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Antifungal activity of cattail (*Typha*) leaf extracts against different fungal species using disc diffusion method

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ABSTRACT

This study evaluated the antifungal potential of *Typha* leaf extracts obtained using ethanol, methanol and aqueous solvents against four fungal species: *Aspergillus niger, Fusarium oxysporum*, *Alternaria* spp., and *Penicillium* spp. The disc diffusion assay was performed with extract concentrations ranging from 10 to 100 mg/ml with fluconazole as a positive control. Ethanol extracts exhibited the highest antifungal activity across all fungi, with *Alternaria* spp. showing the greatest sensitivity (19.50 mm zone of inhibition at 100 mg/ml). Positive control fluconazole showed highest inhibition than all extracts of *Typha*. The lowest inhibition was observed against *Fusarium oxysporum*. These findings suggest that *Typha* leaf extracts, especially ethanolic ones, have significant antifungal properties and potential for development as natural antifungal agents.

Key words: Chemical fungicide, disc diffusion, ethanolic extract, phytopathogenic fungi, *Typha*

Fungal pathogens are among the most destructive agents of crop diseases worldwide, causing significant reductions in agricultural productivity and food quality in cereals, vegetables, and fruits, leading to substantial post-harvest losses (Dean *et al*., 2012; Cheng *et al*., 2022a). Major phytopathogenic fungi such as *Aspergillus niger*, *Fusarium oxysporum*, *Alternaria* spp., and *Penicillium* spp. exhibit aggressive pathogenicity, environmental adaptability, and mycotoxin production, making their control challenging in both open-field and protected systems (Bennett et al., 2017; Kumar *et al.,* 2017; Sridhar et al., 2022; Mishra *et al.,* 2023). Synthetic fungicides remain the primary control measure, but their prolonged use has led to resistant strains, environmental contamination, toxic residues in food, and negative effects on non-target organisms and soil microbiota (Molla *et al.,* 2020; Ali *et al.,* 2021). These drawbacks have driven interest in sustainable, eco-friendly alternatives such as plant-derived bioactive agents, consistent with integrated pest management (IPM) principles (Sarker *et al.,* 2022; Choudhary *et al*., 2023).
India, a major center of medicinal plant resources, provides traditional healthcare benefits to over 500 million people, and more than three-quarters of the world's population relies on plant-based remedies (Bano *et al.,* 2017a). Medicinal plants are rich in compounds such as alkaloids, flavonoids, phenolics, tannins, and terpenoids with proven antifungal, antibacterial, and antioxidant properties (Bano *et al.,* 2017b; Das *et al.,* 2021). Occasionally, synthetic plant growth

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regulators such as ethrel are applied to release ethylene, influencing plant development and stress responses (Lone *et al.,* 2010). *Typha* species (cattails), emergent, wetland (marsh with standing water) macrophytes of the family Typhaceae, occur widely in temperate and tropical regions, and wetlands recognized as biodiversity hotspots require careful management to prevent degradation (Bano *et al.,* 2021, 2025; Mir *et al.,* 2023,2025; Islam *et al.,* 2023, 2024). Traditionally valued for food, fiber, and medicine, *Typha* contains bioactive metabolites with antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties (Sharifi-Rad *et al.,* 2020; Sharma *et al.,* 2021; Liu *et al.,* 2019; Rahman *et al.,* 2022).

Despite this phytochemical richness, the antifungal activity of *Typha* leaf extracts remains underexplored, particularly across different solvents, where polarity strongly influences extraction efficiency and antimicrobial potency (Abdissa *et al.,* 2021; Ezeonu *et al.,* 2023). This study evaluates the antifungal efficacy of ethanol, methanol, and aqueous *Typha* leaf extracts against *A. niger*, *F. oxysporum*, *Alternaria* spp., and *Penicillium* spp. using the disc diffusion method at concentrations of 10–100 mg ml^{-1}, aiming to assess their potential as botanical fungicides for sustainable plant disease management.

