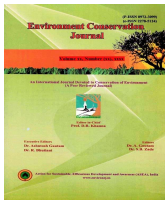
Environment Conservation Journal 24 (4): 96-105, 2023

Journal homepage: https://www.environcj.in/

Environment Conservation Journal

ISSN 0972-3099 (Print) 2278-5124 (Online)



In vitro management of Fusarium wilt of linseed using phytoextract, fungicides and bioagents

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ARTICLE INFO

Received : 01 January 2023
Revised : 18 June 2023
Accepted : 04 July 2023

Available online: 14 November 2023

Key Words:
Mycelial growth
Neem
Pathogen
Propiconazole
Trichoderma

ABSTRACT

Fusarium wilts of linseed caused by *Fusarium oxysporum* f.sp. *lini* have been identified in nearly all linseed-producing countries of the world. A comparison of phytoextract, chemical, and bio control agents against *Fusarium oxysporum* f.sp. *lini* was conducted. Among the phytoextracts tested, Neem extract exhibited the highest antifungal activity in inhibiting the growth of *F. oxysporum* f.sp. *lini* at 5, 15, and 30% concentrations. In terms of biocontrol agents, *T. virens* was identified as the most efficient antagonist against *F. oxysporum* f.sp. *lini.* It significantly inhibited pathogen mycelial growth, displaying the highest level of inhibition. Among the chemical fungicides assessed, propiconazole exhibited the lowest mycelial growth of the pathogen and outperformed the other fungicides, with difenoconazole following as the next most effective.

Introduction

Linseed is an important oilseed crop used largely for commercial oil in India. Linseed (*Linum usitatissimum* L.), also known as Alsi/Flaxseed, is a plant in the Linaceae family that belongs to the genus Linum. The oil content of the seeds varies from 33 to 47 percent depending on the variety. It is high in soluble fiber mucilage and one of the main sources of alpha-linolenic acid (ALA) (Cunnane *et al*., 1993). In terms of yield and acreage, Uttar Pradesh, Maharashtra, Bihar, Rajasthan, Karnataka, and West Bengal follow Madhya Pradesh. Madhya Pradesh and Uttar Pradesh generate more than 70 percent of the nation's linseed (Anonymous, 2015). However, it is hampered by various biotic and abiotic stresses. Considering the present scenario, it is a challenging task to achieve sustainable global food security with the growing human population and shifting global food consumption patterns brought on by climate change (Kumar *et al*., 2021). Among the different diseases, major diseases include Fusarium wilt (*Fusarium oxysporum* f.sp. *lini*), rust (*Melampsora lini*), powdery mildew (*Oidium lini*), Alternaria blight (*Altenaria linicola*), foot rot (*Rhizoctonia solani*, Pythium spp.), damping off of seedlings (Pythium spp.), etc. Wilt caused by *Fusarium oxysporum* f. sp. *lini* is a severe barrier to linseed industry output and productivity (Kishore *et al*., 2021). Sattar and Hafiz (1952) reported crop losses of 80-100 percent due mainly to wilt. *Fusarium oxysporum* f. sp. *lini*, one of the most dangerous fungal pathogens in flax, is a causative agent of Fusarium wilt. It invades the plant through roots and spreads inside the vascular bundle. After germination, it develops microconidia, blocking water and nutrient flow,

