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Seed-borne Fungi of Soybean (*Glycine max* (L.) Merril) and their control.

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Key words: Fungi; Seeds of soybean (*Glycine max* (L.) Merril); control.

RINGKASAN

Sebanyak 27 spesis kulat yang berkaitan dengan 14 jenis dalam 16 sempel biji kacang soya telah diasingkan. Kulat-kulat pathogenik yang sering diasing termasuk Botryodiplodia theobromae, Colletotrichum dematium, Diaporthe phaseolorum, Choanephora cucurbitarum, Fusarium equiseti, F. fusarioides, F. moniliforme, F. semitectum, Macrophomina phaseolina, Myrothecium roridum *dan* Phoma sorghina.

Kulat-kulat lain yang diasing termasuk spesis Aspergillus, Chaetomium, Cladosporium, Curvularia, Nigrospora, Nodulisporium, Penicillium, Rhizopus, Trichoderma *dan* Zygosporium. *Biji-biji yang dirawat dengan racun kulat secara* in vitro *telah memberi percambahan yang terlebeh tinggi jika dibanding dengan kontrol. Benomyl boleh mengawal kebanyakan kulat-kulat pathogenik yang terdapat pada biji kacang soya.*

SUMMARY

Fungi associated with seed of 14 cultivars comprising 16 samples of soybean were investigated and 27 species were isolated. Pathogenic fungi frequently isolated were Botryodiplodia theobromae, Colletotrichum dematium, Diaporthe phaseolorum, Choanephora cucurbitarum, Fusarium equiseti, F. fusarioides, F. moniliforme, F. semitectum, Macrophomina phaseolina, Myrothecium roridum, *and* Phoma sorghina. *Other fungi isolated included species of* Aspergillus, Chaetomium, Cladosporium, Curvularia, Nigrospora, Nodulisporium, Penicillium, Rhizopus, Trichoderma *and* Zygosporium. *Seeds which were treated with fungicides had a higher germination* in vitro *compared to the control. Benomyl eliminated most of the pathogenic fungi associated with soybean seeds.*

INTRODUCTION

Grain legumes, in particular soybean, are attacked by a wide range of diseases many of which are seed-borne. Sinclair (1977) reported that there were at least 66 fungi, 6 bacteria and 8 viruses found to be associated with soybean seeds. These seed-borne micro-organisms have adverse effects on soybean seeds. They can reduce seed germination or seedling emergence or cause blights, leaf spots and other diseases on mature plants. Some of the micro-organisms which were found associated with soybean seeds in the USA include *Cercospora kikuchii, Colleto trichum dematium* f.s.p. *truncata, Corynespora cassiicola, Diaporthe phaseolorum, Macrophomina phaseolina, Myrothecium roridum, Peronospora manshurica* and *Pseudomonas glycinea*(Sinclair and Shurtleff, 1975).

In Malaysia, about 20 pathogens have been recorded on the crop (Singh, 1973; Geh and Lim, 1975; Nik Yusuff, 1977, Abu Kassim, 1979). Fungi which had been recorded to be associated with leaf spot diseases are that of *Ascochyta* sp., *Cercospora canescens, Choanephora cucurbitarum, Mycosphaerella* sp., *Myrothecium roridum,* and *Periconia byssoides.* Other fungal diseases recorded were that of rust caused by *Phakopsora pachyrhizi* and *Phakopsora vignae* and the collar rot and stem rot caused by *Rhizoctonia solani, Sclerotinia sclerotiorum* and *Sclerotium rolfsii.* However, no detailed study of micro-organisms associated with soybean seeds have been reported except that of Wan Zainun *et al.* (1979). This paper reports the frequency of occurrence of pathogenic and saprophytic fungi from 14 cultivars of soybean and the effects of seed treatments on the occurrence of seed-borne fungi and on seed germination.

MATERIALS AND METHODS

Seed source

Seed samples were obtained from the Department of Genetics, University of Malaya and from the Rubber Research Institute of Malaya. A total of 16 samples comprising 14 cultivars of soybean were used viz. K32, Palmetto, G 1612, GG 30279, G 2146, GC 30279-1-168, R 4-13, Disoy, Acadian, KS 437, Calland, KS 535, 66D-1 and Jupiter.

