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Antifungal activity of some leaf extracts against seed-borne pathogenic fungi

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Abstract:
Plant extracts are being used to control the diseases since last several years. Extracts of the various plant parts like leaf, stem, root, fruit and seeds are found to be effective against seed-borne pathogenic fungi. The *in vitro* studies have been performed by using cup-plate method to examine the antifungal activity of some leaf extracts. Leaf extracts of 18 plants were screened against 5 seed-borne pathogenic fungi *viz. Alternaria alternata, Aspergillus niger, Curvularia lunata, Fusarium moniliforme* and *Trichoderma viride.* Out of 18 leaf extracts, 9 leaf extracts showed antifungal activity. The extract of *Azadirachta indica* showed maximum activity; while minimum activity was observed with *Holoptelia integrifolia* against the fungi under investigation. These plant extracts can possibly be exploited in the management of seed-borne pathogenic fungi to prevent biodeterioration of seeds in an eco-friendly way.

Keywords: Antifungal activity, Seed-borne Pathogenic Fungi, Leaf Extracts

INTRODUCTION

Fungal diseases are known to cause great damages all over the world. Different species of *Alternaria, Aspergillus, Ceratobasidium, Cercospora, Cochliobolus, Curvularia, Dreschslera, Fusarium, Gaeumannomyces, Microdochium, Penicillium, Pyricularia, Pythium, Rhizoctonia, Rhizopus, Sclerophthora, Trichoderma* and *Tricoconella* are most common associates of seeds all over the world, causing pre- and post-infections and considerable quality losses *viz.* seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, seedling damage and their nutritive value have been reported (Miller, 1995; Janardhana *et al.,* 1998; Kavitha *et al.,* 2005). Seed treatment is the safest and the cheapest way of control of seed-borne fungal diseases and to prevent biodeterioration of grains (Chandler, 2005; Bagga and Sharma, 2006).

A large number of fungicides are being used in the form of dusting, slurry and soaking treatment (Agrios, 1997). Even though effective and efficient control of seed- borne fungi can be achieved by the use synthetic chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity (Harris *et al*., 2001). It is now realized that chemical fungicides cause serious environmental problems and are toxic to non-target organisms (Anon., 2005). The toxic effect of synthetic chemicals can be overcome, only by persistent search for new and safer pesticides accompanied by wide use of pest control methods, which are eco-friendly and effective (Mohana *et al.,* 2011). Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (Balandrin *et al.,* 1985; Hostettmann and Wolfender, 1997). Leaf extracts of various plants are known to possess antimicrobial activity. Antimicrobial activity of the leaves has been mentioned by Charjan (1995), Abd-Aziz *et al.,* (1994-1996), Suhaila-Mohamed *et al.,* (1996) etc.

Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999). Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trails (Parekh *et al.,* 2006; Aliero and Afolayan, 2006; Buwa and Staden, 2006; Ergene *et al*., 2006; Mohana *et al*., 2008). Exploitation of plant metabolites in crop protection and prevention of biodeterioration caused by fungi appear to be promising. In view of these, the author screened some leaf extracts against seed-borne pathogenic fungi and the data has been presented in this paper.

MATERIALS AND METHODS

Fungal pathogens were isolated on PDA medium from different stored seeds. Identified fungal cultures were isolated and pure cultures of each fungi made separately on PDA slants. These pure cultures were used for further investigation.

a) Preparation of leaf extracts: For the study, fresh leaf extracts were used. The fresh leaves were collected, thoroughly washed with tap water and then rinsed with sterile distilled water. Fresh leaves weighing 1 gm were crushed in mortar and pestle with 10 ml sterile distilled water. Then it

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was centrifuged for 20 min at –4°C at the 11000 rpm speed.

b) Cup Plate Method: 20 ml of PDA media was poured in sterilized petridishes (9 cm diameter) and allowed to solidify. Then pure cultures of fungi were streaked out in regular intervals on the media poured in petridishes. In the centre of the medium, a cup cavity of 8 mm diameter was made with sterilized No. 4 cork borer. This cup was filled with 0.1 ml of the leaf extract (Pawar and Papdiwal, 2010).

