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## *In-vitro* evaluation of different fungicides and bioagents against *Fusarium oxysporum* F. sp. *capsici*

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### Abstract

An experiment was conducted at Plant Pathology Section, College of Agriculture, Nagpur during 2023-2024. Chilli is one of the most important spices used in the world. It is attacked by *Fusarium* wilt and causes huge losses, it is essential to develop effective control strategies. *Fusarium* wilt of chilli caused by *Fusarium oxysporum* f. sp. *capsici* has been emerging as one of the biotic threats in profitable cultivation of chilli crop. This study presents an *in vitro* evaluation of different fungicides and bioagents against *Fusarium oxysporum* f. sp. *capsici*, with specific focus on their potential efficacy of managing *Fusarium* wilt of chilli. The fungicides evaluated *in vitro* against *Fusarium oxysporum* f. sp. *capsici* were significantly reduced the mycelial growth. Among that Iprodione 50% WP, Tebuconazole 25.9% EC and Carbendazim 12% + Mancozeb 63% WP were found most effective with maximum growth inhibition (100%). Antagonist tested against *Fusarium oxysporum* f. sp. *capsici* *in vitro* significantly reduced the growth of test pathogen. Among that *Trichoderma harzianum* inhibited (82.34%) and *Trichoderma viride* (80.91%) found to be most effective with highest mycelial growth.

**Keywords:** *Fusarium*, fungicides, bio-agents, *Trichoderma* spp

### 1. Introduction

Chilli (*Capsicum annum*) is the major economic crop commercially cultivated in India. It is grown in all the regions of country. Production of chilli comes in 400 dissimilar species from all over the world. Chilli crop comes under *solanaceae* family which is a significant vegetable category. The origin is said to be in tropical America specifically from Brazil (Thompson and Kelly, 1957) [13]. Chilli is important spice crop of Indian diet consumed as fresh or processed. Chilli is a tropical and subtropical plant that requires a mix of warm, humid, and dry temperatures to grow. Warm, humid conditions is required during the growth period. India is the largest producer and exporter of chilli, which is grown over an area of 721.15 ha, with production of 1689.52 T and productivity of 2343 kg/ha. (Anonymous, 2019) [1]. The major states growing chilli are Andhra Pradesh, Orisha, Maharashtra, West Bengal, Karnataka, Rajasthan and Tamil Nadu. Andhra Pradesh stands first in chilli production, with maximum acreage under chilli cultivation in the country (Anonymous, 2019) [1]. During the Kharif and Rabi seasons *Fusarium* wilt disease was observed to inflict substantial loss in several Chilli fields. The damage due to *Fusarium* wilt disease are particularly serious in tropical region with symptoms including rolling of leaves, stunting of plants, twig death and withering of upper leaves followed by injury to the vascular system of plant particularly at lower stem and root. The sick plant were found in patches and overall crop production was lowered. The yield loss due to the disease reported were 10-80 percent, worldwide (Loganathan *et al.*, 2013) [4], depending on the prevailing climatic conditions. The pathogen attack on immature root as they grow and stem vessels blocks the water and nutrient transmission as a result the crop plant vascular system is destroyed resulting in plant death. (Suryanto *et al.* 2010) [12]. With these aspects in mind as well as economic importance of the *Fusarium* wilt disease of Chilli present investigation were carried out.

### 2. Materials and Methods

The present investigation on "*In-vitro* Evaluation of different fungicides and bioagents against *Fusarium oxysporum* F. sp. *capsici*" was studied during 2023-2024 at Department of Plant Pathology, College of Agriculture, Nagpur.

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The details of the material and methods used are as follows.

## 2.1 Isolation

Based on visual observation, the plants showing typical symptoms (discolored vascular system) were collected and carried in the laboratory for isolation. Infected roots samples were cut into pieces with sterilized blade and disinfected with sodium hypochlorite solution for two minutes. Pieces were then washed with three changes of distilled water and bits were air dried and placed on sterilized solidified PDA medium. The four bits were placed in each plate. The plates were incubated at  $28 \pm 2$  °C temperature. The plates were examined regularly. When fungal growth starts to appear, the culture was purified by single hyphal tip technique. Identification of fungus was done with the help of compound microscope based on morphological features.

