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RESEARCH ARTICLE

Sustainable management of *Alternaria alternata*-caused okra leaf spot

Repamenla K Imsong[1], Adesh Kumar[1*], Ankit Kumar[2] & Satya Prakash[3]

[1]Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara 144 411, Punjab, India
[2]Regional Plant Quarantine Station, Directorate of Plant Protection Quarantine and Storage, Ministry of Agriculture and Farmers Welfare, Govt of India, Mumbai 400 015, India
[3]Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Phagwara 144 411, Punjab, India

*Correspondence email - adesh.19078@lpu.co.in

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Abstract

The present study evaluated the efficacy of different fungicides, *viz.* Carbendazim 50 % WP, Mancozeb 75 % WP, Hexaconazole 5 % EC, Copper oxychloride 50 % WP, Carbendazim 25 % + Mancozeb 50 % WS, bioagent *Trichoderma viride* and neem oil both individually and in combination for the management of *Alternaria alternata*-induced leaf spot in okra (*Abelmoschus esculentus* L. Moench) under *in vitro* and *in vivo* conditions at Lovely Professional University, Punjab during 2024–25. In laboratory conditions, Dual culture assays on PDA medium revealed that *T. viride* suppressed pathogen growth significantly, achieving 81.1 % inhibition. Neem oil exhibited a concentration-dependent antifungal effect, with inhibition increasing from 29.4 % at 10 ppm to 67.0 % at 50 ppm. Fungicidal treatments using the poisoned food technique showed that Hexaconazole 5 % EC was the "most effective", recording up to 82.47 % inhibition at 750 ppm, followed by Mancozeb 75 % WP (63.79 %). While under *in vivo* condition, Carbendazim 25 % + Mancozeb 50 % WS resulted in the highest disease control among all the fungicides with least average disease incidence (14.18 %) and its highest reduction (60.49 %), disease severity (13.98 %) and its highest reduction up to (51.80 %) while enhancing plant growth parameters such as fruit length (11.26 cm), no. of fruits per plant (39) and plant height (165 cm) followed by Hexaconazole 5 %+ Neem oil, Copper oxychloride 50 % WP and Neem oil respectively. The integration of chemical fungicides, bioagents and botanicals was found to be an effective approach for sustainable management of leaf spot disease in okra.

Keywords: *Alternaria alternata*; disease incidence; fungicides; integrated disease management; neem oil; okra; *Trichoderma viride*

Introduction

Okra (*Abelmoschus esculentus* L. Moench) is an important crop in the Malvaceae family that is believed to have originated in modern-day Ethiopia. It thrives in tropical and subtropical climates, especially on well-drained sandy loam soils that are rich in nutrients. Okra is known as Gumbo in the United States of America and lady s' finger in England, whereas it is called bhinda or bhindi in India. The okra is an erect, herbaceous, annual green plant that grows in arid and semi-arid regions and the cultivation of this crop is mainly done in the rainy season. Its adaptability to a wide range of growing conditions makes it popular among vegetable growers. Okra is a significant vegetable crop that offers a wide range of nutritional and potential health benefits (1). It is widely grown for its immature tender pod, which is used as a vegetable. The okra crop has several biotic and abiotic challenges, including diseases and insect pests. Numerous diseases caused by bacteria, viruses, nematodes and fungi can damage okra, such as Alternaria leaf spot (*Alternaria alternata/A. chlamydospora*), powdery mildew, Cercospora leaf spot (*Cercospora abelmoschi/C. malayensis*), damping off and Yellow vein Mosaic virus (YVMV) (2, 3). Among them, *Alternaria alternata* of leaf spot of okra, is a major fungal disease that results in a major constraint in the economic production of okra crops (4).

The infected symptoms initially include light brown dots that eventually turn into concentric dark brown patches of different sizes, necrosis with concentric rings that initially appeared on older leaves and then became larger and spots with narrow chlorotic edges. Eventually, the spots coalesced together, causing many conidia to form on dead or dying tissues (5). Infected leaves turn brown and eventually die in cases of severe infection (6). Severe infections have occasionally resulted in a complete loss of yield (7).

Application of fungicides (systemic, non-systemic/ contact, combi-fungicides) has been reported to be effective under both *in vitro* and *in vivo* conditions against *A. alternata,* which causes okra leaf spot disease and leaf blight caused by various *Alternaria* spp., earlier by several workers (8, 9). Fungicides play a crucial role in managing fungal diseases in okra, including leaf spot, powdery mildew and wilt. They help prevent, suppress and control infections by inhibiting fungal growth, thereby protecting plant health and ensuring better yields. While fungicides are effective, excessive use can lead to

fungal resistance, environmental pollution and residue accumulation in fruits. Biocontrol agents like *Trichoderma* spp. *Pseudomonas fluorescens, Bacillus subtilis* were reported to be effective against *Alternaria alternata* and other *Alternaria* spp. (10). Numerous essential oils are effective (both *in vitro* and *in vivo*) against a variety of fungal infections due to their bioactive compounds with antifungal properties. It has been observed that essential oils like eucalyptus, clove, mint, neem, garlic and ginger were reported to be effective against *Alternaria* spp. Including *A.alternata* (11). This study aimed to evaluate the comparative efficacy of selected fungicides, bioagents and neem oil against *A. alternata* under laboratory and field conditions.

Materials and Methods

All the experiments, both *in vivo* and *in vitro,* were conducted at the Department of Plant Pathology, School of Agriculture, Lovely Professional University, Punjab. Fungicides and essential oils used in this research were obtained from the local market and biocontrols *T. virens* were obtained as pure culture from our university Lab.

Isolation, purification, identification and pathogenicity test

To isolate the pathogen from infected leaves with typical disease symptoms, the diseased portion of the leaves, as well as healthy tissues, was diced into small pieces. These components were surface sterilised by immersing them in a 1 % sodium hypochlorite solution for one minute. After three washings with sterile distilled water, the pieces were transferred to a sterile potato dextrose agar medium, which was placed in Petri plates and incubated at 25 ± 1 °C in a B.O.D. incubator for seven days. The entire procedure was carried out in a laminar hood under sterile conditions. The fungal pathogen was purified utilising a single hyphal-tip method, identification was done through microscopic examination and pathogenicity through Koch s' postulates (12).

