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Studies on the Seed-borne Fungi of Sugarcane

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ABSTRACT

Eight sugarcane true seed (fuzz) samples of different crosses were screened against seed-borne mycoflora by blotter and water agar plate method. Seventeen different fungi, representing thirteen genera, such as *Alternaria padwickii, Aspergillus flavus, A. niger, Botrytis cinera, Cephalosporium sacchari, Chaetomium globosum, Colletotrichum lindomuthianum, Curvularia lunata, Curvularia* sp., *Cladosporium* sp., *Drechslera sacchari, Fusarium moniliforme, F. oxysporum, F. semitectum, Phoma* sp., *Myrotherium roridum* and *Rhizopus nigricans* were isolated from the said seed samples. All the seventeen fungi isolated from true seeds of sugarcane were new records in Bangladesh. The frequency of fungi recorded in blotter method was higher than the water agar plate method, proving the superiority of blotter method. Out of 17 fungi, *C. lunata, D. sacchari, F. moniliforme* and *Phoma* sp., caused maximum seedling mortality. Germination of seed samples varied from 10 - 15%, and the prevalence of the identified pathogens are considered one of the major factors for low germination.

INTRODUCTION

Seed-borne diseases cause enormous losses to our crops. The determination of fungi that infect and contaminate seeds, causing germination failure and death of seedlings has been carried out in cereal and other crops in the country. In sugarcane, the production of true seeds (fuzz) is restricted to only variety improvement programme. Good seed germination and stand of seedlings are indispensable for a rapid and successful sugarcane breeding programme (Mangelsdorf, 1959). Although germinative capacity of true seed (fuzz) of sugarcane is a genitic character, a considerable amount of seed loose viability for reasons such as physiological, geophysical and pathological one (Sinha and Singh, 1982). During the period 1953 to 1989, eighteen sugarcane varieties have been released by Sugarcane Research and Training Institute (SRTI) by raisings from fuzz produced through hybridization programme. Every year hundred of crosses are made, and thousand of fuzz are sown to raise seedlings in the nursaries, but germination of fuzz is usually obtained very low (8 to 25%). However, information on the role of seed-borne pathogens in impairing germination potential of seeds, and causing seedling mortality is limited. The fungi associated with the fuzz have not yet been identified in the country. In view of the above fact this study was undertaken to investigate the fungi associated with the true seeds of sugarcane, and their involvement in decaying of seeds and seedling mortality.

MATERIALS AND METHODS

Detection and identification of fungi on seeds

Eight true seed samples of sugarcane from different crosses were collected from the Breeding Division of SRTI, Ishurdi during the cropping seson 1987 - 88 and the seed samples were stored in the refrigerator at a temperature of 4°C.

True seeds with and without surface sterilization by 0.1 percent $HgCl_2$ were analysed for the prevalence of fungi by the blotter and water agar method according to

the procedure of Anon (1976) with some modifications, like use of fluorescent tube light during night (for 12 hours) instead of near ultra violet light (NUV) and incubation of plated seeds at room temparature (20 - 22°C) instead of constant 20°C were followed. Each of eight samples collected from separate crosses contained 200 fuzz for each samples and a total of 1600 fuzz for eight samples. The fungi associated with seeds were identified by observing their growth characters on the incubated seeds under sterioscopic microscope, following the keys of Malone and Musket (1964), Benoit and Mathur (1970), Ramnath *et al.* (1970), Kulsharestha *et al.* (1976) and Michail *et al.*, (1977). Fungi which could not be identified under the sterioscopic microscope were identified under the compound microscope with the help of appropriate keys, Malone and Musket (1964), Raper and Fennel (1965), Booth (1971) and Ellis (1971). Decaying of seeds in blotter test by each species of the fungus was recorded as evident from the fungal growth on the seed.

Test tube seedling symptom test

Eight seed samples were tested by the blotter method of which two samples had the maximum representative seed-borne fungi. They were included in the test tube seedling symptom test without any artificial inoculation to determine pathogenic effect. In this method 100 fuzz pretreated with 0.1% $HgCl_2$, at the rate of one fuzz per test tube were placed in the test tube containing 6 ml 1% water agar. The tubes containing seeds were incubated on wooden desk in the laboratory under fluorescent tube light for 14 d. After incubation, the germinating seeds, and seedlings were examined for recording visible symptoms produced, if any, by the fungal pathogens present in the fuzz.

