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Alternaria Blossom-end Rot and Seedling Blight of Cucurbits in Al-Qassim

Mohamed A. El-Meleigi
Plant Protection Department, College of Agriculture, King Saud University, Qassim, Saudi Arabia.

Abstract. Poor stand and blossom-end rot of watermelon (*Citrulus lanatus*), Charleston Gray cultivar were the most serious diseases in several fields at Al Qassim region, central Saudi Arabia, in 1986. *Alternaria* sp. was associated with the infected seedlings and blossom-end rotted fruit. The fungus was internally seedborne in 100 % of the seeds extracted from infected watermelon fruit and in 26 % the seeds in symptomless fruits. Inoculation of cucurbit certified seeds with the *Alternaria* sp. caused seedling blight on two watermelon cultivars, Charleston Gray and Black Diamond; muskmelon (*Cucumis melo*) cvs. Green Flesh Honeydew and Crenshaw; Cantaloupe (*C. melo* var. *canatalupensis*) cvs. Amco Sweet, Jane Conari and Casaba Golden Beauty. Cucumber (*C. sativus*) cv. Amco Sweet MT and Squash (*C. maxima*) cvs. U.E.G. Arrow Lebanese and Caserta. The cucurbit cultivars varied in suscebtibility of their leaves and hypocotyls to infection with the *Alternaria* sp. Bright field microscopic examination of stained sections of infected seeds and seedlings of watermelon revealed the presence of the fungus in seeds and hypocotyls. An analyses of nutrient elements in healthy and infected watermelon fruit showed comparable levels of Cu, Fe, K, Mg and Zn in the blossom and pedicel ends of the fruit regardless of infection. The concentration of Ca in blossom-end of the fruit was significantly (P=0.05) less than in pedicel-end regardless of infection.

Introduction

Watermelon (*Citrulus lanatus* (Thumb.) Mastum & Nakai) is a common cultivated summer vegetable crop in central Saudi Arabia succeeding sprinkler irrigated spring wheat on potentially vast acreage. Other cucurbit vegetables are cultivated in the area but at much smaller scale. In 1986 the production of watermelon in 1986 was threatened by seedling disease that resulted in poor stands in several fields followed by a blossom-end rot that affected watermelon fruits at maturity.

Cephalosporium cucurbitarum Gubler & Grogan, *Fusarium* spp., *Pythium* spp. and *Rhizoctonia solani* Kuhn, cause seedling diseases in cucurbits in many parts of the world [1]. *Alternaria cucumerina* causes leaf blight of cucurbits leading to serious economic losses in many parts of the world [2]. blossom-end rot of watermelon has been identified in other parts of the world as a nonparasitic disease causing 60-80% losses in watermelon fruits under warm dry environment [3,4]. The incidence of the blossom end rot was not affected by irrigation frequency but was significantly reduced when Ca fertilizer was added to the soil [4,5].

The objective of this study was to determine the cause of seedling disease and blossom-end rot of watermelon in Al-Qassim and test the susceptibility of other cucurbit hosts to the pathogen involved.

Materials and Methods

Isolation

Infected seedlings and fruit of watermelon, Black Diamond were collected from fields of infected plants during the 1986 growing season. Diseased seedlings and segments (2 cm) cut just obove the margin of fruit lesions were surface sterilized in 1% solution of sodium hypochlorite for 1 min., blotted dry on sterile filter paper and segments (3 × 3 mm) were transferred onto Acidified potato dextrose agar (APDA) plates (pH 5.0) and incubated at 25°C. Seeds extracted from watermelon seeds from ten cucurbit cultivars were surface sterilized as described above and placed on APDA plates (three to five seeds/ plate and 100 seeds /treatment) and incubated at 25°C.

Pathoginicity tests

A mixture of sandy soil and peat moss (2:1) was autoclaved for 4 h for 2 consecutive days and used for pathogenicity tests in the greenhouse. Conidia of *Alternaria* sp. used for pathoginicity tests were derived from single spore culture. A conidial suspension was prepared by flooding plates (1-week-old cultures) with sterile distilled water, gently rubbing the agar surface with sterile bent glass rod and filtering suspension through two layers of cheesecloth. Hemocytometer counts were used to adjust spore concentrations to 5 × 10 conidial/ml for inoculation of seeds, seedlings, and fruits of cucurbit hosts.

