CAFOBIOS
Cameroon Forum for Biological Sciences

Available Online at http://www.ajol.info/browse-journals.php
and http://cajeb.ifrance.com
Cameroon Journal of Experimental Biology 2009 Vol. 05 N° 01, 21-24.

Short communication *Agronomy*

Incidence and interaction of seed borne micro flora of *Cassia fistula* in the Himalayan region

Navjeet KAUR[1], Shivesh SHARMA*[1], Anchal SOOD[1], Vivek KUMAR[2]

[1]*Department of Microbiology, Division of Life Sciences, SBS (PG) Institute of Biomedical Sciences and Research, Balawala Dehradun. 248161, (UK), (India)*
[2] *Microbiology Section, Department of Soil and Water Research, Public Authority of Agriculture Affairs & Fish Resources, PO Box 21422, Safat- 13075, Kuwait.*
*Corresponding author P: +91-9897138234 Email: dr.shiveshsharma@gmail.com

ABSTRACT
Seed samples of *Cassia fistula* from Uttarakhand and Punjab region of North West Himalayas were evaluated for occurrence of seed micro flora. Several fungal strains and bacterial isolates were isolated from seed sources of Uttarakhand and Punjab regions. Different fungal and bacterial species were identified from the external and internal seed surfaces. Among fungus, *Aspergillus* and among bacteria, *Bacillus* species were found to be dominant with the seeds of *C. fistula.* Varied pattern of percent incidence was shown by bacteria and fungi, *Aspergillus fumigatus* followed by *A. niger*, *Penicillium rubrum* and *Bacillus megaterium*, *B. cereus*, *Pseudomonas fluorescence* and *Erwinia herbicola.* were dominant species. In interaction studies the fungal species behaved antagonistically towards each other and with different bacterial strains.
Key words: *Aspergillus*, *Bacillus*, *C. fistula*, Interaction studies.

INTRODUCTION

The successful regeneration of any plant depends on the quality of seeds, their viability and vigour, which ultimately depends on the storage system for their conservation [1]. With an increasing demand for large quantities of seed to meet the growing demand of seedling for afforestation program, it is essential to screen and evaluate more and more species for their potential germination and viability [2].

One of the factors responsible for reducing the longevity of seeds is seed-borne micro-organisms. Seed borne micro-organisms may reduce germination and seed longevity in storage of all types of seeds. The micro-organisms that are mostly associated with tree seeds are fungi, bacteria and to lesser extent, viruses. The seed borne micro-organisms include the pathogens causing plant diseases, the so called 'field fungi' or 'storage fungi'. Typical examples include *Penicillium spp*., *Aspergillus spp*. Colonization by *Aspergillus*, *Penicillium* and *Fusarium* among others is mainly responsible for production of toxic substances like aflatoxins, ochratoxins, citrinin and zearalenone etc. [3].

Cassia fistula is one among these plants. It is found throughout India in deciduous forests. It is also cultivated as an ornamental tree for attractive yellow blossom in racemes. It is drought resistant and suitable for growing in different areas.

There is scanty information pertaining to seed micro flora of *C. fistula* and considering the significance of this tree, a study on seed borne micro flora was undertaken in different seed sources of Uttarakhand and Punjab of North West Himalayan region.

MATERIALS AND METHODS

Collection of seeds

Seeds were collected from areas of Punjab and Uttarakhand. Trees were selected in the months of Feb/March, at the time of maximum pods. Physiologically mature pods of the tree were collected and mixed properly to maintain uniformity in seeds to be extracted. The collected pods were spread on clean floor for sun drying for 1-2 days and seeds were extracted manually by beating and sieving. After cleaning seeds were further dried for a day and stored in cool and dry place in butter paper bags till the experiment is over.

Detection of seed borne micro flora

For externally seed-borne fungi and bacteria, 20 seeds were plated on potato dextrose agar plate without surface treatment where as for internal seed

borne fungi and bacteria seeds were first washed on running tap water to remove surface contaminants. After thorough washing the seeds were surface sterilized with 0.1 % mercuric chloride solution for 2 minutes and immediately rinsed on sterilized water for 3-4 times to remove all traces of mercury. Excess water of seeds was absorbed by putting the seeds in sterilized blotter paper by forceps. Twenty seeds per plates were placed at equal distances. The plates were incubated for 7 days in an incubator at 25° ± 1°C for fungi and 24-72 h at 37°C for bacteria. The microbial colonies were purified and identified using standard methods and references. The per cent incidence of each micro-organism was calculated using the following formula:

$$\% \text{ Incidence} = \frac{\text{Number of seeds recorded with and organism}}{\text{Total number of seeds examined}} \times 100$$

Interaction Studies

Among fungal isolates

The microbial strains growing in close proximity and making inhibitory zones on plates were tentatively classified as antagonistic agents. For interaction among fungal isolates, 8 mm disc of mycelium was placed in potato dextrose agar plates and another 8 mm disc of second culture was placed at a distance of 2 mm and observed after 3-4days of incubation.

