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Efficacy of various plant extracts against *Fusarium oxysporum* f. sp. *lentis* under in vitro and in vivo conditions.

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KEYWORDS

Lentil, Fusarium oxysporum f.sp. lentis, Plant extract. Mycelial growth, Growth inhibition, Scanning Electron Microscope (SEM), Plant extract, in-vitro evaluation, in-vivo evaluation and plant disease incidence.

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Abstract

The Rabi seasons of 2024–2025 and 2025–2026 were used for the trials. This study was conducted at the Acharya Narendra Deva University of Agriculture and Technology's Department of Plant Pathology in Kumarganj, Ayodhya. The goal of the trials was to develop a module for ecologically friendly management of Fusarium oxysporum. To identify the pathogen causing lentil wilt disease, an investigation was conducted. Fusarium oxysporum, the pathogen responsible for lentil wilt, was identified morphologically after sick plants exhibiting wilting symptoms were collected. In almost every lentil-producing region of Uttar Pradesh, Fusarium oxysporum f.sp. lentis has been shown to cause fusarium wilts of lentils. The current study was conducted to provide an efficient plant extract-based management strategy to counteract the high disease incidence and achieve 100% disease control of *Fusarium oxysporum* f.sp. *lentis* in the lentil crop. First, research was done to assess five plant extracts. Phytoextracts were evaluated against Fusarium oxysporum f.sp. lentis. Neem extract showed the strongest antifungal action among the phytoextracts examined, preventing *Fusarium oxysporum* f.sp. *lentis* from growing at doses of 5, 10, and 20%.

Introduction

One of the earliest legume crops, lentils (*Lens culinaris* Medik), are rich in protein and several other amino acids, including tryptophan and lysine. They date back to 7000 BC in Asia and 13000 BC worldwide. (Zapata *et al*., 2004; Kumar *et al*., 2021; Sandhu and Singh, 2007). Another name for it is poor man's meat, or masoor (Sen and Kapoor, 1975). India's main producers of lentils are Madhya Pradesh (42.50%), Uttar Pradesh (31.25%), West Bengal (9.38%), and Bihar (8.75%). India produced 1.61 million tons of lentils on 1.49 million hectares, with an average yield of 1006 kg/ha. (Ahmad *et al*.,

2018; Abraham, 2015; Anonymous, 2018). Lentil production is hampered by a number of diseases (Nelson *et al*., 1983; Dubey, 2020). Aphanomyces Root Rot (Aphanomyces euteiches), Anthracnose (*Colletotrichum lindemuthianum*), Rust (Uromyces fabae), Ascochyta Blight (*Ascochyta fabae* f.sp. *lentis*), Fusarium wilt (*Fusarium oxysporum*), Collar Rot (*Sclerotium rolfsii*), Root Rot (*Rhizoctonia solani*), Alternaria blight (*Alternaria alternata*), and Botrytis grey mould (Lindbeck, 2009). *Fusarium oxysporum* f. sp. *lentis* can live in soil for multiple seasons thanks to chlamydospores. Despite only infecting lentils, it exhibits significant morphological and pathogenic variation and has a comparatively narrow host range. (Belabid *et al*., 2004; Bayaa *et al*., 1986; Vasudeva and Srinivasan, 1952). Both seedlings and the flowering stage (adult stage) are impacted by the wilt disease, which appears as patches or spots in the field. *Fusarium oxysporum* f. sp. *lentis* infection manifests as sudden drooping of lentil leaves, dry, dull green leaves, and, ultimately, plant death. The vascular system in the roots of infected plants is discoloured brown. (Prasad *et al*., 2019; Lindbeck, 2009). The pathogen is known to produce three different kinds of asexual spores: microconidia, macroconidia, and chlamydospores. Microconidia are usually single-celled, hyaline, ovoid, or kidney-shaped creatures. Macroconidia are lengthy, have two to seven cells, and have a pointed apical cell and a notched basal cell. Chlamydospores are single-celled, thick-walled, oval or spherical creatures that can develop separately in macroconidia or apically or intercalary in hyphae. (Sugha *et al*., 1994; Khare, 1980). Three to four weeks after seeding, wilt developed in the field under optimal circumstances. Crop productivity can be decreased by wilt infection, and vascular wilt is a serious disease in India that can result in losses of over 50% in certain regions. (Kumar *et al*., 2009; Vasudeva and Srinivason, 1952; Khare, 1981). An environmentally safe way to manage different plant diseases and encourage plant growth is to use biocontrol chemicals. (Kumar and others, 2013; Kumar and others, 2014). Secondary metabolites are also helpful in controlling plant diseases (Kumar *et al*., 2010). In addition to acting as antagonistic fungi, they may also act as biofertilizers, promoting plant growth (Srivastava *et al*., 2009; Kumar *et al*., 2019). Thus, the effectiveness of plant extracts in suppressing *F. oxysporum* f. sp. *lentis* was assessed in this study.

