J. Farm Sci., 36(1): (67-70) 2023

RESEARCH PAPER

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

Y. REKHA[1], V. R. KULKARNI[1], M. S. L. RAO[1] AND B. S. PATIL[2]

[1]Department of Plant Pathology, University of Agricultural Sciences, Dharwad, Karnataka, India
[2]IARI- Regional Research Centre, Dharwad - 580 005 Karnataka, India
E-mail: rekhachinna00@gmail.com

(Received: November, 2022 ; Accepted: February, 2023)

Abstract: Dry root rot 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 18 fungal endophytes were isolated from healthy chickpea plant (stem and root). The isolated fungal endophytes were evaluated against *R. bataticola* by dual culture technique under *In vitro* conditions. Isolate IFRE 2 (*Trichoderma yunnanense*) exhibited the maximum inhibition of pathogen growth, followed by BFRE 3 (*Trichoderma simmonsii*) and IFSE 2 (*Trichoderma rifaii*). The least mycelial inhibition was observed in BFRE 2 followed by IFSE 4. The effective endophytes were further evaluated for their bioefficacy against dry root rot under glasshouse conditions as a component of integrated disease management.

Key words: Dry root rot, Endophytes, Inhibition, *Rhizoctonia bataticola*

Introduction

Chickpea (*Cicer arietinum* L.) is an important leguminous crop. India is the largest chickpea producer in the world with production volume amounting to nearly ten million metric tonnes. Chickpea productivity is significantly lowered due to biotic stresses, the most significant disease is dry root rot. Dry root rot (DRR) is caused by soil borne pathogen *Rhizoctonia bataticola* (Taub.) Butler incurs yield loss as high as 50 to 71 per cent. Effective control using fungicide is practically and economically not feasible.Excessive use of synthetic agro-chemicals (particularly pesticides) have created significant environmental and health concerns. Therefore, demand for sustainable agriculture tools is increasing which can support increase in farm productivity without further damaging natural ecosystems. Harnessing plant microbiome is considered a viable emerging approach to sustainably increase agriculture productivity. Endophytes are an attractive alternative to chemical pesticides as they may provide alternatives for plant disease management that contribute to sustainable agriculture. The majority of the endophytes have advantageous effects, including improved biological N_2- fixation, siderophore production and phosphate solubilization, It is also known that endophytes defend plants from diseases by producing antibiosis, competing with other organisms and inducing systemic resistance.

Material and methods

The present study was conducted with the objective of isolating the endophytes from three chickpea genotypes (ICC 14395, BGD 103 and Pusa 212) and assessing their antagonistic effect on the dry root rot causing pathogen, *Rhizoctonia bataticola*. The study was conducted at the Department of Plant Pathology, College of Agriculture, Dharwad, which is located in the transitional tract of Karnataka at an altitude of 678 metres above mean sea level and has a mild tropical rainy climate.

(i) Collection of samples: Root and stem samples of healthy chickpea are collected from IARI Regional Research Centre, Dharwad and other fields. The root and stem samples were collected from the genotypes/ varieties *viz*., ICC-14395 (DRR tolerant), Pusa 212 (DRR susceptible) and BGD 103 (bold seeded variety suitable for irrigated condition).

(ii) Isolation of fungal endophytes and coding of isolates: Root and stem portions were washed in running tap water to remove soil dirt and debris and cut into 1 cm sections. After this, surface sterilization was done with 70 per cent ethanol for a minute followed by one per cent sodium hypochlorite for three minutes. Subsequently the sections were rinsed with sterile distilled water and placed on 9 cm Petri plates containing potato dextrose agar (PDA) medium amended with streptomycin (250 mg/l) to slow down the bacterial growth. Sterilized tissue segments were pressed onto the surface of PDA medium to check the efficacy of surface sterilization procedure and to confirm endophytic isolations only from internal tissues of the plant segments. The absence of growth of any fungi on the medium confirmed that the surface sterilization procedure was effective in removing the surface fungi (Schulz *et al.,*1993). All the plates were incubated at 25± 1°C and observed for fungal growth at three days interval for a duration up-to 7-10 days. Fungi growing out from the plant tissues were transferred on to fresh PDA medium.

