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PHYTOCHEMICAL ASSESSMENT AND ANTIFUNGAL ACTIVITIES OF *MELIA AZEDARACH* L. EXTRACTS FOR THE MANAGEMENT OF SOIL-BORNE PHYTOPATHOGENIC FUNGI

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Abstract *The current study assessed the phytochemical screening and antifungal activities of Melia azedarach fruit and leaf extracts made using aqueous and ethanol solvents against a main soil-borne plant pathogenic mould, comprising Sclerotium rolfsii, Macrophomina phaseolina, Rhizoctonia solani, Alternaria alternata and Fusarium oxysporum. Qualitative p* secondary metabolites *analysis revealed the occurrence of flavonoids, phenols, terpenoids, saponins, tannins and alkaloids in both extracts, with ethanol extracts observing relatively maximum profusion of phytochemicals, chiefly flavonoids and phenols. Antifungal activities were evaluated via reduction of biomass technique at various doses (50–300 mg/mL). All extracts displayed a vibrant concentration-dependent inhibition activity on mould biomass. Amongst all treatments, fruits and leaves ethanol extracts exhibited the maximum fungicidal efficiencies, attaining the highest inhibition of biomass up to 88% against Fusarium oxysporum and over 80% against the greatest experimented mould at 300 mg/mL. The water extracts also presented significant activities nevertheless were relatively lower activity across all doses. Generally, Alternaria alternata and Fusarium oxysporum were the most susceptible fungi, whereas Sclerotium rolfsii revealed comparatively maximum resistance. The research highlights that plant parts and types of solvents meaningfully affect the isolation of bioactive substances and their antifungal properties. The results suggest that Melia azedarach owns powerful capacity as a non-synthetic source of antifungal compounds for controlling soil-borne diseases.*

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Keywords: *Chinaberry tree; plant fungal pathogens; natural fungicide; phytocompounds; fungi biomass inhibition; ethanol and aqueous extracts*

Introduction

Soil-borne ailments are considered a main obstacle in crop production. Soil-borne phyto-pathogens comprises *Phytophthora spp*., *Pythium spp.*, *Verticillium spp.*, *Sclerotinia spp.*, *Fusarium spp.*, and *Rhizoctonia spp.*, might source approximately 50%–75% loss of production for various crops such as ornamentals, fruits, vegetables, maize, cotton, and wheat as documented to date (Vaghasiya and Sumit 2021). Soil-borne mould pathogens occur in the shape of spores or dormant propagules and thrive by developing when micro-surrounds are converted to suitable conditions (Hussain and Khan 2019). The prominent issues caused by soil-borne pathogens in the production of crops comprise higher production costs, decreased yield, and reduced crop performance. The threats of soil-borne ailments waves in crop production, new ailment outbreaks, climate change, development of resistance towards synthetic fungicides, high cost of artificial fungicides, and rising ecosystem worries along with health of the soil are becoming progressively apparent (Vaghasiya and Sumit, 2021).

Artificial fungicides and preservatives have been applied for many years to manage mould rot and decay, while the indiscriminate application of these compounds has created fitness issues for animals and human beings because of their acute toxicity, teratogenicity, and carcinogenicity (Tian et al., 2011), causing resistance to pathogens, besides ecosystem pollution (De Rodriguez et al., 2011). Therefore, scientists are searching for harmless substitutes to replace artificial substances applied as preservatives and fungicides. Ecofriendly antifungal substances, including plant extracts, powder, and oil, have shown countless efficiencies to substitute synthetic fungicides because of their biodegradable nature, lack of toxicity, local availability, and low cost (Maswada et al., 2013). Plant product application for the control of plant pathogenic fungi is an instantly eye-catching vital component of the Integrated Disease Management (I.D.M.) scheme. The natural herbal products are environmentally friendly and biodegradable, and are receiving the attention of the researchers globally. These substances from higher flora are comparatively environmentally safe, feasible, bio-efficacious, and broad-spectrum (Sheema and Durai 2015).

Melia azedarach (M. azedarach) is one of the most valuable folk therapeutic plants (Neycee et al., 2012). It has various general name such as bakain, zarur, zanzalakht, umbrella tree, Indian lilac, Persian lilac, Barbados, Chinaberry, and margosa. This species originates from South China, Iran, Pakistan, and India; it has been broadly grown in Indonesia, Southern Europe, the Middle East, Australia, America, and East-Southern Africa (Jawad and Al Garaawi, 2022). The secondary metabolites of Persian lilac revealed the occurrence of glycosides, triterpens, teranor-triterpenoid, coumarin, phenol, cinnamic acid, benzoic acid, gum, tannins, resins, and alkaloids (Ahmed et al., 2008). The various parts extracts from M. azedarch were investigated as potent antifungal substances for certain plant pathogenic mould such as Sclerotinia sclerotiorum, Fusarium verticillioides, Fusarium solani, Fusarium oxysporum, Diaporthe phaseolorum and Aspergillus flavus (Carpinella et al 2003), while leaves antifungal activities against Ascochyta rabiei (Jabeen et al., 2011) and the methanol extracts exhibited antifungal properties against Rhizoctonia solani, Fusarium oxysporum, Sclerotium spp., and Trichoderma species (Neycee et al., 2012). So, keeping in view the above fact, the current study was designed to assessed that antifungal activities of aqueous and ethanol extracts of M. azedarach in vitro circumstances against soil-borne plant pathogenic fungi viz *Fusarium oxysporum, Alternaria alternata, Rhizoctonia solani, Macrophomina phaseolina and Sclerotium rolfsii*.

