RESEARCH ARTICLE

Effect of AM Fungi on *Mentha spicata* L.

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ABSTRACT

In order to study the effect of various doses of AM fungi on growth of spearmint (*Mentha spicata* L.) plants, a series of experiments were conducted in pot cultures in randomised block design (RBD). A commercial AM fungal consortium 'Rhizagold' was used as the Arbuscular mycorrhizal fungal (AMF) culture. The various growth parameters such as dry weight, chlorophyll content, relative water content, Nitrogen content, Phosphate Phosphorous content and potassium content of spearmint were tested after 60, 90 and 120 DAS. Per cent root colonisation and spore count were also tested. The results were encouraging and demonstrated that there was a significantly positive effect of increasing doses of AMF culture on various growth parameters of spearmint plants throughout the experiment. Amongst the various treatments, the treatment T_2 and T_3 were proved to be better for harvest at 90 DAS than the remaining treatments.

Key words: AM fungi, spearmint (*Mentha spicata* L.), Rhizagold, pot cultures, growth, harvest.

INTRODUCTION

The Arbuscular Mycorrhizal fungi (AMF), which were formerly known as Vesicular Arbuscular Mycorrhiza (VAM) are unique group of ubiquitous soil microorganisms known to form symbiotic association with roots of economically important crop plants (Pragatheswari *et al.*, 2004). The arbuscular mycorrhizal fungi are the associations between higher plants and fungi where the plants normally provide carbon to fungus and fungus provides nutrients and water to plant. The AM fungi infect the root system and invade several layers of the outer root cortex. AM fungal hyphae penetrate the individual cells and form unique structures called arbuscules and vesicles. Arbuscules are the much-branched structures within the cells while vesicles (may be intracellular or intercellular) are balloon-like structures and probably have a function of storage (Harley and Smith, 1983). The fungal hyphae do not penetrate the protoplast, but invaginate the cell membrane. The structure of the arbuscules greatly increases the contact surface area between the hypha and the cell cytoplasm to facilitate the transfer of nutrients between them.

Spearmint (*Mentha spicata* L.), is an annual herb of the family Lamiaceae (Labiatae). Spearmint is chiefly employed in cooking especially as a condiment to flavour the curries, soups, chutneys, sauces, etc. Spearmint is a useful source of essential oil and has been used for a long time in the perfumery, cosmetic, food and pharmaceutical industry. As the spearmint oil is stimulant, antiseptic, restorative, carminative and antispasmodic, it is used in various medicines. Due to its low menthol (0.5 per cent compared to 40 per cent in peppermint) and menthone content, these medicines can be safely given to children and old people. Spearmint has a great export potential (Kumar *et al*., 1997). Thus, there is a need to focus on the cultivation of spearmint plant to enhance its production.

Due to public awareness programs and various government policies, the farmers have realized the hazardous effect of chemical fertilizers and hence, they are increasingly shifting to sustainable agricultural practices which include use of natural resources and organic inputs to get maximum yield without harming the ecosystem. In the past few decades, Arbuscular mycorrhizal (AM) fungi have emerged as potential biofertilizers, a cheap, environmentally friendly alternative to expensive chemical fertilizers (Srivastava *et al*., 1996).

Therefore, in the present experiment, the symbiotic efficiency of AM fungi on growth performance, root colonization and spore density in *Mentha spicata* L. was evaluated. Present study also aimed to select the optimum dose of AM fungal culture in order to harness the maximum benefit from the AMF inoculum at the time of harvest.

MATERIALS AND METHODS

The soil used for the experiment, was procured from Pathare Nursery, Kalyan, India having pH 6.75, electric conductivity (EC) 0.816 mS, water holding capacity 112%, organic carbon 1.031%, nitrogen 0.761 mg/gm soil, phosphate phosphorous 1.763 mg/gm soil and potassium 0.336 mg/gm soil. A commercial AM fungal consortium 'Rhizagold' (200 viable spores per 10 gram) manufactured by 'Biotrack Tech. Pvt. Ltd.' purchased from Tamil Nadu, India was used as the Arbuscular mycorrhizal fungal (AMF) culture. Four doses of AMF culture viz., 1 gm (T_1), 2 gm (T_2), 3 gm (T_3) and 4 gm (T_4) were tested by mixing each dose with 3 kg of sterilized soil separately as each plastic pot could accommodate this much of soil. Four replications of control pots and four replications of each treatment pot were maintained. 10 pieces of spearmint suckers having 1 – 2 inches length were sown in each pot. Pots were watered on alternate days.