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which leads to plant wilt, yellowing of lower parts, and death (Michielse and Rep 2009, Rozhmina and Loshakova 2016). Fusarium is a genus of filamentous, seed, and soil-borne ascomycetes with numerous pathogenic members that have been reported to cause disease in over 100 major crop species worldwide (Ma *et al.,* 2013, Purohit *et al*., 2022). Infection occurs through the roots, invading the water-conducting tissues, which impairs water transport and results in wilting, necrosis, and chlorosis of aerial parts (Ma *et al*., 2013). *Fusarium oxysporum* can persist in the soil for 5–10 years. Fusarium wilt of linseed has management options varying from preventive to curative interventions. To date, various strategies for plant disease management have been advocated, and for eco-friendly management, the use of biocontrol agents has been successfully utilized in different crops against various plant pathogens. Among different bioagents, *Trichoderma* is a potential agent that not only successfully controls plant pathogens (Kumar *et al.*, 2013a; Kumar *et al.*, 2014; Jain *et al.*, 2017; Kharte *et al.*, 2022) but is also used as a biofertilizer (Srivastava *et al*., 2009) and in the production of several secondary metabolites (Kumar *et al*., 2009). They also serve as plant growth-promoting agents (Kumar and Sahu, 2014; Kumar *et al*., 2019) and in bioremediation (Kumar *et al*., 2015). Furthermore, their use as native isolates has proven to have better potential in local areas for successful biocontrol agents after proper identification and characterization (Kumar *et al*., 2013b; Kumar and Sahu, 2015; Kumar *et al*., 2016). They can also be used in combination with fungicides for more effective control of the disease (Kumar *et al*., 2019).Each control mechanism is important, but none can function alone. Identifying the most effective concentration dose of various phytoextracts, as well as the chemical and antagonistic activity of bioagents against the pathogen. The purpose of this study was to determine the most efficient biocontrol and ideal concentrations of phytoextracts, which are fungicides that are widely utilized in disease control.

Material and Methods

In vitro evaluation of leaf extract

The poisoned food method (Sreenu and Zacharia, 2017) was used to assess *F. oxysporum* f. sp. *lini* sensitivity to phytoextracts. Seven phytoextracts, viz., neem, eucalyptus, tulsi, ginger, garlic, gokhru, and mint, were examined to test their effectiveness against Fusarium wilt. By using a wide range of concentrations, the study aims to identify the most effective concentration(s) of these plant extracts for further investigation while also ensuring that any potential toxicity to the plants is minimized. The standard procedure employed by Gerard *et al.* (1994) was utilized to create extracts of plant components such as leaves, bulbs, and clove, among others. Fresh plant parts were cleaned with tap water followed by sterile distilled water before being processed 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 resulting filtrate was the typical plant extract solution. Solutions from stock were used to generate 5, 15, and 30 percent concentrations of plant extract, and 5, 15 and 30 ml were combined with 95, 85, and 70 ml of sterilized molten potato dextrose agar (PDA) medium, respectively. The extracted material was gently shaken to ensure uniform mixing. In sterilized Petri plates, 20 ml of poisoned PDA was poured. The plates were inoculated with a five mm diameter mycelium disc from an actively developing pure culture of *F. oxysporum* f. sp. *lini* and incubated at 27±2°C for 168 hours. The mycelial growth was recorded, and the percent growth inhibition was calculated using the following formula by Vincent, 1947.

$$\mathbf{I = \frac{C-T}{C} \times 100}$$

where I= mycelial growth inhibition (%); C=mycelial growth in control (mm); and T= mycelial growth in treatment (mm)

In vitro evaluation of fungicides

Six fungicides, Mancozeb + Carbendazim, Difenconazole 3% FS, Copper oxychloride, Azoxystrobin 23% SC, Propiconazole, and Fluxapyroxad + Pyraclostrobin, were tested against the pathogen using the poisoned food method, as reported by Sreenu and Zacharia, 2017, at doses of 300 and 500 ppm. In a completely randomized experimental design, Petri plates containing PDA amended with the appropriate concentration of fungicides were infected with five mm mycelial discs of the active culture of fungus and kept at

27±2°C by maintaining a suitable control, and each treatment was replicated three times (More 2016). In each treatment, mycelial growth was measured, and growth inhibition was estimated.

***In vitro* evaluation of bioagents**

Trichoderma viride, T. harzianum, T. virence, Pseudomonas florescence, and *Bacillus subtilis* were tested *in vitro* against *Fusarium oxysporum* f.sp. *lini* utilizing the dual culture method proposed by Vani (2019). Five-millimeter discs of actively growing pathogen and antagonist cultures were placed at a distance of 5 cm on PDA, and plates were incubated at 27±2°C. As a control, PDA medium inoculated with pathogen alone was used. Following the entire development of the pathogen in the control, the mycelial growth of the pathogen and bioagents was recorded, and growth inhibition was calculated.