Among fungal and bacterial isolates

For testing antifungal activity, a test fungus was streaked on yeast extract mannitol agar plates. After 4 days of incubation bacterial isolates were inoculated by using ribbon streak method on these plates. For antagonism experiments the yeast extract mannitol agar was found appropriate as it supported the growth of fungi as well as bacteria. Following incubation at 28^0C for 2-4 days, the plates were observed for zone of inhibition.

RESULTS AND DISCUSSION

In the present study, it was observed that a wide variety of fungal and bacterial species occurred on the seeds of *C. fistula*. A perusal of Table 1 shows the type and percentage of occurrence of different microbes externally and internally. From the seed sources of Uttarakhand, *Aspergillus fumigatus* was found to be most dominant i.e. 75% among both external and internal borne fungi compared to *Penicillium* and *Trichoderma*. *Aspergillus luchensis* (25 %) and *A. flavus* (20 %) dominated internal fungal flora followed by *Penicillium* and *Aspergillus*. Association of fungi as well as their incidence is governed by the nature of the substrates, method of storage and prevailing environmental conditions. Some seed borne fungi may produce aflatoxins and *A. flavus* is one of them. The results of the present investigation draws support from the findings of [4,5,6,7].

Table 1: Percent (%) incidence of fungi and bacteria isolated from different seed sources.

Fungi	Uttarakhand	Punjab
	External	
Aspergillus fumigatus	75±8.16	15±2.41
Aspergillus niger	10±0.81	30±2.48
Aspergillus flavus	-	20±0.40
Penicillium waksmani	10±1.63	10±4.84
Aspergillus funiculosus	-	10±2.04
Aspergillus clavatus	5±0.40	15±2.04
Bacteria		
Pseudomonas fluorescence	20±2.44	65±3.09
Bacillus megaterium	45±4.08	15±2.95
B. cereus	25±1.63	-
Erwinia herbicola	5±1.63	10±3.21
Xanthomonas	5±2.04	10±0.40
Fungi	Internal	
Aspergillus luchensis	25±4.92	20±0.81
Penicillium rubrum	15±2.90	35±3.21
Trichoderma	10±3.20	5±0.40
Aspergillus flavus	20±1.60	15±2.44
Penicillium waksmani	15±2.05	15±1.63
Aspergillus terrus	15±1.60	10±1.25
Bacteria		
B. megaterium	35±2.40	30±3.26
Erwinia herbicola	20±3.21	15±1.63
Achromobacter	5±1.63	10±1.25
Acinetobacter	20±1.63	35±5.71
Brevibacterium	10±3.21	5±1.63
Xanthomonas	10±1.24	5±0.16

Values are Mean ± SD of three determinations.

Externally among bacteria *B. megaterium* was found to be dominant (45 %) and 35 % internally, followed by *B. cereus* (25 %) externally which was not detected internally. The other internal bacteria were *E. herbicola*, *Acenitobacter* and *Brevibacterium* (20 %). *Pseudomonas fluorescence* dominated the Punjab seed samples externally (65

%) followed by *E. herbicola* (20 %) and *B. megaterium* (15 %). The internal bacterial flora was dominated by *Acinetobacter* (35 %) followed by *B. megaterium* (30 %) and other. Other workers also studied the external and internal dominance of bacterial flora of different plant seeds [8,9,10].

Table 2: Interaction studies among fungal isolates

Punjab Seed Source	
Antagonistic Fungal Strains	Sensitive Isolates
A. flavus	*A. clavatus*
Trichoderma	*A fumigatus, A.niger*
A. flavus	*A.niger*
A.niger	*A.clavatus*
Uttarakhand Seed Source	
A. terrus	*Penicillium rubrum*