Materials and Methods

Plant Collection

The *M. azedarach* leaves were collected from the Medicinal Botanical Garden of Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Peshawar, Khyber Pakhtunkhwa, Pakistan. The plant parts were carefully rinsed with running tap H_2O and surface-decontaminated with Sodium hypochlorite solution (1%), meticulously rinsed with sterile H2O and kept in an air cabinet dryer (England) for drying at 40 °C. The dried materials were ground into powder with the help of Standard Model No.3 Wiley Mill, USA. The powder was packed in polyethylene bags and utilized according to the necessity of the investigational work to prepare ethanol and water extracts.

Extraction Preparation

The plant powder (10g) was weighed to produce the ethanol extracts, which were infused with 50g of ethanol (70%) and incubated for seven days in closed glass bottles with ground stoppers, with infrequent shaking (3 times per day). Subsequently, after seven days, the extracts were filtered and made up to ethanol (70%) to the original size formerly filtration. The water extracts were produced by transferring plant powder (2g) into boiling H_2O (100mL), allowing them to cool, and making up the volume with H2O to the initial volume of the previous filtration.

A Buchi Rotavapor R-200 (Switzerland) with a Buchi Heating Bath B-490 Rotary Evaporator was applied for the elimination of the water and ethanol solvents and the concentration of the extracts. The extracts, which were filter was shifted into a round-bottomed flask. The temperature of the water bath was 60 °C, and the flowing water temperature via the condenser was 20 °C. The vacuum was carefully chosen based on the necessity to boil at 40 °C; a vacuum required for H_2O was 72 millibars, while a vacuum needed for ethanol was 175 millibars. The rotation speed of the flask was 200 rotation/min. The procedure might be highly effectively enhanced through increasing the temperature of the water bath, increasing the flask rotation speed, and raising the vacuum; increasing the temperature should be avoided, as it might cause the breakdown of the heat-sensitive ingredients in the extracts. The procedure was stopped when the solvent in the round-bottomed flask vanished, and no more condensate dropped into the waste flask. Afterward, when the solvent evaporation was complete, the round-bottom flask was detached from the Rotary Evaporator, and in the flask the remaining concentrated crude extract was cautiously scraped and transferred using a sterilized spatula into pre-weighed disinfected glass vials. The extracts were kept in a cooled incubator (Gallenkamp- England) at 4 °C till further experiments.

Secondary Metabolites Screening (Qualitative)

The bioactive components in the extracts were determined according to the standard procedures and methods of Harborne (1998).

Soil-Borne Fungal Strains

The soil-borne fungal strains i.e., Fusarium oxysporum (F. oxysporum), Alternaria alternata (A. alternata), Rhizoctonia solani (R. solani), Macrophomina phaseolina (M. phaseolina), and Sclerotium rolfsii (S. rolfsii), were collected from the *Microbiology Laboratory Research Section of Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Peshawar, Khyber Pakhtunkhwa, Pakistan. These moulds* were maintained on potato dextrose agar (PDA) slants preceding experimental work.

Assessment of Antifungal Activity on Soil-Borne Fungi (Measuring Mycelial Growth).

To measure the inhibitory effects of the extracts on soil-borne moulds, a sterilized 100mL Potato Dextrose Broth (PDB) in a 250 mL conical flask was supplemented with numerous doses (50, 100, 200, and 300 mg/mL) of aqueous and ethanol extracts and was seeded with 6mm agar plugs from each pure, actively growing mould culture grown on PDA for 7 days. These flasks were kept in a shaking incubator (180 RPM) for 10 days at 25±2°C. After incubation, the mycelia were filtered and kept in an Electric Drying Oven (Model WOF-050, DAIHAN Scientific, South Korea) at 40°C to achieve a constant weight, and the dry biomass of each fungus was measured. The entire experiments were carried out in a germ-free and

sterile environment using a Laminar Air Flow Cabinet (Lahore, Pakistan). The flask without the plant extracts was used as a control. The inhibitory activities against each mould were measured as per the given formula:

Biomass Inhibition (%) = WC-WT/WC

Where: WT = mycelial dry weight with extract treatment; WC = mycelial dry weight on control (treatment without extract).

Statistical analysis

Each assay was performed in three replicates with a completely randomized design, and the mould biomass was stated as the mean ± standard error.

