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EVALUATION OF ANTIMYCOTIC POTENTIAL OF BOTANICAL EXTRACTS AND BIOAGENT AGAINST MAJOR PLANT PATHOGENIC FUNGI

Nishant Gaurav*, S.K. Biswas, Anju Shukla, Pradyumn Kumar Singh, Akash Kumar, Prabha Siddharth and Saurabh Saini

Department of Plant Pathology, C.S. Azad University of Agriculture and Technology, Kanpur, (Uttar Pradesh), India.
*Corresponding Author: Nishant Gaurav

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ABSTRACT

Fungal diseases threaten plant health, causing economic losses and food insecurity. While chemical fungicides offer effective control, they contribute to resistance, environmental harm, and non-target toxicity, emphasizing the need for sustainable disease management. Botanical extracts and bioagents have emerged as promising alternatives, offering antifungal properties without the adverse effects associated with synthetic chemicals against fungal pathogens, *viz*., *Fusarium oxysporum f. sp. lycopersici*, *Alternaria solani, Fusarium oxysporum f. sp. ciceri*, , *Rhizoctonia solani* and *Phomopsis vexans*. In a series of *in-vitro* experiments, the efficacy of six botanical extracts v*iz*., Neem leaf, Marigold leaf, Garlic bulb, Onion bulb, Neem oil, Karanj oil and the bioagent *Trichoderma harzianum* were determined based on radial mycelial growth of the pathogens. The finding indicates that the Garlic bulb extract (1:1,10%) demonstrated the highest antifungal activity against all the tested pathogens and specifically exhibited an inhibition rate of 95% against *Rhizoctonia solani* with radial mycelial growth of 4.5 mm at 7 days after inoculation (DAI) followed by onion bulb extract (1:1, 10%) which exhibited an inhibition of radial mycelial growth against *Rhizoctonia solani* by 81.3% and Neem oil (1:4, 10%) exhibiting maximum radial growth inhibition against *Fusarium oxysporum f. sp. ciceri* by 74.31%. The bioagent *Trichoderma harzianum* was also effective in achieving its maximum inhibition of radial growth against *Fusarium oxysporum f. sp. ciceri* by 68.3%. In contrast, Marigold leaf extract (1:1, 10%) was least effective, showing its minimum inhibition of 37.81% against *Rhizoctonia solani*.

***Keywords*:** Botanical extracts, Bioagents, antifungal activity, radial mycelial growth.

Introduction

The advent of the Green Revolution in India during the 1960s marked a significant escalation in the application of chemical pesticides and fungicides within the agricultural sector. This intensified usage has led to substantial concerns regarding its adverse effects on non-target organisms such as beneficial insects, birds, soil microorganisms, and the sustainability of agriculture (Gupta *et al.,* 2019).

Among the different alternative management technologies, use of botanicals and bioagents is one of the safe and newly emerging technologies that have not yet been fully explored. Neem (*Azadirachta indica*) is a potent botanical widely used in ayurveda, unani, and homeopathy for its antimicrobial properties (Lakshmi *et al.,* 2015). Karanj (*Pongamia pinnata*) has antifungal and antibacterial properties due to pongamol and karanjin (Rao *et al*., 1982). Marigold flowers contain thiophenes, flavonoids, and triterpenoids with significant antifungal activity (Sikarwar *et al*., 2018; García *et al*., 2017). Onions (*Allium cepa*) and garlic (*Allium sativum*) possess antifungal properties from allicin, sulphur compounds, and flavonoids (Sikkema *et al*., 2018). *Trichoderma* spp. are effective biological control agents, combating pathogens through parasitisation, competition, and antibiosis (Kashyap *et al*., 2017).

Gholve *et al.* (2014) tested ten plant extracts or botanicals at concentrations of 10%, 15%, and 20% and found that Garlic extract was the most effective, exhibiting a significantly high mean mycelial growth inhibition of 94.83% against *Phomopsis vexans.*

Ramaiah *et al.* (2015) evaluated *in vitro* antifungal activity of 15 different plant extracts against *Fusarium oxysporum* f. sp. *Lycopersici* , and found that *Solanum indicum* inhibited growth by 78.33%, *Azadirachta indica* by 75.00%, and *Oxalis latifolia* by 70.33%. Islam *et al.* (2017) investigated the effects of

plant extracts on the radial growth of *Fusarium oxysporum* and *Fusarium solani* at concentrations of 5%, 10%, 15%, and 20%. Among the five plant extracts tested, *Allium sativum* and *Cassia alata* exhibited complete inhibition (100%) of radial growth of *F. oxysporum* at the highest concentration (20%).

Keeping the above points of view, the study entitled "Evaluation of antimycotic potential of botanical extracts and bioagent against major plant pathogenic fungi" has been undertaken in the present investigation.

