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Bio-efficacy of *Trichoderma* isolates against *Colletotrichum geolosporiodes* in onion

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Abstract

Twister blight of onion caused by *Colletotrichum geolosporiodes* is one of the most severe disease in onion-growing areas which results in yield loss up to 50-90% per cent, especially during the rabi season when climatic conditions are more favourable for the pathogen. Chemical management has been proven to be difficult, expensive, and detrimental to the environment for the management of *C. geolosporiodes*. Hence, biological management strategies is one of the best, since it is efficient against a wide range of soil-borne pathogens.*Trichoderma* spp., owing to their complex interactions with plant pathogens, which include parasitizing, secreting antibiotics, and fighting for space and resources were regarded as best antagonists against plant pathogens. In this line, ten different isolates of *Trichoderma* were isolated from rhizosphere soil in different onion growing areas of Tamil Nadu. They were screened against *C. geolosporiodes* under *in-vitro* conditions. Among the isolates, the isolate Tr-2 showed the highest inhibition percentage in Dual culture (82.00%) and poisoned food assay at (87.78%). Least inhibition of pathogen was recorded by the isolate Tr-3 (67.78%). Tr- 2 has recorded the highest inhibition percentage(100%) at 30% concentration of culture filtrate and Tr-4 recorded the least inhibition percentage with (77.62%) at 40% of concentration.

Key word : Bioefficacy, *Trichoderma, Colletotrichum geolosporiodes*. Twister blight.

Onion (*Allium cepa* L.) belonging to the Alliaceae family and often referred to as the "queen of the kitchen" is one of the popular bulbous vegetable crops grown throughout India. It is high in sulphur, potassium, vitamin B6, iron, vitamin C, anthocyanin, and antioxidants, especially quercetin (Cramer 2000). Twister blight, purple blotch, black mould and basal rot are the major diseases that reduce onion yield and productivity. Among these one of the pandemic diseases that has resulted in severe crop loss is onion

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twister blight[15].

Twister disease of onion (*Allium cepa* L.) was first discovered in 1969 at Zaria, Nigeria[6]. Kuruppu[11] reported twister disease on shallot onions, *Allium cepa* var. *ascalonicum*, that caused losses up to 30% in the Kalpitiya Peninsula in Sri Lanka's North Western Province. In 2005-06, this disease wreaked havoc on red onions in several Indonesian onion producing areas[20]. Hegde *et al*.[10] reported that twisting of onion leaves, stems, and bulbs, resulted in the severe production losses varying from 40-60 per cent. Curling, twisting, and chlorosis of the leaves were documented as symptoms. Disease incidence was lower in the kharif season than in the rabi season due to the congenial weather conditions during rabi season which coincides with rainfall. Favourable climatic conditions for pathogen development such as high relative humidity (80 to 90%) and optimum temperature (24±2°C), results in severe incidence of twister blight disease in onion[16]. Various fungicides have been employed earlier to control the twister blight disease of onion, however they havent delivered satisfactory results in the control of this disease due to the creation of new pathogen races. Extensive use of chemicals also leads to development of resistance in the fungal communities and also significantly affects the health of farmers due to drift hazards. So, there is a urgent need to develop an alternate strategy to manage this disease which will be safer for farming community and more environmentally friendly. Accordingly, biological management becomes the need for contemporary agriculture. *Trichoderma* sp. are possible biocontrol agents for a number of soil-borne phytopathogens[9] as they live freely in soil, root habitat and are extremely interacting with pathogens. Galindez *et al*.[8], studied the impacts of *Trichoderma* sp. as a possible biocontrol agent against *C. gloeosporioides*, an onion pathogen. Accordingly, the present study was taken up with an objective to explore the potential of native *Trichoderma* strains from different regions of Tamil Nadu against the twister blight pathogen *C. gloeosporioides* under *in vitro* conditions.

Isolation of onion twister pathogen :

Onion twister disease affected plants were identified based on symptoms, and were collected in separate polythene bags from different onion growing regions of Tamil Nadu. The plant samples were brought to the lab and rinsed in tap water to remove debris that adhere to them. A sterile blade was used to cut the samples into small pieces in such a way that the small piece containing infected portion along with healthy portion.These pieces are surface sterilised for 20 seconds in 1% sodium hypochlorite. Then they're rinsed three times using sterile distilled water to remove the surface sterilising agent, then dried in the laminar airflow chamber's on sterilised filter paper. These pieces were transferred aseptically to Petri-plates containing potato dextrose agar medium (PDA). These plates are incubated for 3-4 days at 25±2!, purified and the resulting pure cultures are sub cultured in PDA slants and kept at 4! for future studies.

