Trichoderma species diversity in Japanese mint field soils

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ABSTRACT

Mentha arvensis L. (Japanese mint) is an aromatic and medicinal herb. So, a little of pesticide residues in the product is objectionable. To overcome this problem in IDM, biofungicide like *Trichoderma* is recommended. Hence, a study on distribution of *Trichoderma* species in the *M. arvensis* field soils revealed 100% occurrence. *Trichoderma* population in the soils ranged between 3 - 18 X 10^3 CFU g^{-1}. The occurrence of several species in this study represents the wide diversity of *Trichoderma* species in field soils of *M. arvensis*. Fifty four *Trichoderma* isolates obtained in this study belong to *T. atroviride, T. aureoviride, T. crassum, T. dorothea, T. gamsii, T. harzianum, H. orientalis, T. intricatum, T. lacteum, T. koningii, T. longibrachiatum, T. ovalisporum, T fertile, T. pubescens, T. pseudokoningii, T. reesei, T. stilbohypoxyli, T. strictipile, T. taiwanense, T. tomentosum, Trichoderma* species. *T. harzianum* is the dominant species followed by *T. ovalisporum*. *T. crassum, T. dorothea, T. lacteum, T. stilbohypoxyli, T. taiwanense* are new records to India; *T. intricatum, T. ovalisporum, T. tomentosum* are new reports to South India.

Key words: *Trichoderma* species, biodiversity, *Mentha arvensis*, Japanese mint

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INTRODUCTION

Trichoderma as a potent fungal biocontrol agent against a wide range of plant pathogens has attracted considerable scientific attention [1], [2], [3], [4]. The selection of potential candidate in biocontrol is an indispensable task to get maximum benefit out of bioaugmented strains. Hence, a careful study of their biodiversity is essential as different isolates of *Trichoderma* species may exhibit significant variation in metabolic activity [5]. Perhaps, this is the reason that has captured the attention of scientists to study the diversity of *Trichoderma* species worldwide.

The global occurrence of *Trichoderma* was thoroughly investigated [6], [7], [8], [9], [10], [11], [12], [13]. Its' diversity studies on Poland soils was attempted in Poland and Egypt [14], [15]. In India, *Trichoderma* isolates were identified from different geographical zones [16], Manipur soils [17], rice field soils of united Andhra Pradesh [18], [19] and Andaman Nicobar islands [20], [21]. However, the studies on the biodiversity of *Trichoderma* species in medicinal and aromatic plants cultivated soils is less attempted so far.

Mentha arvensis (Japanese mint) is an aromatic perennial herb, and commercially cultivated in tropical and subtropical climates. Use of chemical fungicides is discouraged as the products of this plant are used in medicine and pharmaceuticals. [22] proved that the isolates from natural habitats were found to be more effective to be used as bioagents. Hence, soil samples from fields of *Mentha arvensis* were collected to assess the occurrence of *Trichoderma* spp. in the soils of *Mentha arvensis* fields.

MATERIALS AND METHODS

For collecting soil samples, the method was followed [23]. Four soil samples (No. 1, 2, 3 and 4) were collected from *Mentha arvensis* fields of Central Institute of Medicinal and Aromatic Plants (CIMAP), Hyderabad, India and two soil samples (No. 5 and 6) from garden soils of University College of Science, Saifabad (UCSS), O.U, Hyderabad, India. The field soils were dug 5cm deep to collect samples. The subsurface soils from five different places in those fields were collected and pooled in the sterile black polythene bags and used as composite sample from each field. Ten grams of soil was dispensed in 100mL sterile distilled water. The supernatant liquid was taken to estimate the pH of all soil samples using Elico Digital pH meter. Moisture content was carried out by dry oven method by taking 10g of soil.