Materials and Method

Isolation and purification of the pathogen

Roots and leaves of infected plants were cut into small pieces and surface sterilized with 1% Sodium hypochlorite solution for 1 minute. Isolations were made on Petri plates poured with PDA by placing the sterilized root and leaf pieces under aseptic conditions using laminar air flow cabinet. These inoculated Petri plates were incubated at 25 ±1°C in a BOD (Biological Oxygen Demand) incubator. After the growth of pathogens occurred, a hyphal bit from the periphery of the growing fungal colony was transferred with the help of sterilized needle onto a potato dextrose agar slant, in the laminar air flow cabinet to avoid any chance of contamination. For each fungal colony, separate slant was used. The pathogens were isolated by standard tissue isolation method on Potato Dextrose Agar medium (Das *et al*., 2018).

Identification of the pathogen

The pure culture of isolated fungi was examined under a light microscope based on colony color, growth, type of mycelium, sclerotia, and the spores produced. Slides of the pathogens with dark-colored colonies were prepared in lactophenol, while those with cottony white colonies were prepared with lactophenol-cotton blue stain (Das *et al*., 2018).

Maintenance of the cultures

The fungal pathogen was sub cultured on PDA slants and allowed to grow at 25±1°C for 10 days. Such slants were preserved in refrigerator at 4°C and sub cultured once in regular interval of one month.

Preparation of Botanical extract

Botanicals were collected by obtaining fresh and heathy plant parts *viz*., Neem leaves (*Azadirachta indica*), Marigold leaves (*Tagetes erecta*), Garlic bulb (*Allium sativum*), Onion bulb (*Allium cepa*), Neem oil (*Azadirachta indica*) and Karanj Oil (*Millettia pinnata*) from dried seeds of Neem and Karanj respectively. Fresh and healthy plant parts of 100g were collected separately and washed with distilled water, air dried and crushed by motor and pestle in 100 ml of sterile water. In case of neem and karanj oil, 25 ml of pure oil was mixed with 100 ml of sterile water to achieve a 1:4 oil to water concentration. The mixture was then emulsified using a magnetic stirrer for 15 minutes to ensure a uniform emulsion. The obtained product was filtered by using muslin cloth into 150 ml conical flask and were plugged with non-absorbent cotton. Later, the filtered phyto-extract was autoclaved at 15 psi for 20 minutes and then used in Poisoned Food technique. The culture filtrate (100%) was treated as stock solution. Autoclaved extracts were individually added in previously sterilised PDA to form ten per cent extracts and mixed thoroughly at the time of pouring in the previously sterilized Petri plates.

Evaluation of botanicals

The Poisoned Food Technique (Shravelle, 1961) was followed to evaluate the efficacy of botanicals in inhibiting the mycelial growth of different pathogens. The fungus was grown on PDA medium for twelve days prior to setting up the experiment. The PDA medium was prepared and melted. Autoclaved extracts were individually added in previously sterilised PDA to form a concentration of 10 per cent extracts and were mixed thoroughly at the time of pouring in the previously sterilized Petri plates. Mycelial disc of 5mm was taken from the periphery of old colony. The actively growing hyphal tip was removed by cork borer and placed in the centre of Poisoned Petri plates and incubated at 25±1 °C till the control plate is full and three replications were maintained for each treatment.

Antagonistic effect of *Trichoderma harzianum* against different pathogens

The antagonism between fungal antagonist *i.e*., *Trichoderma harzianum* and the pathogen was evaluated using dual culture technique as described by Dennis and Webster (1971). In this method, a mycelial disc of the fungal pathogen and a mycelial disc of the bioagent were placed at opposite ends of a Potato Dextrose Agar (PDA) plate. The plates were incubated at 25°C for 7 days, and the interactions between the bioagent and the pathogen were observed daily. The inhibition zone, where the growth of the pathogen was restricted by the bioagent, was measured to determine the antagonistic potential of *Trichoderma harzianum.* All the experiments were carried out in triplicates and per cent reduction of mycelial growth over control was calculated using the following formula by Vincent (1927).

$$\text{Percent inhibition} = \frac{C - T}{C} \times 100$$

C= Growth of the pathogen in the control plate

T= Radial growth of the pathogen in the treatment or dual culture plate

Statistical analysis

Each treatment was replicated thrice and values were means ± SE. The data were computed using SPSS software version 21.

Results

Effect of Botanical extract and *Trichoderma harzianum* on radial mycelial growth of *Fusarium oxysporium* f. sp. *lycopersici* at different days interval

The inhibition of mycelial growth of *Fusarium oxysporium* f. sp. *lycopersici* was tested using various botanical extracts and *Trichoderma harzianum* and the results are depicted in table 1, figure 1. The result showed that among the different treatments, Garlic bulb extract exhibited highest percentage inhibition of 86.19% followed by Onion bulb extract exhibiting 69.59% inhibition. Neem oil also showed significant inhibition of 68.14% reduction followed by Karanj oil which demonstrated an inhibition of 66.58%. From the table-1, it is clear that Marigold leaf extract was least effective among all the botanicals tested with an inhibition of 58.67%. *Trichoderma harzianum* was not found as much effective as that of botanicals representing 31.80 mm diameter radial mycelial growth against 89.80 mm in case of control. Previous studies, such as those by Smith *et al*. (2008) have also reported varying levels of effectiveness of Marigold extracts against *Fusarium* species.

