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***In vitro* antifungal activity of *Cassia fistula* leaf extracts against major phytopathogenic fungi**

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Abstract

Fungal phytopathogens are responsible for substantial crop losses worldwide, and continued reliance on synthetic fungicides has raised concerns regarding resistance development and environmental safety. This study evaluated the *in vitro* antifungal activity of aqueous, methanolic, and acetone leaf extracts of *Cassia fistula* against four economically important phytopathogenic fungi (*Alternaria*, *Cercospora*, *Colletotrichum*, and *Fusarium*). Antifungal efficacy was assessed using the poisoned food technique on potato dextrose agar, and mycelial growth inhibition was recorded at 3 and 5 days after inoculation (DAI). All extracts inhibited fungal growth in a concentration-dependent manner, with methanolic extract showing the highest inhibitory activity, followed by acetone and aqueous extracts. Inhibition increased with incubation time and was generally greater at 5 DAI. *Alternaria* and *Cercospora* were more susceptible than *Fusarium*. These findings demonstrate that *C. fistula* leaf extracts possess significant antifungal potential and support their further evaluation as botanical fungicides for sustainable management of fungal plant diseases.

Keywords: *Cassia fistula*, antifungal activity, phytopathogenic fungi, poisoned food technique, botanical fungicide

Introduction

Fungal plant pathogens represent a major constraint to global agricultural productivity, causing extensive yield and quality losses. Species of *Alternaria*, *Cercospora*, *Colletotrichum*, and *Fusarium* are widely distributed and responsible for destructive foliar diseases, blights, anthracnose, and wilts in numerous crops [1-3]. Although chemical fungicides remain the primary control strategy, their prolonged use has resulted in resistance development, environmental contamination, and negative impacts on non-target organisms [4, 5].

The search for sustainable alternatives has intensified interest in plant-derived antifungal agents. Botanical extracts are biodegradable, generally less toxic, and often exhibit multi-target modes of action, reducing the likelihood of resistance [6-8]. Several medicinal plants traditionally used for treating microbial infections have demonstrated promising antifungal activity against plant pathogens [6, 9].

Cassia fistula L. (Fabaceae) is a widely distributed medicinal plant with reported antimicrobial properties [10, 11]. However, systematic evaluation of its antifungal efficacy against major phytopathogenic fungi relevant to agriculture remains limited. The present study therefore aimed to evaluate the *in vitro* antifungal activity of aqueous, methanolic, and acetone extracts of *C. fistula* leaves against four important phytopathogenic fungi, with emphasis on solvent-dependent efficacy, concentration response, and incubation time effects.

2. Materials and Methods

2.1 Preparation of leaf extracts: Fresh leaves of *Cassia fistula* were collected, shade-dried, and powdered. Aqueous, methanolic, and acetone extracts were prepared separately. Organic solvent extractions were performed using Soxhlet apparatus, while aqueous extraction was carried out by maceration. Concentrated extracts were dried and stored at 4 °C until use.

2.2 Fungal pathogens: Pure cultures of *Alternaria* spp., *Cercospora* spp., *Colletotrichum* spp., and *Fusarium* spp. were isolated from infected plant tissues and maintained on potato dextrose agar (PDA). Pathogens were identified based on colony morphology and microscopic characteristics.

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2.3 Antifungal assay: Antifungal activity was evaluated using the poisoned food technique. Extracts were incorporated into molten PDA at different concentrations and poured into Petri plates. Control plates contained PDA

without extract. Mycelial discs were placed centrally, and plates were incubated at 25 ± 2 °C. Radial mycelial growth was measured at 3 and 5 days after inoculation (DAI). Percentage inhibition was calculated relative to control.