Results and discussion

Results

Phytochemical Analysis of *M. azedarach*

The qualitative secondary metabolites screening assessed the occurrence of various bioactive components in both fruits and leaves extracts of Chinaberry tree synthesized using aqueous and ethanol solvents (Table 1). The flavonoids, phenols, terpenoids, saponins, tannins, and alkaloids were noticed in all extracts, while their comparative abundance differed according to extraction solvents and plant parts. The ethanol leaf extract showed a maximum richness (+++) of flavonoids and phenols, whereas terpenoids, saponins, tannins, and alkaloids were detected in moderate quantities (++). Likewise, the water extract possessed all test secondary metabolites at moderate quantities (++), while flavonoids and phenols were also moderately detected. Among the fruit extracts, the ethanol extracts showed the highest secondary metabolites, with flavonoids, phenols, terpenoids, and alkaloids present in maximum quantity (+++), while saponins and tannins were found at a moderate degree (++). In contrast, the water fruit extract holds a moderate level of saponins, tannins, and alkaloids (++), whereas flavonoids, phenols, and terpenoids were found only at a small degree (+). Generally, ethanol was verified to be a highly efficient extraction solvent as compared with H_2O, producing greater levels of phytochemicals, predominantly alkaloids, terpenoids, flavonoids, and phenolic substances. The fruit ethanol extracts showed the highest abundance and diversity of secondary metabolites, proposing its capabilities as a rich source of bioactive substances responsible for the antifungal properties of *M. azedarach*.

Fungi Biomass Measurement Using *M. azedarach* Extract

The effect of fungal biomass production activities of *M. azedarach* leaf extracts was assessed against 5 soil-borne plant pathogenic moulds through determination of mycelial dry biomass production at various dosages (50–300 mg/mL), and matched with a control (Table 2). Generally, both aqueous and ethanol extracts documented a vibrant concentration-dependent reduction in mould biomass across all experimented moulds. The degree of suppression increased continuously with raising extract dose, with the highest inhibition recorded at 300 mg/mL. Among the tested moulds, *F. oxysporum* observed 820 ± 0.3 mg biomass in the control, which was meaningfully reduced to 650 ± 0.1 mg and 620 ± 0.5 mg for aqueous and ethanol extracts, respectively, at 50mg/mL. At the maximum dose (300mg/mL), biomass was additionally reduced to 130 ± 0.5 mg (aqueous) and 100 ± 0.3 mg (ethanol), representing powerful anti-biomass production. Correspondingly, at 300mg/mL *A. alternata* exhibited a decrease from 680 ± 0.2 mg (control) to 140 ± 0.5 mg (aqueous) and 100 ± 0.2 mg (ethanol). While in the case of *R. solani*, biomass was reduced distinctly from 950 ± 0.4 mg to 180 ± 0.7 mg (aqueous) and 150 ± 0.5 mg (ethanol) at the maximum dose. Whereas the *M. phaseolina* biomass at 300mg/mL was decrease from 880 ± 0.4 mg (control) to 210 ± 0.5 mg (aqueous) and 175 ± 0.2 mg (ethanol). The maximum primary biomass was displayed in S. rolfsii (1120 ± 0.5 mg), which also observed considerable inhibition at 300mg/mL, attaining 320 ± 0.3 mg (aqueous) and 260 ± 0.4 mg (ethanol). Relatively, ethanol extracts steadily showed powerful antifungal activities as compared with water extracts across all fungal species and doses, proposing that the ethanol extracts are highly effective in isolating bioactive fungicidal substances. Among the experimental moulds, *A. alternata* and *F. oxysporum* were reasonably highly susceptible; however, *S. rolfsii* showed comparatively maximum resistance to both extracts.

Effect of *M. azedarach* Fruit Extract on Mould Biomass Production

The *M. azedarach* fruit extracts' antifungal activities were assessed against 5 soil-borne moulds at doses ranging from 50–300 mg/mL (Table 3). Both water and ethanol extracts considerably minimized mould biomass in a dose-dependent way. Moderate inhibition was observed at 50 mg/mL, although mycelial biomass was reduced decidedly at 100–200 mg/mL. Among the experimental moulds, *A. Alternaria* and *F. oxysporum* were highly vulnerable, while *S. rolfsii* and *R. solani* were relatively highly resistant. The maximum suppression was noted at 300mg/mL, where the ethanol extract minimized the *A. alternata* and *F. oxysporum* biomass to110 ± 0.5 mg, linked with 190 ± 0.6 mg and 170 ± 0.5 mg, respectively, for the water extracts. The least sensitive mould was S. rolfsii, achieving the maximum biomass at this dose. Generally, ethanol extract of fruits holds maximum antifungal activities as compared with water extracts across all treated concentrations and moulds. These findings documented the powerful concentration-dependent anti-mould activities of Chinaberry tree fruit extracts with efficiencies influenced through

Fungal species and solvent type

Biomass Inhibition (%) of Mould Pathogens via Leaves Ethanolic Extract

The biomass inhibition % of 5 soil-borne plant pathogenic moulds treated with *M. azedarach*

ethanolic leaf extracts of ethanol leaves is displayed in Figure 1. Moderate inhibition was measured, ranging from 11% to 24% at the lowest dose (50mg/mL). The highest vulnerability was exhibited by F. oxysporum at this dose (24%), followed by A. alternata (21%), whereas *S. rolfsii* recorded the minimum suppression (11%). With raising extract doses, a constant increase in mycelial suppression was noted. Biomass inhibition was 24%, 27%, 34%, 40%, and 48% against *S. rolfsii, M. phaseolina, R. solani, A. alternata,* and *F. oxysporum,* respectively, at 100mg/mL. This tendency was additionally strengthened at 200mg/mL, where suppression surpassed 60% for wholly mould, with S. rolfsii reaching 60% and F. oxysporum reaching 74%. The maximum antifungal activities were documented at 300mg/mL, where mycelial suppression ranged from 77% to 88%. *F. oxysporum* presented the highest suppression (88%), followed closely by *R. solani* and *A. alternata, at* 84% and 85%, respectively, while *S. rolfsii* persisted relatively less vulnerable (77%).

