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SEED–BORNE PATHOGENS IN OKRA FRUIT ROT

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RINGKASAN

Rhizoctonia sp. dan *Choanephora cucurbitarum* adalah organism penyebab penyakit buah–busuk *Hibiscus esculentus* (bendir). Tanda–tanda penyakit yang disebabkan oleh kulat–kulat ini direncanakan dan kerugian tanaman di plot yang berpenyakit dianggarkan sebanyak 20%. Kedua–dua kulat ini didapati dibawa oleh biji–benih. Dua 'isolate' *Rhizoctonia* sp. telah didapati dari biji–benih yang berpenyakit, satu daripadanya menyerupai 'isolate' dari buah yang berpenyakit dan 'isolate' yang lagi satu mempunyai morfologi yang berlainan. Kedua–dua 'isolate' itu 'pathogenic' kepada buah bendir hijau yang belum dipetik, yang luka atau tidak dan juga pada anak pokok bendir berumur 10 hari. 'Isolate' *C. cucurbitarum* yang dibawa oleh biji–benih pula 'pathogenic' ke atas bunga bendir yang belum dipetik dan buah bendir yang luka; tetapi tidak 'pathogenic' ke atas buah bendir yang tidak luka dan pada anak pokok bendir. Penggunaan air hangat pada suhu 60°C selama 30 minit sangat berkesan untuk menghindarkan kulat yang dibawa oleh biji–benih itu dengan tidak mengurangkan 'viability' biji–benih itu sendiri.

INTRODUCTION

Fruit rot of *Hibiscus esculentus* (okra, ladies' fingers) was observed in a seed multiplication plot at Serdang, Selangor, Peninsular Malaysia during the wet month of May 1974. The organisms associated with the disease were found to be a *Rhizoctonia* sp., the imperfect state of *Thanatephorus cucumeris* (Frank) Donk and *Choanephora cucurbitarum* (Berk and Rav.) Thaxt.

Prior to this there was no record of okra fruit rot caused by *Rhizoctonia* sp. in this country. However, *C. cucurbitarum* has been reported as a weak pathogen of okra leaves, flowers and fruit (THOMPSON and JOHNSTON, 1953; CHUPP and SHERF, 1960). A few species of *Rhizoctonia* are known to be the seed–borne fungi of okra eg. *R. solani* and *R. bataticola* (NOBLE, 1968; VIR and GAUR, 1970). In addition to *Rhizoctonia* spp. other recorded seed–borne pathogens of okra are *Ascochyta abelmoschi* and *Fusarium solani* (SILVEIRA, 1947; JACKSON, 1964; NOBLE, 1968).

This paper reports the symptoms and field assessment of the okra fruit rot, the detection of the causal organisms on the seeds, the pathogenicity of the fungi on various parts of the host plant and the effect of hot water treatment on the seed–borne fungi of contaminated seeds.

MATERIALS AND METHODS

Diseased fruit specimens were examined in the laboratory for detailed symptoms and identification of the causal organisms. The organisms from discoloured seeds of infected green fruit were isolated on potato dextrose agar (PDA) slants. Identification of these organisms were confirmed by the Commonwealth Mycological Institute, England. Alternate rows of okra plants in the diseased plot were inspected to determine the percentage of rotted fruit which included those caused by *Rhizoctonia* sp. or *C. cucurbitarum* or both. A total of 1,531 fruit borne on 368 plants were examined, 2 to 3 weeks before harvest. After harvesting seeds were separated from brown pods (matured fruit) which have been sun–dried for 3 to 4 days. Samples of the seeds were taken, mixed thoroughly and subdivided into lots of approximately 450 seeds per lot. Each seed–lot was placed in a sealed plastic bag (10 x 10 cm) and kept in the laboratory at 22–25°C till required for testing.

Blotter and agar plate tests.

The okra seeds were tested by the blotter and agar (PDA) plate methods to determine if the two fruit rot fungi were seed-borne. Four hundred seeds in replicates of 100 were used in each test. In the blotter test 10 untreated seeds were spaced on 3 layers of wet filter paper (Whatman No. 1) per Petri dish (9.5 cm diameter). The seeds for the PDA plate test were surface sterilized with 1% sodium hypochlorite (clorox) for 10 minutes before plating, 5 seeds per Petri dish. Incubation period for the seeds was 7 days at laboratory temperature, in normal alternating day light and darkness. The number of seeds with colonies of *Rhizoctonia* sp. and *C. cucurbitarum* were recorded on the 4th and 7th day of incubation.