Materials and methods

Isolation, identification, and purification of Test pathogen

Naturally infested plants of lentil showing the characteristic symptoms of wilt were collected in polythene bags and brought to the laboratory for isolation of the causal agent. The injured plant parts were put between two folds of sterile blotting sheets to absorb excess moisture after being properly rinsed under running water to remove adherent dirt. Using a sterilised blade, these contaminated plant components, such as the stem, root, and fruits, which showed half healthy and half sick areas, were chopped into small pieces of 4- 5 mm in size. Following that, these little pieces were surface sterilised for 30 seconds with sodium hypochlorite, followed by 2-3 quick washing with distilled water to eliminate any particles of NaOCl. After that, such bits were put aseptically in previously disinfected Petri plates containing solidified potato dextrose agar medium and 0.001% chloramphenicol. These Petri plates were subsequently incubated in a B.O.D incubator at 20±2°C temperature and the presence of fungal growth, if any, was monitored on a regular basis. Following the development of mycelial growth, the developing hyphal tips were transferred aseptically to other Petri plates. Purification of the fungus was accomplished by transferring freshly developed hyphal tips from sub-cultured Petri plates into culture tubes (Dhingra and Sinclair described 1985). To keep the fungus alive, the pure culture of the pathogen was sub-cultured on every 15 days on potato dextrose agar slants and kept at 4-5°C for subsequent use throughout the study (Kohn, 1992). Based on early macroscopic inspection of radial growth in Petri plates and microscopically in temporary slides made and stained with cotton blue, the fungus was identified. The keys of Alexopoulos and Mims (1979) were used for identification.

In-vitro Evaluation of Plant Extracts

The poisoned food approach (Sreenu and Zacharia, 2017) was used to test Fusarium oxysporum f. sp. lentis sensitivity to phytoextracts. Five phytoextracts, including ginger, tulsi, garlic, onion, and neem, were tested for their efficiency against Fusarium wilt. Using a wide variety of concentrations, the study attempts to determine the best effective concentration(s) of these plant extracts for further exploration while minimizing any potential plant toxicity. Gerard *et al*. (1994) used a standard process to extract plant components such as leaves, bulbs, and cloves, among others. Fresh plant parts were cleansed with tap water, then sterile distilled water, before being ground with a mortar and pestle at 1 mL g-1 of plant

tissue (1:1 v/w) and filtered through a double-layered muslin cloth. The resultant filtrate was the standard plant extract solution. Stock solutions were used to obtain plant extract concentrations of 5, 15, and 30%, and 5, 10, and 20 ml were mixed with 95, 90, and 80 ml of sterilized molten potato dextrose agar (PDA) medium, respectively. The collected material was gently shaken to ensure even mixing. In sterile Petri plates, 20 mL of poisoned PDA was added. The plates were inoculated with a five mm diameter mycelium disk from an actively developing pure culture of *F. oxysporum* f. sp. *lentis*, and incubated at 27±2°C for 7 and 14 days. The mycelial growth was observed, and the percentage growth inhibition was computed using the following formula by Vincent (1947).

Results and Discussion

Effect of Various Plant Extracts on *Fusarium oxysporum* f.sp. *lentis.* (2024-25 and 2025), Neem, Tulsi, garlic, and Neem at 5, 10, and 20 per cent concentrations were evaluated against *Fusarium oxysporum* f. sp. *lentis (Tables* 1, 2,3, 4 and Figures; 2024-25 and 2025-26).

Table 1. Effect of Plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lentis* at 7 days in the year 2024-25

Concentration %	5		10		20	
Treatments	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)
(Ginger)	7.96	68.73	4.22	81.60	05.00	92.15
(Tulsi)	6.22	74.26	3.28	84.45	0.40	92.10
(Garlic)	6.32	73.24	4.55	85.69	16.30	81.88
(Onion)	6.14	69.73	3.85	83.30	16.66	81.48
(Neem)	15.40	64.44	6.12	73.34	05.00	90.00
Control	45	00	45	00	45	00
S.E(m) ±	0.09	0.6	0.10	0.7	0.14	0.40
LSD p ≤ 0.05	0.22	0.18	0.19	0.21	0.30	1.20

Table 2. Effect of Plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lentis* at 14 days in the year 2024-25

Treatments	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)
(Ginger)	48.66	45.92	37.32	58.51	10.00	88.88
(Tulsi)	29.32	67.40	26.00	71.10	15.32	82.96
(Garlic)	40.66	45.58	26.66	70.36	6.00	93.33
(Onion)	29.62	29.62	42.00	53.33	22.00	75.55
(Neem)	40.66	45.58	26.66	70.36	6.00	93.33
Control	90.00	00.00	90.00	00.00	90.00	00.00
S.E(m) ±	0.730	1.814	0.802	1.995	0.471	0.986
LSD p ≤ 0.05	2.300	5.886	2.529	6.472	1.485	3.199

Table 3. Effect of Plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lentis* at 7 days in the year 2025-26

Concentration %	5		10		20	
Treatments	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)
(Ginger)	54.66	39.23	33.33	62.93	14.00	84.40
(Tulsi)	5.23	77.26	6.32	80.45	0.50	94.10
(Garlic)	30.66	65.86	27.33	69.56	20.00	73.73
(Onion)	20	23.67	42.66	52.56	29.33	67.36
(Neem)	20.00	33.30	28.00	68.83	22.66	74.76

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Control	90.00	0.00	90.00	0.00	90.00	0.00
S.E(m) ±	0.09	0.54	0.10	1.43	0.14	0.25
LSD p ≤ 0.05	0.22	0.18	0.19	4.66	0.30	0.75

Table 4 Effect of Plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lentis* at 14 days in the year 2025-26

Treatments	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)
(Ginger)	47.00	44.77	36.33	59.63	15.63	82.60
(Tulsi)	42.66	52.58	26.66	70.36	6.00	93.33
(Garlic)	37.33	58.52	28.33	68.52	9.33	89.63
(Onion)	47.10	47.77	36.33	57.63	15.66	82.50
(Neem)	30.33	66.30	20.33	77.41	3.66	95.93
Control	45.00	00	45.00	00	45.00	00
S.E(m) ±	1.11	1.43	0.47	0.57	0.77	0.96
LSD p ≤ 0.05	3.51	4.66	1.48	1.88	2.44	3.12

