha arvensis* maximum concentration (1.12%) was achieved in Gi followed by MC2 (0.69%) and MC1 (0.81%). Least value of 0.06% was observed in un-inoculated control. Among various monoterpene hydrocarbons, D-limonene showed highest value (0.66%) in MC2 and GI. In *Mentha longifolia*, G1 treated plants displayed complete absence of monoterpene hydrocarbons. A maximum (1.43%) was noted in MC1 followed by MC2 (1.35%). The least (0.12%) contents were observed of D-Limonene in control (Fig. 25).

In both the tested species, D-Limonene concentration was improved in AM treated plants as compared to other compounds. Kapoor *et al.* (2002) also observed that inoculation with *G. macrocarpum* and *G. fasciculatum* significantly increased the concentration of limonene and α -phallendrene, respectively, relative to non mycorrhizal control plants of *Anethum graveolens* L.

3. Sesquiterpenes Hydrocarbons

Mycorrhizal and non-mycorrhizal plants of *Mentha arvensis* and *Mentha longifolia* also contained sesquiterpenes hydrocarbons. Maximum enhancement was seen in *Mentha longifolia* in various treatments (0.54-10.97%) while *Mentha arvensis* had (0.23-2.22%) considerably low concentration (Fig. 26). Among sesquiterpenes hydrocarbons in *Mentha longifolia*, maximum concentrations were seen of beta-farnesene (4.09%), followed by Germacrene-D (3.20%) and caryophyllene (2.99%) as compared to other compounds. While *Mentha arvensis* had very low amount of these compounds. These findings agree with Hussain *et al.* (2008). AM inoculated plants (AMM and AMG) showed a slightly lower level of total sesquiterpenes particularly α -farnesene and α -copaene, in leaf extracts compared to the non-mycorrhizal plants (NAM). Differences in relative proportion were also observed for β -caryophyllene, which was higher in AMM mycorrhizal plants, as previously reported (Nemec & Lund, 1990). α -Farnesene and β -caryophyllene are the most typical inducible terpenes emitted by herbivore damaged plants (Holopainen, 2004). α -Farnesene is also considered a stress metabolite in apple and pear fruits (Rupasinghe *et al.*, 2003). The smaller NAM plants could be at a different growth stage, and their higher content of sesquiterpenes could be due to a different developmental stage. Changes in sesquiterpenes content and composition during development have been noted in many plant species (Chou & Mullin, 1993; Flesch *et al.*, 1992; Kollner *et al.*, 2004), with mature plants frequently exhibiting lower sesquiterpenes content than younger plants (Alonso-Amelot *et al.*, 1992; Haraguchi *et al.*, 1993).

4. Oxygenated Sesquiterpenes

Oxygenated sesquiterpenes exhibited low concentrations in both test species (Fig. 27). Only two chemical compounds caryophyllene oxide and α -cedrol with minor amounts were noticed in *Mentha arvensis* (0.03-0.27%) and *Mentha longifolia* (0.21-1.56%). In both these species maximum concentrations were noted in non inoculated control plants. Recently Karagiannidis *et al.* (2012) also found that the concentrations of borneol and caryophyllene oxide were significantly high in non inoculated plants of *S. chamaecyparissus*.

The mechanism behind changes in essential oil composition is still not clear but it might be related to improved nutrition. Khaosaad *et al.* (2006) concluded that improved P-nutrition is not responsible for yield of essential oil of oregano. The increase of essential oil concentration in AM plants has been attributed either or not to enhanced P-nutrition but depends on the plant species or genotypes (Chaudhary *et al.*, 2008). The present work extend the understanding in a way that AMF can alter the composition of essential oil in *Mentha longifolia* and *Mentha arvensis* However, the significance of the altered composition of essential oil in mycorrhizal aromatic plants requires further investigations.

Furthermore it has been stated by Hussain *et al.* (2008) that the minor variations in the chemical composition of essential oil in different species of *Mentha* might also be attributed to varied agro climatic (climatic, seasonal, geographical) conditions of the regions and adaptive metabolism of plants. In addition phenological status and environmental conditions can influence the regulation of the biosynthesis of essential oils (Kamatou *et al.*, 2008; Masotti *et al.*, 2003).

The mechanism by which AMF trigger changes in phytochemical concentration in plant tissues can be multidirectional and is not quite clear yet (Toussaint, 2007). However, various researchers trend to explain through following possible reasons for such changes.

1. The modification of compounds produced in roots might be the consequence of signaling mechanisms between symbionts and plant response to AMF colonization (Larose *et al.*, 2002; Toussaint, 2007). Moreover, the production of phenolic compounds and terpenoides, the components of essential oils, is considered as a defense response to fungal colonization. Considering the fungicide properties of several constituents of essential oils, and the increase production of these metabolites in mycorrhizal plants, it was suggested that they could be synthesized as a defense response to AMF presence in roots (Copetta *et al.*, 2006).

2. The enhanced production of secondary metabolites may involve several metabolite processes that could be mediated by improved P or N nutrition due to AMF symbiosis (Kapoor *et al.*, 2002a & b). Toussaint *et al.* (2007) suggested that the increased production of terpenoids and phenolic acid could be due to enhanced mineral nutrition, especially P and N, respectively.
3. Copetta *et al.* (2006) found that the increased essential oil yield was associated to a significantly larger number of peltate glandular trichomes (main sites of essential oil synthesis and accumulation) in the leaves *Ocimum* inoculated plants. They suggested that the greater number of trichomes could be related to alterations in the phytohormonal profile induced by AMF. Moreover, studies by Kapoor *et al.* (2007), AMF were found to increase the number of glandular trichomes of *Artemisia annua* and consequently, enhance the concentration of artemisinin in leaves. The authors also pointed out that this could be related to the changes in the hormonal balance of host influenced by AMF.
4. Nevertheless, our findings are in agreement with the observation of Copetta *et al.* (2006) concerning the production of metabolites in *O. basilicum* leaves and confirm earlier observations that functional diversity exists between fungal isolates, even belonging to the same genus. Copetta *et al.* (2006) showed the *G. rosea* increased concentration of camphor and alpha-terpineol, while plants treated with *Gigaspora margarita* significantly decreased eucalyptol, linalool and eugenol contents relative to control. In addition, *Glomus mosseae* did not alter the proportion of the aforementioned compounds (Copetta *et al.*, 2006). Kapoor *et al.* (2002 b) showed increased concentration of geraniol in the fruits of *Coriandrum sativum* L. individuals inoculated with *Glomus macrocarpum*, while the content of linalool was higher in the plants treated with *Glomus fasciculatum*.

Finally the differences in the content of monoterpenes and sesquiterpenes in mycorrhizal and non mycorrhizal *Artemisia annua* plants have been observed by Rapparini *et al.* (2008) and they suggested a possible effect of AMF specific regulation of the different enzymes used in terpene synthesis

Conclusion

The present investigations shows that the high menthol and pipertenone oxides (oxygenated monoterpenes) induced by various AMF inocula. They have very good antioxidants and radical scavenging activities. Moreover they also possess cytotoxic and antimicrobial potential. The study as it was aimed provided interesting and useful information related to oil yield and chromatographic profiles as affected by AMF inoculation. Further on it can be concluded AMF should be managed to help in the reduction of fertilizer and other agrochemical inputs, thus enhancing the sustainability of the commercial cultivation of aromatic plants in low fertile soils.

Future Focus

Generally the mycorrhizal plants performed better than the non mycorrhizal plants in terms of growth performance but the extent to which the growth was affected on mycorrhization varied with AMF used. From experimental results it can be concluded that the plants inoculated with a monospecific culture of *Glomus intraradicus* (commercial inoculum) did not performed better than native AMF inocula (MC1 and MC2). MC1 containing higher (11 species of *Glomus*, *Acaulospora* and *Gigaspora*) AMF species richness exhibited maximum improvement in terms of growth parameter evaluated, mineral nutrition, proximate profile, and essential oil as compared to MC2 and GI. As far as host species response is concerned *Mentha arvensis* performed better in all measured aspects as compared to *Mentha longifolia*. Nevertheless, cultivated *Mentha* species responded well to AM mycorrhization and hence inoculation of these crops with indigenous multiple AMF species would be of potential use for improving their productivity in marginal soils instead of using foreign strains.

Table 18. Experiment 1. Effects of AMF inoculation on Chemical compounds (%) of essential oils of *Mentha longifolia*.

Plants were grown non inoculated (C) or inoculated with single exotic species culture GI (*Glomus intraradicus*) or two multiple mixed culture of Indigenous AMF (MC1 and MC2).

Components	Treatments	C	GI	MCI	MC2	Mode of identification
i. Oxygenated monoterpenes	Total %age	88.48	97.01	94.42	97.93	
	Retention time					
Cineole	13.057	1.32	0.05	4.19	0.56	RT,MS,GC
Beta-linalool	16.521	0.13	—	4.19	0.66	RT,MS,GC
1-terpinen-4-ol	19.406	1.85	0.08	1.68	2.17	RT,MS,GC
Bornyl acetate	21.910	0.45	0.32	1.19	0.78	RT,MS,GC
p-Cymen-2-ol	22.092	0.25	—		0.03	RT,MS,GC
Menthol (Cyclohexanone, 2- (1- (methylethylidene)	23.438	15.19	48.90	6.02	23.96	RT,MS,GC
p-menth-1-en-8-ol	19.897	1.52	0.13	0.85	0.61	RT,MS,GC
Piperitenone oxide		66.44	47.53	75.53	68.90	RT,MS,GC
Nerol acetate	23.294	—	—	0.05	0.01	RT,MS,GC
Alpha limonene		—	—	0.23	—	RT,MS,GC
3-Nananol	22.992	—	—	0.14	—	RT,MS,GC
cis Geraniol	20.573	1.30	—	—	—	RT,MS,GC
o-Cymene	12.678	—	—	0.12	0.16	RT,MS,GC
ii. Monoterpene hydrocarbons	Total	0.12	—	1.13	1.35	
alpha-pinene	8.661	—	—	—	—	RT,MS,GC
Camphene	9.332	—	—	—	0.05	RT,MS,GC
beta-Pinene	10.523	—	—	0.08	0.14	RT,MS,GC
cis-limonene oxide	22.774	—	—	—	—	RT,MS,GC
D-Limonene	12.911	0.12	—	0.61	0.69	RT,MS,GC

Trans- β -Ocimene	13.318	–	–	0.62	0.12	RT,MS,GC
Terpinolene	15.601	–	–	–	–	RT,MS,GC
γ -Terpinene	14.268	–	–	–	0.04	RT,MS,GC
cis β -Ocimene	13.762	–	–	0.05	–	RT,MS,GC
beta-myrcene	11.144	–	–	–	0.23	RT,MS,GC
Alcanfor	18.299	–	–	0.07	0.05	RT,MS,GC
Alpha cedrol	27.560	–	–	–	–	RT,MS,GC
Alpha phellandrene	8.654	–	–	–	0.03	RT,MS,GC
iii. Sesquiterpene hydrocarbons	Total	10.97	2.78	7.57	0.54	
Germacrene D	25.348	3.20	–	1.63	0.13	RT,MS,GC
gamma-Elemene	25.565	–	–	–	–	RT,MS,GC
Farnesene	25.801	–	–	0.081	–	RT,MS,GC
Farnesol	–	0.57	–	–	–	RT,MS,GC
beta-Besabolene	25.672	–	–	–	–	RT,MS,GC
Caryophyllene	24.399	2.99	–	2.73	0.41	RT,MS,GC
beta Farnesene	24.780	4.09	–	3.13	–	RT,MS,GC
iv. Oxygenated sesquiterpenes		1.56	0.21	0.76	0.19	
Caryophyllene oxide	26.834	0.99	0.21	0.76	0.19	RT,MS,GC
Alpha-citral	21.733	0.57	–	–	–	RT,MS,GC

Compounds are listed in order to elution from a HP-5MS column.

RT, identification based on retention time.

MS, GC identification based on comparison of mass spectra.

Table 19. Experiment 1. Chemical composition (%) of essential oils of *Mentha arvensis*.

Plants were grown non inoculated (C) or inoculated with single exotic specie culture Gi (*Glomus intraradicus*) or two multiple mixed culture of Indigenous AMF (MC1 and MC2).

Components	Treatments	C	GI	MC I	MC 2	Mode of identification
i. Oxygenated monoterpenes	Total %age	98.1	96.63	98.79	99.59	
Cineole	Retention time 13.057	—	0.91±	0.21	0.07	RT,MS,GC
beta-Linalool	16.521	—	—	0.35	—	RT,MS,GC
1-Terpinene-4-ol	19.406	0.10	0.09	0.43	0.10	RT,MS,GC
Bornyl acetate	21.910	0.62	0.29	0.28	0.25	RT,MS,GC
p-Cymen-2-ol	22.092	—	—	—	—	RT,MS,GC
Menthol						
Cyclohexanone, 2-(1-methylthylidene)	23.438	59.27	58.07	84.68	71.72	RT,MS,GC
p-Menth-1-en-8-ol	19.897	0.20	0.12	0.35	0.13	RT,MS,GC
Piperitenone oxide		37.91	37.15	12.43	27.32	RT,MS,GC
Nerol acetate	23.294	—	—	0.01	—	RT,MS,GC
alpha-Limonene		—	—	—	—	RT,MS,GC
3-Nanol	22.992	—	—	—	—	RT,MS,GC
cis-Geraniol	20.573	—	—	—	—	RT,MS,GC
o-Cymene	12.678	—	—	—	—	RT,MS,GC
ii. Monoterpenes hydrocarbons		0.06	1.12	0.18	0.69	
α-Pinene	8.661	—	—	—	—	RT,MS,GC
Camphene	9.332	—	0.02	—	—	RT,MS,GC
β-Pinene	10.523	—	0.17	0.04	—	RT,MS,GC
cis-Limonene oxide	22.774	—	—	—	—	RT,MS,GC
D Limonene	12.911	—	0.66	0.11	0.66	RT,MS,GC

trans β -Ocimene	13.318	–	0.14	–	–	RT,MS,GC
Terpinolene	15.601	–	–	–	–	RT,MS,GC
gamma-Terpinene	14.268	–	–	–	–	RT,MS,GC
cis- β -Ocimene	13.762	–	–	–	–	RT,MS,GC
beta-myrcene	11.144	–	0.13	0.03	–	RT,MS,GC
Alcanfer	18.299	–	–	–	0.03	RT,MS,GC
alpha-cedral	27.560	0.06	–	–	–	RT,MS,GC
alpha-phellendrene	8.654	–	–	–	–	RT,MS,GC
Sesquiterpenes		1.57	2.22	0.94	0.23	
hydrocarbons						
Germicene D	25.348	0.39	1.37	0.46	0.03	RT,MS,GC
Γ -Elemene	25.565	0.01	0.02	–	–	RT,MS,GC
Farnasene	25.801	–	0.02	–	–	RT,MS,GC
Farnesol		–	–	–	–	RT,MS,GC
beta- Besabulene	25.672	–	–	0.01	–	RT,MS,GC
Caryophyllene	24.399		0.83	0.48	0.20	RT,MS,GC
beta-Farnesene	24.780	–	–	–	–	RT,MS,GC
Oxygenated		0.27	–	0.03	0.09	
sesquiterpenes						
Caryophyllene oxide	26.834	0.27	–	0.03	0.09	RT,MS,GC
alpha-citral	21.733	–	–	–	–	RT,MS,GC

Compounds are listed in order to elution from a HP-5MS column.

RT, identification based on retention time; MS, identification based on comparison of mass spectra.

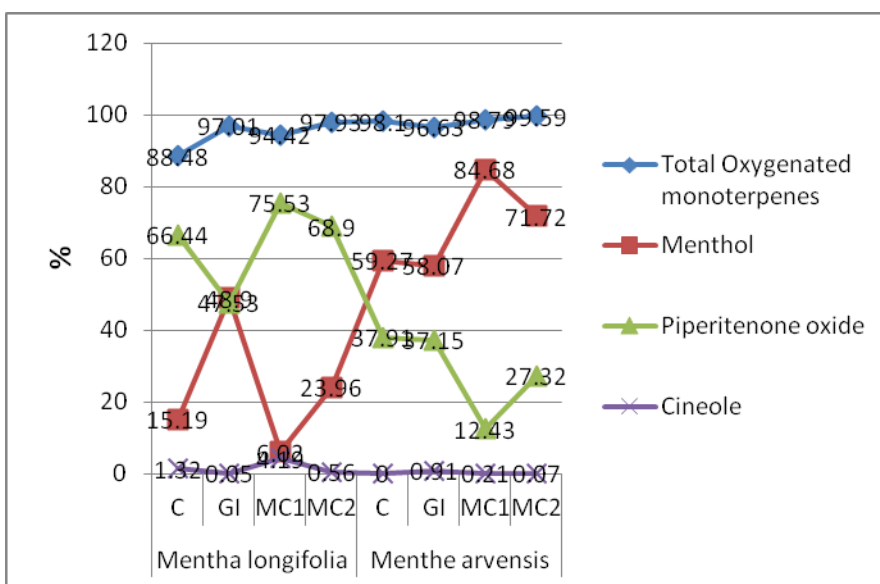


Fig. 24. Effect of AMF species on Oxygenated Monoterpenes in *Mentha arvensis* and *Mentha longifolia*.

C (control), GI (*Glomus intraradicus*), MC1 (Mixed culture), MC2 (Mixed culture).

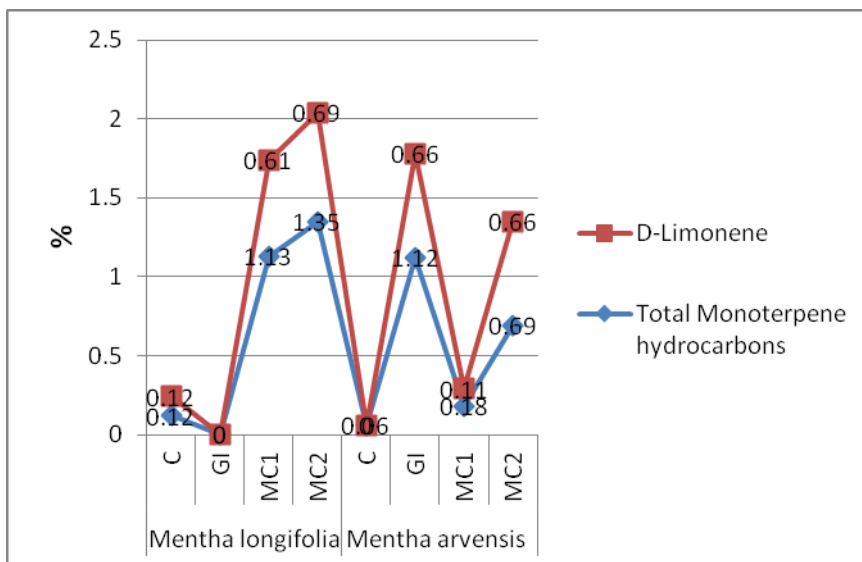


Fig. 25. Effect of AMF species on Monoterpene hydrocarbons in *Mentha arvensis* and *Mentha longifolia*.

C (control), GI (*Glomus intraradicus*), MC1 (Mixed culture), MC2 (Mixed culture).

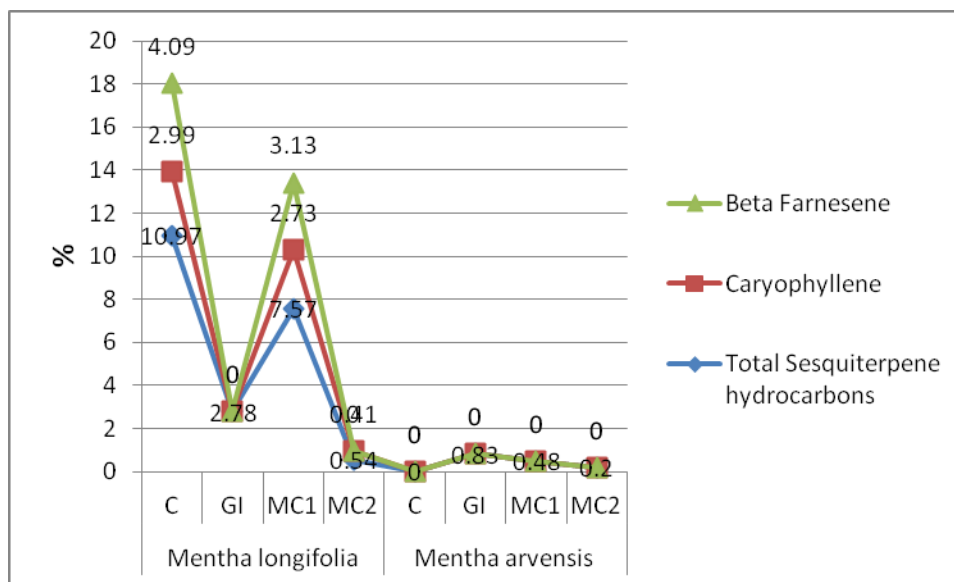


Fig. 26. Effect of AMF species on Sesquiterpene hydrocarbons in *Mentha arvensis* and *Mentha longifolia*.

C (control), GI (*Glomus intraradicus*), MC1 (Mixed culture), MC2 (Mixed culture).

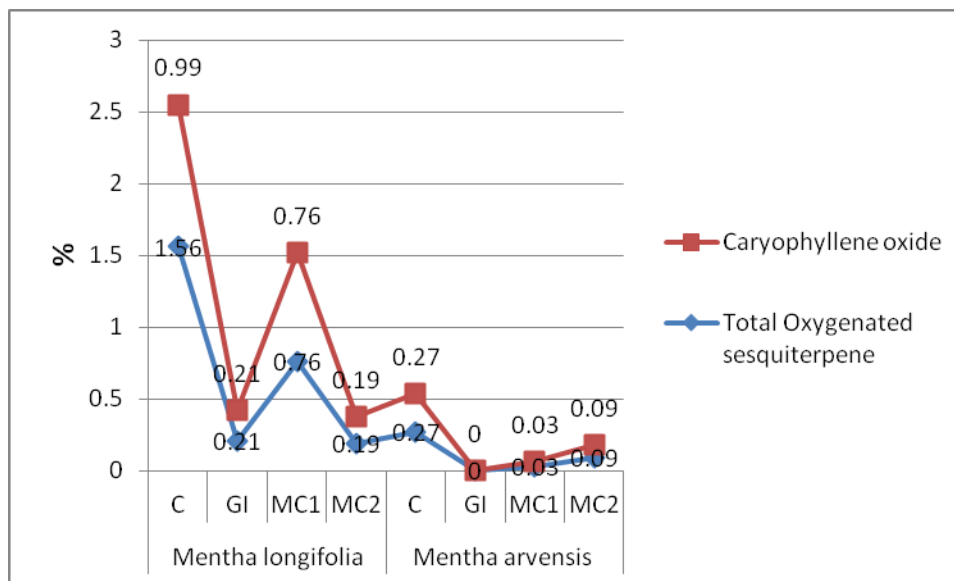


Fig. 27. Effect of AMF species on Oxygenated sesquiterpene in *Mentha arvensis* and *Mentha longifolia*.

C (control), GI (*Glomus intraradicus*), MC1 (Mixed culture), MC2 (Mixed culture).

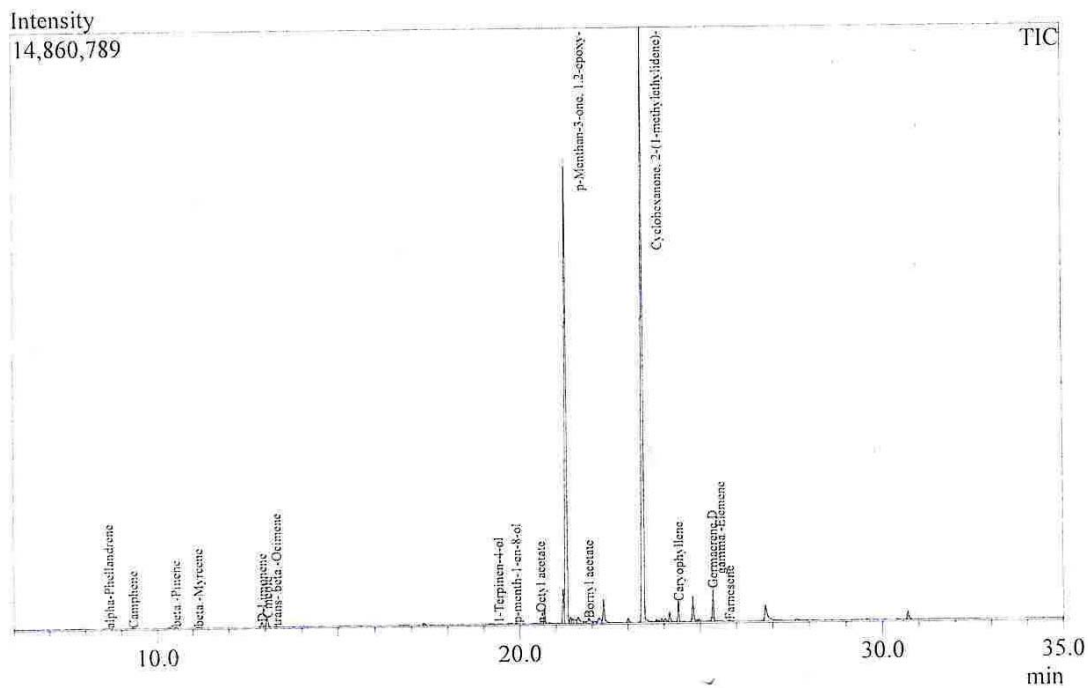


Fig. 28. Typical GC - MS Chromatogram of essential oils from uninoculated (control plant) of *Mentha arvensis*.

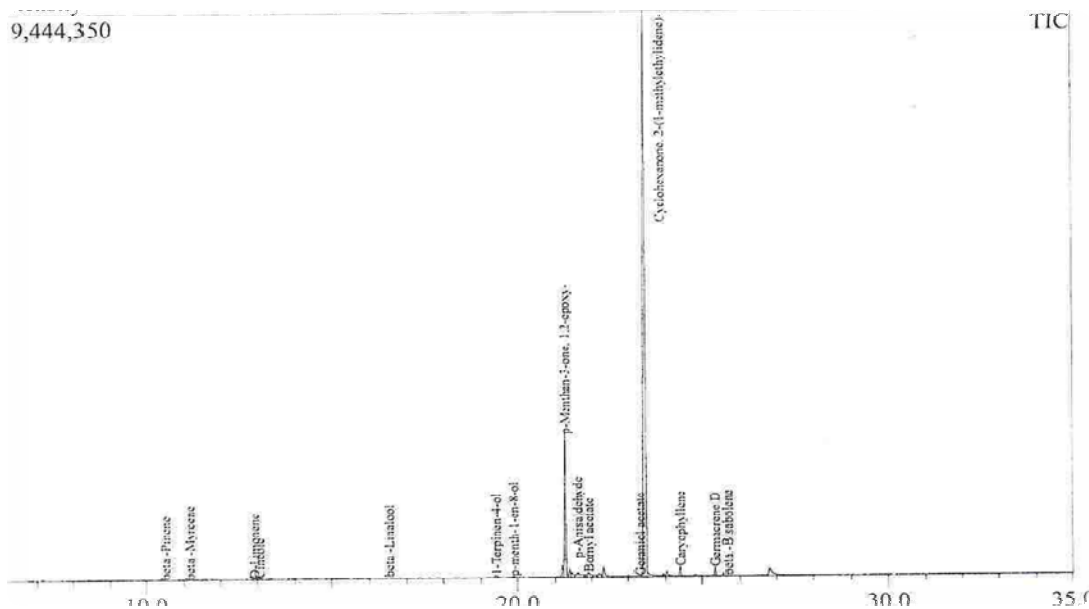


Fig. 29. Typical GC - MS Chromatogram of essential oils from GI inoculated plants of *Mentha arvensis*.

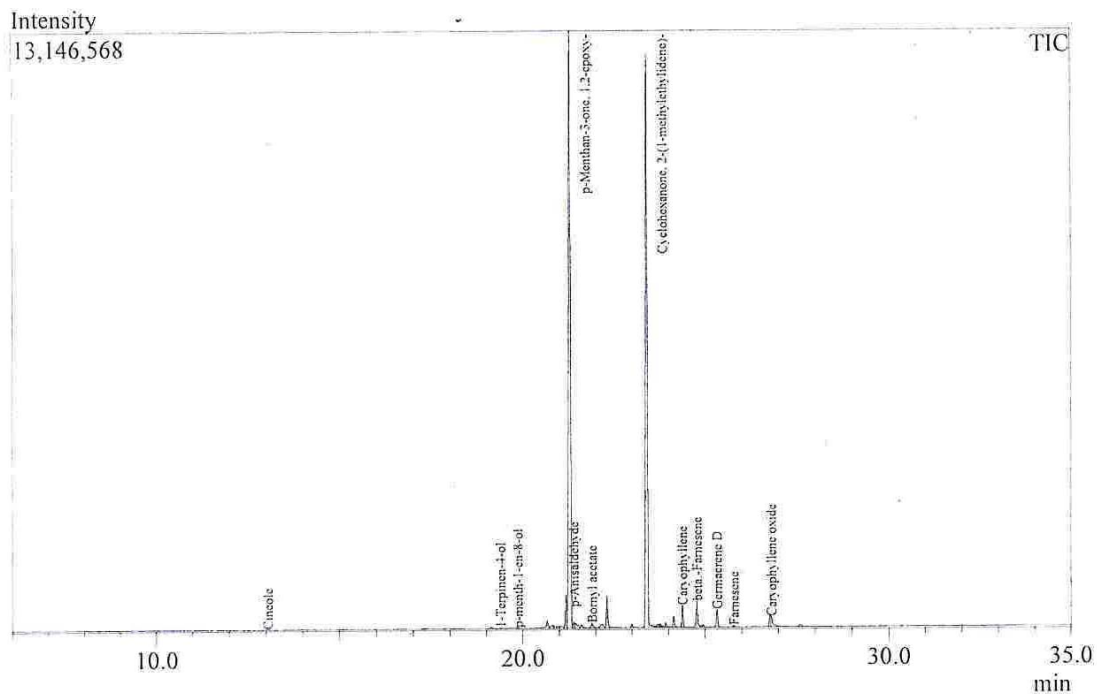


Fig. 32. Typical GC - MS Chromatogram of essential oils from uninoculated (control plant) of *Mentha longifolia*.

