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Isolation of *Trichoderma* spp. from the rhizospheric soils of tomato crop grown in Marathwada region

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Abstract
Sixteen samples were collected from rhizospheric soil of tomato crop of eight (8) districts of marathwada region. From these soil samples 16 isolates were isolated on PDA medium. This rhizospheric soil was isolated by using 10^{-4} to 10^{-5} dilution by dilution plate technique. Out of 16, only 8 rhizospheric soil samples had the population of *Trichoderma* spp. The results of isolation of *Trichoderma* spp from rhizospheric soils of tomato were presented in table.1 (Plate I) by visual observation *Trichoderma* spp. were identified as *Trichoderma viride* isolates, *T. harzianum* isolates and *T. hamatum* isolates.
It is also evident from (Table 1 plate I) that, *T. hamatum* and *T. harzianum* were present in most of the soil samples of Marathwada region. *T. harzianum* was mostly found in Jalna, Parbhani, Osmanabad and Aurangabad districts of Marathwada region. *T. viride* was observed in Beed and Hingoli and *T. hamatum* was observed in Latur and Nanded districts of Marathwada region.

Keywords: collection of rhizospheric soil, isolation, marathwada region

Introduction
Trichoderma species are used as biocontrol agents in agriculture. *Trichoderma*, a genus of asexually reproducing saprophytic fungi, frequently present in nearly all temperate and tropical soils, decaying plant tissues and root ecosystems. The strains of *Trichoderma* spp. are strong opportunistic invaders, fast growing, prolific producers of spores and powerful antibiotic producers.
The antifungal abilities of beneficial microbes have been known since 1930 and there have been extensive efforts to use them for plant disease control. *Trichoderma* able to control a wide range of phytopathogenic fungi as antagonist. In the light of this situation, it is highly essential to formulate integrated disease management strategy. Biological management being cheap, ecofriendly and effective method of management can be very well adopted in such circumstances. It gives appreciable control of diseases without pollution hazards (Cook and Beker. 1974) [2] the mechanism of action of *Trichoderma* consists of hyperparasitisum, antibiosis and production of antibiotics which restricted the growth of the pathogenic fungal organisms (Windham *et al*., 1986) [8].

Materials and Methods
Collection of rhizospheric soil samples
Collection of soil sample was done from eight different districts of marathwada region. Tomato plant is uprooted and soil is collected from rhizospheric area nearly ½ Kg soil was collected in plastic bags. Collected soil was air dried and used for further study.