MATERIALS AND METHODS

Plant material and extraction: Fresh *Typha* leaves were collected from different locations viz. Srinagar, Pulwama, Ganderbal, Budgam and Bandipora of Kashmir valley on the basis of distance. Leaves were washed, shade dried and grounded into fine powder. Extraction was performed by macerating 50 g of leaf powder in 500 ml of ethanol, methanol and aqueous for 72 hours with intermittent shaking. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator at 40°C. Dried extracts were stored at 4°C until use.

Fungal cultures: The fungal strains *Aspergillus niger*, *Fusarium oxysporum*, *Alternaria* spp., and *Penicillium* spp. used in this study were obtained from the Division of Plant Pathology, SKUAST-Kashmir, where they were originally isolated from naturally infected plant materials showing typical symptoms of fungal infection like fruit rot in apple (*A. niger*), vascular wilt in tomato (*F. oxysporum*), leaf spot in brassicas (*Alternaria* spp.), and post-harvest decay in citrus (*Penicillium* spp.). These pathogens are well-recognized for their high virulence and broad host range, causing significant economic losses in horticultural and field crops (Dean et al., 2012; Mishra et al., 2023). Cultures were maintained on Potato Dextrose Agar (PDA) at 25 ± 2 °C, which is considered optimal for the growth of most phytopathogenic fungi (Agrios, 2005; Barnett & Hunter, 1998). Periodic subculturing was performed to maintain viability and pathogenicity.
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Pathogenicity confirmation: Pathogenicity of the isolates were confirmed prior to antifungal assays using Koch's postulates. Healthy, surface-sterilized host tissues (apple fruits for *A. niger*, tomato seedlings for *F. oxysporum*, cabbage leaves for *Alternaria* spp., and citrus fruits for *Penicillium* spp.) were inoculated with spore suspensions (1×10^6 spores/ml) and incubated under conditions favorable for disease development (25 ± 2 °C, >90% RH) (Agrios, 2005; Pitt & Hocking, 2009). Typical symptoms were observed within 5–10 days, and the pathogens were successfully re-isolated and re-identified based on morphological characteristics, confirming their pathogenicity (Dhingra and Sinclair, 1995)

Antifungal assay: Antifungal activity was evaluated by the disc diffusion method (Nostro *et al.,* 2017). Sterile discs (6 mm diameter) were impregnated with 20 µl (The choice of 20 µl per disc was based on the small surface area of the discs, ensuring complete absorption without overflow and maintaining uniform diffusion in the agar medium (Bauer *et al.,* 1966; Balouiri *et al.,* 2016) of *Typha* extracts at concentrations of 10, 30, 50, 80, and 100 mg/ml (the selection of these concentrations were based on preliminary screening assays that indicated measurable inhibition zones within this range while avoiding solvent saturation effects on the discs. Lower concentrations (10 and 30 mg/ml) were included to evaluate the minimal effective dose and detect potential dose–response relationships, whereas intermediate (50 mg/ml) and higher concentrations (80 and 100 mg/ml) were tested to assess maximum inhibitory potential and possible plateau effects. Such a gradient approach is commonly used in antifungal susceptibility testing of plant extracts to provide a comparative evaluation of potency across a range of physiologically relevant doses (Balouiri, Sadiki, and Ibnsouda, 2016; CLSI, 2015). Discs were placed on PDA plates inoculated with fungal spore suspensions (1×10^{6} spores/ml) (Hemocytometer, a specialized counting chamber used under a microscope to determine spore concentration). Plates were incubated at 25 ± 2 °C for 5 days. Fluconazole (25 µg/disc) was used as a positive control, and discs with solvents served as negative controls. Zones of inhibition were measured in millimeters.

