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Isolation and Evaluation of Temperature Tolerant *Trichoderma*

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A B S T R A C T

Trichoderma isolates were collected from the rhizosphere and rhizoplane of Chickpea and Rice ranged from 5°C to 45°C screened for temperature tolerance. Among all the temperature tested at 30°C the isolates showed maximum average mycelial diameter of 8.81cm. Minimum average colony diameter of 0.87 cm was recorded at 40^{0}C. Simultaneously isolates Ta12 showed maximum average colony diameter of 6.36 cm, which did not differ significantly from Ta14 (6.30 cm) and Ta13 (6.16 cm). It was interesting to note that Isolate Ta15 showed maximum colony diameter (2.60 cm) at 40^{0}C, while most of the other isolates were not able to grow at this temperature. Similarly maximum (266.06 mg) average mycelial fresh weight of all the isolates were recorded at 30°C followed by 20°C (229.42 mg). Significantly maximum (143.50 mg) fresh weight was recorded at 40°C by Isolate Ta15. Clearly 30^{0}C is the best temperature for *Trichoderma* to grow and reproduce efficiently using as biocontrol agent.

Introduction

The fungi belonging to *Trichoderma* spp. are in current use as Biocontrol agents due to their antagonistic potential against plant pathogens all over the world. The antifungal properties of *Trichoderma* are attributed to their ability to compete for nutrients, secretions of hydrolytic enzymes and production of antibiotics. However the ability of these fungi to sense, invade and destroy other fungi has been the major driving force behind their commercial success as biopesticides and more than 60% of all registered biopesticides are *Trichoderma* based (Verma *et al.*, 2007).

Most of the *Trichoderma* spp. are mesophilic and the competitive colonization of spp. *viz.*, *T. harzianum* was greatest at degrees lower than the temperature optimum for growth *in vitro* and its saprophytic activity was greatest from 15°C to 21°C (Eastburn and Butler, 1991). A certain amount of work has been also carried out on the temperature relationship of plant virus (Sharma, 2009; Sharma *et al.*, 2012; Sharma *et al.*, 2013; Lavania *et al.*, 2018 and Sharma *et al.*, 2018) and higher *Basidiomycetes*, which also include a majority of edible fungi but most of this is scattered in the literature (Sharma *et al.*, 2010; Suman *et al.*, 2010; Suman *et al.*, 2011; Suman *et al.*, 2012; Sharma *et al.*, 2012; Sharma *et al.*, 2012; Sharma M *et al.*, 2014 and Sharma *et al.*, 2016). Although soils in temperate regions during seeding stage are not often at this temperature, there is a need to identify potential high temperature tolerant *Trichoderma* isolates. Such isolates could sustain and survive the fluctuating temperatures rising due to global warming and naturally antagonize pathogens. Better understanding of the genomics of such isolates will improve and expand their applications (Sharma *et al.*, 2018 and Singh, 2019). In the present investigation, temperature tolerant *Trichoderma* isolates were isolated from natural soils and their biocontrol efficacy was evaluated.

Materials and Methods

A lab experiment was conducted at GBPUAT, Pantnagar 2018-19, for the isolation of potential temperature tolerant isolates of *Trichoderma* from the rhizosphere and rhizoplane of crops from Chickpea and Rice.

Trichoderma selective medium (TSM) was used for the isolation of the *Trichoderma* spp. (Tronsmo and Dennis, 1978) and isolation was done by using serial dilution technique **(**Elad *et al.*, 1981; Askew and Laing, 1993). The experiment was laid in a completely randomized block design and three replications of each dilution.

The extensive collection of samples led to the generation of 20 isolates of *Trichoderma*, coded as Ta-1 to Ta-20. These isolates have been purified, identified and morphologically characterized.

In Vitro evaluation of temperature tolerant *Trichoderma* isolates

To study the effect of various temperature regimes, Petri plates and flasks containing basal medium and inoculum of different *Trichoderma* isolates were incubated at different temperatures ranging between 5°C to 45^{0}C for 5 and 10 days respectively. Kredics *et al.*, (2003) reported that at temperature of 5°C and 45°C, no growth was recorded and hence has been deleted from the statistical analysis for finding out the best temperature for linear colony diameter and average mycelial fresh weight of *Trichoderma* isolates . The data obtained for average colony diameter and mycelial fresh weight of *Trichoderma* isolates were recorded at 10°C to 40°C and are presented in Table 1 and Table 2 respectively.

