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# *In vitro* Efficacy of Fungicides and Bioagents against Early Blight of Tomato caused by *Alternaria solani*

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**A B S T R A C T**

*In vitro* evaluation of three contact and five systemic and two combination was carried out under laboratory condition in 2018-19 at Department of Plant Pathology, College of Horticulture, Mysuru by using Potato Dextrose Agar medium (PDA) as basal medium and six biocontrol agents were evaluated through dual culture technique. The result revealed that, there was significant difference among the fungicides in inhibiting the radial growth of the *A. solani.* Among the different contact fungicides evaluated against test fungi the least mean per cent inhibition of 78.61% was recorded in Mancozeb75%WP and it was found significantly superior over other two fungicides. The least per cent inhibition was noticed in case of Mandipropamid 23.4% SC which has recorded 18.80% inhibition. Among the five systemic and two combination fungicides evaluated against *A. solani*, Hexaconazole @ 0.1% and 0.15% has recorded maximum inhibition of mycelial growth of test fungi and which has recorded 86.22% inhibition followed by 85.00% inhibition was recorded in Trifloxystrobin 25 + Tebuconazole 50% WG @ 0.15% . The least percent inhibition of 53.99% was recorded in Pyraclostrobin 20%WG @ 0.05%. The results also indicated that the per cent inhibition was increased by increasing the concentration of fungicides tested. Among the different antagonistic organisms evaluated maximum inhibition of mycelial growth of *A. solani* was recorded in *Trichoderma asperellum* (69.69 %) followed by *Trichoderma harzianum* (67.51%). Among the four bacterial bio agents *Bacillus vezelensis* has recorded 62.76 % inhibition followed by *Pseudomonas putida* (43.13%) and the least per cent inhibition of mycelial growth was noticed in *Pseudomonas fluorescens* (42.19%) at 5 Days after incubation.

## Introduction

Tomato (*Solanum lycopersicum*) is one of the important and common vegetable grown in world. Tomatoes are the major dietary source of the antioxidant lycopene, which has been linked to many health benefits including reduced risk of heart disease and cancer. They are also great source of Vitamin C, Vitamin K, Potassium and Folate. The crop is grown for table purpose as well as for development of processed products like Ketchup, Juice, soup *etc.* In India it was estimated that it was grown in an area of 0.8 M ha and 19.00 MT

and productivity of 23.75 t/ha (Anonymous, 2018). In Karnataka it was grown mainly in southern parts of the state which include Kolar, Chikkaballapura, Bengaluru rural, Chamarajanagara, Mysuru, Hassan and Mandya. It occupies an area of 0.06 MHa with the production of 2.08 MT and productivity of 32.40 t/ha (Anonymous, 2018).

Tomato early blight caused by *Alternaria solani* (Ellis and martin) Jones and Grout is one of the most common tomato diseases occurring nearly every season in all tomato growing areas. It affects all aerial parts of the plant including leaves, fruits and stem. It was estimated that disease can leads yield loss up to 78% (Datar and Mayee, 1978, Chandravanshi *et al.,* 1994). Irregular brown spots with concentric rings on the lower leaves are the typical symptoms of early blight on leaves (Vloutoglou and Kalogerakis, 2000). It occasionally attacks the fruit, producing large sunken black spots at the stem end, which drop prematurely leading to a sever yield loss.

So, attempt was made to evaluate some fungicides and bio agents under *In vitro* condition to further utilize them in field condition for the integrated management of disease.

