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## EFFECT OF SOME *BACILLUS* SPECIES ON CHOCOLATE SPOT DISEASE OF FABA BEAN IN EGYPT

Ghoneimy E. A<sup>1</sup>.; Abou-Zeid, N. M<sup>2</sup>.; Rania. A. S<sup>2</sup>. and Noher. A. M<sup>2</sup>

<sup>(1)</sup>Faculty of Science., El-Azhar Univ.

<sup>(2)</sup>Plant Pathology Res. Institut., ARC., Giza, Egypt.

### ABSTRACT

A pathogenic fungus causes chocolate spot disease of faba bean was identified as *Botrtis fabae*, it has been isolated from seven Egyptian Governorates {Beheira, Kafr El-Sheikh, Sharkia, Kalyobia, Dakahli, Ismailia and Beni-Sweif}. In case of controlling chocolate spot diseases caused by *Botrtis fabae* under laboratory and greenhouse condition, twenty *bacilli* (*Bacillus subtilis*, *B. megaterium*, *B. licheniformis*, *B. pumilus*, *B. amyloliquificens*, *B. coagulans*, *B. maroccanus*, *B. mycoid* and *B. laevolacticus*) were decreased the mycelial growth of *B. fabae*. While *B. megaterium*, *B. licheniformis* were more effective bioagents followed by *B. subtilis*. All the tested two biocontrol agents (Bio-Arc and Bio-Zeid) and one fungicide Dithen 45M were effective in reducing chocolate spot disease when sprayed 24hrs before inoculation with *B. fabae*. The best percentage of reducing the disease severity was obtained with Dithen 45M followed by Bio-Zeid, Bio-Arc, and then *B. megaterium*, *B. licheniformis* and *B. subtilis* after 14 days.

### INTRODUCTION

Faba bean is considering the most important legume crops in Egypt, it considers the fifth food legume in the world after dry bean, dry pea, chickpea and lentil (Adak, *et al.*, 1998). The total cultivated area of faba bean reached 325000 Fadden with total production 2.645.500 ardab {ardab=155 Kg} at rate of 8,14 ardab/ Fadden. Faba bean suffers from many foliar diseases which are the most common diseases especially in Delta region due to the high humidity, as chocolate spot diseases caused by

*Botrytis fabae* (Morsy, 1993) which consider the most important disease in Egypt lead to serious damage in the crop and losing in the yield for more than 50%. As well as, recent health concerns over pesticides contamination of food public of chemical residues in the food chain especially after 1986. National Academy of Sciences report (USA) (Anonymous, 1987) on pesticides residues, announced fungicide more of a carcinogenic risk than insecticides. All these factors together have generated on urgent need for the development of safer alternative technology. Biological control using antagonistic microbes became widespread to reduce the use of chemical pesticides in a system of integrated plant disease management, (Abou-Zeid *et al.*, 2003).

## MATERIALS AND METHODS

### **1-Pathogenicity test of the isolated fungi under green house conditions:-**

This experiment was done to determine the pathogenic potential of the important fungus isolated from diseased samples of faba bean crop. The Pathogenicity test was studies on detached leaves and whole plants of faba bean. This test was conducted in the greenhouse of "Project of Biological Control of Chocolate Spot Disease on Faba bean" Plant Pathology Research Institute, Agricultural Research Center. Faba bean seeds were sown at beginning of October in 25 cm diameter pots containing loamy soil. The pots were grown for 6-8 weeks in greenhouse at  $23 \pm 2C^{\circ}$ .

#### **a- Preparation of inocula of Chocolate spot pathogen**

Fungal isolates were grown for ten days at  $20C^{\circ}$  using faba bean leaf extract agar (FBLA medium) in order to obtain high number of spores (Leach and Moore, 1966).The medium was autoclaved at 1Lb/inch for 15 minutes and poured before solidification into sterilized Petri plates. Plates were inoculated with equal discs of each of tested isolates and incubated at  $20C^{\circ}$  for 12 days. Three plates were used for each isolate as replicates. Ten milliliters of sterile tap water were added on sporulating plates cultures of *Botrytis fabae*. Conidia were released by

scraping the growth with a glass rode. The spore suspensions were filtering through two layers of sterile muslin to remove the mycelial residues. Then the concentration was estimated by haemocytometer counts and adjusted to  $2.5 \times 10^5$  spore/ ml (Abou-Zeid *et al.*, 1985).

