***In vitro* evaluation of fungicides against *Fusarium spp.* causing wilt disease in pigeon pea**

**Abstract**

*Fusarium* *spp*. a soil and seed-borne pathogen, causes pigeon pea wilt, which causes significant losses in growers' pigeon pea yield. The chemical control method is a widely used and successful strategy for getting rid of disease-causing organisms. The goal of the current study was to evaluate the effectiveness of fungicides against *Fusarium spp.* which causes pigeon pea wilt, in vitro. Copperoxychloride 50%WP, Propineb 70%WP, Mancozeb 75%WP, Captan 75%WP, Carboxin 17.5% + Thiram 17.5% WP, Metalaxyl 4%w/w + Mancozeb 64%w/w, Thiophinate methyl 70%WP, and Carbendazim 50%WP were tested using the poisoned food technique on PDA medium at three different concentrations (100 ppm, 200 ppm, and 500 ppm). All of the fungicides considerably suppressed the fungus's mycelium growth. The maximum 98.96% and 98.88% mycelial growth inhibition was recorded in T7 (Thiophinate methyl 70%WP) and T5 (Carboxin 17.5% + Thiram 17.5% WP), and the minimum 56.92% mycelial growth inhibition was recorded in T3 (Mancozeb 75%WP). All other fungicides significantly inhibited the fungus's mycelial growth, as observed and mentioned in the paper's results.

**Keywords:** Wilt, Fungicides, Evaluation, *In-Vitro*, Pigeon pea.

1. **Introduction**

The legume known as pigeon pea (*Cajanus cajan* L. Millsp.) is a member of the Fabaceae family. "Red gram, Arhar, Tur, Congo pea, Gunga pea, Turvarica, or No-eye pea" are some other common names (Sheela, 2013). In India, pigeon pea is the most significant pulse crop. Numerous diseases, including, Alternaria leaf spot, Phytophthora blight, Sterility mosaic, and wilt, affect it. *F. udum* is the cause of the extremely damaging soil-borne disease pigeon pea wilt. It is the most significant issue in India's pigeonpea-growing regions, particularly in Uttar Pradesh, Madhya Pradesh, Bihar, and Maharashtra. Although the disease first affects immature seedlings, the blooming and podding stages are when the greatest death rate happens. However, if pigeon pea is grown in the same field regularly, the disease may spread throughout the entire field from its initial spots. The fungus *Fusarium udum* causes wilt disease, which seriously reduces pigeon pea productivity. However, wilt in the pre-podding stage caused a loss of nearly 100% of the individual plant, at the podding stage it was 67%, and at the pre-harvest stage it was 29.5% (Kannaiyan J and Nene YL. 1981). *Fusarium* wilt is distinguished by wilting of the afflicted plants and noticeable internal browning or blackening of the xylem vessels that extend from the root system to the stems. Partial wilting and patches of dead plants were observed to be widespread in the fields during the advanced phases of plant development. The current study included an in vitro assessment of fungicides for the treatment of *Fusarium oxysporum f. sp. udum*, which causes wilt disease in Pigeon pea. The *F. udum* is host specific to pigeon pea (Patel et al., 2011) and can survive in soil under wilted plant stubble for a long period. The best way of wilt management is by growing resistant varieties.

**1.1 Symptoms of wilt in pigeon pea**

Characteristic symptoms do not appear until crop developmental stages, despite the infection occurring in the early seedling stage (Reddy *et al*. 1990, Hillocks *et al*. 2000). The afflicted plants exhibit signs of progressive chlorosis and beginning to wilt four to six weeks after planting. Nonetheless, the blooming and podding stages are when wilt symptoms are most noticeable. The illness is characterized by black streaks under the bark and in the vascular area. In afflicted plants, partial wilting is typical. A dark purple stripe that extends from the base to several feet above the ground toward wilting branches is visible on many of these plants. Some genotypes have a purple ring that reaches one of the two main stem/branches or lateral roots. Complete wilting was more frequently induced by infection of the tap root, while partial wilting was more frequently caused by infection that began and spread from one of the two lateral roots (Nene 1980, Reddy *et al*. 1993). However, there were some exceptions. Wilted plants do not shed their dry leaves for a considerable amount of time. Because the typical symptoms of Phytophthora blight and Fusarium wilt are similar, they can be readily confused. The fundamental characteristic that sets the two diseases apart is the browning or blackening of the xylem vessels in wilt disease; in phytopthora, on the other hand, the xylem stays clear and the phloem is smoky gray. Additionally, F. udum-infected plants are easily uprooted, although Phytophthora blight is the opposite. Pande *et al*. (2011) have examined the specific characteristics that set these two illnesses apart.

