J. Mycopathol, Res, ***50(1)*** *: 77-80 (2012)*
© Indian Mycological Society, Department of Botany,
University of Calcutta, Kolkata 700 019, India

Seasonal variation of seeds mycoflora associated with Oak tasar host plants (*Quercus* spp.) in Manipur

N. G. CHANU.T AND G. K. N. CHHETRY
Department of Life Sciences, Manipur University, Canchipur-795003, Imphal, Manipur

Received : 31. 05. 2011 Accepted : 16. 11. 2011 Published : 30.04.2012

As many as fourteen seed mycoflora were isolated using Agar plates and Blotter methods. Seasonal variation of seed borne mycoflora was observed in both the methods. Blotter method was superior over agar plate method in all the three seasons. Fungal species associated with the seed surface were more during summer and least in winter for both varieties of *Quercus* spp, namely *Quercus serrata* and *Quercus griffitti*. Percentage frequency occurrence of *Aspergillus niger* and *Penicillium notatum* was relatively more as compared to other seed mycoflora of all the seeds and it also showed decreasing trend from summer to winter season.

Key words : *Quercus* spp., seed mycoflora, *Quercus serrata, Quercus griffitti*, seasonal variation, Agar plates methods, Blotter methods

INTRODUCTION

Seed is the first and primary requisite either in agriculture or in forestry. The maintenance of good health of seed is a prerequisite for the production of healthy crops. Most of the seeds of angiospermic plants are vulnerable to the attack of microorganisms mostly fungi. As such seed health is important in preventing or controlling certain crop diseases from damaging seedling and ensuring good field establishment. Seed in most of the plant are known to carry one or more pathogen internally or externally. It is called seed borne pathogen and disease caused by it is a seed borne disease. The pathogen may be preventing in the surface of the seed or inside the seed or may be look with the seed. This three types of association of pathogen with the seeds are termed differently as infested, infected and a mixture (Sastry *et.al.,* 2004).

The pathogen may be present anywhere in the seed, in the seed coat, endospern or embryo. The surface of the seed coat is common site of infection for most of the seed transmitted fungi. The damage caused by seed borne fungi includes failure of germination, seedling blight and rot and manifestation of many disease symptoms on adult plants. This point of seed infestation by fungi includes not only the agricultural crops but also the plants related to forestry. *Quercus* seeds are not exception to this and the seeds of *Quercus* spp. are vulnerable to the infestation of many fungi including pathogen. As the *Quercus* seedling are raised from the seeds usually collected from the fallen seed on the soil surface, maintaining healthy seeds free from microbial infestation is an important for a healthy production of *Quercus* seedling.

In view of this present investigation is aimed at seasonal variation of seeds mycoflora associated with Oak tasar host plants (*Quercus* spp.) in Manipur.

MATERIALS AND METHODS

All the seeds samples were collected from forests where *Quercus serrata* and *Quercus griffitti* are naturally grown. Sufficient seed samples were collected in a sterile polythene bag at the time in bulk for the whole period of investigation. The composite samples drawn from the top, middle, bottom and side of bulk storage were used for the assessment of seed mycoflora. While drawing samples from the bulk storage, International Rules for Seed Testing Association (ISTA, 1966) were followed. Care was taken to avoid contamination during sampling by hands. Seed surface mycoflora was assessed seasonally (summer, rainy and winter) for two consecu-

E-mail: gkn2004@yahoo.co.in.

tive years (2006-2008) using the following two standard methods.

Standard blotter methods as recommended by International Seed Testing Association (ISTA, 1966) with certain modifications was used for detection of fungi. In this method three pieces of sterile moisture filter papers with sterile water were placed in a sterile Petridish (9.50 cm dia) containing 5 equispaced seeds in each Petridish. They were incubated at 25±1°C for seven days. One hundred randomly selected seeds were taken for each variety. Plating of seeds was carried out in a laminar flow chamber.

The agar plate method as proposed by the International Seed testing Association (1966) was followed. 5 out of hundred randomly selected seeds of each variety were equispaced using sterile forceps in a Petridish (9.50 cm dia) containing potato dextrose agar (20 ml) and incubated at 25±1°C for 5 days.

