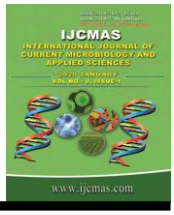
Int.J.Curr.Microbiol.App.Sci (2020) *9*(1): 820-825

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 1 (2020)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2020.901.088

Evaluation of the Efficacy of Culture Filtrate of *Trichoderma* Isolates against *Colletotrichum graminicola* causing Anthracnose of Sorghum

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Keywords

Trichoderma, Colletotrichum graminicola, Anthracnose

Article Info

Accepted:
15 December 2019
Available Online:
20January 2020

A B S T R A C T

In the present study, the efficiency of 15 day old culture filtrate (nonvolatile compound) of 20 *Trichoderma* isolates was evaluated at 10% and 25% concentration using poison food technique against mycelial growth *C. graminicola*. All the 19 isolates of *Trichoderma asperellum* and one isolate of *Trichoderma harzianum* were significantly reduced the mycelia growth of *C. graminicola* over control at 10% and 25% concentration. The maximum reduction of mycelia growth of *C. graminicola* at 10% and 25% of T4 isolate which recorded 68.18% and 80.68% inhibition radial growth respectively.

Introduction

The biocontrol agent like *Trichoderma* sps has the potential to manage the plant pathogens (Elad and Freeman, 2002). Trichodex (*Trichoderma harzianum* isolate T39) has the first biocontrol agent that registered and commercialized and effectively managed *Sclerotinia sclerotiorum*, *Cladosporium fulvum* and *Botrytis cinera* in cucumber, vine yards and Tomato (Elad, 2000).The metabolites are discharged in the metabolic process by fungal bioagents and these metabolites are products of some aromatics, phenols, terpenoids, cyclic peptides, plant growth regulators and amino acids (Madhosing, 1995). Antifungal metabolites produced by *Trichoderma* isolates (Viterbo *et al*., 2002) and these isolates of *Trichoderma* produce lytic enzyme like β 1-3 glucanase and chitinases (El Katary *et al*., 2001) and these enzymes acts on a fungal cell wall during antagonistic process of *Trichoderma* sp. and able to manage plant pathogens. Therefore the present investigation was carried out to evaluate the efficacy of

cultural filtrate of *Trichoderma* isolates on *Colletotrichum graminicola* to manage anthracnose of sorghum under *in vitro* condition.

Materials and Methods

The production of non-volatile diffusible metabolites by the *Trichoderma* isolates against the *Colletotrichum graminicola* was observed using the method given by (Dennis and Webster, 1971). The 5mm diameter mycelial disc of each *Trichoderma* isolate cut with flame sterilized cork borer (5mm) from seven days old actively grown culture was centrally placed and inoculated on the surface of 100 ml Potato dextrose broth (PDB) in a 250 ml conical flask and incubated at 25 ± 1° C for 15 days on a rotary shaker set at 100 rpm. Uninoculated PD broth flasks were maintained as control. After 15 days the liquid culture filtrate of each isolates of *Trichoderma* was obtained by filtering through Whatman filter paper and mycelial mats was removed. After that the cultural filtrate were again sterilized by passing through 0.2 µm pore biological membrane filter. Poisoned Food Technique method (Nene and Thapliyal, 1979) was used to check the bio-efficacy of culture filtrates (500µl/ml) of different *Trichoderma* isolates against *C. graminicola* under *in vitro* condition. To prevent bacterial contamination, streptomycin sulphate @ 50 ppm was added to the potato dextrose medium. Efficacy of non volatile diffusible metabolites of different *Trichoderma* isolates *against C.graminicola was* assessed at 10 and 25 per cent (v/v) concentrations by diluting with required quantity of molten PDA. Pathogen inoculated PDA without culture filtrate of *Trichoderma* isolates were maintained as control. 5 mm mycelial plugs of the *C. graminicola* were inoculated and placed at the centre of PDA plates with culture filtrate of different *Trichoderma* isolates were incubated at 28°C for 7 days. Three replicates were maintained for each treatment. Observations on per cent inhibition of the mycelial growth were recorded after 7 days of inoculation of pathogen mycelial disc.

Statistical analysis

Data obtained on various traits under laboratory experiments were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) using Statistical Product and Service Solution (SPSS) version16.0 software Developed by SPSS Inc., now IBM SPSS. All results were expressed at $P< 0.05$ to compare difference among the treatment means.

Results and Discussion

Evaluation of efficacy of culture filtrate of *Trichoderma* isolates on the mycelia growth of *Colletotrichum graminicola*

In the present study, the efficiency of 15 day old culture filtrate (nonvolatile compound) of 20 *Trichoderma* isolates was evaluated at 10% and 25% concentration using poison food technique against mycelial growth *C. graminicola*. Observations were recorded on percent inhibition of mycelia radial growth and statistically analyzed by DMRT.

The results revealed that all the isolates of *Trichoderma* showed a significant reduction of mycelial growth of test pathogen at 10% and 25% concentration.

