Pak. J. Bot., 45(1): 293-296, 2013.

EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON ESSENTIAL OILS OF TWO PHARMACEUTICALLY IMPORTANT *MENTHA* SPECIES IN MARGINAL SOILS

TANVIR BURNI[1*], FARRUKH HUSSAIN[1] AND M. SHARIF[2]

[1]Department of Botany, Peshawar University, Peshawar
[2]Department of Soil & Environ. Sciences, NWFP Agric. University Peshawar, Pakistan
Corresponding author's email: tanvirburni@yahoo.com

Abstract

Arbuscular mycorrhizal fungi have been used extensively in pharmaceutically important essential oil yielding plants in low fertility soils.The present study aimed at to find out the effects of monospecific GI (*Glomus intraradicus*) and indeginous AMF inocula, MC1, MC2 (with variable species composition) on essential oil yield, root colonization and spore density of *Mentha arvensis* and *M.longifolia* growing in nutrient deficient soil. 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 the inocula used. MC1 containing higher AMF species richness exhibited maximum improvement in terms of growth parameter evaluated, and essential oil. As far as 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 AM fungi would be of potential use for improving their productivity with environment protection

Introduction

In view of the wide spread use of essential oils, aroma chemicals, and other related items, oil yielding plants are now viewed as an important commodity item and there has been a significant increase in industrial turnover, over the last two decades (Kumar *et al*., 2009, Shinwari, 2010). The trade of essential oils, containing pharmaceutically active compounds gained prominence in many industrially developed and under developed countries in the recent time (Singh *et al.,* 2001). Pakistan enjoys a wide variety of soil, climate and altitude, is one of the few countries in the world perfectly suited for the cultivation of a large number of essential oil bearing plants (Shinwari & Qaiser, 2011).

Due to hike in the prices of chemical fertilizer and also with a view to avoid soil pollution, scientists are now focused to compensate the use of chemical fertilizer with biofertilizer like AM fungi which would be an environment friendly approach. 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 and therefore lessen the prerequisite for phosphate-based fertilizers (Khalafallah & Abo-Ghalia, 2008; Roy-Bolduc & Hijri, 2010; Jalaldin & Hamid, 2011) Recently, mycorrhizal inoculation not only promoted the growth of medicinal plants but also improved the productivity and quantity of chemicals.

Therefore in the present experiment symbiotic efficiency of monospecific (GI) and indigenous multispecific AMF inocula (MC1, MC2) were evaluated on growth performance, essential oil yield, composition root colonization and spore density in two species of *Mentha* i.e., *M. arvensis* (MA) and *M. longifolia* (M.L) . Present study also aimed to select symbiotic efficient strains for particular host in order to harness the maximum benefit from the AMF inoculum in herb industry.

Materials and Methods

Soil 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, available Phosphorus (%) 5.25±0.16, texture silt, loam sand 54.2%, silt (38.0%) and clay (17.8%). Stolons (Healthy stolons) of test species *Mentha arvensis*(MA) (Fig. 1) were collected from Herb Garden Qarshi industry Haripur and *Mentha longifolia*(ML) from medicinal garden PFI Peshawer and vocher specimens were deposited in Peshawer university Herbarium. Three AMF inocula i.e., Monospecific inoculum of AMF species *Glomus intraradices* (GI), Multi indeginous AMF inoculum having eleven *Glomus* species (*A. melleae* Spain & Schenck *G. fasiculatum* (Thaxter) *G. etunicatum* Becker and Gerdermann *G. claroides* Schenck & Smith *G. microcarpum* Tul & Tul (Fig. 2) *G.australe* (Berkeley) Berch *G. intaradicus* Shenck & Smith *G. aggregatum* Schenck & Smith *G. constrictum* Trappe G.*mosseae*. Gerdeman & Trappe *Gigaspora gigantea* Gerdemann and Trappe) collected from the rhizosphere soil and roots of *Mentha longifolia* (MC1), Multi indigenous AMF inoculum having 7 species MC2 (*A. leavis* Gerdeman & Trappe *G. microcarpum* Tul & Tul *G. fasiculatum* (Thaxter) G.*mosseae*.Gerdeman&Trappe *G. aggregatum* Schenck & Smith *Glomus inveranum Hall Gigaspora.albidaSchenck&* Smith)collected from the rhizospheric soil of *Mentha arvensis* . For the extraction of spores decanting technique was followed (Gerdemann & Nicolson, 1963). Roots were processed and stained by following the procedure given by Phillips & Hayman, (1970).