In vitro efficacy of test bioagent on mycelial growth of *Alternaria alternata*

Bioagents were determined for their efficacy by the dual culture technique. 5mm diameter PDA discs of the test fungus culture growth and bioagent were taken by using a 5 mm sterilised cork borer. Then the discs of isolated pathogens were placed on one plate containing solidified PDA medium and discs of *Trichoderma viride* were placed at one end of each of the Petri plates. Culture discs inoculated plates only with the test fungus were maintained as an untreated control. The test pathogen s' percentage inhibition of growth was calculated using the formula as per the standard procedure (13).

$$\text{P.I.}(\%) = \frac{C-T}{C} \times 100 \qquad \text{(Eqn. 1)}$$

P.I. = Percent inhibition (%) of pathogen

C = Average radial growth (mm) in control plates

T =Average radial growth (mm) in treated plates

In vitro efficacy of fungicides and essential oils on mycelial growth of *Alternaria alternata*

The poisoned food technique was employed to evaluate the *in vitro* efficacy of five fungicides (Carbendazim 50 % WP, Mancozen 75 % WP, Hexaconazole 5 % EC, Copper oxychloride 50 % WP and Carbendazim 25 % + Mancozeb 50 % WS) and at four concentrations (100, 250, 500 and 750 ppm) and essential oil (neem oil) at (10, 25, 50 ppm) against *Alternaria alternata*. Potato dextrose agar (PDA) was prepared and autoclaved and the respective concentrations of fungicides and neem oil (emulsified with Tween-20) were incorporated into the medium and after cooling to around 45–50 °C. The treated medium was poured into Petri plates and a 5mm mycelial disc from a 3-day-old culture of *A. alternata* was placed at the centre of each plate. Controls were maintained separately for fungicides and neem oil, respectively. The formula proposed by Vincent was used to calculate the growth inhibition percentage (13).

$$\text{P.I.}(\%) = \frac{C-T}{C} \times 100 \qquad \text{(Eqn. 2)}$$

Where,

P.I. = Percent inhibition (%) of pathogen

C = Average radial growth (mm) in control plates

T = Average radial growth (mm) in treated plates

In vivo evaluation of fungicides, bioagents and essential oil against *Alternaria alternata.*

A field experiment was carried out on Rabi cropping seasons at the Agricultural experimental field of Lovely Professional University (31°24' N, 75°69' E), Phagwara, Punjab, to evaluate the efficacy of different fungicides, bio-agent and essential oil against Alternaria leaf spot disease. The experiment was laid out in a randomised block design with 10 treatments and three replications. The okra variety 'Prabhani Kranti', which was obtained from Marathwada Agricultural University, Maharashtra, was sown on 5th March 2024, at the optimum soil moisture level. The standard okra planting procedure was used, with seeds spread at a soil depth of 5 cm and rows and plants spaced 30 x 15 cm apart. Under current *in vivo* experiments, those fungicides, bioagents and essential oils proved effective against *A.alternata* were integrated for the management of Alternaria leaf spot of okra and one plot /treatment /replication was retained as control(unsprayed) as outlined in Table 1.

Disease incidence

Observations on leaf spot occurrence and intensity were recorded beginning with the initial appearance of disease symptoms. The number of plants exhibiting Alternaria leaf spot symptoms and the number of disease-free plants in each of the three treatments were counted to determine the disease incidence. The percentage of disease incidence was then calculated using the formula below.

$$\%\ \text{Disease Incidence} = \frac{\text{No. of plants showing disease symptoms}}{\text{Total No. of plants/plot}} \times 100 \qquad \text{(Eqn. 3)}$$

Disease severity

Three foliage (bottom, middle and top) per plant were selected using the (0-9) disease rating scale in Table 2 and five plants per treatment. Each replication was randomly selected, tagged and observations were made on the severity of Alternaria leaf spots (14). The proportion of disease severity was calculated using the formula below (15).

Table 1. Treatments and their respective dosage rates applied for the management of *Alternaria alternata*-induced leaf spot disease in okra

Code	Treatments	Dosages (g/mil/L.wt)
T_1	Carbendazim 50 % WP	2.0 g
T_2	Mancozeb 75 % WP	2.0 g
T_3	Hexaconazole 5 % EC	1.0 mL
T_4	Copper oxychloride 50 % WP	2.0g
T_5	*Trichoderma viride* (1×10^7 cfu/g)	10g
T_6	Neem oil	2 mL
T_7	Carbendazim 25 % + Mancozeb 50 % WS	2.5 g
T_8	*Trichoderma viride* + Neem oil	10 g + 2.0 mL
T_9	Hexaconazole 5 % EC + Neem oil	1.0 mL + 2.0 mL
T_{10}	Control (Unsprayed)	------

Table 2. Disease rating scale of the okra plant

Scale/Grade	Descriptions
0	No Symptoms
1	> 1 % or less of the leaf area affected
3	1- 10 % of the leaf area is affected
5	11-25 % of the leaf area affected.
7	26-50 % of leaf area affected, slightly sunken in the centre with concentric rings.
9	>50 % leaf area affected

$$\% \text{ Disease Severity} = \frac{\text{Summation of numerical ratings observed}}{\text{No. of leaves/plants observed x maximum rating}} \times 100 \quad \text{(Eqn. 4)}$$

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

$$\% \text{ Disease Control (PDC)} = \frac{\text{PDI in control plot - PDI in Treatment plot}}{\text{PDI in Control plot}} \times 100 \quad \text{(Eqn. 5)}$$

Evaluation of various growth parameters of okra: number of fruits per plant

To analyse the effects of various treatments on fruit production, the total number of fruits per plant was counted at the end of the trial. Both treated and untreated plants were examined and fruit count data were collected consistently and recorded.

Fruit length

The okra fruit of plants both treated and untreated was carefully measured at the end of the experimental trial using a precise wooden scale.

Plant height

Plant height was recorded in centimetres (cm) from the base of the plant at the soil level up to the tip of the main stem using a standard measuring wooden scale.

Statistical analysis

The data obtained under both *in vitro* and *in vivo* experiments were statistically analysed using OPSTAT software, with arcsine transformation applied to percentage values. Standard error (SE ±) and critical difference (CD) were calculated at $p = 0.05$ to determine the significance of treatment effects.

Results

In vitro efficacy of bioagent on the growth of the test pathogen

Dual cultures of both pathogenic fungi and the biological control agent were kept on Potato Dextrose Agar (PDA) medium in order to examine the antagonistic activity. Following a 24 h culture period, the fungal growth parameters were measured. *Trichoderma viride* showed the most growth on the third day, with 12 mm, while the pathogen showed the least amount of growth in all three replicates, with 42 mm. The biological control agents grew at their fastest rate and came into contact with the pathogen on the 4th day of culture, with 16.5 mm. On the 7th, the infection had outgrown the biological control agents with 17.0 mm, which significantly slowed its growth. Based on the radial growth measurements, the percent inhibition of *A.alternata* was calculated using the standard formula and *T.viride* achieved 81.1 % inhibition of the pathogen s' mycelial growth.

