# Efficiency of some bio-inducers in induction of faba bean resistance to chocolate spot disease

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**Abstract-**This study aimed to evaluate the ability of six bioagents and died spores (DS) of *Botrytis fabae* to induce resistance of faba bean plants against *Botrytis fabae*. Among six tested bioagents *in vitro*, *Trichoderma harzianum* and *Trichoderma viride* were the most effective bioagents in inhibiting the growth of *B. fabae*. Under greenhouse conditions, spraying faba bean plants with the tested bio-inducer significantly decreased chocolate spot disease severity. The least disease severity % were recorded on faba bean plants treated with spores exposed to UV light (DS3), *Bacillus subtilis* and *Trichoderma album* at 3 and 5 days post inoculation with *B. fabae*. Under field conditions, all tested bio-inducer significantly reduced the disease severity % of faba bean chocolate spot disease during 2012/13 and 2013/14 seasons. Also, DS3 followed by *B. subtilis* and spores treated with chloroform (DS2) were the best effective treatments in reducing chocolate spot disease severity. Results indicated also that all tested bio-inducer treatments with superiority of DS3 and *Bacillus subtilis* treatments affected positively on the different growth parameters and yield components like plant height, number of pods and seed weight of treated faba bean plants under field conditions. All treatments increased chlorophyll, phenols and flavonoids, content in treated faba bean plants at 0, 3 and 5 days post inoculation of faba bean plants with *B. fabae* spores. The highest increase in the total phenols and flavonoids contents were recorded with (DS3) followed by (DS2) and *Bacillus subtilis* at 0, 3 and 5 days post inoculation with *B. fabae* spores. Also, all treatments increased peroxidase (PO), polyphenoloxidase (PPO), chitinase and β-1, 3- glucanase activities post inoculation of faba bean plants with *B. fabae* spores. The highest activities of all enzymes were recorded with *Trichoderma harzianum* and DS3 treatments.

**Key words** — Faba bean, chocolate spot, bio-inducers, died spores, *Trichoderma*, *Bacillus* , *Ampelomyces* and enzyme activities.

## 1 INTRODUCTION

Faba bean plant is grown mainly for its green pods and dried seeds, which are rich in a protein (18.5 to 37.8%) that can substitute for animal protein in humans, as well as other compounds (El- Sayed *et al*., 1982). Chocolate spot disease caused by *Botrytis fabae* (Sardina) is the most important disease of faba bean in Egypt. It is considered one of the most economically important diseases that damage the foliage, limit photosynthesis activity, and reduce faba bean production globally (Torres *et al*., 2004). Chocolate spot is one of the most important diseases of faba bean worldwide where it is capable of devastating unprotected crops up to 67% (Bouhassan *et al.,* 2004). Also, it is a limiting factor which causes great annual losses and sometimes complete crop failures (Koike, 1998). Chocolate spot disease is controlled mainly using fungicidal treatments although they have great hazards on the human health, environmental pollution and production of pathogen resistant races (Boris, 1997). Different management options have been developed to control chocolate spot disease and reducing the yield losses in faba bean yield worldwide. These include the use of chemical fungicides, resistant/tolerant varieties, use of certain cultural practices such as crop residue management and altering planting date (Dereje, 1999 and Bretag and Raynes, 2004). Recently, one of the new approaches of disease control management is called "induced resistance" which considers a promising modern approach with a broad spectrum in plant disease control. It could be induced in plants by applying chemical elicitors (Reglinski *et al*., 2001) and also using bio-inducers (Cook and Baker, 1983; Abd-El-Moiety and Abu-Zeid, 1985; Ermias *et al*., 2013 and Ramadan (2014). *Pseudomonas fluorescens* isolates possess a variety of promising properties which make it a good plant growth promoting traits (Fekadu and Tesfaye, 2013).Treating faba bean seeds with *Pseudomonas fluorescens* enhanced the accumulation of total phenols and flavonoids compared to untreated infected and untreated uninfected faba bean (Fekadu and Tesfaye, 2013).

