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Chindam Swathi
Department of Plant Pathology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

Dr. Bharati N Bhat
Department of Plant Pathology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

Dr. G Uma Devi
Department of Plant Pathology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

Dr. G Sridevi
Department of Plant Pathology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

Corresponding Author:
Chindam Swathi
Department of Plant Pathology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

Compatability of fungicides with phyllosphere bacteria of maize

Chindam Swathi, Dr. Bharati N Bhat, Dr. G Uma Devi and Dr. G Sridevi

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Abstract
Phyllosphere bacteria plays an important role as biocontrol agents. Phyllosphere microorganisms play important role in suppression of the pathogen and induce growth promoting activities in plants. Compatability of phyllosphere bacteria with Six fungicides *viz*., mancozeb, propiconazole, hexaconozole, tebuconazole, carbendazim + mancozeb and trifloxystrobin + tebuconozole at recommended concentrations were evaluated *in vitro* by turbidomertic method. The bacterial antagonist isolate (P_{16}) recorded maximum growth amended medium with carbendazim + mancozeb OD value of 2.54 and was significantly superior when compared to other treatments. Screening of potential antagonists for their growth promoting activities under in vitro was done. The seedling vigour and germination per cent significantly increased on seed treatment. The germination per cent and seedling vigour to 95.22 per cent and 2394.78 respectively when treated with effective fungicide carbendazim + mancozeb + isolate P_9 + isolate P_{16}.

Keywords: Bacteria, fungicides, maize, phyllosphere microflora

Introduction
The phyllosphere is a term used to refer the total above-ground portions of plants as habitat for microorganisms. Further microbial interactions in the phyllosphere gives protection against pathogens and release of phytohormones to stimulate plant growth & colonization and suppress infection of tissues by plant pathogens. Increase disease resistance and productivity of agricultural crops. Compatability of phyllosphere bacteria with Six fungicides *viz*., mancozeb, propiconazole, hexaconozole, tebuconazole, carbendazim + mancozeb and trifloxystrobin + tebuconozole at recommended concentrations were evaluated *in vitro* by turbidomertic method.

Materials and Methods
Isolation of the pathogen and phyllosphere bacteria
Phyllosphere bacteria was isolated to estimate the bacterial population on adaxial and abaxial leaf surfaces, leaf imprints were made on nutrient agar medium (Melina, 2017) [11]. Selection of single bacterial colonies was done based on morphological variation.

Screening of phyllosphere bacteria against *E. turcicum in vitro*
Antagonism test was performed *in vitro* by dual culture method (Landa *et al*., 1997) [8] on PDA. One loop of 48 hrs old culture of bacterial isolates were streaked one cm from the outer side of 9 cm PDA plates. Five mm discs of actively growing three-day old fungus was placed at the centre of plates, 2.5 cm apart from the bacteria. Plates inoculated with fungus without bacterial isolates served as control. For each isolate three replicates were maintained. These plates were incubated at 28 ± 2 ºC for 3 days. The growth of turcicum blight pathogen in the presence or absence of any bacterial isolates was measured. Observations regarding the zone of inhibition radius was recorded after 9 day of incubation and calculated as per the formula given below (Vincent, 1947) [13].

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

Where, I = Per cent inhibition over control
C = Radial growth of pathogen in control (mm)
T = Radial growth of pathogen in treatment (mm)

Compatibility of fungicides with phyllosphere bacteria
Turbidometric method for bacteria

One ml each of the potential isolate of bacteria was added to 100 ml of nutrient agar broth and amended with six fungicides *viz*., mancozeb, propiconazole, hexaconozole, tebuconazole, carbendazim + mancozeb and trifloxystrobin + tebuconozole at recommended concentration. The experiment was replicated four times. The flask containing NA medium without fungicide served as control and were incubated at 28 ± 1 °C in an orbital shaker. The optical density values of the culture broth were determined in Spectrophotometer at 610 nm after 48 hrs (Archana, 2012) [3].

List of fungicides used in the present study

Common name	Trade name	Concentration (%)
Mancozeb	Dithane M-45	0.25%
Propiconazole	Tilt	0.1%
Hexaconozole	Contaf	0.1%
Tebuconazole	Folicur	0.1%
Carbendazim + Mancozeb	Saaf	0.1%
Trifloxystrobin + Tebuconozole	Nativo	0.1%

Screening of potential bacteria for growth promoting activities *in vitro* Germination (%)

The laboratory test for germination percentage was conducted as per the ISTA rules (ISTA, 2016) [6] by adopting paper towel method with un inoculated and inoculated test pathogen (*E. turcicum*). Three replications of 100 seeds each treated with compatible fungicide carbendazim + mancozeb (0.1%) and effective phyllosphere bacterial isolates (P_9 and P_{16}). The seeds after imposing different treatments were placed in seed germinator and maintained at constant temperature of 25 ± 0.5^{0}C and more than 90 per cent relative humidity. On the day of final count *i.e.* 14th day, the number of seeds germinated was counted and the per cent germination was calculated as follows

$$\text{Germination \%} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds in sample}} \times 100$$

Seedling length (cm)

Ten normal seedlings in each treatment were randomly selected from the germination test for measuring the seedling length on 14th day of germination test. The seedling length was measured from the shoot tip to the primary root tip. The mean of seedling length of ten seedlings was expressed in centimetres.