Table 5. Effect of Seed treatment with Plant Extracts against *Fusarium oxysporum* f.sp. *lentis* in the year 2024-25

TREATMENTS	Per cent disease incidence		
	At 30 DAS	At 45 DAS	At 60 DAS
Ginger Rhizome extract @20%	34.24	41.42	52.00
Tulsi leaf extract @20%	41.35	46.42	54.40
Garlic bulb extract @20%	35.84	46.37	50.21
Onion bulb extract @20%	45.35	55.60	64.72
Neem leaf extracts @20%	39.24	45.20	58.40

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Control	80.17	84.40	90.10
S.E(m) ±	0.52	0.648	1.353
LSD p ≤ 0.05	1.58	1.867	4.078

Table 6. Effect of Seed treatment with Plant Extracts against *Fusarium oxysporum* f. sp *lentis* in the year 2025-26

TREATMENTS	Per cent disease incidence		
	At 30 DAS	At 45 DAS	At 60 DAS
Ginger Rhizome extract @20%	33.24	40.42	50.00
Tulsi leaf extract @20%	40.36	45.40	53.40
Garlic bulb extract @20%	34.83	45.36	51.20
Onion bulb extract @20%	44.35	53.60	62.72
Neem leaf extracts @20%	38.24	48.20	57.40
Control	80.52	86.10	88.00
S.E(m) ±	0.58	0.88	0.94
LSD p ≤ 0.05	2.04	2.53	2.70

Among them, Neem (90.00%) showed the highest inhibition of mycelial growth, followed by Tulsi (93.33%), Garlic (89.63%), and Ginger (82.33%). Onion recorded the minimum growth inhibition (82.50%) of the pathogen. Neem were superior to all other tested Plant extracts at the 5, 10%, and 20% concentrations at 7 days and 14 Days of inoculation. Thus, the results clearly indicated that plant extracts reduced the Radial growth of *Fusarium oxysporum* f.sp. *lentis*. The ability of garlic and neem extract to inhibit mycelial growth may be attributable to the antifungal substances they contain, such as diallyl disulfide, diallyl trisulfide and azadirachtin. Unlike in Tulsi, ginger, and onion, antifungal compounds such as oleanolic acid, ursolic acid, zingiberene, gingerol and catechins have already been reported to have biofungicidal efficacy against various fungi. Each plant extract contains a unique blend of phytochemicals,

and their relative concentrations may differ depending on the plant species, location, and growth stage. Some phytochemicals may exhibit potent antifungal properties, while others may be ineffective or even toxic to the plant. Thus, the variation in the chemical composition of plant extracts could lead to differences in their antifungal activity against Fusarium species. According to Singh *et al*. (2010), *Datura festilosa*, Tagetes erecta, Eucalyptus citridora, Aegle marmelos, and Mimusops elengi were the plants that inhibited the growth of *F. udum* mycelium the least effectively. Similarly, Khaleel *et al.* (2014) investigated the Fungitoxic effects of six methanolic plant extracts at nine different concentrations: garlic, ginger, oak leaf, neem leaf, moringa leaf, and parthenium leaf. The best control was found to be 1000 g/ml Neem leaf extract, followed by Ginger extract. At the highest concentration (1000 g/ml).

Field Experiment

A field experiment was conducted to check the efficacy of seed treatment with botanicals and bio-agents alone combination against Fusarium wilt of lentil var. L 9-12 was carried out during the Rabi season 2024-25and 2025-26 to manage the disease at the Students Instructional Farm of A.N.D. University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.). The experiment had 6 treatments with three replications in a Randomized Block Design (RBD), with each plot measuring 2.5m x 2m. The crop was grown in the field to follow all agronomic packages of practices. Seed germination was counted 25 Days after sowing, along with infected plants with wilt disease. The disease incidence was calculated using the following formula.