Increasing synthesis of chlorophyll and enzymes like peroxidase, chitinase and phenylalanine ammonia lyase inner the plants led to induction of systemic resistance (Lagriminis and Ruthstein, 1987; Hammerschmid, 1999 and Fariduddin *et al.,* 2003), and enhanced phytoalexin production and accumulation in plant tissues (Van peer *et al.,* 1991 and Marley and Hillocks, 1993). Also, they are strengthening the epidermal and cortical cell walls and deposition of newly formed barriers beyond infection sites including callose, lignin and phenolics (Yedida *et al.,* 1999). Peroxidases oxidize phenols to quinines, which are toxic to pathogens (Bowles, 1990). Also, peroxidases participate in a broad range of physiological processes, such as the formation of lignin and suberin, the cross-linking of cell wall components and phytoalexin synthesis (Almagro *et al.,* 2009). Polyphenoloxidases participate in the oxidation of aromatic substrates and dihydroxyphenolic compounds in the presence of oxygen in the host tissues, producing quinones that are toxic to pathogens (Alfred, 2006). Based on the skeleton of flavonoids, some subgroups are flavones, flavonols, isoflavones, chalcones, aurones. The flavonoids are hydroxylated phenolic substances having the ability to disrupt microbial membranes (Dewick, 2001).

This study aimed to evaluate the efficacy of some antagonists and died spores of *Botrytis fabae* treatments in inducing resistance and controlling the chocolate spot disease caused by *B. fabae* pathogen under *in vitro* and *in vivo* conditions and to throw the light on role of some oxidative enzymes like peroxidase, polyphenoloxidase, chitinases and β-1,3-glucanase in induction of systemic acquired resistance and prophylaxes of faba bean plants against chocolate spot infection caused by *Botrytis fabae*.

## 2 Materials and Methods

### Sources of used materials:

Faba bean seeds cv. Giza 429 used in this study were obtained kindly from Legume Crop Res. Dept., Agric. Res. Cent., Giza. Egypt.

The causal organism of faba bean chocolate spot disease was isolated from infected leaf samples that collected from different fields, then, purified and identified as *Botrytis fabae* according to (Morgan 1971 & Barnett and Hunter 1987). One isolate of *Botrytis fabae* was selected from the tested *B. fabae* isolates based on its highly pathogenic ability in inciting chocolate spot infection to use in this investigation.

The tested bioagents i.e. *Ampelomyces qusisqualis Bacillus subtilis, Pseudomonas fluorescens, Trichoderma album, T. harzianum* and *T. viride* used in this study were kindly obtained from the fungal collections bank of Plant Pathology Dept., Fac. of Agric., Benha Univ. Egypt.

### Preparation of *Botrytis fabae* inoculum and its died spores:

The selected pathogenic isolate of *B. fabae* was cultured (disk, 5mm ϕ) onto PDA plates for 7 days, and then 10 mL of sterilized distilled water were added to each one of the cultured plates. The plates were tightly sealed before putting them for 10 minutes into sonar water bath to separate only the conidial spores far from their conidiophores. The crude spore suspension was collected from plates then, adjusted to $2.5\times10^5$ spore/mL (Hassan *et al*. 2006). The prepared spore suspension ($2.5\times10^5$ spore/mL) was divided to 4 equal volumes as follows, the first volume was exposed to temperature degree at 90°C for 30 minutes (DS1) the second volume was treated with chloroform at rate 1mL/L of spore suspension till evaporation chloroform (DS2) while, the third volume was exposed to UV light (1200 nm) for 30 minutes (DS3), also to kill the conidial spores of *B. fabae*. The fourth volume of prepared spore suspension was left without any treatment to use as a source of *B. fabae* inoculum when needed in any artificial inoculation process during this investigation. The first 3 volumes of spore suspension which treated with UV, temperature or chloroform were re-cultured onto PDA plates to ensure that the treated spores of *B. fabae* are completely died and unable to grow again.

