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Integrated evaluation of biocontrol agents, botanical extracts and fungicides for the management of Fusarium wilt of soybean caused by *Fusarium oxysporum* f. sp. *glycines*

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Abstract

An investigation was undertaken for the management of Fusarium wilt disease of soybean caused by *Fusarium oxysporum* f. sp. *glycine* through the integration of biocontrol agents, chemical fungicides and plant extracts possessing medicinal and antifungal properties. *In vitro* evaluations using dual culture and poison food technique were conducted prior to field experiments. Among six bioagents tested, *Trichoderma viride* (T_2) recorded the highest percent mycelial inhibition (70.30 %) with a mean colony diameter of 26.73 mm against the test pathogen. Among six fungicides evaluated, trifloxystrobin (T_2) showed maximum radial growth inhibition (79.59 %) followed by trifloxystrobin 25 % + tebuconazole 50 % (75.07 %). In the evaluation of plant extracts, neem (*Azadirachta indica* A. Juss.) leaf extract recorded the highest inhibition (76.07 %) followed by garlic (*Allium sativum* L.) extract (71.59 %). Pot experiment conducted under randomised block design (RBD) with 7 treatments and 3 replications, among the treatments evaluated, the combination of trifloxystrobin and *T. viride* (T5) was the most effective, recording the lowest disease incidence (6.50, 10.25 and 14.80 %) 30, 45 and 60 days after sowing (DAS), respectively as compared to the untreated control (disease incidence of 51.35 %). The combination of fungicide and biocontrol agent showed promising disease suppression under pot conditions and may have potential for integration into soybean Fusarium wilt management.

Keywords: bioagents; fungicides; Fusarium wilt; integrated disease management; plant extracts; soybean

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Introduction

Soybean (*Glycine max* L.) is one of the most important legume crops in the world, originating in northeastern China approximately 3000–5000 years ago and domesticated from its wild ancestor *Glycine soja* (1). It is widely cultivated in tropical and subtropical regions and has expanded globally to become a key commodity in international trade and agricultural economies. The key soybean producers today are the United States, Brazil, Argentina, China and India (2). In Punjab, soybean is actively promoted to reduce rice-wheat cropping dependence and alleviate groundwater depletion; however, its expansion is limited by several biotic stresses, particularly soil-borne fungal diseases. Among the major fungal diseases affecting soybean, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *glycines* is one of the most destructive soil borne pathogens (3). The pathogen infects soybean primarily through the root system, colonising xylem vessels and producing microconidia that spread systemically, leading to characteristic symptoms including interveinal chlorosis, vascular browning, wilting, stunting and premature plant death. Chlamydospores enable long-term survival of the pathogen in soil and crop residues, making management especially challenging (4).

Application of fungicides, biocontrol agents and plant extracts has been reported to be effective against *F. oxysporum* f. sp. *glycines* and related soil borne pathogens under both *in vitro* and *in vivo* conditions by several workers (5). Biocontrol agents such as *Trichoderma spp*., *Pseudomonas fluorescens* and *Bacillus subtilis* have been widely studied for their antagonistic activity against Fusarium wilt pathogens through mechanisms of mycoparasitism, antibiosis and competition for nutrients and space (6). Without having any adverse effects on the ecosystem or on the health of human beings, *Trichoderma* species supports environmentally safe and sustainable agriculture (7). Growing concerns about pesticide residues, development of fungicide-resistant strains and environmental pollution have necessitated the

exploration of eco-friendly and sustainable alternatives, including botanical extracts, for disease management (8, 9). Although fungicides, biocontrol agents and botanical extracts have each been reported against Fusarium wilt, most studies have evaluated these components individually, often in crops other than soybean and rarely compared them under uniform conditions against the same pathogen isolate. Information on botanicals against *F. oxysporum* f. sp. *glycines* and on whether *in vitro* antagonism translates into disease reduction in planta, remains limited. Moreover, region-specific data from Punjab, where soybean cultivation is expanding, is scarce. Integrating biological and botanical components with reduced fungicide dependence therefore needs systematic evaluation. Hence, the present study was undertaken to (i) evaluate the *in vitro* efficacy of six bioagents, six fungicides and six plant extracts against *F. oxysporum* f. sp. *glycines* and (ii) assess selected individual and integrated treatments for managing Fusarium wilt of soybean under pot conditions.

