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Fungitoxicity of *Cleistanthus collinus* Roxb. extract on selected endemic forest tree seed pathogens

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ABSTRACT

The present study highlights the fungi toxicity of *Cleistanthus collinus extracts which* is known for being toxic and frequently used for homicidal and suicidal purposes contains three identical compounds, viz. Cleistanthin A, B and C (diphyllin glycosides) in addition to other secondary metabolites, Three different concentrations of leaf and bark methanol extract (0.5, 1 and 2%) were tested that inhibit the mycelial growth of the test fungi *F. verticilloides*, *M. phaseolina* (V), *M. phaseolina* (M), *P. guepinii*, *C. gloeosporioides* (M), *C. gloeosporioides* (P) and *P. archeri* which are seed borne pathogens of endemic forest trees. Percentage inhibition of mycelial growth varied with different concentrations barks and leaf extracts.

KEYWORDS

Cleistanthus collinus;
Leaf and bark;
Mycelia;
Fungitoxic;
Forest tree seed.

INTRODUCTION

Seeds are regarded as a highly effective means for transporting plant pathogens over long distances. Various fungi are commonly associated with seeds of many tree species, and these can include pathogens and saprophytes. Some seed borne fungi cause severe damage to the seeds or seedlings. Seed pathogens viz., *Fusarium verticilloides* causing seed rot in *Dysoxylum malabaricum*, *Macrophomina phaseolina* causing seed rot in *Myristica malabarica* and root rot in *Vateria indica*; *Pestalotiopsis guepinii* leaf blight and *Colletotricum gloeosporioides* leaf spot on *Myristica malabarica* seedlings; *Phomopsis archeri* leaf blight and *C. gloeosporioides* leaf spot in *Poeciloneuron indicum* were studied during carrying out work on fungi associated with fruits and seeds of selected endemic trees of Western Ghats of Karnataka. Although the use of synthetic fungicides in plant disease control have shown its fruitfulness in suppressing the disease, several of these have found to have many detrimental effects on surrounding environment. Substitute choice therefore would be of botanical fungicides, which are non-phytotoxic, systemic and biodegradable in nature.

Cleistanthus collinus (Roxb.) Benth. and Hook f. (Euphorbiaceae) is a poisonous plant growing on dry hills in various parts of India[1]. Toxic effect of different parts of this plant against animals like rats and rabbits was reported by[3]. No reports are available on the antimicrobial activity of selected plant on pathogens of forest tree seed. The present study was carried out for the first time to investigate the effect of leaf and bark extract of *C. collinus* against few selected endemic forest seed fungal pathogens.

MATERIALS AND METHODS

Plant material

The plant materials (*Cleistanthus collinus* leaf and bark) were collected from Vishakapatnam forest area in Andhra Pradesh. The collected bark and leaf materials were processed, shade dried and powdered mechanically.

Extraction

The powdered material was subjected to soxhlets extraction with petroleum ether 60-80 grade to remove the fatty materials for 18 hrs, further extracted with methanol for 24 hrs, The solvent fraction thus obtained was cooled and distilled in rotary vacuum evaporator to recover the solvent and extract separately. The extract was dried off completely without any traces of the solvent. The extract thus obtained was weighed (leaf extract 13.4 % and bark extract 23.9%) and transferred to sample bottles for further use. The constituents of the plant extract were identified with primary qualitative analysis and thin layer chromatography (TLC) method for the presence of glycosides (4, 7). The methanol extract was found to have maximum active compounds[2] . Methanol extract of bark and leaf were taken for its evaluation of antifungal activity. The extracts were dissolved in Dimethyl sulfoxide (DMS) solvent and three different concentrations (0.5, % 1%, 2%) were taken for further studies. Parasuraman et al[4] reported that the *Cleistanthus collinus* leaves have many constituents besides cleistanthin A and B, As there are no scientific data available for toxicity of cleistanthin A and B, the isolation of cleistanthin A and B can lead researchers to explore the preclinical profile of this compound.