### Preparation of bioagent inocula:

Two isolates of bacterial bioagents *i.e., Bacillus subtilis, Pseudomonas fluorescens*, were grown on nutrient broth medium for 2 days and then their cell suspensions were adjusted at rate 2.8 $x10^8$ cuf/mL for each one of them. Meanwhile, four fungal bioagents *i.e., Ampelomyces quisqualis*, *Trichoderma album, T. harzianum* and *T. viride* were grown on PDA medium for 7 days and then their spore suspensions were adjusted at rate $2.5x10^5$ spore/mL of each one of them.

### Effect of some bio-inducers on growth of *B. fabae in vitro*.

Six bioagents, *i.e., Bacillus subtilis, Pseudomonas fluorescens*, *Ampelomyces quisqualis*, *Trichoderma album, T. harzianum* and *T. viride* were tested for their antagonistic effects against *Botrytis fabae in vitro*. In this respect, loop of the previously prepared cell suspension was streaked individually of the two tested bacterial bioagents in the opposite side of inoculated *B. fabae* isolate (disk, 5mm ϕ) on PDA plates. Meanwhile, equal disks (5mm ϕ) of the four tested fungal bioagents i.e., *Ampelomyces quisqualis*, *Trichoderma album, T. harzianum* and *T. viride* which grown previously onto PDA plates for 7 days were placed individually in the opposite side of inoculated *B. fabae* isolate (disk, 5mm ϕ) on PDA plates. The inoculated plates were incubated for 7 days at 21 ± 1°C. Three plates were used for each treatment as replicates. The inoculated plates were examined daily and then the linear growth area of *B. fabae* was measured to determine the most effective antagonistic isolate among the tested bioagents (Abou-Zeid and Hassanien, 2000). Percentages of the fungal growth reductions (X) were calculated using the following formula:

$$X = G_1 - G_2 / G_1 \times 100$$

Where:

X= fungal growth reduction.

$G_1$= linear growth of the pathogen grown alone.

$G_2$= linear growth of the pathogen in presence of tested bioagent.

### Effect of some bio-inducers on chocolate spot disease under greenhouse conditions:

In this trail, cell and spore suspension of six bioagents, *i.e. Bacillus subtilis, Pseudomonas fluorescens*, *Ampelomyces quisqualis*, *Trichoderma album, T. harzianum* and *T. viride* in addition to suspensions of three types of died spores (DS1, DS2 and DS3) of *B. fabae* as mentioned above were applied as bio-inducer treatments to test their efficiency in controlling faba bean chocolate spot disease caused by *B. fabae* under greenhouse conditions. In this respect, the grown faba bean plants cv. Giza 429 in plastic pots (20 cm ϕ) under greenhouse conditions at 20–22°C for 40 days were sprayed individually with each one of the tested bio-inducers at rate 20 mL/pot. The antagonistic fungi were used with previously prepared spore suspension as $2.5x10^5$ spore/mL while, the antagonistic bacteria were used as 2.8 $x10^8$ cfu/mL of the previously prepared cell suspension. Whereas, the died spores of *B. fabae* of exposed and treated spore suspensions ($2.5\times10^5$ spore/mL) to UV, temperature or chloroform were also tested. Post 48 h of spraying with bio-inducer treatments, the plants were sprayed with

viable pathogenic spore suspension of *B. fabae* at rate $2.5\times10^5$ spore/mL. Control plants were sprayed with spore suspension of *B. fabae* only. Four pots (2plants/pot) were used for each treatment as replicates and the pots were arranged in a complete randomized design. The plants were covered just spraying with the viable pathogenic spore suspension of *B. fabae* using polyethylene bags for 48h at 20-22°C to keep moist around treated plants under greenhouse conditions. Disease severities were calculated post 3 and 5 days of inoculation with *B. fabae* using the scale of ICARDA (1986) as follows:

