Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(1): 699-702
Received: 22-11-2020
Accepted: 24-12-2020

Prajkta P Wagh
Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

BG Barhate
Department of Plant Pathology and Agricultural Microbiology, College of Agriculture Pune, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Corresponding Author:
Prajkta P Wagh
Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Survey and isolation of *Trichoderma* spp. from rhizosphere soils of Western Maharashtra

Prajkta P Wagh and BG Barhate

DOI: https://doi.org/10.22271/phyto.2021.v10.i1j.13405

Abstract
In this study, the total 45 samples were collected from areas of Western Maharashtra. Out of these only 20 rhizospheric soil samples had the population of *Trichoderma* spp. The *Trichoderma* was isolated from the soil samples collected from the different locations of western Maharashtra by serial dilution technique and plating method. By visual observations 6, *T. hamatum*, 7, *T harzianum* (Rifai), 3, *T. koningii* (Oudem) and 4, *T. asperellum* (Samuels) were identified. *T. harzianum* and *T .hamatum* were present in most of the soil samples collected from Western Maharashtra region. The *T. asperellum* species was obtained from soil samples collected from Dhabewadi, sakri, Pachora and Tasgaon region of western Maharashtra. These isolates of *Trichoderma* spp. were studied and identified on basis of macroscopic i.e. colony characters like growth rates, pigmentation, colony edge, pustule formation showing defined characteristics for that respective species.

Keywords: *Trichoderma*, isolation, biocontrol agent, rhizosphere

1. Introduction
For years various management strategies have been evolved to control the problematic plant diseases including practice of application of chemical pesticides (Pedlowski *et al*., 2012) [15]. Use of synthetic chemical pesticides in long run are uneconomical and hazardous to environment. Pimentel (2009) [6] reported that with price of around $ 40 billion, and near about 3 billion kilogram of pesticides are applied per year globally. Also the excessive use of chemicals in agriculture ecosystem leads to contamination of land and water resources causing threat of death of natural enemies, non –target beneficial flora and fauna and evolution of fungicide resistant variants in nature. Another alternative to chemicals is the use of disease resistant plant varieties which is very time consuming process with some limitations. So why now a days, application of Biological Control Agents in agriculture gained so much attention and popularity as it help to reduce or eliminate use of chemical pesticides (Vinale *et al*., 2008) [21]. *Trichoderma* spp. are widely used as commercial biofungicides the world over. Correct identification is important for successful and safe use of these fungi, comprising of more than 200 defined species. This is especially important since many beneficial and harmful traits are species and often strain-specific. *Trichoderma* taxonomy, relied earlier on morphology and situation prevails till date for many isolates that are widely used, including many that are deposited in type culture collections. *Trichoderma* spp. is well known Biological Control Agent (BCA) because of its ability to reduce the Population of soil borne pathogens showing significant activity against wide range of Plant pathogenic fungi. (Micheli *et al*. 1998) [12]. The mechanisms used by *Trichoderma* spp. in the control of plant pathogens include the competition, mycoparasitism, antibiosis and induced resistance of the plant host.

2. Materials and Methods
2.1 Sample Collection
Total 45 soil samples were randomly collected from Ahmednagar, Dhule, Jalgaon, Kolhapur, Nandurbar, Nasik, Pune, Sangli, Satara and Solapur districts of Western Maharashtra region during June 2017 to October 2017. The soil samples were taken from a depth of 05 to 15cm around the rhizosphere area of crop plants, vegetables, cereals, oilseed and pulses etc. The soil samples were collected in plastic bags, sealed in boxes later labeled with information like name of collection sites, date of collection and origin of samples etc. Then, for further experiment, the samples were transported to the laboratory and exposed to room temperature with humidity of 50 per cent and sieved through a mesh of 2 mm.

