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THE ANTAGONISTIC EFFECT OF FABA BEAN PHYLLOPLANE TO *BOTRYTIS FABAE* SARD.

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Abstract

The numbers of microflora in the phylloplane of the moderately by susceptible cultivar (Giza 3) were greater than in the resistant entry (ILB 938). About 22% of the bacteria isolated in case of the resistant entry and 6% of those in the phylloplane of the moderately susceptible cultivar were antagonistic to *Botrytis fabae*.

Percentage of inhibition area/plate was higher for fungi from the resistant entry ILB 938 (20.67%) compared with those from the moderately susceptible cultivar, Giza 3 (10.11%).

It was found that size of the *B. fabae* spots was reduced as a result of treatment with some isolated antagonistic bacteria (Bacillus spp.) after 2 and 4 days incubation period. However, diameter of the chocolate spots was larger after 6 days compared with the control.

INTRODUCTION

Faba bean is subject to many diseases in Egypt, the most important of these is chocolate leaf spot caused by *Botrytis fabae* Sard. (Mohamed 1982). Several methods of control were attempted including agricultural practices, chemical control and breeding for disease resistance (Khalil *et al.* 1984).

Numerous faba bean entries were, therefore, tested for their reaction to

chocolate leaf spot and few were resistant under natural infection in the field and artificial inoculation in the greenhouses. Nature of disease resistance was studied including the role of the microorganisms in the phylloplane of faba bean leaves (Hanounik and Hassanein 1986, Omar *et al.* 1989 and Habib 1990).

The present study was designed to determine the role of microorganisms in the phylloplane of leaves in two faba bean cultivars differing in their reaction to the chocolate leaf spot organism. Studies were carried out *in vitro* on artificial medium and *in vivo* on faba bean detached leaves.

MATERIALS AND METHODS

Source of seeds:

Seeds of two faba bean entries, namely; Giza 3 and ILB 938 were obtained from the Field Crop Research Institute, Giza. Seeds were grown in pots (20 cm in diameter) at the rate of 5 seeds/pot for 6 weeks.

The pathogen:

The virulent isolate of *B. fabae* used was isolated by Mohamed *et al.* (1987). The isolate was maintained on PDA slants and kept at 5°C.

Isolation of faba bean phylloplane microorganisms:

The method (Plate Count Technique) proposed by Kiraly (1974) was adopted. Fifteen leaf discs 0.5 cm in diameter were taken from 6 weeks old plants around the midrib of leaves at the fourth node. The discs were ground in 8 ml of sterile buffer solution (pH 7) in a mortar. Two dilutions i.e., 10^{-1} and 10^{-5} were prepared from the suspension. Dilution of 10^{-1} was used for counting fungal colonies and the other dilution was used for counting bacterial colonies. Soil extract agar and Waksman's Agar media were used for isolation of bacteria and fungi, respectively. One ml aliquot was streaked on the surface of the medium with the aid of a sterile glass rod. Plates were incubated at 20°C for two and four days to count bacteria and fungi, respectively. The isolated fungi and bacteria were maintained on PDA medium, of pH

7.1. The numbers of colonies were calculated per cm of leaf surface.

The antagonistic effect of some isolated microorganisms:

To evaluate the possible antagonistic effect of the resulting organisms on a virulent isolate of *B. fabae*, one 5 mm disc of the fungus taken from one week-old culture, was placed in the center of each Petri dish containing PDA medium. The isolated bacteria were streaked in different treatments, at four loci, 3 cm apart from *B. fabae*. For the isolated fungi, discs of *B. fabae* were placed near the edges of the dishes, one in each. Other fungi were placed 7 cm apart from the *B. fabae* discs of the tested isolate (Cooper and Chilton 1950). The dishes were incubated at 20°C for one week after which the inhibition zone was measured in mm.

Percentage of inhibition area relative to the plate surface:

A spore suspension ($2.5x10^5$) of *B. fabae* isolate was prepared from growth on a medium consisting of faba bean leaf extract+ sucrose+ sodium chloride and agar. One ml of the spore suspension was added to a test tube containing 9 ml of the forementioned medium, just before solidification. The contents of each tube were poured on the surface of the seeded plates of fungi isolated from the phylloplane. Five replicate dishes were used for each treatment. Plates were incubated at 20°C and examined daily for measuring the diameter of inhibition areas as stated by Barakat *et al.* (1986).