The severity was scored on treated plants using 0-9 scale, where, 0 = no visible leaf infection, 1= less than 10% infection, 2= less than 20% infection, 3= less than 30% infection 4= less than 40% infection, 5= less than 50% infection, 6= less than 60% infection, 7= less than 70% infection, 8= less than 80% infection and 9= infection more than 80% of the foliage using the following formula:

$$\text{Disease Severity \%} = \Sigma (a \times b) / N \times K \times 100$$

Where :a = Number of infected leaves in each category.
b = Numerical value of each category.
N = Total number of examined leaves.
K = The highest degree of infection category.

$$\text{Efficacy (\%)} = \frac{\text{\% Treatment-Control}}{\text{Control}} \times 100$$

## Biochemical changes in treated faba bean plants with the tested bio-inducers:

### Determination of total chlorophyll content:

Total chlorophyll contents were determined in treated faba bean plants with the tested bio-inducers (antagonists and died spores). Ten disks (1 cm ϕ) were taken from leaves of each treatment then weighted and pigments were extracted for 48 h in the dark using tubes each containing 10 mL of 85% acetone according to the method described by Procter (1981). The total chlorophyll pigments were determined by measuring the optical density (OD) at 663 and 645 nm and calculated using the formula reported by Arnon (1949) as follows:

**Total chlorophyll = 8.02 x OD at 663 + 20.20 x OD at 645 nm**

The concentrations of pigments were then expressed in mg/g fresh weight of leaves.

### Determination of total phenols content:

Half gram of fresh plant tissue was ground using a pestle and mortar with 10 mL of 80% ethanol then, filtered and centrifuged at 10,000 rpm for 20 min. The supernatant was evaporated till dryness. The residue was dissolved in 5 mL of 80% ethanol and used as the extract**.** Ten drops of concentrated hydrochloric acid were added to 0.2 mL of the prepared sample in a test tube, then, heated rapidly to boiling point over a free flame, with provision for condensation. Then, the tubes were placed in water bath at 100°C for 10 minutes. After cooling, 1mL of the reagent and 2.5 mL of 20% $Na_2CO_3$ were added to each tube. The mixture was diluted to 50 mL with distilled water and was determined after 20 minutes using Spectrophotometer (SPECTRONIC 20-D) at 520 nm against a reagent blank (Bary and Thorpe, 1954).

### Determination of total flavonoids content:

To quantify the flavonoids, 500 µL of the previously prepared extract for determination of phenol content were transferred to a test tube. Then, 500 µL of the acetic acid solution, 2 mL of the pyridine solution, 1 mL of the reagent aluminium chloride solution and 6 mL of 80% methanol were added. The samples remain at room temperature for 30 minutes. The spectrophotometer should be adjusted to a wavelength of 420 nm and the equipment must be rinsed with distilled water. The flavonoid content is expressed as milligrams of rutin equivalents per gram of sample (mg RE/g) (Peixoto Sobrinho *et al.*, 2008).

### Determination the activities of oxidative and catalyzed enzymes:

Treated leaf samples were ground with 0.2 M Tris HCl buffer (pH 7.8) containing 14 mM $\beta$-mercaptoethanol at the rate 1/3 w/v. The extracts were centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used to determine enzyme activities (Tuzun *et al.*, 1989).

### Determination of Peroxidase (PO):

Peroxidase activity was determined according to the method described by Allam and Hollis (1972), The cuvette contained 0.5 mL of 0.1 M potassium phosphate buffer at pH 7.0, 0.3 mL of enzyme extract, 0.3 mL 0.05 M pyrogallol, 0.1 mL 1.0% $H_2O_2$ and distilled water to bring cuvette contents to 3.0 mL. The reaction mixture was incubated at 25°C for 15 minutes, and then the reaction was inactivated by adding 0.5 mL. of 5.0% (v/v) $H_2SO_4$ (Kar and Mishra, 1976). Peroxides activity was expressed as the increase in absorbance at 425 nm/gram fresh weight/15 minutes.

