

First Attempt on Collection and Isolation of *Trichoderma* Spp. Native Strains from South Gujarat

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The collection and isolation of *Trichoderma* spp. in pocket area of South Gujarat led to the identification of native strains. Six new native strains of *Trichoderma* spp. viz., *Trichoderma fasciculatum* [TFC-1, TFC-2 (Castor)], *Trichoderma viride* [TVS-1, TVS-2 (Sugarcane)], *Trichoderma harzianum* [THCh-1 (Chickpea)] and *Trichoderma atroviride* (TACH-1 (Chickpea)) were first time isolated, identified and reported from the south Gujarat region. *T. fasciculatum* and *T. atroviride* has not been reported hitherto either from South Gujarat or Gujarat or from India.

Key words : *Trichoderma*, Native strains, New records, *T. atroviride*, *T. fasciculatum*.

South Gujarat is heavy rainfall zone and is having sufficient irrigation facilities. These facilitate for extensive cultivation of various crops viz., fruit crops, cash crops, oilseeds, pulses, vegetables and ornamental crops. The disease is the major constraint in economical production as it inflicts heavy crop losses. The drawbacks of chemicals are well known in scientific and farming community. Hence, the farmers have started for eco-friendly plant disease management (EPDM) or biological based Integrated Disease Management (BIDM). *Trichoderma* proved worldwide accepted technology for the biological control of plant diseases particularly for seed borne and soil borne diseases (Pandya *et al.*, 2011). Hitherto, no work on native strains of *Trichoderma*

has been done from this area. As a result this is the first attempt on the collection and isolation on native strains of *Trichoderma* spp.

MATERIALS AND METHODS

Isolation of *Trichoderma* spp. and their enumeration

The isolation was made from soil samples collected from different fields of chickpea, sugarcane and castor. *Trichoderma* spp. were isolated by the employing serial dilution method as mentioned by Ramanujam and Sriram (2009). One sample was collected from castor rhizoplane subjected to tissue isolation. This field was heavily infected with wilt (*Fusarium* sp.). All the isolates were purified by tissue isolation technique and maintained for further studies.

Materials required

Sterilized water	:	100 ml in conical flasks (250ml)
Sterilized water	:	9 ml in test tubes
Sterilized pipettes	:	1ml

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Sterilized petridishes
Rose Bengal Agar medium (RBA)

Magnesium sulphate	:	0.2g
K ₂ HPO ₄	:	0.9g
Ammonium nitrate	:	1.0g
Potassium chloride	:	0.15g
Glucose	:	3.00g
Agar	:	15.00g
Rose Bengal	:	0.15
Chloromphenicol	:	0.25g
Distilled water	:	1 lit.

Methodology and observations

For serial dilution method,

- Take 10 g of soil sample and suspend in 90 ml sterilized distilled water (1:10 or 10⁻¹).
- Shake the tubes thoroughly.
- Take 1 ml suspension and transfer to 9ml sterile water in test tube (1:100 or 10⁻²).
- Make serial dilutions by transferring 1ml of the suspension to the subsequent tubes to get 10⁻⁴.
- Transfer one ml of the desired soil suspension to Petri plates containing RBA in triplicate.
- Rotate the plates gently.
- Incubate at room temperature (27±2°C).

Table 1. *Trichoderma* isolates counts from different rhizospheric soils.

S. No.	Soil samples Crop/Location	No. of <i>Trichoderma</i> colonies (10 ⁻⁴ dilution)	Other colonies	<i>Trichoderma</i> c.f.u. (%)	c.f.u./g
1	Chickpea/PRS*, Navsari	14	7	66.66	1.4 x 10 ⁵
2	Chickpea/ Farmer's field Waghai	16	8	66.66	1.6 x 10 ⁵
3	Sugarcane/ Farmer's field Bardoli	18	7	75.00	1.8 x 10 ⁵
4	Sugarcane,/ Farmer's field Sayan	20	7	74.07	2.0 x 10 ⁵
5	Castor/RRRS**, Vyara	14	7	66.66	1.4 x 10 ⁵

*PRS: Pulse Research Station;

**RRRS: Regional Rice Research Station



Fig. 1. Natural occurrence of *Trichoderma* on castor rhizospheric.

