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In Vitro Efficacy of Tulsi (*Ocimum sanctum*) based Extract against Soil Borne Fungi

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Abstract

In the present study, antifungal effect of different concentrations of aqueous Tulsi (*Ocimum sanctum*) extract and Tulsi-$AgNO_3$ mix was tested *in vitro* against *Alternaria*, *Fusarium* and *Rhizoctonia.* The study was conducted from February to November in the year 2020. Overall results suggested that Tulsi extract significantly inhibited (above 70%) the mycelial growth of soil borne fungi. Highest concentration of Tulsi extract resulted in 77.43% growth inhibition against *Alternaria solani* and 76.75% inhibition of *Rhizoctonia solani*. Tulsi-$AgNO_3$ showed less inhibition of pathogenic fungi compared to the treatments where only Tulsi extract was used. Results show a dose-dependent inhibition of radial growth across all fungi, with *Alternaria* spp. displaying the highest sensitivity, achieving over 58% inhibition at 1000 ppm. In contrast, *Rhizoctonia solani* exhibited the lowest sensitivity, with inhibition percentages peaking at 29.64% at 1000 ppm. These findings suggest that the Tulsi extract is a promising antifungal agent, although its efficacy varies by fungal species, indicating the potential need for targeted or combination treatments for effective pathogen control.

Keywords: *Alternaria, Fusarium, Rhizoctonia*, soil borne, tulsi

1. Introduction

Plant diseases caused by pathogenic microbes lead to the reduction in crop yields around the globe by causing significant economic losses. There are almost over 20,000 species of fungi that are parasitic on different crops plants (Doehlemann et al., 2017). Plant infecting fungi have developed enormous modes of interaction and patterns of pathogenicity with host plants. Some of them produce and secrete posisonous secondary metabolites after colonization. They kill the host cells after successful colonization and survive on the organic compounds. In contrast to them others called biotroophs generally live on living plants and absorb nutrients from them for extended period of time (Mendgen et al., 1996; Horbach et al., 2011). Among fungi, soil-borne fungal pathogens (such as *Alternaria* Spp*., Fusarium* Spp. and *Rhizoctonia solani*) are of serious concern due to their wide host range (Di et al., 2016; Abdel-lateif, 2017). These fungi often damage root and crown tissues of host plants resulting in yield losses. Delgado- Baquerizo and associates (2020) documented that increase in temperature enhances the relative abundance of soil-borne fungal pathogens. It has been reported that *Fusarium oxysporum* individually infects more than 150 plants belonging to different families such as tomato, banana, cotton, carnation etc (Mihajlovic et al., 2017). Persistence of their resting spores in soil, broad host range and limited efficacy of fungicides makes the management of these pathogenic fungi very challenging. It has been documented that soilborne disease epidemics are serious threat` to crop production and extensive use of chemical fungicides along with its implications to environmental and soil microbiome has necessitated the implimenation of other methods (Kowalska, 2021). Farmers use fumigants and other chemicals at regular time intervals during a cropping season to reduce the outbreak of soil borne pathogens. However, it has been proved that extensive use of these chemicals (*e.g.*, methyl bromide and fungicides) disrupt the soil health, ecological balance, cause health hazards and also damage to aquatic ecosystem (Wightwick et al., 2010; Panth et al., 2020; Lin et al., 2020). In addition, cultural control methods viz., soil solarization, crop rotation, biofumigation, soil steam sterilization are

also adopted by farmers. However, these methods give inconsistent results. Ecofriendly management is an effective alternative method over toxic chemicals that are ecologically unsafe (Sharma et al., 2023a). Plants possesses significant antimicrobial activities owing to the presence of serveral bioactive compounds such as flavonoids, phenols, tannins, alkaloids, saponins, glycosides and terpenoids (Altemimi et al., 2017; Sharma et al., 2021; Sharma et al., 2022; Bapat et al., 2023). Tulsi (*Ocimum sanctum*) plant belonging to family Lamiaceae is a good antimicrobial agent against fungal pathogens that are major limitation to food production (Ramteke et al., 2013). This aromatic plant is commonly known as Holy Basil **or** Tulsi. There are two common cultivars of Tulsi one is called *Rama Tulsi* due to its green leaves and other is called *Krishna Tulsi* that contains purple leaves (Vani et al., 2009).