### Determination of Polyphenoloxidase (PPO):

The polyphenoloxidase activity was determined according to the method described by Matta and Dimond (1963). The reaction mixture contained 0.2 ml enzyme extract, 1.0 ml of 0.2 M sodium phosphate buffer at pH 7.0 and 1.0 ml $10^{-3}$ M Catechol and complete with distilled water up to 6.0 ml. The reaction mixture was incubated for 30 minutes at 30°C. Polyphenoloxidase activity was expressed as the increase in absorbance at 420 nm/g fresh weigh/30 min.

### Determination of Chitinase:

Determination the activity of chitinase was carried out according to the method of Boller and Mauch, (1988). In this respect, 1 mL of 1% colloidal chitin was added to 0.05 M citrate phosphate buffer (pH 6.6) in a test tube, then, 1mL of enzyme extract was added and mixed by shaking. The tubes were kept in a water bath at 37°C for 60 minutes, then cooled and centrifuged before assaying. Reducing sugar was determined in 1mL of the supernatant by dinitrosalicylic acid (DNS). Optical density was determined at 540 nm. Chitinase activity was expressed as mM N-acetylglucose amine equivalent released/gram fresh weight tissue/60 minutes.

The substrate colloidal chitin was prepared from chitin powder according to the method described by Ried and Ogryd-Ziak (1981). Twenty five grams of chitin were milted, suspended in 250 mLof 85% phosphoric acid ($H_3PO_4$) and stored at 4°C for 24 h, then blended in 2 Liter of distilled water and the suspension was centrifuged. The washing procedure was repeated twice. The colloidal chitin suspension in the final wash was adjusted to pH 7.0 with (1 N) NaOH, separated by centrifugation and the pelted colloidal chitin was stored at 4°C. Chitinase was expressed as mM N-acetylglucose amine equivalent released / gram fresh weight tissue / 60 minutes.

### Determination of β-1,3-Glucanase:

The enzyme solution (100 µL) was mixed with 200 µL of 0.2 % (w/v) laminarin dissolved in 0.1 M sodium phosphate buffer (pH 6.0) and incubated at 30°C for 30 min. The reaction was terminated by adding dinitrosalicylic acid solution and boiling the reaction mixture for 5 min. The absorbance at 540 nm was measured and the unit was defined as the amount of the enzyme that released reducing sugar equivalent to 1 µg glucose per min under the above conditions (Sun *et al.*, 2006.). β-1,3-glucanase was expressed as mM glucose equivalent released /gram fresh weight tissue /60 minutes.

### Effect of some bio-inducers on chocolate spot disease under field conditions:

In this trail, three types of died spores in addition to six antagonists which prepared as mentioned above were evaluated for their efficiency as bio-inducer treatments in controlling faba bean chocolate spot disease and its productivity under field conditions of natural infection during two growing seasons (2012/13 and 2013/14) at vegetable farm of Hort. Dept., Fac. Agric. Moshtohor, Benha Univ., Egypt. The experiment was designed in a complete randomized design with three replicates (the plot size 3x4 m) for each particular including control treatment. Faba bean cv. Giza 429 was sown in 15th. of November during seasons 2012/13 and 2013/14, respectively. All agricultural practices were performed as usual for all treatments. The prepared suspensions of tested antagonists and died spores were sprayed on faba bean plants three times during the growing season at the beginning of flowering stage at the end of December with 15 days intervals. Chocolate spot disease severity % was recorded 7 days post the last spray as previously described. Also, the vegetative growth parameters and yield components were estimated as average of plant height (cm), number of pods/plant and seed yield weight/plot (kg).

### Statistical analyses:

Statistical analyses of all the previously designed experiments have been carried out according to the procedures (ANOVA) reported by Snedecor and Cochran (1989). Treatment means were compared by the least significant difference test "L.S.D" at 5% level of probability.

## 3 Results

### Effect of bio-inducers on growth of *B. fabae in vitro.*

Results in Table (1) indicate that, all tested bio-inducers reduced significantly the growth of *B. fabae* comparing to control treatment *in vitro*. In this respect, the tested *Trichoderma* isolates were more effective than bacterial isolates. Also, *Trichoderma harzianum* and *Trichoderma viride* were the most effective among the six tested bioagents in inhibiting the growth of *B. fabae* where the reduction percentages were 78.52 and 75.92% respectively, whereas *Pseudomonas fluorescens* was the least effective one where it gave only 57.03% of growth reduction.

