STUDIES OF NEW METHODS FOR CONTROLLING CHOCOLATE SPOT DISEASE OF FABA BEAN IN EGYPT

By

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B.Sc. Agriculture Science, 1996 (Plant Pathology)
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ARABIC SUMMARY

INTRODUCTION

INTRODUCTION

Faba bean (*Vicia fabae* L.) is consider the most important legume crop in Egypt while it is consider the fifth food legume in the world after dry bean, dry pea, chickpea and lentil (**Adak, *et al.* 1998**). In Egypt, it is grown mainly for human food consumption as green pods or dried seeds meanwhile, it is planted in some other parts of the world essentially as animal feed. Seeds of faba bean are rich in protein (28%), carbohydrates (56%) and some other compounds thus, it is a rich available source of food for both human and animals (**Tewati and Virk, 1996**).

The total cultivated area of faba bean in Egypt during season 2004 was 325000 feddan yielded 2.645.500 ardab (ardab = 155 Kg) at rate of 8.14 ardab/feddan. (**Annual Reports of Agricultural Statistics Department, Ministry of Agriculture, ARE, 2003**).

Faba bean is liable to be attacked by many foliar diseases as chocolate spot (*Botrytis fabae* Sard. and *B. cinerea*), rust (*Uromyces fabae*), Ascochyta blight (*Ascochyta fabae*), leaf spots (*Cercospora zonata* and *Alternaria alternata)*, downy mildew (*Peronospora viciae*) and root–rot as well as viral diseases which are responsible to cause considerable losses in the yield and its components.

Foliar diseases are the most common diseases especially in Delta region due to the high humidity, rain fall and favourable temperature which are prevailing during the season. Therefore, chocolate spot disease of faba bean caused by *Botrytis fabae* and

B. cinerea is considered the most important disease in Egypt, which caused serious damage to the crop where the yield losses was more than 50% of the crop according to **(Hussein, 1963 and Mohamed, 1982)**. In the last ten years two severe epidemics of chocolate spot disease were recorded during 1987/88 and 1990/91 growing seasons in Egypt, where the yield was reduced by 50% in both seasons **(Nassib *et al.* 1991).** In low infected years, the yield losses was ranged between 5-15% **(Mansour and Amer 1976)**.

This work aimed to survey the chocolate spot disease at eight Governorates to isolate and identify chocolate spot pathogens and to test the pathological abilities of chocolate spot pathogens. The bossibility of using RAPD-PCR technique as a new method for differentiating between Botrytis isolates was also done. Studying some factors affecting growth, sporulation and sclerotial formation of *Botrytis fabae* and *B. cinerea*, the causal agents of chocolate spot disease such as type of culture media and temperature. Also, factors affecting chocolate spot disease severity like inoculum potential, spore age, aging of plant growth stage and varietal reaction. Also, studying the biochemical changes in plants infected with *Botrytis fabae*. This study also included isolation and identification of faba bean phylloplane microorganisms in order to select some natural antagonists to control chocolate spot disease as biological agents. In addition, chemical control by using different fungicides, cross protection and agricultural practices such as sowing date, varietal reaction and N-P- fertilization was investigated.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Faba bean (*Vicia fabae* L.) is one of the most important field crops in the world. It is susceptible for infection with different leaf spot pathogens which, cause considerable losses to the cultivated plants. Chocolate spot caused by *Botrytis fabae* Sard. is the most important disease attacking faba bean plants and causing a great damage. This disease has been recorded in many countries like Burma, Spain, Japan, Cyprus, England, Morocco, Egypt, Palestine, South Africa, Bermuda, China, Argentine, Italy, Turkey, Australia, USSR and Norway (**Rhind, 1927, Sardina, 1929, Wilson, 1937, Berger, 1937, El-Helaly, 1938, Chorine, 1939, Gorter, 1941, Waterstone, 1942, Yu, 1945, Jouch, 1947, Ciferri, 1949, Karel, 1952, Geard, 1960, Money, 1961, Bikmuhametova, 1963, and Sundheim, 1973**).

The causal organisms:

El-Helaly, (1936) reported that *Botrytis fabae* was the principal causal pathogen for chocolate spot disease on *Vicia faba* in Egypt. It caused severe damages in the northern localities because of the high atmospheric relative humidity, which favour the infection process.

Hegazy, (1968) isolated five isolates of *Botrytis fabae* differing in their infection to faba bean plants; the most virulent isolates were isolated from Ismalia and the weak isolate from Sakha.

Hutson and Mansfield (1980) compared different isolates of *B. fabae* and *B. cinerea* and they found that these isolates

differed in their pathogenicity and therefore both may be heterokaryotic or heteroplasmic for virulence factors.

Mohamed *et al.*, (1981) studied the range of variability within isolates of *B. fabae.* Five isolates were tested from different area, Sakha, Nubaria, Ismalia, Gemmeiza and Alexandria, on different cultivars of faba bean. Nubaria isolate was the most virulent compared with all tested isolates.

Abed El-Latif (1984) survey leaf spot in three governorates, Dakahlia, Kafr El- Sheikh and Sharkia and showed that faba bean plants are attacked by *B. fabae* and *Alternaria alternata* which caused leaf spots in varying degrees of severity. Sixteen isolates of *Botrytis fabae* were obtained during the survey and were almost similar in their virulence.

Mahmoud (1985) showed that the predominant leaf spot during the survey in the northern parts of the Delta was the chocolate leaf spot caused by *Botrytis fabae.*

Harrison (1988) reported that both *Botrytis fabae* and *B. cinerea* can causes chocolate spot disease on fabae bean in the field, but *Botrytis fabae* was the more important pathogen because it is more aggressive than *Botrytis cinerea.*

Habib, Wadiaa (1990) found that isolation trials from diseased faba bean plant collected from surveyed Governorates yielded twenty- eight fungal isolates. The most frequent fungi were *B. fabae* followed by *B. cinerea, Alternaria alternata* and *Helminthosporium* spp. Also, the pathogenicity tests proved that all isolates of *B. fabae* and *B. cinerea* were the most pathogenic isolates.

Heweidy (1993) found that *B. fabae* isolates scored the highest frequency than *B. cinerea.* Also, the pathogenic capabilities of twenty four isolates of *Botrytis* spp. were more varied on 3 cultivars of faba bean, and isolates of *B. fabae* were more virulent than *B. cinerea* isolates.

Morsy (1993) reported that isolation trials from diseased faba bean plants showing chocolate spot symptoms collected from the surveyed Governorates, i.e. Dakahlia, Sharkia, Kafr El-Sheikh, Qalubiya, Menuofia, Beheira and Giza yielded *Botrytis fabae* and *B. cinerea. B. fabae* scored the highest frequency in all Governorates than *B. cinerea,* which was isolated with low frequency. Also, the pathogenicity tests proved that all isolates of *B. fabae* and *B. cinerea* were able to infect faba bean plants and showed typical chocolate spot symptoms.

Abou-Zeid *et al* (1998a) reported that 13 isolates of *Botrytis* spp. from Egypt showed differences in their virulence on different faba bean cultivars.

Akem and Bellar (1999) found that during the 1995-96 cropping season, a quantitative disease survey was conducted in the main faba bean growing regions of Syria, the most important and widespread fungal diseases observed at all locations were chocolate spot (*Botrytis fabae* and *B. cinerea*) and rust (*Uromyces fabae*).

Kuti and Nawer (1999) carried out pathogenicity studies on five isolates of chocolate spot fungi (3 isolates of *B. cinerea* and 2 isolates of *B. fabae),* one isolate of *B. cinerea* originally isolated from faba bean was more pathogenic to faba bean than

the two *B. fabae* isolates. The other two isolates of *B. cinerea* isolated from grapes and eggplant respectively were moderately pathogenic to faba bean.

Wang, (2000) reported that nine leaf diseases and their causative pathogens were identified. *Botrytis fabae and B. cinerea* were the main prevailing pathogens.

Abou- Baker, (2002) isolated two isolates of *B. fabae* and four isolates of *B. cinerea* from faba bean leaves infected with chocolate spot at different growing areas in Egypt. *B. fabae* isolates were the most destructive and virulent.

El-Afifi (2003) found that *Botrytis fabae* was the most frequent than *B. cinerea* within the isolated causal organisms of chocolate spot disease collected from different Governorates. *B. fabae* isolated from EI-Beheira gave the highest disease severity (52.0%).

Characterization of *Botrytis* spp using RAPD-PCR technique:

Abou-Zeid *et al.* (2002d) found different groups of *Botrytis fabae* and *B. cinerea* isolates using RAPD-PCR method for characterization.

Stefania *et al* (2002) used random amplified plolymorphic DNA (RAPD) assays for the identification of 34 fungal strains isolated from strawberry and other host plants, in order to detect polymorphism to consequently identify and isolate molecular markers specific to *Botrytis cinerea*. Among the 10-20 primers tested, one primer mainly amplified a 750-bp cloned with all the

B. cinerea strains and absent in the other species and genera examined.

Factors affecting growth, sporulation and sclerotial formation of *Botrytis* spp.:

Culture media:

El-Neshwy, Saniya (1981) mentioned that *Botrytis fabae* grew and sporulated well on the leaf extract of faba bean agar while no sclerotia were formed. Potato dextrose agar gave satisfactory amount of growth and sclerotial formation. A relatively moderate growth and number of sclerotia were obtained on Richard's and Czapeck's media. Culturing *Botrytis fabae* on Brown's medium resulted in the least amount of fungal growth and few numbers of sclerotia. PDA was the best favourable medium for sclerotial formation.

Gorfu (1986) found that when three isolates of *B. fabae* were grown on faba bean dextrose agar medium, only two of them produced >50 spores/cm.

Hassanein *et al.*, (1990) studied the effect of three solid media i.e. potato dextrose agar (PDA), faba bean leaf agar (FBLA) and faba bean seed agar (FBSA) on twenty five isolates of *Botrytis* spp. incubated at 20°C. They found that FBSA medium was the best among the three media for growth of the different isolates.

Mansour, (1992) found that *Botrytis fabae* gave its best rate of growth on leaf extract of faba bean agar medium, while the lowest rate of growth was on Brown's medium.

Abou-Zeid and Saieda S. Abdel-Rahman (1995) mentioned that forty isolates of *Botrytis* spp collected from different locations were grown on potato dextrose agar (PDA) and faba bean leaf agar (FBLA) media. PDA medium was the best for growth of *Botrytis* spp isolates. In general, isolates of the fabae type were the least in growth and sporulation, while those of the cinerea type were the fastest in growth and the highest in spore production. On the other hand, isolates of fabae type were highest in sclerotial number formed on PDA medium. However, some isolates did not produce any sclerotia, which appeared in cinerea type. Most of the fabae isolates produced small size sclerotia (1-1.5 mm) while isolates of cinerea type produced large sclerotia (2.5-3 mm).

Mahmoud, Nagwa (1996) found that PDA medium was the best for growth of *Botrytis* spp. The best media for sporulation of *Botrytis* spp was faba bean sucrose solid agar, (FSSA) medium. The highest in spore production were *B. cinerea,* while the least in sporulation were the *B. fabae* isolates. Also, isolates of *B. fabae* were the heighest in sclerotial number. Higher number of sclerotia was produced on PDA followed by banana medium.

Abou-Zeid *et al.*, (1998b) found that 40 isolates of *Botrytis* spp from Egypt showed differences in growth rate, spore production number and size of sclerotia on potato dextrose agar (PDA) and faba bean leaf agar (FBLA) media.

Temperature:

Jarvis, (1977) found that the optimum temperature for sporulation of *Botrytis cinerea* on agar was 15°C. Conidia were produced very slowly at 24°C and 10°C.

Harrison (1981) mentioned that the mean optimum temperatures for sporulation of the Scottish and English isolates of *Botrytis fabae* were 17.2 °C and 17.4 °C, respectively.

Harrison (1984) showed that even after 8 days of growth on agar, conidia of *Botrytis fabae* were absent at temperatures above 24.5°C. In contrast, *B. fabae* sporulated on infected leaves in a wider range of temperature. He added that the most spores were produced at 20°C but they were also abundant at 10°C, 15°C and 25°C. A few conidia were produced even at 5°C and 30°C within 8 days from inoculation.

Abou-Zeid *et al.*, (1990) studied the behavior of different isolates of *Botrytis fabae* isolated from non-sprayed and sprayed plots with different fungicides. They found that isolates from treated plots were more affected by change in temperature. Isolate, from non-sprayed plants, grew better at the three temperatures tested i.e. 15°C, 20°C and 30°C. However, the most favorable temperature for growth of all tested isolates was 15°C.

Hassanein *et al.*, (1990) found that the optimum temperature degrees for the growth of twenty five isolates of *Botrytis* spp were between 10°C and 20°C when grown on faba bean leaf agar (FBLA) medium. They added that most isolates covered the Petri dishes within 6 days at 10°C and 20°C. At 25°C, only nine isolates gave the highest amount of growth as they exceeded 8-cm growth within 6 days.

Mahmoud, Nagwa (1996) found that the optimum temperature for mycelial growth and sporulation was 20°C for *Botrytis fabae* and *B. cinerea.*

Factors affecting chocolate spot disease severity:

a- Inoculum potential:

Mansfield and Hutson (1980) attributed the rapid growth of infecting hyphae produced by *B. fabae* on bean leaves to the large number of conidia inoculated. Reducing the inoculum concentration greatly reduced the growth rate, and often led to death of infecting hyphae possibly because low levels of inoculum resulted in few infection sites, which were not sufficient to kill large numbers of bean cells which would be required to suppress active host resistance.

Rossall *et al.*, (1980) reported that at inoculum conc. (20-200 conidia/droplet) virtually all infection hyphae produced by *B. cinerea* died within 3 days after inoculation, whereas those of *B. fabae* were killed only at the lower conc., and even with only 10 conidia/droplet, there was 50% mortality.

Abou-Zeid and Le-Normand (1981) mentioned that the best inoculum suspension of *B. fabae* for artificial inoculation was $250x10^3$ spores/ml.

Hanounik and Hawtin (1981) found that an increase in the inoculum density of *B. fabae* to be $5x10^5$ spores/ml was associated with a corresponding increase in disease severity.

Creighton *et al.*, (1986) showed that the rate of lesion growth has increased with increasing inoculum dose and leaf age.

Abou-Zeid and Mohamed (1987) demonstrated that the optimum concentration for inoculation of faba bean differed according to *B. fabae* isolate, the faba bean cultivar and the

inoculation method used. They found that the reaction of the detached leaflets from different nodes of Giza-3 and Rabaya-40 faba bean plants to *B. fabae* isolated from Nubaria significantly differed.

Habib, Wadiaa (1990) indicated that the highest percentage of infection was produced with highest concentration of conidia (250.000 spore/ml).

Mahmoud, Nagwa (1996) showed that high inoculum of *Botrytis cinerea* ($15x10^4$ conida/ml) may result in the same development of symptoms as the normal inoculum of *Botrytis fabae.* However, development continued for *Botrytis fabae* isolates but not for *Botrytis cinerea* under the prevailing conditions of the test.

b- Spore age:

Harrison, (1983) obtained spreading lesions after inoculation of leaves with *B. cinerea* conidia from young cultures (6 day-old), but spreading lesion was only produced rarely or not at all when older spores were used.

C- Plant age:

Hanounik, (1980) mentioned that susceptibility of faba bean to *Botrytis fabae* and *Ascochyta fabae* increased with plant age from 2 to 7 weeks.

El-Neshwy, Saniya (1981) noted that the highest level of disease severity was observed on leaves of Giza-1 plants, 60 days old, where inoculated with the concentration of 1000 spore/ml.

Khalil *et al.*, (1984) tested 10 genotypes of faba bean to infection with *B. fabae*, in the greenhouse, using either 40 or 80-day-old plants and detached leaves from the same plants, ILB 938 showed least infection and lowest rate of disease development. While, 249/802/80, Seville Giant and 785-49456 showed significantly less infection than Giza-3 when inoculated at 80 days.

Creighton *et al.*, (1986) found that the covered leaf area with *B. fabae* conidiophores was less on 5-day-old than on 17- or 30-day-old leaves. They noted also that there was no interaction between the effects of leaf age and inoculum dose on either lesion growth or sporulation.

Jacqueine and Harrison (1989) found that the oldest leaves developed more lesions than youngest ones on field bean plants inoculated with conidia of *B. fabae* immediately after detaching from stems. On intact plants, the established lesions on young leaves increased in size at only half the rate of those on old ones.

Habib, Wadiaa (1990) found that five week old plants of the tested entries were more susceptible whereas 3 week-old ones, generally, were least infected, other growth stages (7-13 week old) were intermediate in their reaction.

d- Varietal reaction

El-Neshwy, Saniya (1981) stated that Giza-1 and Rebaya-40 faba bean varieties were susceptible to *Botrytis fabae.* However, Giza-1 was more susceptible than Rebaya-40.

Khalil and Harrison (1981) found that the cultivars, Minica and Blaze were markedly resistant to *B.fabae.* All

cultivars were more susceptible at early stages of growth than at flowering and fruiting ones.

Jellis *et al.* (1982) noted that the entry ILB 938 among the tested cvs had superior resistance to that of cv. Maris Beed.

Harrison (1986) noticed that a higher proportion of lesions on old leaves bore conidia than on young leaves, a week after inoculation with *B. fabae*, but leaf age had no effect on number of conidia/mm^2 of lesion area.

Saxena and Stewart (1983) observed that lines ILB 938, RC 39/80 EBWC/787/80 a, BpL 261 and BPL 266, were resistant to leaf spots, caused by *B. faba.*

Mohamed *et al.* (1986) found that Giza-3 variety was susceptible to *Botrytis fabae*, while ILB-938 was resistant.

Habib, Wadiaa (1990) showed differences between eleven isolates of *B. fabae* and their virulence on three faba bean entries. The results showed that ILB-938 was resistant in its reaction, while cv Giza-3 was moderately susceptible and Rebaya-40 was highly susceptible.

Mansour (1992) mentioned that faba cultivars differed in their reaction to *B. fabae.* The tested cultivars were as follows: Giza-2 was moderately susceptible, while, Giza-402 and Equadolce were susceptible and Giza 3 was moderately resistant.

EI-Refai *et al.* (1992) tested 12 local and introduced faba bean cultivars to infection with *B. fabae.* They found that the cultivars MB-39, ILB-4 and 78-S49694 were highly resistant, while Giza-402, Rebaya-40 and Turkish local were highly susceptible.

Mahmoud, Nagwa (1996) reported that faba bean Giza-461 was moderately resistant to infection with *B. fabae*, while Giza-3 was susceptible and Giza-402 was highly susceptible.

Abou-Zeid *et al.* (1998a) found that faba bean cultivars differed in their reaction to *B. fabae* when thirty-five faba bean genotypes from ICARDA were tested for resistance to chocolate spot disease.

El-Afifi, (2003) noticed a significant differences between five tested faba bean cultivars, Giza-3, Giza-402, Giza-461, Giza-674 and Giza-716 against natural infection with chocolate spot. The cultivars Giza-716 and Giza-674 followed by Giza-461 were the most resistant, while cvs. Giza-402 and Giza-3 were highly susceptible.

Phylloplane microorganisms:

Rossall and Mansfield (1981) found that higher levels of nutrients in the droplets apparently allowed *B. cinerea* to overcome the potentially inhibitory effects of both epiphytic-microflora and a biotic factor active on the leaf surface. The author's noticed that symptom less sites and germination of *B. cinerea* was restricted by the combined effects of epiphytic microflora and inhibitors associated with leaf surface wax.

Hanounik and Hassanein (1986) reported that the washing water of faba bean leaflets of the resistant faba bean lines BPL-1179 and 710 significantly suppressed spore germination and germ tube elongation of *B. fabae* compared with those from leaflets of the susceptible line R-40

Omar *et al.* (1987) noted that some of the isolated microorganisms of the phylloplane had an antagonistic effect against *Botrytis fabae*.

Singh *et al.* (1987) mentioned that isolation trials from the diseased and non-diseased leaf surfaces of peas revealed 23 fungi, of which 15 were deteromycetes, 3 were ascomycetes, 2 were phycomycetes, 2 were sterile mycelia and 1 was Actinomycetes. The dominant fungi on diseased leaves were *Cladosporium* sp. and *Alternaria* sp. whereas; *Cladosporium* sp. *Alternaria* sp., *Mucor* sp. and *Penicillium* sp. were dominant on healthy leaves.

Abd El-Moiety *et al.* (1990) found that spraying susceptible variety of faba bean with suspension of *Erwinia herbicola* or *Bacillus subtilis* isolated from the phylloplane of the resistant variety, lead to significant reduction in disease incidence caused by *Botrytis fabae.*

Habib, Wadiaa (1990) reported that the number of microorganisms in the phylloplane of the moderately susceptible cultivar (Giza-3) of faba bean were greater than those in the resistant entry (ILB-938). She also found that the size of the spots caused by *B. fabae* 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 spot was larger after 6 days compared with the control.

Biological control:

Jailloux and Froidefond (1987) found that 7 strains belonging to *Trichoderma hamatum, T. harzianum, T. koningii, T. ngibrocchiatum* and *T. viride* were highly antagonistic against *Botrytis cinerea* growth.

Roulston and Lane (1988) noticed that *Trichoderma viride* inhibited growth of *Botrytis allii, Botrytis cinerea* and *B. fabae* in dual cultures on malt extract agar

Simay (1998) recorded *in vitro* the hyperparasitism of *Gliocladium catenulatum* on *Botrytis cinerea* where the mycelia and conidiophores of *B. cinerea* were parasitized and sclerotia were killed by *G. catenulatum.*

Abd EI-Moity *et al.* (1990) found a significant reduction in chocolate spot disease incidence when a suspension of *Erwinia herbicola* or *Bacillus subtilis* was sprayed on faba bean susceptible cultivars.

Habib, Wadiaa (1990) observed that the inhibition zone was wide between the antagonistic bacteria and *Botrytis fabae* isolated from the resistant entry (ILB-938), while it was narrow for the moderately cultivar (Giza-3).

Vandemark (1995) reported that both *Bacillus subtilis* and *Pseudomonas corrugata* had the ability to inhibit the growth of vegetative mycelia of *Botrytis cinerea. Bacillus subtilis* inhibited the growth of *B. cinerea* by 71.3% and 69.6% on potato dextrose agar (PDA) and tryptic soy agar (TSA) respectively, while *P. corrugata* inhibited the growth on PDA and TSA by 66.3% and 40.8%. Both the two isolates inhibited the germination of *B. cinerea* conidia. Greenhouse tests showed the ability of the two isolates to control the gray mold disease of strawberry.

Zimand *et al.* (1996) mentioned that using *Trichoderma harzianum* (T-39) as a biocontrol agent reduced germination and germ-tube elongation of conidia of *Botrytis cinerea* on bean

leaves. A reduction of 20 to 50% in germ-tube was observed 20 hr. after inoculation. Field experiments showed that Trichoderma spores were able to reduce disease on bean leaves.

Andrew *et al.* (1997) showed the antagonistic potentialities of *Penicillium brevicompactum* (MX1-F62, L32, and L 34) and *Cladosporium dadospor todies* (MB2.F45) against *Botrytis fabae in vitro* and *in vivo*. They found significant reductions in the radial growth of *B. fabae in vitro* as well as discoloration of the pathogen mycelium.

Sharga (1997) suggested that biological control of chocolate spot disease with application of antagonistic bacteria to *Botrytis* spp. may provide a useful alternative to chemicals. Only 14 bacterial strains were able to prevent chocolate spot symptoms from developing *in vivo* and *in vitro.*

Yu and Sutton (1997) found that *Gliocladium roseum* strongly suppressed germination and germ tube growth of *B. cinerea* on faba bean leaf surface. While on faba bean stems, the antagonist moderately suppressed germ tube growth and intensely parasitized the pathogen

Abou-Zeid (2000) isolated 16 isolates of *Trichoderma* spp. from phylloplane of faba bean. *T. harzianum* (T-2), *T. viridi* (T-73) and *T. album* (T-3&T-16) were antagonistic against *Botrytis fabae* on potato dextrose agar medium and reduced disease severity on infected leaves.

Abou-Zeid and Hassanein, (2000) reported that some isolates of *Bacillus* spp. isolated from phylloplane had an antagonistic effect against *B. fabae* on PDA medium. *In vivo,* the

isolates No. 1, 2, 3 and 4 were more effective than others, till the end of experiment, while the isolates No. 5, 6 and 7 were less effective after 7 days from incubation.

El- Shazly (2001) reported that *Trichoderma viride* treatment gave the best control under field conditions to faba bean leaf spot disease with no significant differences with fungicides treatment.

Abou-Zeid *et al.* (2002b) mentioned that treating susceptible faba bean entry with *Bacillus megatherium* decreased severity of chocolate spot disease to the extent that it appeared to be similar to that of the untreated resistant entry.

El-Afifi (2003) indicated that *Trichoderma* spp were the most effective than *Gliocladium* spp in controlling *B. fabae*. While *Bacillus* isolates showed the highest value of relative power than the isolate of *Pseudomonas fluorescens*.

Cross protection by inducing resistance using un-viable *B. fabae* spores:

Abou Zeid and Le Normand (1979) stated that pre-inoculation of faba bean leaves with non-viable heated *B. fabae* spore suspension exhibited a reduction in chocolate spot disease severity.

Abou-Zeid *et al.* (1996) used the un-viable heated spores (UHS) of *Botrytis fabae* for controlling chocolate spot disease on 4 faba bean cvs (Giza-402, Giza-3 , Giza-461 and Giza 716) compared with four fungicides (Dithane M-45, Nemospore, Trimiltox fort and Redomil). Results revealed that Dithane M-45

was more effective than un-viable heated spores. However, spraying with un-viable heated spores reduced disease severity when compared with control.

Abou-Zeid (2002a) showed that faba bean cvs differed in their reaction to *Botrytis fabae* when different means of control used under greenhouse conditions. Faba bean cultivar G-717 showed best response with heated spores of *B. fabae* and Giza-blanka when using plant extract compared with control.

El-Afifi (2003) found that, during 1998/99 growing seasons Dithane M-45 was more effective than the non-viable heated spores. However, spraying with the non-viable heated spores reduced the disease severity when compared with the control treatment after 45 days from application. No effect of the non-viable heated spores after 60 days from application.

Chemical control:

Elliott and Whittington (1980) showed that treating faba bean piants with Benlate (benomyl), Bavistin (carbendazim) or Cercobin reduced rate of leaf loss caused by *B. fabae.*

Mansour (1980) revealed that 4 sprays of Plantvax at 250g, Saprol at 75g, Daconil at 200g and Dithiane M-45 at 250g/100L water controlled leaf spots caused by *B. cinerea, A. tenuis* and *Stemphylium botryosum* and increased the yield.

Abd El-Monem (1981) reported that Dithane M-45, Copper Antracol, Maneb and Zeineb effectively limited infection by *B. fabae.*

Hanounik (1981) found that Ronilan (50% WP vinclozolin) at 2g/L water reduced yield losses from 67.49 % (untreated) to 9.5% in field trials under inoculation of faba bean with *B. fabae.*

Nassib (1983) found that application of Dithane M-45 four times starting from mid-January in two weeks intervals protected faba bean plants against infection with chocolate spot and rust diseases.

Abd El-Latif (1984) showed that Dithane-M45 was the best in controlling chocolate leaf spot more than Trimiltox forte and Plantvax and there was a positive correlation between disease severity and yield.

Creighton *et al.* (1985) revealed that the greatest yields were obtained from faba beans in plots sprayed with benomyl in late May, at the mid-flowering stage of crop growth.

Abou Zeid *et al.* (1990) tested some fungicides against the chocolate spot disease and found that all the used fungicides unless Bavistin gave good results. Rovral, followed by Dithane-M45, Cuprozan Super-311 and Mancozan were the best in this respect.

Omar *et al.* (1990) stated that Dithane-M45, followed by Rovral, Cuprozan and Ridomil were the best fungicides in controlling chocolate spot disease of faba bean.

Zaglol (1991) reported that Dithane-M45 was more effective than Benlate 50 in controlling faba bean leaf spot disease. He also added that Dithane-M 45 improved the yield of plants.

El-Gantiry *et al.* (1991) tested seven different fungicides to control chocolate spot disease and found that Dithane-M45, Mical-M and Tri-Meltox forte were the more successful ones.

Giltrap (1991) found that chocolate spot (caused by *Botrytis fabae* and *B. cinerea*) was severe during 1984-1987 in wet weather conditions. The fungicide spraying with single or combination treatments of benomyl, chlorothalonil, carbendazim, vinclozolin and iprodione significantly increased yield in each of these years.

Mansour (1992) found that the growth of *B. fabae* was completely inhibited when Bavistin-50 and Topsin-M70 were added to PDA medium at the rate of 10 ppm of their active ingredients, while as the fungicide Dithane-M45 inhibited mycelial growth at 50 ppm. Under greenhouse conditions, all the tested fungicides were effective in reducing the severity of leaf spot infection with *B. fabae*. In field experiments, all tested fungicides decreased significantly infection with leaf spot disease than untreated plants. Dithane-M45 at the rate of 2.5% was the best of all in case of Giza-402 in the first season only and for Giza-3 in the second season. The fungicides (Dithane-M45, Bavistin-50 and Topsin-M70) also raised the yield of plants and the weight of 100 seeds.

Khalil *el al.* (1993) showed that chemical control by applying Dithane-M45 decreased the chocolate spot infection to 54.0% compared with control. The yield increased by 92% more than yield of non-treated plants. Chemical control of chocolate spot diseases by applying the fungicide Dithane-M45 markedly increased yield and its components. Also, it increased plant

height, numbers of pods and seeds per plant, 100-seed weight and seed yield per plant.

Hegab and Beshir (1994) showed that the fungicides i.e., Dithane-M45, Kocide, Tri-mltox forte and Topsin-M70 decreased infection percentage of chocolate spot disease.

Khaled *et al.* (1995) mentioned that applying the fungicides i.e., Saprol, Byleton, Ridomil plus, Benlate, Vitavax/Thiram and Plantvax singly or with Dithane-M45 gave a sufficient protection against faba bean chocolate spot, Alternaria leaf spot and rust diseases in field trials during 1989-1990 and 1990-1991 growing seasons and increased seed yield over the control.

Heweidy (1998) showed that applying the fungicides i.e., Plantavax, Copper Acropat and Apron individually 24 hrs before inoculation reduced the percentage of chocolate spot severity of two faba bean entries than those used 24 hrs after inoculation process.

Abou-Zeid *et al.* (2002c) found that spraying faba bean fields at Nubaria and Sakha Research Stations with Diathane-M45 decreased significantly the infection of chocolate spot disease more than other tested fungicides and did not increase weights and seed yields significantly.

El-Afifi (2003) found that addition of the fungicide Topsin-M70 at 10 ppm to the medium completely inhibited the growth of *B. fabae*, while the fungicides i.e. Eminent and Score completely inhibited the fungal growth at 100 ppm while, Ridomil-Cu and Kocide-101were the least effective ones in this respect. Also application of these fungicides to control chocolate spot disease under field conditions gave the same trend.

Relationship between sowing date and chocolate spot infection:

Mohamed *et al.* (1981) observed high infections of chocolate spot and rust especially in earlier planting in Egypt, when faba bean (Giza-3) was planted at Sakha at three different dates i.e., 15th October, 30th October and 15th November.

Hanounik and Hawtin (1982) reported that delaying the date of planting decreased the severity of chocolate spot significantly at Lathakia (Syria).

Saxena and Stewart (1983) found that seed yield and foliar diseases including chocolate spot were affected greatly with sowing date from 1st October till 1st December during the two growing seasons 1979/80 and 1980/81in Egypt.

Abed El-Latif (1984) showed that date of planting affected faba bean disease severity where, early sowing at 15th October decreased severity to large extent. While sowing at 1st November or 15th November resulted in an increase in disease severity compared with the first date of planting.

Salih (1984) stated that sowing faba bean at 20th October gave the highest seed yields due to greater plant survival and more vigorous plant growth, as compared with sowing on 10th or 30th Oct.

Amer (1986) studied the effect of three sowing dates i.e. 15th October, 7th November and 21st November and three plant densities on three faba bean cultivars. He found that leaf spot infection was significantly affected by sowing dates. The rate of

infection decreased by delaying sowing date where, early sowing date resulted in higher infection percentage in two seasons.

Hussein *et al.* (1994) studied the effect of three sowing dates (mid October, first and mid November) on yield and yield components of faba bean cultivar Giza-Blanka at Nubaria Research Station. They indicated that planting Giza Blanka on mid October recorded higher seed yield followed by the first of November whereas the lowest value was obtaining on mid November.

Mahmoud, Nagwa (1996) indicated that the infection with chocolate spot disease was higher in the early sowing date at 1st November than that in 25th November and the third 9th December sowing dates in during the two seasons.

Effect of chocolate spot disease infection on faba bean seed yield:

Elliott and Whittington (1980) showed that chocolate spot disease was not severe in England, except for short periods after artificial inoculation, even when humidity was increased. No yield response was observed under different disease levels in some treatments.

Mohamed (1982) found that natural infection of 10 faba bean entries with leaf spots, rust and downy mildew was high at Sakha in season 1979. Yield losses ranged from 22.8% on the entry 90/1966/72 to 55.7% on Rebaya- 40.

Nassib *et al.* (1991) mentioned that late rains in certain seasons increase relative humidity and wetness, which leads to epiphytotic conditions. Because of such conditions in 1987/88

and 1990/91 growing seasons, chocolate spot and rust epiphytotics reduced faba bean production in the Delta by 50%.

Salh *et al.* (1994) reported that yield losses in the Delta area of Egypt were as high as 50% in faba bean production under chocolate spot epiphytotic conditions. This is mainly due to the high relative humidity (80-90%) and favorable daily temperature (around 15 to 20°C) which prevails in the Delta in winter and early spring.

Effect of N-P fertilizer on chocolate spot infection:

Mansour and Kamel (1975) found that adding calcium superphosphate 15% at the rate of 238 kg/ha and calcium nitrate 15.5% at the rate of 238 kg/ha was the best treatment to provide high yield and to decrease chocolate spot infection.

Zaglol (1991) found that both foliar nutrients, Byfolian and Foksal have no effect in reducing infection percentage on plants receiving one or two sprays. Meanwhile, plants of faba bean cultivars sprayed 3 times with both nutrients showed significant reduction in disease percentage.

Hegab and Beshir (1994) found that adding nitrogen fertilizer at the rate of 15, 30 and 45 kg N/fed. insignificantly increased numbers of branches/plant, seeds/pod and weight of 100 seed. On the other hand, increasing nitrogen fertilizer to increased plant height and seed yield as well as plant infection with chocolate spot disease.