1. **MATERIALS AND METHOD**

*In vitro* experiments were conducted at Department of Plant Pathology, P.G College, Ghazipur, during 2024-25. The Poisoned food technique (Nene and Thapliyal, 1993) was used to assess the effectiveness of several seed dressing fungicides against *Fusarium oxysporum f. sp.udum* at the prescribed doses. Potato Dextrose Agar (PDA) was used as a basal culture medium.

**2.1 Preparation of culture media**

**2.1 (a) PDA (Potato Dextrose Agar)**

PDA (39.0 grams) was mixed in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes, i.e. validated cycle. It was mixed well before dispensing in Petri plate. In specific work, when pH 6.5 is required, acidify the medium with sterile 10% tartaric acid. The amount of acid required for 100 ml. of sterile, cooled medium is approximately 1 ml. Do not heat the medium after addition of the acid.

**2.2 Collection of diseased plant sample**

During survey sample was collected from infected plant part. The infected plant were carefully collected in envelopes or plastic bag and taken to the laboratory for isolation of pathogens and further study.

**2.3 Isolation of Fusarium oxysporum f. sp.udum.**

Infected stem was treated with spirit. Then cut the infected part of the stem along with the some healthy part. Each small pieces dip in 0.1 % mercuric chloride solution for sterilization followed by three washing with sterilized distilled water. These bits were transferred aseptically to 2 percent Potato Dextrose Agar in Petri-dishes separately. Incubation was done 28 ± 10C for 7 days. Sub-culture from uncontaminated peripheral growth was made on PDA slants. Single spore technique was used for the purification of the fungus

**2.4 Identification and characterization of pathogenic fungi**

The pathogen was identified on the basis of its morphological and cultural character, as well as pathogenic behavior towards the host. The morphological characters Viz., shape, size, septation and colour of conidia were recorded. To study the morphological characters particularly asexual organ of the fungus, the temporary slides, were prepared in cotton blue from one week old culture. The morphological character was recorded after growing it on 2% Potato Dextrose Agar medium in Petridis. The inoculated Petri-plates were incubated for 7 days at room temperature 250C to 280C (Simmons 2007).

**2.5 Efficacy of different fungicides.**

Relative efficacy of Five selected chemical fungicides (Copperoxychloride 50%WP, Propineb 70%WP, Mancozeb 75%WP, Captan 75%WP, Carboxin 17.5% + Thiram 17.5% WP, Metalaxyl 4%w/w + Mancozeb 64%w/w, Thiophinate methyl 70%WP, Carbendazim 50%WP) were tested at 100,200 and 500ppm concentration only their inhibition were recorded effect of the growth of the pathogen on 2% Potato dextrose agar medium. The requisite quantities of the above fungicides were thoroughly mixed in 2% sterilized warm unsolidified potato dextrose agar medium and shaken well to make it homogenous. Five mm circular discs from 7 day old culture of the pathogen. The fungus came in direct contact with the medium. A separate check having no fungicide was also maintained. The inoculated plates were incubated for 7 days at 280C with 80% relative humidity for the growth of mycelium. The efficacy of various fungicides was assessed by measuring the radial growth of the fungus colony. The fungicides which were found effective in laboratory evaluation were employed further in two ways, namely seed dressers as well as spray fungicides (Chaudhari *et al.*,2021).

* 1. **Preparation of stock solution**

To determine the ppm concentration, firstly a stock solution of 0.1% of all fungicides was prepared as per the following formula 0.1 into 1000 upon active ingredient (a.i). To make working concentration i.e. ppm from the stock solution of the above concentration, multiply the amount of media required in the petri plate into ppm concentration divided by the 1000 and pour the obtained quantity into the petri plate with the help of a micro pipette.

