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# Seed borne fungi associated with some stored seeds and their bio-control by aqueous medicinal plant extract

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## Abstract

To determine the mycoflora associated with some stored seeds, and their bio-control by aqueous medicinal plant extract. This study aimed to isolate and identify seed-borne fungi associated with seeds in both agar plate method and moist blotter method. The studied seeds were *Oryza sativa* L and *Cajanus cajan* (L.) Millsp. Seven dominative fungal species were isolated from these seeds such as *Aspergillus niger* Van Tieghem, *A. fumigatus* Fresenius, *A. terreus* Thom, *A. Flavus* Link, *Rhizopus nigricans* Ehrenberg, *R. Stolonifer* (Ehrenb.: Fr.)Vuill, *R. oryzae* Went & Prins.Geerl. Percentage of incidents of mycoflora in both agar plate and moist blotter method were studied. Spore suspension of two dominant fungi *A.terreus* and *A.niger* were prepared and frequency of fungal infected seeds was observed to identify the affect of particular fungus on seeds. Four medicinal plants named *Allium cepa* L, *Allium sativum* L, *Zingiber officinale* Roscoe and *Ocimum tenuiflorum* L were screened for their anti-fungal activities. All seeds were treated with aqueous extracts of fresh samples. *Allium sativum* and *Zingiber officinale* exhibited the highest antifungal activity among all plants tested.

Key words: *Aspergillus*, *Rhizopus*, Spore suspension, medicinal plants, antifungal activity.

## Introduction

Cereals and legumes rank among the most important groups of crop plant production. They are an important source of high protein, carbohydrates, minerals, vitamins and fiber and also one of the cheapest food components. Seed-borne fungi have been found to affect the growth and productivity of crop plants including legume and cereal seeds.<sup>1&2</sup> Presence or absence of seed-borne fungi on seed surface is one of the important aspects that determines the quality of seeds. Seed-borne pathogens causes seed rot, germination failure and seedling mortality are the causes for the reduction in the crop production and results enormous losses to our crop. The infected seeds may fail to germinate and they may, transmit disease from seed to seedlings and from seedling to growing plants.<sup>3</sup> Some important seed-borne fungi of pulses which caused reduction in seed germination and they germinate poorly and could be a major source of inoculums for new crops raised from them.<sup>4</sup>

Aflatoxins are poisonous carcinogens that are

produced by certain molds. When contaminated food is processed; aflatoxins enter the general food supply where they have been found in both pet and human foods, as well as in feed stocks for agricultural animals. Children are particularly affected by aflatoxin exposure, which is associated with stunted growth,<sup>[3]</sup> delayed development,<sup>[4]</sup> liver damage, and liver cancer. This work was designed to identify the seed-borne fungi and their bio-control by medicinal plant extracts.

## Materials and methods

### Collection of seed samples

Stored seeds of Rice (*Oryza sativa* L. Poaceae) and Pigeonpea (*Cajanus cajan* (L.) Millsp. Fabaceae) were collected from Triprayar market, Thrissur, Kerala.

### Detection and identification of seed-borne fungi of stored seeds

The samples of Rice and Pigeon pea were assayed for the presence of seed borne

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fungi by using (A) Agar plate method (B) Blotter method as recommended by ISTA (1966)<sup>5,6,7&8</sup>

#### Surface sterilization of seeds

Stored seeds were surface sterilized using 0.1% HgCl<sub>2</sub> (Mercury chloride) for four minutes, rinsed with distilled water for 3-4 times and dried between sterilized filter papers.

#### Agar plate method (PDA method)

25 ml of sterilized PDA medium was poured in a pre-sterilized borosil glass Petri-plates. <sup>9</sup> The Petri-plates were allowed to cool at room temperature 33±0C; then 1 gm of sterilized seeds were plated at equal distances in triplicates and unsterilized seeds were kept as control under aseptic condition. Plates were incubated at room temperature for seven days. On eighth day the seeds were examined under stereoscopic binocular.