Table 1: Effect of botanicals and *Trichoderma harzianum* on radial mycelial growth of *Fusarium oxysporium* f. sp. *lycopersici* at different days interval

Treatment	Radial mycelial growth of *Fusarium oxysporium* f. sp. *lycopersici* at different days interval (diameter in mm)							Inhibition Percentage (%)
	1 Day	2 Day	3 Day	4 Day	5 Day	6 Day	7 Day	
T_1	5	8.8	15.5	21.5	25.8	28.2	31.1	65.35
T_2	4.9	6	7.5	10.3	11.4	12	12.4	86.19
T_3	4.9	7.8	13.6	18.7	22.7	24.6	27.3	69.59
T_4	5	8	13.7	19.1	23	25.3	37.1	58.67
T_5	5	9	15.1	20.7	25.2	27.4	28.6	68.14
T_6	5	9	15.2	21	25.6	27.5	30	66.58
T_7	4.9	15.9	22.1	25	26.1	27.3	31.8	64.57
T_8	6	26	45.1	61.8	75.1	81.9	89.8	
C.D. at 5%	0.27	0.42	0.58	0.85	0.9	1.13	1.47	
SE(m)	0.09	0.14	0.19	0.28	0.3	0.37	0.49	
SE(d)	0.13	0.2	0.27	0.4	0.42	0.53	0.69	
C.V.	3.03	2.12	1.8	1.96	1.77	2.03	2.34	

T_1= Neem leaf extract 1:1 (10%), T_2= Garlic bulb extract 1:1 (10%), T_3= Onion bulb extract 1:1 (10%), T_4=Marigold leaf extract 1:1 (10%), T_5= Neem Oil 1:4 (10%), T_6= Karanj Oil 1:4 (10%), T_7=*Trichoderma harzianum* (Dual Culture), T_8= Control

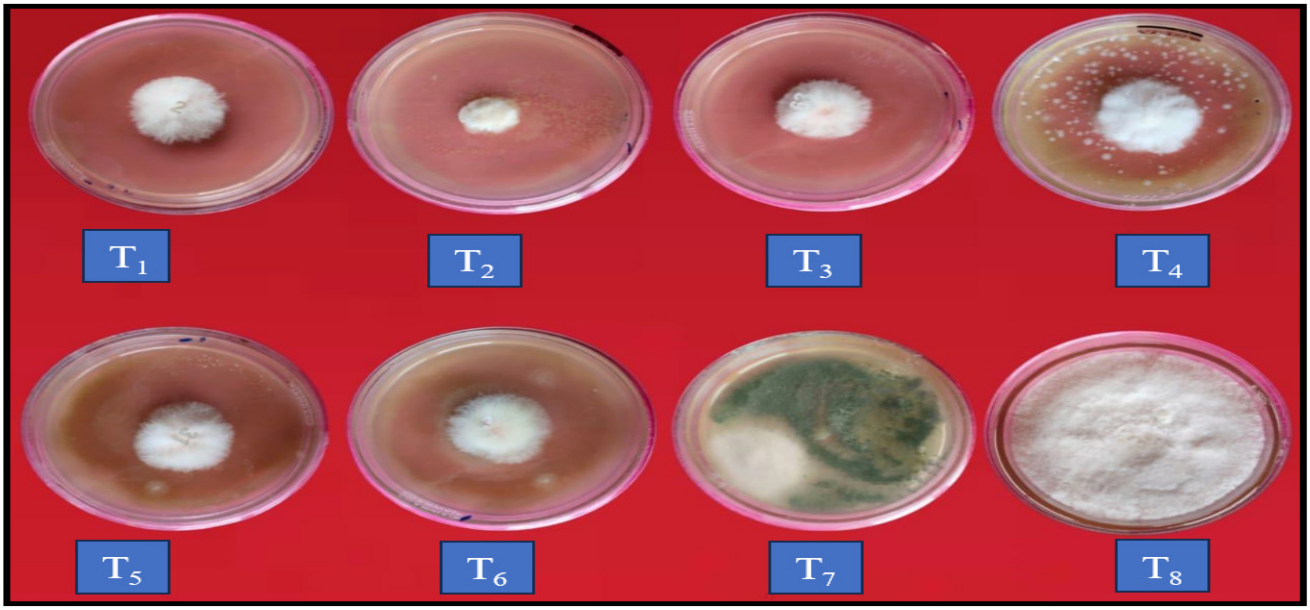

Fig. 1: Effect of Botanicals and *Trichoderma harzianum* on radial mycelial growth of *Fusarium oxysporium* f. sp. *lycopersici*