* 1. **Statistical analysis of data**

The laboratory experiment was conducted with C.R.D. design. The data with appropriate transformations, where ever required, were analysed with the help of analysis of variance techniques. The 'F' value was tested and critical difference (C.D.) was calculated at 5% level of significance for comparing treatment means.

 **2.8 The poisoned food technique**

The efficacy of Copperoxychloride 50%WP, Propineb 70%WP, Mancozeb 75%WP, Captan 75%WP, Carboxin 17.5% + Thiram 17.5% WP, Metalaxyl 4%w/w + Mancozeb 64%w/w, Thiophinate methyl 70%WP, Carbendazim 50%WP fungicide was taken for the study of the inhibiting the radial growth of *Fusarium spp.* through poisoned food technique on three different concentrations viz; 100, 200, 500 ppm. Each treatment was replicated three times. PDA was used and requisite concentration of each fungicide (a.i.g.L-1) was added to get a required concentration. The fungicides were carefully mixed by stirring and about 20 ml poisoned medium was poured to each of the 90 mm petri dishes and allowed for solidification. Three culture plates (90 cm) were poured with PDA for each treatment. After the agar medium has solidified, 3 mm agar plugs containing mycelium of *Fusarium spp.,* were cut from the culture plates using sterilized cork borer and were placed in the center of each agar plate. Suitable control was maintained on PDA having no fungicide. These plates were incubated at 28 ± 1°C. The diameter of mycelium growth was recorded after 7 DAI (Days After Incubation). Corresponding controls were also maintained, simultaneously. Percent inhibition of *Fusarium spp.,* colonies in each treatment was recorded over the control.

The percent inhibition in growth due to various fungicidal treatments at different concentrations was computed as follows (Benicio *et al*., 2003).

$$ PGI \%=\frac{C- T }{C} ×100$$

(PGI = Percent growth inhibition, C = Colony diameter in control plate, T = Colony diameter in intersecting plate.

1. **RESULTS AND DISCUSSION**

A total of eight fungicides such as Copperoxychloride 50%WP, Propineb 70%WP, Mancozeb 75%WP, Captan 75%WP, Carboxin 17.5% + Thiram 17.5% WP, Metalaxyl 4%w/w + Mancozeb 64%w/w, Thiophinate methyl 70%WP, Carbendazim 50%WP at their recommended field dosages were evaluated in vitro with various concentrations by Poisoned food technique against *Fusarium spp.* causing wilt disease in pigeon pea, results obtained on their colony diameter (mm) Table 1. and per cent inhibition of mycelial growth are presented in Table 2.

**Table: 1.** Colony diameter of *Fusarium spp.* at three concentrations with different fungicides.

|  |  |  |  |
| --- | --- | --- | --- |
| **Sr.No** | **Treatment** | **Concentrations (ppm)** | **Mean** |
| **100** | **200** | **500** |
| 1. | **T1** (Copperoxychloride 50%WP) | 46.2 | 26.3 | 8.6 | 27.03 |
| 2. | **T2** (Propineb 70%WP) | 61.3 | 32.7 | 12.8 | 35.60 |
| 3. | **T3** (Mancozeb 75%WP) | 53.5 | 39.2 | 23.6 | 38.76 |
| 4. | **T4** (Captan 75%WP) | 43.4 | 21 | 15 | 26.46 |
| 5. | **T5** (Carboxin 17.5% + Thiram 17.5% WP) | 3 | 0.00 | 0.00 | 1.00 |
| 6. | **T6** (Metalaxyl 4%w/w + Mancozeb 64%w/w) | 24.5 | 21.2 | 7.1 | 17.60 |
| 7. | **T7** (Thiophinate methyl 70%WP) | 2.8 | 0.00 | 0.00 | 0.93 |
| 8. | **T8**(Carbendazim 50%WP) | 13 | 9 | 2 | 8.00 |
| 9. | **T9** Control | 90 | 90 | 90 | 90.00 |
|  | **C.D.** |  |  |  | **22.45** |
|  | **SE(m)** |  |  |  | **7.49** |
|  | **SE(d)** |  |  |  | **10.60** |
|  | **C.V.** |  |  |  | **47.62** |