Isolated fungi from seeds were grown in pure culture on PDA slants for 5 days. Fungi associated with seeds were observed under microscope. Identification was done in pure culture following the keys and manual provided by different workers (Subramanian, 1971; Barnett and Hunter, 1972).

The percentage frequency of occurrence of individual fungus was calculated using following formula : Percentage frequency of occurrence = Total number of individual fungus / Total number of all the species of fungus x 100.

The quantitative data so obtained were the average of two years. The qualitative and quantitative data were presented

RESULTS AND DISCUSSION

A total of fourteen species of fungi belonging to eight genera were isolated from seeds of Oak tasar host plants viz., *Quercus serrata* and *Quercus griffitti* using Agar plates and Blotter methods. More number of fungi were detected using Blotter method than Agar plate method in all the three seasons i.e., summer, rainy and winter.

Seasonal variation of seed mycoflora was observed in both the techniques (Table 1). Isolation of total numbers of fungal species showed a decreasing trend from summer to winter season for two varieties *Q. serrate* and *Q. griffitti*. The various fungal species detected in different seasons by two methods may be categorized into the two categories. (1) 'A' –fungi occurring in all the seasons. (2) 'B'-fungi detected only in a particular season.

Aspergillus flavus, Aspergillus niger, Fusarium solani, Penicillium notatum, Trichoderma spp. etc. came under the category 'A' and *Alternaria alternata, Curvularia lunata, Collectrotrichum* sp. *Penicillium citrinum* etc. came under category 'B'. *Penicillium citrinum* was restricted to summer season, *Alternaria alternata* was restricted to rainy seasons and *Curvularia lunata* to winter season only as presented in Table 2.

Variation of total number of fungal species among the two varieties also showed that maximum number of fungi were isolated from *Quercus griffitti* and least in *Quercus serrata* by both the techniques when all the seasons were considered.

Most of the fungi were detected in all the varieties during summer season and least in winter season by both the methods. This variation among the varieties were clearly depicted together with season and technique.

Screening of seed mycoflora of Oak tasar plants *Quercus serrata* and *Quercus griffitti* using Blotter and Agar methods during summer, rainy and winter season depicted that summer season was more reliable for the growth of seeds mycoflora compared to rainy and winter seasons.

While comparing fours laboratory methods for detection of carrot seed mycoflora, De Tempe (1964) obtained reliable result from the Blotter test experiment and consequently recommended the Blotter test as being reliable, simple and cheap.

In the present investigation, seasonal variation of seed borne mycoflora was observed during summer, rainy and winter seasons. Seasonal changes were statistically significant. Gradual decline of fungi with seasonal changes as observed in the present investigation would possibly be due to storage of seed bulk sample in the laboratory condition.

Qualitative and quantitative differences of fungi between two varieties could possibly be attributed to their physico-chemical nature of the seeds, storage, sampling collection. Isolation of as many as

Table 1 : Seasonal occurrence of seed mycoflora of *Quercus* spp. namely *Quercus serrata* and *Quercus griffitti*

Fungi	Summer season				Rainy season				Winter season			
	Quercus serrata		*Quercus griffitti*		*Quercus serrata*		*Quercus griffitti*		*Quercus serrata*		*Quercus griffitti*	
	Agar M.	Blotter M.	Agar M.	Blotter M.	Agar M.	Blotter M.	Agar M.	Blotter M.	Agar M.	Blotter M.	Agar M.	Blotter M.
Alternaria alternata	-	-	-	-	+	+	+	+	-	-	-	-
Aspergillus flavus	+	+	+	+	+	+	+	+	+	+	+	+
A. fumigatus	+	+	+	+	-	+	+	+	-	+	+	+
A. niger	+	+	+	+	+	+	+	+	+	+	+	+
Cladosporium cladosporodes	-	+	-	+	-	+	-	+	-	+	-	+
Curvularia lunata	+	+	+	+	-	-	-	-	+	+	+	+
Fusarium oxysporum	+	+	+	+	+	+	+	-	-	-	+	+
F. fusaroides	-	+	+	+	-	+	-	-	-	+	-	-
F. solani	+	+	+	+	+	+	+	+	+	+	+	+
F. semitectum	-	+	-	+	-	+	-	+	-	-	-	+
Collectrotrichun sp.	-	+	+	+	-	-	-	-	-	+	-	-
Penicillum notatum	+	+	+	+	+	+	+	+	+	-	+	+
Penicillum citrinum	+	+	+	+	-	-	-	-	-	+	-	-
Trichoderma sp	+	+	+	+	+	+	+	+	+	-	-	-

