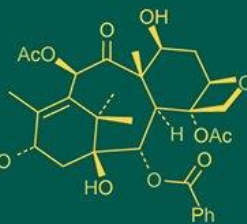
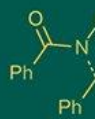


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Evaluation of fungicides against *Fusarium oxysporum* f. sp. *lagenariae* causing wilt of bottle gourd

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Abstract

Bottle gourd (*Lagenaria siceraria* (Mol.) Standl.) is one of the most widely cultivated cucurbitaceous vegetable crops in India and is valued for its nutritional and economic importance. However, its production is severely constrained by Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lagenariae*, which leads to considerable yield losses under favorable environmental conditions. Effective management of this soil-borne pathogen remains a major challenge because of its persistence in soil and ability to survive for prolonged periods. Keeping this in view, the present investigation was conducted to evaluate the efficacy of selected fungicides against the wilt pathogen under *in vitro* conditions and to assess their effectiveness in suppressing disease development under pot culture conditions.

The *in vitro* evaluation, carried out using the poisoned food technique at different concentrations, revealed significant differences among the fungicides tested. Among them, Azoxystrobin + Difenconazole proved to be the most effective, exhibiting 95.02% inhibition of mycelial growth at 500 ppm. This was followed by Carbendazim, which recorded 94.37% inhibition, while Tebuconazole + Trifloxystrobin resulted in 91.57% inhibition at the same concentration. The superior performance of these fungicides indicates their strong inhibitory effect on the growth of *F. oxysporum* f. sp. *lagenariae* under laboratory conditions.

The efficacy of the promising fungicides was further validated under pot culture conditions, where fungicide application significantly reduced wilt incidence and delayed disease development compared with the untreated control. Plants receiving fungicide treatments exhibited better health, reduced symptom expression, and improved protection against Fusarium wilt throughout the experimental period. The findings of the present study demonstrate that the judicious application of effective fungicides, particularly Azoxystrobin + Difenconazole, offers a reliable and practical approach for the integrated management of bottle gourd wilt. These results provide valuable information for developing sustainable disease management strategies and can serve as a useful reference for researchers, extension workers, and farmers aiming to minimize crop losses caused by *Fusarium oxysporum* f. sp. *lagenariae*.

Keywords: Bottle gourd, *Lagenaria siceraria*, *Fusarium oxysporum* f. sp. *lagenariae*, Fusarium wilt, fungicides, disease management, poisoned food technique

Introduction

The family Cucurbitaceae, commonly referred to as cucurbits or gourds, comprises a diverse group of vegetable crops of considerable economic importance throughout the world. The family includes cucumber, muskmelon, watermelon, bottle gourd, bitter gourd, sponge gourd, ridge gourd, snake gourd, pointed gourd, round gourd, ash gourd, pumpkin, summer squash, winter squash, and several other predominantly trailing vegetable crops (Singh, 2017; Agrios, 2005) [15, 1].

Bottle gourd (*Lagenaria siceraria* (Mol.) Standl.) is an important vegetable crop belonging to the family Cucurbitaceae and is widely cultivated for its nutritional, medicinal, and economic value (Singh, 2017; Agrios, 2005) [15, 1]. It is popularly known by various names, including birdhouse gourd, trumpet gourd, calabash gourd, white-flowered gourd, and *dudhi* in India. The plant is a vigorous climbing or trailing annual vine that bears fruits of diverse shapes, including bottle-shaped, oval, cylindrical, and dumbbell-shaped.

Among the fungal diseases affecting bottle gourd, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lagenariae* is one of the most destructive diseases, particularly under protected cultivation, where it causes severe yield losses and adversely affects crop

productivity (Korate *et al.*, 2021; Sharma, 2019) [8, 12]. The pathogen is soil-borne in nature and is capable of infecting several cucurbitaceous hosts, including melon, cucumber, squash, and pumpkin. It survives in the soil for many years in the form of chlamydospores, making disease management extremely difficult (Fravel *et al.*, 2003; Agrios, 2005) [6, 1].

The development and severity of *Fusarium* wilt are influenced by several factors, including inoculum density in the soil, environmental conditions, soil moisture, nutrient status, and the susceptibility of the host plant (Agrios, 2005; Singh, 2017) [1, 15]. Initial symptoms generally appear as yellowing and drooping of older leaves, followed by progressive wilting, vascular discoloration, necrosis, and eventual collapse of the entire plant. Under conditions of high pathogen inoculum or when susceptible cultivars are grown, rapid wilting may occur, leading to complete plant death (Agrios, 2005) [1].

