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In vitro Bioefficacy of Fungal Endophytes against *Rhizoctonia bataticola* (Taub). Butler causing Dry Root Rot of Chickpea

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ABSTRACT: Dry root rot of chickpea is an emerging disease posing threat to chickpea cultivation around the world. It is caused by the soil borne pathogen *Rhizoctonia bataticola*. The current study was conducted to investigate the potential of employing endophytic microorganisms that are antagonistic to *R. bataticola* to manage dry root rot. A total of 20 fungal endophytes were isolated from healthy chickpea plant (roots, stem and leaves). The isolated fungal endophytes were tested against *R. bataticola* by dual culture technique under *in vitro* conditions. Among all *Trichoderma asperellum* (CLEF18) exhibited the maximum inhibition of test pathogen, followed by *Aspergillus fumigatus* (CREF4) and *Acremonium* sp. (CREF1). The least mycelial inhibition of *R. bataticola* was observed in *Colletotrichum* sp. (CLEF6), followed by *Rhizoctonia* sp. (CREF17). These endophytes thus; could be efficient biological control agent in sustainable crop production and offer unique opportunity for crop protection and biological control.

Keywords: Dry root rot, Endophytes, Inhibition, *Rhizoctonia bataticola*, *in vitro.*

INTRODUCTION

Chickpea is one of the important pulse crop grown in over 50 countries of Asia, Africa, America and Oceania in rain-fed environments (Sharma *et al*., 2015). It ranks third among the food legumes after beans and pea. Vavilov (1926) was the first to recognize the near eastern, central Asian, Indian and mediterranean regions as the probable centers of origin for Chickpea. India contributes to a major share of the World's chickpea area (70 %) and production (67 %) and to be the largest chickpea producing nation (Dixit *et al*., 2019). Chickpea is affected by several fungal pathogens among that dry root rot is one of the most destructive soil borne disease of chickpea and it causes a serious damage to the crop. It caused 10-25 per cent crop losses in major chickpea growing states of India (Lakhran and Ahir 2018). Also in Marathwada region of Maharashtra state average 16.12 per cent incidence was found (Kadam *et al*., 2018). Since the disease is soil borme, it is difficult to manage the disease either through crop rotation or application of chemicals. Hence, eco-friendly management with native beneficial organisms is needed. Among beneficial microorganisms, several endophytes are used as bio fertilizers and bio fungicides in agricultural soils to enhance crop growth and to control a wide range of soil-borne fungal plant pathogens. It is also known that endophytes defend plants from diseases by producing antibiosis, competing with other organisms and inducing systemic resistance. Therefore, the present investigation was planned to manage of chickpea dry root rot causing pathogen *in vitro* by dual culture technique and to identify the new effective fungal endophytes, which derive maximum benefit to the farmers.

MATERIAL AND METHODS

A total of 20 endophytic fungal isolates collected from different locations of Marathwada region during the survey were evaluated *in vitro* against *R. bataticola*, applying Dual Culture Technique (Dennis and Webster, 1971). Seven days old cultures of the endophytic fungal isolates and test pathogen grown on respective culture media were used for the study. One each culture disc (5 mm) of the test pathogen and the test fungal endophyte (cut out with sterilized cork borer) were placed at equidistance and exactly opposite to each other, on autoclaved and solidified PDA medium in Petri plates. For each test endophytic fungal isolates, three PDA plates were inoculated and all the treatments replicated thrice. The PDA plates inoculated (in the centre) only with pure culture disc of the test pathogen were maintained as untreated control. The experiment was conducted by using completely randomized design (CRD) manner with 21 treatments replicated thrice.