Pathogenicity test

The pathogenicity of fungi causing heigher percentage of decay of seed and seedlings was tested by water agar test the tube method (Khare *et al.*, 1977) with some modification. The predominant fungi was mixed with sterilized water agar (1%) in the test tube. Seeds treated with formaldehyde (40% formaldehyde, diluted with water 1;20 V/V) for 20 min. and germinated in the petridish. After germination only fungus free seedings were inserted into the test tube against each known pathogens and incubated in the room temperature at 22±2°C. After 14 d the seedlings were examined for disease symptoms and reisolation of the fungus from diseased tissue were carried out and later identified.

RESULTS AND DISCUSSION

Seventeen different fungi covering thirteen genera were identified from eight seed samples (Table 1). The average infection in blotter method by *Curvlaria lunata*, *phoma sp.*, *Cladosprorium sp.*, *Fusarium monilifrome* and *Drechslera sacchari* was 39.4, 19.6, 10.2, 9.7 and 7.6% and by water agar method 36.0, 15.8, 19.7, 7.0 and 5.2% respectively. In blotter method seeds without surface sterilization yielded the highest fungal population (Figure 1), whereas the surface sterilized seeds with $HgCl_2$ resulted the lowest fungal population. In blotter method frequency of each fungus was heigher compared to water agar plate method. Surface sterilized seeds in both the cases showed low frequency of each fungus indicating that the blotter method is superior one for screening fuzz samples. Superiority of the blotter method over agar plate method for detection of seed-borne fungal pathogens from sugarcane and other crops was also

demonstrated by different workers (Mathur *et al.*, 1967; Neergaard *et al.*, 1979; Narendra and Setty, 1979; Sinha and Singh, 1982; Fakir and Kabir, 1988).

In a particular sample, maxium seed-borne fungal colony was developed by *phoma* sp., (55.5%) followed by *Curvularia lunata* (44.0%), *Cladosporium* sp. (34.0%), *Botrytis cinera* (20.5%) *Alternaria padwickii* (20.0%), *Fusarium moniliforme* (18.0%) and *Drechslera sacchari* (17.5%). The highest average infection was recorded by *C. lunata* (39.4%) followed by *Phoma* sp. (19.6%). Cladosporium sp., (10.2%), *F. moniliforme* (9.7%) and *D. sacchari* (7.6%); while the lowest average infection was observed by *Rhizopus nigricans* (0.5%) (Table 1). Comparing two species of *Curvularia*, association of *C. lunata* (Wakker Boedijin, var, aeria (Batista, Lima & Vasconcelos) M.B. Ellis was more (39.4%) than other un-identified *Curvularia* sp. (5.1%). In Sugarcane *C. lunata* is known to cause leaf spot (Singh, 1969) and seedling blight (Khurana and Singh, 1971). Prevalence of *Phoma* sp. and *Cladosporium* sp. with the seed was also high. These fungi had low pathogenicity but they can increase the total mortality in association with other pathogens (Sanigiuino and Tokeshui, 1980). Among three species of *Fusarium* associated with the true seeds *F. moniliforme*, var. *subglutinans* wollenw and Reink, was the highest. Generally *F. moniliforme* causes foot rot of sugarcane (Rao, 1969) and it was isolated from wilted canes (Singh and Singh, 1975). Prevalence of *D. sacchari* (Butler) Subram and Jain with the seeds was relatively lower (7.6%) than *C. lunata*. *D. sacchari* causes important eye spot disease and reported from all sugarcane growing countries (Martin, 1961).

Many of the foliar and seedling diseases of sugarcane are transmitted through infected seeds (Sinha and Singh, 1982). Among seventeen fungi isolated, nine of them caused decay of seeds on blotter (Table 1). Maximum rotting was done by *C. lunata* (27.6%) followed by *Phoma* sp. (7.7%), *D. sacchari* (4.7%), *F. moniliforme* (2.9%), *Curvularia* sp. (2.5%), *F. oxysporum* (1.5%), *Aspergillus flavus* (1.5%), *Cladosporium* sp (0.7%) and *Myrothecium roridum* (0.5%).

In this experiment all the seventeen fungi isolated from true seeds were the first record in Bangladesh.

