MYCOFLORA ASSOCIATED WITH BLUE PINE SEED

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Abstract

A study of 500 blue pine seeds, each collected from Bhurban and Kuzagali, revealed the occurrence of seven fungi and one bacterium on the sample seeds by using the agar plate test method. The Bhurban sample was found to have 80% and 20% infection in non-treated and treated ($HgCl_2$) seeds respectively, while the Kuzagali sample had 100% in the non-treated and 3.3% infection in the treated seeds. The fungi isolated from non-treated seeds comprised: *Diplodia pinea*, *Fusarium moniliforme*, *Penicillium canadense* and *Rhizopus nigricans* whereas those from treated seeds included; *Aspergillus flavus*, *A.janus*, *Rhizopus nigricans* and *R.oryzae*. *Rhizopus nigricans* was the predominant fungus occurring on non-treated as well as treated seeds. *Xanthomonas campestris* was the only bacterium isolated from the non-treated seeds.

Introduction

Blue pine (*Pinus wallichiana*), one of the most valuable species of Pakistan, occurs naturally throughout temperate zone mainly between 2000 and 3000 meters, though sometimes extending beyond these altitudinal limits. At lower elevations, it is associated with chir pine (*Pinus roxburghii*) and at the higher with deodar (*Cedrus deodara*), spruce (*Picca smithiana*), fir (*Abies pindrow*) and broad-leaved species. The wood is normally used for buildings specially interior work, furniture and general carpentry. Apart from its forestry importance, the species adds beauty to the landscape, particularly in tourist resorts.

Artificial regeneration through direct sowing of seeds is preferred to transplanting nursery-raised stock. The seeds sometimes may carry with them harmful fungal pathogens. The infections may not occur on all the seeds. Seeds may also be infected during storage and subsequent handling prior to planting.

Fungi attacking seed in storage usually invade seeds after harvest and cause loss of viability by infecting the embryo. Severely infected seeds in which embryo is affected will not germinate. In the field, such an infection is the culmination point and the disease may disappear along-with the seed. Slightly infected seed will, however, germinate to serve as infection centre from which the disease will spread in the field causing significant loss. The pathogens may be carried within or on the seed, on cones or on inert matter like chaff and straw accompanying the seed.

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No information is available on mycoflora of blue pine seed in Pakistan (Ahmed *et al.*, 1993). However, a list of seed-borne plant diseases including those of forest trees (Noble and Richardson, 1968) and some common moulds attacking seed in storage (Christensen, 1973) have amply shown that a couple of important pathogens such as *Diplodia pinea* and *Lophodermium pinastri* recorded on pine seeds have been transferred through seeds, causing new outbreak of diseases.

Keeping in view the losses reported from the attack by seed-borne fungi during storage, on seedlings and resulting stock, the present study was undertaken to determine the incidence of fungi on blue pine seed and to provide basis for effective control against destructive pathogens.

Material and Methods

The seeds of blue pine, collected from Bhurban (Punjab) and Kuzagali (NWFP) during the year 1992, were obtained from the Tree Seed Centre, Pakistan Forest Institute; Peshawar stored at room temperature.

Agar plate test method was employed for the isolation of fungi borne in or on the seed surface. The composition of the medium was as follows:

Potato dextrose agar (Ainsworth *et al.*, 1971)	
Bactoagar	15.0 gm
Peeled potatoes (without skin and cutup)	200.0 gm
Distilled water	1000.0 ml

The medium was prepared by mixing agar powder in the extract of potatoes. After boiling together it was strained through muslin cloth. The mixture was autoclaved at 15 Ibs pressure/sq. inch for 15 minutes and cooled down to 5°C. This was carefully poured into sterile petridishes (90 mm) by lifting the lid only enough to pour in agar to avoid contamination. It was allowed to cool and solidify for about 20 minutes, after which it was ready for use. The disks were covered with a sterile glass bell jar.

For the determination of deep seated infections, seeds were surface sterilized with 0.1% $HgCl_2$ solution for one minute. They were washed twice with distilled sterilized water to remove the surfactant. This eliminates contamination of the seed coat by saprophytes which tend to develop rapidly on the agar and this may inhibit the growth of comparatively slow growing fungi. Six seeds were plated in each petridish with a sterile forcep. Five dishes each were taken for surface sterilized (treated) seeds and seeds washed with sterile water only (non-treated). These were incubated at room temperature (27-30°C) in the Forest Pathology Laboratory.