#### Moist blotter plate method (BPT)

In moist blotter plate method; the sterilized seeds were placed on water soaked three layered white blotter papers of 8.0 cm diameter and placed in pre sterilized borosil glass petri-dishes. 1 gm sterilized seeds were placed at equal distance in triplicates and unsterilized seeds keep as control in moist blotter paper under aseptic condition. The triplicates were incubated at room temperature 33±0C for seven days. On seventh day the seeds were examined under microscope for the preliminary determination of seed mycoflora. The seed-borne fungi found on each and every seed were isolated and identified, brought in to pure culture and maintained on PDA plates.

In both Agar plate method and Moist blotter method examine the frequency of seed-born fungi during incubation. The result was expressed in percentage.

Frequency of occurrence % =

$$\frac{\text{No. of seed on which fungal species occurs}}{\text{Total no. of seeds}} \times 100$$

#### Preparation of spore suspension and suspension culture:

Spore suspension of dominant seed-borne fungi, *A. terreus* and *A. niger* were prepared separately by adding 10 ml of distilled water in to the sporulating pure culture of seed-borne fungi, maintained on PDA slants for seven days at room temperature 33±0C. The slants were shaken and content filtered through muslin cloth to separate mycelium and spore. The filtrate thus obtained was used as spore suspension. 1 gm of test samples were dipped in the spore suspension prepared from the dominant seed-born fungi for few minutes and placed on the water soaked three layered blotter paper in a pre-sterilized Petri-dishes. The plates were incubated at room temperature 33±0C for seven days. Observed the growth of fungus on test samples.

#### Preparation of Medicinal plant extracts and Effect of medicinal plant extracts on incidence of fungi:

Four fresh samples were used in this study. Samples were Onion bulb, Garlic Bulb, Ginger rhizome and Thulasi Leaves. Water extract of fresh samples were prepared as follow: 10 gm of each plant material were cut in to small pieces using a sharp knife. The cutting plant material was mixed with 25 ml of distilled water by using pestle and mortar. The homogenate was placed in a closed container and was left for 48 hours. <sup>10</sup>After soaking of seeds in medicinal plant extracts for half an hour, they were placed in sterilized Petri-plates on three layer of blotting paper water soaked in distilled water. Each dish contains 1 gm of seeds. Then the Petri-dishes will be kept at 33±0c and data will be recorded seven days after sowing. The percentage of fungal infection and their effects on growth were observed.

## Result and Discussion

Seven fungal species were identified on the basis of colony colour and morphological features from rice and pigeon pea seeds through Agar plate method (PDA) and Blotting paper method (BPT). Dominant fungal species are *Aspergillus niger*, *A. fumigates*,

*A. terreus* , *A. Flavus* , *Rhizopus nigrican*,  
*R. Stolonifer* , *R. oryzae* . The present work reveals the dominance of Aspergillus and Rhizopus sps. (Table .1)

Table 1. Fungal isolates from stored seeds

Sl. No.	Seed sample	Isolated seed mycoflora						
		<i>A.niger</i>	<i>A.fumigatus</i>	<i>A.terreus</i>	<i>A.flavus</i>	<i>R.nigricans</i>	<i>R.stolonifer</i>	<i>R.oryzae</i>
1	Rice	✓	✓	✓	x	✓	✓	✓
2	Pigeon pea	✓	x	✓	✓	✓	✓	x

Note: ✓ Pesent, x Absent

In the present study, the incidents of seed mycoflora were more in controlled seeds than the sterilized seeds. pigeon pea 100 % of incidents of fungi were observed in both types of seeds kept as control and that were subjected to the above mentioned methods . Where as in rice seeds, the percentage of fungal infection were 71.6 % in sterilized seeds of agar plate method and 100 % in control respectively. But in blotter plate method, the infection rate was 15.56 % and 16.7 % observed in sterilized as well as control seed respectively. (Table 2 & Fig 1)

Table.2: Comparative incident of seed borne fungi on different seed in PDA and BPT culture method in percentage

Sl. No.	Seed sample	Incidents of seed borne fungi (%)			
		Agar plate method		Blotter plate method	
		Sterilized seed %	Control seed%	Sterilized seed%	Control seed%
1	Rice	71.6	100	15.56	16.7
2	Pigeon pea	90	100	90	100

