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Antifungal activity of some botanical extracts against seed-borne *Penicillium* species

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Abstract
Seed-borne fungi adversely affect seed germination and seedling vigor and results in economic loss to farmers. In the present study, we screened the antifungal efficacy of 18 extracts from 12 plants against a *Penicillium* species. Isolation of *Penicillium* species from maize seeds was carried out by standard blotter method. Extraction of selected plants was carried out by maceration technique and antifungal activity was evaluated by poisoned food technique. The fungus exhibited varied susceptibility to extracts of selected plants. Out of 18 extracts, 15 extracts caused 50% and higher inhibition of test fungus. Among the selected plants, *Lophopetalum wightianum* and *Argyreia cuneata* showed marked antifungal activity. Highest and least antifungal activity was shown by leaf extract of *L. wightianum* (89.39%) and leaf extract of *C. speciosus* and fruit extract of *S. zeylanica* (36.39%), respectively.

Keywords: Seed-borne fungi, *Penicillium*, maceration, antifungal, poisoned food technique

1. Introduction
Seed is the basic input for production of around 90% of food and other crops. To be used for crop production, seed should be healthy and disease free and the use of such seeds often results in marked productivity. However, seeds are known to carry several pathogenic fungi internally as well as externally. Seeds act as important sources of pathogens that are responsible for deterioration of seed quality and considerable crop loss. Various fungal genera viz. *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Fusarium*, *Curvularia* and *Helminthosporium* are found associated commonly with the seeds of many crops. It is very important to manage seed-borne fungi. Use of botanicals is one of the potent alternatives for control of phytopathogenic fungi [1-6]. *Penicillium* species are reported to be one of the important pathogens of pre and post-harvest diseases of fruits and commercial crops. Some species are known to produce mycotoxins [7-12]. The adverse effect of culture filtrates of some *Penicillium* species in terms of inhibition of germination and seedling growth of sorghum is reported [13]. In the present study, we evaluated the efficacy of 12 plants to inhibit mycelial growth of a seed-borne *Penicillium* species.

Materials and Methods
Collection and identification of plants
A total of 12 plants (Table 1) were collected from different places of Karnataka viz. Haniya (Hosanagara Taluk), Shikaripura (Shivamogga district) and Tarikere (Chikmagalur district) and authenticated by referring standard flora.

Table 1: Plants used in the study

S. No.	Plant name	Family	Habit	Part used	Place of collection
1	*Lophopetalum wightianum* Arn.	Celastraceae	Tree	Leaf, bark	Haniya
2	*Smilax zeylanica* L.	Smilacaceae	Climbing shrub	Leaf, fruit	Haniya
3	*Citharexylum spinosum* L.	Verbenaceae	Tree	Leaf, bark	Haniya
4	*Dichapetalum gelonioides* (Roxb.) Engl.	Dichapetalaceae	Shrub	Leaf, fruit	Haniya
5	*Argyreia cuneata* (Willd.) Ker Gawl.	Convolvulaceae	Suberect shrub	Leaf, flower, stem	Tarikere
6	*Geophila repens* (L.) I. M. Johnst.	Rubiaceae	Creeping herb	Leaf	Haniya
7	*Strobilanthes heyneana* Nees	Acanthaceae	Shrub	Leaf	Haniya
8	*Nilgirianthus ciliatus* (Nees) Bremek	Acanthaceae	Shrub	Leaf	Haniya
9	*Clerodendrum infortunatum* L.	Lamiaceae	Shrub	Leaf	Haniya
10	*Clerodendrum philippinum* Schauer	Lamiaceae	Shrub	Leaf	Shikaripura
11	*Moullava spicata* (Dalzell) Nicolson	Leguminosae	Scandent shrub	Leaf	Haniya
12	*Costus speciosus* (J.Koenig) Sm.	Costaceae	Shrub	Leaf	Haniya

Extraction

The plant materials were dried under shade and powdered in a blender. Extraction of plants was carried out by maceration process. Methanol was used as solvent. Filtrates were evaporated to dryness at room temperature and the crude methanol extract of selected plants was obtained [14].

Test fungus

Penicillium species, used in this study, was isolated from maize seeds by blotter technique and identified based on cultural and microscopic characteristics. The test fungus was maintained on potato dextrose agar (PDA) slants.

Antifungal activity of extracts

The antifungal efficacy of selected plants was evaluated by Poisoned food technique. The extent of inhibition of fungal growth was calculated using the formula:

Inhibition of growth (%) = (Dc – Dt / Dc) x 100, where 'Dc' and 'Dt' represents the diameter of fungal colonies in control and poisoned PDA plates, respectively [14].

Results and Discussion

Chemical agents are widely employed to manage plant pathogenic fungi. However, their extensive and indiscriminate use is associated with certain drawbacks such as environmental pollution and emergence of resistant fungal strains. Botanicals are known to be potential alternatives for chemical agents as they are cheaper, safer and do not cause environmental pollution. Many studies revealed the efficacy of several plant extracts and purified compounds to inhibit a range of fungi including phytopathogenic fungi and seed mycoflora [1, 3, 5, 6, 15-20]. Poisoned food technique was employed to evaluate antifungal potential of 18 extracts obtained from a total of 12 plants. The method is one among the most widely used antifungal assays for plants and the antifungal activity is studied in terms of reduction in mycelial growth of test fungi in poisoned plates on comparing with the fungal growth in control plates[21-25]. Extracts of all plants were effective in causing inhibition of mycelial growth of *Penicillium* species as the extracts amended into the medium (0.5mg extract/ml of medium) caused considerable reduction in fungal growth (Table 2). All extracts, except fruit extract of *S. zeylanica*, leaf extract of *N. ciliatus* and *C. speciosus*, exhibited an inhibitory activity of 50% and higher (Figure 1).