Results and Discussion

Effect of various phyto extracts on *Fusarium oxysporum* f.sp. *lini*

Seven plant extracts, *viz*., Neem, Tulsi, Eucalyptus, Ginger, Garlic, Gokhru and Mint, at 5, 15 and 30 percent concentrations were evaluated against *F. oxysporum* f.sp. *lini* (Tables 1, 2, 3 and Figures 1, 2 and 3). Among them, the maximum percent inhibition of Neem (36.02%) was recorded as the most effective, showing maximum inhibition of mycelial growth of the pathogen, followed by Garlic (34.75%), Tulsi (28.87%) and Ginger (24.95%). Mint recorded minimum growth inhibition (07.18%) of the pathogen. Neem was superior to all other tested plant extracts at the 5, 15 and 30 percent concentrations after 168 hours of inoculation. Thus, the results clearly indicated that plant extracts reduced the radial growth of *F. oxysporum* f. sp. *lini*. 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 Eucalyptus, antifungal compounds such as oleanolic acid, ursolic acid, zingiberence, gingerol, flavonoids, 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), parthenium leaf extract was shown to be the least effective. carbendazim (70.23%). However, copper oxycloride (30.33%) exhibited the least percent growth inhibition over the control, followed by azoxystrobin (46.42%) after 168 hours at 300 ppm (Table 4, plate 4 and figure 4).

Table 1: Effect of plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lini* at a 5 percent concentration

Treatment	72 hours		120 hours		168 hours	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
Neem	16.26	40.90	42.69	40.90	55.50	28.98
Tulsi	21.11	28.46	47.13	34.75	63.37	18.91
Eucalyptus	24.48	17.04	56.30	22.06	72.61	07.08
Ginger	22.54	24.97	50.29	30.38	65.36	16.36
Garlic	17.53	40.59	46.23	36.00	58.14	25.60
Gokhru	26.53	10.09	59.20	18.05	74.41	04.78
Mint	26.99	08.53	60.35	09.53	77.11	01.33
Control	29.51	00.00	72.24	00.00	78.15	00.00
CD (5%)	0.84		1.91		1.63	
SE(m)	0.28		0.63		0.54	

Table 2: Effect of plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lini* at 15 percent concentration

Treatment	72 hours		120 hours		168 hours	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
Neem	12.58	54.69	40.56	43.38	46.51	38.09
Tulsi	16.13	41.91	46.26	35.76	53.38	28.94
Eucalyptus	22.14	20.27	54.39	24.47	59.97	20.17
Ginger	19.78	28.77	49.25	31.62	56.52	24.77
Garlic	13.79	50.34	42.83	40.53	48.52	35.41
Gokhru	23.34	15.95	58.25	19.12	65.12	13.32
Mint	26.36	05.07	60.49	16.00	66.72	11.20
Control	27.77	00.00	72.02	00.00	75.13	00.000
CD (5%)	0.80		2.48		1.48	
SE(m)	0.26		0.82		0.49	

Table 3: Effect of plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lini* at 30 percent concentration

Treatment	72 hours		120 hours		168 hours	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
Neem	11.99	56.62	38.40	36.47	46.37	36.02
Tulsi	15.05	45.54	45.13	25.34	51.55	28.87
Eucalyptus	19.12	30.82	51.73	14.42	59.22	18.29
Ginger	17.13	38.02	47.12	22.05	54.39	24.95
Garlic	12.82	53.61	41.03	32.12	47.30	34.75
Gokhru	21.44	22.43	54.61	09.66	63.36	12.58
Mint	23.22	15.99	57.46	04.94	67.27	07.18
Control	27.64	00.00	60.45	00.00	72.48	00
CD 5%)	1.30		2.24		2.39	
SE(m)	0.43		0.7		0.79	

Table 4: *In vitro* efficacy of systemic fungicides against *Fusarium oxysporum* f. sp. *lini* at 300 ppm

Treatment	72 hours		120 hour		168 hour	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
Mancozeb+ Carbendazim	00.00	100.00	18.00	59.84	22.00	70.23
Difenconazole 3% FS	00.00	100.00	15.50	65.42	19.67	73.39
Copper oxychloride	24.50	11.45	42.00	06.31	51.50	30.33
Azoxystrobin 23% SC	16.33	40.98	28.66	36.06	39.60	46.42
Propiconazole	00.00	100.00	00.00	100.00	00.00	100.00
Fluxapyroxad+ Pyraclostrobin	15.00	45.78	27.00	39.77	36.49	50.63
Control	27.67	00.00	44.83	00.00	73.92	00.00
CD (5%)	1.32		1.62		1.92	
SE(m)	0.43		0.53		0.63	