**Table: 2.** *In vitro* assessment of different fungicides against *Fusarium spp*.

|  |  |  |  |
| --- | --- | --- | --- |
| **Sr.No.** | **Treatment** | **Mycelial growth percent inhibition** | **Mean percent inhibition** |
| **Concentrations (ppm)** |
| **100** | **200** | **500** |
|  |  |  |  |  |  |
| 1. | **T1** (Copperoxychloride 50%WP) | 48.66 | 70.77 | 90.44 | 69.95 |
| 2. | **T2** (Propineb 70%WP) | 31.88 | 63.66 | 85.77 | 60.43 |
| 3. | **T3** (Mancozeb 75%WP) | 40.55 | 56.44 | 73.77 | 56.92 |
| 4. | **T4** (Captan 75%WP) | 51.77 | 76.66 | 83.33 | 70.58 |
| 5. | **T5** (Carboxin 17.5% + Thiram 17.5% WP) | 96.66 | 100.0 | 100.0 | 98.88 |
| 6. | **T6** (Metalaxyl 4%w/w + Mancozeb 64%w/w) | 72.77 | 76.74 | 92.11 | 80.54 |
| 7. | **T7** (Thiophinate methyl 70%WP) | 96.88 | 100.0 | 100.0 | 98.96 |
| 8. | **T8**(Carbendazim 50%WP) | 85.55 | 90.0 | 97.77 | 91.10 |
| 9. | **T9** Control | 0.0 | 0.0 | 0.0 | 0.00 |
|  | **C.D.** |  |  |  | **24.93** |
|  | **SE(m)** |  |  |  | **8.32** |
|  | **SE(d)** |  |  |  | **11.77** |
|  | **C.V.** |  |  |  | **20.69** |

The result revealed that, all the fungicides exhibited significantly mycelial growth inhibition of the *Fusarium spp.* over untreated control. However, the fungicides Thiophinate methyl 70%WP and Carboxin 17.5% + Thiram 17.5% WP resulted in 100 percent inhibition of mycelial growth at 200ppm and 500ppm, the average mycelial growth inhibition of both treatment is 98.88 which is almost the same. The following fungicides significantly inhibited mycelial development of Fusarium oxysporum f. sp. udum, average of all the fungicides at three concentration such as 100ppm, 200ppm and 500ppm :carbendazim 50% WP @ average of (91.10%), Metalaxyl 4%w/w + Mancozeb 64%w/w @ mean of (80.54%), captan 75% WP @ average of (70.58%), Copperoxychloride 50%WP @ average of (69.95%), Propineb 70%WP @ average of (60.43%) and Mancozeb 75%WP @ mean of (56.92%). Except for the indicated dosage of Mancozeb 75%WP, the remaining six fungicides tested were extremely effective against *Fusarium spp*. The current study's findings are consistent with those of previous research by Chaudhary et al. (2019), who assessed eight fungicides—Captan, Blue Copper, Carbendazim, Carbendazim + Mancozeb, Mancozeb, Fipronil, Thiophanate Methyl, and Pyraclostrobin—against pigeonpea wilt. The best fungicide was discovered to be a broadspectrum combination of carbendazim + mancozeb, thiophanate methyl, and carbendazim that totally stopped the test pathogen's development.

**Graph.1:** *In-vitro* efficacy of various fungicides against *Fusarium spp.*

1. **CONCLUSION**

Out of all the combination fungicides that were tested, treatment (T7) (Thiophinate methyl 70%WP) and treatment (T5) (Carboxin 17.5% + Thiram 17.5% WP) showed the greatest suppression of mycelial growth (98.96 and 98.88%), whereas treatment (T3) (Mancozeb 75%WP) showed the least inhibition (56.92%). As the concentration of each fungicide rose, so did the suppression of mycelial growth. According to the results of the in vitro experiment, pigeon pea wilt disease was effectively controlled by T7 therapy (Thiophinate methyl 70%WP) and T5 treatment (Carboxin 17.5% + Thiram 17.5% WP).

**Competing Interest**

Author hereby declare that no competing interest exist.

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