Fungal endophytes were coded by using four letters. First letter indicated the genotype of chickpea (ex. ICC 14395: I), second letter indicated group of endophytes (Fungi: F), third letter indicated the part of plant (S: Stem, R: Root) and last letter indicate endophyte (E).

(iii) *In vitro* evaluation of fungal endophytes against *Rhizoctonia bataticola*: Dual culture technique was adopted for antagonistic activity of the isolated endophytes against pathogen on PDA plates. To test the efficacy of antagonistic

fungi, 20 ml of sterilized melted PDA was plated in Petri plates (9 cm) and allowed to solidify. Mycelial discs measuring 5 mm diameter from three days old cultures of both fungal antagonist and the test pathogen were placed at equidistance on sterile Petri plate containing PDA medium.The Petri plates with pathogen inoculated alone, served as control. The Petri plates were then incubated at 28 ± 2°C until the complete growth of the pathogen in control Petri plate (usually 5-7 days).

After the incubation *i.e.*, after growth of colony in control plate reached 9 cm diameter, the radial growth of pathogen was measured. Per cent inhibition over control was worked out according to formula given by Vincent (1947).

$$I = \frac{C-T}{C} \times 100$$

Where,

I = Per cent inhibition

C = Radial growth in control (cm)

T = Radial growth in treatment (cm)

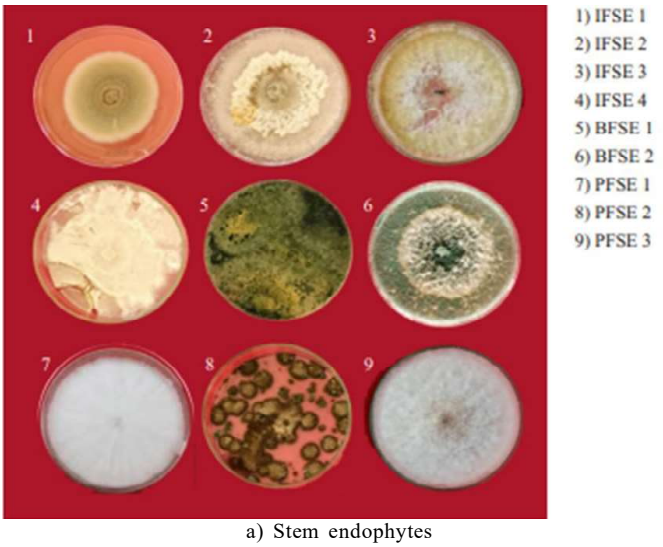
Results and discussion

A total of 18 fungal isolates were obtained from stem and root endosphere and coded them as explained in materials and methods. Endophytes were isolated from three different varieties *viz*., ICC 14395, Pusa 212 and BGD 103 (Table 1).The fungal endophytes isolated were IFSE 1, IFSE 2, IFSE 3, IFSE 4, IFRE 1, IFRE 2, PFSE 1, PFSE 2, PFSE 3, PFRE 1, PFRE 2, PFRE 3, PFRE 4, BFSE 1, BFSE 2, BFRE 1, BFRE 2, and BFRE 3 (Plate 1a and Plate 1b). The coding of isolated fungal endophytes was done as above mentioned in material and methods. The morphological characterization of isolated fungal endophytes was done based on colony color, texture, margin and type of mycelial growth (Table 2). Similar results were obtained by Hadimani (2019), the endophytes from three genotypes of groundnut also showed higher number of endophytes from root tissue when compared to leaf and stem. One of the possible reasons for the difference in the colonization rate between plant parts is the structure and substrate which influences the colonization and distribution of endophytic fungi (Xu *et al*., 2010)

The biocontrol potency of fungal endophytes isolated were tested by dual culture technique against *R. bataticola*. Isolates tested have shown the significant inhibition of pathogen. The isolateswith highest per cent inhibition were again subjected for secondary screening (in present study ten isolates). The extent of per cent inhibition was ranged from 88.51per cent to 25.92 per cent (Plate 2, Table 3). Among all the isolates, the maximum reduction of pathogen growth was showed by isolate IFRE 2 (*Trichoderma yunnanense*) with highest per cent mycelial inhibition of 88.51% followed by BFRE 3 (*Trichoderma simmonsii*) (85.55%), IFSE 2 (*Trichoderma rifaii*) (66.81%) and the least mycelial inhibition was observed in BFRE 2 (25.92%) (Fig 1). The molecular identification of only, the *in vitro* effective endophytes was done by NCIM, Pune with the help of Fungal ITS rRNA gene (600 bp). The results revealed that, isolate IFRE 2 has similarity index of 99.32 per cent to *T. yunnanense*, BFRE 3 has similarity index of 99.02 per cent to *T. simmonsii* and that of IFSE 2 has 99.50 per cent to *T. rifaii*.