Materials and Methods

The laboratory study was done in the Department of Plant Pathology, School of Agriculture, Lovely Professional University. The pot experiment was conducted during the kharif season 2025–26 under controlled/greenhouse conditions. During the experimental period, the optimum temperature ranged from 28–35 °C and the relative humidity from 80–85 %. Plants were maintained under a photoperiod of 14 hr light and 8 hr dark. Temperature and relative humidity were recorded using a thermo-hygrometer.

Isolation, purification, identification and pathogenicity test

Five diseased soybean plants showing characteristic Fusarium wilt symptoms (interveinal chlorosis, vascular browning and wilting) were collected from naturally infected plants at the Agri Farm, Lovely Professional University, Punjab. The cultivar of the sampled plants was SL 958. A 1 % sodium hypochlorite (NaOCl) solution was used to surface-sterilise the diseased stem sections, which were then chopped into small pieces and cleaned three times using sterile distilled water. The pieces were placed on Petri dishes with 100 ppm streptomycin in potato dextrose agar (PDA) medium and cultured for three to seven days at 27 ± 2 °C in a biochemical oxygen demand (BOD) incubator. A single hyphal-tip approach was used to purify the fungal pathogen and conidia and colony morphology were examined under a microscope to identify it.

Assessment of pathogenicity

The pathogenicity of the isolate was tested on healthy soybean (cv. SL 958) under pot conditions. Inoculum was prepared by growing the isolate on sterilised sorghum grains inoculated with a spore suspension (1×10^6 conidia/mL) and incubating for 14 days at 28 ± 2 °C. The inoculum (6 g per pot) was placed 3–4 cm below the surface of sterilised soil in pots (cm) and seeds were sown after 3 days. Control pots received uninoculated sterilised grains moistened with sterile distilled water. Each treatment had 3 replications with five plants per replication. The plants were kept at 28–35 °C and 80–85 % relative humidity under a 14 hr light 12 hr dark photoperiod and observed daily. Typical wilt symptoms such as yellowing, drooping, vascular browning and wilting appeared 10–15 days after inoculation, while control plants remained healthy. The pathogen was re-isolated from inoculated plants on PDA and was identical to the original culture, thereby fulfilling Koch's postulates (Fig. 1).

In vitro efficacy of bioagents on mycelial growth of *Fusarium oxysporum* f. sp. *glycines*

Six bioagents were tested for their antagonistic activity against *F. oxysporum* f. sp. *glycines* using the dual culture method on PDA (Table 1). The assay was carried out on 90 mm Petri plates. A 5 mm disc of the pathogen was cut from the actively growing margin of a 10-day-old culture and placed opposite to a 5 mm disc of the respective bioagent, maintaining 24 mm between the two inocula. Plates containing only the pathogen were maintained as untreated control. Each treatment was replicated three times and arranged in a completely randomised design. The inoculated plates were incubated at 28 ± 2 °C for 7 days. After incubation, the radial growth of *F. oxysporum* f. sp. *glycines* was recorded in millimetres (mm) using a measuring scale. The percentage inhibition of pathogen growth was determined according to Vincent (1947), using the formula: $I = (C - T)/C \times 100$, where C represents the radial growth of the pathogen in the untreated control and T denotes the radial growth in the treatment. The experiment was performed once.

In vitro efficacy of fungicides and plant extracts on mycelial growth of *Fusarium oxysporum* f. sp. *glycines*

The poisoned food technique was employed to evaluate the *in vitro* efficacy of six fungicides (azoxystrobin, trifloxystrobin, difenoconazole, copper oxychloride 50 % WP, trifloxystrobin 25 % + tebuconazole 50 % and kasugamycin). Fresh plant material (100 g each) of neem leaves, tulasi leaves, garlic cloves, onion bulbs, turmeric rhizomes and ginger rhizomes was washed under running tap water, rinsed with sterile distilled water and surface dried. Each sample was crushed in a sterilised mortar and pestle with 100 mL of sterile distilled water (1 : 1 w/v). The macerate was left for 1 hr at room temperature, squeesed through four layers of muslin cloth and filtered through Whatman No. 1 filter paper. The filtrate (about 100 mL) was taken as the 100 % stock solution (w/v). The required quantity of stock was added to autoclaved PDA, cooled to 45–50 °C, to give final concentrations of 1, 3 and 5 % (v/v) and the medium was poured into sterile Petri plates. Extracts were prepared fresh for each experiment and six plant extracts (Turmeric, Neem, Garlic, Tulasi, Onion and Ginger leaf extracts) (Tables 2 and 3). After PDA was