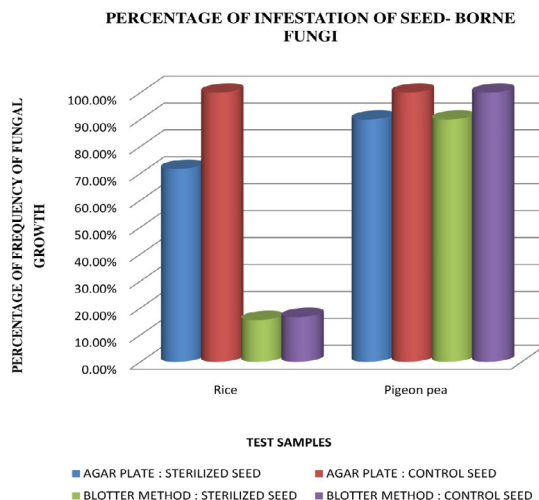


Fig. 1. Comparative incident of seed borne fungi on different seed in PDA and BPT culture method in percentage

Table. 3: Frequency of fungal infection in spore suspension method in percentage

Sl. No.	Seed sample	<i>A. terreus</i>	<i>A. niger</i>
		Fungal incidents	Fungal incidents
1	Rice	100%	100%
2	Pigeon pea	100%	80%

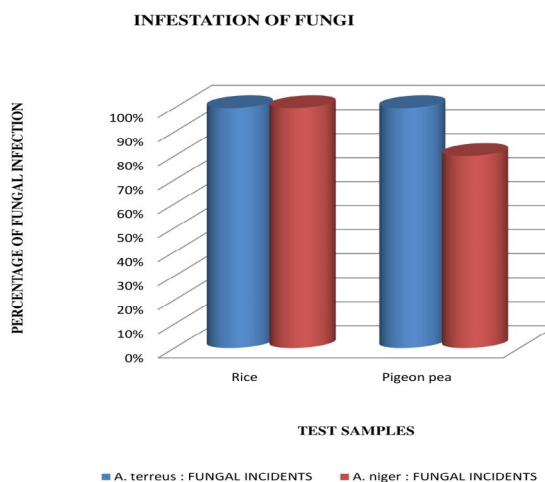


Fig 2. Frequency of fungal infection in spore suspension method in percentage  
*A. terreus* and *A. niger* were commonly found on both seeds. They were isolated and their spore suspensions were prepared. Test

seeds were soaked in the above two suspensions for few minutes and cultured on blotting method. After the treatment of seeds in spore suspension of *A. terreus*, 100% fungal infection is observed in Rice and pigeon pea. Seeds after the treatment of *A. niger* suspension, exhibited 100% infection in both test samples. (Table 3 and Fig 2)

Water extract from four fresh samples onion bulb (*Allium cepa* L. - Amaryllidaceae), garlic bulb (*Allium sativum* L. - Amaryllidaceae), ginger rhizome (*Zingiber officinale* Roscoe.- Zingiberaceae), ocimum leaves (*Ocimum tenuiflorum* L. - Lamiaceae) were prepared and seeds were treated in it as thereafter the frequency of fungal infection was observed after medicinal plant extract treatment. The anti fungal activity of each extract on different samples was tabulated (Table 4 and fig 3).

Table.4. Frequency of fungal incidents after medicinal plant extract treatment

Sl. No.	Test sample	Onion	Garlic	Ginger	Thulasi
		Frequency of fungal incidents	Frequency of fungal incidents	Frequency of fungal incidents	Frequency of fungal incidents
1	Rice	40%	-	-	30%
2	Pigeon pea	50%	-	5%	60%

