Agrosearch
Vol. 1, No. 2, December 1995. pp 123–127

EVALUATION OF SEED HEALTH TESTING METHODS FOR THE DETECTION OF SEED-BORNE FUNGI OF AFRICAN YAM BEAN, *SPHENOSTYLIS STENOCARPA* (Hochst ex. A. Rich) Harms.

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ABSTRACT

The efficiency of four seed health testing techniques namely the blotter, deep- freezing blotter, ragdoll and agar plate methods in detecting seed-borne fungi of African yam bean, *Sphenostylis stenocarpa* (Hochst ex. A. Rich) Harms seeds was evaluated. The blotter method was found to be the most suitable testing technique for detecting *Aspergilus flavus, A niger, A terreus, A. ochraceus, Botryodiplodia theobromae, Colletoritchum dematium, C. gloeosporiodes* and *Curvularia lunata* in seeds pretreated with 1% available chlorine for 5 minute as well as in untreated seeds. The deep-freezing blotter method was superior to the other methods in detecting *Fusarium moniliforme, F. semitectum, Chaetomium* sp. and *Cephalsorium* sp. The incidence of *Macrophomina phaseolina* and *Penicillium* spp. were recorded more in the agar plate method than in the other fungi recorded but gave highest percentage seed germination in both untreated and chlorine pretreated seeds in all the four sampled tested.

KEY WORDS:Sees health, testing techniques, seed-borne fungi, African yam bean.

INTRODUCTION

African yam bean, *Sphenostylis stenocarpa* (Hochst ex. A Rich) Harms seeds have been associated with any seed-borne fungi some of which are of planting and quarantine values (Nwachukwu and Umechrurba, 1991). Systematic investigations to evaluate seed health testing methods for the detection of seed-borne fungi of economic importance of this crop have been done. Maduekwe and Umechruba (1992) evaluated the classical seed-testing methods for the detection of cowpea seed-borne fungi and found the blotter and the agar-plate methods to be better than the deep-freezing blotter method and the ragdoll methods in the detection of *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Fusarium moniliforme, Marcrophomina phaseolina* and *Penicillium* sp., while the blotter method was the only method that detected *Curvularia lunata.* In seed-borne mycoflora studies of sorghum seeds used for malting at the Jos International Brewery, Zuwahu and Akueshi (1989) most frequently isolated *A. flavus* and *P. citrinum* from the seeds tested out of 14 fungi isolated. Michail *et al.* (1979) reported that the blotter method was superior to the agar plate method for detections *Cephalosporium* sp., *Fusarium moniliforme, F. semitectum* and *Myrothecium verrucaria* on seeds of soybean. They also recommended agar plate method for the detection of *Macrophomina phaseolina* in soybean. Detection of *Cochliobolus specifer, F. equiseti* and *F. solani* was equally good on blotters and agar. Scheitza and Kriiger (1980) reported that the deep-freezing blotter method used by Limonard (1966) on tomato seeds was the most useful and reliable. Singh *et al.*, (1974) recommended the deep-freezing method for routine seed health testing of maize. Out of the four methods used by Sinha and Khare (1978), the standard blotter method was found to be the best for the detection of seed-borne fungi of cowpea. Srivastava and Gupta (1981), obtained higher numbers of fungi in the blotter method than in the agar plate method in seed health testing of *Zinnia.*

This present study was undertaken to determine the seed health testing methods for the detection of important seed-borne fungi of African yam bean. Results of this study will be helpful to plant pathologists working on routine

seed health testing in Seed Companies as well as those working in Research and Plant Quarantine Departments, because about 90% of the food crops grown world-wide and the plant germplasm being distributed between and within countries are propagated by true seeds (Neergaard, 1979). Many plant pathogens (bacteria, fungi nematodes and viruses) affecting the food crops and the plant germplasm are seed borne and seed transmitted. Seed transmission is an efficient method of transporting plant pathogens through time and space; and seed transmission has been responsible for the introduction of many diseases into different countries over the years (Neergaard, 1979). Therefore, the quality of seeds must be known before they are sown. Regular seed health testing with recommended method(s) in conjunction with seed health certification schemes will help control spread of seed-borne pathogens from season to season and from one area to another.

MATERIALS AND METHODS

Source of seed samples: Four seed samples used in this study were obtained from Mbaise in Imo State, Nigeria. Mbaise is one of the major towns where African yam bean is grown in South-Eastern State of Nigeria. Seeds from Mbaise were preferred to seeds from other towns because they were found to be heavily infected with different fungal pathogens when compared with seed samples from other towns (Nwachukwu and Umechuruba, 1991).