#### **b-Determination of chocolate spot disease on detached faba bean leaves technique:-**

Leaflets Giza (40) susceptible cultivar to leaf spot disease obtained from 6-8 weeks old. Detached leaves taken from the fifth nodes were placed horizontally on filter papers onto sterilized aluminum trays tray with water soaked filter paper. The detached leaves were inoculated with spores suspension obtained from 12 days old cultures of each isolates in form droplets (10  $\mu$ l) at rate  $2.5 \times 10^5$  spore/ ml and incubated at  $23 \pm 2^\circ\text{C}$ . Three replicates were used for each treatment. The box are covered with transparent polyethylene bags to maintain high humidity. Disease severities were recorded after 1,3,5 and 7 days as table number (1) using scale (0-9) depending on the extent of lesions. (Abou-Zeid *et al.*, 1985).

#### **c-Determination of chocolate spot disease by using whole plants technique**

The seeds of faba bean cultivar (Giza 40), were grown in 25 cm sterilized pots (5seed/pot). Plants (6-8 weeks) have been sprayed with *Botrytis* inoculums at rate of  $2.5 \times 10^5$  spores/ml, plants were covered with polyethylene bags and kept for 24-48 hour in moist chamber at  $23 \pm 2^\circ\text{C}$  in the greenhouse. Three pots were used as replicates for each isolate. Disease severity was recorded on 2,4,7 and 14 days of inoculation by using scale adapted by (Abou-Zeid, 1978) and disease severity was calculated using the following equation:

$$\% \text{ Disease severity} = \frac{nxvx100}{9N}$$

Where, n= number of plants in every grade.

v= number grade

N= total number of examined plants

9= maximum disease grade.

## 2- Biological Control

*Bacillus* isolates were obtained from the Unite of Identification of Microorganisms, Agricultural Research Center (ARC).

### a-The antagonistic effect of *Bacilli* on growth of *Botrytis fabae* under laboratory experiments

20 isolates of *Bacillus* spp. were tested against *B. fabae* using potato dextrose agar PDA medium. Disks (5mm-diam.) taken from 7 day old culture of *B. fabae* (El-Noubaria isolate), were transferred onto PDA plates (9cm) the antagonistic tested *Bacilli* were individually streaked in opposite direction of the *B. fabae*. Three Petri-dishes were used for each isolate and the same number was served as control, while pathogen alone *B. fabae* plated on one side of the Petri-dishes. The inoculated plates were incubated at 20°C until the growth completely covered the plate surface in control treatment. The interaction between *B. fabae* and *Bacilli* were recorded to determine the more effective antagonistic isolates (Abou-Zeid and Hassanien, 2000). The reduction percent in mycelial growth of *B. fabae* was calculated using the formula as follows:

$$\text{Reduction \%} = \frac{C-T}{C} \times 100$$

Where, C = fungal growth of *B. fabae* in control treatment.

T = fungal growth of *B. fabae* in presence of antagonist

### b-The antagonistic effect of *Bacilli* on *B. fabae* using detached leaves technique under laboratory experiments

Detached leaves were taken from the fifth node of 8 weeks old faba bean plants. Giza 40 (susceptible). The leaves were placed on filter papers on sterilized sponge in the aluminum trays. The leaflets were sprayed with the tested bacterial suspension ( $10^8$ cfu). Spore suspension of *B. fabae* ( $2.5 \times 10^5$ spores/ml) was prepared (Mohamed *et al.*, 1994). One drop (10 $\mu$ l) of this suspension ( $10^8$  cfu) was placed near the midrib proper. Control was also prepared with (10 $\mu$ l) of *B. fabae*

spore suspension with out *bacilli*. The trays were covered with polyethylene sheets for 24 hour to maintain high moisture and incubated at 20°C. Disease severity was recorded based on using scale 0-9 (Abou-Zeid, 1978).