Regarding interaction studies among fungus, it is evident from data (Table 2), that most of the species behaved antagonistically towards each other. From the seed source of Punjab both *A. flavus* and *Trichoderma* showed antagonistic behavior towards *A. niger*, while both *A. niger* and *A. flavus* inhibited the growth of *A. clavatus*. *Trichoderma* was found to inhibit two species i.e. *A. fumigatus* and *A. niger*. While from the seed source of Uttarakhand only one fungal species i.e. *A. terrus* showed antagonistic behavior towards *P. rubrum*. *Aspergillus niger* was found to inhibit the growth of bacterial strains *Brevibacterium* and *E. herbicola* and *Trichoderma* showed the antagonistic behavior towards *Xanthomonas*, *E. herbicola* and *Achromobacter* in seed sources of Punjab. While from other seed source, *A. luchensis* inhibited *E. herbicola* and *Achromobacter*, *A. niger* behaved antagonistically against only *Acinetobacter*. The fungus *P. rubrum* inhibited *Achromobacter* and *Xanthomonas* (Table 3). Our results draw support from [11,12], who reported the antagonistic behavior of rhizosperic mycoflora of tea with dominant bacteria. Rhizosphere mycoflora was environmentally competent due to better adaptation (high pH and temperature tolerance) and survival in rhizosphere.

The present study lead to understanding of common myco and bacterial flora and their interaction in seed sources. Further studies are required, particularly regarding how the soil and spermosphere microbial community interact, and how these interactions might be useful for seed survival and effective biological control.

ACKNOWLEDGEMENTS

Authors are thankful to the Management of SBS Post Graduate Institute of Biomedical Sciences & Research, Balawala, Dehradun for providing all necessary facilities required to carry out this study. We are also thankful to Dr. Rajeev Shrivastava (Head, Division of Silviculture, FRI) and Dr. Harsh, Forest Research Institute, Dehradun for identifying some of the fungal isolates.

Table 3: Interaction studies among fungal and bacterial isolates

S.No.	Punjab Seed Source	
	Antagonistic Fungal Strains	Sensitive bacterial Strains
1	*A.niger*	*Brevibacterium*, *E. herbicola*
2	*Trichoderma*	*Xanthomonas*, *E. herbicola, Achromobacter*
	Uttarakhand Seed Source	
1	*A. luchensis*	*Erwinia herbicola, Achromobacter*
2	*P. rubrum*	*Achromobacter, Xanthomonas*
3	*A.niger*	*Acinetobacter*

REFERENCES

1. Atul, Sharma, S., Punam. 2002. Germination studies of some economically important nitrogen fixing tree species of Himalayas. *Indian Journal of Forestry.* 25 (1):104-108.
2. Wang, B.S.P. 1991. Tree and shrubs seeds. *Advances in Research and Technology in seeds*. (Ed: W.T. Bradnoc). Dehradun. pp 34-43.
3. Harsh, N.S.K., Gupta, B,N. 1993. Root diseases in forest nurseries of MP and their control. Tropical Forest Research Institute, Jabalpur, India. pp31.
4. Uniyal, K., Uniyal, D.P. 1996. Mycoflora of New seeds. *Indian Forester*. 122 (5): 423-427.
5. Singh, K.P., Khan, S.N. 2001. Mycoflora and Mycotoxins in medicinal fruit/seed of forestry origin. *Indian Forester*. 127 (7): 770-776.
6. Sahu, R.K.., Agarwal, V.K. 2004. Mycoflora associated with the seeds of *Leucaena leucocephala*. *Indian Forester.* 127: 1040-1044.
7. Singh, K.P., Shukla, A.N. 2005. Studies on mycoflora and mycotoxin infestation in *Shorea robusta* gaertn. *Indian Forester*. 131: 1227-1234
8. Outryvie, V., Gossele, V., Gossele, F., Swings, J. 1988. Composition of the micro flora of Witloof chicory seeds. *Microbial Ecology*. 16: 339-348.
9. Jindal, K.K., Thind, B.S. 1990. Microflora of cowpea seeds and its significance in the biological control of seedborne infection of *Xanthomonas campestris* pv. Viginicola. *Seed Science and Technology*. 18: 393-403.
10. Leichtfried, D., Krist, S., Puchinger, L., Messner, K., Buchbauer, G. 2004. Investigations of the natural micro flora of poppy seeds (*Papaver somniferum*) and hazelnut kernels (*Corylus avellana*) including microbiological decomposition. *European Food Research and Technology*. 219: 282-285.
11. Singh, S., Sood, A., Sharma, S., Kumar, V., Singh, K.D., Pandey, P. 2007. Studies on rhizospheric mycoflora of tea (*Camellia* sinensis) : *In vitro* antagonism with dominant bacteria. *Chinese Journal of Applied and Environmental Biology.* 13 (3): 357-360.
12. Sood, A., Sharma, S., Kumar, V. 2007. Comparative efficacy of diffusible and volatile compounds of tea rhizospheric isolates and their use in biocontrol. *International Journal of Biological and Chemical Sciences*. 1: 28-34.