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Evaluation of Antifungal Activity of Plant Extracts from Selected Plants Against Onion Pathogens Using Different Extractants

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Abstract
This study assessed the antifungal activity of plant extracts from five species: *Ricinus communis*, *Xanthium strumarium*, *Acacia nilotica*, *Ficus racemosa*, and *Tridax procumbens*, collected from various locations in the Kopargaon Tehsil. The extracts were tested against three pathogenic fungi—*Aspergillus niger*, *Stemphylium vesicarium*, and *Alternaria porri*—which cause severe diseases in onion crops. Antifungal effectiveness was evaluated using the food poisoning technique, with both aqueous and methanol extracts at different concentrations. The results demonstrated that all concentrations of the plant extracts significantly inhibited the mycelial growth of the fungi, with higher concentrations showing the most substantial inhibition. Among the extracts, *Ricinus communis* leaf extract, in both aqueous and methanol solvents, proved to be the most effective in controlling fungal growth, followed by the other plant extracts. These findings indicate that the selected plants, particularly *R. communis*, have potential as natural fungicides for managing fungal diseases in onion crops.

Keywords: Antifungal activity, Plant extract, Onion, Pathogenic fungi, Poisoned food technique, mycelial growth.

1. Introduction

Onion (*Allium cepa*) is one of the most widely cultivated and economically important vegetable crops globally. However, its production is significantly hampered by fungal diseases, which affect its yield, quality, and post-harvest shelf life. Common fungal pathogens, including *Fusarium oxysporum*, *Alternaria porri*, *Botrytis allii*, and *Sclerotium cepivorum*, cause major diseases such as purple blotch, white rot, and bulb rot, leading to substantial losses in onion crops (Saharan & Mehta, 2008; Roy et al., 2014). In addition to reducing yield, these pathogens also contribute to storage issues and increase susceptibility to other microbial infestations, further affecting food security and the economic stability of onion growers (Khan et al., 2017).

Traditional methods of controlling these fungal diseases have predominantly relied on synthetic chemical fungicides, which, while effective, have raised several concerns. These concerns include the development of fungicide resistance in pathogens, environmental contamination, non-target organism toxicity, and human health risks (Bali et al., 2020). As a result, there is a growing demand for more sustainable and

environmentally friendly approaches to plant disease management. One promising alternative is the use of plant-derived antifungal agents, which have gained considerable attention due to their bioactive properties, cost-effectiveness, and minimal environmental impact (Kumar et al., 2017).

Plant extracts, especially those from leaves, contain a diverse array of secondary metabolites such as alkaloids, flavonoids, phenolic compounds, terpenoids, and essential oils, many of which exhibit antimicrobial and antifungal activity (Fowler et al., 2015). These natural bioactive compounds have shown significant potential in controlling various plant pathogens, including fungi that affect onion. Furthermore, the use of plant extracts is considered safe and biodegradable, making it a preferable alternative to chemical fungicides (Saqib et al., 2020).

Among the various methods of extracting bioactive compounds from plants, the choice of extractant is critical for determining the efficacy of the extract. Different solvents, such as ethanol, methanol, acetone, and water, possess varying capacities for dissolving plant metabolites, thereby influencing their antifungal activity (Sharma et al., 2013).

Several plant species have been evaluated for their antifungal properties, with many showing promising results. For example, *Azadirachta indica* (neem) leaves are known for their broad-spectrum antifungal activity, particularly against *Fusarium oxysporum* and *Alternaria porri* (Bisht et al., 2016). Similarly, *Ocimum sanctum* (holy basil), *Mentha piperita* (peppermint), *Allium sativum* (garlic), and *Cinnamomum verum* (cinnamon) have been reported to have strong antifungal effects due to their rich content of essential oils and phenolic compounds (Bajpai et al., 2013; Venkatesan et al., 2019). These plants' extracts, when tested using different extractants, have shown varied results, indicating that the extraction method can significantly influence the antifungal potential of these plant materials.

Given the promising results of plant-based antifungals, this study aims to evaluate the antifungal properties of leaf extracts from selected plants against common fungal pathogens affecting onions. Specifically, the study was examined the efficacy of various solvents (methanol and water) as extractants in isolating antifungal compounds from plant leaves. The selected plant species for this study include *Ricinus communis, Xanthium strumarium, Acacia nilotica, Ficus racemosa* and *Tridax procumbens* which are known for their medicinal and antifungal properties. By comparing the effectiveness of different extractants, this research seeks to identify the most potent natural antifungal agents that can be applied to onion disease management in an eco-friendly manner.

2. Materials and Methods

2.1. Collection of Plant material.

The Plant material, *Ricinus communis, Xanthium strumarium, Acacia nilotica, Ficus racemosa* and *Tridax procumbens* were collected from the geographical region of Kopargaon Tehsil. The plant was identified and Authenticated by a plant taxonomist Dr. N. V. Malpure, a distinguished Taxonomist and Assistant Professor in the Department of Botany at S.S.G.M. College, Kopargaon. The voucher specimen was deposited in the herbarium at the department of Botany.