Effect of Botanicals and *Trichoderma harzianum* on radial mycelial growth of *Alternaria solani* at different days interval

The inhibition of mycelial growth of *Alternaria solani* was tested with six botanical extracts and antagonistic fungi *Trichoderma harzianum* and the result are presented in table 2, figure 2. Among various treatments, the Garlic bulb extract was the most effective among all the treatments demonstrating 94.70% growth inhibition followed by *Trichoderma harzianum* demonstrating 65.33% inhibition. Onion bulb extract was also effective showing a 64.89% inhibition rate followed by Neem oil demonstrating notable inhibition of 64.85% representing 3rd and 4th in terms of per cent reduction of mycelial growth of *Alternaria solani*. These findings are supported by Gote *et al.* (2021) who documented substantial mycelial growth inhibition of *Alternaria solani* by *Trichoderma harzianum* and Onion bulb extract. Karanj oil exhibited 60.37% and Neem leaf extract 58.15% inhibition which are superior over control but inferior to rest of the treatments. Marigold leaf extract was least effective with a 47.96% inhibition. Sivagami *et al.* (2011) investigation resembled with this study which resulted in varying levels of effectiveness of Marigold extracts against *Alternaria* species.

Table 2: Effect of botanicals and *Trichoderma harzianum* on radial mycelial growth of *Alternaria solani* at different days interval

Treatment	Radial mycelial growth of *Alternaria solani* at different days interval (diameter in mm)							Inhibition Percentage (%)
	1 Day	2 Day	3 Day	4 Day	5 Day	6 Day	7 Day	
T_1	4.9	11.7	19.7	26.4	31.4	36	37.7	58.15
T_2	4.6	4.6	4.7	4.7	4.7	4.8	4.8	94.7
T_3	4.9	9.8	16.5	22.1	26.3	30.2	31.6	64.89
T_4	5	14.6	24.5	32.8	39	44.8	46.8	47.96
T_5	4.9	9.8	16.5	22.1	26.4	30.2	31.6	64.85
T_6	4.9	11.1	18.6	25	29.7	34.1	35.7	60.37
T_7	5	15	20	23.4	26.6	28.5	31.2	65.33
T_8	5.4	28	47	63	75	86	90	
C.D. at 5%	0.36	0.5	0.75	1.11	1.16	1.69	1.68	
SE(m)	0.12	0.17	0.25	0.37	0.38	0.56	0.55	
SE(d)	0.17	0.23	0.35	0.52	0.54	0.79	0.78	
C.V.	4.18	2.18	2.06	2.32	2.04	2.62	2.48	

T_1= Neem leaf extract 1:1 (10%), T_2= Garlic bulb extract 1:1 (10%), T_3= Onion bulb extract 1:1 (10%), T_4=Marigold leaf extract 1:1 (10%), T_5= Neem Oil 1:4 (10%), T_6= Karanj Oil 1:4 (10%), T_7=*Trichoderma harzianum* (Dual Culture), T_8= Control