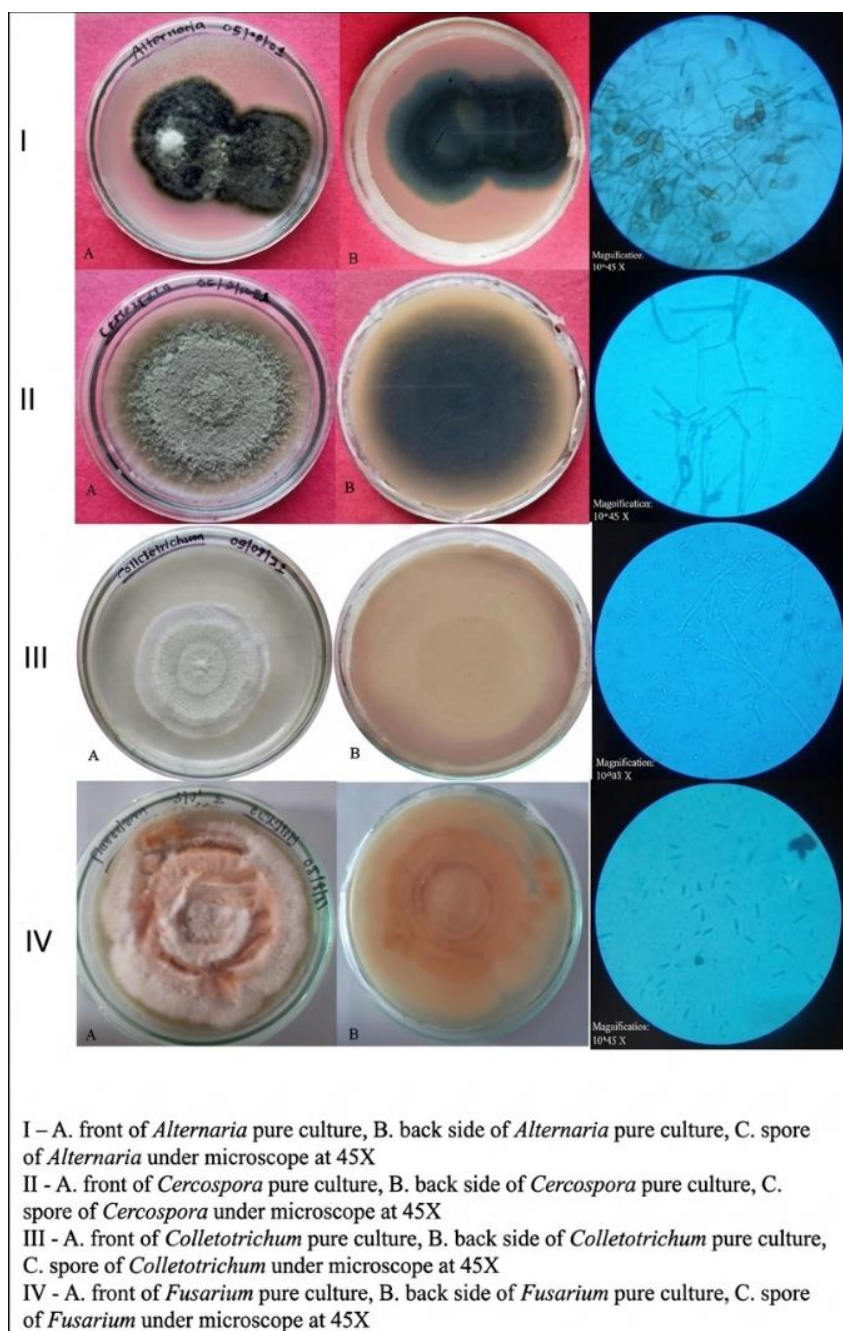


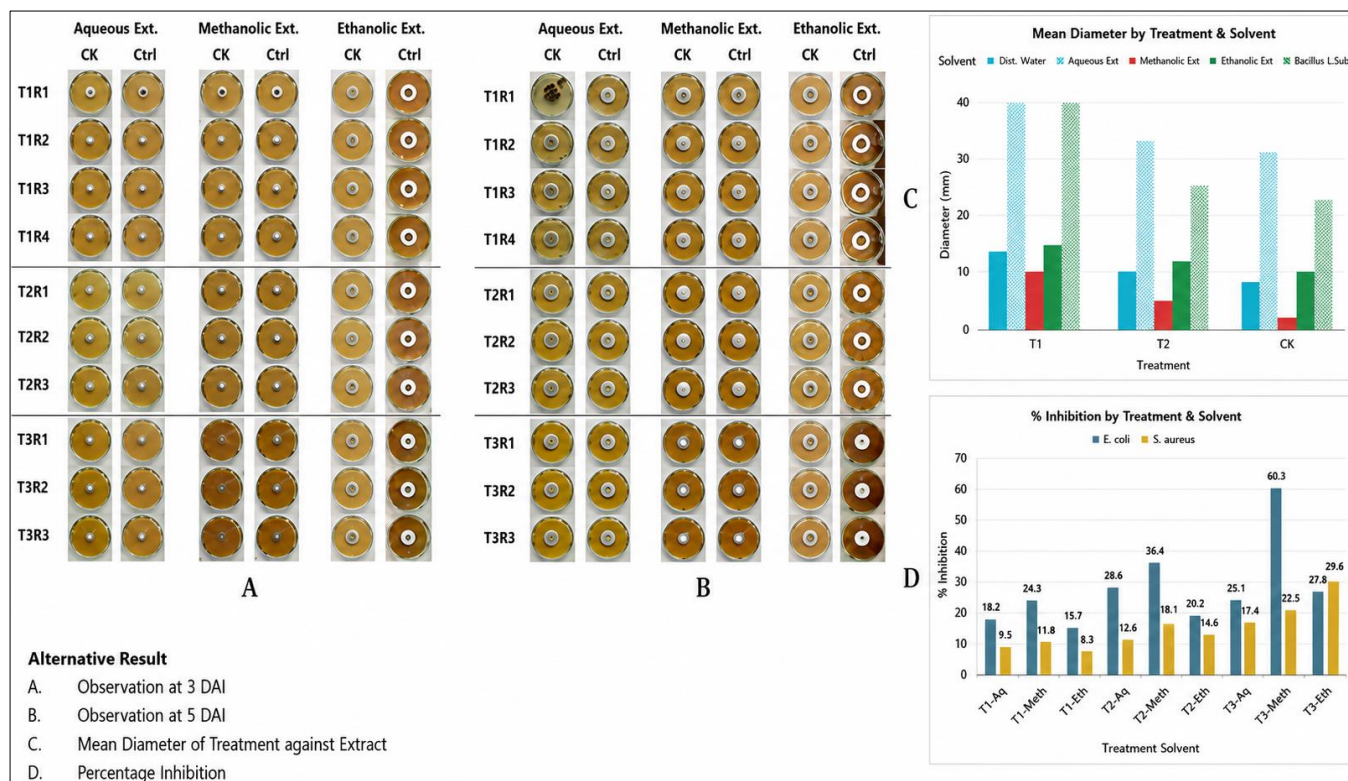
Plate 1: Pure cultures of phytopathogens, *Alternaria*, *Cercospora*, *Colletotrichum* and *Fusarium*

2.4 Statistical analysis: Experiments were conducted in triplicate. Data were analyzed using ANOVA, and treatment means were compared at $p < 0.05$.

3. Results

All *Cassia fistula* leaf extracts significantly inhibited mycelial growth of the tested fungi compared to control.

Antifungal activity varied with solvent, concentration, fungal species, and incubation period. Observed data of *Alternaria*, *Cercospora*, *Colletotrichum* and *Fusarium*, respectively, are presented in Plates 2-5. Mean diameters of colonial growth and percentage inhibition and corresponding ANOVA are presented in Tables 1-8.