Pathogenicity test.

Two isolates of *Rhizoctonia* sp., A and B and an isolate of *C. cucurbitarum* obtained from infected seeds during the previous PDA plate test were used in the pathogenicity experiments. The fungal inocula were prepared from 1 week old cultures, grown on PDA plates. Non-detached flowers, wounded and unwounded, immature, green fruit of potted okra plants were inoculated with culture discs of the *Rhizoctonia* isolates (0.4 cm diameter) and sprays of *C. cucurbitarum* spore suspension (20,000 spores/ml). Wounding was performed by making 5 pricks with a needle at the base and stylar end of each fruit. After inoculation each plant was enclosed in a plastic chamber and placed in the shade. The minimum number of replicates for each treatment was six. For the seedling inoculation, 2 culture discs of the respective isolate were placed at the collar of healthy, 10 day-old seedlings and control seedlings were inoculated with 2 discs of PDA. All the seedlings were grown in plastic pots (6.5 x 8.5 cm) containing sterilized soil mixture (1:1 soil and sand). There were 12 seedlings in each treatment, all of which were placed in a humid chamber.

Hot water treatment.

Seeds which have been kept for 3 months were used in the hot-water treatment. Five seed lots in separate muslin bags were suspended in a hot-water bath at a constant temperature of 55°C. for 10. 20 and 30 minutes and at 60°C and 70°C for 30 minutes respectively. After the treatment they were allowed to dry in the laboratory for 48 hours before planting. The treated and a non-treated seed lots were surface sterilized with sodium hypochlorite and plated out as in the PDA plate test. The percentage of seeds germinated and infected with *Rhizoctonia* sp. and *C. cucurbitarum,* were recorded. A set of the hot-water treated seeds were also germinated in boxes of sterile soil mixture for a period of 14 days. The number of viable seedlings were recorded.

RESULTS

Disease symptoms and assessment.

Fruit rot caused by both the fungi appeared to be similar at the early stage of infection. On immature, green fruit the fungi produced brown, water-soak lesions which spread rapidly from the points of infection, causing the fruit to be partially or completely rotted, depending on environmental conditions *(Fig. 1).* Under favourable humid conditions, *Rhizoctonia* fruit rot usually showed white, cottony mycelium over the rotted area while black fungal heads were often found on the area infected by *C. cucurbitarum (Fig. 2).* With the onset of fine weather, the superficial white mycelium of *Rhizoctonia* sp. and the black fungal heads of *C. cucurbitarum* disappeared, and the partially infected fruit often recovered as the lesions became dry. In the absence of the fungal mycelium and the fruiting bodies, it is difficult to distinguish between the rots caused by the

Figure 1. Detached okra fruit, inoculated with Rhizoctonia sp., showing brown rot symptom.

Figure 2. Non–detached okra fruit, inoculated with Choanephora cucurbitarum, showing brown rot symptom and the black fungal fruiting bodies.

Rhizoctonia sp. and *C. cucurbitarum.* However, the causal organisms could be easily determined by incubating the rotted fruit in moist chamber in the laboratory. *Rhizoctonia* fruit rot is characterised by the formation of sclerotia and *C. cucurbitarum,* by the sporangia formed.

Assessment of the disease in the field showed that 28% of the plants had one or more rotted fruit and 20% of the attached fruit were infected with either or both of the fungi. In most cases,

Rhizoctonia sp. infected the fruit from the basal part and *C. cucurbitarum*, from the stylar end or from wounds made by the fruit borers, *Earias fabia*. Matured fruit were observed to be less susceptible to infection.

Detection of the fruit rot fungi on seeds.

The two fruit rot fungi were detected on the okra seeds in the blotter and agar plate tests. The percentage incidence of the fungi on the seeds is shown in *Table 1.* On blotter, colonies of *Rhizoctonia* sp. from infested seeds developed sclerotia after the 5th day of incubation while black conidial heads and sporangia of *C. cucurbitarum* appeared on the 3rd or 4th day. *Rhizoctonia* colonies on PDA were identified by the characteristic pattern of mycelial growth on the 4th day and the appearance of sclerotia on the 7th day, while the fruiting bodies of *C. cucurbitarum* appeared after the 3rd or 4th day *(Figs. 3 and 4).* Other seed–borne organisms such as *Fusarium* sp. and bacteria were observed but not recorded in this study.