It has been reported that Tulsi plant have different properties like antimicrobial, antioxidant, anti-inflammatory, antidiabetic, immunomodulatory, antifertility, wound healing, antistress and anticancer (Prakash and Gupta, 2000; Suanarunsawat et al., 2010; Singh and Chaudhuri, 2018). Many researchers have attempted synthesis of silver nanoparticles using Tulsi extract against different phytopathogens (Singh et al., 2019; Chauhan and Tapwal, 2023). Keeping in view the antifungal activity of tulsi this study was designed with main objective to check the antifungal effect of locally available Tulsi leaves against common soil borne phytopathogenic fungi.

2. Materials and Methods

2.1. Sample collection and pathogen isolation

Freshly infected leaves showing symptoms of early blight of potato and leaf spot of mustard were collected from experimental farm situated at DAV University, Jalandhar Punjab, (144 012) India during February to November in the year 2020. The infected plant leaves were sliced into minute pieces (measuring about 5 mm) and were surface sterilized by using 1% NaOCl solution for 1–2 minutes, and washed with sterilized distilled water. The leaf slices were then inoculated on PDA medium and incubated for 2–3 days at 25°C. Pure fungal culture was obtained by hyphal tip method of isolation (Papp et al., 2021). Fungus causing black scurf disease of potato was isolated from sclerotia present on infected tubers. *Fusarium* was isolated from rhizosphere soil of wilt infected tomato plants by serial dilution method.

2.2. Preparation of Tulsi extract

The leaves of Rama Tulsi (*Ocimum sanctum*) growing at research farm of DAV University, Jalandhar, Punjab were selected for the preparation of Tulsi leaf extract. Fresh leaves (400 g) were cleaned twice in deionized water and were left for 5–7 days for drying. Dried leaves were further crushed with a sterile pestle and mortar. In 200 ml of deionized water 10 g dried powder was added in a 500 ml conical flask. The mixture was then heated at 60–65°C temprature for 60 min and a condenser was used for vapor recovery. The aqueous extract was cooled and filtered with Whatman paper No. 1. A clear yellow coloured aqueous extract was obtained and stored at 4°C. Tulsi-$AgNO_3$ mix was prepared by adding 5ml of leaf extract to 95 ml of 1 mM aqueous $AgNO_3$ solution and heated to 80°C for 5 min. The solution was further purified by repeated centrifugation (10000 rpm) for 15 min. The supernatant was transferred to sterilized beaker for settlement of particles and repeated centrifugation was carried out using a cooling centrifuge. The sample so obtained was dried in an incubator and was used for bioassay.

2.3. In vitro assay for antifungal activity of extracts

Antifungal activity of prepared extracts (Tulsi leaf extract and Tulsi-$AgNO_3$ mix) was tested by using the poisoned food technique against fungal pathogens *in vitro* on potato dextrose agar (PDA) plates (Dudeja et al., 2023). Before pouring the medium into Petri plates, required amount of extract from the stock solution was added in 100 ml of PDA in sterile flask. Treatments details are as follows - T_1=50 ppm, T_2= 100 ppm, T_3=200 ppm, T_4=500 ppm, T_5=1000 ppm and control=0.0 ppm. To warrant homogeneous dispersion of chemical media was gently shaken and 20ml medium was aseptically poured into Petri plates and was kept for solidification. Mycelial disc (4 mm diameter) from the purified fungal cultures was inoculated on fresh medium and further incubated for 10 days at 25±1°C. Concurrently, proper control was also maintained by culturing the fungal isolates on Tulsi leaf extract and Tulsi-$AgNO_3$ mix free media. The percent inhibition of fungal growth of respective treatments was calculated using the formula given below

Inhibition percentage (I)=(C-T)/C×100

Where I=Percent inhibition, C=Colony diameter in control (mm), T=Colony diameter in respective treatment (mm). The data on percent inhibition were statistically *examined using a completely randomized design (CRD).*

2.4. Statistical analysis

The data obtained from different experiments were subjected to appropriate statistical analysis using standard procedures using the software SPSS (IBM Analytics, Armonk, NY, USA).