Pathogenic effects of isolated fungi

The pathogenic effect on seedling growth of most prevalent fungi, the *Curvularia* spp., *Phoma* sp., *Fusarium* spp., and *D. sacchari* was studied by water agar test tube method. In the test tube seedling symptom test *C. lunata*, *D. sacchari*, *F. moniliforme* and *Phoma* sp. were found to cause seed rot and infection of seedlings. Among these, *C. lunata* was found most pathogenic followed by *D. sacchari*; while *Phoma* sp. was the least pathogenic (Table 2). Similar to the earlier findings (Table 1), by water agar test tube method, seed decay was in large due to *C. lunata*. Seed samples testing showed a very low germination of 20.5% only and none of the seed of seed samples was found free from fungal association. The highest mortality of seedlings was 33.4% due to *C. lunata* after 15 d of incubation at room temperature at 20 - 20°C. Close examination of seedlings showed manifestation blackening and rotting of radicle, lessions on stem base, stem, roots and infection of cotyledonary leves to death of seedlings due to *C. lunata* and *D. sacchari*. *Fusarium moniliforme* and *Phoma* sp. caused seed rot, death of

Table 1. Prevalence of various fungi associated with true seeds (fuzz) and causing seed decay on blotter.

Fungi	Infection on seed samples		Percentage on total fungal infection*	Percentage on total seed infection**	Average seed decaying on blotter (%)
	Range (%)	Average (%)			
Alternaria padwickii	0.5-20.0	6.7	5.2	6.7	—
Aspergillus flavus	0.5-06.0	2.5	1.7	2.1	1.5
Aspergillus niger	0.5-14.0	5.0	2.9	3.7	—
Botrytis cinera	4.0-20.5	7.4	4.4	5.5	—
Cephalosporium sacchari	1.0-04.0	2.1	0.8	1.1	—
Chaetomium globosum	0.5-09.5	3.9	2.7	3.4	—
Colletotrichum lindomuthianum	0.5-04.5	2.6	1.2	1.6	—
Curvularia lunata	32.0-44.0	39.4	31.1	39.4	27.6
Curvularia sp.	2.0-15.5	6.8	4.0	5.1	2.5
Cladosporium sp.	1.0-34.0	10.2	8.0	10.2	0.7
Drechslera sacchari	2.0-17.5	7.6	5.9	7.6	4.7
Fusarium moniliforme	1.0-18.0	9.7	7.6	9.7	2.9
F. oxysporum	0.5-10.0	5.0	3.0	3.8	1.5
F. semitectum	2.0-08.5	4.6	2.7	3.5	—
Phoma sp.	3.0-55.5	19.6	15.3	19.6	7.7
Myrothecium roridum	1.0-11.0	4.4	2.1	2.7	0.5
Rhizopus nigricans	0.5-02.5	0.5	0.2	0.3	—

* Total No. of fungal infection were 2022,

** Percentage on total seed infection was calculated on the basis of 1600 fuzz.

radicle and lessions on roots and stem base, succumbed at later stage. It was observed that the seedlings were affected due to carrying of the fungus over the surface of seeds. Consequently, the fungus grew on the water agar medium and affected the collar region of the seedlings, resulting will and death of seedlings. Sinha and Singh (1982) observed such a situation in sugarcane seeds naturally infected with *Curvularia* spp. and *Drechslera* spp. They also reported similar symptoms in nursery beds. The results are concurrent with the findings of Sinha and Khare (1977), who found *Macrophomina phaseolina* and *F. oxysporum* in cow pea seeds. Kabir and Fakir (1988) who also found blackgram seed to be naturally infected with *Aspergillus flavus, F. oxysporum, F. semitectum, F. solani, M. phaseolina* and *Myrothecium roridum.* It was observed that only 20% seedlings were in good condition, having no collar or root infection but seed coat was associated with few propagules of *Botrytis cinera.*

Pathogenicity of *C. lunata, D. sacchari, F. moniliforme* and *Phoma* sp. isolated from the seeds of sugarcane was confirmed. All of them caused seed decay and seedling mortality. According to Sinha and Singh (1982) and Sanguino and Tokeshi (1980) *Curvularia* spp., *Drechslera* spp., *Fusarium miniliforme, Pestalotia guepini* and *Phoma* sp. were pathogenic and seed-borne on sugarcane seeds/ caryopses.

From this study it can be concluded that the prevalence of the pathogens identified is one of the major factors for low germination and poor stand of sugarcane seedlings in Bangladesh.