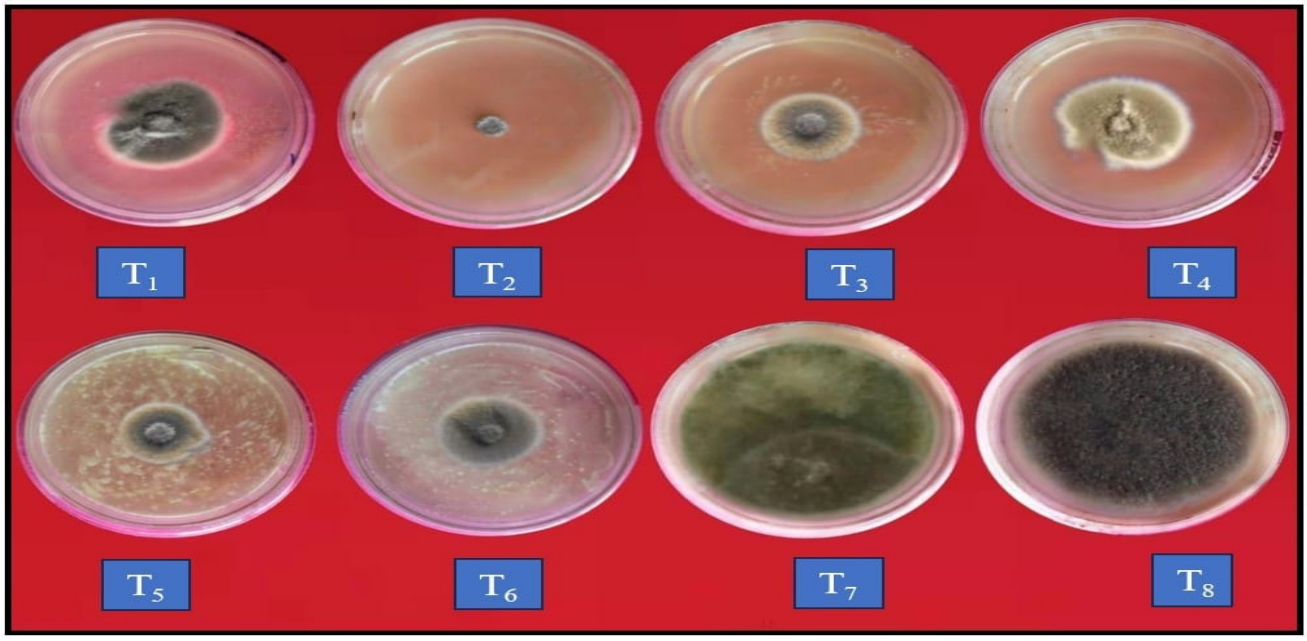

Fig. 2: Effect of botanicals and *Trichoderma harzianum* on radial mycelial growth of *Alternaria solani*

Effect of Botanicals and *Trichoderma harzianum* on radial mycelial growth of *Fusarium oxysporium* f. sp. *ciceri* at different days interval

The inhibition of mycelial growth of *Fusarium oxysporium* f. sp. *ciceri* was tested using six botanical extracts and *Trichoderma harzianum* which are presented in table 3, figure 3. The result showed that among the different treatments, the Garlic bulb extract resulted in highest inhibition of mycelial growth of 90.53% followed by Onion bulb extract exhibiting an inhibition percentage of 76.5%. These results align with the findings of Juwantha *et al.* (2021), who also reported maximum inhibition of *Fusarium oxysporum* f. sp. *ciceri* using Garlic bulb extract. Neem oil demonstrated substantial inhibition of 74.31% followed by Karanj oil showing an inhibition of 69.97%. These finding resembles the work of Gote *et al.* (2021) who observed significant inhibition of mycelial growth by Onion bulb extract and Neem oil against *Fusarium oxysporum*. From the Table-3, it can be concluded that Trichoderma *harzianum* was also effective in per cent reduction of mycelial growth of the tested pathogen by 68.30%. The result is also supported by Nandeesha *et al.* (2021), who documented significant mycelial inhibition of *Fusarium oxysporum* f. sp. *ciceri* by *Trichoderma harzianum*. Marigold leaf extract was least effective among the botanicals with an inhibition of 61.25%. Arzoo *et al.* (2020) similarly reported varying levels of effectiveness of Marigold extracts against *Fusarium* species.

Table 3: Effect of botanicals and *Trichoderma harzianum* on radial mycelial growth of *Fusarium oxysporium* f. sp. *ciceri* at different days interval

Treatment	Radial mycelial growth of *Fusarium oxysporium* f. sp. *ciceri* at different days interval (diameter in mm)							Inhibition Percentage (%)
	1 Day	2 Day	3 Day	4 Day	5 Day	6 Day	7 Day	
T_1	5	7.7	14.7	18.9	23	26.9	28.7	68
T_2	4.9	5.4	5.7	6.1	6.8	8	8.5	90.53
T_3	4.8	5.6	10.8	13.9	16.9	19.7	21.1	76.5
T_4	5	9.3	17.8	22.9	27.9	32.5	34.8	61.25
T_5	5	6.2	11.8	15.2	18.5	21.6	23.1	74.31
T_6	5	7.2	13.8	17.7	21.6	25.2	27	69.97
T_7	5	14	19	23	25.6	27.2	28.5	68.3
T_8	5.7	24	46	59	72	84	89.8	
C.D. at 5%	0.47	0.34	1	1.05	1.31	1.25	1.42	
SE(m)	0.15	0.11	0.33	0.35	0.43	0.41	0.47	
SE(d)	0.22	0.16	0.47	0.49	0.61	0.58	0.66	
C.V.	5.26	1.95	3.29	2.73	2.83	2.33	2.48	

T_1= Neem leaf extract 1:1 (10%), T_2= Garlic bulb extract 1:1 (10%), T_3= Onion bulb extract 1:1 (10%), T_4=Marigold leaf extract 1:1 (10%), T_5= Neem Oil 1:4 (10%), T_6= Karanj Oil 1:4 (10%), T_7=*Trichoderma harzianum* (Dual Culture), T_8= Control

