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Basal Stem Rot of Vegetables in Controlled Environment Greenhouses in Western Saudi Arabia: (B) Disease Characterization

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ABSTRACT. Diseased cucumber (*Cucumis sativus* L.), cantaloupe (Cucumis melo var. cantalopensis) and sweet pepper plants (*capsicum annum* L.) were received for diagnosis from various commercial controlled environment greenhouses at the Dept. of Arid Land Agriculture at King Abdulaziz University. They showed damping off of seedlings or severe wilting and necrosis at the lower parts of the stems of mature fruit-bearing plants. The pathogen was identified earlier as *Pythium aphanidermatum* (Edson) Fitzp. A pathogenicity test was performed by inoculating nine-day old seedlings of three plant genera grown in a conditioned growth room using agar plugs of the pathogen. Inoculated seedlings showed early symptoms of damping off after 8 hr from inoculation for cucumber and 10 hr for sweet pepper and cantaloupe seedlings. The highest percentage of infection of cucumber (90%) and cantaloupe seedlings (80%) was observed within 26 hr from inoculation. However, the highest percentage of infection of sweet pepper seedlings (50%) of sweet pepper seedlings was observed within 18 hr from inoculation. Symptoms started as a water soaking of the hypocotyl for a short period at the soil line, then immediately followed by girdling of the lesions that extended upward turning the hypocotyl into a thread-like organ. In another pathogenicity test in controlled environment greenhouse, lower stem necrosis of cucumber, pepper and cantaloupe plants appeared after 3, 4 and 5 weeks from sowing, respectively. Infected young cucumber and cantaloupe plants developed a severe wilting and a dark yellow lesions at the basal stem and crown area that extended up to 15 cm above the soil line. Pepper plants developed dark purple to dark brown or black discoloration of the basal stems that extended up to the lower leaves. For fruit-bearing

cucumber and cantaloupe plants, on the other hand, the lesions were limited to the crown area and/or few centimeters above the soil line. Pepper plants, however, expressed dark brown or black dry necrosis that extended up to 15 cm above the soil line. Maximum pecentage of infected plants within ten weeks from sowing were 85% for cucumber and cantaloupe plants and 63% for sweet pepper plants.

Introduction

To meet the local demands of vegetables in Saudi Arabia, more than 174 thousand tons of vegetable crops are produced annually in 430 commercial projects of controlled environment greenhouse facilities (Ministry of Agriculture, 1998). Though cooler temperatures and high relative humidity in greenhouses avert the harsh weather conditions prevailing in the open field (Cockshull, 1985). This, however, render plants more conducive to diseases (Jarvis, 1992). Powdery mildew, downy mildew, damping off and root rot are amongst the most prevalent diseases causing extensive losses in commercial greenhouse grown crops in Saudi Arabia (Abu-Jawdah, 1986; Sharif & Abdeen, 1987 and Al-Kherb, 1992).

Basal stem rot and damping off caused by *Pythium aphanidermatum* (Edson) Fitsp. are considered as a limiting factor to the production of many crops grown in the open field (Gottlieb & Butler, 1939; Freeman *et al.,* 1966, Littrell, 1969; McArter & Litterell, 1970; Williams, 1975 and Gullino, 1992) as well as in commercial greenhouses around the world (McArter *et al.,* 1980; Jenkins & Averre, 1983; Bates & Stanghellini, 1984; Gold & Stanghellini, 1985; Favrin *et al.,* 1988 and Menzies *et al.,* 1996).

Diseased samples of vegetable crops from different research and commercial greenhouses in the western region were submitted to the plant pathology lab., Depart. of Arid Land Agr. at King Abdulaziz University for diagnosis. Diseased cucumber (*Cucumis sativus* L.), and sweet pepper plants (*capsicum annum* L.) were collected from Agricultural Experimental Station at Hada-Sham (AESHA). Cucumber plants expressed severe wilt and necrosis at the basal part of the stem of mature plants. Sweet pepper plants showed a dark brown to black dry necrosis of the lower stem. Other diseased samples of cucumber seedlings were received from plastic tunnels from Taif. Seedlings expressed damping off with water soaking and girdling of the hypocotyl at the soil line. Cantaloupe plants (Cucumis melo var. cantalopensis) showing basal stem rot, resemble that in cucumber plants, were received from a large vegetable producing controlled environment greenhouse project in Beesha. The causal agent was previously identified in the lab as *Pythium aphanidermatum* based on the morphological characteristics of the fungus (Sunboul 2001).

