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Evaluation of the efficacy of plant extract and fungicides against *Fusarium oxysporum* f. sp. *lycopersici* under *in vitro* conditions

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Abstract

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop severely affected by *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. The present investigation was carried out to evaluate the efficacy of different plant extracts and fungicides against the pathogen under *in vitro* conditions by poisoned food technique. Seven plant extracts viz., *Azadirachta indica*, *Asparagus racemosus*, *Polyalthia longifolia*, *Withania somnifera*, *Allium cepa*, *Zingiber officinale*, and *Allium sativum* were tested at 10, 20, and 30 per cent concentrations, while seven fungicides viz., Azoxystrobin, Propineb, Thiophanate methyl, Difenconazole, Mancozeb, Mancozeb + Thiophanate methyl, and Boscalid + Pyraclostrobin were evaluated at 250, 500, and 750 ppm concentrations. The experiment was conducted in Completely Randomized Design (CRD) with three replications. Among the plant extracts, *Allium sativum* proved most effective and recorded maximum mycelial growth inhibition of 66.46 per cent at 30 per cent concentration, followed by *Azadirachta indica* with 62.02 per cent inhibition. *Polyalthia longifolia* was found least effective against the pathogen. Among fungicides, Thiophanate methyl exhibited highest inhibition of 95.24 per cent at 750 ppm concentration followed by Mancozeb + Thiophanate methyl with 92.86 per cent inhibition. Azoxystrobin was comparatively less effective than other fungicides tested. The results revealed that garlic extract and Thiophanate methyl were highly effective in suppressing the growth of *F. oxysporum* f. sp. *lycopersici* under laboratory conditions. Therefore, these treatments may be utilized as effective components in integrated management of *Fusarium* wilt of tomato.

Keywords: Tomato, *Fusarium* wilt, *Fusarium oxysporum* f. sp. *lycopersici*, plant extracts, fungicides, poisoned food technique, mycelial inhibition, garlic extract, Thiophanate methyl

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops cultivated worldwide due to its high nutritional value, economic importance, and adaptability under diverse agro-climatic conditions. Among the various diseases affecting tomato, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is one of the most destructive soil-borne diseases, causing severe yield losses under favorable conditions. The pathogen infects the vascular tissues, resulting in yellowing, wilting, vascular browning, and eventual death of plants (Agrios, 2005) ^[1]. The pathogen survives in soil and infected plant debris for many years through resistant structures such as chlamydospores, making its management difficult. Warm temperatures and high soil moisture favor disease development. Although chemical fungicides such as carbendazim and thiophanate-methyl are commonly used for disease management, their indiscriminate use has raised concerns regarding environmental pollution, fungicide resistance, and health hazards. In recent years, plant extracts have gained importance as eco-friendly alternatives for disease management. Botanicals such as neem (*Azadirachta indica*), garlic (*Allium sativum*), onion (*Allium cepa*), and tulsi (*Ocimum sanctum*) possess antifungal properties due to the presence of bioactive compounds that inhibit fungal growth and spore germination (Yadav et al., 2021) ^[4]. Therefore, evaluation of plant extracts and fungicides under *in vitro* conditions is essential to identify effective and sustainable management options against *Fusarium oxysporum* f. sp. *lycopersici*.

Materials and methods

In vitro evaluation of plant extract

The antifungal efficacy of seven plant extracts viz., neem (*Azadirachta indica*), shatavari (*Asparagus racemosus*), ashoka (*Polyalthia longifolia*), ashwagandha (*Withania somnifera*), onion (*Allium cepa*), ginger (*Zingiber officinale*), and garlic (*Allium sativum*) was evaluated against *Fusarium oxysporum* f. sp. *lycopersici* by poisoned food technique (Schmitz, 1930). The extracts were tested at 10, 20, and 30 per cent concentrations. Fresh plant materials were washed, crushed separately with sterilized distilled water in 1:1 (w/v) ratio, and filtered through double-layered cheese cloth. The obtained filtrate was considered as 100 per cent stock solution. Required quantities of extracts were mixed with sterilized molten PDA medium to obtain desired concentrations and poured into sterilized Petri plates. Each plate was inoculated with a 5 mm mycelial disc taken from a seven-day-old culture of *F. oxysporum* f. sp. *lycopersici* and incubated at $25 \pm 1^\circ\text{C}$. PDA plates without plant extract served as control. The experiment was conducted in Completely Randomized Design (CRD) with three replications. Radial mycelial growth was recorded after seven days of incubation.