Table 1. Endophytes isolated from stem and roots of healthy chickpea plant

Genotype	Source	Endophytes isolated
ICC 14395	Stem	IFSE 1, IFSE 2, IFSE 3, IFSE 4
	Root	IFRE 1, IFRE 2
Pusa 212	Stem	PFSE 1, PFSE 2, PFSE 3
	Root	PFRE 1, PFRE 2, PFRE 3, PFRE 4
BGD 103	Stem	BFSE 1, BFSE 2
	Root	BFRE 1, BFRE 2, BFRE 3



a) Stem endophytes

Plate 1a: Fungal endophytes isolated from chickpea stem

b) Root endophytes

Plate 1b: Fungal endophytes isolated from chickpea root

The dual culture method results obtained are in agreement with findings of Hadimani (2019), who evaluated 26 fungal endophytes and 26 bacterial endophytes against *Sclerotium rolfsii*. Among the fungal endophytes, the maximum inhibition

Table 2. Morphological characterization of fungal endophytes isolated from chickpea

Endophyte code	Colony color	Margin	Texture	Mycelial growth
IFSE 1	Light green	Regular	Smooth	Flat
IFSE 2	White	Irregular	Coarse	Raised
IFSE 3	Pale green	Regular	Coarse	Flat
IFSE 4	White	Regular	Smooth	Flat
BFSE 1	Light green	Regular	Smooth	Flat
BFSE 2	Dark green	Irregular	Coarse	Flat
PFSE 1	Pure white	Regular	Smooth	Flat
PFSE 2	Grey	Irregular	Smooth	Raised
PFSE 3	White	Regular	Coarse	Flat
IFRE 1	Whitish orange	Regular	Coarse	Raised
IFRE 2	Dark green	Irregular	Coarse	Raised
BFRE 1	Green	Regular	Coarse	Flat
BFRE 2	Greyish green	Regular	Smooth	Raised
BFRE 3	Dark green	Regular	Coarse	Flat
PFRE1	Light grey	Irregular	Smooth	Raised
PFRE 2	Grey	Irregular	Coarse	Flat
PFRE 3	Light pink	Regular	Smooth	Raised
PFRE 4	Dirty white	Irregular	Coarse	Flat