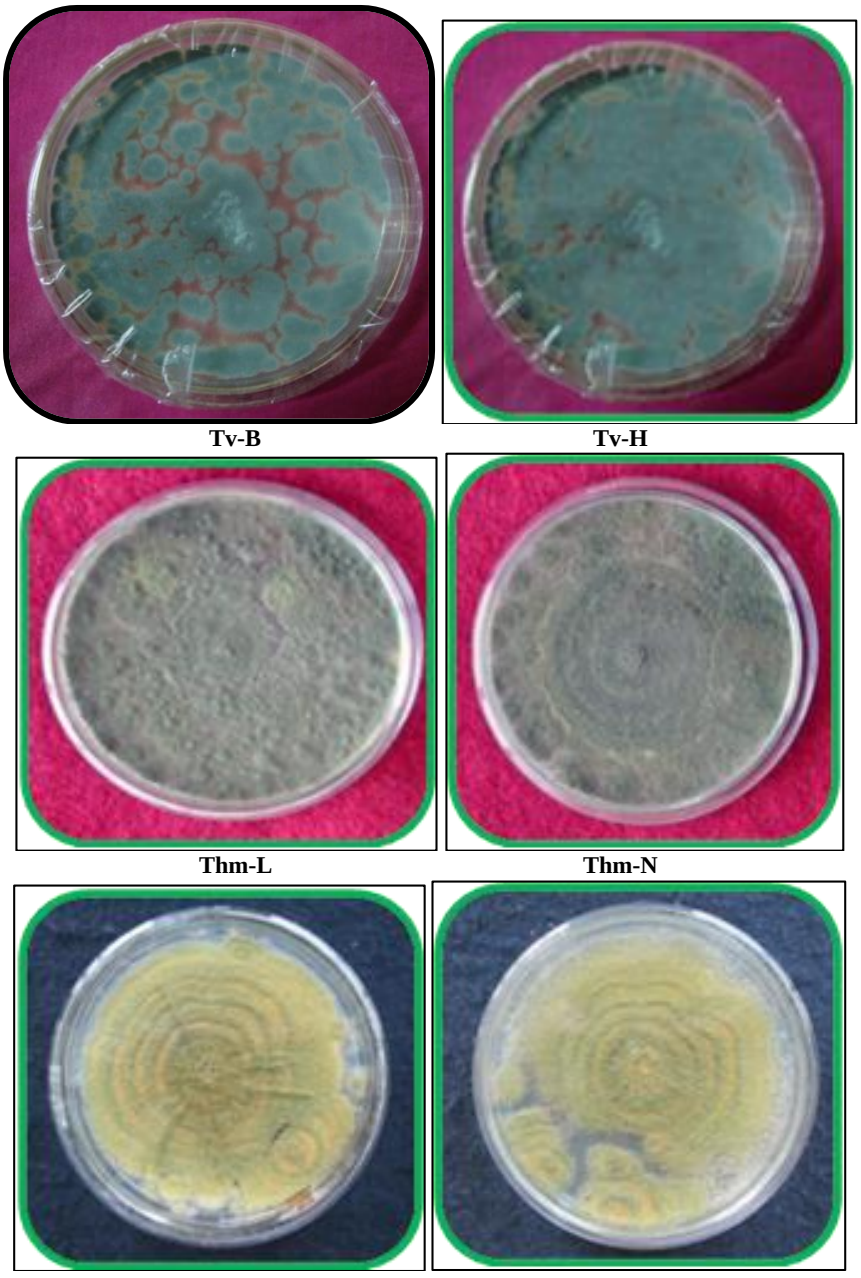
Isolation of *Trichoderma spp.*
Serial dilution and plating technique was used to isolate the *Trichoderma* from the samples collected. The collected samples were air dried in shade and finely grind before serial dilutions. PDA media was used for Isolation.
Test tubes labeled with glass marking pencil as 10^{-1}, 10^{-3} up to 10^{-5}. In each test tube, 9 ml of water were poured. Test tube was plugged with non-absorbent cotton and was sterilized in an autoclave as mentioned earlier. After cooling, initial dilution was prepared in test tube labeled as 10^{-1} by addition of 1 g representative soil sample into the first test tube containing 9 ml of sterilized water. Contents were mixed by rolling the test tubes to and from between palms of hands for 5 minutes to obtain uniform distribution of the soil sample. From the first dilution, 1 ml of suspension while in motion was transferred to the tube labeled as 10^{-2} having 9 ml sterilized water with fresh sterilized pipette. Again same procedure was repeated, till the

original sample will be diluted to 10^{-5}. Each time fresh sterilized pipettes, was used. With the help of sterilized pipette, 1 ml of suspension from each dilution (while rotating the test tubes in palms of hands) was placed in centre of sterilized test tube. Antimicrobial agent was then added to it in required quantity. This medium will be poured over soil water suspension in Petri plate with rotary motion of the plate to mix it thoroughly. Medium was poured at the rate of 20 ml/plate. Plates was labeled with glass marking pencil and inverted on solidification of the medium. These plates were incubated in BOD incubator at 28 ± 1^0 C up to a week's period. Incubated plates were watched every day for the growth of *Trichoderma* spp. Preliminary screening for *Trichoderma* species was carried out by observing both macroscopic and microscopic features of the fungal colonies. For macroscopic screening, the growth rate and colours of colonies were examined. For microscopic screening, slides were prepared. Mycelia from each isolate were taken from PDA plate and spread onto a clean slide mounted with a drop of water, covered with cover slip and then observed under a light microscope using 100X and 400X magnification. The branching patterns of conidiophores and the shapes and sizes of conidia were examined. The macroscopic and microscopic features were compared to the characteristics described by Samuels *et al*., (2002) [6].