Propiconazole exhibited a percent growth inhibition over the control, followed by difenconazole (71.74%),

In vitro evaluation of fungicides

Six fungicides, *viz.,* mancozeb + carbendazim, difenconazole (3% FS), copper oxychloride, azoxystrobin (23% SC), propiconazole, and fluxapyroxad + pyraclostrobin, were evaluated at 300 and 500 ppm each against *F. oxysporum* f.sp. *lini* (Table 4 and 5). Of the six fungicides, no radial growth of the test pathogen was observed with propiconazole, followed by difenconazole (19.67 mm), mancozeb + carbendazim (22.00 mm), and fluxapyroxad + pyraclostrobin (36.49 mm). However, the maximum growth of the test pathogen was observed with copper oxychloride (51.50 mm), followed by azoxystrobin (39.60 mm). Propiconazole exhibited cent percent growth inhibition over the control, followed by difenconazole (73.39%) and mancozeb+

Table 5: *In vitro* efficacy of systemic fungicides against *Fusarium oxysporum* f. sp. *lini* at 500 ppm

Treatment	72 hours		120 hour		168 hour	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
Mancozeb+ Carbendazim	00.00	100.00	15.50	65.28	19.16	68.15
Difenconazole 3% FS	00.00	100.00	09.50	78.72	18.00	71.74
Copper oxychloride	22.83	08.68	40.33	09.67	53.33	11.35
Azoxystrobin 23% SC	9.33	62.68	17.50	60.80	29.00	51.79
Propiconazole	00.00	100.00	00.00	100.00	00.00	100.00
Fluxapyroxad+ Pyraclostrobin	08.00	68.00	21.67	51.46	31.00	48.47
Control	25.00	00.00	44.65	00.00	60.16	00.00
CD(5%)	1.591		2.24		1.29	
SE(m)	0.519		0.73		0.42	

Table 6: Antagonistic efficacy of bioagents against *Fusarium oxysporum* f. sp. *Lini*

Treatment	**72 hours**		**120 hours**		**168 hours**	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
T. virens	21.16	36.49	32.33	42.94	43.16	32.73
T. viride	17.00	48.97	25.00	55.87	35.15	45.21
T. harzianum	28.83	13.47	41.50	26.75	51.66	19.48
P. fluorescence	31.16	06.48	46.83	17.35	57.00	11.15
B. subtilis	26.16	21.48	47.00	17.04	48.17	24.92
Control	33.32	00.00	56.66	00.00	64.16	00.00
CD (5%)	1.62		2.98		1.45	
SE(m)	0.52		0.95		0.46	

mancozeb+carbendazim (68.15%) and azoxystrobin (51.79%); however, copper oxycloride (11.35%) exhibited the least percent growth inhibition over the control, followed by fluxapyroxad+ pyraclostrobin (48.47%) after 168 hours at 500 ppm (Table 5, plate 5 and figure 5). Sharma *et al*. (2002) also investigated the effects of Mancozeb, Thiram, Copper Oxychloride, Rovral, Bavistin, Ridomil, Kavach, Benomyl, and Captan against *F. oxysporum* f.sp. *lini*, they discovered similar results, which caused lins regardless of concentration, and every fungicide outperformed the untreated control in a considerable way. Both carbendazim and benomyl inhibited the mycelial growth of *F. oxysporum* f.sp. *lini*. Taskeen Un-Nisa *et al*. (2011) and Arunodhayam *et al*. (2014) found similar results under *in vitro* assessment of fungicides against *Fusarium* spp. Similarly, fungicide effects against test pathogens were reported by earlier workers; for example, Ravichandran and Hegde (2015) noticed that Carbendazim 12% + Mancozeb 63% inhibited almost half of the growth of *F. oxysporum* f. sp. *ciceri*. Patra and Biswas (2016) indicated that carbendazim, propiconazole, and two combination products (carbendazim + mancozeb and tebuconazole + trifloxystrobin) were most effective in completely inhibiting the mycelial growth of the fungus at different concentrations. Dahal *et al.* (2018) and Niwas *et al.* (2020) found similar results *in an in vitro* assessment of fungicides against *Fusarium* spp. Similarly, Bhujbal *et al*., 2021 reported that Mancozeb + Carbendazim and Thiram + Carbendazim were the most effective, inhibiting 100% and 93.63% of pathogen growth, respectively.