The objective of this study was to characterize the response of cucumber, sweet pepper and cantaloupe plants to the infection with *P. aphanidermatum*.

Materials and Methods

Isolation of the Causal Agent

Pure culture of *P. aphanidermatum* was obtained from a diseased mature cucumber plant from AESHA that showed basal stem rot symptoms. Infected tissues were washed thoroughly under running tap water for 30 min, cut into one cm segments and surface sterilized in 0.5% NaOCl for one min, rinsed three times in sterile distilled water and then blotted against sterile paper towels. Each segment was placed in sterile plastic petri plate containing 1.5% water agar amended with 200 mg/l streptomycin sulfate and 50 mg/l rose bengal (Dhingra and Sinclair, 1985). Plates were incubated in the dark at 33ºC for 48 hr. A hyphal tip of the fungus was transferred into freshly prepared Potato Dextrose Agar (PDA) plates (200 g fresh peeled potato, 15 g dextrose and 15 g agar in 1000 ml distilled water) (Tuite, 1968) which were incubated at 33ºC for 24 h and then stored in the refrigerator for further studies.

Inoculum Preparation

Oospores of *P. aphanidermatum* were produced on a semi solid Vegetable Oil Nitrate Agar (VONA) culture media that consisted of 3 ml vegetable oil, 1.5 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g $MgSO_4$. $7H_2O$, a trace of thiamine-HCl and 3 g agar in 1000 ml distilled water (Tuite, 1968). Culture plates of VONA were seeded by placing a 7-mm disc of a 2-day old PDA culture of the fungus in the middle of each plate. Culture plates were incubated in the dark at 33ºC for ten days. For separation of the oospores, agar was blended in a Waring blender with sterile distilled water for three minutes. Mixture was centrifuged at 4000 g (8,000 rpm) for 30 sec. Pellets were resuspended in distilled water and centrifuged as mentioned above. This step was repeated three to five times until masses of oospores, as yellowish pellets, were formed at the bottom of the tube. Pellets of oospore masses were resuspended in tap water at the rate of 20 oospores/ml and used as inoculum in the pathogenicity test.

Pathogenisity Test: Conditioned Growth Room

Pathogenicity of *P. aphanidermatum* on vegetable host plant seedlings was carried out under white fluorescent light. Temperatures were set at approx. 27º and 32ºC at night and day, respectively. Cucumber (cv. Puma F1 hybrid), cantaloupe (cv. Poli Carp F1 R) and sweet pepper (cv. Rida F1) (Al-Osama Agricultural Company, ASDCO) were germinated in a clean wet paper towels in-

side a plastic bag at room temperature. When rootlets reached 2-3 cm long, they were transferred into 18 × 12 × 6 cm plastic boxes, filled with compost (Super Compost Company) supplemented with macro and micro nutritional elements. A total of 30 seedlings, 6 seedlings/box and 5 boxes of each species were tested. All plants were irrigated to the field capacity with tap water on alternate days. After nine days of planting, 24 seedlings of each species were inoculated with a 5-mm dia. mycelial agar plugs of *P. aphanidermatum* taken from the periphery of a one-day old V-8 Juice agar culture (20% V-8 juice, v/v, 2% agar, and 0.3% $CaCO_3$) (Tuite, 1968) and attached to the base of the hypocotyl below the soil line. The other six plants, left uninoculated, served as a control.

