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RESEARCH PAPER

In vitro evaluation of bioagents against curry leaf spot caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.

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Abstract: A total of four fungal and four bacterial bioagentswere evaluated against *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. causing curry leaf spotby following dual culture technique. Among the bioagents evaluated, commercial formulation of *Trichoderma viride* (84.71%) from Multiplex company and *Trichoderma harzianum* (81.18 %) from Institute of Organic Farming (IOF), UAS Dharwad were found to be effective in inhibiting mycelial growth of the pathogen and were found to be on par with each other. *Trichoderma harzianum* (76.86 %) from Department of Microbiology, UAS Raichur, followed by commercially available *Trichoderma harzianum* (72.55 %). Bacterial bioagents were less effective as compared to fungal bioagents. Among the bacterial bioagents evaluated, *Pseudomonas fluorescens* from IOF Dharwad (43.14 %) and *Pseudomonas fluorescens* (42.35 %) commercial formulation, have shown effective inhibition which are significantly superior and were found to be on par with each other. Least mycelial inhibition was recorded in commercially available *Bacillus subtilis* from IOF Dharwad (29.02 %) and *Bacillus subtilis* (26.27 %) and were on par with each other.

Key words: Bioagent, Curry leaf, Formulation, Leaf spot

Introduction

India is best known for its enormous biodiversity of medicinal plants. Most important among them include curry leaf. The botanical name of *Murraya koenigii* Spreng.refers to 18th century botanists, the Swede Johann Andreas Murray and the German Johann Gerhard Konig. It belongs to family Rutaceae and is commonly found in tropical and subtropical forests of India. It has been originated from the Tarai region of Uttar Pradesh (India) and is found growing in West Bengal, Madhya Pradesh, Kerala, TamilNadu, Karnataka and Andhra Pradesh.In Karnataka, total area under curry leaf cultivation is about 1141 hactare with totalproduction of about 9233 tonnes with productivity of about 6.55 tonnes per hectare. In Karnataka maximum area under curry leaf production is reported from Belagavi district with 203 hactare and maximum production of about 1146 tonnes in Ballari district (Anon., 2019).

Curry leaf,a medicinal plant is susceptible to only a few fungal pathogens. Epidemics of a fungal leaf spot disease with characteristic necrotic spots were evident during the onset of monsoon season every year.It has been regularly infected by leaf spot caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. The disease severely affects the qualityand reduces the yield of leaves.It impacts on its market price and also if severity of disease is high, then total loss of crop may occur (Hande and Gaikwad, 1999)

Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. is one of the commonest plant-pathogenic fungi of the genus*Colletotrichum* to occur in the tropics and subtropics and is found worldwide. *Glomerellacingulata* is the sexual stage of the fungus while the asexual stage or anamorph is called *Colletotrichum gloeosporioides*.The fungus produces hyaline, one- celled, ovoid to oblong, slightly curved or dumbbell shaped conidia, 10-15 µm in length and 3-7 µm in width. Masses of conidia appear pink or salmon colored. The waxy acervuli, that are produced in infected tissue, are sub-epidermal, typically with setae, and simple, short, erect conidiophores (Padman and Janardhana, 2011).

Curry leaf is being consumed as leafy vegetableand it isvulnerable to several foliar diseases. Since leaf is an economically important part and it is consumed as raw, chemical management of the disease is not suitable because of its residual toxicity. In this context, suitable eco- friendly management of disease is at most importance.Biological control through the use of antagonistic microorganisms is a potential non-chemical way of reducing plant diseases by reducing inoculum levels of the pathogens. These are safe and cheaper means of controlling the disease which in turn reduces toxicity hazards and also helps in eco-friendly management of disease approach as compared to use of chemicals.

Material and methods

Isolation of *Colletotrichum gloeosporioides*

The infected plants showing typical symptoms of the disease were used for the isolation of the pathogen. The standard tissue isolation procedure was followed to isolate the pathogen. The infected tissues of leaves were cut into small bits and were surface sterilized with one per cent sodium hypochlorite solution for 60 seconds and washed in sterilized distilled water to remove the traces of the chemical if any and then transferred to sterilized Petri plates containing potato dextrose agar (PDA).

In vitro evaluation of bioagents against *Colletotrichum gloeosporioides*

The antagonistic micro-organisms were evaluated for their antagonistic effect under *in vitro*conditions against the

pathogenby dual culture technique (Dennis and Webster, 1971). The bioagents used are *Trichoderma viride* (Multiplex Nisarga), *Trichoderma harzianum* (IOF, UASD isolate), *Trichoderma harzianum* (UAS, Raichur isolate), *Trichoderma harzianum* (Commercial formulation), *Pseudomonas fluorescens* (IOF UASD isolate), *Pseudomonas fluorescens* (Commercial sample),*Bacillus subtilis* (IOF UASD isolate), *Bacillus subtilis* (Commercial sample). The efficacy of these bio agents was tested against the pathogen for per cent radial mycelial growth inhibition on the potato dextrose agar media.