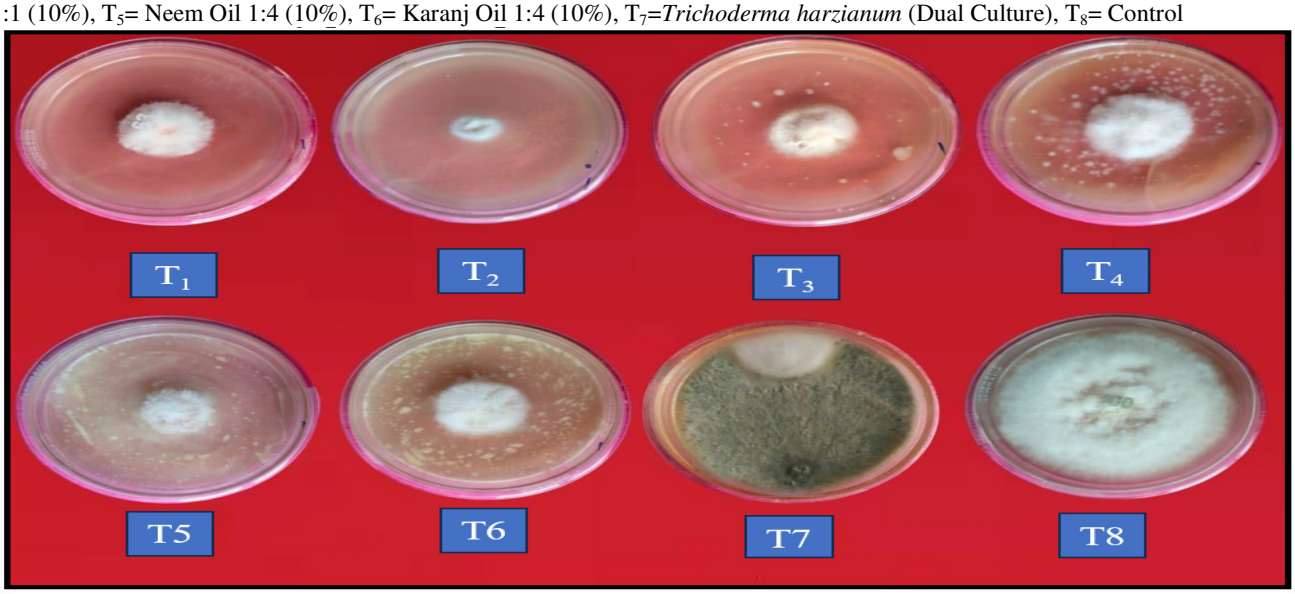

Fig. 3: Effect of botanicals and *Trichoderma harzianum* on radial mycelial growth of *Fusarium oxysporium* f. sp. *ciceri*

Effect of Botanicals and *Trichoderma harzianum* on radial mycelial growth of *Rhizoctonia solani* on different days interval

The inhibition of mycelial growth of *Rhizoctonia solani* was assessed using six botanical extracts and *Trichoderma harzianum*. The results are presented in table 4, figure 4 which showed that Garlic bulb extract proved to be the most effective treatment, consistently maintaining minimal mycelial growth achieving 95% inhibition. Onion bulb extract also demonstrated second highest efficacy resulting in 81.30% inhibition. Neem oil showed strong antifungal properties and a 73.04% inhibition rate followed by Neem leaf extract exhibiting a 68.33% inhibition rate. This effectiveness is resembled by Singh *et al*. (2020) study who noted similar results regarding Neem oil's impact on *Rhizoctonia solani*. Karanj oil demonstrated moderate effectiveness with 67.48% inhibition rate followed by *Trichoderma harzianum* exhibiting 64.48% inhibition rate. Prasad *et al*. (2019) supported this finding by demonstrating the antagonistic properties of *Trichoderma harzianum* against various pathogens.

Table 4: Effect of botanicals and *Trichoderma harzianum* on radial mycelial growth of *Rhizoctonia solani* at different days interval

Treatment	Radial mycelial growth of *Rhizoctonia solani* at different days interval (diameter in mm)							Inhibition Percentage (%)
	1 Day	2 Day	3 Day	4 Day	5 Day	6 Day	7 Day	
T_1	4.9	8.9	14.3	20.6	24.1	27.6	28.5	68.33
T_2	4.3	4.3	4.3	4.4	4.5	4.5	4.5	95
T_3	4.8	5.2	8.4	12.2	14.2	16.3	16.8	81.3
T_4	4.9	17.4	28	40.4	47.3	54.1	56	37.81
T_5	5	7.5	12.1	17.5	20.5	23.5	24.3	73.04
T_6	5	9.1	14.6	21.1	24.7	28.3	29.3	67.48
T_7	5	14	19	23.2	27.1	29	32	64.48
T_8	5.7	28	45	65	76	87	90	
C.D. at 5%	0.32	0.6	0.88	0.96	0.99	1.35	2.11	
SE(m)	0.11	0.2	0.29	0.32	0.33	0.45	0.7	
SE(d)	0.15	0.28	0.41	0.45	0.46	0.63	0.99	
C.V.	3.67	2.9	2.78	2.14	1.9	2.3	3.43	