Evaluation of bioagents against *Fusarium oxysporum* f.sp. *lini*

T. viride, T. harzianum, T. virens, P. fluorescens, and *B. subtilis* were evaluated *in vitro* for their bio efficacy against *F. oxysporum* f.sp. *lini.* It was observed that all bioagents exhibited antifungal activity against *F. oxysporum* f.sp. *lini* and significantly inhibited its mycelial growth. *T. viride* was shown to be the most efficient pathogen inhibitor, with considerably less mycelial growth (35.15 mm) and the greatest mycelial growth

inhibition (45.21%) after 168 hours, because *T. viride* interacts directly with pathogens by hyperparasitism or antibiosis. Hyperparasites attack and kill the mycelium, spores and resting structures of pathogens. The pathogen's growth suppression was lowest in *P. fluorescens* (11.15%), followed by *B. subtilis* (19.48%) after 168 hours (Table 6, plate 6 and figure 6). Similarly, *T. viride* and *T. harzianum* were also tested *in vitro* for antagonistic activity against *F. oxysporum* in a dual culture experiment, and *T. viride* inhibited the mycelial development of all pathogens. These studies are supported by Jagraj *et al*., 2018, who reported that *T. harzianum* inhibited the maximum radial growth of *F. oxysporum,* followed by *T. viride.*

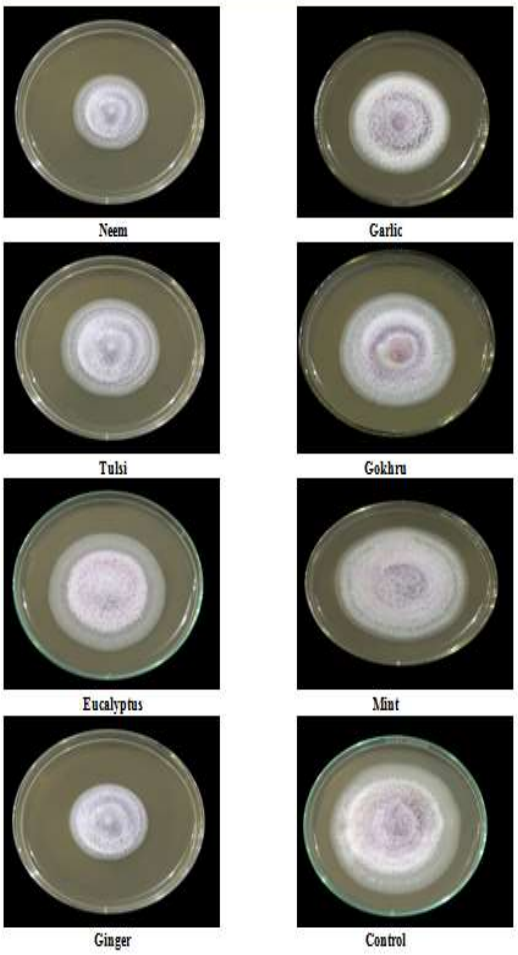

Plate 1: Effect of plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lini* at a 5 percent concentration after 168 hours

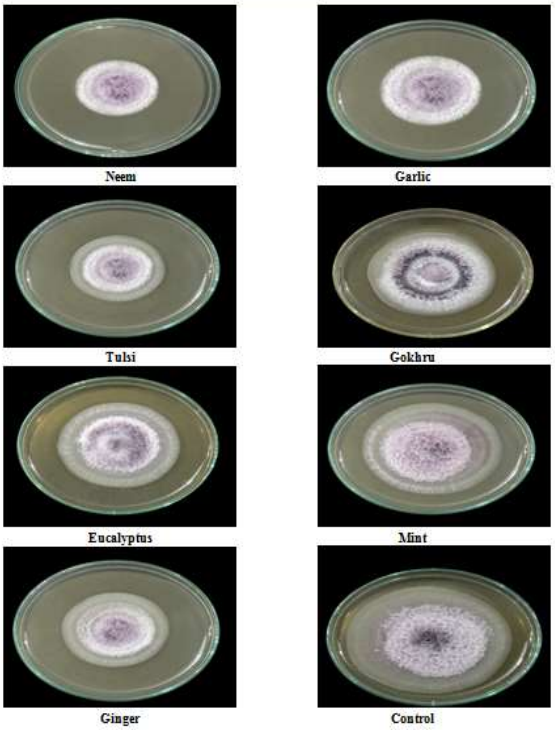

Plate 2: Effect of plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lini* at a 15% concentration after 168 hours