Mahmoud, Nagwa (1996) reported that increasing nitrogen fertilizer rate from N_0 to N_{30} increased chocolate spot

disease and decreased the yield. But phosphorus decreased the percentage of infection by chocolate spot and increased the yield. The higher infection was observed under zero phosphorus application than15 kg P_2O_5/fed. and 30kg P_2O_5/fed., while under P_{30}, the N_{15} level showed higher yield than N_0 or N_{30}.

Biochemical changes associated with chocolate spot infection:

1-Sugar content:

EI-Neshwy, Saniya (1981) showed that there was an increase in total soluble sugars content of faba bean leaf exudates with the increase of infected leaves age with chocolate spot and no clear variation was noticed between leaves of both tested cvs. Giza-1 and Rebaya-40 at the same age.

Habib, Wadiaa (1990) studied sugar content in faba bean infected with chocolate spot. The resistant entry ILB-938 extract contained higher content of each of the reducing, non-reducing and total sugars if compared with the extract of the moderately susceptible Giza-3 cv.

Mahmoud (1992) mentioned that the healthy leaves of faba bean cultivars (Giza-402 and Giza-2) contained more total and reducing sugars than the leaves infected with chocolate spot.

Mansour (1992) mentioned that healthy leaves of both faba bean cvs (Giza-402 and Giza-2) contained more total and reducing sugars than the diseased one.

Abou-Baker (2002) studied sugar content in faba bean infected with chocolate spot. He found that the susceptible entry Giza-429 extract contained higher content of each of reducing,

non reducing and total sugars if compared with the extract of the resistant Giza-461 cv. Moreover, the amount of sugar contents in infected plants of Giza-429 and Giza-461 were higher than the healthy plants.

2- Phenolic content:

Younis (1989) reported that faba bean varieties Giza-3 and NA-112 irradiated treatments recorded decrease in free phenols content. On the contrary S. Giant cv. irradiated treatment recorded higher content of free phenols than the control.

Habib, Wadiaa (1990) found that total phenols in the extract of faba bean leaves were to somewhat higher in the extract of resistant entry ILB-938 than in the moderate susceptible Giza-3.

Mansour (1992) found that free and total phenols were produced and accumulated at a faster rate in Giza-2 (moderate susceptible) than that of Giza-402 (susceptible). As regards the conjugated phenols, an opposite trend in both tested cvs was noticed

Heweidy (1993) found that all Botrytis isolates caused an increase in the total phenols relative to the control. When cultivars were inoculated with the more virulent isolates of *B .fabae,* ILB-938 entry plants showed moderate increase of phenols compared with either cv. Giza-402 but when *B. cinerea* was used, ILB-938 entry contained the highest increase of total phenols.

Abou- Baker (2002) mentioned that free and total phenols in the extract of faba bean leaves were to somewhat higher in the extract of resistant entry Giza-461 than in the susceptible Giza-429. While, inoculated both cultivars with *B. fabae* caused an

increase in free and total phenols from 6 h to 24 h from inoculation. After 24 h of inoculation, the free and total phenols content was decreased.

3- Amino acids content:

Farahat (1980) reported that the levels of free amino acids decreased with plant age and the infection increased free amino acid contents in leaves and stems of three pea varieties.

El-Neshwy, Saniya (1981) found that the free amino acids increased in faba bean plants (Giza-1 and Rebaya-40) infected with *B. fabae,* particularly infected leaves of 30 days old as compared with 45 and 60 days old plants.

El-Beih *et al.* (1988) detected some amino acids, i.e. glycine, proline, glutamic acid, D-valine and histidine in the leaves of faba bean of cv. Rebaya-40 (more susceptible to *B. fabae*).

Habib, Wadiaa (1990) found that amino acids content of the resistant entry ILB-938 extract was higher compared with the extract of the moderate susceptible Giza-3 cv.

Mansour (1992) found that total free amino acids were higher in diseased leaves than in healthy ones. Also, the susceptible cultivar Giza-402 contains higher levels of total free amino acids than the moderate susceptible cv Giza-2.

Abou- Baker (2002) showed that total free amino acids were higher in infected leaves with *Botrytis fabae* than in healthy ones. The increase of amount in resistant cultivar Giza-461 was higher comparing with the amount in Giza-429 susceptible cultivar.

MATERIAL AND METHODS

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1- Survey of chocolate spot disease:

Surveying of chocolate spot disease was carried out on faba bean plantations during the end of February of the growing season 1998/1999 at eight Governorates, i.e. Kafr-El-Sheikh, El-Beheira, Gharbia, Dakahlia, Sharkia, Menoufia, Qualubia and Beni-Swief. Three to four locations were inspected in each Governorate and each location was represented by three randomly villages with three dispersed fields in each village. Three samples (each one 100 leaves) were selected randomly from each field. The leaves were carefully examined and classified into categories as devised by **Abou-Zeid (1978)** in order to calculate the average percentage of disease severity for each Governorate.

2- Isolation and identification of chocolate spot pathogens:

Samples of naturally infected faba bean leaves showing leaf spot symptoms were collected from different Egyptian locations (Governorates) at flowering stage during two growing seasons1998/1999 and 1999/2000. The infected leaves with chocolate spot disease symptoms were cut into small pieces (5 mm ϕ), each piece contain single lesion. The infected tissues were sterilized by soaking in 5% sodium hypochlorite for 2 minutes, 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±1 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 Biolog-System technique which belonged to the biological control of faba bean chocolate spot disease project, Plant Pathology Research Institute, A.R.C., Giza, Egypt. The pure cultures of each isolate were maintained on PDA slants at 4°C for the further studies.

3- Pathogenicity test:

Twenty isolates of *Botrytis* spp (9 isolates of *B. fabae* and11 isolates of *B. cinerea*), isolated during two growing seasons1998/1999 and 1999/2000, were chosen based on their high frequency and distribution in the different locations to tested for their pathogenic potentialities under greenhouse conditions as followes:

Inoculum preparation:

The obtained Botrytis isolates were grown onto faba bean leaf extract agar medium (FBLA) which consists of 250g faba bean leaves, 30g sucrose, 20g sodium chloride and 20g agar in one liter of distilled water 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 (5mm ϕ) of each of the tested isolates and incubated at 20°C±1 for 12 days **(Last and Hamley, 1956)** under alternating light and darkness regime (12-h/12-h) to enhance spore production. Five

plates were used for each isolate as replicates. The plates were flooded with 10 ml of sterilized distilled water and brushed thoroughly. The suspension was filtered through three layers of cheesecloth to remove the mycelial residues. Number of spores/ml was counted in the collected spore suspension by using a Spencer haemacytometer slide to about 2.5×105 spores/ml for either *B. fabae* or *B. cinerea*. The prepared inocula were used as follow:

a- Determination of chocolate spot disease under greenhouse conditions:

The tested isolates were evaluated for their pathogenic abilities on intact leaves of faba bean plants. Seeds of the most susceptible faba bean Giza-40 cv were sown in sterilized potted soil (15 cm ϕ pots, each one was sown with 5 seeds). Forty-five days after sowing, the grown plants were sprayed with Botrytis inoculum of each of the tested isolates at rate 2.5×105 spores/ml using a sterilized atomizer and then covered with polyethylene bags for 24h to maintain high level of relative humidity which necessary for fungal infection. Control plants were sprayed with sterilized water. 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 for each isolate after 1, 3, 5, 7 and 14 days of inoculation using the devised scale of **Abou-Zeid *et al.*, (1978)** as illustrated in **Table(1)**. The Disease severity was calculated after 1, 3, 5, 7 and 14 days, using the following equation:

$$\% \text{ Disease severity} = \frac{n \times v}{9\,N} \times 100$$

Where, n = number of plants in every grade.

v = numerical grade.

N = total number of examined plants.

9 = maximum disease grade.

Table (1): Rating scale for disease assessment of chocolate spot disease caused by *B. fabae* (Abou-Zeid *et al.*, 1978).

Score	Leaves	Flowers	Stems
0	No infection	No infection	No infection
1	Few localized lesions on some leaves. Percentage of infected leaf area (1-5%).	Few lesions on some flowers	No lesions
2	Some few lesions on 1/2-3/4 of infected leaves. Percentage of infected leaf area (5-10%).	Some lesions on 1/2-3/4 of flowers	Few lesions on lower part of stem
3	Large lesions on more than 3/4 of infected leaves. Infected leaf area is less than 25%.	Striped flowers	Many lesions on stem
4	Some coalesced lesions on infected leaves. Percentage of infected leaf area is larger than 25%.	Lower flowers turned to black colour	Coalesced lesions on stem.
5	Coalesced lesions on ½ of the infected leaves. Drop of the lower leaves. Percentage of infected leaf area ranged from 25 – 50%.	Drop of earlier flowers.	Spreading lesions on stem.
6	Coalesced lesions on 1/2– 3/4 of the leaves. Percentage of infected leaf area is more than 50%.	Drop 1/4 of the flowers.	Mottled stems.
7	All leaves infected by large coalesced lesions. Percentage of infected leaf area reached to 75%.	Drop > 3/4 of the flowers	Blackness of flower part of stem.
8	Whole plant died except the apex.		
9	Death of the whole plant.		

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

Faba bean plants (Giza-40 cv) were grown in polyethylene pots (15 cm ϕ) under greenhouse conditions for 40 days. Detached leaves taken at from the fifth nodes were placed horizontally on filter papers onto sterilized polyethylene boxes (25 ×15×15 cm) contained water soaked filter paper in order to obtain high relative humidity **(Abou Zeid *et al.*, 1985).** Ten Botrytis isolates including eight Botrytis fabae and two B. cinerea were used as follows.

The inoculum of each isolate were prepared as mentioned before was added onto incubated faba bean leaflets at rate 2.5 ×105 spores/ml in form of droplets (10 µl). The boxes are covered with transparent polyethylene bags to maintain high humidity. Three replicates of each treatment were used (each replicate contains 5 faba bean leaflets in each box). Disease severity data were recorded as described above after 24h using scale (0-9) depending on the extent of lesions **(Abou-Zeid *et al.*, 1985)** as shown in **Table (2) and Fig. (1).**

Table (2): Chocolate spot disease scale (0-9) on detached faba bean leaves (Abou-Zeid *et al.*, 1985).

Score	Description
0	Healthy leaflets
1	Less than 10 small spots appeared, 0.5mm in diameter
2	Increasing spots number with the same previous size
3	Large spots, 1mm in diameter
4	Coalesced spots together
5	Dark spots
6	Diameter of necrotic areas ranged from 1 – 2 mm
7	Diameter of the necrotic areas lies between 2–4 mm
8	Diameter of the necrotic areas ranged from 4–6 mm
9	Diameter of the necrotic areas is more than 15 mm

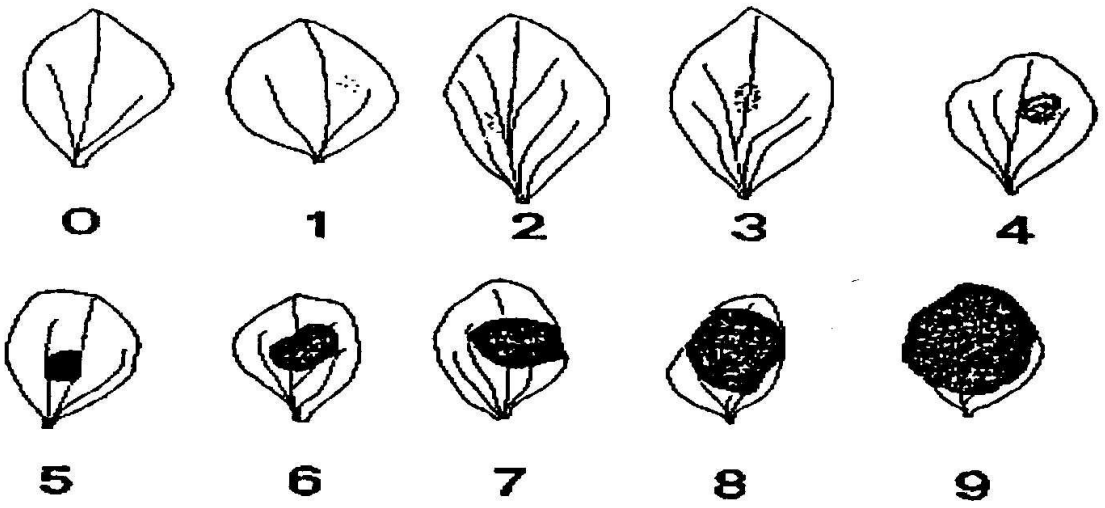

Fig (1): Chocolate spot disease scale (0-9) on detached faba bean leaves (after Abou-Zeid *et al.*, 1985).

4- RAPD-PCR technique (Random amplified polymorphism DNA) for detecting similarity or diversity between Botrytis isolates:

This experiment was done in order to detect similarity or diversity between 10 Botrytis isolates identified as B. fabae and B. cinerea before using later in the further studies.

Extraction of Botrytis DNA:

Botrytis fabae or *B. cinerea* DNA was extratid from 50 mg mycelium of each of the tested fungi where samples were frozen using liquid nitrogen and ground into a fine powder with a mortar and pestle. The powder was transferred into 1.5-ml microfuge tube and dispersed in 1 ml of extraction buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 100mM EDTA), mixed thoroughly and 0.06 ml of 20% sodium dodecyl sulphate (final concentration 1%) was added. The mixture was gently

shaken for 1 h at about 20oC, mixed with 0.15 ml of 5 M NaCl (final concentration 0.8M) and 0.13 ml of CTAB/NaCl solution (10% CTAB in 0.7 M NaCl, final concentration 1%), and kept at 65oC for 20 min. The mixture was divided into two 1.5 ml microfuge tubes and extracted with chloroform/isoamyl alcohol (24:1). The top aqueous phase was transferred to a clean tube, and about 360 µl of cold isopropanol was added. After 20 min of incubation at 4oC, the solution was centrifuged for 10 min at 10,000 rpm at 20oC to precipitate the nucleic acid. The pellet was rinsed twice with cold 70% ethanol, dried in vacuum, and dissolved in 0.5 ml of TE buffer (10mM Tris-HCl and 1 mM EDTA, pH 8.0). One microliter of ribonuclease from stock 10mg/ml was added (final concentration 20 µg/ml) and kept at 4oC overnight to completely digest the RNA. The DNA was reprecipitated, rinsed with cold 70% ethanol, dried, and dissolved in 40 µl of TE (stock DNA), then DNA was quantified by the minigel method using Gene Quantum system–Pharmacia Biotech **(Sambrook *et al.*, 1989)**. After quantification, the stock DNA was kept at –20oC for later use.

RAPD-PCR technique:

Thirty ng/ml of extracted DNA were used for amplification reaction. The polymerase chain reaction (PCR) mixture contained PCR beads tablet (manufactured by Amessham Pharmacia Biotech), which containing all of the necessary reagents except the primer and the DNA which add to the tablet in order to start reaction. The kits of Amessham Pharmacia Biotech were also included the following primers. Five microliter of the primer (10 mer) was added. The sequence

of used specific primer according to **Abou-Zeid *et al.* (2002d)** was primer 2: 6-d (GTTTCGCTCC)-3 for differentiating between B. fabae and B. cinerea isolates. The total volume was completed to 25 µl using sterile distilled water. Amplification was carried out using PCR unit II Biometra programmed for 5 min at 95oC for initial denaturation and 45 cycles that consisted of 1 min at 95oC, 1 min at 36oC, and 2 min at 72oC, followed by a final 5 min extension at 72oC after then holding at 4oC over night. Then, 7µl of 6X tracking buffer (manufactured by Qiagen Kit) were added to 25 µl of the amplification product. After amplification, 15µl of the solution for each sample was electrophoresed in a 1% Agarose gel in 0.5X TBE buffer (0.089 M Tris-borate, 0.089 M boric acid, and 0.002 M EDTA) using electrophoresis unit (WIDE mini-sub-cell GT Bio-RAD). A 1-kb DNA ladder (0.15 µg) (Gibco BRL, Bethesda, MD) was used to estimate the size of each amplified DNA fragment. The gel was run for 90 min at 75 volts, stained with ethidium bromide (0.5ug/ml) for 30 min, and photographed under ultraviolet light (Transilluminator). The test primer was repeated at least twice to ensure the consistency of each RAPD band **(Pieter *et al.* 1995).**

5- Factors affecting growth, sporulation and sclerotial formation of *Botrytis fabae* and *B. cinerea*:

a-Types of Culture media:

Twenty isolates of chosen *Botrytis fabae* and *B. cinerea*, which isolated from faba bean plants, were cultured on Petri plates (9 cm ϕ) of four different media with an equal disc (5 mm ϕ) of 7 days old cultures of the tested isolates. The observations

on mycelial growth, sporulation and sclerotial formation (number and size) were daily recorded after inoculation.

The media used were:

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

(Extract of 200 g of peeled potato, 20g dextrose and 20g agar, 1000ml distilled water). Sterilisation was at 1.5 lb/inch^2 for 20 minutes.

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

(Extract of 250 g faba bean leaves, 30g sucrose, 20g sodium chloride and 20g agar, 1000 ml distilled water). Sterilisation was at 1 Ib/inch^2 for 15 minutes.

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

(Extract of 250g faba bean seeds, 30g sucrose, 20g sodium chloride and 20g agar, 1000 ml distilled water). Sterilisation was at 1.5 Ib/inch^2 for 20 minutes.

4- Czapek's medium **(Levine and Schoenlein, 1930):**

(30g sucrose, 2g $NaNO_3$, 1g K_2HPO_4, 0.5g KCl, 0.5 MgCl, 0.01g Fe_2SO_4-$7H_2O$ and 20g agar in one litter distilled water). Sterilisation was at 1.5 Ib/inch^2 for 20 minutes.

In this experiment, Petri plates (9 cm ϕ) contained media were inoculated each with an equal disk (5 mm ϕ) of each one of *Botrytis fabae* or *B. cinerea* isolates grown previously on PDA

media (7days old culture). The inoculated Petri plates were incubated at 20°C±1. The growth was measured daily for each particular medium until the diameter of fungal growth reached 9 cm. The sporulation was estimated after 12 days by adding 10 ml distilled water to each plate, then exposed to electric sonar water bath to separate the conidiospores out their conidophores by falling in the added water. The spore suspension was filtered using clean sterilized cheesecloth and the spore suspension was received in a test tube. Number of spores was counted using the haemacytometer 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/cm^2 were recorded for each of *Botrytis fabae* and *B. cinerea* isolates.

b- Temperatures:

In this experiment, poured Petri plates (9 cm ϕ) with FBLA medium were inoculated individually with an equal disc (5mm ϕ) of 8 *Botrytis fabae* and 2 *B. cinerea* isolates taken from 7 days-old cultures and incubated at different temperature degrees i.e., 10, 15, 20, 25 and 30 °C until the diameter of fungal growth reached 9 cm. Three plates were used as replicates for each treatment. Linear growth of each isolate was measured daily while spore number was counted after 12 day post incubation.

6- Factors affecting chocolate spot disease severity:

a- Inoculum potential:

This experiment was carried out to evaluate chocolate spot disease on faba plants as a result for infection with different

inoculum densities of *B. fabae*. Spore suspension of *B. fabae* isolated from El-Nubaria district (the most virulent isolate) was prepared as mentioned before. Different inoculum densities of spore suspension i.e., 0.75×10^5, 1.25×10^5, 2.5×10^5, 5.0×10^5 and 6.0×10^5 spores/ml were made and tested on faba bean plants (Giza-40) grown in pots (15cm ϕ, 5 plants/pot). Inoculation and incubation were made as mentioned previously in pathogenicity test. Control plants were sprayed with sterilized water. 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. All plants were examined for chocolate spot disease after 2, 4, 7 and 14 days from inoculation using the devised scale of **Abou-Zeid *et al.*, (1978)**

b- Spore age:

The inoculated FBLA plates (9 cm ϕ) with *B. cinerea* (Etay-El Baroud isolate) or *B. fabae* (El-Nubaria isolate) were incubated at 20°C for 1, 2 and 3 weeks for producing sopre suspension. Spore suspension was prepared as mentioned before for each particular period. The prepared spore suspension at rate 2.5×10^5 spore/ml of each tested *B. fabae* or *B. cinerea* isolates was placed in form of droplets (10 µl) on the lower surface of faba bean leaflets detached from 40 days old faba plants cv Giza-40 grown in pots. The inoculated leaflets were placed in sterilized polyethylene boxes (25 × 15 × 15 cm) contained water soaked filter paper in order to obtain high relative humidity. Three replicates of each treatment were used (each replicate contains 5 faba bean leaflets in each box). Incubation of boxes was at 20°C. Disease severity was recorded after 1, 3, 5 and 7

days using the scale (0-9) depending on the extent of lesions (**Abou-Zeid *et al.*, 1985**).

c- Plant age:

Potted faba bean plants of Giza-40 cv. at different ages i.e. 17, 29, 41, 53 and 65 days old were sprayed with spore suspension of *Botrytis fabae* (El-Nubaria isolate) at the rate of 2.5×10^5 spore/ml. 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 severity after 2, 4, 7 and 14 days post inoculation using the devised scale of **Abou-Zeid *et al.*, (1978)**

d- Varietal reaction:

In this experiment, eight faba bean cultivars i.e., Giza-3, Giza-40, Giza-429, Giza-461, Giza-667, Giza-717, Giza-716 and Giza-Blanka were evaluated for their reaction against chocolate spot disease caused by ten Botrytis spp 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-Zeid *et al.*, (1978)**

7- Biochemical changes due to infection with *Botrytis fabae*:

Preparation of plant extracts:

In this experiment, three different faba bean varieties (Giza-40, Giza-3 and Giza-716) were artificially inoculated with *B. fabae* (El-Nubaria isolate) at three different plant ages (30, 45 and 60 days). Fresh samples of infected and non-infected faba bean leaves were taken after1, 2 and 7 days from inoculation.

The leaf samples were extracted for chemical analysis. Five grams from each treatment were cut into small pieces and immediately plunged into 95% boiling ethanol for ten minutes to kill the tissue. The samples were then resumed for 8-10 hrs. in Soxhelt apparatus by using 75% ethanol as an extractant until the percolate was colorless. The combined ethanolic extracts were filtered and evaporated on a mild water bath (60-70°C) to near dryness. The dried residues were redissolved in a known volume (10 ml of 50% isopropanol) and stored in sample vials at 1°C and used for the determination of sugars content, total free amino acids and phenolic compounds. All chemical determination values were calculated on fresh weight basis.

a- Determination of sugar contents:

Total and reducing sugars were determined spectrophotometrically with picric acid method as described by **Thomas and Dutcher, (1924)**. The sugar content was calculated as glucose from standard curve prepared for glucose. Two solutions were used for determination of reducing and total sugars.

(1) Preparation of picrate-picric solution:

Thirty-six grams of picric acid were added to 500 ml of 1% solution of sodium hydroxide and 400 ml of hot distilled water, mixture was shaken occasionally until the picric acid was dissolved after cooling and diluted to one liter.

(2) Preparation of sodium carbonate solution:

Twenty grams of sodium carbonate were dissolved in 100 ml of distilled water.

For determination of total soluble sugars, 1ml of each sample was placed in 70 ml test tube, containing 5 ml distilled water plus 4 ml picrate–picric solution, then the mixture was boiled for 10 minutes on water bath. After cooling, 1 ml of sodium carbonate was added and the mixture was boiled again for 10 minutes then cooled and completed to 50 ml with distilled water. The optical density of the developed color was measured using spectrophotometer (SPECTRONIC 20-D) in the presence of blank at 540 nm.

The above technique was applied also for determination of reducing sugars except that picrate–picric and sodium carbonate were added together at the same time and boiled only for 10 min.

Non–reducing sugars were determined as the difference between the total and reducing sugars. All these determinations were expressed as milligram of glucose per one-gram fresh weight of plant sample according to the standard curve of glucose.

b- Determination of phenolic compounds:

Phenolic compounds were determined using colorimetric method of analysis as described by **Snell and Snell, (1953).**

Preparation of Folin-Ciocalteu reagent:

Phenol reagent (Folin-Ciocalteu reagent) was prepared by boiling 100 g sodium tungstate, 25 g sodium molybdate and 700 ml distilled water into a 1500 ml flask, 50 ml of 85% phosphoric acid and 100 ml of concentrated hydrochloric acid (HCL) under reflux condenser for 10 h in a water bath. Then, 150 g of lithium sulfate, 50 ml distilled water and few drops of liquid bromine were added to the mixture and boiled again for 15 minutes without a reflux condenser to remove excess bromine, then cooled and diluted to one liter using distilled water and filtered.

The free phenols were determined by adding one ml of the phenol reagent and 5 ml of 20% sodium carbonate solution to the isopropanol sample (0.2 ml sample), and then diluted to 10 ml with warm distilled water (30-35°C).

The mixture was let to stand for 20 minutes and read at 520 nm against a reagent blank using spectrophotometer (SPECTRONIC 20-D).

For total (free and conjugated) phenols determination, drops (about 0.25 ml) of concentrated HCL were added to the isopropanol samples (0.2 ml) in a test tube, heated rapidly to boiling over free flame with provision for condensation and placed in boiling water bath for 10 min. After cooling, 1 ml of the Folin-Ciocalteu reagent and 2.5 ml of 20% sodium carbonate (Na_2Co_3) were added. The mixture was diluted to 50 ml with

warm distilled water and after 20 minutes readings were measured using spectrophotometer (SPECTRONIC 20-D) at 520 nm against a reagent blank.

Conjugated phenols were determined by subtracting free phenols from the total phenols. All these determinations of the phenolic compounds were expressed as mg catechol/1g fresh weight of plant sample based on standard curve for catechol.

Preparation of standard curve:

One gram of catechol was dissolved in distilled water and the volume was made up to one liter. Different volumes from catechol solution were taken and raised to 100 ml using distilled water. One ml of the different catechol concentrations were taken separately in test tube, 1 ml distilled water, 1 ml reagent and 3 ml sodium carbonate were added and then the mixture was completed to 10 ml using distilled water and then treated as shown in the determination of free phenols. Finally, the readings of standard curve of catechol were measured at 520 nm using spectrophotometer (SPECTRONIC 20-D).

c- Determination of total free amino acids:

The total free amino acids were determined in the previous isopropanolic extract according to the method described by **Rosen, (1957).**

The reaction mixture contained 0.5 ml of the sample extract, 1 ml of 0.1% ninhydrin in actone (0.1g/100ml acetone) and 0.5 acetate buffer (pH 5.4), brought to 10 ml with distilled water and placed in a boiling water bath for 10 minutes.A blank containing all reagents without sample extract was also prepared

and read at 570 nm. Results were expressed as milligrams from a leucine standard curve.

9- Biological control:

a- Isolation and identification of faba bean phylloplane micro-organisms:

The plate count method described by **Kiraly (1974)** was used for isolation and identification of faba bean phylloplane microorganisms. One gram faba bean leaf discs (5 mm ϕ) were taken from 8 weeks old plants around the midrib of leaves at fifth node. The discs were cut with a sterile cork borer into small pieces and then placed in 10 ml of sterile distilled water in a mortar. Serial dilutions from 10^{-1} to 10^{-6} were prepared from the suspension by using sterile distilled water as dilution to study the phylloplane microorganisms. One ml of both dilutions (10^{-3} and 10^{-6}) were used for the isolation of fungi and bacteria on Petri plates containing PDA medium + streptomycin or King's agar medium for isolation fungi and bacteria respectively. One ml of each desired dilution was placed and streaked on the surface of each plate using special sterile glass rod. The plates were incubated at 25-28°C for 2-3 days until developing of single colonies. Different separated fungal and bacterial colonies were picked up, then transferred on a new medium, and re-incubated again.

After 2-7 days, the bacterial and fungal isolates were purified by hyphal tip method (**Brown, 1924**) or single spore technique (**Hansen, 1926**) respectively.The resulted bacterial and fungal isolates were maintained on nutrient agar and PDA

slant media respectively and kept in a refrigerator at 5-10°C till achieving identification. The isolated fungi were identified based on their morphological features according to **Ellis (1971)** and **Barnett and Hunter (1987)**. The isolated micro-organisms were identified again using Biolog-System technique which belonged to the biological control of faba bean chocolate spot disease project, Plant Pathology Research Institute, A.R.C., Giza, in order to ensure identification.

The antagonistic microorganisms (fungi and bacteria) previously isolated from faba bean phylloplane were tested for their antagonistic effects on the growth of *Botrytis fabae in vitro* and *in vivo* under greenhouse and field conditions.

b- Effect of antagonists against *B. fabae* on PDA plates:

In this experiment, inoculum of the virulent isolate of *B. fabae* (El-Nubaria isolate) was prepared by growing on PDA medium at 20°C ±2 for 7 days. An equal disc (5 mm ϕ) of *B. fabae* was placed onto PDA plates (9 cm ϕ) 1cm from the periphery in opposite to another disc of each one of antagonistic tested fungi. Meanwhile the tested antagonistic bacteria were placed as individual streak in the opposite of *B. fabae* disc. Three plates were used as replicates for each treatment. The control plates were inoculated with the pathogen alone (*B. fabae*) onto one side of the plates. All inoculated plates were incubated at 20±2°C until the growth completely covered the plate surface in control treatment (after 6 days). The plates were then examined and linear growth of *B. fabae* was measured to determine the more effective antagonistic isolate of fungus or bacteria (Abou-

Zeid and Hassanien, 2000b). The inhibition 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

c- Effect of antagonistic culture filtrates against *B. fabae* on PDA plates:

In this experiment, culture filtrates of four antagonists (*T. hamatum, T.harzianum, Bacillus subtilis* and *Pseudomonas fluorescens*) were examined for their effect on growth of *B. fabae* on PDA plates. Culture filtrates of both *T. hamatum* and *T. harzianum*, were prepared as described by **Mukherjee *et al.* (1995)**.

In this respect, flasks 250 ml containing 50 ml of potato dextrose broth (PDB) medium were inoculated with an equal disc (5 mm ϕ) of each of *Trichoderma* isolates which taken from 3 days old culture and then incubated at 25-30°C for 10 days in the dark. Then they were filtered using filter paper (Watman No.1) and centrifuged at 3000 rpm. The resulted supernatants of *Trichoderma* isolates were sterilized by filtration using sterilized Center glass funnel (G5). While, culture filtrates of *B. Subtilis* and *P. fluorescens* were obtained by growing tested bacteria on sterilized nutrient agar broth medium (50 ml/250 ml flasks).

The cultures were incubated at 25°C for 3 days using shaker-incubator at 150 shake/Min. The cultures were centrifuged at 3000 rpm for 10 minutes and then the supernatants were collected and sterilized by filtration through a bacteria proof filter (Center glass funnel G5). Four different concentrations i.e., 12.5, 25, 37.5 and 50% of each of sterilized culture filtrates of the four antagonists were prepared and added to PDA medium then poured into Petri plates. Control treatment was sterile distilled water PDA medium. The prepared plates were centrally inoculated with individual equal disc (5 mm ϕ) of *B. fabae* (7 day old culture) and incubated at 20°C±2. Three plates were used as replicates for each treatment. Linear growth of grown *B. fabae* was measured when the growth reached 9.0 cm after 6 days in control treatment.

d- Effect of antagonists against *B. fabae* on detached leaves:

The antagonistic effects of the previously tested antagonists were tested *in vitro* also onto detached faba bean leaves inoculated with *B. fabae* in the same time. In this experiment, faba bean plants (Giza-40 cv) grown in polyethylene pots (15 cm ϕ) under greenhouse conditions at 20–22°C for 40 days then the detached leaves were taken from the fifth nodes of faba bean plants. The detached leaves were placed horizontally on filter papers onto sterilized clean aluminum trays. Spore suspension of *B. fabae* (2.5×10^5 spore/ml) was prepared as described previously according to the method of **Mohamed *et al*, (1994)**. One drop (10 µl) of each of bacterial suspensions (10^8 cfu), *Trichoderma* isolates ($6x10^6$ spore /ml), *Gliocladium virens* ($5x10^6$ spore/ml) and *Pacellomyces* spp ($7x10^6$ spore/ml) were

were dropped on each one of leaflets and the spore suspension (10µl) of *B. fabae* was was placed on dropped antagonists after 24h. Control treatment was treated with 10µl of *B. fabae* spore suspension. The trays were covered with polyethylene sheets for 24 hours to maintain high moisture and then incubated directly after covering at 20°C. Disease severity was recorded based on type of infection using scale 0-9 (**Abou-Zeid, 1985**) after 1, 3, 5 and 7 days post inoculation.

e- Effect of antagonists filtrates against *B. fabae* on detached leaves:

In this experiment, four different concentrations i.e., 25, 50, 75 and 100% of each of the sterilized culture filtrates of the four antagonists were prepared as mentioned before and tested for their efficacy on chocolate spot disease caused by *B. fabae* (El-Nubaria isolate) using detached leaves. Detached faba bean leaves were placed horizontally on filter papers into sterilized clean aluminum trays. The culture filtrates at different concentrations of each antagonist were dropped (10µl) on each one of leaflets and the spore suspension (10µl) of *B. fabae* was pipetted on dropped culture after 24h. Control treatment was treated with 10µl of *B. fabae* spore suspension. The trays were covered with polyethylene sheets for 24 h. to maintain high moisture and incubated at 20°C. Disease severity was recorded based on type of infection using scale 0-9 as described before after 7 days post inoculation.

f- Effect of antagonists on chocolate spot disease under greenhouse conditions:

In this experiment, nine antagonists were tested for their efficiency in controlling faba bean chocolate spot disease caused by *B. fabae* (El-Nubaria isolate) under greenhouse conditions. Faba bean plants (Giza-40 cv) grown in polyethylene pots (15 cm ϕ) under greenhouse conditions at 20–22°C for 40 days were sprayed until dropping with an individual spore suspension of the desired antagonists (5 isolates of *Trichoderma* spp, *Gliocladium virens*, *Pacellomyces* spp. and 2 bacterial isolates) prepared at certain concentrations as mentioned above. The pots were arranged in a randomized complete block design. After 24 h post spraying with antagonists, the plants were sprayed with spore suspension of *B. fabae* (2.5×10^5 spore/ml). Control plants were sprayed with *B. fabae* only. All pots (plants) were covered with polyethylene bags for 48h in moist chamber at 20-22C° in greenhouse. Disease severity was recorded after 1, 3, 7 and 14 days as mentioned before.

g- Effect of antagonistic culture filtrates on chocolate spot disease:

In this experiment, four antagonistic culture filtrates prepared as mentioned above were tested for their efficiency in controlling faba bean chocolate spot disease caused by *B. fabae* under greenhouse conditions. Faba bean plants (Giza-40 cv) grown as mentioned above were sprayed individually with culture filtrate (100% conc.) of each of the tested antagonists 24 h before or after spraying with spore suspension of *B. fabae* (2.5×10^5 spore/ml). Control plant was sprayed only with spore

suspension (without culture filtrate). All plants were covered with plastic bags for 24-48 h to maintain suitable relative humidity around the plants, then kept under greenhouse conditions. Three pots for each treatment were used as replicates. Disease severity was determined after 2, 4, 7 and 14 days post inoculation with *B. fabae* as described before.

