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**Kamalini Thakur**  
Department of Plant  
pathology, Dr. Panjabrao  
Deshmukh Krishi Vidyapeeth,  
Akola, Maharashtra, India

**Dr. SS Mane**  
Department of Plant  
Pathology, Dr. Panjabrao  
Deshmukh Krishi Vidyapeeth,  
Akola, Maharashtra, India

**Dr. SB Brahmkar**  
Department of Plant  
Pathology, Dr. Panjabrao  
Deshmukh Krishi Vidyapeeth,  
Akola, Maharashtra, India

**Dr. ST Ingle**  
Department of Plant  
Pathology, Dr. Panjabrao  
Deshmukh Krishi Vidyapeeth,  
Akola, Maharashtra, India

**Corresponding Author:**  
**Kamalini Thakur**  
Department of Plant  
Pathology, Dr. Panjabrao  
Deshmukh Krishi Vidyapeeth,  
Akola, Maharashtra, India

## *In vitro* evaluation of fungicides efficacy against *Pythium aphanidermatum* causing damping off of tomato

**Kamalini Thakur, Dr. SS Mane, Dr. SB Brahmkar and Dr. ST Ingle**

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### Abstract

Damping-off caused by *Pythium aphanidermatum* is one of the most serious diseases of tomato in nursery and field which causes seedlings to topple down and die consequently. In the present study different fungicides were tested for their efficacy against *Pythium aphanidermatum* *in vitro*. Among the eleven fungicides evaluated, Tebuconazole 25.9% W/W, Carbendazim 50% WP and Carbendazim 12%+Mancozeb 63% WP were most effective as they completely inhibited the mycelial growth of *P. aphanidermatum* at all concentrations (0.1%, 0.2%, 0.3%). The least effective was found to be Tetraconazole 3.8% W/W (49.66%, 61.52%, 65.11) and Mancozeb 75% WP (51.11%, 72.72%, 87.57%) at (0.1%, 0.2%, 0.3%) respectively.

**Keywords:** *Pythium aphanidermatum*, damping off, tomato, fungicides

### Introduction

Tomato is one of the most important warm season vegetables grown all over the world. It belongs to Solanaceae family and is a self-pollinated crop. Tomato is considered as 'protective food' as it is rich in vitamins (A, B1, B2, B6, biotin, folic acid, C, E and K), antioxidants and carbohydrates. Tomato is grown worldwide on an area of 49,17,735 hectares and annual production is estimated around 18,61,07,972.48 tonnes. (FAOSTAT, 2022) <sup>[1]</sup>. The largest producer of tomato is China followed by India, Turkey, United States and Egypt. In India the tomato covers an area of 8,43,000 hectares and annual production is around 2,06,94,000 tonnes. (FAOSTAT, 2022) <sup>[1]</sup>.

More than 200 pests and diseases are known to attack tomato crop and among them damping off is one of the most important diseases that cause serious losses in nurseries. Damping off causes 25-75% losses per year, depending on the host type and environmental variables (Adhikari *et al.*, 2024) <sup>[2]</sup>. Damping-off disease caused by *Pythium* species kills more than 60% of seedlings in the nursery and main field (Manoranjitham *et al.*, 2001) <sup>[8]</sup>.

### Materials and Methods

#### Isolation of pathogen

Tomato seedlings showing damping off symptoms were collected from field for isolating the pathogen. Diseased collar portion were cut into small bits of 2-3mm size and rinsed in tap water. Bits were then dipped in 0.1% sodium hypochlorite for 30 seconds for surface sterilization. This was followed by rinsing bits three times in sterilized distilled water and then placing them on paper towel to remove excess moisture. These bits were placed on potato dextrose agar medium contained in petri plates. The plates were incubated at 25 ± 2°C and checked regularly. The fungal colonies developed around each bit were examined and sub cultured. Based on the morphological and cultural characteristics the fungus was identified as *Pythium aphanidermatum*. By hyphal tip method the pure culture of *P. aphanidermatum* was maintained on PDA slants and used for further studies.

