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RESEARCH ARTICLE

"Effect of *Trichoderma* species on yield of *Mentha arvensis* L."

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Abstract

Mentha arvensis L. (Family Lamiaceae) commonly called Japanese mint. Two strains of *Trichoderma* were tested for its effect on the growth and productivity of mint plants without using any chemical fertilizers. *Trichoderma harzianum* (NFCCI 2241) and *T. ovalisporum* (NFCCI2689) were isolated from soil samples of Mentha growing fields of CIMAP, Hyderabad. The herb, oil yield and menthol content were highest and a significant increase with NFCCI 2241 in both crops and it was more than with NFCCI 2689.

Introduction

Mentha arvensis L. (Family Lamiaceae) commonly called Japanese mint is an aromatic perennial herb and is commercially cultivated in tropical and subtropical climates. It is extensively grown in India to meet about 70% of the international annual requirement. The oil and by-product (menthol and dementholised oil) of this plant have the highest share in the global mint trades (Singh and Singh, 2004).

To increase the production of mint plants, generally chemical fertilizers are applied. But, the application of nitrogen fertilizers may deplete soil organic carbon in the long run (Khan et al., 2007). Alternative methods have been devised to overcome this problem with sustainable productivity. In turn, the type of ecofriendly biofertilizers increase yield and reduce environmental pollution (Mia and Shamsuddin, 2010). Among various microbial products employed for this purpose, the fungal genus *Trichoderma* is more popular worldwide (Harman et al., 2012; Kulkarni et al., 2007). A number of successful commercialized products based on different species of *Trichoderma* have been marketed in India, Asia, Europe and USA for use on a wide range of crops (Mohiddin et al., 2010).

Trichoderma is considered as a multifunctional plant symbiont (Harman, 2011; Hermosa et al., 2012; Nagamani and Sarojini, 2012; Saba et al., 2012). The interaction of *Trichoderma* strains with the plant may promote growth, enhance plant productivity, improves crop yield, increase nutrient availability and enhance disease resistance (Benitez et al., 2004; Harman et al., 2004; Shoresh and Harman, 2008; Shoresh et al., 2010), induce resistance in the host plant (Howell, 2003; Harman et al., 2004; Shoresh et al., 2010; Yedidia et al., 2000); stimulate plant defenses against biotic and abiotic damage and increase nutrient uptake capacity (Shoresh et al., 2010). Hence, the species of *Trichoderma* gained popularity because of multifaceted efficacy among several microbial biofertilizers and biopesticides. In view of this, two strains of *Trichoderma* were tested to study its effect on the growth and productivity of Japanese mint plants without using any chemical fertilizers.

Material and Methods:

Trichoderma harzianum (NFCCI 2241) and *T. ovalisporum* (NFCCI 2689) were isolated from soil samples of *Mentha arvensis* growing fields of Central Institute of Medicinal and Aromatic Plants (CIMAP), Hyderabad, India. Mass cultures of *Trichoderma* were prepared on sorghum and sand (1:3) medium. Sorghum and sand (1:3) was filled into 2 kg polypropylene bags up to one third. Bags were autoclaved twice at 15 lb pressure for 30 min. Five mm diameter discs of actively growing mycelium of *T. harzianum* and *T. ovalisporum* were cut from the margins of 3 days old cultures and inoculated into the sorghum and sand contained bags. These bags were incubated at 27 ± 2^0C for 30 days following the procedure adopted by Singh and Singh (2004). Plant material Kosi variety was procured from Central Institute of Medicinal and Aromatic Plants (CIMAP), Pantnagar, Uttaranchal for this study.

Soil–compost mixture (50:50) was prepared for pot experiments. Soil application method was followed to apply *Trichoderma* (Singh and Singh 2004). About 20g of *T. harzianum* (NFCCI 2241) and *Trichoderma ovalisporum* (NFCCI 2689) inoculum having 2 x 10^7 cfu/g (colony forming units) was mixed with the soil for T1 and T2 treatments respectively. Sorghum - sand sterilized mixture (20g) without any *Trichoderma* was added to control pots (T3 treatment). Twelve suckers having around 4 nodes of *Mentha arvensis* were planted in each replicate. The pots were kept open to provide natural conditions and required moisture in the pots was maintained. For each treatment, four replicates were prepared and conducted during December 2011 to July 2012 under natural conditions.

The mint herb was harvested after 105 DAP by cutting whole plant 2 cm above the ground. The experiment was continued as second crop and the herb was again harvested after 210 DAP. The fresh herb yield was calculated by weighing the plants. The mint oil was extracted from the harvested herb by using Clevenger apparatus (Singh and Singh, 2004).

