Current Biotica 5(4): 454-464, 2012 ISSN 0973-4031

Fungicide compatible potential biocontrol agents against *Colletotrichum gloeosporioides* Penz. causing mango anthracnose

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ABSTRACT

Pathogenic studies were conducted under greenhouse conditions to test the virulence of *Colletotrichum gloeosporioides* Penz. isolates collected from different regions of Chittoor district, Andhra Pradesh. The results indicated that the isolate PTR6 was highly virulent (76.30%) and the isolate VDM2 (12.55%) was found to be least virulent. Of all the six fungicides evaluated, the systemic fungicide thiophanate-methyl (100%) and the non-systemic fungicide mancozeb (100%) proved to be effective in inhibiting the mycelial growth of the highly virulent pathogen at 50 ppm and 500 ppm concentrations, respectively. Among the 21 bacterial biocontrol agents screened in dual culture, the potential biocontrol agents *viz.,* BP5 (100%), BL5 (100%), BL4 (97.11%) and BL6 (90.44%) were superior in arresting the growth of the pathogen. Compatibility studies revealed that the potential bacteria BL5 from leaf endophyte habitat was highly compatible with thiophanate-methyl (100%) followed by mancozeb (98.33%) which can be exploited for field evaluation.

KEY WORDS: Anthracnose, biocontrol agents, fungicides, mango

INTRODUCTION

Mango (*Mangifera indica* L.) called "King of Fruits", an important crop in India is affected by several diseases reducing its production and productivity. Among the diseases, mango anthracnose caused by *Colletotrichum gloeosporioides* Penz., is the most devastating and highly destructive pathogen responsible for the losses up to 60%. In India, Andhra Pradesh ranks first in production and Chittoor district stands first in the Rayalaseema region of Andhra Pradesh. This fruit has become an essential fruit crop in Chittoor District as well as in many parts of Rayalaseema region. Because of diverse production conditions and the vast area grown, mango suffers from a number of diseases, some of them taking heavy toll on the crop. To understand the present disease situations, it is mandatory to identify the potential biocontrol agents and effective fungicide against the pathogen with respect to particular geographical region and on the other hand, the compatibility of antagonists with effective fungicides should also be traced out which plays a vital role in inhibiting the pathogen without damaging the agroecosystem. The fungicides are the most common tools for controlling disease losses. In recent years, there has been growing concern over their use because of their potential hazardous effect on biotic activity of soil biota particularly antagonists and chemical residues in the soil adding to pollution. These factors have led to search for new

and innovative approaches to plant disease management. Biological control of plant pathogens is a distinct possibility for the future and can be successfully exploited in the modern agriculture, especially within the frame work of integrated disease management (IDM) system which is needed to hold disease below economic threshold without damaging the agro ecosystem (Papavizas, 1985). So far, the biocontrol agents were isolated and characterized mainly from rhizosphere. The information on fructoplane biocontrol agents is scanty and as such it is worthwhile to isolate microflora having antagonistic activity from the new habitats. Therefore, the present research has been mainly focused on characterization of pathogenic variability and identification of fungicide compatible potential biocontrol agent against *C.gloeosporioides*.

MATERIALS AND METHODS

The studies were carried out in the Department of Botany, S.V. University College of Sciences, Sri Venkateswara University, Tirupati, Chittoor District, Andhra Pradesh during 2009-2011.

Isolation and maintenance of pathogen

Survey was conducted in major mango growing mandals of Chittoor district in Andhra Pradesh to assess the per cent disease incidence. The pathogen was isolated from the mango leaves showing typical anthracnose symptoms by tissue segment method and purified by single spore isolation method (Rangaswami and Mahadevan, 1999) on potato dextrose agar medium (PDA). The pathogen was identified based on its mycelial, conidial characteristics following standard mycological keys (Barnett and Hunter, 1972) and were maintained separately on potato dextrose agar medium for further experiments.

Pathogenicity of C.gloeosporioides isolates

The variation in pathogenicity of different isolates of *C.gloeosporioides* was tested by spray inoculation method. One year old Baneshan mango grafts were chosen and wounds were made by pin prick method (Bhuvaneswari and Rao, 2001). Spore suspension containing a load of 2 x 10^4 conidia ml^{-1} was inoculated on the wounded areas. Alcohol washed hand automizer was used for spraying inoculum suspension of each isolate. After inoculation, the seedlings were covered with polythene bags for two days to ensure high humidity by spraying sterile distilled water to provide congenial conditions for conidial germination and infection. The lesions were observed on the inoculated leaves within eight to ten days of inoculation. The fungus was reisolated from the infected leaves showing typical symptoms and its identity was confirmed.