In vitro efficacy of essential oil on the growth of the test pathogen

The results revealed that at 10 ppm, neem oil exhibited a relatively low inhibition percentage of fungal growth was moderately restricted to 60 mm, indicating 29.4 % inhibition. While at 25 ppm, radial growth dropped to 42 mm, corresponding to 50.6 % inhibition. The highest concentration, 50 ppm, demonstrated the greatest antifungal activity, limiting growth to just 28 mm with 67.0 % inhibition. These results confirm that neem oil effectively suppresses *A. alternata* in a concentration-dependent manner, with significant growth suppression at 25 ppm and above.

In vitro efficacy of fungicides on the growth of the test pathogen

The effects of two systemic fungicides (Carbendazim 50 % WP, Hexaconazole 5 % EC), two non-systemic fungicides (Mancozeb 75 % WP, Copper oxychloride 50 % WP) and one combined fungicide (Carbendazim 25 % + Mancozeb 50 % WS) against *Alternaria alternata* growth at varying concentrations (100, 250, 500 and 750 ppm) were examined in laboratory experiments using the poisoned food technique. The percentage of growth inhibition of *A.alternata* over the untreated control was computed using data on mycelial radial growth.

Results shown in Table 3 and Fig. 1-2 indicate that the maximum radial of mycelial growth was higher in the control with 34.8 mm and mycelial growth ranged from 12 to 25 mm under various treatments at 100 ppm. However, Hexaconazole 5 % EC recorded the maximum inhibition at all concentrations. At 100 ppm, it achieved 65.52 % inhibition, which increased to 82.47 % at 750 ppm, followed by Mancozeb 75 % WP with 52.3 % at 100 ppm and 63.79 % at 750 ppm. The least inhibition was seen in Carbendazim 25 % + Mancozeb 50 % WS, which ranged from 28.16 % at 100 ppm to 42.53 % at 750 ppm.

Table 3. Effect of different fungicides on the mycelial growth inhibition of *Alternaria alternata* under *in vitro* conditions

Fungicides	Mean radial growth (mm)							
	Concentration (ppm)							
	100	% Inhibition	250	% Inhibition	500	% Inhibition	750	% Inhibition
Carbendazim 50 % WP	18.2	47.7	17.6	49.43	16.8	51.72	14.2	59.2
Mancozeb 75 % WP	16.6	52.3	15.6	55.17	13.7	60.63	12.6	63.79
Hexaconazole 5 % EC	12.0	65.52	10	71.26	9.2	73.56	6.1	82.47
Copper oxychloride 50 % WP	17.9	48.56	17.1	50.86	14.7	57.76	13.8	60.34
Carbendazim 25 % + Mancozeb 50 % WS	25.0	28.16	23	33.91	21.2	39.08	20	42.53
Control	34.8		34.8		34.8		34.8	
SE (m) ±	0.04		0.05		0.03		0.02	
CD at 0.05 %	0.14		0.15		0.11		0.09	

Mean of three replications

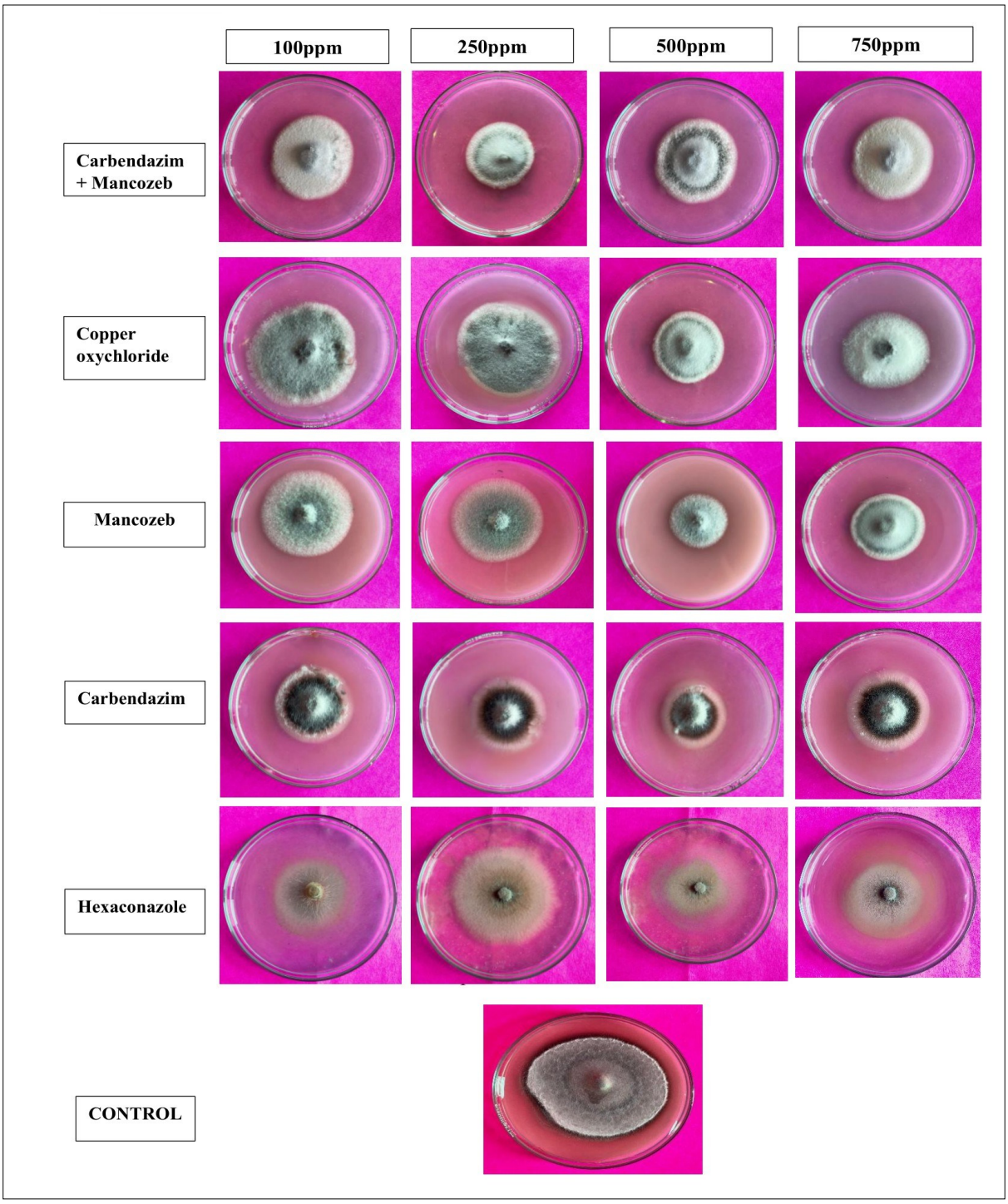

Fig. 1. Effect of different fungicides at varying concentrations (100, 250, 500 and 750ppm) on the mycelial growth inhibition of *Alternaria alternata* after 120 h of incubation under *in vitro* conditions.