***In vitro* evaluation of fungicides:** The eleven fungicides *viz.* Tetraconazole, Tebuconazole, Azoxystrobin, Propiconazole + Difenconazole, Mancozeb, Carbendazim, Thiophanate methyl, Propineb, Metalaxyl, Carbendazim + Mancozeb, Benalaxyl-M 4% + Mancozeb were

evaluated at 3 concentrations viz., 0.1%, 0.2% and 0.3%, *in vitro* by poisoned food technique for their efficacy to inhibit the fungal growth.

Potato Dextrose Agar (PDA) medium was used as basal culture medium. 100 ml of PDA was poured in each 250 ml Erlenmeyer flask and then autoclaved. The sterilized PDA media was amended using three concentration (0.1%, 0.2% and 0.3%) of each fungicide. The flasks containing molten poisoned media were shaken well to mix fungicides uniformly. Poisoned PDA from each flask was then poured in sterilized petri plate (15-20 ml / plate), each replicated thrice and allowed to solidify at room temperature. After solidification, a 5 mm disc of about one week old fungus culture was placed at the center of the PDA plates. Poisoned PDA plates were incubated at 25±2 °C for seven days. The petri plates without the poisoned PDA served as control and incubated in same conditions as poisoned PDA plates were incubated.

Observation on radial mycelial growth / colony diameter of the *P. aphanidermatum* was recorded at 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> DAI. Percent of mycelial growth inhibition of the pathogen with the test fungicides on 7<sup>th</sup> DAI over the untreated control were calculated (Vincent, 1947) <sup>[10]</sup>.

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,

C = Growth of the test fungus in untreated control plates.