Isolation of fungal antagonist Trichoderma:

Rhizosphere soil samples were collected from various onion growing areas of Tamil Nadu. These samples were serially diluted for the isolation of *Trichoderma* spp.

using *Trichoderma* specific medium (TSM)[7]. These cultures were stored at 4°C in slants for further studies. The cultural and morphological characters such as colony mophology, colony colour, colony diameter, conidiophores, conidial colour and size of the conidia were determined on PDA.

Effect of Trichoderma spp. against Colletotrichum spp. by Dual culture :

Through a dual culture approach, the antagonistic capability of *Trichoderma* spp. was assessed against a virulent *C. gloeosporioides* in *in-vitro* conditions,[5]. 5 mm disc of seven-day-old culture of pathogen and antagonistic fungi were inoculated on PDA plates on either ends. Only test fungus was transferred to the control plates. and incubated at 25±2ºC. Three replications were maintained. After incubation, radial growth was observed (mm) and Percentage inhibition of pathogen growth was calculated using the formula given by Pandey *et al*.[15].

Percent inhibition (I) = C-T/C × 100

Where, I = Per cent inhibition in growth of test pathogen

C = Radial growth in control

T = Radial growth in treatment

Preparation of the culture filtrates of Trichoderma :

Based on the antagonist activity in dual culture technique, five *Trichoderma* isolates were selected and cultured in Erlenmeyer flasks with 50 ml of sterilised potato dextrose broth for 15 days in a shaker incubator at 25±2ºC. The culture filtrate was obtained by filtering the resultant broth and filtered through a bacteriological filter in vacuum.

Poisoned food technique :

PDA media amended with culture filtrate at different concentrations 5, 10, 15, and 20%. 15 ml of the altered medium were transferred to sterilised Petri dishes and allowed to solidfy. The plate without antagonist culture filtrate serve as control. Three replications were maintained and were incubated at 25±2ºC. After 7 days of incubation, the diameter of *C. gloeosporioides* (mm) was measured and the percent inhibition was calculated as per the formula given by Vincent[19].

Percent Inhibition = C-T/C × 100

Where,

C = Radial Growth in Control

T = Radial Growth in treatment

Cultural and morphological characteristics of ten distinct *Trichoderma* isolates were isolated from rhizospheric soil samples which was collected from various onion growing regions. The results revealed that all the isolates entirely covered the petriplates by the end of seventh day. Colony colour of different isolates varied from whitish green, dull green, light green, dark green, pale white and pale green in colour. Colony morphology was circular and growth pattern varied from powdery to cottony was observed.

Microscopic observations revealed that conidiophores were highly branched to regular or irregular pattern, and some species were found to be moderately branched (Tr-1,4).

Table-1. Morphological and cultural characteristics of different *Trichoderma* spp.

S. No	Isolate number	Colony colour	Colony morphology	Colony in diameter (mm)	Conidio-phore	Conidia	Conidial colour	Conidial size (µm)
1.	Tr-1	White to pale green	Circular as a ring, cottony	87.12	Moderately branched, Regular	Globose	Pale green	2.57 x 3.42
2.	Tr -2	Dark green	Circular at the centre, powdery and spread over	90.00	Highly branched, Regular	Ellipsoi-dal	Dark green	3.23 x 4.75
3.	Tr -3	Dark green	Thick, highly aerial	86.43	Highly branched	Sub globose to obovoid	Dark green	2.80 x 3.48
4.	Tr -4	Light Green to bright green	Circular as a ring, cottony	89.00	Moderately branched, Regular	Globose	Light Green	2.43 x 3.16
5.	Tr -5	Dark green	Circular, solid, aerial	88.54	Few lateral branches at apex, Irregular	Obovoid	Dark green	1.02 x 3.12
6.	Tr -6	Whitish green to dull green	Circular at the centre, powdery and spread over	86.08	Highly branched, Regular	Sub globose to obovoid	Pale green	1.16 x 3.54
7.	Tr -7	Light Green to bright green	powdery, circular	88.56	Regularly Branched	Ellipsoi-dal	Light Green	2.23 x 3.16
8.	Tr -8	Off whitish green to dull green	Circular at the centre, powdery and spread over	83.23	Rarely branched, Regular	Globose to ellip-soidal	dull-green	1.76 x 2.45
9.	Tr -9	Light Green to bright green	Cottony, pustule	89.10	Few lateral branches at apex, Irregular	Obovoid to ellipsoid	bright green	2.05 x 3.14
10.	Tr -10	Pale green	powdery, circular	85.78	Highly branched	Narrow ellipsoidal	Pale green	1.98 x 2.76

