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Rhizoctonia solani, a seed-borne pathogen of French Bean in Malaysia

WAN ZAINUN WAN NIK and M. Y. YAP[1]

Key words: *Rhizoctonia solani;* **Seed-borne pathogen; French bean; Malaysia.**

RINGKASAN

Penyelidekan dalam aspek aspek biologi, patogenesiti dan kawalan Rhizoctonia solani *Kuhn (perengkat tak lengkap kulat* Thanatephorus cucumaris *(Frank) Donk telah dijalankan dengan menggunakan asingan kulat daripada biji kacang buncis. Kulat ini telah didapati dibawa bersama biji pada kacang buncis tempatan dan kacang buncis yang diimport. Kadar tumbesar kulat berbeza mengikut suhu dan media yang digunakan. Agar 'malt extract' memberikan tumbesar yang kurang sekali manakala agar kacang lima memberi tumbesar yang paling baik pada suhu 28°C. Kulat ini menjangkiti kacang buncis pada bahagian bahagian diatas permukaan tanah. 'Strain' kulat ini didapati mempunyai perumah yang luas. Penyelidikan 'in vitro' untuk mengkaji kemujraban racun kulat terhadap kulat* Rhizoctonia *menunjukkan yang pentachlorontirobenzene pada paras 50 ppm memberi kawalan yang baik.*

SUMMARY

A study on some aspects of the biology, pathogenicity and control of Rhizcotonia solani *Kuhn (the imperfect state of* Thanatephorus cucumaris *(Frank) Donk was undertaken using an isolate from infected French bean seed. The fungus was found to be seed-borne on both the imported and some local varieties of French bean. The rate of growth of the fungus varied with temperature and the medium used. Malt extract agar gave poor growth and lima bean agar supported the best growth of the fungus at 28°C. The fungus infects the aerial parts of the French bean plant. The strain was found to have a wide host range. An* in vitro *study to test the efficacy of fungicides against* Rhizoctonia *showed that pentachloronitrobenzene at 50 ppm gave a good control.*

INTRODUCTION

Rhizoctonia solani Kuhn (the imperfect state of *Thanatephorus cucumaris* (Frank) Donk is an ubiquitous soil-borne pathogen capable of inciting diseases of many crop plants. The fungus has been reported to cause seed decay of many crop plants (Weber, 1939; Walker, 1960; Ramsey and Smith, 1961), damping off of beans (Baker, 1947; Person, 1944), foliage diseases of beans (Atkins and Lewis, 1954; Weber, 1939; Onesirosan, 1977; Nene, 1978) and diseases of other crop plants (Ramsey and Smith, 1961; Ho, 1971; Tai and Musa, 1975). In Malaysia, apart from the study of Tai and Musa (1975), there are no other published records of such transmission on other seeds. A study on the biology and pathogenic potential of the fungus was undertaken using an isolate from infected French bean seeds. This is the first record of seed-borne transmission of *R. solani* on French bean in Malaysia.

MATERIALS AND METHODS

Isolation of Fungus

Two methods were employed to ensure the isolation of *R. solani* and other fungi present on or in seeds. The two methods were incubation of seeds on blotter and incubation on potato dextrose agar (PDA) plates.

The Blotter Method

This involved placing 10 non-sterilised seeds on three layers of moistened (9 cm Whatman No. 1 filter papers) in each petri dish. Two hundred seeds were used. The plates were incubated in the dark at 25°C for 7 days. Fungi developing on seeds were isolated onto PDA tubes for identification and pathogenicity tests. Confirmation of the fungus was done by the Commonwealth Mycological Institute, England.

[1]Selborne Estate Padang Tengku, Kuala Lipis, Pahang.

Key to authors' names: Wan Zainun and Yap, M.Y.

Potato Dextrose agar method

Ten surface sterilised seeds were placed on each PDA plate and incubated at 25°C in the dark. A total of 200 seeds were used for each seed sample. Fungi developing from each seed were transferred to fresh PDA tubes for storage and morphological study.

Growth, Morphology and Pathogenicity

Growth of the fungus was compared on seven artificial media at temperatures ranging from 10 to 40°C. The seven media used were potato-dextrose agar (PDA), corn meal agar (CMA), lima bean agar (LBA), bean pod agar (BPA), Yeast extract agar (YEA), Malt extract agar (MEA) and V-8 juice agar (V-8). Growth measurements were given as the radial diameter on the above media after five days of growth at 28°C. Morphological characteristics of the hyphae and sclerotia were based on cultures grown on autoclaved cellophane pieces placed on PDA plate.