9- Effect of applying the un-viable heated spores of *B. fabae* on chocolate spot disease severity:

This experiment was carried out to study the effect of un-viable-heated spores of *B. fabae* as cross protection against chocolate spot disease of faba bean.

a- On detached leaves:

Spore suspension of un-viable heated spore (UHS) of *B. fabae* was prepared by growing *B. fabae* (El-Nubaria isolate) on faba bean leaf agar (FBLA) medium for 12 days and incubated at 20°C for 12 days. Ten ml of distilled water was poured in each Petri plate. Spore suspension was prepared and counted to $2.5X10^5$ spores/ml as mentioned before then heated for 10 minutes using water bath at 100°C **(Abou-Zeid and Le Normand 1979).** Detached faba bean leaves prepared as mentioned above were placed on filter papers and put in aluminum trays. Heated spore suspension (UHS) was dropped (10 µl) on each leaflet and then, 10 µl of the viable spore suspension of *B. fabae* was placed on the UHS drop after 24h. In control treatment viable spore suspension of *B. fabae* was placed on detached leaflets. All treatments were incubated at 20°C. Disease severity was recorded as mentioned before after 1, 3, 5 and 7 days post inoculation.

b– Under greenhouse conditions (in pots):

In this experiment, faba bean plants (Giza-40 cv) prepared as mentioned before were sprayed with the spore suspension of un-viable- heated spores (UHS) of *B. fabae* one day before spraying again with viable spore suspension of *B. fabae.* Control treatment was sprayed only with viable spore of *B. fabae.* All pots kept in polyethylene pages for one day then the pots were exposed to mist conditions under greenhouse at 20-22°C. Disease severity was recorded after 2, 4, 7 and 14 days as mentioned before.

10- Chemical Control of *Botrytis fabae* using different fungicides:

a- On PDA plates:

Four different fungicides were tested to study their effect on growth of *B. fabae* on PDA medium *in vitro.* The tested fungicides were Dithane M-45, Tridex, Polyram-D.F. and Kocide-101. Five different concentrations (0, 25, 50, 100, 150 and 200 ppm) were done based on their active ingredient according to **Horsfall, (1956)**. Each desired concentration of the four tested fungicides was added to the autoclaved PDA medium before poured into three Petri plates, and then inoculated with an equal disc (5mm ϕ) of *B. fabae* taken from a virulent isolate of *B. fabae* (El-Nubaria isolate) 7 day-old culture. Medium free of fungicides was used as control. All plates were incubated at 20°C. Linear growth was recorded when plates of control were completely covered with fungal growth.

b- On detached leaves:

The effect of different concentrations of fungicides on chocolate spot disease on detached leaflets of faba bean was tested. Detached faba bean leaves onto sterilized clean aluminum trays, inoculum of *Botrytis fabae* at the desired concentration (2.5×10^5 spores/ml) and the different concentrations of the four tested fungicides (Dithane-M45, Tridex, Polyram-DF and Kocide-101) were prepared as mentioned before. The detached leaves were treated with 10 µl of each concentration of the four tested fungicides and then 10 µl of *B. fabae* spore suspension was placed on the fungicide drops after 24h. Five treated leaflets were used as replicates for each concentration of fungicide. Control treatment was leaflets treated with 10 µl of *B. fabae* spore suspension. The trays were covered with polyethylene sheets for 24 hours only and incubated at 20°C. Disease severity data were recorded after 7 days as mentioned before according to the scale of **Abou-Zeid** *et al*, **(1985)**.

c- Under greenhouse conditions (in pots):

The effect of some fungicides (Dithane-M45, Tridex, Polyram-DF and Kocide-101) on disease severity of chocolate spot disease of faba bean under green house conditions was tested. Seeds of faba bean (Giza-40 cv) were sown in plastic pots (15 cm ϕ). Five seeds were sown in each pot and three pots were used for each treatment. After 45 days from sowing, plants were sprayed with the recommended dose of the for mentioned fungicides, 24 h before inoculation. The plants were sprayed 24 h after fungicidal spraying with the adjusted spore suspension of

B. fabae. The untreated plants in the control pots were inoculated only with spore suspension of the *B. fabae.* All plants were covered with plastic bags for 24h and kept in the greenhouse at 20°C.

The disease severity was estimated using the disease scale (0-9) suggested by **(Abou-Zeid *et al.*, 1978)** after 2, 4, 7 and 14 days from inoculation with *B. fabae.*

11- Field experiments for controlling chocolate spot disease:

All following experiments were done during the 2000/2001 and 2001/2002 growing seasons at El-Bagour, Minoufia Governorates, to study the effects of chemical and biological control, sowing date, cultivars reaction and fertilization on severity of faba bean chocolate spot disease. Chocolate spot disease was determined under field conditions according to the adopted scale of **Abou-Zeid (1978)**.

a- Effect of P and N fertilization onto chocolate spot disease:

This experiment aimed to investigate the effect of nitrogen and phosphorus fertilizer rates on chocolate spot disease severity, yield and yield components of faba bean (Giza-40) during two successive seasons 2000/01 and 2001/02. The experiment was designed as factorial design with three plots (replicates). Plant density was 17 plant/m^2. The field was divided into plots 2.4 × 3.75 m^2. Each plot consisted of four rows; each row contained 10 hills on the eastern side. Seeds were sown as two seed/hill, two edges of each hill 20cm between holes. The phosphorus levels were 0, 15, 30 kg P_2O_5/fed in form of

calcium-super phosphate (15% P_2O_5) and added during land preparation to the main plots. Nitrogen levels were 0, 7.5, 15, 30 kg N/fed in form of ammonium nitrate (33.6% N) which added to the sub-plots at 25 days after sowing, just before the second irrigation. Seeds were sown at 10th November 2000 for the first season and 7th November, 2001 for the second season. The agricultural practices were applied as recommended. The chocolate spot disease severity under natural infection was determined as mentioned before at 15th January in 2000/01 season, while the second season was at 20th January in 2001/02 and repeated every four weeks interval up to 3 scores. At harvest, ten plants were taken randomly from each plot to determine the following parameters i.e., plant height, number of branches, number of pods/plant, 100–seed weight (gm), number of seeds/pod. Meanwhile, at full maturity, plants in each replicate were harvested and left to dry about ten days later, pods were picked from the plants, left to dray then seeds for each replicate were weighted as seed yield/plot and seed yield (ton/fed).

b- Effect of chemical and biological control treatment onto chocolate spot disease:

In this experiment, four fungicides and four antagonists were tested under field conditions to know their effect on chocolate spot disease of faba bean (Giza-40) and consequently, yield and yield components during 2000/01 and 2001/02 growing seasons.

The tested fungicides and dose of their application are shown in **Table (3)**. As for antagonists spore suspension (6×10^6

spore/ml) for*T. Hamatum* and *T. harzianum* or bacterial cell suspension (10^8 cfu cell/ml) for *Bacillus subtilis* and *P. fluorescens*) were prepared as mentioned before. The experiment was designed as a complete randomized block design with three replicates for each particular including control treatment. Faba bean (Giza-40) was sown on 12th and 14th November during seasons 2000 and 2001, respectively. All agricultural practices were done as normal for all treatments. The fungicide prepared was plants were sprayed four times started at 15th January and every15- days intervals. while biological preparations were sprayed on faba bean plants four times during the growing season, beginning at the flowering stage (15th January) with 15 days intervals.Disease severity , yield and yield components were estimated as mentioned before.

Table (3) List of tested fungicides show the Common name, chemical structure, active ingredients and rates of application.

Common name	Active ingredient in chemical structure	Application doses in greenhouse and field
Dithane- M45	Mancozieb (zinc, manganese ethylene bis dithcarbomate related maneb and zineb 80%).	250 g / 100L
Tridex -80%	Mancozieb (manganese ethylene bisdithocarbomate) polymeric complex with zinc salt (80%).	250 g / 100L
Polyram-DF	Metiram complex (Tris(amine (ethylen bis (dithocarbomate)) zine II (tetrahydro-b2,4,7-dithiadiazocine –3, 8- dithione) polymer 80%).	200 g / 100L
Kocide-101	Cooper hydroxide (77%).	150 g / 100L

c- Effect of varietal reaction on chocolate spot disease:

In this experiment, eight faba bean cultivars, i.e. Giza-3, Giza-40, Giza-429, Giza-461, Giza-667, Giza-717, Giza-716 and Giza-Blanka were evaluated against natural infection with chocolate spot disease under field conditions at El-Bagour, Minoufia during 2000/01 and 2001/02 growing seasons. These faba beanj cultivars were obtained kindly from Legume Department, Field Crops Institute, A.R.C., Giza. This experiment was designed in complete randomized block design with three replicates, each plot 10 m^2. Faba bean cultivars were sown on 12^{th} and 14^{th} November during 2000/2001 and 2001/02 respectively. All other recommendations were followed. Disease severity, yield and yield components were estimated as mentioned before.

d- Effect of sowing date on chocolate spot disease:

This experiment was carried out to know the effect of sowing date on faba bean (Giza-40) chocolate spot disease during seasons 2000/01 and 2001/02. The experiment was conducted in complete randomized block design with four sowing dates started at 1^{st} November and repeated then every two weeks. The first reading of the chocolate spot infection was recorded at 15th and 20^{th} January 2001 and 2002 respectively. Meanwhile, the rest other readings were recorded every 4 weeks interval for three times. Yield and yield components were estimated and recorded as mentioned before.

Statistical analysis procedure:

Statistical analysis was done according to the procedures "*ANOVA*" reported by **Sneddecor and Cochran (1980).**

EXPERIMENTAL RESULTS

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1- Survey of leaf spot disease:

As shown in **Table (4)** surveying of faba bean chocolate spot disease in 128 fields distributed in 26 different locations during two successive growing seasons, i.e. 1998/1999 and 1999/2000 at seven Governorates in the northern parts of the delta and one Governorate in the middle Egypt reveal that severity % of chocolate spot disease was higher in 1999/2000 (5.0-23.5%) than in 1998/99 growing season (2.5-17.0%), with average of disease severity 11.14 and 6.59% respectively. The highest percentage of disease severity was recorded at El-Beheira Governorate in all surveyed fields during both growing seasons where the averaged disease severity during the first season was 12.38% and 19.9% during the second season. On the other hand, the highest disease severity was recorded in El-Nubaria location followed by Damanhour, Koum-Hamada and Metobus in season 1999/2000 while, it was the highest in El-Nubaria and Metobus in season 1998/1999. Meanwhile, Qualubia and Beni-Swief Governorates showed the least disease severity in both seasons where they were 3.25 and 6.75% for Qualubia while, it was 3.0 and 5% for Beni-Swief during the two seasons, respectively.

Table (4): Survey of faba bean chocolate spot disease in eight Governorates during two growing seasons 1998/1999 and 1999/2000.

Governorates	Locations	Number of surveyed fields	%Average of disease severity of chocolate spot	
			98/1999	99/2000
Kafr-El Sheikh	Kleen	3	6.5	13.0
	Sakha	4	7.5	16.5
	Fowa	4	6.0	(*)--
	Sidy-Salem	2	12.0	15.5
	Metobus	4	14.5	20.0
Mean			**9.3**	**16.25**
El-Beheira	El-Nubaria	4	17.0	23.5
	Etay-El Baroud	5	10.0	16.0
	Damanhour	5	12.5	21.5
	Kafr-El Dawar	3	(*)--	18.0
	Koum-Hamada	4	10.0	20.5
Mean			**12.38**	**19.9**
Gharbia	Kafr-El Zayat	6	6.5	12.0
	Tanta	5	8.5	9.5
Mean			**7.5**	**10.75**
Dakahlia	Meet-Ghamr	6	6.5	11.5
	El-Mansoura	4	5.5	14.5
	Aga	3	8.0	13.0
Mean			**6.67**	**13.0**
Sharkia	Faquos	6	3.0	6.0
	El-Hussinia	3	4.0	7.0
	Hehia	4	5.0	7.5
	El-Gaafariya	4	4.5	11.5
Mean			**4.13**	**8.0**
Menuofia	Shebeen El Koum	6	8.0	10.0
	El-Bagour	14	7.5	10.5
	Qewisna	8	4.0	8.0
Mean			**6.5**	**9.5**
Qualubia	Toukh	11	3.0	6.0
	Kafr-Shokr	4	3.5	7.5
Mean			**3.25**	**6.75**
Beni Seawif	Beni-Swief	3	3.5	5
	Beba	3	2.5	--
Mean			**3.0**	**5.0**
Grand Total or Mean	**26**	**128**	**6.59**	**11.14**

(*) -- = The survey is not made in this area.

2- Isolation and identification of chocolate spot pathogens:

a- Frequency % and number of isolated chocolate spot pathogens:

The obtained results in **Table (5)** show the frequency % of fungi isolated from spotted faba bean leaves during two growing seasons 98/99 and 99/2000 in different location and Governorates. The results indicate that Botrytis isolates were the most frequent (%) followed by *Alternaria alternata,* while *Stemphylium botryosum* showed the least frequency during the two seasons.

In this respect, the frequency of *Botrytis* spp was higher in 1998/1999 than in 1999/2000 season with frequency 72.3 and 70.6 % respectively, meanwhile the frequency of *Alternaria alternata* and *Stemphylium botryosum* was low during the two seasons comparing to *Botrytis* spp. Concerning the different Governorates, it is clear that the highest frequency of Botrytis isolates was recorded at El-Menuofia (91.3%) during the first season followed by El-Gharbia (76.9%), Beni-Swief (75.0%), El-Sharkia (72.4%), El-Beheira (72.0%) and El-Qualubia (71.0%) respectively. Meanwhile, at the second season (1999/2000), the highest frequency of Botrytis isolates was recorded at Dakahlia (76.2%), Kafr-El Sheikh (75%), El-Menuofia (73.5%), El-Qualubia (71.9%) and El-Beheira (71.0%) respectively. On the other hand, the least frequency % of Botrytis isolates was recorded at Kafr-El Sheikh (61.2%) during the first season and at El-Gharbia (57.1%) during the second season. Also, *Stemphylium botryosum* was very low in its number

Table (5): Number and Frequency of isolated fungi from spotted faba bean leaves at different Governorates during two growing seasons 1998/1999 and 99/2000.

Governo-rates	Locations	Seasons of isolation							
		1998/1999			Total	1999/2000			Total
		*B	*A	*S		*B	*A	*S	
Kafr El-Sheikh	Kleen	6	9	0	15	13	2	0	15
	Sakha	11	3	0	14	15	1	0	16
	Fowa	9	5	0	14	-	-	-	-
	Sidy-Salem	5	4	2	11	8	0	4	12
	Metobus	10	3	0	13	6	7	0	13
Total		41	24	2	67	42	10	4	56
% Frequency		61.2	35.8	3.0	100	75	18.9	7.1	100
El Beheira	El-Nubaria	14	2	0	16	17	2	0	19
	Etay-El Baroud	9	5	0	14	7	6	0	13
	Damanhour	5	3	0	8	13	2	0	15
	Kafr-El Dowwar	-	-	-	-	2	7	5	14
	Koum-Hamada	8	0	4	12	15	0	0	15
Total		36	10	4	50	54	17	5	76
% Frequency		72	20.0	8.0	100	71.0	22.4	6.6	100
Gharbia	Kafr-El Zayat	11	2	0	13	13	4	0	17
	Tanta	9	4	0	13	13	0	8	11
Total		20	6	0	26	16	4	8	28
% Frequency		76.9	23.1	0	100	57.1	14.3	28.6	100
Dakahlia	Meet-Ghamr	8	4	3	15	14	0	0	14
	El-Mansoura	7	5	0	12	6	7	0	13
	Aga	12	0	2	14	12	3	0	15
Total		27	9	5	41	32	10	0	42
% Frequency		65.9	22.0	12.1	100	76.2	23.8	0	100
Sharkia	Faquos	8	2	0	10	13	0	0	13
	El-Hussinia	11	5	0	16	3	3	9	15
	Hehia	13	3	0	16	16	1	0	17
	El-Gaafaria	10	6	0	16	8	7	0	15
Total		42	16	0	58	40	11	9	60
% Frequency		72.4	27.6	0	100	66.7	18.3	15.0	100
Menuofia	Shebeen-El-kum	15	1	0	16	6	7	3	16
	El-Bagour	15	0	0	15	16	0	0	16
	Qewisna	12	3	0	15	14	3	0	17
Total		42	4	0	46	36	10	3	49
% Frequency		91.3	8.7	0	100	73. 5	20.4	6.1	100
Qualubia	Toukh	12	5	0	17	13	2	0	15
	Kafr-Shokr	10	4	0	14	10	4	3	17
Total		22	9	0	31	23	6	3	32
% Frequency		71.0	29.0	0	100	71. 9	18.8	9.3	100
Beni Swief	Beni-Swief	10	4	0	12	11	6	0	17
	Beba	8	2	0	12	-	-	-	-
Total		18	6	0	24	11	6	0	17
% Frequency		75.0	25.0	0	100	64.7	35.3	0	100
Grand Total		248	84	11	343	254	74	32	360
Grand % Frequency		72.3	24.5	3.2	100	70.6	20.5	8.9	100

* B = *Botrytis* spp. A = *Alternaria* spp. S = *Stemphylium botyrosum.*

and frequency in most tested locations at different Governorates comparing with *Alternaria alternata* and *Botrytis* spp where it is not surveyed in most locations during the two seasons. Furthermore, *Alternaria alternata* ranked the second after *Botrytis* spp in its frequency and number during the two growing seasons where its highest frequency was 35.8 % at Kafr-El Sheikh in the first season and 35.3% in the second season. As for tested locations, the highest frequency number of Botrytis isolates was recorded at El-Nubaria, Hehia and El-Bagour during season 1999/2000 respectively. While it was recorded at Shebeen-El Koum, El-Bagour and El-Nubaria at season 1998/1999 respectively.

b- Frequency % and number of Botrytis isolates:

The previous results presented in **Table (5)** indicated that Botrytis isolates were the highest frequent comparing with the other isolated fungi. Identification of isolated Botrytis isolates indicated that these isolates include *Botrytis fabae* and *B. cinerea* and these isolates were differ also in their distribution, appearance and frequency according to its Governorate, location and field. Thus, the frequency % and number of isolates were recorded individually for each *Botrytis spp*. In this respect, data in **Table (6)** indicate that out of 248 Botrytis isolates obtained during the first season1998/1999, *B. cinerea* occurred at the highest frequency (86.7%); meanwhile, *B. fabae* was less frequency (13.3%). However, this turned was slightly varied during the second season 1999/2000 where *B. fabae* and *B. cinerea isolates* recorded frequency 55.5% and 44.5%, respectively.

Table (6): Frequency of *Botrytis fabae* and *Botrytis cinerea*, which identified as the main causal agents of chocolate spot disease of faba bean at seven Governorates during 1998/1999 and 1999/2000 growing seasons.

Governo-rates	Locations	Seasons of isolation									
		1998 / 1999					1999 / 2000				
		Total isolates	*B. fabae*		*B. cinerea*		Total isolates	*B. fabae*		*B. cinerea*	
			F	%	F	%		F	%	F	%
Kafr El - Sheikh	Kleen	6	6	100	0	0	13	10	77.0	3	23
	Sakha	11	5	45.0	6	54.5	15	13	87.0	2	13
	Fowa	9	0	0.0	9	100	-	-	-	-	-
	Sidy-Salem	5	0	0.0	5	100	8	0	0.0	8	100
	Metobus	10	2	20	8	80	6	0	0.0	6	100
Mean		8.2	2.6	31.7	5.6	68.3	8.4	4.6	54.8	3.8	45.2
El-Beheira	El-Nubaria	14	7	50	7	50	17	14	82.4	3	17.6
	Etay-El Baroud	9	0	0.0	9	100	7	0	0.0	7	100
	Damanhour	5	0	0.0	5	100	13	12	92.3	1	7.7
	Kafr-El Dowwar	-	-	-	-	-	2	0	0.0	2	100
	Koum-Hamada	8	0	0.0	8	100	15	14	93.3	1	6.7
Mean		9.0	1.8	19.4	7.3	80.6	10.8	8.0	74.1	2.8	25.9
Gharbia	Kafr-El Zayat	11	0	0.0	11	100	13	10	76.9	3	23.1
	Tanta	9	0	0.0	9	100	3	0	0.0	3	100
Mean		10.0	0	0.0	10.0	100	8.0	5.0	62.5	3.0	37.5
Dakahlia	Meet-Ghamr	8	0	0.0	8	100	14	14	100	0	0.0
	El-Mansoura	7	0	0.0	7	100	6	0	0.0	6	100
	Aga	12	0	0.0	12	100	12	0	0.0	12	100
Mean		9.0	0	0.0	9.0	100	10.7	4.7	43.8	6.0	56.2
Sharkia	Faquos	8	0	0.0	8	100	13	9	69.2	4	30.8
	El-Hussinia	11	0	0.0	11	100	3	0	0.0	3	100
	Hehia	13	0	0.0	13	100	16	13	81.3	3	18.7
	El-Gaafaria	10	0	0.0	10	100	8	8	100	0	0.0
Mean		10.5	0	0.0	10.5	100	10.0	7.5	75	2.5	25
Menuofia	Shebeen-El Koum	15	13	86.7	2	13.3	6	0	0.0	6	100
	El-Bagour	15	0	0.0	15	100	16	13	81.3	3	18.7
	Qewisna	12	0	0.0	12	100	14	0	0.0	14	100
Mean		14.0	4.3	31.0	9.7	69.0	9.0	4.3	36.1	7.7	63.9
Qualubia	Toukh	12	0	0.0	12	100	13	11	84.6	2	15.4
	Kafr-Shokr	10	0	0.0	10	100	10	0	0.0	10	100
Mean		11.0	0	0.0	11.0	100	11.5	5.5	47.8	6.0	52.2
Beni-Swief	Beni-seawif	10	0	0.0	10	55.6	11	0	0.0	11	100
	Beba	8	0	0.0	8	44.4	-	-	-	-	-
Mean		9.0	0	0.0	18	100	11	0	0.0	11	100
Grand Total		248	33	13.3	215	86.7	254	141	55.5	113	44.5

F= Frequency % = Frequency %

B. fabae recorded the highest frequency (100%) at Kleen (Kafr-El Sheikh) and Shebeen-El Koum (86%) Menuofia during season 1998/1999. *B. fabae* not be detected in Gharbia, Dakahlia, Shakia Qualubia and Beni-Swief Governorates. Meanwhile, the highest frequency of *B. cinerea* (100%) was recorded at Sharkia, Dakahlia, Menuofia, Qualubia and Beni-Swief Governorates while the least frequency was recorded at Kafr-El Sheikh (68.3%). Also, it is pronounced from the obtained results that *B. cinerea* was isolated from all tested locations in all Governorates except Kleen location. In the second growing season 1999/2000, the highest frequency of *B. fabae* isolate was recorded at Sharkia (75%) and El-Beheira (74.1%), meanwhile the least isolation frequency was at Menuofia Governorate (36.1%). On the other hand, the highest isolation frequency of *B. cinerea* was recorded at Menuofia (63.9%) followed by Dakahlia (56.2%), meanwhile the least isolation frequency was at Sharkia Governorate (25%).

3- Pathogenicity test:

a- Determination of chocolate spot disease under greenhouse conditions:

Twenty of *Botrytis* isolates (9 isolate of *Botrytis fabae* and 11 isolate of *B. cinerea*) were chosen based on their frequency at different locations in seasons 98/99 and 99/2000 in order to test their pathogenic abilities on faba bean leaves of Giza-40 under greenhouse conditions. Data in **Table (7)** show that all tested *Botrytis* isolates were differed in their virulence onto faba bean leaves (Giza-40) after 1, 3, 5, 7, and 14 days from inoculation.

Table (7): Virulence of 20 *Botrytis* isolates on faba bean plants (Giza-40) under greenhouse conditions using scale (0-9).

Isolates code No.	Governorates	Locations	Mean of disease severity after days				
			1	3	5	7	14
B. cinerea							
6M-00	**Kafr-El Sheikh**	Metobus	3.3	9.1	13.3	16.6	19.1
5SS-99	**Kafr-El Sheikh**	Sidy-Salem	1.1	4.4	8.8	14.4	17.7
6Sa-99	**Kafr El Sheikh**	Sakha	7.7	18.8	24.4	29.1	38.9
13H-99	**Sharkia**	Hehia	1.1	3.3	5.5	8.8	11.1
5BS-99	**Beni Seawif**	Beni-Swief	1.1	3.3	6.6	11.1	12.2
10KZ-99	**Gharbia**	Kafr-El Zayat	4.4	11.1	14.4	17.7	22.2
14QE-00	**Monofia**	Qewisna	6.6	13.3	16.6	19.1	24.4
12AG-00	**Dakahlia**	Aga	7.7	12.2	16.6	21.1	28.8
9EB-99	**El-Beheira**	Etay-El Baroud	9.9	19.9	23.3	31.1	39.9
7NB-99	**El-Beheira**	El-Nubaria	9.9	14.4	16.6	23.3	27.7
10KS-00	**Qualubia**	Kafer- shokr	3.3	7.7	11.1	15.5	17.7
B. fabae							
11T-00	**Qualubia**	Toukh	14.4	18.8	42.2	61.1	76.6
13KZ-00	**Gharbia**	Kafr-El Zayat	22.2	27.7	52.2	61.1	78.8
9FQ-00	**Sharkia**	Faquos	17.7	25.5	54.4	69.1	81.1
14NB-00	**El-Beheira**	El-Nubaria	34.4	63.3	81.1	92.2	98.8
12D-00	**El-Beheira**	Damanhour	24.4	42.2	63.3	77.7	86.6
6K-99	**Kafr-El Sheikh**	Kleen	27.7	52.2	67.7	81.1	92.2
13Sa-00	**Kafr-El Sheikh**	Sakha	29.1	58.8	72.2	85.5	94.4
14MGh-00	**Dakahlia**	Meet-Ghamr	25.5	45.5	65.5	83.3	88.8
13Ba-00	**Monofia**	El-Bagour	32.2	48.8	65.5	78.8	87.7
L.S.D at 5%			1.46	1.41	1.3	1.4	1.61

Also the results clearly indicate that chocolate spot severity incited by *B. fabae* was higher than those incited by *B. cinerea.* Moreover, the disease severity of the isolates of the two Botrytis types was increased gradually by increasing period post inoculation from 1to 14 days.

In this respect, out of 11 *B. cinerea* isolates, Etay-El Baroud isolate (Beheira) gave the highest disease severity being 39.9% after 14 days followed by Sakha isolate (Kafr-El Sheikh) being 38.9% while, the least chocolate spot disease severity 11.1% was recorded by Hehia isolate (Sharkia). As for chocolate spot disease severity incited by tested isolates of *B. fabae*, El-Nubaria isolate recorded the highest disease severity (98.8%), followed by Sakha and Kleen isolates which gave disease severity of 94.4 and 92.2% respectively with significant difference between them. Meanwhile, Qualubia isolate (Toukh) recorded the least disease severity 76.6%.

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

Data in **Table (8)**, indicate that the ten tested Botrytis isolates were differ clearly in their pathogenicity on faba bean leaflets of Giza-40 cv. In this respect, isolates of *Botrytis fabae* were more virulent than those of *B. cinerea* where, *B. fabae* (El-Nubaria isolate) was the most virulent followed by Sakha isolate that resulted in the highest average of disease severity after 7 days being 8.91 and 8.19 respectively. Meanwhile, the least pathogenic isolate was *B. cinerea* (Sakha isolate) where its average disease severity was 1.19. Generally, the disease severity of the two types of tested Botrytis isolates were increased gradually post inoculation from the first day to reach maximum after 7 days post inoculation. Other Botrytis isolates were intermediate in their virulence.

Table (8): Virulence of 2 isolates of *Botrytis cinerea* and 8 isolates of *B. fabae* on faba bean plants (Giza-40) using detached leaves technique (scale 0-9).

Isolates code No.	Locations	Mean of disease severity after days			
		1	3	5	7
B. cinerea					
6Sa-99	Sakha	0.24	0.57	0.81	1.19
9EB-99	Etay-El Baroud	0.43	0.95	1.19	1.57
B. fabae					
11T-00	Toukh	1.24	1.52	2.0	2.81
13KZ-00	Kafr-El Zayat	1.43	1.86	2.34	3.28
9FQ-00	Faquos	1.62	2.86	3.55	4.43
14NB-00	El- Nubaria	4.24	5.91	7.19	8.91
12D-00	Damanhour	2.0	3.91	4.71	5.52
13Sa-00	Sakha	3.33	5.09	6.48	8.19
14MGh-00	Meet-Ghamr	3.00	4.62	5.95	7.62
13Ba-00	El-Bagour	2.43	4.34	5.43	6.91
LSD at 5%		0.103	0.147	0.172	0.139

3- Detecting similarity and diversity between 10 Botrytis isolates using RAPD-PCR technique (Random amplified polymorphism DNA):

As shown in **Figures (2 & 3)**, the cluster analysis of similarity coefficients based on RAPD profiles revealed discrete clusters of isolates, possibly representing different linkages among 10 isolates of *Botrytis* spp isolated from faba bean. These corresponded to differences in pathogenicity characteristics. DNA of ten isolates of Botrytis spp. (2 isolates of *B. cinerea* and 8 isolates of *B. fabae*) were amplified using RAPD analysis of specific primer-2-6dto characterize *Botrytis* spp isolates.

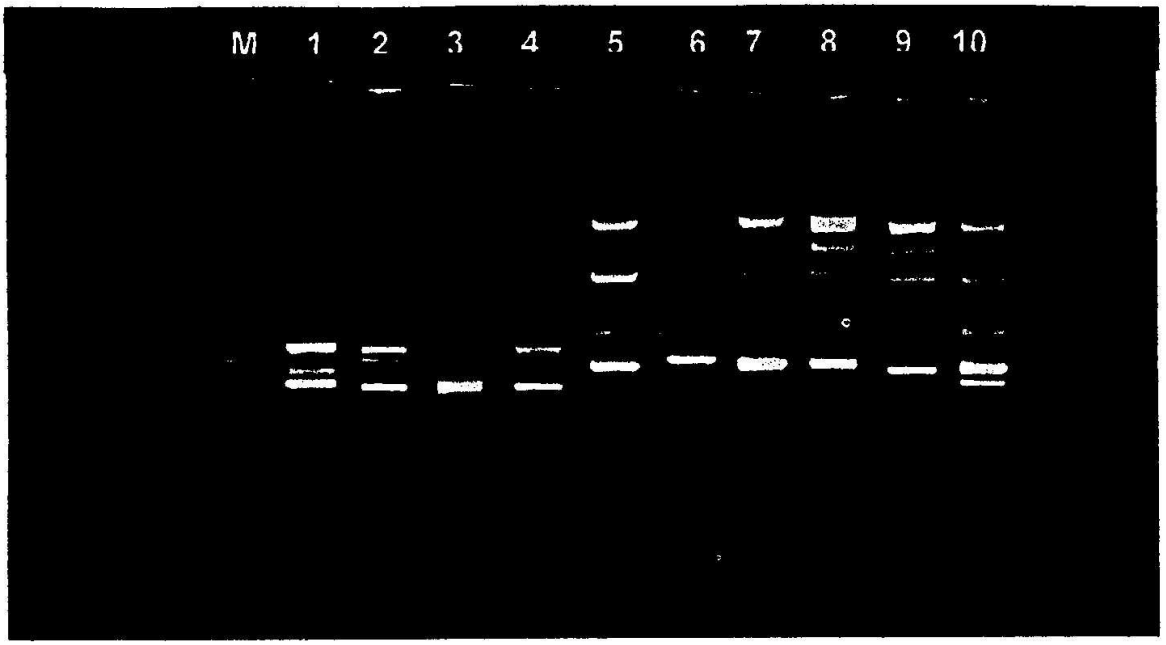

Fig. (2): RAPD-PCR analysis of 10 Botrytis isolates using the specific primer-2-6-d

Botrytis fabae (Lanes 3,4,5,6,7,8,9 and 10)

Botrytis cinerea (Lanes 1 & 2)

(M): Molecular weight of the Marker

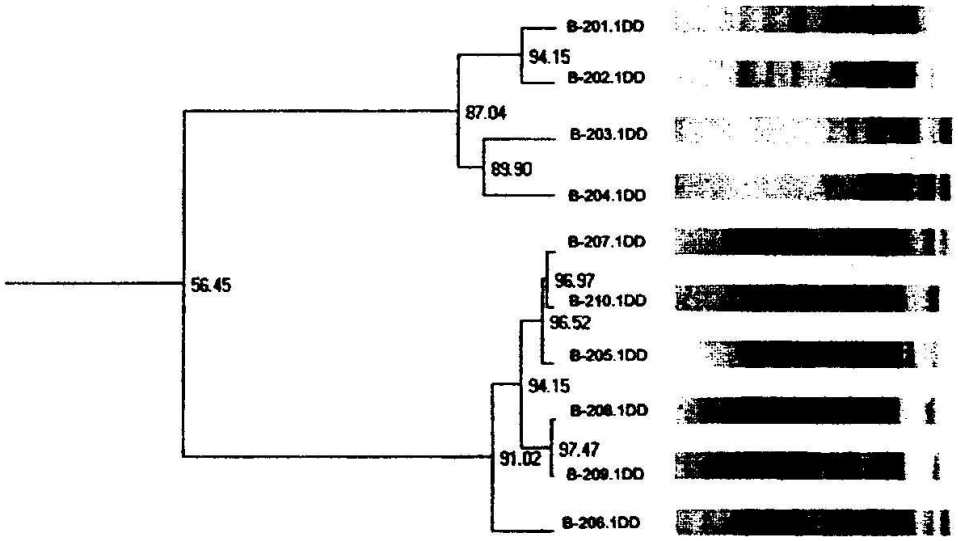

Fig. (3): Dendogram cluster analysis of 10 Botrytis isolates using the specific primer-2-6-d similarity values are indicated, and final linkages for the subclusters are marked.

1-Sakha isolate
2-Etay-El Baroud isolate
3-Toukh isolate
4-Kafr-El Zayat isolate
5-Faquos isolate
6-El- Nubaria isolate
7-Damanhour isolate
8-Sakha isolate
9-Meet-Ghamr isolate
10-El-Bagour isolate

The RAPD amplification analysis revealed that the ten Botrytis isolates could be subdivided into main clusters with similarity 56.45% in between. In this respect, two *Botrytis cinerea* isolates were distinguished as single strain where the similarity between them was 94.15%. As for the eight *B. fabae* which revealed different clusters with primer-2-6d. In the first cluster, the similarity between isolates 7and10 was 96.97% while, it was 96.52% between them and isolate 5. The second cluster was for isolates 8 and 9 with similarity between them 97.47%.

