

VARIATION AMONG ISOLATES OF *Botrytis* spp. AND INTERACTION BETWEEN *B. fabae* ISOLATES AND SOME FABA BEAN VARIETIES

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ABSTRACT

Isolates of *Botrytis* spp. collected from faba bean growing governorates of Northern and Middle Egypt were compared for their morphological and physiological characters.

Botrytis spp. isolates showed differences in growth rate and spore production as well as number and size of sclerotia while, growth of *Botrytis cinerea* isolates was faster than those of *Botrytis fabae* onto all tested media and most of the *fabae* type isolates produced number of spores less than that produced the *cinerea* type. Also, *B. fabae* isolates were the highest number of small sclerotia.

Eight faba bean varieties varied in their reaction to chocolate leaf spot disease caused by *B. fabae* Sard. Giza 402 is highly susceptible cv. (74.2%) while, Giza-Blanka was 402 the highly resistant (40.0%) whereas, Giza-3 was moderately resistant.

Also, isolate obtained from Nubaria was more virulent than those obtained from other governorates.

Keywords: Faba Bean, *Botrytis fabae*, *Botrytis cinerea*, sclerotia

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 favor 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 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 and Hassanein *et al.*, 1990).

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

MATERIALS AND METHODS

Isolation and identification of chocolate spots pathogen

Diseased samples were collected from different faba bean growing areas of northern Egypt; Kafr El-Sheikh, El-Beheira, Gharbia, Dakahlia, Damietta, Menoufia, Qualubia and Beni-Seawif governorates in Middle Egypt, at flowering stage during two seasons 2004/2005 and 2005/2006. The infected leaves with chocolate spots disease symptoms were cut into small pieces (5mm), each piece contain single lesion. The infected tissues were sterilized by soaking in 5% sodium hypochlorite for 2 min., then washed thoroughly several times with sterilized distilled water and dried between two layers of sterilized filter paper. The sterilized pieces were transferred onto potato dextrose agar (PDA) plates at rate of five pieces/plate. All plates were incubated at 20°C for 5-7 days. The isolated fungi were purified using single spore method (Riker and Riker, 1936) and then identified as described by Jarvis (1977) and Barnett and Hunter, (1987) according to their morphological and microscopical characters. The identification was confirmed by using Biology-System technique which belonged to the biological control of faba bean chocolate spots disease project, Plant Pathology Research Institute, A.R.C., Giza, Egypt. The pure cultures of each isolate were maintained on PDA slants at 20°C for further studies.

Effect of different media on radial growth, sporulation and sclerotial formation of *B. fabae* and *B. cinerea*

Three different media i.e.:

1-Potato dextrose agar (PDA) medium (Riker and Riker, 1936):

(Extract of 200g of peeled potato, 20g dextrose and 20g agar in 1000ml distilled water). Sterilization was at 1.5 lb/inch² for 20min.

2-Faba bean leaf agar (FBLA) medium (Leach and Moore, 1966):

(Extract of 250g faba bean leaves, 30g sucrose, 20g sodium chloride and 20g agar in 1000ml distilled water. Sterilization was at 1 lb/inch² for 15min.

3-Faba bean seed agar (FBSA) medium (Hanounik and Hawiin, 1979):

(Extract of 250g faba bean seeds, 30g sucrose, 20g sodium chloride and 20g agar in 1000 ml distilled water. Sterilization was at 1.5 lb/inch² for 20min.

In this experiment, 18 isolates of chosen *B. fabae* and *B. cinerea* were cultured on Petri dishes of 9cm in diameter of three different media with an equal disk (5mm) of *B. fabae* or *B. cinerea* grown previously on PDA media (7 days old culture). The cultivated Petri dishes were incubated at 20°C in precision incubator. The growth was measured daily until the diameter of fungal growth reached 9 cm. The sporulation was estimated after 12 days by adding 10ml distilled water to each plate, then exposed to electric sonar water bath to separate the conidiospores out their conidiophores by falling in the added water. The spore suspension was filtered using clean sterilized cheesecloth and the filtrate was received in a test tube. Number of spores

was counted using the haemocytometer slide. Sclerotial formation was measured in 21 days old cultures of each medium for each isolate. Plates were examined; the number and size of sclerotia/cm² were recorded for each of *B. fabae* and *B. cinerea* isolates.