TABLE 1. DETECTION OF *RHIZOCTONIA* SP. AND *C. CUCURBITARUM* ON OKRA SEEDS BY THE BLOTTER AND AGAR PLATE TESTS.

Fungi	Blotter Test Infected seeds (%)	Agar Test Infected seeds (%)
Rhizoctonia sp.	16.5	18.5
C. cucurbitarum	37.5	17.0

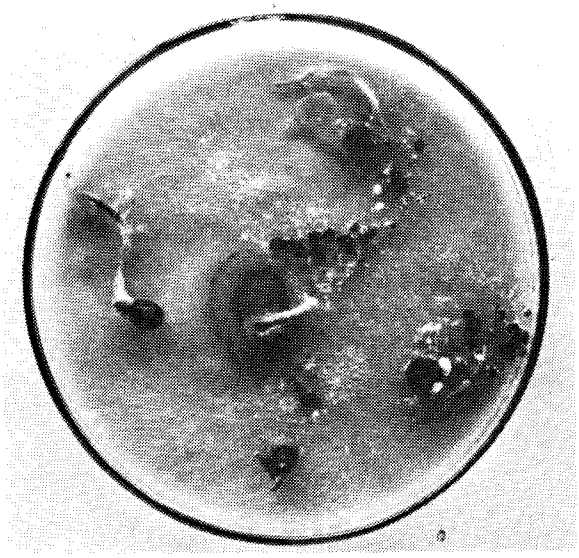

Figure 3. Rhizoctonia sp. with white to brown sclerotia on potato dextrose agar plate.

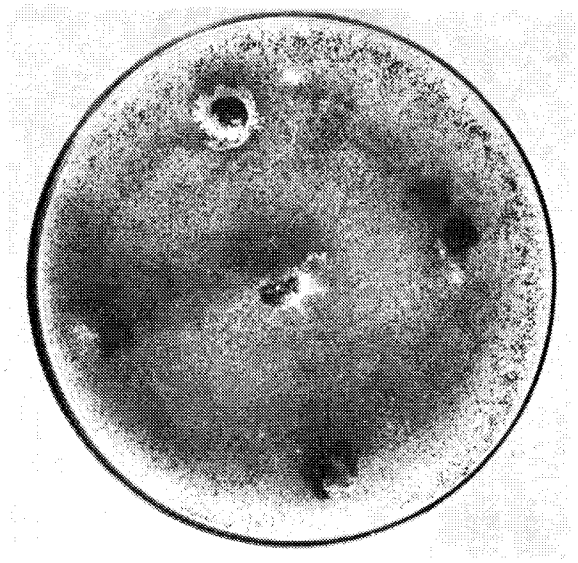

Figure 4. Choanephora cucurbitarum with black conidial heads and sporangia on potato dextrose agar plate.

Two different isolates of *Rhizoctonia* sp. (A and B) and an isolate of *C. cucurbitarum* were observed and isolated from the seeds tested on PDA plates. Isolate A resembled the *Rhizoctonia* sp., isolated from naturally infected fruit while isolate B was morphologically diffirent. Isolate B produced compact mycelium and much smaller sclerotia on PDA medium. Both isolates gave rise to seedling rot. The *C. cucurbitarum* isolated from seeds was similar to that of naturally infected fruit. It grew profusely on PDA but did not infect healthy germinating seeds.

Pathogenicity of the seed–borne fungi.

Non–detached wounded and unwounded immature fruit and 10–day old seedlings of okra were susceptible to the 2 isolates of *Rhizoctonia* sp. A and B. All the inoculated fruit showed the symptoms of wet, brown rot, similar to that observed in the field, and all the inoculated seedlings produced symptoms of damping off within 1 week of inoculation. The non–inoculated fruit and seedlings were normal and healthy. The two types of *Rhizoctonia* sp. were re–isolated from the inoculated fruit and seedlings.

The seed–borne isolate of *C. cucurbitarum* was pathogenic to flowers and wounded fruit only. All the 10 inoculated flowers rotted and were covered with black fungal fruiting bodies within 3–4 days, after which they dropped off from the plants. Three of the 10 non–inoculated flowers were also affected. This showed that aerial spore contamination might have occurred during the experiment. The inoculated fruit were infected from the wounded stylar end but not from the wounded basal area. Unwounded fruit were not infected with the exception of one which rotted from the wounds made by the fruit borer. Old, yellowing leaves were also observed to be infected. A summary of the pathogenicity tests is shown in *Table 2.*

Hot water treatment.