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prepared, autoclaved and cooled to 45–50 °C, the appropriate amounts of fungicides and plant extracts were added to the medium. A 5 mm mycelial disc from a 3-day old pathogen culture was positioned in the middle of each Petri plate after the treatment media was added.

Table 1. List of fungal and bacterial bio control agents used under *in vitro*

Sl. No	Bio-control agent	Type
1.	*Trichoderma harzianum*	Fungal
2.	*Trichoderma viride*	Fungal
3.	*Trichoderma asperellum*	Fungal
4.	*Aspergillus niger*	Fungal
5.	*Bacillus subtilis*	Bacterial
6.	*Pseudomonas fluroscence*	Bacterial

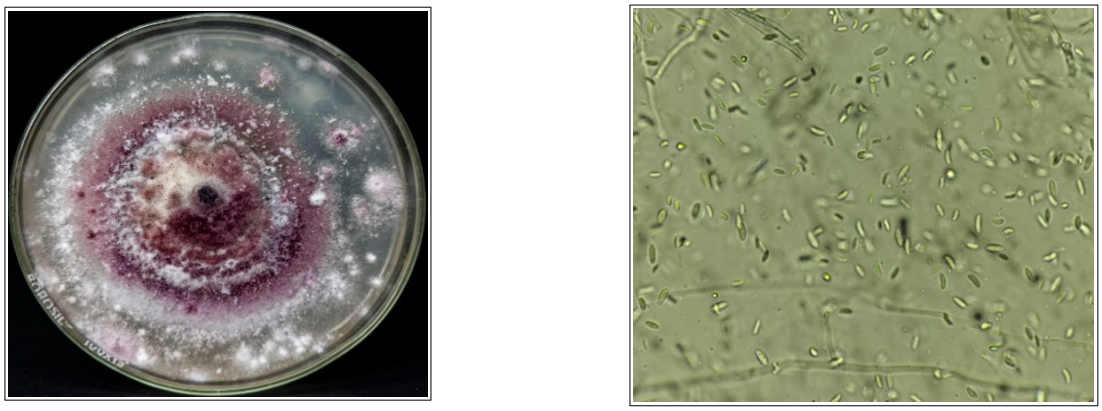


Fig. 1. (A). Cultural characteristics of *F. oxysporum* f. sp. *glycine* on potato dextrose agar (PDA) (B) Microscopic view of microconidia.

Table 2. List of fungicides and concentrations used for *in vitro* evaluation

Sl. No	Common name	Trade name	Concentration (ppm)
1.	Azoxystrobin	Amistar	100, 300, 500
2.	Trifloxystrobin	Flint	100, 300, 500
3.	Trifloxystrobin 25 % + Tebuconazole 50 %	Nativo 75 WG	100, 300, 500
4.	Copper oxychloride 50 % WP	Blitox	100, 300, 500
5.	Difenoconazole	Score	100, 300, 500
6.	Kasugamycin	Kasumin 2 L	100, 300, 500

WP: wettable powder.

Table 3. List of plant extracts and concentrations used for *in vitro* evaluation

Sl. No	Name of plants	Plant parts used	Concentration (%) *in vitro*
1	*Curcuma longa* (Turmeric)	Rhizomes	1, 3, 5
2.	*Allium sativum* (Garlic)	Cloves	1, 3, 5
3.	*Zingiber officinale* (Ginger)	Rhizomes	1, 3, 5
4.	*Ocimum tenuiflorum* (Tulasi)	leaves	1, 3, 5
5.	*Allium cepa* (Onion)	Bulb	1, 3, 5
6.	*Azadiracta indica* (Neem)	Leaves	1, 3, 5

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In vivo evaluation of integrated treatments

Details of integrated management treatments and their concentrations against Fusarium wilt of soybean presented in Table 4. Observation on per cent disease incidence was recorded at 30, 45 and 60 DAS.