### **c-The antagonistic effect of *Bacillus* spp. against *B. fabae* in vitro conditions under greenhouse**

Faba bean plants Giza (40) were grown in polyethylene pots (25 cm diameter) under greenhouse conditions at 20-22°C for 40 days, five seeds were planted per pot. Twenty antagonist *Bacilli* suspension were papered by growing culture on (TSB) medium for 48 hr. at 30°C with shaking at 120 rpm. Each antagonistic *Bacilli* suspension were sprayed on faba bean plants 24 hours before sprayed with spore suspension of *B. fabae* (El-Nubaria) isolate. Three pots were sprayed with *B. faba* only to represent the control. All pots kept in polyethylene bags in moist chamber at 20°C in greenhouse for 48hr. Disease severity was recorded after 2,4,7 and 14 days.

### **d-Effect of biocontrol agents, fungicide and the most effective bacilli on chocolate spot diseases of faba bean under greenhouse conditions.**

The treatment with bioagents (Bio-Arc) and (Bio-Zeid) and fungicide Diathen (M45) on disease severity of chocolate spot disease of faba bean under greenhouse conditions were tested as comparison to *bacilli* effects. Five seeds of faba bean plants Giza 40 (susceptible) were grown in polyethylen pots. Plants were sprayed with the recommended dose of the mentioned fungicides at the rate of 2.5 g/ liter 24 hours and bioagents at rat ( $10^8$  cfu) before inoculation with *B. fabae* ( $2.5 \times 10^5$  spore/ml). The bioagents and fungicide preparation were sprayed on faba bean plants four times during the growing season. Three pots were sprayed with spore suspension of *B. fabae* only to represent the control. All pots kept in plastic bags in moist chamber at 20°C in greenhouse for 48 hr. Disease severity was recorded after 2,4,7 and 14 days.

## RESULTS AND DISCUSSION

### 1- The pathogenicity test

#### a-Determination of chocolate spot disease on detached leaves of faba bean:-

Pathogenicity test was carried out using detached leaves technique. As shown in Table (1) all isolates of *Botrytis fabae* (15 isolates) were differs in their aggressiveness on Giza 40 faba bean leaves. The isolate no. (1) which isolated from El-Behaira (Nubaria) recorded the highest disease severity (98.8%) after 7 days followed by isolate no. (5) obtained from Kafer El-Sheikh (Sakha) which represented disease severity reached 95.5%. While the least virulent isolate no. (15) which isolated from Bene-Sweif it caused disease severity reached 33.3%. In general all *Botrytis* isolated from El-Behira were the most virulent than other isolates.

**Table 1. Pathogenicity test of *Botrytis fabae* on faba bean using detached leaves technique (scale 0-9).**

<i>Botrytis</i> isolates	Places of isolate	Mean of disease severity (%)			
		1day	3day	5day	7day
B.1	El- Behira (Nubaria)	34.4	77.7	89.9	98.8
B.2	El- Behira (Etay-El Baroud)	24.4	35.5	73.3	91.1
B.3	El- Behira (Damanhour)	26.6	57.7	75.5	93.3
B.4	El- Behira (Koum-Hamada)	23.3	44.4	71.1	90.0
B.5	Kafr-El Sheikh (Sakha)	32.2	69.9	83.3	95.5
B.6	Kafr-El Sheikh (Dosok)	27.7	51.1	71.1	89.9
B.7	Kafr-El Sheikh (Kleen)	26.6	32.2	73.3	87.7
B.8	Kafr-El Sheikh (Sidy-Salem)	18.8	35.5	67.7	85.5
B.9	Dakahlia (Aga)	22.2	37.7	65.5	82.2
B.10	Dakahlia (Meet-Ghamer)	20.10	29.9	59.9	76.6
B.11	Sharkia (Fauques)	23.3	27.7	45.5	67.7
B.12	Sharkia (Hehia)	21.1	23.3	31.1	65.5
B.13	Kalyobia (Kafr-Shokr)	17.7	20.0	37.7	60.0
B.14	Kalyobia (Toukh)	14.4	19.9	35.5	58.8
B.15	Beni-Sweif (Beni-Swif)	9.2	12.2	12.2	33.3