Testing Procedures:

Blotter methods:- Ten seeds, both untreated and 1% chlorine-pretreated (obtained from 10% sodium hypochlorite) for 5 mins were plate per Petri dish (9 cm diam) containing three layers if water-soaked blotters. The dishes were incubated for seven days at 20± 2°C in complete darkness.

Agar plate method:- Untreated seeds and seeds pretreated with 1% chlorine for 5 min were plated on potato dextrose agar (PDA) medium in Petri dishes at the rate of ten seeds per dish. Seeds were incubated as described as in the standard blotter method. Recording of fungi was made on the fourth and seventh days of incubation.

Deep-freezing blotter method:- The seeds (untreated and 1% chlorine pretreated for 5 mins) were plated as described in the blotter method. The dishes containing the seeds were first incubated at 20 ± 2°C for 24 hours, then transferred to the deep-freezer (-20°C) for 24 hours only and incubated again at 20 ± 2°C in complete darkness for five days.

Ragdoll (rolled towel) method:- One hundred seeds were placed on two layers of water soaked towels (48 x 48cm) and then covered with another layer of water-soaked towel of the same size. The towels were rolled and the end closed with rubber bands. The rolls were then wrapped with alunminium foil and incubated at 20 ± 2°C in complete darkness for seven days. At the end of unrolled and the seeds examined carefully under the sterobinocular microscope for seed-borne fungi. Four replicates of 100 seeds in each towels were made. In each of the methods, seeds were examined under sterobinocular microscope (6 - 50x) for identification of fungi. Whenever necessary slides were prepared and examined under a compound microscope for confirmation of fungal identification. A total of 400 seeds were used in each treatment, per sample, in each method.

RESULTS

The results of the comparative study of four incubation methods (agar plate, ragroll, standard blotter and deep-freezing blotter) for the detection of seed-bornes fungi associated with African yam bean seeds are shown in Table 1a and 1b. The four methods were effective in the recovery of *Aspergillus flavus, A niger, A terreus, Botryodiplodia theobromae, Fusarium moniliforme, F. semitectum, Chaetomium* sp. and *Cephalosporium* sp. The blotter method was found to be the most suitable testing technique for the detection of *A. Flavus, A terreus, A ochraceus, B. theobromae, Colletrotrichum dematium, C. gloeosporides* and *Curvularia lunata* (Table 1a and 1b). The highest records of *Macrophomina phaseolina* and *Penicillium* spp. were made by the agar plate method in both pretreated and untreated seeds. Ragdoll method

gave the lowest percentage recovery of all the fungi recorded and highest percentage seed germination in both untreated and pretreated seeds.

In general, 1% available chlorine reduced the infection percentages of all the fungi in the four sample tested while the untreated seeds gave higher counts of all the fungi in the four methods (Table 1a and 1b).

DISCUSSION

The results of this study revealed that the standard blotter method and the deep-freezing blotter method were both satisfactory in detecting fungi infection in African yam bean seeds. The blotter method was capable of detecting most seed-borne fungi at their maximum frequencies while deep-freezing blotter method was more sensitive in detecting some fungi with very low infections. The deep-freezing method also suppressed the incidence of some saprophytic fungi such as *Penicillium spp.* and *Rhizopus stolonifer.* The effectiveness of these two methods in the detection of seed-borne fungi in various crops has been reported by many workers (Michail *et al.*, 1979; Scheita and Kriiger 1980; and Srivastava and Gupta, 1981). We therefore, recommended that the blotter method be used when testing African yam bean seed sampled for planting value and for testing the efficacy of seed dressing chemicals on seed-borne fungi,while the deep-freezing blotter method should be used for quarantine purposes. The agar plate method should be used specifically for the detection of *M. phaseolina* and *Penicillium spp* since this method is very sensitive in the detection of the two fungi. On the other hand the Ragdoll method should be considered when measuring percentage seed germination of African yam bean seeds.

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Table 1a. Mean percentage incidence of seed-borne fungi recorded in four seed samples of untreated African yam bean seeds by four testing methods. 400 seeds were tested per sample in each method.