Varietal reaction

Interaction between *B. fabae* isolates and some faba bean cultivars

In this experiments, eight faba bean cultivars i.e., Giza 3, Giza 402, Giza 429, Giza 461, Giza 667, Giza 716, Giza 717 and Giza Blanka were evaluated for their reaction against chocolate spot disease caused by ten *B. fabae* isolates. Different cvs of faba bean plants (45 days old) were sprayed with spore suspension (2.5×10^5 spore/ml) of each isolate. Three pots were used as replicates for each treatment. All pots were kept in the greenhouse for 48h at 20° C under high relative humidity. The inoculated plants were examined for chocolate spot disease and the data were recorded after 7 days post inoculation using the devised scale of Abou-Zaid *et al.*, (1978).

RESULTS

Variation among isolates

Effect of different media

1- On rate of growth

Results in Table (1) show that faba bean seed agar (FBSA) medium was the test favorable medium for growth of all tested Botrytis isolates followed by the PDA and faba bean leaf agar (FBLA) media. Also, growth of *B. cinerea* isolates was faster than those of *B. fabae* onto all tested media.

Table (1). Linear growth rate of different isolates of *B. fabae* and *B. cinerea* as affected by different media.

No.	Location	Linear growth after 5 days (mm)			Mean
		PDA	FBLA	FBSA	
B. fabae					
1	El-Nubaria	56.3	32.7	51.3	46.8
2	Etay-Elbaroud	42.7	36.3	45.7	41.6
3	Damanhour	45.0	36.7	47.3	44.0
4	Sakha	54.3	23.7	54.3	44.1
5	Sidy-Salem	52.7	27.3	51.3	43.8
6	Sherbean	34.0	37.7	41.3	37.7
7	Mahala	47.3	34.7	60.3	46.2
8	Kafr-Saad	33.7	32.7	56.7	41.0
9	Sheleen El-koum	41.3	35.3	59.7	45.4
10	Qualub	32.3	36.7	30.0	33.0
B.cinerea					
1	Etay-Elbaroud	70.3	53.4	78.3	67.3
2	Sidy-Salem	79.7	56.3	74.7	70.2
3	Sinbilawen	69.3	51.3	62.0	60.9
4	Mahala	64.0	52.7	80.0	65.6
5	Dammietta	54.3	48.0	83.0	61.8
6	El-Bagour	48.0	37.3	68.3	51.2
7	Kafr-Shokr	81.7	60.3	87.3	76.4
8	Beni-Swief	43.7	41.7	57.3	47.6
Mean		52.8	41.0	61.0	-

L.S.D at 5% for Isolates Media IXM

2-Spore production

Results in Table (2) indicated that isolates differed in the number of spores per Petri-dish. Most of the faba type isolates produced number of spores less than that produced the cinerea type isolates. Also, FBLA medium was the best favorable medium for spore production which produced 7.38×10^6 spore/ml followed by FBSA and PDA media which produced 0.57×10^6 and 0.29×10^6 spore/ml, respectively.