After 7-8 days, the fungi growing around the seeds were grown into pure cultures. These were identified on the basis of growth (vegetative and spore characteristics) by using "Illustrated Genera of imperfect fungi" (Barnett, 1960) and "A Manual of Soil Fungi" (Gilman, 1957).

Results

The study of two samples consisting of 500 seeds each, indicated the occurrence of seven fungi and one bacterium when plated on Potato dextrose agar at room temperature (27-30°C) for 7-8 days. 250 non-treated seeds from Bhurban sample showed 80% infection while the equal number of treated ($HgCl_2$) seeds had 20% infection. In the Kuzagali sample, the non-treated seeds exhibited 100% infection and it was 3.3% in the treated seeds as presented in Table 1.

Table 1. Percent seed infection by fungi in two blue pine (*Pinus wallichiana*) seed samples

Seed sample	Collection year	Number of seeds tested		Infection (%)	
		(Non-treated)	(Treated)	(Non-treated)	(Treated)
Bhurban	1992	250	250	80.0	20.0
Kuzagali	1992	250	250	100.0	3.3

Seven fungi were isolated from both non-treated and treated seeds; whereas one bacterium was isolated only from non-treated seeds. The results are shown in Table 2.

Table 2. Fungal and bacterial isolates obtained from non-treated and treated blue pine seeds

Fungi and Bacterium isolated from	
Non-treated seeds	Treated seeds
Diplodia pinea	*Aspergillus flavus*
Fusarium moniliforme	*A. janus*
Penicillium canadense	*Rhizopus oryzae*
Rhizopus nigricans	*R. nigricans*
*Xanthomonas campestris**	

* Bacterium

The fungal and bacterial isolates were grown into pure cultures on Potato dextrose agar to describe their characteristics for identification as follows:

1. ***Aspergillus flavus*** Link
Class: Deuteromycetes
Order: Moniliales
Family: Moniliaceae

Colony widely spreading with flocossity limited to scanty growth of a few aerial hyphae in older areas. Conidial areas ranging in color from sea foam yellow to lime green. Reverse and agar yellow. Conidiophores arise separately from the substratum, 400-1000 μ × 5-15 μ. Heads vary from small with a few chains of conidia to large columnar masses. Conidia pyriform to almost globose, colorless to yellow-green. Sclerotia at first white, then brown, hard, parenchymatous. Cleistothecia not found.

2. ***Aspergillus janus*** Rapor and Thom
Class: Deuteromycetes
Order: Moniliales
Family: Moniliaceae

Colony spreading irregularly, consisting of a central floccose mass, 1-2 cm deep, pale yellow-buff bearing few to abundant fruiting structures, surrounded by irregular zone of crowded dark green heads adjacent to the substrate with numerous long stalked white heads projecting above this layer. Reverse dull yellow to light brown. Conidiophores long, thin, 2-3.5 mm x 8-10.5 μ in diameter. Conidia smooth, colorless, globose to subglobose, 2-2.5 μ in diameter. Green conidial heads compact, becoming olive grey in age.

3. ***Diplodia pinea*** (Desm.) Kickx
Class: Deuteromycetes
Order: Sphaeropsidales
Family: Sphaeropsidaceae

Pycnidia black, single, globose, immersed, erumpent, ostiolate, conidiophores slender, single conidia dark, 2-celled, ellipsoid or ovoid. Colour of colony white first then dark brown to black, pin head sized pycnidia measuring 136.6-200.3 μ.

4. ***Fusarium moniliforme*** Sheldon Var. minus Wollenweber
Class: Deuteromycetes
Order: Moniliales
Family: Tuberculariaceae

Pinnotal and sporodochial slime absent. Colony floccose. Microconidia in long chains or in false heads, then scattered in whitish rosy aerial mycelium, oval-spindle form, 1-2 celled. Macroconidia sparingly scattered (1%), 3-5 septate, lenciform, seldom spindle-sickle shaped, slightly curved, generally pointed at the tip, truncate at the base.

Dark blue globose sclerotia sometime present.