T = Growth of the test fungus in treated plates

## Results and discussion

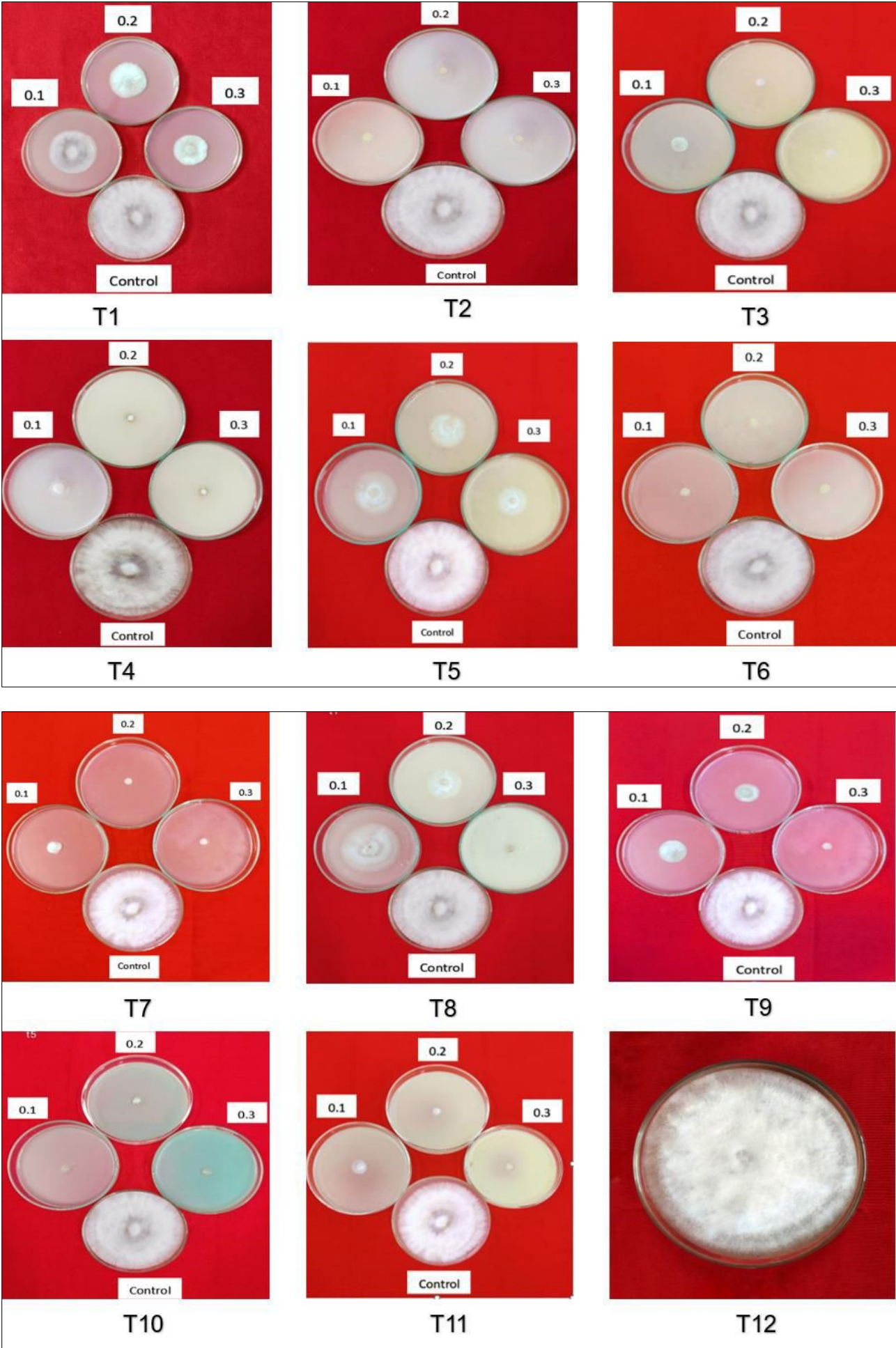
***In vitro* evaluation of fungicides:** *In vitro* efficacy of the fungicides was evaluated against *Pythium aphanidermatum*. The efficacy of fungicides was evaluated at 0.1%, 0.2% and 0.3% concentration based on the colony diameter and reduced mycelium growth on PDA. The results presented in Table 1 showed that among the eleven fungicides evaluated, T<sub>2</sub> (Tebuconazole 25.9%), T<sub>5</sub> (Carbendazim 50%), T<sub>12</sub> (Carbendazim 12%+Mancozeb 63%) were most effective as they inhibited the mycelial growth of *P. aphanidermatum* at all concentrations (0.1, 0.2, 0.3). The next effective treatments that inhibited mycelial growth at 0.2% and 0.3% conc. were T<sub>3</sub> (Azoxystrobin 23%) T<sub>7</sub> (Thiophanate methyl 70%) and T<sub>4</sub> (Propiconazole 13.9% + Difenconazole 13.9%). Fungicides that completely inhibited the mycelial growth at 0.3% conc. are T<sub>8</sub> (Propineb 70%), T<sub>9</sub> (Metalaxyl 35%) and T<sub>11</sub> (Benalaxyl-M4%+Mancozeb 63%). The least effective fungicide against *P. aphanidermatum* was found to be T<sub>1</sub> (Tetraconazole 3.8%) which showed 49.66%, 61.52% and 65.11% inhibition of mycelial growth at 0.1%, 0.2% and 0.3% respectively. T<sub>1</sub> was followed by T<sub>5</sub> (Mancozeb 75%) in least effectiveness against *P. aphanidermatum* which gave 51.11%, 72.72% and 87.57% inhibition at 0.1, 0.2 and 0.3% conc. respectively.

The results discussed above are in conformity with the Archana *et al.* (2020) <sup>[3]</sup> and Avinash *et al.*, (2018) <sup>[4]</sup> who reported cent percent inhibition by Tebuconazole 25.9%. Chavan *et al.* (2017) <sup>[6]</sup>, Avinash *et al.* (2018) <sup>[4]</sup> and Chaithra *et al.* (2019) <sup>[5]</sup> reported Carbendazim 50% to give cent percent inhibition of mycelial growth. Carbendazim 12%+Mancozeb 63% was reported to give cent percent inhibition of mycelial growth by Chavan *et al.* (2017) <sup>[6]</sup>, Chaithra *et al.* (2019) <sup>[6]</sup> and Archana *et al.* (2020) <sup>[3]</sup>.