#### b-Determination of chocolate spot disease of faba bean on whole plants under greenhouse conditions:-

Fifteen isolates of *B. fabae* were chosen based on their frequency at different locations in order to test their pathogenic abilities on faba

bean leaves of Giza (40) under greenhouse conditions. Data in Table (2) showed that all the isolated *Botrytis* from different governorates were differ in their aggressiveness on faba bean leaves after 1,3,5,7,14 days from inoculation. The most virulent isolate no.(1) obtained from El- Behaira (Nubaria) which gave highest disease severity reached 99.9% followed by isolate no. (2, 3) from El-Behaira (Etay-Elbaroud, Damanhour) which recorded the disease severity reach 97.7,96.6%, While the least virulent. Isolate was from Beni-Sweif isolate no. (15) which gave disease severity about 35.5%. In general, the disease severity of *Botrytis* isolates was increased gradually from the first day to reach maximum after 14 days post inoculation. El-Behaira which gave highest disease severity followed by Kafr-El Sheikh and the least virulent isolate was from Beni-Sweif.

**Table 2. Pathogenicity test of *Botrytis fabae* on faba bean under greenhouse conditions scale (0-9).**

<i>Botrytis</i> isolates	Places of isolate	Mean of disease severity				
		1 day	3 days	5 days	7 days	14 days
B.1	El- Behira (Nubaria)	35.5	69.9	85.5	97.7	99.9
B.2	El- Behira (Etay-El Baroud)	28.8	45.5	71.1	87.7	97.7
B.3	El- Behira (Damanhour)	31.1	40	62.2	85.5	96.6
B.4	El- Behira (Koum-Hamada)	25.5	26	43.3	71.1	86.6
B.5	Kafr-El Sheikh (Sakha)	30.0	53	77.7	93.3	95.5
B.6	Kafr-El Sheikh (Dosok)	24.4	34.4	53.3	73.3	87.7
B.7	Kafr-El Sheikh (Kleen)	27.7	52	66.6	87.7	92.2
B.8	Kafr-El Sheikh (Sidy-Salem)	25.5	49	53.3	72.2	88.8
B.9	Dakahlia (Aga)	20.0	33	49.9	68.8	82.2
B.10	Dakahlia (Meet-Ghamer)	19.9	22.2	38.8	62.2	78.8
B.11	Sharkia (Faques)	18.8	24.4	31.1	58.8	75.5
B.12	Sharkia (Hehia)	16.6	21.1	28.8	53.3	70.0
B.13	Kalyobia (Toukh)	15.5	22.2	26.6	49.9	65.5
B.14	Kalyobia (Kafr-Shokr)	13.3	19.9	21.1	37.7	62.2
B.15	Beni-Swif (Beni-Swif)	11.1	14.4	20.0	27.7	35.5

## 2-Biological control

### a-Effect of antagonistic *bacilli* on mycelial growth of *B. fabae* under laboratory experiment

Twenty of *bacilli* isolates were tested *In Vitro* (laboratory experiment) on *B. fabae*. Data in Table (3) showed the different effects of bacterial isolates on the inhibition of radial growth of *B.*

*fabae* on PDA plates compared to control. Among antagonistic *Bacilli*, *B. megaterium* no. (1, 2) isolated from Kalyobia (Toukh) and Sharkia (Faques) were the most effective, they recorded the highest reduction in the growth of *Botrytis fabae* causing chocolate spot disease i.e 87.7 and 86.6% followed by *B. licheniformis* no. (7, 8, 9) from Behaira (Etay-El Baroud) Dakahlia (Aga) were the most effective, 85.5, 84.4% compare to other *bacilli*. On the other hand, *B. maroccanu*, *B. mycoid* and *B. coagulans* had the lowest effects as they reduced *B. fabae* growth by 43.3 and 44.4 respectively.