## 2.2 In-vitro evaluation of fungicides

Poisoned food technique was used to evaluate the efficiency of fungicides against pathogens. Potato dextrose agar medium was prepared and distributed at the rate of 100 ml in 250 ml conical flask, autoclaved  $1.05 \text{ kg/cm}^2$  for 15 min. Then before solidification of media different fungicides with desired concentration were incorporated aseptically in different flasks. These flasks shaken thoroughly and poured in Petri plates 20 ml/plate likewise three plates for each treatment were poured. One set of three plates was poured without any fungicides to serve as a control. After solidification of medium, the plates inoculated with eight days old pathogens separately. five mm diameter mycelial disc selected from peripheral growth of the plate by sterilized cork borer were used for inoculating the plates by keeping one disc per plate in the centre in inverted position, so as to make the mycelia growth touch the surface of medium. The inoculated plates were incubated at room temperature for seven days. The colony diameter of the fungal pathogens on medium was recorded and percent inhibition in each treatment was calculated (Vincent, 1947) [15] by using following formula.

$$\text{Percent inhibition (I)} = \frac{C - T}{T} \times 100$$

Where,

I = Percent inhibition of mycelial growth

C = Growth of mycelium in control (mm)

T = Growth of mycelium in treatment (mm)

## 2.3 In vitro evaluation of bio-agents by dual culture technique

Efficacy of four bioagents viz, *Trichoderma viride*, *Trichoderma harzianum*, *Psuedomonas fluorescens*, *Bacillus subtilis* were evaluated by dual culture technique. 15 ml of autoclaved and sterilized PDA was poured in sterilized petri plates and were allowed to solidify. Seven days old cultures of the antagonist and the pathogen were used for the study. Five mm diameter discs of culture of the test fungus and bioagents were cut out with sterilized cork borer. Then the two growth culture discs, test fungus and bioagents were placed at equidistance and exactly opposite with each other on sterilized solidified PDA medium in petri plates. Petri dishes with no antagonist served as control for the pathogen. These plates were incubated at  $27 \pm 2$  °C for seven days. For each treatment four replications were maintained. The extent antagonistic activity by bioagents were recorded after incubation period of 7 days by measuring the growth of the test pathogen in dual culture and in control plates. In case of bacterial bioagents pathogen was kept in the middle of bacterial antagonist.

Percent inhibition over control was worked out according to formula given by Vincent (1947) [15].

$$\text{Percent inhibition (I)} = \frac{C - T}{T} \times 100$$

Where,

I = Percent inhibition of mycelial growth

C = Growth of test fungus (mm) in control plate,

T = Growth of test fungus (mm) in treatment plates

## 3. Results and Discussions

The present study was carried out on chilli wilt caused by *Fusarium oxysporum* f. sp. *capsici*. The study includes isolation of pathogen, identification of the isolated pathogen, proving Koch's postulates (Pathogenicity test). In-vitro evaluation of fungicides and bioagents against *Fusarium oxysporum* f. sp. *capsici*. The results of the experiments were presented here under.

### 3.1 In-vitro evaluation of fungicides

Efficacy of six fungicides at respective concentration was tested in-vitro by following poison food technique for mycelial growth of *Fusarium oxysporum* f. sp. *capsici*.