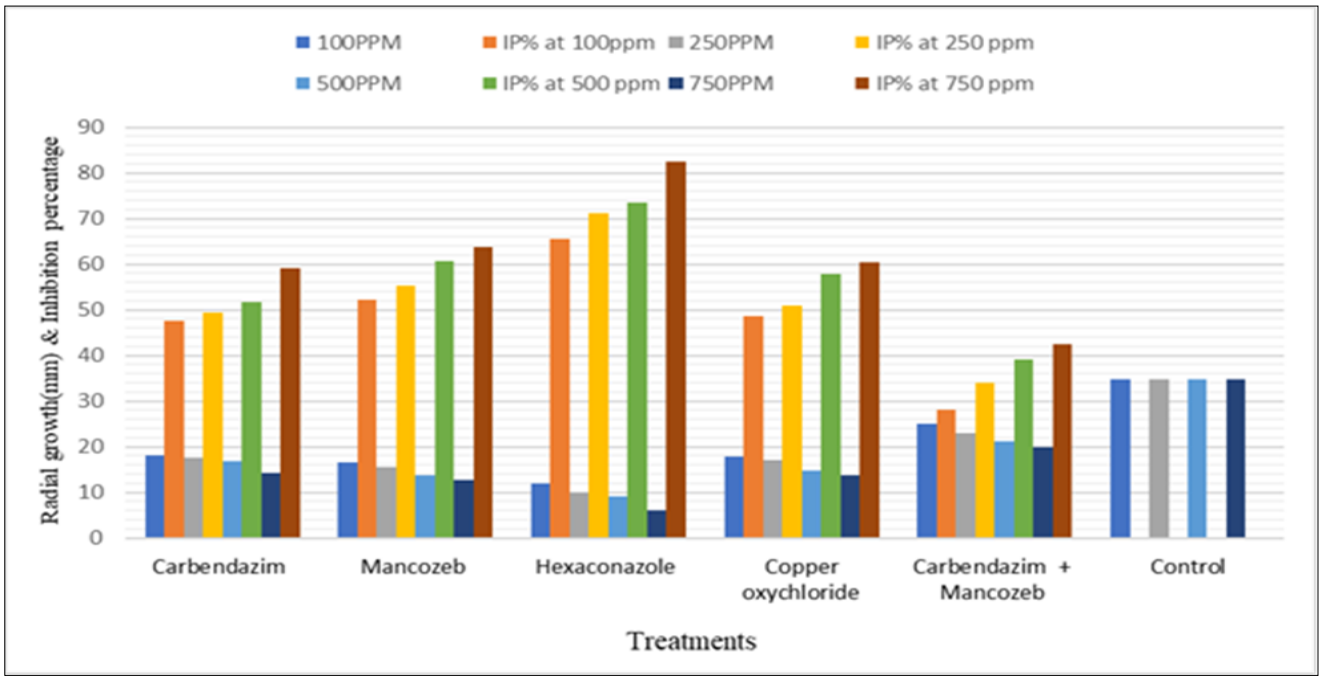

Fig. 2. Bars represent the average radial growth and corresponding inhibition percentage of the test fungus at 120 h after inoculation, as influenced by different fungicide treatments under the poisoned food technique, each applied at concentrations of 100, 250, 500 and 750 ppm.

Field evaluation of fungicides, bioagents and essential oils against *Alternaria alternata*

Disease incidence

The incidence of okra leaf spot disease was significantly impacted by all treatments, according to the results displayed in Table 4 and Fig 3. Three consecutive sprays were used to assess the efficacy of different treatments against *Alternaria alternata*, which causes okra leaf spot disease. Disease symptoms first appeared 55-60 days after sowing and gradually increased in intensity until the application of the second spray, after which a marked decline was observed across most treatments. At the initial appearance of symptoms, the lowest percent disease incidence (PDI) was recorded in plants treated with Hexaconazole 5 % EC (9.80 %), whereas the untreated control showed the highest PDI (13.90 %). Overall, the initial PDI among treatments ranged from 9.80 % to 13.90 %, indicating the early effectiveness of fungicidal applications in suppressing disease establishment. Disease incidence ranged from 14.90 % to 30.90 % after three spray administrations, compared to 72.40 % in the control. Among the treatments, Carbendazim 25 % + Mancozeb 50 % WS was found to be the "most effective", with the lowest average disease incidence (14.68 %) and the highest average percent disease control (57.97 %) followed by Copper oxychloride 50 % WP (15.88 % PDI and 54.54 % PDC), Hexaconazole 5 % EC (16.58 % PDI and 52.54 % PDC), Carbendazim 50 % WP (16.83 % and 51.83 %), Mancozeb 75 % WP and Hexaconazole 5 % EC+ Neem oil also showed moderate effectiveness, recording 17.65 % and 15.85 % PDI and 49.48 % and 54.62 % PDC, respectively. Biological treatments like *Trichoderma viride* (19.80 % PDI and 43.34 % PDC) and its combination with Neem oil (19.75 % PDI and 43.47 % PDC) showed better results than Neem oil, which was found to be the "least effective" treatment with the highest disease incidence (23.60 %) and lowest percent disease control (32.45 %).

Table 4. Effect of various treatments on the percent disease incidence (PDI) of okra leaf spot caused by *Alternaria alternata* under field conditions