Endemic forest tree seed pathogens

F. verticilloides (Fv) isolated from *D. malabaricum* seeds causing rot; two isolated of *M. phaseolina* (Mp V) from *V. indica* seeds causing root rot and *M. phaseolina* (Mp M) *M. malabarica* causing charcoal seed rot; Seed borne fungi *P. guepinii* (Pg) and *C. gloeosporioides* (Cg M) causing leaf blight and spot on *M. malabarica* seedlings; *P. archeri* (Pa) and *C. gloeosporioides* (Cg P) seed borne fungi of *P. indicum* causing leaf blight and spot were maintained on Potato Dextrose Agar plates at 26 – 28^{o}c. (TABLE 1)

TABLE 1 : Seed borne fungi isolated from endemic forest trees and its symptoms

Fungus	Host	Disease
Fusarium verticilloides	Dysoxylum malabaricum	Seed rot
Macrophomina phaseolina 1	Vateria indica	Root rot
Macrophomina phaseolina 2	Myristica malabarica	Charcoal seed rot
Pestalotiopsis guepinii	---do---	Seedling blight
Colletotricum gloeosporioides 1	---do---	Seedling leaf spot
Colletotricum gloeosporioides 2	Poeciloneuron indicum	Seedling leaf spot
Phomopsis archeri	---do---	Seedling blight

Antifungal activity of plant extracts

Antifungal mechanism of plant extracts was studied by Poisoned food technique[1]. Three different concentrations of leaf and bark extract (0.5, 1 and 2%) were prepared by adding the requisite quantity of each extract dissolved little amount of solvent to warm potato dextrose agar after autoclaving. The media was poured into Petri plates and allowed to cool. Young fungal pathogenic cultures of 7-day-old of 5 mm diameter culture discs were placed at the centre of each treatment. Each treatment was maintained in triplicate. Media without extract served as a control. Radial growths of the mycelial colonies were recorded till the growth in control plates was maximum.

The percentage growth inhibition was calculated using the formula,

$$I = 100 \times (C - T)/C$$

Where I is percentage inhibition, C is growth of fungus in the, T is growth of fungus in the treatment.

Data was analyzed statistically by analysis of variance (P at 0.01)

RESULTS AND DISCUSSION

Effect of *C. collinus* bark and leaf extract against *F. verticilloides, M. phaseolina* (V), M. *phaseolina* (M), *P. guepinii, C. gloeosporioides* (M), *C. gloeosporioides* (P) and *P. archeri* showed significant difference from control in radial mycelial growth and percentage growth inhibition irrespective of concentrations at P=0.01.

Bark extract was more inhibitive of fungal mycelial growth than leaf extract (Figure 1). As shown in figure

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2 & 3 there is significant difference in fungitoxic activity of bark and leaf extracts. Percentage inhibition of mycelial growth varied with different concentrations of extracts. Different fungal pathogens showed varying degree of susceptibility to the plant extracts. Among the three concentrations (0.5, 1 & 2 %), maximum inhibition of the pathogens was observed with highest concentration i.e., 2% of bark and leaf extract. 2 % concentration of *C. collinus* bark extract completely hindered mycelial growth (100 % inhibition) of all tested pathogens compared to the control (TABLE 2). Highest concentration of leaf extract showed 100 % inhibition of mycelial growth of *F. verticilloides*, *P. guepinii*, *C. gloeosporioides* (M), *C. gloeosporioides* (P) and *P. archeri*. Mycelial growth of *M. phaseolina*, seed borne fungus of *V. indica* and *M. malabarica* was inhibitied by 96% (TABLE 3).

