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FREQUENCY PERCENTAGE OF SOIL FUNGI AND DIVERSITY OF *TRICHODERMA* SPP. IN THE RHIZOSPHERE SOIL OF SELECTED VEGETABLE CROP FIELDS

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Key words: Frequency percentage, Soil fungi, *Trichoderma* spp, Vegetable crops

Abstract

An attempt was undertaken to detect the soil fungi and diversity of *Trichoderma* spp in the rhizosphere soil of selected vegetable crops. A total of fifteen fungi *viz*., *Alternaria* sp., *Aspergillus flavus, A. fumigatus*, *A. niger*, *A. ochraceus*, *Aspergillus* sp., *Colletotrichum* sp., *Curvularia* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizoctonia solani*, *Rhizopus* sp., *Trichoderma* sp. and *Syncephalastrum* sp. were isolated from rhizospheric soil of brinjal, chili, cucumber, cabbage and onion in Naogaon district. Except *Syncephalastrum* sp., all the above mentioned fungi including *Monilia* sp., were isolated from rhizospheric soil of brinjal, cabbage, chili and tomato of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. A total of eight types of *Trichoderma* spp belonging to four species *viz*., *Trichoderma harzianum*, *T. koningii*, *T. reesei* and *T. viride* were isolated from nine vegetable fields from Naogaon district and BARI. These T*richoderm*a spp. may be used in the management of soil borne diseases of vegetable crops.

Introduction

The rhizosphere is a micro ecological zone in direct proximity of plant roots. It is functionally defined as the particulate matter and microorganisms that cling to roots after being gently shaken in water. The theoretical extent of the rhizosphere is dependent on the zone of influence of the plant roots and associated microorganisms. The rhizosphere is a metabolically busier, faster moving, more competitive environment than the surrounding soil(1).

The genus *Trichoderma* is a diverse group of free-living fungi present in soil(2-7). *Trichoderma* are characterized by rapid growth and abundant production of conidial spores as well as the capacity to produce sclerotia. These species produce several pigments, ranging from a greenish-yellow up to a reddish tinge, although some colourless specimens are also present. The conidia may also have diverse colouration,

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genus *Trichoderma* belongs to the phylum Ascomycetes, class Sordariomycetes, order Hypocreales, family Hypocreaceae. The systematics and taxonomy of these fungi have evolved since 1794 when Persoon introduced the name *Trichoderma*[8]. In 1865, Tulasne and Tulasne showed that *Hypocrea rufa* is the teleomorph of *Trichoderma viride* Pers[9]. Up to 1969 it was reported that within the genus *Trichoderma* there was only one species, namely *T. viride*[10]. Then Rifai distinguished nine "aggregate species" i.e., *T. harzianum* Rifai, *T. viride, T. hamatum* (Bonord.) Bainier, *T. koningii* (Oudem.) Duché & R. Heim, *T. polysporum* (Link) Rifai, *T. piluliferum* J. Webster & Rifai, *T. aureoviride* Rifai, *T. longibrachiatum* Rifai, and *T. pseudokoningii* Rifai based on morphological characteristics. After that Bissett identified five sections and 27 biological species within the genus *Trichoderma*[11]. These ascomycetous fungi are opportunistic, avirulent, plant symbionts inhabiting in root ecosystems [4,12] but parasites on other groups of fungi[2].

Trichoderma spp. is the most promising and effective antagonistic fungi against several soil borne plant pathogenic fungi[13,14]. The advantage of using *Trichoderma* in controlling soil borne plant pathogens are eco-friendly, effective, ease of mass culturing with less cost of production and growth promoting effect. At present *Trichoderma* genus is comprised of more than 200 species. Very little information is available about the presence of *Trichoderma* spp. in the rhizosphere soils of Bangladesh. Hence, the present study was undertaken to find out the frequency percentage of soil fungi and diversity of *Trichoderma* spp. in the rhizospheric soils of selected vegetable crop fields of BARI, Joydebpur and Naogaon, Bangladesh.