Isolation Techniques

Two generalised isolation procedures were employed for the isolation of pathogenic and saprophytic fungi (Neergaard, 1977; Wan Zainun and Parbery, 1978). The two methods were the moist blotter and the potato dextrose agar (PDA) method. All isolates were sent to CMI for confirmation of identities.

Isolation on Moist Blotting Paper

Ten non-sterilized seeds were evenly placed on three layers of moistened 9 cm diameter filter paper (Whatman No. 1) in plastic petri dishes to allow for the penetration of light. A total of 200 seeds were used for each sample. The plates were incubated at 24 $\pm$ 2°C for 8 days in an alternating cycle of 12 hours NUV (near ultra violet light) and 12 hours darkness regime. Fungi developing on seeds were examined and transferred to PDA for identification and pathogenicity studies.

Incubation on PDA

Ten seeds surface sterilized for 10 minutes in 1 percent solution of sodium hypochlorite as a pre-treatment were evenly spaced on PDA plate. The plates were incubated at 24 $\pm$ 2°C for 8 days in an alternating cycle of 12 hours NUV and 12 hours darkness. A total of 200 seeds per sample was used. Fungi developing on seeds were identified as in the previous experiment.

Seed treatment

Soybean seeds cv. Palmetto were treated at recommended rates with five different fungicides according to the method of Lal and Mathur (1967) and Ellis *et al.* (1975). Fungicides were applied at 0.1 g active ingredients/100g seeds for captan [(N-[(trichloromethyl) thio]-4-cyclohexene-1,2-dicarboximide, Captan 500, 50% WP)] and dithane M 45 (manganese ethylene bisdithiocarbamate, 80% WP) and at 0.2g active ingredient/100g seeds for thiram [bis (dimethylthio-carbamoyl) disulfide, thiram 80% WP], benomyl [methyl 1-(butyl-carbomyl)-2 benzimidazole carbamate, Benlate 50% WP] and busan [thiocyano-methylthio benzothiazole, Busan 25% WP]. Seeds were shaken for 45 min. on a mechanical shaker to give a uniform coating and then kept for two days in sealed bottles at 25°C. Another lot of seeds was subjected to hot water treatment for 10 minutes at 55°C. Two hundred seeds per treatment per lot were plated at 10 seeds per plate on moist Whatman No. 1 filter paper and on PDA plates. Nontreated seeds served as controls. The plates were incubated as previously described.

RESULTS

Altogether 27 species of fungi were isolated from 16 samples of 14 cultivars of soybean. Twenty-five species were isolated by the PDA method and 21 were isolated by the blotter method. Fungal species which were not isolated by the blotter method were *Zygosporium echinosporium, Sterile mycelia, Trichoderma* and *Nodulisporium* sp. Pathogenic species encountered are shown in italics (Table 1). Some of the pathogenic species were isolated at low percentages, for instance that of *Myrothecium roridum* (Table 1). *Colletotrichum dematium* occurred in 75, and 56.2 percent of the samples tested on blotter and PDA respectively while *Diaporthe phaseolorum* occurred in 43.7 percent of the samples tested on blotter and 56.2 percent of the samples tested on PDA. Another important pathogen, *Macrophomina phaseolina* occurred in 25 and 18.7 percent of the samples tested on blotter and PDA respectively. Fusaria form an accumulative total of 137.4 percent of the samples tested on blotter and 93.1 percent of the samples on PDA. Other fungi isolated included species of *Aspergillus, Chaetomium, Choanephora, Cladosporium, Curvularia, Nigrospora, Nodulisporium, Penicillium, Rhizopus* and *Trichoderma*. The frequency of isolation of pathogenic fungi in terms of percent isolate is also given in Table 1. Percent isolates for pathogens such as *Colletotrichum dematium, Diaporthe phaseolorum* and *Macrophomina phaseolina* are higher on PDA compared to that of the blotter. However, percent isolate for *Myrothecium roridum* was zero on PDA and 1.8 on blotter. *Myrothecium* was isolated only from cultivar, G2146 by the blotter method. Fungi such as species of *Aspergillus, Cladosporium* and *Curvularia* occur in higher percentages on blotter compared to those of the PDA because they were removed by surface sterilization. Tables 2 and 3 show the percentage isolation of nine pathogenic fungi from 16 samples comprising 14 cultivars of soybean. The most frequently isolated pathogens