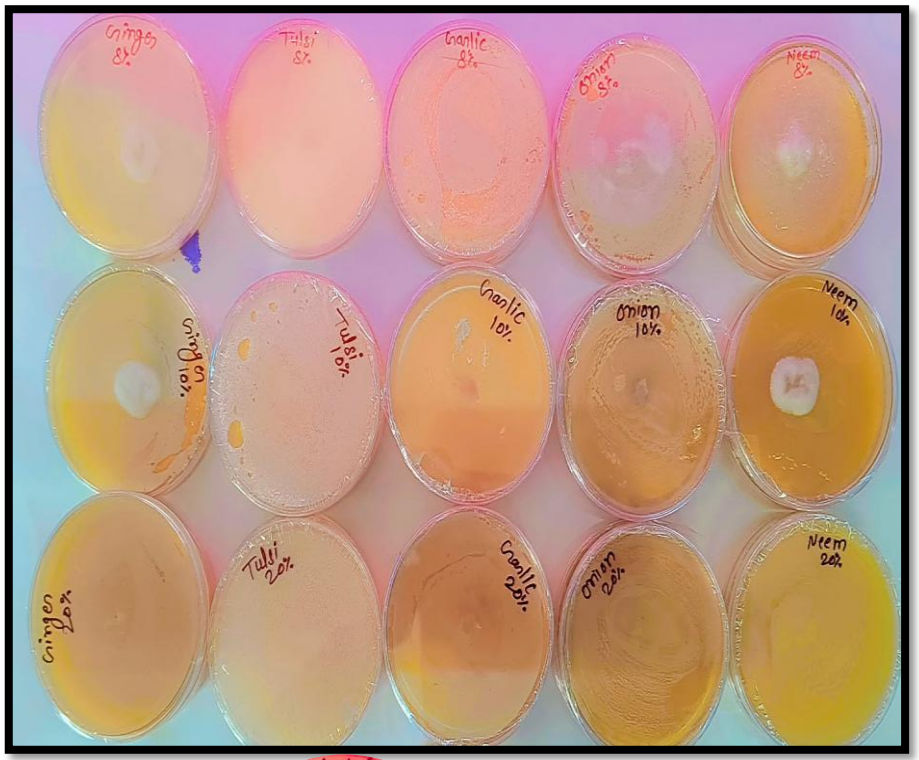
Disease incidence (%) =

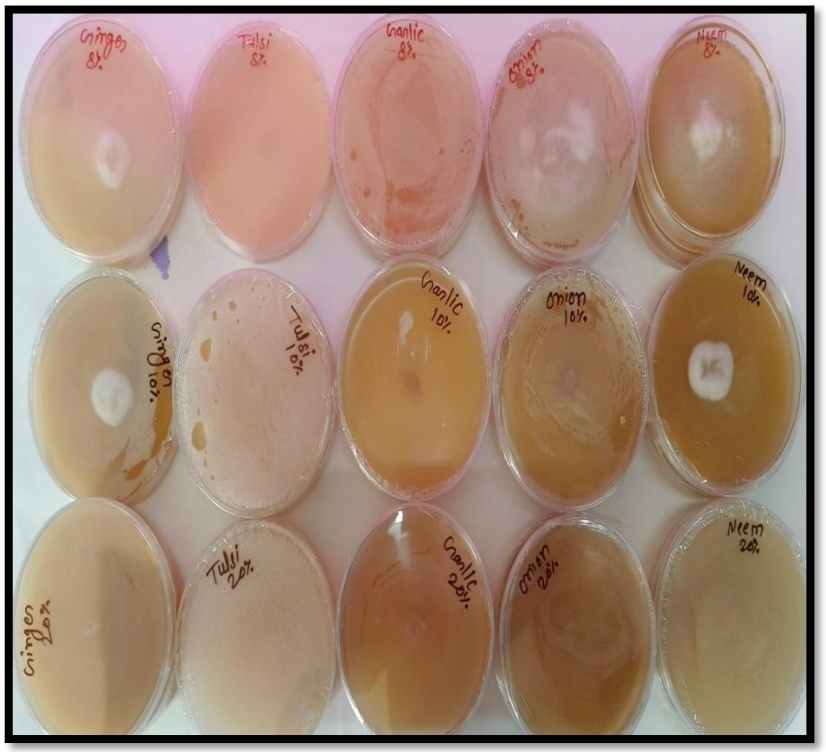
$$\frac{\text{Number of Infected Plants observed}}{\text{Total number of Plants observed}} \times 100$$

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	GINGER	TULSI	GARLIC	ONOIN	NEEM	GINGER	TULSI	GARLIC	ONOIN	NEEM
5%										
10%										
20%										
Control										