AM inoculum was applied by the method described by Kapoor *et al*., (2002). Harvesting of plants was done after 90 days. Mycorrhizal inoculum preparation, placement and application were done by the method given by Gaur & Adholeya, (2002). A modified Clevenger type apparatus were used for the extraction of essential oil from the leaves of *Mentha* species through hydro- steam distillation. Experimental data was statistically analyzed by applying ANOVA test the means were subjected to LSD test.

Results and Discussion

Mentha species have long been used as medicine by the indigenous communities and have also been exposed to modern studies like molecular systematics (Shinwari *et al*., 2011; Jabeen *et al*., 2012). However, little work had been to study the effect of mycorrhizal species on their growth and oil yield .The results of present investigations revealed that both the studied species of *Mentha* inoculated with indeginous multispecies inocula MC1 and MC2 performed better than G1 The research work showed better performance of mycorrhizal plants than non inoculated and non-inoculated (Control) Different plant species (oregano and mint) showed different reactions to the same isolates of mycorrhizal fungi (Karagiannidis *et al*., 2011).In addition not only the plant species, but also the host variety may influence the response to AM inoculation (Gupta *et al*., 2002).

Essential oils yield: Essential oils are volatile, lipophilic mixtures of secondary compounds, mostly containing monoterpenes, sesquiterpenes and phenylpropanoids (Khaosaad *et al.*, 2007). There is a growing volume of research that indicates that AM may influence 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 fugus and plant accession.(Chaudary *et al*., 2008; Kapoor *et al*., 2004). During present investigations we also noticed the superior performance of mycorrhizal fungi regarding oil yield as campared to non inoculated control (Fig. 3).

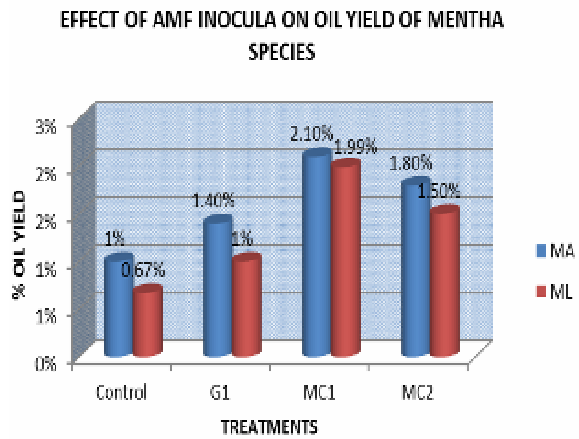

Fig. 3. Oil yield (%) of *Mentha longifolia* (ML) and *Mentha arvensis* (MA).Plants were grown non inoculated(C) or inoculated with GI (*Glomus intraradicus*) Indeginous AMF (MC1 and MC2). Treatments were tested with two way ANOVA.

Fig. 1. Plants of MA inoculated with MC1.

Fig. 2. Spores of *Glomus claroides*.

Mentha arvensis inoculated with MC1 was also found to contain significantly higher amount of essential oil (2.0/100 gm. herb), as compared to uninoculated control plants (1.00 ml/100gm herb). In *Mentha longifolia* oil yield was 2.0ml/100 gm. in the same treatment. There are reports in *Mentha* species where the root colonization by AM fungi improve the quality and quantity of essential oils and it also depends upon plant variety (Gupta *et al.*, 2002; Frietas *et al.*, 2004). Karagiannidis *et al.*, (2010) reported essential oil yield in different species of *Mentha* ranged from1.5 to 3.6%.from different locations of Northern Greece. Our results also matched with Karagiannidis *et al.*, (2012) who noticed the significant increase in essential oil production in mycorrhizal plants ranged from 24%-56.95%.

Root colonization and AMF spore density: The data on percent colonization and spore count of studied test species are given in (Table 1). Mycorrhizal plants of *M.arvensis* treated with MC1 showed significantly maximum colonization (84.96%) (Fig. 4). While significantly lowest colonization level (34.70 %) was observed in plants inoculated with GI. Similar trend of root colonization was noticed in *M.longifolia* maximum being in MC1 (65%) (Fig. 5) and minimum in GI (38.7%).

Both *Mentha* species investigated, 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 MC1consequently the association enhanced biomass production as compared to the other two inocula of AM fungi. Therefore, a degree of selective effectiveness is evident among the three VAM fungi inocula used for inoculating *Mentha* species.our results are also supported by (Khaliq & Janardhanan, 1997) who studied the influence of three *Glomus* species on growth performance of six *Mentha* species.They observed the significant variations in effectiveness of tested AM species on mints. Moreover, they suggested that variation in the degree of the symbionts effectiveness of AMF should be considered before large scale inoculation programme.