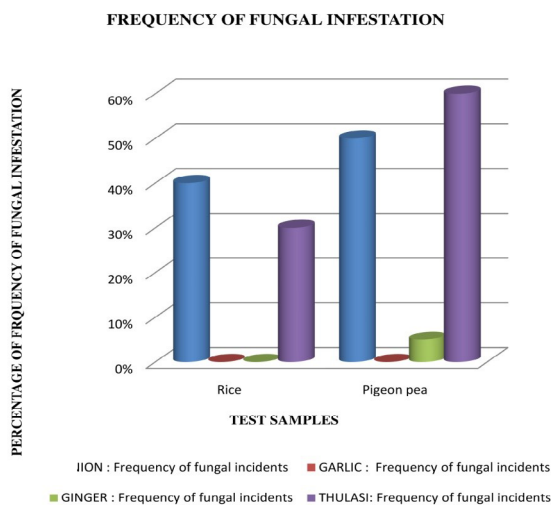


Fig. 3. Frequency of fungal incidents after medicinal plant extract treatment

No fungal infection was observed in seeds, soaked in Garlic extract unlike other three. Seeds treated with Onion extract showed maximum rate of fungal invasion.

Healthy seeds play a vital role in the production of healthy crops. Healthy seed is the foundation of healthy plant; necessary conditions for good yields.<sup>11</sup> Many pathogenic fungi are seed transmitted, often reduce the germination ability or killed the infected plants or substantially reduce the productivity. Therefore, control of seed-borne fungi is extremely important and the damaging effects can be revealed through integrated approaches.<sup>12</sup> This study was conducted to determine the prevalence of seed borne fungi of stored seeds were collected from the Tripprayar market for the experiment, different methods were carried out simultaneously employing BPT and PDA method.

Seed health test in two methods yielded seven different fungi *Aspergillus niger*, *A. fumigatus*, *A. terreus*, *A. flavus*, *Rhizopus nigricans*, *R. stolonifer*, *R. oryzae*. A considerable number of seed borne fungi belonging to the two genera *Aspergillus* and *Rhizopus*.<sup>13</sup>

All the seven fungal species were observed in both methods. In PDA culture availability of nutrients continuously along with moisture may be the reason for increased fungal growth.

The present work reveals that after treatment in the both seeds, spore suspension of *A. terreus* and *A. niger* possess 100% infection. Deteriorations of seeds by fungi is due to unhygienic condition of storage and this in turn is associated with initial high moisture content of stored products or absorption of moisture during storage due to defects in the storage system.<sup>15</sup> In present study, the predominant fungi can cause deterioration in stored seeds and reduce the seed quality.

After medicinal plant extracts treatment, showed reduction in seed borne fungus. Similar studies were conducted in

various ways to control seed borne fungi in biological method.<sup>16</sup> intensive.

This study revealed that Ocimum and Garlic are most suitable plants to make extract to control fungi pathogen. Because it had high antifungal activity and make the seed fungal free after treatment. According to Zakaria., 2014.<sup>17 & 18</sup> all samples extracted from fresh samples, only Garlic bulb exhibited the highest anti-fungal activity against isolated fungi. The most abundant sulfur compound in Garlic is allicin (S-allylcysteine sulfoxide), which is present at 10 mg/g fresh Garlic or 30 mg/g dry weight, water extract of Ginger also suitable one and exhibited anti-fungal activity against all tested fungi.<sup>19</sup> Reported that the gingerols and gingerdiol are the main anti-fungal activity against all tested fungi.<sup>20</sup>

Onion extract treated seeds were contaminated by fungus when compared to other plant extract . . Medicinal plant extract preparation is very simple and economically and environment friendly approach. 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

Thus, there is a need to search for alternative approaches to store seeds, cereals for human consumption without toxicity problems that are eco friendly and not capital

## Conclusion

Seed-borne fungi appear in sterilized and unsterilized condition. They cause toxicity in human consumption. Moisture rich condition is favorable for their growth and their occurrence on stored seed adversely affect the germination rate and also inhibit the plant growth and also adversely affect the productivity of crop. High seed quality is essential in any crop production venture to attain higher yield and good quality products. Present work reveals that aqueous medicinal plant extracts have antifungal property. The extract obtained from Ocimum, Garlic and Ginger plants. They are more suitable alternatives to minimize the seed-borne fungus on the stored seeds. It is a less expensive and an eco-friendly approach to reduce the severity of seed-borne fungus in agriculture and human consumption. This study recommends the use of natural plant extract because they are safer than chemical fungicides. The use of these extracts cans also increase seed germination during agriculture. Ocimum, Garlic and Ginger extract are recommended as anti-fungal agent for preserving seeds.

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