Table (1): Effect of some bio-inducers on growth of *Botrytis fabae in vitro.*

| Tested bioagent | Growth (mm) | %Reduction |
|---|---|---|
| *Bacillus subtilis* | 29.67 | 67.03 |
| *Pseudomonas fluorescens* | 38.67 | 57.03 |
| *Ampelomyces quisqualis* | 31.33 | 65.19 |
| *Trichoderma album* | 24.33 | 72.97 |
| *Trichoderma harzianum* | 19.33 | 78.52 |
| *Trichoderma viride* | 21.67 | 75.92 |
| Control | 90 | 0.00 |
| L.S.D. at 5% | 1.87 | |

### Effect of some bio-inducers on chocolate spot disease under greenhouse conditions:

Data in Table (2) show that spraying faba bean plants with any of the tested bio-inducer treatments significantly decreased chocolate spot disease severity (7.5 - 12.4%) compared with control treatment (60.5%). In this respect, DS3, *Bacillus subtilis* and DS2 were the most effective treatments respectively in reducing chocolate spot disease severity where they recorded 87.6, 86.4 and 84.8% respectively, followed by *Trichoderma album, T. harzianum* and *Ampelomyces quisqualis,* which recorded 83.5, 83.7 and 82.2% respectively. Also, it is clear from the obtained results that the determined disease severity % of all tested bio-inducers and control treatments at 5 days

post inoculation with *B. fabae* were higher in their values than those at 3 days. The least determined disease severity % at 3 and 5 days post inoculation with *B. fabae* were recorded on faba bean plants treated with DS3 and *Bacillus subtilis* as bio-inducer treatments. On the other hand, *Trichoderma viride* was the least effective treatment in reducing chocolate spot disease severity caused by *B. fabae* compared with the other tested bio-inducers under greenhouse conditions.

## Biochemical changes in treated faba bean plants with the tested bio-inducers:

### Changes in total chlorophyll content:

It is well known that chlorophyll content is a good parameter for reflecting the health condition of any plant. Data in Table (3) indicate that all bio-inducer treatments increased chlorophyll content in treated faba bean plants compared with control treatment at 0, 3 and 5 days post inoculation. The highest recorded increase in chlorophyll content among all tested bio-inducer treatments was in case of treating faba bean plants with DS3 at 0, 3 and 5 days post inoculation with *B. fabae* with efficacy% being 103,0, 102.2 and 113.7% respectively, followed by *Ampelomyces quisqualis* treatment at 0 time and DS2 treatment at 0, 3 and 5 days post inoculation of faba bean plants with *B. fabae*.

Table (2): Effect of some bio-inducers on chocolate spot disease under greenhouse conditions

| Treatment | Disease severity % at days | | | Reduction % at days | | |
|---|---|---|---|---|---|---|
| | 3 | 5 | Mean | 3 | 5 | Mean |
| Died spores (DS1) | 10.0 | 13.3 | 11.7 | 79.5 | 81.5 | 80.7 |
| Died spores (DS2) | 7.5 | 10.9 | 9.2 | 84.7 | 84.9 | 84.8 |
| Died spores (DS3) | 6.1 | 8.9 | 7.5 | 87.4 | 87.7 | 87.6 |
| *Bacillus subtilis* | 6.7 | 9.7 | 8.2 | 86.2 | 86.6 | 86.4 |
| *Pseudomonas fluorescens* | 9.9 | 12.3 | 11.1 | 79.6 | 82.9 | 81.6 |
| *Ampelomyces quisqualis* | 9.4 | 12.1 | 10.8 | 80.8 | 83.2 | 82.2 |
| *Trichoderma album* | 8.5 | 11.6 | 10.0 | 82.7 | 84.0 | 83.5 |
| *Trichoderma harzianum* | 9.3 | 11.7 | 10.5 | 81.0 | 83.8 | 82.7 |
| *Trichoderma viride* | 10.6 | 14.2 | 12.4 | 78.3 | 80.3 | 79.5 |
| Control | 48.8 | 72.2 | 60.5 | 0.0 | 0.0 | 0.0 |
| LSD 0.05 | 1.37 | 1.84 | | | | |