Treatment details:

Tr. No.	Treatments	Tr. No.	Treatments
1.	*Acremonium* sp. (CREF1)	11.	*Nigrospora sphaerica* (CSEF11)
2.	*Alternaria alternata* (CSEF2)	12.	*Paecilomyces lilacinus*(CLEF12)
3.	*Aspergillus flavus* (CLEF3)	13.	*Paecilomyces* sp. (CREF13)
4.	*Aspergillus fumigatus* (CREF4)	14.	*Penicillium* sp. (CLEF14)
5.	*Aspergillus niger* (CSEF5)	15.	*Phoma* sp. (CSEF15)
6.	*Colletotrichum* sp. (CLEF6)	16.	*Phomopsis* sp. (CLEF16)
7.	*Curvularia lunata* (CREF7)	17.	*Rhizoctonia* sp. (CREF17)
8.	*Fusarium oxysporium* (CSEF8)	18.	*Trichoderma asperellum* (CLEF18)
9.	*Fusarium verticiloides* (CLEF9)	19.	*Trichoderma virens*(CREF19)
10.	*Macrophomina phaseolina* (CREF10)	20.	*Verticillium leccani* **(**CLEF20)
		21.	Control (untreated)

Observations on linear colony growth / diameter (mm) of the test pathogen and the test endophytic fungal isolate was recorded at an interval of 24 hrs of incubation and continued up to seven days or till the untreated control plates were fully covered with mycelial growth of the test pathogen. Based on cumulative data, per cent mycelial growth inhibition of the test pathogen with the test fungal endophyte, over untreated control was calculated by applying following formula (Arora and Upadhay 1978).

$$\text{Per cent Growth Inhibition} = \frac{\text{Colony growth in control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

RESULTS

Data shown in (Table 1, Plate I and Fig. 1) revealed that in dual culture test, all 20 fungal isolates inhibited the mycelial growth of *R. bataticola* with per cent growth inhibition was recorded from 10.37 to 90.30 per cent. Among all the twenty endophytic fungal isolates tested, significant per cent growth inhibition of *R. bataticola* was observed in eight isolates and it was ranged from 78.12 to 90.30 per cent with the radial growth ranged from 10.69 to 22.08 mm.

Of these eight fungal isolates, *Trichoderma asperellum* (CLEF18), *Aspergillus fumigatus* (CREF4), *Acremonium* sp. (CREF1), *Aspergillus niger* (CSEF5), *Verticillium leccani* **(**CLEF20), *Fusarium oxysporium* (CSEF8), *Paecilomyces lilacinus* (CLEF12) and *Penicillium* sp. (CLEF14) were found to exhibit antagonistic effect against *R. bataticola*.

Table 1: Effect of endophytic fungal isolates on radial growth of *R. bataticola in vitro* using dual culture technique.

Sr. No.	Fungal endophytic Isolate	Colony diameter of test pathogen*(mm)	% inhibition
1.	*Acremonium* sp. (CREF1)	14.01	86.21 (68.20)
2.	*Alternaria alternata* (CSEF2)	40.15	55.38 (48.08)
3.	*Aspergillus flavus* (CLEF3)	28.67	75.90 (60.59)
4.	*Aspergillus fumigatus* (CREF4)	12.36	88.15 (69.86)
5.	*Aspergillus niger* (CSEF5)	15.70	85.66 (67.74)
6.	*Colletotrichum* sp. (CLEF6)	80.66	10.37 (18.78)
7.	*Curvularia lunata* (CREF7)	36.45	59.50 (50.47)
8.	*Fusarium oxysporium* (CSEF8)	19.66	81.48 (64.51)
9.	*Fusarium verticiloides* (CLEF9)	45.36	49.60 (44.77)
10.	*Macrophomina phaseolina* (CREF10)	35.39	60.67 (51.16)
11.	*Nigrospora sphaerica* (CSEF11)	53.74	40.28 (39.39)
12.	*Paecilomyces lilacinus* (CLEF12)	20.80	80.33 (63.67)
13.	*Paecilomyces* sp. (CREF13)	35.49	60.56 (51.09)
14.	*Penicillium* sp. (CLEF14)	22.08	78.12 (62.11)
15.	*Phoma* sp. (CSEF15)	73.75	18.05 (25.14)
16.	*Phomopsis* sp. (CLEF16)	69.84	22.40 (28.24)
17.	*Rhizoctonia* sp. (CREF17)	75.56	16.04 (23.60)
18.	*Trichoderma asperellum* (CLEF18)	10.69	90.30 (71.85)
19.	*Trichoderma virens* (CREF19)	32.16	64.26 (53.28)
20.	*Verticillium leccani* **(**CLEF20)	17.86	83.37 (65.93)
21.	Control (untreated)	90.00	00.00 (00.00)
SE(m)±		**0.74**	**0.76**
C.D. (P=0.01)		**2.13**	**2.19**
C.V.		**3.56**	**2.20**