Results and Discussion

It is clear from the data recorded in Table 1, that significantly maximum average growth of all the isolates was recorded at 30°C, followed by 20°C and 35°C. A temperature of 30°C was significantly best for the growth of all the isolates of *Trichoderma*, which recorded 8.81 cm average colony diameter in 5 days. Minimum average colony diameter of 0.87 cm was recorded at 40°C. Simultaneously isolates Ta12 showed maximum average colony diameter of 6.36 cm, which did not differ significantly from Ta14 (6.30 cm) and Ta13 (6.16 cm).

The interaction study revealed that isolate Ta18 recorded maximum (5.70 cm) colony diameter at 10°C which was significantly different from other isolates. Isolates Ta3 and Ta18 showed maximum (9.00 cm) colony diameter at 20°C which did not differ significantly from other isolates viz., Ta11 (8.8 cm), Ta12 (8.8 cm), Ta15 (8.8 cm), Ta17 (8.8 cm) and Ta19 (8.8 cm). Isolates Ta1 recorded maximum (9.00 cm) colony diameter at $30^{\circ}C$ which did not differ significantly from other isolates. Isolates Ta14 recorded maximum (8.80 cm) colony diameter at $35^{0}C$ which did not differ significantly from Ta 13 (8.30 cm). Isolates Ta15 showed maximum colony diameter (2.60 cm) at $40^{0}C$, while most of the other isolates were not able to grow at this temperature. It is obvious from the data presented in Table 2, that significantly maximum (266.06 mg) average mycelial fresh weight of all the isolates was recorded at $30^{0}C$ followed by $20^{\circ}C$ (229.42 mg). Irrespective of temperature isolate Ta 12 recorded maximum (203.40 mg) average fresh weight of mycelium followed by Ta 19 (201.96 mg) and these two differed significantly among themselves. Minimum (162.06 mg) average fresh weight of mycelium was recorded by Ta3.

Fig.1 Isolates of *Trichoderma* isolated from the rhizosphere and rhizoplane of Chickpea and Rice