Biomass Inhibition (%) of Fungal Pathogens by Aqueous Leaf Extract

The biomass inhibition (%) of 5 soil-borne plant pathogenic moulds treated with water leaf extracts of *M. azedarach* is depicted in Figure 2. At 50mg/mL dosage, comparatively minimal to moderate suppression was measured, ranging from 8% to 21%. *F. oxysporum* displayed the maximum suppression (21%), tracked through A. alternata (61%), whereas S. rolfsii revealed the minimum vulnerability (8%). AT 100mg/mL, suppression augmented to 41%, 34%, 31%, 23%, and 21% for *F. oxysporum, A. alternata, R. solani, M. phaseolina,* and *S. rolfsii,* respectively. At 200mg/mL dose, suppression surpassed 60% for most moulds, with numbers ranging from 56% (*S. rolfsii)* to 71% (*F. oxysporum*). The highest biomass inhibition was measured at 300 mg/mL, where antifungal activities reached 71%, 76%, 79%, 81%, and 84% for *S. rolfsii, M. phaseolina, A. alternata, R. solani,* and *F. oxysporum,* respectively.

Biomass Inhibition (%) of Mould Pathogens via Fruit Ethanolic Extract

The fruit ethanolic antifungal activities of *M. azedarch* against 5 soil-borne plant pathogenic moulds are shown in Figure 3. At 50mg/mL concentration, modest suppression was recorded, ranging from 9% to 23%; *F. oxysporum* unveiled the maximum vulnerability (23%), trailed by *A. alternata* (19%), whereas *S. rolfsii* presented the lowest inhibition (9%). With growing doses, a noticeable improvement in biomass inhibition was observed. At 100mg/mL concentration, suppression level raised to 46%, 38%, 33%, 25%, and 23% for *F. oxysporum, A. alternata, R. solani, M. phaseolina,* and *S. rolfsii,* respectively. At 200mg/mL concentration, every mould displayed extensive suppression, ranging from 58% to 73%, with *F. oxysporum* again observing the highest response. The highest biomass inhibition (%) was recorded at 300mg/mL, where antifungal activities touched 76%, 78%, 83%, 84%, and 87% against *S. rolfsii*, *M. phaseolina*, *R. solani, A. alternata*, and *F. oxysporum*, respectively.

Biomass Inhibition (%) of Mould Pathogens via Fruit Aqueous Extract

The biomass inhibition (%) of water extracts of *M. azedarach against 5 soil-borne plant pathogenic moulds is presented in Figure 4. At 50 mg/mL concentration, lower to modest suppression was determined, ranging from 4% to 16%. F. oxysporum observed the maximum suppression (16%), followed* by *A. alternata (10%), whereas S. rolfsii observed the minimum vulnerability (4%). At 100mg/mL concentration, suppression values augmented to 18%, 19%, 25%, 28%, and 37% for S. rolfsii, M. phaseolina, R. solani, A. alternata, and F. oxysporum, respectively. At 200mg/mL concentration, the suppression surpassed 50% for most moulds, excluding S. rolfsii, with numbers* ranging *from 47% to 66%. The maximum biomass inhibition (%) was measured at 300mg/mL, where antifungal activities touched 60%, 64%, 69%, 72% and 79% for S. rolfsii, M. phaseolina, R. solani, A. alternata and F. oxysporum, respectively.*

The M. azedarach all extracts showed dose-dependent biomass inhibition (%) against the experimental moulds. The ethanol extracts exhibited significant efficiency as compared with water extracts, showing that the ethanol extracts were more active in isolating anti-mould components. Amongst the moulds, F. oxysporum was commonly the most vulnerable, while S. rolfsii showed the maximum resistance. Generally, fruit and leaf extracts, mainly the ethanol extracts, exhibited significant powerful wide-range biomass inhibition, prominence their appropriateness as non-synthetic biofungicides for controlling soil-borne mould pathogens.

Table 1. Phytochemicals Screening of *M. azedarach*.

Plant Parts	Bioactive Agents	Ethanol	Aqueous
Leaves	Alkaloids	++	++
	Tannins	++	++
	Saponnins	++	++
	Terpenoids	++	++
	Phenols	+++	++
	Flavonoids	+++	++
Fruit	Alkaloids	+++	++
	Tannins	++	++

	Saponnins	++	++
	Terpenoids	+++	+
	Phenols	+++	+
	Flavonoids	+++	+

+++ = highly present, ++ = moderately present, + = low present, – = absent.