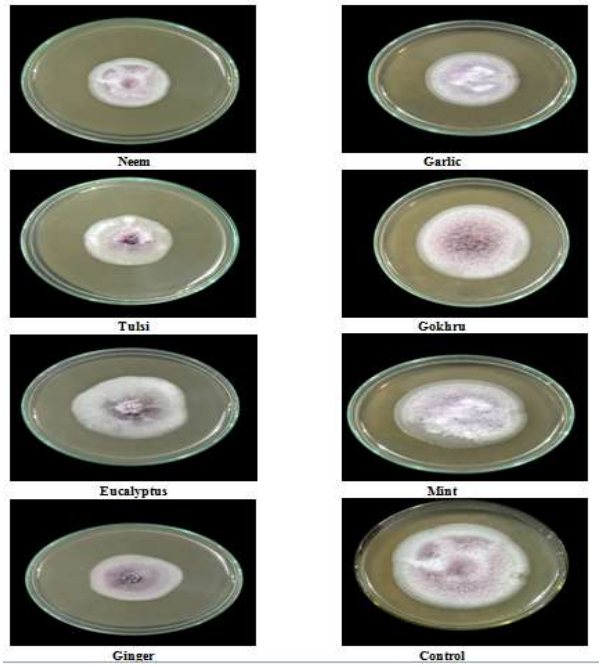

Plate 3: Effect of plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lini* at a 30% concentration after 168 hours

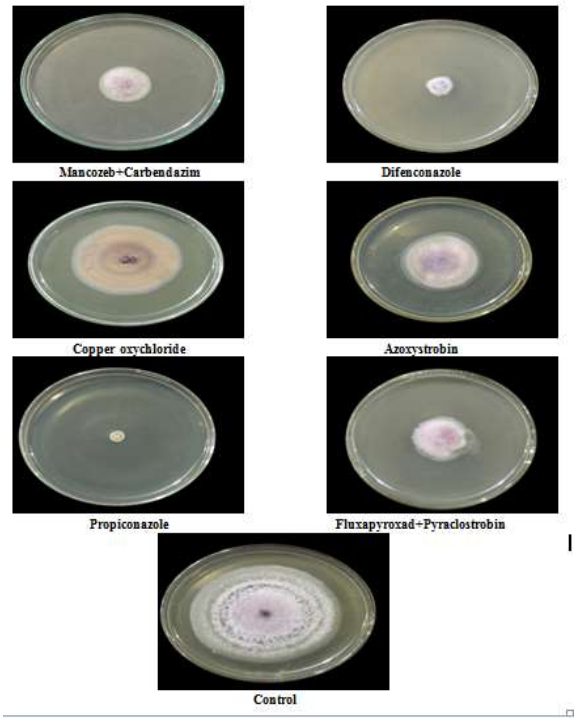

Plate 4: Efficacy of different fungicides against *Fusarium oxysporum* f. sp. *lini* at 300 ppm after 168 hours