2.2 Isolation of *Trichoderma* spp. from rhizosphere soils

The *Trichoderma* was isolated from the soil samples collected from the different locations of western Maharashtra by serial dilution technique and plating method. The *Trichoderma* selective medium (TSM) (Elad *et al.*, 1981) [10] was used for the isolation of *Trichoderma* spp. The antibiotic and fungicides were suspended in 50 ml of 30 per cent ethyl alcohol and added to the medium prior to pouring into petriplates. Isolation was carried out under aseptic conditions of laminar air flow cabinet to avoid contamination. Test tubes were labeled with glass marking pencil as 10^{-1}, 10^{-2} up to 10^{-5} for respective dilutions. In each test tube, 9 ml of sterilized water was poured and these were plugged with non-absorbent cotton. Later sterilized in an autoclave as recommended for 15-20 min. at 15lbs pressure. After cooling, initial dilution was prepared by addition of 1 g representative soil sample into the first test tube contain 9 ml of sterilized water in test tube labelled as 10^{-1}. In order to obtain uniform distribution of the soil sample, solution contents were mixed for 5 min, by rolling the test tubes to and fro between palms of hands. From the first dilution, 1 ml of suspension while in motion were transferred to the tube as 10^{-2} having 9 ml sterilized water with digital micropipette. Same labelled procedure was repeated till the original sample was diluted to 10^{-5}. Medium were poured at the rate of 20 ml/plate. Petriplates were labelled with permanent marker pen and inverted on solidification of the medium. These plates were incubated in BOD incubator at ± 28 ^{0}C for a week. Observations were recorded for growth of *Trichoderma* spp. every day.

2.3 Selection of *Trichoderma* colony

After incubation, well separated individual colonies with yellow green and whitish green pigments were identified and marked. The individual colonies were picked up with sterile loop of needle and transferred to Potato Dextrose agar media plates and pure culture so obtained were stored in a refrigerator for further studies (Arumugam *et al*., 2013) [2]

2.4 Purification of fungal cultures

Isolated cultures of fungi from rhizosphere soil samples were transferred on petriplates containing sterilized Potato Dextrose Agar (PDA) media under aseptic condition. After growth of fungi, purification was done by transferring growing hypha of the fungus devoid of contamination to PDA slants in test tube. Purification of fungal isolates was done by hyphal tip method as per procedure described by Lelliott and Stead.

2.5 Maintenance of fungal cultures

Isolated fungal cultures were maintained on sterilized PDA slants in the refrigerator. Periodic transfers of cultures were carried out on sterilized PDA slants in order to keep the cultures in viable active growth condition.

2.6 Stock Culture

Half-strength PDA slants prepared in test tube were used for the storage of fungal cultures. Small mycelial growth were picked up by using an inoculating needle from each pure culture and inoculated on the surface of PDA slants. The inoculated slants were incubated at ± 28 ºC for 5 days. After 5 days, the inoculated slants were stored at 4 °C.

2.7 Identification and characterization of *Trichoderma* isolates

Preliminary screening for *Trichoderma* species was carried out by observing both macroscopic and microscopic features of the fungal colonies. The *Trichoderma* isolates were identified on the basis of cultural and microscopic features followed by the method of Subramanian (Subramanian, 1971) [20]. The colonial morphology of fungal isolates was examined on PDA medium and microscopic appearance by lactophenol cotton blue staining technique which determined the type of reproductive mycelium i.e. conidiophores.

3. Result and Discussions

3.1 Sample collection and isolation

The total 45 soil samples were randomly collected from different districts of Western Maharashtra during June 2017 to Oct. 2017. The soil samples were taken from a depth of 05 to 15 cm around the rhizospheric area of crop plants. Details of the rhizosphere soil samples which had population of *Trichoderma* spp. collected from different locations of western Maharashtra presented in (Table 2). On the basis of morphological and cultural characters the *Trichoderma* spp. were identified.

The total 45 samples were collected from areas of Western Maharashtra. Out of these only 20 rhizospheric soil samples had the population of *Trichoderma* spp. By visual observations 6, *T. hamatum*, 7, *T harzianum* (Rifai), *3, T. koningii* (Oudem) and 4, *T. asperellum* (Samuels) were identified. It is also evident from (Table. 3) that *T. harzianum* and *T .hamatum* were present in most of the soil samples collected from Western Maharashtra region. The *T. asperellum* species was obtained from soil samples collected from Dhabewadi, sakri, Pachora and Tasgaon region of western Maharashtra. *Trichoderma* isolates were recovered according to Elad *et al*. (1981) [10] method using *Trichoderma* selective medium (TSM). Cultures were then subcultured on PDA for purification. The similar results for isolation of *Trichoderma* spp. from rhizosphere soils on TSM were found by workers (Papavizas and Lumsben 1982; Elad and Chet 1983; Sitansu *et al*., 2009; Pan and Bhagat 2007; Rajkonda and Bhale 2011; Attitalla *et al.*, 2012; Adhikari *et al*, 2014, Dehariya *et al.*, 2015 and Iqbal *et al*., 2017) [14, 9, 13, 17, 3, 1, 8, 11].