Disease incidence

From the moment sickness signs first appeared, observations were made about the frequency and severity of wilting. The disease incidence was calculated by counting the number of plants in each of the 7 treatments that showed signs of Fusarium wilt and the number of plants that were free of the disease. The following formula was then used to get the percentage of disease incidence:

$$\% \text{ Disease incidence} = \frac{\text{No. of plants showing disease symptoms}}{\text{Total No. of plants/plot}} \times 100$$

Percent disease control (PDC) over untreated control was calculated by using the following formula.

$$\text{Per cent disease control} = \frac{[(\text{Disease incidence in untreated control} - \text{Disease incidence in tratments}}{\text{Disease incidence in untreated control}]} \times 100$$

Statistical analysis

The data generated from both *in vitro* and *in vivo* experiments were subjected to statistical analysis using OPSTAT software. Percentage data were transformed using the arcsine transformation prior to analysis. The standard error (SE±) and critical difference (CD) were calculated at the 5 % level of significance (p = 0.05) to assess the significance of differences among treatments.

Results

Isolation, purification, identification and pathogenicity assessment of causal organism

The pathogen was successfully isolated from disease soybean plant samples on PDA medium. Fungal growth appeared within 3–5 days of incubation as white, cottony colonies which later turned pale pink to violet. The purified culture showed uniform and profuse mycelial growth. Microscopic examination revealed hyaline, septate mycelium along with abundant microconidia and macroconidia. Microconidia were single celled, oval to elliptical, while macroconidia were multi celled slightly curved and fusiform. Chlamydospores were also observed, confirming the identity of the pathogen as *F. oxysporum* f. sp. *glycines*. Pathogenicity of isolated fungus was confirmed by artificial inoculation on healthy soybean plants. Typical wilt symptoms such as yellowing, drooping, vascular browning and plant wilting appeared within 10–15 days after inoculation. The pathogen was successfully re-isolated from infected plants and was found to be identical to the original culture, thereby fulfilling Koch's postulates (10).

In vitro evaluation of bioagents, fungicides and plant extracts against *Fusarium oxysporum* f. sp. *glycines*

The *in vitro* efficacy of six bioagents (dual culture technique), the concentrations were calculated based on the active ingredient concentration of each commercial formulation and six plant extracts (poisoned food technique at 1, 3 and 5 % concentration) was evaluated separately against *F. oxysporum* f. sp. *glycines.* All three group of treatments were evaluated independently with no combinations tested *in vitro* conditions. The results are presented below.

In vitro efficacy of bioagents on the mycelial growth of *Fusarium oxysporum* f. sp. *glycines* (dual culture method)

The antagonistic activity of six bioagents against *F. oxysporum* f. sp. *glycines* was assessed by dual culture technique on PDA medium. The radial growth of the pathogen and the corresponding percent inhibition were recorded at 12 hr (5 days) after inoculation. The control plates recorded a maximum radial growth of 90 mm. Among all the bioagents tested, *Trichoderma viride* (T_2) recorded the maximum percent mycelial inhibition of 70.30 % with the lowest mean colony diameter of 26.75 mm was found to be statistically superior over all other bioagents evaluated (Table 5). It was followed by *Trichoderma harzianum* (T_3) with 56.41 % inhibition (mean colony diameter 39.23 mm), *Bacillus subtilis* (T_6) with 53.96 % inhibition (41.43), *Trichoderma asperellum* (T_1) with 50.30 % inhibition (44.73 %), *Aspergillus niger* (T_4) with 49.93 % inhibition (45.07 mm) and *Pseudomonas fluorescens* (T_5) with 47.85 % inhibition (46.93 mm).