Plate 2: Observation data of *Alternaria*Table 1: Mean Colony Diameter and Percentage Inhibition (*Alternaria*)

Solvent	Treatment	Mean Diameter (mm), 3 DAI	Mean Diameter (mm), 5 DAI	Percentage Inhibition 3 DAI (%)	Percentage Inhibition 5 DAI (%)
Aqueous	T ₁	11.00	25.88	30.47	13.75
Aqueous	T ₂	9.73	22.60	38.95	24.67
Aqueous	T ₃	8.58	21.20	46.35	29.33
Methanolic	T ₁	9.82	25.88	38.64	13.75
Methanolic	T ₂	6.78	22.60	57.61	24.67
Methanolic	T ₃	1.00	21.20	93.75	29.33
Acetone	T ₁	12.13	25.97	24.22	13.44
Acetone	T ₂	10.77	18.50	32.71	38.33
Acetone	T ₃	9.58	16.40	40.10	45.33

Table 2: ANOVA Table -Mean Colony Diameter for *Alternaria*

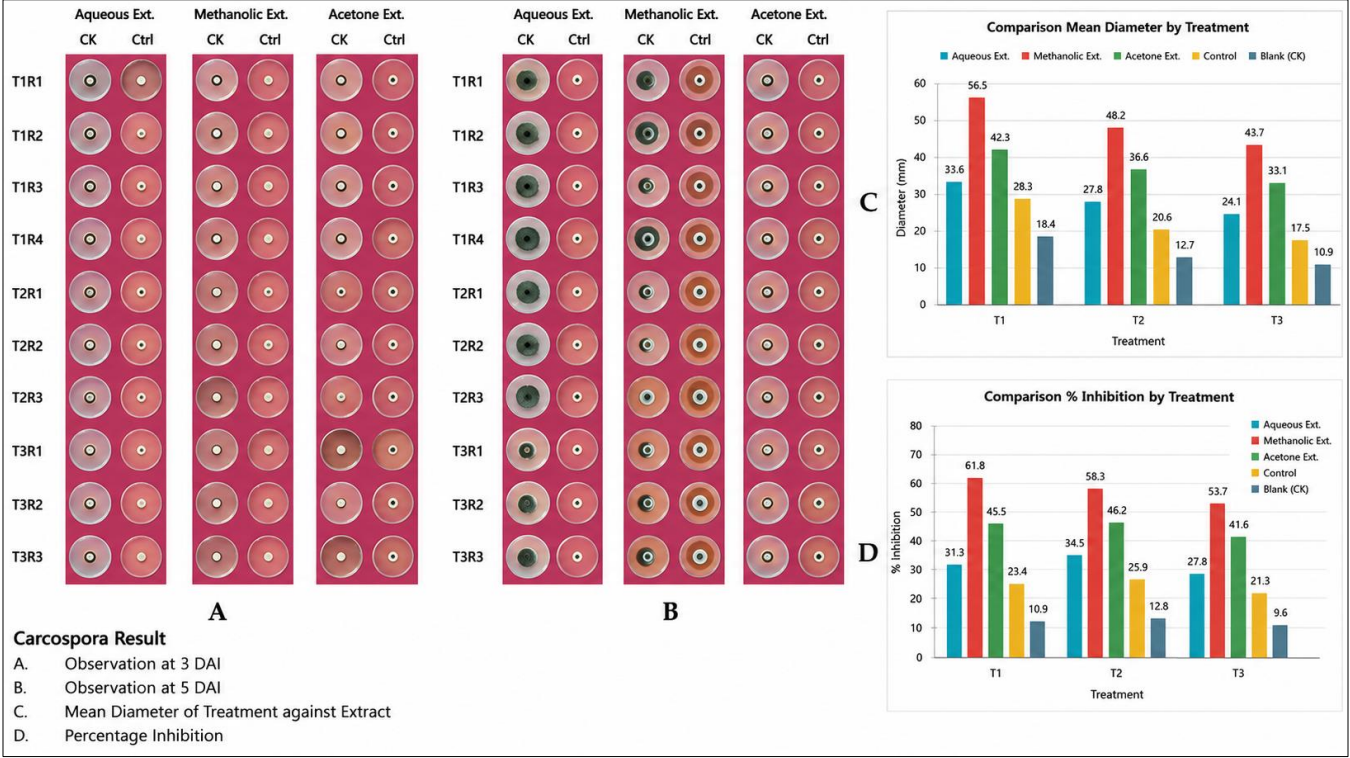
Solvent	Day	F-statistic	p-value
Aqueous	3 DAI	197.96	<0.001
Aqueous	5 DAI	194.14	<0.001
Methanolic	3 DAI	19698.82	<0.001
Methanolic	5 DAI	194.14	<0.001
Acetone	3 DAI	197.96	<0.001
Acetone	5 DAI	1739.03	<0.001