*: Mean of three replications, Figures in parentheses are arcsine transformed values

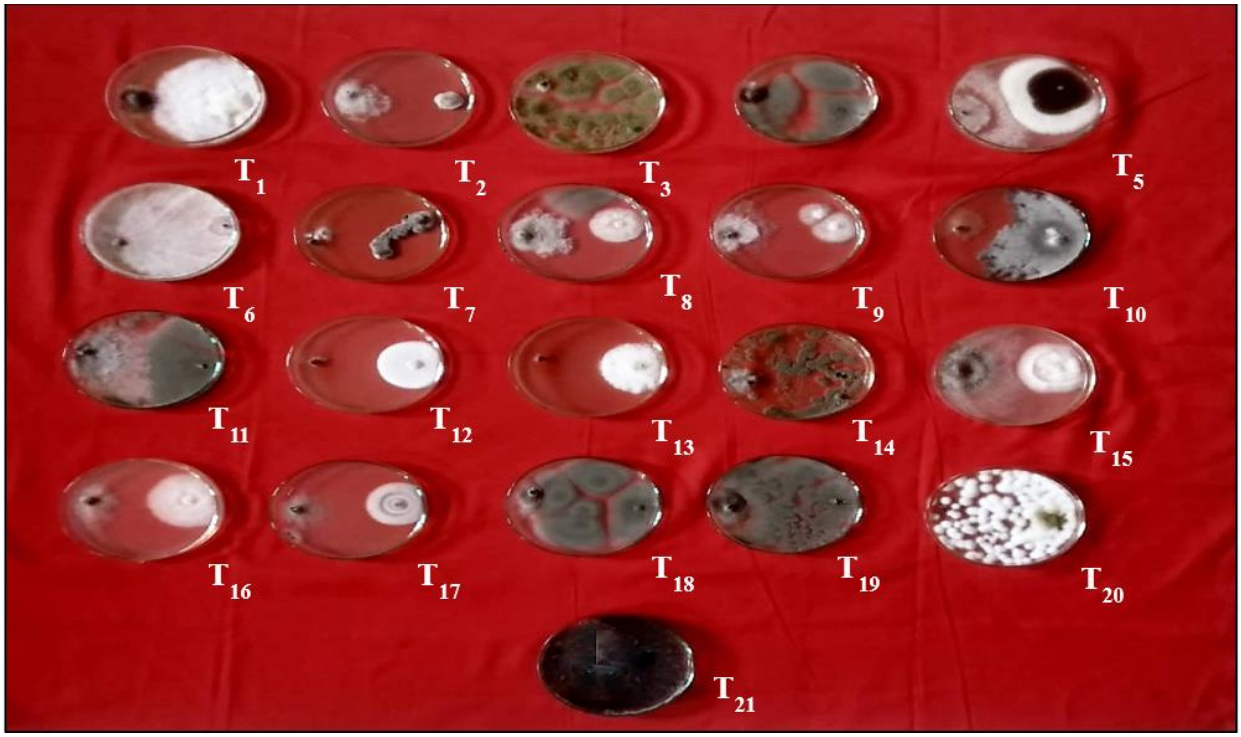

PLATE I. *In vitro* evaluation of the fungal endophytes against *Rhizoctonia bataticola* (Taub). Butler causing dry root rot of chickpea.