Statistical analysis: Experiments were performed in triplicates. Data are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

The present study investigated the antifungal potential of *Typha* leaf extracts prepared using ethanol, methanol and aqueous solvents against four major phytopathogenic fungi: *Alternaria* spp., *Fusarium oxysporum*, *Aspergillus niger*, and *Penicillium* spp. The evaluation was performed using the disc diffusion method at different concentration of 10, 30, 50, 80 and 100 mg/ml of *Typha* leaf extract. The antifungal activity of *Typha* leaf extracts showed significant variation depending on solvent type, fungal strain, and concentration. Ethanol extracts demonstrated the highest antifungal efficacy across all fungi. The maximum zone of inhibition was observed against *Alternaria* spp. (19.50 ± 0.75 mm) at 100 mg/ml, closely followed by methanol (18.53 ± 0.81 mm) and aqueous extracts (13.37 ± 0.55 mm). *Fusarium oxysporum* exhibited the least sensitivity, with the smallest inhibition zones across all extracts and concentrations. *Penicillium* spp. showed inhibition zone of 16.97±0.47 mm with ethanol, 15.53±0.78 mm with methanol and 11.43±0.61 mm with aqueous extract at 100 mg/ml. Also *Aspergillus niger* showed inhibition zone of 15.53±0.81 mm with ethanol, 14.40±0.72 mm with methanol and 10.10±0.53 mm with aqueous extract at 100 mg/ml. Inhibition zones increased proportionally with extract concentration for all fungi, indicating a dose-dependent antifungal effect. Fluconazole produced larger inhibition zones ranging 15.63 mm to 20.35 mm, confirming assay reliability (Table 1, 2 and Fig. 1, 2, 3).

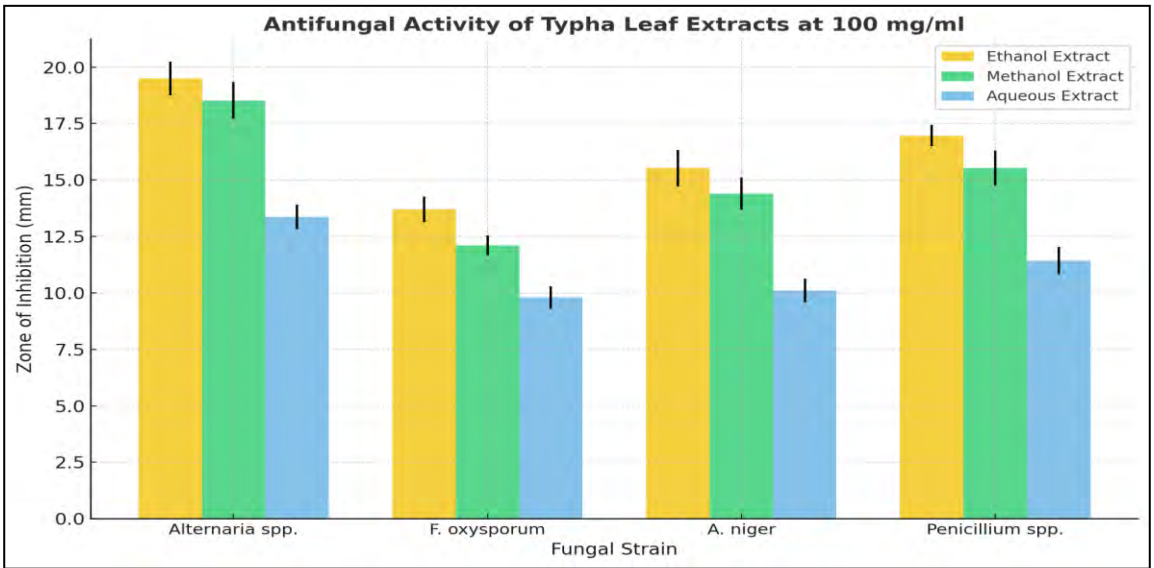

Fig. 1: Bar graph showing the antifungal activity of *Typha* leaf extracts at 100 mg/ml against four fungal strains (*Alternaria* spp., *Fusarium oxysporum, Aspergillus niger* and *Penicillium* spp.) using ethanol, methanol and aqueous extracts

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Table 1: Antifungal property of leaf extract of *Typha* spp. against different fungal strains