Disease Severity

The disease intensity was calculated based on the disease rating scale as described by Agostini *et al.,* (1992). Disease severity was recorded at eight to ten days after inoculation. Each treatment was replicated thrice, keeping suitable control by just spraying sterile water. Disease rating scale on mango leaves after inoculation is given hereunder:

Numerical rating	Disease severity
0	No disease
1	Isolated chlorotic spots of 1-3 mm in diameter
2	Numerous small necrotic spots
3	Large confluent necrotic areas on the leaves
4	Defoliation and necrosis of the shoot tip

Per cent disease incidence (PDI) was computed by using the formula:

$$PDI = \frac{\text{Sum of the numerical ratings}}{\text{No.of leaves observed x maximum rating}} \times 100$$

The isolates were classified as highly virulent, moderately virulent and less virulent as given below:

Group	Per cent disease incidence
Highly virulent	> 40
Moderately virulent	> 30-40
Less virulent	< 30

Evaluation of fungicides against C. gloeosporioides isolates by poisoned food technique

The systemic and non-systemic fungicides *viz.,* carbendazim, thiophanate-methyl, propioconazole, hexaconazole, mancozeb and copper oxychloride were (ppm) used (Nene and Thapliyal, 1993). A control was maintained without fungicide Three replications were maintained for each treatment.

S No.	Concentration (ppm)	Common Name	Active ingredient
1.	50	Carbendazim	50% wp
2.	50	Thiophanate-methyl	70% wp
3.	25	Hexaconazole	50% wp
4.	25	Propioconazole	25% wp
5.	500	Mancozeb	75% wp
6.	500	Copper oxychloride	50% wp

Per cent reduction in radial growth over control was calculated by using the following formula:

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

Where,

I = Per cent reduction in growth of test pathogen
C = Radial growth (mm) in control
T = Radial growth (mm) in treatment

Isolation and screening of biocontrol agents

Isolation of native biocontrol agents was made from washings of healthy mango leaves and fruits following serial dilution plate technique (Zenichi *et al.,* 2003). The antagonistic activity of biocontrol agents against *C. gloeosporioides* was determined by dual culture technique under *in vitro* (Thahir Basha *et al.,* 2010). The Petri plates were then incubated at 28±2°C. Three replications were maintained in each treatment with suitable controls and the per cent inhibition was tabulated.

Compatibility studies

The fungicides were evaluated for compatibility with potential bacterial biocontrol agents by spectrophotemtric method (Kishore *et al.,* 2005). Five hundred microliters of antagonistic bacterial cultures grown in Nutrient Broth (NB) for overnight at 28±2°C and 180 rpm were added to 50 ml of NB in 250 ml flasks containing different fungicides. Inoculated flasks were incubated at 28±2°C and 180 rpm. Bacterial growth was determined by measuring optical density (OD) at 600 nm after 24 hours of incubation. Each treatment consisted of three flasks as individual replications. The nutrient broth without bacteria served as control.

Statistical analysis

Completely Randomized Design (CRD) was used for radial growth, per cent disease incidence, poisoned food technique and dual culture technique (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Survey, isolation and identification of pathogen

Roving survey was conducted in 7 mandals of Chittoor district in Andhra Pradesh and mango leaves showing typical anthracnose symptoms were collected. Symptoms on the leaves appears as oval, brown to black spots with greyish centre. The maximum per cent disease incidence was recorded in in the area of Rangampeta (RPT1) 47.90% followed by Sri kalahasti (SKT3) 40.25% and the least PDI was recorded in Vadamalapeta (VDM2) 12.55%. The total per cent disease incidence of 37.99% was recorded in Chittoor district.

A total of seven isolates were collected in the regions where the disease incidence is severe according to the information given by the local farmers during the survey. The colonies of *Colletotrichum gloeosporioides* isolates on PDA were initially white and later became dull grey to dark grey. Mycelium was sparsely septate and dark brown in colour. Conidia were hyaline, aseptate, cylindrical with rounded ends containing one or two oil globules. On reverse the colonies produced pink to orange colour with or without concentric rings. The morphological characteristics of the pathogen were in accordance with the reports given by earlier researchers Afanador Kafuri *et al.,* (2003), Sampath Kumar (2007) and Thahir Basha (2010).