TABLE 1

Frequency of isolation of seed-borne fungi from seed of *Glycine max* according to method of isolation

Fungal species	Blotter		PDA	
	1† 1†	S†	I	S
Aspergillus flavus Link	24.6	81.2	11.9	31.2
Aspergillus niger Van Teigh	9.7	75.0	4.2	5.0
Aspergillus wentii Wehmii	0.7	31.2	0	0
* *Botryodiplodia theobromae* Pat	0.5	37.5	4.6	43.0
Chaetomium globosum kuhze ex steud	0.86	12.5	0.26	6.0
Chaetomium sp.	0	0	0.26	6.0
* *Choanephora cucurbitarum* (Berk. & Rav.) Thaxt.	0.49	6.2	0	0
* *Cladosporium* spp.	18.2	62.5	0.5	12.5
* *Colletotrichum dematium* Arx	7.0	75.0	10.8	56.2
Curvularia lunata (Wakker) Boedijn	4.0	62.5	2.2	37.5
Curvularia sp.	2.8	12.5	1.2	12.5
* *Diaporthe phaseolorum* (Cooke & Ellis) Sacc.	3.15	43.7	19.3	56.2
* *Fusarium equiseti* (Corde) Sacc.	1.3	25.0	4.0	31.2
* *Fusarium fusarioides* (Frag. & Cif) Booth	0	0	2.2	18.7
Fusarium moniliforme Sheldon	0.8	31.2	0.26	6.0
* *Fusarium oxysporum* Schlecht.	0.2	12.5	0.1	6.0
* *Fusarium semitectum* Berk & Rav.	7.8	68.7	7.0	31.2
* *Macrophomina phaseolina* (Tassi) Goid	4.5	25.0	14.0	18.7
* *Myrothecium roridum* Tode ex Fro	1.8	6.2	0	0
* *Nigrospora oryzae* (Berk. & Br.) Petch	1.8	12.5	1.4	31.2
Nodulisporium sp.	0	0	0.26	6.0
Penicillium sp.	5.1	37.5	4.5	37.5
* *Phoma sorghina* (Sacc.) Boerema, Dorenbosch & Van Kest	1.1	37.5	4.3	31.2
Rhizopus nigricans (Ehrenb. ex Fr.) Lind	5.0	56.2	0.4	12.5
Trichoderma spp.	0	0	0.5	18.7
Zygosporium echinosporium	0	0	3.1	12.5
Sterile Mycelia	0	0	0.5	6.0

* Potentially pathogenic species
I† Percent isolate
S† Percent infected sample

are *C. dematium, Diaporthe phaseolorum, Phoma sorghina* and *Fusarium semitectum. Macrophomina phaseolina* occurred only in 4 of the 14 cultivars tested; however, its occurrence on cv. KS437 is high (Tables 2, 3).

Effect of seed treatment on the isolation of fungi and on germination of soybean

All fungicide treatments and the hot water treatment reduced the percentage of fungi recorded on Blotter and on PDA compared to

TABLE 2

Percent isolation of potentially pathogenic seed borne fungi from 16 samples of *Glycine max*. (Isolation on PDA)

Cultivar	Fungus	*Colletotrichum dematium* Arx	*Diaporthe phaseolorum* (Cook & Ellis) Sacc.	*Phoma sorghina (Sacc.)* Boerema, Dorenbosch & Van kest.	*Macrophomina phaseolina* (Tassi) Goid	*Botryodiplodia theobromae* Pat.	*Fusarium equiseti* (Corda) Sacc.	*F. semitectum* Berk. & Rav.	*Myrothecium roridum* Tode ex Fr.	*F. Moniliforme* Sheldon
K 32			4							
Palmetto		2	12	2		2	3			
G 1612			4	3.5		0.5				
GG 30279		5.5	23.5	1		0.5	6			
G 2146		7	2	8		0.5	3.5			
GC 30279-1-168		6					1			
R 4 – 13		12.5	11.5	2			2			
Disoy					8	8		2		
Palmetto						1				
Acadian		1						3		1
KS 437					38					
Calland		1	1							
Jupiter			14			5		9		
KS 535		3.5			2.5			5.5		
66D-1		3			5			7.5		
Jupiter			2							

the control (Table 4). The frequency of isolation of fungi was lowest from the benomyl treated seed compared to the rest of the treatments. Benomyl was the only fungicide which eliminated most of the pathogens. Hot water treatment was also effective against those pathogens but seed germination was impaired.

