

ISOLATES OF *BOTRYTIS* SPP. AND THE CHOCOLATE SPOT OF FABA BEAN IN EGYPT

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Abstract

Forty isolates of *Botrytis* spp. collected from faba bean growing governorates of Northern and Middle Egypt were compared for their morphological and physiological characters, as well as for their virulence and aggressiveness on four faba bean cultivars, namely Giza 402, Giza 3 Giza 461 and Giza 716.

Botrytis spp. isolates showed differences in growth rate, spore production, number and size of sclerotia, and aggressiveness on the 4 different faba bean cultivars.

B. fabae isolates which showed the least amount of growth, spore production, and the highest number of small sclerotia were the most virulent on the 4 tested cultivars, while *B. cinerea* isolates which were the fastest in growth rate, the highest in spore production and the least in number of large sclerotia were the least virulent on such tested cultivars. Moreover, results showed differences in virulence among isolates of *B. fabae* themselves, where isolates obtained from Nubaria were more virulent than those obtained from other governorates.

INTRODUCTION

Chocolate leaf spot is considered the most destructive diseases on faba beans in Egypt, causing serious damage to the crop, especially in the northern parts of the Delta, where low temperature and high relative humidity favour its spread and severity (Mohamed, 1982). Losses in seed yield were estimated by 49.4% in Giza 3 and 57.5% in Giza 402 cultivars, when plants were artificially inoculated with *B. fabae* (Mahmoud, 1996). The disease is either controlled by spraying certain fungicides (Mohamed, 1982) or by resistant cultivars (Khalil *et al.*, 1984).

For breeding and screening programs for resistant cultivars to chocolate spot disease, it is inevitable to determine the aggressiveness of the isolates of the causal pathogen. It is well known that isolates of *B.fabae* are more virulent than those of *B.cinerea* (Harrison, 1988). Moreover, isolates of *B.cinerea* varied significantly in their pathogenicity on faba bean leaves (Paul, 1929). Also, Deverall *et al.* (1968) compared the pathogenicity of four isolates of *B.fabae*, and found three of them highly pathogenic and one inducing slow-spreading lesions. Huston and Mansfield (1980) found no differences in pathogenicity between eight *B.fabae* isolates from France and the UK. Hanounik and Maliha (1986) classified 12 isolates of *B.fabae* into 4 groups which they named as races 1,2,3 and 4 depending on disease severity rates induced by each group. In Egypt, limited studies started earlier (Mohamed *et al.*, 1981 b and Hassanein *et al.*, 1990).

Therefore, this work was aimed to study variation and aggressiveness among isolates of *Botrytis* spp. collected from different faba bean growing governorates of Egypt.

MATERIALS AND METHODS

Isolation and identification :

Diseased samples were collected from different faba bean growing areas of northern Egypt; Damietta, Kafr El-Sheikh, Dakahlia, Gharbia, Beheira, Nubaria and Sharkia as well as Beni-Swef governorates in Middle Egypt, during 1994/95 season. Leaves of each sample were cut into small pieces, surface disinfested with 3% sodium hypochlorite for 2 minutes, rinsed with sterilized distilled water, dried on filter paper and plated on Potato Dextrose Agar (PDA) medium in Petri dishes. After incubation for 7 days at 20°C, suspected isolates were purified onto PDA plates, and re-incubated at 20°C for 12 days. Single sclerotia were picked and placed on Faba Bean Leaf Agar (FBLA) medium in Petri dishes. Cultures were then single spored before identification and kept on PDA slants for further studies. Isolates were identified to species level according to keys of Ellis (1976).

Variation among isolates:

Effect of different media:

Isolates were compared by culturing on two media and recording observations on mycelial growth, sclerotial presence, number, and size, as well as spore produc-

tion. The two media used were:

- a- Potato Dextrose Agar (PDA) medium: 200g peeled potato, 20g dextrose, 20 g agar, completed to 1000 cc with distilled water, then sterilized at 1.5 lbs/inz for 20 minutes.
- b- Faba Bean Leaf Agar (FBLA) medium : 250g faba bean leaves, 30 g sucrose, 20g sodium chloride, 20g agar, completed to 1000 cc with distilled water, then sterilized at 1 lb/inz for 15 minutes.