Dual culture test

Twenty ml.of sterilized and cooled potato dextrose agar (PDA) was poured into sterilized Petri plates. Fungal antagonists was evaluated by inoculating the pathogen at one side of Petri plate and the antagonist inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. For this, actively growing culturewasused. In case of evaluation of bacterial antagonist, the bacterium is streaked at one end of the Petri plate and the test fungus was placed at the other end.

After required period of incubation *i.e.* after control plate reached 90 mm. diameter, the radial growth of pathogen was measured. Per cent inhibition over control was calculated by using the formula (Vincent, 1947).

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

Where,

I = Per cent inhibition of mycelial growth

C = Radial growth in control (cm)

T = Radial growth in treatment (cm)

Results and discussion

Isolation of the pathogen was done from infected curry leaf plant having typical symptoms of the disease. Standard tissue isolation method was followed after disinfection. Repeated isolations from diseased samples yielded fungus and it was identified as *Colletotrichum gloeosporioides*. The culture showed whitish pink colour mycelium with concentric rings at lower surface of the Petri plate. The fungus was sub cultured on the PDA slants and allowed to grow at 28 ± 1^{0}C temperature for one week and later the culture was stored in refrigerator at 4 ^{0}C for further studies and was sub cultured periodically (Plate 1).

A total of eight bioagents(four fungal and four bacterial)were evaluated against

C. gloeosporioides. Among the bioagents evaluated, commercial formulation of *Trichoderma viride* (84.71%) from Multiplex company and *Trichoderma harzianum* (81.18 %) from Institute of Organic Farming (IOF), UAS Dharwad which were on par with each other and were found to be statistically superior over other treatments. Next best was Trichoderma harzianumfrom Department of Microbiology, UAS, Raichur with which exhibited 76.86 percent inhibition,this was followed by commercially available *Trichoderma harzianum* (72.55 %) respectively (Table 1 and Plate 2).

Bacterial bioagents were less effective when compare with fungal bioagents. Among the bacterial bioagents evaluated, *Pseudomonas fluorescens* from IOF Dharwad (43.14 %) and *Pseudomonas fluorescens* (42.35%) commercial formulation have shown effective inhibition which are significantly superior and were found to be on par with each other. Least mycelial inhibition was recorded in commercially available *Bacillus subtilis* from IOF Dharwad (29.02 %) and *Bacillus subtilis* (26.27 %) were on par with each other.

The results from *In vitro* evaluation of bioagents against *C. gloeosporioides* revealed that fungal bioagents were excellent when compared to bacterial bioagents in inhibiting the mycelial growth of the test pathogen. Inhibition range of 72.55 to 84.71 per cent was recorded from fungal bioagents while it was 26.27 to 43.14 per cent for bacterial bioagents.

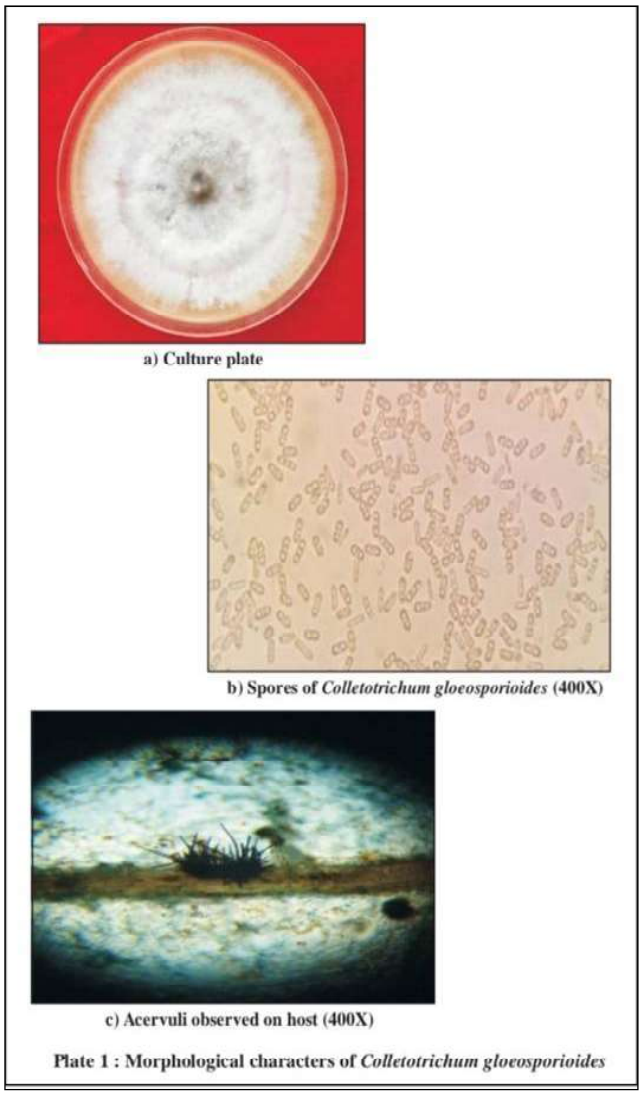
These results were confirmative with Shirshikar (2002) who reported that, *Trichoderma viride* was found to be most effective in inhibiting the mycelial growth of *Colletotrichum gloeosporioides* followed by *T. harzianum.* Further the results are in agreement with Borras *et al.* (1993) and Rocha *et al.* (1998) who reported the antagonistic properties of *T. viride* and *Trichoderma* spp. (especially *T. harzianum)* against pathogen *C.gloeosporioides.*