2% Potato dextrose agar medium (PDA) was used to isolate *Trichoderma* isolates. Dilution plate method [24] was followed and 10^{-3} and 10^{-4} dilutions were used for isolations. The plates were incubated for 4 days at 25 ± 2^0C. The total fungal and *Trichoderma* colonies appeared in the plates were noted. The occurrence and percentage frequency of *Trichoderma* isolates was calculated. *Trichoderma* colonies appeared after incubation were subculture. The pure cultures of all isolates were obtained by single spore culture method and preserved on PDA slants for further investigation. Taxonomy of *Trichoderma* is currently based on morphological characters recorded on both Malt extract agar (MA) (composition of MA: Malt extract agar-20g; Agar agar-20g; Water-1000mL) and PDA plates. *Trichoderma* species were characterized with the quantitative and qualitative parameters. The quantitative parameters include size and shape of the conidiogenous cells (phialides) and conidia, the time of initiation of spore production and the radial growth rate at 27 ± 2^0C. The qualitative parameters include texture of the colony, presence of odour and excreted pigments, spore colour, ornamentation and chlamydospores formation. For identification, at least 30 conidia (L/W) and phialides were measured and the average of minimum and maximum size was noted. *Trichoderma* isolates were identified up to species level based on taxonomic keys, monographs and available literature [25], [26], [27], [28], [29], [30], [31], [32], [33], [34], [35], [6]. Phenotypic identity was also established with the TrichoKey program (http://nt.ars-grin.gov/ taxa description /keys/TrichodermaIndex.cfm). New species records to India or south Indian states of India have been deposited at National Fungal Culture Collection Institute (NFCCI), Pune, Maharashtra, India.

RESULTS

The moisture content of the soil samples ranged between 10 - 25% and pH 6.5 for CIMAP field soils and 7.0 for college field soil samples. The total fungal population ranged between 13 - 74 x 10^3 cfu g^{-1}. The total fungi and *Trichoderma* occurrence showed variation among the samples. The soils from CIMAP field were colonized with more fungal population than the college field soils. All soil samples harboured *Trichoderma* and 100% occurrence was noticed in this study. The percentage frequency of *Trichoderma* spp. in soil samples is presented in Table 1. The *Trichoderma* population ranged from 3 to 18 x 10^3 cfu g^{-1} in the soil samples and an average CIMAP field soils recorded 10.18 x 10^3 cfu g^{-1} and college field

soils showed 3 - 4 x 10^3 cfu g^{-1}. The percentage frequency of *Trichoderma* in the samples is in between 5.66 - 30.77 (Table 1). The percentage frequency of *Trichoderma* in the college field soils is low. Sample 1 and 3 harboured maximum *Trichoderma* population. The sample 2 is having maximum total fungal population but least *Trichoderma* population. A total of 54 colonies of *Trichoderma* were obtained from six soil samples. The isolates were identified up to species level.

Table 1. Percentage frequency of total fungal and *Trichoderma* isolates in field soils of *Mentha arvensis*

Sample No.	Total fungal colonies	Total *Trichoderma* colonies	% frequency of fungi other than *Trichoderma*	% frequency of *Trichoderma*	% moisture content	pH
1	74	17	77.03	22.97	20	6.5
2	53	3	94.34	5.66	20	6.5
3	77	19	75.33	24.67	15	6.5
4	67	8	88.06	11.94	10	6.5
5	13	4	69.23	30.77	25	7.0
6	22	3	86.36	13.64	25	7.0

Table 2. Percentage frequency of *Trichoderma* species in field soils of *Mentha arvensis*