Pathogenicity Test: Controlled Environment Greenhouse

Another pathogenicity test was held in a controlled environment greenhouse at AESHA in June, 1996. The greenhouse was divided into two blocks, with three rows in each block. Cucumber (cv., Sahara F1 hybrid), cantaloupe (cv., Poli Carp F1 R), and sweet pepper (cv., Reema F1) were each planted in a row in 40 cm distance. Hills within rows were one meter apart, with 40 plants/row. The soil beds of one block were artificially infested with the pathogen, one week prior to sowing, by mixing the soil bed with 20 oospores/ml suspension of *P. aphanidermatum* prepared as described above. The other block, left uninfested, served as control. For cucumber and cantaloupe, seeds were planted directly. Sweet pepper seeds, however, were germinated first in a peat moss pots, and then two week old seedlings were transplanted into the greenhouse soil. Three nutrient regimes were applied to fertilize the plants at different growth stages. Plants were fertilized with (6-6-19) mixture at the rate of 28 kg/ha at the seedling stage, (18-6-17) mixture at the rate of 84 kg/ha at the flowering stage, and (15-30-15) at the rate of 112 kg/ha at the fruiting stage. Irrigation was applied twice a day using drip irrigation.

Plants were monitored for symptom expression and diseased plants were collected at three-day intervals and were placed in plastic bags in a refrigerator. Isolation of the causal agent from diseased tissues was achieved from the basal stem, crown area, and roots, as described above.

Results and Discussion

Pathogenisity Test: Conditioned Growth Room

Damping off symptoms started as water soaking lesions in the hypocotyl with the soil line (Fig. 1-a). Water soaking could hardly be seen since it lasts for very short time. It followed by restriction and girdling of the hypocotyl that extended by time to the upper part turning it to a thread-like lesion without any visible discoloration.

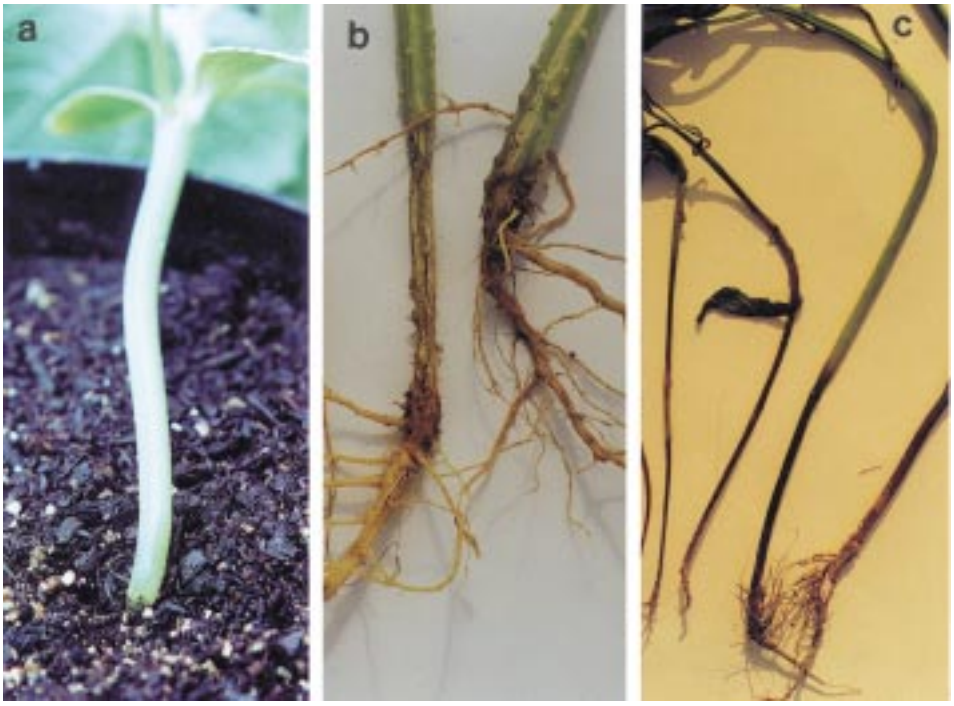

FIG. 1. Symptoms caused by *Phythium aphanidermatum;* a, water soaking and girdling of hypocotyl of a cucumber seedling; b, dark yellow necrosis of crown and basal stem of cucumber plants; dark brown-black necrosis of basal stem of pepper plants at different growth stages.