Mycelial growth inhibition of 43.6 and 89.3 % of *F. verticilloides* was exhibited by 0.5 and 1 % bark extract followed by 32 and 59.8 % of *M. phaseolina* (V), 55.5 and 73% of *M. phaseolina* (M), 37.4 and 59.8 % of *P. guepinii*, 87.3 and 89.1 % of *C. gloeosporioides* 9M0, 81.2 and 86.7 % of *C. gloeosporioides* (p) and 45.7 and 62.3 % of *P. archeri*.

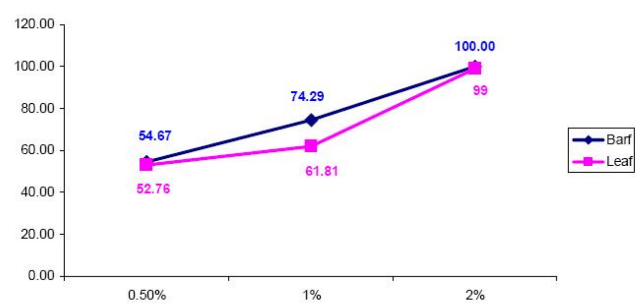

Figure 1 : Average mycelial growth inhibition of test fungi using *C. collinus* bark and leaf extract

0.5% concentration of extract showed high percentage mycelial growth inhibition of *C. gloeosporioides* isolates, followed by *M. phaseolina* (M), *P. archeri*, *F. verticilloides*, *P. guepinii* and *M. phaseolina* (V). Like-

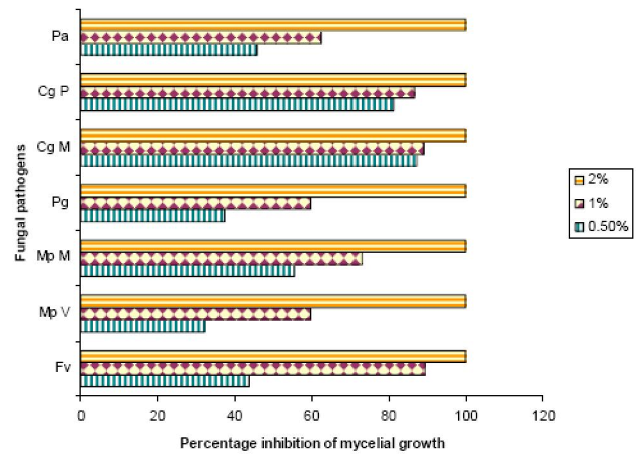

Figure 2 : Variations in fungitoxicity of *C. collinus* bark extract in inhibiting different forest tree seed pathogens

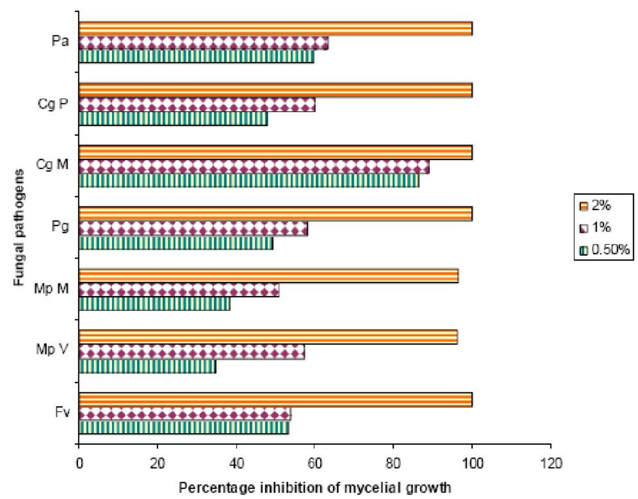

Figure 3 : Variation in the fungitoxicity of *C. collinus* leaf extract in inhibiting different forest tree seed pathogens

TABLE 2 : Effect of different concentrations of bark extract of *C. collinus* on forest tree seed pathogen