The effect of various concentrations of AM culture on the vegetative growth of spearmint plants was studied separately after 60, 90 and 120 days of sowing (DAS) with respect to various parameters viz., Dry weight, Chlorophyll content (Arnon, 1949), Relative Water Content (Noggle and Fritz, 1983), Nitrogen by micro-kjeldahl (Sadasivam and Manickam, 2008), Phosphate phosphorous by colorimetry (Bhargava and Raghupathi, 1993) and Potassium by flame photometry (Bhargava and Raghupathi, 1993). Per cent root colonization (Phillips and Hayman, 1970; Koske and Gamma, 1989 and Grace and Stribley, 1991) and spore count (Gerdemann and Nicolson, 1963) were also studied after 60, 90 and 120 days of sowing.

Statistical analysis

The experiment was laid in randomized block design (RBD) with four replicates of control pots and four replicates of each treatment pot. Data were expressed as mean value of these four replicates. The mean values were subjected to statistical analysis and the one way Analysis of Variance (ANOVA) was constructed. The test was carried out by referring the 'F' value obtained to the standard 'F' value at 5% level of significance. The standard error and critical differences were also calculated. All the calculations were made by using data analysis tool pack for Microsoft Excel 2007 and Windows 7.

RESULTS AND DISCUSSION

Dry weight of aerial shoot

The treatments T_2 and T_4 produced significant effect on dry weight of shoots throughout the study (Table 1.A). At 120 DAS, all the treatments produced significant effect on mean dry weight of arial shoot of spearmint plants. The probable reason for the significant increase in dry weight, is the increase in supply of nutrients (Furlan *et al*., 1983; Marschner and Dell, 1994; Habte and Soedrajo, 1996) and enhanced nutrient absorption especially phosphorous and greater rates of photosynthesis in inoculated plants

(Cooper, 1984; Sankaran, 2004). Increase in shoot biomass of spearmint plants by mycorrhiza was also reported by Kumar (2012). Similar significant increase in dry weight of shoot was also reported by Khaliq *et al*. (2001) in peppermint, Zolfaghari *et al*. (2013) in *Ocimum basilicum*, by Padmavathi (2009) in *Ocimum sanctum*, Rasouli-Sadaghiani *et al.* (2010) in Basil and by Rashmi and Roy (2003) in *Eleusine coracana* (Finger millet).

Chlorophyll content

The present study showed that the AMF treatments had significant impact on chlorophyll content of spearmint leaves (Table 1.A). The treatments T_3 and T_4 were proved themselves to be more promising by exhibiting significant impact on total chlorophyll content throughout the experiment. The treatment T_2 produced significant positive effect at later stage of development i.e. at 90 and 120 DAS. This may be due to relatively lower root colonization in treatment T_1 and T_2 at 60 and 90 DAS. The increase in chlorophyll content under the influence of AMF may be due to the increase in stomatal conductance, photosynthesis, transpiration and enhanced plant growth (Levi and Krikun, 1980; Hayman, 1983). Rate of photosynthesis is susceptible to the inorganic phosphate concentration in the chloroplast (Beever and Burns, 1980). The AM fungi not only help the plants in better absorption of phosphorous but also help indirectly in increased uptake of other macro and micro nutrients (Safir *et al*., 1972) which are essential for chlorophyll synthesis. Thus, improved phosphorous nutrition through mycorrhizal application may have contributed for the enhanced chlorophyll content in the present investigations.