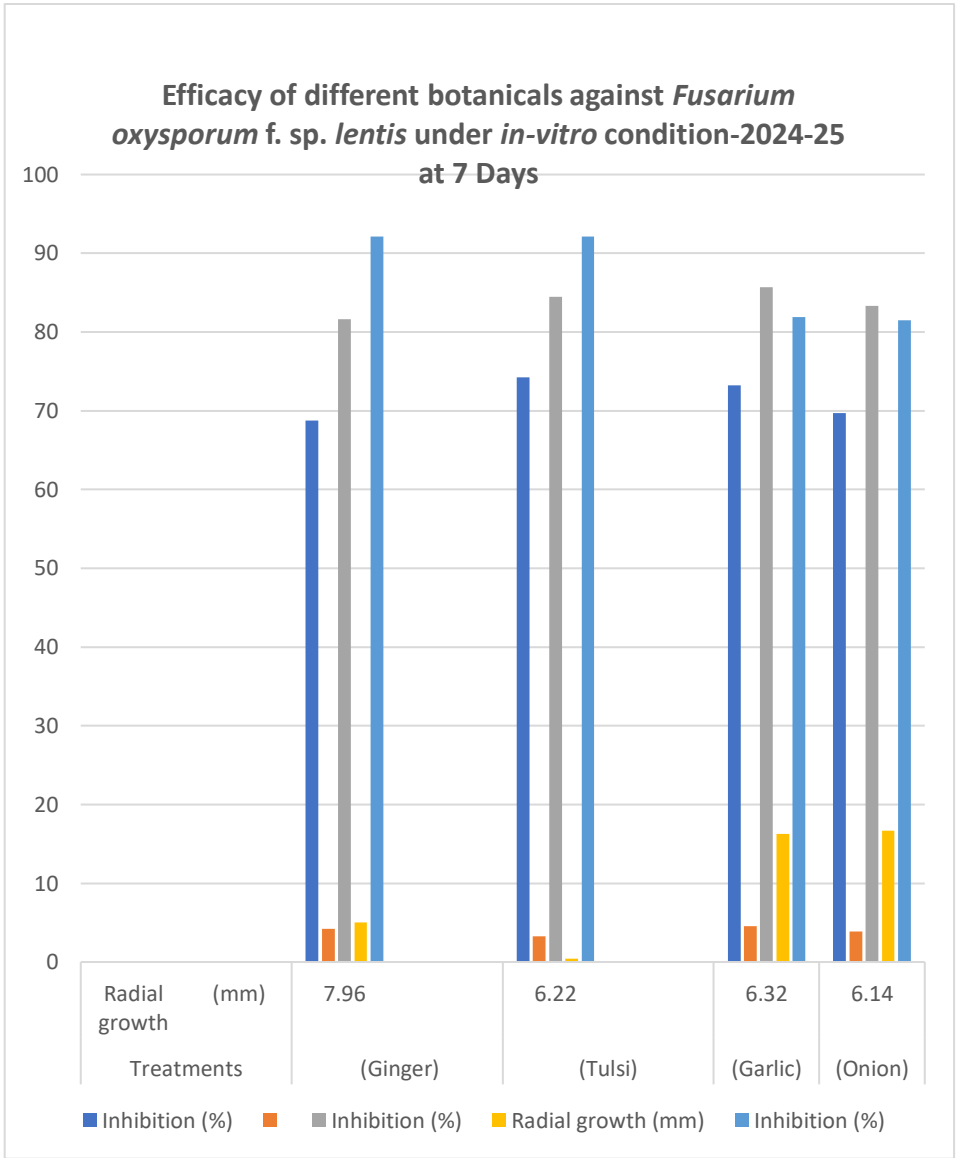
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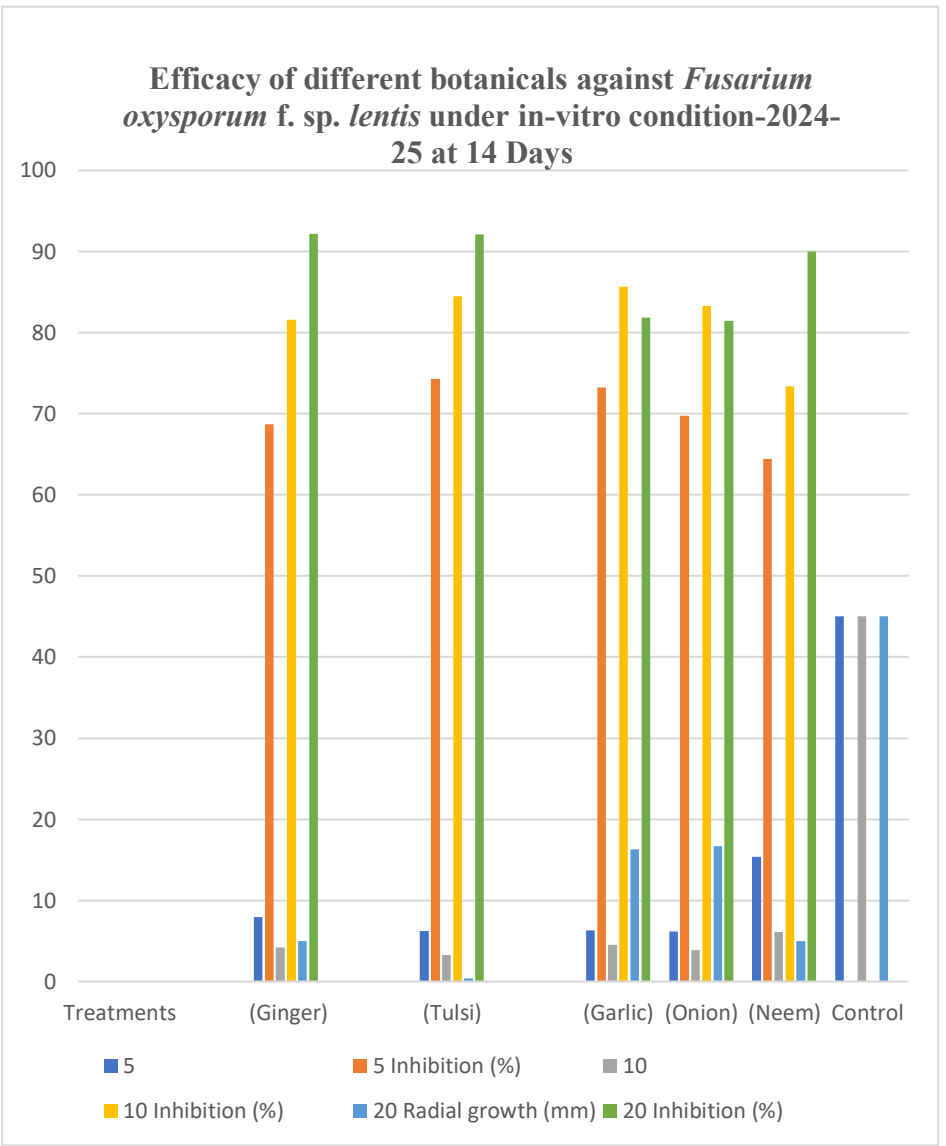
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Efficacy of different botanicals against *Fusarium oxysporum* f. sp. *lentis* under *in-vitro* condition-2024-25 at 7 Days	Efficacy of different botanicals against *Fusarium oxysporum* f. sp. *lentis* under in-vitro condition-2024-25 at 14 Days