Pathogenecity

Pathogenecity of seven isolates of *C.gloeosporioides* was tested by spray inoculation method on one year old Baneshan mango grafts purchased from local nursery. The pathogen was reisolated from the infected leaves and found to be the same there by proved Koch's postulates. The variability among the *C.gloeosporioides* isolates were observed with respect to per cent disease incidence (PDI). Based on PDI, the isolates were classified as less virulent, moderately virulent and highly virulent. The maximum incidence of disease was recorded in isolate PTR6 (76.30%) followed by RPT1 (47.90%), SKT3 (40.25%), APG5 (37.65%), ITP7 (31.90%), DMC4 (19.68%), and the least PDI was recorded in VDM2 (12.55%). There is a significant difference with respect to pathogenecity among the seven isolates. The findings were similar to the reports given by Mathur *et al.,* (2001) in sorghum, Giblin & Coates (2007) in avocado, Sampath Kumar (2007) and Thahir Basha (2010) in mango.

Group	Isolates
Highly virulent	PTR6
Moderately virulent	RPT1, SKT3, APG5, ITP7
Less virulent	DMC4, VDM2

Efficacy of fungicides against *C.gloeosporioides*

Different approaches may be used to prevent, mitigate the disease beyond the good agronomic and horticultural practices. Among the six fungicides tested against *C.gloeosporioides* isolates in poisoned food technique, the systemic fungicide *viz.*, thiophanate methyl (100%) @ 50 ppm and non-systemic fungicide mancozeb (100%) @ 500 ppm were highly effective in inhibiting the mycelial growth of pathogen. The excessive use of

fungicides causes environmental pollution, resistance towards chemical fertilizers and pesticides which are nature warnings to agriculture to shift towards other practices. Biological control continues to inspire research and development in many fields. However, the interrelationships of many environmental variables can result in multiple interactions among organisms and their environment, several of which might contribute to effective biological control. This biological control which is promising practice along with the use of low concentration of fungicide is our further research which may prove to be best for green agriculture.

Screening for potential biocontrol agents

Biological control can result from many different types of interactions between organisms and pathogens and characterizing the mechanisms may be different in different experimental situations. In all cases, pathogens are antagonized by the presence and activities of other organisms that they encounter. Dual culture technique was adopted to identify the potential biocontrol agent. Among the 21 bacterial biocontrol agents screened for its antagonistic activity, the antagonists, BP5 (100%), BL5 (100%), BL4 (97.11%) and BL6 (90.44%) were proved to be best in inhibiting the mycelial growth of the test pathogen, *C.gloeosporioides*. The use of biological control methods to reduce disease incidence caused by plant pathogens is continually being developed and is being used in a variety of crops (Soytong *et al.,* 2001). This inhibition may be due to the secretion of metabolites and siderophores which diffuses through the medium and arrests the pathogen.

Compatibility studies

Compatibility of potential biocontrol agents with fungicides revealed that the potential bacterial biocontrol agent BL5 was highly compatible with thiophanate methyl (100%) followed mancozeb (98.33%). In this case of phylloplane bacterial biocontrol agent BP5, the fungicides thiophante methyl and mancozeb were compatible to the extent of 98.50% and 89.01% respectively. The combination of antagonistic biocontrol agents and fungicides may also influence biocontrol activity by metabolizing antibiotic substances and inhibiting the growth and development of plant pathogenic fungi. Similar reports were reported by Thahir *et al.,* (2010) which are in agreement with present findings. The bioproducts from endophytic bacterial biocontrol agent BL5, which showed 100% inhibition may have potential efficacy to colonize and get distributed in leaves, propagates, and survives for long time, increasing colonization and reduction of pathogens when compared with chemical control. Biological control of plant pathogens using antagonists are eco-friendly, economical and efficient method and can be successfully exploited in the frame work of integration disease management. The spread of plant diseases in natural ecosystems may preclude successful application of biocontrol agents, because of the scale to which such applications might have to be applied. The future studies were focused on exploiting suitable methods for mass production as well as to test the potentiality of few carrier based material for enhancing the effectiveness with biocontrol agents under field conditions.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial grant and support provided by the University Grants Commission (UGC), Government of India, New Delhi and also extend the same to Mr. Shaik Thahir Basha, Research Scholar in Microbiology for his contribution in execution of experiments.

Table 1: Survey and isolation of *C.gloeosporioides* isolates collected from Chittoor district of Andhra Pradesh

S.No.	Isolate No.	Place of collection	Per cent disease incidence (%)
1.	RPT1	Rangampeta	47.90
2.	VDM2	Vadamalapeta	12.55
3.	SKT3	Sri Kalahasti	40.25
4.	DMC4	Damalacheruvu	19.68
5.	APG5	Appalayagunta	37.65
6.	PTR6	Puttur	76.03
7.	ITP7	Ithepalli	31.90
Overall PDI			**37.99%**

Table 2: Evaluation of fungicides against *C.gloeosporioides* isolates by poisoned food technique