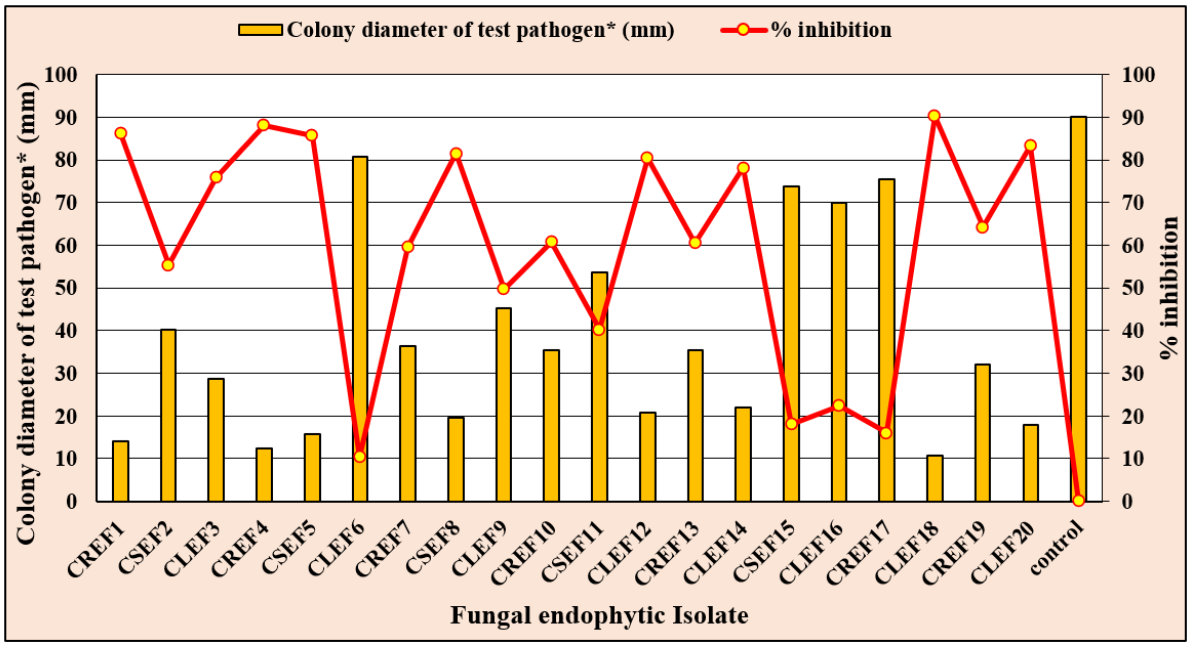

Fig. 1. *In vitro* evluation of the fungal endophytes against *Rhizoctonia bataticola* (Taub). Butler causing dry root rot of chickpea.

Among all the eight fungal endophytic isolates *T. asperellum* (CLEF18) was proved most effective with maximum inhibition (90.30%) of *R. bataticola* with radial growth of 10.69 mm, followed by *A. fumigatus* (CREF4) with 88.15 per cent inhibition and radial growth of 12.36 mm, *Acremonium* sp. (CREF1) with 86.21 per cent inhibition and radial growth of 14.01 mm and *A. niger* (CSEF5) with 85.66 per cent inhibition and radial growth of 15.70 mm in the order of merit. These were followed by *V. leccani* **(**CLEF20) with 83.37 per cent inhibition and radial growth of 17.86 mm, *F. oxysporium* (CSEF8) with 81.48 per cent inhibition and radial growth of 19.66 mm and *P. lilacinus* (CLEF12) with 80.33 per cent inhibition and radial growth of 20.80 mm both were on par. These were followed by *Penicillium* sp. (CLEF14) with 78.12 per cent inhibition and radial growth of 22.08 mm. rest of the isolates showed <70 per cent growth inhibition of *R. bataticola*. The least antifungal activity against the test pathogen was exhibited by *Colletotrichum* sp. (CLEF6) 10.37 per cent. Based on results recorded *in vitro* dual culture test, per cent growth inhibition of *R. bataticola* was found significantly greater in isolates *viz.*, *Trichoderma asperellum* (CLEF18), *Aspergillus*