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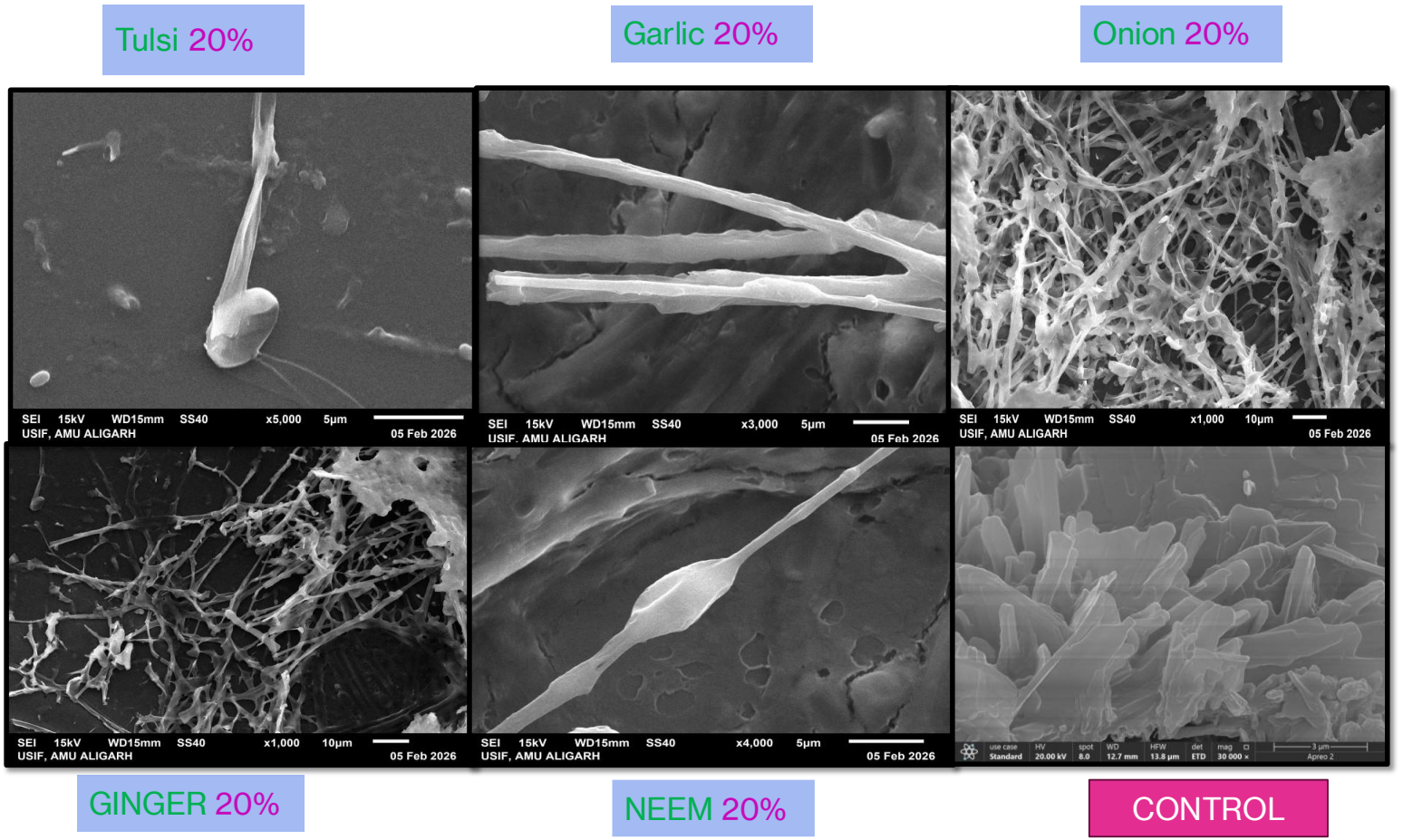
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Efficacy of different botanicals against *Fusarium oxysporum* f. sp. *lentis* under *in-vitro* condition. 2025-26