**Table 1:** Comparative efficacy of different fungicides on the growth of *P. aphanidermatum* at various conc. *in vitro*.

| Sr. No. | Fungicides                                   | Mycelial growth(mm)* |       |       | Percent growth inhibition* |       |       |
|---------|----------------------------------------------|----------------------|-------|-------|----------------------------|-------|-------|
|         |                                              | Concentration        |       |       | Concentration              |       |       |
|         |                                              | 0.1%                 | 0.2%  | 0.3%  | 0.1%                       | 0.2%  | 0.3%  |
| 1.      | Tetraconazole 3.8% W/W                       | 45.33                | 34.63 | 31.40 | 49.66                      | 61.52 | 65.11 |
| 2.      | Tebuconazole 25.9% W/W                       | 0.00                 | 0.00  | 0.00  | 100                        | 100   | 100   |
| 3.      | Azoxystrobin 23% SC                          | 8.35                 | 0.00  | 0.00  | 90.72                      | 100   | 100   |
| 4.      | Propiconazole 13.9% + Difenconazole 13.9% EC | 13.55                | 0.00  | 0.00  | 85.00                      | 100   | 100   |
| 5.      | Mancozeb 75% WP                              | 44.00                | 24.55 | 11.18 | 51.11                      | 72.72 | 87.57 |
| 6.      | Carbendazim 50% WP                           | 0.00                 | 0.00  | 0.00  | 100                        | 100   | 100   |
| 7.      | Thiophanate methyl 70% WP                    | 8.44                 | 0.00  | 0.00  | 90.62                      | 100   | 100   |
| 8.      | Propineb 70% WP                              | 52.75                | 8.17  | 0.00  | 41.38                      | 90.92 | 100   |
| 9.      | Metalaxyl 35% WS                             | 16.42                | 10.45 | 0.00  | 81.75                      | 88.38 | 100   |
| 10.     | Carbendazim 12% + Mancozeb 63% WP            | 0.00                 | 0.00  | 0.00  | 100                        | 100   | 100   |
| 11.     | Benalaxyl- M 4% + Mancozeb 65% WP            | 8.26                 | 6.55  | 0.00  | 90.82                      | 92.72 | 100   |
| 12.     | Control                                      | 90.00                | 90.00 | 90.00 |                            |       |       |
|         | F test                                       | Sig                  | Sig   | Sig   |                            |       |       |
|         | SE(m) ±                                      | 0.35                 | 0.14  | 0.12  |                            |       |       |
|         | CD (P= 0.01)                                 | 1.42                 | 0.56  | 0.49  |                            |       |       |