It is clear also that similarity between the isolates of first and second clusters was high being 94.15%. The third cluster was isolate 6, which revealed similarity 91.02 with the isolates of clusters 1 and 2. Isolates 3 and 4 of *B. fabae* represented the fourth cluster were similar to each with 89.90% but those two isolates were more similar to the *B. cinerea* isolates with 87.04% than other *B. fabae* isolates. The fourth cluster of *B. fabae* and the cluster of two *B. cinerea* had low similarity with the other clusters of *B. fabae* being 56.45%.

5- Factors affecting growth, sporulation and scleroial formation of *Botrytis fabae* and *B. cinerea* isolates:

a- Effect of type of media:

This experiment was carried out to study the effect of nutrition in form of different media (synthetic and semi-synthetic) on growth, sporulation and sclerotial formation of the tested Botrytis isolates *in vitro*

Results in **Table (9a)** show that faba bean seed agar media (FBSA) was the best favourable medium for growth of all

tested *Botrytis* isolates followed by the PDA and Faba bean leaf agar media (FBLA). Meanwhile, culturing Botrytis isolates on Czapek's medium (synthetic) was not favourable for fungal growth. Also, growth of *B. cinerea* isolates was faster than those of *B. fabae* onto all tested media. On the other hand, the highest linear growth was produced in case of *B. cinerea* isolated from Qualubia (Kafer-shoker) on FBSA followed PDA and FBLA media.

As for spore formation, results indicated that the tested Botrytis isolates were differed in their behavior by differing the source of nutrition where FBLA medium was the best favourable medium for spore production which produced 7.93×10^6 spore/ml followed by FBSA and media PDA which produced 0.47×10^6 and 0.24×10^6 spore/ml respectively while Czapek's media resulted the least number of spore production which produced 0.11×10^6 spore/ml.

Most isolates of *B. fabae* produced number of spores less than that produced by the *B. cinerea*. The highest number of spores was produced by *B. cinerea* Sakha isolate followed by Ety- El Baroud isolate while the least was Beni-Swief isolate on FBLA medium.

Regarding sclerotial formation and their size, Data in **Table (9b)** show that PDA was the best favourable medium for sclerotial formation followed by FBSA medium. Relatively very few number of sclerotia was recorded when *Botrytis* isolates was cultured on Czapek's medium.

Table (9a): Linear growth rate and sporulation of different isolates of *B. fabae* and *B. cinerea* as affected by different media.

Isolates code No.	Location	Linear growth after 3 days (mm)				Mean	No. of spores/ml x 10^6				Mean
		PDA	FBLA	FBSA	Cz		PDA	FBLA	FBSA	Cz	
B. cinerea											
6M-00	Metobus	64.0	52.3	81.3	43.7	60..3	0.2	18.4	0.0	0.2	4.7
5SS-99	Sidy-Salem	41.0	43.0	76.0	32.0	48.0	0.4	16.3	1.5	0.0	4.6
6Sa-99	Sakha	71.7	61.0	86.3	40.7	64.9	0.1	23.2	1.3	0.5	6.3
13H-99	Hehia	68.7	51.3	61.3	26.0	51.8	0.0	2.0	0.3	0.0	0.6
5BS-99	Beni-Swief	42.3	45.3	57.0	22.0	41.7	0.0	0.5	0.0	0.0	0.13
10KZ-99	Kafr-El Zayat	46.0	30.0	68.3	28.0	43.1	0.0	16.8	0.0	0.0	4.2
14QE-00	Qewisna	74.0	56.3	71.7	38.7	60.2	0.7	14.0	1.6	0.7	4.3
12AG-00	Aga	54.3	48.0	83.7	43.3	57.3	0.3	13.1	0.0	0.1	3.4
9EB-99	Etay-El Baroud	61.0	51.3	78.0	31.0	55.3	1.8	19.0	3.0	0.0	5.95
7NB-99	El-Nubaria	54.3	49.0	74.3	19.3	49.2	0.0	1.3	0.0	0.0	0.33
10KS-00	Kafer- shokr	80.3	60.7	87.7	52.0	70.2	0.1	19.8	0.0	0.3	5.1
B. fabae											
11T-00	Toukh	32.3	42.0	31.0	9.30	28.7	0.0	0.7	0.1	0.0	0.2
13KZ-00	Kafr-El Zayat	49.7	34.3	63.0	21.0	42.0	0.3	0.4	0.1	0.0	0.2
9FQ-00	Faquos	41.3	44.7	47.3	14.0	36.8	0.0	1.2	0.4	0.07	0.42
14NB-00	El-Nubaria	51.3	36.0	50.3	16.0	38.4	0.2	2.0	0.5	0.15	0.71
12D-00	Damanhour	34.0	37.7	43.3	10.3	31.3	0.1	1.5	0.2	0.08	0.47
6K-99	Kleen	41.0	24.7	38.3	7.30	27.8	0.3	3.0	0.1	0.06	0.87
13Sa-00	Sakha	52.3	21.3	52.7	12.3	34.7	0.2	1.6	0.0	0.0	0.45
14MGh-00	Meet-Ghamr	33.53	32.7	56.7	18.7	35.41	0.0	2.8	0.3	0.0	0.78
13Ba-00	El-Bagour	40.3	37.3	60.0	17.0	38.7	0.0	1.0	0.0	0.0	0.25
Mean		51.7	42.95	63.4	25.13	-	0.24	7.9	0.5	0.11	-

LSD at 5% for:	Isolates	0.922	0.027
	Media	2.061	0.061
	I x M	4.123	0.121

PDA= Potato dextrose agar medium FBLA= Faba bean leaf agar medium FBSA= Faba bean seed agar medium Cz= Czapek's medium

Table (9b): Number and size of sclerotia of different isolates of *B. fabae* and *B. cinerea* as affected by different media.

Isolates code No.	Location	No. of sclerotia in cm^2				Mean	Size of sclerotia(mm)				Mean
		PDA	FBLA	FBSA	Cz.		PDA	FBLA	FBSA	Cz.	
B. cinerea											
6M-00	Metobus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5SS-99	Sidy-Salem	1.1	0.0	0.0	0.0	0.28	6.0	0.0	0.0	0.0	1.5
6Sa-99	Sakha	1.6	0.0	1.3	1.2	1.03	7.0	0.0	4.0	3.5	3.63
13H-99	Hehia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5BS-99	Beni-Swief	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10KZ-99	Kafr-El Zayat	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14QE-00	Qewisna	2.8	0.0	1.1	0.0	0.98	4.5	0.0	3.5	0.0	2.0
12AG-00	Aga	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9EB-99	Etay-El Baroud	3.9	0.0	0.0	0.0	0.98	3.6	0.0	0.0	0.0	0.9
7NB-99	El-Nubaria	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10KS-00	Kafer- shokr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B. faba											
11T-00	Toukh	43.9	0.0	42.0	0.0	21.5	2.0	0.0	1.5	0.0	0.9
13KZ-00	Kafr-El Zayat	60.0	0.0	25.6	15.3	25..23	1.3	0.0	1.1	1.2	0.9
9FQ-00	Faquos	20.7	0.0	38.2	8.2	16. 8	1.5	0.0	1.9	1.0	1.1
14NB-00	El-Nubaria	28.7	0.0	32.2	18.9	19..95	2.0	0.0	1.6	1.3	1.23
12D-00	Damanhour	44.4	0.0	26.7	12.7	21.0	2.3	0.0	1.3	2.8	1.6
6K-99	Kleen	41.8	0.0	24.0	13.3	19.8	1.1	0.0	1.3	1.0	1.3
13Sa-00	Sakha	39.2	0.0	22.8	16.7	19.7	1.1	0.0	1.8	1.1	1.0
14MGh-00	Meet-Ghamr	56.7	0.0	53.6	1.1	27.9	1.1	0.0	2.0	1.5	1.2
13Ba-00	El-Bagour	40.0	0.0	48.0	6.9	23.73	2.8	0.0	2.8	1.8	1.9
Mean		19..24	0.0	15.8	4.72	-	1.82	0.0	1.14	0.76	-

LSD at 5% for:	Isolates	0.922	0.027
	Media	2.061	0.061
	I x M	4.123	0.121

PDA= Potato dextrose agar medium FBLA= Faba bean leaf agar medium FBSA= Faba bean seed agar medium Cz= Czapek's medium

The isolates of fabae type produced small size of sclerotia in large number, while the isolates of cinerea type did not produce sclerotia or produce sclerotia large in size but less in number.

b- Effect of temperature:

Results in **Table (10)** reveal that temperature degrees affecting growth and sporulation of tested Botrytis isolates. All isolates were able to grow at all temperature degrees ranging between 10-30°C. In general, temperature ranging from 15 to 20°C gave the best linear growth where, the average growth rate at these degrees of temperature ranged from 41.8-53.3mm after 4 days post inoculation. While it was 19.0, 37.8 and 31.2 mm post inoculation at 10, 25 and 30°C respectively. Also, *B. cinerea* (Sakha isolate), followed by Etay ELBaroud isolate gave the best growth, being 61.6 and 50.7mm respectively, while lowest growth (21.1mm) was produced by *Botrytis fabae* (Sakha isolate).Also, the results show that all tested Botrytis isolates were significantly differed in their capability to produce spores as affected by temperature. Generally, isolates of *B. cinerea* sporulated more abundantly than *B. fabae* ones. Botrytis isolates grown at 10°C on FBLA medium could not be able to sporulate. Maximum sporulation being 127.9×10^5spores/ml was produced by *B. cinerea* Sakha isolate.

The fewer one was 1.0×10^5 spores/ml that recorded with *B. fabae* isolated from Kafer EL Zayat. Other tested isolates were intermediate in this respect.

Table (10): Effect of different temperature degrees on linear growth and sporulation of *Botrytis* isolates on FBLA medium.

Isolates code No.	Locations	Linear growth (mm) after 4 days of incubation at °C					X̄	Average number of spore/ml (x10^5) at °C					X̄
		10	15	20	25	30		10	15	20	25	30	
B. cinerea													
6Sa-99	Sakha	36.0	65.7	86.3	72.0	48.0	61.6	0.0	162.0	232.0	176.0	69.6	127.9
9EB-99	Etay El Barod	32.0	58.0	68.0	55.0	40.3	50.7	0.0	132.0	190.0	137.8	47.0	101.4
B. fabae													
11T-00	Toukh	20.0	45.3	54.0	38.0	33.3	38.1	0.0	5.6	7.0	1.8	0.14	2.9
13KZ-00	Kafer-El Zayat	13.0	31.7	44.7	28.0	25.7	28.6	0.0	0.2	4.0	0.7	0.08	1.0
9FQ-00	Faqous	22.3	49.0	57.7	41.3	36.3	41.3	0.0	8.6	12.0	2.0	0.24	4.6
14NB-00	El-Nubaria	16.3	34.3	48.3	29.7	27.3	31.2	0.0	14.6	19.5	4.8	0.39	7.9
12D-00	Damanhour	18.0	41.0	52.2	35.0	30.3	35.3	0.0	11.0	15.0	2.8	0.20	5.8
13Sa-00	Sakha	8.3	26.3	30.7	21.3	19.0	21.1	0.0	11.8	16.0	3.0	0.22	6.2
14MGh-00	Meet-Ghamr	10.3	29.3	41.0	25.3	24.0	26.0	0.0	20.8	27.0	5.9	0.45	10.9
13Ba-00	El bagour	13.7	37.7	50.0	32.7	28.0	32.4	0.0	0.7	10.0	0.2	0.01	2.2
X̄		19.0	41.8	53.3	37.8	31.2	-	0.0	36.8	53.0	33.5	11.8	-

LSD at 5% for:

Isolates (I)	0.22	0.365
Temperature (T)	0.31	0.516
I xT	0.71	1.153

No. of spores as an average of 3 replications, after 12 days of incubation.

6- Factors affecting chocolate spot disease severity:

a- Inoculum potential:

In this experiment, spore suspension of *Botrytis fabae* isolated from EL-Nubaria at five different concentrations (0.75×10^5, 1.25×10^5, 2.5×10^5, 5.0×10^5 and 6.0×10^5 spores/ml) were tested for their efficacy in inciting faba bean chocolate spot disease.

Data in **Table (11) and Figure (3),** reveal that increasing spore density increased gradually disease severity with clearly significant differences between treatments. The highest infection (100%) was observed 14 days post spraying faba bean plants cv Giza-40 with the highest inoculum density of spore suspension (6.0×10^5 spores/ml). Meanwhile the lowest reading of severity (48.9%) was recorded with the lowest spore density (0.75×10^5spores/ml). Results also showed that 2.5×10^5 spores/ml could be considered the most convenient inoculum density for inoculation.

Table (11): Effects of inoclum potential of *Botrytis fabae* (Nubaria isolate) on chocolate spot disease of faba bean under green house conditions.

Inoclum potential (spores/ml)	Disease severity after (days)			
	2 d	4 d	7 d	14 d
0.75×10^5	14.8	22.2	34.1	48.9
1.25×10^5	26.7	30.3	47.4	67.4
2.5×10^5	34.7	48.1	68.1	88.9
5.0×10^5	42.2	60.0	83.7	94.8
6.0×10^5	54.1	72.6	92.6	100.0
L.S.D at 5%	1.53	2.81	2.28	2.75

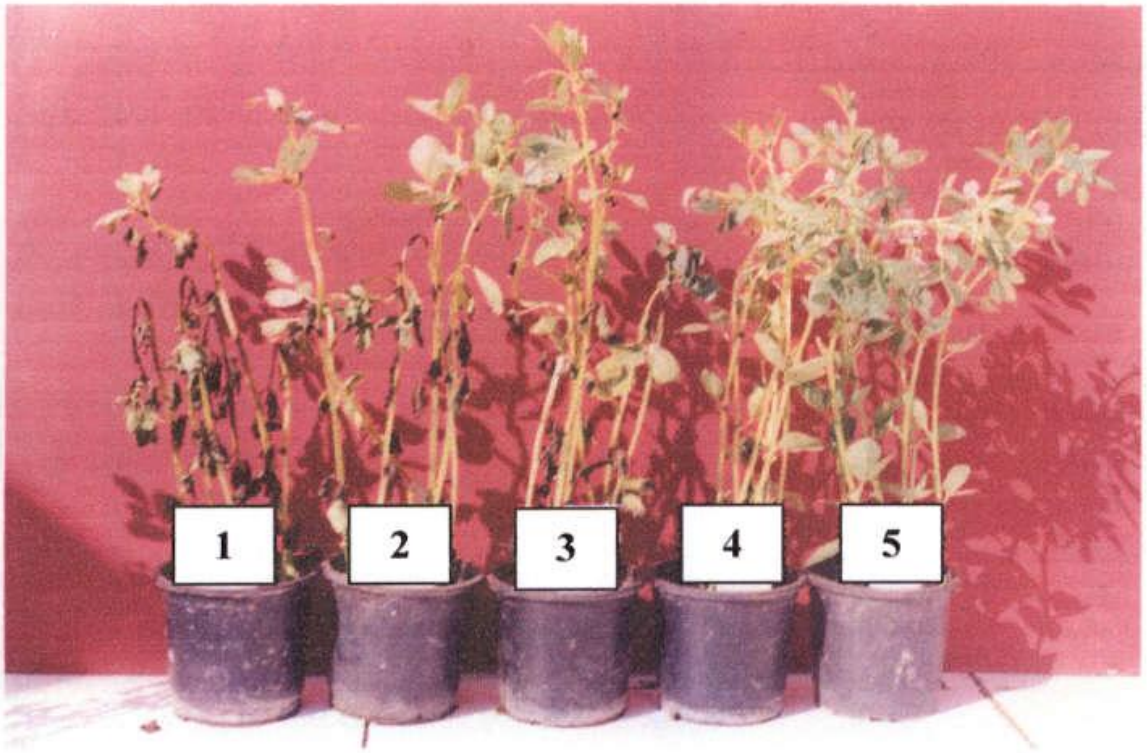

Fig (4): show the effect of different levels of *Botrytis fabae* inoculum potential (EL-Nubaria isolate) on chocolate spot infection of Giza-40.

1 = 6.0×10^5 spores/ml 2 = 5.0×10^5 spores/ml 3 = 2.5×10^5 spores/ml

4 = 1.25×10^5 spores/ml 5 = 0.75×10^5 spores/ml

b- Spore age:

In this experiment, *B. fabae*-14NB-00 (El-Nubaria isolate) and *B. cinerea*-9EB-99 (Etay-El Baroud isolate) were tested to know the best spore age incited highest chocolate spot disease severity on detached leaves of faba bean Giza-40.

It is clear from results in **Table (12)** that the effect of spore age on disease severity is pronounced. In this respect, the highest average of disease severity (1.97 and 8.73) was obtained from one week (young spores) in case of tested *B. cinerea* isolate and 2 week old spores of *B. fabae* after 7 days post inoculation.

Inoculation of detached faba bean leaflets with 3 weeks old spores caused significant reduction in average disease severity of *Botrytis cinerea* and *B. fabae* isolates where they were 1.07 and 6.47 respectively.

Table (12): Effect of spore age of *Botrytis fabae* and *B. cinerea* on chocolate spot disease disease severity using detached leaves technique.

Spore age (weeks)	Isolates code No.	Isolates	Average Disease severity after days			
			1	3	5	7
1	9EB-99	*B. cinerea*	0.66	1.23	1.63	1.97
	14NB-00	*B. fabae*	3.60	5.03	5.83	7.73
2	9EB-99	*B. cinerea*	0.43	1.0	1.33	1.58
	14NB-00	*B. fabae*	4.13	5.83	7.17	8.73
3	9EB-99	*B. cinerea*	0.29	0.64	0.86	1.07
	14NB-00	*B. fabae*	3.27	4.43	5.23	6.47
L.S.D at 5%			0.18	0.16	0.20	0.23

c- Plant age:

Data in **Table (13)** show that plant aged 41 days were the most suitable age for infection with *Botrytis fabae* followed by 65 days old plants when the disease severity was scored after 7 days post inoculation, where it was 92.55% and 87.44% respectively. While the least disease severity was 31.88% when recorded onto 17 days-old faba plants. Infection has significantly increased by increasing plant aging from 17 to 41 days then decreased at 53 and increased again. Also, increasing incubation days increased gradually disease severity.

Table (13): Effect of faba bean age on infection with *Botrytis fabae* under greenhouse conditions using scale (0-9).

Plants age (days)	Disease severity after (days)			
	2	4	7	14
17	12.55	17.77	31.88	43.0
29	16.33	25.22	43.66	59.44
41	43.0	76.33	92.55	99.22
53	31.11	50.33	82.22	95.55
65	38.55	69.66	87.44	98.44
L.S.D at 5%	2.11	2.55	2.22	2.66

d- Varietal reaction:

Eight faba bean cultivars were tested for their reaction to *Botrytis* isolates. Results in **Table (14)** and **Figures (5 & 6)**, showed that all tested cvs differed in their reaction by different Botrytis isolates. Giza-40 cv. showed the highest disease severity (66.6%) followed by Giza-667 (65.2%) and Giza-429 (63.8%). This result indicates that Giza-40 is highly susceptible cv. whereas, Giza-Blanka was the least susceptible one (38.8%). Meanwhile, Giza-3 was moderately resistant.

In general, the varietals reaction against chocolate spot disease was significantly affected by the interaction between cvs and isolates. In this respect, *Botrytis fabae* (EL-Nubaria isolate) caused the highest disease severity on Giza-40 cv. (92.6%) while *B. cinerea* (Sakha isolate) caused the least disease severity on Giza-Blanka (20.8%).

Table (14): Interaction between 2 isolates of *B. cinerea* and 8 isolates of *B. fabae* and eight faba bean cultivars using scale 0-9 after 7 days from inoculation.

Isolates code No.	Locations	Average disease severity on cultivars								
		G3	G40	G429	G461	G667	G717	G716	G. Blanka	Mean
B .cinerea										
6Sa-99	Sakha	23.0	30.3	31.9	20.8	31.1	25.9	24.4	19.2	25.9
9EB-99	Etay-El Baroud	28.9	36.3	28.1	23.0	31.9	28.1	23.7	25.2	28.1
B .fabae										
11T-00	Toukh	41.4	49.7	44.4	27.4	57.0	35.6	37.8	31.9	40.7
13KZ-00	Kafr-El Zayat	44.4	61.4	55.6	34.8	68.8	41.4	37.8	34.1	47.3
9FQ-00	Faquos	51.1	69.7	76.3	37.0	65.9	51.1	45.9	41.4	54.8
14NB-00	El- Nubaria	64.4	92.6	88.1	62.2	86.7	69.7	57.0	54.1	71.8
12D-00	Damanhour	56.3	77.8	74.1	48.9	73.3	48.1	40.8	35.6	56.9
13Sa-00	Sakha	62.2	85.9	86.7	50.3	80.0	57.8	59.2	48.1	66.3
14MGh-00	Meet-Ghamr	57.8	83.0	83.7	49.7	83.7	52.6	38.6	53.3	62.8
13Ba-00	El-Bagour	64.4	79.2	68.9	48.1	73.3	45.9	35.6	44.4	57.5
Mean		49.5	66.6	63.8	40.2	65.2	45.7	40.1	38.8	-

L.S.D. 5% level for:

Isolates (I)	0.58
Cultivars (C)	0.65
I * C	1.84

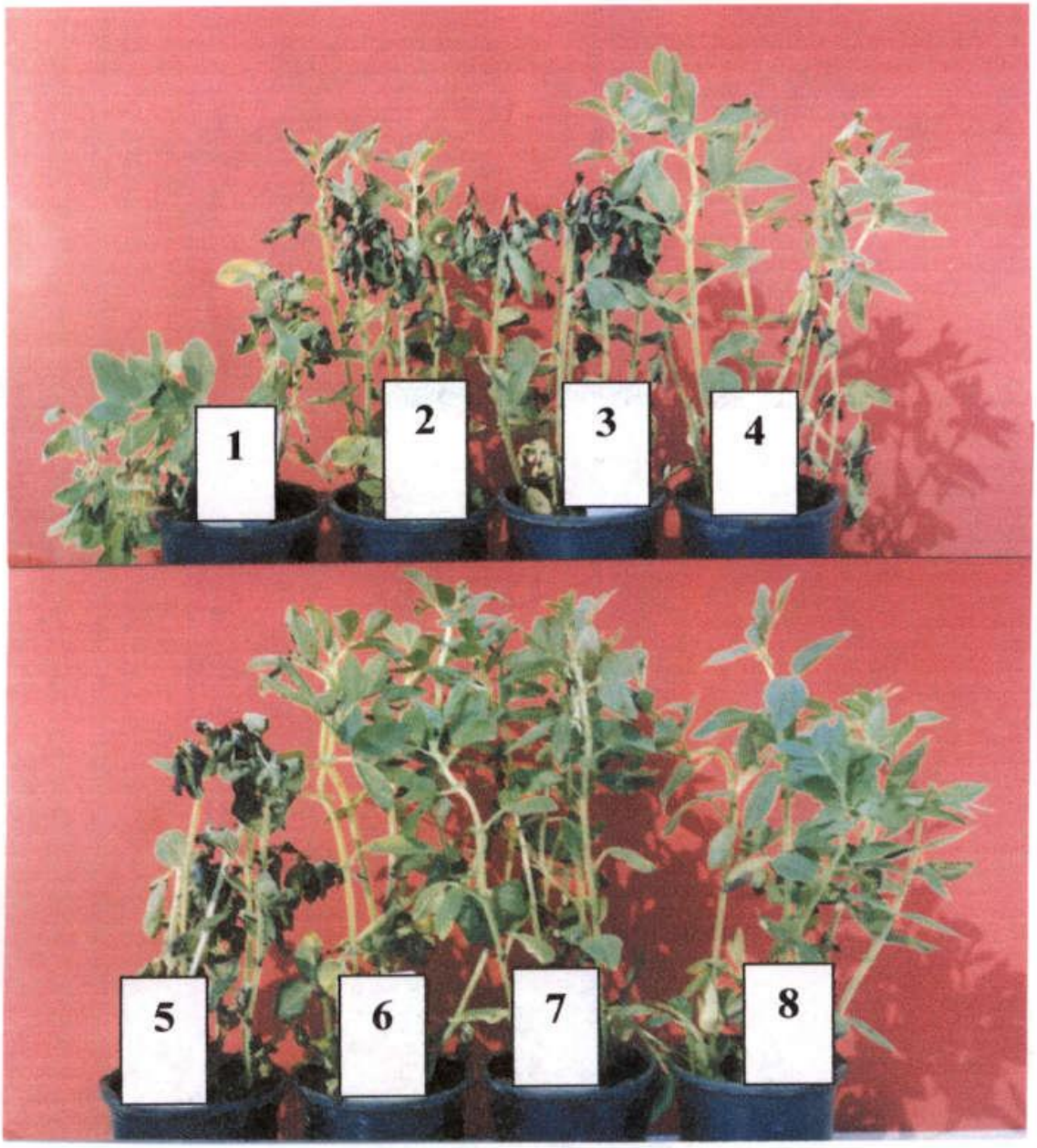

Fig (6): Varietal reaction of eight faba bean cvs to infection with Nubaria isolate of *B. fabae*.

1-Giza-3 2- Giza-40 3- Giza-429

4- Giza-461 5- Giza-667 6- Giza-717

7- Giza-716 8- Giza-Blanka

Fig (7): Varietal reaction of faba bean cv. Giza-40 to two isolates of *B. cinerea* (1-2) and eight isolates of *B. fabae* (3-10) under greenhouse conditions.

C= control	1-Sakha isolate	2-Etay-El Baroud isolate
3-Toukh isolate	4-Kafr-El Zayat isolate	5-Faquos isolate
6-El- Nubaria isolate	7-Damanhour isolate	8-Sakha isolate
9-Meet-Ghamr isolate	10-El-Bagour isolate	

7- Biochemical changes due to infection with *Botrytis fabae*:

a- Sugars content:

Total, reducing and non-reducing sugars were determined in healthy and infected leaves of faba bean cultivars (Giza-40, Giza-3 and Giza-716) at thee different plant ages (30, 45 and 60 days), after 1, 2 and 7 days from inoculation under greenhouse conditions.

Data presented in **Table (15)** show that healthy (non infected) leaves of the high susceptible cultivar (Giza-40) was higher in its total, reducing and non-reducing sugars content comparing with either moderately resistant cultivar (Giza-3) or resistant cultivar (Giza-716) at 45 and 60 days after planting. On the other hand, the infected faba bean leaves with *B. fabae* revealed higher sugar content comparing with healthy leaves in the thee tested varieties at all plant ages (30, 45 and 60 days) compared with the healthy ones. The total sugar content in infected plants of the thee cultivars (Giza-40, Giza-3 and Giza-716) were greatly increased particularly after the 1and 2 days post inoculation compared with healthy leaves. Also, it is clear from results that the total sugars content in the infected leaves of all cvs. was decreased gradually to reach the similar leaves in the healthy leaves after 7 days from inoculation.

Table (15): Sugars content (mg glucose/g fresh weight) of thee faba bean cultivars infected with *Botrytis fabae* (Nubaria isolate) at different plant ages:

*Faba bean cvs	plant age (days)	Days after inoculation	Sugars content (mg/g fresh weight)					
			Total sugars		Reducing sugars		Non reducing sugars	
			Infected	Non infected	Infected	Non infected	Infected	Non infected
Giza-40 (S)	30	1	45.14	19.15	9.19	3.85	35.95	15.30
		2	37.7	19.17	7.62	4.02	30.08	15.15
		7	20.11	20.50	4.12	4.27	15.99	16.23
	45	1	51.63	24.29	10.11	5.42	41.52	18.87
		2	41.12	25.0	8.49	5.49	32.63	19.51
		7	25.28	26.05	5.46	5.55	19.82	20.5
	60	1	52.20	32.65	10.55	6.21	41.65	26.44
		2	42.71	32.76	8.67	6.23	34.04	26.53
		7	33.12	35.09	6.08	6.81	27.04	28.28
Giza-3 (MS)	30	1	43.86	20.57	9.10	4.32	34.76	16.25
		2	36.04	21.09	7.42	4.67	28.62	16.42
		7	22.33	22.10	4.67	4.75	17.66	17.35
	45	1	44.06	23.50	9.12	5.09	34.94	18.41
		2	36.61	23.95	7.45	5.42	29.16	18.53
		7	23.35	25.58	5.07	5.53	18.28	20.05
	60	1	48.92	27.74	10.01	5.74	38.91	22.0
		2	40.02	27.93	8.25	5.81	31.77	22.12
		7	30.24	30.28	5.77	5.94	24.47	24.34
Giza-716 (R)	30	1	34.80	16.54	6.31	3.47	28.49	13.07
		2	29.94	16.48	5.86	3.61	24.08	12.87
		7	18.79	20.41	3.68	4.21	15.11	16.20
	45	1	42.93	22.37	8.73	4.76	34.20	17.61
		2	35.64	22.53	7.11	4.90	28.53	17.63
		7	22.59	23.27	4.97	5.00	17.62	18.27
	60	1	46.57	26.40	9.42	5.62	37.15	20.78
		2	39.0	26.48	7.99	5.64	31.01	20.84
		7	29.24	29.07	5.69	5.83	23.55	23.24

*S = susceptible MS = moderately susceptible R = resistant

b- Phenolic compounds:

Data in **Table (16)** show that the amounts of total, free and conjugated phenols were higher in faba bean leaves of cvs Giza-40, Giza-3 and Giza-716 due to infected with *B. fabae* compared with the healthy leaves.

In this respect, the total and free phenols contents were higher either in healthy or infected leaves of the resistant variety (Giza-716) compared with the moderate and high susceptible cvs (Giza-3 and Giza-40), this turned was true at different plant ages and days after inoculation. In all cases, the highest increase in total and free phenols content was recorded at 60 days after planting after one day from inoculation. Both total and free phenols were decreased gradually in the infected leaves and reach approximately some level in healthy leaves after 7 days from inoculation. While the least increase in total and free phenols was recorded in the infected leaves of 30 days old plants and 7 days post inoculation. As for conjugated phenols, it is pronounced that infection with *B. fabae* increased almost this kind of phenols at 30 and 60 days after planting comparing with the healthy ones while it decreased in the thee varieties almost at 45 days after planting. The conjugated phenols content were higher in the resistance cv Giza-716 compared with the moderate and high susceptible cvs Giza-3 and Giza-40 particulary at 1 day post inoculation. The greatest values of the conjugated phenols were recorded in infected leaves of all tested faba bean cvs at one day post inoculation while the least values were at 7 days. The highest amount of conjugated phenols was 14.63 mg catechol/g fresh weight at one day post inoculation of the 60 day

sowing period in infected leaves of Giza-716.

Generally, in the present study, it was found that phenolic compounds accumulated faster in resistant cultivar than in susceptible cultivars as a result of infection.

Table (16): Phenolic content (mg catechol/g fresh weight) of thee faba bean cvs. infected with *Botrytis fabae* (Nubaria isolate) at different plant ages.

Faba bean cvs	plant age (days)	Days after inoculation	Phenolic content (mg/g fresh weight)					
			Total phenols		Free phenols		Conjugated phenols	
			infected	Non infected	infected	Non infected	infected	Non infected
Giza-40 (S)	30	1	24.70	10.45	17.47	6.64	7.23	3.81
		2	20.0	11.03	13.75	7.76	6.25	3.36
		7	12.41	12.96	9.79	10.0	2.62	2.96
	45	1	22.01	15.32	19.62	12.10	2.39	3.22
		2	17.89	15.75	16.61	12.24	1.28	3.51
		7	16.41	16.47	13.24	12.82	3.17	3.65
	60	1	30.98	17.47	21.62	14.25	10.36	3.22
		2	23.15	18.04	17.90	14.25	5.25	3.79
		7	18.76	18.90	14.62	15.75	4.10	3.15
Giza-3 (MS)	30	1	29.71	11.57	19.47	8.93	10.24	2.64
		2	22.41	11.53	16.39	9.28	6.02	2.25
		7	13.63	14.18	10.74	11.0	2.89	3.18
	45	1	24.35	16.62	22.33	13.03	2.02	3.59
		2	19.10	16.79	17.19	13.18	1.91	3.61
		7	17.66	17.34	13.96	13.96	3.70	3.38
	60	1	33.11	18.35	21.33	14.97	11.78	3.38
		2	23.76	18.91	18.59	15.11	6.19	3.80
		7	19.62	19.73	14.90	15.90	4.72	3.83
Giza-716 (R)	30	1	31.52	14.37	22.04	10.86	9.48	3.51
		2	23.46	14.48	18.61	11.67	4.85	2.81
		7	14.96	15.04	11.67	11.96	3.29	3.08
	45	1	28.64	16.33	24.83	12.46	3.81	3.87
		2	22.45	16.57	19.61	12.6	2.84	3.97
		7	16.90	17.47	14.25	12.83	2.65	4.64
	60	1	37.67	20.05	23.04	15.61	14.63	4.44
		2	25.05	20.62	19.04	15.52	6.01	5.10
		7	20.33	21.48	15.61	16.23	4.72	5.25

*S = susceptible MS = moderately susceptible R = resistant

c- Total free amino acids content.

Total free amino acids were determined in healthy and infected faba bean cvs (Giza-40, Giza-3 and Giza-716) infected with *B. fabae* (Nubaria isolate) at thee different plant ages (30, 45 and 60 days) after 1, 2 and 7 days from inoculation. Data presented in **Table (17)** show that faba bean cv., plant age, and days post inoculation affected the change level in the total free amino acids.

Table (17): Total free amino acids content (mg lucine/g fresh weight) of thee faba bean cvs infected with *Botrytis fabae* (Nubaria isolate).

Faba bean cvs	plant age (days)	Days after inoculation	Total free amino acid (mg/g fresh weight)	
			Infected	Non infected
Giza40 (S)	30	1	3.41	2.23
		2	3.19	2.29
		7	2.29	3.0
	45	1	3.34	2.52
		2	3.17	2.64
		7	2.55	2.69
	60	1	3.28	2.80
		2	3.11	2.95
		7	3.01	2.96
Giza-3 (MS)	30	1	3.31	1.94
		2	3.15	1.96
		7	1.94	2.04
	45	1	3.20	2.36
		2	3.11	2.39
		7	2.56	2.66
	60	1	3.02	1.15
		2	2.74	1.17
		7	1.83	1.86
Giza-716 (R)	30	1	2.99	2.18
		2	2.71	2.12
		7	2.00	2.02
	45	1	2.30	1.83
		2	2.20	1.78
		7	1.29	1.18
	60	1	2.77	1.43
		2	2.66	1.31
		7	1.21	0.96

*S = susceptible MS = moderately susceptible R = resistant

In all cases, the total amino acids was higher in the infected than healthy leaves and maximized 1day after inoculation then decreased gradually to its minimum values 7 days after inoculation. Total free amino acids were increased in both susceptible (Giza-40) and moderately resistant (Giza-3) cvs as a result of infection with *B. fabae*. Meanwhile, they decreased slightly in resistant cv Giza-716 .This turned was true in all tested faba bean cvs. The highest increase in total free amino acids (3.41 mg lucine/g fresh weight) was detected in infected faba bean leaves of Giza-40 plants (30 days old) at one day post inoculation while, the least amount of total free amino acids (1.21 mg lucine/g fresh weight) was found in infected faba bean leaves of Giza-716 plants (60 days old) at 7 days post inoculation. It is clear from results that healthy plants were low in their inner total free amino acids comparing with infected ones.