Table 2. Effect of *M. azedarach* Leaves Extract on Biomass of Fungi

Fungal Species	Control Biomass (mg)	Extracts	Extracts Concentration (mg/mL)			
			50	100	200	300
F. oxysporum	820 ± 0.3	Ethanol	620±0.5	430± 0.1	210±0.5	100±0.3
		Aqueous	650±01	480±0.5	240±0.5	130±0.5
A. alternata	680 ± 0.2	Ethanol	540±0.1	410±0.5	200±0.8	100±0.2
		Aqueous	570±02	450±0.7	230±0.6	140±0.5
R. solani	950 ± 0.4	Ethanol	790±0.4	630±0.2	330±0.4	150±0.5
		Aqueous	820±01	660±0.8	360±01	180±0.7
M. phaseolina	880 ± 0.4	Ethanol	750±0.6	640±0.4	320±0.2	175±0.2
		Aqueous	790±02	680±0.5	345±02	210±0.5
S. rolfsii	1120 ± 0.5	Ethanol	995±0.1	850±0.2	450±0.1	260±0.4
		Aqueous	1030±01	880±0.4	490±0.7	320±0.3

Values represent the dry mycelial biomass (mg) of fungi. Control cultures received no extract treatment. Data are expressed as mean ± standard error.

Table 3. Effect of *M. azedarach* Fruits Extract on Biomass of Soil-Borne Plant Pathogenic Fungi

Fungal Species	Control Biomass (mg)	Extracts	Extracts Concentration (mg/mL)			
			50	100	200	300
F. oxysporum	820 ± 0.3	Ethanol	630±01	440± 02	220±0.5	110±0.5
		Aqueous	690±01	520±01	280±0.5	170±0.5
A. alternata	680 ± 0.2	Ethanol	550±01	420± 01	210±0.5	110±0.6
		Aqueous	610±02	490±01	280±0.8	190±0.6
R. solani	950 ± 0.4	Ethanol	800±02	640±01	340±0.7	160±0.4
		Aqueous	870±01	710±02	410±0.9	290±0.6
M. phaseolina	880 ± 0.4	Ethanol	770±01	660± 02	325±0.5	190±0.6
		Aqueous	840±01	710±02	390±0.2	320±0.4
S. rolfsii	1120 ± 0.5	Ethanol	1020±02	860± 02	470±0.8	270±0.5
		Aqueous	1070±02	920±01	590±0.5	450±0.6

Values represent the dry mycelial biomass (mg) of fungi. Control cultures received no extract treatment. Data are expressed as mean ± standard error.

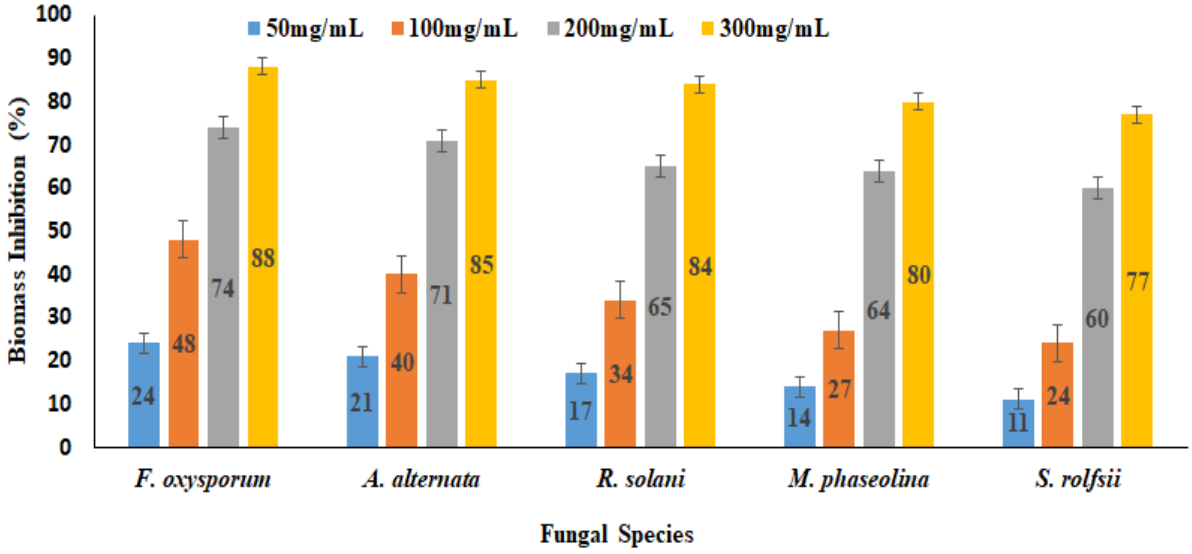

Figure 1. Biomass Inhibition (%) of Fungi Using Leaves Ethanolic Extracts of *M. azedarach*

