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# Evaluation of Fungicides against Anthracnose Disease in Green Gram under *in vitro* and Pot Conditions

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## ABSTRACT

**Background:** Green gram (*Vigna radiata* L.) is one of the most important legume crops which is a rich source of protein (24.5%), fat (1.3%), Minerals (3.5%) and Carbohydrates (56.7%). Anthracnose disease of green gram caused by *Colletotrichum truncatum* is a major fungal disease causing serious loss of 24 to 67% both qualitative and quantitatively. Fungicide-resistant strains can develop due to excessive use of fungicides with similar actions. To avoid this, it is necessary to use fungicides having novel modes of action to lessen selection pressure, develop resistance and successfully manage infections over the long term. The current study aimed to test the efficacy of some fungicides against anthracnose of green gram under *in vitro* and pot conditions.

**Methods:** A total of 10 different fungicides were tested at three different concentrations (0.10, 0.15 and 0.20%) against the pathogen by poisoned food technique and a pot culture experiment was also conducted in protected structure (poly house) on green gram susceptible variety 'MH-421' against this disease during *Kharif* season of 2023 at Hi-Tech Nursery, S.K. Rajasthan Agricultural University Bikaner, Rajasthan, India.

**Result:** Among 11 treatments of fungicides, propiconazole at all concentrations, tebuconazole at 0.15 and 0.20% and carbendazim+ mancozeb at 0.20% gave 100% inhibition of growth of the pathogen. In the pot culture experiment, the least disease incidence was recorded in the treatment sprayed with propiconazole (0.10%) among tested fungicides.

**Key words:** Anthracnose, *Colletotrichum truncatum*, Fungicides, Green Gram.

## INTRODUCTION

Green gram (*Vigna radiata* L.) is one of the most important pulse crops, belongs to the family *Leguminosae* commonly known as mungbean is an important legume of Asian origin (Marak *et al*., 2019). It is also known as "Golden gram" because of its nutritional richness (Kaur *et al*., 2023) and suitability for increasing soil fertility by way of the addition of 30 kg/ha/annum nitrogen (Guan *et al*., 2013). It is a rich source of protein (24.5%), fat (1.3%), minerals (3.5%) and carbohydrates (56.7%). It also provides high quality essential amino acids like lysine (460 mg/g N) and tryptophane (60 mg/g N). It can provide some antioxidants in the form of ascorbic acid when sprouted and also contains riboflavin (0.21 mg/100 gm) and minerals (3.84 g/100 g) (Mekkara nikarthil Sudhakaran and Bukkan, 2021). It is a fast-growing, short duration pulse crop cultivated in summer and *Kharif* season with the least input requirement and performs well under heat and drought conditions. It grows in the range of temperatures of about 20 to 40°C (Lambrides and Godwin, 2007).

In recent years, there have been several challenges encountered in the successful cultivation of green gram, including the presence of pests and diseases.These issues are significant and can have a serious impact on the crop. Its cultivation is adversely affected by several major diseases namely, powdery mildew, anthracnose, *Cercospora* leaf spot, web blight, dry root rot *etc*. Among them, anthracnose caused by *Colletotrichum truncatum* (Schw.) is one of the economically important diseases. Fungicide-resistant strains can develop due to excessive use of fungicides with similar actions. To avoid this, it is necessary to use fungicides that have novel modes of action to lessen selection pressure, develop resistance and successfully manage infections over the long term (Thind, 2022). In such cases, the only viable options for

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treating diseases are either new fungicides with unique mechanisms of action or a combination of new and traditional fungicides (Ekabote *et al*., 2024). Keeping in view, the present investigation was undertaken to test the efficacy of some fungicides against anthracnose of green gram under *in vitro* and pot conditions.

## MATERIALS AND METHODS

### Isolation of pathogen

Infected leaf samples collected from the farmers' field showing typical symptoms of sunken lesions with black margins were collected and then washed with tap water and rinsed with sterile distilled water. Diseased along with healthy portions were cut into small pieces and then surface sterilized in 1% sodium hypochlorite (NaOCl) for 30 seconds and repeatedly washed with sterilized distilled water to remove traces of mercury and then transferred to potato dextrose agar media and incubated at 27±1°C. Fungal mycelium developed from the infected tissue in water agar media was finally transferred to PDA slants and incubated at 27±1°C to obtain a pure culture of *C. truncatum* and was identified based on morphology and microscopically.