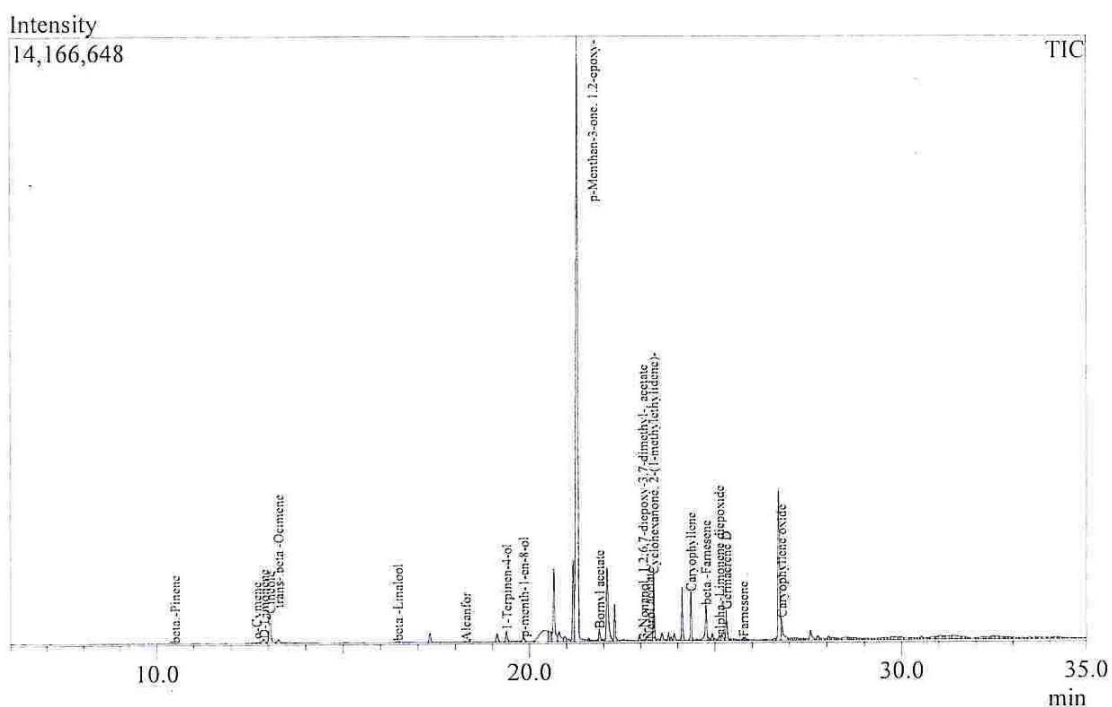


Fig. 33. Typical GC - MS Chromatogram of essential oils from GI inoculated plants of *Mentha longifolia*.

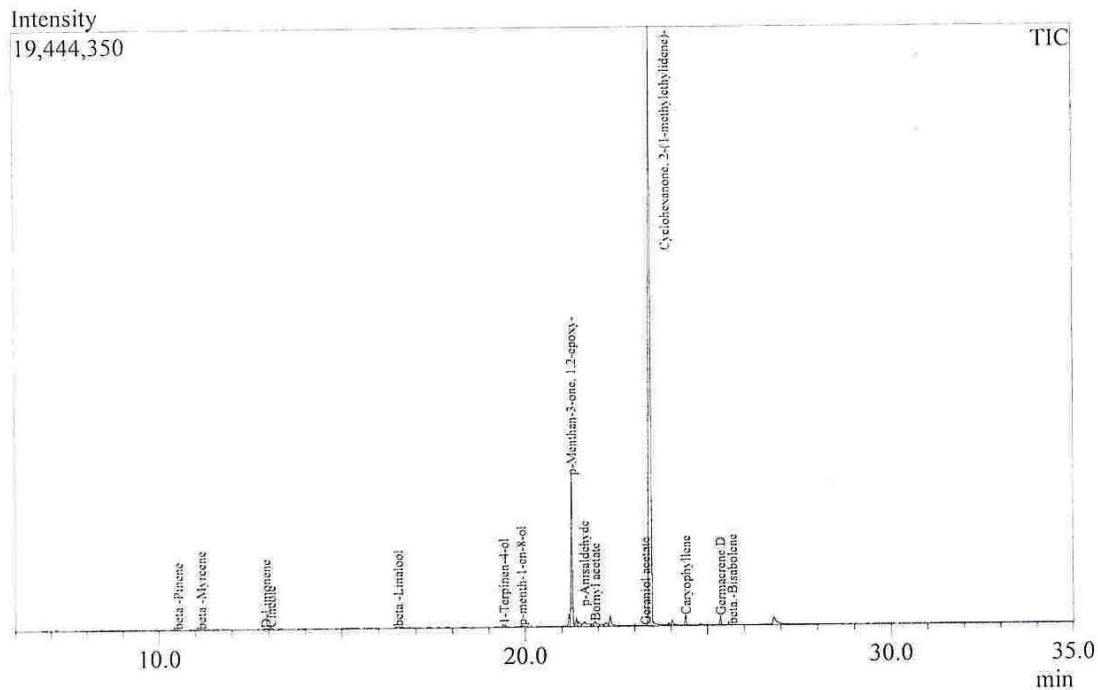


Fig. 34. Typical GC - MS Chromatogram of essential oils from MC1 inoculated plants of *Mentha longifolia*.

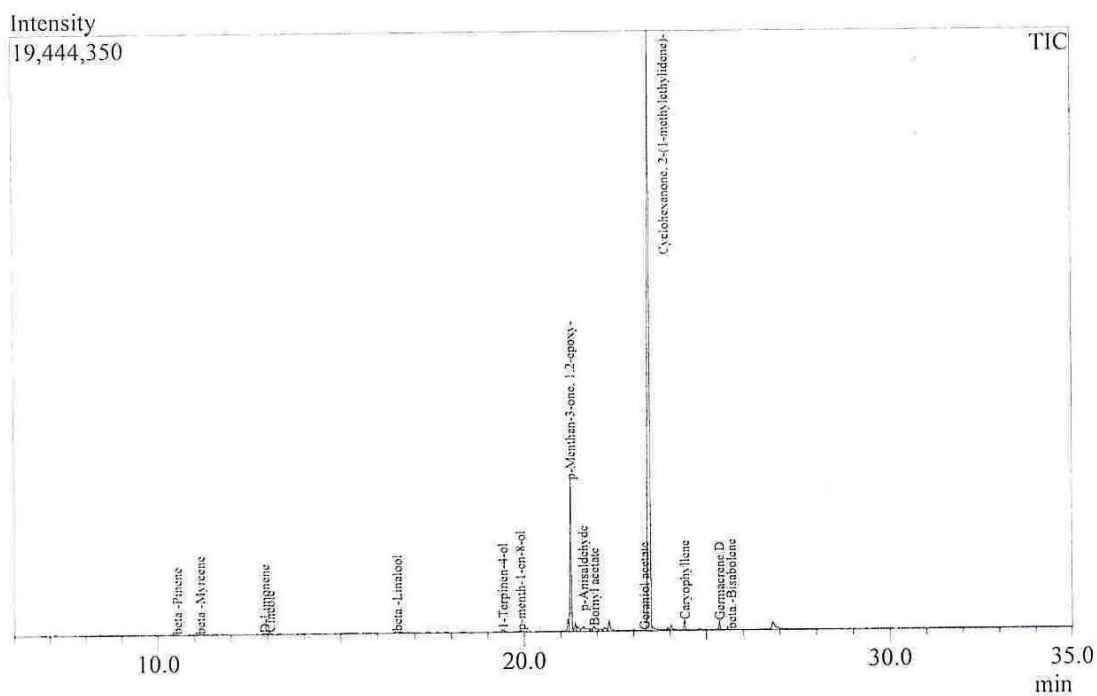


Fig. 35. Typical GC - MS Chromatogram of essential oils from MC2 inoculated plants of *Mentha longifolia*.

EFFECT OF PHOSPHORUS FERTILIZER ON GROWTH OF AM INDUCED *MENTHA* SPECIES

In the second phase of study, experiments were designed to find out the symbiotic efficiency of mixtures (MC1 and MC2) of indigenous AMF fungi (species of *Glomus*, *Acaulospora* and *Gigaspora*) with single exotic strain (Gi) on growth performance of *Mentha* species. In last experiment MC1 came up with comparatively high symbiotic efficiency, therefore was selected in further experimentation. Experiments were designed to find out the host growth performance of *Mentha* species and development of AMF endophytes under various regimes of fertilizers in terms of growth parameters and below ground fungal attributes.

i. Effect of indigenous AMF species

The average P contents soil was 0.05% but only 0.1% of the total P was available to plants due to its low solubility and its fixation in soil. Phosphorus is the least mobile macronutrient in soil, because it is readily sorbed (e.g., to metal oxides), occurs in mineral forms of low solubility (e.g., calcium phosphates such as apatite), and can be incorporated into organic molecules which are poorly available to plants (Marschner, 95). Under these circumstances micro-organisms offer an opportunity to acquire sufficient phosphorus (Sharma & Adholey 2004). Among microbial groups that could solubilize mineral phosphates and improve phosphorus nutrition are AM fungi be an environment friendly approach.

The data regarding various growth parameters including shoot length, root length, fresh weight, dry weight, MD values, essential oil yield, AM infections and spore density are presented below.

Values of LSD and ANOVA test showed that species and effects of various treatments were significant ($P < 0.0001$) (Tables 20, 21, 22) interaction between species and treatments was also significant.

It was seen that the mycorrhizal plants performed better than non-mycorrhizal plants in various parameters (Table 20), *Mentha arvensis* responded better to AM inoculation as compared to *Mentha longifolia* in all evaluated parameters (Figs. 36, 37). Our results agree with others (Moremmanni *et al.*, 2003; Gupta *et al.*, 2002; Freitas *et al.*, 2004; Silveira *et al.* 2006), who also proved positive relationship between *Mentha* and AM. The present results are also strengthened by the findings of Faboodi *et al.* (2011) who reported positive effects of mycorrhiza on vegetative characteristics of maize. Like the present results Karthikeyan *et al.* (2008) observed positive response with AM application in *Catharanthus* plants. The present study revealed a statistically increased growth in all mycorrhizal plants. Similar results were also reported by Earanna *et al.* (2001) for *Coleus aromaticus* and in *C. forskholii* (Sailo & Bagyaraj, 2005). Laei *et al.* (2011) recorded increased dry weight in mycorrhizal sorghum plants due to AM application. AM fungi are known to be of great importance due to their great capability to increase growth, yield and crop quality through efficient nutrient acquisition in infertile soils & therefore lessen the prerequisite for phosphate-based fertilizers (Khalafallah & Abo-Ghaila, 2008; Roy-Bolduc & Hijri, 2010).

The perusal of the data regarding the effect of various levels of phosphate fertilizers with AM showed that the mycorrhizal responses were promising at low levels of applied P which minimized at higher levels of P. In the present study mycorrhizal plants excelled non-mycorrhizal plants grown at Po and P1 fertilizer level significantly ($P < 0.001$) and non-significantly at high P levels. Mycorrhizal plants at P1 fertilizer level showed maximum enhancement in growth as compared to non-mycorrhizal ones. This may be due to the combined effect of applied fertilizers and VAM. Ayoob *et al.* (2011) and Thingstrup *et al.* (1998) also suggested that the mycorrhizal responses were most pronounced at low and intermediate levels of applied fertilizers. It is suggested that the possible reason for improved growth might be due to enhanced uptake of nutrient by inoculated plants (Al-Karaki, 2000; Kerur & Lakshman, 2004; Rapparini *et al.*, 2008; Rydlova *et al.*, 2010). The AM fungi enhances the nutrient up later, especially phosphate, micronutrients (Bresinsky *et al.*, 2008) and N and K (Porrás-Soriano *et al.*, 2009) that promotes the growth of mycorrhizal plants. Mycorrhizal inoculation increases

transpiration, photosynthetic rate and chlorophyll concentration and hormones production in plant (Manoharan *et al.* 2008). In nutrient deficient soils, the amount of phosphorus applied to non-mycorrhizal plants is very high. Howeler *et al.* (1987) observed that non-mycorrhizal plants need 1,600kg. Phosphorus/ha for their growth to equal to mycorrhizal plants.

A number of factors can affect the growth response of host plants to AMF colonization, includes, the genotypes of host plants and environmental conditions (Sensory *et al.*, 2007). However, increased growth of mycorrhizal plants may be linked to overcome two types of inhibitions of photosynthetic activity. First, by drawing upon the starch reserves in the leaves of the host plant, the VAMF reduces the starch-sink inhibition in leaves. Secondly, increasing the concentration of inorganic P in the chloroplast reduces the ADP/ATP limitation of photosynthesis (Harris & Paul, 1987).

Table 20. Effects of AMF species on growth parameters of *Mentha longifolia* and *Mentha arvensis*.

Plants were grown non inoculated (NM) or inoculated (M) with four levels of fertilizers (PO, P1, P2, P3) Treatments were compared with ANOVA.

Treatments	Shoot length (cm)	Root length (cm)	Fresh weight (gm.)	Dry weight (gm.)
<i>Mentha arvensis</i>				
NMPO	21.20a	5.20h	12.00a	4.3b
MPO	38.61b	6.52b	18.00b	6.3d
NMP1	40.31bc	9.60c	31.23c	10.80g
MP1	48.28d	11.70d	33.21d	7.5h
NMP2	50.45de	12.30e	27.42e	8.78g
MP2	56.50e	13.25f	39.00f	13.56e
NMP3	60.73f	13.50f	45.31g	18.00c
MP3	61.70f	13.75f	45.60g	18.80c
<i>Mentha longifolia</i>				
NMPO	18.20h	4.75g	9.12h	3.20a
MP O	30.67i	5.52a	12.42a	4.5b
NMP1	38.21b	8.50h	17.38b	6.5d
MP1	42.38j	9.70c	28.40e	9.5g
NMP2	47.45d	10.20d	26.22e	14.00e
MP2	50.70de	12.70e	38.00f	16.50i
NMP3	55.70e	11.50d	44.12g	17.00cd
MP3	56.61e	12.90e	44.50g	17.90c
LSD at 5%				
	2.435	1.477	1.455	0.4786
P VALUE				
T	0.0000	NS	0.0001	NS
S	0.0000	0.0000	0.0000	0.0000
(T × S)	0.0026	0.0794	0.0037	NS

Each value is a mean of five replicates. Values followed by different letters are significantly different ($p < 0.05$). P (Probability), NS (Non significant), LSD (Least Significant Test), T (Treatment), S (species).

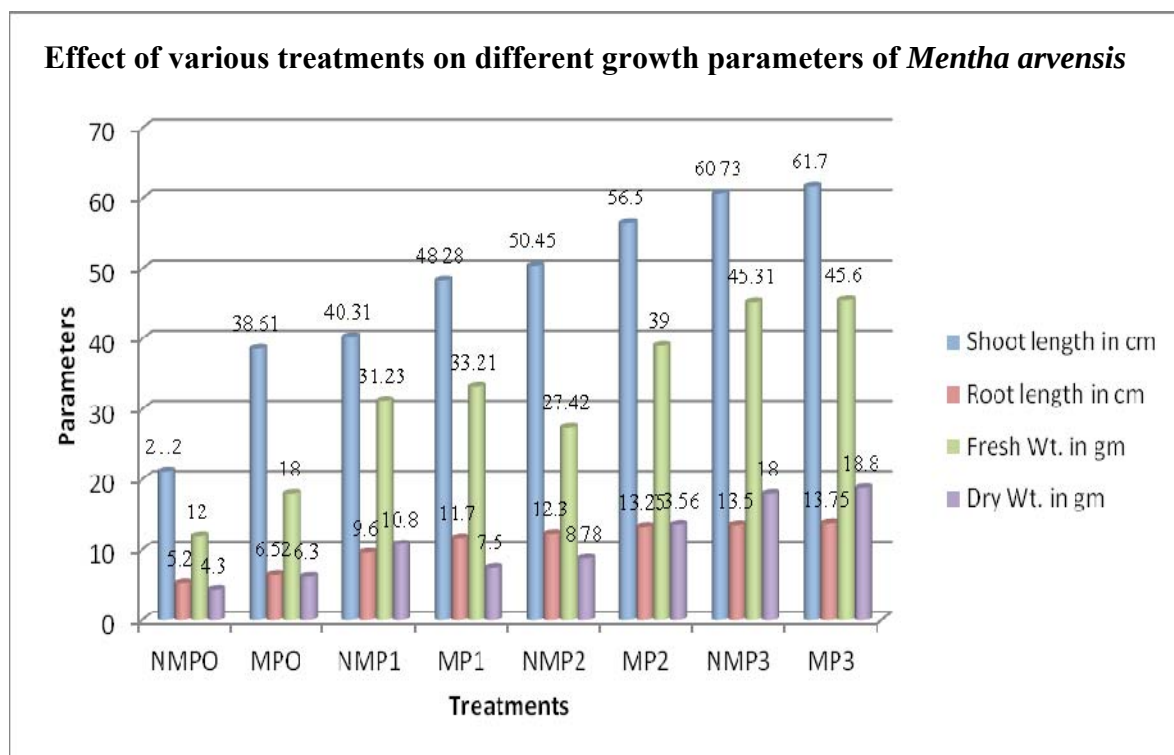


Fig. 36. Effects of AMF species on growth parameters of *Mentha arvensis*.

Plants were grown non inoculated (NM) or inoculated (M) with four levels of fertilizers (PO, P1, P2, P3).

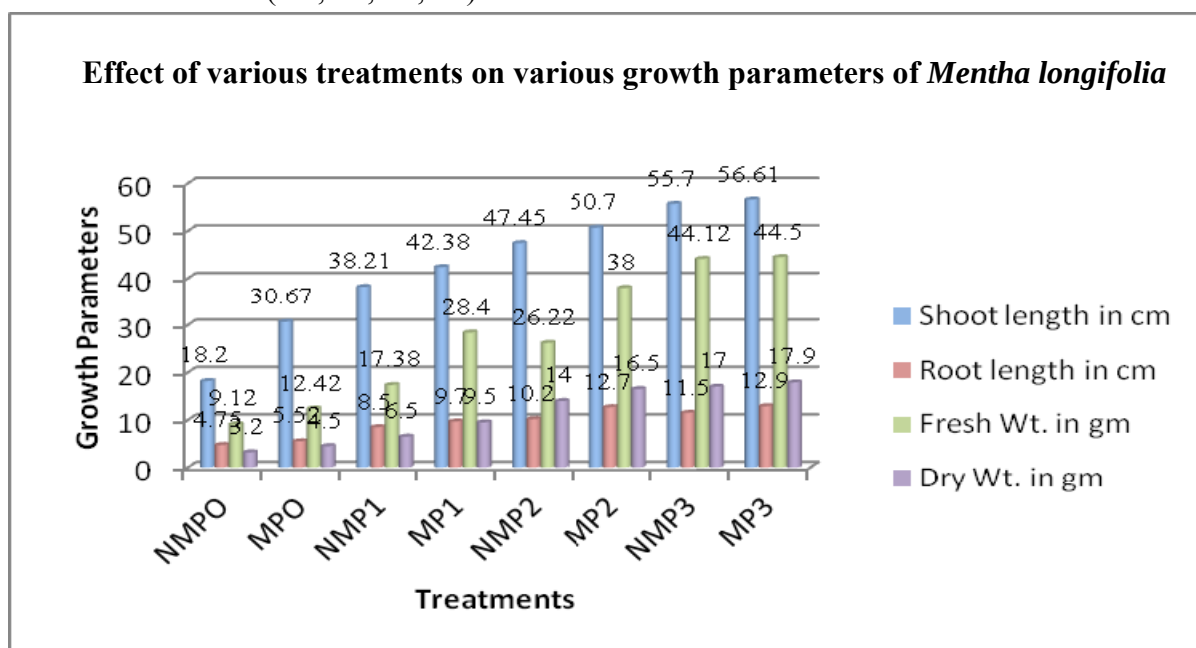


Fig. 37. Effects of AMF species on growth parameters of *Mentha longifolia*.

Plants were grown non inoculated (NM) or inoculated (M) with four levels of fertilizers (PO, P1, P2, P3).

ii. Mycorrhizal dependency (MD)

Mycorrhizal dependency is the ratio of the dry mass of a plant exhibiting mycorrhizal to that of non mycorrhizal plants. (Lambers., 2008) Mycorrhizal species involved in the association and nutrient levels of the soil directly affect the mycorrhizal dependency that in turn controls the response of the plant to mycorrhizas to their natural selection (Costa *et al.*, 2005; Zangaro *et al.*, 2007; Nogueira & Cardoso, 2007). The mycorrhizal dependency values of *Mentha species* at various phosphorus levels (Table 23) showed that the values of mycorrhizal dependency decreased with increasing concentration of phosphorus in the soil beyond P₀ level. Maximum values being 38.28% and 31.50% for *Mentha arvensis* and *Mentha longifolia* respectively at MP₁. While at higher fertilizer level i.e. MP₂ and MP₃ mycorrhizal dependency values declined significantly up to 4.2% and 3.5% in *Mentha arvensis* and *Mentha longifolia* respectively (Fig. 38). The comparison revealed that *Mentha arvensis* was more dependent than *Mentha longifolia*. Sawers *et al.* (2008) stated that mycorrhizal dependency varies in the species of same genus. The results also revealed that the last plants were more dependent on AM partnership at low nutrients level compared to high. Mycorrhizal activity and dependency was maximum at moderate P-applications for different agricultural crops; intermediate fertilizer doses with controlled concentration of phosphorus induced higher growth in AM colonized plants than non-mycorrhizal plants Mukerji *et al.* (2000). The present results also agree with Yao & Chritie *et al.* (2001) who stated that AMF increased dependence of mycorrhizal plants at low phosphorus level. The degree of root branching affects dependence on the symbiosis (Smith & Read, 1997; Barakh & Heggo, 1998). Oliveira *et al.* (2006) reported that the AMF species have high extra radical mycelium lengths that benefited the plant species with greater mycorrhizal dependency. This may be further supported by the fact that in nutrients deficient condition plants need more nutrients supplied by AM fungus through external mycelium. At high P-concentration the effect of mycorrhiza is either nil or negative and dependency on AMF was negatively correlated with soil phosphorus contents (Ryan *et al.* 2004).

Data on mycorrhizal dependency of the crop at different P- level might facilitate to decide suitable level of phosphate application for maximum mycorrhizal benefits. However, ready supply of soluble P - fertilizer decreases mycorrhizal colonization in soil except in P- deficient soils. Application of P after planting reduced mycelial growth and root colonization more than if applied before planting (De Miranda & Haris, 1994).

Table 23. Effects of phosphorus fertilizers on Mycorrhizal Dependency (MD) % of *Mentha arvensis* and *Mentha longifolia*.

Treatments	MD value (%)	MD value (%)
	<i>Mentha arvensis</i>	<i>Mentha longifolia</i>
MPO No fertilizer	31.74 ± 0.02	28.81 ± 0.03
MP1 (25 % P)	38.38 ± 0.03	31.57 ± 0.01
MP2 (50 % P)	11.00 ± 0.02	10.23 ± 0.04
MP3 (100%P)	4.25 ± 0.01	3.51 ± 0.02

MPO (no fertilizer) MP1 (25% phosphate) MP2 (50% phosphate), MP3 (100%)
 ± Standard error.

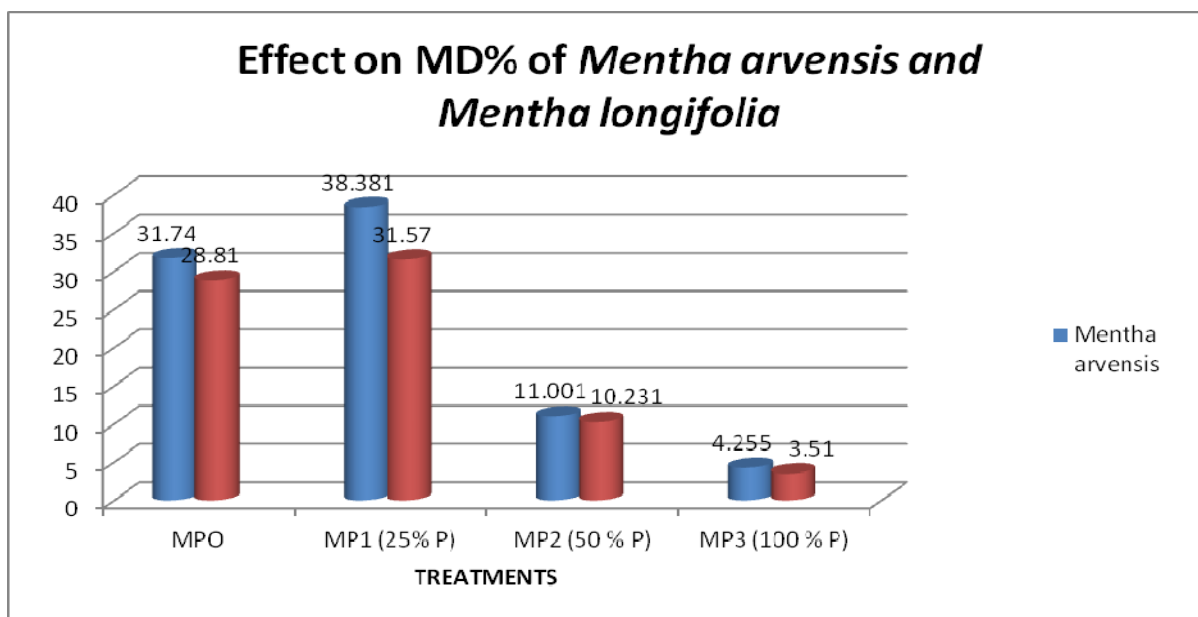


Fig. 38. Effects of phosphorus fertilizers on Mycorrhizal Dependency (MD) % of *Mentha arvensis* and *Mentha longifolia*.

MPO (no fertilizer) MP1 (25% phosphate) MP2 (50% phosphate), MP3 (100%)

iii. Macromineral acquisition

The results showed (Tables 24, 25) that mycorrhizal colonization enhanced the acquisition of macronutrients like, P, N, K and Mg compared to control plants. However, results at various levels of P fertilizer varied. At low level P1 significant differences were noted between M and NM plants while at high levels (P2 and P3) they were insignificant in both test species. Mycorrhizal enhancement regarding various nutrients followed the trend. $P_0 > P_1 > P_2 > P_3$ at various levels of treatments. N, K and Mg contents also followed the similar pattern (Figs. 39, 40). The present results agree with George (2000), who observed that the colonization of plant roots by arbuscular mycorrhizal (AM) fungi significantly promoted the plant uptake of mineral nutrients. Schreiner (2007) reported increase in P by AM fungi in grapevine. Likewise, Yaseen *et al.* (2011) reported that AM inoculated Cowpea (*Vigna unguiculata*) varieties performed better than non-inoculated plants. There was positive symbiotic response of *Phyllanthus emblica* and Chakaiya to different AM fungi for P uptake (Batnal & Lakshman, 2011). Karagiannidis *et al.* (2011) also revealed positive effect of AMF on nutrient acquisition in oregano and mint. They noticed the enhanced uptake of both macro and micronutrients in AM inoculated plants in P deficient soils. Contrarily, Al-Karaki (2000) observed that AMF-inoculated garlic (*Allium sativum* L.) had less P use efficiency than uninoculated plants. Inoue *et al.* (2001) reported non significant interaction ($P > 0.05$) between phosphorus fertilizer and AMF inoculation on nitrogen phosphorus and potassium uptake.

This might be due to the fact that mycorrhizal fungi release chemicals like lytic enzymes and organic acids, into the soil that dissolve hard to capture nutrients such as P, Fe and other tightly bound soil nutrients (Lester, 2009; Jansa *et al.*, 2011). The possible explanation for mycorrhizal role might be wider physical exploration of soil by mycorrhizal fungi than by roots. Besides this, various other mechanisms might be responsible for P uptake by AMF, such as the kinetics of P

uptake into hyphae differ from those of roots either through a higher affinity (lower K_m) or a lower threshold concentration at which influx equals efflux (C_{min}) (Joner & Jakobsen, 1995).

Table 24. Effect of AMF species and fertilizers on macronutrient acquisition of *Mentha longifolia* and *Mentha arvensis*.

Plants were grown non-inoculated (C) or inoculated with Indigenous AMF.

Treatments were compared with ANOVA.