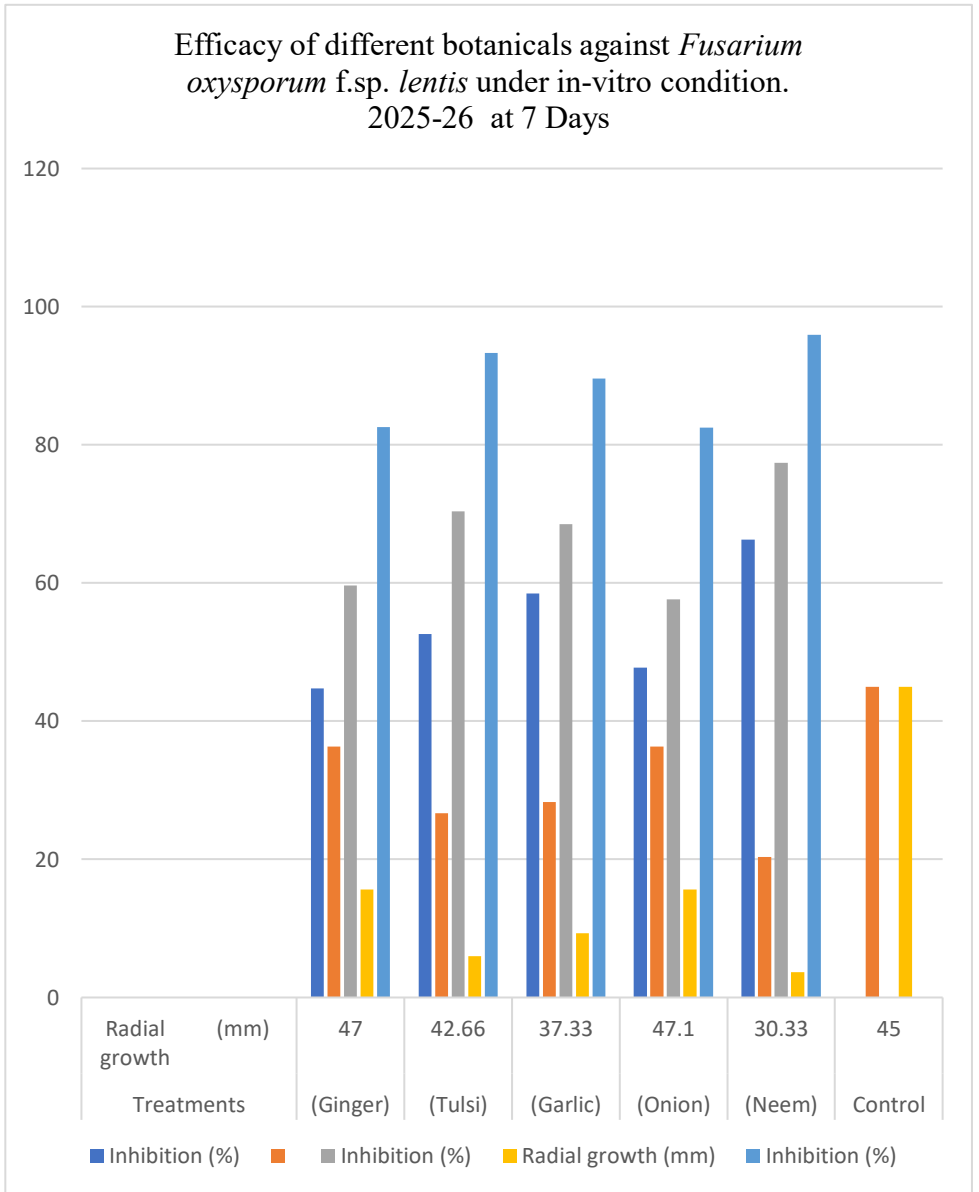
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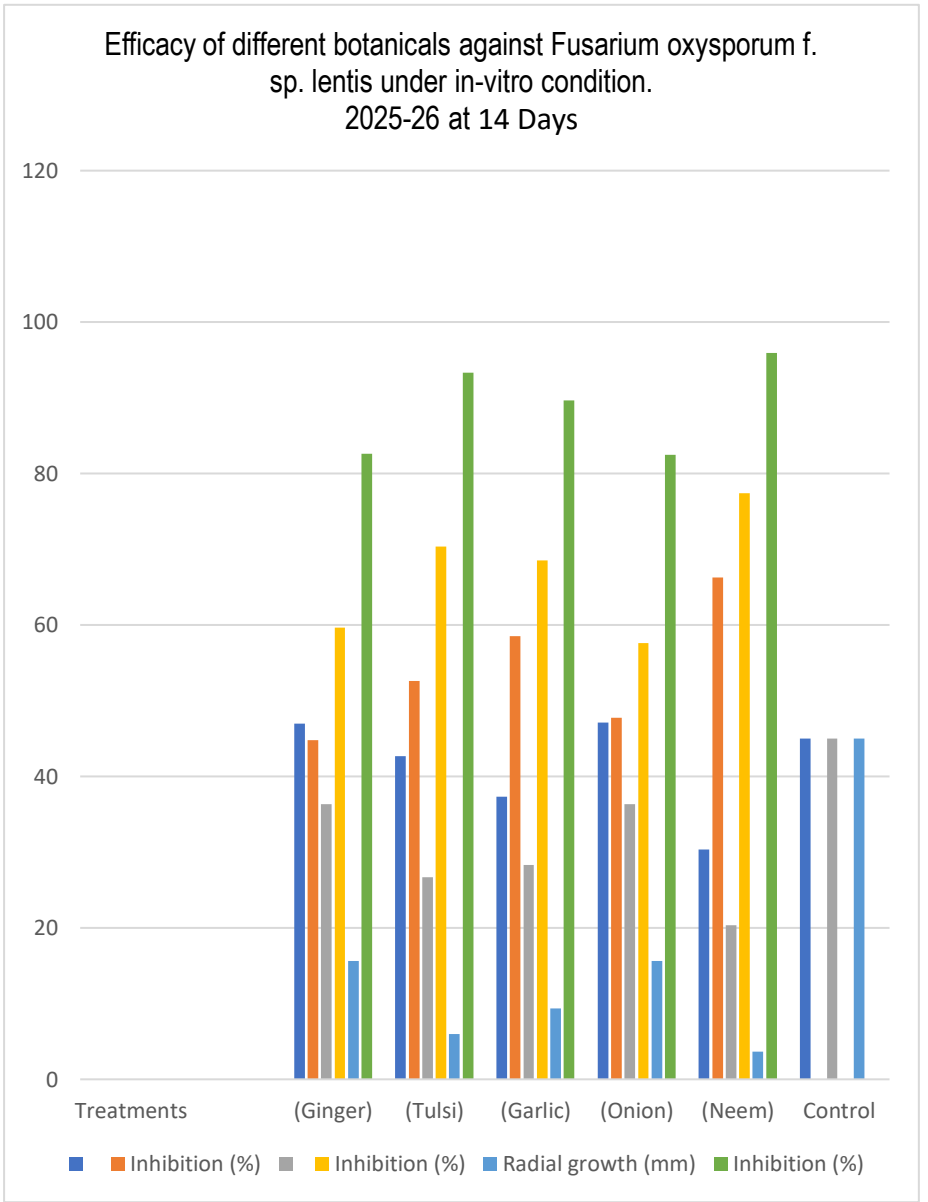
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Plant extracts concentration

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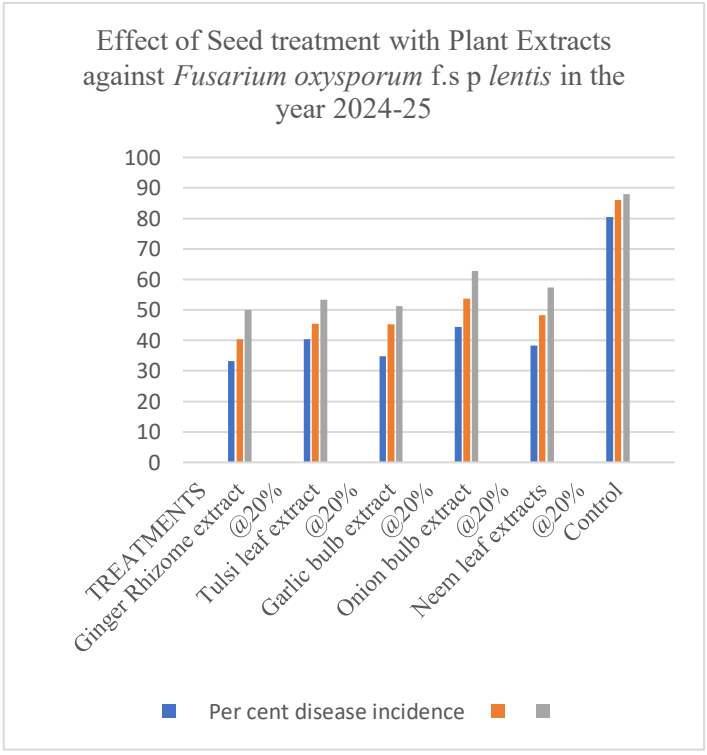
Effect of Seed treatment with Plant Extracts against *Fusarium oxysporum* f. sp. *lentis*