## Materials and Methods

The present study was carried out under laboratory condition in 2018-19 at Department of Plant Pathology, College of Horticulture, Mysuru. *In vitro* evaluation of three contact and five systemic and two combination fungicides were evaluated at different concentrations through poison food technique (Nene and Tapliyal, 1993; Roopa *et al.,* 2014) by using Potato Dextrose Agar medium (PDA) as basal medium and six biocontrol agents were evaluated through dual culture technique. 100 ml of potato dextrose agar medium was prepared in 250ml conical flask and medium was sterilized at 15lbs for 15 minutes at 121°C. Required quantity of test fungicides were calculated and added in the sterilized medium separately. Flasks containing poisoned medium were shaken well to have even and uniform distribution of the fungicides. About 20ml of poisoned PDA was poured in each of the sterilized petriplates and allowed to solidify. The plates were inoculated by pure culture of *Alternaria solani* by placing 5mm disc of 1week old pure culture. The disc was transferred aseptically to the petriplates containing the medium with test fungicides. The seven plates were maintained in contact fungicides for each treatment whereas; five plates were maintained in systemic and combination fungicides for each treatment.. The control plates without fungicide were also inoculated and incubated. The plates were kept for incubation at 26 ± 2°C temperature. The observations on colony diameter were recorded after 10 days.

The antagonistic potential of Bioagents *viz*., *Trichoderma asperellum, Trichoderma harzianum, Pseudomonas fluorescens, Pseudomonas putida, Bacillus subtilis and Bacillus vezelensis* were tested by dual culture technique (Utkhede and Rahe, 1983). For this 20 ml of sterilized, melted and cooled medium was poured in each petriplates and allowed to solidify and the plates were inoculated with 5 mm disc of 7 days old growth of fungal biocontrol agents with the help of sterilized cork borer and subsequently inoculated with 5 mm disc of 7 days old culture of *Alternaria solani* a opposite corner of the plates, keeping 15 mm distance from periphery. The bacterial antagonists were streaked with the help of sterilized inoculating loop at one end of the PDA petriplates. After 24 hrs of incubation just opposite to the bacterial streak 5mm disc of pathogen was

placed with the help of sterilized cork borer. The inoculation of pathogen alone on the center in the plates serves as control. Three replication of each treatment including the control were maintained. These plates were incubated at 26± 2°C in BOD incubator.

The percent inhibition mycelial growth of fungus was calculated by using the formula given by Vincent (1947).

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

Where,

I- Percent inhibition of mycelial growth
C- Growth of mycelium in control
T- Growth of mycelium in treatment

## Results and Discussion

In the absence of resistant cultivars, use of fungicides to manage the disease is the best alternative when the disease is epidemic. The fungicides has to be used judiciously according to need of the situation and pathogen. Availability of new group of chemicals necessitates evaluation under *in vitro* conditions to know their efficacy and initiate spray schedule in field conditions.

The result clearly indicates that there was significant difference among the contact fungicides in inhibiting the radial growth of the *A. solani*. Among the different contact fungicides evaluated against test fungi Mancozeb (78.61%) was significantly superior over other two fungicides. Chlorothalonil has recorded 36.57 % inhibition and the least inhibition was noticed in case of Mandipropamid which has recorded 18.80% inhibition. Among the different concentration of contact fungicides evaluated against *A. solani*, Mancozeb at 0.25% recorded maximum inhibition of 80.83% followed by Chlorothalonil (40.56%) and least was recorded in Mandipropamid (26.39%) (Fig. 1–5 and Table 1–3).

**Table.1** *In vitro* evaluation of contact fungicides against *Alternaria solani*

| Fungicides | Per cent inhibition of mycelial growth | | | Mean |
|---|---|---|---|---|
| | Concentration | | | |
| | 0.15 % | 0.20% | 0.25% | |
| Chlorothalonil 75% WP | 29.72 (32.94) | 39.44 (38.81) | 40.56 (39.56) | 36.57 (37.10) |
| Mancozeb 75% WP | 75.28 (60.40) | 79.72 (63.13) | 80.83 (64.04) | 78.61 (62.52) |
| Mandipropamid 23.4% SC | 9.44 (17.77) | 20.56 (26.73) | 26.39 (30.90) | 18.80 (25.13) |
| Mean | 38.15 (37.04) | 46.57 (42.89) | 48.52 (44.40) | |

| | Fungicides | Concentration | Fungicides x Concentration |
|---|---|---|---|
| S Em± | 0.14 | 0.14 | 0.23 |
| CD @1% | 0.51 | 0.51 | 0.88 |