8-Biological control:

a-Isolation and identification of faba bean phylloplane bioagents:

Fifteen bacterial and seven fungal isolates were isolated from faba bean phylloplane and tested *in vitro* for their antagonistic activity against *B. fabae* (Nubaria isolate) on PDA plates.The seven isolates of the antagonistic fungi were identified as *Trichoderma harzianum* (3 isolates), *T. hamatum* (2 isolates) and one isolates of each *Gliocladium virens and Pacellomyces* spp. Meanwhile, the 15 bacterial isolates were found belonging to 4 genus and six species. These bacterial isolates were identified as *Acinetobacter calcoaceticas* (2

isolates), *Bacillus subtilis* (3 isolates), *Pseudomonas fluorescens* (one isolate), *Pseudomonas aeruginosa* (4 isolates), *Pseudomonas chlororaphis* (one isolate),and *Stenotrphomonas maltophilia* (4 isolates).

b- Effect of antagonists against *B. fabae* on PDA plates:

Data in **Table (18)** indicate that all tested bio-agents decreased the mycelial growth of *B. fabae* isolate markedly to high extent on PDA plates compared with the control. Among antagonistic fungi, *Trichoderma harzianum*-II was the most effective followed by *T. hamatum*-II and *T. harzianum*-III since they recorded the highest inhibition in growth of *B. fabae* i.e 79.6, 67.8 and 64.1% respectively. However, among bacterial isolates *Bacillus subtilis*-I was the most effective to reduced the growth of *B. fabae* (64.1%) compared with the other isolates. On the other hand, *Pacellomyces* spp and *Stentrphomonas maltophilia*-IV followed by *Gliocladium virens* were weakly effective as they reduced *B. fabae* growth by 13.3, 30.8 and 31.5% respectively.Generally, *Trichoderma* isolates were found to be the most effective bio-agents followed by *Bacillus subtilis*–I while *Pacellomyces* spp was the least effective bio-agent in this respect.

c- Effect of culture filtrates against *B. fabae* on PDA plates:

This experiment was carried out to investigate the inhibitory effect of culture filtrates of *Bacillus subtilis*-I, *Ps. fluorescens, T. hamatum*-II and *T .harziunum*-II at different concentrations on linear growth of *B. fabae*.

Table (18): Effect of different antagonists on growth of *B. fabae* (Nubaria isolate) onto PDA plates 6 days post inoculation.

Tested antagonists	Linear growth (mm) of *B. fabae*	Reduction %
Acinetobacter calcoaceticas-I	51.3	43.0
Acinetobacter calcoaceticas-II	54.0	40.0
Bacillus subtilis-I	32.3	64.1
Bacillus subtilis-II	41.3	54.11
Bacillus subtilis-III	34.3	61.9
Stenotrophomonas maltophilia-I	45.0	50.0
Stenotrophomonas maltophilia-II	53.0	41.1
Stenotrophomonas maltophilia-III	44.0	51.8
Stenotrophomonas maltophilia-IV	62.3	30.8
Pseudomonas aeruginosa-I	47.7	47.0
Pseudomonas aeruginosa-II	46.0	48.9
Pseudomonas aeruginosa-III	49.3	45.2
Pseudomonas aeruginosa-IV	50.00	44.4
Pseudomonas chlororaphis	43.3	51.9
Pseudomonas fluorescens	38.0	57.8
Trichderma hamatum-I	37.0	58.9
Trichderma hamatum-II	29.0	67.8
Trichderma harzianum-I	34.7	61.5
Trichderma harzianum-II	18.3	79.6
Trichderma harzianum-III	32.3	64.1
Gliocladium virens	61.7	31.5
Pacellomyces spp.	78.0	13.3
Control	90.0	0.0
L.S.D at 5%	0.108	1.195

Data in **Table (19)** show that all tested culture filtrates at the different concentrations significantly decreased the linear growth of *B. fabae* (Nubaria isolate) in comparison with control treatment.

Increasing concentration significantly increased the inhibitory effect of all tested culture filtrates. In this respect, culture filtrates of *B. subtilis*-I was the most effective in reducing liner growth of *B. fabae* followed by *Ps. Fluorescens*, *T. harzianum*-II and *T. hamatum*-II, as they recorded averages of *B. fabae* growth 57.58, 64.92, 67.92 and 74.42 mm respectively comparing with control treatment. This turned was true for the interaction between antagonists and concentration of culture filtrates.

Table (19): Effect of different concentrations of culture filtrate of antagonists on linear growth (mm) of *B. fabae in vitro* at 20°C after 5 days of incubation.

Tested antagonists	Average linear growth (mm) after 5 days of incubation				Mean
	Concentration %				
	50	37.5	25	12.5	
***Bacillus subtilis*-I**	38.67	56.0	62.67	73.0	57.58
Pseudomonas fluorescens	52.33	61.33	69.33	76.67	64.92
***Trichderma hamatum*-II**	63.33	70.33	78.33	85.67	74.42
***Trichderma harzianum*-II**	57.0	63.67	70.67	80.33	67.92
Control	90.0	90.0	90.0	90.0	90.0
Mean	60.27	68.27	74.20	81.33	-

L.S.D. at 5% for	
Antagonists (B)	0.43
Concentration (C)	0.40
B x C	0.90

d- Effect of antagonists against *B. fabae* on detached leaves:

In this experiment, twenty-two of isolated bacterial and fungal isolates were tested for their antagonistic potentialities against *B. fabae* (Nubaria isolate) the most virulent isolate on faba bean (Giza-40) detached leaves.

Data in **Table (20)** show that all tested antagonists significantly decreased chocolate spot disease severity caused by *B. fabae* on detached leaves after 1, 3, 5 and 7 days of inoculation compared with control. In this respect, the tested bacteria ingeneral had less antagonistic effect than the antagonistic fungi comparing with control (*B. fabae* alone). While, *T. harzianum*-II (0.8), *T. hamatum*-II (0.87), *T. hamatum*-I (1.07) and *Bacillus subtilis*-I (1.27) were the most effective antagonists to reduced infection degree of chocolate spot disease severity after 7 days post inoculation. However, *Pacellomyces* spp, *Stenotrphomonas maltophilia*-IV and *Gliocladium virens* were the least effective in this respect. in most cases, increasing incubation period till 7 days post inoculation increased gradually average disease severity .

e- Effect of antagonists culture filtrates against *B. fabae* on detached leaves:

Different concentrations (100, 75, 50 and 25%) of culture filtrates of four antagonists were used to study their antagonistic reaction on disease severity of *B. fabae* (Nubaria isolate) on faba bean (Giza-40) detached leaves after 7 days post inoculation.

Table (20): Effect of different antagonists on chocolate spot disease severity using faba bean detached leaves technique (scale 0-9).

Tested antagonists	Average Disease severity after (days)			
	1	3	5	7
Acinetobacter calcoaceticas-I	0.73	2.20	2.87	3.33
Acinetobacter calcoaceticas-II	1.40	2.33	3.13	3.73
Bacillus subtilis-I	0.0	0.67	1.0	1.27
Bacillus subtilis-II	0.33	1.13	1.53	1.80
Bacillus subtilis-III	0.07	8.0	1.27	1.40
Stenotrophomonas maltophilia-I	0.67	1.20	1.67	2.13
Stenotrophomonas maltophilia-II	1.07	2.40	3.0	3.47
Stenotrophomonas maltophilia-III	1.60	2.60	3.40	4.07
Stenotrophomonas maltophilia-IV	1.73	2.87	3.80	4.47
Pseudomonas aeruginosa-I	0.60	1.40	2.07	2.40
Pseudomonas aeruginosa-II	0.53	1.27	1.87	2.27
Pseudomonas aeruginosa-III	0.47	1.67	2.33	2.53
Pseudomonas aeruginosa-IV	0.80	1.80	2.60	2.80
Pseudomonas chlororaphis	0.40	1.27	1.40	1.67
Pseudomonas fluorescens	0.20	1.07	1.20	1.47
Trichderma hamatum-I	0.53	0.67	0.80	1.07
Trichderma hamatum-II	0.40	0.47	0.67	0.87
Trichderma harzianum-I	0.80	0.93	1.07	1.27
Trichderma harzianum-II	0.27	0.40	0.60	0.80
Trichderma harzianum-III	1.0	1.40	1.73	2.67
Gliocladium virens	1.40	1.87	2.53	4.27
Pacellomyces spp.	1.60	2.13	2.93	5.0
Control	3.93	5.93	7.87	9.0
L.S.D at 5%	0.107	0.136	0.111	0.112

Data in **Table (21)** indicate that increasing concentration of antagonists culture filtrates from 25% to 100% reduced significantly average disease severity of *B. fabae* from 6.44 to 2.81, respectively. In this respect, culture filtrates of *B. subtilis*-I

was the most effective ones in reducing average disease severity of *B. fabae* followed by *T. harzianum*-II, *Ps. fluorsecens* and *T. hamatum* filtrates where they reduced average disease severity of *B. fabae* to 2.3, 3.24, 3.28 and 4.77 respectively after 7 days post inoculation compared with control treatment 8.50.

Table (21): Effect of some antagonists culture filtrates at different concentrations on chocolate spot disease severity caused by *B. fabae* (Nubaria isolate) using detached leaves technique (scale 0-9).

Tested antagonists	Average Disease severity after 7 days				Mean
	Concentration %				
	100	75	50	25	
***Bacillus subtilis*-I**	0.95	1.71	2.19	4.34	2.30
Pseudomonas fluorescens	1.29	2.19	3.76	5.86	3.28
***Trichderma hamatum*-II**	2.05	3.38	5.76	7.91	4.77
***Trichderma harzianum*-II**	1.29	2.38	3.71	5.57	3.24
Control	8.47	8.47	8.53	8.53	8.50
Mean	2.81	3.63	4.79	6.44	-

L.S.D. at 5% for	
Antagonists (B)	0.07
Concentration (C)	0.08
B x C	0.16

f- Effect of antagonists on chocolate spot disease severity under greenhouse conditions:

Data presented in **Table (22)** indicate that spraying faba bean plants 24 h with any of the tested antagonists before inoculation with *B. fabae* under greenhouse conditions significantly decreased chocolate spot disease severity (9.22-51.11%) compared with control (98.9%) after 14 days from inoculation.

In this respect, *T. harzianum*-II, *T. hamatum*-II and *T. hamatum*-I were the best effective treatments respectively in controlling chocolate spot disease after 14 days post inoculation which recorded 9.22, 13.33 and 16.33 respectively followed by *Bacillus subtilis*-I, *T. harzianum*-I and *Pseudomonas fluoresecens,* which recorded 18.56, 19.22 and 25.22% disease severity respectively. While, *Pacllomyces* spp and *Gliocladium virnes* were the least effective ones in decreasing chocolate spot disease severity caused by *B. fabae* compared with the other tested antagonists tested under greenhouse conditions.

Table (22): Effect of different antagonistes on faba bean chocolate spot disease severity caused by *B. fabae* under greenhouse condition (scale 0-9).

Tested antagonists	Disease severity after days			
	2	4	7	14
Bacillus subitils -I	7.33	14.11	17.0	18.56
Pseudomonas fluoreseens	9.67	16.33	20.0	25.22
Trichoderma hamatum -I	11.11	8.68	13.33	16.33
Trichoderma hamatum -II	5.88	9.66	10.33	13.33
Trichoderma harzianum -I	8.89	13.33	16.33	19.22
Trichoderma harzianum -II	4.44	6.67	7.44	9.22
Trichoderma harzianum-III	10.33	16.33	24.44	30.33
Gliocladium virens	13.33	23.0	31.89	41.11
Pacellomyces spp.	16.33	28.11	36.33	51.11
Control	46.6	70.3	92.5	98.8
L.S.D at 5%	1.49	1.58	1.56	3.38

g- Effect of antagonistic culture filtrates on chocolate spot disease severity:

Data in **Table (23)** indicate that spraying faba bean plants with tested antagonistic culture filtrates either 24 h before or after inoculation with *B. fabae* resulted in significant decrease in chocolate spot disease severity comparing with control treatment. However, spraying antagonistic culture filtrates 24 h before inoculation with *B. fabae* was more effective in reducing disease severity than applying culture filtrates 24 h after inoculation. This turned was true at 2, 4, 7 and 14 days after inoculation. The filtrate of *B. subtilis* was the most effective in this respect recorded the highest decrease in disease severity i.e. 25.22 and 56.33% after 14 days when sprayed 24 h before and after 24 h inoculation with *B. fabae* respectively, followed by *T. harzianum*-II and *P .fluorescens* respectively. While, *T. hamatum*-II was the least effective either used 24h before or after inoculation with *B. fabae* since recoded 59.22 and 70.33 respectively after 14 days from inoculation.

Furthermore, increasing days post inoculation till 14 day increased gradually disease severity.

9- Effect of applying the un-viable heated spore of *B. fabae* as cross protection on chocolate spot disease severity:

This experiment was carried out to study the effect of spraying with un-viable-heated spores of *B. fabae* (Nubaria isolate) on incidence of faba bean chocolate spot disease on both detached leaves *in vito* and whole plants under greenhouse conditions.

Table (23): Effect of spraying antagonists culture filtrates on chocolate spot disease severity under greenhouse conditions (scale 0-9).

Tested antagonists	Filtrates sprayed 24 h. before inoculation				Filtrates sprayed 24 h. before inoculation			
	Disease severity after (days)				Disease severity after (days)			
	2	4	7	14	2	4	7	14
Bacillus subtilis-I	13.33	17	23	25.22	23.66	28.11	39.22	56.33
Pseudomonas fluorescens	15.55	20.77	25.22	31.88	31.88	43	50.33	65.22
Trichderma hamatum-II	21.44	36.33	39.22	59.22	36.33	48.11	56.33	70.33
Trichderma harzianum-II	14.77	17.77	24.44	27.44	26.66	31.11	45.22	62.22
Control	39.22	65.22	89.6	98.77	39.22	65.22	89.6	98.8
L.S.D at 5%	2.22	1.44	1.44	1.11	2.55	1.55	2.22	1.44

Data in **Table (24)** indicate that spraying faba bean detached leaves (under lab condition) with suspension of un-viable heated spores of *B. fabae* spores scored a remarkable depression in chocolate spot disease severity comparing with unsprayed one (control) which being 2.07 and 8.87, respectively after 7 days from inoculation. Similar turned was found on the whole plants under greenhouse conditions.

Table (24): Effect of un-viable spores of *B. fabae* (UHS) on faba bean chocolate spot disease severity on detached leaves and whole plants.

Treatment	Detached leaves				Whole plants			
	Disease severity after (days)				Disease severity after (days)			
	1	3	5	7	2	4	7	14
*UHS	0.93	1.20	1.60	2.07	6.67	20.78	28.11	32.56
Control	4.20	6.33	7.33	8.87	50.33	80.0	92.56	98.89

UHS = Un-viable heated spores of *B. fabae.*

Plant sprayed with un-viable heated spores showed less disease severity of chocolate spot disease after 14 days (32.65%) compared with the control (98.89%).

10- Chemical control of *Botrytis fabae*:

a- On PDA plates:

This experiment revealed the inhibitory effect of tested fungicides on the *in vitro* growth of *Botrytis fabae* was significantly increased as concentration increased from 25 to 200ppm. Results in **Table (25)** indicate that all the tested fungicides suppressed the fungal growth. In this respect, Dithane M-45 was the most effective fungicide in this concern followed by Tridex and Polyram-DF respectively with significant differences among the thee fungicides while, Kocide-101 was the least effective one in this respect.

Table (25): Effect of fungicides on linear growth (mm) of *B. fabae* (Nubaria isolate) at 20°C after 6 days from inoculation *in vitro*.

Fungicides	Average linear growth (mm). Concentration (ppm)						Mean
	0	25	50	100	150	200	
Dithane M.45	90.0	12.33	10.33	8.33	6.0	5.0	22.20
Tridex	90.0	14.0	12.33	9.0	7.33	5.67	23.06
Kocide-101	90.0	70.33	56.33	43.67	37.0	26.67	54.0
Polyram- DF	90.0	60.67	43.0	36.0	27.0	15.33	45.33
Mean	90.0	39.33	30.50	24.25	19.33	13.17	-

L.S.D. at 5% for

Fungicides (F)	0.39
Concentration (C)	0.48
F x I	0.96

b- On detached leaves:

Data in **Table (26)** indicate that the tested fungicides at the different concentrations were differed clearly in their effect against *B. fabae* infection on faba bean leaflets (Giza-40). In this respect, Dithane M-45, Tridex, Kocide-101 were more effective followed by Polyram-DF, the respective average of disease severity recorded after 14 days from inoculation were 0.72, 0.91, 1.75 and 2.24, respectively.

The highest concentration (200 ppm) of all tested fungicides gave the best results especially of Dithane M-45 where recorded the lowest average disease severity after 7 days (0.14).

Table (26): Effect of different fungicides on faba bean chocolate spot disease severity using detached leaves technique (scale 0-9).

Fungicides	Disease severity after 7 days					Mean
	Concentration (ppm)					
	25	50	100	150	200	
Dithane-M45	1.29	1.05	0.71	0.43	0.14	0.72
Tridex	1.52	1.24	0.91	0.57	0.29	0.91
Kocide-101	3.76	2.34	1.43	0.81	0.43	1.75
Polyram-DF	4.71	3.05	1.76	1.05	0.62	2.24
Control	8.01	8.01	8.01	8.01	8.01	8.01
Mean	3,86	34.88	2.56	2.17	1.90	-

L.S.D. at 5% for

Fungicides (F)	0.06
Concentration (C)	0.05
F x I	0.13

c- Under greenhouse condition (in pots):

Four fungicides (Dithane-M45, Tridex, Polyram-DF and Kocide-101 were tested at their recommended doses for their efficacy in controlling faba bean chocolate spot disease under greenhouse conditions.

Data in **Table (27)** show that all tested fungicides were effective in reducing the disease severity of faba bean chocolate spot disease incited by *B. fabae* (Nubaria isolate) when those fungicides were sprayed 24 h before inoculation. The tested fungicides were differed in their effect on disease severity where the highest remarkable reduction in disease severity was recorded onto plants sprayed with Dithane-M45, followed by Tridex, Kocide-101 and Polyram-DF, respectively. The disease severity recorded by these fungicides was 2.99, 7.44, 9.66 and 18.55 respectively after 14 days from inoculation.

Table (27): Effect of some fungicides at recommended doses on faba bean chocolate spot disease under greenhouse conditions (scale 0– 9).

Fungicides	Recommended dose	Disease severity after days			
		2	4	7	14
Dithane-M45	2.5g/l	0.0	0.77	1.44	2.99
Tridex	2.5g/l	0.77	2.99	5.22	7.44
Kocide-101	1.5g/l	4.44	5.88	8.11	9.66
Polyram-DF	2.0g/l	9.55	14.77	16.99	18.55
Control	$2.5x10^5$spore/ml	50.33	79.99	92.55	98.8
L.S.D 5%		2.22	1.53	1.07	1.86

11- Field experiments

a- Effect of P and N fertilization onto chocolate spot disease:

This experiment was carried out in two successive seasons 2000/01 and 2001/02 to investigate the effect of fertilization onto infection with faba bean leaf chocolate spot, yield and yield components.

At the first season 2000/01, data in **Table (28)** indicate that chocolate spot disease severity was affected by different levels of nitrogen and phosphor fertilizers. Plants received of P fertilizer at the highest levels at 30 kg P_2O_5 (in form of supper phosphate 15%) combined with N fertilizer at the levels of 7.5 or 15 Kg N/fed. (in form of ammonium nitrate 33%) showed the highest reduction in chocolate spot disease severity comparing with plants received the highest N level alone (30 Kg N/fed.) or control plants (No fertilization). This turned was true in scored readings during January, February and March. The above turned was noticed also in case of yield and yield components. While, the highest effect of P and N fertilizers on the plant height was at P_0N_{15} and P_0N_{30} levels compared with P_0N_0 and $P_{30}N_0$. The interaction between P and N fertilizers each alone or in combination at different levels had no effect significant on branches number, pods number, weight of 100 seeds and seeds number/pod.

The results in the second season (2001/02) as shown in **Table (29)** were parallel with those above described in the first season (2000/01).

Table (28): Effect of Nitrogen and Phosphorus fertilization on faba bean chocolate spot disease severity, plant height, No. of branches and yield component under field condition at El-Bagour (Menoufya), season 2000/2001.

Main-Treatment (P-levels)	Sub-treat (N. levels)	Disease severity %			Plant height (cm)	No. of branches/ plant	No. of bods/ plant	100seed weight (g)	No. of seeds/ plant	Seed yield	
		Jen.	Feb.	March						Kg/plot	Ard/fed
Mean of P	P_0	6.3	20.4	27.6	133.2	2.62	13.70	56.0	2.8	2.32	7.0
	P_{15}	4.4	13.1	22.5	128.6	2.83	16.4	63.6	3.0	2.74	8.24
	P_{30}	3.6	10.9	20.7	126.9	2.70	17.3	65.7	3.1	2.87	8.64
Mean of N	N_0	5.1	15.2	24.6	118.9	2.64	15.2	60.3	2.9	2.58	7.76
	$N_{7.5}$	4.0	12.1	21.3	124.9	2.78	16.4	62.7	3.1	2.73	8.24
	N_{15}	4.4	13.1	22.4	136.0	2.82	17.2	64.1	3.1	2.83	8.52
	N_{30}	6.9	18.8	26.3	138.5	2.62	14.4	60.0	2.9	2.43	7.33
P_0	N_0	6.3	18.5	28.2	116.7	2.60	13.0	55.1	2.7	2.30	6.93
	$N_{7.5}$	5.9	17.8	26.3	122.2	2.67	13.9	56.8	2.9	2.37	7.15
	N_{15}	7.0	21.1	27.0	146.3	2.73	15.0	58.9	2.8	2.47	7.44
	N_{30}	8.1	24.1	28.9	147.7	2.47	12.9	53.3	2.8	2.15	6.48
P_{15}	N_0	4.8	14.4	23.0	120.3	2.73	16.0	63.3	2.9	2.67	8.04
	$N_{7.5}$	3.7	11.5	20.4	126.7	2.80	16.9	64.8	3.1	2.81	8.46
	N_{15}	3.3	9.6	21.1	132.1	2.93	17.6	65.0	3.1	2.93	8.82
	N_{30}	5.6	16.7	25.6	135.2	2.87	15.0	61.1	2.9	2.54	7.65
P_{30}	N_0	4.1	12.6	22.6	119.7	2.60	16.5	62.3	3.1	2.76	8.31
	$N_{7.5}$	2.2	7.0	17.1	125.8	2.87	18.5	66.4	3.2	3.02	9.10
	N_{15}	3.0	8.5	18.9	129.6	2.80	18.9	68.4	3.3	3.09	9.30
	N_{30}	5.2	15.6	24.4	132.6	2.53	15.5	65.6	3.0	2.61	7.85
L.S.D 5% level for:											
Phosphorus (P)		0.415	0.461	0.456	1.557	N.S	0.446	1.621	0.116	0.016	0.048
Nitrogen (N)		0.84	0.532	0.527	1.798	N.S	0.515	1.87	0134	0.018	0.055
P *N		0.83	0.922	0.912	3.114	N.S	N.S	N.S	N.S	0.031	0.095

N_0 = 0 Kg N/fed $N_{7.5}$ = 7.5 Kg N/fed N_{15} = 15 Kg N/fed N_{30} = 30 Kg N/fed

P_0 = 0 Kg P_2O_5/fed P_{15}= 7.5 Kg P_2O_5/fed P_{30} = 15 Kg P_2O_5/fed

Table (29): Effect of Nitrogen and Phosphorus fertilization on faba bean chocolate spot disease severity, plant height, No. of branches and yield component under field condition at El-Bagour (Menoufya), season 2001/2002.

Main-Treatment (P-levels)	Sub-treat (N. levels)	Disease severity %			Plant height (cm)	No. of branches/ plant	No. of bods/ plant	100seed weight (g)	No. of seeds/ plant	Seed yield	
		Jen.	Feb.	March						Kg/plot	Ard/fed
Mean of P	P_0	5.7	16.1	22.8	132.8	2.8	13.7	61.4	2.9	2.82	8.47
	P_{15}	4.0	12.4	17.8	130.8	2.9	16.5	65.8	3.1	3.25	9.80
	P_{30}	3.4	11.7	16.7	127.1	2.8	16.9	69.6	3.3	3.28	10.17
Mean of N	N_0	4.8	14.1	19.8	118.7	2.7	15.4	62.7	3.0	3.09	9.31
	$N_{7.5}$	3.5	11.7	17.5	126.1	2.8	16.3	69.3	3.2	3.25	9.79
	N_{15}	3.8	12.8	18.0	135.6	2.9	16.8	67.0	3.2	3.32	9.99
	N_{30}	5.4	14.9	21.0	140.6	2.9	14.3	63.5	3.0	2.94	8.84
P_0	N_0	5.9	16.7	23.3	116.0	2.7	13.5	58.2	2.9	2.81	8.46
	$N_{7.5}$	5.2	14.8	20.7	126.9	2.7	14.0	62.3	3.0	2.88	8.67
	N_{15}	5.6	15.6	22.2	141.6	2.8	14.7	62.9	3.0	2.96	8.87
	N_{30}	6.3	17.4	24.8	146.6	2.9	12.7	62.3	2.9	2.62	7.89
P_{15}	N_0	4.4	13.0	18.1	121.8	2.8	16.2	64.3	3.0	3.21	9.69
	$N_{7.5}$	3.0	10.7	17.0	127.9	2.6	17.0	67.2	3.2	3.33	10.03
	N_{15}	3.3	11.5	16.3	134.4	3.0	17.5	68.1	3.1	3.41	10.27
	N_{30}	5.2	14.4	19.6	139.2	3.1	15.3	63.6	3.0	3.06	9.21
P_{30}	N_0	4.1	12.6	17.8	118.2	2.6	16.5	65.7	3.2	3.25	9.79
	$N_{7.5}$	2.2	9.6	14.8	123.5	3.1	17.9	78.3	3.3	3.54	10.66
	N_{15}	2.6	11.5	15.6	130.9	3.0	18.3	69.9	3.4	3.59	10.82
	N_{30}	4.8	13.0	18.5	136.0	2.6	15.0	64.5	3.2	3.13	9.42
L.S.D 5% level for:											
Phosphorus (P)		0.364	0.429	0.367	1.143	N.S	0.407	3.744	0.122	0.021	0.066
Nitrogen (N)		0.420	0.496	0.424	1.320	N.S	0.47	4.323	0.141	0.024	0.076
P *N		0.337	1.134	0.734	2.287	N.S	N.S	N.S	N.S	0.042	0.132

N_0 = 0 Kg N/fed $N_{7.5}$ = 7.5 Kg N/fed N_{15} = 15 Kg N/fed N_{30} = 30 Kg N/fed

P_0 = 0 Kg P_2O_5/fed P_{15}= 7.5 Kg P_2O_5/fed P_{30} = 15 Kg P_2O_5/fed

b- Effect of chemical and biological control onto chocolate spot disease:

As shown in **Tables (30 & 31)**, spraying faba bean plants under field conditions with different fungicides (Dithane M-45, Tridex, polyram-DF and Kocide-101) and antagonists (*Bacillus subtilis*, *Pseudomonas fluorecens*, *Trichoderma hamatum* and *Trichoderma harzianum*) significantly reduced chocolate spot disease severity and increased plant height and seed yield components and some other growth characters of faba bean during two successive seasons compared with control treatment.

At the first season 2000/2001 as shown in **Table (30)**, spraying with fungicides reduced, in general the disease severity to levels better than that sprayed ones with antagonists at different scored readings during January, February and March. The only exception was using *T. harzianum* where it was more effective in reducing disease severity than Polyram fungicide. Moreover, using fungicides and antagonists increased the growth of faba bean plant height where the highest increase was in case of Kocide-101 followed by *T. harzianum* and *T. hamatum* with significant differences between them and other treatments, while least plant height was recorded with control treatment and Polyram fungicide. The rest of other treatments whether fungicides or antagonists had moderate effect in this respect. Also, using fungicides or antagonists did not affect the branching of plants but affected the pod numbers/plant, seed numbers/pod, 100 seed weight (g) and seed yield components. In this respect, the highest pod number/plant was produced by using the fungicides (Dithane-M45, Tridex and Kocide-101, respectively)

Table (30): Effect of some fungicides and biocontrol agents on disease severity of chocolate spot of faba bean, plant height, No. of branches and yield components under field condition, during 2000/2001 at (El Bagour) Minufiya governorate.

Control type	Treatments	Disease severity %			Plant height (cm)	No. of branches /plant	No. of pods /plant	100seed weight (g)	No. of seeds / pod	Seed yield	
		Jen.	Feb.	March						Kg/plot	Ard/fed
Chemical control	Dithane M.45	1.1	3.0	5.9	135.2	2.7	18.1	66.3	3.3	2.99	9.00
	Tridex	1.1	3.7	6.7	136.0	3.0	17.2	67.2	3.3	2.97	8.94
	Polyram- DF	1.9	3.3	13.3	127.6	2.7	15.4	64.3	3.1	2.84	8.55
	Kocide-101	1.5	4.8	9.3	142.6	3.1	16.2	64.7	3.2	2.87	8.64
Biological control	*Bacillus subtilis*-I	2.6	8.2	15.6	136.4	2.8	14.5	63.2	3.0	2.75	8.28
	Pseudomonas fluorescens	3.0	8.9	18.9	133.8	2.6	14.2	63.1	2.9	2.70	8.13
	Trichderma hamatum-II	2.2	5.9	14.8	141.8	2.9	15.0	68.0	3.0	2.80	8.43
	Trichderma harzianum-II	1.9	5.6	8.1	141.9	3.0	16.5	68.5	3.3	2.91	8.76
Control (Untreated)		4.4	10.0	21.5	126.2	2.6	14.2	62.7	2.9	2.64	7.95
L.S.D at 5%		0.740	1.192	0.961	1.614	N.S	0.792	1.529	0.211	0.028	0.330

Table (31): Effect of some fungicides and bio control agents on faba bean chocolate spot disease severity, plant height, No. of branches and yield components under field condition, during 2001/2002at (El Bagour) Minufiya governorate.

Control type	Treatments	Disease severity %			Plant height (cm)	No. of branches/ plant	No. of pods /plant	100seed weight (g)	No. of seeds / pod	Seed yield	
		Jen.	Feb.	march						Kg/plot	Ard/fed
Chemical control	Dithane M.45	0.7	2.6	5.6	131.3	2.8	19.2	70.4	3.4	3.95	11.89
	Tridex	0.7	3.0	5.9	134.5	3.1	18.1	69.8	3.2	3.88	11.68
	Polyram- DF	1.9	4.8	8.2	128.1	2.8	16.0	68.8	3.1	3.43	10.34
	Kocide-101	1.5	4.4	7.4	141.1	2.9	16.7	69.1	3.3	3.77	11.35
Biological control	*Bacillus subtilis*-I	3.0	8.2	11.5	132.6	2.9	14.9	67.9	3.1	3.24	9.76
	Pseudomonas fluorescens	3.3	9.3	13.0	137.1	2.6	14.3	68.1	3.0	3.17	9.54
	Trichderma hamatum-II	2.6	7.4	10.4	140.3	2.7	15.7	71.0	3.1	3.61	10.87
	Trichderma harzianum-II	2.2	6.3	9.6	138.9	2.9	16.9	72.5	3.3	3.81	11.47
Control (Untreated)		4.1	11.1	14.8	125.2	2.7	14.0	67.2	2.9	3.03	9.12
L.S.D at 5%		0.64	0.704	0.649	1.651	0.310	0.783	2.019	0.241	0.023	0.069

and *T. harzianum* while, the highest increase in 100 seed weight was recorded with Trichodema (*T. harzianum* & *T. hamatum*) followed by Tridex and Dithane-M45 treatments.

The latter treatments, however recorded the highest increased in seed number/pod compared with control. Consequently, seed yield components increased by using fungicides and antagonists while the highest increase (ard/fedd.) was recorded by using Dithane-M45, Tridex or *T. harzianum.*

At the second season 2001/02 (**Table 31**), all fungicides and antagonists affected chocolate spot disease severity, plant height and seed yield components and other growth characters of faba bean to similar extends with same trend of the first season.

c- Effect of varietal reaction on chocolate spot disease:

Eight faba bean cultivars, i.e. Giza-3, Giza-40, Giza-429, Giza-461, Giza-717, Giza-767, Giza-716 and Giza-blanka were tested for their reaction to chocolate spot disease and their yield and yield components during 2000/2001 and 2001/2002 growing seasons.

As shown in **Tables (32 & 33)**, the tested cultivars were differed significantly in their reaction to chocolate spot disease severity, yield and yield components of faba bean during the two successive seasons. At the first season, data in **Table (32)** indicate that the least infection with chocolate spot disease was recorded onto Giza-716 cv followed by Giza-blanka, Giza-461 and Giza-717, respectively. Meanwhile, faba bean cvs; Giza-667 followed by Giza-40 and Giza-429 showed susceptible reaction as they recorded the highest infection. The same data reveal that, Giza-667 gave the highest plant height followed by Giza-461

Table (32): Cultivars reaction to chocolate spot disease and its effect on plant height, No. of branches and yield components under field condition at El-Bagour (Menoufya), season 2000/2001.

