Antifungal Activity of Some Seed 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 seed extracts. Seed 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 seed extracts, 5 seed extracts showed antifungal activity. The extract of Azadirachta indica showed maximum activity; while minimum activity was observed with Annona squamosa against the fungi under investigation. While seed extract of Brassica juncea, Citrus aurantifolia and Terminalia muelleri also showed good activity against the same fungal pathogens. 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, Seed Extracts.

Received 05.01.2015 Accepted 12.02.2015 © 2015 Society of Education

Citation of this article

INTRODUCTION
Fungal diseases are known to cause great damages all over the world. Different species of Alternaria, Aspergillus, Ceratobasidium, Cercospora, Cochliobolus, Curvularia, Dreschlera, Fusarium, Gaeumannomyces, Microdochium, Penicillium, Pyricularia, Pythium, Rhizoctonia, Rhizopus, Sclerotinia, 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 [1-3]. Seed treatment is the safest and the cheapest way of control of seed-borne fungal diseases and to prevent biodeterioration of grains [4, 5].

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 [6]. 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 [7]. Green plants represent a reservoir of effective chemotherapeutics and can provide valuable sources of natural pesticides [8, 9]. Seed extracts of various plants are known to possess antimicrobial activity. Several workers have observed the antifungal activity of seed extracts [11-14]. Satish et al. [15] have tested some aqueous extract of fifty-two plants from different families were tested for their antifungal potential against eight important species of Aspergillus such as A. candidus, A. columnaris, A. flavipes, A. flavus, A. fumigatus, A. niger, A. ochraceus, and A. tamarii which isolated from sorghum, maize and paddy seed samples. Dellavalle et al. [16] evaluated the antifungal activity of extracts of 10 plant species used in traditional Uruguayan medicine against the phytopathogenic fungus Alternaria spp. Kiran et al. [13] studied antifungal activity of aqueous and solvent extract of seeds of P. corylifolia against five seed borne fungi of maize viz., Curvularia lunata, Dreschlera halodes, Alternaria alternata, Cladosporium cladosporioides and Rhizopus sp. were tested in vitro.
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 seed extracts: The seeds were collected, thoroughly washed with tap water and then rinsed with sterile distilled water. Seeds 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.

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 seed extract [17]. 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 DISCUSSION
The antifungal activity of 9 seed 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 seed extracts, 5 seed extracts showed antifungal activity; out of which Azadirachta indica showed maximum activity (Mean activity zone 20.796 mm), followed by Terminalia muelleri (Mean activity zone 18.464 mm) and minimum activity was observed with seed extract of Annona squamosa (Mean activity zone 15.664 mm). The seed extracts of Brassica juncea, Citrus aurantifolia and Terminalia muelleri also showed good antifungal activity; however, seed extracts of Albizia lebbeck, Capsicum annuum, Cyamopsis tetragonoloba and Lantana camera could not show any antifungal activity against the fungi under investigation.

Gupta et al. [18] reported antibacterial and antifungal efficacy of crude leaf and seed extract and seed oil of Seabuckthorn (Hippophae salicifolia D. Don) on different microbial cultures causing infections/diseases was investigated by agar disc diffusion method. The methanol extract of Cassia fistula seeds was investigated by Subramanion et al. [19] for potential antimicrobial activity against medically important bacterial, yeast and fungal strains. Adam et al. [12] tested antimicrobial activity of the petroleum ether, methanol and water extracts of Lepidium sativum seed extracts against six opportunistic pathogens namely taphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa and one fungus Candida albicans was assessed using the concentrations of 2.5, 5 and 10%. Antibacterial and antifungal activity of Syzygium jambolanum seeds has been reported by Chandrasekaran and Venkatesalu [20].

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

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of the Plant</th>
<th>Alternaria alternata</th>
<th>Aspergillus niger</th>
<th>Curvularia lunata</th>
<th>Fusarium moniliforme</th>
<th>Trichoderma viride</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Albizia lebbeck (L.) Willd.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Annona squamosa L.</td>
<td>15.33</td>
<td>16.00</td>
<td>15.66</td>
<td>15.00</td>
<td>16.33</td>
<td>15.664</td>
</tr>
<tr>
<td>4</td>
<td>Brassica juncea Czern et. Coss.</td>
<td>18.00</td>
<td>18.66</td>
<td>17.66</td>
<td>18.00</td>
<td>17.66</td>
<td>17.996</td>
</tr>
<tr>
<td>5</td>
<td>Capsicum annuum L.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Citrus aurantifolia (Christm.) Sw.</td>
<td>16.33</td>
<td>16.66</td>
<td>16.66</td>
<td>17.00</td>
<td>16.00</td>
<td>16.530</td>
</tr>
<tr>
<td>7</td>
<td>Cyamopsis tetragonoloba (L.) Taub.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Lantana camera L.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Terminalia muelleri Benth.</td>
<td>18.00</td>
<td>18.66</td>
<td>18.33</td>
<td>18.33</td>
<td>19.00</td>
<td>18.464</td>
</tr>
</tbody>
</table>

− : No Activity.

REFERENCES