Table 3: Mean Colony Diameter and Percentage Inhibition (*Cercospora*)

Solvent	Treatment	Mean Diameter (mm), 3 DAI	Mean Diameter (mm), 5 DAI	Percentage Inhibition 3 DAI (%)	Percentage Inhibition 5 DAI (%)
Aqueous	T ₁	23.25	43.08	3.13	4.26
Aqueous	T ₂	20.15	41.4	16.04	8
Aqueous	T ₃	16.45	34.95	31.79	22.33
Methanolic	T ₁	21.18	43.1	11.74	4.22
Methanolic	T ₂	18.5	35.75	22.92	20.56
Methanolic	T ₃	12.5	32.5	47.92	27.78
Acetone	T ₁	16.6	31.68	30.83	29.59
Acetone	T ₂	13.9	28.9	42.08	35.78
Acetone	T ₃	7.36	22.28	69.31	50.48

Table 4: ANOVA Table -Mean Colony Diameter for *Cercospora*

Extract	Day	F-statistic	p-value
Aqueous	3 DAI	1303.88	<0.001
Aqueous	5 DAI	240.99	<0.001
Methanolic	3 DAI	864.3	<0.001
Methanolic	5 DAI	2166.67	<0.001
Acetone	3 DAI	918.54	<0.001
Acetone	5 DAI	1027.74	<0.001



Comparison Mean Diameter by Treatment

Diameter (mm)

Treatment

Aqueous Ext.

Methanolic Ext.

Acetone Ext.

Control

Blank (CK)

Treatment	Aqueous Ext.	Methanolic Ext.	Acetone Ext.	Control	Blank (CK)
T1	33.6	56.5	42.3	28.3	18.4
T2	27.8	48.2	36.6	20.6	12.7
T3	24.1	43.7	33.1	17.5	10.9

Comparison % Inhibition by Treatment

% Inhibition

Treatment

Aqueous Ext.

Methanolic Ext.

Acetone Ext.

Control

Blank (CK)

Treatment	Aqueous Ext.	Methanolic Ext.	Acetone Ext.	Control	Blank (CK)
T1	31.3	61.8	45.5	23.4	10.9
T2	34.5	58.3	46.2	25.9	12.8
T3	27.8	53.7	41.6	21.3	9.6