2.2. Collection of diseased plant material

Different infected onion sample have been collected from different localities in the region of Kopargaon Tehsil. The collected sample were transported to the laboratory in sterile polyethene bag for examination and kept at temperature of 25ºc for identification.

2.3. Isolation of pathogenic fungi.

Different onion sample were taken and infected part inoculated on potato dextrose agar medium (PDA)

plate by direct plate method and plates were incubated at 28-30º c for 7 days. After incubation the macroscopic and microscopic distinct colonies by using lactophenol cotton blue were done. The fungal culture is purified by the single hyphal tip method. Pure culture of isolated fungi will be used for further study.

Sr.no	Botanical name	Family	Local name	Plant part used.
1	*Ricinus communis*	Euphorbiaceae	Yarand	Leaves
2	*Xanthium strumarium*	Malvaceae	Rough cocklebur	Leaves
3	*Acacia nilotica*	Fabaceae	Babul	Bark
4	*Ficus racemosa*	Moraceae	Umber	Bark
5	*Tridax procumbens*	Asteraceae	Tantani	Leaves

Table 1. Plants collected from the region of Kopargaon Tehsil and used for extraction preparation.

2.4. Preparation of Plant Crude extracts

The part of selected plants were washed with distilled water, air-dried at room temperature for 15-20 days, and then grinded into a uniform powder. Aqueous and methanol extracts were prepared by soaking 50 g of the powdered plant material in 500 mL (1:10w/v) of each solvent at room temperature for 72 hours. Each mixture was stirred at 24 h interval using a sterile glass rod (Alagesaboopathi, 2011). The plant samples squeezed and then filtered with the help of triple- layered cotton cloth. Water content of distilled water filtrate was evaporated on using water bath till the solution reduced to semisolid form (Bhattarai & Shrestha, 2009) and warmed on water bath at the methanol extract at 50°C, resulting in semi-solid residues. The crude extracts were weighted and made the bottles air tight and stored in a refrigerator at temperature 4◦ C until further use (Mahida & Mohan, 2007) & prepared different concentrations of the extracts (5, 10, 15, and 20%) were made in both solvents i.e., methanol and distilled water separately. Methanol and distilled water solvents were used as control.

2.5. Effect of plant extract on Radial growth of mycelium.

The poisoned food technique (Nene and Thapliyal 1968) was used for fungicidal and fungi-static activity screening in vitro. The selected plant extracts were incorporated into a sterilized PDA 20 ml (Potato dextrose agar) medium and mixed thoroughly to make them homogeneous and poured into sterilized Petri-dishes. The Petri-dishes containing poisoned PDA medium was allowed to solidify. Control was maintained without plant extracts. Each Petri-dish was inoculated by placing a 3 mm mycelium disc taken from a pure fungal culture of selected fungi in the centre with the help of a cork borer and incubate at 28±1^{0}c temp. for three days. Radial growth of the mycelium was recorded and examined their inhibitory effect after three days of inoculation. The three replicates were kept for each treatment. The inhibitory effect of plant extracts of selected plant species on pathogenic fungi was mentioned in table. Fungus toxicity of each plant extracts was expressed in terms of the percentage of mycelium growth inhibition, calculated according to the formula of Vincent *et al., (*1927)

C-T /C ×100.

C = Average diameter of a fungal colony with control.

T =Average diameter of a fungal colony with treatment

3. Results

Table 3. Effect of different concentrations of Aqueous plant extracts on radial mycelial growth of phytopathogenic fungi after three days of inoculation at 28±1^{0}c.

		Inhibition (%) of mycelial growth											
Sr. No.	**Plant Extract**	***Aspergillus niger***				***Stemphylium vesicarium***				***Alternaria porri***			
		5%	**10 %**	**15 %**	**20 %**	**5%**	**10 %**	**15 %**	**20 %**	**5%**	**10 %**	**15 %**	**20 %**
1	*R. communis*	23.76	41.49	53.01	65.26	25.93	41.86	55.93	67.79	26.68	43.32	60.13	71.75
2	*X. strumarium*	21.14	38.57	48.57	60.00	27.69	44.81	55.38	67.69	26.56	40.62	51.56	64.06
3	*A. nilotica*	21.30	31.94	44.4	56.94	17.33	30.7	42.6	50.6	20.27	31.08	41.89	52.70
4	*F. racemosa*	16.0	26.7	38.7	49.3	13.1	24.32	36.48	47.29	13.33	24.00	30.22	47.12
5	*T. procumbens*	12.34	20.98	30.86	40.74	7.40	23.45	31.69	44.03	11.11	22.12	32.09	39.92

= All values in percentage.

Table 4. Effect of different concentrations of Methanolic plant extracts on radial mycelial growth of phytopathogenic fungi after three days of inoculation at 28±1^{0c}.