**Table 1:** In-vitro efficacy of fungicides against *Fusarium oxysporum* F. sp. *Capsici*

| Treatment      |                                                  | Conc (%) | <i>Fusarium oxysporum</i> F. sp. <i>capsici</i> |       |       |                           |        |        |
|----------------|--------------------------------------------------|----------|-------------------------------------------------|-------|-------|---------------------------|--------|--------|
|                |                                                  |          | Mean Colony diameter (mm)                       |       |       | Percent growth inhibition |        |        |
|                |                                                  |          | 3 DAI                                           | 5 DAI | 7 DAI | 3 DAI                     | 5 DAI  | 7 DAI  |
| T <sub>1</sub> | Copper oxychloride 50% WP                        | 0.2      | 23.33                                           | 39.38 | 54.33 | 32.70                     | 33.81  | 35.70  |
| T <sub>2</sub> | Iprodione 50% WP                                 | 0.2      | 0.00                                            | 0.00  | 0.00  | 100.00                    | 100.0  | 100.00 |
| T <sub>3</sub> | Tebuconazole 25.9% EC                            | 0.1      | 0.00                                            | 0.00  | 0.00  | 100.00                    | 100.00 | 100.00 |
| T <sub>4</sub> | Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC | 0.1      | 8.83                                            | 14.33 | 19.33 | 74.53                     | 75.91  | 77.12  |
| T <sub>5</sub> | Carbendazim 12% + Mancozeb 63% WP                | 0.25     | 0.00                                            | 0.00  | 0.00  | 100.00                    | 100.00 | 100.00 |
| T <sub>6</sub> | Metiram 55% + Pyraclostrobin 5% WG               | 0.3      | 12.83                                           | 20.83 | 28.50 | 62.99                     | 64.99  | 66.27  |
| T <sub>7</sub> | Control                                          | -        | 34.67                                           | 59.50 | 84.50 | -                         | -      | -      |
| F test         |                                                  |          | Sig                                             | Sig   | Sig   |                           |        |        |
| S.E(±m)        |                                                  |          | 0.19                                            | 0.47  | 0.63  |                           |        |        |
| C.D (P=0.01)   |                                                  |          | 0.83                                            | 2.00  | 2.67  |                           |        |        |

The data presented in Table. 1 revealed that among the six fungicides, the most promising fungicides which restricted 100% growth of the test pathogen was Iprodione 50% WP, Tebuconazole 25.9% EC and Carbendazim 12% + Mancozeb 63% WP, followed by Azoxystrobin 18.2% + Difenconazole 11.4% SC and Metiram 55% + Pyraclostrobin 5% WG which recorded 77.12 and 66.72 percent inhibition respectively. Whereas the least control of the pathogen or minimum percent inhibition was observed in Copper oxychloride 50% WP having 35.70 percent inhibition.

In the present investigation, the data revealed that Iprodione 50% WP, Tebuconazole 25.9% EC and Carbendazim 12% +

Mancozeb 63% WP completely inhibited the mycelial growth at all the concentrations of 0.2%, 0.1% and 0.25% respectively. These fungicides were highly effective even at low concentration.

In a similar way Pimpale *et al.*, (2023) <sup>[7]</sup> reported that Carbendazim 12% + Mancozeb 63% WP @ 0.20% concentration were found most effective against *Fusarium oxysporum* F. sp. *capsici*. The results are also in conformity with the findings of several other workers in case of *F. oxysporum* i.e. Shah *et al.*, (2006) <sup>[10]</sup>; Madhavi and Bhattiprolu (2011) <sup>[5]</sup>; Tewari *et al.*, (2012) <sup>[14]</sup>; Wani and Nazar (2012) <sup>[17]</sup> and Bashir and Chakma (2014) <sup>[3]</sup> and Rao *et al.*, (2021) <sup>[9]</sup>.