Table 5 shows the effect of various seed treatments on the germination of soybean seed.

TABLE 3

Percent isolation of potentially pathogenic seed borne fungi from 16 samples of *Glycine max* (Isolation on blotter)

Cultivar	Fungus *Colletotrichum dematium* Arx	*Botryodiplodia theobromae* Pat.	*Diaporthe phaseolorum* (Cook & Ellis) Sacc.	*Phoma sorghina* (Sacc.) Boerema, Dorenbosch & Van Kest.	*Macrophomina phaseolina* (Tassi) Goid	*Fusarium equiseti* (Corda) Sacc.	*F. semitectum* Berk & Rav.	*Myrothecium roridum* Tode ex Fr.	*F. moniliforme* Sheldon
K 32		7.5							
Palmetto	12	11				7	2		
Palmetto	1						2		
G 1612	7	3							
GG 30279	15		2		1	6	16		
G 2146	13	1	7		2		3	2	
GC 30279-1-16-8	6	1.5							
R4 – 13	5	7	2			2	34		1
Disoy				1			12		
Acadian	1								4
KS 437	2		1	49		1			
Calland	4								1
Jupiter		7					34		3
KT 535	13		1	1			15		
66D-1	6			4			3		
Jupiter			1				3		1

All fungicide treated seeds except those of Busan had a higher percent seed germination compared to the control (Table 5). No significant difference in percent germination was obtained between fungicide treated seeds and the control for seeds incubated on blotter. However, significant differences were obtained for seeds which were incubated on PDA.

DISCUSSION

The present study has demonstrated that seed of *Glycine max* frequently carry a number of pathogenic fungi which can cause serious diseases in the field. Most of the fungi isolated have been recorded in the United States except for *Fusarium fusarioides, F. moniliforme, Nodulisporium* sp. and *Zygosporium echinosporium*. Altogether, 27 species of fungi were isolated, and included among the 27 species were 12 which were potential pathogens and species like *Aspergillus, Chaetomium, Curvularia, Nigrospora,* and *Rhizopus* have been associated with seed diseases of soybean (Sinclair and Shurtleff, 1975). Others, including species of *Fusarium, Penicillium, Cladosporium* and *Pithomyces* have been associated with mycotoxicoses in animals fed on contaminated grain (Brook and White, 1966).

TABLE 4

Percent isolation of fungi from seeds of *Glycine max* vc. Palmetto subjected to fungicidal and hot water treatment

Fungus \ Seed treatment	Control		Hot water		Thiram		Benomyl		Captan		Dithane M 45		Busan	
	B*	P*	B	P	B	P	B	P	B	P	B	P	B	P
Aspergillus spp.	47.0	53.0	0.5	1.0	2.5	4.0	–	–	–	1.0	–	0.5	–	1.0
Aspergillus niger Van Teigh	6.0	8.5	–	0.5	–	–	–	–	–	–	–	–	–	–
Chaetomium globosum Kuhze ex Steud	0.5	0.5	–	–	–	–	–	–	–	–	–	–	–	–
Colletotrichum dematium f. sp. *truncata* Arx.	3.0	0.5	–	–	0.5	1.0	–	–	–	1.0	–	0.5	0.5	–
Corynespora cassicola (Berk. & Curt.) Wei	–	0.5	–	–	–	0.5	–	–	–	0.5	–	0.5	–	0.5
Curvularia sp.	6.0	7.5	–	–	–	–	–	3.5	–	1.0	–	–	–	–
Diaporthe phaseolorum (Cook & Ellis) Sacc.	3.0	3.0	0.5	–	–	1.0	–	–	0.5	3.0	0.5	2.0	–	1.5
Fusarium spp.	18.0	11.0	2.5	2.5	3.0	9.0	–	1.5	3.0	10.0	4.0	5.5	0.5	7.0
Macrophomina phaseolina (Tassi) Goid	0.5	–	–	–	–	–	–	–	–	–	–	–	–	–
Penicillium sp.	0.5	2.5	–	–	–	1.5	–	–	–	–	–	–	–	–
Rhizopus nigricans (Ehrenb. ex. Fr.) Lind.	1.0	3.5	–	–	–	0.5	–	1.0	–	–	–	–	–	–
Syncephalastrum sp.	–	–	–	1.0	–	–	1.5	13.0	–	1.0	–	–	–	–