Significantly higher amount of total chlorophyll content in the leaves of AMF inoculated *Vigna unquiculata* plants than that in control plants was observed by Rajasekaran and Nagarajan (2005) while that in Mulberry plants was reported by Baqual *et al*. (2005). Jadhav (2011) had also got parallel results while working on Patchouli and Ashwagandha plants. Haripriya *et al*. (2010) reported ameliorative effect of AM inoculation on chlorophyll content of Ashwagandha.

Table 1: Effect of various levels of AMF culture on growth of spearmint plants

A. Dry weight, chlorophyll content and relative water content (RWC) under various levels of AMF culture									
	Dry weight (gm)			**Total Chlorophyll content** (mg per gm fresh leaves)			**RWC of leaves** (per cent)		
Treatments	**60 DAS**	**90 DAS**	**120DAS**	**60 DAS**	**90 DAS**	**120DAS**	**60 DAS**	**90 DAS**	**120DAS**
C	0.053	0.041	0.058	1.325	1.578	1.561	85.284	89.310	90.452
T_1	0.065	0.044	0.062	1.560	1.830	1.696	88.852	93.552	93.552
T_2	0.075	0.070	**0.147**	1.617	1.841	2.151	88.402	**94.316**	92.036
T_3	0.066	**0.083**	0.131	1.821	2.055	2.187	**90.597**	94.087	**95.788**
T_4	**0.086**	0.063	0.096	**1.898**	**2.093**	**2.297**	89.512	93.534	93.742
F Test	Significant	Significant	Significant	Significant	Significant	Significant	Non-Significant	Significant	Significant
S.E.	±0.009	±0.010	±0.032	±0.182	±0.102	±0.252	–	±1.601	±1.410
C.D.	0.020	0.022	0.067	0.389	0.216	0.537	–	3.410	3.004
B. NPK content of spearmint plants under various levels of AMF culture									
	Nitrogen content (mg per gm dry plant material)			**Phosphatephosphorous content** (mg per gm dry plant material)			**Potassium content** (mg per gm dry plant material)		
Treatments	**60 DAS**	**90 DAS**	**120DAS**	**60 DAS**	**90 DAS**	**120DAS**	**60 DAS**	**90 DAS**	**120DAS**
C	22.584	20.442	20.466	0.105	0.216	0.255	2.476	2.700	2.291
T_1	22.811	27.579	32.848	0.156	0.353	0.406	2.532	4.815	**4.154**
T_2	28.434	34.561	34.596	0.169	0.482	0.475	4.454	4.562	3.879
T_3	**32.406**	35.187	**35.382**	**0.255**	**0.537**	**0.555**	**5.174**	**5.647**	3.482
T_4	29.926	**35.747**	30.633	0.236	0.345	0.419	4.376	4.423	3.963
F Test	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant
S.E.	±3.427	±5.027	±4.595	±0.067	±0.078	±0.059	±0.450	±0.406	±0.474
C.D.	7.299	10.708	9.787	0.142	0.166	0.126	0.959	0.866	1.011

Relative water content

Though the AMF treatments were non-significant for relative water content at 60 DAS, all of them gave significant results at 90 DAS while at 120 DAS, the treatments T_1, T_3 and T_4 were significantly effective (Table 1.A). Poor response with respect to relative water content at 60 DAS may be the result of lower root colonization at 60 DAS. Maiti *et al*. (2009) found out that higher AM fungi colonization in rice roots maintained elevated leaf RWC over that of treatments with lower colonization. Higher RWC due to AMF inoculation was also reported by Shivaputra *et al*. (2004[b]) in papaya plant and by Devachandra *et al*. (2009) in *Syzygium cuminii* Skeel. (Jamun). According to Singh (2004), AM inoculated plants showed improved resistance to wilting and recover their leaf turgor faster than that in non-mycorrhizal plants.

