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Screening of Seven Medicinal Plants for Antifungal Activity Against Seed Borne Fungi of Maize Seeds

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Abstract: Antifungal activity of different concentrations of aqueous extract of seven medicinal plants viz., *Acalypha indica* L.(Leaf), *Anisomeles malabarica* (L.) Sims (Leaf), *Amaranthus spinosus* L. (Leaf), *Eupotorium odoratum* L.(Leaf), *Psoralea corylifolia* L.(Seed), *Tribulus terrestris* L (Whole Plant) and *Alternanthera pungens* Kunth. (Whole Plant) were tested against six seed borne fungi of maize seeds viz., *Aspergillus flavus Aspergillus niger, Fusarium moniliforme, Fusarium graminearum, Penicillium chrysogenum and Penicillium notatum* employing poison food technique. Among the seven medicinal plants tested, *P. corylifolia* seed extract recorded a significant activity against all the test pathogens and other six plants did not showed any antifungal activity even at highest concentration of aqueous extract.

Key words: *Aspergillus · Fusarium · Penicillium · Antifungal activity · P. corylifolia*

INTRODUCTION

Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country [1]. Traditional and folklore medicines play important role in health services around the globe. About three quarter of the world's population relies on plants and plant extracts for healthcare. India has an extensive forest cover, enriched with plant diversity [2]. The roles of plants in maintaining human health is well documented. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. The active principles of many drugs found in plants are secondary metabolites [3]. Therefore, basic phytochemical investigation of these plant extracts for their major phytoconstituents is also vital. Most of the molecules in plants are secondary metabolites, of which at least 12,000 have been isolated and the number estimated to be less than 10% of the total [4]. Herbal medicines represent one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants which have folklore reputation in a more intensified way. Over the past 20 years, there has been an increased interest in the investigation of natural materials as a source of new antifungal agents. Different extracts and essential oils from traditional medicinal plants have been tested to identify the source of therapeutic effects. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action [5]. In the present study, antifungal activity of seven important medicinal plants viz, *Acalypha indica* L.(Leaf) (Euphorbiaceae)., *Anisomeles malabarica* (L.) Sims.(Lamiaceae)(Leaf), *Amaranthus spinosus* L.(Amaranthaceae)(Leaf), *Eupotorium odoratum* L.(Asteraceae)(Leaf), *Psoralea corylifolia* L. (Fabaceae) (Seed), *Tribulus terrestris* L (Zygophyllaceae)

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(Whole Plant) and *Alternanthera pungens* Kunth. (Amaranthaceae)(Whole Plant) were tested for their antifungal activity against two species of *Aspergillus*, *Penicillium* and *Fusarium* a potent seed borne fungi of Maize seeds.

MATERIALS AND METHODS

Collection of Seed Samples: Naturally infected seed samples of maize (*Zea mays* L.) were collected from seed market, Mysore, Karnataka, India. The seeds were surface sterilized with 0.1% sodium hypochloride and rinsed with distilled water for four to five times, air dried and subjected for standard blotter method.

Isolation and Identification of Biodeterioration Causing Fungi in Maize: Standard blotter method was employed for isolation of seed borne biodeterioration causing fungi. Three layers of blotters equivalent to the size of the petridish were soaked in distilled water, the surplus water is drained from the blotters and placed in the lower lid of the petridish. Four hundred seeds of each of the samples were placed on the blotters at the rate of ten seeds per plate. These plates were incubated for seven days at 22±2°C under alternating cycles of 12/12 hrs of NUV light and darkness. After the period of incubation the seeds were observed under stereobinocular microscope and the fungi associated with these seeds were identified based on their growth habit, mycelial structure and spore morphology using standard manuals. The diversity of the fungal species were recorded and the percentage of infection of each of the fungi were determined [6]. All the fungi associated with the seeds were isolated on Czapek Dox Agar (CDA) medium and their pure cultures were maintained on specific media. The fungi were subcultured periodically.