Pathogenicity studies

The pathogenic potential of the fungus was assessed in a series of inoculation studies of French bean pods. Inoculum was prepared by macerating a week old pure culture of *R. solani* growing on PDA. To each PDA plate 20 ml of sterile distilled water was added. Inoculation was made by brushing the inoculum with a soft camel brush over the whole surface of the pod.

Efficacy of some fungicides against R. solani

Five commercial fungicidal compounds were used. They were captan 500 (a.i: n-trichloromethyl thio 4-cyclohexene-1, 2-dicaboximide; 50% w/w); thiram (a.i: tetramethylthiuram disulphide; 80% w/w); benomyl (a.i: (butylcarbamyl 1-2-benzimidazole carbamate; 50% w/w); brassicol (a.i: pentachloronitrobenzene; 75% w/w); Vitigran blue (a.i: copper oxychloride; 60% w/w). Concentrations of each fungicide at 10, 50, 100, 500 and 1000 ppm active ingredients were added aseptically to molten sterile PDA. A *R. solani* mycelial disc of 5 mm diameter was placed centrally on the solidified agar medium in petri dishes. The plates were incubated in the dark at 28°C. The diameter of the colonies was observed daily for a period of one week.

RESULTS

Morphological characteristics

On PDA, the colony of *R. solani* grew radially to a diameter of 9 cm in three days. Growth rate was 1.2 – 1.5 mm per hour. The mycelium was generally hyaline with dense cytoplasm and slight constriction at each septum.

Young vegetative hyphal branches at the periphery of the colony branched out at an acute angle approaching 45° to the main hyphae. In some cases the young branches arose at approximately right angles to the main hyphae. Branches arose from mature hyphae both at right angles and at acute angles (approx, 45°).

Young white sclerotia were formed from masses of monilioid cells in four day-old culture. The sclerotia turned light brown after 24 – 48 hours. Mature sclerotia were brown in colour with no definite form.

Growth on different media

Radial growth of the fungus on all media except that of malt extract agar (MEA) was rapid *(Fig. 1)*. The rate of growth varied with temperature and medium. Growth rate of the fungus on different media were as follows: 1.39 mm/hr on PDA; 0.90 mm/hr on MEA; 1.34 mm/hr on V-8; 1.56 mm/hr on LBA; 1.39 mm/hr on BPA; 1.45 mm/hr on YEA and 1.46 mm/hr on CMA. Malt extract agar gave poor growth and lima bean agar (LBA) supported the best growth of the isolate. Detailed cultural characteristics of the isolate are summarised in Table 1.

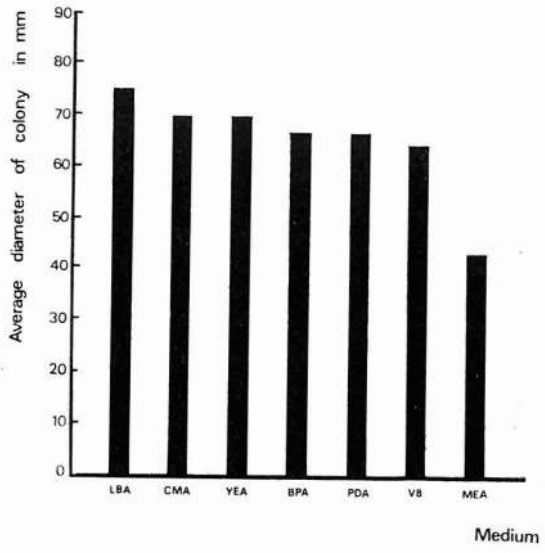

Fig. 1: Influence of agar media on the growth of R. solani, *2 days after incubation at 28°C.*

Effect of R. solani *on pod inoculation*

One day after inoculation, sclerotia began to produce threads of hyphae over the surface of the pods. Dark brown lesions were formed on the pods after three days. The mycelium grew rapidly and colonized shoots, flower buds, pods, stems and leaves.

