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Comparison Between Two Detection Techniques of Seed-Borne Pathogens in Cucurbits in Bangladesh

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Fifty four seed samples of three cucurbits namely sweet gourd (*Cucurbita moschata* Duch. Ex Poir.), white gourd (*Benincasa hispida* Cogn.) and bitter gourd (*Momordica charantia* L.) were collected from six different districts of Bangladesh, to find out a suitable detection technique of associated seed-borne fungi in laboratory conditions. Three different detection methods namely, dry inspection, blotter test and seedling symptom test were compared in present study. Among the tested three detection techniques, more infection rates were observed for *Aspergillus flavus* and *Penicillium* in all cucurbit seeds in blotter test method, whereas higher infection caused by *Fusarium* and *Rhizopus* were recorded in test tube seedling symptom test. The higher germination percentage of cucurbit seeds was observed in blotter test and it is less expensive, quick and useful for the detection of most of the infectious fungi.

Key words: Seed-borne pathogens, Cucurbits, Detection techniques, Dry inspection, Blotter test

Introduction

In Bangladesh, cucurbits are one of the most popular and cheap vegetables. A commendable area of highland specially the homestead area (during rainy season) and crop fields (in winter) comes under cucurbit cultivation and the production of cucurbits is 7.78 to 9.66% of total vegetable production in Bangladesh (BBS 1986). Though cucurbits are the main sources of vegetables in summer, their production rate is not sufficient. They suffer from more than 24 different diseases, out of which 14 are seed-borne (Richardson 1979; Peregrine and Ahmad 1983; Peregrine *et al* 1984).

Detection of pathogen(s) is prerequisite for improvement, protection and certification of seed in trade. Different types of detection techniques such as blotter method, agar plate method, washing test, florescence test, ELISA and test tube seedling symptom test methods are more familiar among the scientists (Moslem and Parvez 1993; Alkashim 1996; Lagerberg 1996). However, these methods or techniques mainly depend on the type of crop as well as the type of associated pathogens. Several criteria have to be considered in selecting a suitable routine seed health testing procedure. Primarily, it should be capable of revealing maximum pathogen infection and should also be versatile and can detect a range of pathogens (Singh *et al* 1974). Introduction of new, quick, economic and reliable technique(s) can save a lot of crops from damage caused by seed-borne pathogens. In Bangladesh, it is very necessary to develop economic and quick techniques of detection of seed-borne pathogens of cucurbits. Keeping this in view, the present experiment was outlined to find out a suitable, efficient and economic technique for seed-borne pathogens detection in cucurbits in Bangladesh.

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Materials and Methods

A. Collection of seed samples. Fifty four seed samples of three cucurbits namely sweet gourd (*Cucurbita moschata*), white gourd (*Benincasa hispida*) and bitter gourd (*Momordica charantia*), were collected from six different districts of Bangladesh *viz*. Dhaka, Mymensingh, Kishoregonj, Bogra, Dinajpur and Rangpur were included for the survey of the prevalence of seed-borne fungi. The samples were collected in paper bags from farmers house of the Sadar thana of the above-mentioned districts. After collection, all seeds were brought to the laboratory, put in paper bags and stored in a refrigerator (at 4°C) untill used for the experiment.

B. Fungi identification keys. Most of the fungi were detected by observing the growth characters on the incubated seeds in plates under stereoscopic microscope following the keys outlined by different workers (Malone and Muskett 1964; Benoit and Mathur 1970; Nath *et al* 1970; Chidambaram *et al* 1973). *Aspergillus* spp were cultured on Czapek's solution agar and identified by using Raper and Fennel's (1965) keys. For primarily unknown fungi, temporary slides were

prepared and examined under the compound microscope to identify them by using the keys of Ellis (1971) and Booth (1971).

C. Comparison of different detection methods. The effects of three different methods, namely dry inspection, blotter test and test tube seedling symptom test, on the growth of different seed-borne fungi on cucurbit seeds, were tested for detecting associated seed-borne fungi, as follows:

(i) Dry inspection test. Seed samples of collected three cucurbits were analyzed in the Department of Plant Pathology, Bangladesh Agricultural University. One hundred seeds from each sample were taken on random basis and were examined under a stereobinocular microscope for checking abnormalities, discolouration and presence of fungal fruiting bodies.