Seeds which have been exposed to 60°C and 70°C for 30 minutes were free from the seed–borne fungi, *Rhizoctonia* sp. and *C. cucurbitarum.* The viability of the seeds as noted in the

TABLE 2. PATHOGENICITY OF *RHIZOCTONIA* SP. AND *C. CUCURBITARUM.*

Parts of okra plants	Infection by *Rhizoctonia* sp	Infection by *C. cucurbitarum*
Flowers	–	+
Wounded fruit	+	+
Unwounded fruit	+	0
Seedlings	+	0

+ = positive infection 0 = negative infection

agar plate test was not lowered by the heat treatment at 60°C for 30 minutes, but it was reduced to 2% at 70°C for the same period of exposure *(Table 3).* Results obtained from the germinatioı test, carried out on sterile soil mixture confirmed that hot–water treatment at 55°C and 60°C did not affect the viability of the seeds. In this test, the percentage viability of treated seeds was apparently higher than those of non treated seeds. All the emerged seedlings, including those of the controls were healthy up to the 14th day.

TABLE 3. EFFECT OF HOT WATER TREATMENT OF OKRA SEEDS ON *RHIZOCTONIA* SP. AND *C. CUCURBITARUM.*

Treatment	Incidence of the seed–borne fungi and germination of okra seeds			
	% incidence of *Rhizoctonia* sp.	% incidence of *C. cucurbitarum*	% seed germination on PDA[1]	% seed germination in soil mixture.[2]
70°C for 30 min.	0.00	0.00	2.0	–
60°C for 30 min.	0.00	0.00	95.0	78
55°C for 30 min.	0.25	2.25	95.0	65
55°C for 20 min.	1.25	0.00	96.0	–
55°C for 10 min.	5.70	1.00	96.0	–
Control	15.50	8.00	96.5	60

[1]Germination count was based on the appearance of radicles.

[2]Germination count was based on the emergence of seedlings.

DISCUSSION

Results of the investigation show that fruit rot of okra can be caused by *Rhizoctonia* sp. or *C. cucurbitarum.* The disease has not been observed during fine weather but in wet period its incidence

could cause a 20% loss in crop yield. Of the two fungi, the former is the more virulent pathogen as it infects wounded as well as unwounded fruit and it also causes damping–off of okra seedlings; whereas, the latter fungus only infects flowers, fading leaves and fruit which have been injured or damaged by insect borers.

Both the fungi can be detected on okra seeds by the blotter and agar plate tests. They are found to be seed–borne for the first time in Peninsular Malaysia. In other countries *Rhizoctonia* sp. has been known to be seed–borne (NOBLE, 1968; VIR and GAUR, 1970) but not *C. cucurbitarum.*

Sclerotia of *Rhizoctonia* sp. have not been observed on the seeds used for testing. The positive isolation of the fungus from seeds which have been pretreated with sodium hypochlorite and the absence of superficial sclerotia, indicate that the fungus is probably present within the seed. In the case of *C. cucurbitarum* the minute fungal spores which adhere to the seed coat may survive the chemical pretreatment.

Seed transmission of the two pathogens has yet to be investigated. However, it is to be noted that the use of infected seeds may directly or indirectly initiate an outbreak of the fruit rot disease in the field. As a preventive measure of disease control, known infected seed lots should be treated before sowing, particularly in disease – free areas. Hot water treatment has been found to be effective against the seed–borne pathogens, but it is a more cumbersome method compared to chemical treatment. In view of this, it will be feasible to evaluate for effective fungicides, especially those of the systemic types to disinfect and protect contaminated okra seeds.

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SUMMARY

Rhizoctonia sp. and *Choanephora cucurbitarum* were the causal organisms of *Hibiscus esculentus* (okra) fruit rot. The symptoms produced by these fungi were described and crop loss was estimated to be 20% in the diseased plot. Both the fungi were found to be seed–borne. Two isolates of *Rhizoctonia* sp. were obtained from infected seeds, one of which resembled the isolate from naturally infected fruit while the other was morphologically different. They were both pathogenic to non–detached, wounded and unwounded green fruit and 10–day old seedlings of okra. The seed–borne isolate of *C. cucurbitarum* on the other hand was pathogenic to non–detached flowers and wounded fruit but not unwounded fruit and seedlings. Hot water treatment at 60°C for 30 minutes was effective in eliminating the seed–borne fungi without affecting the viability of the seeds.

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