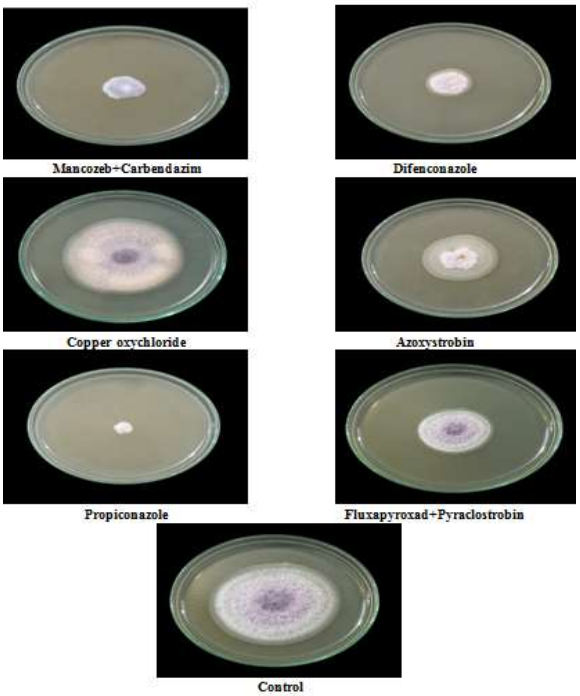

Plate 5: Efficacy of different fungicides against *Fusarium oxysporum* f. sp. *lini* at 500 ppm after 168 hours.

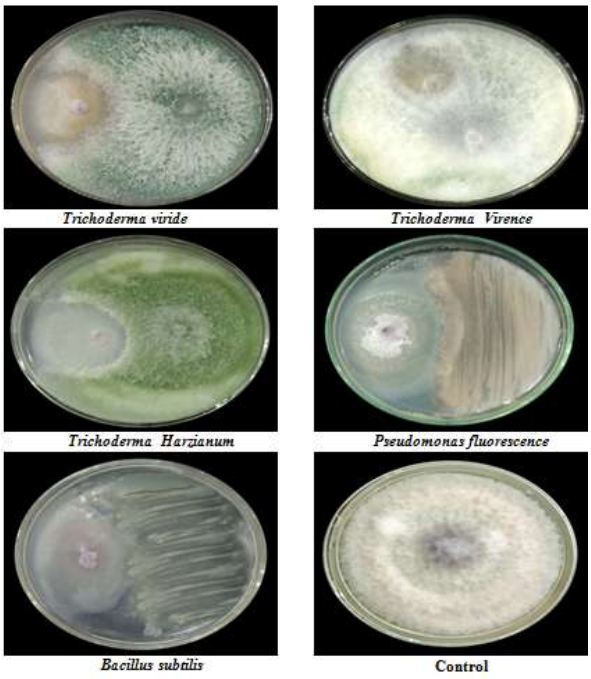

Plate 6: Antagonistic efficacy of bioagents against *Fusarium oxysporum* f. sp. *Lini*

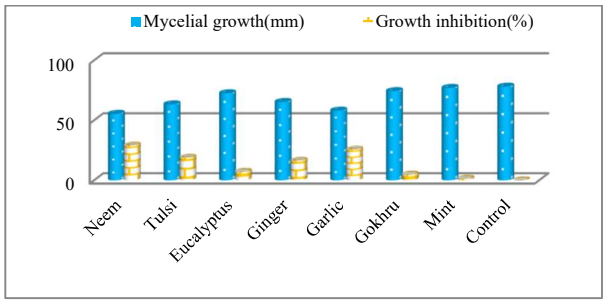

Figure 1: Influence of plant extracts on mycelial growth and growth inhibition of *Fusarium oxysporum* f.sp. *lini* after 168 hours at a 5 percent concentration

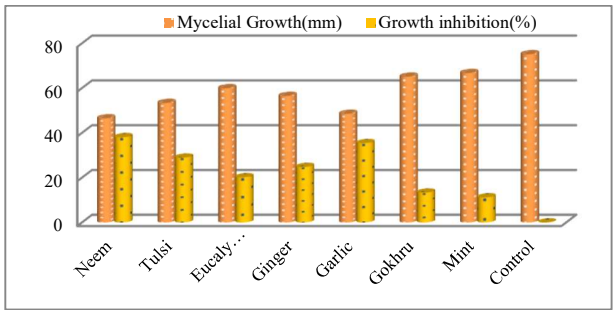

Figure 2: Influence of plant extracts on mycelial growth and growth inhibition of *Fusarium oxysporum* f.sp. *lini* after 168 hours at 15 percent concentration