S. No	*Trichoderma* species	CIMAP soils				College soils	
		1	2	3	4	5	6
1	*T. atroviride*	5.88	-	-	-	25	
2	*T. aureoviride*	-	-	-	12.5		-
3	*T. crassum*	-	-	-	-	25	-
4	*T. dorothea*	5.88	-	5.26	-	-	-
5	*T. fertile*	5.88	-	-	-	-	-
6	*T. gamsii*	11.77	-	-	-	-	-
7	*T. harzianum*	17.64	-	68.42	-	-	66.67
8	*T. intricatum*	-	-	-	-	25	33.33
9	*T. koningii*	-	-	-	12.5	-	-
10	*T. lacteum*	5.88	-	-	-	-	-
11	*T. longibrachiatum*	11.77	-	5.26	-	-	-
12	*T. ovalisporum*	5.88	-	21.05		-	-
13	*T. pseudokoningii*	11.77	-	-		-	-
14	*T. pubescens*	-	-	-	25	-	-
15	*T. reesei*	-	-	-	12.5	-	-
16	*T. stilbohypoxyli*	5.88	-	-	12.5	-	-
17	*T. strictipile*	-	-	-	25	25	-
18	*T. taiwanense*	-	33.33	-	-	-	-
19	*T. tomentosum*	5.88	-	-	-	-	-
20	*H. orientalis*	5.88	-	-	-	-	-
21	*Trichoderma* spp.	-	66.67	-	-	-	-

Twenty one *Trichoderma* species (Fig 1) were encountered namely *T. atroviride* Karsten (2), *T. aureoviride* Rifai (1), *T. crassum* Bissett (1), *T. dorothea* Samuels Dodd (2),

T. fertile Bissett (1), *T. gamsii* Samuels & Druzhinina (2), *T. harzianum* Rifai (19), *H. orientalis* Samuels & O. Petrini (1), *T. intricatum* Samuels & Dodd (2), *T. lacteum* Bissett (1), *T. koningii* Oudem (1), *T. longibrachiatum* Rifai (3), *T. ovalisporum* Samuels & Schroers (5), *T. pubescens* Bissett (2), *T. pseudokoningii* Rifai (2), *T. reesei* E.G. Simmons (1), *T. stilbohypoxyli* Samuels & Schroers (2), *T. strictipile* Bissett (2), *T. taiwanense* Samuels & M.L. Wu (1), *T. tomentosum* Bissett (1), *Trichoderma* species (2). The number of isolates obtained under each species is given in the parenthesis.