Seeds of watermelon (cvs, Black Diamond and Charleston Gray), muskmelon (cvs, Honey Dew Green Flesh and Cranshaw), cantaloupe (cvs Amco Sweet, Jane Conari and Casaba Golden Beauty), cucumber (cvs Amco Sweet MT) and squash (cultivars U.E.G. Arrow Lebanese and Caserta) supplied by Harris Moran Seed Company, Salines, California U.S.A., were used in this study. Seeds were surface sterilized in 1% sodium hypochlorite for 2 min., rinsed twice in sterile distilled water and dipped in the conidial suspension of *Alternaria* sp. for 30 sec. then removed and sown in plastic pots (15 cm in diameter) with autoclaved soil mixture (10 seeds/pot) and ten replicates for each treatment. Naturally-infected Charleston Gray watermelon seeds and noninoculated seeds from all cucurbit cultivars were sown in sterile soil mixture. Seedlings of all cultivars were grown in the greenhouse for 3 weeks at 28°C. The percentage of diseased seedlings was determined 2 weeks after emergence and the organisms associated with infected tissues were isolated on APDA plates.

Three-week-old seedlings of the cucurbit cultivars, grown in autoclaved soil mixture, were inoculated by placing 0.1 ml of the spore suspension on the hypocotyl or first true leaf. Thirty seedlings were used for each treatment. Inoculated seedlings were incubated at near 100% relative humidity for 48 h in growth chamber at 28°C and then transferred to the greenhouse for 2 weeks. Disease severity on leaves and hypocotyls of all seedlings was recorded. The severity of infection was based on a scale of 0 to 5 where 0= no disease symptoms or fungal growth, 1= minor growth of the fungus on the inoculated tissues, 2= small lesion, 3= medium size lesion and extensive sporulation of the fungus, 4= large size lesion, 5= severe infection collapse of inoculated tissues. The roots, stems and leaves of diseased and healthy seedlings of Charleston Gray watermelon were dissected (3 mm long), surface sterilized in 1 % sodium hypochlorite for 1 min., placed on APDA and incubated at 25°C.

Symptomless ripe fruits of all the forementioned cucurbit hosts except Cranshaw muskmelon, Casaba Golden Beauty Cantaloupe and U.E.G. Arrow Lebanese squash cultivars, were tested for their susceptibility to *Alternaria* fruit rot. Twelve fruits from each cucurbit cultivar were surface treated with 1% sodium hypochlorite for 2 min and rinsed with sterile distilled water. The blossom-end was wounded by a sterile scalpel and nine fruits were inoculated with 0.25 ml of spore suspension while three fruits were inoculated with boiled spore suspension.

Histological studies

Naturally-infected seeds and segments of infected or healthy seedlings (3- week old) of Charleston Gray watermelon were fixed in a solution of formalin, acetic acid and ethyl alcohol 50% (5 : 5 : 90 %) (FAA) for 48 h., washed in ethyl alcohol 50%, then dehydrated in a series of ethyl alcohol concentrations, cleaned in xylol, and embedded in paraffin wax [6]. Microtome sections (10-15 μm thick) were mounted on glass slides and stained with toluidine for 1 min. [6,7], and examined with bright field microscope and photographed with Zeiss MC 63 camera system.

Nutrient elements

Samples (400g each) were taken from the terminal ends of diseased and healthy ripe fruits of Charleston Gray watermelon (10 fruits/ treatment). The tissues were oven dried for 48 h. at 105°C, ashed in a muffle furnace and extracted for elemental analysis [8-10]. Concentrations of Ca, Cu, Fe, K, Mg and Zn were determined using Atomic Absorption (AA 875) manufactured by Varian, Zug, Switzerland.

A randomized complete block design was used in this study and means were separated according to Duncan's multiple range test.