fumigatus (CREF4), *Acremonium* sp. (CREF1), *Aspergillus niger* (CSEF5), *Verticillium leccani* (CLEF20), *Fusarium oxysporium* (CSEF8), *Paecilomyces lilacinus* (CLEF12) and *Penicillium* sp. (CLEF14) and these potential isolates were selected for further studies (Plate 4.11). The difference in per cent inhibition of mycelial radial growth indicated differences in antagonistic activity among all the isolates of endophytic fungi against *R. bataticola*. The variability in antagonistic ability of endophytic fungal isolates against pathogen showed clearcut difference in results obtained. It may also due to the production of volatile or non-volatile toxic metabolites like antibiotics, aldehydes, ketones, cyanide, ethylene, organic acids and other factors like siderophores, secretion of lytic enzymes, etc. which played an indirect role in inhibition of pathogen growth.

DISCUSSION

These results of the present study are in consonance with the reports of several earlier workers. Veena *et al.* (2014) who evaluated *in vitro* efficacy of 10 endophytic isolates of *Trichoderma* sp. against *R. bataticola* causing dry root rot of chickpea and reported significantly highest mycelial growth inhibition with *Trichoderma* isolate-7 (CT7) (83.33%), followed by *Trichoderma* isolate-4 (CT4) (81.11%), *Trichoderma* isolate-10 (CT10) (74.44%), *Trichoderma* isolate-1 (CT1) (71.11%). Kadam *et al*. (2021b) also evaluated *in vitro* efficacy of fourteen fungal endophytes against *M. phaseolina* and reported that highest mycelial inhibition found in *P. lilacinus* (61.11 %), followed by *A. niger* (53.87 %), *Penicillium* sp. (51.48 %), *Phomopsis* sp. 2 (49.75 %), *C. lunata* (47.41 %), *N. sphaerica* (46.05 %), *F. oxysporum* (44.94 %), *Aspergillus* sp. (44.82 %), *Chaetomium* sp. (44.57 %), *M. phaseolina* (43.58 %), *Phomopsis* sp. 1 (42.96 %) and *A. alternata* (41.61 %). Whereas, *C. cladosporioides* (41.00 %) and *Rhizoctonia* sp. (40.86 %) were found least effective. Similarly, Rekha *et al*. (2023) also reported significantly highest mycelial growth inhibition with isolate *Trichoderma yunnanense* (IFRE 2) (88.51%), followed by *Trichoderma simmonsii* (BFRE 3) (85.55%), *Trichoderma rifaii* (IFSE 2) (66.81%), IFSE 3 (65.63%), IFRE 1 (60.74%), PFSE 3 (51.50%), PFRE 3 (42.56%), IFSE 1 (38.43%), IFSE 4 (33.70%) and BFRE 2 (25.92%).

Similar findings were also reported by Lahlali and Hijri (2010), Dalal *et al*. (2014), Zuhria *et al.* (2016), Nuraini *et al*. (2017), Talapatra *et al*. (2017), Mmbaga *et al*. (2018) and Wati *et al.* (2019).

CONCLUSIONS

The chickpea endophytic microbes *viz*., *Trichoderma asperellum* (CLEF18), *Aspergillus fumigatus* (CREF4) and *Acremonium* sp. (CREF1) showed the significant inhibition of mycelial growth of pathogen. This is a novel and preliminary research on chickpea endophytes as biocontrol agent against *Rhizoctonia bataticola*. Endophytic strains as they possible dual ability of antagonizing fungal pathogen and plant growth promotion; with the view of plant health and productivity. Therefore, these promising endophytes as a bio-control agents can be used as a component in the integrated disease management for enhancing crop productivity and for safe, eco-friendly and for sustainable management of this economically important disease.

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