Species	Treatments	P (%)	N (%)	K (%)	Mg (%)
<i>Mentha arvensis</i>	NMPO	0.180a	0.260g	0.560b	0.180a
	MPO	1.120c	2.00b	0.750b	0.880c
	NMP1	1.990e	2.330b	0.990c	0.890c
	MP1	2.110g	3.350c	1.121a	0.900d
	NMP2	2.501i	3.550c	1.351a	0.912d
	MP2	2.751h	2.741d	1.421b	0.955d
	NMP3	2.811h	2.900d	1.755m	1.000a
	MP3	2.990	3.005h	1.782m	1.120b
<i>Mentha longifolia</i>	NMPO	0.161a	0.240g	0.520w	0.170a
	MPO	1.760d	1.980a	0.701q	0.770g
	NMP1	1.800s	2.220b	0.920b	0.790g
	MP1	1.990e	2.290c	1.003a	0.890
	NMP2	2.001f	2.451k	1.251d	0.900k
	MP2	2.301r	2.552k	1.321d	0.961k
	NMP3	2.401r	2.723m	1.652g	0.991k
	MP3	2.521i	2.852m	1.792g	0.993k
LSD at 5%		2.435	1.477	1.455	1.345
P VALUE	T	0.0000	0.0001	0.0001	0.001
	S	0.0000	0.0000	0.0000	0.0000
	(T × S)	0.0026	0.0794	0.0037	0.000

Each value is a mean of five replicates. Values followed by different letters are significantly different ($p < 0.05$). P (Probability), NS (Non significant), LSD (Least Significant Test), T (Treatment), S (species).

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
ANOVA-K						
1	Replication	4	0.003	0.001	1.4097	0.2417
2	Factor A	1	0.064	0.064	115.9267	0.0000
4	Factor B	7	13.389	1.913	3473.0306	0.0000
6	AB	7	0.054	0.008	14.0799	0.0000
-7	Error	60	0.033	0.001		
	Total	79	13.543			

Coefficient of Variation: 2.00%

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
ANOVA-Mg						
1	Replication	4	0.054	0.013	1.0458	0.3913
2	Factor A	1	0.543	0.543	42.3495	0.0000
4	Factor B	7	7.714	1.102	85.9154	0.0000
6	AB	7	1.067	0.152	11.8887	0.0000
-7	Error	60	0.770	0.013		
	Total	79	10.148			

Coefficient of Variation: 12.89%

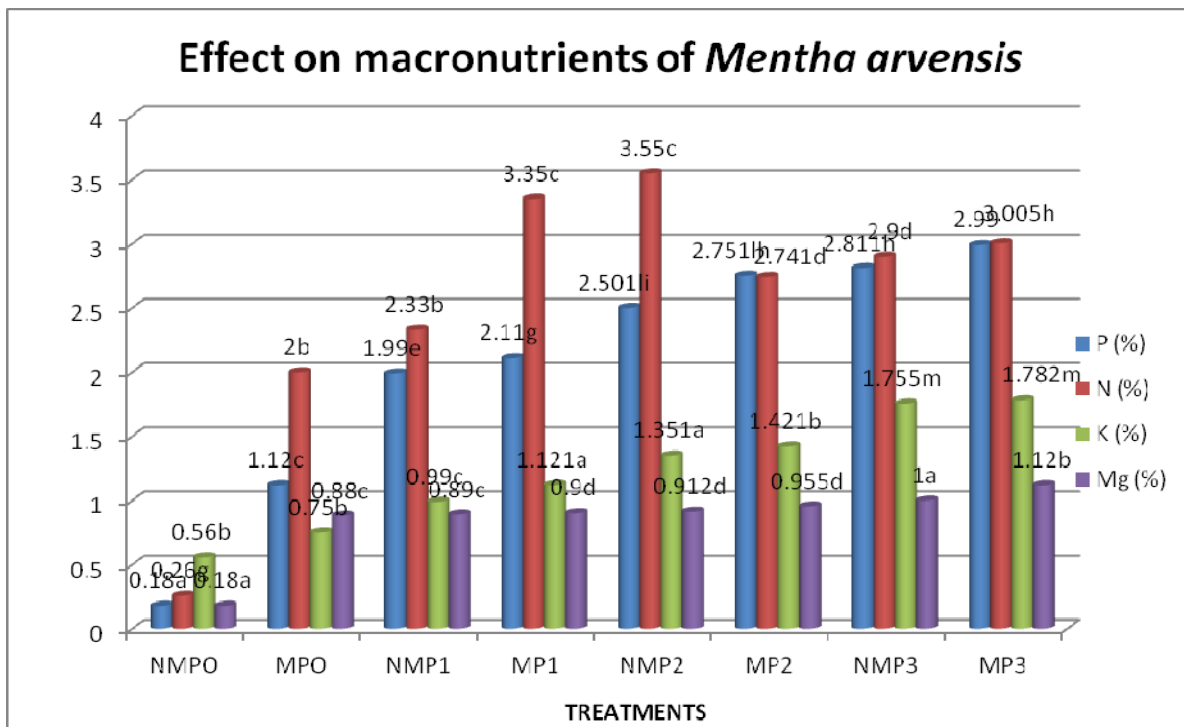


Fig. 39. Effect of AMF species and fertilizers on macronutrient acquisition of *Mentha arvensis*.

Plants were grown non-inoculated (C) or inoculated with Indigenous AMF. Plants were grown non inoculated (NM) or inoculated (M) with four levels of fertilizers (PO, P1, P2, P3)

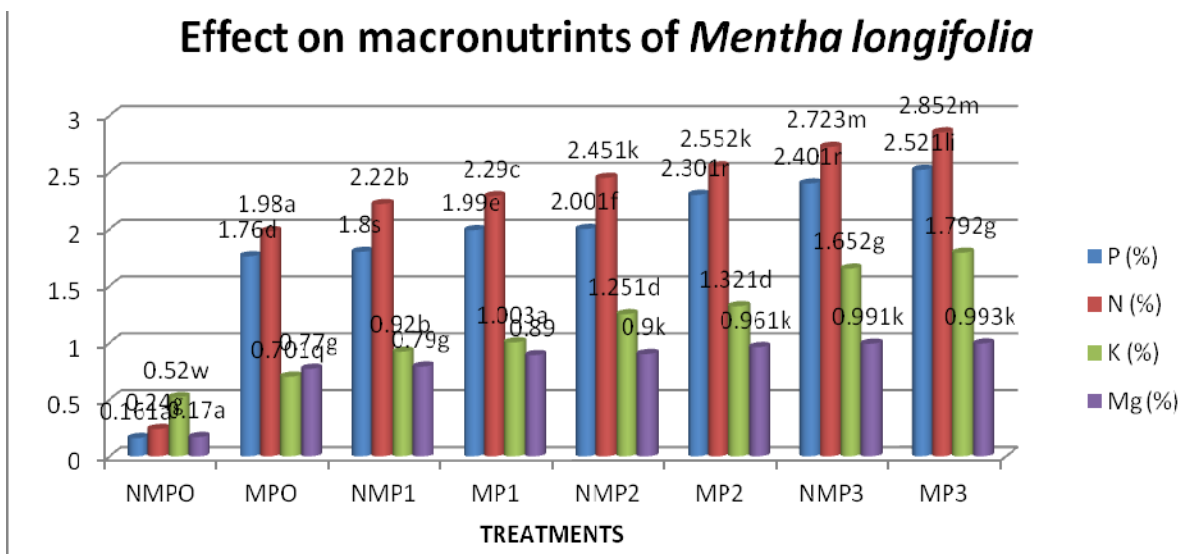


Fig. 40. Effect of AMF species and fertilizers on macronutrient acquisition of *Mentha longifolia*.

Plants were grown non-inoculated (C) or inoculated with Indigenous AMF. Plants were grown non inoculated (NM) or inoculated (M) with four levels of fertilizers (PO, P1, P2, P3)

iv. Micromineral Acquisition

Mycorrhizal symbiosis provides balanced nutrients to its host including macro and micronutrients. In the present case it was revealed that there was a positive role of AMF in the uptake of analyzed micronutrients (Fe, Cu, Zn). The findings agree with many workers, who observed improved uptake of copper (Li *et al.*, 1991; George, 2000), iron (Caris *et al.*, 1998), and zinc (Jamal *et al.*, 2002; Chen *et al.*, 2003). However, inoculation effects were significant ($P < 0.001$) (Tables 26, 27) at nil (MPO) or at low P levels (MPI), as compared to their non inoculated plants.

The results were non-significant at high levels or sometime even inhibited in the growth (Figs. 41, 42). This convergence has been attributed to the decreased mycorrhizal colonization which occurs at high P levels (Vander-Zang *et al.*, 1979; Vaast *et al.*, 1997). Jansa *et al.* (2003) observed a correlation of P and Zn uptake by maize plants colonized by *Glomus intraradices*. AM inoculated plants show enhanced acquisition of mineral nutrients with low mobility such as Zn, Cu and Fe by extending their fungal hyphae far from the rooting zone (Al-Karaki, 2000). Non-significant micronutrient uptake might be due to the fact, as explained by Gilroy & Jones (2000), that most nutrients in plants is driven by electrochemical gradient across the plasma membranes, of which a major part is induced by the H^+ - ATPase activity, which is also responsible for the uptake of Zn. This may also be true for Fe and Cu. Further as explained by Gahoonia & Nielsen (1998) and Ma *et al.* (2001) that the main sites of H^+ -ATPase activity and nutrients uptake are the root hairs, as in *Arabidopsis* that increased density extremely on P –deficient soil and might inhibited at P luxury levels.

Table 26. Effects of AMF species on micronutrient acquisition of *Mentha longifolia* and *Mentha arvensis*.

Plants were grown non inoculated (NM) or inoculated (M) with four levels of fertilizers (PO, P1, P2, P3) Treatments were tested with ANOVA.

Species	Treatments	Fe (ppm)	Cu (ppm)	Zn (ppm)	Oil%
<i>Mentha arvensis</i>	NMPO	55.20	6.200	42.50	0.50
	MPO	64.55	8.002	50.73	0.60
	NMP1	56.50	6.501.	43.52	0.78
	MP1	57.23	6.911	43.63	0.95
	NMP2	57.15	6.901	43.95	1.10
	MP2	57.50	6.311	42.11	1.30
	NMP3	57.70	6.500	43.21	2.21
	MP3	56.24	6.6 01	40.23	2.30
<i>Mentha longifolia</i>	NMPO	50.20	6.990	40.50	0.50
	MPO	61.10	5.200	46.55	0.56
	NMP1	55.75	7.5001	42.35	0.70
	MP1	56.20	5.932	43.23	0.81
	NMP2	56.99	6.512	43.99	0.90
	MP2	57.00	6.021	44.21	0.99
	NMP3	57.65	6.320	45.33	2.01
	MP3	55.25	6.751	44.56	2.30
LSD at 5%		1.334	6.801	1.433	1.245
P VALUE	T	0.0000	6.990	0.0001	NS
	S	0.0000	0.0000	0.0000	0.0000
	(T × S)	0.0026	0.0794	0.0037	NS

Each value is a mean of five replicates. Values followed by different letters are significantly different ($p < 0.05$). P (Probability), NS (Non significant), LSD (Least Significant Test), T (Treatment), S (species).

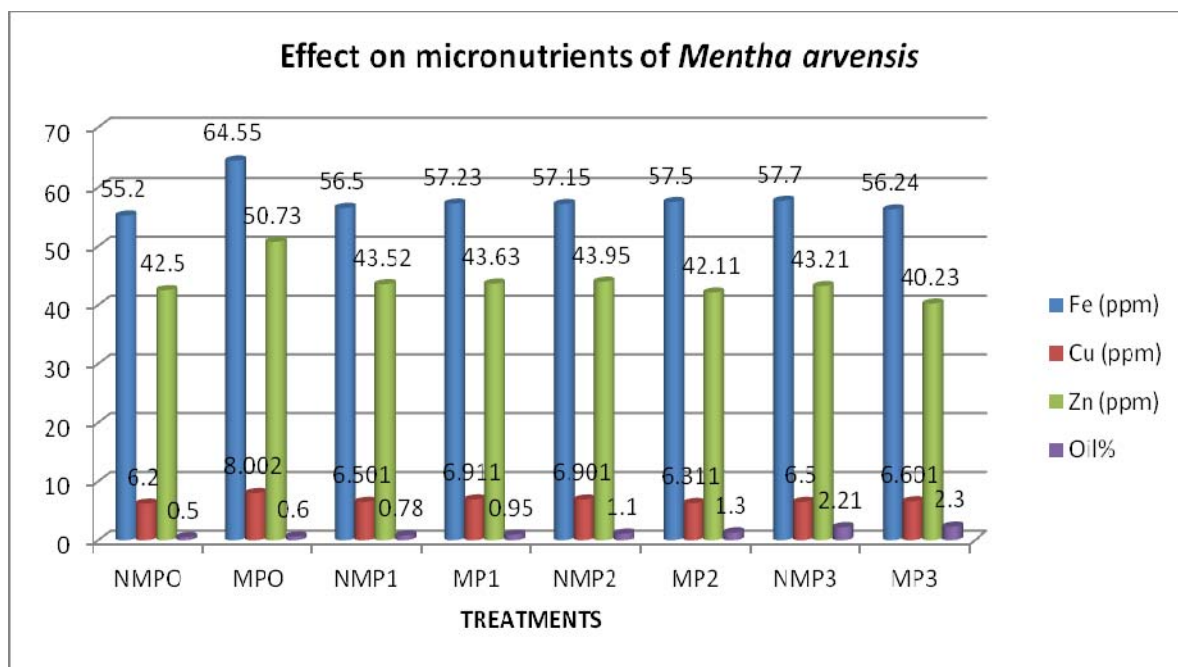


Fig. 41. Effects of AMF species on micronutrient acquisition of *Mentha arvensis*.

Plants were grown non inoculated (NM) or inoculated (M) with four levels of fertilizers (PO, P1, P2, P3)

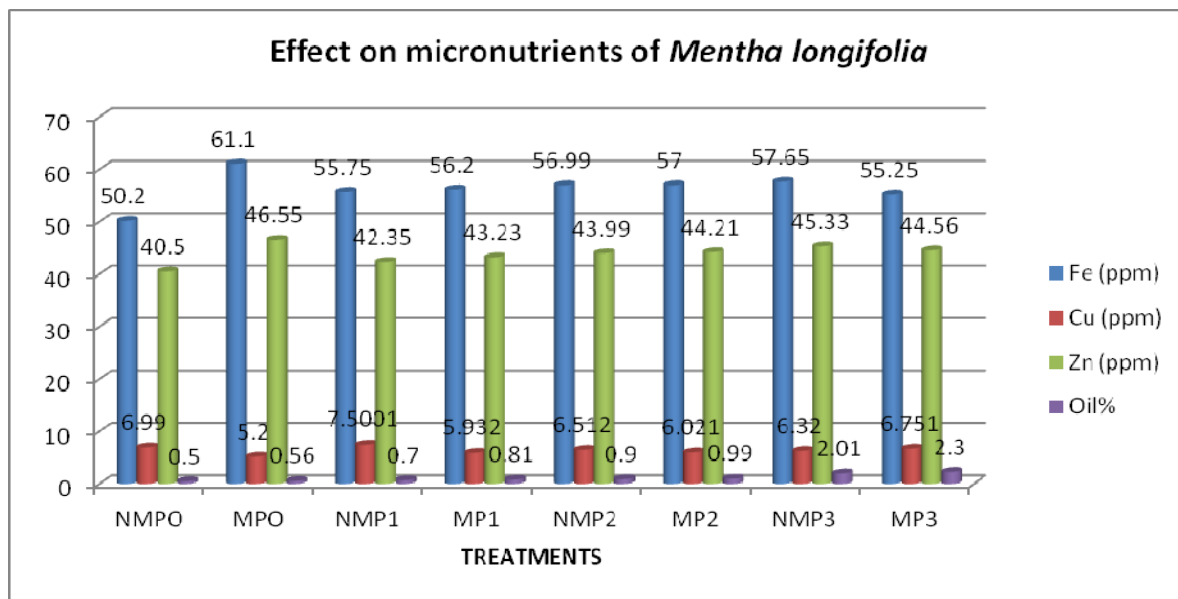


Fig. 42. Effects of AMF species on micronutrient acquisition of *Mentha longifolia*.

Plants were grown non inoculated (NM) or inoculated (M) with four levels of fertilizers (PO, P1, P2, P3)

v. Essential oils

Fertilization also influences constituents of medicinal and aromatic plants this practice also affect oil yield by enhancing the amount of biomass yield per unit area (Malik *et al.*, 2011). Our results regarding the effect of AMF and fertilizer (Table 26) revealed that at low P levels treatments significantly enhanced oil yield (%) as compared to their un-inoculated counterparts. Maximum oil yield was obtained at MP3 (2.30%) smoothly and non-significantly followed by NMP3 (2.10%) thereafter significantly followed by P2 and P1 counterparts (Fig. 43). Least figures were achieved at MPO (0.50%) in both species understudy. Generally *Mentha arvensis* outperformed *Mentha longifolia* but some values were statistically at par .our results agreed with the findings of many workers who shown that yield and composition of essential oil vary considerably on application of fertilizers (Tiwari & Banafar, 1995; Figueira, 1996).

Addition of P- fertilizers with AMF in case of *Foeniculum vulgare* has also affected the yield and composition of essential oils. Our results also agreed with El-Sherbeny *et al.* (2007) on *Ruta graveolens*, Kumar *et al.* (2009) on *Artemisia pallens* and Rehman *et al.* (2003) on *Mentha arvensis*. Our results also agreed with Prasad *et al.* (2011) who found a significant increase in the essential oil yield per plant by the AMF inoculation alone or in combination with phosphorus may be attributed to the increase in plant biomass. The main components of essential oils are terpenoids based on C_5H_8 subunits (isoprenoid). Isoprenoid biosynthesis require acetyl COA, ATP and NADPH and is dependent on inorganic P concentration in the plant (Loomis& Croteau, 1972). Kim *et al.* (1998) reported that improved P uptake in plants was due to an enhanced number of uptake sites per unit area of roots and improved ability for these roots to explore the soil for nutrients. Moreover another reason might be responsible for improved P uptake is due to improved dissolution of Ca apatite through elevated concentration of CO_2 in the roots.

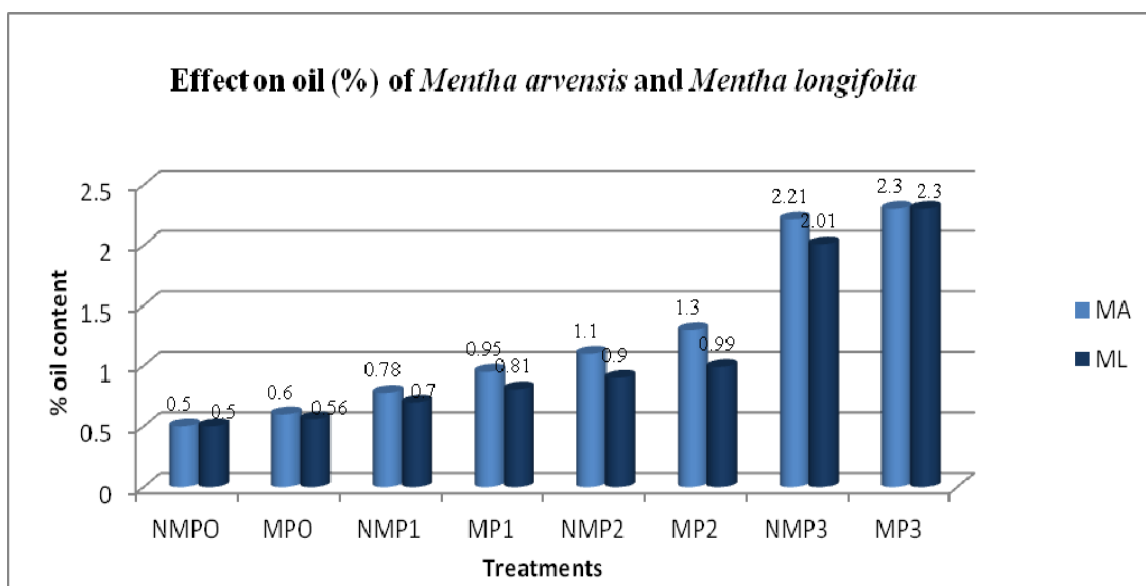


Fig. 43. Effects of AMF species on oil yield of *Mentha longifolia* and *Mentha arvensis*.

Plants were grown non inoculated (NM) or inoculated (M) with four levels of fertilizers (PO, P1, P2, P3).

vi. Effect of Fertilizers on AMF infections and the densities of AM spores

The results given in Table 28 showed the effect of various phosphorus treatments on the AMF spore density and on the root infections in the roots of *Mentha arvensis* and *Mentha longifolia*. It has been observed that with increasing fertility level the number of spore density and percentage infection decrease in both the species. High number of spore densities (361 ± 0.01 and 254 ± 1.11 /100 grams of soil) and percentage infection (86 ± 1.3 and 84 ± 0.1) were noticed at MP₀ level for *Mentha arvensis* and *Mentha longifolia* respectively. Janaki & Manoharachary (1994), Korthari & Singh (1996) and Panwar & Tarafdar (2006) also found negative correlation between AMF spores and soil phosphorus. Sharif *et al.* (2011) showed that low availability of soil phosphorus increases VAM colonization. But our results negate the finding of Chandrasekara *et al.* (2005), who did not found any significant relationship with available phosphate.

Root infections and spore densities in both species got declined sharply at high P levels and least values were encountered at MP₃ levels in both species (Figs. 44, 45). Lower colonization at higher P rates has also been reported (Muhammad *et al.*, 2003) and this might be attributed to the fact that under P-stress the plant roots release organic compounds that could enhance spore germination and colonization, while such exudation under high soil P-level is absent. AMF are usually considered to play an important role and it is assumed that they can compensate for the reduced use of P.

Regarding the effect of various fertilizer levels on the morphological parts of AM colonization results elucidated that all the parts got declined or failed to develop with increasing p levels (Table 29). Maximum percentage of external hyphae was noted at MPO level (70 ± 0.00) and (65 ± 0.24) in *Mentha arvensis* and *Mentha longifolia* respectively. Least percentage (2 ± 0.002) in *Mentha arvensis* were noticed at MP₃ levels. 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 & Bago, 2005). Similar response was also noted for other parts. Arbuscules are considered as key site where symbiotic phosphate is delivered (Yang & Paszkowski, 2011). Arbuscules were found to be most sensitive parts, either absent or poorly developed in both studied species (Figs. 46, 47).

Conclusion

In conclusion the results of present study reveals that AM fungi are effective in promoting the productivity of *Mentha* species of their role as an agent of bio-fertilizer should be exploited for improving the yield of *Mentha*. Inoculation with indigenous AMF species i.e. *Glomus*, *Asaulospora* and *Gigaspora* were found to be significantly effective in enhancing the herb yield in P-deficient soil over non inoculated plants. From these investigation it can easily be inferred that in *Mentha* species better yield can be obtained by using these natural alternatives instead of relying on expensive fertilizers. Moreover lower doses of P-fertilizers with AMF are more effective than higher levels. Due to hike in the prices of chemical fertilizers and also with a view to protect the health of soils and avoid soil pollution, scientists are now focused to compensate the use of chemical fertilizers with biofertilizers like AM fungi which would be an environment friendly approach. The use of biological tools and organisms to sustain plant growth and to maintain ecosystem balance is receiving attention. Efforts are currently focused on developing technology for the utilization of mycorrhizal fungi as bio-fertilizer.

Table 28. Root infection (%) and spore density/100gms soil of *Mentha longifolia* and *Mentha arvensis*.

Species	Treatments	Spore density/ 100gm soil	% age infection
<i>Mentha arvensis</i>	MP0 (NOP)	361±0.01	86 ± 1.3
	MP1 (25%P)	245 ± 0.12	82 ± 1.1
	MP2 (50%P)	135 ± 1.01	50 ± 0.3
	MP3 (100%P)	30 ± 1.02	40 ± 0.2
<i>Mentha longifolia</i>	MP0 (NOP)	254 ± 1.11	84 ± 0.1
	MP1 (25%P)	230 ± 2.1	80 ± 0.21
	MP2 (50%P)	161 ± 0.003	41 ± 2.3
	MP3 (100%P)	31 ± 0.21	25 ± 1.12

± Standard error

Table 29. Effects of various levels of fertilizers on AM infection Morphologies, external hyphae, internal hyphae, Arbuscules, vesicles, (%) in roots of mycorrhizal *Mentha arvensis* and *Mentha longifolia*.

Species	Treatments	External hyphae %	Internal Hyphae %	Arbuscules %	Vesicles %
<i>Mentha arvensis</i>	MP0 (NOP)	70 ± 0.00	60 ± 1.1	61 ± 0.002	40 ± 2.30
	MP1 (25%P)	50 ± 0.00	30 ± 1.1	25 ± 1.04	30 ± 0.01
	MP2 (50%P)	25 ± 0.001	20 ± 2.4	2 ± 1.88	35 ± 0.00
	MP3 (100%P)	2 ± 0.002	10 ± 1.3	*	30 ± 0.01
<i>Mentha longifolia</i>	MP0 (NOP)	65 ± 0.24	45 ± 0.12	70 ± 0.001	55 ± 0.02
	MP1 (25%P)	40 ± 0.08	20 ± 0.34	25 ± 0.01	65 ± 0.03
	MP2 (50%P)	20 ± 0.05	15 ± 0.23	3 ± 0.009	30 ± 0.06
	MP3 (100%P)	*	*	*	*

± Standard error *(absent).

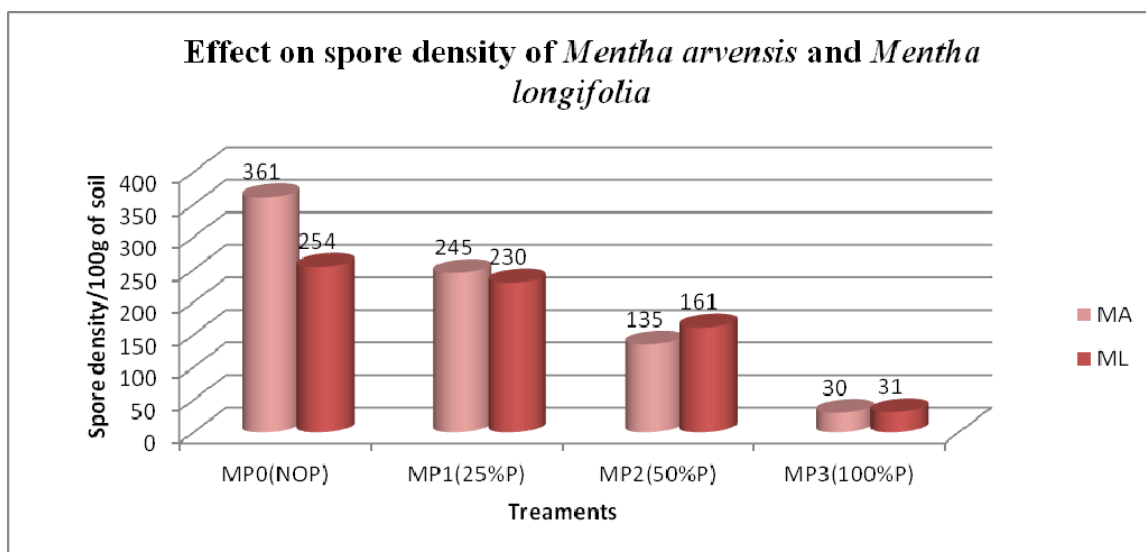


Fig.. 44. Spore density/100gms soil of *Mentha longifolia* and *Mentha arvensis*.

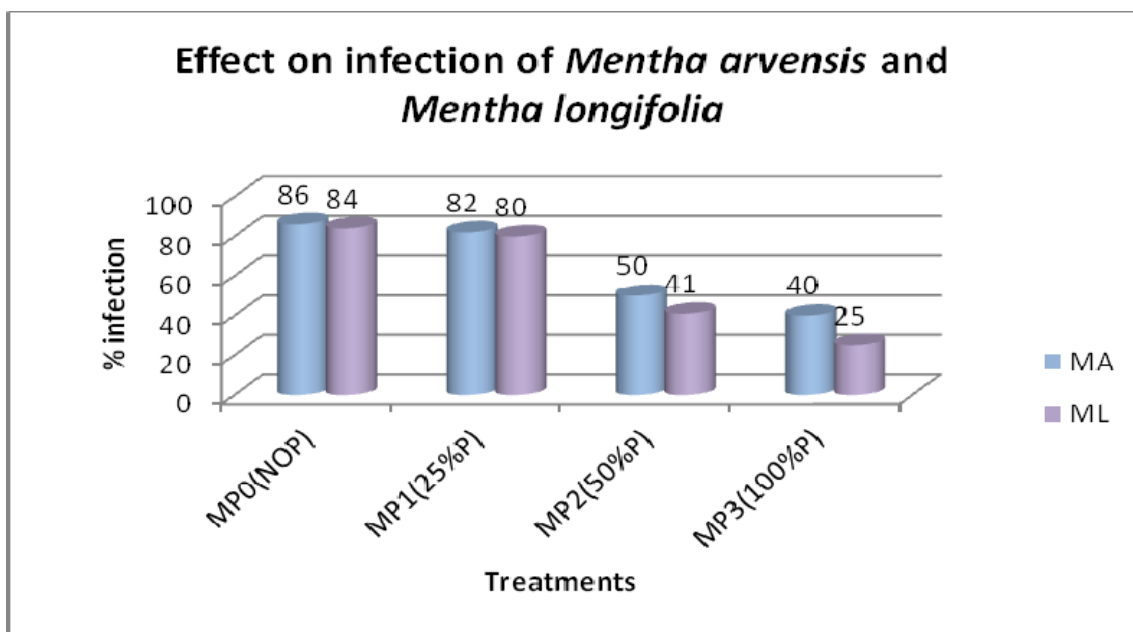


Fig. 45. Root infection in soil of *Mentha longifolia* and *Mentha arvensis*.