**Table 3. Effect of antagonistic *bacilli* on growth *Botrytis fabae* causing chocolate spot diseases of *faba bean* under laboratory experiment**

<i>Bacillus</i> isolates	<i>B. fabae</i>	
	Radial growth (cm)	Growth reduction (%)
(049) <i>B. megaterium</i> (1)	1.1	87.7
(240) <i>B. megaterium</i> (2)	1.2	86.6
(029) <i>B. subtilis</i> (3)	1.6	82.2
(232) <i>B. subtilis</i> (4)	1.7	81.1
(241) <i>B. subtilis</i> (5)	1.6	82.2
(043) <i>B. licheniformis</i> (6)	1.5	83.3
(039) <i>B. licheniformis</i> (7)	1.3	85.5
(160) <i>B. licheniformis</i> (8)	1.4	84.4
(235) <i>B. licheniformis</i> (9)	1.4	84.4
(066) <i>B. amyloliquefaciens</i> (10)	4.3	52.2
(069) <i>B. amyloliquefaciens</i> (11)	4.2	53.3
(158) <i>B. amyloliquefaciens</i> (12)	4.3	52.2
(239) <i>B. amyloliquefaciens</i> (13)	4.2	53.3
(032) <i>B. pumilus</i> (14)	2.0	77.7
(236) <i>B. pumilus</i> (15)	2.0	77.7
(156) <i>B. laevolacticus</i> (16)	4.7	47.7
(238) <i>B. laevolacticus</i> (17)	4.8	46.6
(194) <i>B. maroccanus</i> (18)	5.1	43.3
(118) <i>B. mycoid</i> (19)	5.1	43.3
(034) <i>B. coagulans</i> (20)	5.0	44.4
Control	9.0	100
LCD at 5%	0.12	

**b- Evaluation of fungicide and biocontrol agents in comparison with active *bacilli* for controlling faba bean chocolate spot disease under greenhouse conditions:-**

Data in Table (4) demonstrated that the effective *bacilli*, two biocontrol agents (Bio-Arc and Bio-Zeid) and one fungicide Dithan 45M were effective in reducing chocolate spot disease



when sprayed 24 hour before inoculation with *B. fabae*. *B. megaterium* no. (1, 2) were the best effective antagonists which reduced infection degree of chocolate spot disease severity after 48 hour post inoculation comparing to the control (*B. fabae* alone) they causes disease severity (18.8, 19.9) %, followed by *B. licheniformis* no. (7,6,8) which recorded (20.0, 21.1, 22.3)%, respectively. However when compared two biocontrol agents and one fungicide, the best percentage of reducing the disease severity was obtained with Dithan 45M (14.34)% and Bio-Zeid (16.6)% followed by Bio-Arc and *B. megaterium* with percentage of disease reduction (18.8 %) to both of them, and then by *B. licheniformis* and *B. subtilis* which recorded (20.0, 23.2)% after 14 days. However, *B. amylololiquefaciens* were the least effective antagonists to which reduced disease severity compared to the other tested.

**Table 4. Evaluation the effect of fungicides and biocontrol agents for controlling chocolate spot diseases of faba bean under greenhouse conditions.**

<i>Bacillus</i> isolates	Mean of disease severity			
	2 days	4 days	7 days	14 days
<i>B. megaterium</i> (1)	10.0	12.2	14.4	18.89
<i>B. megaterium</i> (2)	11.2	12.5	15.5	19.9
<i>B. subtilis</i> (3)	14.46	16.6	19.9	23.2
<i>B. subtilis</i> (4)	15.6	19.9	23.3	26.6
<i>B. subtilis</i> (5)	15.4	18.67	22.2	25.56
<i>B. licheniformis</i> (6)	12.2	15.45	18.78	21.1
<i>B. licheniformis</i> (7)	11.5	14.4	17.7	20.0
<i>B. licheniformis</i> (8)	13.2	15.5	19.9	22.3
<i>B. licheniformis</i> (9)	14.56	17.7	20.0	24.4
<i>B. amylololiquefaciens</i> (10)	18.8	24.4	30.0	44.4
<i>B. amylololiquefaciens</i> (11)	19.3	27.7	33.3	45.5
<i>B. amylololiquefaciens</i> (12)	19.9	29.9	39.9	55.6
<i>B. amylololiquefaciens</i> (13)	21.1	30.0	41.1	59.9
<i>B. pumilus</i> (14)	17.7	22.2	29.9	37.7
<i>B. pumilus</i> (15)	17.3	20.0	27.7	35.5
Bio-Arc	18.8	15.65	13.3	10.0
Bio-Zeid	16.6	14.4	11.23	9.9
Dithan 45M	14.34	11.1	10.0	8.78
Control	95.5	80.0	67.7	45.5
LCD at 5%	0.34			