### *In vitro* efficacy of fungicides against the pathogen

The efficacy of ten fungicides namely, carbendazim (Bavistin 50% WP), difenoconazole (Score 25% EC), propiconazole (Tilt 25% EC), hexaconazole (Contaf 5% EC), tebuconazole (Folicur 25.9% EC), mancozeb (Indofil M45 75% WP), carbendazim + mancozeb (Starlet 75% WP), fenamidone + mancozeb (Sectin 60% WG), azoxystrobin + difenoconazole (Magnite 29.6 % SC) and captan + hexaconazole (Taqat 75% WP) were tested at three different concentrations (0.10, 0.15 and 0.20%) against the pathogen by Poisoned food technique as given by Sharvelle (1961). The per cent inhibition was calculated by following the method described by Vincent (1947) as given below:

$$\text{Inhibition}(\%) = \frac{C - T}{C} * 100$$

Where,
C = Radial growth of pathogen in control (cm).
T = Radial growth of pathogen in treatment (cm).

### Pot culture studies

A pot culture experiment was also conducted in a protected structure (poly house) on green gram susceptible variety 'MH-421' against anthracnose during the *Kharif* season of 2023 at Hi-Tech Nursery, S.K. Rajasthan Agricultural University Bikaner, Rajasthan, India. The pathogen was mass multiplied on the PDA in Petri dishes. Spore-cum-mycelial suspension of the test pathogen was prepared by harvesting freshly sporulating 7-8 days old culture in plates by flooding with 5-10 ml sterile distilled water. The resultant spore-cum-mycelial suspension was suitably diluted with sterile distilled water to get an inoculum concentration of $1\times10^6$ conidia/ml. Twenty days old seedlings of green gram cultivar MH-421 were artificially inoculate by spraying with automizer the conidial suspension ($1\times10^6$ conidia/ml) of the test pathogen. Seedlings sprayed with sterile water (without inoculum) was also maintained as a suitable control. Similarly, the best *in vitro* concentrations of fungicides and plant extracts were sprayed along with the pathogen sprayed. Garden soil was collected and sterilized in an autoclave for three days consecutively at 15 lb/inch$^2$ for 2 hours. 100 g of sterilized soil was filled per pot and watered regularly.

### Statistical analysis

Observations were recorded on disease severity and per cent disease index (PDI) and per cent disease reduction (PDR) were calculated by using the standard formula given by McKinney (1923). The angular transformed and statistically analysed by off campus CCSHAU, Hisar (Haryana) OPSTAT statistical analysis software in RBD design:

$$\text{PDI} = \frac{\text{Sum of numerical disease rating}}{\text{Total no.of samples} \times \text{Maximum disease grade}} \times 100$$

$$\text{PDR} = \frac{\text{\% disease index in control} - \text{\% disease index in treatment}}{\text{\% disease index in control}} \times 100$$

## RESULTS AND DISCUSSION

### Efficacy of fungicides against the pathogen *in vitro* conditions

Data (Table 1 and Fig 1) revealed the efficacy of ten fungicides at three different concentrations @ 0.10, 0.15 and 0.20% against this fungal growth. It was observed that propiconazole gave 100% inhibition of growth of the pathogen at all the concentrations (0.10, 0.15 and 0.20%) similarly tebuconazole also gave 100% inhibition at 0.15 and 0.20% concentrations and carbendazim+ mancozeb gave 100% inhibition at 0.20% concentration only and was found significantly different from other tested fungicides. Except for carbendazim, all other fungicides gave >80% inhibition of the pathogen at 0.20% concentration. A similar finding was reported by Kadam *et al*. (2014) who evaluated the different fungicides *in vitro* for their efficacy against *Colletotrichum* leaf blight of turmeric and found that the fungicides hexaconazole, propiconazole, tricyclazole, thiophanate methyl and carbendazim + mancozeb each at 0.1% were completely inhibited the growth and sporulation of *C. gloeosporioides.* The result of the present study was also supported by the works of past researchers (Jagtap *et al.,* 2013; Fitson *et al.,* 2014; Gurav *et al.,* 2015) who evaluated different fungicides against *Colletotrichum* under *in vitro* conditions.

