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Macrophomina phaseolina Detected in Seeds of *Sesamum indicum* and It's Pathogenicity

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참깨 種子에서 檢出된 *Macrophomina phaseolina*와 그의 病原性에 關하여

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ABSTRACT

Out of 12 seed samples of *Sesamum indicum* L. tested, *Macrophomina phaseolina* (Tassi.) Goid was detected in 7 samples for the first time in Korea. Detailed descriptions of the habit character and pycnidial and pycnospore morphology of this fungus were described. Pycnidia of this fungus were not formed on agar media but they were formed on Water Agar Leaf Media under fluorescent light. *M. phaseolina* aused heavy reduction in seed germination and seedling stand of sesame and produced charcoal rot symptom on potato tubers. It was also detected from over wintered plant debris and diseased seedlings in the field.

INTRODUCTION

Macrophomina phaseolina(Tassi.) Goid,(Syn. *Macrophomina phaseoli* (Maub.) Ashby, *Sclerotium bataticola* Taub) is a pathogen of worldwide importance, inciting several types of symptom on the hosts. The most usual symptoms are charcoal rot, root rot,wilt,damping-off and stem necrosis[2,13,16,17]

M. phaseolina is seed-borne in several crops[11] and the seedborne nature of this fungus for sunflower[3] and kidney bean[17] has been studied. Meiri and Sole[10] stated that *Sclerotium bataticola* is seedborne in sesame and a high degree of seed infection might account for low field germination. So far, this pathogen has not been described for any crop in Korea.

In this investigation results were presented on the incidence of the pathogen in sesame seed samples, pycnidial formation by this fungus, pathogenicity to sesame seedlings and potato tubers and it's occurence.

MATERIALS AND METHODS

Twelve seed samples of sesame used were collected from Chungnam Provincial Office of Rural Development and Crop Experiment Station in Korea. The samples were harvested in September 1979 and tested in March and May 1980 by th methods given below.

1. Blotter method. Two hundred of each sample were tested. Twenty-five seeds were plated on three water soaked blotters in plastic petri dishes of 9cm diameter. The dishes were incubated at 24°C for seven days under 12hr. alternating cycles of fluorescent light(2500~3000 Lux) and darkness. Seeds were examined under stereoscopic microscope

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and the seedlings and ungerminated seeds were observed for pycnidial and tiny sclerotial production.

Chlorine pretreated(2% in 5 minutes) seeds were plated on blotters in a subsequent experiment and the infection percentages were compared with those of untreated seeds.

2. Pycnidium formation by *M. phaseolina*. Five isolates of this fungus were used. Cultures were first grown on PDA at 26°C.

Mycelial disks from cultures 48hr. old were transferred to autoclaved leaf bits(2~3cm long) of barley, wheat and sesame placed on 1.5% water-agar in plastic petri dishes as described by Srini vasan *et al*[14] and Chidambaram *et al*[2] Plates were incubated in either darkness or fluorescent light for 12hr. each day. Observations were recorded by stereoscopic microscope 7 days after incubation and compared with respective isolates on PDA and Oatmeal Agar(OA) under similar incubation conditions of light and darkness.

3. Pathogenicity test. Pathogenicity of *M. phaseolina* isolates was determined by the techniques of Thirumalachar *et al*[16]

(1). Pre-emergence and post-emergence damping-off. Petri plates with PDA were inoculated with *M. phaseolina* and incubated at 26±1°C for two, four and eight days. After incubating the young and old mycelial mats were overlaid with sterilized soil 1/2-1cm thick and planted with surface sterilized sesame seeds. The plates were incubated at room temperature. In the controls the plates were without the fungal growth and had soil overlaid on PDA.

(2) Charcoal rot symptoms. Two days old mycelium from PDA was inserted into a potato tuber using a sterilized tooth pick, which was left in the place of inoculation. The tubers were surface sterilized with alcohol before inoculation and the inoculated tubers were incubated at room temperature.

RESULTS

1. Growth of *Macrophomina phaseolina* on seed.

Mycelium was very scanty, dark brown to black pycnidia and tiny sclerotia were appeared on the seed(Fig. 1). Mostly the pycnidia with a well defined neck were embedded in the seed coat, only the upper part with a protruding neck being visible. The pycnidial body might also be seen on the seed surface, and they might be formed in the mycelium. Sometimes the white to dull white ooze from the pycnidia formed a small cirrus. On the blotter, light coloured and very loose mycelium interspersed with dark brown to black, tiny scler-