Disease percentage of the three plant genera is presented in Fig. (2). Disease response was obvious as early as 8-10 hr after inoculation. Early symptoms of damping off of cucumber seedlings started after eight hours from inoculation. It had the highest infection rate compared to the other tested crops, reaching about 80% infected plants in 14 hr. The maximum level of infected cantaloupe seedlings reached 90% after 26 hr from inoculation. For cantaloupe and pepper plants, almost 50% of the plants were infected after 16 hr from inoculation (Fig. 2). The maximum percentage of infected cantaloupe seedlings reached 80% after 26 hr from inoculation. For sweet pepper seedlings, on the other hand, no increase in the number of infected plants were observed after 18 hr, remaining at the 50% level (Fig. 2).

Pathogenicity Test: Controlled Environment Greenhouse

Pythium aphanidermatum was isolated from all plants showing basal stem necrosis and wilting. The fungus was isolated from infected stem and crown areas. This isolate, however, did not cause any root rot and could not be isolated from the roots of infected plants. Some reports however, indicated that isolates of *P. aphanidermatum* were affecting stems, crowns and roots of cucumber plants (Stanghellini & Phillips ,1975, Favrin *et al.,* 1988 and Sonogo & Moorman, 1993).

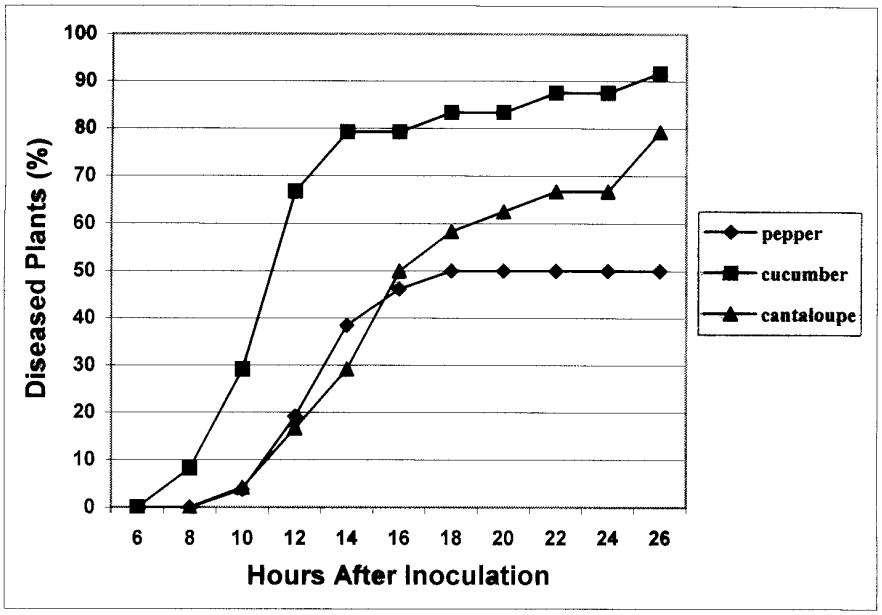

FIG. 2. Percentage of damping off disease over time in pepper (cv Rida F1), cucumber (cv Puma F1 hybrid) and cantaloupe (cv Poli Carp F1 R), when nine days old seedlings were inoculated with agar plugs of *Phythium aphanidermatum* in conditioned growth room.
LSD (0.05) for plant species = 12.71
LSD (0.05) for time after inoculation = 27.46

Disease percentage of the three plant species in the controlled environment greenhouse is presented in Fig. (3). Symptoms of cucumber, pepper, and cantaloupe plants appeared 3, 4, and 5 weeks after sowing, respectively (Fig. 3). Cucumber and cantaloupe plants have revealed the highest disease percentage, reaching 85% infected plants as compared to 63% for pepper plants, ten weeks after sowing.