Detached leaves experiment:

This experiment was carried out to test the effect of four highly antagonistic isolates of bacteria (White and cream colonies) against the tested isolate of *B. fabae*. Two different concentrations of each bacterial isolate, i.e. 10^8 and 10^6 were tested. Detached leaves were taken from the fourth node of 6 weeks old faba bean plants cv. Giza 3 (moderately susceptible to *B. fabae*). The leaves were placed on filter paper on sterilized sponge in the aluminium trays. The leaflets were deliberately contaminated with the tested bacterial suspension (10^8 and 10^6 cfu). Spore suspension of *B. fabae* isolate ($2.5x10^5$ spores/ml) was prepared as mentioned before. One drop (10 μl) of this suspension was placed near the midrib. Two drops were placed on each leaflet. Proper control was also prepared. The trays were covered with poly ethylene sheets for 12 hr. to maintain high moisture and incubated at 20°C. The diameter of spots in mm was recorded 2, 4 and 6 days after inoculation (Mansfield and Deverall 1974).

EXPERIMENTAL RESULTS

Isolated epiphytic fungi and bacteria from leaf surface of two entries of faba bean:

Data in Table 1 show that the numbers of microflora (bacteria and fungi) were greater in the phylloplane of the susceptible Giza 3 than the resistant entry, ILB 938. Most of the fungi that accompanied Giza 3 were *Aspergillus niger*, *Epicocum* sp. and *Penicillium* sp. For the resistant entry, only *Aspergillus flavus* and *Cladosporium* sp. were common. On the other hand, data revealed that most colonies of bacteria in the entry ILB 938 were cream and white (not pigmented), whereas in the cv. Giza 3, the bacterial colonies were red and yellow (pigmented). Also, it was found that all the isolated bacteria antagonistic to the tested isolate were spore-formers, gram positive and in chains or in duplicate rods. The antagonistic bacteria were identified as *Bacillus subtilis*, *B. cereus* and *B. magatharium*. It was noticed that about 22% of the isolated bacteria from the resistant entry (ILB 938) were antagonistic, while 6% from the moderate susceptible cultivar (Giza 3) were antagonistic.

Results in Fig. (1) also indicate that *Aspergillus flavus* and *Cladosporium* sp. were able to inhibit the growth of the tested isolate of *B. fabae*. On the other hand, it was found that the non-pigmented bacteria (white and cream) were antagonistic to the *B. fabae* isolate (Fig. 2), while the pigmented ones (red and yellow) had no adverse effect on the growth of *B. fabae*.

Table 1. Number of microorganisms on faba bean leaf surface.

Tested plants	cfu/cm^2 of leaflets	
	Bacteria	Fungi
ILB 938	18,134,385	42,759
Giza 3	27,360,651	60,575

Results (Fig. 2) show that, there was a large inhibition zone between the antagonistic bacteria from the resistant entry and *B. fabae* isolate (16-18 m), while the zone was narrow (8 mm) for those from the moderately susceptible cultivar.

Percentage of inhibition area relative to plate surface:

It was noticed that the percentage of inhibition area/plate was higher in the resistant entry of faba bean ILB 938 (being 20.67%) than the moderately susceptible cultivar Giza 3, being 10.11% (Table 2).

Antagonistic effect of the epiphytic bacteria to *B. fabae* on the detached leaflets of faba bean:

In this experiment, four different isolates of the highly antagonistic bacteria were tested with a virulent isolate of *B. fabae* on the detached leaves of faba bean cv. Giza 3.

Data in Table (3) show that size of the spots, as a result of the tested antagonistic bacteria, were less than the control after 2 and 4 days of incubation, what ever the bacterium isolate and the concentration used. However, diameter of the spots gradually increased with increasing the incubation period to 6 days when they became larger than the control treatment.

Table 2. Antagonistic effect of the tested fungi against the growth of *B. fabae* virulent isolate.

Tested plants	% inhibition area/plate
ILB 938	20.67*
Giza 3	10.11

* Figure represents average of five plates.

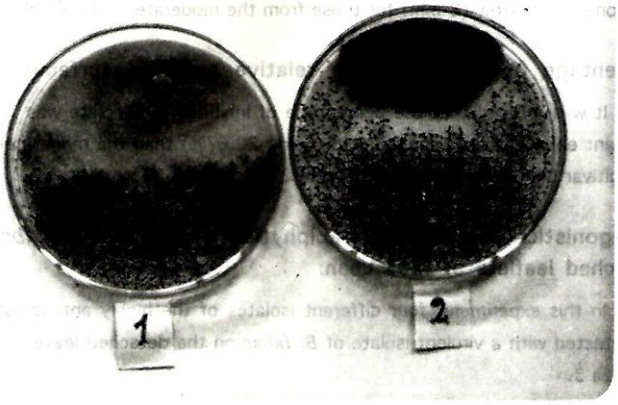

Fig. 1. The simple antagonistic phenomenon for the isolated fungi from the resistant entry of faba bean.
Note: Plate No. 1 contains *Aspergillus flavus*, and
Plate No. 2 contains *Cladosporium* sp.