Faba bean is subjected to attacks by several diseases. The chocolate spot disease of faba bean caused by *Botrytis fabae* is considered the most important disease in Egypt. Mohamed, (1982). The result of Pathogenicity test showed that *Botrytis fabae* recorded the highest frequency (99.6) at El-Behira (Nubaria) and not be detected in Kalubia, Sharkia and Beni-Sweif Governorates. Abou-Baker, (2002) and El-Afifi, (2003) found that *Botrytis fabae* isolated from El-Behira gave the highest disease severity (52%). While Morsy, (1993) reported that *B. fabae* recorded the highest frequency in all Governorates than *B. cinerea*. Under greenhouse conditions. *B. fabae* isolates were differed in their virulence on faba bean leaves after 1,3,5,7 and 14 days, and the disease severity was increased gradually post inoculation and reach the maximum after 14 days from inoculation. However, *B. fabae* isolated from El-Nubaria followed by Sakha gives the highest disease severity respectively; these results are in harmony with the Huston and Mansfield (1980) they reported the *B. fabae* differed in their Pathogenicity on faba bean. While, Mohamed *et al.*, (1981) found that Nubaria isolate was the most virulent compared with all tested isolates.

In case of controlling of chocolate spot disease, our results indicated that all the tested biocontrol agents and fungicide were effective in reducing chocolate spot disease when sprayed 24hour before inoculation with *B. fabae*. The best percentage of reducing the disease severity was obtained with fungicide and followed by biocontrol agents. But they have recently come under special scruting as potential monogenic risks when applied. There are still many pesticides including fungicides currently under review by the environmental protection agency of USA FAO. Microorganisms were tested for potential as biocontrol agent against chocolate spot; these bacterial isolates showed increase of the width of inhibition zone, also inhibition of the radial growth. It is therefore important to extend the work *in vitro* to *in vivo* studies. The effective isolates were tested for their efficacy under greenhouse conditions, most of the isolates showed antagonism preventive effect against pathogens of chocolate spot disease.

The biocontrol of plant diseases as an alternative strategy has received increasing attention in recent years Handelsman and Stabb, (1996); Larkin and Fravel, (1998). Bacterial species have been used as

biocontrol agents against various plant pathogens Cook and Baker, (1983).

Twenty *Bacillus* isolates were tested *in vitro* for their antagonistic activity against *B. fabae* on PDA plates. The isolates were subjected to characterization according to their physiological characteristics using the Biolog system described by Bochner, (1989); Jones *et al.*, (1993); Harris and Gudmestad, (1996). The results of this investigation indicated that, the bacterial isolates under study were belonged to the species of *Bacillus subtilis*, *B. megaterium*, *B. licheniformis*, *B. pumilus*, *B. coagulans*, *B. amyloliquefaciens*, *B. laevolacticus* and *B. mycoid*. Data of antagonistic and inhibitory effect of *Bacillus* isolates (*in Vitro*) was explained by Matar *et al.*, (2009). All *bacillus* bio agents decreased the mycelial growth of pathogens on PDA plates compared with the control (*B. fabae* alone). *B. megaterium* and *B. licheniformis* were the most effective, they recorded the highest inhibition in the growth of *Botrytis fabae* for chocolate spot disease.

The nutrient competition is intense on aerial plant surface and is important aspect of antagonism toward pathogens including *B. fabae* (Blackman and Fokkema, 1982).

On the other hand, testing of *Bacillus* isolates revealed that all tested antagonists decreased chocolate spot disease severity caused by *B. fabae* (El-Nubaria) isolate on detached leaves after 1, 3, 5, and 7 days post inoculation. In our investigation, the result clearly indicated that the bioagents (*Bacillus* isolates) produced the largest zone of inhibition. However, Sharga, (1997) found that, the *Bacillus* cells were able to stick to *B. fabae*, especially on the sites of leaching and damaged regions of hyphae and then multiply there. These results in competition among themselves and between bacterial cells and fungus for nutrient, *B. megaterium* and *B. licheniformis* were the highly effective antagonists.