Management of *Fusarium* wilt and other soil-borne diseases remains a major challenge because the pathogen survives in soil for prolonged periods and is difficult to eradicate completely using a single control measure (Fravel *et al.*, 2003; Alabouvette *et al.*, 2006) [6, 2]. Therefore, integrated disease management approaches involving chemical fungicides, biological control agents, organic amendments, botanicals, and suitable cultural practices have been widely recommended for sustainable suppression of soil-borne pathogens (Baker and Cook, 1974; Katan, 2000; Lewis and Papavizas, 1991; Punja and Utkhede, 2003) [3, 7, 9, 10]. Among these strategies, fungicides continue to play an important role in reducing pathogen populations and minimizing disease incidence, particularly during severe disease outbreaks (Agrios, 2005) [1].

Chemical fungicides continue to play an important role in the management of *Fusarium* wilt because they provide rapid suppression of pathogen growth and disease development. Several studies have reported the superior efficacy of systemic fungicides and combination fungicides against *Fusarium oxysporum* under laboratory and field conditions (Korate *et al.*, 2021; Sharma *et al.*, 2020; Chaudhari and Patel, 2024) [8, 12, 5].

Keeping these facts in view, the present investigation was undertaken to evaluate the efficacy of different fungicides against *Fusarium oxysporum* f. sp. *lagenariae* under *in vitro* conditions with the objective of identifying effective fungicides for the management of bottle gourd wilt.

Materials and methods

In vitro Evaluation of Fungicides Against *Fusarium oxysporum* f. sp. *lagenariae*

The efficacy of different fungicides against *Fusarium oxysporum* f. sp. *lagenariae*, the causal organism of bottle gourd wilt, was evaluated under laboratory conditions by employing the poisoned food technique described by Schmitz (1930) [11]. This technique is one of the most widely accepted methods for assessing the fungitoxic effect of chemicals against plant pathogenic fungi and has been extensively used in laboratory screening studies (Agrios, 2005) [1]. The experiment was conducted under aseptic conditions to determine the inhibitory effect of selected fungicides on the radial growth of the pathogen.

A total of seven fungicides representing different chemical groups and modes of action were selected for the study. These included Azoxystrobin, Carbendazim, Azoxystrobin + Difenconazole, Metalaxyl + Difenconazole,

Thiophanate methyl, Copper oxychloride, and Tebuconazole + Trifloxystrobin. Each fungicide was evaluated at four concentrations, namely 50, 100, 200, and 500 ppm, in order to determine the influence of increasing concentration on the suppression of fungal growth. Potato Dextrose Agar (PDA) medium without the addition of any fungicide was maintained as the untreated control for comparison.

The required quantity of each fungicide was accurately calculated and incorporated separately into sterilized molten Potato Dextrose Agar (PDA) medium after it had cooled to approximately 45-50 °C to avoid thermal degradation of the fungicidal compounds. The amended medium was mixed thoroughly to ensure uniform distribution of the fungicide throughout the medium. Approximately 20 ml of the fungicide-amended medium was then poured aseptically into sterilized 90 mm Petri plates and allowed to solidify under laboratory conditions.

After solidification, each Petri plate was inoculated at the centre with a 5 mm diameter mycelial disc cut from the actively growing peripheral region of a seven-day-old pure culture of *F. oxysporum* f. sp. *lagenariae*. The mycelial discs were carefully transferred using a sterile cork borer and inoculation needle to avoid contamination and to ensure uniformity among treatments. Three replications were maintained for each fungicide concentration, and the entire experiment was conducted following a Completely Randomized Design (CRD). The inoculated plates were incubated in a Biological Oxygen Demand (BOD) incubator maintained at 27±2 °C, which is considered favourable for the growth of the pathogen.

Observations on fungal growth were recorded at 24, 48, 72, 96, and 120 hours after inoculation. The radial growth of the fungal colony was measured in millimetres by taking observations along two mutually perpendicular directions, and the average colony diameter was calculated for each treatment. The growth recorded in fungicide-treated plates was compared with that of the untreated control to determine the relative effectiveness of each fungicide in suppressing mycelial growth. As the incubation period progressed, the inhibitory effect of each fungicide at different concentrations was carefully monitored and recorded.

The fungitoxic effect of each treatment was expressed as the percentage inhibition of mycelial growth over the untreated control. The percent inhibition was calculated using the formula proposed by Vincent (1947) [17], which is widely adopted in plant pathology studies for evaluating the efficacy of fungicides.