Code	Treatments	Rate/ Conc.	Disease incidence (%)				Av. DI (%)	Av. Red. (%)
			At 1st Appear	After 1st spray	After 2nd spray	After 3rd spray		
T_1	Carbendazim 50 % WP	2.0 g	11.90 (20.18)	16.20 (23.73)	19.90 (26.49)	17.30 (24.58)	16.33 (23.83)	54.49 (47.49)
T_2	Mancozeb 75 % WP	2.0 g	12.00 (20.27)	16.10 (23.66)	22.50 (28.32)	20.00 (26.57)	17.65 (24.84)	50.80 (45.44)
T_3	Hexaconazole 5 % EC	1.0 mL	09.80 (18.24)	17.00 (24.35)	21.10 (27.35)	18.40 (25.40)	16.58 (24.03)	53.80 (47.14)
T_4	Copper oxychloride 50 % WP	2.0 g	11.60 (19.91)	15.00 (22.79)	18.80 (25.70)	16.10 (23.66)	15.38 (23.09)	57.14 (49.04)
T_5	*Trichoderma viride* (1×10^7 cfu/g)	10g	10.60 (19.00)	16.60 (24.04)	26.00 (30.66)	24.00 (29.33)	19.30 (26.06)	46.20 (42.86)
T_6	Neem oil	2 mL	10.70 (19.09)	19.30 (26.06)	33.50 (35.37)	30.90 (33.77)	23.60 (29.06)	34.22 (35.69)
T_7	Carbendazim 25 % + Mancozeb 50 % WS	2.5 g	10.10 (18.53)	13.90 (21.89)	17.80 (24.95)	14.90 (22.71)	14.18 (22.12)	60.49 (51.22)
T_8	*Trichoderma viride* + Neem oil	10 g + 2.0 mL	12.50 (20.70)	20.00 (26.57)	24.80 (29.87)	21.70 (27.76)	19.75 (26.39)	44.95 (42.20)
T_9	Hexaconazole 5 % EC + Neem oil	1.0 mL + 2.0 mL	11.00 (19.37)	14.80 (22.63)	20.60 (26.99)	16.00 (23.58)	15.60 (23.26)	56.52 (48.71)
T_{10}	Control (Unsprayed)	------	13.90 (21.89)	29.70 (33.02)	37.50 (37.36)	62.40 (52.18)	35.88 (36.80)	
	S.E.±		**0.06**	**0.05**	**0.33**	**0.09**		
	C.D. at 0.05 %		**0.17**	**0.14**	**1.01**	**0.26**		

Mean of three replications, Conc.: Concentration, Av.: Average, DI: Disease incidence, Red.: Reduction. Figures in parentheses are arcsine-transformed values

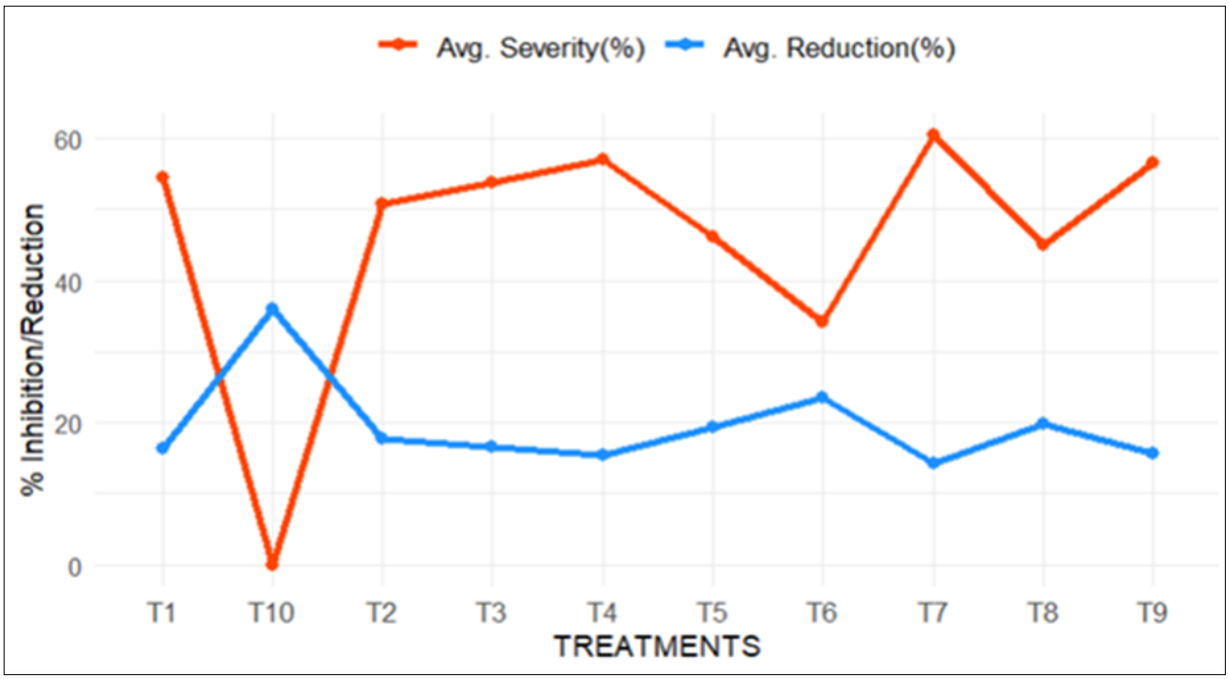

Fig. 3. Effect of different treatments (T_1 to T_{10}) on average disease incidence and average disease reduction in okra plants, illustrating the relative effectiveness of each treatment in managing the disease under *in vivo* conditions.

Disease severity

Results observed that all treatments considerably reduced the severity of leaf spot disease compared to the control (untreated) (Table 5 and Fig. 4). Disease symptoms were first observed 55-60 days after sowing and throughout the cropping season, with a substantial reduction in disease severity recorded following the third spray across all treatments. At the initial appearance of symptoms, disease severity ranged from 11.25 % to 16.20 %, with the highest severity observed in plants treated with neem oil (16.20 %) and the lowest in those treated with Carbendazim 25 % + Mancozeb 50 % WS (11.25 %). After the third spray, the percent disease severity (PDI) among treatments declined gradually, ranging from 13.97 % to 31.72 %, compared with 42.00 % in the untreated control. Therefore, Carbendazim 25 % + Mancozeb 50 % WS was the "most effective" treatment, with the lowest average disease severity (13.97 %) and the highest percent disease control (55.27 %) followed by Hexaconazole 5 % EC + Neem oil (16.29 % PDI, 47.82 % PDC), Copper oxychloride 50 % WP (17.12 % PDI, 45.18 % PDC), Hexaconazole 5 % EC(18.93 % PDI, 39.33 % PDC) and Carbendazim 50 % WP (19.75 % PDI, 36.70 % PDC), *Trichoderma viride* + Neem oil (20.88 % PDI, 33.18 % PDC), *Trichoderma viride* (20.73 % PDI, 33.60 % PDC) and Mancozeb 75 % WP (21.73 % PDI, 30.40 % PDC) were moderately effective. While Neem oil was the "least effective", with the highest disease severity (27.27 %) and the lowest disease control (12.29 %), over the untreated control (31.76 % PDI).

Evaluation of various growth parameters of okra

Results in Table 5 showed that the treatments used to control leaf spot disease had a major impact on both the okra plant growth and yield parameters.