		Inhibition (%) of mycelial growth											
Sr. No.	**Plant Extract**	***Aspergillus niger***				***Stemphylium vesicarium***				***Alternaria porri***			
		5%	**10 %**	**15 %**	**20 %**	**5%**	**10 %**	**15 %**	**20 %**	**5%**	**10 %**	**15 %**	**20 %**
1	*R. communis*	25.86	45.34	56.89	70.68	33.33	47.82	57.97	69.56	40.50	50.00	61.16	72.83
2	*X. strumarium*	28.98	40.57	52.17	63.76	29.68	42.18	56.25	68.75	31.37	46.03	57.14	69.84
3	*A. nilotica*	29.16	40.27	50	61.11	21.33	32.0	43.56	53.33	20.77	32.46	44.15	54.50
4	*F. racemosa*	21.33	32.0	44.0	53.3	18.66	29.33	41.33	52.00	16.00	25.33	36.00	48.00

5	*T. procumbens*	12.82	21.79	32.05	43.58	17.94	24.88	37.61	45.73	14.10	24.35	33.33	42.30

= All values in percentage.

Effect of plant extract on Radial growth of mycelium.

The effects of aqueous and methanolic extracts on the radial growth of mycelium in the studied pathogenic fungi were assessed through in vitro tests, as presented in Table 3 and Table 4 The results demonstrated that all treatments effectively inhibited the radial mycelial growth of the fungi when compared the control group. Notably, the percentage reduction in mycelial growth increased with higher concentrations of the extracts, indicating a dose-dependent response.

The results presented in Table 3 indicate that the aqueous plant extracts had the highest effect on mycelial growth inhibition, with *Alternaria porri* showing an inhibition of 71.75% at a 20% concentration of *Ricinus communis* plant extract. Conversely, the lowest inhibition was observed in *Stemphylium vesicarium*, with just 7.40% inhibition at a 5% concentration of *Tridax procumbens* plant extract.

In Table 4, the methanolic plant extracts demonstrated a more pronounced effect on mycelial growth inhibition, with *Alternaria porri* exhibiting 72.83% inhibition at a 20% concentration of *Ricinus communis* plant extract. The lowest inhibition was recorded for *Aspergillus niger*, with only 12.82% inhibition at a 5% concentration of *Tridax procumbens* plant extract.

4. Discussion

The study provides strong evidence supporting the antifungal potential of plant extracts from selected plants against major fungal pathogens that affect onion crops, including *Aspergillus niger*, *Stemphylium vesicarium*, and *Alternaria porri*. Notably, extracts from *Ricinus communis* demonstrated the highest antifungal activity, significantly inhibiting the mycelial growth of these fungi at varying concentrations. These findings align with previous research indicating that plant extracts can be effective in controlling fungal pathogens due to the presence of bioactive compounds, such as alkaloids and phenolic compounds, known for their potent antimicrobial properties (Fowler et al., 2015; Kumar et al., 2017).

The choice of solvent for the extraction process was found to be a critical factor in determining the efficacy of the antifungal agents. Methanol and aqueous solvents yielded varying results, underscoring the importance of selecting the appropriate extraction medium to optimize the solubility and bioavailability of active compounds (Sharma et al., 2013). Additionally, the increased antifungal activity observed at higher concentrations of the extracts suggests a dose-dependent relationship, where larger quantities of bioactive compounds result in stronger inhibitory effects.

The study also highlights the significant implications of transitioning from synthetic fungicides to plant-based alternatives for sustainable agriculture. Chemical fungicides are associated with risks such as environmental contamination and the development of resistance in fungal pathogens (Bali et al., 2020; Khan et al., 2017). The results of this study reinforce the potential of utilizing natural products as safe, biodegradable alternatives to synthetic chemicals, which aligns with the growing demand for more sustainable and environmentally friendly agricultural practices (Saqib et al., 2020).

Finally, the study emphasizes the need for further research to investigate the specific mechanisms of action of the bioactive compounds in these plant extracts. Isolating and identifying these compounds will be

crucial in developing more targeted antifungal treatments, further supporting the role of plant extracts in crop disease management.

5. Conclusion

The antifungal screening of plant part extracts from selected plants revealed their potential effectiveness as natural fungicides against pathogenic fungi affecting onion crops. The results indicate that *Ricinus communis* is particularly potent, suggesting that it may be an excellent plant for further development in biological control settings. The varying effectiveness based on the solvent used for extraction emphasizes the need for detailed evaluations of extraction methods to maximize antifungal efficacy.

The study advocates for adopting plant-derived antifungals as a sustainable alternative to synthetic chemicals, thereby contributing positively to agricultural practices and reducing environmental risks. This research opens up avenues for future investigations into the isolation and characterization of specific antifungal compounds, which could enhance integrated pest management strategies and support sustainable farming initiatives (Bajpai et al., 2013; Venkatesan et al., 2019)

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