Faba bean cultivars	Disease severity %			Plant height (cm)	No. of branches /plant	No. of pods /plant	100seed weight (g)	No. of seeds / pod	Seed yield	
	Jen.	Feb.	March						Kg/plot	Ard/fed
GIZA-3	3.0	7.0	15.6	126.5	2.8	11.9	71.5	3.6	2.92	8.79
GIZA-40	3.3	8.1	21.9	123.4	2.7	16.2	64.4	2.8	2.69	8.10
GIZA-429	3.3	8.9	19.6	146.6	2.4	15.1	67.3	3.1	2.98	8.98
GIZA-461	2.6	5.6	9.6	150.6	3.1	12.9	76.7	3.3	3.04	9.16
GIZA-667	3.7	9.6	28.5	151.2	2.7	13.0	69.1	3.2	2.51	7.56
GIZA-717	2.2	4.4	8.9	148.8	3.1	12.0	78.3	3.6	3.06	9.22
GIZA-716	1.9	4.1	6.7	137.2	3.0	14.6	72.3	3.4	3.15	9.49
GIZA-Blanka	2.2	5.2	8.2	124.2	3.9	9.2	116.8	4.1	3.11	9.37
L.S.D at 5%	0.671	0.938	1.114	1.962	0.285	1.521	1.136	0.261	0.033	0.095

Table (33): Cultivars reaction to chocolate spot disease and its effect on plant height, No. of branches and yield components under field condition at El-Bagour (Menoufya), season 2001/2002.

Faba bean cultivars	Disease severity %			Plant height (cm)	No. of branches/plant	No. of pods /plant	100seed weight (g)	No. of seeds / pod	Seed yield	
	Jen.	Feb.	March						Kg/plot	Ard/fed
GIZA-3	4.0	7.8	11.9	129.5	2.6	13.2	89.8	3.5	3.44	10.37
GIZA-40	5.2	10.7	14.8	126.9	2.9	19.0	69.3	2.9	3.04	9.15
GIZA-429	4.8	9.6	14.1	144.7	2.3	17.8	72.1	3.0	3.49	10.51
GIZA-461	3.3	5.6	7.0	149.7	3.1	12.5	94.2	3.4	3.78	11.38
GIZA-667	5.9	12.2	16.7	153.9	2.7	13.9	77.6	3.3	2.88	8.67
GIZA-717	3.7	6.3	8.2	146.9	3.1	12.0	96.9	3.7	4.14	12.47
GIZA-716	3.0	3.7	4.8	139.7	2.9	16.7	91.7	3.3	4.37	13.16
GIZA-Blanka	3.3	4.4	6.3	133.2	4.1	9.4	152.3	4.6	4.79	14.42
L.S.D at 5%	0.689	0.985	0.658	2.178	0.355	1.948	5.646	0.209	0.019	0.058

and Giza-717 while, Giza-40 was the least. The highest number of branches/plant was obtained with Giza-blanka followed by Giza-461 and Giza-717, while the least number of branches was recorded with Giza-429. Also, Giza-40 and Giza-429 produced the highest number of pods/plant while, Giza-blanka produced the least number of pods/plant.

As for the weight of 100-seed, Giza-blanka was the greatest one followed by Giza-717 while Giza-40 was the least one. The highest number of seeds/plant was recorded with Giza-blanka followed by Giza-717 and Giza-3, while Giza-40 cv was the least in this respect. Concerning the yield/fed, Giza-716 followed by Giza-blanka and Giza717 in the first season were the highest respectively while, Giza-667 was the least one.

At the second season 2001/02, data in **Table (33)** indicate that all tested cultivars were also differed significantly in their reaction to chocolate spot disease severity, plant height, seed yield components and other growth characters of faba bean similarly to that obtained in the first season. On the other hand, the highest yield/fed was recorded with Giza-Blanka followed by Giza-716 and Giza-717 respectively while, the least yield/fed was recorded with Giza-667 cv.

d- Effect of sowing date on chocolate spot disease:

In this experiment faba bean variety Giza-40 were sown at four sowing dates during 2000/2001 and 2001/2002 seasons, to study the sowing date effect on chocolate spot disease severity, yield and yield component.

Results in **Tables (34 & 35)** indicate that sowing date greatly affected chocolate spot disease severity. In this respect, delaying sowing date from 1st November till 15th December in both seasons decreased significantly the disease severity from 25.6 to be 13.0% during 2000/2001 and 23.3 to be 10.0 during season 2001/2002. Also, results reveal that delaying sowing date in the both seasons decreased significantly all growth parameters such as yield and yield components, plant hight, number of branches/plant, number of pods/plant, 100-seed weight, number of seeds/pod and seed yield/fed. For example, the highest seed yield/fed was recorded at 1st November and then decreased gradually to reach its minimum values at 15th December in both growing seasons. The recorded values were 8.49, 8.01, 5.33 and 2.83 ard/fed during season 2000/2001 respectively, while they were 10.18, 8.88, 6.65 and 4.49 ard/fed during season 2001/2002 respectively.

Table (34): Effect of Sowing date on faba bean chocolate spot disease severity, plant height and yield component under field condition at El-Bagour (Menoufya), season 2000/2001.

Sowing date	Disease severity %			Plant height (cm)	No. of branches /plant	No. of pods /plant	100seed weight (g)	No. of seeds / pod	Seed yield	
	Jen.	Feb.	March						Kg/plot	Ard/fed
November 1	4.8	13.0	22.2	132.1	2.9	17.1	68.3	3.1	2.82	8.49
November 16	5.6	11.1	20.0	125.1	2.5	14.3	63.4	3.0	2.66	8.01
November 30	2.2	9.6	17.8	115.1	2.3	12.1	56.9	2.9	1.77	5.33
December 15	1.5	4.8	10.4	99.9	1.8	9.9	49.6	2.6	0.95	2.83
L.S.D at 5%	0.981	1.229	1.174	2.177	0.275	0.807	1.737	0.188	0.029	0.108

Table (35): Effect of Sowing date on faba bean chocolate spot disease severity, plant height and yield component under field condition at El-Bagour (Menoufya), season 2001/2002.

Sowing date	Disease severity %			Plant height (cm)	No. of branches /plant	No. of pods /plant	100seed weight (g)	No. of seeds / pod	Seed yield	
	Jen.	Feb.	March						Kg/plot	Ard/fed
November 1	4.1	10.4	18.5	137.5	2.8	17.5	71.33	3.10	3.38	10.18
November 16	3.0	8.9	16.3	128.3	2.6	13.9	64.7	2.9	2.95	8.88
November 30	2.2	7.4	14.4	111.5	2.4	13.2	61.7	2.9	2.21	6.65
December 15	1.1	4.8	9.6	94.3	1.9	10.7	58.7	2.7	1.49	4.49
L.S.D at 5%	0.739	0.826	0.64	5.117	0.258	0.791	2.902	0.240	0.013	0.038


DISCUSSION

DISCUSSION

Faba bean (*Vicia fabae* L.) is considering the most important legume crop in Egypt. It is attacked by many foliar diseases as chocolate spot (*Botrytis fabae* sard. and *B. cinerea*), rust (*Uromyces fabae*), Ascochyta blight (*Ascochyta fabae*), leaf spots (*Cercospora zonata* and *Alternaria alternata)*, downy mildew (*Peronospora viciae*) and root-rot as well as viral diseases which are responsible to cause considerable losses in the yield and its components (**Sardina, 1929, El-Helaly, 1938, Sundheim, 1973, Mansour and Amer, 1976 and Nassib *et al.* 1991**).

Foliar diseases are the most common diseases especially in Delta region due to the high humidity, rain fall and favourable temperature which are prevailing during the season. Therefore, chocolate spot disease of faba bean caused by *Botrytis fabae* and *B. cinerea* is considered the most important disease in Egypt **(Hussein, 1963 and Mohamed, 1982)**.

Surveying the faba bean chocolate spot disease in the tested locations during seasons1998/99 and 1999/00 reveal that severity % of chocolate spot disease was higher in 1999/2000 than in 1998/99 growing seasons. The highest disease severity was recorded at El-Beheira Governorate in El-Nubaria location followed by tobus in season 1999/2000 while, it was the highest in El-Nubaria and Metobus in season 1998/199Damanhour, Koum-Hamada and Me9. Meanwhile, Qualubia and Beni-Swief Governorates show the least disease severity in both seasons. In this respect, similar results were obtained by **Abed El-Latif,**

(1984) who surveyed leaf spot in three Governorates, Dakahlia, Kafr El- Sheikh and Sharkia and showed that faba bean plants are attacked by *B. fabae* and *Alternaria alternata* which caused leaf spots in varying degrees of severity. Also, **Mahmoud, (1985)** reported that the predominant leaf spot during the survey in the northern parts of the Delta, was the chocolate leaf spot caused by *Botrytis fabae.*

Isolation of fungi from spotted faba bean leaves during seasons 1998/99 and 99/2000 revealed that Botrytis isolates were the most frequent in their number and frequency % followed by *Alternaria alternata,* while *Stemphylium botryosum* was the lesser one during the two seasons. In this respect, the frequency of *Botrytis* spp was higher in 1998/99 than in 1999/00 season comparing to *Alternaria alternata* and *Stemphylium botryosum.* It is clear that the highest frequency of Botrytis isolates was recorded at El-Menuofia during the first season followed by El-Gharbia, Beni-Swief, El-Sharkia, El-Beheira and El-Qualubia respectively whereas, the highest frequency % of Botrytis isolates was recorded at Dakahlia, Kafr-El Sheikh, El-Menuofia, El-Qualubia and El-Beheira at the second season respectively. Also, *Stemphylium botryosum* was very low in its number and frequency % in most tested locations at different Governorates comparing with *Alternaria alternata* and *Botrytis* spp where it is not surveyed in most locations during the two seasons. As for tested locations, the highest frequency number of Botrytis isolates was recorded at El-Nubaria, Hehia and El-Bagour during season 1999/00 respectively. While it was at Shbeen-El Koum, El-Bagour and El-Nubaria at season 1998/99 respectively. These

results are in agreement with the findings of **Habib, Wadiaa (1990)** who found that isolation trial from diseased faba bean plant collected from surveyed Governorates yielded twenty-eight fungal isolates. The most frequent fungi were *B. fabae* followed by *B. cinerea, Alternaria alternata* and *Helminthosporium* spp.

Identification of isolated Botrytis isolates indicated that these isolates include *Botrytis fabae* and *B. cinerea* isolates and these isolates were differed also in their distribution, appearance and frequency according to differing the Governorate, location and field. In this respect, out of 248 Botrytis isolates obtained during the first season1998/1999, *B. cinerea* occurred at the highest frequency (86.7%); meanwhile, *B. fabae* was less frequency (13.3%). However, this turned was slightly varied during the second season 1999/2000 where *B. fabae* and *B. cinerea isolates* recorded frequency 55.5% and 44.5%, respectively. *B. fabae* recorded the highest frequency (100%) at Kleen (Kafr-El Sheikh) and Shebeen-El Koum (86%) Menuofia during season 1998/1999. *B. fabae* not be detected in Gharbia, Dakahlia, Shakia Qualubia and Beni-Swief Governorates. Also, it is pronounced from the obtained results that *B. cinerea* was isolated from all tested locations in all Governorates except Kleen location. In the second growing season 1999/2000, the highest frequency of *B. fabae* isolate was recorded at Sharkia and El-Beheira Governorates. On the other hand, the highest isolation frequency of *B. cinerea* was recorded at Menuofia followed by Dakahlia. In this respect, **El-Helaly, (1936)** reported that *Botrytis fabae* was the principal causal pathogen for chocolate spot disease on *Vicia faba*. Also **Hegazy, (1968)**

isolated five isolates of *Botrytis fabae* differing in their infection to faba bean plants; the most virulent isolates were isolated from Ismailia and the weak isolate from Sakha. While, **Harrison, (1988)** reported that both *Botrytis fabae* and *B. cinerea* can causes chocolate spot disease on fabae bean in the field, but *Botrytis fabae* was the more important pathogen because it is more aggressive than *Botrytis cinerea.* Also, **Heweidy, (1993)** found that *B. fabae* isolates scored the highest frequency than *B. cinerea* where all isolates of *B. fabae* were more virulent than *B. cinerea* isolates. **Morsy, (1993)** reported that *B. fabae* scored the highest frequency in all Governorates than *B. cinerea.* **Abou-Baker, (2002)** and **El-Afifi, (2003)** reported that *B. fabae* was the most destructive pathogen and most virulent to faba bean leaves than *B. cinerea* collected from different Govemorates. *B. fabae* isolated from EI-Beheira gave the highest disease severity (52.0%).

The tested *Botrytis* isolates were differed in their virulence onto faba bean leaves (Giza-40) after 1, 3, 5, 7, and 14 days from inoculation under greenhouse conditions. Also, chocolate spot severity incited by *B. fabae* was higher than those incited by *B. cinerea.* The disease severity of the two types of tested isolates was increased gradually post inoculation and reach the maximum after 14 days from inoculation. In this respect, out of the 11 *B. cinerea* isolates, Etay-El Baroud isolate (Beheira) gave the highest disease severity followed by Sakha isolate (Kafr-El sheikh). However, among *Botrytis fabae* isolates (9 isolates) El-Nubaria isolate followed by Sakha and Kleen isolates gave the highest disease severity respectively with

significant difference between them. These results are in harmony with the findings of **Hutson and Mansfield (1980)** who reported that *B. fabae* and *B. cinerea* isolates differed in their pathogenicity on faba bean. While, **Mohamed *et al.*, (1981)** mentioned that Nubaria isolate was the most virulent compared with all tested isolates. Also, **Heweidy (1993)** found that all isolates of *B. fabae* were more virulent than those of *B. cinerea* isolates.

RAPD-PCR analysis of ten isolates of *Botrytis* spp. (two isolates of *B. cinerea* and eight isolates of *B. fabae*) using the specific primer-2-6-d revealed that the ten Botrytis isolates were subdivided into main clusters with similarity 56.45% in between. In this respect, the two *Botrytis cinerea* isolates were distinguished as single strain with high similarity between them. The eight *B. fabae* revealed different clusters with primer-2-6-d, where the similarity was high between isolates 7, 10 and isolate 5. Also, similarity was high between isolates 8 and 9. Isolates 3 and 4 of *B. fabae* were similar to each but those two isolates were more similar to the *B. cinerea* isolates than other *B. fabae* isolates. Similar results were obtained by **Abou-Zeid *et al.* (2002d)** who found different groups of *Botrytis fabae* and *B. cinerea* isolates using RAPD-PCR method.

As for the effect of type of media (synthetic and semi-synthetic) on growth, sporulation and sclerotial formation of tested Botrytis isolates *in vitro*, faba bean seed agar media (FBSA) was the best favourable medium for growth of all tested *Botrytis* isolates followed by PDA medium and faba bean leaf agar media (FBLA). Also, growth of *B. cinerea* isolates was

faster than those of *B. fabae* onto all tested media. As for spore formation, FBLA medium was the best for sporulation followed by FBSA and media PDA media respectively. Most isolates of *B. fabae* produced number of spores less than isolates of *B.* cinerea. Regarding sclerotial formation and their size, PDA was the best favourable medium for sclerotial formation followed by FBSA medium. The isolates produced small size of sclerotia in large number were belong to fabae type, while the isolates not producing sclerotia or producing sclerotia large in size but less in numbers were of the cinerea type. 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. **Mansour, (1992)** found that *Botrytis fabae* gave its best rate of growth on leaf extract of faba bean agar medium. Also, **Abou-Zeid and Saieda S. Abdel-Rahman (1995)** mentioned that forty isolates of *Botrytis* spp collected from different locations were grown on potato dextrose agar (PDA) and faba bean leaf agar (FBLA) media. PDA medium was the best for growth of *Botrytis* spp. Isolates of the fabae type were the least in growth and sporulation while those of the cinerea type were the fastest in growth and highest in spore, sclerotial production. **Mahmoud, Nagwa (1996)** found that PDA medium was the best for growth of *Botrytis* spp. The best media for sporulation of *Botrytis* spp was FSSA. The highest in spore production were *B. cinerea,* while the least in sporulation were the *B. fabae* isolates. Also, isolates of *B. fabae* were the highest in sclerotial number.

Concerning effect of temperature, all tested isolates were able to grow at all temperature degrees ranging between 10-

30°C. In general, temperature ranging from 15 to 20 °C showed the best linear growth comparing with 10, 25 and 30°C respectively. Also, *B. cinerea* (Sakha isolate), followed by Etay EL-Baroud isolate gave the best growth respectively, while minimum growth was produced by *Botrytis fabae* (Sakha isolate). Generally, isolates of *B. cinerea* have sporulated more abundantly than *B. fabae* ones. *Botrytis* isolates grown on FBLA medium could not be able to sporulate at 10°C. The highest main average of spore number was at 20°C. These obtained results are in agreement with the findings of **Jarvis, (1977), Harrison (1981), Abou-Zeid *et al.*, (1990) Hassanein *et al.*, (1990), Mahmoud, Nagwa (1996)** who found that the optimum temperature for mycelial growth and sporulation was 20°C for both *Botrytis fabae* and *B. cinerea.* While, **El-Afifi (2003)** found that PDA media was the pest for growth of Botrytis isolates than FBLA medium. Most of *B. fabae* type isolates produced small sclerotia, while most of *B. cinerea* type produced large ones.

Studies on inoculum density of *B. fabae* indicated that, increasing spore density increased gradually disease severity. These findings are in agreement with **Hanounik and Hawtin (1981), Creighton *et al.*, (1986), Abou-Zeid and Mohamed (1987)** who found that an increase in the inoculum density of *B. fabae* was associated with a corresponding increase in disease severity. Also, **Habib, Wadiaa (1990)** indicated that the highest percentage of infection was produced with highest concentration of conidia.

Concerning the effect of spore age on disease severity, the spores of 1and 2 weeks produced the highest average of disease

severity in case of tested isolates of *B. cinerea* and *B. fabae*, respectively. Inoculation of detached faba bean leaflets with 3 weeks old spores caused significant reduction in disease severity of *Botrytis cinerea* and *B. fabae* isolates. Similar results were obtained by **Harrison, (1983)** who found spreading lesions after inoculation of leaves with *B. cinerea* conidia from young cultures (6 day-old), but spreading lesion was only produced rarely or not at all when older spores were used.

The most suitable plant age for infection with *Botrytis fabae* was 41 days followed by 65 days old plants when the disease severity was scored 7 days post inoculation. Infection has significantly increased by increasing plant age from 17 to 41 days then decreased at 53 and increased again. The author's results suggested that once the pathogen in a lesion is sporulating, leaf age had no effect on the density of conidia produced. **Hanounik, (1980), Creighton *et al.*, (1986)** who mentioned that susceptibility of faba bean to *Botrytis fabae* increased with plant age from 2 to 7 weeks. Also, **Jacqueine and Harrison (1989)** found that the oldest leaves developed more lesions than youngest ones. While, **Habib, Wadiaa (1990)** found that five week old plants of the tested entries were more susceptible whereas 3 week-old ones, generally, were least infected, other growth stages (7-13 week old) were intermediate in their reaction.

The tested faba bean cvs differed in their infection reaction by differing the kind of isolate. The highest disease severity was recorded on leaves of cv Giza-40, whereas, the least infection was recorded on leaves of cv Giza-Blanka. Meanwhile,

Giza-3 showed moderately resistant reaction. Also, the results indicated that faba bean cvs i.e., Giza-40, Giza-667 and Giza-429 are consider susceptible whereas, Giza-3 is consider moderately resistant as well as Giza-Blanka, Giza-716, Giza-461 and Giza-717 are consider resistant. *Botrytis fabae* (EL-Nubaria isolate) caused higher disease severity. Also, the tested faba bean cultivars, were significantly differed in their reaction to chocolate spot disease, yield and yield components during the both growing seasons 2000/2001 and 2001/2002. The lowest infection rate was noticed on Giza-716 cv. followed by Giza-Blanka, Giza-461 and Giza-717 during the two seasons. The highest infection was noticed on the most susceptible cv. Giaz-667 followed by Giza-40 and Giza-429. Meanwhile, Giza-3 was moderately resistant in the two seasons respectively. All faba bean cultivars were differed also in their yield and yield components where Giza-667 gave the highest plant height followed by Giza-461 and Giza-717 in both seasons. These results could be interpret in line with the findings of **El-Neshwy, Saniya (1981), Mohamed *et al.* (1986), Habib, Wadiaa (1990), Mansour (1992)** and **El-Afifi, (2003).**

Regarding sugar contents, the healthy susceptible cultivar (Giza-40) was higher in its total, reducing and non-reducing sugars content comparing with the moderately resistant cultivar (Giza-3) or resistant cultivar (Giza-716) at 45 and 60 days after planting while the reverse was remarkable at 30 days. On the other hand, the faba bean leaves infected with *B. fabae* revealed higher sugar content comparing with healthy leaves in the three varieties at all examined periods (30, 45 and 60 days) after

planting compared with the healthy ones. The total sugars content was decreased gradually by increasing incubation period from 1-7 days. The obtained results are in harmony with those of **EI-Neshwy, Saniya (1981), Habib, Wadiaa (1990), Mahmoud (1992)** who mentioned that the healthy leaves of faba bean cultivars (Giza-402 and Giza-2) contained more total and reducing sugars than that infected with chocolate spot. Also, **Mansour (1992)** mentioned that both healthy leaves of faba bean cvs (Giza-402 and Giza-2) contained more total and reducing sugars than the diseased one. While, **Abou-Baker (2002)** found that the susceptible entry Giza-429 extract contained higher content of each of reducing, non reducing and total sugars if compared with the extract of the resistant Giza-461 cv. Moreover, the amount of sugar contents in infected plants of Giza-429 and Giza-461 were higher than the healthy plant.

The content of total phenolic compounds in healthy resistant variety (Giza-716) was higher than that recorded in moderately resistant or susceptible cvs (Giza-3 and Giza-40). On the other hand, infection with *B. fabae* led to an increase in total and free phenols in the three tested cultivars almost at all examined periods as compared with the healthy ones. The highest increase in total and free phenols content was recorded at 60 days after planting when measured at the first day post inoculation in resistant cv (Giza-716) comparing with the moderately resistant and susceptible cvs (Giza-3 and Giza-40). While the least increase in total and free phenols was recorded at 30 days after planting when the infected plants were incubated 7

days post inoculation. As for conjugated phenols, it is pronounced that infection with *B .fabae* increased almost this kind of phenols at 30 and 60 days after planting comparing with the healthy ones while it decreased in the three varieties almost at 45 days after planting. The conjugated phenols content were higher in the resistance cv Giza-716 compared with the moderate and susceptible cvs Giza-3 and Giza-40. Generally, it was found that phenolic compounds accumulated faster in resistant cultivar than in susceptible cultivars as a result of infection. In this respect, the results of **El-Neshwy, Saniya (1981)** are in agreement with the obtained results where she found that free and total phenols in faba bean leaf exudates were higher in infected leaves with *Botrytis fabae* than in healthy of cv Giza-1. While, **Mansour (1992)** found that free and total phenols were produced and accumulated at a faster rate in Giza-2 (moderate susceptible) than that of Giza-402 (susceptible). Also, **Abou-Baker (2002)** mentioned that free and total phenols in the extract of faba bean leaves were to somewhat higher in the extract of resistant entry Giza-461 than in the susceptible Giza-429. While, inoculated both cultivars with *B. fabae* caused an increase in free and total phenols from 6 h to 24 h from inoculation. After 24 h of inoculation, the free and total phenols content was decreased.

Total free amino acids were increased in both susceptible (Giza-40) and moderately resistant (Giza-3) cvs as a result of infection with *B. fabae*. Meanwhile, they decreased slightly in resistant cv Giza-716 by increasing age of leaves from 30 to 60 days old. It is clear from results that healthy plants were low in their inner total free amino acids comparing with infected ones.

These results could be interpret in light the findings of **Farahat (1980)** who reported that infection increased free amino acid contents in leaves of three pea varieties. Also, **El-Neshwy, Saniya (1981)** found that free amino acids increased in faba bean plants (Giza-1 and Rebaya-40) infected with *B. fabae* particularly infected leaves of 30 days old as compared with 45 and 60 days old. Furthermore, **Habib, Wadiaa (1990), Mansour (1992)** and **Abou- Baker (2002)** verified our obtained results where they found that total free amino acids were higher in infected leaves with *Botrytis fabae* than in healthy ones and in resistant cultivar Giza-461 than in susceptible cultivar Giza-429.

Fifteen bacterial and seven fungal isolates were isolated from faba bean phylloplane and tested *in vitro* for their antagonistic activity against *B. fabae* on PDA plates. The seven isolated antagonistic fungi were identified as *Trichoderma harzianum, T. hamatum, Gliocladium virens* and *Pacellomyces* spp. Meanwhile, the bacterial isolates were found belonging to 4 genus and six species. These bacterial isolates were identified as *Acinetobacter calcoaceticas, Bacillus subtilis, Pseudomonas fluorescens, Pseudomonas aeruginosa, Pseudomonas chlororaphis* and *Stenotrphomonas maltophilia*. The results of **Hanounik and Hasanin (1986), Omar *et al.* (1987), Abd El-Moiety *et al.* (1990), Habib, Wadiaa (1990)** emphasized these obtained results.

All tested bio-agents decreased the mycelial growth of *B. fabae* to different extent on PDA plates compared with the control. *Trichoderma harzianum*-I, *T. hamatum*-II and *T. harzianum*-III were the highly antagonistis to *B. fabae*. In this

respect, they over lapped and inhibited the growth of the pathogen while *Bacillus subtilis*-I reduced greatly the growth of *B. fabae*. Also, *Pacellomyces* spp and *Stentrphomonas maltophilia*-IV followed by *Gliocladium virens* were less effective. Also, culture filtrates of *Bacillus subtilis*-I, *Ps. fluorescens, T. hamatum*-II and *T.harziunum*-II at the different concentrations significantly decreased the linear growth of *B. fabae* in comparison with control treatment. Furthermore, increasing concentration increased the inhibitory effect of the tested culture filtrates. In this respect, culture filtrates of *B. subtilis*-I and *Ps. fluorescens* were more effective in reducing liner growth of *B. fabae* followed by *T. harzianum*-II then the filtrate of *T. hamatum*-II if comparing with control treatment. On the other hand, testing twenty-two of the bacterial and fungal 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 this respect, the tested bacteria had less antagonistic effect than the antagonistic fungi comparing with control (*B. fabae* alone). *T. harzianum*-II, *T. hamatum*-II and *Bacillus subtilis*-I were the highly effective antagonists. Increasing concentration of antagonists culture filtrates reduced significantly disease severity of *B. fabae* comparing with control.

Spraying faba bean plants 24-hour 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, *T. harzianum*-II, *T. hamatum*-II and *T. hamatum*-I were the best effective

treatments respectively in controlling chocolate spot disease after 14 days post inoculation followed by *Bacillus subtilis*-I, *T. harzianum*-I and *Pseudomonas fluoresecens* respectively. Spraying faba bean plants with tested antagonistic culture filtrates 24 hours before inoculation with *B. fabae* resulted in significant decrease in chocolate spot disease severity comparing with control treatment. On the other hand, spraying faba bean plants with the tested antagonistic culture filtrates 24 hours after inoculation with *B. fabae* was less effective in reducing disease severity. Also, filtrates of *B. subtilis*-I was the most effective one before and after 24 hours post inoculation with *B. fabae* at 14 days respectively, followed by *T harzianum*-II and *Ps fluorescens* respectively. Furthermore, increasing days post inoculation till 14 day increased gradually disease severity. These results are in harmony with those obtained by **Jailloux and Froidefond (1987), Simay (1988), Habib, Wadiaa (1990), Zimand *et al* (1996), Sharga (1997), Abou Zeid *et al.* (2000), Abou-Zeid and Hassanein (2002)** and **El-Afifi (2003)** where all of them verified the success role of antagonistic fungi like *Trichoderma* spp., *Gliocladium roseum* and the antagonistic bacteria isolated from phylloplane of faba bean in controlling *Botrytis cinerea* and *Botrytis fabae in vitro* and *in vivo*.

On the other hand, spraying detached leaves (under lab condition) of faba bean with suspension of un-viable heated spores of *B. fabae* scored a remarkable depression in chocolate spot disease severity comparing with unsprayed ones (control) after 7 days post application. On the other hand, spraying whole plants under greenhouse conditions with the un-viable heated

spores reduced the disease severity of chocolate spot disease when compared with the control after 14 days post inoculation. In this respect, the results of **Abou Zeid and Le Normand (1979), Abou-Zeid *et al.* (1996)** and **Abou Zeid *et al.* (2002a)** support our obtained findings where they stated that pre-inoculation of faba bean leaves with non-viable heated *B. fabae* spore suspension exhibited a reduction in chocolate spot disease severity.

In this study fungicides different in their active ingredient, were evaluated for their effect on fungal growth in vitro, and also on disease severity under greenhouse and field conditions. All the tested fungicides suppressed *in vitro* the growth of *Botrytis fabae* at concentration; 25, 50,100,150 and 200 ppm. In this respect, the most effective fungicide in this concern was Dithane M-45 followed by Tridex and Polyram-DF respectively while, Kocide-101 was the least effective one. All tested fungicides at the different concentrations were differed clearly in their effect against *B. fabae* on faba bean leaflets (Giza-40). Dithane M-45, Tridex and Polyram-DF were more effective than Kocide-101 respectively, after 14 days post inoculation. The best concentration was 200 ppm of all tested fungicides especially of Dithane-M-45. Also, all tested fungicides were effective in reducing faba bean chocolate spot disease severity incited by *B. fabae* when those fungicides were sprayed 24h before inoculation under greenhouse conditions. The tested fungicides were differed in their effect on disease severity where the highest remarkable reduction in disease severity was onto faba pots sprayed with Dithane-M45, followed by Tridex, Kocide-101 and

Polyram-DF respectively when sprayed 24h before inoculation after 14 days post inoculation.

Spraying faba bean plants in the open field during two successive growing seasons 2000/01 and 2001/02 with different fungicides and different antagonists affected chocolate spot disease severity of faba bean. Also, using fungicides and antagonists increased the growth of faba bean plant height where the highest increase was in case of Kocide-101 followed by *T. harzianum* and *T. hamatum* with significant differences between them and other treatments. Also, using fungicides or antagonists did not affect the branching of plants but affected the pod numbers/plant, seed numbers/pod, 100 seed weight (g) and seed yield component. In this respect, the highest increase in seed yield component was recorded with using Dithane-M45, Tridex and *T. harzianum.* Similar results were obtained by **Elliott and Whittington (1980), Abd El-Monem (1981), Hanounik (1981), Abed El-Latif (1984), Abou Zeid *et al.* (1990), Omar *et al.* (1990), Hegab and Beshir (1994),** and **Abou-Zeid *et al.* (2002b)** who verified our obtained results.

As for effect of fertilization during two successive growing seasons 2000/01 and 2001/02, chocolate spot disease severity was affected by different levels of nitrogen and phosphor fertilizers. Plants received of P fertilizer at the highest levels at 30 kg P_2O_5 combined with N fertilizer at the levels of 7.5 or 15 Kg N/fed. showed the highest reduction in chocolate spot disease severity comparing with plants received the highest N level alone (30 Kg N/fed.) or control plants (No fertilization). The above turned was noticed also in case of yield and yield

components. While, the highest effect of P and N fertilizers on the plant height was at P_0N_{15} and P_0N_{30} levels compared with P_0N_0 and $P_{30}N_0$. The interaction between P and N fertilizers each alone or in combination at different levels had no effect significant on branches number, pods number, weight of 100 seeds and seeds number/pod.These results could be interpret in line with the findings of **Mansour and Kamel (1975)**, who found that adding calcium superphosphate 15% at the rate of 238 kg/ha and calcium nitrate 15.5% at the rate of 238 kg/ha was the best treatment to provide high yield and to decrease chocolate spot infection. While, **Hegab and Beshir (1994)** and **Mahmoud, Nagwa (1996)** found that nitrogen fertilizer insignificantly increased numbers of branches/plant, seeds/pod and weight of 100 seed.

As for effect of sowing date, delaying sowing date from 1 November to 15 December, during seasons 2000/01 and 2001/02 significantly decreased disease severity. Also, yield and yield component i.e. plant high, No. of branches/plant, No. of pods /plant, 100 seed weight, No. of seed/pod and seed yield/fed were significantly decreased by delaying sowing date in the both seasons. The results of **Mohamed *et al.* (1981), Hanounik and Hawtin (1982), Saxena and Stewart (1983)** and **Mahmoud, Nagwa (1996)** are in harmony with our obtained results where all of them observed that delaying the date of planting decreased the severity of chocolate spot significantly, where seed yield and chocolate spot affected greatly with sowing date from 1st October till 1st December.

SUMMARY

SUMMARY

Faba bean (*Vicia fabae* L.) is considering the most important legume crops in Egypt. It is attacked by many foliar diseases as chocolate spot (*Botrytis fabae* and *B. cinerea*), rust (*Uromyces fabae*), Ascochyta blight (*Ascochyta fabae*), leaf spots (*Cercospora zonata*, *Stemphylium botryosum* and *Alternaria alternata*), downy mildew (*Peronospora viciae*) and root rot as well as viral diseases which are responsible to cause considerable losses in the yield and its components.

The obtained results of the present study could be summarised as follows:

1- Surveying of faba bean chocolate spot disease during seasons1998/99 and 1999/00 reveal that, severity % of chocolate spot disease was higher in 1999/2000 than in 1998/99 growing seasons. The highest disease severity was recorded at El-Beheira Governorate (El-Nubaria) while, Qualubia and Beni- Swief Governorates show the least disease severity in both seasons.

2- Isolation of fungi from spotted faba bean leaves during seasons 1998/99 and 99/2000 revealed that Botrytis isolates were the most frequency followed by *Alternaria alternata,* while *Stemphylium botryosum* was the lesser one during the two seasons. The highest frequency of Botrytis isolates was recorded at El-Menuofia during the first season whereas; it was recorded at Dakahlia, at the second season. As for tested locations, the highest frequency number of Botrytis isolates

was recorded at El-Nubaria during the secaned season and Shbeen-El Koum at the first season.

3-Identification of isolated Botrytis isolates indicated that these isolates include *Botrytis fabae* and *B. cinerea* isolates. In the first season 1998/1999, *B. cinerea* occurred at the highest frequency (86.7%); meanwhile, *B. fabae* was less frequency (13.3%). However, this turned was slightly varied during the second season 1999/2000 where *B. fabae* and *B. cinerea isolates* recorded frequency 55.5% and 44.5%, respectively. The highest frequency of *B. fabae* was recorded at Kleen (Kafr-El Sheikh) during season 1998/1999. In the second growing season 1999/2000, the highest frequency of *B. fabae* isolate was recorded at Sharkia and El-Beheira Governorates. On the other hand, the highest isolation frequency of *B. cinerea* was recorded at Menuofia followed by Dakahlia.

4-The tested *Botrytis* isolates were differed clearly in their virulence onto faba bean leaves (Giza-40) under greenhouse conditions. All isolates of *B. fabae* were more virulent than *B. cinerea* ones irrespective of variation within isolates. In this respect, *B. fabae* (El-Nubaria isolate) was the most virulent followed by Sakha isolate.