Table 5. Effect of different treatments on the disease severity of okra leaf spot caused by *Alternaria alternata* under field conditions

Tr, No	Treatments	Rate/ Conc.	Disease Severity %				Av. PDI (%)	Av. PDC (%)
			At 1st Appear	After1st spray	After2nd spray	After3rd spray		
T_1	Carbendazim 50 % WP	**2.0 g**	13.80 (21.78)	19.20 (25.62)	23.00 (28.05)	21.00 (26.47)	19.25 (25.77)	33.66 (35.26)
T_2	Mancozeb 75 % WP	**2.0 g**	15.60 (23.25)	21.00 (27.08)	25.20 (30.37)	23.40 (28.69)	21.30 (27.09)	26.57 (31.09)
T_3	Hexaconazole 5 % EC	**1.0 mL**	14.20 (22.18)	18.50 (24.87)	22.00 (27.83)	20.10 (26.44)	18.70 (25.48)	35.54 (36.57)
T_4	Copper oxychloride 50 % WP	**2.0 g**	13.05 (20.86)	16.63 (23.65)	20.30 (26.71)	18.50 (25.10)	17.12 (24.23)	40.99 (40.04)
T_5	*Trichoderma viride* ($1x10^7$ cfu/g)	**10g**	15.10 (22.77)	20.50 (26.16)	24.50 (29.70)	23.10 (28.74)	20.80 (26.77)	28.27 (31.67)
T_6	Neem oil	**2 mL**	16.20 (23.60)	26.20 (31.05)	30.00 (33.21)	31.40 (34.16)	25.95 (31.61)	10.54 (18.80)
T_7	Carbendazim 25 % + Mancozeb 50 % WS	**2.5 g**	11.25 (19.47)	14.40 (23.16)	16.20 (25.02)	14.05 (22.26)	13.98 (21.99)	51.80 (45.75)
T_8	*Trichoderma viride* + Neem oil	**10 g + 2.0 mL**	14.90 (22.61)	20.00 (26.57)	24.00 (29.43)	22.60 (28.23)	20.38 (26.85)	29.77 (32.84)
T_9	Hexaconazole 5 % EC + Neem oil	**1.0 mL + 2.0 mL**	12.50 (20.50)	16.33 (23.45)	19.15 (25.81)	17.20 (24.49)	16.30 (23.48)	43.81 (41.41)
T_{10}	Control (Unsprayed)	------	15.25 (22.62)	27.60 (31.00)	35.20 (36.38)	38.00 (38.82)	29.01 (33.46)	
	S.E.±		**0.09**	**2.40**	**0.16**	**0.18**		
	C.D. at 0.05 %		**0.27**	**0.21**	**0.48**	**0.54**		

Mean of three replications, Conc.: Concentration, Av.: Average, PDI: Percent disease intensity, PDC: Percent disease control. Figures in parentheses are arcsine-transformed values.

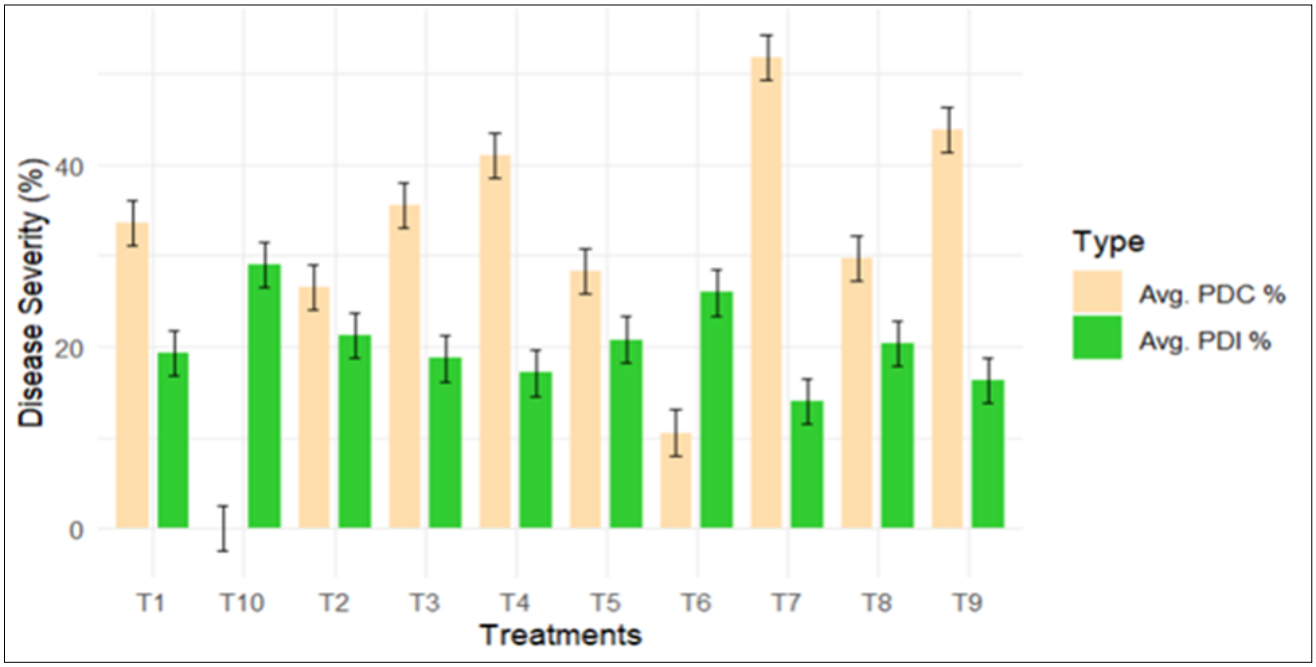

Fig. 4. Comparison of disease severity based on the average percent disease index (PDI) and percent disease control (PDC) across ten treatments(T_1-T_{10}).

Fruit length

Table 5 and Fig. 5 is plotted against the total number of fruits and various treatments, where fruit length in okra reveals that the combined fungicide Carbendazim 25 % + Mancozeb 50 % WS recorded the highest fruit length (11.26 cm), followed by Hexaconazole 5 % EC + Neem oil (10.76 cm) and the untreated control (10.79 cm). Among individual fungicides, Copper oxychloride 50 % WP (10.54 cm) and Hexaconazole 5 % EC (10.41 cm) performed moderately well, while Carbendazim 50 % WP (10.37 cm) and Mancozeb 75 % WP (10.02 cm) showed slightly lower effectiveness. In contrast, bioagents and botanicals such as Neem oil (9.07 cm), *Trichoderma viride* (9.11 cm) and their combination (9.24 cm) recorded the least fruit lengths, likely due to their slower and less systemic action.