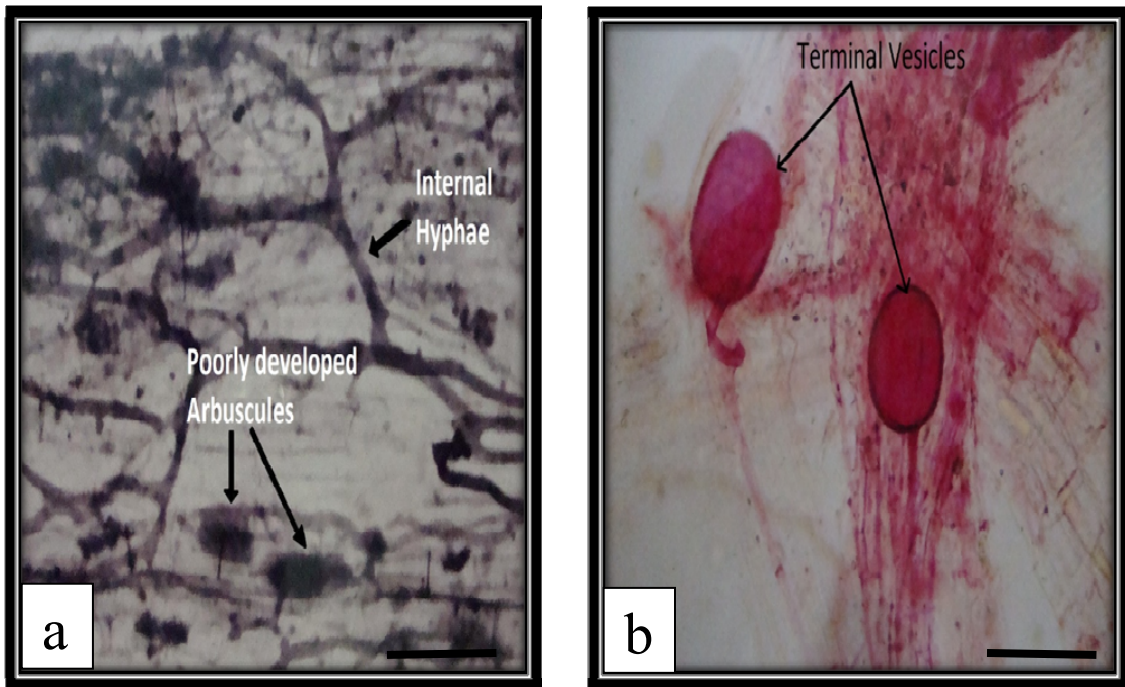


Fig. 46. a. Poorly develop arbuscules b. Terminal vesicles in roots of MP3 treated plants of *Mentha arvensis*.

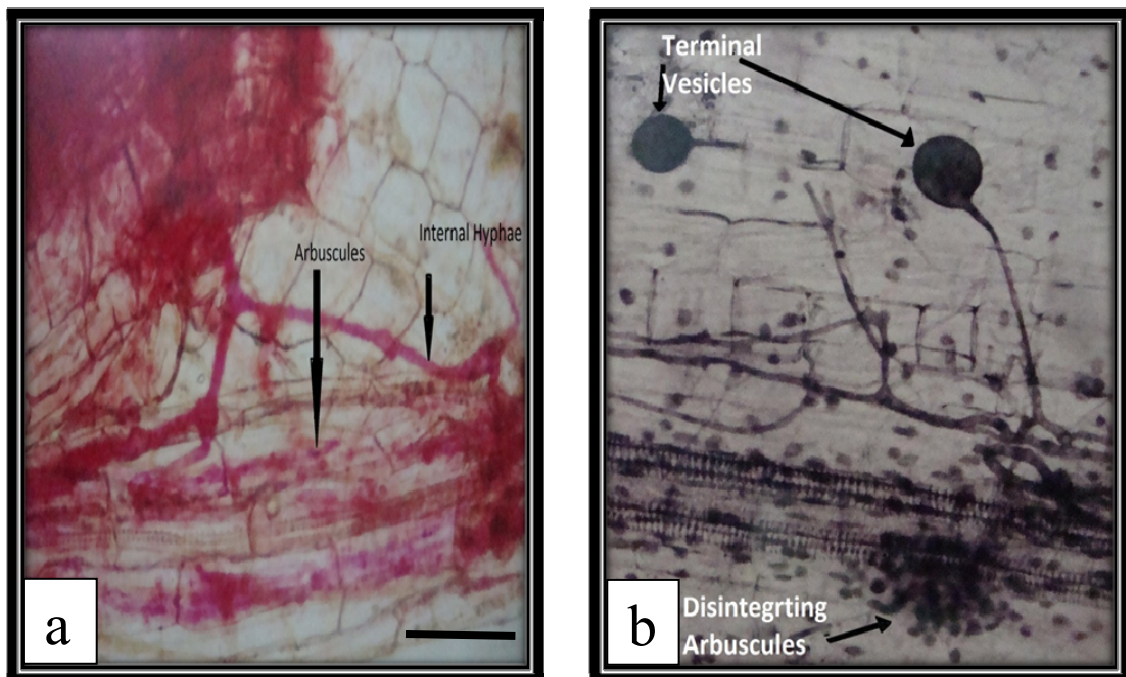


Fig. 47. a. Poorly develop b. disintegrating arbuscules in roots of MP3 treated plants of *Mentha longifolia*.

COMBINED EFFECT OF AMF SPECIES APPLICATION ROCK PHOSPHATES (RP) AND ORGANIC MATTER (FYM) FERTILIZERS ON GROWTH PERFORMANCE & CHEMICAL BEHAVIOUR OF *MENTHA ARVENSIS*.

The worlds richer grade phosphate reserves are being rapidly exhausted. Continuous efforts are being made every where for utilizing locally available impure and low grade rocks. Rock phosphate can be solubilized using micro-organisms. Throughout the world scientists are now focused in developing alternative technology involving organic farming to minimize the dependence on chemical fertilizers. The present experimental work was done with an objective to find out the effect of indigenous AMF in combination with inorganic (Rock phosphate levels and organic (Farmyard manure) fertilizer on the growth of *Mentha arvensis* .The data regarding growth performance in term of dry weight, MD values and essential oil yield (Tables 30, 31), AM infections and spore density were recorded as below.

Mycorrhizal plants performed significantly than nonmycorrhizal plants. Mycorrhizal inoculation increased dry weight (16.51gms) by 281% over non inoculated control plants (4.00 gms) at RP1 level. Then followed by RP2 level that exhibited 16.90 gms showed increase by 285% over respective control (Fig. 48). Our results agree with those of Darzi *et al.*, (2009) who reported maximum vegetative growth of fennel plants at 100% applied rockphosphate level as compared to non inoculated plants. These results are also in agreement with those obtained by Abdel Hafez & Abdel Monsief (2006) on Cantaloupe and cucumber. They found that AM inoculated plants with p fertilization significantly improved growth compared to application of each separately.

Regarding the growth responses of *Mentha arvensis* to AM inoculation with organic fertilizers with AMF (Table 30) were pronounced as compared to non inoculated counterparts. At both fertilizers levels (F1 & F2) mycorrhizal plants had better dry weight.

It was (17.90gms) at MF2 level followed by MF1 (16.90gms). Gaur & Adholeya (2002) also observed that inoculation with arbuscular mycorrhizal (AM) fungi amended with organic matter promoted shoot and root dry weights and accelerated total uptake of P and N compared to their respective control. The present findings are also in line with other workers (Caravaca *et al.*, 2004; Aryal *et al.*, 2003). Evidence indicated that AMF are capable of compensating for low inputs of P fertilizer in organic system. Kahiluoto & Vestberg (1998) found that AMF effectively at increased available P as superphosphate. However, this does not always translate into higher yields (Ryan *et al.*, 1994; Galvez *et al.*, 2001) even when phosphorous use efficiency is higher (Galvez *et al.*, 2001). Prolific AM colonisation in organic systems may even be associated with reduced yield in some cases because of the carbon drain by the AMF (Dann *et al.*, 1996).

i. Mycorrhizal dependency

Mycorrhizal dependency values of *Mentha arvensis* (Table 30) under rock phosphate treatments was significantly high (40.82%) at MRP2 level as compared to non inoculated plants (30.00%). The results are strengthened by Radovich & Habte (2009) and Yaseen *et al.* (2011) who observed that in cowpea (*Vigna unguiculata*) arbuscular mycorrhizal fungal inoculation significantly influenced the mycorrhizal dependency (MD). They found that china variety is more dependent than watni cowpea.

Among organic fertilizer treatments with AMF, highest MD value (46.92%) was observed at F2 that was statistically at par with F1 (45.45%) (Fig. 49). The increased plant growth might be due to increased rhizosphere aggregate stability that might have favored the beneficial microbes that in turn contributed to the improved biomass. (Caravaca *et al.*, 2002). Aryal *et al.*, (2003) reported higher dependence of bean plants treated with organic fertilizers as compared to plants treated with chemical fertilizers. (Oliveira *et al.*, 2006) reported that the AMF species with greater extra radical mycelium and root branching determines plant dependence on the symbiosis. The relationship between P level and mycorrhizal dependence has also been confirmed for a wide range of plant species (Menge *et al.*, 1978; Krishna & Bagyaraj, 1982; Saif, 1987).

ii. Oil yield

Fertilization also influences the volatile oil contents and constitution of medicinal and aromatic plants. The production of essential oils not only depends upon the metabolic state and preset developmental differentiation programme of the synthesizing tissue, but also is highly integrated with the physiology of the whole plant (Malik *et al.*, 2011). Data regarding oil yield in rockphosphate and AMF treated plants are given in (Table 30). Minimum oil yield was noticed at MPO level (0.53%) followed by increased in MRP1 (0.66%) and MRP2 (0.70%). The results revealed the positive response of *Mentha arvensis* to AMF inoculation at both applied levels of rock phosphates. In large scale production of plants containing essential oils even relatively small increase of their essential oil contents can result in enhanced product yield of economic interest. Thus any agricultural practice or treatment increasing essential oil level is of practical interest, (Khaosaad *et al.*, 2006).

The results are also in accordance with Abou-Zeid *et al.* (2005) who reported that the volatile oil yield of fennel increased due to using rock phosphate with AMF.

Results regarding the combine effect of AMF and essential oil yield of *Mentha arvensis* are given in (Fig. 50). Maximum oil yield (1.8%) was obtained of MF2 level statistically at par with MFI level (1.7%). Minimum figure (0.53%) was noticed at non-inoculated control our results showed the promoting effects of combined treatment of AMF with organic fertilizer. Our results agreed with Tanu *et al.* (2004) also treated the positive effect of different organic manures/composts on the herbage and essential oil yield of varieties of *Cymbopogon winterianus*.

Table 30. Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on dry weight (gms) and oil yield (%) of *Mentha arvensis*.

Treatments	Dry weight (gms)	Oil yield (%)	MD value %
NM	4.001a	0.53a	
M	6.00b	0.56a	38a
NMRP1	10.50c	0.59a	
MRP1	16.51d	0.66b	37a
NMRP2	10.71c	0.70b	
MRP2	16.90d	0.80c	40.82b
NMF1	9.01e	1.20d	
MF1	16.90d	1.5e	45.45
NMF2	9.5e	1.7f	
MF2	17.90d	1.8f	46.92

Each value is a mean of five replicates. Values followed by different letters are significantly different ($p < 0.05$). P (Probability), NS (Non significant), LSD (Least Significant Test), T (Treatment).

M (mycorrhizal no fertilizer) NM (Non Mycorrhizal), MRP1 (50% rockphosphate) MRP2 (100% rockphosphate) MF1 (50% Farmyard Manure) MF2 (100% Farm yard manure).

Table 31. ANOV for Dry weight

Source	Degree of Freedom	Sum of Squares	Mean Square	F-value	Prob
Replications	4	15.96	5.322	1.42	0.2643
Treatments	9	84.06	12.008	3.21	0.0179
Error	21	78.56	3.741		
Total	31	178.58			
Oil yield					
Replications	4	0.83	0.276	0.83	0.4930
Treatments	9	3.52	0.503	1.51	0.2181
Error	21	6.99	0.333		
Total	31	11.34			

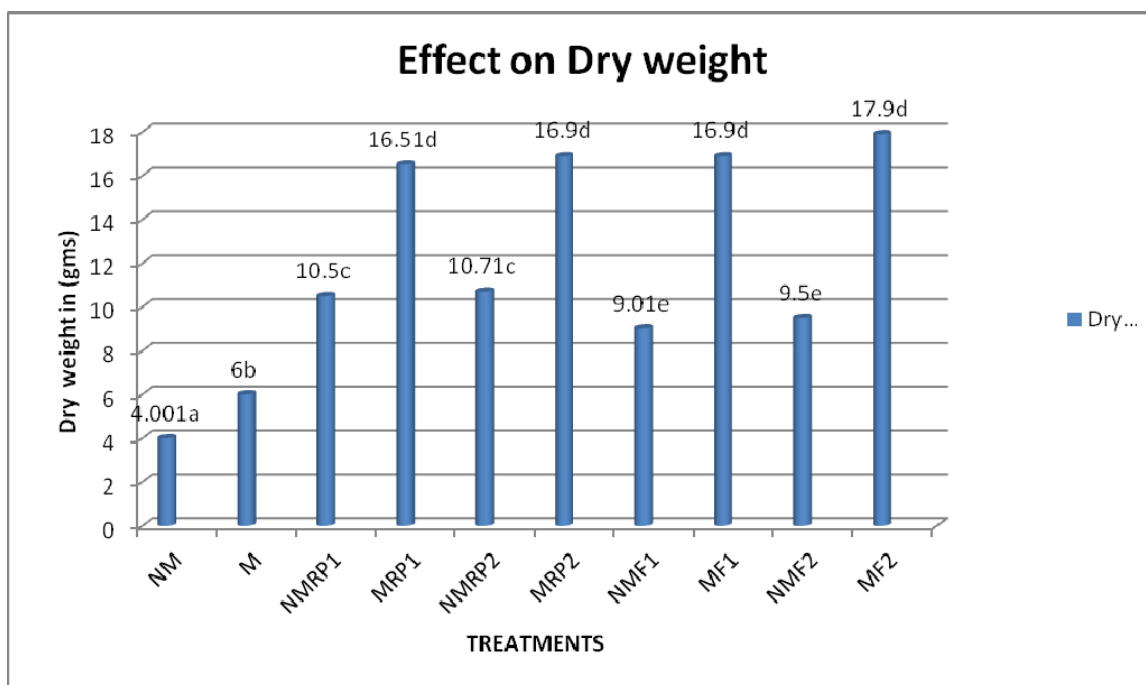


Fig. 48. Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on dry weight (gms) of *Mentha arvensis*.

M (mycorrhizal no fertilizer) NM (Non Mycorrhizal), MRP1 (50% rockphosphate) MRP2 (100% rockphosphate) MF1 (50% Farmyard Manure) MF2 (100% Farm yard manure).

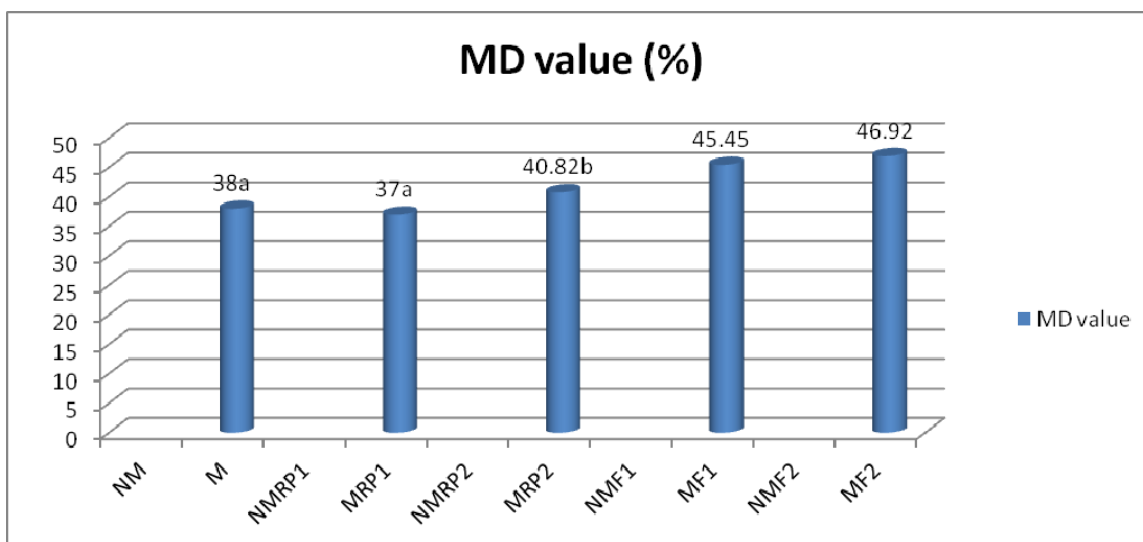


Fig. 49. Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on Mycorrhizal dependency (gms) of *Mentha arvensis*.

M (mycorrhizal no fertilizer) NM (Non Mycorrhizal), MRP1 (50% rockphosphate) MRP2 (100% rockphosphate) MF1 (50% Farmyard Manure) MF2 (100% Farm yard manure).

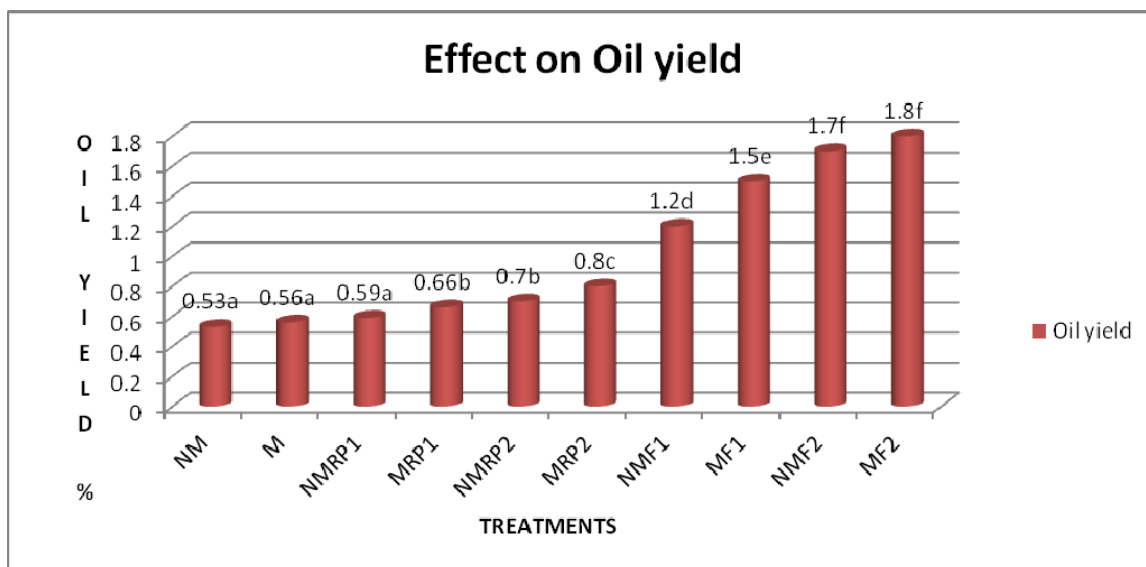


Fig. 50. Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on oil yield (%) of *Mentha arvensis*.

M (mycorrhizal no fertilizer) NM (Non Mycorrhizal), MRP1 (50% rockphosphate) MRP2 (100% rockphosphate) MF1 (50% Farmyard Manure) MF2 (100% Farm yard manure).

Nutrient acquisition

Regarding nutrients uptake by mycorrhizal plants it was observed that there was significant ($p > 0.005$) enhancement in macro nutrients (P, N, K, Mg) (Tables 32, 33; Fig. 51) and micronutrients (Fe, Cu, and Zn) (Tables 34, 35; Fig. 52) all tested in mycorrhizal as compared plants to non treated plants at both RP levels. The present results are also in harmony with Wani & Konde (1998) who observed that, applied rock phosphate enhanced dry weight and nutrient uptake in garlic. Abou El- Salehein & Ahmed (1998) stated that applied rock phosphate enhanced the dry matter accumulation. Sharif *et al.* (2011) reported that millet crop inoculated with AMF enhanced root and shoot dry matter by 21% and 20% respectively. Sabnavar & Lakshman (2009) also concluded that AMF promoted the growth and nutrient uptake of varieties of sesamum at high RP levels (100%). Zaki *et al.* (2010) indicated the combined effect of AMF and RP caused significant increase in growth and chemical contents at 100% rock phosphate in *Foeniculum vulgare*.

This growth promotion and nutrient uptake can be attributed to the fact that mycorrhizal fungi are capable of dissolving weakly soluble soil minerals, especially phosphate by releasing acids or increasing CO₂ partial pressure. Therefore, they have the ability to enhance host plant uptake of relatively immobile nutrients particularly P and Zn (Thompson, 1987). Available literature also support our results (Caravaca *et al.*, 2004; Zaki *et al.*, 2011; Darzi *et al.*, 2009).

AM fungi can be very efficient in exploiting rock phosphate (Barea *et al.*, 2002; Duponnois *et al.*, 2005). Mechanisms for mobilization of P may include lowering of pH in the growth substrate (Son *et al.*, 2006), excretion of α -ketoglutaric acid (Duponnois *et al.*, 2005), or phosphatases excretion. The excretion of phosphatases as a mobilization mechanism for hydrolyzing organic phosphates is similar for plants and AM fungi (Joner & Johansen, 2000; Koide & Kabir, 2000), rock phosphate can be solubilized by acid phosphatases. The advantage of mycorrhizal roots compared to non-mycorrhizal roots is that the hyphae exploit a higher soil volume than the root by penetrating soil pores that are not reachable by plant roots (Drew *et al.*, 2003). Mycorrhizal external hyphae extend the absorptive surface area of the root and help in the take up of mobilized P quickly before it is bound again. Some bacteria associated with AM spores such as *Bacillus pabuli* have the ability to enhance AMF root colonization therefore nutrient mobilization (Xavier & Germida, 2003).

Table 32. Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on macronutrient acquisition (%) of *Mentha arvensis*.

Treatments	P (%)	N (%)	K (%)	Mg (%)
NM	0.16	0.230	0.130	0.10
M	1.00	1.63	0.350	0.55
NMRP1	1.10	1.78	0.480	0.65
MRP1	1.30	1.85	0.68	0.70
NMRP2	1.50	1.90	0.75	0.80
MRP2	1.61	1.99	0.90	0.82
NMF1	1.62	1.85	0.70	0.73
MF1	1.85	1.90	0.85	0.85
NMF2	1.88	1.95	0.90	0.95
MF2	1.99	2.00	0.97	0.96

Each value is a mean of five replicates. Values followed by different letters are significantly different ($p < 0.05$). P (Probability), NS (Non significant), LSD (Least Significant Test), T (Treatment).

M (mycorrhizal no fertilizer) NM (Non Mycorrhizal) MRP1 (50% rockphosphate) MRP2 (100% rockphosphate) MF1 (50% Farmyard Manure) MF2 (100% Farmyard manure).

Table 33. ANOVA FOR MACRONUTRIENTS

P Analysis of Variance						
K value	Source	Degree of freedom	Sum of squares	Mean square	F value	Prob
1	Replication	4	0.10	0.024	0.41	0.8004
2	Treatments	9	2.76	0.307	5.17	0.0002
-3	Error	36	2.13	0.059		
	Total	49	4.99			
N Analysis of Variance						
1	Replication	4	0.88	0.034	0.56	0.500
2	Treatments	9	1.76	0.309	3.12	0.0001
-3	Error	36	2.19	0.043		
	Total	49	5.22			
K Analysis of Variance						
1	Replication	4	0.22	0.099	0.87	0.000
2	Treatments	9	1.99	0.200	4.99	0.000
-3	Error	36	1.22	0.098		
	Total	49	3.96			
Mg Analysis of Variance						
1	Replication	4	0.10	0.011	0.34	0.004
2	Treatments	9	2.76	0.304	5.20	0.000
-3	Error	36	2.13	0.076		
	Total	49	2.88			

Table 34. Effect of two levels of Rock Phosphate (RP) fertilizers with AM (M) and without AM (NM) on micronutrient acquisition of *Mentha arvensis*.

Treatments	Fe (ppm)	Cu (ppm)	Zn (ppm)
NM	51.00	5.25	40.26
M	55.00	6.12	45.30
NMRP1	57.21	6.75	41.20
MRP1	57.98	6.85	42.30
NMRP2	58.90	7.00	43.36
MRP2	59.00	7.50	43.90
NMF1	56.00	6.50	41.25
MF1	56.50	6.70	42.60
NMF2	56.80	6.85	43.70
MF2	56.90	6.99	43.99

Each value is a mean of five replicates. Values followed by different letters are significantly different ($p < 0.05$). P (Probability), NS (Non significant), LSD (Least Significant Test), T (Treatment).

M (mycorrhizal no fertilizer) NM (Non Mycorrhizal) MRP1 (50% rock phosphate) MRP2 (100% rockphosphate) MF1 (50% Farmyard Manure) MF2 (100% Farmyard manure).

Table 35. ANOVA Micronutrients

Zn ANOVA Micronutrients						
K value	Source	Degree of freedom	Sum of squares	Mean square	F value	Prob.
1	Replication	4	120.78	30.194	1.48	00.2273*
2	Treatments	9	758.36	84.262	4.14	0.0010*
-3	Error	36	732.31	20.342		
	Total	49	1611.45			

Cu Analysis Of Variance						
1	Replication	4	0.50	0.124	0.19	0.9438
2	Treatments	9	44.64	4.960	7.45	0.0000**
-3	Error	36	23.97	0.666		
	Total	49	69.12			

Fe Analysis Of Variance						
1	Replication	4	628.200	157.050	0.8513	
2	Treatments	9	9743.300	1082.589	5.8686	0.0001*
-3		36	6641.000	184.472		
	Total	49	17012.500			

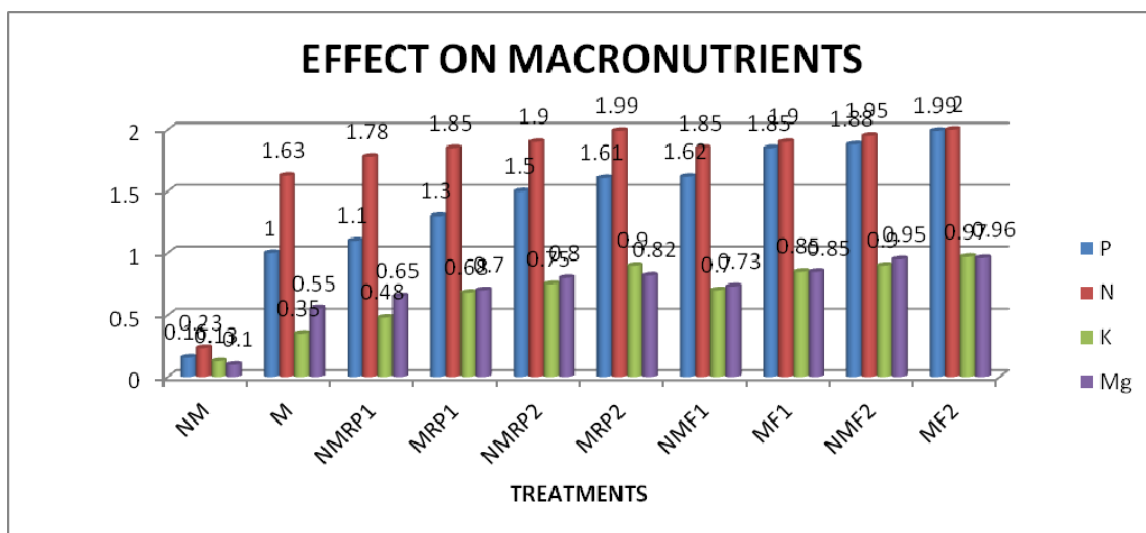


Fig. 51. Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on macronutrients of *Mentha arvensis*.

M (mycorrhizal no fertilizer) NM (Non Mycorrhizal), MRP1 (50% rockphosphate) MRP2 (100% rockphosphate) MF1 (50% Farmyard Manure) MF2 (100% Farm yard manure).