In vitro evaluation of fungicide

Seven fungicides viz., Azoxystrobin, Propineb, Thiophanate methyl, Difenconazole, Mancozeb, Mancozeb + Thiophanate and Boscolid + pyraclostrobin were evaluated against *F. oxysporum* f. sp. *lycopersici* causing wilt in tomato *in vitro* by applying poisoned food technique using potato dextrose agar as basal medium. An appropriate quantity of concentration of the fungicides was added in previously sterilized 100 ml PDA separately in 250 ml conical flasks. The flasks were shaken well to ensure uniform distribution of fungicides in the basal medium. Twenty ml of the medium containing fungicides was poured into sterilized petri dishes. After solidification, the plates were inoculated with the fungal disc of 5 mm diameter cut out from seven days old culture and incubated at $25 \pm 1^\circ\text{C}$ for seven days. The experiment was conducted in completely randomized design (CRD) with three replications for each treatment with one control.

Observations

Observations on radial mycelia growth was recorded in all the replicated treatments. Per cent growth inhibition of the test pathogen was calculated by applying the formula given by Vincent (1947) and the data obtained were averaged and analyzed statistically.

$$\text{Inhibition of mycelial growth} = \frac{C-T}{C} \times 100$$

Where,

C = Growth of pathogen mycelium in control

T = Growth of pathogen mycelium in Treatment

Result and Discussion

In vitro evaluation of plant extract

The efficacy of different plant extracts against *Fusarium oxysporum* f. sp. *lycopersici* was evaluated under *in vitro* conditions by poisoned food technique at different concentrations viz., 10, 20 and 30 per cent. The results presented in Table 1 clearly indicated that all the tested plant

extracts significantly inhibited the radial growth of the pathogen as compared to control. Further, increase in concentration of plant extracts resulted in corresponding increase in inhibition of fungal growth. Among all the botanicals tested, *Allium sativum* (T₇) proved to be the most effective treatment against the pathogen. It recorded minimum radial growth of 46.35, 39.24 and 30.18 mm at 10, 20 and 30 per cent concentrations, respectively. The corresponding inhibition percentages were 48.50, 56.40 and 66.46 per cent. The superior efficacy of garlic extract may be attributed to the presence of antifungal compounds such as allicin and sulphur containing constituents which inhibit fungal growth effectively. The next best treatment was *Azadirachta indica* (T₁), which recorded radial growth of 48.24, 42.12 and 34.18 mm with inhibition percentages of 46.40, 53.20 and 62.02 per cent at respective concentrations. *Allium cepa* (T₅) and *Zingiber officinale* (T₆) were also found effective in reducing fungal growth. *Allium cepa* recorded inhibition of 42.08, 49.78 and 58.67 per cent, whereas *Zingiber officinale* recorded inhibition of 39.63, 46.34 and 55.93 per cent at 10, 20 and 30 per cent concentrations, respectively. Among the tested plant extracts, *Polyalthia longifolia* (T₃) was found least effective and recorded comparatively higher radial growth of 65.33, 57.20 and 50.66 mm at 10, 20 and 30 per cent concentrations, respectively. The corresponding inhibition percentages were 27.41, 36.44 and 43.71 per cent. Similarly, *Asparagus racemosus* (T₂) and *Withania somnifera* (T₄) showed moderate inhibitory effect against the pathogen. The control treatment recorded maximum radial growth of 90 mm without any inhibition. Thus, the results clearly indicated that garlic extract was most effective in suppressing the growth of *F. oxysporum* f. sp. *lycopersici* under laboratory conditions. The present findings are in close conformity with Barhate *et al.* (2024), who reported maximum inhibition of *F. oxysporum* by garlic extract under laboratory conditions. Similarly, Gulya *et al.* (2023) [5] observed that garlic and neem extracts significantly reduced mycelial growth and sporulation of *Fusarium* species. Sharma and Gupta (2022) [8] also reported higher antifungal activity of garlic extract due to presence of volatile sulfur compounds and antimicrobial metabolites. The effectiveness of neem extract observed in the present study is also supported by findings of Hassan (2020) [6], who reported that neem contains azadirachtin and several bioactive compounds capable of suppressing fungal pathogens. Similar reports were also given by Rakesh *et al.* (2023) [7], who observed significant inhibition of tomato wilt pathogen by neem and onion extracts under *in vitro* conditions. The comparatively lower efficacy of *Polyalthia longifolia* and *Asparagus racemosus* may be due to lower concentration of antifungal compounds. Similar observations were reported by Kumar *et al.* (2022) [13], who observed moderate suppression of *Fusarium* wilt pathogen by these botanicals.