Growth rate:

Growth rate of the obtained isolates was determined on both PDA and FBLA media. A small disk from the culture was placed in the center of the plate. Diameter of each isolate's growth was determined daily by averaging two diameters. Average diameters of 8 days growth were estimated, then growth rate of each isolate was calculated on both media.

Spore production:

Isolates were grown on FBLA medium and incubated at 20°C for 12 days. Ten ml of distilled water were poured in each Petri dish. Spores were separated using a brush and filtered through cheese cloth. Spores were counted using a haemocytometer and calculated to number of spores per ml, then number of spores per Petri dish.

Production of sclerotia :

Isolates were grown on PDA medium at 20°C for 30 days. Plates were examined for the presence, number, and size of sclerotia. Sclerotia were calculated in terms of sclerotia per cm² in the Petri dish. Presence and size of sclerotia were determined according to the following rates:

O = absent, a ≥ 1 mm, b = <1-1.5 mm, c = <1.5-2.5mm, and d = <2.5-3.0 mm.

Virulence test :

During 1995/96 season, the forty isolates of *Botrytis* spp were tested for their virulence on four faba bean cultivars, Giza 461, Giza 402, Giza 3 and Giza 716, using the detached leaf technique (Abou-Zeid, 1978). Plants of the tested cultivars were grown in pots, 25 cm in diameter, in a plastic house. Leaflets were collected from plants when 35 days old, arranged in sterilized trays on sterilized blotting paper moistened with distilled water. Droplets of the spore suspension were

placed on each of the leaflets, then trays were covered with polyethylene sheets to maintain high humidity. Spore suspensions were prepared from 12 days old cultures and adjusted to 15×10^4 spores/ml. Each treatment was replicated 10 times and treatments were completely randomized. Data were recorded on the type of infection after 48 hours using scale (0-9) depending on the extent of lesions (Abou-Zeid, 1985).

RESULTS

Identification and distribution of *Botrytis* isolates :

Forty isolates of *Botrytis* collected from different growing areas of faba bean in Egypt were identified to the species level. Identification of the obtained isolates proved that, 16 isolates were *B.cinerea*, while the other 24 were *B.fabae*. Also, both species of *Botrytis* were found in all faba bean growing governorates (Table 1).

Effect of different media on rate of growth :

Two solid media were used to study the differences among these isolates as to their rate of growth at 20°C. PDA medium was the best for growth of all *Botrytis* isolates; growth of most of the isolates covered the Petri dishes within 5 days. On FBLA medium, growth rate of all isolates was less than on PDA, only 16 isolates covered the Petri dishes with their mycelial growth within the same period.

On both media, the fastest isolates in growth were the isolates belonging to *B.cinerea*, while the least in growth were the remaining twenty isolates of the *fabae* species.

Spore production :

Ten ml distilled water were added to a 12 days old culture of each isolate grown on FBLA medium. Spores were counted, calculated per ml, then per the whole Petri dish. Average of the 4 replicates was calculated for each isolate (Table 1).

Data show that isolates differed in the number of spores per Petri dish. Most of the *fabae* type isolates produced number of spores less than 13×10^6 per dish, while most of the *cinerea* type produced numbers of spores more than 17×10^6 per dish.

The least levels of spore production, which ranged between 2×10^6 and

Table 1. Type, growth rate, and morphological characters of different isolates of *Botrytis* spp. isolated from Faba bean plants.