* Figures in the parenthesis are angular transformed values

**Table.2** *In vitro* evaluation of systemic and combination fungicides against *Alternaria solani* at 10DAI

| Fungicides | Per cent inhibition of mycelial growth at 10DAI | | | Mean |
|---|---|---|---|---|
| | Concentration % | | | |
| | 0.05 | 0.10 | 0.15 | |
| **Difenoconazole 25%EC** | 74.10 (59.41) | 76.94 (61.31) | 77.78 (61.88) | 76.28 (60.87) |
| **Hexaconazole 5% EC** | 86.11 (68.12) | 86.22 (68.21) | 86.22 (68.21) | 86.15 (68.15) |
| **Pyraclostrobin 20%WG** | 53.99 (47.29) | 58.33 (49.80) | 58.89 (50.12) | 57.07 (49.07) |
| **Tetraconazole 3.8%EW** | 77.66 (61.81) | 80.83 (64.06) | 82.78 (65.50) | 80.42 (63.79) |
| **Thiafluzamide 24%SC** | 56.76 (48.91) | 71.39 (57.67) | 70.56 (57.14) | 66.23 (54.57) |
| **Fluxapyraxad 250 + Pyraclostrobin 250 SC** | 76.57 (61.06) | 79.72 (63.24) | 80.56 (63.84) | 78.95 (62.72) |
| **Trifloxystrobin 25 + Tebuconazole 50% WG** | 82.39 (65.21) | 83.33 (65.91) | 85.00 (67.23) | 83.57 (66.11) |
| **Mean** | 72.53 (58.84) | 76.67 (61.45) | 77.38 (61.97) | |

| | Fungicides | Concentration | Fungicides x Concentration |
|---|---|---|---|
| **S Em±** | 0.20 | 0.31 | 0.54 |
| **CD @1%** | 0.76 | 1.16 | 2.01 |

* Figures in the parenthesis are angular transformed values

**Table.3** *In vitro* evaluation of bio agents against *Alternaria solani*

| Fungicides | Per cent inhibition of mycelial growth | |
|---|---|---|
| | **3 DAI** | **5 DAI** |
| ***Bacillus subtilis*** | 29.74 (33.02) | 42.49 (41.91) |
| ***Bacillus vezelensis*** | 46.79 (43.16) | 62.76 (55.36) |
| ***Pseudomonas fluorescens*** | 21.00 (27.22) | 42.19 (40.50) |
| ***Pseudomonas putida*** | 38.46 (38.30) | 43.13 (41.05) |
| ***Trichoderma asperellum*** | 62.98 (49.11) | 69.69 (56.65) |
| ***Trichoderma harzianum*** | 63.91 (53.30) | 67.51 (55.27) |
| **SEm±** | 1.22 | 1.10 |
| **CD @ 1%** | 4.90 | 4.41 |