TABLE 1

Cultural Characteristics of 7-day-old culture of *R. solani* growing on various agar media at 28°C

Medium	Cultural Characteristics
PDA	Brown on both sides. Moderately dense hyaline-white cottony mycelium. Dense lateral branching. Dense brown sclerotia (> 600). Globose to suglobose sclerotia with tufted mycelium. Sclerotia size: 0.3 – 3 mm.
BPA	Light brown on both sides. Mycelium adpressed. Dense lateral branching. Scanty dark brown sclerotia (> 100). Globose to subglobose sclerotia. Sclerotia size: 0.4 – 2 mm.
LBA	Light brown on both sides. Mycelium adpressed. Dense lateral branching. Moderately dense brown sclerotia (300 – 600). Globose to irregularly elongated sclerotia. Sclerotia size: 0.2 – 2 mm.
YEA	Light hyaline-brown on surface. Olive yellow at reverse. Mycelium adpressed. Moderately dense lateral branching. No sclerotia.
CMA	Hyaline-white on surface. Light yellow at reverse. Mycelium adpressed. Moderately dense lateral branching. Scanty reddish brown sclerotia (100). Subglobose to flat sclerotia. Sclerotia size: 0.5 – 3 mm.
MEA	Yellowish brown on surface. Light yellowish brown at reverse. Moderately dense hyaline-white cottony aerial mycelium. Dense lateral branching. Dense yellowish brown sclerotia (> 600). Loose masses to subglobose sclerotia. Sclerotia size : 1 1.5 mm.
V-8	Brownish yellow on surface. Yellow at reverse. Mycelium adpressed. Moderately dense lateral branching. No sclerotia.

Isolation of R. solani *and other fungi from French bean seeds*

High incidence of *Rhizoctonia* (27 – 39.75%) was found in seeds of the pioneer variety harvested from UPM vegetable farm (Table 2). However, the infection of *Rhizoctonia* from a commercial sample of pioneer variety was low (Table 3). Isolation from one sample of local French bean seeds gave only 0.50% of *Rhizoctonia* infected seeds (Table 4).

Efficacy of some fungicides against R. solani

The relative efficacy of the five fungicides in inhibiting the growth of *R. solani* is shown in *Fig. 2.* The degree of inhibition of growth of the fungus varies with the types of fungicides used. It was found that with the exception of vitigran blue, all other fungicides evaluated were significant at 5 per cent level (Table 5).

TABLE 2

Frequency of isolation of some fungi from French bean seeds (FB-P2)*

Fungi	% Isolation	
	Blotter	PDA
Aspergillus spp.	32.50	4.75
Botryodiplodia sp.	0.75	6.25
Choanephora cucurbitarum	6.00	2.50
Colletotrichum sp.	11.75	10.75
Corynespora sp.	1.00	0.75
Cunninghamella sp.	0.0	0.25
Diaporthe phaseolorum	0.25	0.50
Fusarium spp.	55.50	32.25
Drechslera sp.	0.0	0.25
Monilia sp.	0.0	0.25
Penicillium spp.	0.0	1.25
Rhizoctonia solani	39.75	27.00
Rhizopus stolonifer	1.75	5.75

* FB-P2 = Pioneer variety

TABLE 3

Frequency of isolation of some fungi from French bean seeds (FB-P1)*

Fungi	% Isolation	
	Blotter	PDA
Aspergillus spp.	–	32.00
Curvularia spp.	–	0.50
Fusarium spp.	–	5.00
Penicillium spp.	–	3.00
Rhizoctonia solani	–	0.25
Rhizopus stolonifer	–	1.75
Unidentified fungus	–	9.50

* FB-P1 = Pioneer variety

Pentachloronitrobenzene (PCNB) gave the highest percentage of inhibition at 50 ppm. Benlate, captan and thiram were found to have intermediate fungicidal effect on the growth of *R. solani.* An analysis of variance showed that there is a significant difference at 1 per cent level between the fungicides; between the various concentration levels and between interaction of fungicides and concentrations.

TABLE 4

Frequency of isolation of some fungi from French bean seeds (FB-L1)*

Fungi	% Isolation	
	Blotter	PDA
Alternaria sp.	8.50	8.00
Aspergillus spp.	37.00	0.0
Botryodiplodia sp.	0.0	0.50
Corynespora sp.	0.0	2.50
Curvularia spp.	0.0	1.00
Fusarium spp.	4.00	2.50
Rhizoctonia solani	0.0	0.50