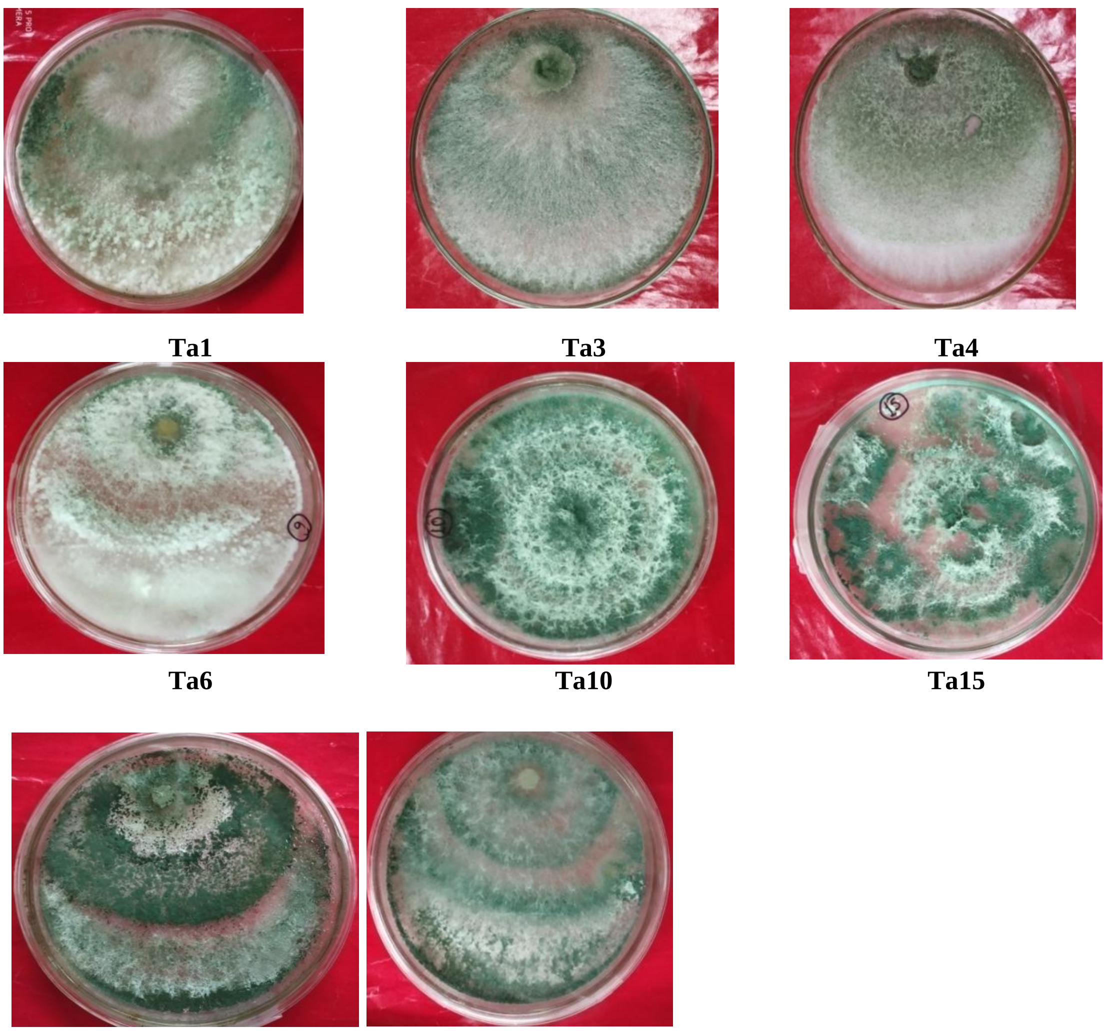

Table.1 Colony diameter of different *Trichoderma* isolates at various temperatures on solid medium (PDA)

Isolates	Average colony diameter (cm) of *Trichoderma* isolates after 5 days					
	10°C	20°C	30°C	35°C	40°C	Mean A
Ta1	3.73	6.10	9.00	7.30	0.00	5.22
Ta2	3.46	7.60	8.80	4.40	1.80	5.21
Ta3	3.76	9.00	8.80	3.90	0.00	5.09
Ta4	3.63	7.70	8.80	4.20	0.00	4.86
Ta5	3.30	8.10	8.80	8.10	0.00	5.66
Ta6	3.66	8.30	8.80	2.80	2.30	5.17
Ta7	2.83	7.30	8.80	2.40	0.00	4.26
Ta8	3.10	7.50	8.80	3.40	0.00	4.56
Ta9	3.63	7.20	8.80	8.00	0.00	5.52
Ta10	4.13	8.20	8.80	3.00	2.50	5.32
Ta11	3.36	8.80	8.80	6.70	0.00	5.53
Ta12	4.20	8.80	8.80	8.30	1.70	6.36
Ta13	3.80	8.10	8.80	7.60	2.50	6.16
Ta14	4.00	7.90	8.80	8.80	2.00	6.30
Ta15	4.16	8.80	8.80	3.20	2.60	5.51
Ta16	2.96	7.40	8.80	4.50	0.00	4.73
Ta17	4.66	8.80	8.80	3.50	0.00	5.15
Ta18	5.70	9.00	8.80	5.00	0.00	5.70
Ta19	3.63	8.80	8.80	8.00	0.00	5.84
Ta20	2.86	8.00	8.80	7.50	2.10	5.85
Mean B	3.73	8.07	8.81	5.53	0.87	
Effect		**CD (0.05)**				
Factor(A) (Colony diameter)		**0.24**				
Factor(B) (Temperature)		**0.12**				
Factor (A X B)		**0.54**				

*Diameter of Petri plates, 9.0 cm. Each value is an average of three replications.