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

Where:

- I = Percent growth inhibition
- C = Colony diameter in control (mm)
- T = Colony diameter in treatment (mm)

The recorded observations on colony diameter and percentage inhibition were subjected to statistical analysis to compare the efficacy of the different fungicides tested against *F. oxysporum* f. sp. *lagenariae*. The results obtained from this experiment served as the basis for identifying the

most effective fungicides for subsequent evaluation under pot culture conditions.

Results

In vitro evaluation of fungicides against *Fusarium oxysporum* f. sp. *lagenariae*

The efficacy of seven fungicides against *Fusarium oxysporum* f. sp. *lagenariae* was evaluated under *in vitro* conditions using the poisoned food technique at four concentrations (50, 100, 200, and 500 ppm). The results revealed that all the fungicides significantly reduced the radial growth of the pathogen compared to the untreated control. A gradual increase in inhibition of mycelial growth was observed with increasing fungicide concentration, indicating a concentration-dependent response.

Among the fungicides tested, Azoxystrobin + Difenconazole was the most effective treatment at all concentrations. At 500 ppm, it recorded the highest inhibition of mycelial growth (95.02%), followed by Carbendazim (94.37%) and Tebuconazole + Trifloxystrobin (91.57%). These fungicides consistently restricted fungal growth and exhibited superior fungitoxic activity compared to the remaining treatments.

Carbendazim maintained high efficacy throughout the concentration range, with inhibition increasing from 56.21% at 50 ppm to 94.37% at 500 ppm. Tebuconazole + Trifloxystrobin also showed excellent inhibitory activity, with inhibition increasing progressively as the concentration increased. Thiophanate methyl produced appreciable inhibition and recorded 87.35% inhibition at 500 ppm.

Metalaxyl + Difenconazole exhibited moderate effectiveness against the pathogen, with inhibition ranging from 45.95% at 50 ppm to 81.63% at 500 ppm. Azoxystrobin applied alone was comparatively less effective than its combination with Difenconazole. Copper oxychloride was the least effective fungicide among the treatments evaluated, showing only 50.81% inhibition even at the highest concentration.

Overall, the results clearly demonstrated that increasing fungicide concentration significantly enhanced the inhibition of mycelial growth. Among all the fungicides tested, Azoxystrobin + Difenconazole, Carbendazim, and Tebuconazole + Trifloxystrobin were found to be the most effective in suppressing the growth of *Fusarium oxysporum* f. sp. *lagenariae* under laboratory conditions.

The percent inhibition of mycelial growth was calculated using Vincent's formula (1947) ^[17]:

$$I = [(C - T)/C] \times 100$$

where *I* is the percent inhibition of mycelial growth, *C* is the radial growth of the fungus in the untreated control, and *T* is the radial growth in the fungicide-treated plates.

Discussion

The present investigation demonstrated that all the fungicides tested significantly inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *lagenariae*, although their efficacy varied considerably among fungicides and concentrations. The inhibition of mycelial growth increased progressively with increasing fungicide concentration, indicating a clear concentration-dependent fungitoxic effect. Similar dose-dependent inhibition of *Fusarium* species has also been reported by Sharma (2019) ^[12], Korate *et al.*

(2021) ^[8], and Soni *et al.* (2024) ^[16], who observed that higher fungicide concentrations resulted in greater suppression of fungal growth under *in vitro* conditions.

The present findings are in close agreement with those of Chaudhari and Patel (2024) ^[5], who reported Carbendazim and Propiconazole among the most effective fungicides against *F. oxysporum* f. sp. *lagenariae* under *in vitro* conditions. Similar observations were reported by Sharma *et al.* (2020) ^[8, 12], who found Carbendazim to completely inhibit the pathogen, while Trifloxystrobin + Tebuconazole provided excellent disease control. Likewise, Korate *et al.* (2021) ^[8] demonstrated the superior efficacy of systemic fungicides against bottle gourd wilt under both laboratory and pot culture conditions. Singh and Jain (2020) ^[14] also reported the excellent performance of Carbendazim and the combination fungicide Trifloxystrobin + Tebuconazole against *Fusarium* spp. associated with bottle gourd diseases. Collectively, these studies support the present findings and further confirm the effectiveness of systemic and combination fungicides for the management of *Fusarium* wilt.