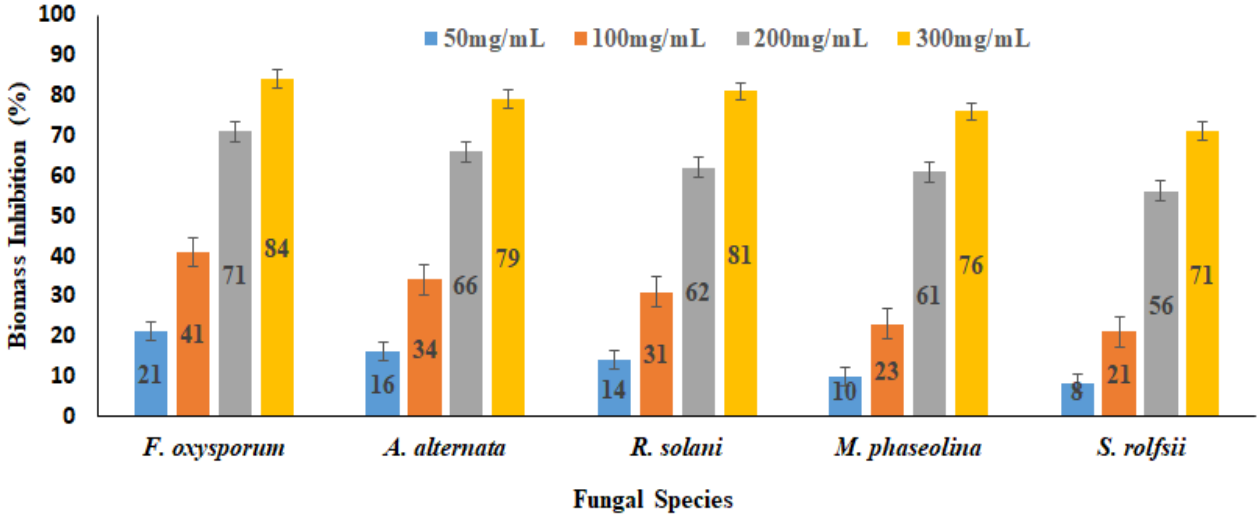

Figure 2. Biomass Inhibition (%) of Fungi Using Leaves Aqueous Extracts of *M. azedarach*

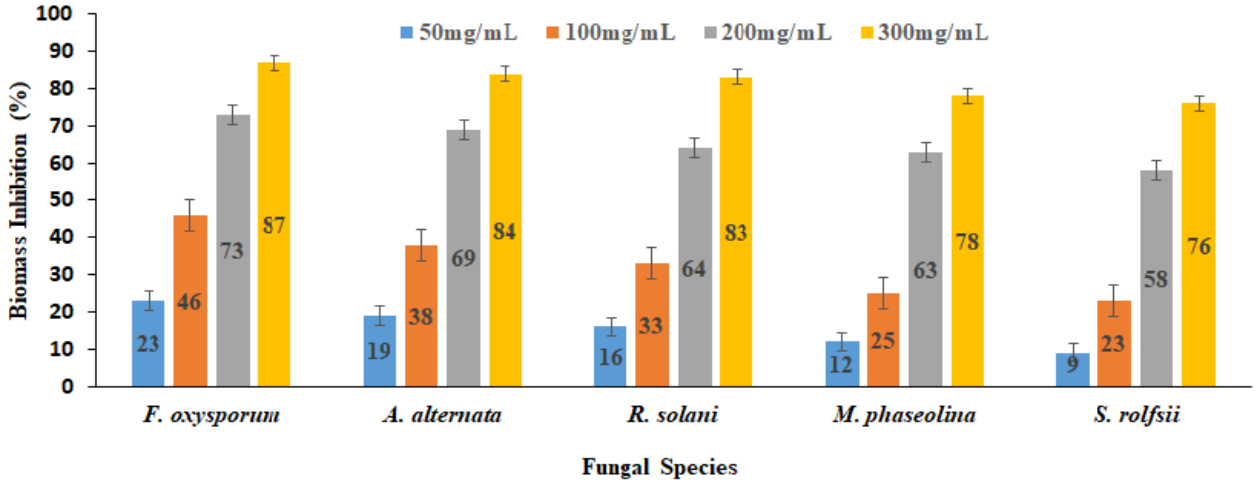

Figure 3. Biomass Inhibition (%) of Fungi Using Fruits Ethanolic Extracts of *M. azedarach*

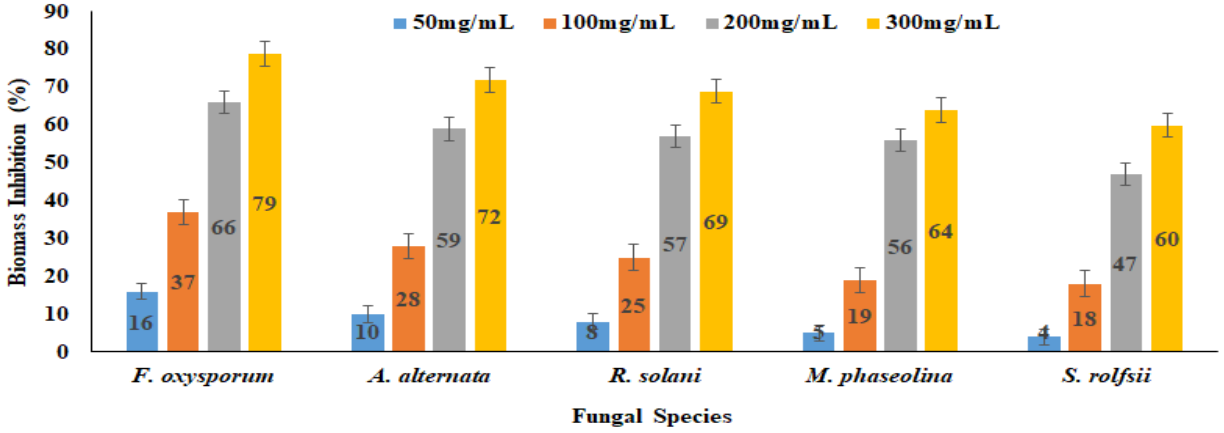

Figure 4. Biomass Inhibition (%) of Fungi Using Fruits Aqueous Extracts of *M. azedarach*