3. Results and Discussion

In the present study, Tulsi based extract was tested *in vitro* against soil borne fungi. *Rhizoctonia solani* was isolated and purified from black scurf infected potato tuber on PDA plates. At optimum temperature, the colonies were radially grown at an average rate of 32.50 mm d^{-1}. Isolate formed white to greyish mycelium on PDA medium. After two weeks of incubation, gray to black spherical sclerotia were produced on medium. *Alternaria solani* was isolated from early blight infected potato leaves specifically with concentric dark brown spots. On PDA medium, fungus showed greenish black, circular and smooth growth. Conidiophores of fungi were generally pale-brown to olivaceous-brown in colour.

These conidiophores arose either singly or in groups on fungal hyphae and were up to 110 µm long, 6–10 µm thick, with one or more distinct conidial scars. Conidia were formed solitary, slightly flexuous or muriform with transverse (5–9) and longitudinal (0–4) septa. *Fusarium* sp. isolated on PDA showed light pink to white pigmentation and formed micro and macroconidia. The conidiophores are laterally formed on aerial hyphae. They branch into thin and elongated monophialides that further form conidia. In culture, *Alternaria alternata* generaly grows as long chains with dark brown conidiophores. Fungal colonies were olivaceous black and woolly. Obclavate conidia with short beaks were formed on acropetal chains of conidiophores.

Results from antifungal activity of Tulsi extract revealed that the radial growth of *A. solani* decreased significantly with increasing concentrations of Tulsi extract. At 50 ppm, the radial growth was 28.00 mm, which reduced to 7.66 mm at 1000 ppm. The percent inhibition increased with the concentration of Tulsi extract, starting from 17.62% at 50 ppm and reaching 77.43% at 1000 ppm. This indicates a strong inhibitory effect of Tulsi extract on *A. solani*. Similar to *A. solani*, the radial growth of *A. alternata* also reduced with increasing Tulsi extract concentration. The growth decreased from 28.00 mm at 50 ppm to 9.33 mm at 1000 ppm. Similar to *A. solani*, the radial growth of *A. alternata* also reduced with increasing Tulsi extract concentration. The growth decreased from 28.00 mm at 50 ppm to 9.33 mm at 1000 ppm. The inhibition ranged from 16.74% at 50 ppm to 72.21% at 1000 ppm, showing that Tulsi extract effectively inhibits *A. alternata*. The radial growth of *Fusarium spp.* was also reduced as the concentration of Tulsi extract increased, from 36.66 mm at 50 ppm to 10.00 mm at 1000 ppm. The inhibition increased from 5.98% at 50 ppm to 74.32% at 1000 ppm. This suggests that *Fusarium spp.* is sensitive to Tulsi extract, especially at higher concentrations. The radial growth of *R. solani* decreased notably with increasing Tulsi extract concentrations, from 25.66 mm at 50 ppm to 7.66 mm at 1000 ppm (Table 1). The inhibition ranged from 22.26% at 50 ppm to 76.75% at 1000 ppm, indicating a strong inhibitory effect of Tulsi extract on *R. solani*. Across all four fungi, there is a clear trend where increasing the concentration of Tulsi extract results in a significant reduction in radial growth and an increase in percent inhibition. This suggests that Tulsi extract has broad-spectrum antifungal properties, effective against multiple pathogenic fungi. Among the four fungi, *Alternaria solani* and *Rhizoctonia solani* showed the highest inhibition at 1000 ppm, with percent inhibitions of 77.43% and 76.75%, respectively. *Fusarium spp.* also demonstrated substantial inhibition (74.32%), although it started with a lower inhibition rate at 50 ppm compared to the others.