Among the fungicides evaluated, Azoxystrobin + Difenconazole proved to be the most effective treatment against the pathogen. The superior performance of this combination may be attributed to the complementary modes of action of its active ingredients. Azoxystrobin, a strobilurin fungicide, inhibits mitochondrial respiration by blocking electron transfer at the Qo site of cytochrome *b*, thereby suppressing spore germination and mycelial growth (Bartlett *et al.*, 2002) ^[4]. The inhibition of fungal growth through disruption of essential physiological processes is consistent with the findings of Xu *et al.* (2025) ^[18], who demonstrated that antifungal agents not only suppressed the growth of *F. oxysporum* but also altered the expression of genes associated with fungal development and pathogenicity. Difenconazole, a triazole fungicide, inhibits ergosterol biosynthesis, an essential component of fungal cell membrane formation. This disruption impairs membrane integrity and restricts fungal growth, thereby enhancing the overall efficacy of the combination fungicide. The combined action of these two active ingredients provides broad-spectrum antifungal activity and improves disease suppression.

Carbendazim also exhibited excellent fungitoxic activity throughout the study. Its high efficacy may be attributed to its systemic nature and its ability to interfere with fungal cell division by inhibiting β -tubulin synthesis, thereby preventing normal mitosis and mycelial development (Agrios, 2005; Singh, 2017) ^[1, 15]. The excellent performance of Carbendazim observed in the present investigation corroborates the findings of Korate *et al.* (2021) ^[8], who also reported its high efficacy in suppressing the growth of the bottle gourd wilt pathogen under both laboratory and pot culture conditions.

The combination fungicide Tebuconazole + Trifloxystrobin also exhibited excellent inhibition of the pathogen. The high efficacy of this treatment may be attributed to the dual modes of action of its constituent fungicides, wherein Tebuconazole inhibits sterol biosynthesis while Trifloxystrobin disrupts mitochondrial respiration. Such combination fungicides not only enhance fungicidal efficacy but also broaden the spectrum of disease control and help delay the development of fungicide resistance (Bartlett *et al.*, 2002) ^[4].

Thiophanate methyl also demonstrated substantial antifungal activity against *F. oxysporum* f. sp. *lagenariae*. As a systemic benzimidazole fungicide, it interferes with fungal nuclear division by inhibiting microtubule formation, thereby restricting mycelial development. However, its efficacy was comparatively lower than that of Azoxystrobin + Difenconazole and Carbendazim. In contrast, Metalaxyl + Difenconazole exhibited only moderate inhibition of the pathogen. Copper oxychloride was comparatively the least effective fungicide among all the treatments evaluated. The comparatively lower efficacy of Copper oxychloride may be attributed to its contact mode of action and limited systemic movement, which restricts its ability to suppress actively growing fungal mycelium under laboratory conditions (Agrios, 2005) [1]. Similar observations have also been reported in studies evaluating fungicides against soil-borne

Fusarium pathogens, where systemic fungicides consistently outperformed contact fungicides (Sharma *et al.*, 2020) [8, 12]. Overall, the present study clearly demonstrated that Azoxystrobin + Difenconazole, Carbendazim, and Tebuconazole + Trifloxystrobin were the most effective fungicides against *F. oxysporum* f. sp. *lagenariae* under *in vitro* conditions. Their high fungitoxic activity indicates that these fungicides have considerable potential for incorporation into integrated disease management programmes for bottle gourd wilt. However, further validation under pot and field conditions is essential before recommending their large-scale use under diverse agro-climatic conditions. The findings of the present investigation provide valuable information for the selection of effective fungicides and contribute to the development of sustainable management strategies for *Fusarium* wilt of bottle gourd.

Table 1: *in vitro* evaluation of Fungicides against wilt pathogen

Fungicides	Colony diameter (mm) at 50 ppm	PI (%)	Mean radial growth (mm) at 100 ppm	PI (%)	Mean radial growth (mm) at 200 ppm	PI (%)	Mean radial growth (mm) at 500 ppm	PI (%)
Azoxystrobin	42.47	31.12	36.53	40.76	28.53	53.73	19.87	67.78
Carbendazim	27.00	56.21	18.93	69.30	10.93	82.27	3.47	94.37
Azoxystrobin + Difenconazole	24.20	60.75	16.40	73.40	9.33	84.87	3.07	95.02
Metalaxyl + Difenconazole	33.33	45.95	26.73	56.65	18.73	69.62	11.33	81.63
Thiophanate methyl	30.33	50.81	22.60	63.35	15.00	75.67	7.80	87.35
Copper oxychloride	49.53	19.67	44.33	28.10	37.53	39.13	30.33	50.81
Tebuconazole + Trifloxystrobin	28.13	54.38	20.33	67.03	12.00	80.54	5.20	91.57
Control	61.66	0.00	61.66	0.00	61.66	0.00	61.66	0.00
SEm ($\pm$)	0.598							
CD (P = 0.05)	1.662							
CV (%)	3.375							