* Figures in the parenthesis are angular transformed values

**Fig.1** *In vitro* evaluation of non systemic fungicides against *Alternaria solani*


**Fig.2** *In vitro* evaluation of systemic fungicides against *Alternaria solani*

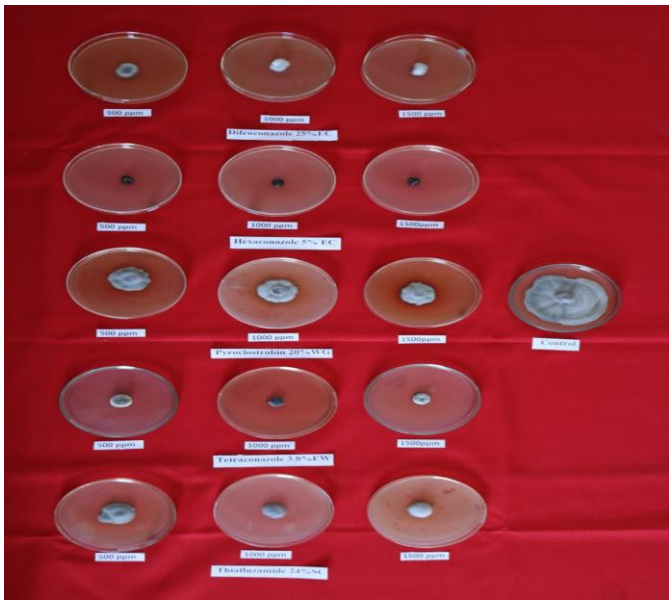


**Fig.3** *In vitro* evaluation of combination fungicides against *Alternaria*

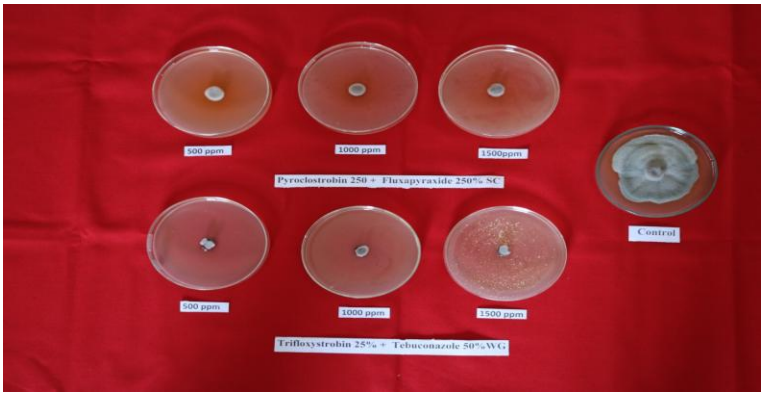

**Fig.4** *In vitro* evaluation of fungal bio agents against *Alternaria solani*


**Fig**.**5** *In vitro* evaluation of bacterial bio agents against *Alternaria solani*


Among the five systemic and two combination fungicides evaluated against *A.solani* Hexaconazole @ 0.1% and 0.15% has recorded maximum inhibition of mycelial growth of test fungi (86.22%) followed by 85.00% inhibition was recorded in Trifloxystrobin 25 + Tebuconazole 50% WG @ 0.15%. Among the other systemic and combination fungicides evaluated Tetraconazole 3.8%EW @ 015% recorded 82.78% and Fluxapyraxad 250 + Pyraclostrobin 250 SC @ 0.15% recorded 80.56% per cent inhibition. The least percent inhibition of 53.99% was recorded in Pyraclostrobin 20%WG @ 0.05%. In overall among the different systemic and combination fungicides maximum mean inhibition of mycelia of *A. solani* was recorded in Hexaconazole (86.15%), followed by 83.57% in Trifloxystrobin 25 + Tebuconazole 50% WG and least of 57.07% was recorded in Pyraclostrobin20%WG (Table. 2). The results also indicated that the inhibition percentage was increased by increasing the concentration fungicides tested. The results are in agreement with results of Roopa *et al.*, 2014, Arun kumar 2006, reported the effectiveness of Mancozeb@ 0.2% and Hexaconazole @0.1% in inhibiting the mycelial growth of test fungi.

Six antagonistic agents evaluated against *A. solani* through dual culture technique as mentioned in material and methods. The results of the study were presented in Table 3.

Results clearly shows that among the different antagonistic organisms evaluated maximum inhibition of mycelial growth of *A. solani* was recorded in *Trichoderma asperellum* (69.69 %) followed by *Trichoderma harzianum* (67.51%). Among the four bacterial bio agents evaluated against *A. solani*, *Bacillus vezelensis* has recorded 62.76 % followed by *Pseudomonas putida* (43.13%) and the least per cent inhibition of mycelial growth was noticed in *Pseudomonas fluorescens* (42.19%) at 5 days after incubation. The effectiveness of *Trichoderma harzianum*, *Trichoderma viride* against *A. solani* was reported by Babu *et al.*, 2000 and Yadhav *et al.*, 2018.

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