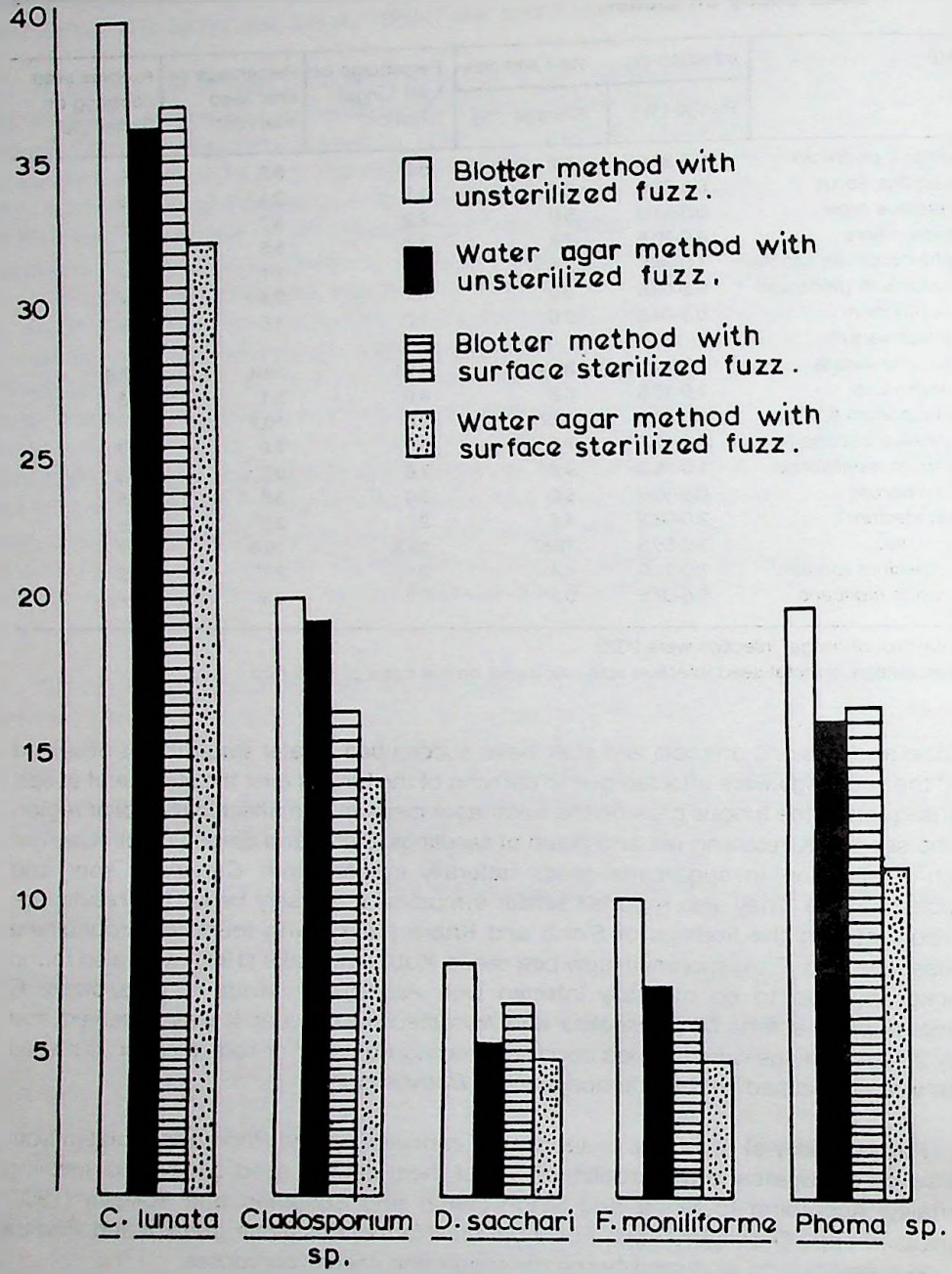

Figure 1. Prevalence of predominant fungi associated with sugarcane seeds by surface sterilized and unsterilization of seeds by $HgCl_2$ in blotter and water agar testing methods.

Table 2. Pathogenic effect of five different fungi on seeds and seedlings of sugarcane in seedling symptom test.

Fungi	Total seed-borne infection (Nos.)	Seed decay (%)	Post emergence mortality (%)
Curvularia lunata	72	31.5	33.4
Drechslera sacchari	28	7.0	22.1
Fusarium moniliforme	20	6.5	12.9
Fusarium oxysporum	06	1.0	—
Phoma sp.	55	14.0	12.1

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