The data on percent inhibition of radial growth of *C. graminicola* by 10% and 25% culture filtrate of *Trichoderma* isolates in presented in Table 1; Figure 1 and Plate 1

Table.1 Antifungal activity of culture filtrates of the antagonist *Trichoderma* isolates against *Colletotrichum graminicola*

Sl. No.	Isolates	Percent inhibition of mycelial growth	
		10% concentration	25% concentration
1.	T1	50.68±0.41^{g} (45.39)	70.08±1.00^{e} (56.84)
2.	T2	25.76±1.00^{j} (30.49)	40.91±0.66^{g} ((39.76)
3.	T3	61.37±0.66^{b} (51.56)	80.30±1.00^{ab} ((63.66)
4.	T4	68.18±0.66^{a} (55.66)	80.68±0.66^{a} (63.93)
5.	T5	46.97±1.00^{h} (43.26)	37.88±0.38^{h} ((37.98)
6.	T6	53.65±0.79^{ef} (47.09)	75.38±1.00^{d} (60.26)
7.	T7	12.50±0.66^{l} (20.69)	13.64±0.66^{k} (21.66)
8.	T8	14.02±0.76^{l} (21.97)	13.64±0.66^{k} (21.66)
9.	T9	6.44±1.00^{n} (14.60)	6.82±0.66^{l} (15.10)
10.	T10	58.58±0.61^{bc} (49.94)	78.88±0.51^{abc} (62.64)
11.	T11	46.59±1.31^{h} (43.04)	34.85±1.00^{i} (36.17)
12.	T12	53.79±1.00^{ef} (47.17)	71.59±0.66^{e} (57.79)
13.	T13	7.58±1.37^{lm} (15.85)	8.33±1.00^{l} (16.72)
14.	T14	25.76±1.00^{j} (30.49)	57.97±0.66^{f} (49.57)
15.	T15	50.96±1.17^{fg} (45.55)	78.03±1.00^{bc} (62.06)
16.	T16	56.81±2.15^{cd} (48.91)	80.86±1.06^{a} (64.06)
17.	T17	9.47±1.37^{m} (17.82)	9.09±0.66^{l} (17.52)
18.	T18	17.42±1.00^{mn} (24.65)	22.73±0.66^{j} (28.46)
19.	T19	55.47±1.26^{de} (48.14)	76.89±1.00^{cd} (61.28)
20.	T20	39.77±0.66^{i} (39.09)	40.15±1.00^{g} (39.31)
	Control	-	-
	C.D at 1%	2.88 (1.87)	2.36 (1.66)
	SE(m)	1.00 (1.28)	0.82 (1.01)
	C.V.	5.17 (3.05)	3.32 (2.29)

Fig.1 Efficacy of culture filtrates of the antagonist *Trichoderma* isolates against *Colletotrichum graminicola*