3.2 Screening and identification of *Trichoderma* isolates

Trichoderma isolates (Trc-1, Trc-2, Trc-3, Trc-4, Trc-5, Trc-6, Trc-7, Trc-8, Trc-9, Trc-10, Trc-11, Trc-12, Trc-13, Trc-14, Trc-15, Trc-16, Trc-17, Trc-19 and Trc-20) were placed into different groups. Microscopic examination was carried out according to Bissett (1984, 1991 a,b, c) [4-7] classification method. For the identification of *Trichoderma* spp. 3-7 days old culture grown on PDA was used. The *Trichoderma* strains were morphologically identified by using cultural characters like colony growth rate, colony colour, growth pattern, colony edge, mycelial form, mycelial colour and presence or absence of chlamydospores etc. Accordingly 20 *Trichoderma* isolates were grouped into 4 different *Trichoderma* spp. i.e. *T. hamatum* (6 isolates), *T harzianum* (7 isolates), *T. koningii* (3 isolates) and *T. asperellum* (4 isolates)

Table 1: Abstract of *Trichoderma* spp. isolated from soils of Western Maharashtra

Sr. No	Name of the District	Number of *Trichoderma* Isolates
1.	Ahmednagar	03
2.	Dhule	01
3.	Jalgaon	02
4.	Kolhapur	01
5.	Nandurbar	01
6.	Nasik	02
7.	Pune	03
8.	Sangli	03
9.	Satara	03
10.	Solapur	01
	Total	20

Table 2: Details of *Trichoderma* isolates recovered from soils of different districts of the Western Maharashtra

Sr. No.	Name of village	Taluka	District	Isolate No.	Month/ Year
1.	Chopda	Chopda	Jalgaon	Trc-1	Aug.2017
2.	Dhabewadi	Patan	Satara	Trc-2	July.2017
3.	Bhor	Bhor	Pune	Trc-3	Aug.2017
4.	Sinnar	Sinnar	Nasik	Trc-4	Aug.2017
5.	Indapur	Indapur	Pune	Trc-5	Sept.2017
6.	Vita	Khanapur	Sangali	Trc-6	Aug.2017
7.	Kavathe Yemai	Shirur	Pune	Trc-7	June.2017
8.	Malshiras	Malshiras	Solapur	Trc-8	Aug.2017
9.	Parner	Parner	Ahmednagar	Trc-9	Aug.2017
10.	Niphad	Niphad	Nasik	Trc-10	Aug.2017
11.	Sakri	Sakri	Dhule	Trc-11	Aug.2017
12.	Man	Man	Satara	Trc-12	July.2017
13.	Palus	Palus	Sangali	Trc-13	Aug.2017
14.	Pathardi	Pathardi	Ahmednagar	Trc-14	June.2017
15.	Rahuri	Rahuri	Ahmednagar	Trc-15	June.2017
16.	Pachora	Pachora	Jalgaon	Trc-16	Sept.2017
17.	Tasgaon	Tasgaon	Sangali	Trc-17	Oct.2017
18.	Khatav	Khatav	Satara	Trc-18	Aug.2017
19.	Shahada	Shahada	Nandurbar	Trc-19	Aug.2017
20.	Radhanagari	Radhanagari	Kolhapur	Trc-20	Aug.2017

Table 3: List of *Trichoderma* spp. obtained from different geographical locations of Western Maharashtra

Isolate No.	*Trichoderma* species	Place (Soil sample)
Trc-1	*T. harzianum*	Chopda
Trc-2	*T. asperellum*	Dhabewadi
Trc-3	*T. harzianum*	Bhor
Trc-4	*T. harzianum*	Sinnar
Trc-5	*T. koningii*	Indapur
Trc-6	*T. harzianum*	Vita
Trc-7	*T. hamatum*	Kavathe Yemai
Trc-8	*T. hamatum*	Malshiras
Trc-9	*T. harzianum*	Parner
Trc-10	*T. hamatum*	Niphad
Trc-11	*T. asperellum*	Sakri
Trc-12	*T. harzianum*	Man
Trc-13	*T. koningii*	Palus
Trc-14	*T. hamatum*	Pathardi
Trc-15	*T. hamatum*	Rahuri
Trc-16	*T. asperellum*	Pachora
Trc-17	*T. asperellum*	Tasgaon
Trc-18	*T. hamatum*	Khatav
Trc-19	*T. harzianum*	Shahada
Trc-20	*T. koningii*	Radhanagari

4. Conclusions

In the respective study, we isolated and identified 20 isolates of *Trichoderma*. Among them, isolates were grouped into 4 different *Trichoderma* spp. i.e. *T. hamatum* (6 isolates), *T harzianum* (7 isolates), *T. koningii* (3 isolates) and *T. asperellum* (4 isolates).