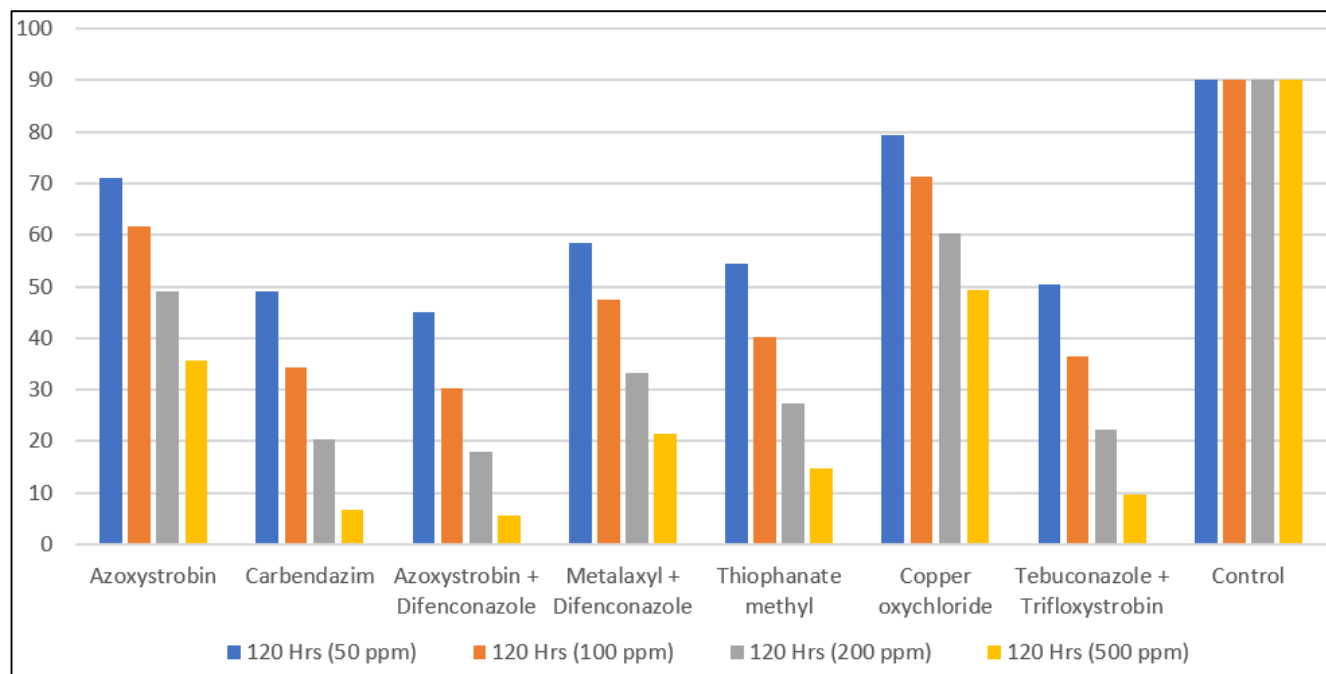


Fig 1: *in vitro* evaluation of Fungicides against wilt pathogen

Conclusion

The present study demonstrated significant differences in the efficacy of the fungicides tested against *Fusarium oxysporum* f. sp. *lagenariae*, the causal agent of bottle gourd wilt. All the fungicides exhibited inhibitory effects on the radial growth of the pathogen, with the degree of inhibition

increasing as the concentration increased, indicating a clear concentration-dependent response. Among the treatments evaluated, Azoxystrobin + Difenconazole proved to be the most effective, followed by Carbendazim and Tebuconazole + Trifloxystrobin, which consistently suppressed the mycelial growth of the pathogen under *in vitro* conditions.

In contrast, Copper oxychloride exhibited comparatively lower efficacy, suggesting limited effectiveness against the pathogen under laboratory conditions.

The findings of the present investigation indicate that systemic and combination fungicides possess greater fungitoxic activity than contact fungicides against *F. oxysporum* f. sp. *lagenariae*. The superior performance of Azoxystrobin + Difenconazole highlights its potential as an effective component of integrated disease management programmes for bottle gourd wilt. Although the present study was conducted under controlled laboratory conditions, the promising results provide a strong basis for further validation under pot and field conditions to optimize disease management strategies under diverse agro-climatic situations.

Overall, the study contributes valuable information on the comparative efficacy of fungicides against the wilt pathogen and provides a scientific basis for selecting effective fungicides for the management of *Fusarium* wilt in bottle gourd. The adoption of suitable fungicides, along with integrated disease management practices, may help reduce disease incidence, improve crop health, and enhance sustainable bottle gourd production.

Competing Interests

Authors have declared that no competing interests exist.

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