Discussion

Phytochemical Profile and Its Antifungal Basis

The current study documented that *M. azedarach* holds a wide spectrum of bioactive components such as phenolic compounds, terpenoids, saponins, tannins, flavonoids, and alkaloids, with relatively maximum levels in ethanol extracts as compared with water extracts (Table 1). Likewise, outcomes have been documented before, where Chinaberry tree was observed to be rich in coumarin derivatives, vanillin and scopoletin, which are strongly linked with fungicidal properties (Carpinella et al., 2005). Similarly, alkaloids, cardiac glycosides, tannins, saponins, and flavonoids have been present in considerable amounts in *M. azedarach* leaves (Shumirai et al., 2020; Kaushik et al., 2018). Flavonoids like kaempferol and quercetin are well reported for their antimicrobial activities and their

capabilities to hinder membrane integrity and microbial enzyme systems (Sultana et al., 2018). In addition, various phytochemicals such as β-sitosterol derivatives, quercitrin, and rutin have been originated form Chinaberry tree, various of which hold antifungal and antimicrobial activities (Khan et al., 2008; Jabeen et al., 2011). The maximum levels of flavonoids and phenolics in ethanol extracts, as shown in the current study are probably attributed to their significant antifungal activities. Saponins, in specific are reported to play a vital job in herbs' protection against mould pathogens because of their capabilities to disrupt mould cell membranes (Osbourn, 1996). Steroidal saponins have also been documented to deliver defence against a comprehensive spectrum of pathogenic fungi of plants (Sprag et al., 2004; Chong-Ren et al., 2006). Likewise, phenolic acids (syringic acid, chlorogenic acid and gallic acid) and tannins have documented powerful antibacterial and antifungal activities against Candida, Aspergillus and Fusarium species (Alves et al., 2014; Nguyen et al., 2015; Suarez-Quiroz et al., 2013). Consequently, the synergistic properties of these secondary metabolites are probably responsible for the recorded biomass inhibition effects in the current study.

Plant Extracts Antifungal Potential

Soil-borne mould pathogens signify a main constraint to agricultural yield globally. Amongst these, R. solani is a damaging pathogen creating stem canker, root rot, damping-off, seed decay and other diseases in a broad range of horticultural crops (Farr et al., 1989; Parameter, 1970). The pathogen is chiefly challenging in rigorous farming systems because of its resistance to conventional management procedures and persistence in soil, subsequent in considerable financial losses and a thoughtful danger to sustainable food security and crop production (Shahzaman et al., 2015). Similarly, *F. oxysporum* is one of the most extensive and financially significant soil-borne mould pathogens producing vascular wilt and root rot diseases under both field and greenhouse circumstances (Hartman and Fletcher, 1991). Correspondingly, the causative agent of charcoal rot is *M. phaseolina*, which contaminates more than five hundred floral species belonging to about a hundred families, comprising various financially imperative crops such as fiber crops, fruits, vegetables, sunflower, maize and soybean
(Iqbal et al., 2014; Iqbal and Mukhtar, 2020b; Ghosh et al., 2018). Under heat stress and drought circumstances, production losses created through this mould might attain up to fifty percent (Iqbal et al., 2014). In addition, A. alternata produces fruit rots, seedling blights, and leaf spots in various horticulture and field crops, upsetting both crop post-harvest quality and crop productivity (Bibi et al., 2023). An additional significant soil-borne pathogen, *S. rolfsii*, causes blight disease and holds a remarkably wide host spectrum exceeding five hundred plant species. The mould lives in soil for various decades by the creation of sclerotia, which act as hibernating structures and initial seed bases.
(Hegde et al., 2010; Iqra and Javid, 2013). Amongst the treated pathogens, *A. alternata* and *F. oxysporum* were commonly highly vulnerable to both fruit and leaf extracts, whereas S. rolfsii observed comparatively more resistance. This difference in vulnerabilities might be linked to variances in the fungal spores' survival strategies, enzymatic detoxification mechanisms, and cell wall composition. The findings of the current study reported significant antifungal activities of *M. azedarch* extracts against the treated plant pathogenic fungi. Similar results have been documented by numerous investigators. Khajista et al. (2014) documented that methanol leaf extracts of Chinaberry tree considerably inhibited the A. solani growth, with a 5% extract dosage minimizing mould growth by up to 90%. Similarly, aqueous leaf extracts revealed considerable inhibition of *A. solani*, creating radial biomass development decreases of 73.18%, 68.72% and 64.08% at doses of 20%, 15% and 10%, respectively (Asma et al., 2020). The *M. azedarach* antifungal activities against Fusarium species have also been widely reported. Akacha et al. (2016a) described that leaf extracts inhibited biomass growth of *F. oxysporum* f. sp. *lycopersici, F. sambucinum and F. oxysporum* f. sp. *melonis.* The reported antifungal activities were attributed to the occurrence of flavonoids and phenolic compounds found in the extracts (Akacha et al., 2016a; Akacha et al., 2016b). Moreover, Neycee et al. (2012) confirmed antifungal activities of fruit-seed, flower and leaves extracts of *M. azedarach* against a species of *Trichoderma, Sclerotium, Geotrichum, R. solani* and *F. oxysporum* at doses of 200, 150 and 75 mg/mL. The results of the current study are also verified by Tuba et al. (2016), who described that fruit methanol extracts of *M. azedarach induc*ed 81.99%, 49.97% and 100% mycelial inhibition of *M. phaseolina, F. oxysporum* and *R. solani*, respectively. Likewise, Iffat et al. (2014) reported variable levels of antifungal activities among fruit, stem-bark and leaves extracts of *M. azedarach* against *R. solani.* The maximum efficiencies were observed with aqueous fruit extracts, methanol stem-bark extracts and chloroform leaves extracts, followed by biomass decreases ranging from 28–70%, 4–85% and 20–89%, respectively.
The antifungal activities of *M. azedarach* against *S. rolfsii* have also been documented before. The fruit methanol extracts and their fractions considerably minimized mould biomass at a concentration of 3.125mg/mL was 41–65% (Iqra and Javid, 2013). Similarly, Nighat et al. (2017) documented the fungicidal activities of leaves methanol extracts against S. rolfsii. These results verify the conclusion of Iqra and Arshad (2013), who described that the fruit methanol extracts and their portions hold significant antifungal activities against this mould. The wide

