

## Prevalence of *Trichoderma* spp. in crop niches with variable water need and their tolerance to high temperature

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### ABSTRACT

This study was conducted on native *Trichoderma* spp. from different crop niches with variable water need of Kanpur and its adjoining districts to assess their comparative tolerance towards the high temperature. Out of 97 soil samples from 17 crop niches, only 28 samples yielded *Trichoderma* spp., out of which, 21 isolates were of *T. harzianum*, 6 *T. viride* and one of *T. virens* (*Gliocladium virens*). Crop wise analysis revealed that in low water requiring crops (pigeonpea, chickpea lentil, sorghum, barley) *Trichoderma* prevalence was 34.1%, in medium water requiring crops (mustard, wheat, field pea) recovery was 11.2% while in high water requiring crops (lucerne, garlic, cauliflower, coriander and *rajmash*), its recovery was as low as 6 per cent. All 28 native isolates along with two checks were evaluated for high temperature tolerance (50 + 3°C) in respect of colony growth, conidia and chlamydospore production in relation to optimum temperature (28 + 1°C). Two isolates showed full colony growth (90 mm), two isolates between 38-56 mm growth while 26 isolates exhibited 30 mm colony growth at 72 hrs of incubation at high temperature. One isolate of *T. viride* from lentil did not show any growth reduction at high temperature and was comparable to *T. harzianum*. In others, colony growth inhibition ranged between 38.3-79.2 per cent. Same isolates again exhibited least reduction in conidia production (5.1%) against 29.9% in *T. harzianum* and 99.9% in *T. viride* as checks. Reduction in chlamydospore production at high temperature varied between 56.6-100%, least (56.6-58.3%) being in *T. harzianum* isolated from garlic and mustard, respectively. Species wise there was no major difference in growth characters between the three species of *Trichoderma*.

**Key words:** Bioagent. Crop niches. Pulses. Temperature tolerance. *Trichoderma*

Pulses such as chickpea (*Cicer arietinum*), pigeonpea (*Cajanus cajan*), mungbean (*Vigna radiata*), urdbean (*V. mungo*), lentil (*Lens culinaris*), fieldpea (*Pisum sativum*) and *rajmash* (*Phaseolus vulgaris*) suffer from a number of soil borne diseases caused by *Fusarium* spp, *Rhizoctonia solani*, *R. bataticola*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii* and *Choenephora cucurbitarum* etc., and cause heavy yield losses. Biological control of these pathogens is an eco-friendly approach and appears to be attractive. Though a large number of antagonists and mycoparasitic fungi have been reported as potential bio-control agent, none could show consistent and desirable result under North Indian conditions. Recent works of De and Chaudhary (1999), Chaudhary and Kumar (1999) and Chaudhary *et al.*(2003) showed some

promise in managing soil borne diseases of pulses through bioagents. However, it was felt necessary to isolate native *Trichoderma* spp. and evaluate their ability to tolerate high temperature, so as to identify isolates that are effective during the summer and rainy seasons of this agro-ecological area.

### MATERIALS AND METHODS

The study was conducted during 2003-05 at Indian Institute of Pulses Research, Kanpur. Total 97 soil samples were collected from Kanpur Nagar, Kanpur Dehat, Fatehpur and Hamirpur districts of Uttar Pradesh from standing crop fields. The collections were made from 17 crop niches. Isolation of *Trichoderma* spp. was done on potato dextrose agar (PDA) medium using soil dilution technique (Singh 1970). The *Trichoderma* colonies were aseptically transferred and purified by adopting single spore culture. Identification was done at Indian Type Culture Collection, I.A.R.I., New Delhi. Studies on colony growth, sporulation and chlamydospore formation were under taken on PDA medium using 90 mm Petri-plates, which after inoculation with *Trichoderma* isolates were maintained under two separate temperature regimes each replicated thrice. One regime was of high temperature at 50 + 3°C and another of optimum temperature at 28 + 1°C in B.O.D. incubator. Observations were recorded at 72 hrs for colony growth, at 7 days for sporulation and at 15 days for chlamydospore formation. Quantitative estimation of sporulation and chlamydospore formation was done with the help of haemocytometer. Per cent inhibition at high temperature regime was calculated in relation to the growth of individual isolate at optimum temperature.

### RESULTS AND DISCUSSION

Ninety-seven soil samples collected from 17 crop niches of 4 districts of central Uttar Pradesh showed the recovery of 28 *Trichoderma* isolates from 28.8% of the samples. Crop wise analysis revealed its recovery in all soil samples collected from sorghum fields. This was followed by 46.1% from pigeonpea. Recovery from mustard, chickpea, lentil, cauliflower, garlic and lucerne grown soils ranged between 20-36.4% while from wheat field it was only 8.3 per cent. Grouping of these crops into 3 moisture regimes further indicated that crops requiring high moisture of 4-6 irrigations (lucerne, garlic, cauliflower) showed lowest *Trichoderma* recovery of only 6 per cent, crops requiring medium moisture

of 2-3 irrigations (mustard, pea, wheat) showed 11.2% recovery while crops like (chickpea, lentil, pigeonpea, sorghum) requiring low moisture showed 34.1% recovery of *Trichoderma* spp. (Table 1). Burgess and Keane (1997) indicated poor recovery of bioagents from continuously wet soil and our studies confirm these findings. Biswas (1999) enumerated *Trichoderma harzianum* from different districts of West Bengal and observed variability in respect to population, pigmentation, conidia production and antagonistic activity. The role of native *Trichoderma* in the management of disease has been emphasized by several workers (Bankole and Adebajo 1996, Sumitha and Gaikwad 1995).