5. Acknowledgement

The authors thank Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri -413722, Maharashtra, India for providing all possible facilities for present study in partial fulfilment of Ph. D research.

6. References

1. Adhikari AN, Datta SS, Bhattacharya L, Mandal T. Study of morphology and mycoparasitism of some antagonists of *Trichoderma* sp. from West Bengal, India. Internat. J. Res 2014;1(9):593-606.
2. Arumugam K, Ramalingam P, Appu M. Isolation of *Trichoderma viride* and *Pseudomonas fluorescens* organism from soil and their treatment against rice pathogens. J. Microbiol. Biotech. Res 2013;3(6):77-81.
3. Attitalla IH, Abdelrawaf SS, Omar KS, El-komy HM, Sarwar M. Occurrence and microbiological characteristics of *Trichoderma* in al jabal al akhdar region, Libya. J. Biol. Sci 2012;12:209-217.
4. Bissett J. A revision of the genus *Trichoderma:* 1. Section *Longibrachiatum*, new section. Can. J. Bot 1984; 62:924-931.
5. Bissett J. A revision of the genus *Trichoderma.* II. Infrageneric classification. Can. J. Bot 1991a;69:2357-2372.
6. Bissett J. A revision of the genus *Trichoderma*. III. Section *Pachybasium*. Can. J. Bot 1991b;69:2373-2417.
7. Bissett J. A revision of the genus *Trichoderma*. IV. Additional notes on section *Longibrachiatum*. Can. J. Bot 1991c;69:2418–2420.
8. Dehariya K, Shukla A, Ganaie MA, Vyas D. Individual and Interactive role of *Trichoderma* and *Mycorrhizae* in controlling wilt disease and growth reduction in *Cajanus cajan* caused by *Fusarium udum*. Archives of Phytopathology and Plant Protection 2015;48:50-61.
9. Elad Y, Chet I. Improved selective media for isolation of *Trichoderma* spp. Phytoparasitica 1983;11(1):55-58.
10. Elad Y, Chet I, Henis Y. A selective medium for improving quantitative isolation of *Trichoderma* spp from soil. Phytoparasitica 1981;9(1):59-67.
11. Iqbal S, Muhammad A, Malik AH, Inam-ulhaq, Khan KS, Mathews P. Isolation, preservation and revival of *Trichoderma viride* in culture media Jounal of Entomology and Zoology Studies 2017;5(3):1640-1646.
12. Migheli Q, Gonzalez-Candelas L, Dealessi L, Camponogara A, Ramon-Vidal D. Transformants of *Trichoderma longibrachiatum* over expressing the β-1,4-endoglucanase gene egl1 show enhanced biocontrol of *Pythium ultimum* on cucumber. Phytopathology 1998;88:673-677.
13. Pan S, Bhagat S. Antagonistic potential of *Trichoderma* and *Gliocladium* spp. from West Bengal. J. Mycol. Plant Path 2007;37:235-239.
14. Papavizas GC, Lumsden RD. Improved medium for isolation of *Trichoderma* spp. from soil. Pl. Dis 1982; 66(11):1019-1020.
15. Pedlowski, Marcos, Canela, Maria, Terra, Maria *et al*, Rogeria Modes of pesticides utilization by Brazilian smallholders and their implications for human health and the environment. Crop Protection 2012;31:113-118.

16. Pimentel D. Environmental and economic cost of the application of pesticides primarily in the United States. In: Peshin, R. and Dhawan, A. K. (eds.). Integrated Pest Management: Inovation Development Process, Springer Netherlands. 2009, 89-111.
17. Rajkonda JN, Bhale UN. Report on two species of *Trichoderma* from black soil of Marathwada region of Maharashtra State. J Mycol. Plant Path 2011;8(32):102-106.
18. Samuels GJ. *Trichoderma*: a review of biology and systematics of the genus. Mycol. Res. 1996;100: 923-935.
19. Sitansu, P., Jennyfer, B. and Amrita, D. Effect of seed bio priming with some antagonistic isolates of *Trichoderma* species. J Mycol Pl Pathol. 2011;41(1): 43-48.
20. Subramanian CV, Hypomycetes. ICAR, New Delhi 1971, 930.
21. Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ, Li H, *et al*. *Trichoderma* -plant –pathogen interactions. Soil Boil. Biochem. 2008;40:1-10.