+ Presence of fungal species
– Absence of fungal species

Table 2: Percentage frequency occurrence of fungal species isolated from two varieties of oak tasar host *Quercus serrata* and *Quercus griffittii* in three seasons (summer, rainy and winter) using Blotter and PDA methods.

Fungal species	Summer season				Rainy season				Winter season			
	Quercus serrata		*Quercus griffittii*		*Quercus serrata*		*Quercus griffittii*		*Quercus serrata*		*Quercus griffittii*	
	Agar M.	Blotter M.	Agar M.	Blotter M.	Agar M.	Blotter M.	Agar M.	Blotter M.	Agar M.	Blotter M.	Agar M.	Blotter M.
Alternaria alternata	-	-	-	-	4.35	3.45	2.44	5.62	-	-	-	-
Aspergillus flavus	11.55	17.62	13.52	24.67	5.06	10.15	8.15	9.36	4.65	7.20	5.62	5.00
A. fumigatus	7.12	16.46	9.20	19.04	-	3.15	3.05	5.65	-	1.70	2.15	3.61
A. niger	22.69	24.16	17.62	26.15	14.15	11.15	8.00	12.115	12.15	9.62	9.15	5.62
Cladosporium cladosporoides	-	4.20	-	5.67	-	2.15	-	2.65	-	1.22	-	3.15
Curvularia lunata	3.17	5.62	3.61	4.06	-	-	-	-	2.75	1.65	2.46	0.62
Fusarium oxysporum	6.34	7.15	5.61	8.15	3.45	3.20	2.10	-	-	-	2.46	4.02
F. fusaroides	-	5.24	3.61	3.65	-	2.15	-	-	-	3.19	-	-
F. solani	7.21	2.61	5.12	5.62	3.65	5.60	2.45	4.20	4.20	2.65	3.05	2.15
F. semitectum	-	3.67	-	2.67	-	4.20	-	3.65	-	-	-	1.02
Collectrotrichum sp	-	4.46	2.45	5.36	-	-	-	-	-	15.20	-	-
Pencillium notatum	15.11	22.65	12.15	29.41	10.16	20.15	19.40	24.62	6.95	-	12.15	15.15
P. citrinum	1.20	2.40	4.65	2.49	-	-	-	-	-	5.20	-	-
Trichoderma sp	11.60	10.15	12.12	21.42	10.40	7.65	7.12	10.15	7.15	-	6.21	11.15
Total	78.34	132.39	89.66	158.36	53.37	73.00	54.57	84.015	39.07	47.69	44.50	49.54

Average of two years (2006-2008)

fourteen fungi without any pathogen showed that *Quercus* seeds are free from pathogenic fungi in all two varieties though seeds of *Quercus* spp. harvoured quite a good number of saprophytic fungi. Similar observation was made while observing the seeds mycoflora of French bean.

REFERENCES

Barnett H.L and Hunter, B.B. 1972. *Illustrated genera of Imperfect fungi.* 2nd Ed. Burges Publ. Minneapolis. 241pp.

De Tempe, J. 1964. Recent developments in seed health testing. *Proc. Inter. Seed test Assn.* **29**: 479-486.

ISTA, 1966. International Rules for seed testing. *Proc. Inter. Seed test Assn.* **31**:1-152.

Sastry Mrs, Muralidharan K and Prasad, G.S.V. 2004. *Production and Marketing of seed and seed materials.* Padama Publications, Hyderabad 500044, INDIA.

Subramanian, C.V. 1971. Hyphomycetes: An account of the Indian species except Cercosporae. ICAR, New Delhi; 930 pp.