5- RAPD-PCR analysis of the ten isolates of *Botrytis* spp. (two isolates of *B. cinerea* and eight isolates of *B. fabae*) using the specific primer-2-6-d revealed that the ten Botrytis isolates were subdivided into main clusters with similarity 56.45% in between. In this respect, two *Botrytis cinerea* isolates were distinguished as single strain with high similarity between them. The eight *B. fabae* revealed different clusters with

primer-2-6-d, where the similarity was high between isolates 7, 10 and 5. Also, similarity was high between isolates 8 and 9. Isolates 3 and 4 of *B. fabae* were similar to each but those two isolates were more similar to the *B. cinerea* isolates than other *B. fabae* isolates.

6- As for the effect of different media on growth, sporulation and sclerotial formation of tested Botrytis isolates in vitro, faba bean seed agar media (FBSA) was the best favourable medium for growth of all tested *Botrytis* isolates followed by PDA media and Faba bean leaf agar media (FBLA). Also, growth of *B. cinerea* isolates was faster than those of *B. fabae* onto all tested media. As for spore formation, FBLA medium was the best favourable medium for spore followed by FBSA media and PDA media respectively. Most isolates of *B. fabae* produced number of spores less than isolates of *B.* cinerea. Regarding sclerotial formation and their size, PDA was the best favourable medium for sclerotial formation followed by FBSA medium. The isolates produced small size of sclerotia in large number were belong to fabae type, while the isolates not producing sclerotia or producing sclerotia large in size but less in numbers were of the cinerea type.

7- All tested isolates were able to grow at all temperature degrees ranging between 10-30°C. In general, temperature ranging from 15 to 20 °C showed the best linear growth comparing with 10, 25 and 30°C respectively. Also, *B. cinerea* (Sakha isolate), followed by Etay EL-Baroud isolate gave the best growth respectively, while minimum growth was produced by *Botrytis fabae* (Sakha isolate). Generally,

isolates of *B. cinerea* have sporulated more abundantly than *B. fabae* ones. *Botrytis* isolates grown on FBLA medium could not be able to sporulate at 10°C. The highest main average of spore number was at 20°C. Increasing spore density increased gradually disease severity. Concerning the effect of spore age on disease severity, the spores of 1and 2 weeks produced the highest average of disease severity in case of tested isolates of *B. cinerea* and *B. fabae*, respectively. Inoculation of detached faba bean leaflets with 3 weeks old spores caused significant reduction in disease severity of *Botrytis cinerea* and *B. fabae* isolates. The most suitable plant age for infection with *Botrytis fabae* was 41 days followed by 65 days old plants when the disease severity was scored 7 days post inoculation. Infection has significantly increased by increasing plant age from 17 to 41 days.

8- The tested faba bean cvs have significantly differed in their reaction to different isolates. The highest disease severity was recorded on leaves of cv Giza-40, whereas, the least infection was recorded on leaves of cv Giza-Blanka. Meanwhile, Giza-3 showed moderately resistant reaction. *Botrytis fabae* (EL-Nubaria isolate) caused higher disease severity.

9- The healthy susceptible cultivar (Giza-40) was higher in its total, reducing and non-reducing sugars content comparing with the moderately resistant cultivar (Giza-3) or resistant cultivar (Giza-716) at 45 and 60 days after planting. Faba bean leaves infected with *B. fabae* revealed higher sugar content comparing with healthy leaves in the three varieties at all examined periods (30, 45 and 60 days) after planting. The

total sugars content was decreased gradually by increasing incubation period from 1-7 days.

10- The total and free phenols contents were higher either in healthy or infected leaves of the resistant cultivar (Giza-716) compared with the moderate and high susceptible cvs (Giza-3 and Giza-40), this turned was true at different plant ages and days after inoculation. The highest increase in total and free phenols content was recorded at 60 days after planting after one day from inoculation. While the least increase in total and free phenols was recorded at 30 days after planting when the infected plants were incubated 7 days post inoculation. As for conjugated phenols, it is pronounced that infection with *B. fabae* increased almost this kind of phenols at 30 and 60 days after planting comparing with the healthy ones while it decreased in the three varieties almost at 45 days after planting. The conjugated phenols content were higher in the resistance cv Giza-716 compared with the moderate and susceptible cvs Giza-3 and Giza-40 from at 1 day post inoculation.

11- Total free amino acids were increased in both susceptible (Giza-40) and moderately resistant (Giza-3) cvs as a result of infection with *B. fabae*. Meanwhile, they decreased slightly in resistant cv Giza-716 by increasing age of leaves from 30 to 60 days old. It is clear from results that healthy plants were low in their inner total free amino acids comparing with infected ones.

12- Fifteen bacterial and seven fungal isolates were isolated from faba bean phylloplane. The seventh isolated antagonistic fungi

were identified as *Trichoderma harzianum, T. hamatum, Gliocladium virens* and *Pacellomyces* spp. Meanwhile, the bacterial isolates were found belonging 4 genus and six species. These bacterial isolates were identified as *Acinetobacter calcoaceticas, Bacillus subtilis, Pseudomonas fluorescens, Pseudomonas aeruginosa, Pseudomonas chlororaphis* and *Stenotrphomonas maltophilia.*

13- All tested bio-agents decreased the mycelial growth of *B. fabae* on PDA plates compared with the control. *Trichoderma harzianum*-II, *T. hamatum*-II, *T. harzianum*-III and *Bacillus subtilis*-I were the highly antagonists to *B. fabae* while *Pacellomyces* spp and *Stentrphomonas maltophilia*-IV followed by *Gliocladium virens* were weakly effective.

14- Also, all tested culture filtrates of *Bacillus subtilis*-I, *Ps. fluorescens, T. hamatum*-II and *T. harziunum*-II at the different concentrations were significantly decreased the linear growth of *B. fabae* in comparison with control. Culture filtrates of *B. subtilis*-I and *Ps. fluorescens* were more effective in reducing liner growth of *B. fabae* followed by *T. harzianum*-II than the filtrate of *T. hamatum*-II comparing with control treatment.

15- All tested antagonists of 22 bacterial and fungal isolates were decreased chocolate spot disease severity caused by *B. fabae* on detached leaves after 1, 3, 5 and 7 days inoculation. *T. harzianum*-II, *T. hamatum*-II and *Bacillus subtilis*-I were the highly effective antagonists.

16- Spraying faba bean plants 24-hour before inoculation with *B. fabae* under greenhouse conditions with all tested antagonists decreased significantly chocolate spot disease severity comparing with control. In this respect, *T. harzianum*-II was the best effective treatment in controlling chocolate spot disease after 14 days post inoculation followed by *T. hamatum*-II and *T. hamatum*-I, *Bacillus subtilis*-I respectively.

17- Spraying faba bean plants with tested antagonistic culture filtrates 24 hour before inoculation with *B. fabae* resulted in significant decrease in chocolate spot disease severity comparing with control treatment.

18- Spraying faba bean plants with tested antagonistic culture filtrates 24 hour after inoculation with *B. fabae* was less effective in reducing disease severity. Also, filtrate of *B. subtilis*-I was the most effective one before and after 24 hour post inoculation with *B. fabae* at 14 days respectively, followed by *T. harzianum*-II and *Ps fluorescens* respectively.

19-Also, spraying detached leaves (under lab condition) of faba bean with suspension of un-viable heated spores of *B. fabae* spores scored a remarkable depression in chocolate spot disease severity comparing with unsprayed one (control) after 7 days post application.

20- Spraying whole plants under greenhouse conditions with the un-viable heated spores reduced the disease severity of chocolate spot disease when compared with the control after 14 days post inoculation.

21- All the tested fungicides suppressed *in vitro* the growth of *Botrytis fabae* at concentration; 25, 50,100,150 and 200 ppm. Dithane M-45 was the most effective fungicide followed by Tridex and Polyram-DF respectively while, Kocide-101 was the least effective one in this respect.

22- All tested fungicides at the different concentrations were differed clearly in their effect against *B. fabae* on faba bean leaflets (Giza-40). Dithane M-45, Tridex and Polyram-DF were more effective than Kocide-101 respectively. Also, under greenhouse conditions, the highest reduction in disease severity was onto faba pots sprayed with Dithane-M45, followed by Tridex, Kocide-101 and Polyram-DF respectively when sprayed 24-h before inoculation after 14 days post inoculation.

23- As for field trails, during two successive growing seasons 2000/01 and 2001/02, chocolate spot disease severity was affected by different levels of nitrogen and phosphor fertilizers. Plants received of P fertilizer at the highest levels at 30 kg P_2O_5 combined with N fertilizer at the levels of 7.5 or 15 Kg N/fed. showed the highest reduction in chocolate spot disease severity comparing with plants received the highest N level alone (30 Kg N/fed.) or control plants (No fertilization). The above turned was noticed also in case of yield and yield components. While, the highest effect of P and N fertilizers on the plant height was at P_0N_{15} and P_0N_{30} levels compared with P_0N_0 and $P_{30}N_0$. The interaction between P and N fertilizers each alone or in combination at different levels had no effect

significant on branches number, pods number, weight of 100 seeds and seeds number/pod.

24- Spraying faba bean plants in the open field with different fungicides and different antagonists affected on chocolate spot disease severity of faba bean. Moreover, using fungicides and antagonists increased the growth of faba bean plant height where the highest increase was in case of Kocide-101 followed by *T. harzianum* and *T. hamatum* with significant differences between them and other treatments, while least plant height was recorded with control treatment and Polyram fungicide. Also, using fungicides or antagonists did not affect the branching of plants but affected the pod numbers/plant, seed numbers/pod, 100 seed weight (g) and seed yield component. In this respect, the highest increase in seed yield component was recorded with using Dithane-M45, Tridex and *T. harzianum.*

25- Eight faba bean cultivars, i.e. Giza-3, Giza-40, Giza-429, Giza-461, Giza-717, Giza-767, Giza-716 and Giza-blanka were significantly differed in their reaction to chocolate spot disease and its effect on yield and yield components during the both growing seasons 2000/2001 and 2001/2002. The lowest infection was noticed on Giza-716 cv. followed by Giza-blanka, Giza-461 and Giza-717 during the two seasons. The highest infection was noticed on the most susceptible cv. Giaz-667 followed by Giza-40 and Giza-429. Meanwhile. Giza-3 was moderately resistant in the two seasons respectively. All faba bean cultivars were differed also in their yield and yield compound where Giza-667 gave the highest

plant height followed by Giza-461 and Giza-717 in both seasons.

26- As for effect of sowing date, delaying sowing date from 1 November to 15 December, during seasons 2000/01 and 2001/02 significantly decreased disease severity. Also, yield and yield component i.e. plant high, No. of branches/plant, No. of pods /plant, 100 seed weight, No. of seed/pod and seed yield/fed were significantly decreased by delaying sowing date in the both seasons.

REFERENCES

REFERENCES

Abd EI-Moity, T.H., N.M. Abou-Zeid and A.E. Tawfik. (1990): Correlation between microbial populations in phylloplane of faba bean varieties and their reaction to *Botrytis fabae.* Agric. Res. Rev., **68**: 433- 441.

Abd EI-Monem, A.M. (1981): Studies on chocolate spot of broad bean in Libya. Ann. Agric. Sci., Moshtohor, **16**:119-131.

Abed El-Latif, M.A. (1984): Studies on leaf spot of faba bean. M.Sc. Thesis, Fac. Agric., Moshtohor, Zagazig Univ., Egypt.

Abou-Baker, M.E. (2002): Biochemical studies on important fungal disease of some major food crops. M. Sc. Thesis, Fac. Agric. Sci. (Biochemistry), Ain shams Univ., Egypt, 120 pp.

Abou-Zeid, N.M. (1978): Contribution a la connaissance de la resistance naturelle de *Vcia faba* L. contre les Botrytis. These de Docteur-Ingenieur en Sciencees Agronomiques. E.N.S.A. de Rennes Universite de Rennes I, France, 154 pp.

Abou-Zeid, N.M. (2000): Biological control of chocolate spot disease caused by *Botrytis fabae* on faba bean using *Trichoderma* spp. Proceedings of the sixth international

conference on plant disease, AFPP, 6-8, December 2000, Tours, France, 521-528.

Abou-Zeid, N.M. (2002a): Interaction between varieties of faba bean and bioagents to control chocolate spot disease in Egypt. Proc. of the sixth Iinternat. Conf. on Agricultural Biotechnology Research, (ICABR): New avenues for production and technology, 11-14, July 2002, Transfeer Ravello-Italy, 1 pp.

Abou-Zeid, N.M. and A.M. Hassanien. (2000): Biological control of chocolate spot disease (*Botrytis fabae* Sard.) in faba bean in Egypt. Phytophathology, **90**:1182.

Abou-Zeid, N. M. and H. A. Mohamed. (1987): Factors affecting severity of *Botrytis fabae* in bean in Egypt. 5th. Proc. Cong. Egypt. Phytopathol. Soc. Giza, 13-26.

Abou-Zeid, N.M. and M. Le-Normand. (1979): Varietal variability measurement of *Vicia faba* susceptibility to *Botrytis fabae* by a rapid method. ISSN0003-4177 V. **11**, 134 -135.

Abou-Zeid, N.M. and M. Le-Normand. (1981): Analysa de la réponse de la féverole a l' inoculation in vitro par les *Botrytis* 20 colloque de la société Francaise de Phyto-Pathologie. Agrononmic, **I**: 821-826.

Abou-Zeid, N.M. and Saieda S. Abdel-Rahman. (1995): Variability and distribution of Botrytis isolates in Egypt. Nile Valley Regional Program on Cool Season Food

Legumes and Cereals. Food Legumes Report, Seven Annual National Coordination Meeting Cairo, Egypt, 10-14 September1995: 146-151.

Abou-Zeid, N.M., H.A. Mohamed and M. Le-Normand. (1985): Effect of geographical origin of *Botrytis* spp their virulence on *Vcia faba* L. and spread in different varieties.1st Nat. Conf. of Pests and Disease of Vega. And Field Crops in Egypt, Ismailia, Proc. 862-871.

Abou-Zeid, N.M., M. Abdalla and Y.EL-Gammal. (2002b). Integrated control of chocolate spot disease of faba bean caused by *Botrytis faba Sard.* In: Wilt/Root-Rot Disease in Faba bean, Lentil and Chickpea and chocolate spot disease in Faba bean. Nile Valley and Red Sea Regional Program. International Center for Agricultural Research in Dry Areas. ICARDA/ NVRSRP, Cairo, Egypt, 53-57.

Abou-Zeid, N.M., M. El-Hady and Saieda S. Abdel-Rahman. (1998a): Pathogenic, morphological and physiological variability in *Botrytis* spp. affecting faba bean in Egypt.3rd European conference on grain legumes. Part II. 225-226.

Abou-Zeid, N.M, A.M. El-Garhy, M.A. Abd El-Aal and A. El-Afifi. (2002c): Effect of chemical and biological control of chocolate spot disease in faba bean. In: Wilt/Root-Rot Disease in Faba bean, lentil and chickpea and chocolate spot disease in Faba bean. Nile Valley and Red Sea Regional Program. International Center for

Agricultural Research in Dry Areas. ICARDA/ NVRSRP, Cairo, Egypt, 57-60.

Abou-Zeid, N.M; D.I. Harfoush, M. Abdalla and M.S. Khalil. (2002d): Application of Molecular for characterization of *Botrytis fabae* and *Botrytis cinerea* in faba bean. In: Wilt/Root-Rot Disease in Faba bean, Lentil and Chickpea and chocolate spot disease in Faba bean. Nile Valley and Red Sea Regional Program. International Center for Agricultural Research in Dry Areas. ICARDA/ NVRSRP, Cairo, Egypt, 15-21.

Abou-Zeid, N.M., M.S.H. Moustafa., A.M. Hassanien and I. Ez-Eldin. (1990): Control of chocolate spot disease of faba bean and the effect of fungicides on the behaviour of the causal fungus. Agric. Res. Rev., **68**: 411-421.

Abou-Zeid, N.M., A.M. Hassanein., G.A. El-Morsy., Saieda S. Abdel-Rahman and M. El-Hady. (1996): Cross protection as a new control measure for chocolate spot disease (*Botrytis fabae* Sard) of faba bean. Nile Valley and Red Sea Regional Program on cool-season food legumes, cereals, and resource management ICARDA /NVRSRP Annual Report, Cairo, Egypt Doc.045, 31-33.

Abou-Zeid, N.M., G.A. EI-Morsy., A.M. Hassanein., M.M. EI-Hady, and **Saieda S. Abdel-Rahman. (1998b):** Isolation of *Botrytis* spp and the chocolate spot of Egypt. J. Argic. Res. Review, **76**: 479-489.

Adak, M.S., H. Ulukan and M. Guler. (1998): Determination of some agronomic traits in Turkish faba bean (*Vicia fabae* L.) Lines. FABIS, Newsletter, **42**: 29-31.

Akem, C and M. Bellar. (1999): Survey of faba bean diseases in the main faba bean growing regions of Syria. Arab Journal of Plant Protection, **17**:113-116.

Amer, M.I. (1986): Effect of some agronomic practices on productivity of some broad bean varieties. Ph.D. Thesis, Faculty of Agric., Zagazig University, Egypt.

Andrew, J.J., D.R. Waiter and G. Marshall. (1997): Antagonstic interaction between the foliar pathogen, *Botrytis fabae* and isolates of *Penicillium brevicompactum* and *Cladosporium cladosporiodes* on faba beans. Biological Control, **8**, 1997 Acad Press.

Barnett, H.L. and B.B. Hunter (1987): Illustrated Genera of Imperfect Fungi. (4^{th} ed.). MacMillan Pub. Co., New York, U.S.A., 218 PP.

Berger, G. (1937): A serious disease of broad bean in Moracco (*Botrytis fabae* Sard.). Rev. Path. Veg., **24**:101-111(c.f Rev. Appl. Mycol., **16**:651).

Bikmuhametova, R.N. (1963): Diseases of broad beans. Trud. bashkir. Sel. Khoz. Inst., **11**: 19-22. (c.f. Rev. Appl. Mycol., **44**: 1325).

Brown W. (1924): Two mycological methods: 11.A method of isolating single strains of fungi by cutting a hyphal tip. Ann. Bot.**38**: 404.

Chorine, M. (l939): The chocolate spot disease of beans.Palest. J. Bot., R. Ser., **2**: 291-293. (c.f. Rev. Appl. Mycol., **19**:l88).

Ciferri, R. (1949): Co-existence of *Botrytis fabae* and *Botrytis cinerea* on broad bean. Notiz. Malatt .Painte, **1**: 3-5. (c.f. Rev. Appl. Mycol.,**29**:72).

Creighton, N.F., A. Bainbridge and D.L. Fitt. (1985): Epidemiology and control of chocolate spot (*Botrytis fabae*) on winter field bean *Vicia faba.* Crop Protection, **4**: 235-243.

Creighton, N.F., A. Bainbridge and D.L. Fitt. (1986): Effects of leaf age, inoculum dose and freezing on development of chocolate spot (*Botrytis fabae*) lesions on field bean (*Vicia faba*) leaves. Phytopathology, **76**: 108-115.

N. J. (1982): Potenial for biological control of plant diseases on the phylloplane. Ann. Rev. Phytopath., **20**: 167- 192.

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.

El-Beih, F. M., M.R.A. Saady, K.A. Youssef and A.I.A. Kader. (1988): Changes in amino acids and sugar

content of broad bean leaves following infection with *Botrytis fabae*. Phytologia, **64**:367-378.

EI-Gantiry, S.M., S.A.M. Omer, N. EI-Safawany and M.M. Rahal. (1991): Chemical control of foliar diseases of faba bean. (Subpublished) in Agriculture Research Centre Giza, Egypt.

EI-Helaly, A.F. (1936): Studies on the brown spot disease of field bean *(Vicia faba)* in Egypt. Report Agric. Research, Min. Agric. (March), 105.

EI-Helaly, A.F. (1938). A chocolate spot disease of beans (*Vicia faba*) part 1. Bull. Minist. Agric., Egypt, **19**: 8.

Elliott, J.E. and W.J. Whittington. (1980): The control of chocolate spot (*B. fabae*) infection of field beans (*V. faba* L.) by the fungicides Benlate, Bavistin, Cercobin and BAS 325 F. J. Agric. Sci., Camb. **94**: 461-464.

Ellis, M.B. (1971): Dematiaceous Hyphmycetes. Commonwealth Mycol. Inst., 608 pp.

EI-Neshwy, Saniya, M. (1981): Studies on chocolate spot of broad bean in A. R. E. M. Sc. Thesis, Faculty of Agric., Ain Shams Univ., Cairo, Egypt, 91pp.

EI-Refai, I.M., A.M. Zedan and O.A. Alia, (1992): Evaluation of certain faba bean cultivars against *Botrytis fabae* with special reference to role of hydrolytic enzymes in the cell death. Delta J. Sci., **16**: 231- 254.

EI-Shazly, A.M. (2001): Control of leaf spot diseases by fungicides and phyllospheric microflora. El-Azhar J. Agric. Res., **331**: 189- 203.

Farahat, A. A. (1980): Studies on powdery mildew in some legumes, Ph.D. Thesis, Fac. Agric., Ain Shams Univ. Egypt.

Geard, I.D. (1960): Diseases of broad beans and tick beans. Tasmaniam Journal of Agriculture **31**, 68-73.

Giltrap, N. J. (1991): Comparison of fungicides for chocolate spot control winter field beans. Aspects of Applied Biology, **27**:123-128. (c.f. Rev. PI. Path., **71**: 8).

Gorfu, D. (1986): Sporulation of *Botrytis fabae* as affected by dextrose and faba bean leaf extract. FABIS Newsletter, **16**:44-45.

Gorter, G.J.M.A. (1941): Chocolate Spot of broad beans. Fmg. S. Afr., xvi, **182**: 167-168 (c. f. Rev. Appl. Mycol., **20**: 441).

Habib, Wadiaa, F. (1990): Studies on leaf spots of faba beans. Ph.D. Thesis, Fac. of Agric., Cairo Univ., Egypt, 128 pp.

Hanounik, S.B. (1980): Susceptibility of faba beans of different ages to infection by *Botrytis fabae* and *Ascochyta fabae*. Faba Bean Information Service Newsletter, **2**:46. (c.f. Rev. Pl. Path., **60**: 562).

Hanounik, S.B. (1981): Influence of Ronilan on the severity of chocolate spot and yield in faba bean. FABIS Newsletter **3**:50-51.

Hanounik, S.B. (1986): Screening techniques for disease resistance in faba beans. International Center for Agricultural Research in the Dry Areas (ICARDA), Alepp, Syria, 59 pp.

Hanounik, S. B. and Hasanin, M. A. (1986): Inhibition of - *Botrytis fabae* in the phyllosphere of *Vicia faba* leaves. FABIS Newsletter, **16**: 41- 44.

Hanounik, S.B. and G.C. Hawtin (1979): Disease of major food legume crop in syria. In food legume improvement and development ed. G.C. Hawtin and G.J. Chancellar, IDRC Pub 126 ottawa

Hanounik, S.B. and G.C. Hawtin. (1981): Breeding for resistance to chocolate spot caused by *Botrytis fabae.* International Conf. on faba bean, 7-11 March, Cairo.

Hanounik, S.B. and G.C. Hawtin. (1982): Screening for resistance to chocolate spot caused by *Botrytis fabae.* Faba Bean Improv., Proc. Cairo, Egypt, 7-11March, Ed. by G. Hawtin and C. Webb. 243-250.

Hansen, H.N. (1926): A simple method of obtaining single spore cultures. Science (N.S.), **64**: 384.

Harrison, J.G. (1981): Chocolate spot of field beans in Scotland . Plant Pathol., **30**: 111-115.

Harrison J.G. (I983): Survival of *Botrytis fabae conidia* in air. Trans. of the British Mycol. Soc., 80:263-269.

Harrison J.G. (1984): Effects of environmental factors on sporulation of *Botrytis fabae*. Trans. of the British Mycol. Soc., **83:** 295-298.

Harrison, J.G. (1986): Epidemiology and aetiology of *Botrytis* on field bean. Scottish Crop Res. Inst. Ann. Rep. for 1985, 87pp.

Harrison, J.G. (1988): The biology of *Botrytis* spp. on Vicia beans and chocolate spot disease. Plant Pathology, **37**: 168-201.

Hassanein, A.M., N.M. Abou-Zeid and H.A. Mohamed (1990): Correlation between morphological and pathological characters of *Botrytis* spp. the incitant of chocolate spot. Agric. Res. Rev., **68**: 423-432.

Hegab, M.C. and M.A. Beshir (1994): Effect of nitrogen and fertilizer and application fungicides on chocolate spot. Rust diseases. Yield and component of field bean under calcareous soil, Annales of Agric. Sc. Moshtohor, **32**: 717-729.

Hegazy, M.F. (1968): Effect of some factors affecting infection with *Botrytis fabae* Sard. on broad bean with special

reference to nutrition. Ph.D. Thesis, Fac. Agric., Ain Shams Univ. Egypt.

Heweidy, M.A.M. (1993): Variation in *Botrytis fabae* in relation to its virulence and disease control. Ph. D. Thesis, Faculty of Agric., Cairo Univ.Egypt, 108 pp.

Heweidy, M.A.M. (1998): Pathological studies on chocolate spot disease of faba bean (*Vicia faba* L.). Egypt. J. Appl. Sci., **13**:88-105.

Horsfall, J.G. (1956): Princopals of fungicidal action, Waltham, Mas: Chronica Botanica. (c.f. Trans. Brit. Mycol. Soc., **45:** 463-473, 1962).

Hussein, A.H.A., R.F. Dossoky, M.A. El-Deeb and M.M. El-Morsy (1994): Effect of sowing dates and plant densities on yield and yield components of new faba bean cultivar (Giza Blanka) in newly reclaimed land. J. Agric. Sci. Mansoura Univ., **19**: 47-45.

Hussein, M.R.B. (1963): Diseases of legume and oil crops. Publ. No.39 Plant Pathol.Res. Dept., Egypt, Min. Agric., 45 pp. (in Arabic).

Hutson, R.A. and J.W. Mansfield. (1980): A genetical approach to the analysis of mechanism of pathogenicity in *Botrytis/ Vicia faba* interaction. Physiological Plant Pathology, **17**: 309-317.

Jacqueine, H and J.G. Harrison. (1989): Effects of bean leaf Age on Pathogenicity by *Botrytis fabae.* Phytopathology, **126:**272-278.

Jailloux, F. and G. Froidefond. (1987): Evaluation of the antagonistic properties of 7 strains of *Trichoderma* on *Botrytis cinerea* with the help of several laboratory methods. Bulletin OEPP, **17**: 541-547. (c.f. Rev. of Pl Path., **68**: 223).

Jarvis, W. R. (1977): Botryotinia and Botrytis species: Taxonomy, Physiology and Pathogenicity, pp. 33-34. Monograph 15, Research Branch, Canada Dept. of Agric. Ottawa.

Jellis, G.J., D.A. Bond and J. Old (1982): Resistance to chocolate spot (*Botrytis fabae*) in ICARDA accessions of *Vicia faba.* FABIS Newsletter **4**: 53-54.

Jouch, C. (1947): Chocolate spot of broad beans. Rev. Invest. Agric., B. Aires, **2**:65-80 .(c.f. Rev. Appl. Mycol., **26**: 522).

Karel, G. (1952): Broad bean disease caused by Botrytis.Bit. Kor. Bult., **2**: 16-22 (c.f. Rev. Appl.Mycol., **21**: 588).

Khaled, A.A., Abd EI- Moity, M.H. Samiha and S.M. Omar. (1995): Chemical control of some faba bean diseases with fungicides. Egypt. J. Agric. Res., **73**: 45 - 55.

Khalil, S.A. and J.G. Harrison. (1981): Methods of evaluating faba bean materials for chocolate spot. FABIS Newsletter **3**: 51-52.

Khalil, S.A., A.M. Nassibe, H.A. Mohamed and Wadiaa F. Habib. (1984): Identification of some sources of resistance for chocolate spot and rust diseases in faba beans. Systems for cytogenetic analysis in *Vicia faha* L. (Ed. by G.P. Chapman & S.A. Tarawali), pp: 9-13. Martinus Nijhoff, Dordrecht.

Khalil, S.A., R.F. Dissouky, M.I. Amer, M.M. EI-Hady and M. W.A. Hassan (1993): Performance of yield and yield components of two faba bean *(Vicia faba* L.) cultivars as affected by two plant densities and foliar diseases control in the new reclaimed land. J. Agric. Sci. Mansoura Univ. **18**: 1306-1314.

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

Kuti, J.O. and H. F. Nawer (1999): Factors affecting growth sporulation and pathogenicity of chocolate spot fungi *(Botrytis* spp). Phytopathology, **89**: 23-28.

Last, F.T. and R.E. Hamley. (1956): A local-lesion technique for measuring the infectivity of conidia of *Botrytis fabae* Sard. Ann. Appl. Biol. **44**: 410-418.

Leach, R. and K.G. Moore. (1966): Sporulation of *Botrytis fabae* on agar cultures. Trans. of the British Mycol. Soc., **49**: 593-601.

Levine, M., and H.W. Schoenlein. (1930): A Compilation of culture media for the cultivation of microorganisms. 969 PP. Baltimore.

Mahmoud, M.R.M (1985): Studies on leaf spots of faba bean. M.Sc. Thesis, Fac. Agric., Kafr El sheikh, Tanta. Univ., Egypt.

Mahmoud, Nagwa. M. (1996): Studies on chocolate spot disease of broad bean and loss occurrence. Ph.D. Thesis, Fac. Agric., Minufiya Univ., 133pp.

Mahmoud, T.M.M. (1992): Studies on leaf spots of field bean (*Vicia faba* L.). M. Sc. Thesis Fac. Agric., Moshtohor, Zagazig Univ., Benha Branch, Egypt, 121.

Mansfield, J.W. and R.A. Hutson. (1980): Microscopical studies on fungal development and host responses in broad bean and tulip leaves inoculated with five species of Botrytis. Physiological Plant Pathology, **17**: 131-144.

Mansour, K. (1980): Chemical control of rust and leaf spots of field beans (*Vicia faba* L.). Agric. Res. Rev., **58**: 49-56.

Mansour,K. and B. Kamel. (1975): Studies on the effects of phosphorus and nitrogen fertilization and spraying with the fungicide Diathane M-45 on the control of chocolate

spot and amount of yield of horse beans. Agr. Res. Rev. **53**: 123-134.

Mansour, K. and S. Amer. (1976): Severity of chocolate spot disease of horse beans and search for resistance. Agric. Res. Rev., **54**:101.

Mansour, M.T. (1992): Studies on leaf spots of field bean (*Vicia faba* L.). Thesis M.Sc. Fac. Agric., Ain Shams Univ.

Mohamed, B.A., A. Nassib, M.E. EI-Rafei and S.A. Khalil. (1981): Effect of planting date and number of fungicide sprays on foliar diseases and yield of faba beans. FABIS Newsletter, **3**:40-41.

Mohamed, H.A. (1982): Major disease problems of faba bean in Egypt. In Faba Bean Improvements, Edited by G. Hawtin and C. Webb, 213-225.

Mohamed, H. A., N. M. Abou-Zeid and Wadiaa F. Habib. (1981): Variation within the fungus *Botrytis fabae* Sard. FABIS Newsletter, **3**: 49-54.

Mohamed, H.A.,S.A.M. Omer and S. M. EI-Gantiry. (1986): Interaction between Botrytis isolates and faba bean strains with special reference to the effect of diffusion from leaves on the fungus conidia germination. Agric. Res. Rev., **64**: 233- 243

Mohamed, H.A., H.Y. Aly 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.

Money S.P. (1961): Vegetable growing for home and market gardeners. Fungus, bacterial, and virus diseases of crops. New Zealand Journal of Agriculture, **103:** 541-551.

Morsy, K.M.M. (1993): Pathological and genetical studies from improving resistance of faba bean to chocolate spot disease. M. Sc. Thesis, Fac. Agric., Cairo Univ.Egypt, 119 pp.

MukherJee P.K., M.P. Haware and S. Jayanthi (1995): Preliminary investigations in integrated bio-control of *Botrytis gray* mold of chickpea. Indian Phytopath. **48**: 141-149.

Nassib, A.M. (1983): On farm trails in Egypt. Pages 38-47 in faba bean in the Nile Valley. Report on the first phase of ICARDA / IFAD Nile Valley project (M.C. Saxena and R.A. Stewart, eds.) Martinus Nijhoff, The Hague, the Netherlands.

Nassib, A.M., S.A.Khalil, M.A. El-Botai and M.M. Radi. (1991): In: Nile Valley Regional program on Cool Season Food Legumes and Cereals.1990/91.Annual Report, Egypt. Cairo: ARC /ICARDA.

Omar, S.A.M., E.S. Dorreiah and S.M. El-Gantiry. (1987): Methods for estimating microorganisms population in faba bean phylloplane. 2nd Nat. Conf. of Pests & Dis. of Veg. & Fruits, Ismailia, Oct., 1987.

Omar, S.A., N.M. Abou-Zeid, S.M. El-Gantiry, E.S. Dorreiah, A.M. Hassanein and G.A. Morsy. (1990): Reports on fungicides project No.22, 7, seasons 1987-1990. Reports of applied problems researches support count for control of agricultural pests. Agricultural Research Centre, Giza, Egypt.

Pieter. V., R. Hogers, M. Bleeker, M. Reijans, T.V.D. Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau. (1995): AFLP: new technique for DNA fingerprinting. Nucleic Acids Research **23**: 4407-4414.

Rhind, D. (1927): Annual report of the mycologist, Burma, for the year ending June 30, 1926. Rangoon, Burma. (c.f. Yu, T .F., 1945. Phytopathology, **35**: 945 – 954).

Riker, A.J. and R.S. Riker. (1936): Introduction to research on plant diseases. John S. Swift Co. Inc. Iouis, Chicago, New York, 117pp.

Rosen, H. (1957): A modified ninhydrine colorimetric analysis for amino acids. Arch. Biochem. and Biophysiol., **67**: 10-51.

Rossall, S. and J. W. Mansfield. (1981): Nutrients and lesion formation by *Botrytis cinerea* on leaves of *Vicia faba*. Trans. of the British Mycol. Soc., 76:172-175.

Rossall, S., J. W. Mansfield and R.A. Huston. (1980): Death of *Botrytis cinerea* and *B. fabae* following exposure to wyerone derivatives in vitro and during infection development in broad bean leaves. Physiological Plant Pathology, **16**: 135-146. (c.f. Rev. PI. Path., **59**: 4838).

Roulston, S. and S.D. Lane. (1988): Observations on the interaction between *Trichoderma · viride* and three *Botrytis* species. Mycologist, **1**: 176 - 177. (c.f. Rev. Pl. Path., **68**: 2524).