Table 1. Prevalence to *Trichoderma* spp. in crop niches requiring different moisture

Moisture regime	% Recovery of <i>Trichoderma</i> spp.	Group mean (%)
<b>High</b>		
Lucern	20.0	6.0
Coriander	0.0	
Garlic	33.3	
Bean	0.0	
Cauliflower	25.0	
<b>Medium</b>		
Mustard	36.4	11.2
Pea	0.0	
Wheat	8.3	
<b>Low</b>		
Pigeonpea	46.1	34.1
Chickpea	33.3	
Lentil	25.0	
Sorghum	100.0	

All 28 *Trichoderma* isolates when grown at two temperature regimes showed a wide range of variation in respect of colony growth, conidia production and chlamyospore formation (Table 2). At optimum temperature (28 + 1°C) colony growth of these isolates ranged between 75-90 mm and at high temperature (50 + 3°C) it ranged between 21.5-90 mm. Colony growth of isolate from lentil and *Trichoderma harzianum* (check) remained unaltered at high

temperature. Again, these two isolates produced comparable quantity of conidia at high temperature 4720 x 10<sup>4</sup> and 5025 x 10<sup>4</sup> conidia per 5 mm disc) as against 4487 x 10<sup>4</sup> and 7175 x 10<sup>4</sup> conidia/disc, respectively at optimum temperature. Under optimum temperature regime, all *Trichoderma* isolates produced very high number of conidia ranging between 3852 x 10<sup>4</sup>– 6775 x 10<sup>4</sup> /disc. Chlamyospore production at high temperature was drastically reduced in all isolates. In general reduction in colony growth, conidia production and chlamyospore formation under high temperature regime was between 0-76.1 per cent, 5.1-99-9% and 56.6-100%, respectively (Table 2). The role of temperature and humidity in the efficacy of bio-agents against bean diseases has been emphasized by Hannush and Boland (1996 a and b) who found that higher temperature and lower humidity which were conducive for the disease also improved the efficacy of bioagents.

Identification of 28 *Trichoderma* isolates (Table 3) revealed 22 isolates as *T. harzianum*, 7 as *T. viride* and one as *T. virens* (*Gliocladium virens*). While colony growth (85.4-90.0 mm), conidia production (4425-5373 x 10<sup>4</sup>/5 mm disc) and chlamyospore formation (184.9-216.9/5 mm disc) at normal temperature (28+ 1°C) was at par among the three species, their performance at high temperature (50 + 3°C) was highly variable. *T. harzianum* and *T. viride* showed higher colony growth (30.4-34.2 mm) than *T. virens* (18.7 mm). On the other hand, *T. virens* showed more chlamyospore formation at high temperature (12.5 /disc) than *T. harzianum* and *T. viride* (2.6-3.5 /disc). Trutmann and Keane (1990) found that *T. koningii* had better potential to control *S. sclerotiorum* in beans when applied during warmer climates. Therefore, the native isolates derived from rainfed or low moisture area and tolerant to higher temperature are expected to show better performance in the management of soil borne diseases.

Table 2. Performances of *Trichoderma* spp. at high temp for colony growth, conidia and chlamyospore production

Crop	Optimum temperature (28+1°C)			High temperature (50+ 3°C)			% Reduction at high temperature		
	Colony growth (mm)	No. of conidia (10 <sup>4</sup> /5 mm disc)	No. of chlamyospore (5 mm Disc)	Colony growth (mm)	No. of conidia (10 <sup>4</sup> /5 mm disc)	No. of chlamyospore (5 mm disc)	Colony growth	Conidia	Chlamyospore
Lucern (1)	82.05	4720	68.5	25.5	61.0	0.0	69.0	98.7	100.0
Garlic (1)	75.0	4612	30.0	22.5	43.0	13.0	70.0	99.1	56.6
Cauliflower (1)	90.0	6775	47.5	30.7	20.0	0.0	65.8	99.6	100.0
Mustard (4)	86.8	4670	46.7	25.0	67.0	1.2	71.1	98.4	97.4
Wheat (1)	90.0	4787	47.5	21.5	48.0	2.0	76.1	98.9	95.8
Pigeonpea (12)	86.0	5370	216.7	29.4	59.0	2.4	65.8	98.9	98.8
Chickpea (6)	85.8	4895	277.7	23.9	32.0	4.5	72.1	99.3	98.3
Lentil (1)	90.0	4487	135.0	90.0	4720.0	14.2	0.0	5.1	89.5
Sorghum (1)	87.5	3852	125.0	29.7	85.0	0.0	66.0	97.7	100.0
<i>T. viride</i> check	87.5	5005	270.0	22.5	0.80	2.0	74.3	99.9	99.2
<i>T. harzianum</i> check	90.0	7175	600.0	90.0	5025.0	15.7	0.0	29.9	97.3
CD at 5%	5.8	6.3	10.1	14.9	1.7	0.47			

Figures in parentheses are number of isolates

Table 3. Species wise performance of *Trichoderma* isolates at high and optimum temperature

<i>Trichoderma</i> Spp.	At (28±1°C)			At (50±3°C)			% Reduction at high temperature		
	Colony growth (mm)	No. of conidia (10 <sup>4</sup> /5 mm disc)	No. of chlamydo-spore (5 mm disc)	Colony growth (mm)	No. of conidia (10 <sup>4</sup> /5 mm disc)	No. of chlamydo-spore (5 mm disc)	Colony growth	No. of conidia	No. of chlamydo-spore
<i>T. harzianum</i> (22)	85.4	5128	184.9	30.4	282	3.5	64.4	94.5	98.1
<i>T. viride</i> (7)	88.5	5373	216.9	34.2	718	2.6	61.3	86.6	98.8
<i>T. virens</i> (1)	90.0	4425	191.5	18.7	13	12.5	79.2	99.9	93.4

Figures in parentheses are number of isolates

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