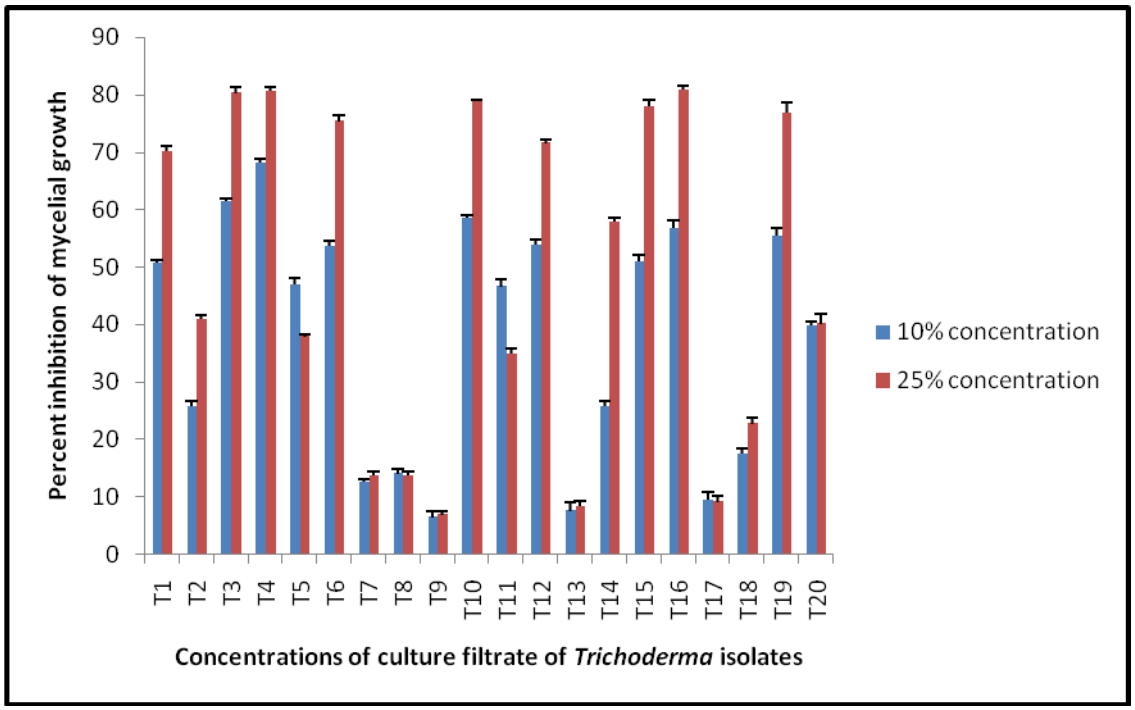
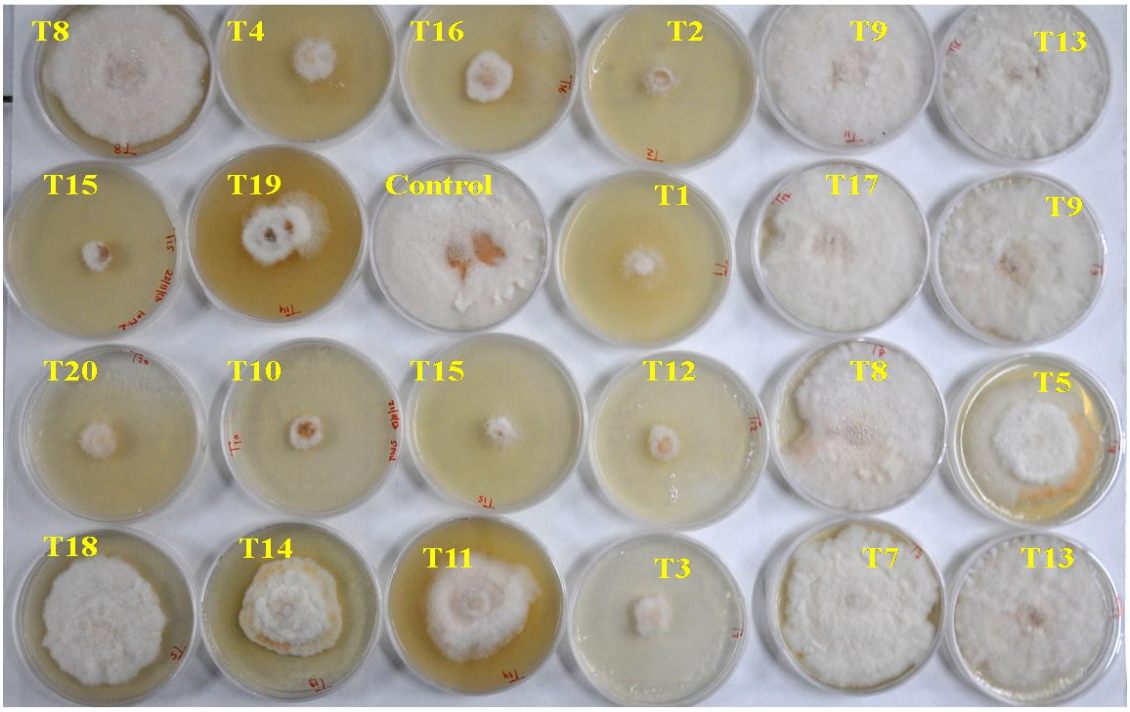


Plate.1 Antifungal activity of culture filtrates of the antagonist *Trichoderma* isolates against *C. graminicola*



The maximum reduction of mycelial growth of *C. graminicola* was observed on 10% culture filtrate of T4 isolate which recorded 68.18% inhibition radial growth followed by T3 isolate (61.37% inhibition).Least reduction was observed on T9 isolate which recorded 6.44% inhibition of mycelia growth of *C.graminicola*.

The maximum reduction of mycelia growth of the pathogen was observed on 25% culture filtrate of T4 isolate which recorded 80.68% inhibition of mycelia radial growth followed by T3 which showed 80.30% inhibition radial growth which is statistically at par with T4 isolate.

The results showed the similarity with Michereff *et al*., 1993 who reported that cultural filtrate of *T.viride, Pseudomonas fluorescens, T.harzianum* and *T.koningi* showed significant reduction of mycelial radial growth of *C. graminicola*. Iqbal *et al*., 1994 reported that cultural filtrate of 17 fungal isolates belongs from *T. harzianum* and *Acremonium* sp. showed significant reduction of mycelial radial growth of *C. falcatum*. Weindling, 1934 first reported that cultural filtrate of *Trichoderma* isolates produced toxic metabolites. Kaur *et al*., 2006 evaluated the efficacy of cultural filtrate of *T.viride* and showed a significant reduction of mycelial growth and spore germination of *C. capsici*. Padder *et al*., 2011 evaluated the efficacy of culture filtrate of *T. harzianum, Trichoderma viride, Gliocladium virens* and *Trichoderma hamatum* against *Colletotrichum lindemuthianum* and found that that cultural filtrate at 25% and 50% of *T. viride* showed a significant reduction of mycelial growth and spore germination of the pathogen.

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How to cite this article:

Nazia Manzar and Yogendra Singh. 2020. Evaluation of the Efficacy of Culture Filtrate of *Trichoderma* Isolates against *Colletotrichum graminicola* causing Anthracnose of Sorghum. *Int.J.Curr.Microbiol.App.Sci.* 9(01): 820-825. doi: https://doi.org/10.20546/ijcmas.2020.901.088