The antagonistic properties of *Trichoderma* spp. may be attributed to its ability to produce antibiotics. The production of secondary metabolites like alkaloides, phenols, which can affect the interaction of pathogen with the host there by reducing aggressiveness of the pathogen which in turn reduces the disease (Shirshikar, 2002). The survival ability of

Table 1. *In vitro* evaluation of bioagents against *Colletotrichum gloeosporioides*

Sl. No.	Bioagents	Inhibition of mycelial growth (%)
1	*Trichoderma viride* (Multiplex Nisarga)	84.71(66.95)*
2	*Trichoderma harzianum* (IOF, UASD isolate)	81.18(64.26)
3	*Trichoderma harzianum* (UAS Raichur isolate)	76.86(61.22)
4	*Trichoderma harzianum* (Commercial formulation)	72.55(58.38)
5	*Pseudomonas fluorescens* (IOF UASD isolate)	43.14(41.04)
6	*Pseudomonas fluorescens* (Commercial sample)	42.35(40.58)
7	*Bacillus subtilis* (IOF Dharwad)	29.02(32.58)
8	*Bacillus subtilis* (Commercial sample)	26.27(30.82)
9	*Control*	-
	S.Em.±	0.82
	C.D. at 1 %	3.23

* Angular transformed value

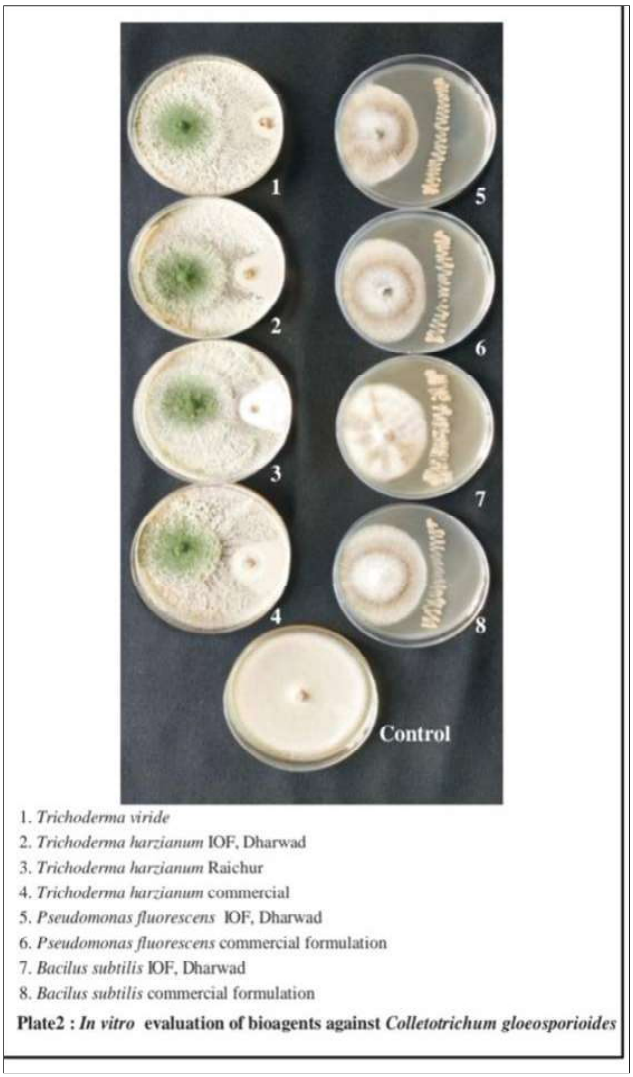


a) Culture plate

b) Spores of *Colletotrichum gloeosporioides* (400X)

c) Acervuli observed on host (400X)

Plate 1 : Morphological characters of *Colletotrichum gloeosporioides*



1. *Trichoderma viride*
2. *Trichoderma harzianum* IOF, Dharwad
3. *Trichoderma harzianum* Raichur
4. *Trichoderma harzianum* commercial
5. *Pseudomonas fluorescens* IOF, Dharwad
6. *Pseudomonas fluorescens* commercial formulation
7. *Bacilus subtilis* IOF, Dharwad
8. *Bacilus subtilis* commercial formulation

Plate2 : *In vitro* evaluation of bioagents against *Colletotrichum gloeosporioides*

Trichoderma spp. on the phylloplane was found to be more because of the close canopy of the crop.

Conclusion

The results from *in vitro* evaluation of bioagents revealed that fungal bioagents were better than bacterial bioagents in inhibiting the mycelial growth of the leaf spot pathogen. Among the fungal bioagents tested, *Trichoderma viride* from Multiplex company showed maximum inhibition of mycelial growth (84.71 %). Among bacterial bioagents tested *Pseudomonas fluorescens* IOF Dharwad showed maximum mycelial inhibition (43.14 %).

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