Karagiannidis *et al.,* (2010) found root colonization in various *Mentha* species ranged from (8-100%) but Karagiannidis *et al*., (2011) while working on mint and oregano found non significant differences between each other in root colonization. The effect of AM fungi on the plants depends strongly on the exent of colonization (Copetta *et al*., 2006). Moreover, studies on three genotypes of oregano showed that essential oils in mycorrhizal plants is not rely on available P in the soil,but on the association with mycorrhizae (Khaosaad *et al*., 2006). In the present study there were variations in root colonization in two studied species of *Mentha*. Our findings are supported by Karangiannidis *et al.,* (2011) and Chaudary *et al., (*2012) there may be differences in the responsiveness to mycorrhization among varieties of the same plant.

Results of present Investigations have shown that number of spores present in the rhizosphere follow the same pattern significantly high spore count 350/100gm soil was noticed in MC1followed by MC2 and least130/100gm encountered in GI. Moreover we also found correlation of AMF spore population with root colonization. Contrarily, Khaliq & Janardhanan, (1997) did not find any correlation between spore count and root colonization, they found maximum spore count in *G. aggregatum* inoculated pots (600/100 gm soil) where the root colonization was only 61%.

Table 1. Infection and Spores of two mentha species. Each value is a mean of five replicates. Values followed by different letters are significantly different ($p<0.05$) (Two way ANOVA).

Species	Treatments	Infection%	Spores/100g. soil
	Gi	34.70[f]	150.8[e]
ML	MC1	84.96[a]	357.0[b]
	MC2	58.04[d]	180.2[c]
	Gi	38.76[e]	130.0[f]
MA	MC1	80.00[b]	380.0[a]
	MC2	65.00[c]	170.0[d]
LSD at 5%		1.278	8.624
	T	0.0000	0.2800
P VALUE	S	0.0000	0.0000
	(T × S)	0.0000	0.0000

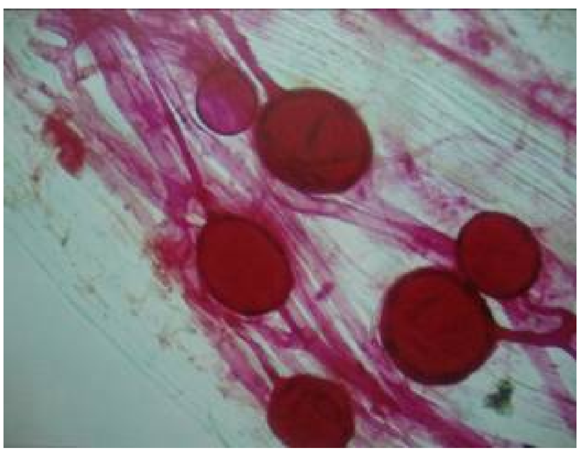

Fig. 4. AM root infection in M.A.

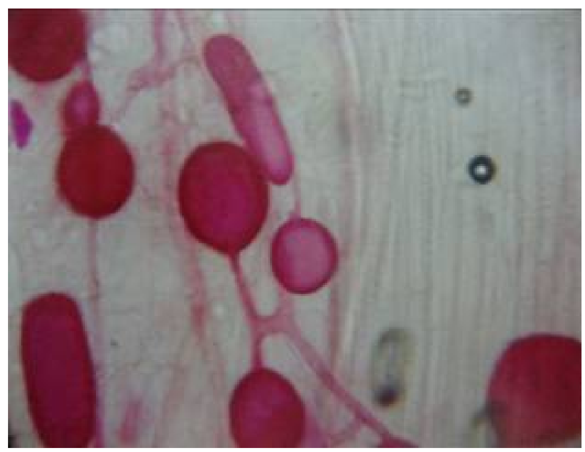

Fig. 5. AM root infection in M.L.

Conclusion

Results of present investigation supports the new trend of using eco-friendly AMF as natural alternatives to improve the production of *Mentha* essential oils instead of using synthetic fertilizers in order to achieve clean environment with minimum pollution

Acknowledgments

The grants of University of Peshawer and HECIslamabad to Tanvir Burni Ph.D. scholar is gratefully acknowledged. This paper is a part of Ph.D. research work sponsored by university of Peshawer Pakistan

References

Copetta, A., G. Lingua and G. Berta G. 2006. Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in Ocimum basilicum L. var. Genovese. *Mycorrhiza*, 16: 485-494.