In vitro efficacy of fungicides on the mycelial growth of *Fusarium oxysporum* f. sp. *glycines*

The efficacy of six fungicides at three concentrations (100, 300 and 500 ppm) against *F. oxysporum* f. sp. *glycine* was assessed by poison food technique. Required concentration of each fungicide were incorporated into molten PDA medium before pouring into Petri plates. A 5 mm mycelial disc of test pathogen from 5 –7 days old culture was placed at the centre of each treated plate. The untreated control recorded a maximum radial growth of 90 mm. Among the fungicides evaluated, trifloxystrobin (T_2) recorded the highest percent inhibition of 79.67 % (mean colony diameter 18.37 mm) at the recommended concentration and was statistically superior over all other treatments (Table 6). It was followed by trifloxystrobin 25 % + tebuconazole 50 % (T_5) with 75.07 % inhibition, kasugamycin (T_6) with 69.33 % inhibition, azoxystrobin (T_1) with 63.52 % inhibition, difenoconazole (T_3) with 50.56 % inhibition and copper oxychloride 50 % WP (T_4) with 41.85 % inhibition.

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Table 4. Details of integrated management treatments and their concentrations against Fusarium wilt of soybean

Sl. No	Treatment number	Treatment details	Concentration
1.	T1	*Trichoderma viridae*	10 g/kg of soil (Soil drench)
2.	T2	Trifloxystrobin	0.1 % at 1 g/L of water
3.	T3	Neem leaf extract	5 % at 50 g/L of water
4.	T4	*Trichoderma viride* + Neem	10 g/kg soil + 5 % (Soil drench + Foliar spray)
5.	T5	Trifloxystrobin + *Trichoderma viride*	0.1 % + 10 gm/kg soil (Foliar spray + Soil drench)
6.	T6	Trifloxystrobin + tebuconazole (nativo)	0.1 % at 1 g/L of water (Foliar spray)
7.	Control	-	-

Table 5. Effect of different bio-control agents on the radial growth and percent inhibition of *Fusarium oxysporum* f. sp. *glycine in vitro*

Sl. No	Treatments	Mean colony diameter (mm)	Percent inhibition (%)
T_1	Trichoderma asperellum	44.73	50.30[b]
T_2	Trichoderma viride	26.73	70.30[a]
T_3	Trichoderma harzianum	39.23	56.41[b]
T_4	Aspergillus niger	45.07	49.93
T_5	Pseudomonas fluorescens	46.93	47.85
T_6	Bacillus subtilis	41.43	53.96[b]
T_0	Control	90.00	0.00
	SE (m) ±	1.33	
	CD at 0.05 %	4.04	

Table 6. *In vitro* efficacy of fungicides against the radial growth of *Fusarium oxysporum* f. sp. *glycine*

Fungicides	Mean radial growth (mm) 100 ppm	% inhibition	Mean radial growth (mm) 300 ppm	% inhibition	Mean radial growth (mm) 500 ppm	% inhibition
Azoxystrobin	32.50	63.89	26.80	70.22	20.10	77.67
Trifloxystrobin	18.37	79.59	14.20	84.22	11.30	87.44
Difenoconazole	44.50	50.56	38.20	57.56	32.10	64.33
Copper oxychloride 50 % WP	52.30	41.89	46.70	48.11	40.50	55.00
Trifloxystrobin + Tebuconazole 50 % WP	22.40	75.11	18.10	79.89	13.80	84.67
Kasugamycin	27.60	69.33	22.30	75.22	18.50	79.44
Control	90.00	0.00	90.00	0.00	90.00	0.00
SE (m) ±	0.39	-	0.42	-	0.38	-
CD at 0.05 %	1.17	-	1.27	-	1.13	-

WP: wettable powder.

Effect of different plant extracts on the mycelial growth inhibition of *Fusarium oxysporum* f. sp. *glycines* under *in vitro* conditions

The efficacy of six plant extracts at three concentrations (1, 3 and 5 %) against *F. oxysporum* f. sp. *glycines* was evaluated by poison food technique. Untreated control recorded a maximum radial growth of 90 mm.

Among the plant extracts evaluated, Neem leaf extract (T_2) recorded the highest percent inhibition of 76.07 % at 5 % concentration with a mean colony diameter of 21.53 mm and was not statistically significant compared with Garlic clove extract (T_3) 71.5 9 %, however both were significantly superior to all other treatments (Table 7). These were followed by onion bulb extract (T_5) with 66.07 % inhibition, turmeric rhizomes extract (T_1) with 61.30 % inhibition, ginger rhizome extract (T_6) with 35.81 inhibition and tulasi leaf extract (T_4) with 31.11 % inhibition at 5 % concentration. The antifungal activity efficacy of neem is attributed to its bioactive compounds such as azadirachtin, nimbin and nimbidin, which disrupt fungal cell membranes.