Symptoms on both cucumber and cantaloupe plants were identical. Diseased plants were recognized easily by the appearance of wilting that is very similar to vascular wilts. They show dry soft dark yellow to orange necrosis at the lower stem and/or crown area of infected plants (Fig.1-b). Length of lesions varied depending on age of the plant. At earlier stages prior to flowering, lesions were extend 7-12 cm above the soil line and plants were severely wilted and usually die soon. At adult fruit-bearing stage, however, necrosis was limited to the crown area or extended few centimeters above the soil line. At this stage, wilting appears later, but infected plants rarely die compared to young plants, which are usualy stunted as compared to the healthy ones.

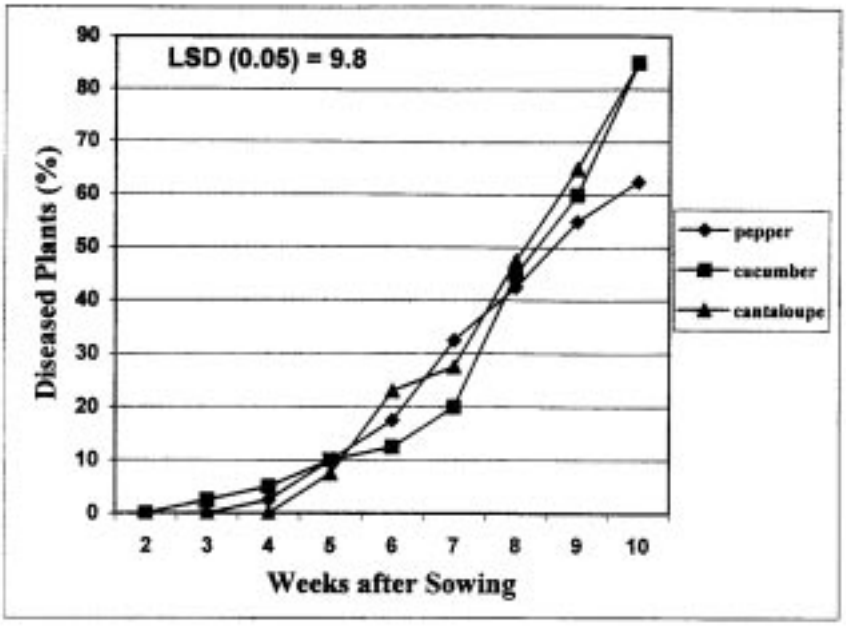

FIG. 3. Percentage of basal stem rot disease over time in pepper (cv Reema F1), cucumber (cv Sahara F1) and cantaloupe (cv Poli Carp F1 R), when soil in controlled environment was infested with 20 oospores/ml of *Pythium aphanidermathum.*

Infected sweet pepper plants with *P. aphanidermatum* showed severe wilting accompanied by dry rot with dark purple, dark brown to black discoloration of the lower stem at the early stages of plant growth (Fig. 1-c). At this stage, however, infected area of the stem was extended up to the lower leaves. Mature plants, on the other hand, express less wilting response to infection, and necrosis is characterized as dry hard black necrosis, but not soft rot, of the lower stem that can extend up to 15 cm above the soil line.

In general, no damping off of vegetables was observed in the environmentally controlled greenhouses in the last seven years at Hada-Sham, or specifically in the greenhouse pathogenicity test. This is most probably due to the low number of propagules present naturally or introduced artificially in the greenhouse soil. It is also possible that the fungal isolate has a low agressiveness (Favrin *et al.,* 1988).

It has been observed (not published data) that when plants were inoculated with high inoculum density of propagules (> 200 oospores/ml), plants expressed damping off at the early stages of seedlings. However, at lower inoculum densities (< 50 oospores/ml) basal stem rot and wilting is expressed at later growth stages of plant species.