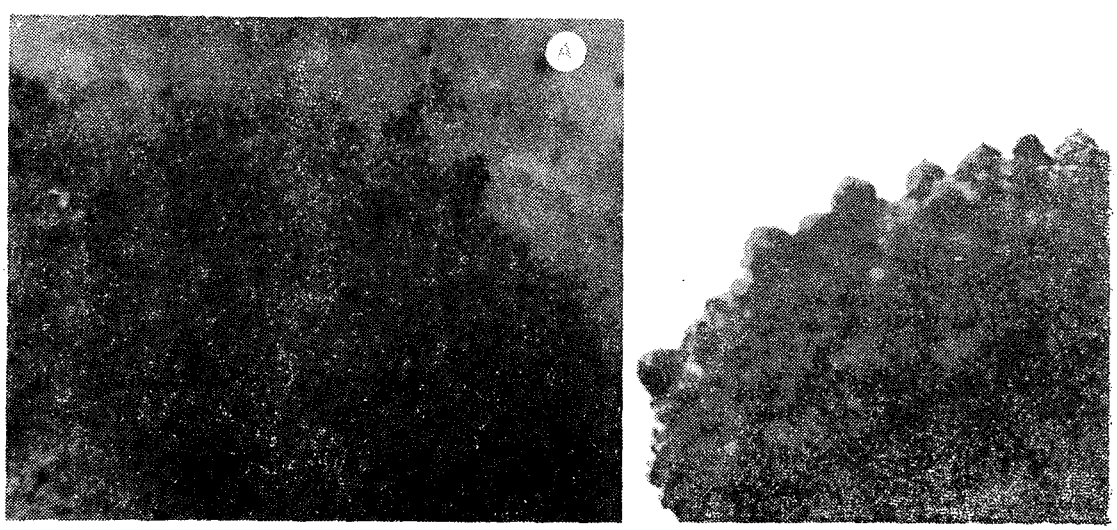

Fig. 1. Pycnidia and sclerotia of *Macrophomina phaseolina* on seed surface in blotter. (A) X25; (B) X40.

otia.

Heavily infected seeds usually did not germinate. A few of the seeds germinated and the seedlings being invariably killed.

2. Detection in seed samples.

Out of 12 seed samples tested 7 samples were found infected with *M. phaseolina*. The percentage infection was low in most of the samples, ranged 0.5~8.0% (Table 1). Chlorine pre-treatment reduced and in most samples completely wiped out the infections.

Table 1. Percentage infection of *Macrophomina phaseolina* in seed samples of *Sesamum indicum* recorded by blotter method

Sample No.	Untreated seeds	Pretreated seed (2% Chlorine 5 minutes)
1.	4.0	0.5
2.	0	0
3.	0.5	0
4.	1.5	0
5.	1.0	0
6.	0	0
7.	3.0	0
8.	0	0
9.	8.0	0.5
10.	0	0
11.	2.0	0
12.	0	0

3. Production of pycnidia by *M. phaseolina*.

All isolates produced abundant mycelium and sclerotia on PDA and OA. On Water Agar Leaf Medium(WALM) all isolates tested produced pycnidia and scanty mycelium and sclerotia under light.(Fig. 2) Leaves of barley and wheat appeared better substrates for pycnidial production than those of sesame. Two isolates of this fungus produced more pycnidia than sclerotia and others produced more sclerotia than pycnidia on WALM under fluolescent light.

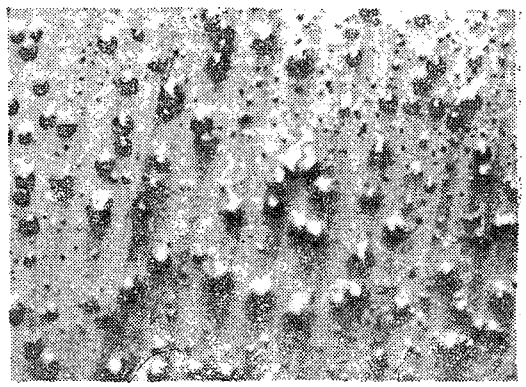

Fig. 2. Pycnidia and sclerotia of *Macrophomina phaseolina* on WALM. (25).

4. Measurements of pycnidia and pycnidiospores.

Demensions of pycnidia and pycnidiospores of three isolates of *M. phaseolina* were compared (Table 2). The length and width of pycnidia varied from 123 to 266μ and 115 to 245μ, respectively. Much variation in size of pycnidiospores within the same isolate was noticed. The measurements for pycnoidispores were 8.2~31.0×4.2~10.5μ. Pycnidiospores were hyaline, ellipsoid to obovoid, without septa and had a thin wall(Fig. 3.)

Table 2. Pycidial and pycnidiospore measurements of isolates of *Macrophomina phaseolina* based on 50 pycnidia and 100 pycnidiospores of the respective isolate.