Isolate No.	Origin	Type	Growth rate (mm) on:		No.spores/Petri dish (x610)	No.Sclerotia Cm2	Size of sclerotia*
			PDA	FBLA			
1	Domiatta I	cinerea	8.6	6.4	10.0	0.0	-
2	DM. II	cinerea	10.8	8.3	56.0	1.0	c
3	Nubaria (NB 716)	fabae	7.5	6.7	6.5	11.3	b
4	NB. Mostafa K.I	cinerea	10.8	8.3	39.5	2.5	d
5	NB. Mostafa K.II	cinerea	10.9	8.6	15.75	0.0	-
6	NB. Kafah	fabae	8.2	6.6	10.25	18.7	c
7	NB. West	fabae	8.4	5.9	5.5	18.3	c
8	NB. Sid	fabae	8.5	5.9	28.0	13.7	b
9	NB. 10.000	fabae	8.0	5.9	9.5	20.3	b
10	NB. 461 Jan	fabae	8.5	5.3	6.75	13.7	b
11	NB. Twx 402	fabae	8.2	6.1	8.0	11.0	c
12	NB. 402	fabae	7.7	6.4	12.75	13.0	b
13	Beni Sweif	fabae	8.1	5.1	2.0	16.7	a
14	SAKHA 717	fabae	7.5	5.9	17.5	24.0	a
15	SAKHA G3	cinerea	10.8	9.0	46.5	0.0	-
16	SKH 461	cinerea	10.0	8.1	21.75	1.7	d
17	SKH 402	cinerea	10.9	8.5	35.0	2.0	c
18	ARIMON	cinerea	8.0	6.1	15.25	14.0	d
19	Sidi Salem	cinerea	11.0	9.0	39.5	2.0	d
20	MAHALA	cinerea	11.0	9.0	48.75	2.0	b
21	Hamoul	fabae	6.6	5.1	21.0	7.5	b
22	NB. Blanka	fabae	10.7	5.4	8.5	15.3	b
23	NB. T.W.	fabae	7.0	5.4	11.0	10.0	c
24	NB. T.W. 225	fabae	7.7	6.3	5.75	18.0	b
25	NB. 461 Feb.	fabae	6.9	5.6	11.5	22.0	a
26	NB. T.W. 234	fabae	6.4	5.2	20.25	12.0	b
27	NB. T.W. 230	fabae	7.4	5.6	9.75	10.7	c
28	Kafr Saad	fabae	7.7	5.4	15.25	18.3	c
29	Taef	cinerea	10.0	8.2	9.0	1.0	d
30	Mansora	cinerea	11.0	8.7	17.25	0.0	-
31	Meseel	cinerea	9.6	8.6	56.5	0.0	-
32	NB. 94	fabae	8.0	5.6	10.5	14.3	b
33	DM. 94	fabae	6.8	5.7	5.75	12.0	c
34	Gemmeiza I	fabae	8.4	5.3	7.75	15.7	b
35	Gemmeiza II	cinerea	10.8	9.0	21.25	1.0	d
36	Sinbilawen	fabae	7.6	5.8	16.0	15.7	c
37	Barkin	cinerea	11.0	9.0	33.5	0.0	-
38	Belbis	fabae	7.4	4.4	13.5	13.0	b
39	Tranis	cinerea	11.2	9.0	17.25	0.0	-
40	Zarzura	fabae	7.0	5.3	8.0	13.0	b

a, b : Small sclerotia
c, d : Large sclerotia

7.7×10^6 were found in isolates No. 3,7,10,13,24,33 and 34. All these isolates were of the *fabae* type, while the highest spore production, ranging between 33.5×10^6 and 56.5×10^6 and 56.5×10^6 , were characteristic for isolates No. 2,4,15,17,19,20,31, and 37. These isolates were of the *cinerea* type.

Presence, number and size of sclerotia :

Data in Table (1) show that, isolates No. 1,5,15,30,31,37 and 39 did not produce any sclerotia, while the others produced sclerotia of different types. Also, number and size of sclerotia were widely varied among the isolates. Isolates No. 6,7,9,13,14,22,24,25,28,34 and 36 produced the highest numbers of sclerotia (15.3 - 24 sclerotia / cm^2), while isolates No. 2,4,16,17,19,20,29 and 35 produced the least number of sclerotia (1 - 2.5 sclerotia/ cm^2). Moreover, some isolates produced small sclerotia (types a and b), while others produced large ones (types c and d).