Result and Discussion

Sixteen samples were collected from rhizospheric soil of tomato crop of eight (8) districts of marathwada region. From these soil samples 16 isolates were isolated on PDA medium. This rhizospheric soil was isolated by using 10^{-4} to 10^{-5} dilution by dilution plate technique. Out of 16, only 8 rhizospheric soil samples had the population of *Trichoderma* spp. The results of isolation of *Trichoderma* spp from rhizospheric soils of tomato were presented in table.1 (Plate I) By visual observation *Trichoderma* spp. were identified as *Trichoderma viride* isolates, *T. harzianum* isolates and *T. hamatum* isolates.

It is also evident from (Table 1 plate I) that, *T. hamatum* and *T. harzianum* were present in most of the soil samples of Marathwada region. *T. harzianum* was mostly found in Jalna, Parbhani, Osmanabad and Aurangabad districts of Marathwada region. *T. viride* was observed in Beed and Hingoli and *T. hamatum* was observed in Latur and Nanded districts of marathwada region.



Tv-B **Tv-H**

Thm-L **Thm-N**

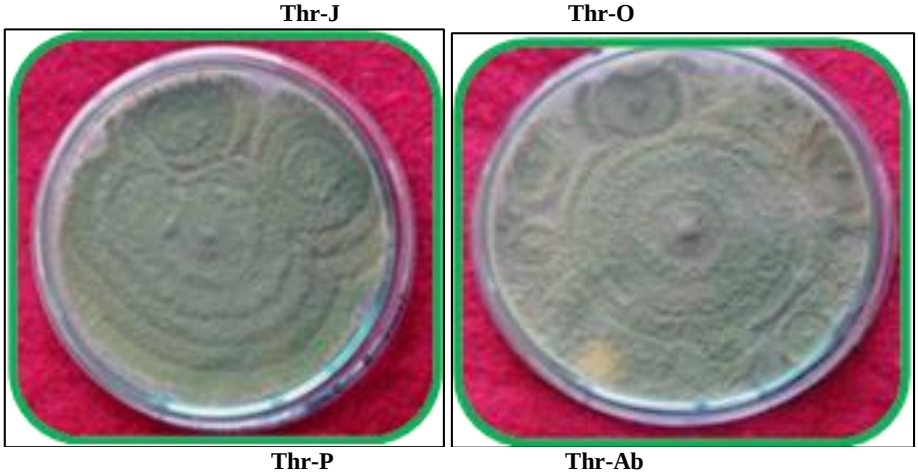


Plate I: Isolates of *Trichoderma* spp obtained from rhizospheric soil of tomato from marathwada region

Table 1: Details of isolates of *Trichoderma* spp. obtained from rhizosphere soil collected from Marathwada region.

Sr. No	Name of District	Name of Taluka	Isolate No.	*Trichoderma* spp. isolated
1.	Jalna	Bhokardan	Thr-J	*T. harzianum*
2.	Aurangabad	Sillod	Thr-Ab	*T. harzianum*
3.	Latur	Ausa	Thm-L	*T. hamatum*
4.	Hingoli	Sengaon	Tv-H	*T. viride*
5.	Osmanabad	Tuljapur	Thr-O	*T. harzianum*
6.	Parbhani	Parbhani	Thr-P	*T. harzianum*
7.	Nanded	Nanded	Thm-N	*T. hamatum*
8.	Beed	Kaij	Tv-B	*T. viride*

Similar results for isolation of *Trichoderma* spp. from rhizosphere soils of marathwada region was reported by Rajkonda and Bhale 2011 [5] and from other region on TSM were found by earlier workers (Elad *et al* 1983; Sitansu *et al.*, 2009; Adhikari *et al.*, 2014 and Dehariya *et al.*, 2015) [4, 7, 1, 3].

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