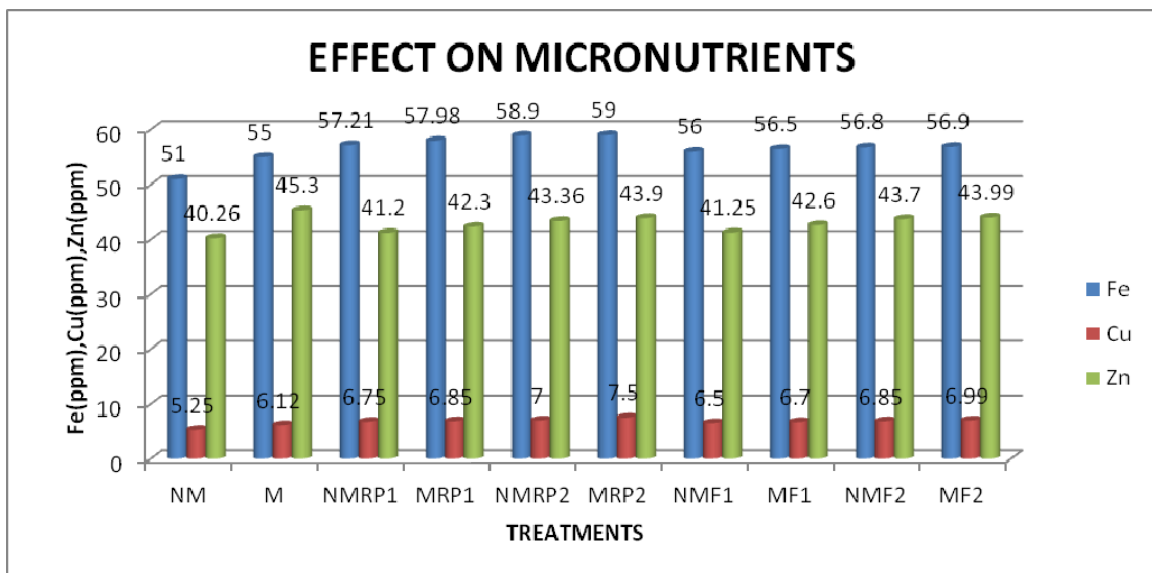


Fig. 52. Effect of two levels of Rockphosphate (RP) fertilizers with AM (M) and without AM (NM) on micronutrients of *Mentha arvensis*.

M (mycorrhizal no fertilizer) NM (Non Mycorrhizal), MRP1 (50% rockphosphate) MRP2 (100% rockphosphate) MF1 (50% Farmyard Manure) MF2 (100% Farm yard manure).

iii. Effect on AMF infections and spore densities

Table 36; Fig. 54 showed that applied rockphosphate treatments had higher colonization (77.89%) especially at RP2 (100%), followed by (70.51%) at RP1. Maximum (82.21%) colonization was exhibited at M (no fertilizer). Rock phosphate treatments exerted positive effects on AM colonization. The development of symbiotic efficient parts, external hyphae and arbuscules showed higher percentages and intensity (Figs. 55, 56). Graham & Timmer (1985) and Manjunath *et al.* (1989) stated that rock phosphates slowly released P with beneficial effect on AMF colonization and activity.

During present investigation, it was seen that in organic fertilizer treatments highest AMF colonization percentage (95.23%) was noticed at MF2 (100% FYM), followed by least (80.88%) at F2 (50% FYM) level as compared to MPO (control). Similar trend was also noticed in spore density. Joner (2000) observed that FYM had less adverse effects on AM than NPK fertilizers. Similar findings have been reported by many workers (Gour & Adholeya, 2002; Aryal *et al.*, 2003; Linderman & Davis, 2004). On the contrarily, Javaid & Riaz (2008) and Hameeda *et al.* (2007) found negative response of organic matter on root colonization of maize and soghum, respectively. AMF are usually play an important role by compensating for the reduced use of P fertilizers (Galvez *et al.*, 2001).

Prolific AM colonization in organic systems may even be associated with reduced yield in some cases because of the carbon drain by the AMF (Dann *et al.*, 1996). Muthukumar & Udaiyan (2002) reported the higher AMF production cowpea under organic farming than in the non-amended soils. They also reported that the mycorrhizal response highly correlated with manure type. Other worker also agreed with our findings. (Kumar *et al.*, 1995; Harinikumar *et al.*, 1990; Ryan *et al.*, 1994). Our results of present investigation (Table 36; Fig. 53) revealed the spore density range from 150 -190/100gm soil at various low and high applied levels of rock phosphate and FYM. Tanu *et al.*, 2004 also reported the stimulatory effects of various organic amendments on AMF spore number in soil. Maximum spore number were recorded in leaf compost treatments. Regarding the effects on morphological parts of AMF colonization we found a very rich and intensified development of external hyphae,

internal hyphae, arbuscules and vesicles (Figs. 57, 58). Gryndler *et al.* (2002) found the enhancement in mycelium length in organic fertilizer. Aryal *et al.* (2006) found that arbuscular formation is always high in organic fertilized soils than in chemical fertilized plants. Our results are also favoured by (Galvan *et al.*, 2009).

Table 36. Effects of various levels of fertilizers on AM infection (%), spore density, external hyphae, internal hyphae, Arbuscules and vesicles (%) in roots of mycorrhizal *Mentha arvensis*.

Treatments	AM infection (%)	Spore density / 100gms soil	External hyphae (%)	Internal hyphae (%)	Arbuscules (%)	Vesicles (%)
M	82.21 $\pm$ 0.001	150 $\pm$ 0.001	50 $\pm$ 0.02	60 $\pm$ 0.00	61 $\pm$ 1.00	40 $\pm$ 00.9
MRP1	70.51 $\pm$ 0.004	160 $\pm$ 1.01	70 $\pm$ 0.05	60 $\pm$ 0.01	65 $\pm$ 0.001	50 $\pm$ 0.1
MRP2	77.89 $\pm$ 0.02	165 $\pm$ 2.11	65 $\pm$ 1.50	45 $\pm$ 00.8	70 $\pm$ 1.8	55 $\pm$ 0.2
MF1	80.88 $\pm$ 0.04	170 $\pm$ 0.03	80 $\pm$ 0.06	70 $\pm$ 0.6	75 $\pm$ 1.2	65 $\pm$ 0.33
MF2	95.23 $\pm$ 0.04	190 $\pm$ 1.23	80 $\pm$ 0.01	75 $\pm$ 0.8	80 $\pm$ 1.30	70 $\pm$ 0.11

$\pm$ Standard error

M (no fertilizer), MRP1 (50% rock phosphate) MRP2 (100% rockphosphate).

MF1 (50% Farmyard Manure) MF2 (100% Farmyard manure).

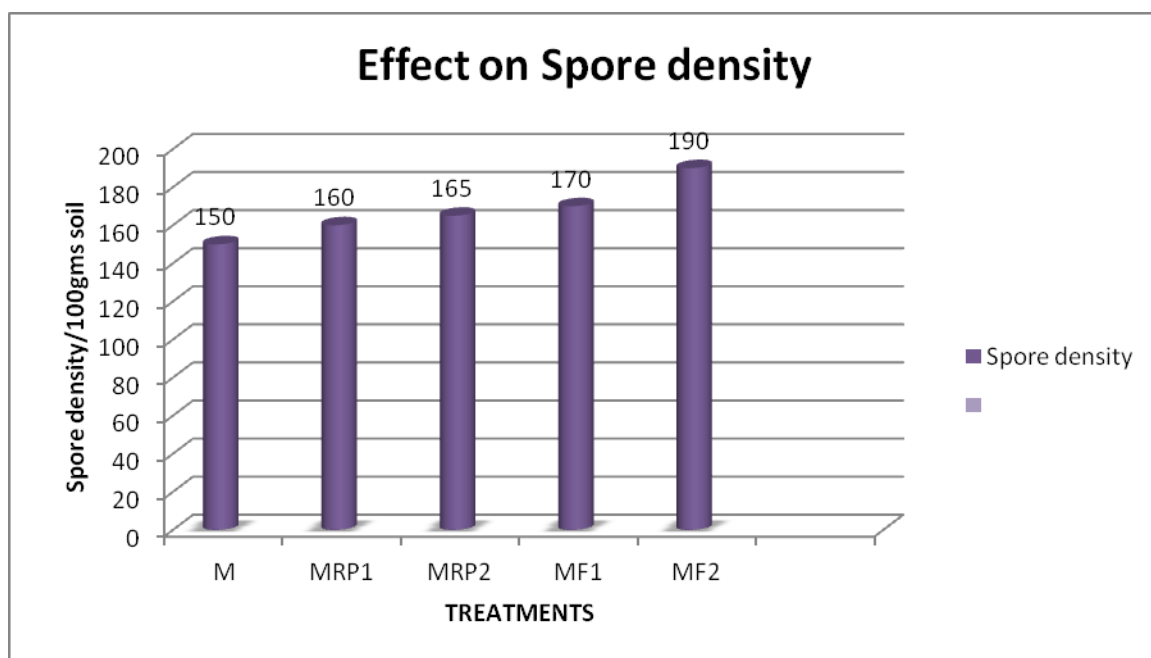


Fig. 53. Effects of various levels of fertilizers on spore density in *Mentha arvensis*.

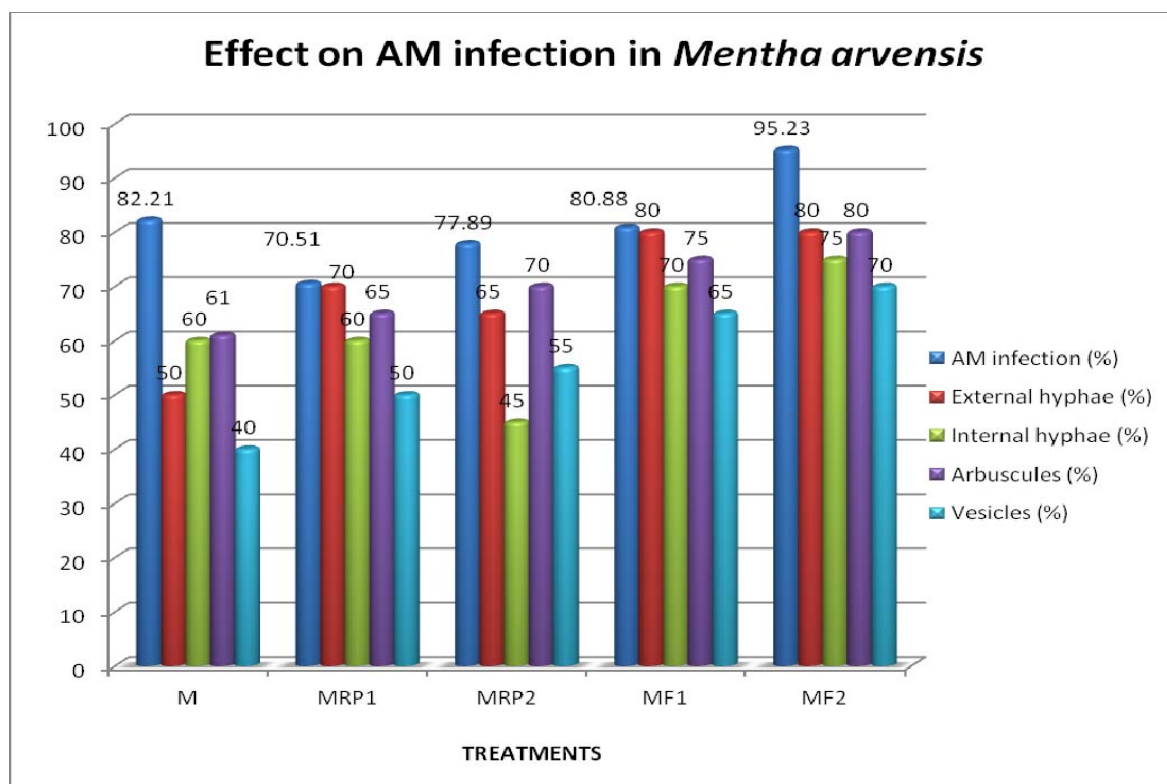


Fig. 54. Effects of various levels of fertilizers on AM infection (%), external hyphae, internal hyphae, Arbuscules and vesicles (%) in roots of mycorrhizal *Mentha arvensis*.

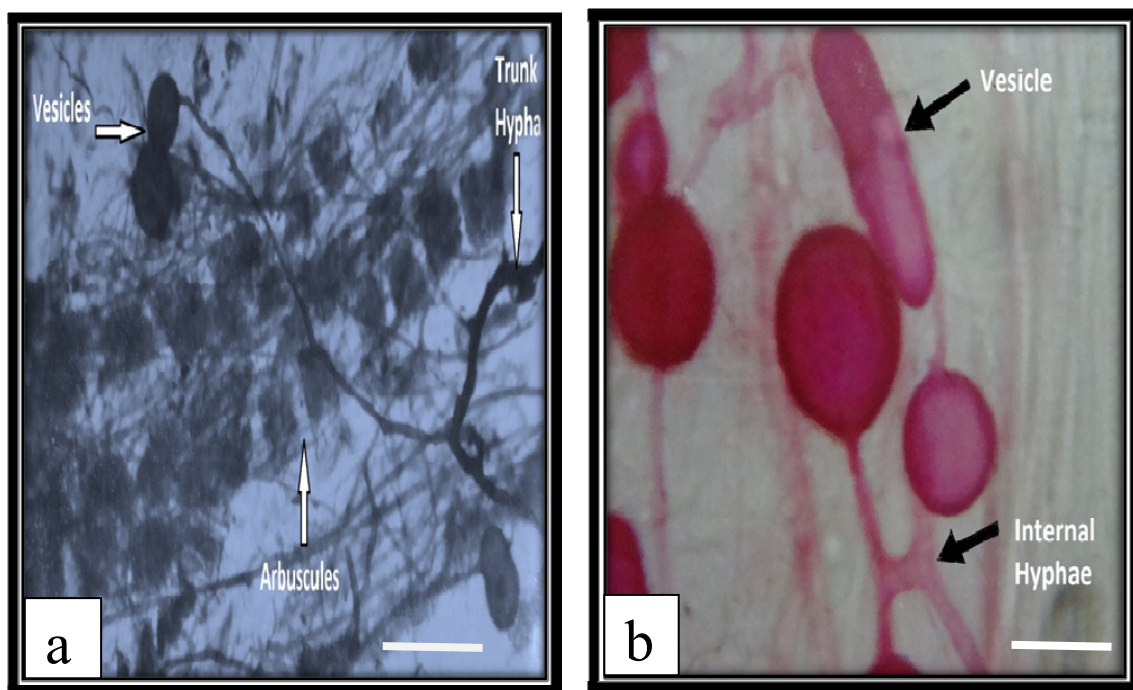


**Fig. 55 a, b. Heavy AM infection in RP1 treated plants of *Mentha arvensis*.
Bar = 10μm**

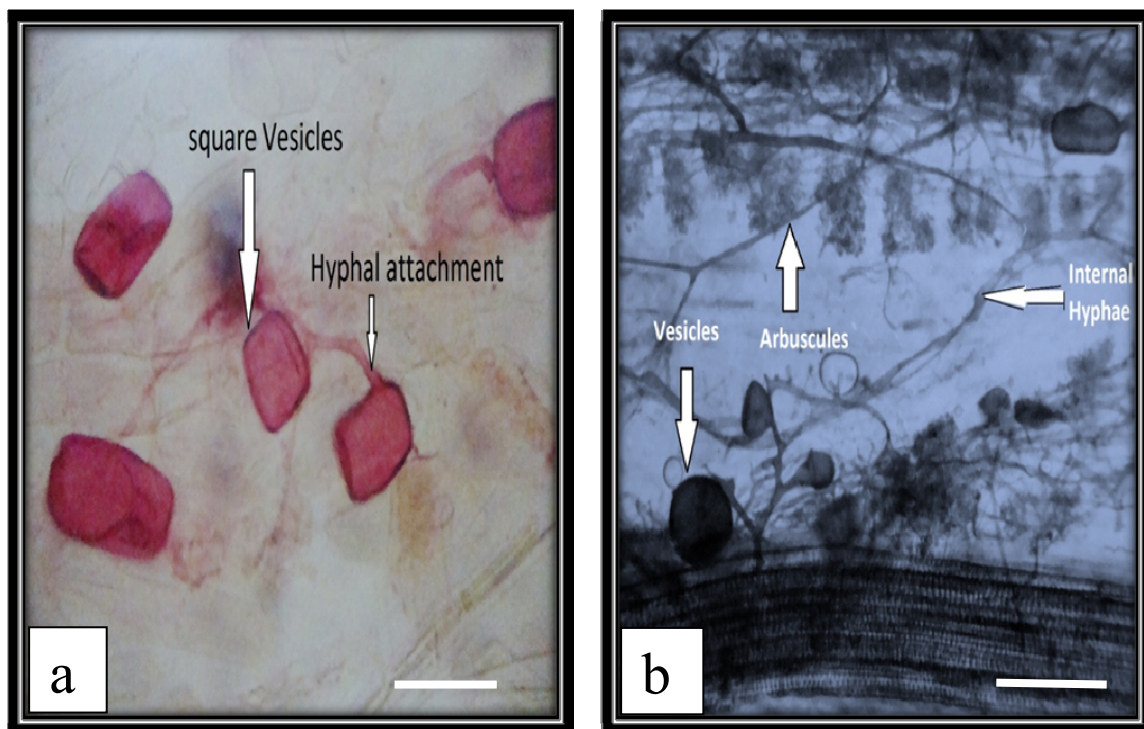


**Fig. 56 a, b. Heavy AM infection RP3 treated plants of *Mentha arvensis*.
Bar = 10μm**

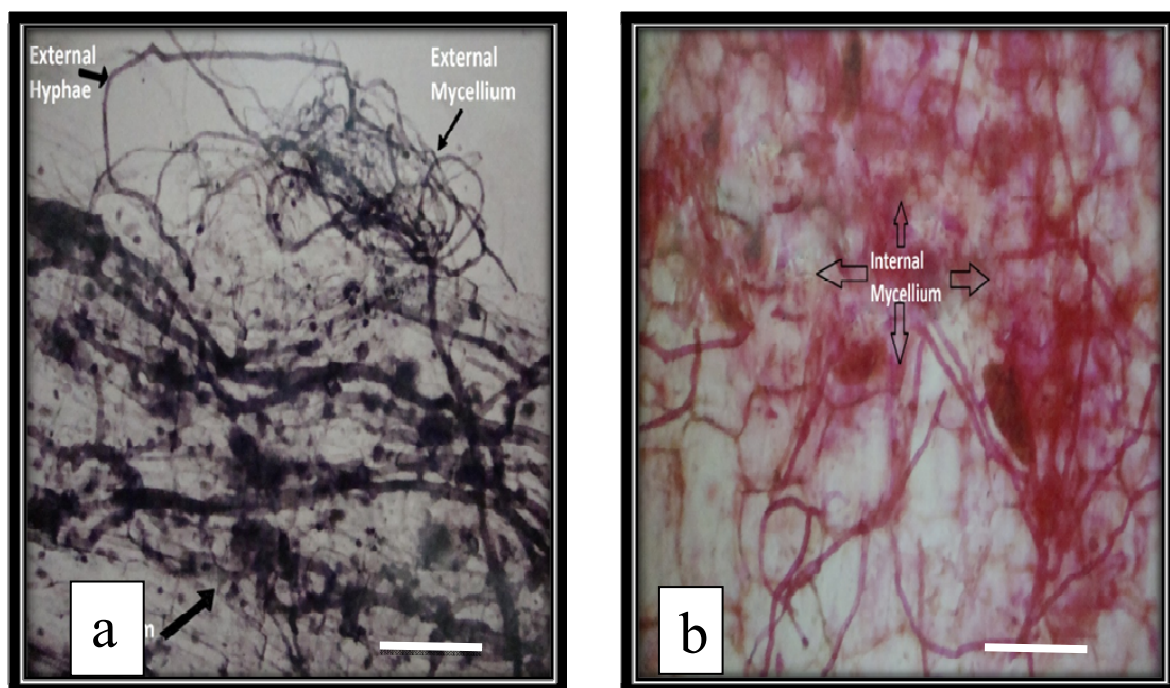


Fig. 57 a, b. Heavy AM infection in F1 treated plants of *Mentha arvensis*.
Bar = 10 μ m

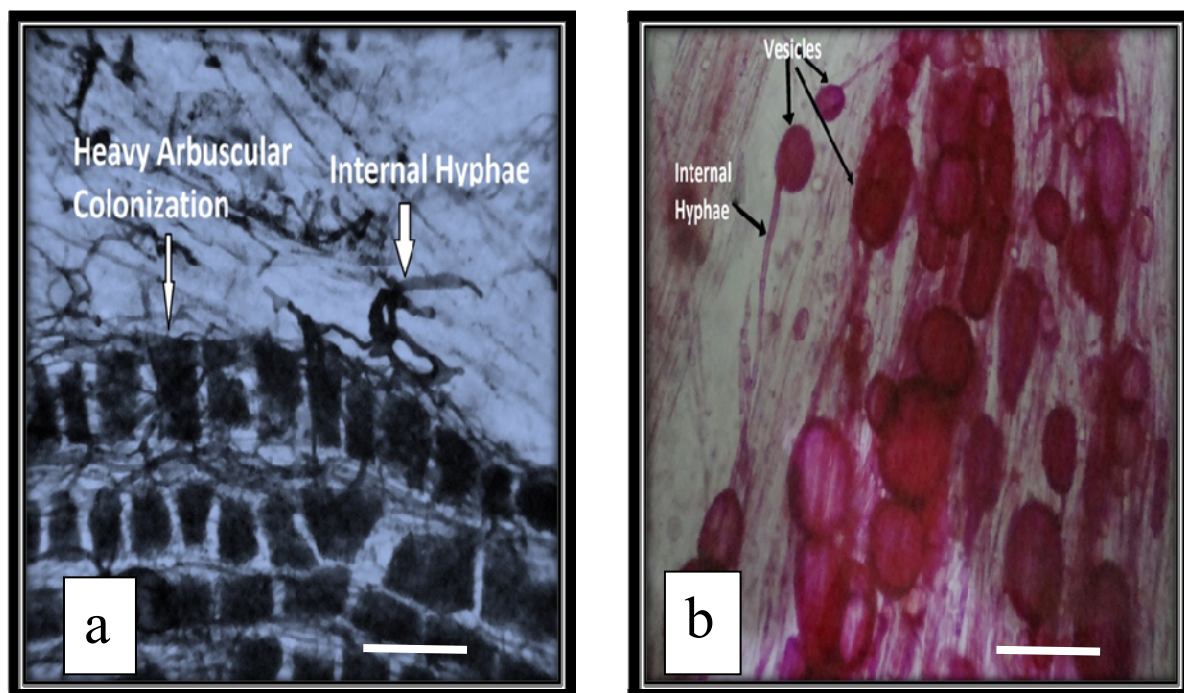


Fig. 58 a, b. Heavy AM infection in F2 treated plants of *Mentha arvensis*.
Bar = 10 μ m

CONCLUSIONS

Findings revealed the effective performance of indigenous AMF fungi on growth, oil yield and biological mobilization of nutrients in *Mentha arvensis* in combination with natural alternatives (Rockphosphate and organic matter). Results suggest that AMF species have abilities to weather rock phosphate and selected native fungi (species of *Glomus*, *Acaulospora* and *Gigaspora*) were efficient to mobilize and provide nutrients for improved oil yield. It is known that these fungi are explore in more area of soil than roots alone and are expert for nutrients greater area. Moreover, the use of indigenous mixture of AMF might improve the chance of including one very effective fungal isolate, but also create a more favourable environment for the development of ecosystem processes and it would be better strategy to improve plant growth in nutrient deficient soil.

Repeated use of bio-fertilizer leads to build up of agriculturally useful microbial population in soil which helps in maintaining soil fertility. Use of biofertilizers (AMF) production is gaining momentum as they are environmentally friendly as compared to chemical fertilizers. Bio fertilizer remain in soil, multiply and benefit crops. They are not lost due to depletion as chemical fertilizers. Being biological their effect is spectacular as compared to chemical fertilizers.

CONCLUSIONS

Following conclusion could be drawn from the present investigation.

1. It can be concluded that all studied 15 medicinal plants of family lamiaceae were positively mycotrophic, enjoying the symbiotic partnership. *Mentha* species *Mentha arvensis* and *Mentha longifolia* showed heavy AMF colonization and AMF species richness in the rhizospheric soil.
2. In conclusion the results of the experimental work revealed that AM fungi are effective in growth promotion and essential oil yield in nutrient deficient soils.
3. Native AMF (species of *Glomus*, *Acaulospora* and *Gigaspora*) fungi in multiple inocula are better than monospecific foreign strains for maximizing the productivity of medicinal herbs.
4. Moreover from the results of the present research it is concluded that lower doses of phosphorus fertilizer with AMF are effective than its higher levels.
5. Rock phosphate is cheap and within the reach of poor farmers of undeveloped country like Pakistan. For the results of present investigations it is concluded that AM fungi holds promise as an efficient agent of utilizing indigenous rock phosphate resources.
6. Use of AMF would be an integral part of organic farming ensure sustainable development not only in terms of production of medicinal herbs, but also in ecological and socio-economic parameters.

RECOMMENDATIONS

1. Considering the importance of AM it is essential that more attention should be given on soil monitoring and development of endophytes during the process of cultivation. The present investigation suggests the possibility of AMF application in order to maximize this herb production and quality.
2. The soils of Pakistan are phosphorus deficient; therefore our farmers hesitate to grow many economically important crops which demand costly input of P-fertilizer.

It is recommended that AMF could be exploited as biofertilizer instead of using synthetic chemicals. It is most important and need of the hour to develop bio-friendly (AM) technology to increase the active ingredients in medicinal plants.

3. It is suggested that inoculation of medicinal plants with mixed AMF inocula should be adapted. Their use is also useful for soil health, soil biodynamic and sustainability. Use of Indigenous AMF mixture would be a better strategy to improve herb productivity.
4. The use of AMF as biofertilizer is suggested to fulfill the increasing demand of food, fibre and fuel due to expanding population in the country. Besides this we can also earn handsome amount of foreign exchange by exporting our indigenous medicinal herb, as KPK is rich in aromatic medicinal flora.
5. It is recommended to produce and preserve the inocula of efficient AMF strain in bulk & supply to farmers with inoculation methodologies for low fertility regime areas and reduce the use of agrochemicals which are costly and harmful for the environment.
6. To establish plant with AMF along with integrated use of rock phosphates and organic fertilizers. Further medicinal plants cultivation require a fine-tuning between the application of AMF and organic nutrition.

REFERENCES

- Abdel-Hafez, A. A. M. and A. R. Abbdel-Monsief. 2006. Effect of VAM Inoculation on growth, yield and nutrient content of Cantaloupe and Cucumber under different water regime. *Res. J. Agri. & Biol. Sci.*, 29(6): 503-508.
- Abov El – Salehain and Ahmad. 1998. Effect of some bio and chemical fertilizers on growth, chemical composition & yield of snap bean (*Phaseolus vulgaris*, L.) *Egypt. J. Appl. Sci.*, 13(12): 228-246.
- Abov Zeid, M. H., H. E. Asfovr and M. I. Eid. 2005. Effect of nitrogenous and phosphorous fertilization on volatile oil percentage and volatile oil composition of *F. vulgare* Mill. (Fennel). *Egypt. J. Appli. Sci.*, 20(1): 226-240.
- Abu-Zeyad, R. A., G. Khan and C. Khoo. 1999. Occurrence of arbuscular mycorrhiza in *Castanospermum australe* and effects on growth and production of castanospermine. *Mycorrhiza*, 9(2): 111-117.
- Adewole, M. B. and A. O. Ilesanmi. 2011. Effects of soil amendments on the nutritional quality of okra (*Abelmoschus esculentus* [L.] Moench). *J. Soil Sci. & Pl. Nutri.*, 11(3): 45-55.
- Aher, R. K. 2009. Effect of AM fungi on mycorrhizal colonization, growth and nutrition of *Arachis hypogea* L. *Internet. J. Agricultural Sci.*, 4(1): 245-247.
- Ahiabor, B. D. and H. Hirataz. 2003. Associate influence of soluble phosphate Rock phosphate and arbuscular mycorrhizal fungus on plant growth and phosphorus uptake of three tropical legumes. *West Afric. J. Appl. Ecol.*, 75(4): 1-9.
- Akiyama, K. and H. Hayashi. 2002. Arbuscular fungus-promoted accumulation of two new triterpenoids in cucumber roots. *Biosci. Biotech. & Biochem.*, 66(4): 762-769.
- Alguacil, M. D., F. Caravaca, G. Diaz, P. Marin and A. Roldan. 2004. Establishment of *Retama sphaerocarpa* L. seedlings on a degraded semiarid soil as influenced by

- mycorrhizal inoculation and sewage-sludge amendment). *J. Plant Nutrition & Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde*. 167(5): 637-644.
- Alizadeh, A., M. Khoshkhui, K. Javidnia, O. Firuzi, E. Tafazoli and A. Khalighi. 2010. Effects of fertilizer on yield, essential oil composition, total phenolic content and antioxidant activity in *Satureja hortensis* L. (Lamiaceae) cultivated in Iran. *J. Medicinal Plants Res.*, 4(1): 033- 040.
- Al-Karaki, G. N. 2000. Growth of mycorrhizal tomato and mineral acquisition under salt stress. *Mycorrhiza*, 10(2): 51-54.
- Al-Karaki, G. N. 2002. Field response of garlic inoculated with arbuscular mycorrhizal fungi to phosphorus fertilization. *J. Pl. Nutri.*, 25(4): 747-756.
- Alloush, G. N. and R. B. Clarke. 2001. Growth, Mineral acquisition and water use by mycorrhizal when growth under stress. *J. Plant Nutri.*, 21: 263-276.
- Alonso-Amelot, M. E., M. Perez-Mena, M. P. Calcagno, R. Jaimes-Espinoza and U. Castello. 1992. Ontogenic variation of Biologically Active Metabolites of *Pteridium aquilinum* (L., Kuhn), Pterosins A. and B. and Ptaquiloside in a Beaken population of the tropical land. *Journal Chem. Ecology*, 18: 1405-1420.
- Amaranthus, M., D. Perry, J. Anderson and Z. Amaranthus. 2009. Building soil organic matter Biologically. A power full sink for the greenhouse Gas Co₂. *Ecology*. 39(1): 3p.
- Amaya, L. C., F. T. Davies Jr, T. Fox and C. He. 2009. Arbuscular mycorrhizal fungi and organic fertilizer influence photosynthesis, root Phosphatase activity, Nutrition and growth of *Ipomoea carnea*. *Photosynthetica*, 47(1): 1-10.
- Andrade, G., R. G., Linderman and G. J. Bethlen Falvay. 1998. Bacterial associations with the Mycorrhizosphere and Hyphosphere of the Arbuscular mycorrhizal fungus *Glomus mosseae*. *Plant & Soil*, 202. 79-87.
- AOAC (Association of official analytical Chemists). 2000. Official methods of analysts, (Gaithersburg, MD, Washington, USA).