### Pot culture studies

Results of the pot experiment indicated that all the treatments of fungicides were found superior over control for reducing the anthracnose disease of green gram (Table 2).

**Table 1:** *In vitro* efficacy of fungicides against green gram anthracnose pathogen *C. truncatum*.

| Treatments | Radial growth (cm) | | | Inhibition % | | |
|---|---|---|---|---|---|---|
| | 0.10% | 0.15% | 0.20% | 0.10% | 0.15% | 0.20% |
| $T_1$ (Carbendazim) | 2.93±0.86 | 2.3±0.2 | 1.87±0.25 | 67.4±9.57 | 74.44±2.22 | 79.25±2.79 |
| $T_2$(Difenconazol) | 1.57±0.15 | 1.33±0.35 | 0.37±0.63 | 82.59±1.69 | 85.18±3.89 | 97.04±5.13 |
| $T_3$ (Propiconazol) | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 100±0.0 | 100±0.0 | 100±0.0 |
| $T_4$(Hexaconazol) | 1.33±0.23 | 1.06±0.25 | 0.67±0.58 | 85.18±2.56 | 88.14±2.79 | 92.59±6.51 |
| $T_5$(Tebuconazol) | 0.93±0.15 | 0.0±0.0 | 0.0±0.0 | 89.63±1.70 | 100±0.0 | 100±0.0 |
| $T_6$ (Mancozeb) | 5.2±0.17 | 4.93±0.20 | 4.5±0.0 | 42.22±1.92 | 45.18±2.31 | 50±0.0 |
| $T_7$(Carbendazim+ Mancozeb) | 1.77±0.05 | 0.93±0.15 | 0.0±0.0 | 80.37±0.64 | 89.63±1.70 | 100±0.0 |
| $T_8$(Fenamidone+ Mancozeb) | 1.47±0.30 | 1.37±0.25 | 1.13±0.05 | 83.7±3.39 | 84.81±2.79 | 87.4±0.64 |
| $T_9$ (Azoxystrobin + Difenoconaz) | 1.47±0.15 | 1.2±0.36 | 0.97±0.20 | 83.7±1.69 | 86.66±4 | 89.25±2.31 |
| $T_{10}$ (Captan + Hexaconazol) | 1.13±0.05 | 1.07±0.11 | 0.37±0.63 | 87.4±0.64 | 88.14±1.28 | 95.92±7.06 |
| $T_{11}$ (Control) | 9±0.0 | 9±0.0 | 9±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| SE(m) | 0.174 | 0.123 | 0.195 | 1.931 | 1.367 | 2.002 |
| C D | 0.513 | 0.363 | 0.577 | 5.699 | 4.036 | 5.909 |
| CV | 12.355 | 10.109 | 19.733 | 4.585 | 3.093 | 4.278 |