Carcospora Result

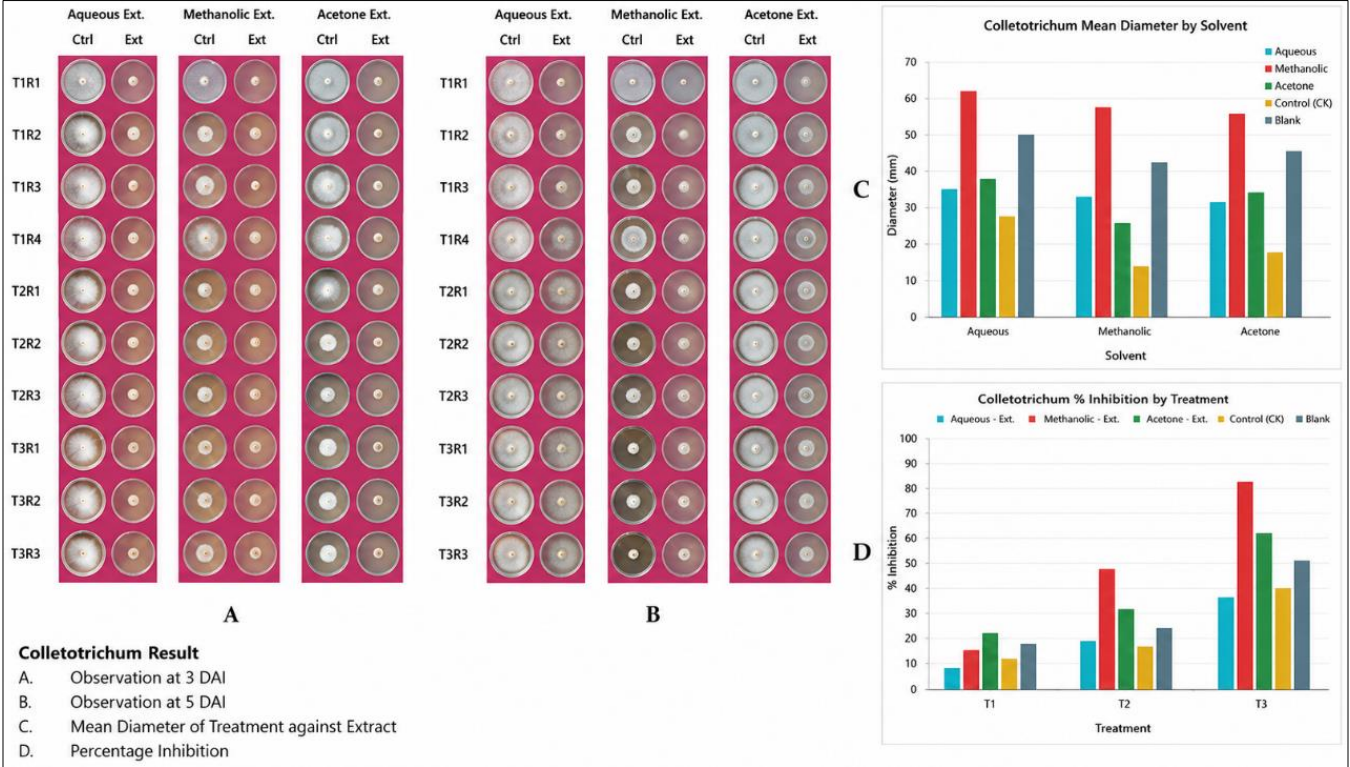
A. Observation at 3 DAI

B. Observation at 5 DAI

C. Mean Diameter of Treatment against Extract

D. Percentage Inhibition

Plate 3: Observation data of *Cercospora*



Colletotrichum Mean Diameter by Solvent

Diameter (mm)

Solvent

Aqueous

Methanolic

Acetone

Control (CK)

Blank

Solvent	Aqueous	Methanolic	Acetone	Control (CK)	Blank
Aqueous	35	62	38	28	50
Methanolic	32	58	25	15	42
Acetone	31	55	34	18	45

Colletotrichum % Inhibition by Treatment

% Inhibition

Treatment

Aqueous - Ext.

Methanolic - Ext.

Acetone - Ext.

Control (CK)

Blank

Treatment	Aqueous - Ext.	Methanolic - Ext.	Acetone - Ext.	Control (CK)	Blank
T1	10	15	22	12	20
T2	20	48	32	18	25
T3	38	82	62	40	50