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Table 7. Effect of different plant extracts on the mycelial growth inhibition of *Fusarium oxysporum* f. sp. *glycine* under *in vitro* conditions (poisoned food technique)

Plant extract	Radial growth (mm) 1 %	% inhibition	Radial growth (mm) 3 %	% inhibition	Radial growth (mm) 5 %	% inhibition
Turmeric	64.33	28.52	49.67	44.81	34.83	61.30
Neem	49.67	44.81	35.40	60.67	21.53	76.07
Garlic	53.33	40.74	39.17	56.48	25.57	71.59
Tulasi	79.33	11.85	70.67	21.48	62.00	31.11
Onion	67.00	25.56	48.33	46.30	30.53	66.07
Ginger	74.34	17.41	66.00	26.67	57.77	35.81
Control	90.00	0.00	90.00	0.00	90.00	0.00
SE (m) ±	0.31	-	0.29	-	0.27	-
CD at 0.05 %	0.95	-	0.89	-	0.83	-

In vivo evaluation - Effect of treatments on disease incidence (%) of Fusarium wilt of soybean at 30, 45 and 60 days after sowing

The incidence of Fusarium wilt disease was significantly impacted by all treatments at all observation dates, as presented in Table 8.

Disease incidence at 30 days after sowing

At 30 DAS, the lowest disease incidence was recorded in T_5 (trifloxystrobin + *T. viride*) at 6.50 %, which was statistically on par with T_6 (Nativo) at 8.15 %. Both were significantly superior over all other treatments. These were followed by T2 (trifloxystrobin, 10.85 %) and T_4 (*T. viride* + neem extract, 11.20 %), which were statistically similar to each other. T_1 (*T. viride*) recorded 13.45 % and T_3 (Neem Extract) recorded the highest incidence among treated plots at 15.30 %.

Disease incidence at 45 days after sowing

A similar trend was observed at 45 DAS. T_5 (trifloxystrobin + *T. viride*) continued to be the most effective, recording the minimum disease incidence of 10.25 %, significantly superior over all other treatments. This was followed by T_6 (nativo, 14.30 %), T_2 (trifloxystrobin, 18.55 %) and T_4 (*T. viride* + Neem extract, 19.40 %). T_1 (*T. viride*) and T_3 (neem extract) recorded 22.80 and 26.15 % respectively.

Disease incidence at 60 days after sowing

At the final observation (60 DAS), T_5 (trifloxystrobin + *T. viride*) remained the most effective treatment with the lowest disease incidence of 14.80 %, recording 71.18 % disease control over the untreated control. T_6 (nativo) followed with 20.65 % (59.79 % control). T_2 (trifloxystrobin, 25.40 %) and T_4 (*T. viride* + neem extract, 27.10 %) were grouped together based on CD value (0.973). T_1 (*T. viride*) and T_3 (neem extract) recorded 31.55 and 38.25 % respectively.

Table 8. Effect of various treatments on the percent disease incidence (PDI) of Fusarium wilt in soybean at 30, 45 and 60 days after sowing under *in vivo* conditions

Sl. No.	Treatment	30 DAS (%)	45 DAS (%)	60 DAS (%)	% disease control (60 DAS)
1.	Trichoderma viridae	13.45	22.80	31.55	38.58
2.	Trifloystrobin	10.85	18.55	25.40	50.54
3.	Neem extract	15.30	26.15	38.25	25.51
4.	*T.viridae* + Neem extract	11.20	19.40	27.10	47.23
5.	Trifloxystrobin + *T. viride*	6.50	10.25	14.80	71.18
6.	Nativo (trifloxystrobin + tebuconazole)	8.15	14.30	20.65	59.79
7.	Control	19.10	34.60	51.35	-
	SE (m) ±	0.144	0.231	0.318	-
	CD at 0.05 %	0.442	0.707	0.973	-

DAS: days after sowing.