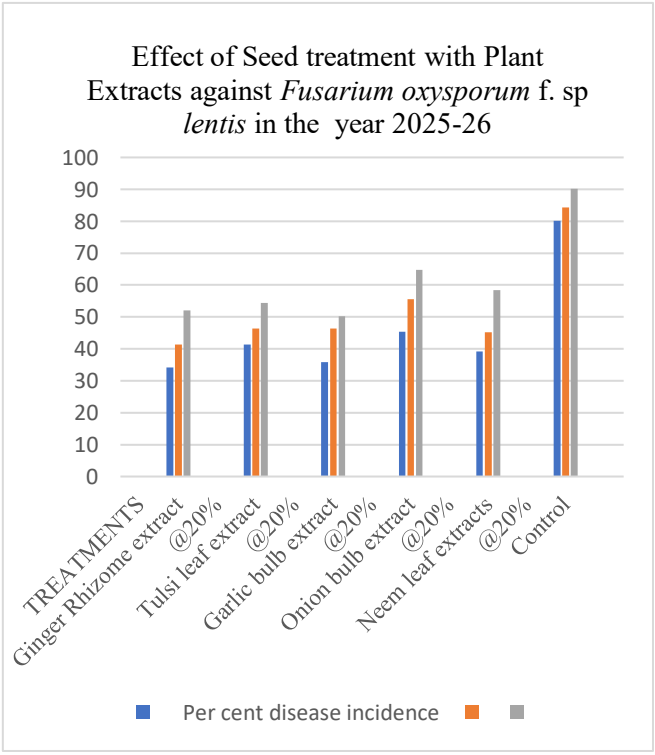
In-vivo Evaluation of Plant Extracts 2024-25

Minimum Disease incidence at all 3 dates: Ginger Rhizome extract @20% (33.24%, 40.42%,50.00%) and Second best: Garlic bulb extract @20% at 30 DAS (34.83%); maximum disease incidence (least effective): Onion bulb extract @20% (44.35%, 53.60%, 62.72%)

In-vivo Evaluation of Plant Extracts 2025-26

From the present investigation on the effect of botanicals against wilt of lentil caused by *Fusarium oxysporum* f. sp. *lentis* under pot culture conditions, it was observed that all the botanicals tested @20% significantly reduced the per cent disease incidence compared to the control. Among the five botanicals, Ginger rhizome extract @20% was found most effective in reducing the disease, recording the minimum disease incidence of 34.24% at 30 DAS, 41.42% at 45 DAS and 52.00% at 60 DAS. It was followed by Garlic bulb extract @20%, which recorded 35.84%, 46.37% and 50.21% disease incidence at 30, 45 and 60 DAS, respectively, which was the best at 60 DAS. Onion bulb extract @20% was found least effective, recording the maximum disease incidence of 45.35%, 55.60% and 64.72% at 30, 45 and 60 DAS, respectively. Neem leaf and Tulsi leaf extracts were moderately effective. Therefore, Ginger rhizome extract and Garlic bulb extract @20% can be recommended as eco-friendly and economical botanicals for the management of lentil wilt.





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Conclusion

Among the botanical extracts, Neem leaf extract recorded the highest inhibition of mycelial growth (95.93%) at 20% concentration, followed by tulsi (93.33%), Garlic leaf extract (89.63%),%), Ginger extract 82.33%) and onion (82.20%). Minimum Disease incidence at all 3 dates: Ginger Rhizome extract @20% (33.24%, 40.42%,50.00%) and Second best: Garlic bulb extract @20% at 30 DAS (34.83%); maximum disease incidence (least effective): Onion bulb extract @20% (44.35%, 53.60%, 62.72%) Based on the overall results, it can be concluded that integration of chemical seed treatment with biological control agents, particularly *Trichoderma*, is an effective and eco-friendly approach for the management of Fusarium wilt of lentil. Such integrated practices reduce disease incidence, improve plant growth and increase yield, and can be recommended as a suitable IDM strategy for lentil cultivation. Overall, the present investigation clearly demonstrates that no single management component alone can provide complete and sustainable control of Fusarium wilt. Instead, integration of compatible chemical, biological and cultural approaches offers superior disease suppression while promoting healthier plant growth and improved yield. The combination of fungicidal seed treatment with beneficial bioagents not only reduced disease incidence but also contributed to better crop establishment and productivity, thereby reducing dependence on repeated chemical applications.

Recommendations: Selection of lentil varieties for wilt disease (the susceptible variety L9-12 is suitable for *Fusarium oxysporum* f. sp. *lentis*-affected areas in Uttar Pradesh, for the Research purpose of assessing the percentage incidence of the disease).

Ethics declarations: The authors declare that ethical standards have been followed and that no human participants or animals were involved in this research.

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