Materials and Methods

A total of twelve rhizospheric soil samples from twelve locations were collected from four vegetable crop fields *viz*. cabbage, brinjal, tomato and chili of BARI, Joydebpur, Gazipur. Another six soil samples from six locations were collected from six vegetable crop fields *viz*. brinjal, chili, cucumber, cabbage, tomato and onion from Naogaon district. The samples were collected between the months of April 2018 to May 2019. Soil samples were collected from a depth of 15 cm and kept into sterilized cellophane bags[18]. Fungi associated with soil samples were isolated individually following Serial dilution technique[19].

Collected soil samples were air dried and ground into powder. At first 1.0 g soil was added to 99 ml of distilled water in a conical flask and mixed it very well with a glass rod and marked as mother suspension. Then five test tubes each containing 9 ml sterilized distilled water were taken. 1 ml of mother suspension was added into the 1st test tube and made it 10 ml. Then it was mixed well, 1 ml of the suspension from the 1st test tube was added into the 2nd test tube and made it 10 ml. This process was performed for rest of the test tubes and diluted the mother suspension 10, 100, 1000, 10000 and 100000 times.

For each dilution, 1 ml of suspension was poured into a sterilized Petri plate and then about 15 ml of sterilized melted PDA medium (about 50°C) was added. One drop of Lactophenol solution was added to 15ml medium for preventing bacterial growth, before pouring into Petri plates. The plate was moved gently on the Laminar air flow table to get a homogenous distribution of the suspension. Five replications were maintained for each dilution. All the Petri plates were incubated into 25 ± 2°C temperature. After 3 days, individual fungal colonies belonging to the genera *Rhizoctonia* and *Trichoderma* were sub-cultured on PDA slants randomly, from the culture plates and stored at 4°C in an incubator for future studies.

Population density expressed in terms of Colony Forming Unit (CFU) per gram of soil with dilution factors. The per cent frequency of each isolate was calculated by

$$\% \text{ Frequency} = \frac{\text{Total No.of CFU of an individual sp.}}{\text{Total No.of CFU of all spp}} \times 100$$

Identities of soil fungi were determined following the standard literature [16-18]. Detail morphological studies of the fungal isolates were made in order to determine their identity. For microscopic observations fungal structures like mycelia, spore bearing structures and spores were scrapped off from the surface with a scalpel or picked up with a needle and was mounted in lacto phenol over a clean slide. In case of hyaline structures, a little amount of cotton blue was added to the mounted fluid. A clean cover glass was placed over the material. Excess fluid was removed by shaking with blotting paper and examined under microscope. The microscopic structural view of the fungi was taken by a digital camera. All the specimens, included in the present study were preserved in the slant at 4-10°C in refrigerator in the Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka, Bangladesh.

Antagonistic fungal colony had key characteristics that was used to identify them as *Trichoderma*, including growth pattern, growth rate and colour. Species level identification of *Trichoderma* isolates was done based on colony colour, formation of chlamydospores, conidiophores, phialide characters and shape of conidia [19-21]. Isolated *Trichoderma* types were divided into several types according to their vegetable field of BARI and Naogaon district.

Results and Discussion

In Naogaon district, a total of fifteen fungi were isolated from rhizospheric soil of brinjal, chili, cucumber, cabbage and onion. They were *Alternaria* sp., *Aspergillus* sp., *A. flavus, A. fumigatus*, *A. niger*, *A. ochraceus*, *Colletotrichum* sp., *Curvularia* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizoctonia solani*, *Rhizopus* sp., *Trichoderma* sp. and *Syncephalastrum* sp. (Table 1).

Table 1. Frequency percentage of fungi associated with rhizospheric soil of vegetable crop fields of Naogaon district.