The petridishes were incubated for 6 days at 30±2°C temperature and the observations were recorded as diameter of inhibitory zone in mm. Cup plate filled with sterile distilled water was used as control in all the experiments. All the experiments were in triplicate and mean has been considered in observation table.

RESULTS AND DISCUSSIONS

The antifungal activity of 18 leaf extracts against 5 seed-borne fungi is presented in table 1 as zone of inhibition (in mm). It was observed from table 1 that out of 18 leaf extracts, 9 leaf extracts showed antifungal activity; out of which *Azadirachta indica* showed maximum activity (Mean activity zone 22.996 mm), followed by *Terminalia thorelii* (Mean activity zone 21.396 mm) and minimum activity was observed with leaf extract of *Holoptelia integrifolia* (Mean activity zone 14.996 mm). The leaf extracts of *Callistemon rigidus, Capsicum annum, Datura inoxia, Dolichandrone falcata, Lantana camera* and *Vitex negundo* also showed good antifungal activity; however, leaf extracts of *Adhatoda zaylanica, Annona sqamosa, Butea monosperma, Caesalpinia pulcherrima, Citrus aurantifolia, Cordia sebestena, Lawsonia inermis, Mimusops elengi* and *Santalum album* could not show any antifungal activity against the fungi under investigation.

Many reports revealed that, plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999; Harborne, 1998; Gottlieb *et al.*, 2002). Eventhough effective and efficient control of seed borne pathogenic fungi can be achieved by the use of synthetic fungicides, the same cannot be applied to grains for reasons of pesticide toxicity (Wodageneh *et al*. 1997; Harris *et al*., 2001). Thus, there is a need to search for alternative approaches to store grains/cereals for human consumption without toxicity problems that are eco-friendly and not capital intensive. Considering these as first step in the present investigation 18 leaf extracts were screened against 5 important seed-borne phytopathogenic fungi isolated from stored seeds.

Table 1: Antifungal activity of Leaf Extracts against Seed-borne Pathogenic Fungi

Sr. No	Name of the Plant	Zone of Inhibition (in mm)					
		Alternaria alternata	*Aspergillus niger*	*Curvularia lunata*	*Fusarium moniliforme*	*Trichoderma viride*	Mean
1.	*Adhatoda zaylanica* Medic.	–	–	–	–	–	–
2.	*Annona sqamosa* L.	–	–	–	–	–	–
3.	*Azadirachta indica* A.Juss.	23.33	22.66	23.66	23.33	22.00	22.996
4.	*Butea monosperma* (Lamk.) Taub.	–	–	–	–	–	–
5.	*Callistemon rigidus* R. Br.	15.33	16.33	14.00	16.33	14.33	15.264
6.	*Capsicum annum* L.	16.00	18.33	19.66	17.66	15.00	17.330
7.	*Caesalpinia pulcherrima* (L.) Swartz.	–	–	–	–	–	–
8.	*Citrus aurantifolia* (Christm.) Sw.	–	–	–	–	–	–
9.	*Datura inoxia* Mill.	18.33	19.33	17.00	19.66	16.33	18.130
10.	*Dolichandrone falcata* (Wall ex DC) Seem.	15.33	18.33	16.66	15.00	15.33	16.130
11.	*Cordia sebestena* L.	–	–	–	–	–	–
12.	*Holoptelea integrifolia* (Roxb.) planch.	15.33	15.33	14.66	15.66	14.00	14.996
13.	*Lantana camera* L.	20.33	21.66	20.33	22.00	20.33	20.930
14.	*Lawsonia inermis* L.	–	–	–	–	–	–
15.	*Mimusops elengi* L.	–	–	–	–	–	–
16.	*Santalum album* L.	–	–	–	–	–	–
17.	*Terminalia thorelii* Ganep	22.33	22.66	21.00	21.66	19.33	21.396
18.	*Vitex negundo* L.	17.33	19.66	19.33	18.00	18.66	18.596

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