Results

All the diseased seedlings of Charleston Gray watermelon cultivar were colonized with an *Alternaria* sp.. Colonies of *Alternaria* sp. were dark brown with gray to light brown edges. Conidiphores were single or in small groups. Conidia were oblavate, ovoid or ellipsoidal, formed in chains (from four to six conidia) with up to six transverse and one to three longitudinal or oblique septa. The overall lengths of conidia were 18-54 (32)μ, 8-16 (11)μ thick in the broadest part. *Fusarium* spp, *Pythium* sp. and *Rhizoctonia* sp. were isolated from less than 10% of the infected seedlings. The blossom-end rotted fruits of watermelon collected from the field (Plate 1 - a) were colonized with *Alternaria* sp. at 100 %. *Choenophora cucurbitarum* and *Fusarium* sp. were occasionally isolated from the infected fruit. *Alternaria* sp. was found in 100% of the seeds of infected from symptomless fruit. The imported certified seeds of all the tested cucurbit cultivars were free of fungal infection.

The germination of seeds extracted from infected fruit of Charleston Gray watermelon cultivar in sterilized soil mixture in greenhouse was 83%. White corky lesions (5-38 mm long and 2-3 mm wide) were developed along the hypocotyles of 73% of the emerged seedlings. The infected seedlings collapsed between 7-10 days (Plate 1-c). Leaf spots (2-5 mm in diameter) developed occasionally on cotyledonally leaves of infected seedlings. The hypocotyls and cotyledonal leaves of diseased seedling were colonized with *Alternaria* sp., Roots, crowns, the epicotyls, shoot apeces and first true leaves of infected seedlings and all parts of healthy seedlings were free of fungal colonization.

Seed inoculation of various cucurbits with the conidial suspension of *Alternaria* sp. caused pre and/or post emergence damping off in all cultivars (Table 1). Casaba Golden Beauty cantaloupe was the most tolerant to *Alternaria,* while Charleston Gray watermelon was the most susceptible (Table 1).

Inoculation of hypocotyls with conidial suspension of *Alternaria* showed that cucumber, squash and watermelon seedlings were highly susceptible (Plate 1-d). The severity of hypocotyle infection was high in all cultivars but moderate in Charleston Gray watermelon. The hypocotyls of muskmelon and Jane Conari cantaloupe were extremely resistant to infection, while that of Casaba Golden Beauty cantaloupe were moderately susceptible (13%). The leaves of squash cultivars were highly susceptible (82-93%) to *Alternaria* sp. while the leaves of the other cucurbit hosts were extremely resistant (Table 1).

Fruits of cucumber, squash and watermelon were highly susceptible to infection with *Alternaria* sp causing severe blossom-end rot after 5-7 days after inoculation (Table 1 & Plate 1-a). Cantaloupe and muskmelon fruit appeared.