Salh, M.B., H.M. Halila, G. Hernandez-bravo, B.A. Malik, M.I Mihov and B. Sardi. (1994): Biotic and abiotic constraining the productivity of cool season food legumes in different farming systems: Specific examples. (c.f.: Expanding the production and use of cool season food legumes, Edited by F.J. Muehibauer and W.J. Kaiser. Proc. Of the Sec. Int. Food Legume Res. Conf., Cairo, Egypt, 12-16 April, 1992, 991 pp.

Salih, F.A. (1984): Influence of sowing date, seed rate and seed size on yield and its components of faba beans. FABIS Newsletter (1983) 7, 32 Shambat Res. Sta. Khartoum, North, Sudan.

Sambrook, J., E.F. Fritsch and Maniatis.L. (1989): Molecular cloning a laboratory manual. Cold Spring Harbor Laboratory Press. 1989.

Sardina, J.R. (1929): A new species of *Botrytis* attacking broad beans. Men. R. Soc. Espanola Hist. Nat., **1**: 251-292. (c.f. Rev. Appl. Mycol.,**9**:424).

Saxena, M.C. and R.A. Stewart. (1983): Faba bean in Nile Valley, Report on the first phase of the ICARDA/IFAD Nile Valley Project, P.70-74, Martinus Nijhoff Publishers, The Hague Boston London.

Sharga, B.M. (1997): *Bacillus* isolates as potential bio-control agents against chocolate spot and faba beans. Canadian J. microbiology,**43** :915 -924.

Simay, E.L. (1988): In vivo occurrence of hyper parasitism of *Botrytis cinerea* pers. by *Gliocladium catenulatum* Gilman et Abbott. Acta. Phytopathologica et Entomologica Hungarica, **23**: 1-2, 133-135.

Singh, N.L., M. Pangabam and M. Devi. (1987): Leaf surface mycoflora of diseased and non-diseased pea (*Pathisum sativum*). Entomol. et Phytopathol. Appl., **54**: 57-66. (c.f. Rev. Plant Pathol., **69**: 794).

Sneddecor, G.W. and W.G. Cochran. (1980): Statistical methods. Oxford & J.BH Pubishing com. 6 *th*. edition.

Snell, F.D. and C.T. Snell. (1953): Colorimetric methods of analysis including some turbidimetric and nephelometric methods. D. Van Noster and Company,

Inc. Toronto-New York- London. Vol.III. Organic-I: 116-117.

Stefania, R., K. Gindro, H. Richter and O. Viret. (2002): Characteriz-ation of molecular markers for specific and sensitive detection of *Botrytis cinerea* using PCR .Microbiology letters, **209:** 169-174.

Sundheim, L. (1973): *Botrytis fabae*, *B. cinerea* and *Ascochyta fabae* on broad bean (*Vicia faba*) in Norway. Acta Agriculture Scandinavica **23,** 43-51.

Tewati, B. and A.S. Virk. (1996): Nutritional potential of faba bean for improved productivity in ruminants. FABIS Newsletter, **38/39**: 2-11.

Thomas, W. and R.A. Dutcher. (1924): The colorimetric determination of carbohydrates in plants by the picric acid reduction method. I. The estimation of reducing sugars and sucrose. J. Amer. Chem. Soc., **46**: 1663-1669. (c.f. El-Shiekh, M.M., M.Sc. Thesis, Fac.Agric., Al-Azhar Univ., Cairo, 1985).

Vandemark, G.J. (1995): Biological control of *Botrytis cinerea* by use of *Pseudomonas corrugata* and *Bacillus subtilis.* Phytopathology, 85, **20**:117.

Wang, S.C. (2000): Pathogen identification of leaf diseases and the study on the main in spring broad bean in Gansu province. Acta- Phytophylacica- sinica. **27**: 121-125.

Waterstone, J.M. (1942): Plant Pathology. Res. Dep. Agric. Bermuda, pp. 8-9 (c.f. Rev. Appl. Mycol., 21: 321.

Wilson, A.R. (1937): The chocolate spot disease of beans *(Vicia faba* L.) caused by *Botrytis cinerea* Pers. Ann.of Appl. Biol., **24**: 258-288.

Younis, N.A. (1989): The pathological response of broad beans produced from gamma irradiated seeds. Ph.D Thesis, Fac. Agric., Ain Shams Univ., Egypt.

Yu, H. and J.C. Sutton (1997): Morphological development and interactions of *Gliocadium roseum* and *Botrytis cinerea* in raspberry. Can. J. of Plant Pathology, **19**: 237-246.

Yu, T.F. (1945): The red-spot disease of broad beans (*Vicia faba* L.) caused by *Botrytis fabae* Sardina in China. Phytopathology, **35:** 945-954.

Zaglol, H.H.A. (1991): Studies on the effect of some fungicides and foliar spray with nutrients on some leaf spot diseases of Brood bean plant in A.R.E. M.Sc Thesis, Fac. of Agric. at Moshtohor, Zagazig Univ. Benha Branch. Egypt.

Zimand, G, Y. Elad and I. Chet (1996): Effect of *Trichoderma harzianum* on *Botrytis cinerea* pathogenicity. Phytopathology, **86**:1255-1260.

الملخص العربى

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يعتبر الفول البلدى من أهم المحاصيل البقولية فى مصر حيث يصاب بالعديد من الأمراض، خاصة تحت الظروف المصرية أهمها أمراض المجموع الخضري ومنها أمراض التبقعات مثل التبقع البنى فى الفول البلدى الناتج عن الفطر بوترايتس فابى والفطر بوترايتس سينريا وهو من أهم الأمراض واسعة الانتشار التى تصيب الفول البلدى وكذلك الصدأ المتسبب عن الفطر يورميسس فابى ولفحة الأسكوكيتا المتسببة عن الفطر أسكوكيتا فابى وتبقعات الأوراق المتسببة عن الفطر سركوسبورا زاناتا والفطر ألترناريا الترناتا والبياض الزغبى المتسبب عن الفطر برونوسبورا فشيا بالإضافة إلى فطريات الذبول والأمراض الفيروسية والتى تسبب نقصا فى المحصول ومكوناته. و ترجع أهمية مرض التبقع البنى إلى انه يسبب نقصا كبيرا فى إنتاج المحصول وخاصة تحت الظروف البيئية من انخفاض درجة الحرارة وارتفاع الرطوبة النسبية كما قد تسبب الإصابة الشديدة بهذا المرض نقصا خطيرا فى المحصول قد يصل الى١٠٠% وعموما تقدر الخسارة فى محصول الفول البلدى الناتجة عن الإصابة بالتبقع البنى بأكثر من ٥٠% من المحصول فى السنوات الوبائية أما فى الحالة التى تكون فيها الإصابة عادية فتتراوح ما بين ٥-١٥% من المحصول.

وقد هدف هذا العمل إلى حصر الإصابة بالتبقع البنى على الفول البلدى فى ثمانى محافظات وعزل المسببات المرضية وإجراء اختبار القدرة المرضية لهذه العزلات والتفرقة بينها بإستخدام تقنية RAPD-PCR ودراسة بعض العوامل المؤثرة على نمو وتجرثم وتكوين الاسكلورشيات لفطري التبقع البنى (بوترايتس فابى وبوترايتس سينريا) من المغذيات ودرجة الحرارة. . . كذلك دراسة العوامل المؤثرة على شدة الإصابة بالتبقع البنى مثل عمر الجراثيم – الطاقة اللقاحية- عمر النبات – تفاعل الأصناف كذلك دراسة التغيرات البيوكيميائية نتيجة الإصابة بالتبقع

البنى. . . كما هدفت هذه الدراسة الى التعرف على طرق مقاومة مرض التبقع البنى فى الفول البلدى عن طريق عزل الكائنات الحية الدقيقة من المتواجدة على سطح أوراق الفول البلدى (الفلوبلان) لاستخدمها فى مجال المقاومة البيولوجية إلى جانب طرق المقاومة الأخرى عن طريق استخدام المقاومة الكيماوية والمقاومة المستحثة باستخدام الجراثيم المقتولة حراريا بالإضافة إلى المقاومة باستخدام المعاملات الزراعية مثل ميعاد الزراعة – تفاعل الأصناف ودراسة مستويات التسميد الأزوتى والفوسفاتى.

وتتلخص النتائج المتحصل عليها فى الأتى :

١- تم حصر مرض التبقع البنى فى الفول البلدى فى المواقع والحقول المختلفة خلال الموسمين ١٩٩٨/١٩٩٩ ، ٩٩ /٢٠٠٠ وقد تبين أن شدة الإصابة كانت أعلى خلال موسم النمو ٩٩/٢٠٠٠ عنه فى الموسم ١٩٩٨/٩٩ و قد سجلت أعلى شدة إصابة فى منطقة النوبارية بمحافظة البحيرة يليها دمنهور وكوم حمادة ومطوبس فى الموسم ٩٩/٢٠٠٠ بينما كانت أعلى شدة إصابة فى النوبارية ومطوبس خلال الموسم ١٩٩٨/٩٩. . . فى حين سجلت أقل شدة إصابة فى محافظة القليوبية وبنى سويف خلال الموسمين.

٢- أظهرت نتائج عزل المسببات المرضية من أوراق الفول المصابة خلال الموسمين١٩٩٨/١٩٩٩ ،٩٩ /٢٠٠٠ أن عزلات الفطر بوترايتس كانت الأكثر عدد ا وتكررا يليها عزلات الألترناريا بينما كان أقلها عزلات الفطر استيمفليم بوتريوزم خلال الموسمين. وقد سجلت أعلى نسبة ظهور لفطريات البوترايتس فى محافظة المنوفية خلال الموسم الأول فى حين قد سجلت فى محافظة الدقهلية خلال الموسم الثانى. وقد سجلت أعلى نسبة ظهور لفطريات البوترايتس بالمناطق المختلفة فى منطقة النوبارية خلال الموسم الثانى فى حين قد سجلت فى منطقة شبين الكوم خلال الموسم الأول .

٣- وجد أن عزلات البوترايتس عند تعريفها تشمل البوترايتس فابى والبوترايتس سينيريا والتى تختلف فى توزيعها ونسبة ظهورها وتكرارها باختلاف المحافظات والمواقع والحقول وقد تم الحصول على ٢٤٨ عزلة من جنس البوترايتس تضم ٢١٥ عزلة بوترايتس سينريا بنسبة ظهور٨٦,٧% بينما يوجد ٣٣ عزلة بوترايتس فابى بنسبة ظهور ١٣,٣% خلال موسم العزل الأول ٩٨/ ١٩٩٩ ..أما خلال الموسم الثانى ٩٩/٢٠٠٠ حيث تم الحصول على٢٥٤ عزلة بوترايتس منها ١٤١ عزلة من البوترايتس فابى بنسبة ظهور٥٥,٥% و١١٣ عزلة من البوترايتس سينيريا بنسبة ظهور ٤٤,٥%. وقد وجد أن أكبر عدد من عزلات البوترايتس فابى قد سجلت فى كفر الشيخ والمنوفية خلال الموسم الأول ٩٨/١٩٩٩ فى حين أنه لم يتواجد الفطر فى محافظات الغربية والدقهلية والشرقية والقليوبية وبنى سويف بينما عزل البوترايتس سينيريا من مختلف المواقع فى جميع المحافظات ما عدا منطقة قلين . وفى موسم النمو ٩٩/٢٠٠٠ سجل أكبر عدد من عزلات البوترايتس فابى فى البحيرة وخاصة فى منطقتي النوبارية وكوم حمادة فى حين أن أكبر عدد من عزلات البوترايتس سينيريا قد سجل فى محافظة المنوفية يليها كفر الشيخ والدقهلية.

٤- اختلفت كل عزلات البوترايتس فى قدرتها المرضية على أوراق الفول البلدى للصنف جيزة-٤٠ بعد ١،٣،٥،٧،١٤ يوم من العدوى تحت ظروف الصوبة. وكانت شدة الإصابة المتسببة عن الفطر بوترايتس فابى أعلى من تلك المتسببة عن الفطر بوترايتس سينيريا. وتزداد شدة الإصابة تدريجيا من اليوم الأول لتصل إلى أقصى شدة إصابة بعد ١٤ يوم من العدوى . وقد سجلت عزلة ايتاى البارود وعزلة سخـــا أعلى شدة إصابة فى العزلات الـــ١١ امختبرة من البوترايتس سينيريا فى حين كانت أعلى عزلات البوترايتس فابى فى شدة الإصابة هى عزلة النوبارية يليها سخا وقلين على التوالى .كما اختلفت أيضا ١٠ عزلات من جنس البوترايتس فى قدرتها المرضية على أوراق الفول

المفصولة من الصنف جيزة-٤٠ وقد كانت عزلات البوترايتس فابى هى الأعلى فى قدرتها المرضية عن عزلات البوترايتس سينيريا وكانت عزلة البوترايتس فابى(النوبارية) هى الأعلى فى قدرتها المرضية تليها عزلة سخا وكانت شدة الإصابة تزداد تدريجيا نتيجة للعدوى بفطريات البوترايتس من اليوم الأول لتصل إلى أقصى شدة إصابة بعد ٧ أيام من العدوى.

٥- أمكن التفرقة بين ١٠ عزلات من جنس البوترايتس (عزلتين من البوترايتس سينيريا وثماني عزلات من البوترايتس فابى) عن طريق تقنية RAPD-PCR باستخدام بادئ متخصص رقم(٢-٦-د) حيث وجد أن العزلات قد انقسمت إلى مجاميع مختلفة وقد تميزت عزلتى البوترايتس سينيريا إلى سلالة مفردة بدرجة تشابه عالية بين العزلتين .وقد أوضح البادئ أن هناك أيضا مجاميع مختلفة بين الثمانى عزلات من البوترايتس فابى وقد وجدت درجة تشابه عالية بين العزلتين رقم ٧ , ١٠ والعزلة رقم ٥ كذلك وجدت درجة تشابه عالية بين العزلتين رقم٨ , ٩ كذلك العزلتين٣, ٤ واللتان تتشابهان مع باقى عزلات البوترايتس فابى وان كانت درجة تشابههما أكبر مع عزلتى البوترايتس سينيريا.

٦- تم دراسة تأثير المغذيات تحت ظروف المعمل على بيئات مختلفة (طبيعية وصناعية ونصف صناعية) على النمو والتجرثم وتكوين الاسكلورشيات للعزلات المختبرة من جنس البوترايتس.

وقد وجد أن بيئة مستخلص بذور الفول هى أفضل البيئات لنمو كل عزلات البوترايتس المختبرة يليها بيئة البطاطس دكستروز أجار وبيئة مستخلص أوراق الفول. وقد كانت عزلات البوترايتس سينيريا هى الأسرع نموا عن عزلات البوترايتس فابى. وقد اختلفت عزلات البوترايتس فى تكوينها للجراثيم باختلاف البيئات وكانت بيئة مستخلص أوراق الفول هى أفضل بيئة للتجرثم يليها بيئة بيئة مستخلص بذور الفول وبيئة مستخلص البطاطس دكستروز أجار.

وجد أن معظم عزلات البوترايتس فابى تنتج عددا أقل من الجراثيم عند مقارنتها بعزلات البوترايتس سينيريا ولكن فيما يختص بتكوين الاسكلورشيات وحجمها كانت بيئة بذور الفول هى أفضل بيئة لتكوين الاسكلورشيات يليها بيئة مستخلص البطاطس دكستروز أجار . وقد وجدت علاقة بين عدد وحجم الاسكلروشيات ونوع عزلات البوترايتس، فالعزلات التى تنتج اسكلورشيات قليلة الحجم كثيرة العدد كانت من نوع الفابى وفى حين كانت العزلات التى لا تكون اسكلورشيات أو ذات اسكلورشيات قليلة العدد كبيرة الحجم فكانت من نوع البوترايتس سينيريا.

٧- أمكن لجميع العزلات النمو على درجات الحرارة المختلفة من ١٠-٣٠ م، وكانت درجة الحرارة المثلى للنمو وتكوين الجراثيم هى ٢٠ م بالمقارنة بباقي درجات الحرارة المختبرة وكانت عزلة سخا للفطر بوترايتس سينيريا هى أكثر العزلات نموا فى حين كانت عزلة سخا للفطر بوترايتس فابى أقل العزلات نموا. وجد أن عزلات الفطر بوترايتس سينيريا قد تجرثمت بدرجة أكثر من عزلات الفطر بوترايتس فابى وقد وجد أن عزلات البوترايتس كانت غير قادرة على إنتاج الجراثيم عند نموها على بيئة أوراق الفول على درجة ١٠ م . وقد درست بعض العوامل المؤثرة على شدة الإصابة بفطريات البوترايتس اشتملت على الطاقة اللقاحية، عمر الجراثيم وعمر النبات. ازدادت شدة الإصابة تدريجيا بزيادة الطاقة اللقاحية كذلك دلت النتائج أن أعلى شدة إصابة تم الحصول عليها عندما استعملت طريقة الأوراق المفصولة باستخدام الجراثيم صغيرة العمر (عمر أسبوع واحد) فى حالة البوترايتس سينيريا .بينما كانت من جراثيم عمرها أسبوعين فى حالة البوترايتس فابى وبزيادة العمر إلى ثلاثة أسابيع لوحظ انخفاض معنوي فى شدة المرض. .كما لوحظ أن شدة الإصابة تزداد بزيادة عمر النبات من ١٧ إلى ٤١ يوم ثم تقل عند ٥٣ يوم ثم تزيد بعد ذلك. وقد

وجد أن عمر ٤١ يوم هو أفضل عمر لإصابة نباتات الفول بالبوترايتس فابى، يليه ٦٥ يوم عندما سجلت شدة الإصابة بعد ٧ أيام من العدوى.

٨- اختلفت أصناف الفول المختبرة فى شدة إصابتها باختلاف نوع العزلات، وكان الصنف جيزة-٤٠ أكثر الأصناف قابلية للإصابة بينما كان الصنف جيزة-بلانكا مقاوما فى حين أن الصنف جيزة-٣ كان متوسط القابلية للإصابة. وقد انقسمت الأصناف المختبرة إلى أصناف شديدة القابلية للإصابة وتشمل الأصناف: جيزة-٤٠ ، جيزة-٦٦٧ وجيزة-٤٢٩ بينما يعتبر الصنف جيزة-٣ متوسط القابلية للإصابة ،فى حين تعتبر الأصناف جيزة-بلانكا ،جيزة-٧١٦ ،جيزة-٤٦١ وجيزة-٧١٧ أصناف مقاومة.

٩- أدت الإصابة بالبوترايتس فابى (عزلة النوبارية) إلى حدوث بعض التغيرات البيوكيميائية. ووجد أن الصنف جيزة-٤٠ القابل للإصابة كان الأعلى فى محتوى السكريات المختزلة والغير مختزلة مقارنة بالصنف المتوسط القابلية للإصابة جيزة-٣ والصنف المقاوم جيزة-٧١٦ عند عمر ٤٥ ،٦٠ يوم من الزراعة .وقد وجد أن إصابة أوراق الفول بالبوترايتس فابى تزيد من محتوى السكريات فى الأصناف الثلاثة فى كل الفترات بعد ٣٠، ٤٥ ،٦٠ يوم من الزراعة مقارنة بالنباتات السليمة. تقل السكريات الكلية تدريجيا بزيادة فترة التحضين بعد العدوى من ١-٧ أيام بعد ٣٠ ،٤٥، ٦٠ يوم من الزراعة على التوالى.

١٠- وجد أن محتوى الفينولات الكلية فى الصنف المقاوم جيزة-٧١٦ أعلى من الصنف المتوسط القابلية للإصابة جيزة-٣ والصنف القابل للإصابة جيزة-٤٠. وقد وجد أن الإصابة بالبوترايتس فابى تزيد من الفينولات الحرة والكلية فى الأصناف الثلاثة تقريبا خلال فترات الدراسة مقارنة بالنباتات السليمة. وكانت أعلى زيادة فى محتوى الفينولات الحرة والكلية على عمر ٦٠ يوم من الزراعة بعد يوم واحد من العدوى فى الصنف المقاوم جيزة-٧١٦ مقارنة بالصنف

المتوسط القابلية للإصابة جيزة-٣ والصنف جيزة-٤٠. بينما كان أقل زيادة فى محتوى الفينولات الحرة والكلية قد سجل بعد ٣٠ يوم من الزراعة بعد ٧ أيام من العدوى . تزيد الإصابة بفطر البوترايتس فابى بدرجة كبيرة من الفينولات المرتبطة بعد ٣٠ ، ٦٠ يوم من الزراعة مقارنة بالنباتات السليمة بينما تقل فى الأصناف الثلاثة تقريبا بعد ٤٥ يوم من الزراعة . يزداد محتوى الفينولات المرتبطة فى الصنف المقاوم جيزة-٧١٦ بالمقارنة بالصنف المتوسط القابلية للإصابة والصنف القابل للإصابة (جيزة-٣ ،جيزة-٤٠) بعد يوم واحد من العدوى. وقد وجد أن المركبات الفينولية تتراكم بسرعة فى الصنف المقاوم عن الصنف القابل للإصابة نتيجة لحدوث الإصابة .

١١- تزداد الأحماض الأمينية الكلية فى الصنف الحساس جيزة-٤٠ والصنف متوسط القابلية للإصابة جيزة-٣ نتيجة لحدوث الإصابة بالبوترايتس فابى بينما تقل فى الصنف المقاوم جيزة-٧١٦ بزيادة عمر النبات من ٣٠ إلى ٦٠ يوم . وقد أوضحت النتائج أن النباتات السليمة كانت أقل فى محتواها من الأحماض الأمينية مقارنة بالنباتات المصابة.

١٢- أوضحت نتائج عزل الكائنات الحية الدقيقة من أوراق الفول السليمة (من منطقة الفلوبلان) أنه تم الحصول على ١٥ عزلة بكتيرية و٧ عزلات فطرية ،تم اختبارها تحت ظروف المعمل ككئنات تضادية للبوترايتس فابى. وقد عرفت العزلات الفطرية المستخدمة فى المقاومة الحيوية إلى ترايكودرما هارزيانم، ترايكودرما هماتم ، جليوكلاديم فرني وعزلة باسلوميسيس. بينما تتبع العزلات البكتيرية ٤ أجناس و٦ أنواع وقد عرفت إلى أسينتوبكتر كالكوسيتكس، باسيلس ساتيلس، سيدوموناس فلورسينس، سيدوموناس ايروجينوزا، سيدوموناس كلوروفيس وعزلة ستينوتروفوموناس مالتيفيليا.

١٣- قللت كل الكائنات التضادية المعزولة النمو الميسليومى للبوترايتس فابى على بيئة البطاطس دكستروز آجار بالمقارنة بالكنترول. وكانت عزلات الترايكودرما

هارزيانم-٢ ،ترايكودرما هماتم-٢، ترايكودرما هارزيانم-٣ الأعلى فى مقاومتها الحيوية للفطر بوترايتس فابى حيث غطت وثبطت نمو المسبب المرضى. كما ثبطت عزلة باسيلس ساتيلس-١ نمو المسبب المرضى بدرجة كبيرة فى حين كانت عزلتى باسلوميسيس ، ستينوتروفوموناس مالتيفيليا يليها عزلة الجليوكلاديم فرني هم الأقل تأثيرا.

١٤- أظهرت النتائج أن كل التركيزات المختلفة من رواشح الباسيلس ساتيلس، سيدوموناس فلورسينس، ترايكودرما هارزيانم-٢، ترايكودرما هماتم-٢ قد قللت من نمو البوترايتس فابى مقارنة بالكنترول. ويزداد التأثير التثبيطي للراشح المزرعى بزيادة تركيز الراشح. وكان الراشح المزرعى لـــ باسيلس ساتيلس-١ وسيدوموناس فلورسينس هو الأكثر تأثيرا فى تقليل النمو الميسليومى لفطر البوترايتس فابى يليها الراشح المزرعى لفطر الترايكودرما هرزيانم-٢ مقارنة بالكنترول.

١٥- أظهر اختبار ٢٢ عزلة بكتيرية وفطرية كعوامل مقاومة حيوية أن كل العزلات قد قللت من شدة الإصابة بالتبقع البنى المتسبب عن الفطر بوترايتس فابى على الأوراق المفصولة بعد١،٣،٥،٧ يوم من العدوى . وقد أظهرت النتائج أن العزلات البكتيرية هى الأقل تأثيرا فى قدرتها على المقاومة الحيوية عن العزلات الفطرية بالمقارنة بالكنترول. وكانت عزلات الترايكودرما هارزيانم-٢، ترايكودرما هماتم-٢ ، باسيلس ساتيلس-١هى الأكثر فاعلية فى مجال المقاومة الحيوية . كما وجد أيضا أن زيادة تركيز راشح عامل المقاومة الحيوية يقلل بوضوح شدة الإصابة بالفطر بوترايتس فابى مقارنة بالكنترول.

١٦- وجد أن رش نباتات الفول قبل العدوى بـــ٢٤ ساعة بأي من عوامل المقاومة الحيوية يقلل بدرجة معنوية شدة الإصابة بالتبقع البنى مقارنة بالكنترول وقد كانت المعاملة بالترايكودرما هارزيانم-٢، ترايكودرما هماتم-١ و ترايكودرما هماتم-٢ الأفضل تأثيرا فى مقاومة مرض التبقع البنى بعد ١٤ يوم من العدوى

يليها المعاملة بالباسيلس ساتيلس-١، ترايكودرما هارزيانم-١ و سيدوموناس فلورسينس على التوالى.

١٧- أظهرت النتائج أن رش نباتات الفول البلدى براشح العوامل الحيوية قبل العدوى بالبوترايتس فابى بـــ٢٤ ساعة يقلل بدرجة معنوية من الإصابة بالتبقع البنى مقارنة بالكنترول.

١٨- دلت النتائج أن رش نباتات الفول البلدى بالعوامل الحيوية بعد العدوى بالبوترايتس فابى بـــ٢٤ ساعة كان أقل تأثيرا فى تقليل شدة الإصابة وكان راشح الباسيلس ساتيلس هو الأكثر تأثيرا قبل أو بعد العدوى بالمسبب المرضى بوترايتس فابى وذلك بعد ١٤ يوم من التحضين يليه راشح ترايكودرما هارزيانم و سيدوموناس فلورسينس على التوالى. وتزداد شدة الإصابة تدريجيا بزيادة فترة التحضين بعد العدوى حتى ١٤ يوم.

١٩- وجد أن رش الأوراق المفصولة بمعلق جراثيم البوترايتس فابى (عزلة النوبارية) المقتولة حراريا يقلل الإصابة بالتبقع البنى بشكل ملحوظ مقارنة بالكنترول بعد العدوى بـــ٧ أيام.

٢٠- على الجانب الأخر وجد أن رش نباتات الفول البلدى بمعلق جراثيم الفطر البوترايتس فابى المقتولة حراريا تحت ظروف الصوبة يقلل الإصابة بالتبقع البنى عند مقارنتها بالكنترول بعد ١٤يوم من العدوى.

٢١- أظهرت النتائج أن كل تركيزات المبيدات المستخدمة (٢٥،٥٠،١٠٠،١٥٠ ، ٢٠٠ جزء فى المليون) قد قللت نمو البوترايتس فابى تحت ظروف المعمل وقد أعطى مبيد الدياثين م-٤٥ أفضل نتيجة يليه الترايدكس والبوليرام د-ف على التوالى فى حين كان الكوسيد-١٠١ أقلها تأثيرا.

٢٢- اختلفت المبيدات المستخدمة بتركيزاتها المختلفة فى تأثيرها على شدة الإصابة بالبوترايتس فابى على الأوراق المفصولة للصنف جيزة-٤٠ وكانت مبيدات

الدياثين م- ٤٥ والترايدكس والكوسيد-١٠١ هى الأكثر تأثيرا يليها البولييرام د- ف بعد ١٤ يوم من العدوى وكان تركيز ٢٠٠ جزء فى المليون هو أفضل تركيز فى كل المبيدات خاصة دياثين م- ٤٥. وقد قللت كل المبيدات الإصابة بالتبقع البنى عند رشها قبل العدوى بـــ٢٤ ساعة تحت ظروف الصوبة . ولقد اختلفت المبيدات المستخدمة فى تأثيرها على شدة الإصابة بالبوترايتس فابى وقد كان أعلى نقص فى شدة الإصابة عند رش النباتات قبل العدوى بـــ٢٤ ساعة بالدياثين م- ٤٥ يليها الترايدكس و الكوسيد١٠١ ثم البولييرام د- ف على التوالى.

٢٣- أوضحت التجارب الحقلية لدراسة تأثير التسميد الأزوتى والفوسفورى على الإصابة بالتبقع البنى والصفات المحصولية خلال الموسمين ٢٠٠٠/٢٠٠١ ، ٢٠٠١/٠٢ أن شدة الإصابة بالمرض تتأثر نتيجة لاستخدام مستويات مختلفة من التسميد الأزوتى والفوسفورى وقد وجد أن إضافة التسميد الفوسفاتي والنيتروجيني مفردا أو خليط منهما يؤثر على طول النبات ، مكونات المحصول بدرجة معنوية بين بعض المعاملات وبدرجة غير معنوية بين البعض الأخر. وبالرغم من أن التسميد الفوسفاتي والنيتروجيني يزيد من مكونات المحصول إلا أن أفضل النتائج تم الحصول عليها نتيجة للتسميد الفوسفاتي فقط أو مختلطا مع التسميد النيتروجيني. وقد وجد أن إضافة التسميد الفوسفاتي والنيتروجيني ليس له تأثير معنوي على بعض صفات النمو مثل عدد الفروع ،عدد القرون ، وزن ١٠٠ بذرة وعدد البذور بالقرن.

٢٤- وجد أن رش نباتات الفول البلدى باستخدام المبيدات وعوامل المقاومة الحيوية المختلفة خلال موسمي النمو ٢٠٠٠/٢٠٠١ ، ٢٠٠١/٠٢ يؤثر على شدة الإصابة بالتبقع البنى كما يؤثر على الصفات المحصولية الأخرى حيث يزيد طول النبات بدرجة معنوية وتكون أعلى زيادة فى حالة الرش بمبيد كوسيد-١٠١ يليها ترايكودرما هرزيانم ثم ترايكودرما هماتم. وجد أيضا أن الرش

بالمبيدات وعوامل المقاومة الحيوية المختلفة يؤثر بدرجة معنوية على عدد القرون بالنبات ، وعدد البذور بالقرن ، ووزن١٠٠ بذرة بالجرام و محصول البذور (أردب/ للفدان) وكان أفضل معاملة للدياثين م- ٤٥ يليها الترايدكس ثم ترايكودرما هرزيانم.

٢٥- اختلفت ثمانى أصناف: جيزة- ٣ ، جيزة- ٤٠ ، جيزة- ٤٢٩ ، جيزة- ٤٦١ ، جيزة-٦٦٧ ، جيزة- ٧١٧ ، جيزة-٧١٦ و جيزة- بلانكا فى درجة حساسيتها للإصابة بالتبقع البنى وتأثيرها على المحصول ومكوناته خلال موسمي النمو ٢٠٠٠/٢٠٠١ ، ٢٠٠١/٠٢ وقد لوحظت أقل شدة إصابة فى الصنف جيزة-٧١٦ يليها جيزة- بلانكا ، جيزة- ٤٦١ والصنف جيزة- ٧١٧ خلال الموسمين فى حين لوحظت أعلى شدة إصابة فى الصنف جيزة- ٦٦٧ يليها الصنفين جيزة- ٤٠ والصنف جيزة- ٤٢٩ فى حين أن الصنف جيزة- ٣ كان متوسط القابلية للإصابة خلال الموسمين. كما اختلفت كل أصناف الفول المختبرة فى إنتاجيتها وفى مكونات المحصول. وقد أعطى الصنف جيزة- ٦٦٧ أعلى طول للنبات يليه الصنف جيزة- ٤٦١ والصنف جيزة- ٧١٧ خلال الموسمين.

٢٦-أوضحت النتائج أن شدة الإصابة بمرض التبقع البنى تنخفض تدريجيا بتأخير ميعاد الزراعة من ١ نوفمبر حتى ١٥ ديسمبر خلال موسمي النمو ٢٠٠٠/ ٢٠٠١ ، ٢٠٠١/٠٢ . ووجد أيضا أن المحصول ومكوناته من طول النبات ، عدد الفروع /نبات ، عدد القرون/نبات ،وزن ١٠٠ بذرة ، عدد البذور/قرن وإنتاجية البذور/ للفدان يقل بدرجة معنوية بتأخير ميعاد الزراعة فى كلا الموسمين.

صفحـــة الموافقـــــة

دراسات على الطرق الجديدة لمقاومة مرض التبقع البني في الفول البلدي في مصر

رسالة مقدمة من

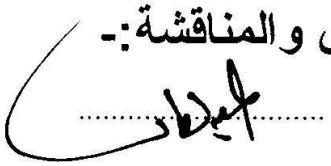
ياسر حسن السيد الجمال

بكالوريوس أمراض النبات

كلية الزراعة بمشتهر – جامعة الزقازيق/ فرع بنها (١٩٩٦)

للحصول على درجة الماجستير في العلوم الزراعية
(أمراض النبات)

تمت الموافقة على هذه الرسالة من قبل لجنة الفحص والمناقشة:-



أ.د/ عيد محمد أبو طالب .....

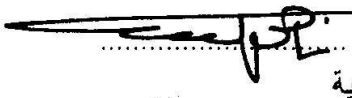
أستاذ ورئيس قسم أمراض النبات – كلية الزراعة - جامعة الإسكندرية

أ.د/ نوال عبد المنعم عيسى .....

أستاذ أمراض النبات - كلية الزراعة بمشتهر - جامعة الزقازيق (فرع بنها)

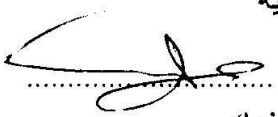
أ.د/ عبد المنعم إبراهيم إسماعيل الفقى .....

أستاذ أمراض النبات - كلية الزراعة بمشتهر - جامعة الزقازيق (فرع بنها)



أ.د/ ناجى محمد أبو زيد .....

أستاذ أمراض النبات ومدير معهد بحوث أمراض النبات – مركز البحوث الزراعية



د/ جهاد محمد دسوقى الهباء .....

أستاذ أمراض النبات المساعد - كلية الزراعة بمشتهر - جامعة الزقازيق (فرع بنها)

تاريخ الموافقة : ٢/٦ / ٢٠٠٥

دراسات علي الطرق الجديدة لمقاومة مرض التبقع البنى فى الفول البلدى فى مصر

رسالة مقدمة من

ياسر حسن السيد الجمال

بكالوريوس أمراض النبات

كلية الزراعة بمشتهر – جامعة الزقازيق/ فرع بنها (١٩٩٦)

للحصول على

درجة الماجستير فى العلوم الزراعية

(أمراض النبات)

قسم النبـــات الزراعـــى
كليـــة الزراعـــة بمشتهـــر
جـــامعة الزقازيـــق/ فرع بنها

٢٠٠٥