T_1= Neem leaf extract 1:1 (10%), T_2= Garlic bulb extract 1:1 (10%), T_3= Onion bulb extract 1:1 (10%), T_4=Marigold leaf extract 1:1 (10%), T_5= Neem Oil 1:4 (10%), T_6= Karanj Oil 1:4 (10%), T_7=*Trichoderma harzianum* (Dual Culture), T_8= Control

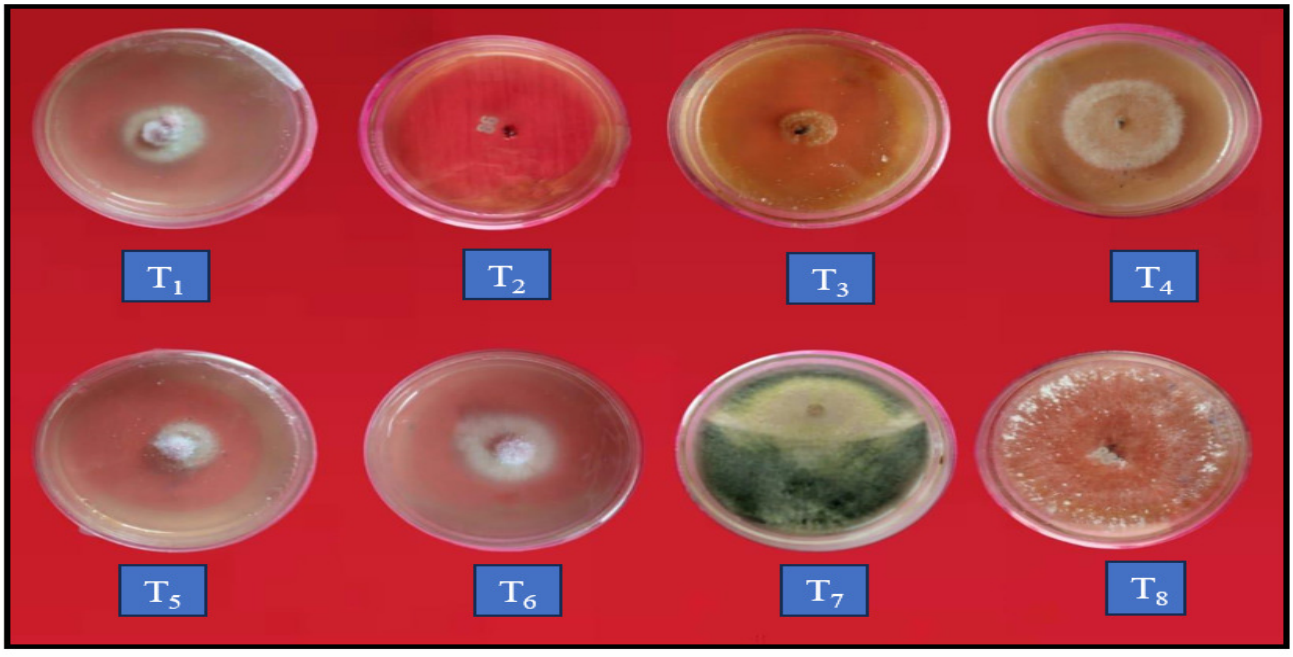

Fig. 4: Effect of botanicals and *Trichoderma harzianum* on radial mycelial growth of *Rhizoctonia solani*

Effect of Botanicals and *Trichoderma harzianum* on radial mycelial growth of *Phomopsis vexans* at different days interval

The inhibition of mycelial growth of *Phomopsis vexans* was tested using six botanical extracts and *Trichoderma harzianum* and the results are presented in table 5, figure 5 and the result showed that the Garlic bulb extract exhibited the highest inhibition percentage of 83.69%. This finding is consistent with the study by Noor *et al*. (2021), who also observed significant inhibition of *Phomopsis vexans* mycelial growth with Garlic bulb extract. The Onion bulb extract exhibited significant efficacy with an inhibition percentage of 72.43%. This result aligns with Prashant *et al*. (2017) noted a significant reduction in *Phomopsis vexans* mycelial growth using Onion bulb extract. Neem oil also resulted in marked inhibition percentage of 66.67% followed by *Trichoderma harzianum* with an inhibition percentage of 66.37%. Other treatments showed varying degrees of effectiveness with Neem leaf extract and Karanj oil exhibiting an inhibition percentage of 61.35% and 61.72%, respectively. Marigold leaf extract was least effective with an inhibition percentage of 53.40%. Kumar *et al*. (2023) reported varying levels of effectiveness of Marigold extracts against *Phomopsis vexans* which align with the current findings.