Table-2. The effect of antagonist *Trichoderma spp.* in Dual culture technique against *C. gloeosporioides*

S.no	Isolate number	Mycelia growth (mm)		Percent Inhibition over control
		Trichoderma spp.	*Colletotrichum. Gloeosporioides*	
1.	Tr-1	63.1	26.9	70.11 (56.85)
2.	Tr -2	73.7	16.2	82.00* (64.89)
3.	Tr -3	60.9	29.0	67.78 (55.48)
4.	Tr -4	65.6	24.4	72.89 (58.62)
5.	Tr -5	69.5	20.4	77.33 (61.56)
6.	Tr -6	61.4	28.5	68.33 (55.75)
7.	Tr -7	67.0	22.9	74.56 (59.71)
8.	Tr -8	61.2	28.7	68.11 (55.61)
9.	Tr -9	71.7	18.1	79.89 (63.35)
10.	Tr -10	63.0	26.8	70.22 (56.93)
11.	Control	90	-	-
	C.D Value	2.11	-	-
	SE(m)	0.71	-	-

Table-3. Effect of culture filtrate of promising fungal antagonist *Trichoderma* spp. against *C. gloeosporioides* (C 2)

Sl. No	Isolate Number	Radial growth (mm)				Per cent inhibition over control			
		Conc. of culture filtrate				Con. of culture filtrate			
		10%	20%	30%	40%	10%	20%	30%	40%
1	Tr-2	22.45	10.12	0.00	0.00	75.11	88.76	100.00	100.00
2	Tr-4	46.04	37.24	28.90	20.14	40.33	58.67	67.89	77.62
3	Tr-5	42.33	32.50	22.32	12.13	45.56	63.89	75.22	86.52
4	Tr-7	44.82	35.46	24.71	15.86	50.22	60.44	72.55	82.38
5	Tr-9	39.11	27.62	17.41	03.15	47.22	69.33	80.67	96.50
6	Control	90.00	90.00	90.00	90.00	—	—	—	—
	C.D Value	3.24	2.03	1.36	1.23	—	—	—	—
	SE(m)	1.041	0.64	0.43	0.39	—	—	—	—

Conidia are dark or pale green in colour and were globose, ellipsoidal, and obovoid in nature. The length of the conidia ranged from 1.16 to 2.80 µm and breadth ranged from 2.45 to 4.75 µm respectively. Similar findings were reported by Shah *et al*.,[18].

The effect of antagonist *Trichoderma* species against the radial growth of test fungus *in vitro* (*C. gloeosporioides*) were presented in the (Table-2). Twister blight causing pathogen *C. gloeosporioides* isolate is deposited in the NCBI Gene bank with the Accession number **ON619419.** Among the isolates Tr- 2 has recorded the highest inhibition percentage of 82.00% and is followed by Tr-9 with 79.89%. And Tr -3 recorded the least inhibition percentage with 67.11%. Among them five virulent *Trichoderma* spp. were chosen to investigate their antagonistic capability against *C. gloeosporioides* using the poison food technique. When compared to control, all five isolates strongly suppressed Colletotrichum mycelial development. Similar findings were reported by Patil[16]. Similar experiment was earlier performed by Abhishek Mishra *et al*., (2017) and found that *Trichoderma harzianum* isolates CA-06 and CA-07 showed highest inhibition of mycelial growth at all concentrations against the tested pathogens.

Effect of culture filtrate of five best isolates *Trichoderma* spp. against *C. gloeosporioides* was presented in (table-3). Among the isolates Tr- 2 has recorded the highest inhibition percentage(100%) at 30% concentration of culture filtrate and is followed by Tr-9 with (96.50%) at 40% concentration. And Tr-4 recorded the least inhibition percentage with (77.62%) at 40% of concentration. Ahsanur Rahman *et al*.,[2] have studied the effect of non-volatile compounds of different *Trichoderma* species on the mycelial growth.