In vitro evaluation of fungicide

The efficacy of different fungicides against *Fusarium oxysporum* f. sp. *lycopersici* was evaluated under *in vitro* conditions by poisoned food technique at different concentrations viz., 250, 500 and 750 ppm. The results presented in Table 2 revealed that all the fungicides significantly inhibited the radial growth of the pathogen as compared to untreated control. Further, an increase in fungicidal concentration resulted in a corresponding

decrease in radial growth and increase in per cent inhibition of the pathogen. Among all the fungicides tested, Thiophanate methyl (T_3) proved to be the most effective treatment against the pathogen. It recorded minimum radial growth of 18.36, 10.54 and 4.28 mm at 250, 500 and 750 ppm concentrations, respectively. The corresponding inhibition percentages were 79.60, 88.29 and 95.24 per cent. The superior efficacy of Thiophanate methyl may be attributed to its systemic mode of action and strong fungitoxic activity against fungal pathogens. The next best treatment was Mancozeb + Thiophanate methyl (T_6), which recorded radial growth of 21.84, 14.63 and 6.42 mm with corresponding inhibition percentages of 75.73, 83.74 and 92.86 per cent at 250, 500 and 750 ppm concentrations, respectively. Difenconazole (T_4) was also found highly effective and recorded radial growth of 24.52, 17.36 and 10.85 mm with inhibition percentages of 72.76, 80.71 and 87.94 per cent at respective concentrations. Mancozeb (T_5) also showed good inhibitory effect against the pathogen and recorded radial growth of 27.35, 19.82 and 15.24 mm with inhibition percentages of 69.61, 77.98 and 83.06 per cent at increasing concentrations. Similarly, Boscalid + Pyraclostrobin (T_7) recorded inhibition of 63.91, 73.15 and 80.18 per cent at 250, 500 and 750 ppm concentrations, respectively. Propineb (T_2) exhibited moderate fungitoxic effect and recorded radial growth of 36.46, 28.35 and 25.26 mm with corresponding inhibition percentages of 59.49, 68.50 and 71.93 per cent. Among all the fungicides tested, Azoxystrobin (T_1) was found comparatively less effective against the pathogen. It recorded comparatively higher radial growth of 41.72, 35.64 and 30.53 mm at 250, 500 and 750 ppm concentrations, respectively. The corresponding inhibition percentages were 53.64, 60.40 and 66.07 per cent. However, it still performed significantly better than untreated control. The untreated control recorded maximum radial growth of 90 mm without any inhibition of the pathogen. The results clearly indicated that Thiophanate methyl and Mancozeb + Thiophanate methyl were highly effective in suppressing the growth of *Fusarium oxysporum* f. sp. *lycopersici* under laboratory conditions.

The present findings are in close agreement with Dholu *et al.* (2022) ^[9], who reported maximum inhibition of *F. oxysporum* f. sp. *lycopersici* by Thiophanate methyl under in vitro conditions. Similar results were also obtained by El-Aswad *et al.* (2023) ^[10], who observed strong fungitoxic activity of systemic fungicides against tomato wilt pathogen. Patel and Singh (2024) ^[11] further reported that combination fungicides such as Mancozeb + Thiophanate methyl provide better disease suppression due to combined protective and systemic action. The effectiveness of Difenconazole observed in the present study is supported by findings of Sharma *et al.* (2023) ^[12], who reported significant inhibition of mycelial growth and sporulation of *Fusarium oxysporum* by triazole fungicides.

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