Table (2). Sporulation levels of different isolates of *B. fabae* and *B. cinerea* as affected by different media

No.	Location	No. of spores/ml×10 ⁶			Mean
		PDA	FBLA	FBSA	
B. fabae					
1	El-Nubaria	0.1	2.8	0.2	1.0
2	Etay-Elbaroud	0.3	2.0	0.4	0.9
3	Damanhour	0.2	3.0	0.3	1.2
4	Sakha	0.0	0.4	0.1	0.2
5	Sidy-Salem	0.0	0.2	0.4	0.5
6	Sherbean	0.1	1.6	0.2	0.6
7	Mahala	0.2	1.0	0.2	0.5
8	Kafr-Saad	0.0	1.5	0.1	0.5
9	Sheleen El-koum	0.0	0.8	0.3	0.4
10	Qualub	0.0	0.7	0.0	0.2
B. cinerea					
1	Etay-Elbaroud	0.3	19.5	1.3	7.0
2	Sidy-Salem	1.2	22.3	1.5	8.3
3	Sinbilawen	0.4	16.5	0.7	5.9
4	Mahala	0.7	18.4	1.3	6.8
5	Dammietta	0.0	14.2	0.0	4.7
6	El-Bagour	0.0	0.5	0.0	0.2
7	Kafr-Shokr	1.8	25.2	3.0	10.0
8	Beni-Swief	0.0	1.3	0.3	0.5
Mean		0.29	7.38	0.57	-

L.S.D at 5% for Isolates
Media
IXM

3-Presence, number and size of sclerotia

Data in Table (3) show that PDA was the best favorable medium for sclerotial formation followed by FBSA medium. Also, number and size of sclerotia were widely varied among the isolates. The isolates produced small size of sclerotia in large number were of the faba type while, the isolates not producing sclerotia or producing sclerotia large size but less in numbers were of the cinerea type.

Table (3). Number and Size of Sclerotia of different isolates of *B. fabae* and *B. cinerea* as affected by different media

No.	Location	No.of Sclerotia in cm ²			Mean	Size of sclerotia (mm)			Mean
		PDA	FBLA	FBSA		PDA	FBLA	FBSA	
B. fabae									
1	El-Nubaria	39.2	0.0	22.0	20.4	1.5	0.0	1.9	1.1
2	Etay-Elbaroud	40.0	0.0	24.0	21.3	1.3	0.0	1.1	0.8
3	Damanhour	43.4	0.0	26.7	23.4	2.0	0.0	1.9	1.3
4	Sakha	41.8	0.0	28.7	23.5	1.1	0.0	1.3	0.8
5	Sidy-Salem	40.1	0.0	31.0	23.7	2.8	0.0	1.8	1.5
6	Sherbean	51.2	0.0	42.8	31.3	1.1	0.0	0.7	0.6
7	Mahala	54.3	0.0	48.0	34.1	1.3	0.0	0.9	0.7
8	Kafr-Saad	56.7	0.0	53.0	36.6	1.3	0.0	1.0	0.8
9	Sheleen El-koum	42.3	0.0	38.2	26.8	2.0	0.0	1.5	1.2
10	Qualub	52.3	0.0	40.3	30.9	1.6	0.0	2.0	1.2
B.cinerea									
1	Etay-Elbaroud	3.9	0.0	0.0	1.3	6.7	0.0	0.0	2.2
2	Sidy-Salem	1.2	0.0	1.0	0.7	0.6	0.0	4.0	1.5
3	Sinbilawen	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	Mahala	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	Dammietta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	El-Bagour	1.6	0.0	1.3	1.0	4.5	0.0	3.5	2.7
7	Kafr-Shokr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	Beni-Swief	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean		26	0.0	19.8	-	1.5	0.0	1.2	-

L.S.D at 5% for Isolates
Media
IXM

Varietal reaction

Eight faba bean cultivars were tested for their reaction against chocolate spot disease caused by ten *B. fabae* isolates. Results in Table (4) showed that Giza 402 is highly susceptible cv. (78.2%) while, Giza-Blanka was the least susceptible one (40.0%) whereas, Giza 3 was moderately resistant. Also, isolate obtained from Nubaria was more virulent than those obtained from other governorates.