Isolate No.	Pycnidia,(μ)	Pycnidiospores,(μ)
A	123.0−266.0 × 120.0−205.0	9.5−28.6 × 5.2−10.3
B	143.5−266.0 × 123.0−245.0	10.5−30.0 × 6.0−10.5
C	123.0−256.5 × 115.0−220.0	8.2−31.0 × 4.2− 9.5

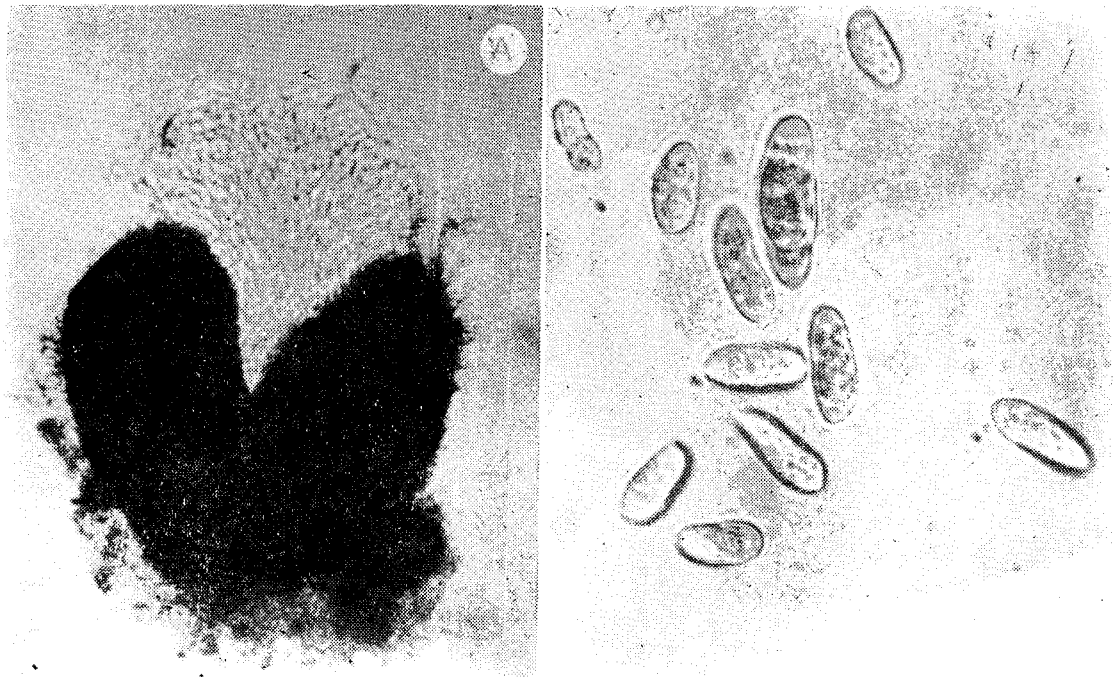

Fig. 3. Pycnidium and pycnidiospoes of *Marophomina phaseolina*.
(A)exuding pycnidiospores from a pycnidium (X100).
(B)pycnidiospores (X400).

5. Pathogenicity test.

(1) Pre-and post-emergence damping-off.

Seven days after sowing all seeds had grown in the control and the seedlings appeared completley healthy. In the inoculated plates with young mycelium cultured for 2days, 88~93% of damped-off seedlings were produced(Fig. 4, 5), while old mycelium cultured for 8 days produced only from 0 to 3.0% damping-off(Table 3).

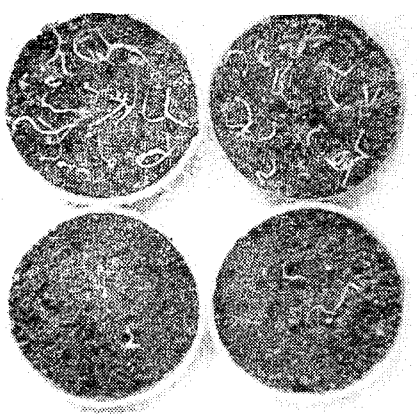

Fig. 4. Damping-off symptom in sesame seedlings due to *Macrophomina phaseolina* infection. (A) non-inoculated; (B) inoculated.

