EFFEect of ARBUCULAR MYCORRHIZAL FUNGI ON ESSENTIAL OILS OF TWO PHARMACEUTICALLY IMPORTANT MENTHA SPECIES IN MARGINAL SOILS

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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 intraradices) and indigenous 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-Ghelia, 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 (ML). 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 voucher specimens were deposited in Peshawer university Herbarium. Three AMF inocula i.e., Monospecific inoculum of AMF species Glomus intraradices (GI), Multi indigenous 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. interadices Shenck & Smith G. aggregatum Schenck & Smith G. constrictum Trappe G.mosseae. Gerderman & Trappe Gigaspora gigantea Gerdermann and Trappe) collected from the rhizosphere soil and roots of Mentha longifolia (MC1), Multi indigenous AMF inoculum having 7 species MC2 (A. leavis Gerderman & Trappe G. microcarpum Tul & Tul G. fasciulatum (Thaxter) G.mosseae Gerderman&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 (Gerdermann & 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 indigenous 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 fungus 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 compared to non inoculated control (Fig. 3).

![Fig. 1. Plants of MA inoculated with MC1.](image1)

![Fig. 2. Spores of Glomus claroides.](image2)

*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 (1.0 ml/100gm herb). In *Mentha longifolia* oil yield was 2.0 ml/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 from 1.5 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%.

![Effect of AMF inocula on oyle yield of Mentha species.](image3)

![Fig. 3. Oil yield (%) of Mentha longifolia (ML) and Mentha arvensis (MA). Plants were grown non inoculated (C) or inoculated with G1 (Glomus intraradicus). Indegenous AMF (MC1 and MC2). Treatments were tested with two way ANOVA.](image4)

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 MC1 consequently 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 Karagianndis 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 extent 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 MC1 followed by MC2 and least 130/100gm 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).

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatments</th>
<th>Infection%</th>
<th>Spores/100g. soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gi</td>
<td>MC1</td>
<td>34.70</td>
<td>150.8</td>
</tr>
<tr>
<td></td>
<td>MC2</td>
<td>58.04</td>
<td>180.2</td>
</tr>
<tr>
<td></td>
<td>Gi</td>
<td>38.76</td>
<td>130.0</td>
</tr>
<tr>
<td></td>
<td>MC1</td>
<td>80.00</td>
<td>380.0</td>
</tr>
<tr>
<td></td>
<td>MC2</td>
<td>65.00</td>
<td>170.0</td>
</tr>
<tr>
<td>MA</td>
<td>Gi</td>
<td>34.96</td>
<td>150.6</td>
</tr>
<tr>
<td></td>
<td>MC1</td>
<td>37.86</td>
<td>130.0</td>
</tr>
<tr>
<td></td>
<td>MC2</td>
<td>31.00</td>
<td>100.0</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>T</td>
<td>1.278</td>
<td>8.624</td>
</tr>
<tr>
<td>P VALUE</td>
<td>S</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td></td>
<td>(T × S)</td>
<td>0.0000</td>
<td>0.0000</td>
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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

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