Test Fungi: Two species of *Fusarium* viz., *Fusarium moniliforme* and *Fusarium graminearum and* two species of *Aspergillus viz., Aspergillus flavus* and *Aspergillus niger* and two species of *Penicillium viz., Penicillium chrysogenum* and *Penicillium notatum* which were frequently associated in maize seeds with higher percentages were selected for antifungal activity assay of aqueous extract of test plants.

Test Plants: Apparently healthy plant parts of *Acalypha indica* L.(Leaf) (Euphorbiaceae)., *Anisomeles malabarica* (L.) Sims.(Lamiaceae)(Leaf), *Amaranthus spinosus* L. (Amaranthaceae)(Leaf), *Eupotorium odoratum* L. (Asteraceae)(Leaf), *Psoralea corylifolia* L. (Fabaceae)(Seed), *Tribulus terrestris* L (Zygophyllaceae) (Whole Plant) and *Alternanthera pungens* Kunth. (Amaranthaceae)(Whole Plant) used in traditional medicine were selected for the study (Table 1). The plant parts were washed thoroughly with running tap water 2-3 times and once in sterile distilled water. The plant materials were air dried at room temperature on a sterile blotter under shade [7].

Preparation of Aqueous Extract: One hundred grams of each of the of the air dried healthy plant materials selected and were macerated with 100ml sterile distilled water in a waring blender (Waring international, new hart-ford, CT, USA) for 5min. The macerate was filtered through double layered muslin cloth and then centrifuged at 4000g for 30 min. The supernatant was filtered through WhatMan No.1 filter paper and sterilized at 120°C for 10min. The filtrate served as the mother extract (100%). The extracts were preserved aseptically in a brown bottle at 5°C until further use [7,8] Different concentrations were prepared with sterile water as dilutant.

Antifungal Activity Assay by Poisoned Food Technique: Different concentrations of aqueous extracts of the test plants viz., 10, 15, 20, 25, 30, 35 and 40% were achieved in CDA medium for antifungal activity against species of *Fusarium*, *Aspergillus* and *Penicillium* respectively. The media with the test plant extracts were sterilized at 120°C for 15 minutes at 15 lb pressure. The sterilized media were

Table 1: Plants evaluated for antifungal activity assay

Sl. No.	Name of the plant	Family	Plant parts used
1.	*Acalypha indica* L.	Euphorbiaceae	Leaves
2.	*Anisomeles malabarica* (L.)Sims.	Lamiaceae	Leaves
3.	*Amaranthus spinosus* L.	Amaranthaceae	Leaves
4.	*Eupotorium odoratum* L.	Asteraceae	Leaves
5.	*Psoralea corylifolia* L.	Fabaceae	Seeds
6.	*Tribulus terrestris* L.	Zygophyllaceae	Whole plant
7.	*Alternenthera pungens* Kunth.	Amaranthaceae	Whole plant

poured on to the petriplates allowed to cool and solidify. Five mm discs from the margin of the seven day old cultures of the test fungi were placed aseptically at the center of the petriplates and incubated at 22±2°C for seven days. After the period of incubation the colony diameter was measured in mm. Petriplates without the test plant extracts but with the same concentrations of water served as control. Percentage inhibition of mycelial growth if any was calculated in relation to growth in control using the formulae C-T/C X 100 [9]. The data was subjected to Tukey's HSD statistical analysis.

RESULT

Antifungal Activity Assay by Poisoned Food Technique: Among the seven plants tested, only *P. corylifolia* showed antifungal activity. Whereas the others plants did not show any activity. The activity was observed against all the test fungi in all the different concentrations of the aqueous extracts of seeds of *P. corylifolia*. The antifungal activity increased with increased concentration of the extract. Highly significant activity was observed against all the test fungi compared with control. The percentage of mycelial inhibition was highest against species of *Fusarium* followed by species of *Penicillium* and species of *Aspergillus*. The activity was observed more than 50% mycelial inhibition even at 10% concentration against *A. flavus, F. moniliforme* and *P. notatum*. Total inhibition of mycelial growth was observed only in case of *F. graminearum* at and above 15% concentration of the extract, whereas no inhibition was observed at 10% concentration of the extract. Total mycelial growth inhibition was also observed in case of *F. moniliforme* at 40% concentration.