Fig. 2. The highly antagonistic phenomenon for the most frequent bacteria from the resistant entry of faba bean.
Note: Plates No. 1 & 3 show different degrees of antagonism, while Plate No. 2 indicates a simple degree of the antagonism, whereas Plate No. 4 shows more antagonism with the tested isolate of *B. fabae*.

Table 3. Leaf spot diameter (mm) produced by *B. fabae* on detached leaflets in presence of epiphytic bacteria.

Incubation period	Control *B. fabae* (mm)	Treatments							
		*B.F. + **isolate 1		B.F. + isolate 2		B.F. + isolate 3		B.F. + isolate 4	
		10^8	10^6	10^8	10^6	10^8	10^6	10^8	10^6
2 days	6	2.8	2.8	2.1	2.8	2.2	2.6	2.2	2.6
4 days	20	19.0	17.0	15.2	14.4	17.4	14.6	14.8	14.2
6 days	36.3	38.0	40.0	40.0	40.0	39.0	40.0	40.0	40.0

* B.F. = *B. fabae* (2.5×10^5 spore suspension).

** initial concentration = 10^9 cells/ml.

DISCUSSION

Microorganisms in the environment around the plant may lodge on the foliage of these plants. Some of these microorganisms may play a role in the mechanism of resistance of these plants to foliar diseases. Previous studies indicated the presence of a strong inhibitory phyllosphere effect on *B. fabae* before penetration into leaf tissue (Hanounik and Hasanain, 1986 and Omar *et al.* 1989).

Results obtained *in vitro* showed that the number of microflora was greater in the phylloplane of the moderately susceptible faba bean cultivar (Giza 3) than the resistant one (ILB 938). This indicates that the foliage of susceptible cultivar acts as a suitable court for air microflora probably due to its chemical composition. Stout (1960) stated that the distribution of microorganisms mainly depend upon the microorganism itself, host plant and to some extent leaf age. Also, data proved that 22% of the isolated bacteria from ILB 938 line (resistant) were antagonistic, while 6% of the bacteria from the susceptible were antagonistic. Such bacteria may create ecosystems with different degrees of suitability for the pathogen.

In vitro studies showed a simple antagonistic effect between isolate of *B. fabae* and fungi isolated from the leaf surface of ILB 938 entry. In the meantime, there was a strong effect of antagonism with respect to the isolated bacteria. Using the isolated fungi, the percentage of inhibition area/plate for growth of *B. fabae* was 20.67% with fungi from ILB 938 compared with those from Giza 3 (being 10.11%). this may explain the role of antagonism in reducing disease incidence in the resistant entry. Generally, it was noticed that the inhibition zone between the antagonistic bacteria and the tested isolate of *B. fabae* was wider in the resistant line (ILB 938) compared with that obtained with those obtained from the moderately susceptible one (Giza 3). This conforms with results of Omar *et al.* (1989) on the *in vitro* relationship between *B. fabae* and phylloplane microorganisms.

Further studies *in vivo* using four different isolates of highly antagonistic bacteria against an isolate of *B. fabae* on the detached leaves of Giza 3 of faba bean showed that after 2 and 4 days of incubation the size of the resulting spots was limited and less than the control treatment. However, after 6 days incubation (the end period of the experiment), the size of the spots increased more than the control. This indicates that the inhibitory effect is not persistent with the same efficacy or otherwise the pathogen growth overcome the activity of the antagonist under the experimental conditions. Also, it may indicate that the operating inhibitory agent is not always the same as that operating on leaf surface. Other factors such as the inoculum potential of the antagonistic bacteria, the nature of interaction of antagonist with others and/or the variability within the tested isolates of the pathogen can also play a role in such situations. Last and Deighton (1965) and Fokkema *et al.* (1979) found no evidence for the acceleration of leaf senescence by phyllosphere fungi in wheat and hence no objection to their being used as biological control agents.