Fruits per plant

The number of fruits per plant in okra varied substantially between treatments, with Carbendazim 25 % + Mancozeb 50 % WS having the greatest fruit count, *i.e.* 39, followed by Hexaconazole 5 % EC + Neem oil and Copper oxychloride 50 % WP, Hexaconazole 5 % EC and Carbendazim 50 % WP provided moderate outcomes. On the other hand, bioagents and botanicals, such as *Trichoderma viride*, Neem oil and their combination, resulted in fewer fruits (Table 5 and Fig. 6), most likely due to slower action and restricted systemic effects.

Plant height

The analysis of various treatments on plant height in okra demonstrated significant differences as shown in Table 6 and Fig. 7, with the tallest plants recorded in the plot treated with

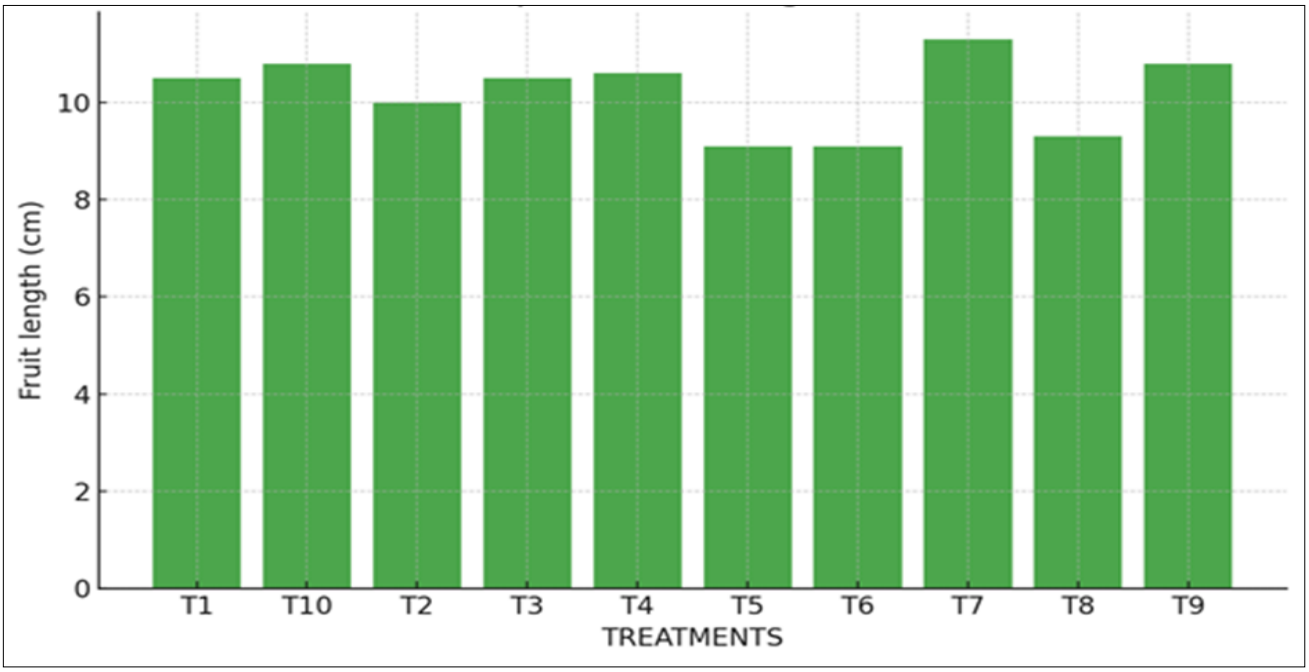

Fig. 5. Effect of different treatments (T_1-T_{10}) on mean fruit length in okra. Bars represent the average fruit length under each treatment, showing significant variation among treatments.

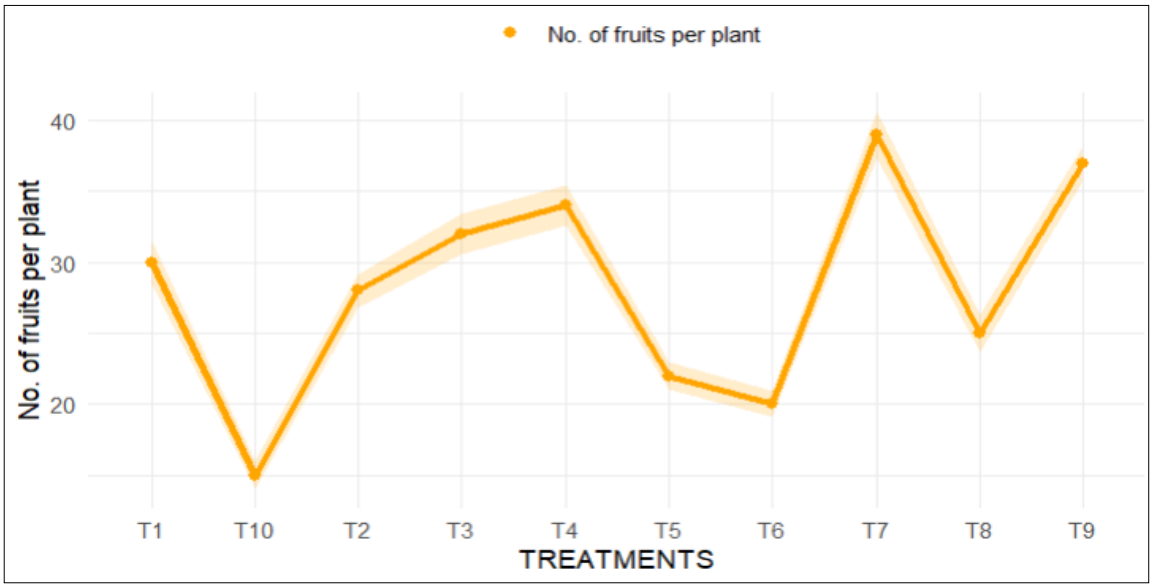

Fig. 6. Effect of different treatments (T_1-T_{10}) on the mean number of fruits per plant in okra.

Table 6. Effect of various treatments on growth parameters of okra

Code	Treatments	Rate/ Conc.	Fruit length (cm)	No. of fruits per plant	Plant height (cm)
T_1	Carbendazim 50 % WP	2.0 g	10.37	30	140
T_2	Mancozeb 75 % WP	2.0 g	10.02	28	135
T_3	Hexaconazole 5 % EC	1.0 mL	10.41	32	148
T_4	Copper oxychloride 50 % WP	2.0 g	10.54	34	152
T_5	*Trichoderma viride* (1 x 10^7 cfu/g)	10g	9.11	22	122
T_6	Neem oil	2 mL	9.07	20	115
T_7	Carbendazim 25 % + Mancozeb 50 % WS	2.5 g	11.26	39	165
T_8	*Trichoderma viride* + Neem oil	10 g + 2.0 mL	9.24	25	128
T_9	Hexaconazole 5 % EC + Neem oil	mL + mL	10.76	37	160
T_{10}	Control (Unsprayed)	------	10.79	15	90
	S.E.±		0.06	0.60	0.59
	C.D. at 0.05 %		0.19	1.81	1.77

Mean of three replications, Conc.: Concentration.