Table.2 Fresh mycelial weight of different *Trichoderma* isolates at various temperature on liquid medium (Potato Broth)

Isolates	**Average mycelial fresh weight (mg) of *Trichoderma* isolates after 10 days**					
	10°C	**20°C**	**30°C**	**35°C**	**40°C**	**Mean A**
Ta1	129.83	235.50	272.33	225.83	0.00	201.40
Ta2	125.66	240.50	275.83	225.50	0.00	173.50
Ta3	124.16	225.83	249.83	210.50	0.00	162.06
Ta4	120.83	238.50	251.50	209.83	0.00	164.13
Ta5	124.50	216.16	279.00	230.00	123.83	194.70
Ta6	128.50	219.66	248.83	223.66	0.00	164.13
Ta7	125.50	234.50	274.00	230.16	0.00	172.83
Ta8	124.16	226.00	257.83	207.16	0.00	163.03
Ta9	121.16	227.50	275.00	224.50	124.33	194.50
Ta10	129.16	231.16	268.50	219.16	0.00	169.60
Ta11	126.50	220.83	259.16	213.50	119.00	187.80
Ta12	149.16	228.33	282.50	229.16	127.83	203.40
Ta13	127.50	230.16	255.50	209.83	125.16	189.63
Ta14	126.50	231.50	279.16	228.50	128.50	198.83
Ta15	124.50	227.00	258.33	213.66	143.50	164.70
Ta16	130.00	225.33	267.66	219.83	0.00	168.56
Ta17	153.50	236.00	270.00	218.50	0.00	175.60
Ta18	155.83	234.83	262.00	218.00	0.00	174.13
Ta19	129.00	230.66	278.83	230.16	141.16	201.96
Ta20	126.83	228.50	255.50	224.16	129.16	192.83
Mean B	130.14	229.42	266.06	220.58	58.12	
Effect		**CD (0.05)**				
Factor(A) (Colony diameter)		**0.67**				
Factor(B) (Mycelial Fresh Weight)		**0.34**				
Factor (A X B)		**1.51**				

The interaction of temperatures with the fresh weight of mycelium revealed that maximum (155.83 mg) mycelial fresh weight was recorded by Ta18 at a temperature of 10◦C, which was significantly different from other isolates. Isolates Ta2 showed maximum (240.50 mg) fresh weight at 20◦C, which was significantly different from other isolates. Significantly maximum mycelial fresh weight (282.50 mg) was recorded at 30˚C by isolate Ta12. Isolate Ta 7 and Ta 19 showed maximum (230.16 mg) fresh weight at 35^0C, followed by Ta 5 (230.00 mg) at the same temperature and these were not differing significantly among themselves. Significantly maximum (143.50 mg) fresh weight was recorded at 40^0C by Isolate Ta15

These results are concurrent with the findings of Duzniewska (2004) who investigated the effect of different temperature on mycelial growth and spore germination of *Trichoderma* spp. and found that temperature between 5 to 15˚C inhibited mycelium growth and sporulation. Singh and Kumar (2009) conducted a similar study to find optimum temperature for growth of *Trichoderma*. They found that most *Trichoderma* isolates grew best at temperature range of 25˚C to 30˚C. Similar results were also obtained by other workers. Srivastava *et al.,* (2014a) reported that excellent mycelial growth of *Trichoderma harzianum* was at 30˚C followed by 25˚C. Mishra and Khan (2015) reported that *Trichoderma viride* could grow at a wide range of temperature ranging between 20 to 30˚C. However 28˚C was found best for the growth and sporulation of the fungus. Domingues *et al.*, (2016) reported that at 7^0C, the mycelial growth of all the isolates was inhibited. The increase in mycelial growth was proportional to the increase in temperature ranging from 12 to 27˚C and thereafter decreased until 37˚C and it was completely inhibited at 42˚C. Mishra *et al.*, (2018) assessed the growth of *Trichoderma* at five different temperatures ranging from 15 to 35˚C. They found that growth of all the isolates increased up to 25˚C temperature, thereafter start decreasing. The maximum mycelial growth was recorded at 25˚C. The growth of *Trichoderma* isolates was significantly affected either by increase or decrease in the temperature.

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