Pathogenic microorganisms poses a serious threat to production of agriculture commodities. A number of fungi, bacteria and nematodes are regarded as notorious pathogens that inhabit soil. Plants infected with these pathogenic agents suffer from wilting, rotting of roots, damping off, blackening, and stunting of plants. Detection and sustainable management of these pathogens is foremost important to reduce their population (Sharma et al., 2021b). Several eco-friendly management strategies adopted worldwide has shown better results against different pathogens (Kaur et al., 2023a; Sharma et al., 2023b; Sharma et al., 2024a). The efficacy of Tulsi extract, particularly at higher concentrations, in inhibiting the growth of *Alternaria solani*, *Rhizoctonia solani*, and *Fusarium spp.*, aligns with previous research highlighting the antimicrobial properties of medicinal plants (Gupta et al., 2021a; Narware et al., 2023).

The strong inhibitory effects observed in this study are likely due to the presence of phytochemicals such as eugenol, ursolic acid, and rosmarinic acid, which have been documented for their antimicrobial properties (Kaur et al., 2023b). These compounds disrupt the cellular processes of pathogens, leading to reduced growth and viability (Sharma

Table 1: Effect of Tulsi extract on radial growth and percent inhibition of the plant pathogenic fungi

Tulsi extract (ppm)	Pathogenic fungi							
	Alternaria solani		*Alternaria alternata*		*Fusarium* Spp.		*Rhizoctonia solani*	
	Radial growth (mm)	Percent inhibition (%)	Radial growth (mm)	Percent inhibition (%)	Radial growth (mm)	Percent inhibition (%)	Radial growth (mm)	Percent inhibition (%)
50	28.00	17.62	28.00	16.74	36.66	5.98	25.66	22.26
100	24.33	28.44	20.00	40.57	28.66	26.48	21.00	36.29
200	18.00	47.05	14.00	58.41	21.66	44.44	13.66	58.65
500	13.33	60.67	12.00	64.27	12.00	69.26	13.00	60.55
1000	7.66	77.43	9.33	72.21	10.00	74.32	7.66	76.75
Control	34.00	0.00	33.66	0.00	39.00	0.00	33.00	0.00
CD (p=0.05)	1.94	5.99	2.03	6.17	2.07	4.88	2.07	6.13
SEm±	0.62	1.87	0.65	1.93	0.66	1.52	0.66	1.92

et al., 2023b). Thus, Tulsi extract is effective in inhibiting the growth of several pathogenic fungi, with its efficacy increasing with concentration. This suggests its potential as a natural antifungal agent in managing fungal diseases in crops. The data supports the hypothesis that Tulsi extract can be a valuable tool in integrated disease management strategies, especially at higher concentrations.

Table 2 presents data on the effect of a Tulsi-$AgNO_3$ mix at various concentrations (measured in ppm) on the radial growth and percent inhibition of four different pathogenic fungi: *Alternaria solani*, *Alternaria alternata*, *Fusarium spp.*, and *Rhizoctonia solani*. The radial growth of *A. solani* decreases with increasing concentrations of the Tulsi-$AgNO_3$ mix, from 32.33 mm at 50 ppm to 23.33 mm at 1000 ppm. The percent inhibition of *A. solani* increases from 42.23% at 50 ppm to 58.31% at 1000 ppm. This indicates a moderate inhibitory effect of the Tulsi-$AgNO_3$ mix, with its efficacy improving at higher concentrations. The radial growth of *A. alternata* shows a decline from 50.33 mm at 50 ppm to 37.67 mm at 1000 ppm. The percent inhibition rises from 42.23% at 50 ppm to 58.31% at 1000 ppm, indicating a significant reduction in fungal growth with increasing concentration. *Fusarium* spp. exhibits a decrease in radial growth from 54.67 mm at 50 ppm to 37.00 mm at 1000 ppm. The percent inhibition starts at 18.78% at 50 ppm and increases to 45.04% at 1000 ppm. While the inhibition is notable, it is less effective compared to the other fungi, suggesting that *Fusarium spp.* might be less sensitive to the Tulsi-$AgNO_3$ mix. The radial growth of *R. solani* reduces from 50.33 mm at 50 ppm to 45.00 mm at 1000 ppm, showing a less pronounced decrease compared to other fungi. The inhibition ranges from 21.35% at 50 ppm to 29.64% at 1000 ppm. The inhibition percentage is relatively low, indicating that *R. solani* is less affected by the Tulsi-$AgNO_3$ mix compared to the other fungi. *Alternaria solani* and *Alternaria alternata* are more sensitive to the Tulsi-$AgNO_3$ mix, with percent inhibition above 58% at 1000 ppm. *Fusarium spp.* shows moderate sensitivity, with inhibition reaching 45.04% at 1000 ppm. *Rhizoctonia solani* demonstrates the lowest sensitivity, with inhibition percentages only reaching 29.64% at 1000 ppm, indicating that it is less affected by the mix.