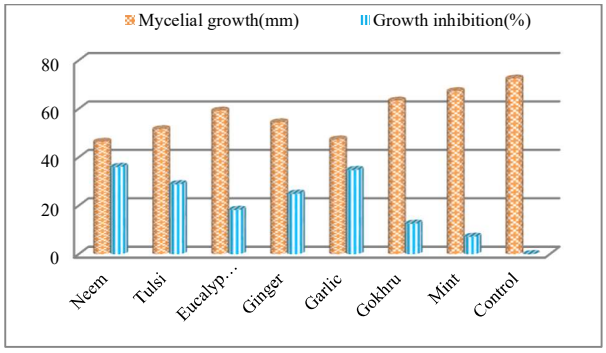

Figure 3: Influence of plant extracts on mycelial growth and growth inhibition of *Fusarium oxysporum* f.sp. *lini* after 168 hours at a 30 percent concentration

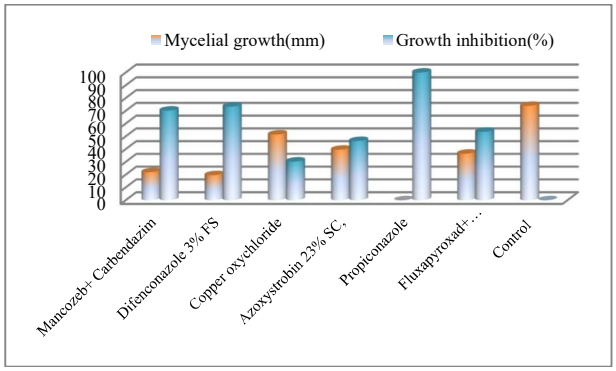

Figure 4: Efficacy of different fungicides on mycelial growth and growth inhibition of *Fusarium oxysporum* f. sp. *lini* at 300 ppm

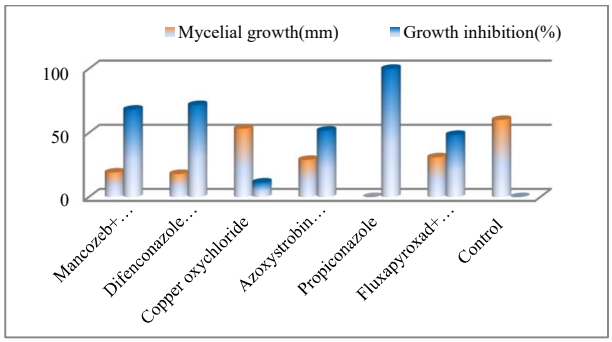

Figure 5: Efficacy of different fungicides on mycelial growth and growth inhibition of *Fusarium oxysporum* f. sp. *lini* at 500 ppm

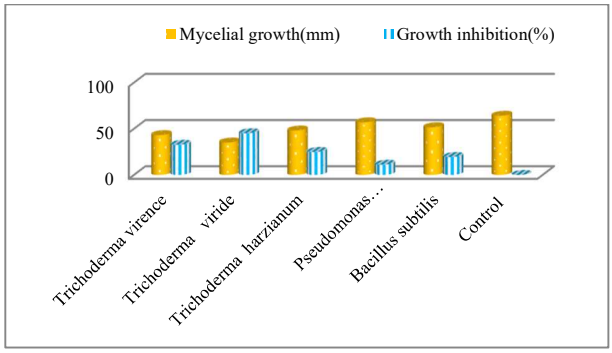

Figure 6: Antagonistic efficacy of bioagents against *Fusarium oxysporum* f.sp. *lini*

Conclusion

In conclusion, this study investigated the effectiveness of different bioagents, phosphoextracts and fungicides against *F. oxysporum* f. sp. *lini*. The findings demonstrated that Neem extract exhibited high efficacy as an antifungal agent, effectively inhibiting the growth of the pathogen *in vitro*. Propiconazole fungicide was also identified as a potent inhibitor of Fusarium wilt, with recommended concentrations of 300 ppm and 500 ppm, respectively. Among the bioagents tested, *T. viride* showed the highest effectiveness in inhibiting the test pathogen. These findings suggest that Neem extract, propiconazole, difenoconazole, and *T. viride* can be considered valuable options for controlling Fusarium wilt of linseed. However, further research is needed to validate their efficacy under field conditions and evaluate potential environmental impacts before implementing them as best practices for disease management.

Acknowledgement

I extend my sincere thanks to my guide and Head of the Department Plant Pathology JNKVV, Jabalpur for supporting my research work.

Conflict of interest

The authors declare that they have no conflict of interest.

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