Name of fungi	Frequency percentage of fungi in rhizospheric soil of different vegetables					
	Brinjal	Cabbage	Chili	Cucumber	Onion	Tomato
Alternaria sp.	1.39	19.23	14.20	10.22	21.55	12.28
Aspergillus sp.	-	-	-	-	14.37	12.86
Aspergillus flavus	1.39	12.18	13.11	1.70	-	1.17
A. fumigatus	13.98	7.69	4.37	-	-	26.90
A. niger	24.47	-	19.67	18.75	30.38	6.43
A. ochraceus	-	-	4.36	-	-	0.58
Colletotrichum sp.	-	14.11	0.55	19.89	-	-
Curvularia sp.	2.09		14.75		9.95	5.85
Fusarium sp.	13.99	17.95	**11.48**	13.99	-	1.75
Mucor sp.	4.89	-	-	-	-	3.50
Penicillium sp.	10.48		**1.63**	1.14	-	2.92
Rhizoctonia solani	-	-	-	-	7.73	21.6
Rhizopus sp.	4.89	-	-	17.05	-	-
Syncephalastrum sp.	1.39	-	-	-	-	-
Trichoderma sp.	4.88	3.21	3.27	3.21	-	1.75

'–' represents absence of respective fungi.

On the other hand, a total of 15 fungi were isolated from rhizospheric soil of brinjal, cabbage, chili and tomato collected from BARI, Joydebpur, Gazipur. They were *Alternaria* sp., *Aspergillus flavus, A. fumigatus*, *A. niger*, *A. ochraceus, Aspergillus* sp., *Colletotrichum* sp., *Curvularia* sp., *Fusarium* sp., *Monilia* sp., *Mucor* sp., *Penicillium* sp., *Rhizoctonia solani*, *Rhizopus* sp. and *Trichoderma* sp. (Table 2).

Among the isolated soil fungi, *Trichoderma* spp. were selected as antagonistic fungi due to their novel biological control activity against notorious pathogens. Taxonomic identification of eight different isolates of *Trichoderma* spp. up to species level were done based on colony morphology and microscopic observation by using compound microscope (Table 3, Figs 1-3).

Table 2. Frequency percentage of fungi associated with rhizospheric soil of vegetable crop fields of BARI, Joydebpur, Gazipur.

Name of fungi	Frequency percentage of fungi in rhizospheric soil of different vegetables			
	Brinjal	Cabbage	Chili	Tomato
Alternaria sp.	-	20	16.66	10.73
Aspergillus sp.	12.96	12.41	5.56	13.66
Aspergillus flavus	-	10.34	16.67	-
A. fumigatus	14.81	4.83	4.32	-
A. niger	20.37	0.69	7.41	23.41
A. ochraceus	-	1.37	7.43	-
Colletotrichum sp.	-	15.17	1.23	-
Curvularia sp.	-	**1.40**	10.49	10.73
Fusarium sp.	18.51	20.69	7.41	-
Monilia sp.	3.70	-	0.62	-
Mucor sp.	1.85	0.70	-	4.39
Penicillium sp.	5.56	1.39	-	4.38
Rhizoctonia solani	-	-	-	21.97
Rhizopus sp.	11.11	-	-	-
Trichoderma sp.	9.26	2.76	-	8.78

'–' represents absence of respective fungi.

Trichoderma harzianum Rifai, Mycological Papers 116: 38 (1969)

Isolate type-7, colony showed light greenish producing tufts or pustules fringed by sterile white mycelium, colony reverse showed dull yellowish. Conidiophores showed frequent branching and were verticillate. Phialides were ampulliform and convergent. Conidia were sub-globose to ovoid shape. Formation of chlamydospore was infrequent and produced terminally and intercalary. Based on these characters this isolate was identified as *Trichoderma harizanum* (Figs 1A and 2A).

Specimen examined: Isolates were from rhizospheric soil of damping off brinjal field, Naogaon district. Priyanka Bhattacharjee 11, 8 October 2018.

Trichoderma koningii Oudem., Archives Néerlandaises 7: 291 (1902)

Isolates type-1, type-2 and type-3 colony showed dull green to bluish green sporulation. Colony reverse was colourless to pale yellow. Conidiophores were broad or

narrow, verticillate branching frequently. Phialides showed lageniform or ampulliform, shape divergent and terminal phialide more elongated. Conidia shape was sub cylindrical to narrow ellipsoidal. Formation of chlamydospore was infrequent or frequently producing intercalary and terminally. Based on these characters the isolates were identified as *Trichoderma koningii*. They were classified into *Trichoderma koningii* 1, *T. koningii* 2 and *T. koningii* 3 (Figs 1B-D and 2B-D).