*P PDA method
*B Blotter method

TABLE 5

Effect of seed treatment on seed germination

Treatment	†Average germination on blotter	†Average germination on PDA
Captan	9.15 a*	8.95 a*
Benomyl	9.05 a	9.50 a
Thiram	8.75 a	8.60 bc
Dithane M45	8.65 a	9.15 a
Control	8.35 a	8.15 c
Busan	7.15 b	8.35 c
Hot water	1.10 c	5.5 d

† Based on combined data from 20 replications of 10 seed per treatment.
* Values followed by similar letters in the same column are not significantly different at P = 0.05 by New Duncan Multiple Range Test.

In comparing the two isolation procedures, it was found that more isolates were obtained from the blotter method; however, in terms of the number of genera, a total of 18 against 15 was isolated on PDA. This is to be expected since seeds which were plated on PDA had been subjected to surface sterilization and PDA is more sensitive in detecting even traces of infection (Neergaard, 1977). However, one species, *Myrothecium roridum*, was not isolated by the agar method. This is to be expected since the fungus occurred only on the surface of the testa; therefore, it is removed by surface sterilization. (Wan Zainun and Parbery, 1977; 1980).

One of the factors which lowers seed quality and seed germination is seed-borne fungi. Our study has shown that a total of eighteen genera of fungi had been isolated from 14 different cultivars of soybean and six were potential pathogens belonging to genera, *Colletotrichum, Diaporthe, Fusarium, Macrophomina, Myrothecium* and *Phoma*. Wan Zainun *et al.* (1979) reported 13 species of pathogens from 34 cultivars of soybean grown in Selangor and most of the pathogens were new records for Malaysia.

The frequent occurrence of such potentially pathogenic fungi on the cultivars of soybean poses a potential threat to the grain legume establishment in this country. The grain legume development programmes here are based on cultivars introduced from overseas such as the United States of America and the Asian Vegetable Research and Development Centre (AVRDC), Taiwan. Thus, failure to observe strict quarantine procedures during the importation of such seeds could lead to the introduction of a variety of destructive diseases.

All fungicide treatments and hot water treatment reduced the percentage of fungi present on soybean seeds. Among the five fungicides used only benomyl was effective in eliminating potential pathogens viz. *Colletotrichum dematium, Corynespora cassicola, Diaporthe phaseolorum* and *Macrophomina phaseolina*. Ellis *et al.* (1975) showed that benomyl was taken up by both the seed coats and embryo tissues and this may explain total eradication of the above pathogens from the benomyl-treated seeds. Other fungicides such as thiram and captan were found by Ellis *et al.* (1975) to move into the seed coats of treated seeds but not into the embryo tissues. This is why captan and thiram were not effective against the internally seed-borne pathogens such as *Colletotrichum, Diaporthe* and *Macrophomina*. However, captan and thiram were effective against *Aspergillus, Chaetomium* and *Penicillium*.

This study also showed that seed germination, except for those which had been Busan-treated, was significantly increased with fungicidal-seed treatment. However, seeds which were treated with benomyl and dithane M 45 gave the highest seed germination. Bolkan *et al.* (1976) found that benomyl was most effective in reducing the frequency of total fungal recovery and increasing seed germination. Ellis *et al.* (1975) found that seed germination was slightly reduced in vermiculite and soil when compared to germination on PDA. Our results showed a similar trend for soybean seeds which were incubated on PDA when compared to those germinated on the blotter. These differences cannot be fully explained. It could be due to the nature and composition of PDA which seem to induce better germination of soybean than the blotter, vermiculite or soil.

Soybean seeds which were subjected to hot water treatment gave the lowest percent seed germination. Seed became soft and decayed resulting in a loss in viability. According to Neergaard (1977), hot water treatments are effective on seeds of cereals and vegetables such as onions, tomato, crucifers and celery. This study showed that benomyl is among the fungicides most effective against the potentially pathogenic fungi of soybean seeds. However, this research on the seed treatments is based only on *in vitro* experiments and hence conclusion for practice cannot be drawn. A similar caution is expressed in regard to seed germination.

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