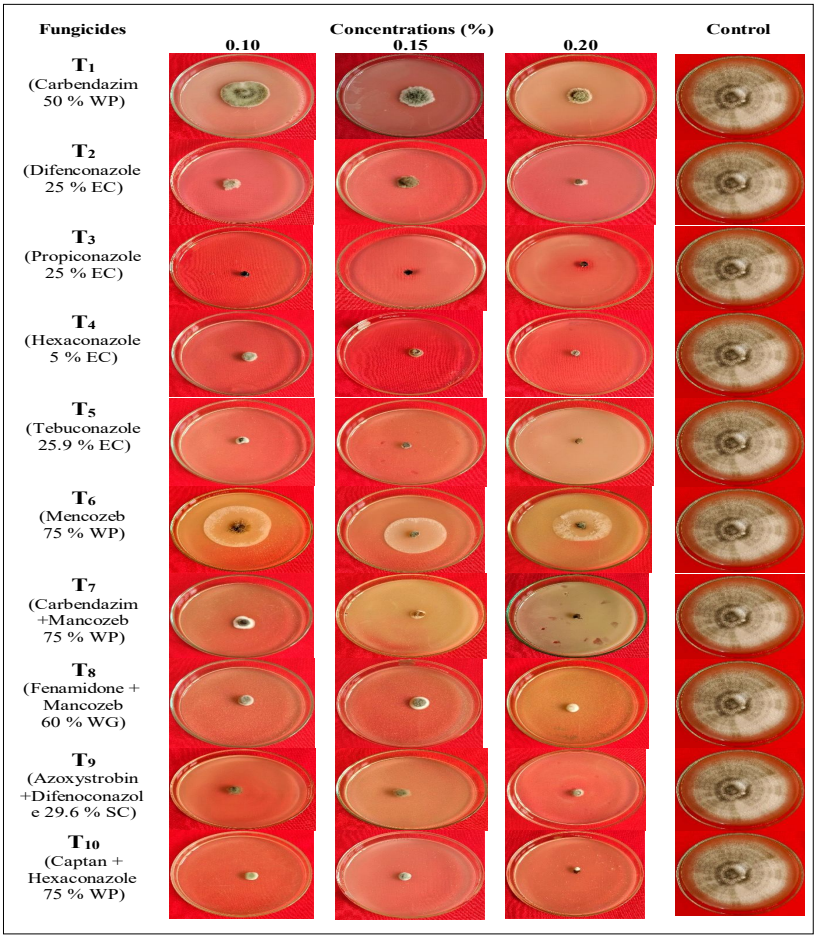


**Fig 1:** *In vitro* evaluation of fungicides against green gram anthracnose pathogen *C. truncatum* through poison food technique.

**Table 2:** Efficacy of fungicides against *C. truncatum* in pot culture studies.

| Treatments | Doses (%) | Per cent disease index (PDI) @ 35 DAS* | Per cent disease reduction (%) @ 35 DAS | Per cent disease index (PDI) @ 50 DAS | Per cent disease reduction (%) @ 50 DAS |
|---|---|---|---|---|---|
| $T_1$ (Carbendazim 50% WP) | 0.10 | 31.50 | 29.21 | 25.56 | 28.18 |
| $T_2$ (Difenconazole 25% EC) | 0.10 | 27.72 | 37.70 | 22.21 | 37.59 |
| $T_3$ (Propiconazole 25% EC) | 0.10 | 22.10 | 50.33 | 16.98 | 52.58 |
| $T_4$ (Hexaconazole 5% EC) | 0.10 | 29.31 | 34.13 | 23.10 | 35.09 |
| $T_5$ (Tebuconazole 25.9% EC) | 0.10 | 24.85 | 44.15 | 19.49 | 45.23 |
| $T_6$ (Mancozeb 75% WP) | 0.10 | 30.33 | 31.84 | 23.99 | 32.59 |
| $T_7$ (Carbendazim +Mancozeb 75% WP) | 0.10 | 23.71 | 46.71 | 17.95 | 49.56 |
| $T_8$ (Fenamidone + Mancozeb 60% WG) | 0.10 | 29.57 | 33.55 | 23.21 | 34.78 |
| $T_9$ (Azoxystrobin + Difenoconazole 29.6% SC) | 0.10 | 30.03 | 32.51 | 23.59 | 33.71 |
| $T_{10}$ (Captan + Hexaconazole 75% WP) | 0.10 | 27.14 | 39.01 | 21.14 | 40.60 |
| $T_{11}$ (Control) | - | 44.50 | - | 35.59 | - |
| S. Em.± | 1.12 | | 1.13 | | |
| C.D. at 5% | 3.30 | | 3.33 | | |
| C.V. | 6.64 | | 8.50 | | |

*DAS= Days after sowing.

However, among 11 treatments, propiconazole (0.1%) was found the most effective treatment against anthracnose disease with a minimum disease index (PDI) of 22.10 and 16.98% at 35 and 50 DAS respectively with 50-52% disease reduction (PDR), followed by tebuconazole. Nandeesha *et al*. (2023) got the minimum per cent disease intensity of anthracnose (33.91%) in green gram when sprayed with carbendazim 25% + mancozeb (50% WS) at 0.15% concentration at 15 days interval. The present experimental results are also supported by several other research works and conform with the findings of Marak *et al*. (2020) and Kulkarni (2019).

## CONCLUSION

From the *in vitro* and pot experiment study, it is concluded that a foliar spray of propiconazole at 0.1% can effectively control the green gram anthracnose disease.

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### Conflict of interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest and authors does not have any conflict of interest.

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