Table 3. Morphological and microscopic characteristics of *Trichoderma* spp.

Sl. No.	Isolate	Colony colour	Colony reverse colour	Conidiophore character	Phialide character	Conidia shape
1	Type 1	Dull green to bluish green	Colour less	Broad, frequent branching	Terminal phialide more elongated	Cylindrical to ellipsoidal
2	Type 2	Dull green to bluish green	Pale yellowish	Broad, verticillate, frequent branching	Cylindrical	Globose
3	Type 3	Bluish green	Colourless	Broad, frequent branching	Sub cylindrical to narrow ellipsoid	Globose
4	Type 4	Scattered in minute tufts, pale yellow green	Pale yellowish	Rarely branched	Cylindrical or slightly inflated, divergent	Ellipsoidal
5	Type 5	Dark green producing tufts or pustules fringed by sterile white mycelium	Dull yellowish	Frequent branching	Ampulliform, convergent	Sub globose to ovoid
6	Type 6	Dark bluish green	Colour less	Infrequent branching, verticillate	Convergent	Globose to ellipsoidal
7	Type 7	Bluish green	Colour less	Infrequent branching	Convergent	Globose to ellipsoidal
8	Type 8	Dark green, Scattered in minute tufts	Colour less	Rarely branched, verticillate	Cylindrical or slightly inflated, divergent	Globose to ellipsoidal

Specimen examined: Isolate type 1 from damping off rhizospheric soil of chili field, Naogaon district. Priyanka Bhattacharjee 17, 9 September 2018, type 2 from damping off rhizospheric soil of chili field. Naogaon district. Priyanka Bhattacharjee 29, 16 October 2018 and type 3 from damping off rhzospheric soil of brinjal field. BARI, Gazipur district. Priyanka Bhattacharjee 43, 8 February 2019.