Table 2: Colony diameter of *Penicillium* sp. in control and poisoned plates

Treatment	Colony diameter in cm
Control	2.83±0.05
G. repens leaf	1.20±0.00
D. gelonioides leaf	1.20±0.00
D. gelonioides fruit	1.40±0.00
L. wightianum leaf	0.30±0.00
L. wightianum bark	0.53±0.05
S. zeylanica leaf	1.30±0.00
S. zeylanica fruit	1.80±0.00
C. spinosum leaf	0.93±0.05
C. spinosum bark	1.40±0.00
A. cuneata leaf	0.40±0.00
A. cuneata flower	0.53±0.05
A. cuneata stem	1.20±0.00
C. infortunatum leaf	0.96±0.11
C. philippinum leaf	1.40±0.00
S. heyneana leaf	0.60±0.00
N. ciliatus leaf	1.63±0.05
M. spicata leaf	1.23±0.05
C. speciosus leaf	1.80±0.17

Among plants, *L. wightianum* and *A. cuneata* showed marked antifungal activity. Leaf extract of *L. wightianum* revealed highest activity (89.39% inhibition) while leaf extract of *C. speciosus* and fruit extract of *S. zeylanica* exhibited least inhibitory activity (36.39% inhibition). In case of *L. wightianum*, both leaf and bark extracts caused an inhibitory activity of >80% against the fungus. Leaf extract of *S. zeylanica* exhibited higher activity when compared to fruit extract of *S. zeylanica*. Among extracts of *C. spinosum*, leaf extract showed higher inhibitory activity (67.13%) than bark extract (50.53%). In case of *A. cuneata*, the activity observed was in the order: leaf extract (85.86%) > flower extract (81.27%) > stem extract (57.59%). In case of *D. gelonioides*, Leaf extract caused higher inhibitory activity (57.59%) than fruit extract (50.53%). Among the remaining plants, marked antifungal activity was observed in case of *S. heyneana* (78.79%) followed by *C. infortunatum* (66.07%), *G. repens* (57.59%), *M. spicata* (56.53%), *C. philippinum* (50.53%), *N. ciliatus* (42.40%), and *C. speciosus* (36.39%). Earlier studies have shown the antifungal effect of *S. zeylanica* [14], *L. wightianum* [26], *D. gelonioides* [27], *G. repens* [28], *C. spinosum* [2] against seed mycoflora viz. *Aspergillus niger* and *Bipolar is* sp. Antifungal activities of *C. infortunatum* [29], *C. philippinum* [5], *C. speciosus* [30], *N. ciliatus* [31], *M. spicata* [32], *G. repens* [33] and *C. spinosum* [34] have been investigated.

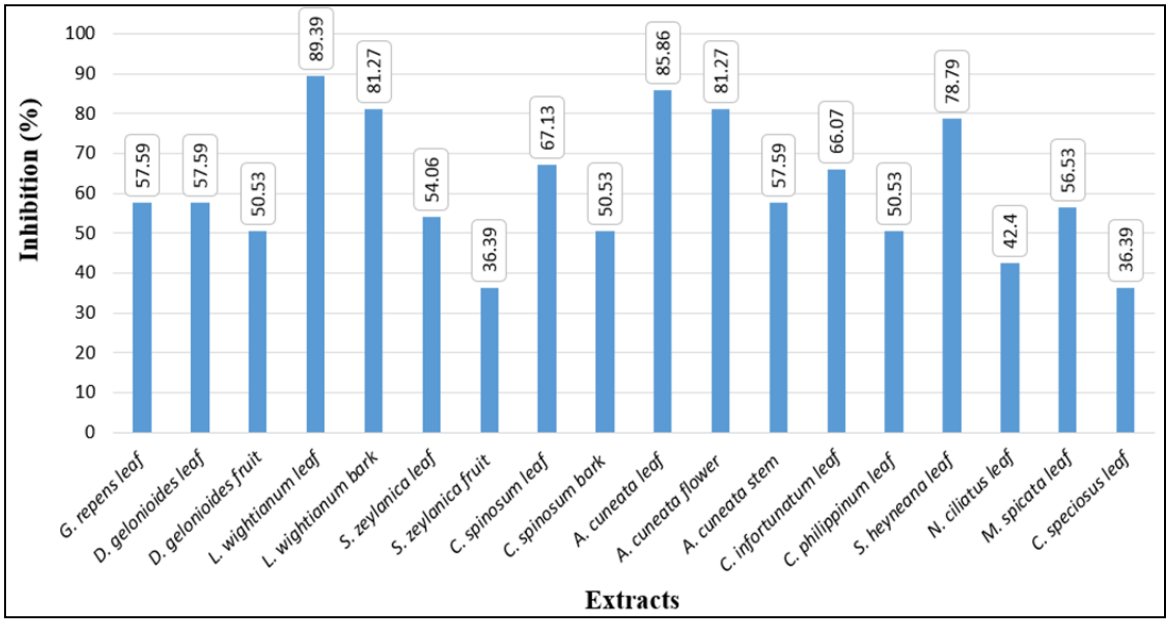

Fig 1: Inhibition of *Penicillium* sp. by extracts of selected plants

Conclusion

The results obtained in this study indicates the potential of selected plants as antifungal agents against seed-borne *Penicillium* species among plants selected, *L. wightianum* and *A. cuneata* were effective in causing inhibition of the fungus to maximum extent. These plants may be employed in certain formulations in order to manage infections, subsequent damage and crop losses resulting from seed mycoflora especially species of *Penicillium*.

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Conflicts of Interst

None declared

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