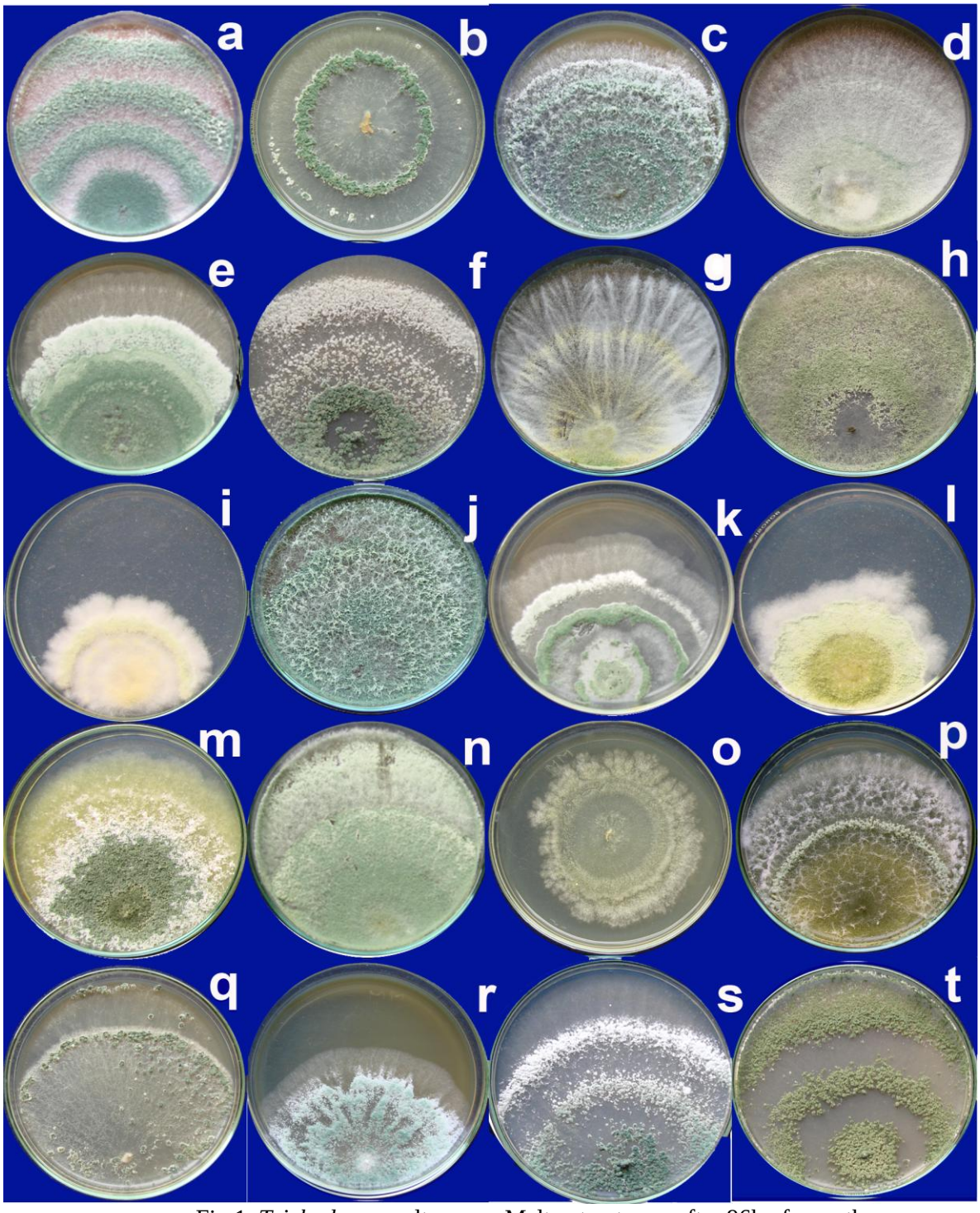

Fig 1. *Trichoderma* cultures on Malt extract agar after 96h of growth

a. *T. atroviride*; **b.** *T. aureoviride*; **c.** *T. crassum*; **d.** *T. dorothea*; **e.** *T. fertile*; **f.** *T. gamsii*; **g.** *T. harzianum*; **h.** *T. koningii*; **i.** *T. intricatum*; **j.** *T. lacteum*; **k.** *T. longibrachiatum*; **l.** *T. ovalisporum*; **m.** *T. pseudokoningii*; **n.** *T. pubescens*; **o.** *T. reesei*; **p.** *T. stilbohypoxyli*; **q.** *T. strictipile*; **r.** *T. taiwanense*; **s.** *T. tomentosum*; **t.** *Hypocrea orientalis*

The isolates encountered in this study showed variation in culture characters as well as in microscopic parameters. Based on these results the isolates were assigned to different species. In general, *T. harzianum* is a dominant species wherever it was occurring i.e. in samples 1, 3 and 6. In other samples no species showed the dominance. *T. ovalisporum* is predominant next to *T. harzianum* and is followed by *T. longibrachiatum* (Table 2). All other species found to be with low frequency of occurrence. Among 21 species, 5 species namely *T. crassum, T. dorothea, T. lacteum, T. stilbohypoxyli, T. taiwanense,* are new records to India and while 4 viz., *T. gamsii, T. intricatum, T. ovalisporum and T. tomentosum,* are new records south India. All cultures of new records were deposited in National Fungal Culture Collection of India (NFCCI) (Table 3).