range antifungal activities reported in the current study agree with earlier research describing the efficiency of *M. azedarach* extracts against a broad range of mould pathogens. Carpinella et al. (2003) reported antifungal activities against *Sclerotinia sclerotiorum*, *F. verticillioides*, *F. solani*, *Fusarium oxysporum*, *Diaporthe phaseolorum* var. *meridionales* and *Aspergillus flavus.* Likewise, Sen and Batra (2012) described the antifungal activities of aqueous leaves, petroleum ether, ethanol and methanol leaf extracts against *Rhizopus stolonifer*, *F. oxysporum*, *A. flavus* and *Aspergillus niger*. Jabeen et al. (2011) similarly confirmed the *M. azedarach* antifungal activities against *Ascochyta rabiei*. In addition, Arshad and Hafiza (2011) detected noteworthy biomass reductions of *M. phaseolina* when treated with ethyl acetate, chloroform and methanol leaf extracts of *M. azedarach*. Extracts of chloroform were found to be most efficient, producing biomass reductions of 57–78%, whereas ethyl acetate and methanol extracts minimized biomass by 56–75% and 38–60%, respectively. Additionally, Seepe et al. (2021) documented powerful antifungal activities of leaf extracts of *M. azedarach* against *F. proliferatum* on the seeds of maize and spotlighted the efficiency of herbal-originated fungicidal components for disease control without creating phytotoxic effects.

While further research verified the fungicidal activity of *M. azedarch*, differences in activities have been documented. Neycee et al. (2012) detected relatively inferior activities of leaf extracts against *R. solani*, *F. oxysporum*, and *Sclerotium* species under sure experimental circumstances. Such inconsistencies could stand up in phytochemicals composition, fungal isolates, solvent system, extraction techniques, environmental circumstances, and plant origin. Earlier research has reported significant dissimilarities in the chemical components of *M. azedarach* procured from various topographical areas, which might considerably affect antimicrobial activities (Gottlieb et al., 2001; Szewczuk et al., 2003; Orhan et al., 2012). The *M. azedarach* antifungal activities are mainly attributed to its varied range of bioactive phytochemicals. Limonoids, which comprise the main ingredients of the fruits, have been documented as effective fungicidal ingredients and are principally responsible for the suppression of mould development (Roy and Saraf, 2006; Carpinella et al., 2005). Regardless of these differences, the general tendency reliably verifies the antifungal activities of *M. azedarach*. The rising antifungal activities with doses propose a concentration-dependent buildup of bioactive components at the mould interface, causing to boosted mould growth processes disruption. The ethanol extracts were steadily more active compared with aqueous extracts, which might be attributed to the good solubility of limonoids, flavonoids, and phenolic compounds in organic solvents.

C**onclusion**

The current study reported that *M. azedarch* holds considerable biomass inhibition against soil-borne plant pathogenic fungi. The phytochemical qualitative screening verified the occurrence of main bioactive components, such as terpenoids, saponins, tannins, flavonoids, phenols and alkaloids, with maximum degree commonly documented in ethanol extracts as compared with water extracts. The assays of biomass inhibition (%) showed vibrant dose-dependent antifungal activity of both fruit and leaf extracts. Amongst all experiments, ethanol extracts of leaves and fruits showed the maximum degree of activity, obtaining the highest level of inhibition at 300mg/mL against all tested moulds. In contrast, water extracts showed relatively inferior but still considerable biomass inhibition effects. Generally, A. alternata and F. oxysporum were reported to be more vulnerable, while *S. rolfsii* was steadily observed to be more resistant to all treatments. The heightened biomass inhibition of ethanol extracts might be attributed to their maximum efficacy in extracting phenolic substances and other secondary metabolites accountable for antifungal activities. In conclusion, *M. azedarch* signifies a competent source of natural fungicide components and might be further explored for the formulation of environment-friendly bio-fungicides. Future research should focus on the extraction of bioactive components on an industrial scale and assessment under field circumstances to authenticate their practical application in the management of soil-borne disease.

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