Menthol content was calculated in the mint oil by using the GC (Singh et al., 2005). Average of four replicates is presented in the data and analysis of variance (ANOVA) was carried out by using WINDOSTAT 9.2**.**

Results and discussion:

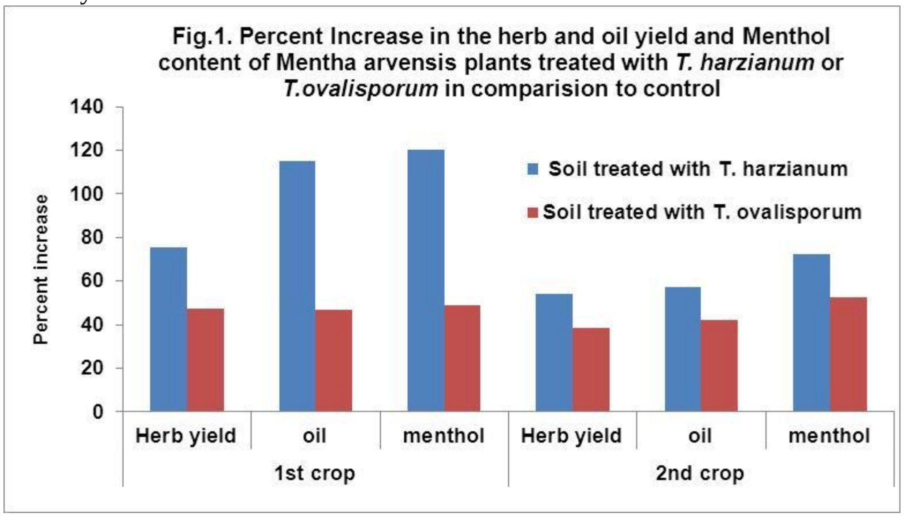
An increase in herb yield was noticed in *Trichoderma* inoculated treatments, i.e., T1 and T2; 144 gm and 120.67 gm from first crop; 160gm and 144 gm in second crop respectively (Table 1). In addition, herb, oil and menthol content of mint was maximum in T1 treatment in comparison to other treatments. Similar results were reported with *Trichoderma* spp. (Singh and Singh 2004). *T. harzianum* alone or in combination *Paecilomyces lilacinus* and *Pseudomonas fluorescens* also significantly increased plant growth and oil yield of this plant (Perveen et al., 2007).

Maximum oil yield and menthol content was noticed in T1 treatment in first and second crops. In T1 treatment, the herb yield was highest and a significant increase (75.6 %) was noticed in first crop (Fig.1). A similar result was noticed in second crop also but percentage (53.84) increase was lower than first crop. More than 100% increase in the oil yield and menthol content was recorded with T1 treatments when compared with control.

Table 1. Influence of *Trichoderma* isolates (NFCCI 2241, NFCCI 2689) as soil treatments on herb yield, oil and menthol production of mint plants

	First crop			Second crop		
Treatments	Herb Yield (gm) / pot	% Oil	% Menthol	Herb Yield (gm) /pot	% Oil	% Menthol
T1 (NFCCI 2241)	144.00	0.49	90.60	160.00	0.93	94.20
T2 (NFCCI 2689)	120.67	0.40	89.60	144.00	0.93	92.50
T3 (Control)	82.00	0.40	88.70	104.00	0.91	86.00
C.V.	7.64	2.17	0.35	6.51	0.18	0.18
S.E.M.	4.23	0.01	0.18	4.23	0.00	0.09
C.D. 5%	13.33	0.02	0.56	13.34	0.00	0.29

In T2 treatment, the herb yield increased up to 47.15% in first crop; 38.46% in second crop in comparison to control. But, the herb yield was lower in treatment T2 than in T1. In T2 treatment, menthol content was more than T3 (Control) but less than T1. This shows there is a variation in the effect of different strains of *Trichoderma* on the mint yield.



The positive effect of *Trichoderma* on plant growth was proved by other workers and the reason for this effect was recognized as rhizosphere competence of the *Trichoderma* (Harman, 2006; Harman et al., 2004; Shoresh et al., 2010; Whipps, 2001). Earlier discoveries showed that the genus *Trichoderma* is opportunistic, avirulent plant symbionts, as well as being parasites of other fungi (Harman et al., 2004). Harman (2006) stated that there is a complex interactions occur with the best-known rhizosphere-competent fungus *T. harzianum*, which is used as a biocontrol agent because it can parasitize plant fungal pathogens and establishes a symbiotic relationship with the roots of some plants, increasing plant growth and productivity. Complex interactions between multiple strains of *Trichoderma*, the roots of plants and other fungal pathogens suggest an established molecular cross-talk between them (Woo et al., 2006). Thus, the *Trichoderma* as soil treatments in this study might have affected the aerial plant parts of mint which might have induced growth response of the plants through various unregulated mechanisms. It has also been reported that *T. harzianum* 1295-22 could improve nitrogen use efficiency and could solubilize a number of poorly soluble nutrients, such as Mn^{4+}, Fe^{3+} and Cu^{2+} etc. leading to plant growth and development (Altomare et al., 1999). This could be one of the reasons for increasing yield of mint plants of present investigation.

This investigation clearly indicates that there is an enhancement of oil yield and menthol content is possible with *Trichoderma* application as soil treatment without supplementing with any chemical fertilizers. The present investigation proved that the strain *T. harzianum* (NFCCI 2241) is more efficient than the strain *T. ovalisporum* (NFCCI 2689) in enhancing the plant growth, oil and menthol yield.

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