Chaudhary, M.S., M. Saeed, A. Khan, N. Siali and M. Jamil. 2012. Moorphological diversity of Arbuscular Mycorrhiza colonizing two aromatic grasses *Veteviria zizanioides* and *Cymbopogon jawarancusa*.*Pak. J. Bot.*, 44(4): 1479-1485.

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.

Gaur, A. and A. Adholeya. 2002. Arbuscular-mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended organic matter. *Biol. Fertil. Soils,* 35: 214-218.

Gerdemann, J.W. and T.H. Nicolson. 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Trans. Brit. Mycol. Soc*., 46: 235.

Gupta, M.L., A. Prasad, M. Ram and S. Kumal. 2002. Effect of the AM Fungus *G.fasiculatum* on the essential oil yield related characters and nutrient acquisition in the crops of different cultivars of menthol under field condition. *Luknow.Bioresourse technol.,* 81: 77-79.

Jabeen A., B. Guo, B. H. Abbasi, Z. K. Shinwari1 and T. Mahmood. 2012. Phylogenetics of selected *Mentha* species on the basis of *rps*8, *rps*11 and *rps*14 chloroplast genes. *J. of Medicinal Plants Research,* 6(1): 30-36.

Jalaluddin, M. and M. Hamid. 2011. Effect of adding inorganic, organic and microbial fertilizers on seed germination and seedling growth of sunflower. *Pak. J. Bot.,* 43(6): 2807-2809.

Kapoor, R., B. Giri and K.G. Mukergi. 2004. Improved growth and essential oil yield and quality in *Foeniculun vulgare* Mill mycorrhizal inoculation supplemented with P fertillizer. *Bioresource Technol*., 93l: 309-311.

Kapoor, R., B. Giri and K.G. Mukergi. 2002. *Glomus macrocarpum*: a potential bioinoculant to improve essential oil quality and concentration in Dill (*Anethum graveolens* L.) and Carum (*Trachyspermum ammi* (Linn.) Sprague). *World J. Microbiol. & Biotech.*, 18(5): 459-463.

Karagiannidis, N., H. Panou-Filotheou, D. Lazari, I. Ipsilantis and C. Karagiannidou. 2010. Essential oil content and composition, nutrient and mycorrhizal status of some aromatic and medicinal plants of Northern Greece. *Natural Product Communications*, 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 Horticulturae*, 129: 329-334.

Karagiannidis, N., T. Thomidis, D. Lazari, E. Panou-Filotheou and C. Karagiannidou. 2012. Response of three mint and two oregano species to Glomus etunicatum inoculation, *Astralian. J. Crop. Sci*., 6(1): 164-169.

Khaliq, A. and K.K. Janardhanan. 1997. Influence of VAM Fungi on the productivity of cultivated mints. Lucknow. *J. of Medi. and Arom. Pl. Sci*., 19: 7-10.

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.

Khaosaad, T., H. Vierheilig, M. Nell, K. Zitterl-Eglseer and J. Novak. 2006. Arbuscular mycorrhiza alter the concentration of essentialoils in oregano (*Origanum* sp., Lamiaceae). *Mycorrhiza*, 16: 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 Biol. & Biochem.,* 39: 727-734.

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.). *EJEAFChe*, 8(2): 86-95.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Transaction British Mycological Society,* 5: 158-161.

Roy-Bolduc, A. and M. Hijri. 2010. The Use of Mycorrhizae to Enhance Phosphorus Uptake, A Way Out the Phosphorus Crisis. *J. Biofertil. Biopestici.*, 2: 104.

Shinwari, Z.K. 2010. Medicinal Plants Research in Pakistan. *J. Med. Pl. Res*., 4(3): 161-176.

Shinwari, Z.K., S. Sultan and T. Mehmood. 2011. Molecular and Morphological Characterization of Selected *Mentha* Species. *Pak. J. Bot*., 43(3): 1433-1436.

Shinwari, Z.K. and M. Qaiser. 2011. Efforts on conservation and sustainable use of medicinal plants of Pakistan. *Pak. J. Bot*., 43 (Special Issue): 5-10.

Singh, M. 2001. Effect of nitrogen, phosphorus and potassium nutrition on herb, oil and artemisinin yield of Artemisia annua under semi-arid tropical condition. *J.Med.Aro.Pl.Sci.*,23(1):368-369.

(Received for publication 31 August 2011)