Spraying faba bean plants 24 hours before inoculation with *B. fabae* under greenhouse conditions with any of the tested antagonists decreased significantly chocolate spot disease severity comparing with control. In this respect, *B. megaterium* and *B. licheniformis* were the best effective treatments in controlling chocolate spot disease after 14 days post inoculation.

## REFERENCES

- Abou-Baker, M.E. (2002): Biochemical studies on important fungal disease of some major food. M. Sc. Thesis, Fac. Agric. Sci. (Biochemistry), Ain shams Univ., Egypt, 122pp.
- Abou-Zeid N. M. and Le-Normand, M. (1979): Mesure de variable de *Vicia faba* L. Vis a Vis de *Botrytis fabae* par une methode rapide. Ann phytopathologia I: 134 – 135.
- Abou-Zeid, N. M., H. A. Mohamed and M. Le- Normand. (1985): Effect of geographical origin of *Botrytis* spp their virulence on *Vicia faba* L. and spread in different varieties. 1st Nat. Conf. of Pests and disease of Vega. And Field Crop in Egypt, Ismailia, Proc. 862-871.
- Abou-Zeid, N.M. and Hassanein, A.M. (2000): Biological control of chocolate spot disease (*Botrytis fabae* sard.) in faba bean in Egypt. Phytology 90: 1182 (Abstract).
- Abou-Zied, N. M.; Hassanein, A. M. and EL-Behery, M. H. (2003): Biological control of damping off and root rot diseases of faba bean caused by *Rhizoctonia* in faba bean, lentil and chickpea and chocolate spot disease in faba bean / ICARDA / NVR SRP. Cairo. Egypt. viii-66p. ISBN : 977-302-073-8pp-49-52.
- Adak, M,S,; Ulukan, H. and Guler, M. (1998): Determination of some agronomic traits in Turkish fababean (*Vicia fabae* L.) Lines. FABIS, Newsletter, 42:29-31.
- Anonymous, (1987): Research Council, Board of Agriculture Regulating Pesticides in food. The Delaney paradox. Natinal Academy Press Washington, D.C.
- Blakman, J. P. and Fokkema, N. J. (1982): Potential for biological control of plant diseases on the phlloplane. Annu. Rev. Phytopathol. 20: 167-192.
- Bochner, B. R. (1989): Sleuthing out bacterial identities. Nature,339:157 – 158.
- Cook, R. J. and Baker, K. F. (1983): The nature and practice of biological control of plant pathogens. Amer. Phytopathol. Soci, St. Paul, Minn., 539 pp.

- El-Afifi, A. M. M. (2003): Pathological studies on some fungal diseases of faba bean and their control in Egypt. M. Sc. Thesis, Fac. Agric. Al-Azhar univ. Egypt, 121pp.
- Handelsman, J. O. and Stabb, E. V. (1996): Biocontrol of soil borne plant pathogens. *The plant cell*, 8: 1855-1869.
- Harris, B. A. and Gudmestad, N. C. (1996): Identification of phytopathogenic coryneform bacteria using the Biolog automated microbial identification system. *Plant Dis.*, 80: 874 -878.
- Huston, R. A. and Mansfield, J.W. (1980): A genetical approach to the analysis of mechanism of pathogenicity in *Botrytis/ Vicia/ faba* interactions. *Physiol. Plant Pathol.* 17:309-317.
- Jones, J. B.; Chase, A. R. and Harris, G. K. (1993): Evaluation of the Biolog GN Micro-plate system for identification of some plant pathogenic bacteria. *Plant Dis.*, 77:553-558.
- Larkin, R. P. and Fravel, D. R. (1998): Efficacy of various fungal and bacterial biocontrol organisms for control of *Fusarium* wilt of Tomato. *Plant Dis.*, 82:1022-1028.
- Leach, R. and K. G. Moore. (1966): Sporylation of *Botrytis fabae* on agar cultures. *Trans. Of British Mycol. Soc.*, 49:593-601.
- Matar, S. M.; El-Kazzaz, S. A.; Wagih, E. E.; El- Diwany, A. I.; El-Saadani, M. A.; Hafez, E. E.; Moustafa, H. E.; Abd- Elsalam, H. E.; Abo-Zaid, G. A. and Serour, E. A. (2009): Molecular characterization and batch fermentation of *Bacillus subtilis* as biocontrol agent, II. *Biototechnology*, 1-9.
- Mohamed, H. A.; Aly, H.Y. and Wadiaa, F. Habib. (1994): The antagonistic effect of faba bean phylloplane to *Botrytis fabae* Sard. *Egyptian Journal of Agricultural Research* 72 (3):645-654.
- Mohamed. M. S. (1982): Integrated control of root-rot disease in faba bean. *Egypt. J. Agric. Res.*, 73: 543-549.
- Mohamed, H. A.; Abou-Zeid, N. M. and Habib, W. F. (1981): Variation within the fungus *Botrytis fabae* Sard. *FABIS Newsletter* 3:46-47.
- Morsy, K. M. M. (1993): Pathological and genetic studies for improving resistance of faba bean to chocolate spot disease. M. Sc. Thesis, Fac. Agric., Cairo univ. Egypt, 119pp.