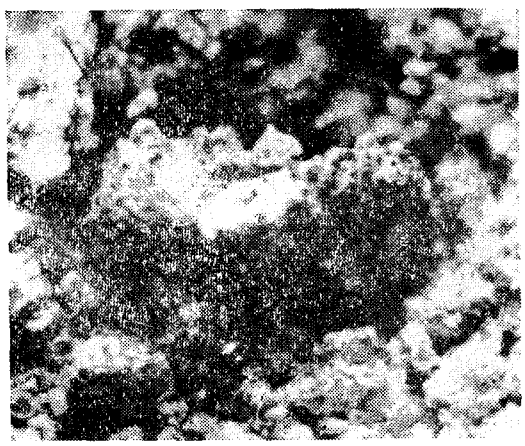

Fig. 5. Pycnidia and sclerotia of *Macrophomina phaseolina* on sesame seedling after damping-off. (X10).

Table 3. Percentage of pre-and post-emergence damping-off of sesame seedings raised from inoculated soils with *Macrophomina phaseolina*.

Age(days) of cultures at *inoculation*	% of pre-and post-emergence damping off		
	Isolate A	Isolate B	Isolate C
2	88.0	80.0	92.0
4	10.0	5.0	18.0
8	0.0	0.0	3.0
Check	0.0	0.0	1.0

(2) Charcoal rot symptoms.

When the potato tubers were cut through the point of inoculation 7 days after incubation, the blackening and charcoal rot type of disease was apparent with the hyphae traversing over the entire tuber tissues

6. Occurence

Attempts were made to isolate the pathogen from overwintered plant debris collected from the field of Yuseong in March 1980. *M. phaseolina* was detected sporadically and other fungi such as *Corynespora cassicola, Alternaria sesami, A. sesamicola* and *Fusarium* spp. were also recorded.

During the period from May to July 1980, seedlings of sesame showing damping-off(both pre-and post-emergence), root rot and wilt symptoms were collected from several locations of Chungnam district and subjected to investigate the causal organisms. *M. phaseolina* has been isolated from the diseased seedling obtained at Daejon, Yuseong, Nonsan and Keumsan. *C.cassicola* and *Fusarium* spp. have also been isolated occassionally from the diseased seedlings. These observations indicate the possibility that the disease may occur throughout sesame growing area in Korea.

DISCUSSION

Macrophomina phaseolina is world-w de in distribution, attacking many hosts and being seed-borne in several crops[11]. Association of this pathogen with seeds of sesame has been shown earlier by Meiri and Solel[10] but it was detected from the seeds of sesame for the first time in Korea. The pathogen is capable of affecting adversly the seed health. Seeds showing heavy infection usually did not germinate and rot, while seeds having light infection showed initial germination but soon radicle and hypocotyl were invaded by the fungus.

It is reported that there are numerous strains of this fungus, both morphologically and physiologically. Some of these produced both sclerotia and pycnidia, others only sclerotia[2,18]. Environmental factors such as light and media also greatly influenced pycnidial formation. In ordinary media, it does not form pycnidia,it may, however, sporulate when plated on special media[1,2,4,6,7,8,9,12,18]. According to Chidanbaram *et al*[2], only 20 out of 58 isolates originated from seeds of different hosts produced pycnidia on WALM under near ultraviolet light, and Watanabe[18] found that only 19 out of 50 different isolates originated from field soil, kidney bean seed, or contaminated dirt of seed formed pycnidia on dried hypocotyl segments placed on 1.5% water agar under different environmental conditions. In this study, 5 isolates tested from the seeds of sesame produced pycnidia on WALM under fluorescent light.

Inoculation experiments showed that the young cultures of the isolates of *M. phaseolina* tested were highly pathogenic, causing damping-off of germinating sesame seeds and charcoal rot symptom in potato tubers but old cultures bearing sclerotia did not readily bring about infection. Similar results have been reported by Thirumalachar[15].

Incidence of damping-off, wilt and stem rot caused by this pathogen in the fields and detection of pycnidia and sclerotia from the overwintered plant debris indicate that healthy seed also may be infected or contamined with the fungus when planted in such the fungus-infested field. In USA, Kinman and Martin[5] suggested that charcoal rot, *M. phaseoli*, will cause a stem rot of sesame and this disease is favored by droughtly conditions.

摘 要

참깨種子의 種子傳染菌을 調査하던中, 우리나라에서는 未記錄인 *Macrophomina phaseolina*가 12개 sample中 7개 sample에서 檢出되었다. 本 菌의 種子上에서의 生育相 및 柄子殼과 柄胞子의 形態를 記述하였다

本 菌의 柄子殼形成에는 光線이 必須條件이었으나 PDA나 OA培地上에서는 柄子殼이 形成되지 않았고 물한천잎培地上에서 柄子殼이 많이 形成되었다.

本 菌은 참깨種子의 심한 發芽沮害와 立枯가 原因이 되었고 감자塊莖에 炭腐病徵을 나타내었다.

本 菌은 越冬後의 참깨植物의 殘滓와 圃場의 罹病幼苗에서도 檢出되었다.

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