The silver ions in $AgNO_3$ are known for their broad-spectrum antimicrobial properties, particularly in nanoparticle form, where they can penetrate microbial cells and disrupt vital functions (Gupta et al., 2021b). Our results indicated that the Tulsi-$AgNO_3$ mix exhibits antifungal properties, effectively inhibiting the growth of the tested pathogenic fungi, though its efficacy varies depending on the fungal species. *Alternaria spp.* (both *solani* and *alternata*) are the most affected by the mix, while *Rhizoctonia solani* shows the least susceptibility. These differences could be due to the distinct structural and metabolic characteristics of each fungal species, which affect their susceptibility to antifungal agents (Sharma et al., 2024 b). Further, this variation suggests that while the Tulsi-$AgNO_3$ mix could be a useful antifungal agent, its application may need to be targeted or combined with other treatments for comprehensive control of different fungal pathogens. It has been documented by many reserchers that Silver (Ag) is one of the well known materials that is used in synthesis of nanoparticles. It is hignly stable and impart significant antimicrobial properties against phytopathogens (Gupta et al., 2021a; Narware et al., 2023).

Table 2: Effect of Tulsi-$AgNO_3$ mix on radial growth and percent inhibition of the plant pathogenic fungi

Tulsi-$AgNO_3$ mix (ppm)	Pathogenic fungi							
	Alternaria solani		*Alternaria alternata*		*Fusarium* Spp.		*Rhizoctonia solani*	
	Radial growth (mm)	Percent inhibition (%)	Radial growth (mm)	Percent inhibition (%)	Radial growth (mm)	Percent inhibition (%)	Radial growth (mm)	Percent inhibition (%)
50	32.33	42.23	50.33	42.23	54.67	18.78	50.33	21.35
100	32.33	42.25	48.33	42.25	48.00	28.71	48.33	24.48
200	30.00	46.44	48.67	46.44	42.67	36.66	48.33	24.50
500	26.33	52.98	43.33	52.98	39.33	58.72	43.33	32.27
1000	23.33	58.31	37.67	58.31	37.00	45.04	45.00	29.64
Control	56.33	0.00	65.00	0.00	67.33	0.00	64.00	0.00
CD (p=0.05)	2.36	4.79	2.61	5.50	2.88	24.68	3.23	5.83
SEm±	0.76	1.50	0.84	1.72	0.92	7.73	1.04	1.83

4. Conclusion

Green synthesis of nanoparticles using tulsi and other medicinal plants with bioactive potential has been proven environmentally safe, non-toxic and economically feasible. This study highlights the potential of ecofriendly approaches, specifically the use of Tulsi extract and Tulsi-$AgNO_3$ mix, as antifungal agents against soilborne fungi. The results demonstrate that Tulsi extract significantly inhibits the

growth of multiple pathogenic fungi, with the highest efficacy observed at increased concentrations. Although the Tulsi-$AgNO_3$ mix also exhibited antifungal properties, its efficacy was lower compared to Tulsi extract alone, particularly against *Rhizoctonia solani*. These findings suggest that Tulsi extract could serve as a valuable component in integrated disease management strategies, offering a natural and sustainable alternative to chemical fungicides.

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