Recorded minimum and maximum temperatures in the controlled environment greenhouses at Hada-Sham were avg. 29-42ºC. It has been observed by many researchers that diseases caused by *P. aphanidermatum* are more severely affected when temperatures exceeds 30ºC (Litterell & McCarter, 1970 and Von Bretzel *et al.,* 1988). It has been observed in the greenhouses in AESHA, in general, that more damage appears in vegetables due to infection by the fungus to plants that received high moisture than lower moisture level. Hine & Ruppel (1969) and Kaiser *et al.,* (1971) reported that excessive soil moisture increased the incidence of diseases caused by *P. aphanidermatum*.

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تعفن قاعدة الساق في الخضر المزروعة في البيوت المحمية في غرب المملكة العربية السعودية : (ب) توصيف المرض

يحيى حمزة سنبل

قسم زراعة المناطق الجافة ، كلية الأرصاد والبيئة وزراعة المناطق الجافة

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المستخلص. تم استلام عينات مريضة من نباتات خيار وشمام وفلفل في معمل أمراض النبات بكلية الأرصاد بجامعة الملك عبد العزيز من عدة بيوت محمية لإنتاج الخضار ، وذلك لفحصها. وقد ظهر على النباتات المريضة الذبول الطري للبادرات أو ذبول شديد وتعفن لقاعدة الساق على النباتات في مرحلة حمل الثمار. وقد كانت هذه الأمراض سببا في خسائر كبيرة في محاصيل الخضر ، مما أدى إلى توقف زراعتها في بعض الأحيان. وقد تم تعريف المسبب المرضي *Pythium aphanidermatum* (Edson) Fitzp. كما أظهرت اختبارات الإصابة ، والتي تمت بتلقيح بادرات ثلاثة أجناس من النباتات في غرفة الإنبات المكيفة بوضع أقراص من بيئة الآجر للفطر المسبب في اسفل الساق للبادرات بعمر ٩ أيام ، أن البادرات ظهرت عليها الأعراض الأولى لمرض الذبول الطري بعد ٨ ساعات بالنسبة للخيار و١٠ ساعات من التلقيح بالنسبة لبادرات الفلفل والشمام . كما بلغ أعلى معدل للإصابة ٩٠٪ للخيار والشمام خلال ٢٦ ساعة من التلقيح ، أما أعلى نسبة إصابة بالنسبة للفلفل فكانت ٥٠٪ خلال ١٨ ساعة من التلقيح. وقد ظهر على النباتات المصابة بوادر التشبع المائي للسويقة الجنينية عند التقائها مع سطح التربة ، والتي لم تدم إلا لفترة قصيرة جدا ، ثم تبعها بعد ذلك اختناق أو تخصر ، ثم امتد الاختناق إلى أعلى بعد فترة لتصبح السويقة الجنينية خيطية الشكل. أما بالنسبة لاختبار الإصابة الآخر، والذي أجري في أحد البيوت المحمية ، فقد أظهرت النتائج أن نباتات الخيار والفلفل والشمام أصيبت بمرض تعفن قاعدة الساق بعد ٣ و٤ و٥ أسابيع من الزراعة ، على التوالي. وقد تكشف عن نباتات الخيار المريضة حديثة السن ظهور ذبول شديد وتعفن أصفر داكن لقاعدة الساق

ومنطقة التاج ، والذي امتد إلى مسافة ١٥ سم فوق سطح التربة. أما بالنسبة لنباتات الفلفل ، فقد ظهر عليها تعفن بنفسجي داكن إلى بني داكن أو أسود ، والذي امتد إلى الأوراق السفلية . بالنسبة لنباتات الخيار والشمام في مرحلة الإثمار ، فإن الإصابة كانت محصورة في منطقة التاج ، أو امتدت بضع سنتمترات فوق سطح التربة . أما نباتات الفلفل في المراحل المتأخرة ، فقد ظهر عليها عفن بني داكن أو أسود امتد إلى مسافة ١٥سم فوق سطح التربة . وقد بلغت أعلى نسبة إصابة ٨٥٪ بالنسبة لنباتات الخيار و٦٣٪ بالنسبة لنباتات الفلفل والشمام خلال ١٠ أسابيع من الزراعة .