Table (4). Interaction between 10 isolates of *B. fabae* and 8 Faba Bean cultivars after 7 day from inoculation

Isolates code No.	Location	Average disease severity on cultivars								
		G3	G402	G429	G461	G667	G716	G717	Giza Blanka	Mean
10Nu 2006	El-Nubaria	61.3	89.2	86.2	62.3	84.0	67.4	57.5	52.1	70.0
9EB2006	Etay-Elbaroud	58.4	80.3	69.3	41.1	73.3	51.6	50.3	44.2	53.0
10 Da2006	Damanhour	59.3	83.7	78.2	51.4	75.3	48.1	42.8	36.6	59.4
15Sa 2006	Sakha	63.2	86.3	83.2	53.1	80.1	55.6	59.0	44.3	66.0
4 SS 2006	Sidy-Salem	66.4	81.5	70.0	52.1	78.2	51.1	45.0	41.3	60.7
10 Sn 2006	Sherbean	65.3	83.3	71.6	49.7	79.6	42.1	40.3	36.1	58.5
10 Ma2006	Mahala	57.4	70.9	63.3	38.4	76.4	41.4	36.2	35.6	52.5
5 KS 2006	Kafe-Saad	53.3	69.1	53.4	44.2	58.6	42.3	37.4	34.1	79.1
9 SK2005	Shebeen El-koum	51.9	76.3	64.1	45.3	65.2	44.3	36.1	40.3	54.1
5 Qu 2006	Qualub	48.0	61.3	44.2	29.1	57.3	35.6	37.8	32.2	43.2
Mean		58.5	78.2	59.7	46.7	72.8	48.0	44.2	40.0	-

L.S.D at 5% for Isolates

**Cultivars
IXC**

DISCUSSION

Faba bean seed agar (FBSA) medium was the best favorable medium for growth of all tested *Botrytis* isolates. Also, isolates of *B. fabae* were the least in growth rate, spore production and produced small sclerotia in high number while, isolates of *B. cinerea* were the fastest in growth rate, the highest in spore production and did not produce or produced large sclerotia in fewer number.

These results are agree with Hassanein *et al.* (1990) who found that FBSA medium was the best among the three media for growth of the different *Botrytis* isolates.

The tested faba bean cvs differed in their infection reaction by differing the kind of isolate. Giza 402 was the most susceptible one, where average disease severity was (78.2), whereas, the least infection was recorded on leaves of cv. Giza-Blanka.

Meanwhile, Giza 3 cultivar was moderate in average disease severity (58.5). These results could be interpret in line with the findings of El-Neshawy, Saniya (1981), Mohamed *et al.*, (1986), Habib, Wadiaa (1990), Mansour (1992) and El-Afifi (2003).

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تقدير الاختلافات بين عزلات أنواع الفطر بوترايتس *Botrytis spp.* والتداخل بين عزلات الفطر *Botrytis fabae* وبعض أصناف الفول البلدى
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تم مقارنة عزلات من نوعى بوترايتس فابى وبوترايتس سيناريا تم عزلها من الفول البلدى من محافظات شمال ووسط مصر بالنسبة لخواصها المورفولوجية والفسولوجية. وكانت هناك فروق واضحة بين العزلات المختلفة من حيث معدل النمو على البيئات الثلاث، كمية الجراثيم، عدد وحجم الأجسام الحجرية المتكونة. حيث تميزت عزلات الفطر بوترايتس سيناريا بالسرعة فى النمو على البيئات المختلفة وإنتاج أكبر عدد من الجراثيم وأقل عدد من الأجسام الحجرية ذات الأحجام الكبيرة مقارنة بعزلات الفطر بوترايتس فابى. كما تبين وجود إختلافات بين ثمانية أصناف من الفول البلدى فى درجة حساسيتها لمرض التبقع البنى المتسبب عن الفطر بوترايتس فابى حيث كان الصنف جيزة 402 أشد الأصناف قابلية للإصابة بينما كان الصنف جيزة بلانكا أكثرها مقاومة والصنف جيزة 3 متوسط القابلية للإصابة. كما وجد أن عزلة النوبارية للفطر بوترايتس فابى كانت أكثر العزلات شراسة مقارنة بالعزلات الأخرى.

قام بتحكيم البحث

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