- Aryal, U. K., H. L. Xu and M. Fujita. 2003. Rhizobia & AM fungal inoculation improve growth & nutrient uptake of bean plants under organic fertilization. *Sustain Agric.*, 21: 29-41.
- Aryal, U. K., S. K. Shah, H. L. Xu and M. Fujita. 2006. Growth, nodulation and mycorrhizal colonization in bean plants improved by rhizobial inoculation with organic and chemical fertilizer. *Journal Sustainable Agriculture*, 29(2): 71-83.
- Assaf, T. A., M. A. Turk and K. M. Hameed. 2009. Impact of olive pomace wastes and fungicide treatment on indigenous arbuscular mycorrhizal fungi associated with chickpea (*Cicer Arietinum* L.) under field conditions. *Australian J. Crop Science*, 3(1): 6-12.
- Auge, R. M. 2004. Arbuscular mycorrhizae and soil/plant water relations. *Can J. Soil Sci.*, 84: 373-381.
- Auge, R. M., D. M. Sylvia, S. J. Park, B. R. Bulteog, A. M., Saxton, J. L., Moor and K. Chol. 2004. Partitioning mycorrhizas influence on water relations of *phaseolus vulgaris* into soil and root components. *Canadian J. Botany*, 82: 503-514.
- Auge, R. M., J. C. Moore, D. M. Sylvia and K. Cho. 2004b. Mycorrhizal promotion of host stomatal conductance in relation to irradiance and temperature. *Mycorrhizae*, 14: 85-92.
- Ayoob, M., I. Aziz and P. K. Jite. 2011. Interaction effects of arbuscular mycorrhizal fungi and different phosphate levels on growth performance of *Catharanthus roseus* Linn. *Not. Sci. Biol.*, 3(3): 75-79.
- Bahadur, A., Singh, J., K. P. Singh, A. K. Upadhyay and M. Rai. 2009. Effect of organic amendments and biofertilizers on growth, yield and quality attributes of Chinese Cabbage (*Brassica pekinensis*). *Indian J. Agricultural Sciences*, 76(10): 596-598.
- Baird, J. M., F. L. Walley and S. J. Shirtliffe. 2010. Arbuscular mycorrhizal fungi colonization and phosphorus nutrition in organic field pea and lentil. *Mycorrhiza*, 20(2): 541-549.

- Barakh, F. N. and A. M. Heggo. 1998. Moisture stress bradyrhizobia, vesicular arbuscular mycorrhiza and P-Fertilizers effect of soybean growth, nutrient content and phosphate activity under calcareous soil. *Ann. Agric. Sci. Carrio*, 43(2): 261-475.
- Barea, J. M., M. Tot, M. O. Orozco, E. Campos and R. Azcón. 2002. The application of isotopic (^{23}P and ^{15}N) dilution techniques to evaluate the interactive effect of phosphate- solubilizing rhizobacteria, mycorrhizal fungi and *Rhizobium* to improve the agronomic efficiency of rock phosphate for legume crops, *Nutrient cycling in Agro-ecosystems*, 63: 35-42.
- Batnal, B. M. and H. C. Lakshman. 2011. Symbiotic response of phyllanthus emblica L. vars. wild and Chakaiya to different arbuscular mycorrhizal fungi. *Libyan Agriculture Research Center Journal International*, 5(1): 313-319.
- Bellgard, S. E. and S. E. Williams. 2011. Response of mycorrhizal diversity to current climatic changes. *Diversity*, 3(1): 8-90.
- Bennett, A. E. and J. D. Bever. 2007. Mycorrhizal species differentially alter plant growth and response to herbivory. *Ecology*, 88(1): 210-218.
- Bertea, C. M., A. Voster, F. W. A. Verstappen, M. Maffel, J. Beekwilder and H. J. Rouwmeester. 2006. Isoprenoid Biosynthesis in *Artemisia Annua*. Cloning and heterologous Expression of a germacrene A synthase from A glandular trichomes, DNA Library, *Achieves Biochemistry & Biophysics*, 448: 3 – 12.
- Bethylenfalvay, G. J. and R. N. Ames. 1987. Comparison of two methods for quantifying extra radical medium of VAMF. *Soil Sci. American J.*, 51: 834-836.
- Bidartondo, M. I. and T. D. Bruns. 2002. Fine-level mycorrhizal specificity in the Monotropoideae (Ericaceae): specificity for fungal species groups. *Mol. Ecol.* 11: 557-569.
- Black, G. A. 1965. Methods of Soil analysis Agronomy 9 III. *Amer. Soc. Agron. Inc. Medison*. USA.

- Bouarmri, R., Y. Dalpe, M. N. Serrhini and A. Bennani. 2006. Arbuscular mycorrhizal fungi species associated with rhizosphere of *Phoenix dactylifera* L. in Morocco. *Afric. J. Biotech.*, 5(6): 510-516.
- Boureima, S., M. Diouf, T. A. Diop, M. Diatta, E. M. Leye, F. Ndiaye and D. Seck. 2008. Effects of arbuscular mycorrhizal inoculation on the growth and the development of sesame (*Sesamum indicum* L.). *Afric. J. Agric. Res.*, 3(3): 234-238.
- Bremner, J. M. and C. S. Mulvaney. 1982. Nitrogen-total. In: A. L. page, R. H. Miller and D. R. Keeney (ed.). Methods of soil analysis part 2. 2nd ed. *Agronomy*, 9: 595-621.
- Bresinsky, A., C. Korner, J. W. Kadereit, G. Neuhaus and U. Sonnewald. 2008. Strasburger -Lehrbuch der Botanik. 36. Auflage. Spektrum Verlag, Heidelberg, 1175 p.
- Brickell, C. and T. Chistopher. 2002. The American Horticultural society: Encyclopedia of plants and flowers. N. Y, USA. DK. Publishing. Inc. pp. 605.
- Brundrett, M. and B. Kendrick. 1988. The mycorrhizal status, root anatomy and phenology of plants in a sugar maple forest. *Can. J. Bot.*, 66: 1153-1173.
- Brundrett, M. and B. Kendrick. 1990. The root and mycorrhizas of herbaceous woodland plants. 11. Structural aspects of morphology. *New Phytol.*, 114: 457-468.
- Burni, T. and F. Hussain. 2011. Diversity in arbuscular mycorrhizal morphology in some medicinal plants of family Lamiaceae. *Pak. J. Bot.*, 43(3): 1789-1792.
- Burni, T. and I. Ilahi. 2004. Quantification and correlation of VAM spores with the soil characteristics of wheat fields of NWFP. *Pak. J. Pl. Sci.*, 10: 139-144.
- Burni, T., F. Hussain and M. Sharif. 2011. Arbuscular mycorrhizal fungi (AMF) associated with the rhizosphere of *Mentha Arvensis* L., and *Mentha longifolia* Huds. *Pak. J. Bot.*, 43(6): 3013-3019.
- Burni, T., M. Shah and F. Hussain. 2007. Occurrence and characterization of VAM infections in *Mentha longifolia* (L.) and *Nepeta cataria* L. *Pak. J. Pl. Sci.*, 13(2): 147-150.

- Burni, T., N. Aktar, D. Afshan and S. B. Zainab. 2008. Mycorrhizal studies in plants of saline habitats of District Kohat, *Pak. J. Pl. Sci.*, 14(2): 77-83.
- Burni, T., Z. Muhammad and A. Hussain. 1993. Vesicular arbuscular mycorrhizal association in *Targionia hypophylla*. *Sci. Khyber*, 5(1): 65-70.
- Caglar, S. and A. Akgun. 2006. Effects of vesicular-arbuscular mycorrhizal (VAM) fungi on the seedling growth of three *Pistacia* species. *J. Environ. Biol.*, 27(3): 485-489.
- Calvet, C., J. Pinochet, A. Hernandez-Dorrego, V. Estaun and A. Camprubi. 2001. Field micro plot performance of the Peach-Almond hybrid GF-677 after inoculation with AM Fungi in a replant soil infested with root-knot Nematodes. *Mycorrhiza*, (10): 295-300.
- Cano, C. and A. Bago. 2005. Competition and substrate colonization strategies of three polyxenically grown AM fungi. *Mycologia*, (97): 1201-1214.
- Caputi, H. and E. Aprea. 2011. Recent patent on food. *Nutrition & Agriculture*, 3(1): 9-16.
- Caravaca, F., C. Garcia, M. T. Hernandez and A. Roldan. 2002. Aggregate stability changes after organic amendment and mycorrhizal inoculation in the afforestation of a semiarid site with *Pinus halepensis*. *Applied Soil Ecology*, 19(3): 199-208.
- Caravaca, F., J. M. Barea and A. Roldan. 2002. Synergistic influence of an arbuscular mycorrhizal fungus and organic amendment on *Pistacia lentiscus* L. seedlings afforested in a degraded semiarid soil. *Soil Biology & Biochemistry*, 34(8): 1139-1145.
- Caravaca, F., M. M. Alagnacil, R. Azcon, G. Diaz and A. Roldan. 2004. Comparing the effectiveness of mycorrhizal inoculation and amendment with sugar beet, rock phosphate and *Aspergillus niger* to enhance field performance of the leguminous shrub *Dorycnium pentaphyllum* L. *Applied Soil Ecology*. 25(2): 169-180.
- Cardoso, I. M. and T. W. Kuyper. 2006. Mycorrhiza and tropical soil fertility. *Agriculture Ecosystem & Environment*, 116: 72-84.

- Caris, C., W. Hoerd, H. J. Hawkins, V. Roenheld and E. George. 1998. Studies on the iron transport by arbuscular mycorrhizal hyphae from soil to peanut and sorghum plants. *Mycorrhiza*, 8: 35-39.
- Cavagnaro, T. R., F. A. Smith, M. F. Lorimer, K. A. Haskard, S. M. Ayling and S. E. Smith. 2001. Quantitative development of Paris-type arbuscular mycorrhizas formed between *Asphodelus fistulosus* and *Glomus coronatum*. *New Phytol.*, 149: 105-113.
- Cavender, N. C. D., R. M. Atiyeh and M. Knee. 2003. Vermicompost stimulates mycorrhizal colonization of roots of *Sorghum bicolor* at the expense of plant growth. *Pedobiologia*, 47(1): 85-89.
- Celik, I., I. Ortas and S. Kilic. 2004. Effects of compost mycorrhiza, manure and fertilizer on some physical properties of a Chromo expert soil. *Soil Till Res.*, (78): 59-67.
- Chandanie, W. A., M. Kubota and M. Hyakumachi. 2009. Interaction between the AM Fungus *G. mosseae* and plant growth promoting fungi and their significance for enhancing plant growth and suppressing damping off of cucumber. *Applied Soil Ecol.*, 41(5): 571-578.
- Chandra, K. K., N. Kumar and G. Chand. 2010. Studies on mycorrhizal inoculation on dry matter yield and root colonization of some medicinal plants grown in stress and forest soils. *J. Environmental Biology*. November, 31(6): 975-979.
- Chandrasekara, C. M. C. P., H. M. S. P. M. Weerasinghe, I. A. U. N. Gunatilleke and G. Seneviratne. 2005. Spatial distribution of arbuscular mycorrhizas along an elevation and adaphic gradient in the forest dynamics plot at Sinharaja, Srilanka. *Cey. J. Sc.*, 34: 47-67.
- Chaudhary, V., R. Kapoor and A. K. Bhatnagar. 2008. Effectiveness of two arbuscular mycorrhizal fungi on concentrations of essential oil and artemisinin in three accessions of *Artemisia annua* L. *Appl. Soil Ecol.*, 40(1): 174-181.

- Chen, B. D., X. L. Li, H. Q. Tao, P. Christie and M. H. Wong. 2003. The role of arbuscular mycorrhiza in zinc uptake by red clover growing in calcareous soil spiked with various quantities of zinc. *Chemosphere*, 50(6): 839-846.
- Chou, J. C. and C. A. Mullin. 1993. Phenologic and tissue distribution of sesquiterpenes lactones in cultivated sunflowers (*Helianthus annus* L.) *Journal Plant Physiology*, (142): 657-663.
- Chand, S., M. Anwar and D. D. Pazra. 2001. Influence of combine application of farmyard manure and inorganic fertilizers on herb essential oil yield and nutrients accumulation in menthol mint (*Mentha Arvensis*). *Journal Medicinal & Aromatic Plant Sciences*, 23: 29-33.
- Cigar, S., N. Sari and I. Ortas. 2000. The effect of AM Fungi on the plant growth and nutrient uptake of cucumber. *Turk. J. Agric. Forest.*, 24(5): 571-578.
- Comis, D. 2002. Glomalin. Hiding place for a third of the world's stored soil carbon. *Agricultural Research*, 50(9): 47.
- Cooper, K. M. and D. M. Losel. 1978. Lipid physiology of vesicular arbuscular mycorrhiza 1. Composition of lipids in roots of onion clover and ryegrass infected with *Glomus mosseae*. *New Phytol.*, 80: 143.
- Cooper, K. M. and P. B. Tinker. 1987. Translocation and transfer of nutrients in VAM Fungi. Effect of environmental variable on movement of Phosphorus. *New Phytol.*, (88): 327-398.
- Copetta, A., G. Lingua, and G. Berta. 2006. Effects of three AM Fungi on growth. Distribution of glandular Hair, and of essential oil production in *Ocimum basilicum* L. var. Genovese. *Mycorrhiza*, 16: 845-494.
- Copetta, A., G. Lingua, L. Bardi, G. Masoero and G. Berta. 2007. Influence of arbuscular mycorrhizal fungi on growth and essential oil composition in *Ocimum basilicum* var. Genovese. *Caryologia*, 60(1-2): 106-110.
- Cordier, C., M. J. Pozo, J. M. Barea, S. Gianinazzi and P. V. Gianinazzi. 1998. Cell defence response associated with localized and systemic resistance to

- Phytophthora parasitica* induced in tomato by an AM fungi. *Molecular Plant Microbe Interaction*, 11: 1017-1028.
- Costa, C. M. C., U. M. T. Cavalcante, B. T. Goto, V. F. dos Santos and L. C. Maia. 2005. Arbuscular mycorrhizal fungi and phosphorus supply on seedlings of *Mangabeira pesquisa*, *Agropecuaria Brasileira*, 40(3): 225-232.
- Cumming, J. and J. Ning. 2003. AM Fungi enhance Aluminum resistance of Broom sedge. *J. Exp. Bot.*, 54(386): 1477-1459.
- Cwala, Y., C. P. Laubscher, P. A. Ndakidemi and A. H. Meyer. 2010. Mycorrhizal root colonisation and the subsequent host plant response of soil less grown tomato plants in the presence and absence of the mycorrhizal stimulant. *Mycotech. Afr. J. Microbial. Res.*, 4(5): 414-419.
- da Silveira, S. V., P. V. D. de Souza, R. J. Bender and O. C. Koller. 2002. Effect of arbuscular mycorrhizae on cv. Carmem avocado plants. *Communications in Soil Sci. & Pl. Analysis*, 33(7-8): 1323-1333.
- Daei, G., M. R. Ardekani, F. Rejali, S. Teimuri and M. Miransari. 2009. Alleviation of salinity stress on wheat yield, yield components and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. *J. Plant Physiology*, 166: 617-625.
- Dann, P. R., J. W. Derrick, D. C. Dumareso and M. H. Ryan. 1996. The response of organic and conventionally grown wheat to super phosphate and reactive phosphate rock, *Aust. J. Exp. Agric.*, 36: 71-78.
- Darzi, M. T., A. Ghalavand, F. Rejali and F. Sefidkon. 2006. Effects of biofertilizers application on yield and yield components in fennel (*F. vulgare* Mill.) *Iranian J. Medicinal & Aromatic Plants*, 22(4): 274-292.
- Darzi, M. T., A. Ghalavand, F. Sefidkon and F. Rejali. 2009. The effect of mycorrhiza, vericompost & phosphatic biofertilizers application on quantity & quality of essential oil in fennel (*Foeniculum vulgare* Mill.) *Iranian J. Medicinal & Aromatic plants*, 24(4): 396-413.

- Das, K., R. Dang, T. N. Shivananda and N. Sekeroglu. 2008. Influence of bio-fertilizer on the biomass, yield and nutrient contents. *J. Med. Pl. Res.*, 1(1): 5-8.
- Dave, S. and J. C. Tarafdar. 2011. Stimulatory synthesis of saponin by mycorrhizal fungi in safed musli (*Chlorophytum borivillianum*) Tubers. *International Research J. Agric. Science & Soil Science*, 1(4): 137-141.
- Davies, F. T., V. Olalde-Portugal, M. J. Alvarado, H. M. Escamilla, R. C. Ferrera-Cerrato and J. I. Espinosa. 2000. Alleviating phosphorus stress of Chile ancho pepper (*Capsicum annuum* L. 'San Luis') by arbuscular mycorrhizal inoculation. *J. Horticultural Sci. & Biotechnol.*, 75(6): 655-661.
- de Boulois, H., D. Delvoux and B. S. Declerck. 2005. Effects of arbuscular mycorrhizal fungi on the root uptake and translocation of radiocaesium. *Environmental Pollution*, (134): 515-524.
- Declerck, S., C. Plenchette and D. G. Strullu. 1995. Mycorrhizal dependency of banana, *Musa acuminata*. *Plant Soil*, (176): 183-187.
- Dell-Amico, J., A. Torrecillas, P. Rodriguez, A. Morte and M. J. Sanchez-Blanco. 2002. Responses of tomato plants associated with the arbuscular mycorrhizal fungus *Glomus clarum* during drought and recovery. *J. Agric. Sci.*, (138): 387-393.
- De-Miranda and Harris. 1994. The effect of soil phosphorus on the external mycelium growth of Arbuscular mycorrhiza fungi during the early stages of mycorrhiza formation. *Plant & Soil*, (166): 271-280.
- Dickson S., P. Schweiger F. A. Smith, B. Soderstrom and S. Smith. 2003. Paired arbuscules in the Arum-type arbuscular mycorrhizal symbiosis with *Linum usitatissimum*. *Can. J. Botany*, 81(5): 457-463.
- Dickson, S. 2004. The Arum-paris continuum of mycorrhizal symbioses, *New Phytol.*, (163): 187-200.
- Dighton, J. 2009. Evaluation of mycorrhizal symbiosis as defense in extreme environment. In defensive Mutualism in Microbial Symbiosis. CRC. Newyork. 199.

- Drew, E. A., R. S. Murray and S. E. Smith. 2003. Beyond the photosphere growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes, *Plant & Soil*. 25(1): 105-114.
- Duponnois, R., A. Colombet, V. Hien and J. Thioulouse. 2005. The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericeae*, *Soil Biology & Biochemistry*, 36(1): 1460-1468.
- Earanna, N., R. R. Mallikartuniah, D. J. Bagyaraj and C. K. Suresh. 2001. Response of *Coleus Aromaticus* to *Glomus fasciculatum* and other beneficial soil Micro flora. *Journal Spices & Aromatic Crops*, 10(2): 141-143.
- Elsheikh, E. A. E. and E. M. M. Mohamedzein. 1998. Effect of Bradyrhizobium, VA mycorrhiza and fertilizers on seed composition of groundnut. *Annals Appl. Bio.*, 132(2): 325–330.
- El-Sherbeny, S. E., M. S. Hussein and M. Y. Khalil. 2007. Improving the production of *Ruta graveolens* L. plants cultivated under different compost levels and various sowing distance. *American – Eurasian J. Agric. & Environmental Sciences*, 2(3): 271-281.
- Essien, E. P., J. P. Essien, B. N. Ita and G. A. Ebong. 2008. Physiochemical properties and fungi toxicity of the essential oil of *Citrus medica* L. against Groundnut storage Fungi. *Turk. J. Bot.*, 32: 161-164.
- Estrada-Luna, A. A., F. T. Davies and J. N. Egilla. 2000. Mycorrhizal fungi enhancement of growth and gas exchange of micro-propagated guava plantlets (*Psidium guajava* L.) during ex vitro acclimatization and plant establishment. *Mycorrhiza*, 10(1): 1-8.
- Faboodi, M., M. Alfarmani, A. Famarzi and S. Shahrokhi. 2011. Phosphorus levels effects on quantitative and qualitative characteristics of Corn in presence and absence of a Biofertilizer. (IPCBE) 2nd International Conference on Agriculture and Animal Science, 22: 129-132.

- Farahani, A., H. Lebaschi, M. Hussein, S. A. Hussain, V. A. Reza and D. Jahanfar. 2008. Effects of arbuscular mycorrhizal fungi, different levels of phosphorus and drought stress on water use efficiency, relative water content and proline accumulation rate of coriander (*Coriandrum sativum* L.). *J. Med. Pl. Res.*, 2(6): 125-131.
- Farkoosh, S. S., M. R. Ardakani, F. Rejali, M. T. Darzi and A. H. Faregh. 2011. Effect of mycorrhizal symbiosis and *Bacillus coagulans* on qualitative and quantitative traits of *Matricaria chamomilla* under different levels of phosphorus, *Middle-East J. Scientific Research*, 8(1): 1-9.
- Fedderman, N. R., T. Finally, F. Boller and Elfstr. 2010. Functional diversity in arbuscular mycorrhiza the role of gene expression, phosphorus nutrition and Symbiotic efficiency. *Fungal Ecol.*, (3): 1-8.
- Feng, G., F. S. Zhang, X. L. Li, C. Y. Tian, C. Tang and Z. Rengel. 2002. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza*, 12(4): 185-190.
- Figueira, G. M. 1996. Mineral nutrition, production and artemisinin content in *Artemisia annua* L. *Hort.*, 426: 573-577.
- Fisher, J. B. and K. Jayachandran. 2002. Arbuscular mycorrhizal fungi enhance seedling growth in two endangered plant species from South Florida. *Internat. J. Pl. Sci.*, 163(4): 559-566.
- Frank, A. B. 1887. Uber new mycorrhiza-formen Ber dt. Bot. Ges., 5: 395.
- Frank, A. B. 1885. Uber de auf werzel symbiose beruhende Ernahrung Gewisser Baume durch unterirdische pilze, *Bar. dt. bot. Ges.*, 3: 128-145.
- Freitas, M. S. M., M. A. Martins and E. I. J. C. Vieira. 2004. Yield and quality of essential oils of *Mentha arvensis* in response to inoculation with arbuscular mycorrhizal fungi. *Pesquisa Agropecuaria Brasileira*, 39(9): 887-894.
- Fuchs, B. and K. Haselwandter. 2004. Red list plants colonization by arbuscular mycorrhizal fungi and dark septate endophytes. *Mycorrhiza*, 14: 277-281.

- Fusconi, A., A. M. Tagliasacchi and G. Berta. 2001. Root apical meristems of Leek (*Allium porrum*) as affected by AM Fungi and phosphorus. Department of Biology University of Toronto.
- Gahoonia, T. S. and N. E. Nielsen. 1998. Direct evidence on participation of root hairs in phosphorus (^{32}P) uptake from soil. *Plant & Soil*, 198: 147-152.
- Gallaud, J. 1905. Etude sur les mycorrhizas endotrophes. *Rev. Gen. Bot.*, 17: 5-48.
- Galvan, G. A., I. Paradi, K. Burger, J. Baar, T. N. Kuyper, O. E. Scholten and C. Kik. 2009. Molecular diversity of arbuscular mycorrhizal fungi in onion roots from organic and conventional farming systems in the Netherlands. *Mycorrhiza*, 19(5): 317-328.
- Galvez, L., D. D. Douds, L. E. Drinkwater and P. Wagoner. 2001. Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. *Plant & Soil*, 228(2): 299-308.
- Garampalli, R. H., S. Deene and C. N. Reddy. 2005. Infectivity and efficacy of *Glomus aggregatum* and growth response of (*Cajanus cajan* L.) Millsp in flyash Amended sterile soil. *Journal Environmental Biology*, 26(4): 705-708.
- Gaur, A. and A. Adholeya. 1994. Estimation of AMF spores in soil. A modified *Mycorrhiza News*, 6: 10-11.
- Gaur, A. and A. Adholeya. 2002. Arbuscular- mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter. *Biology & Fertility of Soils*, 35(3): 214-218.
- Gaur, A. and A. Adholeya. 2004. Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. *Current Science*. 86(4): 528-534.
- Geneva, M. P., I. V. Stancheva, M. M. Boychinova, N. H. Mincheva and P. A. Yonova. 2010. Effects of foliar fertilization and arbuscular mycorrhizal colonization on *Salvia officinalis* L. growth, antioxidant capacity and essential oil composition. *Journal Science, Food & Agriculture*, 90: 696-702.

- George, E. 2000. Nutrient uptake. In: Arbuscular mycorrhizas: physiology and function. (eds.). Y. Kapulnick, and D. D. Douds. J. R. Kluwer Academic Press. pp. 307-344.
- Gerdemann, J. W. and T. H. Nicolson. 1963. Spores of mycorrhizal endogene species extracted from soil by wet sieving decanting. *Trans. Br. Mycol. Soc.*, 46: 235-244.
- Gilroy, S. and D. L. Jones. 2000. Through form to function: root hair development isolate from soybean rhizosphere, *Bio-resource Technology*, 97: 204-210.
- Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques to measure vesicular – arbuscular mycorrhizal infection in roots. *New Phytol.*, 84: 489-500.
- Giri, B., R. Kapoor and K. G. Mukerji. 2005. Effect of the arbuscular mycorrhizae *Glomus fasciculatum* and *G-macrocarpum* on the growth and nutrient content of *Cassia siamea* in a semi-arid Indian wasteland soil. *New Forests*, 29(1): 63-73.
- Gogoi, P. and R. K. Singh. 2011. Differential effect of some arbuscular mycorrhizal fungi on growth of *Piper longum* L. (Piperaceae), *Indian J. Science & Technology*, 4(2): 119-125.
- Gonzalez-Chavez, C., J. Dhaan. J. Vangronsved and J. C. Dodd. 2002. Copper absorption and accumulation by the extra radical mycelium of different *Glomus* spp (AMF) isolated from the same polluted soil. *Plant & Soil*, 240(2): 287-297.
- Goo, A. X. and X. L. He. 2007. Ecological study on AM Fungi around roots of medicinal plants in the middle area of Hebei province, *Agric. Res. in the Arid Areas*, 25(3): 196-202.
- Gorsi, M. S. 2002. Studies on mycorrhizal Association In some Medicinal plants of Azad Jammu AN Kashmir *Asian J. Plant Sciences*, 1: 383–387.
- Govindarajulu, M., P. E. Pfeffer, H. Jin, J. Abubaker, D. D. Douds, J. W. Allen, H. Bucking, P. J. Lammers and Y. Shachar-Hill. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature*, 435: 819-823.
- Gracy and Bagyaraj. 2005. Influence of different AM-fungi on the growth, nutrition & forskolin content of *Coleus forskohlii*. *Mycological Research*, 109(7): 795-798.

- Graham, D. J. H. and L. W. Timmer. 1985. Rock phosphate as a source of phosphorus for VAM development and growth of citrus in a soil less medium. *J. Am. Soc. Hortic. Sci.*, 110(4): 489-492.
- Gryndler, M., H. Hrselova and M. Vosatka. 2001. Organic fertilization changes the response of mycelium of arbuscular mycorrhizal fungi and their sporulation to mineral NPK supply. *Folia Microbial.*, 46(6): 540-542.
- Gullace, M., F. Sahin, M. Sokmen, H. Ozer, D. Daferera, A. Sokmen, M. Polissiou, A. Adiguzel and H. Ozken. 2007. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha Longifolia* L. *Food Chem.*, 103: 1449-1456.
- Gupta, M. L., A. Prasad, M. Ram and S. Kumar. 2002. Effect of the AM fungus *Glomus fasciculatum* on the essential oil yield related characters and nutrient acquisition in the crops of different cultivars of menthol under field conditions Lucknow. *Bioresource Technology*, (81): 77-79.
- Gyaneshwar, P., G. N. Kumar, L. J. Parech and P. S. Poole. 2002. Role of microorganism in improving P nutrition of plants. *Plant & Soil*, 245: 83-93.
- Hameeda, B., G. Harini O. P. Rupela G. Reddy. 2007. Effect of composts or vermicomposts on sorghum growth and mycorrhizal colonization. *Afric. J. Biotechnol.*, 6(1): 9-12.
- Haraguchi, H., R. Matsuda and K. Hashimoto. 1993. High performance liquid chromatographic determination of sesquiterpene dialdehydes and antifungal activity from *Polygonum hydropiper*. *Journal Agric. & Food Chemistry*, 41: 5-7.
- Harinikhmar, K. M. and D. J. Bagyaraj. 1990. Effect of crop rotation on native VAM propaule in soil. *Plant & Soil*, 110(1): 77-80.
- Harries, L. A. and C. A. Watson. 2004. The potential role of AM fungi in the bio-protection of plants against soil borne pathogens in organic and or other sustainable forming systems. *Pest Management Sci.*, 60: 149-157.