(ii) Blotter test. For observing the growth of certain fungi on seeds and infection of the emerged seedlings, 200 seeds from each cucurbit were selected and immersed in 2% sodium hypochlorite solution for 15 min, for surface-sterilization. The sterilized seeds were washed 3 times with distilled water and then air-dried overnight. Next day the seeds were placed on plates (10 seeds per plate) and tested by the method of ISTA with some modifications (ISTA 1976). In this method, three layers of filter papers (9 cm, Whatman No.1) soaked in sterilized water, were placed at the bottom of plates. Then two hundred randomly selected seeds of each cucurbit were placed on the moistened paper in twenty replications at the rate of 10 seeds per plate. The plate were then incubated for seven days at room temperature on the laboratory desk under fluorescent tube light. For keeping the blotting papers moistened, watering was done whenever necessary. Data were recorded on the 8th day of incubation for the detection of the presence of pathogens.

(iii) Test tube seedling symptom test. For this test, 100 surface-sterilized seeds from each of the cucurbit were randomly selected and placed one seed per test tube containing 6 ml of 1 per cent sterile water agar. The test tubes containing cucurbits seeds were then incubated on wooden desk in the laboratory for 15 days. The mouth of test tubes were plugged with cotton for first 48 h of incubation, and then replaced at same time interval, with new cotton plugs to avoid surplus moisture accumulation from water agar media. Regular inspection was done to observe any possible abnormalities of seedlings due to the attack of seed-borne pathogens. After completion of the incubation period, seedlings in all test tubes were thoroughly examined and all visible disease symptoms were recorded.

D. Design of the experiment. The present experiment was set up following the Completely Randomized Design (CRD). The Data were converted to arcsine (Y=Arcsine x) value before their ANOVA test. The mean differences among the different values were adjudged with Duncan's Multiple Range Test or DMRT (Duncan 1951).

Results and Discussion

The results showed that tested three detection techniques, dry inspection test has no major impact on the disease detection in cucurbits. Therefore, it was excluded from the present results. The other two methods are presented in tables 1-4.

A. Disease development in blotter test. The analyzed results of natural disease development rates in the blotter tests are presented in tables 1-3. The data revealed that the presence of *Aspergillus flavus* and *A.niger* was frequent in all

Table 1

Disease development percentage in sweet gourd as observed in blotter test

Name of Fungi	Percentage of infection on infected samples of different districts					
	Dhaka	Mymensingh	Kishoregonj	Bogra	Dinajpur	Rangpur
Aspergillus flavus	17.33bc	33.33a	16.00b	15.00b	24.67b	31.67a
Aspergillus niger	28.00a	32.67a	26.33a	32.00a	13.67c	37.33a
Chaetomium sp	12.00c	12.00bc	4.00d	6.00c	7.00cd	14.33c
Penicillium sp	21.67ab	16.67b	11.67bc	18.67b	20.67b	7.33d
Fusarium sp	6.67d	7.00bc	3.67d	7.33c	4.00d	6.00d
Rhizopus sp	27.67a	20.33ab	15.67b	13.67b	35.33a	22.33b
Ceratocystis sp	4.67d	1.67c	4.33cd	6.00c	3.00d	4.67d
Doratomycetes sp	6.00d	6.67c	10.67bcd	6.00c	7.00cd	8.00d
Corynospora sp	3.33d	2.33c	6.00cd	2.67c	4.00d	4.67d
Seed rot %	10.67	20.50	25.33	33.33	30.50	37.67
Seedling infection	6.05	9.90	10.55	12.67	12.00	17.30
Germination rate	60.00	66.33	55.67	49.33	62.67	52.67

N B Within a column, mean values followed by different letters are significantly different (at 5% level).

three cucurbit seeds tested in all districts.

(i) Sweet gourd (Cucurbita moschata). In the case of sweet gourd seeds, *Aspergillus niger* was more prevalent among the infectious fungi (Table 1). In seeds of Dhaka district, *Rhizopus* was second prevalent fungus (27.67% infection), whereas *Cornyospora* was the least frequent fungus (3.33% infection). More or less same trend was observed in seeds of Mymensingh, Kishoreganj and Rangpur districts. In the case of seeds, collected from Dinajpur, *Rhizopus* was the most abundant fungus. The seed rot rate in blotter test was more or less high. Among the tested sweet gourd seeds, the highest seed rot rate (37.67%) was observed in seeds of Rangpur followed by seeds of Bogra district (33.33%), whereas the lowest seed rot was found in seeds of Dhaka (10.67%). In the case of seedling infection, same trend was observed. The highest rate of seedling infection was observed in the seeds of Rangpur (17.30%) whereas the least infection was in seeds of Dhaka (6.05%). *Aspergillus flavus, A.niger* and *Fusarium* were associated with these seedling infection, though pecific infection rate on seedlings were not separately presented.