**DS1= *B. fabae* spores exposed to temperature**
**DS2 = *B. fabae* spores treated with Chloroform**
**DS3= *B. fabae* spores exposed to UV light**

Table (3): Changes in total chlorophyll content in treated faba bean plants with the tested bio-inducers as mg/g fresh weight of leaves.

| Treatment | Days post inoculation | | | Efficacy % Days post inoculation | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 5 | 0 | 3 | 5 |
| DS1 | 6.6 | 6.1 | 5.8 | 42.0 | 35.3 | 38.6 |
| DS2 | 8.3 | 8.0 | 8.1 | 78.5 | 78.8 | 95.7 |
| DS3 | 9.4 | 9.1 | 8.9 | 103.0 | 102.2 | 113.7 |
| *B. subtilis* | 8.0 | 8.0 | 7.8 | 72.0 | 78.4 | 86.8 |
| *Ps. fluorescens* | 5.9 | 5.6 | 4.8 | 26.3 | 25.7 | 16.1 |
| *A. quisqualis* | 9.2 | 7.6 | 6.2 | 98.7 | 69.0 | 49.9 |
| *T. album* | 7.3 | 6.8 | 6.0 | 56.5 | 50.9 | 44.6 |
| *T. harzianum* | 6.9 | 6.1 | 5.5 | 48.3 | 35.3 | 33.0 |
| *T. viride* | 7.3 | 7.2 | 6.6 | 58.0 | 61.4 | 57.8 |
| Control | 4.6 | 4.5 | 4.2 | 0.0 | 0.0 | 0.0 |

### Changes in total phenols and flavonoids contents:

Data in Table (4) reveal that the spraying faba bean plants with the different tested bio-inducer treatments affected greatly on phenols and flavonoids contents at days post inoculation. In this respect, all tested treatments increased the total phenols content compared with control treatment. The highest increase in the total phenols was recorded in treated faba bean plants with DS3 followed by DS2 and *Bacillus subtilis* at 0, 3 and 5 days post inoculation with *B. fabae*. On the other hand, the least increase of total phenols content was recorded with *Ampelomyces quisqualis* treatment at 0, 3 and 5 days post inoculation. As for flavonoids content, the highest increase was scored in case of treating faba bean plants with DS3 followed by DS2 and *Bacillus subtilis* respectively at 0, 3 and 5 days post inoculation with *B.*

*fabae* with highly efficacy % also of the three treatments comparing to the other tested bio-inducer treatments and control. However, the least increase in the flavonoids content was recorded with *Trichoderma harzianum* treatment at all tested inoculation times.

Table (4): Changes in Phenols and total Flavonoids contents in treated faba bean plants with the tested bio-inducers as mg/g fresh weight of leaves.