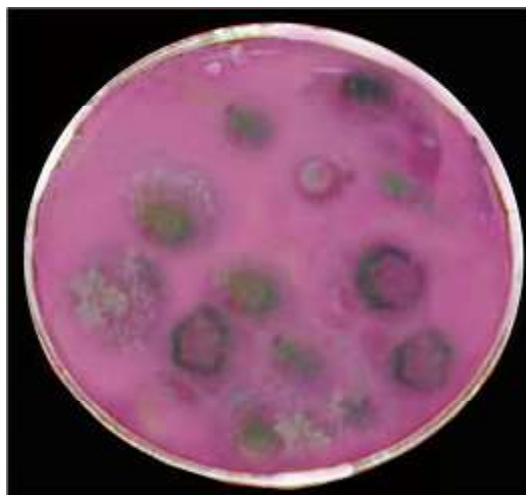


Fig. 2. Isolation of *Trichoderma* on Rose Bengal (RBA) medium.

For tissue isolation,

- Collect the samples of castor roots along with visually observed *Trichoderma* colonization. That colonized area was subjected to tissue isolation.
- The colonized area was cut into small bits. The bits were surface sterilized with mercuric chloride (HgCl₂) solution @ 0.1 per cent for 30 seconds followed by three washing with sterilized distilled water and then transferred aseptically, under laminar air flow system (Cabinet manufactured by Klenzoid contamination control Ltd.), on sterilized Petriplates containing 20 ml Rose Bengal Agar (RBA) medium.
- These petriplates were incubated at room temperature (27±2°C).
- The fungal hyphae developing from the bits were first examine microscopically and then sub-cultured aseptically on RBA slants for the purification of culture.

Observations

- Count the number of colonies of *Trichoderma* spp. and other microorganisms developing in individual plates.
- The percentage of *Trichoderma* colonies were calculated as suggested by Siddiquee *et al.* (2009):

$$\% \text{ of } Trichoderma \text{ cfu} = \frac{\text{No. of } Trichoderma \text{ colonies per plate}}{\text{Total no. of colonies per plate}}$$

- Number of cfu per gram of soil were recorded by following formulae suggested by Bhat *et al.* (2009): cfu/g of soil sample = Average colonies x Dilution factor

RESULTS

The isolation from soils at 10⁻⁴ dilution resulted into 14 to 20 colonies of *Trichoderma* and 7-8 colonies of other fungi. Maximum colonies of *Trichoderma* were recorded from soil sample collected from the farmer's field of sugarcane at Sayan²⁰. This was followed by farmer's field of chickpea at Waghai¹⁶, chickpea field at Navsari and castor field at Vyara¹⁴. The percentage of *Trichoderma* colony forming unit was ranging from 66.66 to 75.00. The number of colony forming unit of *Trichoderma* in 1 g of soil was ranging from 1.4

x 10⁵ to 2.0 x 10⁵ c.f.u./g (Table 1). One isolate of *Trichoderma* was also isolated from the rhizoplane of castor. Natural occurrence of *Trichoderma* was observed profusely in castor crop at Navsari which was heavily infected by wilt (Fig 1). *Trichoderma* colonies were initially observed as white specks on agar which then enlarged within six days. By this time, the white colony turned yellowish to green on Rose Bengal Agar medium. *Trichoderma* colonies grew rapidly and developed their typical green colour, which aided in their identification from other soil-borne fungi (Fig 2). The isolates of *Trichoderma* spp. obtained from the different rhizospheric soils and rhizoplane (castor) were purified. The pure culture was sent to Indian Type Culture Collection (I.T.C.C.), Division of Mycology and Plant Pathology, I.A.R.I., New Delhi – 110 012 and was identified as *Trichoderma fasciculatum* (ID No. 6929.08 & 6930.08), *Trichoderma viride* Pers. (ID No. 7120.08 & 7122.08), *Trichoderma harzianum* Rifai (ID No. 7121.08) and *Trichoderma atroviride* Bissett (ID No. 7123.08). Isolates were coded as TFC-1, TFC-2 (Castor); TVS-1, TVS-2 (Sugarcane); THCh-1 and TACH-1 (Chickpea). All the isolates were first time reported from the south Gujarat region. Among them, *T. fasciculatum* and *T. atroviride* were recorded for the first time from Gujarat.

DISCUSSION

Results revealed that there is ample amount of *Trichoderma* population present in the soils tested. Also the quantification of *Trichoderma* cfu/g are in line with earlier workers (Papvizas and Lumsden, 1982 and Rahman *et al.*, 2009). The intensification of this should be done so as to eradicate soil borne plant pathogens biologically and to maintain soil health. Such extensive survey to create database and making a map showing the status of useful microorganisms in the area is suggested. This information can also be incorporated in "Soil Health Card" prepared by the Government of Gujarat.

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