Fungi	Blotter method				Deep-freezing method				Agar Plate method				Ragdoll method			
	Sample number															
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
A. flavus	84.0	5.15	80.0	35.0	66.0	49.0	62.0	45.0	53.3	46.8	46.0	44.0	35.0	38.0	40.0	21.0
A. niger	60.0	37.0	62.0	20.0	33.0	46.0	31.0	35.0	45.0	38.0	50.0	35.0	30.0	22.0	32.0	30.0
A ochraceus	15.3	0	10.0	0	5.0	0	2.8	0	0	0	0	0	0	0	0	0
A terreus	28.5	15.0	21.0	13.0	10.5	21.8	15.0	16.0	15.0	5.0	5.0	7.8	11.3	2.3	3.3	5.0
B theobromae	58.0	16.3	30.0	15.0	20.3	30.0	10.5	24.0	29.0	16.0	25.0	11.5	20.0	10.3	20.0	11.3
Chaetomium sp.	0	0	0	0	2.3	0	2.5	0	0	0	0	0	0	0	0	0
C. dematium	12.3	1.0	15.8	2.0	1.3	0.5	1.0	4.0	0	0	0	0	0	0	0	0
C. gloesporiodes	8.0	0	0	0	0	2.5	0	0	0	0	0	0	0	0	0	0
Curvularia lunata	5.8	1.0	1.0	0	4.0	0	0	0	0	0	0	0	0	0	0	0
Cephalosporium sp.	0	0	0	0	10.5	0	0	0	0	0	0	0	0	0	0	0
F. moniliforme	35.0	17.5	32.3	22.0	42.8	39.0	25.3	36.3	30.5	20.0	30.0	31.8	25.0	18.0	25.0	10.0
F. semitectum	6.3	1.0	1.5	0	15.3	1.5	8.0	0.5	0	0	0	0	0	0	0	0
M. phaseolina	0	0	0	0	1.0	0	0.5	0.8	2.0	1.0	3.0	5.3	0	0	0	0
Penicillium spp.	30.0	14.0	23.0	21.0	8.5	15.3	18.0	10.5	38.8	40.0	33.0	36.5	20.8	22.1	12.0	20.5
Phomopsis sp.	5.8	0	0	0	2.3	0	0	0	0	0	0	0	0	0	0	0
L S D (0.05)	6.5	4.2	5.3	3.8	5.2	3.9	4.7	4.8	8.0	5.5	4.3	3.9	4.9	3.7	3.1	4.0
% Seed germination	48.0	50.0	43.0	52.0	0	0	0	0	40.0	58.0	45.0	51.0	59.5	60.0	65.0	55.0

Table 1b. Mean percentage incidence of seed-borne fungi recorded in four seed samples of 1% chlorine pretreated seeds of African yam bean seeds by four testing methods. 400 seeds were tested per sample in each method.

Fungi	Blotter method				Deep-freezing method				Agar Plate method				Ragdoll method			
	Sample number															
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
A. flavus	55.0	50.0	51.0	29.5	36.8	20.3	33.8	23.3	30.8	38.0	29.8	24.0	37.3	35.3	28.5	31.3
A. niger	28.8	20.0	56.0	17.5	12.5	28.0	34.0	26.8	28.5	40.3	41.8	29.5	21.0	26.5	28.8	18.3
A. ochraceus	6.5	0.5	5.3	0	2.3	0	0	0	0	0	0	0	0	0	0	0
A. terreus	12.0	5.5	18.0	15.0	5.5	8.0	13.3	3.5	10.8	3.3	6.5	3.0	8.5	5.3	4.0	2.8
B. theobromae	40.0	30.0	31.0	12.3	10.8	20.0	22.3	18.0	15.8	10.3	9.5	17.0	10.0	10.8	5.0	6.3
Chaetomium sp.	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0
C. dematium	0	5.0	1.0	0.5	0	0.5	2.0	1.0	0	0	0	0	0	0	0	0
C. gloeosporiodes	5.0	0	0	0	3.0	0	0	0	0	0	0	0	0	0	0	0
Curvularia lunata	3.0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0
Cephalosporium sp.	0	0	0	0	4.0	0	0	0	0	0	0	0	0	0	0	0
F. moniliforme	13.0	20.0	26.0	10.5	18.8	31.5	30.0	18.3	10.3	15.0	11.3	10.0	10.0	11.3	14.3	18.8
F. senitectum	0	0	0	0	0.5	1.0	0	0	0	0	0	0	0	0	0	0
M. phaseolina	0	0	0	0	0	0	0	0	0.5	0.5	0.3	2.5	0	0	0	0
Penicillium spp.	9.5	0	13.0	10.0	3.3	5.0	6.3	3.5	13.5	10.0	12.0	11.5	6.8	15.0	0	7.3
Phomopsis sp.	2.0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0
L S D (0.05)	3.6	3.5	4.3	4.5	4.0	3.2	4.1	3.0	3.3	4.2	3.1	3.6	5.3	2.6	2.8	3.9
% Seed germination	66.0	70.0	59.0	75.0	0	0	0	0	72.0	69.0	61.5	66.8	77.0	84.0	80.0	81.0