Table 5: Effect of botanicals and *Trichoderma harzianum* on radial mycelial growth of *Phomopsis vexans* at different days interval

Treatment	Radial mycelial growth of *Phomopsis vexans* at different days interval (diameter in mm)							Inhibition Percentage (%)
	1 Day	2 Day	3 Day	4 Day	5 Day	6 Day	7 Day	
T_1	5	10	17.8	24.7	29.8	33.2	34.7	61.35
T_2	4.8	5.4	7.5	10.4	12.6	14	14.6	83.69
T_3	4.9	7.2	12.7	17.6	21.2	23.7	24.7	72.43
T_4	5.1	12.1	21.4	29.8	35.9	40.1	41.8	53.40
T_5	5	8.7	15.3	21.3	25.7	28.7	29.9	66.67
T_6	5	10	17.6	24.5	29.5	32.9	34.3	61.72
T_7	5	15	21.2	24.1	26.2	28	30.2	66.37
T_8	5.3	26	46	64	77	86	89.7	
C.D. at 5%	0.25	0.7	0.72	0.95	1.16	1.47	1.75	
SE(m)	0.08	0.23	0.24	0.31	0.38	0.49	0.58	
SE(d)	0.12	0.33	0.34	0.44	0.54	0.69	0.82	
C.V.	2.86	3.38	2.06	2.01	2.06	2.36	2.68	

T_1= Neem leaf extract 1:1 (10%), T_2= Garlic bulb extract 1:1 (10%), T_3= Onion bulb extract 1:1 (10%), T_4=Marigold leaf extract 1:1 (10%), T_5= Neem Oil 1:4 (10%), T_6= Karanj Oil 1:4 (10%), T_7=*Trichoderma harzianum* (Dual Culture), T_8= Control

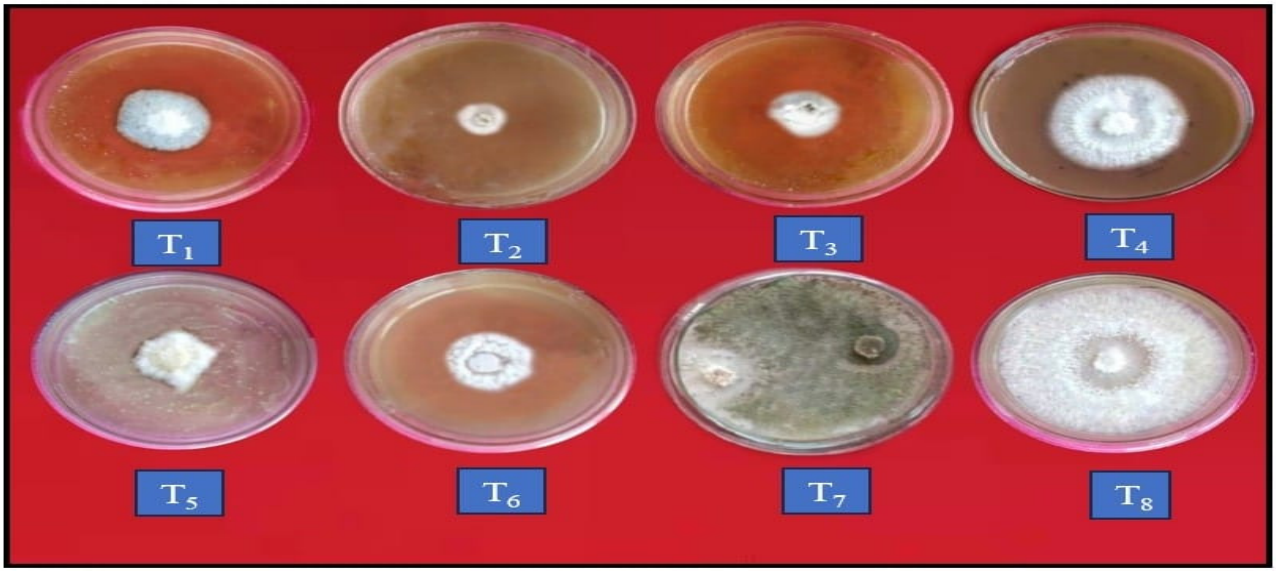

Fig. 5: Effect of botanicals and *Trichoderma harzianum* on radial mycelial growth of *Phomopsis vexans*

Conclusion

Garlic (*Allium sativum*) was found most effective at 10 % concentration of 1:1 mixture of bulb extract among all the pathogen tested which proved garlic as the most effective botanical which possess antifungal properties against most of the major plant pathogenic fungi. Also, a 10% (1:1) concentration is found suitable for all the five-pathogen tested and hence validating its use as a broad-spectrum antifungal agent. After Garlic, Onion bulb (1:1, 10%) extract seems effective among most of fungal plant pathogen tested. Neem Oil consistently showed higher inhibition percentages across all tested pathogens compared to Karanj oil. This suggests that Neem oil is generally more effective at inhibiting the mycelial growth of the tested plant pathogens than Karanj oil. Also, the antagonistic effect of *Trichoderma harzianum* proved to be effective for major fungal pathogen tested in this experiment with good efficacy varying with pathogen. The Marigold Leaf Extract was the least effective treatment in all cases, indicating limited antifungal activity at the tested concentration.

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