Compatibility of fungicides with phyllosphere bacteria of maize

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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. Compatibility of phyllosphere bacteria with Six fungicides viz., mancozeb, propiconazole, hexaconazole, tebuconazole, carbendazim + mancozeb and trifloxystrobin + tebuconozole at recommended concentrations were evaluated in vitro by turbidometric method. The bacterial antagonist isolate (P16) 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 percentage significantly increased on seed treatment. The germination percentage and seedling vigour to 95.22 percent and 2394.78 respectively when treated with effective fungicide carbendazim + mancozeb + isolate P9 + isolate P16.

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. Compatibility of phyllosphere bacteria with Six fungicides viz., mancozeb, propiconazole, hexaconazole, tebuconazole, carbendazim + mancozeb and trifloxystrobin + tebuconozole at recommended concentrations were evaluated in vitro by turbidometric 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 °C 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

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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, hexaconazole, tebuconazole, carbendazim + mancozeb and trifloxystrobin + tebuconazole 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].

| Common name      | Trade name    | Concentration (%) |
|------------------|---------------|-------------------|
| Mancozeb         | Dithane M-45  | 0.25%             |
| Propiconazole    | Tilt          | 0.1%              |
| Hexaconazole     | Contal        | 0.1%              |
| Tebuconazole     | Folicur       | 0.1%              |
| Carbendazim + Mancozeb | Saaf     | 0.1%              |
| Trifloxystrobin + Tebuconazole | 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 (P16 and P16). The seeds after imposing different treatments were placed in seed germinator and maintained at constant temperature of 25 ± 0.5°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 P16 by turbidimetric method. The bacterial antagonist isolate (P16) recorded maximum growth amended medium with carbendazim + mancozeb OD value of 2.54 and was significantly superior when compared to other treatments. Propiconazole + isolate P16, hexaconazole + isolate P16, mancozeb + isolate P16, and native + isolate P16 were on par with each other. Lowest turbidity was observed in hexaconazole treatment (OD value 0.02).

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

| S. No | Treatments                        | Conc. (%) | Optical density value at 610 nm* |
|-------|----------------------------------|-----------|---------------------------------|
| 1.    | Mancozeb + P16                   | 0.25%     | 1.75                            |
| 2.    | Propiconazole + P16              | 0.1%      | 0.06                            |
| 3.    | Hexaconazole + P16               | 0.1%      | 0.02                            |
| 4.    | Tebuconazole + P16               | 0.1%      | 2.08                            |
| 5.    | Carbendazim + Mancozeb + P16     | 0.1%      | 2.54                            |
| 6.    | Trifloxystrobin + Tebuconazole + P16 | 0.1% | 1.70                            |
| 7.    | Control + P16                    | -         | 1.39                            |

*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 P9 + isolate P16. The treatments isolate P9 + isolate P16, carbendazim + mancozeb + isolate P9 and carbendazim + mancozeb + isolate P16 were found on par with each other with respect to germination per cent. Treatments isolate P9 + isolate P16 and treatment carbendazim + mancozeb + isolate P9 + isolate P16 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 P9 + isolate P16 (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 P9 + isolate P16 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 (PFI 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

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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 P₉ | 92.16* (64.63)  | 21.50                | 1981.44      |
| 2.    | Isolate P₁₆ | 91.33 (63.42)   | 20.22                | 1846.69      |
| 3.    | Isolate P₉ + isolate P₁₆ | 94.66 (67.50) | 24.65                | 2333.36      |
| 4.    | Carbendazim + Mancozeb + isolate P₉ | 93.33 (66.78) | 23.00                | 2146.59      |
| 5.    | Carbendazim + Mancozeb + isolate P₁₆ | 92.66 (65.14) | 22.91                | 2122.84      |
| 6.    | Carbendazim + Mancozeb + isolate P₉ + Isolate P₁₆ | 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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