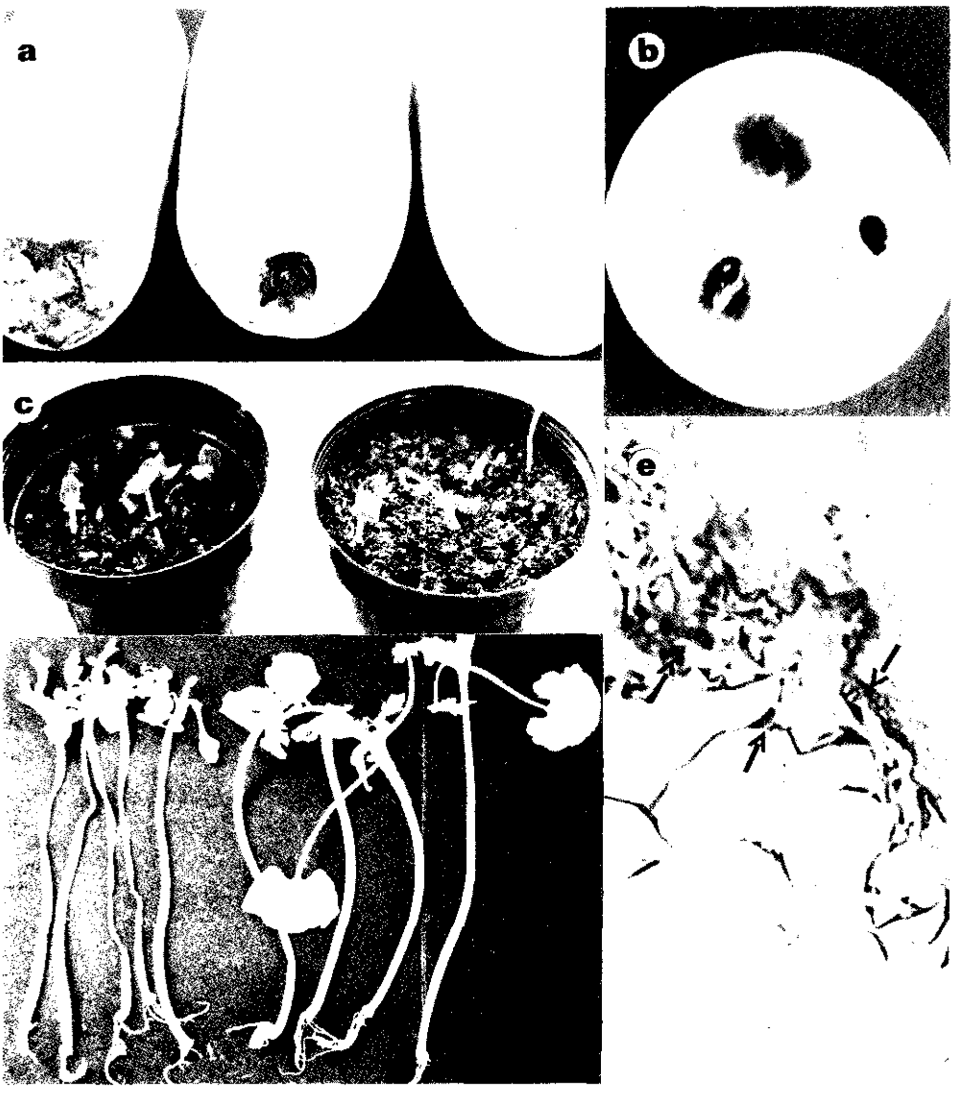

Plate 1. a) Symptoms of blossom-end rot in Charleston Gray watermelon fruit, infected in the field (left), inoculated with *Alternaria* sp. in the laboratory (middle) and inoculated with boiled spore suspension (right); b) *Alternaria* sp. developed from seeds of naturally infected blossom-end rotted watermelon fruit of Charleston Gray cultivar on acidified potato dextrose agar; c) Charleston Gray watermelon seedlings grown from healthy seeds (left) and seeds infected with *Alternaria* sp. (right); d) Diseased seedlings (left) and healthy seedlings (right) of squash Ceserta cultivar; and e) Intercellular mycelia of *Alternaria* sp. in tissues of watermelon seeds.

Table 1. Pathogenicity of *Alternaria* sp. on seeds, hypocotyls, leaves or fruit of various cucurbit hosts

Cucrurbit cultivar	Suceptibity of tissues[a]								
	Seeds			Hypocotyl		Leaves		Fruit	
	Postemergence	Preemergence	Total[b] %	I[L]	D.S.[c]	I	D.S.	I.	D.S.
Watermelon:									
Black Diamond	65	0	65	57	5	00	00	100	5
Charleston Gray	40	60	100	67	2	00	00	100	5
Muskmelon:									
Green Flesh Honeydew	35	11	46	00	00	00	00	00	00
Cranshaw	30	0	30	00	00	00	00	-	-
Cantalope:									
Amco Sweet	15	36	51	00	00	00	00	00	00
Jane Conari	20	35	55	00	00	00	00	00	00
Casaba Golden Beauty	10	7	17	13	4	00	00	-	-
Cucumber:									
Amco Sweet MT	0	65	65	90	5	00	00	100	5
Squash:									
U.E.G. Arrow Lebanese	20	66	86	83	4	85	5	-	-
Caserta	5	21	26	71	4	93	5	100	5

a) Mean of 100 seeds, 30 seedlings or ten fruit per treatment

b) Represent the sum of pre- and postemeregence damping off

c) The severity was based on a scale of 0 to 5 where 0 = No disease symptoms; 1 = minor fungal growth on infected tissues; 2 = small lesion; 3 = mediam size lesion and extensive sporulation of the pathogen; 4 = large lesion; 5 = severe infection and collapse of inoculated tissues.

L) I = Infection, D.S. = Disease severity.

Bright-field microscopic examination of stained sections of infected seeds showed that the fungal mycelia were associated with the interior of the seed coat near the radical. Fungal mycelia also were associated with hypocotyl lesion (Plate 1-e) and in leaf mesophyll of infected seedlings.

The assay of nutrient elements in the terminal ends of healthy or diseased Charleston Gray watermelon showed comnparable levels of Cu, Fe, K, Mg and Zn in both ends with the concentration of Ca in pedicel end being significantly higher than in blossom-end (Table 2).