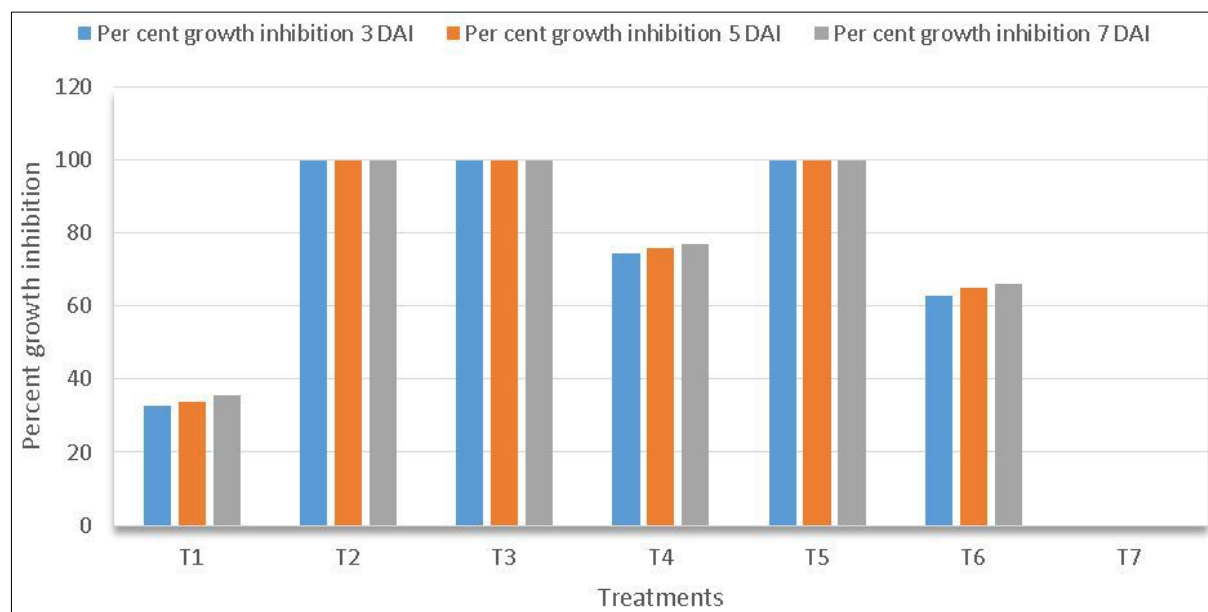


**Fig 1:** Effect of fungicides on percent growth inhibition of *Fusarium oxysporum* F. sp. *capsici*

### 3.2 In vitro evaluation of bio-agents against *Fusarium oxysporum* F. sp. *capsici*

Efficacy of bio-agent against *Fusarium oxysporum* f. sp. *capsici* was tested by dual culture technique. From the data presented in Table. 2, it was evident that all the bioagents were significantly superior in restricting the growth of *Fusarium oxysporum* f. sp. *capsici* in dual culture technique. Efficacy of four bioagents viz., *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were tested against *Fusarium oxysporum* F. sp. *capsici* in vitro by dual culture technique.

The minimum growth of the *Fusarium oxysporum* F. sp. *capsici* was recorded by *Trichoderma harzianum* i.e., 15.38

mm followed by *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* recording a growth of 16.63 mm, 21.13 mm and 24.00 mm respectively. Similarly, maximum percent growth inhibition was recorded in *Trichoderma harzianum* (82.34), *Trichoderma viride* (80.91), *Pseudomonas fluorescens* (75.74) and *Bacillus subtilis* (72.45). These findings are in consonance with reports of several earlier workers. Rini and Sulochana (2007) <sup>[8]</sup> reported *T. harzianum* as more effective than *P. fluorescens* against *F. solani* and *F. oxysporum*. Similarly *Trichoderma* spp. were reported as efficient antagonist against several *Fusarium* spp. Wani *et al.*, (2014) <sup>[17]</sup>; Nagendran *et al.* (2016) <sup>[6]</sup>.

