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Antifungal activity of some stem 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 stem extracts. Stem extracts of 9 plants were screened against 5 seed-borne pathogenic fungi *viz. Alternaria alternata, Aspergillus niger, Curvularia lunata, Fusarium moniliforme* and *Trichoderma viride.* Out of 9 stem extracts, 6 stem extracts showed antifungal activity. The extract of *Azadirachta indica* showed maximum activity; while minimum activity was observed with *Callistemon rigidus* 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, Stem 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).

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). 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). Stem extracts of various plants are known to possess antimicrobial activity. Several workers have observed the antifungal activity of stem and its bark extracts (Boughalleb *et al*, 2005; Vats *et al*, 2009; Upadhay *et al*, 2010; Sule *et al*, 2011). Pandey *et al* (2011) have studied antimicrobial activity of *Ruta graveolens* stem extract by disc diffusion method.

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; Buwa and Staden, 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 stem 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.

Preparation of stem extracts

The young stems were collected, thoroughly washed with tap water and then rinsed with sterile distilled water. Stems weighing 20 gm were crushed in electric mixer grinder with 50 ml sterile distilled water. Then it was centrifuged for 20 min at –4°C at the 11000 rpm speed.

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 stem extract (Pawar and Papdiwal, 2010).

The petridishes were incubated for 6 days at 30±2°C

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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 9 stem 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 9 stem extracts, 6 stem extracts showed antifungal activity; out of which *Azadirachta indica* showed maximum activity (Mean activity zone 22.262 mm), followed by *Lantana camera* (Mean activity zone 21.130 mm) and minimum activity was observed with stem extract of *Callistemon rigidus* (Mean activity zone 15.130 mm). The stem extracts of *Capsicum annum, Datura inoxia,* and *Terminalia thorelii* also showed good antifungal activity; however, stem extracts of *Citrus aurantifolia, Lawsonia inermis,* and *Santalum album* could not show any antifungal activity against the fungi under investigation.

Table 1. Antifungal activity of Stem Extracts against Seed-borne Pathogenic Fungi.

Sl. No	Name of the Plant	Zone of Inhibition (in mm)					
		Alternaria alternata	*Aspergillus niger*	*Curvularia lunata*	*Fusarium moniliforme*	*Trichoderma viride*	Mean
1	*Azadirachta indica* A.Juss.	21.33	21.66	22.33	22.66	23.33	22.262
2	*Callistemon rigidus* R. Br.	12.66	16.00	15.33	15.33	16.33	15.130
3	*Capsicum annum* L.	16.33	17.66	18.66	17.33	16.00	17.196
4	*Citrus aurantifolia* (Christm.) Sw.	–	–	–	–	–	–
5	*Datura inoxia* Mill.	18.33	18.66	19.00	18.33	17.66	18.396
6	*Lantana camera* L.	21.33	22.33	20.33	20.66	21.00	21.130
7	*Lawsonia inermis* L.	–	–	–	–	–	–
8	*Santalum album* L.	–	–	–	–	–	–
9	*Terminalia thorelii* Ganep	20.66	21.66	20.00	21.33	20.66	20.862

– : No Activity.

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). Antifungal activity and preliminary phytochemical analysis of stem bark extracts of *Juglans regia* linn. has been studied by Upadhyay *et al* (2010). Studies on antifungal activity of different extracts of *Cassia fistula* and bioactivity guided isolation and identification of antifungal agent has been performed by Shilpakala *et al* (2009). Igbinosa *et al* (2009) studied antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas.* Tang *et al* (2010) isolated sphignolipids from the stems of Cucumber (*Cucumis sativus* L.) and observed antimicrobial activity. Priya *et al* (2010) studied antifungal activity of different extracts of *Cassia fistula* and bioactivity guided isolation and identification of antifungal agent. Recently, Londokar *et al* (2011) reported potential antibacterial and antifungal activity of A*chyranthes aspera*. 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.

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