It was interesting to observe the negative correlation between the number and size of sclerotia for each isolate, and also the correlation between the number and size of sclerotia and type of *Botrytis* isolate. The isolates produced small size of sclerotia in large number were of the *fabae* type, while the isolates not producing sclerotia or producing sclerotia large in size but less in numbers were of the *cinerea* type.

Aggressiveness and virulence of different isolates :

The forty *Botrytis* isolates were tested to determine their aggressiveness on four commercial faba bean cultivars. Results in Table (2) show clear differences among the isolates in their aggressiveness on the four cultivars. The twenty four isolates of the *fabae* type (*Botrytis fabae*) were more virulent than those of the *cinerea* type (*Botrytis cinerea*). The most virulent were isolates No. 10,22,23 and 32 resulting in an average disease severity ranging from 4.75 to 5.10. These isolates which are of the *fabae* type, were obtained from faba bean plants grown in the Nubaria area. The least virulent on the 4 cultivars were isolates No. 5 and 17 resulting in an average disease severity of 1.850 and 1.875, respectively. These isolates which are of the *cinerea* type, were obtained from plants grown in Nubaria and Sakha regions. Other isolates were intermediate in their virulence.

Regarding the 4 tested cultivars, Giza 402 (susceptible) was the most susceptible one, where average disease severity was 4.16. Both cultivars Giza 461 and

Table 2. Aggressiveness of *Botrytis* spp. isolates on four faba bean cultivars using detached leaf technique (scale 0-9).

Isolate No.	Average disease severity on cultivars				Mean
	Giza 461	Giza 402	Giza 3	Giza 716	
1	1.6	3.2	2.2	1.7	2.175
2	4.4	5.0	4.9	4.4	4.675
3	2.5	4.4	3.8	3.3	3.500
4	2.6	3.1	5.0	4.6	3.825
5	1.3	2.6	2.2	1.3	1.850
6	2.4	3.3	3.3	1.9	2.725
7	3.3	3.5	4.1	3.8	3.675
8	3.6	4.9	4.5	2.6	3.900
9	3.0	4.5	4.3	3.3	3.775
10	5.0	5.3	5.5	4.6	5.100
11	2.9	3.9	3.5	3.3	3.400
12	4.1	5.1	4.1	3.8	4.275
13	1.7	3.4	3.1	3.7	2.575
14	3.9	4.6	4.4	4.3	4.300
15	2.2	4.1	3.0	2.3	2.900
16	2.6	3.8	2.2	1.9	2.625
17	1.6	2.3	2.1	1.5	1.875
18	3.6	4.5	4.0	3.7	3.950
19	3.2	4.2	3.4	2.6	3.350
20	2.3	2.3	3.0	1.7	2.325
21	4.2	4.3	4.9	4.4	4.450
22	4.6	6.0	4.5	3.9	4.750
23	4.3	5.2	5.1	4.5	4.775
24	3.9	3.8	4.3	3.0	3.750
25	3.2	3.2	3.4	2.3	3.025
26	4.2	4.2	3.2	4.9	4.125
27	3.5	4.2	4.2	3.8	3.925
28	4.2	4.6	3.7	3.2	3.925
29	4.9	5.1	3.0	4.7	4.425
30	3.3	4.6	3.7	3.6	3.800
31	3.1	4.7	4.3	4.6	4.175
32	4.6	5.5	5.5	4.3	4.975
33	2.7	4.0	3.6	3.4	3.425
34	4.9	4.7	4.9	4.3	4.700
35	2.1	3.3	3.4	4.4	3.300
36	4.5	4.3	3.9	3.3	4.000
37	2.7	4.1	3.1	2.6	3.125
38	4.2	4.8	4.8	4.6	4.600
39	4.5	4.6	4.0	4.3	4.350
40	4.0	3.3	4.3	3.4	4.75
Mean	3.385	4.163	3.860	3.445	3.713

L.S.D. at 1% 5%
 for Cultivars (C) = 0.99 = 0.130
 for Isolates (I) = 0.312 = 0.410
 for (C) X (I) = 0.624 = 0.821

Giza 716 (resistant) were the least in average disease severity (3.86), while Giza 3 cultivar was moderate in average disease severity (3.86).