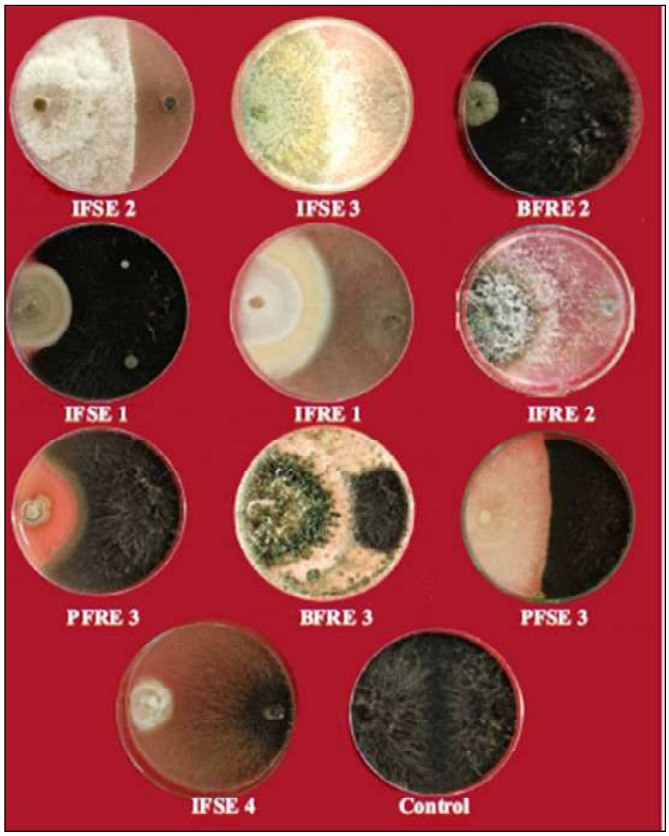


Plate 2: *In vitro* evaluation of fungal endophytes against *R. bataticola*

Table 3. *In vitro* evaluation of fungal endophytes against *R. bataticola*

Isolates	Mycelial inhibition over control (%)
IFSE 1	38.43(38.29)*
IFSE 2	66.81(54.81)*
IFSE 3	65.63(54.09)*
IFSE 4	33.70(35.47)*
IFRE 1	60.74(51.19)*
IFRE 2	88.51(70.19)*
BFRE 2	25.92(30.56)*
BFRE 3	85.55(67.64)*
PFRE 3	42.56(40.69)*
PFSE 3	51.50(45.84)*
S Em±	0.86
C D @ 1%	2.56

* Arcsine values

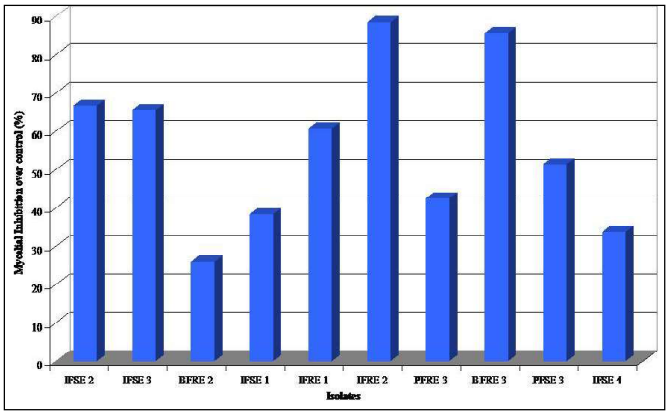


Fig. 1. *In vitro* evaluation of fungal endophytes against *R. bataticola*

of pathogen was observed in 4 pr 2 (54.28%) followed by 4 ps 1 (40%). Similarly, Veena (2013) isolated ten *Trichoderma spp* from chickpea *rhizosphere* and root endophytic region among which, ten isolates were tested against *Rhizoctonia bataticola*. *Trichoderma* isolate-7 showed highest inhibition percentage (83.33 %).

It is reported that, endophytes generally employ the mechanisms such as antibiosis, competition, mycoparasitism *etc*., to combat the pathogen growth. It is also noted that, endophytes isolated from variety ICC-14395 showed much inhibition of pathogen under *in vitro* conditions than other two varieties, Pusa 212 and BGD-103. Thus, the presence of these endophytes might be one of the reasons for the tolerance of ICC-14395 to dry root rot (*Rhizoctonia bataticola*).

Conclusion

The chickpea endophytic microbes *viz*., *Trichoderma yunnanense* (IFRE 2) and *Trichoderma simmonsii* (BFRE 3) 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*. Such 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.

References

Hadimani R H, 2019, Identification and characterization of potential endophytes against *Sclerotium rolfsii* Sacc. A stem rot causing pathogen of groundnut, *Doctoral dissertation,* University of Agricultural Sciences*,* GKVK, Karnataka, India*.*

Schulz B, Wanke U, Draeger S and Aust H J, 1993, Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. *Mycological Research*, 97 (12): 1447-1450.

Veena G A, Reddy N E, Reddy B B and Prasanthi L, 2014, Potential of *Trichoderma* spp. as biocontrol agents against *Rhizoctonia bataticola* causing dry root rot of chickpea. *International Journal of Plant, Animal and Environmental Sciences*, 4(1): 78-81.

Vincent J M, 1947, Distribution of fungal hyphae in the presence of certain inhibitors. *Nature*, 150: 850-853.

Xu L, Wang J and Zhao J, 2010, Beauvericin from the endophytic fungus, *Fusarium redolens*, isolated from *Dioscorea zingiberensis* and its antibacterial activity. *National Production Community*, 5:811-814.