The percentage of mycelial inhibition at different concentrations of the extracts ranged between 55 to 64 in case of *A. flavus* and 48 to 68 in case of *A. niger*. Similarly the mycelial percentage inhibition ranged between 45 to 86 in case of *P. chrysogenum* and 57 to 91 in case of *P. notatum*. Total inhibition of species of *Aspergillus* and species of *Penicillium* was not observed in any of the concentrations tested (Table 2).

Table 2: Antimicrobial activity of different plant materials against seed borne fungi of maize

	Percent inhibition																				
	Aspergillus flavus							*Aspergillus niger*							*Fusarium moniliforme.*						
	Plant materials							Plant materials							Plant materials						
Concentration (%)	*A.i*	*A.m*	*A.p*	*A.s*	*E.o*	*P.c*	*T.t*	*A.i*	*A.m*	*A.p*	*A.s*	*E.o*	*P.c*	*T.t*	*A.i*	*A.m*	*A.p*	*A.s*	*E.o*	*P.c*	*T.t*
10%	0.0	0.0	0.0	0.0	0.0	55.66	0.0	0.0	0.0	0.0	0.0	0.0	48.45	0.0	0.0	0.0	0.0	0.0	0.0	55.50	0.0
15%	0.0	0.0	0.0	0.0	0.0	58.12	0.0	0.0	0.0	0.0	0.0	0.0	53.57	0.0	0.0	0.0	0.0	0.0	0.0	82.50	0.0
20%	0.0	0.0	0.0	0.0	0.0	58.61	0.0	0.0	0.0	0.0	0.0	0.0	61.23	0.0	0.0	0.0	0.0	0.0	0.0	84.80	0.0
25%	0.0	0.0	0.0	0.0	0.0	60.09	0.0	0.0	0.0	0.0	0.0	0.0	63.25	0.0	0.0	0.0	0.0	0.0	0.0	87.75	0.0
30%	0.0	0.0	0.0	0.0	0.0	62.55	0.0	0.0	0.0	0.0	0.0	0.0	63.25	0.0	0.0	0.0	0.0	0.0	0.0	89.10	0.0
35%	0.0	0.0	0.0	0.0	0.0	64.52	0.0	0.0	0.0	0.0	0.0	0.0	67.32	0.0	0.0	0.0	0.0	0.0	0.0	93.55	0.0
40%	0.0	0.0	0.0	0.0	0.0	64.52	0.0	0.0	0.0	0.0	0.0	0.0	68.50	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0

Acalypha indica(*A.i*)(Leaf), *Alternanthera pungens*(*A.p*)(Leaf), *Anisomeles malabarica*(*A.m*)(Leaf), *Amaranthus spinosus*(*A.s*)(Leaf), *Eupotorium odoratum*(*E.o*)(Leaf), *Psoralea corylifolia*(*P.c*)(Seed), *Tribulus terrestris*(*T.t*)(Leaf),.

	Percent inhibition																				
	Fusarium graminearum							*Penicillium chrysogenum*							*Penicillium notatum*						
	Plant materials							Plant materials							Plant materials						
Concentration (%)	*A.i*	*A.m*	*A.p*	*A.s*	*E.o*	*P.c*	*T.t*	*A.i*	*A.m*	*A.p*	*A.s*	*E.o*	*P.c*	*T.t*	*A.i*	*A.m*	*A.p*	*A.s*	*E.o*	*P.c*	*T.t*
10%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	45.70	0.0	0.0	0.0	0.0	0.0	0.0	57.60	0.0
15%	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	64.75	0.0	0.0	0.0	0.0	0.0	0.0	70.30	0.0
20%	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	65.71	0.0	0.0	0.0	0.0	0.0	0.0	70.97	0.0
25%	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	68.57	0.0	0.0	0.0	0.0	0.0	0.0	71.91	0.0
30%	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	68.57	0.0	0.0	0.0	0.0	0.0	0.0	76.86	0.0
35%	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	80.00	0.0	0.0	0.0	0.0	0.0	0.0	85.90	0.0
40%	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	86.30	0.0	0.0	0.0	0.0	0.0	0.0	91.30	0.0