* FB-L1 = Local variety

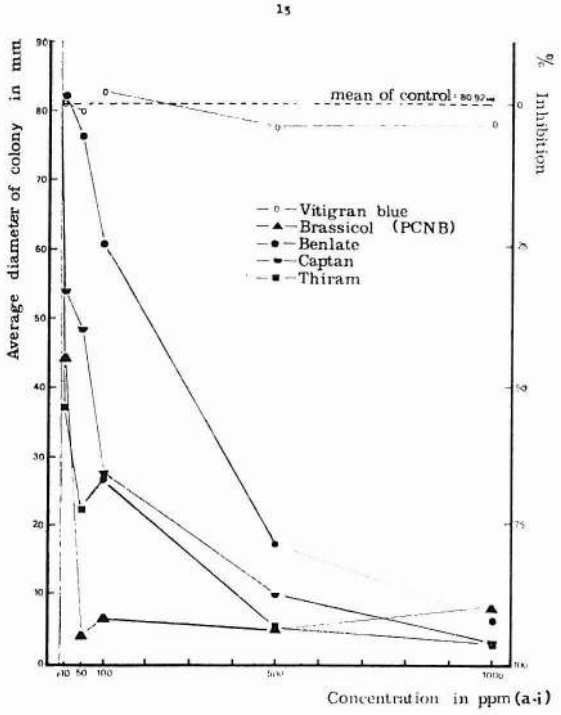

Fig. 2: Effect of fungicides in the control of R. solani *2 days after incubation at 28°C.*

DISCUSSION

The morphology of hyphae and sclerotia resembles that recorded by Peltier (1915), Palo (1961) and Saksena and Vaartaja (1961, 1971). However, Saksena and Vaartaja (1971) referred to monilioid cells of sclerotia as chlamydospores and suggested that their morphology is characteristic for each species of *Rhizoctonia*. Growth of the isolate varied with temperature and medium used. Ullstrup (1930) reported that the growth rate of a given isolate at a given temperature differs on different media and Le Clerg (1934) reported that the growth rate of a particular isolate on PDA was not always correlated with that of malt extract agar. The fungal mycelium appeared whitish on V-8 agar and light brown on all other media. This could be due to variations in pigmentation on different media.

TABLE 5

Frequency of isolation of *R. solani* on PDA from inoculated and uninoculated French bean seeds

Fungi	% Isolation	
	Uninoculated seed	Inoculated seeds
Botryodiplodia theobromae	24.2	3.6
Colletotricum sp.	2.50	2.4
Curvularia spp.	1.7	6.0
Diaporthe phaseolorum	0.0	4.8
Fusarium spp.	28.3	3.6
Rhizoctonia solani	0.0	12.05

TABLE 6

Effectiveness of several fungicides on the radial growth inhibition of *R. solani*

Treatment	Mean Growth Rate (mm)[1,2]	
Control	80.9	A
Brassicol	13.3	B
Captan 500	28.3	C
Vitigran blue	79.9	A
Benlate	48.3	D
Thiram	18.5	E

[1]Values of colony diameter at 2-day-old and at 28°C
[2]Values with similar letter denotes no significant difference at 5% level as determined by Duncan's New Multiple Range Test.

The lower percentage of infection in French bean var. pioneer could be due to the fact that the seeds had been treated with fungicides before. However, pioneer seeds of the next generation which were harvested from the University farm gave a much higher percentage of infection (27 – 39.75 per cent). Baker (1947) reported that pepper seeds having 0.3% *Rhizoctonia* – infected seeds had a much higher percentage of infection in seeds of the next generation.

Seed transmission of *R. solani* in French bean was demonstrated in the glasshouse. *R. solani* occurred on as many as 12.05 per cent of seeds. Baker (1947) reported that bean pods in contact with soil were invaded and the mycelium grew through pod into the seed coat or cotyledons. Such transmission ensures the continued association of pathogenic strain of *Rhizoctonia* with the appropriate host.

Most of the fungicides tested *in vitro* against the strain of *R. solani* were moderately effective in inhibiting mycelial growth except that of vitigran blue. PCNB gave the greatest reduction in mycelial growth compared to all other fungicides used. Mc Carter and Barksdale (1977) reported that PCNB was one of the most effective chemicals in controlling *Rhizoctonia* rot of tomato fruit in the greenhouse and in the field. Benomyl was found to give a significant reduction in mycelial growth of *Rhizoctonia*. Batson (1973), Mc Carter and Barksdale (1977) reported similar findings although some of the results varied among the tests. Thiram and captan gave better control compared to benomyl in inhibiting the growth of *R. solani* strain. However, Mc Carter and Barksdale (1977) found that captan gave poorer control compared to other fungicides in reducing *Rhizoctonia* rot of tomato fruit. Differences in results could be due to the differences in methods of testing the fungicides, one being the *in vitro* and the other being the *in vivo* test. It could also be due to the difference in *Rhizoctonia* strains used in the two tests.

Although the study shows that *Rhizoctonia* can be controlled *in vitro,* the economic feasibility of applying fungicides in the field is still questionable since the fungus thrives well in soil. Therefore control should be aimed at eradicting the inoculum on the host plants as well as avoiding attack of inoculum from the soil on to the host plants. The use of *Rhizoctonia* tolerant cultivars of *Phaseolus vulgaris* in conjunction with the most effective fungicides may provide good control.

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