Nitrogen content

The effect of AMF culture on nitrogen content was found to be commendable throughout the experiment (Table 1.B). At 60 DAS only treatments T_3 and T_4 of AMF exhibited significant results while at 90 DAS, the T_2 also started producing significantly higher nitrogen content whereas at 120 DAS, all the treatments were significantly praiseworthy. The higher plant nitrogen content in AMF inoculated plants could be attributed to hyphal uptake. Extra radical hyphae permit the transfer of nutrients such as nitrogen (Marschner and Dell, 1994). Besides, inorganic form of ammonium nitrogen can be absorbed by AM fungi (Ames *et al*., 1983). The increasing response of spearmint plants over a period may be attributed to increasing root colonization and activity of AMF. Increase in nitrogen content in AMF inoculated plants was also reported by many workers (Rasouli-Sadaghiani *et al*., 2010; Shivaputra *et al*., 2004[a]; Kessel *et al*., 1985).

Phosphate-phosphorous content

Mycorrhizal fungi are known to have better access to the pools of phosphorous which otherwise not readily available to the plants (Sharma *et al*., 2014). In the present study also, the effect of AMF culture on phosphate phosphorous was noteworthy (Table 1.B). Each treatment exhibited significant results at least at one stage of the development. The AMF treatment T_3 was successful enough for producing significant increase in phosphate phosphorous content throughout. Increase in phosphate uptake by mycorrhiza leads to increase in plant phosphate concentration (Filter, 1991). Previous work has shown that arbuscular mycorrhizal fungi increase plant phosphate content (Rasouli-Sadaghiani *et al*., 2010; Bolan, 1991). McGonigle and Miller (1993) have reported higher amount of phosphorous in the leaves of AM associated maize plants than that in control maize plants.

A number of factors may contribute to the increased rate of phosphorous uptake measured in mycorrhizal plants (Smith and Read, 1997). Mycorrhizal fungi increase the availability of phosphorous either by organic acids production or by enhancing the phosphatase activity in the rhizosphere (Sharma *et al*., 2014). An extensive network of hyphae, extending from roots enables the plants to explore a greater volume of soil, thereby overcoming limitations imposed by the slow diffusion of phosphorous in the soil. The mycorrhizal fungi may also be able to scavenge phosphorous from the soil solution more effectively than other soil fungi. The plant fungus association could, therefore, enables the plant to compete more effectively with soil microorganisms for the limited amount of available soil phosphorous. Mycorrhizal fungi may also be able to acquire phosphorous from organic sources that are not available directly to the plants (Jayachandran and Shetty, 2003).

Potassium content

The potassium content of spearmint plants was significantly affected by AMF treatments. Almost all the treatments showed significantly higher potassium content of spearmint plants throughout the experiment (Table 1.B). This higher nutrient uptake in mycorrhizal plants might be attributed to the contribution of fungal external mycelia which explore a large volume of soil and thus absorb more nutrients (Gupta and Janardhanan, 1991). Khaliq *et al*. (2001) reported significant increase in potassium uptake by peppermint due to application of VAM fungi at 90 DAS. Besides, Balasubramaniun and Nambisan (1989) marigold seedlings, Gupta *et al*. (1990) in palmrosa, Kennedy and Rangarajan (2001) in papaya proved the efficiency of AMF inoculum in potassium uptake.

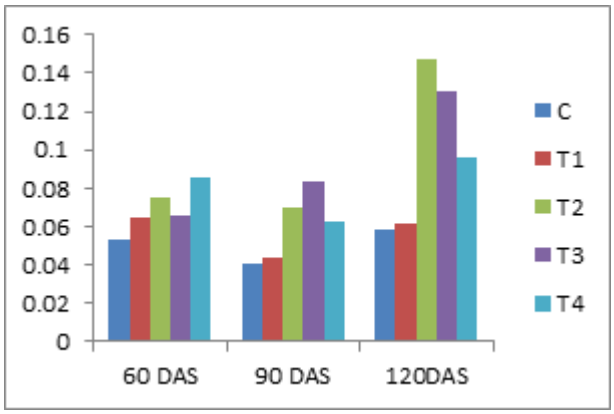
Spore count and Root colonization in AMF inoculated plants

The data showed that throughout the study, the increase in dose of AMF culture successfully resulted in increase in spore count of mycorrhizal fungi in soil (Table 2). The treatment T_1 which received lowest