- Harris, D. and E. A. Paul. 1987. Carbon requirement of VAM in eco-physiology of VA mycorrhizal plants. Safir, G.R. (ed.) 93-106. CRC Press. Boca Raton. Fl.
- Hartwig, U. A., P. Wittmann, R. Braun, B. Hartwig-Räz, J. Jansa, A. Mozafar, A. Lüscher, A. Leuchtmann, E. Frossard and J. Nösberger. 2002. Arbuscular mycorrhiza infection enhances the growth response of *Lolium perenne* to elevated atmospheric CO₂. *J. Exp. Bot.*, 53(371): 1207-13.
- Henrike, P., S. Dietmar, B. Christian, M. Paul and G. Eckhard. 2007. Effect of arbuscular mycorrhizal colonization and two levels of compost supply on nutrient uptake and flowering of pelargonium plants. *Mycorrhiza*, 17: 469-474.
- Hildebrandt, U., M. Regvar and H. Bothe. 2007. Arbuscular mycorrhiza and heavy metal tolerance. *Phytochem.*, 68: 139-146.
- Hindumathi, A., Bhumi, Ni. Reddy. 2011. Occurrence and distribution of arbuscular mycorrhizal fungi and microbial flora in the rhizosphere soil of mungbean (*Vigna radiata* (L.) Wilczek and soybean (*Glycine max* (L.) Merr) from Adilabad, Nizamabad and Karimnagar districts of Andhra Pradesh state, India. *Advances in Bioscience & Biotechnology*, 2: 275-286.
- Hinsinger, P., G. R. Gobran and P. J. W. W. Wenzel. 2005. Rhizosphere geometry heterogeneity arising from root mediated physical and chemical processes. *New Phytologist*, 168(2): 293-303.
- Hodge, A., T. Helgason and A. H. Fitter. 2010. Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecology*, 1-7.
- Holopainen, J. K. 2004. Multiple functions of inducible plant volatiles. *Trends in Plant Science*, 9: 529-533.
- Howeler, R. H., E. Sieverdina and S. R. Saif. 1987. Practical aspects of mycorrhizal technology in some tropical crops and pastures. *Plant & Soil*, 100: 249-283.
- Howkins, H. J., A. Johansen and E. George. 2000. Uptake and transport of organic and inorganic nitrogen by AM Fungi. *Plants & Soil*, 226: 275-285.

- Hussain, A. I., F. Anwar, S. T. H. Sherazi and R. Parzbylski. 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on Seasonal variations. *Food Chemistry*, 108: 986-995.
- Illmer, P. and F. Schinner. 1992. Solubilization of inorganic phosphate by micro-organisms isolated from Forest Soil. *Soil Biol. Biochem.*, 24: 389-395.
- Imhof, S. 2007. Specialized mycorrhizal colonization pattern in achlorophyllous *Epirixanthes* Spp. (Polygalaceae). *Plant Biol.*, 9: 786-792.
- Imhof, S. 2009. Arbuscular, ecto-related, orchid mycorrhizas-three independent structural lineages towards mycoheterotrophy: implications for classification. *Mycorrhiza*, 19: 357-363.
- Inoue, S., I. Kheoruenrome, S. Anchalea and T. Supicha. 2001. Effect of AM fungi on phosphorus uptake and growth of baby corn on a sand soil. Kaset Sart University of Bangkok.
- Jacobsen, I., L. K. Abbott and A. Robson. 1992. External hyphae of AM Fungal association with *Trifolium subterraneum* L. spread of hyphae and P inflow into roots. *New Phytologist*, 120: 371-380.
- Jakobsen, I., B. Chen, L. Munkvold, T. Lundsgaard and Y.G. Zhu. 2005. Contrasting phosphate acquisition of mycorrhizal fungi with that of root hairs using the root hairless barley mutant. *Plant Cell and Environment*, 28: 928-938.
- Jalaluddin, M. and Q. M. Anwar. 1991. VAM fungi in wheat and rice fields. *Pak. J. Bot.*, 22(1): 115-122.
- Jalal-ud-Din, M. N. B. Jajra, K. Firoza and F. Shahina. 2008. Effect of *Glomus callosum* *Meloidogyne incognita* and soil moisture on growth and yield of Sunflower. *Pak. J. Bot.*, 40(1): 391-396.
- Jamal, A., N. Ayub, M. Usman and A. G. Khan. 2002. AM Fungi enhance Zinc and Nickle uptake from contaminated soil by soybean and lentil. *Int. J. Phytoremed.*, 4(3): 203-221.

- Janaki, R. and C. Manoharachary. 1994. Occurrence and distribution of VAM fungi associated with safflower Indian. *Phytopathol.*, 47: 263-265.
- Janouskova, M., M. Vosatka, L. Rossi and N. Lugon-Moulin. 2007. Effects of Arbuscular mycorrhizal inoculation on cadmium accumulation by different tobacco (*Nicotiana tabacum* L.) Types. *Applied Soil Ecology*, 35: 502-510.
- Jansa, J., A. Mozafar, G. Kuhn, T. Anken, R. Ruh, I. R. Sanders and E. Frossard. 2003. Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Applications*, 13(4): 1164-1176.
- Jansa, J., R. Finlay, H. Wallander, F. A. Smith and S. E. Smith. 2011. Role of mycorrhizal symbioses in phosphorus cycling, *Soil Biol.*, 100(2): 137-168.
- Javaid, A. 2009. Arbuscular mycorrhizal mediated nutrition in plants. *J. Pl. Nutri.*, 32(10): 1595-1618.
- Javaid. A. and T. Riaz. 2008. Effect of application of green leaf manure on growth and mycorrhizal colonization of maize. *Allelopathy Journal*, 21(2): 339-347.
- Jayachandran, K. and K. G. Shetty. 2003. Growth response and phosphorus uptake by arbuscular mycorrhizae of wet prairie sawgrass. *Aquatic Bot.*, 76(4): 281-290.
- Johnson, N. C., D. Tilman and D. Wedin. 1992. Plant and soil controls on mycorrhizal fungal communities. *Ecology*, 73: 34-42
- Joner, E. J. 2000. The effect of long term fertilization with organic or inorganic fertilizers on mycorrhiza mediated phosphorus uptake in subterranean clover. *Biology & Fertility Soils*, 32(5): 435-440.
- Joner, E. J. and A. Johansen. 2000. Phosphatase activity of external hyphae of two arbuscular mycorrhizal fungi. *Mycological Research*, 104(1): 81-86.
- Joner, E. T. and I. Jakobsen. 1995. Growth and extra cellular phosphates activity of arbuscular mycorrhizal Hyphae as influence by soil organic matter. *Soil Biology & Biochemistry*, 27: 1153-1159.

- Jones, E. J. and C. Levylal. 1997. Uptake of roots by root and hyphae of *Glomus mossese* in *Trifolium subterraneum* from soil amended with high and low concentration of Cadmium. *New Phytol.*, 135: 353-360.
- Kafkas, S. and I. Ortas. 2009. Various mycorrhizal fungi enhance dry weights, P and Zn uptake of four *Pistacia* species. *J. Pl. Nutrition.*, 32(1): 146-159.
- Kahiluoto, H. and M. Vestberg. 1998. The effect of arbuscular mycorrhiza on biomass production and phosphorus uptake from sparingly soluble sources by leek (*Allium porrum* L.) in Finnish field soils. *Biol. Agric. Hortic.*, 16(1): 65-85.
- Kamal, P. and K. Prasad. 1995. Physico-chemical characteristic of soil in relation to VAM (*Glomus fasciculatum*) colonization in *Saccharum officinarum*. *J. Phytol Research*. 8(2): 201-205.
- Kamatou, G. P. P., R. L. V. Zyl, S. F. V. Vuuren, A. C. Figueiredo, J. G. Barroso, L. G. Pedro and A. M. Viljoen. 2008. Seasonal variation in essential oil composition, oil toxicity and the biological activity of solvent extracts of three South African *Salvia* species. *South African J. Botany*, 74: 230–237.
- Kapoor, R. and K. G. Mukerji. 2000. Mycorrhizal technology to enhance the growth and yield of aromatic plants. In: “Glimpses in Botany” (Eds. K.G. Mukerji, B.P. Chamola and A.K. Sharma) APH Publishing Corporation. New Delhi, 391-405.
- Kapoor, R. B. Giri and K. G. Mukerji. 2004. Improved growth and essential oil yield and quality in *Foeniculum vulgare* Mill. on mycorrhizal inoculation supplemented with P fertilizer. *Bio-resource Technology*, 93(3): 307-311.
- Kapoor, R., B. Giri and K. G. Mukerji. 2002b. Mycorrhization of coriander (*Coriandrum sativum* L.) to enhance the concentration and quality of essential oil. *Journal Science Food & Agriculture*, 82(4): 339-342.
- Kapoor, R., B. Giri and K. G. Mukerji. 2002a. *Glomus macrocarpum*: potential bio-inoculants to improve essential oil quality and concentration in Dill (*Anethum graveolens* L.) and Carum (*Trachyspermum ammi* (Linn.) Sprague). *World J. Microbiology & Biotechnology*, 18(5): 459-463.

- Kapoor, R., G. Bhoopander and G. M. Krishna. 2003. Improved growth and essential oil yield and quality in *Foeniculum vulgare* on mycorrhizal inoculation supplemented with P fertilizer. University of Delhi, India. 110-120.
- Kapoor, R., V. Chaudhary and A. K. Bhatnagar. 2007. Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in *Artemisia annua* L. *Mycorrhiza*, 17(7): 581-587.
- Karagiannidis, N. T. Thomidis, E. Panou-Filotheou and Christina Karagiannidou. 2012. Response of three mint and two oregano species to *Glomus etunicatum* inoculation, *Australian J. Crop. Sci.*, 6(1): 164-169.
- Karagiannidis, N., H. Panou-Filotheou, D. Lazari, I. Ipsilantis and C. Karagianidou. 2010. Essential oil content and composition, nutrient and mycorrhizal status of some aromatic and medicinal plants of northern Greece. *Nat. Prod. Commun.*, 5(5): 823-830.
- Karagiannidis, N., T. Thomidis, D. Lazari, E. Panou-Filotheou and C. Karagiannidou. 2011. Effect of three greek arbuscular mycorrhizal fungi in improving the growth, nutrient concentration and production of essential oils of oregano and mint plants. *Scientia Horticulture*, 129: 329-334.
- Karagiannidis, N., D. Velmis and N. Stravropoulos. 1997. Root colonization and spore population by VA mycorrhizal fungi in four grapevine root stocks. *Vitis*, 36: 57-60.
- Karthikeyan, B., C.A. Jaleel, C. Zhao, M. M. Joe, J. Srimannarayan and M. Deiveekasundaram. 2008. The effect of A.M. fungi and phosphorus level on the biomass yield and Ajmalicine production in *Catharanthus Roseus*. *Eurasian Journal of Biosciences*, 2: 26-33.
- Karthikeyan, B., M. M. Joe, C. A. Jaleel. 2009. Response of some medicinal plants to VAM inoculations. *J. Sci. Res.*, 1(1): 381-386.
- Kerur, A. S. and Lakshman. 2004. Effect of phosphate fertilizer and VA Mycorrhiza on two floricultural plants. *J. Curr. Sci.*, 5(1): 321-326.

- Khade, S. W. and B. F. Rodrigues. 2009. Application of arbuscular mycorrhizal fungi in agro system. *Tropical & Subtropical Agrosystems*, 10: 337-354.
- Khalafallah, A. A. and H. H. Abo-Ghalia. 2008. Effect of arbuscular mycorrhizal fungi on the metabolic products and activity of antioxidant system in wheat plants to short term water stress, followed by recovery at different growth stages. *J. Appl. Sci. Res.*, 4(5): 559-569.
- Khaliq, A. and K. K. Janardhanan. 1997. Influence of VAM Fungi on the productivity of cultivated mints. *Lucknow. J. Med. & Arom. Pl. Sci.*, 19: 7-10.
- Khan, A. G., C. Kuek., T. M. Chaudhry, C. Khoo and W. J. Hayes. 2000. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere*, 41: 197-207.
- Khanam, D. 2007. Assessment of arbuscular mycorrhizal association in some fruit plants in Bangladesh. *Bangladesh Journal of Microbiology*, 24: 34-37.
- Khaosaad, T., H. Vierheilig, M. Nell, K. Zitterl-Eglseer and J. Novak. 2006. Arbuscular mycorrhiza alters the concentration of essential oils in oregano (*Origanum* sp., Lamiaceae). *Mycorrhiza*, 16(6): 443-446.
- Khaosaad, T., J. M. Garcia Garrido, S. Steinkellner and H. Vierheilig. 2007. Take all disease is systemically reduced in roots of mycorrhizal Barley plants. *Soil Biology & Biochemistry*. 39: 727-734.
- Khaosaad, T., L. Krenn, S. Medjakovic, A. Ranner, A. Lossel, M. Nell, A. Jungbauer, and H. Vierheilig. 2008. Effect of mycorrhization on the isoflavone content and the phytoestrogen activity of red clover. *J. Pl. Physiology*, 165(11): 1161-1167.
- Kim, K. Y., D. M. C. Jordan and G. A. Donald. 1998. Effect of phosphate solubilizing bacteria and vesicular - arbuscular mycorrhizae on tomato growth and soil microbial activity. *Biol Fertil Soils*, 26: 79-87.
- Kitajima, K., S. R. Bray and D. M. Sylvia. 2003. Mycorrhizae differentially alter growth, physiology and competitive ability of an invasive shrub. *Ecol. Appl.*, 13: 565-574.

- Klironomos. 2003. Variation in plant response to native & exotic arbuscular mycorrhizal fungi. *Ecology*, 84: 2292-2301.
- Knight, W. G., M. F. Allen, J. J. Turinak and L. M. Dudley. 1989. Elevated carbon dioxide and solution phosphorus in soil with vesicular arbuscular mycorrhizal western wheat grass. *Soil Sci. Soc. Amer.*, 53: 1075-1098.
- Koide, R. T. and Z. Kabir. 2000. Extra radical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. *New Phytologist*, 148: 511-517.
- Kollner, T. G., C. Schnee, J. Gershenzon and J. Degenhardt. 2004. The variability of sesquiterpenes emitted from two Zeamays cultivated varieties as controlled by Allelic variation of two terpene synthase genes encoding stereo selective, multiple product enzymes. *Plant Cell*, 16: 1-17.
- Kormanik, P. P. 1982. Quantification of VAM in plant roots. Ed. Methods and Principles of mycorrhizal research. *American Phytopathol Soc.*, 37-45.
- Koske, R. E. and J. N. Gemma. 2006. Arbuscular mycorrhizae effects on growth of two Hawaiian species: Indigenous *Osteomeles anthyllidifolia* (Rosaceae) and invasive *Psidium cattleianum* (Myrtaceae). *Pacific Science*, 60(4): 471-482.
- Kothari, S. K. and U. B. Singh. 1996. Response of citronella Java (*Cymbopogon winterianus* Jowitt) to VA mycorrhizal fungi and soil compaction in relation to P supply. *Plant & Soil*, 178(2): 231-237.
- Krishna, K. R. and D. J. Bagyaraj. 1982. Interaction between a vesicular arbuscular fungus and streptomyces cinnamomeous and their effects on finger Millet. *New. Phytol.*, 92: 401-405.
- Kristine, A. N. 2008. Indirect contributions of AM fungi and soil aggregates to plant growth and protection. *Mycorrhizae*, 18: 177-194.
- Kubato, M, T. P. McGonigle and M. Hyakumachi. 2005. Co-occurrence of arum and paris-type morphologies of arbuscular mycorrhiza in cucumber and tomato. *Mycorrhiza*, 15: 73-77.

- Kumar, A., C. Mangla, A. Aggarwal and V. Parkas. 2010. Arbuscular mycorrhizal fungal dynamics in the rhizospheric soil of five medicinal plant species. *Middle East J. Scientific Research*, 6(3): 281-288.
- Kumar, D., K. Rai and J. Jayaraman. 1995. Effect of mushroom –mycorrhizal fungi interactions on yield and quality of pearl millet seeds. In mycorrhiza: Biofertilizers for the future. (Eds. Adholya, A. & Sing) pp. 378-380.
- Kumar, K. V. C., K. R. Chandrashekar and A. N. Balakrishna. 2007. Arbuscular mycorrhizal activity in *Solanum nigrum* in different regions of Karnataka. *Asian Jour. Microbial. Biotech. & Environmental Sciences*, 9(3): 633-635.
- Kumar, T. S., V. Swaminathan and S. Kumar. 2009. Influence of nitrogen, phosphorus and biofertilizers on growth, yield and essential oil constituents in ratoon crop of davana (*Artemisia pallens* Wall.). *Electronic J. Environ., Agric. & Food Chemistry*, 8(2): 86-95.
- Lackie, S. M., M. Gorriock, L. Peterson and Bowled. 1987. Influence of host plant on the morphology of vesicular arbuscular mycorrhizal fungus *Glomus versiforme* Berch. *Symbiosis & Plant Pathol.*, 67(7): 357.
- Laei, G., M. H. Khajehzadeh, Hossein Afshari, Abdol Ghaffar Ebadi and Hossein Abbaspour. 2011. Effect of mycorrhiza symbiosis on the NaCl salinity in *Sorghum bicolor*. *Afric. J. Biotechnol.*, 10(40): 7796-7804.
- Lambers, H., F. S. III. Chapin and J. L. Pons. 2008. Plant physiological ecology. Second edition. Springer-Verlag, New York. pp. 604.
- Lanfranco, L., A. Bolchi, E.C. Ros, S. Ottonello and P. Bonfante. 2002. Differential expression of a metallothionein gene during the presymbiotic versus the symbiotic phase of an arbuscular mycorrhizal fungus. *Plant Physiol.*, 130: 58–67
- Larose, G., R. Chenevert, P. Moutoglis, S. Gagne, Y. Piche and H. Vierheilig. 2002. Flavanoid levels in roots of medicago sativa are modulated by developmental stage of symbiosis and the root colonizing arbuscular mycorrhiza fungus. *J. Plant Physiol.*, 159: 1329-1339.

- Leigh, J., A. Hodge and A. H. Fitter. 2009. AM Fungi can transfer substantial amount of nitrogen to their host plant from organic material. *New Phyt.*, 181: 199-207.
- Lester, D. 2009. Buying and applying mycorrhizal fungi. Max. Yield. USA., pp: 126-131. http://www.maximumyield.com/article_shdb.php?articleID=483).
- Li, X. L., H. Marschner and E. George. 1991. Acquisition of phosphorus and copper by VA-mycorrhizal hyphae and root-to-shoot transport in white clover, *Plant & Soil*. 136: 49-57.
- Linderman, R. G. and A. E. Davis. 2004. Varied response of marigold (*Tagetes* spp.) genotypes to inoculation with different arbuscular mycorrhizal fungi. *Sci. Hort.*, 99: 67-78.
- Linderman, R. G. and E. A. Davis. 2001. Vesicular mycorrhiza and plant growth response to soil amendment with composted grape pomace or its water extract. *Phyton-annales Rei Botanicae*, 11(3): 446-450.
- Linderman, R. G. and E. A. Davis. 2003. Arbuscular mycorrhiza and growth responses of several ornamental plants grow in soil less peat and baid medium amended with coconut dust (Coir). *Hort. Technology*, (13): 482-487.
- Liu, A., C. Hamel, A. Elmi, C. Costa, B. Ma and D.L. Smith. 2002. Concentrations of K, Ca and Mg in maize colonized by arbuscular mycorrhizal fungi under field conditions. *Canad. J. Soil Sci.*, 82(3): 271-278.
- Liu, A., C. Hamel, R. I. Hamilton, B. L. Ma and D. L. Smith. 2000. Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. *Mycorrhiza*, 9(6): 331-336.
- Liu, J. N., L. J. Wu, S. L. Wei, X. Xiao, C. X. Su, P. Jiang, Z. B. Song, T. Wang and Z. L. Yu. 2007. Effects of arbuscular mycorrhizal fungi on the growth, nutrient uptake and glycyrrhizin production of licorice (*Glycyrrhiza uralensis* Fisch). *Plant Growth Regulation*, 52(1): 29-39.
- Loomis, W. D. and R. Croteau. 1972. Essential oil biosynthesis *recent Adv. Phytochem*, 6: 147-185.

- Louis, I. and G. Lim. 1987. Spore density and root colonization of VA mycorrhiza in tropical soil. *Trans. Br. Mycol. Soc.*, 88(2): 207-212.
- Ma, Z., D. G. Bielenberg, K. M. Brown and J. P. Lynch. 2001. Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. *Plant Cell & Environment*, 24: 459-467
- Mader, P. H., Vierheilig and R. Streitwolf-Engel. 2000. Transport of in from a soil compartment separated by a polytetrafluoroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. *New Phytologist*, 146: 155-161.
- Maicon, F., Dasilva, P. Rosete, A. Ricardo, Robelo and L. S. Sidney. 2008. The effect of VAM Fungal isolates on the development and oleoresin production of micropropagated *Zingiber officinalis*. *Braz. J. Pl. Physiol.*, 20(2): 119-130.
- Maier, W., J. Schmidt, V. Wray, M. H. Walter and D. Strack. 1999. The arbuscular mycorrhizal fungus, *Glomus intraradices*, induces the accumulation of cyclohexanone derivatives in tobacco roots. *Planta*, 207(4): 620-623.
- Malik, A. A., S. Suryapani and J. Ahmad. 2011. Chemical vs organic cultivation of medicinal and aromatic plants. *Int. J. Med. From. Plants*, 1: 5-13.
- Manjunath, A., N. V. Hue and M. Habte. 1989. Response of *Leucaena Leucocephala* to vesicular arbuscular mycorrhizal colonization and rock phosphate fertilization in an oxisol. *Plant & Soil*, 114(1): 127-133.
- Manoharan, P. T., M. Pandi, V. Shanmugaiah, S. Gomathinayagam and N. Balasubramanian. 2008. Effect of vesicular arbuscular mycorrhizal fungus on the physiological and biochemical changes of five different tree seedlings grown under nursery conditions. *Afric. Jour. Biotech.*, 7(19): 3431-3436.
- Mansfield-Giese, K., J. Larsen and L. Badker. 2002. Bacterial populations associated with mycellium of the arbuscular mycorrhizal fungus *Glomus intraradices*. *FEMS Microbial. Ecol.*, 41: 133-140.
- Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press. UK. London.

- Marshner, P. and S. Timonen. 2005. Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere. *Appl. Soil. Ecol.*, 28: 23-36.
- Martin. J., I. Sampedro, I. Garcia Romera, J. M. Garcia-Garrido and J. A. Ocampo. 2002. Arbuscular mycorrhizal colonization and growth of soybean (*Glycine max*) and lettuce (*Lactuca sativa*) and phototoxic effects of olive mill residues. *Soil Biol. & Biochem.*, 34(11): 1775.
- Masotti, V., F. Juteau, J. M. Bessiere and J. Viano. 2003. Seasonal and phenological variations of the essential oil from the narrow endemic species *Artemisia cxxli molinieri* and its biological activities. *Jour. Agric. & Food Chemistry*, 51: 7115-7121.
- Mathur, N. and A. Vyas. 2000. Influence of arbuscular mycorrhizae on biomass production, nutrient uptake and physiological changes in *Ziziphus mauritiana* Lam. under water stress. *J. Arid Environ.*, 45(3): 191-195.
- Meding, S. M. and R. J. Zhsoki. 2008. Hyphal mediated transfer of nitrate, arsenic, cesium, rusidium and strontium between arbuscular mycorrhizal forbs and grasses from a California Oak woodland. *Soil Biol. & Biochem.*, (40): 126-134.
- Mehrvarz, S. and M. R. Chaichi. 2008. Effect of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on forage and grain quality of barely (*Hordeum vulgare* L.). *American-Eurasian J. Agric. & Environ. Sci.*, 3(6): 855-860.
- Menge, J. A., D. Steirle, D. J. Bagyaraj, E. L. V. Johnson and R. T. Leonard. 1978. Phosphorus concentration in plans responsible for inhibition of mycorrhizal infection. *New Phytol.*, (80): 575-578.
- Mengel, K., and E. A. Kirkby. 2001. Principles of plant nutrition, 5th edn. Kluwer, Dordrecht.
- Miransari, M. 2010. Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stresses. Review article. *Plant & Biol.*, 12: 563-569.

- Miransari, M., H. A. Bahrami, F. Rejali, M. J. Malakouti and H. Torabi. 2007. Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on corn (*Zea mays*) growth. *Soil Biol. & Biochem.*, 39(8): 2014-2026.
- Mohammad, M. J., S. R. Hamad and H. I. Malkawi. 2003. Population of arbuscular mycorrhizal fungi in Semiarid environment of Jordan as influenced by biotic and abiotic factors. *J. Arid Environm.*, 53: 409-417.
- Moora, M., M. Opik and M. Zobel. 2004. Native arbuscular mycorrhizal fungi communities differentially influence the seedling performance of rare and common *Pulsatilla* species. *Funct. Ecol.*, (18): 554-562.
- Moremmani, A., S. Bedini, I. Matosevic, P. E. Tomei and M. Giovannetti. 2003. Type of mycorrhizal association in two coastal nature reserves of the Mediterranean Basin. *Mycorrhiza*, 13: 33-40.
- Morton, J. B. 1988. Taxonomy of VAM Fungi, classification nomenclature and identification. *Mycotaxon*, xxxii: 267-324.
- Motosugi, H., Y. Yamamoto, T. Naruo, H. Kitabayashi and T. Ishii. 2002. Comparison of the growth and leaf mineral concentrations between three grapevine rootstocks and their corresponding tetraploids inoculated with an arbuscular mycorrhizal fungus *Gigaspora margarita*. *Vitis*, 41(1): 21-25.
- Mridha, M. A. U. and P. P. Dhar. 2007. Biodiversity of Arbuscular Mycorrhizal colonization and spore population in different Agroforestry trees and crop species growing in Dianan-jpur Bangladesh. *J. Forestry Res.*, 18(2): 91-96.
- Mukerji, K. G., B. P. Chamola and J. Singh. 2000. Mycorrhizal biology. Springer, NewYork. Pp. 336.
- Muthukumar, T. and K. Udaiyan. 2002. Growth and yield of cowpea as influenced by changes in arbuscular mycorrhiza in response to organic manuring. *J. Agron. Crop Sci.*, 1882: 123-132.
- Muthukumar, T. and S. Prakash. 2009. Arbuscular mycorrhizal morphology in crops and associated weeds in tropical agro-ecosystem. *Mycorrhizae*, 50: 233-239.

- Muthukumar, T., M. Senthikumar, M. Rajangam and K. Udaiyan. 2006. AM morphology and dark septate fungal associations in medicinal and aromatic plants of western ghats, Southern India. *Mycorrhiza*, 17: 11-24.
- Nair, L. N. 2007. Vesicular arbuscular mycorrhiza. A review in organic farming and mycorrhizal in agriculture I.K. *International India*. 135-160.
- Nasim, G. 2010. The role of arbuscular mycorrhizae in resistance to drought and salinity stress in crops. *Plant Adaptation & Phytoremediation*, 1: 119-141.
- Nasim, G., S. Irum, S. Ali, A. Wahid and S. Sheikh. 1999. Allelopathic effects of four local grasses on their VAM status and dynamics of endogonaceous spore flora. *Sci. Khyber*, 12(1): 1-14.
- Nasrullah, M. Sharif, K. Robina and T. Burni. 2010. Occurrence and distribution of AMF in wheat and maize crops of Malakand division of North West Frontier Province. *Pak. J. Bot.*, 42(2): 1301-1312.
- Ndiaye, F., A. Manga, G. Diagnelaye, S. N. A. Samba and T. A. Diop. 2009. Effects of rock phosphate and AM fungi on growth and nutrition of *Sesbania sesban* and *Gliricida sepium*. *Afric. J. Micro. Res.*, 3(5): 305-309.
- Nelson, D. W. and L. E. Sommer. 1982. In: Total carbon, organic carbon and organic matter. (Eds.): A.L. Page, R.H. Miller and D.R. Keeney. *Methods of soil analysis*, part 2.2nd (ed). *Agron.* 9: 574-577.
- Nelson, R. and P. N. Achar. 2001. Stimulation of growth and nutrient uptake by VAM fungi in *Brassica oleracea* var. capitata. *Biologia Plantarum*, 44(2): 277-281.
- Nemec, S. and E. Lund. 1990. Leaf volatiles of mycorrhizal and non-mycorrhizal citrus Jamshiri. Lush. *J. Essential oil Research*, 2: 287-297.
- Ning, J. C. and J. R. Cumming. 2001. Arbuscular mycorrhizal fungi alter phosphorus relations of broomsedge (*Andropogon virginicus* L.) plants. *Journal Experimental Botany*, 52(362): 1883-1891.