Fungal pathogens	Control Growth (mm)	Bark Extract					
		0.50%		1%		2%	
		Growth (mm)	% IHT	Growth (mm)	% IHT	Growth (mm)	% IHT
Fv	56	31.6±1.8	43.6±3.3d	6±0	89.3±0	0	100±0
Mp V	90	61.2±3.5	32±3.9e	36.2±1.4	59.8±1.6	0	100±0
Mp M	90	40±18	55.5±20b	24.3±2.7	73±3	0	100±0
Pg	73.8	46.2±2.5	37.4±3.3	29.7±7.9	59.8±10.7	0	100±0
Cg M	55.3	7±0	87.3±0a	6±0	89.1±0	0	100±0
Cg P	45.2	8.5±0	81.2±0a	6±1	86.7±0	0	100±0
Pa	85	46.2±2.02	45.7±2.4c	32.1±5.6	62.3±6.5	0	100±0

Values are mean inhibition zone (mm) ± S.D of three replicates CD = 6.83

TABLE 3 : Effect of different concentrations of leaf extract of *C. collinus* on forest tree seed pathogen

Fungal pathogens	Control Growth (mm)	Leaf Extract					
		0.50%		1%		2%	
		Growth (mm)	% IHT	Growth (mm)	% IHT	Growth (mm)	% IHT
Fv	56	26.1±2.7	53.4±4.8	25.8±2.2	53.9±4	0	100±0
Mp V	90	58.8±1.0	34.6±1.1	38.5±3.9	57.2±4.3	3.3±5.8	96.3±6.4
Mp M	90	55.7±5.1	38.1±5.7	44.2±0.3	50.9±0.3	3.0±5.2	96.7±5.7
Pg	73.8	37.5±5.2	49.2±7.07	31±1.9	58±2.5	0	100±0
Cg M	55.3	7.5±0	86.4±0	6±0	89.1±0	0	100±0
Cg P	45.2	23.5±1.3	48±2.9	18.1±0.9	60±2.08	0	100±0
Pa	85	34.3±1.6	59.6±1.9	30.9±1.1	63.6±1.3	0	100±0

Values are mean inhibition zone (mm) ± S.D of three replicates CD = 6.83

phaseolina (V) by lowest concentration i.e., 0.5%. Though there is increase in percentage mycelial inhibition of fungal pathogens by 1% leaf extract highest inhibited pathogen was *C. gloeosporioides* (M), followed by, *P. archeri*, *C. gloeosporioides* (P), *P. guepinii*, *M. phaseolina* (V), *F. verticilloides*, and *M. phaseolina* (M).

CONCLUSION

Present study emphasizes the presence of chemical components in bark and leaf extract of *C. collinus* that inhibit the mycelial growth of the test fungi *F. verticilloides*, *M. phaseolina* (V), *M. phaseolina* (M), *P. guepinii*, *C. gloeosporioides* (M), *C. gloeosporioides* (P) and *P. archeri* which are seed borne pathogens of endemic forest trees. The fungitoxicity was found to be variable with bark and leaf extracts. However, the 2% concentration of C. collinus bark and leaf extracts could be recommended for seed treated prior to germination. The present work is to be extended further involving more analytical processes and test plants to yield results useful in raising healthy seedlings of endemic forest trees.

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REFERENCES

[1] O.D.Dhingra, J.B.Sinclair; 'Basic Plant Pathology Methods' (CRC Press, Inc.: Boca Raton, FL). Organic Irritant Poisons (I), Section II Toxicology. In: B.V.Subrahmanyam, Editors. Modi's Medical Jurisprudence & Toxicology, New Delhi: Butterworths S India, 250-2 **(1999)**.

[2] S.H.Jain, C.Soumya, G.Ravikumar; NPAIJ, **6(1)**, 39-42 **(2010)**.

[3] M.Jayanthi, R.Raveendran, Debdatta Basu; Indian Journal of Medical Research, **130**, 467-474 **(2009)**.

[4] S.Parasuraman, R.Raveendran, V.Gopal; Arch.Pharm.Sci.& Res., **1(2)**, 199-202 **(2009)**.