Fungal strains	Strain solvents	Plant extract Concentration (mg ml^{-1}) 10	30	50	80	100	Mean St X So	Fluconazole (+C)	Factor mean of solvent
Aspergillus niger	Methanol	7.87 ±0.49	8.53 ± 0.65	9.60 ± 0.62	11.43 ± 0.61	14.40 ±0.72	10.37	18.71	
	Ethanol	9.50 ±0.60	10.70 ± 0.46	12.30 ±0.62	13.60 ± 0.66	15.53 ±0.81	12.33	19.63	
	Aqueous	6.27 ±0.49	6.93 ± 0.25	8.60 ± 0.53	9.50 ± 0.85	10.10 ±0.53	8.28	16.91	
Sub mean St X		**7.88**	**8.72**	**10.17**	**11.51**	**13.34**	**10.33**	**18.42**	
Fusarium oxysporum	Methanol	6.63 ±0.49	7.50 ± 0.53	8.47 ± 0.60	10.37 ± 0.67	12.10 ±0.44	9.01	18.52	
	Ethanol	7.30 ±0.95	9.97 ± 0.40	11.47 ±0.60	12.47 ± 0.85	13.70 ±0.56	10.98	19.51	Methanol=11.20 Ethanol =12.93 Aqueous =8.77 Fluconazole= 17.94
	Aqueous	6.11 ±0.55	6.23 ± 0.31	8.33 ± 0.68	9.20 ± 0.30	9.80 ± 0.50	7.78	15.63	
Sub mean St X C		**6.42**	**7.90**	**9.42**	**10.68**	**11.87**	**9.26**	**17.89**	
Penicillium spp.	Methanol	8.90 ±0.46	9.77 ± 0.55	11.27 ±0.85	12.93 ± 0.32	15.53 ±0.78	11.68	19.12	
	Ethanol	10.00 ±0.70	11.73 ± 0.42	13.83 ±0.35	15.40 ± 0.85	16.97 ±0.47	13.59	20.21	
	Aqueous	7.03 ±0.21	7.70 ± 0.46	8.73 ± 0.47	10.07 ± 0.25	11.43 ±0.61	8.99	17.82	
Sub mean St X C		**8.64**	**9.73**	**11.28**	**12.80**	**14.64**	**11.42**	**19.05**	
Alternaria spp.	Methanol	9.70 ±0.46	11.60 ± 0.89	13.27 ±0.50	15.53 ± 0.71	18.53 ±0.81	13.73	19.43	
	Ethanol	10.53 ±0.65	12.77 ± 0.38	14.47 ±0.96	16.80 ± 0.35	19.50 ±0.75	14.81	20.35	
	Aqueous	7.73 ±0.40	8.73 ± 0.40	9.20 ± 0.46	11.03 ± 0.67	13.37 ±0.55	10.01	18.12	
Sub mean St X C		**9.32**	**11.03**	**12.31**	**14.45**	**17.13**	**12.85**	**19.30**	
Mean		**8.07**	**9.35**	**10.79**	**12.36**	**14.25**	**10.97**	**18.67**	

*St: Strain, C: Concentration, So: Solvent and +C: Positive control

Table 2: Antifungal activity of *typha* leaf extracts at 100 mg/ml

Fungal Strain	Ethanol extract	Methanol extract	Aqueous extract
***Alternaria* spp.**	19.50 ± 0.75	18.53 ± 0.81	13.73 ± 0.55
Fusarium oxysporum	13.70 ± 0.56	12.10 ± 0.44	9.80 ± 0.50
Aspergillus niger	15.53 ± 0.81	14.40 ± 0.72	10.10 ± 0.53
***Penicillium* spp.**	16.97 ± 0.47	15.53 ± 0.78	11.43 ± 0.61