Carbendazim 25 % + Mancozeb 50 % WS (165 cm) followed by Hexaconazole 5 % EC + Neem oil (160 cm) and Copper oxychloride 50 % WP (152 cm), Hexaconazole 5 % EC (148 cm) and Carbendazim 50 % WP (140 cm) produced taller plants, demonstrating effective performance. Moderate plant heights were observed with Mancozeb 75 % WP at 135 cm and the combination of *Trichoderma viride* and Neem oil at 128 cm. In contrast, *Trichoderma viride* alone measured (122 cm), while Neem oil recorded 115 cm and the control treatment measures (90 cm), indicating the least effectiveness.

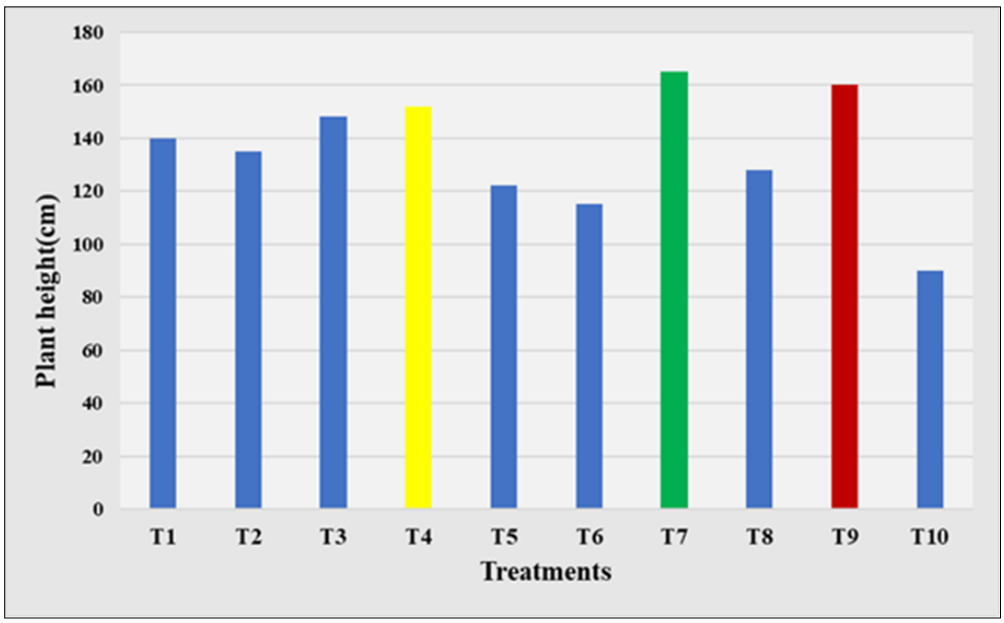

Fig. 7. Effect of different treatments (T_1–T_{10}) on plant height in okra. Bars represent the mean plant height recorded under each treatment, showing significant variation in vegetative growth.

Discussion

The present investigation demonstrated significant differences among treatments in suppressing *Alternaria alternata* leaf spot of okra, with varying efficacy under *in vitro* and *in vivo* conditions. Although all treatments effectively reduced disease incidence and severity compared to the untreated control, their relative performance differed between laboratory and field evaluations, indicating the influence of environmental and physiological factors on treatment efficacy.

Under *in vitro* conditions, Hexaconazole 5 % EC exhibited the highest mycelial inhibition, attributable to its systemic demethylation-inhibiting (DMI) action that disrupts ergosterol biosynthesis, thereby restricting fungal cell membrane development. In contrast, the combination fungicide Carbendazim 25 % + Mancozeb 50 % WS proved "most effective" under field conditions. This discrepancy between laboratory and field performance can be attributed to factors such as photodegradation, wash-off during rainfall, systemic versus protectant activity and the enhanced persistence and rainfastness of combination fungicides. The dual action formulation of Carbendazim (systemic benzimidazole fungicide) and Mancozeb (protectant dithiocarbamate) provides both curative and protective effects, ensuring longer residual activity and broader pathogen control under variable environmental conditions. Similar findings have been reported by earlier studies that identified Hexaconazole and Pyraclostrobin as highly effective *in vitro*, whereas Mancozeb- and Carbendazim-based combinations performed better in field disease suppression (16-21)

Trichoderma viride showed strong antagonism (81.1 % inhibition) *in vitro*, likely due to mechanisms such as mycoparasitism, antibiosis and competition for nutrients and space. However, its efficacy under field conditions was moderate, which may be attributed to fluctuating temperature, humidity, UV exposure and competition with native microflora in the phyllosphere, factors that commonly limit the establishment of biocontrol agents. Similar observations have been made by other researchers, who reported that *Trichoderma* spp. Effectively suppressed foliar fungal pathogens under controlled conditions but exhibited reduced activity in field environments (22, 24). Neem oil demonstrated limited efficacy in the field, primarily due to rapid photodegradation and low residual persistence. Neem oil, integrating with *T. viride* or fungicides, enhanced disease control. Several essential oils, including neem, eucalyptus, clove, karanj and cinnamon, have been reported to inhibit various *Alternaria* spp. When used alone or in combination with bioagents or fungicides (25).

Furthermore, the combination fungicide Carbendazim 25 % + Mancozeb 50 % WS not only reduced disease incidence and severity but also improved growth parameters such as fruit length, fruit number per plant and plant height. This may be attributed to reduced biotic stress and enhanced physiological activity due to effective disease suppression. Similar growth-promoting effects of fungicidal treatments on okra and other crops have been documented in previous studies (26).

Conclusion

The present study concluded that among all the treatments tested against *Alternaria alternata,* the causal agent of leaf spot in okra, *Trichoderma viride* showed antagonistic activity with 81.1 %, neem oil demonstrated significant antifungal activity in a dose-dependent manner and Hexaconazole 5 % EC emerged as the "least effective" fungicide, achieving up to 82.47 % growth inhibition. Field trials confirmed that these treatments significantly reduced disease incidence and severity while improving plant growth parameters such as fruit number, fruit length and plant height. Thus, integrating effective fungicides, bioagents and essential oils provides a promising and sustainable approach to managing Alternaria leaf spot in okra.

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Authors' contributions

RKI contributed to conceptualization, methodology, investigation and writing-original draft and figure preparation. AK contributed to conceptualization, methodology, reviewing and editing- original draft. KA and SP contributed to review and editing. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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