(ii) White gourd (Benincasa hispida). In the case of white gourd (Table 2), the highest number of infection was caused by *Penicillium* in seeds of Dhaka district (36.67%), *A.niger* in Mymensingh and Bogra (34.67%), Dinajpur (25.67%), Rangpur (20.67%) and Kishoreganj (19.33%) which was followed by *A.flavus* in Mymensingh, Dhaka and Kishoreganj; *Penicillium* in Bogra, *Rhizopus* in Dinajpur and *Penicillium* in Rangpur. The highest rate of seed rot was observed in the seeds of Mymensingh (42.67%) followed by those of Dinajpur (40.10%), whereas the least seed rot was observed in the seeds of Rangpur (33.50%), though these rates were greater than the rate of seed rot in sweet gourd (Table 1). Seedling infection was the highest in the seeds of Dinajpur (17.50%), followed by the seeds of Mymensingh (15.00%), whereas the lowest infection was recorded in the seeds of Kishoreganj (7.90%).

iii) Bitter gourd (Momordica charantia). Results of disease development in bitter gourd is presented in Table 3. The data revealed that *Aspergillus flavus* was the most infectious among the recorded fungi. In the seeds of Dhaka district, infection of *A.flavus* was the highest (27.00%) followed by *A.niger* as well as *Penicillium* (22.33%), whereas Corynospora was the least prevalent seed-borne pathogen. In the seeds of Mymensingh, *Aspergillus flavus* (31.67%) was followed by *Penicillium* (26.33%) and the least infection was caused by *Doratomycetes* (2.33%). The same trend was observed in the seeds of Kishoreganj and Rangpur. On the other hand, the bitter gourd seeds collected from Dinajpur showed a different type of trend. In that case, *Penicillium* was the most prevalent seed-borne fungi (33.00% infection) followed by *A.flavus* (25.33%) and the least infection was caused by *Ceratocystis* (2.67%). The highest seed rot of bitter gourd was observed in the seeds of Dhaka (37.60%) followed by those of Rangpur (29.67% rot), whereas the lowest amount of seed rot was recorded in the seeds of Dinajpur (23.80%). Seedling infection followed the same trend as in seed rot. In the case of seeds of Dhaka 15.55% seedling infection was observed, followed by Mymensingh (11.33%) and the least infection was recorded in the case of seeds of Dinajpur (7.90%).

B. Test tube seedling symptom test. As a result of this test, five fungi, namely *Aspergillus flavus, Curvularia, Fusarium, Penicillium,* and *Rhizopus* appeared as pathogenic for all of

Table 2

Disease development percentage in white gourd as observed in blotter test

Name of Fungi	Percentage of infection on infected samples of different districts					
	Dhaka	Mymensingh	Kishoregonj	Bogra	Dinajpur	Rangpur
Aspergillus flavus	22.00b	31.00a	17.33ab	14.67b	14.00cd	14.33bc
Aspergillus niger	12.00cdc	34.67a	19.33a	34.67a	25.67a	20.67a
Chaetomium sp	13.33bcd	8.33c	6.67cd	1.67c	7.67def	5.67de
Penicillium sp	36.67a	29.33ab	7.00cd	15.33b	18.33bc	16.67ab
Fusarium sp	14.67bc	11.67bc	12.67bc	13.33b	9.67de	10.33cd
Rhizopus sp	5.33cde	7.00c	10.33c	14.00b	20.67ab	11.67b
Ceratocystis sp	14.33bcd	3.00c	1.33d	3.33c	5.67ef	2.67e
Doratomycetes sp	4.00e	5.67c	12.00b	5.67c	2.00f	1.67e
Corynospora sp	5.00de	3.00c	7.33c	4.00c	3.67ef	3.67c
Seed rot %	35.33	42.67	35.30	35.00	40.10	33.50
Seedling infection	13.33	15.00	7.90	12.67	17.50	10.67
Germination rate	59.67	54.67	59.33	61.67	51.67	60.00

N B Within a column, mean values followed by different letters are significantly different (at 5% level).