Acalypha indica(*A.i*)(Leaf), *Alternanthera pungens*(*A.p*)(Leaf), *Anisomeles malabarica*(*A.m*)(Leaf), *Amaranthus spinosus*(*A.s*)(Leaf), *Eupotorium odoratum*(*E.o*)(Leaf), *Psoralea corylifolia*(*P.c*)(Seed), *Tribulus terrestris*(*T.t*)(Leaf)..

DISCUSSION

Plants are a repository of various biomolecules responsible for different biological activities. India is endowed with rich plant biodiversity and Karnataka is one of the hot spots of plants diversity. Many plants have been evaluated for different biological activities world over [10-13]. Considering the biodiversity of higher plants, the number of plants screened for various biological activities is negligible and the number of plants screened for antifungal activities is negligible small percentage. Further, the number of plants evaluated for antifungal activity against phytopathogenic fungi in general and biodeterioration causing fungi in particular is infitisimly small. Considering these, in the present investigation a systematic investigation was envisaged to evaluate the antifungal potency of seven plants against important biodiversity causing fungi of maize.

Among the seven plants evaluated for the activity, results revealed that only one plants viz., *P. corylifolia* was antifungally active against all the test fungi. Where as the other six plants viz., *Acalypha indica*, *Anisomeles malabarica*, *Amaranthus spinosu*, *Eupotorium odoratum*, *Tribulus terrestris* and *Alternenthera pungens* did not showed any antifungal activity even at 40% concentration suggesting that *P. corylifolia* is an important candidate plant for further evaluation with regard to its antifungal potency. The antibacterial potential of the seed extract of *P. corylifolia* has been demonstrated. However none of the earlier reports have demonstrated the antifungal potency of *P. corylifolia* against phytopathogenic fungi in general and biodeterioration causing fungi in particular [14-16] Thus in the present investigation, for the first time the antifungal potency of the plant against phytopathogenic fungi in general and biodeterioration causing fungi of maize in particular has been demonstrated.

The seeds of *P. corylifolia* are used in indigenous medicine as laxative aphrodisiac, anthelmintic, diuretic and diaphoretic in febrile conditions. They have been specially recommended in the treatment of leucoderma, leprosy, psoriasis and inflammatory diseases of the skin and are prescribed both for oral administration and for local external application in the form of a paste or ointment [17]. A number of preparations made from the seeds have been tried in numerous cases of leucoderma and other skin diseases. Oral administration of the powdered seeds to the patients has generally resulted in side reactions such as nausea, vomiting, malaise, headache and sometimes purging. The seed extracts inhibit the growth of *Staphylococcus citreus*, *S. aureus* and *S. albus* including strains resistant to Penicillin. The seeds possesses anthelmintic activity against earthworms, Psoralen being the active principle [18].The seeds are also used locally in the preparations of certain types of medicated oils and incense preparations. The root is useful in the dental caries of teeth and leaves are used in diarrhoea [19].

Further experiments conducted to evaluate the antifungal potency at different concentrations of aqueous extract of seeds of *P. corylifolia* revealed highly significant antifungal activity against all the test fungi in all the concentrations tested compared with control. Highly significant activity could be observed even at 10% concentration against species of *Aspergillus*, species of *Penicillium* and *F. monoliforme*. While no activity was observed at this concentration against *F. graminearum*. It is interesting to note from the present investigation that total inhibition of *F. graminearum* was observed at 15% concentration itself. However total mycelial growth inhibition was not observed against any of the other test fungi in any of the concentrations except *F. moniliforme* at 40% concentration.The findings of the present investigation clearly demonstrates that *P. corylifolia* is as an important for further work to isolate and identify the active principle responsible for antifungal activity and further evaluation of the same against a large number of fungal species known to cause biodeterioration in maize during storage.

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