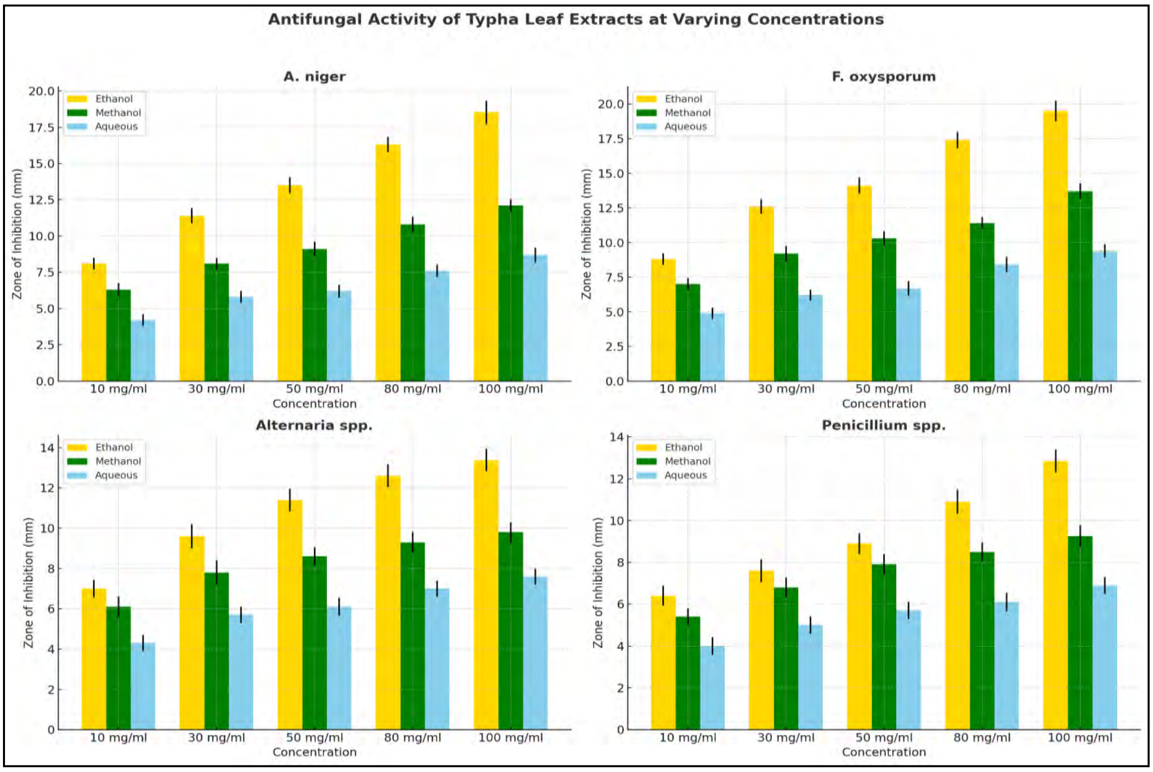

Fig. 2: Antifungal activity of *Typha* leaf extracts (ethanol, methanol and aqueous) at different concentrations (10, 30, 50, 80, and 100 mg/ml) against four phytopathogenic fungi (*Aspergillus niger, Fusarium oxysporum, Alternaria* spp. and *Penicillium* spp.) using the disc diffusion method