DISCUSSION

Growing the 40 isolates of *Botrytis* spp. on two different media PDA and FBLA showed differences among them in growth rate, spore production, and number and size of sclerotia. Isolates of *B.fabae* (24 isolates) were the least in growth rate, spore production, and produced small sclerotia in high number, while isolates of *B.cinerea*, (16 isolates) were the fastest in growth rate, the highest in spore production and did not produce or produced large sclerotia in fewer number.

Testing disease causing ability of these isolates on the detached leaflets of faba bean cultivars indicated that isolates of *B.fabae* were more virulent and aggressive than those of *B.cinerea*. Moreover, results showed differences in virulence among isolates of *B.fabae* where isolates obtained from Nubaria were more virulent than those obtained from other governorates.

Similar results were previously observed by several investigators. El-Helaly (1938), Naguib (1948), and Abou-Zied *et al.*, (1985) mentioned that chocolate spot disease of faba bean can be caused by either *B.cinerea* or *B.fabae*. The spots caused by *B.cinerea* are inconspicuous with those of *B.fabae*; only epidermal cells being affected by *B.cinerea*, whereas *B.fabae* always causes necrosis of the mesophyll (Leach, 1955 and Harrison, 1983). Moreover, Sardina (1929), and Wilson (1937) stated that, the pathogenic behaviour of both *B.cinerea* and *B.fabae* are similar. During dry weather, lesions caused by both pathogens remain small and non-aggressive, but in humid conditions lesions caused by *B.fabae* become aggressive, spread rapidly and lead to a blight which eventually kills the plants. Also, Hassanein *et al.*, (1990) mentioned that isolates of *B.fabae* obtained from Nubaria were, in general, more virulent than those isolated from Kafr El-Sheikh.

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عزلات أنواع فطر بوتريتس ومرض التبقع البنى فى الفول البلدى فى مصر

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تم مقارنة الصفات المورفولوجية والفسولوجية لأربعين عزلة من الفطر بوترايتس تم عزلها من الفول البلدى من محافظات شمال الجمهورية فى موسم ٩٤ / ١٩٩٥ م و ٩٥ / ١٩٩٦ م تم دراسة القدرة المرضية لهذه العزلات بأستخدام ٤ أصناف من الفول البلدى وهى جيزة ٤.٢ (حساس)، جيزة ٣ (متوسط الحساسية) وكل من جيزة ٤٦١ وجيزة ٧١٦ (مقاوم).

لوحظ وجود فروق واضحة بين العزلات المختلفة من حيث معدل النمو على البيئات المختلفة، كمية الجراثيم، عدو حجم الأجسام الحجرية وأيضاً القدرة المرضية على أصناف الفول الأربعة المختبرة.

أوضحت النتائج أن عزلات الفطر بوترايتس فابى والتي تميزت بالبطئ فى النمو، إنتاج أقل كمية من الجراثيم، إنتاج أكبر عدد من الأجسام الحجرية الصغيرة الحجم كانت هى الأشد ضراوه من حيث القدرة المرضية على أصناف الفول البلدى الأربعة التى تم اختبارها.

من ناحية أخرى فان عزلات الفطر بوترايتس سيناريا والتي تميزت بالسرعة فى النمو على البيئات المختلفة، إنتاج أكبر كمية من الجراثيم الكونيدية وأيضاً إنتاج أقل عدد من الأجسام الحجرية ذات الأحجام الكبيرة كانت أقل ضراوه من حيث القدرة المرضية على أصناف الفول المختبرة.

وجد أيضاً أن عزلات الفطر بوترايتس فابى المعزولة من منطقة النوبارية كانت أشد ضراوه من حيث قدرتها المرضية مقارنة بعزلات نفس الفطر المعزولة من المحافظات الأخرى.