5. ***Penicillium canadense*** Smith
Class: Deuteromycetes
Order: Moniliales
Family: Moniliaceae

Colony spreading, thin at first, pale straw colour, darkening to pale fawn with a slight greenish cast, indistinctly zonate, with fimbriate margin. Drops not seen. Reverse uncoloured. Penicilli short, very compact, usually with four stages of branching, with the elements successively smaller in diameter. Conidiophores upto 600 μ long, stiff, with granular contents, 5-5.6 μ in diameter, but occasionally more slender and then bearing small Penicilli, conidia globose to sub-globose, smooth, 3-4 $\times$ 3-3.5 μ.

6. ***Rhizopus nigricans*** Ehrenberg
Class: Zygomycetes
Order: Mucorales
Family: Mucoraceae

Stolons creeping, recurving to the substrate in the form of arachnoid hyphae, which are strongly raised and distant from the substrate and implanted at each node by means of rhizoids. The internodes often attain a length of 1-3 cm and the hyphae are more or less branched. Sporongiophore rarely single, united in groups of 3-5 or more, 0.5-4 mm in height, 24-42 μ in diameter. Sporangia hemispheric 100-300 μ. Columellae broad, hemispheric, depressed, 70 μ in diameter by 90 μ in height. Spores un-equal, irregular, round or oval, angular, striate, 9-12 μ $\times$ 7.5-8 μ. Zygospores round, 160-220 μ in diameter. Exine brown black, verrucose. Suspensors swollen, usually unequal. Azygospore present. No chlamydospores.

7. ***Rhizopus oryzae*** Went and Gerlings
Class: Zygomycetes
Order: Mucorales
Family: Mucoraceae

Stolons creeping, recurving to the substrate in the form of arachnoid hyphae, which are strongly raised and distant from the substrate and implanted at each node by means of rhizoids. Sporangiophores rarely single, united in group of 3-5 or more, 0.5-4 mm in height $\times$ 24-42 μ in diameter. Apoplyses broad, sporangia hemispheric, 100-350 μ. Columella broad, hemispheric, depressed, 70 μ in diameter and 90 μ in height. Spores unequal, irregular, round or oval, angular, striate, 7-9 μ long. No chlamydospore.

* ***Xanthomonas campestris*** (Pammel) Dawson
Order: Eubacteriales

Cells usually monotrichous. A yellow, non water-soluble pigment is produced on agar. A diffusible, brown colour infrequently occurs in potato dextrose agar. Proteios are usually readily digested. Milk usually becomes alkaline. Hydrogen sulphide is produced. Asparagine is not sufficient as a source of carbon and nitrogen. Mostly plant pathogens causing necrosis. Rods 0.3-0.5 × 0.7-20 μ. Motile with a polar flagellum. Encapsulated. Gram-Negative.

Discussion

The study has clearly shown that the highest seed infection (100%) was recorded in non-treated seeds and the lowest (3.3%) in the treated ones. This further shows that majority of the infections were externally seed-borne. The lowest infection in the treated seed may be indicative of a few deep-seated infections (Mittal, 1983).

As many as 7 different fungi: *Aspergillus flavus, A. Janus, Diplodia pinea, Fusarium moniliforme, Penicillium canadense, Rhizopus nigricans* and *R. oryzae* were isolated from blue pine seeds. These fungi may attack the embryo causing loss of viability in the infected seeds and mortality in the seedlings. *Penicillium, Fusarium* and *Aspergillus* have been reported most frequently on spruce seed (Peno, 1983). *Rhizopus nigricans* was the fungus found infecting non-treated as well as treated seeds. This fungus has also been reported most common on various seeds of agricultural crops in Pakistan (Ahmed *et al.*, 1993). Almost all the fungi isolated from blue pine seeds have been reported by various workers on coniferous seeds (Mason and Arsdal, 1978; Urosevic, 1979; Mittal, 1983; Anderson *et al.*, 1984).

The lowest number of fungi encountered in the present study may be attributed partly to agar plate test method and partly to room temperature (27-30°C) used for incubating seed in the laboratory. The isolation of *Xanthomonas campestris*, a bacterium, from only non-treated seeds indicates that this does not cause deep-seated infection. The present study, therefore, warrants proper handling of seeds by providing suitable storage facilities and finding out effective seed dressing fungicides.

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