- Nogueira, M. A. and E. J. N. Cardoso. 2007. Phosphorus availability changes the internal and external endo-mycorrhizal colonization and effects symbiotic effectiveness. *Scientia Agricola.*, 64(3): 295-300.
- Nowak, J. 2004. Effects of AM fungi and organic fertilization on growth, flowering, nutrients uptake, photosynthesis and transpiration of Geranium (*Pelargonium hartorum*). Poland. *Symbiosis*, 37(1-3): 259-266.
- Nwoke, O. C., J. Diels, R. Abaidoo, G. Nziguheba and R. Merckx. 2008. Organic acids in the rhizosphere and root characteristics of soybean (*Glycine max*) and cowpea (*Vigna unguiculata*) in relation to phosphorus uptake in poor savanna soils. *Afric. J. Biotechnol.*, 7(20): 3620-3627.
- Ohtomo, R. and M. Saito. 2005. Phosphate dynamics in mycorrhizal roots during colonization of an AM fungus. *New Phytol.*, 167: 571-578.
- Oliveira R. S., P. M. L. Castro, J. C. Dodd and M. Vosátka. 2006. Different native arbuscular mycorrhizal fungi influence the coexistence of two plant species in a highly alkaline anthropogenic sediment. *Plant & Soil*, 287: 209-221.
- Omomowo, I. O., I. O. Ola, A. K. Akintokun, M. O. Bankole and Babalola. 2009. Direct and residual influence of inoculation with *Glomus mosseae* and *Bradyrhizobium japonicum* on proximate and nutrient element content of Cowpea seeds. *Internat. J. Food Safety*, 10: 85-91.
- Orlowska, E., S. Zubek, A. Jurkiewicz, G. Szarek-Lukaszewska and K. Turnau. 2002. Influence of restoration on arbuscular mycorrhiza of *Biscutella laevigata* L. (Brassicaceae) and *Plantago lanceolata* L. (Plantaginaceae) from calamine spoil mounds. *Mycorrhiza*, 12(3): 153-160.
- Ortiz, E. 1992. The encyclopedia of herbs, spices and flavorings. London. Dorling Kindersley. 36-37.
- Osorio, N. W., J. M. Alzate and G. A. Ramirez. 2002. Coffee seedling growth as affected by mycorrhizal inoculation and organic amendment. *Communications in Soil Science & Plant Analysis*. 33(9-10): 1425-1434.

- Ouahmane, L., J. Thioulouse, M. Hafidi, Y. Prin, M. Ducousso, A. Galiana, C. Plenchette, M. Kisa and R. Duponnois. 2007. Soil functional diversity and P solubilization from rock phosphate after inoculation with native or allochthonous arbuscular mycorrhizal fungi. *Forest Ecol. & Management*, 241(1-3): 200-208.
- Pagano, M. C., M. N. Cabello, A. F. Bellote, N. M. Sa and M. R. Scotti. 2008. Intercropping system of tropical leguminous species and *Eucalyptus camaldulences*, inoculated with rhizobia and or mycorrhizal fungi in semi arid Brazil. *Agro Forestry Systems*, 74(3): 231-242.
- Pande, M. and J. F. Tarafdar. 2004. Arbuscular Mycorrhizal fungal diversity in Neem based Agroforestry Systems in Rajistan. *Applied Soil Ecology*, 26: 233-241.
- Panwar, J. and J. C. Tarafdar. 2006. Distribution of three endangered medicinal plant species and their colonization with arbuscular mycorrhizal fungi. *J. Arid Environments*, 65(3): 337-350.
- Perner, H., D. Schwarz, C. Bruns, P. Mader and E. George. 2007. Effect of arbuscular mycorrhizal colonization and two levels of compost supply on nutrient uptake and flowering of pelargonium plants. *Mycorrhiza*, 17(5): 469-474.
- Phillips, J. M. and D. S. Hayman. 1970. Improved procedures for cleaning roots and staining parasitic and vesicular – arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.*, 55: 158-161.
- Plenchette, C., J. A. Fortin and V. Furlan. 1983. Growth response of several plant species to mycorrhiza in a soil of moderate P fertility. In: Mycorrhizal dependency under field conditions. *Plant Soil*, 70: 199-209.
- Porras-Soriano, A., M. L. Soriano-Martin, A. Porras-Piedra and R. Azcon. 2009. Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *J. Plant Physiol.*, 166(13): 1350-1359.
- Posta, K., H. Marshner and V. Romheld. 1994. Manganese reduction in the rhizosphere of mycorrhizal and non-mycorrhizal maize. *Mycorrhiza*, 5: 119-124.

- Prasad, A. S., Kumar, A. Khalid and A. Pandey. 2011. Heavy metals and arbuscular mycorrhizal (AM) fungi can alter the yield and chemical composition of volatile oil of sweet basil (*Ocimum Basilicum* L.). *Biology & Fertility Soils*, 47(8): 853-861.
- Purohit, S. S. and S. P. Vyas. 2005. Medicinal plants cultivation. A Scientific Approach Agrobios, India.
- Quilambo, O. A. 2003. The vesicular- arbuscular mycorrhizal symbiosis. *Afr. J. Biotech.*, 2(12): 539-546.
- Radhika, K. P. and B. F. Rodrigues. 2010. AM fungal diversity in some commonly occurring medicinal plants of western Ghat (Goa) region. *J. Forest. Res.*, 2(1): 45-52.
- Radovich, T. J. K. and M. Habte. 2009. Arbuscular Mycorrhizal Dependency of three Moringa Genotypes. *Hort. Science*, 44(4): 1025-1026.
- Rahman, M., A. Islam and K. T. Osman. 2003. Effect of N, P and K fertilizers on growth and herb and oil yield of *Mentha arvensis* in Bangladesh. *Jour. Med. & Aromatic Plant Sciences*, 25: 661-667.
- Rai, M., D. Acharya, A. Singh and A. Varma. 2001. Positive growth responses of the medicinal plants *Spilanthes calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. *Mycorrhiza*, 11(3): 123-128.
- Rajeshkumae, V., V. Thangapandian, T. Muthukumar and C. S. Sumathi. 2008. Influence of bioinoculants on growth, nutrient uptake and yield of green gram [*Vigna radiata* (L.) Wilczek]. *J. Sustainable Agriculture*. 31(3):85-109.
- Raju, P.S., R. B. Clark, J. R. Ellis and J. W. Maranville. 1987. Vesicular-arbuscular mycorrhizal infection effects on Sorghum growth, phosphorus efficiency and mineral elements uptake. *J. Pl. Nutrition.*, 75: 255-278.
- Rao, A. V. and R. Tak. 2001. Influence of mycorrhizal fungi on the growth of different tree species and their nutrient uptake in gypsum mine spoil in India. *Soil Ecol.*, 17(3): 279-284.

- Rapparini, F., J. Liousia and J. Penuelas. 2008. Effect of AM colonization on terpene emission and content of *Artemisia annua*. L. German botanical society and the Royal botanical society of Netherlands. *Plant Biology*, 10: 108-122.
- Ratti, N., H. N. Verma and S. P. Gautam. 2010. Effect of *Glomus* species on physiology and biochemistry of *Catharanthus roseus*. *Indian J. Microbiol.*, 50: 355-360.
- Redecker, D. 2005. Glomeromycota. AM Fungi and their relatives. In the tree of web projects. <http://tolweb.org/Glomeromycota/28715>.
- Redecker, D. and R. Phillips. 2006. Phylogeny of the Glomeromycota (Arbuscular mycorrhizal fungi). Recent development and new gene marks. *Mycologia*, 98(6): 885-895.
- Reissek, S. 1847. Endophyten der pflanzenzelli Nature. Abh. Edt. W. Haidinger, 1: 31.
- Rejali, F. A., M. Alizadeh, J. Malakuti and N. Salehrastin. 2008. Efficiency Mycorrhizal relationship on growth, quality and mineral uptake absorption in drought stress on wheat. *Soil Water Sci.*, 21(2): 241-256.
- Rilling, M. C. 2004. Arbuscular mycorrhiza, glomalin and soil aggregation. Microbial ecology program, Division of Biological Weed Science. University of Montana, Missoula, USA.
- Rilling, M. C., S. F. Wright and V. Eviner. 2002. The role of Am fungi and glomalin in soil aggregation: Comparing effects of five spp. *Plant & Soil*, 238: 325-333.
- Rivera-Becerril. F. F., D. Van Tuinen, F. Martin-laurent, A. Metwally, K. J. Dietz, S. Gianinazzi and V. Gianinazii-Pearson. 2005. Molecular changes in *Pisum sativum* L. roots during arbuscular mycorrhiza buffering of cadmium stress. *Mycorrhiza*, (16): 51-60.
- Robert, M. 1990. Indigenous healing plants. Southern Book Publisher, Pretoria.
- Robson, A. D., G. W. O. Hara and L. K. Abbott. 1981. Involvements of phosphors I Nitrogen fixation of *Trifolium subterraneum* L. *Aust. J. Pl. Phys.*, 8: 427-436.
- Rogers, J., A. B. Dowsett, P. J. Dennis, J. V. Lee and C.W. Keevil. 1994. Influence of temperature & plumbing material selection on biofilm formation & growth of

- Legionella pneumophila* in a Model potable water system containing complex microbial flora. *Applied & Environmental Microbiology*, 60(5): 1585-1592.
- Rose, F. 1981. The Wild Flower Key. Fredrick warne and co. pp. 310.
- Rowe, C. S., V. P. Brown and Claassen. 2007. Comparisons of mycorrhizal responsiveness with field soil & commercial inoculum for six native montane species & *Bromustectorum*, *Roster. Ecol.*, 15: 44-52.
- Roy-Bolduc, A. and M. Hijri. 2010. The use of mycorrhizae to enhance phosphorus uptake: a way out the phosphorus crisis. *Jour. Biofertilizers & Biopesticides*, 2: 104.
- Rufykiriri, G., Y. Thiry, L. Wang, B. Delvaux and S. Declerk. 2002. Uranium uptake and translocation by the arbuscular fungi *G. intraradices*, under root organ culture conditions. *New Phytol.*, 156(2): 275-281.
- Ruiz-Lozano, J. M. 2003. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. *Mycorrhiza*, 13: 309-317.
- Ruiz-Lozano, J. M., R. Porcel and R. Aroca. 2006. Does the enhanced tolerance of arbuscular mycorrhizal plants to water deficit involve modulation of drought-induced plant genes? *New Phytol.*, 171(4): 693-698.
- Rupam, K., G. Bhoopander and K. G. Mukerjii. 2004. Improved growth and essential oil yield and quality in *F. vulgare* Mill on Mycorrhizal inoculation supplemented with P – fertilizer. *Bioresourse Technology*, 93(3): 307-311.
- Rupasinghe, H. P. V., G. Paliyath and D. P. Murr. 2003. Biosynthesis of isoprenoids in higher plants. *Physiology & Molecular Biology of Plants*, 9: 19-28.
- Ryan, M. H., D. R. Small and J. E. Ash. 2004. Phosphorus controls the level of colonization by arbuscular mycorrhizal fungi in conventional and biodynamic irrigated dairy pastures. *Aust. J. Exp. Agric.*, 40: 663-670.
- Ryan, M. H., G. A. Chilvers and D. C. Dumaresq. 1994. Colonization of wheat by mycorrhizal fungi was found to be higher in an organic manured farm than on a conventional neighbour. *Plant & Soil*. 160: 33-44.

- Rydlova, J., D. Puschel, R. Sudova, M. Gryndler, O. Mikanova and M. Vosatka. 2010. Interaction of AM Fungi and Rhizobia and its effect on flax yield in spoil bank clay. *Czech Republic. J. Pl. Nut. & Soil Sci.*, 253.
- Sabannavar. S. J. and H. C. Lakshman. 2009. Effect of Rock Phosphate Solubilization using Mycorrhizal fungi and Phosphobacteria on two high yielding varieties of *Sesamum indicum* L. *World J. Agri. Sci.*, 5(4): 470-479.
- Saif, R. 1986. The influence of soil aeration on the efficiency of VAM. Effect of soil oxygen on the growth and mineral uptake of *Eupatorium odoratum* L. inoculated with *Glomus macrocarpus*. *New Physiologist*, 88: 649-659.
- Saif, S. R. 1987. Growth responses of tropical forage plant to VAM. *Plant & Soil*, 97: 25-35.
- Saito, M. 2000. Symbiotic exchange of nutrients in arbuscular mycorrhizas: transport and transfer of phosphorus. In: *Arbuscular mycorrhizas: physiology and function*. (Eds.). Kapulnick, Y. and D. D. Douds. Jr. Kluwer Academic Press. pp. 85-106.
- Salio, G. L. and D. J. Bagyaraj. 2005. Influence of different AM fungi on the growth nutrition and forskolin content of *Coleus Forskohl*. *Mycological Research*, 109: 795-798.
- Satter, M. A., M. M. Hanafi, T. M. M. Mehmud and H. Azizah. 2006. Influence of AM Fungi and Rock Phosphate on uptake of major nutrients by *Acacia mangium*. *Biol. & Fertility of Soils*, 42(4): 345-349.
- Sawers, R. J. H., S. Y. Yang, C. Gutjahr and U. Paszkowski. 2008. The molecular components of nutrient exchange in arbuscular mycorrhizal interactions. In: *Siddiqui, Z. A., Akhtar, M. S. and Futai, K. (Eds.). Mycorrhizae: Sustainable Agriculture and Forestry*. Springer, Dordrecht, the Netherlands. pp. 37-60.
- Schenck, N. C. and Y. Perez. 1990. Manual for the identification of VA mycorrhizal fungi INVAM, University of Florida, Gainesville, USA. 1-283.

- Schreiner, R. P. 2007. Effects of native and non-native AMF on growth and nutrient uptake of *Vitis vinifera* L. in two soils with contrasting levels of phosphorus. *Appl. Soil Ecol.*, 36: 205-215.
- Schubler, A., D. Schwarzott and C. Walker. 2001. A new fungal phylum, the Glomeromycota, phylogeny and evolution. *Mycological Research*, 105: 1413-1421.
- Sensory, S., S. Demir, O. Turkmen, C. Erdinc and O. B. Savur. 2007. Responses of some different pepper (*Capsicum annuum* L.) genotypes to inoculation with two different arbuscular mycorrhizal fungi. *Sci. Hort.*, 113: 92-95.
- Sharda, W. K and B. F. Rodrigues. 2009. Application of AM Fungi in agro-ecosystems. *Tropical & Sub-tropical Agrosystem*, 10: 337-354.
- Sharif, M., E. Ahmad, M. S. Sarir, D. Muhammad, M. Shafi and J. Bakht. 2011. Response of different crops to arbuscular mycorrhiza fungal inoculation in phosphorus deficient soil. *Communications in Soil Science & Plant Analysis*, 42(19): 2299-2309.
- Sharif, M., T. Burni and Saima. 2005. Arbuscular mycorrhizal incidence and infectivity in wheat and maize crops of Bannu and D.I. Khan areas, Pakistan. *Pak. J. Pl. Sci.*, 11(1): 67-77.
- Sharma, D., R. Kapoor and A. K. Bhatnagar. 2008. Arbuscular mycorrhizal (AM) technology for the conservation of *Curculigo orchoides* Gaertn; an endangered medicinal herb. *World J. Microbial. Biotechnol.*, 24: 395-400.
- Sharma, D., R. Kapoor and A. R. Bhaytnagar. 2009. Differential growth response of *Curculigo orchoides* to native AMF communities varying in number and fungal components. *European J. Soil Biology*, 45(4): 328-333.
- Sharma, M. P. and A. Adholey. 2004. Effect of AM Fungi and P fertilization on the micro propagated strawberry grown in a sandy loam soil. *Can. J. Bot.*, 82: 322-328.

- Shinwari, Z. K., T. Watanabe, M. U. Rehman and T. Yoshikawa. 2006. A pictorial Guide to Medicinal Plants of Pakistan. Kohat University of Science and Technology, Pakistan. 273.
- Silveira, S. A., R. Lorscheiter, I. B. I. Barrow, S. F. Schwarz and P. W. D. Souza. 2006. *Mentha piperata* as a multiplying of AM fungi. *Rev. Bras. Pl. Med.*, 8: 91-97.
- Singh, A. K., V. K. Raina, A. A. Naqvi, N. K. Patra, B. Kumar, P. Ram and S. P. S. Khanuja. 2005. Essential oil composition and chemoarrays of menthol mint (*Mentha arvensis* L. f. *Piperascens* Malinvaud ex. Hoimes) cultivars. *Flavour & Fragrance Journal*, 20: 302-305.
- Smith, E. E., E. Facelli, S. Pope and F. A. Smith. 2010. Plant performance in stressful environments. Interpreting new and established knowledge of the roles of arbuscular mycorrhizas. *Plant & Soil*, 326: 3–20.
- Smith, F. A. and S. E. Smith. 1997. Structural diversity in VAM symbiosis. *New Phytol.*, 137: 373-388.
- Smith, S. E. and D. J. A. P. Read. 2008. Mycorrhizal Symbiosis Academic Press, London.
- Smith, S. E., F. A. Smith and I. Jakobsen. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiol.*, 133: 16-20.
- Soltanpour, P. N. and A. P. Schwab. 1977. A new soil test for simultaneous extraction of macro and micro nutrients in alkaline soils comm. *Soil. Sci. Plant Anal.*, 8: 195-207.
- Son, H. J., G. T. Park, M. S. Cha and M. S. Heo. 2006. Solubilization of insoluble inorganic phosphates by a novel salt- and pH-tolerant *Pantoea agglomerans* R-42 isolate from soybean rhizosphere. *Bioresourse Technology*, 97: 204-210.
- Strack, D. and T. Fester. 2006. Isoprenoid metabolism and plastid reorganization in arbuscular mycorrhizal roots. *New Phytologist*, 172: 22-34.

- Strack, D., T. Fester, B. House, W. Sehliemann and M. H. Walter. 2003. Arbuscular mycorrhizae, Biological, chemical and molecular aspects. *Journal Chemical Ecology*, 29: 1955-1979.
- Subramanian, K. S. and C. Charest. 1998. Arbuscular mycorrhizae and nitrogen assimilation in maize after drought and recovery. *Physiology Plants*, 102: 285-296.
- Sumana, D. A. and D. J. Bagyaraj. 2003. Influence of VAM fungi on growth response of neem (*Azadirachta Indica*). *Journal Tropical Forest Science*, 15(4): 531-538.
- Sylvia, D. M., L. C. Hammond, J. M. Bennett Haas and S. B. Linda. 1993. Field response of maize to a VAM fungus and water management. *Agron. J.*, 85: 193-198.
- Tanu, A. Prakash and A. Adholeya. 2004. Effect of different organic manures / composts on the herbage & essential oil yields of cymbopogon winterianus and their influence on the native AM population in a marginal alfisol. *Bio-resource Technology*, 92(3): 311-319.
- Tao, L., L. Jianping and Z. Zhiwei. 2004. Arbuscular mycorrhiza in valley type Savanna in South West China. *Mycorrhiza*, 14: 323-327.
- Tarafdar, J. C. and H. Marschner. 1995. Dual inoculation with *Aspergillus fumigates* and *Glomus mosseae* enhances biomass production and nutrient uptake in wheat (*Triticum aestivum* L.) supplied with organic phosphorus as Na-phytate. *Plant Soil*, 173: 97-102.
- Tawaraya, K., M. Turjaman and H. A. Ekamawanti. 2007. Effect of arbuscular mycorrhizal colonization on nitrogen and phosphorus uptake and growth of *Aloe vera* L. *Hortscience*, 42(7): 1737-1739.
- Thenmozhi, R., K. Rejino, K. Madusu Dhanan and A. Nagasathya. 2011. Study of various biofertilizer on the growth & biomass production of selected medicinal plants. *Research J. Agric. & Biol. Sci.*, 6(3): 296-301.
- Thingstrup, I., G. Rubeak, E. Sibbesen and I. Jakobsen. 1998. Flax (*Linum usitatissimum* L.) depends on arbuscular mycorrhizal fungi for growth and p uptake at

- intermediate but not high soil phosphorous levels in the field. *Plant & Soil*, 203(1): 37-46.
- Thompson, J. P. 1987. Decline of vesicular – arbuscular mycorrhizae in long fallow disorder of field crops and its expression in phosphorus deficiency of sunflower, *Aust. J. Agric. Res.*, 38: 847-867.
- Tian, C. Y., G. Feng, X. L. Li and F. S. Zhang. 2004. Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants. *Applied Soil Ecology*, 10: 161-168.
- Tiwari, R. J. and R. N. S. Banafar. 1995. Application of nitrogen and phosphorus increases seed yield and essential oil of corrian der. *Ind. J. Cocoa Arfcavt species*. 19: 51-55.
- Torelli, A., A. Trotta, L. Acerbi, G. Arcidiacono, G. Berta and C. Branca. 2000. IAA and ZR content in leek (*Allium porrum* L.) as influenced by P nutrition & arbuscular mycorrhizae, in relation to plant development. *Plant Soil.*, 226: 29-35.
- Toussaint, F. A., S. E. Smith and F. Smith. 2007. Arbuscular mycorrhizal can induce the production of phytochemical in sweet basil irrespective of phosphorus nutrition. *Mycorrhiza*, 17: 291-297.
- Toussaint, J. P. 2007. Investigating physiological changes in the aerial of AM plants what do we know and where should we be heading? *Mycorrhiza*, 17: 349-353.
- Toussaint, J. P., M. Kraml, M. Nell, S. E. Smith, F. A. Smith, S. Steinkellner, C. Schmiderer, H. Vierheilig and J. Novak. 2008. Effect of *Glomus mosseae* on concentrations of rosmarinic and caffeic acids and essential oil compounds in basil inoculated with *Fusarium oxysporum* f. sp. basilici. *Plant Pathology*, 57(6): 1109-1116.
- Treeby, M. T. 1992. The role of mycorrhizal fungi and nonmycorrhizal microorganism in iron nutrition of citrus. *Soil Biology and Biochm.*, 24: 857-864.

- Trindade, A. V., J. O. Siqueira and S. L. Sturmer. 2006. Arbuscular mycorrhizal fungi in papaya plantations of Espirito Santo and Bahia, Brazil. *Brazilian J. Microbial.*, 37(3): 283-289.
- Turkmen, O., S. Sensoy, S. Demir and C. Erdinc. 2008. Effect of two different AMF species on growth and nutrient content of Pepper seedlings grown under moderate salt stress. *Afric. J. Biotechnol.*, 7(4): 392-396.
- Umma, E., T. Muthukumae, K. Satheiyadash and V. Munia Ppan. 2010. Mycorrhizal and septate fungal associations in gingers and spiral gingers. *Botany*, 88(5): 500-511.
- Vaast, P., R. J. Zasoski and C. S. Bledsoe. 1997. Effect of VA mycorrhizal inoculation at different soil P availabilities on growth and nutrient uptake of *in vitro* propagated coffee plants. *Mycorrhiza*, 6: 493-497.
- Van Der Heijden, T., A. Boller and I. R. Wiemken. 1979. Sanders different arbuscular mycorrhizae fungal species are potential determinants of plant community structure. *Ecology*, 79: 2082-2091.
- Vance, C. P. 2003. Phosphorus acquisition and use: Critical adaptation by plants for securing a non-renewable resources. *New Phytol.*, 157: 423-447.
- VanderHeijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf Engel, T. Boller, A. Wiemken and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant diversity, ecosystem variability and productivity. *Nature*. 396(6706): 69-72.
- Vender-Zang, P. R., L. Fox, R. S. De La Pena and R. S. Yost. 1979. P nutrition of *Cassava* including mycorrhizal effects on P, K, S Zn and Ca uptake. *Field Crops Research*, 2: 253-263.
- Venkateshwar Rao, G., Manoharachary and B. R. Rajeswara Rac. 2002. Beneficial influence of arbuscular mycorrhizal fungal association on growth, yield and nutrient uptake of rose-scented geranium (*Pelargonium replices*). *Philippine Journal Science*, 131: 49-58.

- Venkateswarlu, B., M. Pirat, N. Kishore and A. Rasul. 2008. Mycorrhizal inoculation in Neem (*Azadirachta indica*) enhances azadirachtin content in seed kernels. *World J. Microbial. & Biotech.*, 24(7): 1243-1247.
- Viljoen, A.M., S. Petkar, S. F. Van-Vuuren, A. Cristina Figueiredo, L.G. Pedroad and J.G. Barroso. 2006. Chemo-Geographical variation in essential oil composition and the antimicrobial properties of “Wild mint” *Mentha longifolia sub sp. Polyadena* (Lamiaceae) in Southern, Africa. *J. Essential oil Research*, 18: 60-65.
- Wang and Shi. 2006. Biodiversity of arbuscular mycorrhizal fungi in China. A review. *Adv. Environ. Biol.*, 2(1): 31-39.
- Wang, B., D. M. Funakoshi, Y. Dalpe and C. Hamel. 2002. Phosphorus 32 absorption and translocation to host plants by arbuscular mycorrhizal fungi at low root – zone temperature. *Mycorrhiza*, 12: 93-96.
- Wang, S. F., X. L. He. T. S. Chen and J. Wang. 2006. Temporal and spatial distribution and colonization of AM Fungi round the rhizosphere of raspberry. *Agri. Res. In the Arid Areas*, 24(3): 214-219.
- Wani, P. V. and B. K. Konde. 1998. Effect of *Glomus mosseae* using with p source on garlic. *Journal Maharashtra Agriculture Universities*, 231: 39-42.
- Wright, S. F. and A. Upadyhaya. 1998. A survey of soils for aggregate stability and Glomalin, a glycoprotein produced by hyphae of arbuscular fungi, *Plant & Soil*, 198: 97-107
- Wu, O. S. and R. X. Xia. 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *J. Pl. Physiol.*, 163: 417-425.
- Wu, Q. S., Y. H. Peng, Y. N. Zou and C. Y. Liu. 2010. Exogenous polyamines affect mycorrhizal development of *Glomus mosseae* colonized citrus (*Citrus tangerine*) seedlings. *Science Asia*, 36: 254-258.
- Xavier, L. J. C. and J. J. Germida. 2003. Bacteria associated with *Glomus clarum* spores influence mycorrhizal activity. *Biochem.*, 35: 471-478.

- Yamato, M. 2004. Morphological types of arbuscular fungi in roots of weeds on vacant land. *Mycorrhiza*, 14: 127-131.
- Yang, A. N., L. Lu and N. Zhang. 2011. The diversity of arbuscular mycorrhizal fungi in the subtropical forest of Huangshan (Yellow Mountain), East-Central China. *World J. Microbial. & Biotechnol.*, 40(2): 1–8.
- Yang, S. Y. and U. Paszkowski. 2011. Phosphate Import at the Arbuscule: Just a Nutrient. *Molecular Plant-Microbe Interactions*, 24(11): 1296-1299.
- Yao, O. and X. Li. Chritie. 2001. Factors affecting mycorrhizal dependency of wheat genotypes with different phosphorus deficiencies. *Plant Nutrition*, 24: 1409-1419.
- Yaseen, T., T. Burni and F. Hussain. 2011. Effect of arbuscular mycorrhizal inoculation on nutrient uptake, growth and productivity of cowpea (*Vigna unguiculata*) varieties. *Afric. J. Biotechnol.*, 10(43): 8593-8598.
- Zainab, S. B. and T. Burni. 2005. Vesicular arbuscular mycorrhizal studies in weeds of wheat fields of Peshawar, Pakistan. *Pak. J. Pl. Sci.*, 11: 93-101.
- Zaki, M. F., A. A. M. Abdel Hafez and Camiliay El-Dewiny. 2010. Influence of applying phosphate Bio-fertilizers and different levels of phosphorus sources on the productivity, quality and chemical composition of sweet fennel (*Foeniculum vulgare* Mill.) *Aust. J. Basic. Appl. Sci.*, 4(2): 334-347.
- Zaki, M. F., A. M. Hoda, E. H. Habib, E. H. Abd, E. L. Samad and Z. A. Lashine. 2011. Effect of different sources and rates of phosphorus on vegetative growth, antioxidant and mineral contents of two sweet fennel cultivars *European J. Scientific Research*, 51(4): 499-511.
- Zandavalli, R. B., L. R. Dillenburg and P. V. D. de Souza. 2004. Growth responses of *Araucariaangustifolia* (Araucariaceae) to inoculation with the mycorrhizal fungus *Glomus clarum*. *Appl. Soil Ecol.*, 25(3): 245-255.
- Zanearo, W., J. Nishidate G. Vandressen, Andrade and M. A. Nogueiva. 2007. Root mycorrhizal colonization and plant responsiveness are related to root plasticity,

soil fertility and successional status of native wood species in Southern Brazil. *J. Trop. Ecol.*, 23: 53-62.

Zang, Y., L. D. Guo and R. J. Liu. 2003. Diversity and ecology of Arbuscular mycorrhizal fungi in Dujiangyan. *Acta. Phytoecol. Sinica*, 27: 537-544.

Zubek, S. and J. Błaszowski. 2009. Medicinal plants as hosts of arbuscular mycorrhizal fungi and dark septate endophytes. *Plant-Microbial Interactions*, 8(3): 571-580.

Zubek, S., S. Mielcarek and R. Turnav. 2011. Hypericin and pseudohypericin concentrations of a valuable medicinal plant *Hypericum perforatum* L. are enhanced by arbuscular mycorrhizal fungi. *Mycorrhiza*, 22: 149-156.