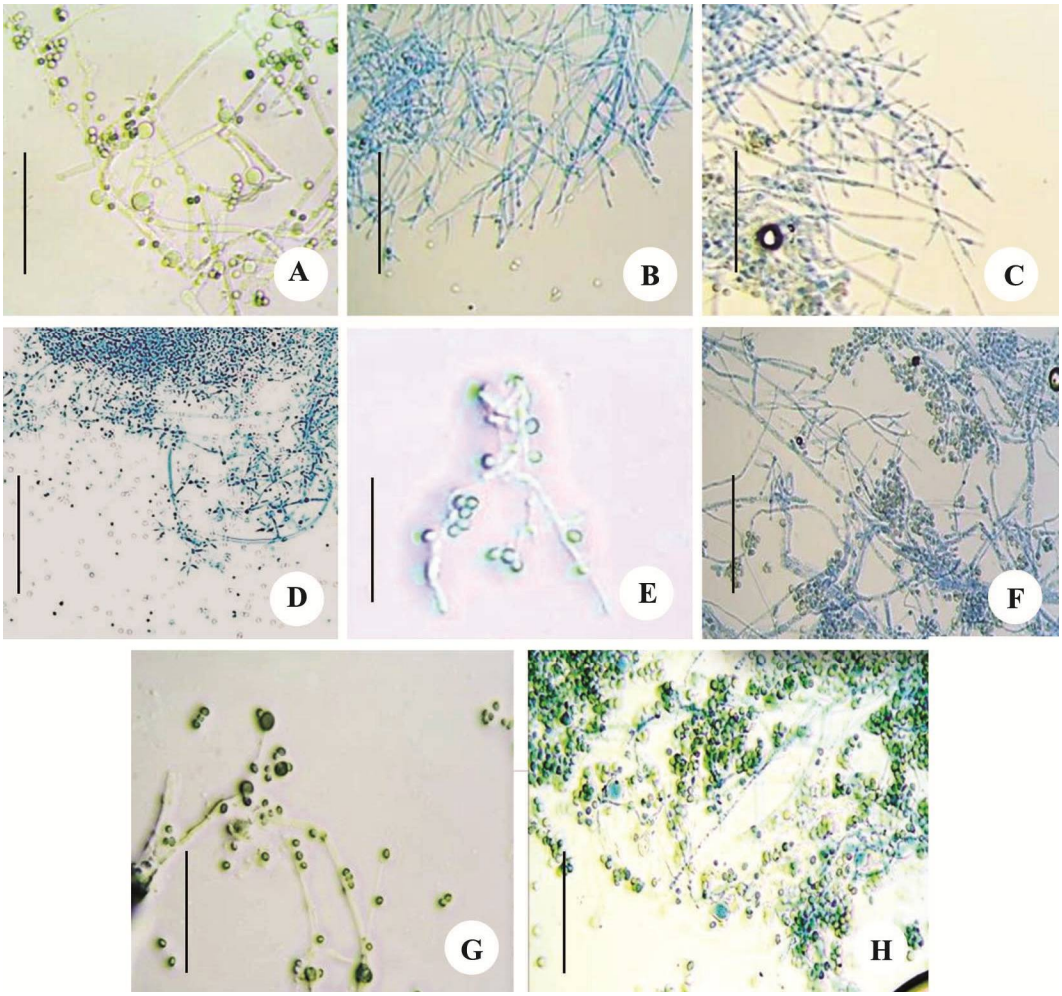

Fig. 1. Conidiophores, phialides and conidia of different isolates of *Trichoderma* spp. A *Trichoderma harzianum,* B. *T. koningii* type 1, C. *T. koningii* type 2, D. *T. koningii* type 3, E. *T. reesei*, F. *T. viride* type 5, G. *T. viride* type 6, and H. *T. viride* type 8. (Bar = 50 µm).

Trichoderma reesei Simmons EG (1977) in Bigelow & Simmons, Abstracts, 2nd International Mycological Congress (Tampa) 2: 618.

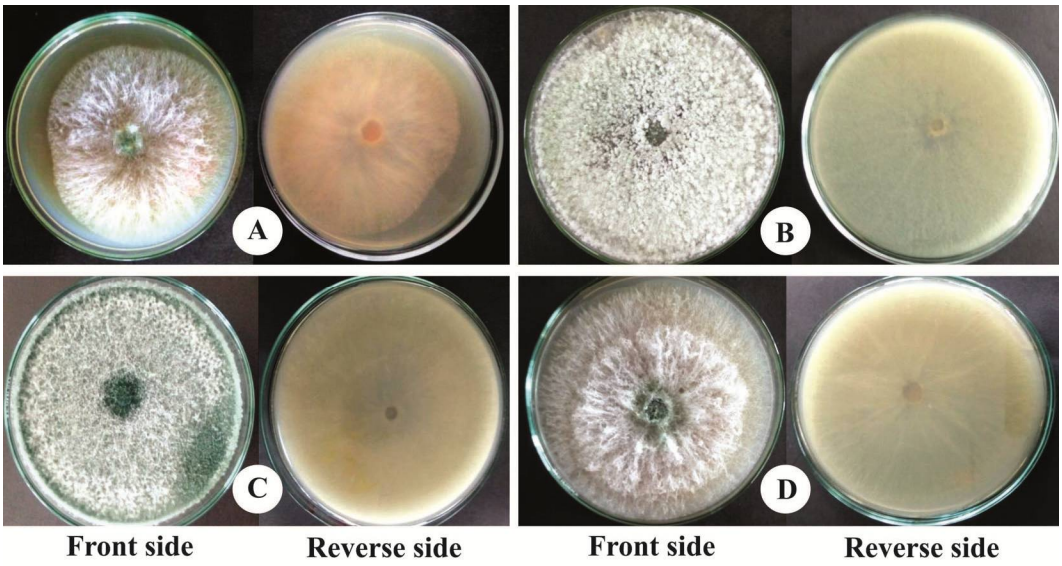

Plate 1. Colonies show front and reverse side of different isolates of *Trichoderma* spp. on PDA medium after 5th day of inoculation. A. *T. harzianum* type 7, B. *T. koningii* type 1, C. *T. koningii* type 2 and *T. koningii* type 3.