Table 3. List of *Trichoderma* cultures deposited at NFCCI, Pune, India

S. No	Culture No	NFCCI Accession	Name of the *Trichoderma* species
1	M49	NFCCI-2691	*Trichoderma crassum* Bissett*
2	M40	NFCCI-2694	*Trichoderma dorothea* Samuels Dodd*
3	M51	NFCCI-2693	*Trichoderma intricatum* Samuels & Dodd**
4	M10	NFCCI-2688	*Trichoderma lacteum* Bissett*
5	M14	NFCCI-2689	*Trichoderma ovalisporum* Samuels & Schroder's**
6	M31	NFCCI-2697	*Trichoderma stilbohypoxyli* Samuels & Schroers*
7	M43	NFCCI-2690	*Trichoderma taiwanense* Samuels & Wu*
8	M6	NFCCI-2685	*Trichoderma tomentosum* Bissett**

*New records to India **New records to South Indian states

DISCUSSION

The species encountered in this study were reported earlier either from India or elsewhere. In this study, *T. harzianum* dominated the soils of *Mentha arvensis* fields. Studies on investigation of 76 isolates from Russia, Nepal, India [11] reported 7 species in which *T. harzianum* represented majority of isolates. *T. harzianum* reported as the most dominant species among the 22 *Trichoderma* spp. from the soils of Manipur, India [17]. *T. harzianum* was reported as most dominant taxon [14], [8], [36], [10], [12], [13] and truly cosmopolitan [37]. In addition to the soils, *T. harzianum* is also commonly associated with rhizosphere of cultivated plants [38]. In our study also more isolates (20 out of 54 isolates) were of *T. harzianum*. The next abundant species identified in the present study was *T. ovalisporum*. It was first reported from the soils of Andaman and Nicobar Island of India [21] and also reported from Egypt soils [15]. [39] identified this species as an endophyte of *Theobromae cocoa* and as an apparent saprophyte from soil in Panama [38]. Ours is the first report from the main land of India.

T. longibrachiatum was isolated from CIMAP field soils only and was reported as dominant species of rice field soils and forest soils [19]. This species was also reported from various places of India [40], [16]. *T. aureoviride* was reported from the forest soils and rhizosphere soils of Meghalaya of India [40] and rice fields of Philippines [41]. It was a rarely encountered species in the present investigation. *T. pubescens* was frequently isolated from the rhizosphere soils of groundnut [42] in Karnataka and Andhra Pradesh, but it was of

rare occurrence in this study. *T. fertile* was found in only one sample and was earlier reported from groundnut soils [43]; from rhizosphere soil of peanut [42] and polluted soil [30].

T. atroviride was found only one sample in this investigation. Earlier it was reported from rice field soils of Andhra Pradesh, Telangana states of India and from Himalayan Soil [11]. *T. reesei* and *T. strictipile* were isolated from polluted [30] and rice field soils [44]. *T. koningii* was isolated from garden and field soils of Punjab, Orissa, Madhya Pradesh, Uttar Pradesh, Meghalaya [40] and rice field soils of Andhra Pradesh [19]. *T. pseudokoningii* was reported from rice field soils of Andhra Pradesh [19] was found to be rare in the present study. *T. gamsii*, *T. tomentosum* were reported from soils of Manipur, India [17]. *T. gamsii* was reported from soil of Italy, USA, and Central Russia [45]. *T. crassum, T. dorothea, T. lacteum, T. stilbohypoxyli, T. taiwanense* were identified elsewhere other than India. *T. dorothea, T. intricatum, T. ovalisporum and T. taiwanense* were encountered earlier from Australia, Thailand, South America and China respectively [46]. *H. orientalis* reported from South America, Europe, East Asia and North America [46]. *T. stilbohypoxyli* reported from plant materials of Costa Rica Puerto [35]. *T. crassum* and *T. tomentosum* were reported from Canada [26] and elsewhere.

CONCLUSION

In the present study, 20 *Trichoderma* species are encountered from Telangana state, India from mint cultivated soils with rare occurrence. The survey of available literature indicates that this is a pioneer study explored the biodiversity of *Trichoderma* in field soils of Medicinal and Aromatic plants. The occurrence of several species in this study represents the wide diversity of *Trichoderma* species in field soils of *M. arvensis*. This clearly explains that there is a possibility of occurrence wide variety of *Trichoderma* species from these unexplored substrates. Occurrence of several species encountered from a single sample reveals the existence of diversity of *Trichoderma* species in the soil samples of mint cultivated fields.

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