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Data represent mean inhibition zone diameters (mm) ± standard deviation from triplicate assays. The antifungal efficacy of *Typha* leaf extracts observed in the present study underscores the potential of this underutilized wetland plant as a source of natural bioactive agents. The consistent superiority of the ethanol extract across all tested fungal strains and concentrations corroborates earlier findings which suggest that ethanol's intermediate polarity makes it an ideal solvent for extracting a broad spectrum of phytochemicals, especially phenolics, flavonoids, and other lipophilic antimicrobial compounds (Borges *et al*., 2021; Patil *et al*., 2023). These classes of compounds have been widely reported to exert antifungal action by targeting fungal cell wall synthesis, disrupting membrane integrity, or interfering with essential metabolic pathways (Cowan, 1999; Ma *et al*., 2022). Methanol extracts, while moderately effective, demonstrated slightly reduced antifungal activity in comparison to ethanol extracts. This variation could stem from differences in the solvent's ability to extract specific bioactive profiles or their concentration. Although methanol is also a polar solvent, its selectivity may limit its efficiency in extracting certain antifungal compounds present in *Typha* leaves (Kaur *et al*., 2020). On the other hand, the aqueous extracts showed the least inhibition across all fungal strains, which is consistent with prior research noting the limitations of water in extracting hydrophobic phytoconstituents such as terpenoids and some flavonoid aglycones (Abdissa *et al*., 2021; Nostro *et al*., 2017). These findings emphasize the critical role of solvent choice in phytochemical extraction protocols and the resulting bioactivity. Among the tested fungal strains, *Alternaria* spp. exhibited the highest susceptibility to all extract types, with inhibition zones reaching up to 19.50 ± 0.75 mm in the ethanol treatment. This high sensitivity might be linked to the relatively more permeable cell wall structure of *Alternaria*, allowing easier penetration of active compounds (Cheng *et al*., 2022b). Phenolic compounds and flavonoids are known to induce oxidative stress and inhibit fungal spore germination, which may explain the substantial inhibition observed (Nazzaro *et al*., 2013; Sharma *et al*., 2021). In contrast, *Fusarium oxysporum* was the least inhibited among the test organisms, showing only moderate susceptibility even at

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the highest concentration (13.70 ± 0.56 mm with ethanol extract). *Fusarium* species are notorious for their inherent resistance mechanisms, including thick chitinous walls, multidrug efflux transporters, and enzymatic detoxification systems, which collectively reduce susceptibility to antifungal agents (Molla *et al*., 2020; Wang *et al*., 2019). These intrinsic defense mechanisms likely contributed to the reduced efficacy observed in this study. Importantly, the antifungal activity of all extracts was dose-dependent, with inhibition zones increasing progressively with rising extract concentrations (from 10 to 100 mg/ml). This dose-response trend is well-documented in phytochemical studies and supports the quantitative relationship between bioactive compound concentration and antimicrobial potency (Kaur *et al*., 2020; Sarker *et al*., 2022). Such findings reinforce the assertion that the antifungal effects observed are not incidental but are the result of specific interactions between fungal cellular structures and phytochemicals, likely involving membrane disruption, ROS induction, or enzymatic inhibition (Abdel-Rahman *et al*., 2019). The use of fluconazole as a positive control further validated the relative effectiveness of the plant extracts. While the synthetic antifungal demonstrated the highest zone of inhibition, the ethanolic *Typha* extract displayed competitive efficacy, particularly against *Alternaria* and *Penicillium*, suggesting a viable alternative in specific agricultural or storage contexts. The present findings align with a growing body of literature supporting the utility of wetland plants and traditional medicinal flora in integrated pest and pathogen management strategies. The identification of potent antifungal activity in *Typha*—a widely distributed and sustainable species—opens promising avenues for the development of eco-friendly fungicides that reduce chemical inputs in agriculture (Sharifi-Rad *et al*., 2020; Nostro *et al*., 2017). However, while the in vitro data is compelling, further work is essential to isolate and characterize the active compounds responsible for the antifungal effects. Additionally, mechanistic studies are needed to determine the precise mode of action against different fungal targets. Field-based trials should also be conducted to evaluate the practical applicability, efficacy under natural conditions, phytotoxicity to crops, and long-term impacts on soil and microbial health.

CONCLUSION

This study demonstrates that *Typha* leaf extracts, particularly ethanolic preparations, exhibit strong antifungal activity against key phytopathogens, with *Alternaria* spp. showing the highest susceptibility. The efficacy is attributed to the presence of phenolics and flavonoids extracted efficiently by ethanol. These findings support the potential use of *Typha* as a natural source of fungicidal agents. Future research should focus on isolating active compounds, understanding their mode of action, and evaluating their effectiveness through field trials and formulation development. Such efforts can pave the way for eco-friendly, plant-based alternatives to synthetic fungicides in sustainable agriculture.

CONFLIT OF INTEREST

All the authors affirm that there is no conflict of interest among them. All research activities comply with relevant legal, institutional and ethical standards.

AUTHOR CONTRIBUTION

All the authors contributed equally in the present study.

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