In Dual culture, Tr-2 showed the highest inhibition and least inhibition is seen in Tr-3 (Table-3)[17]. Data presented in table-3 revealed that among the isolates Tr-2 showed the maximum inhibition percentage and Tr-4 isolate showed the least inhibition percentage in culture filtrate when compared to the control. The results suggest that the action by each Trichoderma strain is dependent on the species and the kind of released compounds, such as antibiotics or volatile compounds that can be spread through the medium limiting the normal growth. The presence of lytic enzymes followed by mycoparasitism it is the most feasible mechanism used by Trichoderma strains. Depending on the Trichoderma system nature, it is possible to produce 5-7 individual chitinases and 1 to 7 b-1,3-glucanases and these enzymes can act complementarily[12,17]. Galindez *et al*.[8] studied the impacts of *Trichoderma* sp. as a possible biocontrol agent against *C. gloeosporioides*, an onion pathogen.

References :

1. Agrios, G. N. (1997). Plant Pathology. 4th Ed. Academic Press, San Diego and London.
2. Ahsanur Rahman M., M Anowar Razvy, and M. Alam (2013). *International Journal of Microbiology and Mycology*. *1*(1): 7-22.
3. Alberto R.T., (2014). *Plant Pathology & Quarantine 4* (1): 23–31.
4. Consolo, V., C. Monaco, C. Cordo, and G. Salerno, (2012). *World J. Microbiol.*

Biotechnol. 28(4): 1389-1398.
5. Dennis C., Webster J. (1971). *T. Brit. Mycol. Soc, 57:* 25-39.
6. Ebenebe, A. C. (1980). *Pl. Dis. 64 :* 1030-1032.
7. Elad Y. and I. Chet (1983) *Phytoparasite. 11:* 55-58.
8. Galindez, H.J.A., L. L. M. A. Lopez, S.P. Kalaw, K. G. D. Waing and J. L. Galindez, (2017). *Advances in Environmental Biology. 11*(6): 62-68.
9. Hassan M.M., A Gaber and EI. El-Hallous (2014). *Wulfenia Journal, 21:* 80-96.
10. Hegde, G. M., G. R. Rajkumar, and Jaware Gouda (2012). *Ext. Bull. Uni. Agric. Sci. Dharwad* (India).
11. Kuruppu, P. U., (1998). *Thai Phytopath. 8* (3-4): 97-104.
12. Markovich, N., and G. Kononova (2003). *Appl. Biochem. Microbiol. 39* (4) : 341e351.
13. Nargund, V. B., H. Gurudath, G. V. Nayak, V. I. Benagi, P. Suresh, P. R. Dharmatti, and S. Ravichandran (2013). Management of twister disease in sweet onion -a strategy for livelihood improvement and welfare of mankind. Proc. *Int. Symp. Human health effects of fruits and vegetables, Univ. Agril. Sci., Dharwad,* 205.
14. Nischwitz C., D. Langston, Jr., and H. F. Sanders, (2008). *The American Phyto-pathological Society. J., 92*(6).
15. Panday, S. S., R. T. Alberto, and M. S. Labe, (2012). *Pl. Path. and Quarantine. 2*(2): 168–177.
16. Patil Suresh V.B., K. Nargund, Hariprasad, Gurudatth Hegde, S. Lingaraju and V. I. Benagi (2018). *Int. J. Curr. Microbiol. App. Sci. 7*(12): 3644-3657.
17. Reynaldo De la Cruz-Quiroz, Sevastianos Roussos, Rau´l Rodríguez-Herrera, Daniel Hernandez-Castillo, Cristobal N. Aguilar, (2018). *Karbala International Journal of Modern Science 4:* 237-243.
18. Shah S., S. Nasreen, and P.A. Sheikh (2012). *Research Journal of Microbiology, 7:* 139-144. 18.
19. Vincent, J.M. (1927). *Nature, 59:* 850.
20. Wiyono, S. (2007). Climate change and pests and diseases explosion. Pro. Biodiversity in the middle of global warming. Kheti Foundation, Jakarta, Indonesia.