Colletotrichum Result

A. Observation at 3 DAI

B. Observation at 5 DAI

C. Mean Diameter of Treatment against Extract

D. Percentage Inhibition

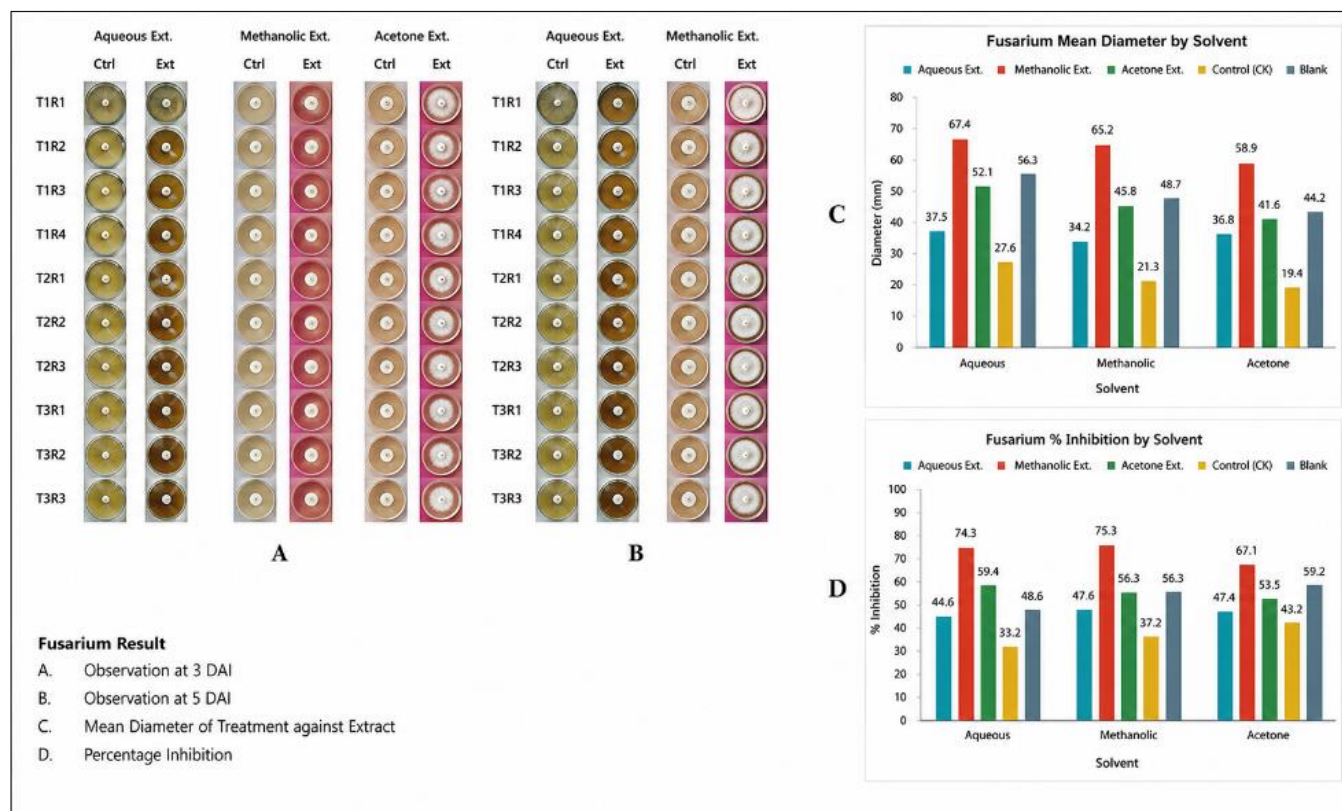
Plate 4: Observation data of *Colletotrichum*

Table 5: Mean Colony Diameter and Percentage Inhibition for *Colletotrichum*

Solvent	Treatment	Mean Diameter (mm), 3 DAI	Mean Diameter (mm), 5 DAI	Percentage Inhibition 3 DAI (%)	Percentage Inhibition 5 DAI (%)
Aqueous	T ₁	31	53.1	7.7	2.4
Aqueous	T ₂	27	46.7	18.5	13
Aqueous	T ₃	24.5	41.8	27	23.4
Methanolic	T ₁	28.4	48	15.7	8.7
Methanolic	T ₂	18.5	31.7	44.9	41.8
Methanolic	T ₃	7.9	17	75.6	67.4
Acetone	T ₁	26	44.3	21.8	19.3
Acetone	T ₂	26.4	40.5	21.3	25.6
Acetone	T ₃	15.2	27.2	54.3	49.9