Phae, C. G., M. Shoda, and H. Kubota. (1990): Suppressive effect of *Bacillus subtilis* and its products on phytopathogenic microorganisms. J. Ferment. Bioeng. 69:1-7.

Sharga, B. M. (1997): *Bacillus* isolates as potential biocontrol agents chocolate spot on faba beans. Can. J. Microbial. 43:915-924.

### تأثير بعض انواع الباسيلس لمرض التبقع البنى فى الفول البلدى فى مصر

(1) ا.د/ اجلال عبداللة الغنيمي، (2) ا.د/ ناجى محمد أبو زيد ، (2) رانيا عباس صالح ،  
(2) د/ نهير عبد النظر محمود

(1) كلية العلوم (فرع النبات) - جامعة الازهر

(2) مركز البحوث الزراعية - معهد بحوث أمراض النباتات

يعتبر محصول الفول البلدى من أهم المحاصيل البقولية فى مصر وكثير من دول العالم. يصاب محصول الفول البلدى بالعديد من الامراض ومن اهمها امراض المجموع الخضرى (التبقع البنى) ومسببة فطر *Botrytis fabae*. وقد تم دراسة القدرة المرضية على اوراق الفول المفصولة لصنف حساس جيزة 40 وكانت عزلة النوبارية هى اقوى العزلات فى القدرة المرضية وتسبب شدة اصابة (99.9%) اما عزلة بنى سوف كانت تسجل اقل نسبة اصابة (35.5%). وقد اظهرت نتائج اختبار 20 عزلة *Bacillus* كعوامل مقاومة حيوية ان جميع العزلات قد قللت من نمو الفطر المسبب للمرض، كما اوضحت نتائج القدرة التضادية فى المعمل ان عزلات *B. megaterium* هي الأكثر تثبيطاً للنمو الميسليومي لفطر *Botrytis fabae* بنسبة (87.7%) تليها عزلات *B. licheniformis* (85.5%). بينما كانت عزلات *B. maroccanus*, *B. mycoid* and *B. laevolactic* هم الاقل تثبيطاً. كذلك اظهرت نتائج اختبار عزلات *Bacillus* كعوامل مقاومة حيوية ان جميعها قللت من شدة الاصابة بمرض التبقع البنى على الاوراق المنفصلة وكانت عزلات *B. megaterium* هي الأكثر فاعلية فى مجال المقاومة حيث قللت نسبة الاصابة الى (16.2%) تليها عزلات *B. licheniformis* (18.5%). وتبين من نتائج مقارنة استخدام المبيدات الحيوية (بيو زيد وبيوارك) والمبيد الكيمايى (دياسين ام 45) وكذلك اقوى عزلات *Bacillus* فى الصوبة ان جميع المعاملات قد قللت من شدة الاصابة ولكن المبيد الكيمايى سجل افضل نتيجة فى تقليل الاصابة بالمرض الى (14.34%) ويلية مباشرة المبيد الحيوى الفطرى بيوزيد بنسبة (16.6%) ثم المبيد الحيوى البكتيرى بيوارك و *B. megaterium* بنسبة (18.8%) يليهم *B. licheniformis* و *Bacillus subtilis* بنسبة (20.0, 23.2%) بعد 14 يوم من العدوى.