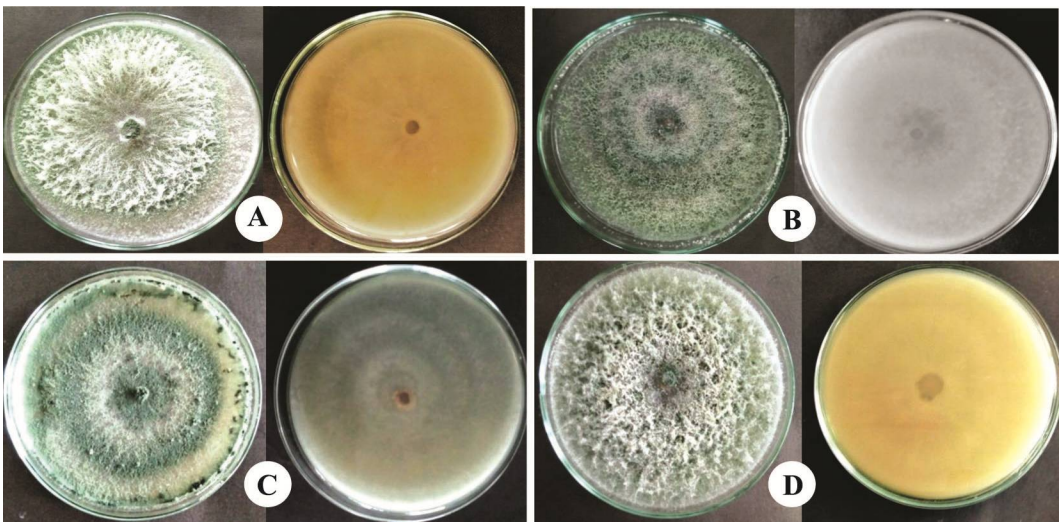

Plate 2. Colonies show front and reverse side of different isolates of *Trichoderma* spp. on PDA medium after 5th day of inoculation. A. *T. reesei* type 4, B. *Trichoderma viride* type 5, C. *T. viride* type 6, and D. *T. viride* type 8.

Colony of isolate type 4 showed scattered minute tufts, pale yellowish-green in colour, colony reverse was pale yellowish. Conidiophores were rarely branched and verticillate. Phialides shape were cylindrical or slightly inflated and divergent phialides. Conidia shape was ellipsoidal. Chlamydospore was frequently produced both terminally

and intercalary. Based on these characters this isolate was identified as *Trichoderma reesei* (Fig. 1E and 3A).

Specimen examined: Isolate type 4 from damping off rhizospheric soil of tomato field. BARI Gazipur district. Priyanka Bhattacharjee 67, 17 October 2019.

Trichoderma viride Pers., Neues Magazin für die Botanik 1: 92 (1794)

Isolates type-5, type-6 and type-8, colony showed dark green to dark bluish green sporulation, colony reverse was amber or colourless. Conidiophore usually long, infrequently branched, verticillate conidiophores. Phialides were frequently paired, convergent. Conidial shape was globose to ellipsoidal. Formation of chlamydospore was infrequent or frequently produced terminally and intercalary. Based on these characters these isolates were identified as *Trichoderma viride* 1, *T. viride* 2 and *T. viride* 3 (Fig. 1F-H and 3B-D).

Specimen examined: Isolates type 5 from damping off rhizospheric soil of cabbage field, BARI Gazipur district. Priyanka Bhattacharjee 81, 19 November 2019. Type 6 from damping off rhizospheric soil of chili field, Naogaon district. Priyanka Bhattacharjee 55, 11 October 2019 and type 8 from damping off rhizospheric soil of tomato field, BARI Gazipur district. Priyanka Bhattacharjee 58, 11 October 2019.

Trichoderma spp. isolated and identification in the present investigation may be used in the management of soil borne diseases of vegetable crops.

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