Table 6: ANOVA Table -Mean Colony Diameter for *Colletotrichum*

Extract	Day	F-statistic	p-value
Aqueous	3 DAI	674.28	<0.001
Aqueous	5 DAI	861.52	<0.001
Methanolic	3 DAI	2192.43	<0.001
Methanolic	5 DAI	4299.84	<0.001
Acetone	3 DAI	466.2	<0.001
Acetone	5 DAI	5452.7	<0.001

**Plate 5:** Observation data of *Fusarium***Table 7:** Mean Colony Diameter and Percentage Inhibition for *Fusarium*

Solvent	Treatment	Mean Diameter (mm), 3 DAI	Mean Diameter (mm), 5 DAI	Percentage Inhibition 3 DAI (%)	Percentage Inhibition 5 DAI (%)
Aqueous	T ₁	16.27	35.35	32.22	11.63
Aqueous	T ₂	13.15	25.32	45.21	36.71
Aqueous	T ₃	9.48	21.23	60.49	46.92
Methanolic	T ₁	16.28	35.18	32.15	12.04
Methanolic	T ₂	11.05	26.13	53.96	34.67
Methanolic	T ₃	6.57	16	72.64	60
Acetone	T ₁	17.27	24.87	28.06	37.83
Acetone	T ₂	14.15	20.92	41.04	47.71
Acetone	T ₃	10.48	17.12	56.32	57.21

Table 8: ANOVA Table -Mean Colony Diameter for *Fusarium*

Extract	Day	F-statistic	p-value
Aqueous	3 DAI	1617	<0.001
Aqueous	5 DAI	3412.69	<0.001
Methanolic	3 DAI	1995.49	<0.001
Methanolic	5 DAI	6543.8	<0.001
Acetone	3 DAI	1617	<0.001
Acetone	5 DAI	1150.28	<0.001

Methanolic extract consistently exhibited the highest antifungal activity against all fungi, followed by acetone extract, while aqueous extract showed comparatively lower inhibition. Inhibition increased with extract concentration and was generally higher at 5 DAI than at 3 DAI.

Among the fungi tested, *Alternaria* and *Cercospora* were more susceptible to extract treatment, whereas *Fusarium* exhibited relatively lower sensitivity.

4. Discussion

The present study demonstrates clear *in vitro* antifungal activity of *Cassia fistula* leaf extracts against major phytopathogenic fungi. The superior efficacy of methanolic and acetone extracts compared to aqueous extract suggests that solvent selection plays a critical role in extracting antifungal constituents, consistent with previous reports on botanical antifungal agents [6, 12].

The observed concentration- and time-dependent inhibition indicates sustained antifungal activity during incubation. Differential sensitivity among fungal species may be attributed to variations in cell wall composition, membrane permeability, and physiological responses [1, 2]. The relatively lower susceptibility of *Fusarium* aligns with earlier findings reporting higher tolerance of this genus to plant-derived antifungal compounds [8, 13].

Although the study was limited to *in vitro* assays using crude extracts, the consistent inhibitory patterns observed across multiple fungi highlight the potential of *C. fistula* leaves as a source of botanical antifungal agents.

5. Conclusions

Cassia fistula leaf extracts exhibit significant *in vitro* antifungal activity against *Alternaria*, *Cercospora*, *Colletotrichum*, and *Fusarium*. Methanolic extract was the most effective, followed by acetone, while aqueous extract showed lower activity. Antifungal effects were concentration-dependent and increased with incubation time. These findings support further investigation of *C. fistula* leaf extracts for development of botanical fungicides aimed at sustainable management of fungal plant diseases.

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