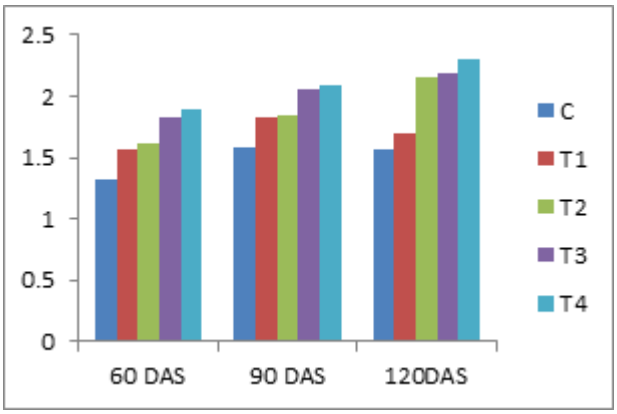
dose of AMF culture showed lowest mean spore count whereas treatment T_3 and T_4 showed almost two folds higher spore count than that of treatment T_1. At 120 DAS, the highest spore count was recorded in the soil from treatment T_4. The per cent root colonization followed a similar trend. Throughout the experiment, the treatment T_1 showed lowest root colonization. Treatments T_3 and T_4 showed almost same extent of root colonization. At 120 DAS, all the treatments possessed higher root colonization.

Table 2: Spore count and per cent root colonization in AMF treated plants

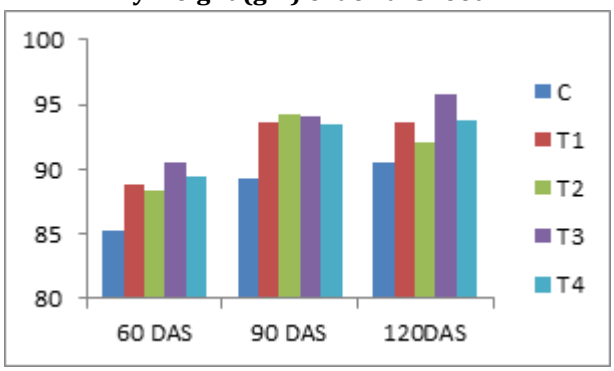
	Spore count			% Root colonization		
Treatments	**60 DAS**	**90 DAS**	**120 DAS**	**60 DAS**	**90 DAS**	**120 DAS**
T_1	15.75	28.00	62.75	27.33	33.35	55.67
T_2	25.75	43.25	70.75	36.00	58.33	73.33
T_3	31.25	64.50	105.25	43.67	68.67	88.05
T_4	33.75	60.75	118.75	45.00	76.01	90.89



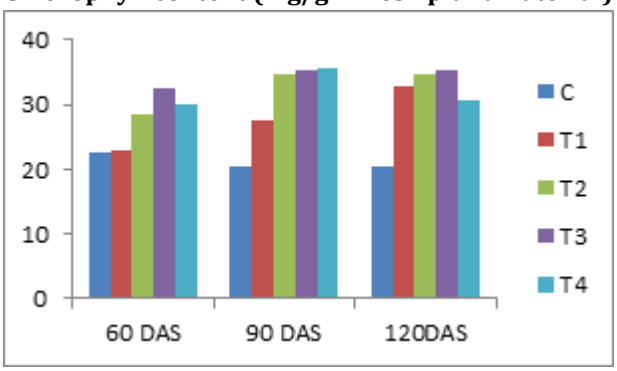
Dry weight (gm) of aerial shoot



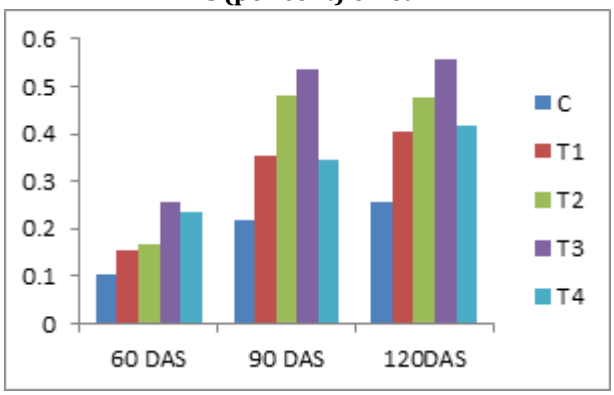
Chlorophyll content (mg/gm fresh plant material)