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Discussion

The present study showed that all treatments differed significantly in managing Fusarium wilt of soybean under laboratory and pot conditions and that integrating a fungicide with a bioagent was more effective than any individual treatment, as reported earlier (11). The better performance of the combination can be explained by the complementary modes of action of its components: trifloxystrobin disrupts fungal respiration, whereas *Trichoderma viride* acts through mycoparasitism, antibiosis and sustained disease suppression (12–13). *Trichoderma* spp. showed strong antagonism against the pathogen through direct and indirect mechanisms (14) and they are also known to improve seed germination, plant establishment and grain yield in soybean (15, 16), largely by enhancing nutrient uptake (17). This supports their use as sustainable bioinoculants that reduce dependence on chemical fertilisers (18). Their lytic enzymes, secondary metabolites and rapid colonisation of the rhizosphere restrict pathogen growth, as observed in the biological management of other soil-borne pathogens (19).

A recent study demonstrated strong antifungal activity of *T. harzianum* against *F. oxysporum* and showed simultaneous improvement in plant growth and defence responses, highlighting the dual biocontrol and plant-growth-promoting potential of *Trichoderma*. Similarly, recent work on soybean has shown that *Trichoderma* application can enhance root and shoot biomass, nodulation and primary and secondary metabolism, ultimately contributing to improved plant performance (20). These findings support the present observation that *T. viride* was the most effective bioagent and indicate that its beneficial effects may extend beyond direct inhibition of *F. oxysporum*. The comparatively better performance of the integrated trifloxystrobin + *T. viride* treatment in the present study is also in agreement with the increasing emphasis on combining chemical and biological approaches for sustainable disease management. Recent research indicates that biological treatments can reduce dependence on synthetic fungicides while maintaining effective suppression of biotic stress, whereas excessive chemical application may adversely affect plant health (21).

Botanical extracts also showed antifungal activity, with neem and garlic performing best, since plant secondary metabolites are toxic to microbial pathogens (22). The effectiveness of neem is associated with azadirachtin and related compounds that interfere with fungal growth and cellular processes (23). *In vitro* and pot results were largely in agreement, as treatments effective in the laboratory were also effective in pots (24). The antifungal activity of garlic may be attributed to sulfur-containing compounds, particularly allicin, which can inhibit fungal growth by affecting cellular enzymes and membrane functions (25). Allicin and other organosulfur compounds have been reported to interfere with essential metabolic processes and destabilise fungal cellular structures, thereby contributing to their broad-spectrum antifungal activity (24). The comparatively better performance of these extracts may also be related to the presence of multiple bioactive compounds acting synergistically. Similar responses have been reported where plant-derived treatments not only suppressed pathogen development but also improved plant growth and physiological performance under biotic stress (21). Recent studies have further highlighted the potential of combining biological and botanical approaches as safer alternatives for reducing dependence on synthetic fungicides and improving disease management under field conditions. Overall, the study reinforces the value of integrated approaches that combine chemical, biological and botanical components for sustainable management of Fusarium wilt in soybean (26), while reducing reliance on synthetic fungicides.

Conclusion

Fusarium oxysporum f. sp. *glycines*, the cause of Fusarium wilt of soybean, was significantly influenced by the control approaches tested. *In vitro, Trichoderma viride* (70.30 % inhibition) was the most effective bioagent, trifloxystrobin (79.59 % at 100 ppm) followed by its combination with tebuconazole was the most effective fungicide and neem leaf extract (76.07 % at 5 %) followed by garlic extract was the most effective botanical. In the pot experiment, the integrated treatment of trifloxystrobin + *T. viride* recorded the lowest disease incidence (14.80 % at 60 DAS) and the highest disease control (71.18 %), outperforming the individual treatments. Integrating a fungicide with a bioagent is therefore a promising, eco-friendly approach for managing Fusarium wilt of soybean under pot conditions and field validation is recommended.

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Authors' contributions

CBD led the conceptualisation, design, material preparation and data collection as well as data analysis for this research as part of her MSc dissertation. SM served as the major advisor, providing invaluable guidance and support, including help with securing funding. BCN, MD and SS contributed to the study through critical review and editing of the manuscript. All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

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