Isolates	Carbendazim (50 ppm)			Thiophanate methyl (50 ppm)			Propiocoanzole (25 ppm)		
	Mycelial growth (mm)*		Per cent inhibition over control	Mycelial growth (mm)*		Per cent inhibition over control	Mycelial growth (mm)*		Per cent inhibition over control
	Control	Treatment		Control	Treatment		Control	Treatment	
RPT1	79	9.91	87.45	79	13.27	83.20	83	14.6	82.40
VDM2	83	14.5	82.53	78	10.95	85.96	86	19.8	76.97
SKT3	85	11.9	86	84	9.86	88.26	81	12.4	84.69
DMC4	87	10.75	87.64	78	12.6	83.84	80	11.41	85.73
APG5	88	12.12	86.22	80	12.3	84.62	86	10.73	87.52
PTR6	81	10.4	87.16	**82**	**0.00**	**100**	80	13.67	82.91
ITP7	84	9.17	89.08	89	10.87	87.78	77	10.27	86.66
	SEm		1.4066	SEm		1.0949	SEm		1.7930
	CD (5%)		1.0749	CD (5%)		1.2032	CD (5%)		1.5899

Isolates	Hexaconazole (25 ppm)			Mancozeb (500 ppm)			Copper oxychloride (500 ppm)		
	Mycelial growth (mm)*		Per cent inhibition over control	Mycelial growth (mm)*		Per cent inhibition over control	Mycelial growth (mm)*		Per cent inhibition over control
	Control	Treatment		Control	Treatment		Control	Treatment	
RPT1	81	19.7	75.67	85	7.9	90.70	88	19.8	77.5
VDM2	86	23.4	72.79	86	9.5	88.95	71	17.55	75.28
SKT3	77	16.39	78.71	88	8.74	90.06	79	13.54	82.86
DMC4	79	16.2	79.49	88	11.98	86.38	83	14.20	82.89
APG5	84	15.0	82.14	87	12.31	85.85	89	10.99	87.65
PTR6	82	10.22	87.53	**75**	**0.00**	**100**	76	13.7	81.97
ITP7	83	11.74	85.85	70	10.62	84.82	87	12.93	85.13
	SEm		1.0842	SEm		1.0082	SEm		1.7195
	CD (5%)		1.6241	CD (5%)		1.2356	CD (5%)		1.1011

* Mean of three replications

Table 3: *In vitro* evaluation of the efficacy of antagonistic microflora against growth of *C.gloeosporioides* in dual culture technique

S.no.	Biocontrol agents	*Mycelial growth (mm)	Per cent inhibition over control
1.	BP1	18.05	79.44
2.	BP2	53.65	40.38
3.	BP3	28.98	67.08
4.	BP4	48.80	45.77
5.	**BP5**	**0.0**	**100**
6.	BP6	15.10	83.22
7.	BP7	65.35	27.38
8.	BF1	35.95	60.05
9.	BF2	31.21	65.32
10	BF3	42.15	53.16
11.	BF4	65.29	27.45
12.	BF5	59.73	33.63
13.	BF6	73.14	18.73
14.	BF7	60.24	30.06
15.	BL1	37.19	58.67
16.	BL2	12.90	58.66
17.	BL3	29.77	66.92
18.	**BL4**	**2.6**	**97.11**
19.	**BL5**	**0.00**	**100**
20.	**BL6**	**8.6**	**90.44**
21.	BL7	5.4	94.0
	Control	90.00	---
	SEm	1.1361	0.3716
	CD (0.05)	2.5165	0.7165

*Mean of three replications

Table 4: Compatibility of potential biocontrol agents with fungicides by poisoned food technique

Fungicides	Concentration (ppm)	*Mycelial growth (mm)				*Per cent compatibility over control			
		BP5	BL4	BL5	BL6	BP5	BL4	BL5	BL6
Carbendazim	50	71.47	72.64	33.56	64.97	20.58	19.28	62.71	27.81
Thiophanate methyl	50	1.35	65.49	0.0	51.94	98.50	27.23	100	42.28
Hexaconazole	25	29.34	83.42	46.79	75.46	67.4	7.31	48.01	16.15
Propioconazole	25	59.92	59.37	69.51	84.25	33.42	34.03	22.76	6.38
Mancozeb	500	9.89	1.25	1.5	51.84	89.01	98.61	98.33	42.4
Copper oxychloride	500	10.47	3.75	14.81	11.87	88.36	95.83	83.54	86.81
Control	---	90.00	90.00	90.00	90.00	---	---	---	---
SEm	---	0.3771	0.5879	0.8974	0.3730	0.42228	1.3575	1.3470	0.4424
CD (0.05)	---	1.1124	1.1874	1.9521	1.1004	0.8883	0.7916	0.5498	0.9294

*Mean of three replications

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[MS received 8 January 2012;
MS accepted 12 March 2012]