\*Mean of three replications



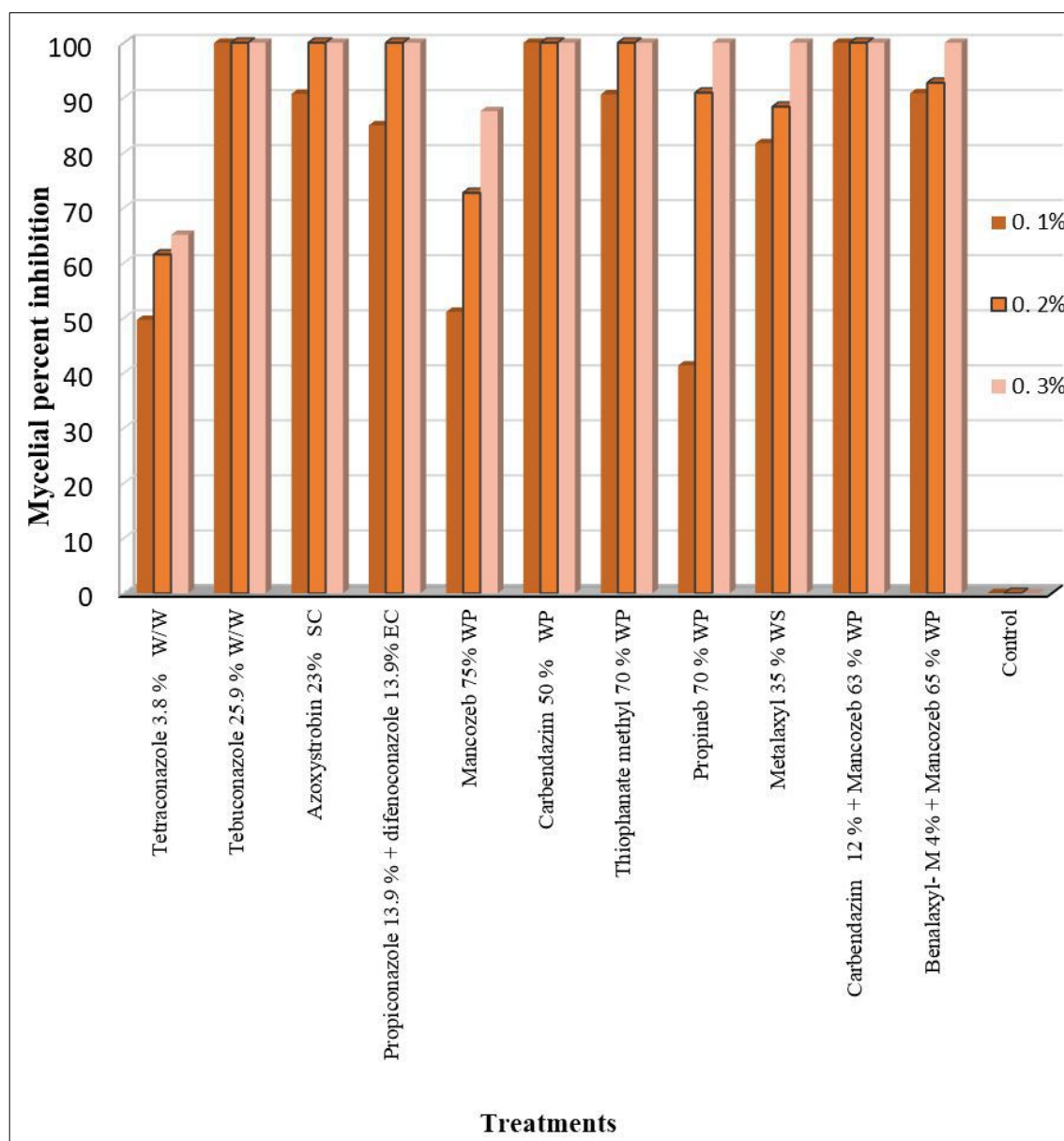


**Fig 1:** Efficacy of fungicides *in vitro* against *P. aphanidermatum* by poisoned food technique

Similar results were found by Prasanna *et al.* (2019) [9] who reported Metalaxyl M to show 83.89% and 90.56% inhibition at 0.1% and 0.2% concentration. Jyoti *et al.* (2019) [7] observed 82.94% average inhibition of mycelial growth by Thiophanate methyl. Chavan *et al.* (2017) [6] reported Mancozeb to give 64.96%, 70.72% and 76.12% inhibition at 0.15%, 0.2% and 0.3% conc. respectively and Azoxystrobin to give 90.70%, 92.09% and 100% inhibition at 0.05%, 0.1% and 0.2% conc. respectively.

### Conclusion

In the research carried it was found that among the eleven fungicides evaluated *in vitro* against *Pythium aphanidermatum* Tebuconazole 25.9% W/W, Carbendazim 50% WP and Carbendazim 12%+Mancozeb 63% WP were most effective as they completely inhibited the mycelial growth of *P. aphanidermatum* at all concentrations (0.1%, 0.2%, 0.3%). The least effective was found to be Tetraconazole 3.8% W/W (49.66%, 61.52%, 65.11) and Mancozeb 75% WP (51.11%, 72.72%, 87.57%) at (0.1%, 0.2%, 0.3%) respectively.

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