Discussion

Our results suggest that the seedling and blossom-end rot diseases of watermelon in Al-Qassim fields were caused by *Alternaria* sp. This unidentified *Alternaria* is an epiphytotic fungus that attacks seeds or seedlings at early stages of growth or invade the blossom-end of fruit in the field. The lower content of Ca in blossom-end compared to the pedical-end, wounds induced by floral dropping, and insects damage to the fruit may contribute to the development of the blossom-end rot. Moreover, the cultivation of large acreages of watermelon under sprinkle irrigation and the high temperature in central Saudi Arabia during the watermelon growing season (May to July) may contribute to the prevalence of seedling and fruit diseases.

The high incidence of *Alternaria* sp. in seeds of infected and, to some extent of symptomless watermelon fruit, collected from infected fields suggested that the fruit infection occurs during early stages of their development leading to seed infection. This indicated that locally produced watermelon seeds may become highly infected with *Alternaria* sp. without expressing external disease symptoms on the fruit.

Most of the tested cucurbit hosts were susceptible to seedborne inoculum. However, hypocotyles of certain hosts and leaves of the majority of the cucurbits tested were tolerant to the foliar inoculum of *Alternaria* sp. The infection of the blossom-end was most likely to occur during the flowering stage by air-and soilborne inoculum, a suggestion that seedling and fruit infection occurred independent of each other. The results of this study showed that fruit of the cucurbit hosts varied in their susceptibility to infection. Therefore, replacing highly susceptible cucurbit hosts with resistant ones would help reduce losses from seedling and blossom-end rot diseases.

Table 2. Concentration of certain macro- and micronutrient elements in the terminal ends of blossom-end rotted and healthy fruits of Charleston Gray watermelons.

Tissue		Elements (μg/ml)					
		Ca	Cu	Fe	K	Mg	Zn
Blossom-end	Healthy	40.300* a	110 a	670 a	5.170 a	2.380 a	70 a
Blossom-end	Infected	54.500 a	130 a	850 a	4.820 a	1.950 a	70 a
Pedicle-end	Healthy	81.700 a	80 a	600 a	4.730 a	2.320 a	70 a
Pedicle-end	Infected	88.000 b	130 a	750 a	4.800 a	2.270 a	90 a

* Mean of 10 replicate per treatment.
Means within a column followed by a common letter do not differ significantly, according to Duncan's multiple range test at P = 0.05

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عفن الطرف الزهري ولفحة البادرات في القرعيات بالقصيم المتسبب عن *Alternaria*

محمد عبدالستار المليجي

قسم وقاية النبات، كلية الزراعة والطب البيطري، جامعة الملك سعود، فرع القصيم، المملكة العربية السعودية

ملخص البحث. كان موت البادرات وعفن الطرف الزهري في البطيخ *(Citrulus lanatus)* صنف شارلستون جراي من أهم الأمراض في حقول القصيم في ١٩٨٦م. عزل الفطر *Alternaria* من البادرات والثمار المصابة كما وجد في ١٠٠٪ من البذور المستخلصة من ثمار مصابة وفي ٢٦٪ من البذور المستخلصة من ثمار لا تظهر عليها الأعراض.

تم اختبار العدوى على صفنين من البطيخ وصنفين من الشمام وثلاثة أصناف من الكنتالوب وصنف واحد من الخيار وصنفين من الكوسة وقد وجد أن عدوى البذور بالفطر يؤدي إلى موت البادرات في كل الأصناف السابقة بينما اختلفت درجة العدوى بين الأصناف عندما لقحت الأوراق أو السويقة تحت الفلقية بالفطر.

بفحص قطاعات رقيقة مصبوغة من البذور والبادرات المصابة شوهد ميسليوم الفطر بين خلايا العائل. وبتحليل العناصر الغذائية في الطرف الزهري وطرف العنق في الثمار الاسطوانية للبطيخ المصاب أو السليم وجدت كميات متساوية في كلا الطرفين من كل من النحاس والحديد والزنك والمنجنيز والبوتاسيوم بينما وجد الكالسيوم في الطرف الزهري بنسبة أقل معنويا من طرف العنق ولم يكن هناك تأثير للاصابة على تركيز العناصر المختلفة.