Seedling vigour index

The seedling vigour indices were calculated as per the method suggested by Abul-Baki and Anderson (1973) as given below.
Seedling vigour index = Germination (%) × Seedling length (cm)

Results and Discussion
Compatibility of fungicides with phyllosphere bacteria
Compatibility of phyllosphere bacteria *in vitro*

Six fungicides at recommended concentrations were evaluated *in vitro* for its compatibility with the bacterial biocontrol agent, isolate P_{16} by turbidomertic method.

The bacterial antagonist isolate (P_{16}) recorded maximum growth amended medium with carbendazim + mancozeb OD value of 2.54 and was significantly superior when compared to other treatments. Propiconozole + isolate P_{16}, hexaconozole + isolate P_{16}, mancozeb + isolate P_{16}, and native + isolate P_{16} were on par with each other. Lowest turbidity was observed in hexaconazole treatment (OD value 0.02).

These results are in agreement with Archana *et al.*, 2012 [3] who reported that compatibility of azoxystrobin with bacterial biocontrol agents could be attributed for the reason that it acts on mitochondria as an inhibitor of electron transfer and mitochondria are not present in bacteria.

Kataria *et al.* (2002) [7] reported that lower rates of azoxystrobin applied as seed treatment in combination with *P. fluorescens* (W36) showed better antagonist interaction against *Rhizoctonia solani* Kuhn. in bean and cucumber.

Compatibility study of potential phyllosphere bacteria (P16) with fungicides by turbidometric method

S. No	Treatments	Conc. (%)	Optical density value at 610 nm*
1.	Mancozeb + P_{16}	0.25%	1.75
2.	Propiconazole + P_{16}	0.1%	0.06
3.	Hexaconozole + P_{16}	0.1%	0.02
4.	Tebuconazole + P_{16}	0.1%	2.08
5.	Carbendazim + Mancozeb + P_{16}	0.1%	2.54
6.	Trifloxystrobin + Tebuconozole + P_{16}	0.1%	1.70
7.	Control + P_{16}	-	1.39
	C.D.		0.07
	SE(m) ±		0.02
	C.V.		3.55

*Average of four replications

Screening of potential antagonists for their growth promoting activities under *in vitro*
Vigour index

The seedling vigour and germination per cent significantly increased on seed treatment. The germination per cent and seedling vigour in control (water) was 76.66 per cent and 1353.81 which significantly increased 95.22 per cent and 2394.78 respectively. when treated with effective fungicide carbendazim + mancozeb + isolate P_9 + isolate P_{16}. The treatments isolate P_9 + isolate $P_{16,}$ carbendazim + mancozeb + isolate P_9 and carbendazim + mancozeb + isolate P_{16} were found on par with each other with respect to germination per cent. Treatments isolate P_9 + isolate P_{16} and treatment carbendazim + mancozeb + isolate P_9 + isolate P_{16} were found on par with respect to vigour index.

In general, the seeds when inoculated with the pathogen showed reduction in vigour index and germination per cent compared to un inoculated treatments. Seedling vigour was highest in *E. turcicum* + carbendazim + mancozeb + isolate P_9 + isolate P_{16} (1283.92) which was significantly superior over other treatments. Lowest vigour index was recorded in the treatment *E. turcicum* + carbendazim + mancozeb (716.84) compared to inoculated control (421.68).

In the present study, significant increase in seedling vigour was recorded in seed treatment with carbendazim + mancozeb + isolate P_9 + isolate P_{16} which may be attributed to increase in root and shoot length by production of growth stimulating factors.

Nandakumar *et al.* (2001) [12] found two strains of *P. fluorescens* (PF1 and PF7), inhibiting the mycelia growth of rice sheath blight fungus, *R. solani*, besides enhancing the seedling vigour of rice plants *in vitro*.

Similar results were also reported by Bharathi *et al*. (2004) [4] in chillies crop. The efficacy of thirteen plant growth promoting antagonistic rhizobacterial strains were evaluated against chilli fruit rot and dieback incited by *Colletotrichum capsici*. Among them *P. fluorescens* (Pf1) and *B. subtilis* were found to be effective in increasing the seed germination and seedling vigour.

Screening of potential antagonists for their growth promoting activities *in vitro*

S. No	Treatments	Germination (%)	Seedling length (cm)	Vigour Index
1.	Isolate P_9	92.16* (64.63)	21.50	1981.44
2.	Isolate P_{16}	91.33 (63.42)	20.22	1846.69
3.	Isolate P_9 + isolate P_{16}	94.66 (67.50)	24.65	2333.36
4.	Carbendazim + Mancozeb + isolate P_9	93.33 (66.78)	23.00	2146.59
5.	Carbendazim + Mancozeb + isolate P_{16}	92.66 (65.14)	22.91	2122.84
6.	Carbendazim + Mancozeb + isolate P_9 + Isolate P_{16}	95.22 (70.61)	25.15	2394.78
7.	Carbendazim + Mancozeb	88.66 (61.56)	19.66	1743.05
8.	Control	76.66 (48.42)	17.66	1353.81
	C.D.	3.86	1.89	190.54
	SE (m) ±	1.27	0.62	63.01
	C.V.	2.44	4.96	5.48

*Figures in parentheses indicate angular transformed value.

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