REFERENCES

1. Barakat, F.M., K.A. Sabet and Wadiaa F. Habib. 1986. Varietal reaction of faba bean and lentil to specific strains of *Fusarium solani* in relation to antagonistic rhizosphere microflora. Agric. Res. Rev., Cairo. 64: 245-256.
2. Cooper, W.E. and S.J. Chilton. 1950. Studies on antibiotic soil microorganisms 1. Actinomycetes antibiotic to *Pythium arrhenomanes* in sugar-cane soils of Louisiana. Phytopathology, 40: 544-552.

3. Fokkema, N.J., P. Kastelein and J. post. 1979. No evidence for acceleration of leaf senescence by phyllosphere saprophytes of wheat. Trans. Br. Mycol. Soc., 72: 312-315.

4. Habib, Wadiaa F. 1990. Studies on leaf spots of faba bean. Ph.D. thesis, Fac. Agric., Cairo University 128 pp.

5. Hanounik, S. and A.M. Hasanain. 1986. Inhibition of *Botrytis fabae* in the phyllosphere of *Vicia faba* leaves. Fabis Newsletter, 16: 41-44.

6. Khalil, S.A., A.M. Nassib, H.A. Mohamed and Wadiaa F. Habib. 1984. Identification of some sources of resistance for chocolate spot and rust in Faba bean. In G.P. Chapman and S.A. Tarawall (eds) System for cytogenic analysis in *Vicia faba* L. p. 80-94.

7. Kiraly, Z. 1974. Methods in plant pathology with special reference to breeding for disease resistance. Elsevier Scientific Publishing Company, Inc., New York, 509 pp.

8. Last, F.T. and P.C. Deighton. 1965. The non-parasitic microflora on the surfaces of living leaves. Trans. Br. Mycol. Soc., 48: 83-99.

9. Mansfield, J.W. and B.J. Deverall. 1974. The rates of fungal development and lesion formation in leaves of *Vicia faba* during infection by *Botrytis cinerae* and *Botrytis fabae*. Ann. Appl. Biol., 76: 77-89.

10. Mohamed, H.A. 1982. Major disease problems of faba beans in Egypt. In G. Hawtin and C. Webb (eds.) Faba Bean Improvement. Netherland, p. 213-225.

11. Mohamed, H.A., M.A. Kararah, Olfat Mousa and Wadiaa F. Habib. 1987. Virulence of *Botrytis fabae* on faba beans. 5th Cong. Phytopathol. Soc., 1: 27-44. Egypt.

12. Omar, S.A.M., Dorreiah E. Salem and S.M.M. El-Gantiry. 1989. Methods for estimating microorganisms population in faba bean phylloplane. Fabis Newsletter, 24: 16-20.

13. Stout, J.D. 1960. Bacteria of soil and pasture leaves at Claudelands showground. N.Z.J. Agric. Res., 3: 413-430.

التأثير التضادى لبعض الكائنات الدقيقة على أوراق الفول البلدى

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أوضحت نتائج تأثير الكائنات الدقيقة السطحية لأوراق الفول البلدى ان أعداد تلك الكائنات كانت أكثر بكثير على الصنف متوسط القابلية للإصابة جيزة ٣ عن الصنف المقاوم ILB 938، وقد لوحظ أن ٢٢٪ من البكتريا المعزولة من الصنف المقاوم كانت مضادة فى حين كان هناك فقط ٩٪ منها عزل من الصنف متوسط القابلية للاصابة. وقد لوحظ أن عرض منطقة التثبيط بين البكتريا المضادة وعزلة الفطر بوتريتس فابى كانت كبيرة بالنسبة للبكتريا التى عزلت من الصنف المقاوم فى حين أن هذه المنطقة كانت أقل عرضا بالنسبة لبكتريا الصنف المتوسط القابلية للإصابة.

لوحظ أن النسبة المئوية للتثبيط مقارنة بمساحة الطبق كانت كبيرة للفطريات المعزولة من الصنف المقاوم إذا ما قورنت بمثيلتها من الصنف القابل للإصابة حيث كانت النسب المتحصل عليها هى ٢٠,٦٧ ، ١٠,١١٪ على التوالى.

وقد وجد أن مساحة البقع الناتجة بواسطة الفطر بوتريتس فابى تقلصت نتيجة لتأثير بعض أنواع من البكتريا المختبرة المضادة عن معاملة المقارنة بعد ٢ ، ٤ أيام من التحضين فى حين أنه بعد ٦ أيام إنعكس الوضع و إزدادت البقع فى حجمها إذا ما قورنت بالمقارنة.