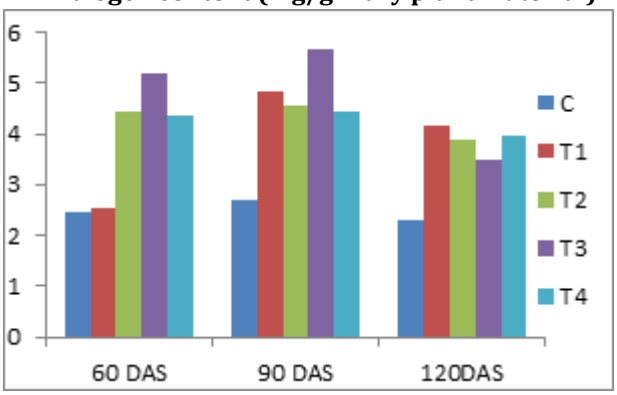
RWC (per cent) of leaf



Nitrogen content (mg/gm dry plant material)



Phosphate P content (mg/gm dry plant material)



Potassium content (mg/gm dry plant material)

The spore count and root colonization studies indicate the extent of association between host plants and AM fungi. The present investigations showed that the higher dosage of mycorrhiza gave better establishment of mycorrhiza in terms of spore count and root colonization in spearmint plants. At 60 DAS, the spore count and root colonization was low which increased considerably at 90 and at 120 DAS. Draft and Nicolson (1972) demonstrated that the higher root colonization allows more mycorrhiza – host contact and more exchange of nutrients, hence, the better plant growth. The low level of infection at 60 DAS might be due to the time taken by AM fungi for initial colonization of the roots as reported by Ramirez *et al*. (1975) and Kennedy and Rangarajan (2001).

Determination of optimum dose of AMF culture

From the present study, it is apparent that the AMF culture had a stimulating effect on growth, productivity and quality of spearmint plants throughout the course of study. 90 DAS stage is considered as very vital from harvest point of view (Sud and Kumar, 2004), hence, to determine the optimum dose of AMF culture, the results obtained at 90 DAS were analyzed, compared and significant results were counted (Table 3).

Table 3: Determination of optimum dose of AMF culture

Parameters Tested	AMF			
	T_1	T_2	T_3	T_4
1. Dry Weight	-	S	S	-
2. Total Chlorophyll	S	S	S	S
3. RWC	S	S	S	S
4. Nitrogen	-	S	S	S
5. Phosphorous	-	S	S	-
6. Potassium	S	S	S	S
Total	3	6	6	4
Best Treatments	**T_2 and T_3**			

'S' : Significant, '–' : Non-significant

Treatment T_2 and T_3 showed a remarkable effect on spearmint plants as all growth parameters were significantly influenced by the same at 90 DAS. It was very surprising that in spite of highest dose, treatment T_4 was not as effective as T_3. Kapooria (2006), in his study, also noticed the same behavior and reported that beyond a certain inoculum level, the mycorrhizal fungus failed to produce desired stimulatory effect.

CONCLUSION

It is evident from the data that application of AM Fungi played a vital role in improving plant biomass, photosynthetic activity in terms of chlorophyll content, water uptake and enhanced uptake and accumulation of nutrient elements in spearmint plants. This must have improved the host nutrition by increasing the delivery of phosphorous and other minerals to roots of the plant. It was also observed that to obtain better growth and yield of spearmint plants, high inputs of biological fertilizers were not needed. In fact, spearmint plants flourished well even in lower doses of AMF culture. AMF treatment T_2 and T_3 (2 gm and 3gm AMF culture per pot respectively) were proved to be the better than the remaining treatments from harvest point of view. Thus, the application of AMF for enhancing the growth of spearmint plants has promising future. This will not only reduce the dependence of spearmint plants on hazardous chemical fertilizers but will also ensure the maintenance of soil health, owing to eco-friendly nature of AM fungi.

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