**Table 2:** In vitro efficacy of bioagents against *Fusarium oxysporum* F. sp. *capsici*

| Treatment      |                                | <i>Fusarium oxysporum</i> F. sp. <i>capsici</i> |       |       |                           |       |       |
|----------------|--------------------------------|-------------------------------------------------|-------|-------|---------------------------|-------|-------|
|                |                                | Mean Colony diameter (mm)                       |       |       | Percent growth inhibition |       |       |
|                |                                | 3 DAI                                           | 5 DAI | 7 DAI | 3 DAI                     | 5 DAI | 7 DAI |
| T <sub>1</sub> | <i>Trichoderma viride</i>      | 11.63                                           | 14.50 | 16.63 | 66.89                     | 75.52 | 80.91 |
| T <sub>2</sub> | <i>Trichoderma harzianum</i>   | 10.50                                           | 13.38 | 15.38 | 70.11                     | 77.41 | 82.34 |
| T <sub>3</sub> | <i>Pseudomonas fluorescens</i> | 16.00                                           | 19.13 | 21.13 | 54.45                     | 67.71 | 75.74 |
| T <sub>4</sub> | <i>Bacillus subtilis</i>       | 19.25                                           | 22.00 | 24.00 | 45.21                     | 62.86 | 72.45 |
| T <sub>5</sub> | Control                        | 35.13                                           | 59.25 | 87.13 | -                         | -     | -     |
|                | F test                         | Sig                                             | Sig   | Sig   |                           |       |       |
|                | S.E(+m)                        | 0.27                                            | 0.43  | 0.46  |                           |       |       |
|                | C.D (P=0.01)                   | 1.15                                            | 1.79  | 1.92  |                           |       |       |

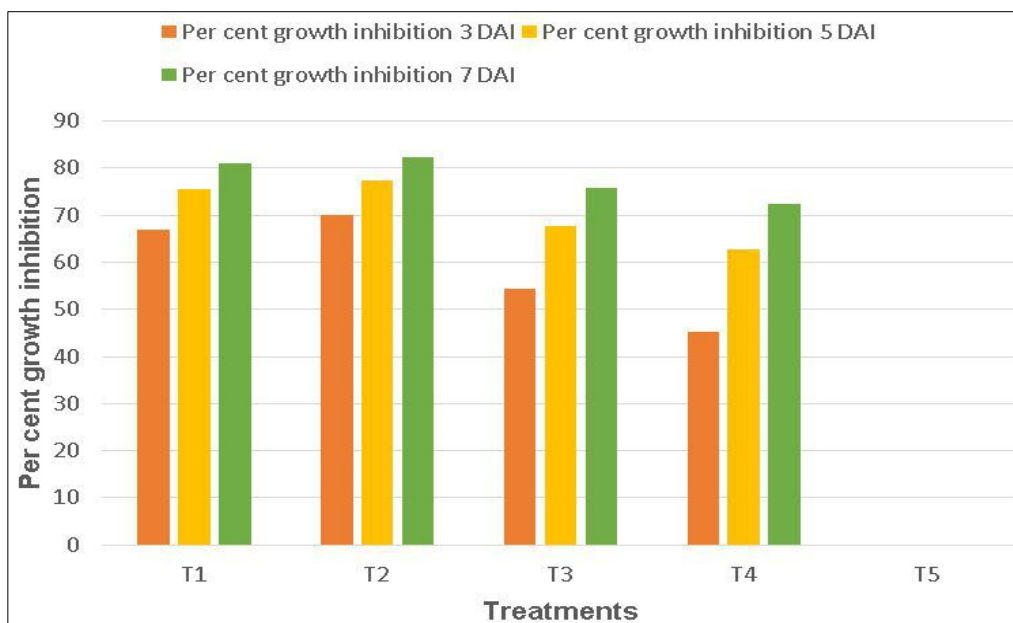


**Fig 2:** Effect of bioagents on percent growth inhibition of *Fusarium oxysporum* F. sp. capsici

#### 4. Conclusion

##### 4.1 The salient features of the present investigation are as under

1. The most promising fungicides which restricted 100% growth of the *Fusarium oxysporum* F. sp. capsici was Iprodione 50% WP, Tebuconazole 25.9% EC and Carbendazim 12% + Mancozeb 63% WP.
2. Bio-agents *Trichoderma harzianum* and *Trichoderma viride* were found most effective against *Fusarium oxysporum* F. sp. capsici.

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