Table 3

Disease development percentage in bitter gourd as observed in blotter test

Name of Fungi	Percentage of infection on infected samples of different districts					
	Dhaka	Mymensingh	Kishoregonj	Bogra	Dinajpur	Rangpur
Aspergillus flavus	27.00a	31.67a	20.33a	18.00a	25.33b	13.00a
Aspergillus niger	22.33a	13.67bc	16.00ab	22.00a	16.67c	12.67a
Chaetomium sp	6.67b	14.00b	10.00bc	6.33bc	5.33d	9.67ab
Penicillium sp	22.33a	26.33a	17.00a	9.67b	33.00a	11.33a
Fusarium sp	8.00b	14.67b	14.67ab	4.67bc	5.00d	0.67c
Rhizopus sp	2.67b	11.67b	13.00ab	4.00bc	23.67bc	5.33bc
Ceratocystis sp	6.00b	6.67cd	10.67bc	3.67c	2.67d	1.33c
Doratomycetes sp	4.33b	2.33d	4.33c	4.33bc	4.33d	0.00d
Corynospora sp	3.67b	2.67d	10.67bc	2.67c	4.67a	0.00d
Seed rot %	37.60	27.55	25.67	27.05	23.80	29.67
Seedling infection	15.55	11.33	8.70	12.50	7.90	11.00
Germination rate	59.00	52.67	60.33	61.67	59.67	55.33

N B Within a column, mean values followed by different letters are significantly different (at 5% level).

Table 4

Infection percentage due to seed borne-fungi in the test tube seedling symptom test and blotter method

Treatment	Sweet gourd (%)	White gourd (%)	Bitter gourd (%)
A.Test tube seedling symptom:			
Aspergillus flavus	18.33ab	26.67a	33.33a
Curvularia sp	28.33ab	16.67ab	28.67ab
Fusarium sp	21.67ab	11.67b	26.67ab
Pencillium sp	6.67b	20.67a	19.00b
Rhizopus sp	38.33a	16.67ab	11.67c
Ascochyta sp	0.00c	1.67c	0.00d
Germination rate	60.00	33.33	28.33
Seed rot	26.23	62.67	60.33
Poor seedlings	33.33	14.00	13.00
Vigour seedling	36.67	19.33	15.33
Seedling infection	20.50	12.75	10.90
B.Blotter method			
Aspergillus flavus	23.00	26.80	33.80
Fusarium sp	6.65	12.28	9.28
Penicillium sp	16.87	20.94	20.50
Rhizopus sp	29.50	14.80	10.33
Germination rate	60.00	58.17	53.60

N B Mean values in a column, followed by different letters are significantly different (at 5% level)

the three cucurbit seeds and another fungus, *Ascochyta* was found to be slightly pathogenic only for white gourd seeds (Table 4A). *Aspergillus flavus* infected seeds were easily recognized by the presence of grey colony of fungus on the cotyledons of seedlings. Infection of *Fusarium* was identified by the presence of pinkish or greyish fluffy colony on the seeds and around the base of seedlings, which caused rotting of roots and base of seedlings and finally the seedlings died. In sweet gourd, the highest percentage of infection was caused by *Rhizopus* (38.33%) followed by *Curvularia* (28.33%), whereas, the least infection was recorded for *Penicillium* (6.67%). In the case of white gourd, the highest infection was caused by *Aspergillus flavus* (26.67%), which was followed by *Penicillium* (20.67%) and *Ascochyta* produced the least infection (1.67%). Relatively higher infection rate was observed in bitter gourd seeds. The highest infection was recorded for *Aspergillus flavus* (33.33%) followed by *Curvularia* (28.67%). The lowest amount of infection was observed for *Rhizopus* (11.67%) though it was considerably high infection.

Germination rates of three cucurbits tested were greatly variable for seedling symptom test. In case of sweet gourd, 60% seeds germinated, but that was very much low in other two seeds, where only 33.33% and 28.33 seeds germinated in white gourd and bitter gourd, respectively. A considerable amount of seeds were damaged by seed rot. The rate was 26.33% for sweet gourd, 62.67% for white gourd and 60.33% for bitter gourd. Among the germinated seedlings, 20.50%, 12.75% and 10.90% seedling infections were encountered in sweet gourd, white gourd and bitter gourd, respectively.

C. Evaluation of detection techniques. The results of comparison of two detection techniques (blotter test and test tube seedling symptom test) for four seed-borne fungi of cucurb-

its are presented in tables 4A & 4B. More or less higher percentages of infection of all pathogens were recorded in both the blotter test and test tube seedling symptoms test. However, trend of more infection rate was observed for *Aspergillus flavus* and *Pencillium* (in all cucurbit seeds) in blotter test method, whereas higher infection caused by *Fusarium* and *Rhizopus* were recorded in test tube seedling symptom test than the blotter test method. Again, higher germination rate was obtained in blotter method in comparison to test tube seedling symptom tests for all of the three cucurbit seeds tested. Between the two detection techniques, blotter method was less expensive and all infectious fungi could be easily and quickly recorded under stereoscopic microscope by the technique of observing the distinct growth characteristics of pathogens on incubated seeds in blotter (Barma and Fakir 1980; Kabir 1985; Moslem and Parvez 1993).

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