| Treatment | Phenols | | | Flavonoids | | | Efficacy (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days post inoculation | | | Days post inoculation | | | Phenols | | | Flavonoids | | |
| | | | | | | | Days post inoculation | | | Days post inoculation | | |
| | 0 | 3 | 5 | 0 | 3 | 5 | 0 | 3 | 5 | 0 | 3 | 5 |
| DS1 | 2.5 | 3.0 | 6.7 | 1.9 | 2.3 | 12.3 | 171.4 | 187.4 | 192.1 | 110.9 | 74.6 | 739.7 |
| DS2 | 3.7 | 4.6 | 9.2 | 3.0 | 6.3 | 23.5 | 311.0 | 341.8 | 301.8 | 222.8 | 387.7 | 1512.3 |
| DS3 | 3.9 | 6.0 | 9.3 | 4.8 | 12.0 | 25.4 | 324.2 | 482.5 | 306.1 | 422.8 | 826.2 | 1638.4 |
| *B.subtilis* | 3.6 | 4.2 | 7.3 | 2.5 | 5.1 | 21.7 | 291.2 | 306.8 | 218.0 | 176.1 | 294.6 | 1387.0 |
| *Ps. fluorescens* | 1.4 | 2.4 | 6.2 | 1.3 | 1.6 | 2.5 | 48.4 | 131.1 | 171.5 | 39.1 | 24.6 | 69.9 |
| *A. quisqualis* | 1.2 | 2.1 | 3.4 | 1.5 | 1.8 | 2.8 | 27.5 | 104.9 | 48.3 | 58.7 | 41.5 | 92.5 |
| *T. album* | 1.8 | 2.4 | 6.3 | 1.7 | 1.9 | 6.8 | 94.5 | 132.0 | 175.0 | 81.5 | 45.4 | 362.3 |
| *T. harzianum* | 1.3 | 2.2 | 4.0 | 1.2 | 1.5 | 1.7 | 42.9 | 114.6 | 74.6 | 34.8 | 16.2 | 18.5 |
| *T. viride* | 2.7 | 3.1 | 7.4 | 2.3 | 5.1 | 21.3 | 201.1 | 204.9 | 224.1 | 146.7 | 290.8 | 1357.5 |
| Control | 0.9 | 1.0 | 2.3 | 0.9 | 1.3 | 1.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

## Changes in activities of oxidative and catalyzed enzymes:

### Changes in peroxidase and polyphenoloxidase activity:

Data in Table (5) show that all treatments increased peroxidase and polyphenoloxidase activity compared with control treatment at all days post inoculation.

As for PO, the highest activities were recorded with *Trichoderma harzianum* followed by DS3 and *Ampelomyces quisqualis* treatments at 0, 3 and 5 days post inoculation respectively. On the other hand, the least increase in the determined peroxidase activities at the three times post inoculations were with *Trichoderma album.* Generally, it could be concluded that *Trichoderma harzianum* was the most effective bio-inducer on peroxidase among the other tested bio-inducers while, DS3 was the best compared with DS2 and DS1.

As for PPO, the highest activities were recorded with *Trichoderma harzianum* followed by *Ampelomyces quisqualis* and DS3 comparing with the other tested bio-inducer treatments and control at zero time post inoculation. At 3 and 5 days, the highest increase in activities of PPO was recorded with the treatments of *Trichoderma harzianum* followed by DS3 and *Ampelomyces quisqualis* respectively. Whereas, the least increase in activity of PPO at zero time and 5 days post inoculation were recorded with DS1 treatment, however, it was recorded with *Trichoderma album* at 3 days post inoculation.

### Changes in chitinase and β-1,3-glucanase activity.

Data in Table (6) reveal that *Trichoderma harzianum*, DS3 and *Trichoderma album* respectively, were the most effective bio-inducers among the others tested in increasing chitinase activities at 0, 3 and 5 days post inoculation of faba bean plants with *B. fabae* the causal pathogen of chocolate spot disease. However, DS2 and *Trichoderma viride* came in the second rank in this respect. On contrary, *Pseudomonas fluorescens* was the least effective bio-inducer treatment on chitinase activity at the three times post inoculation with *B. fabae*.

As for Activity of β-1,3-glucanase, DS3, *Trichoderma harzianum* and DS2 were the most effective bio-inducer treatments in increasing the activities of β-1,3-glucanase at zero time post inoculation with *B. fabae.* However, *Trichoderma harzianum*, DS3 and DS2 respectively were the most effective bio-inducer treatments in increasing the activities of β-1,3-glucanase at 3 and 5 days post inoculation with *B. fabae*. On the other hand, *Pseudomonas fluorescens* was the least effective treatment on β-1,3-glucanase activity at the three times post inoculation.

Table (5): Changes in peroxidase and polyphenoloxidase activities in treated faba bean plants with the tested bio-inducers.

| Treatment | PO | | | PPO | | | Efficacy (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days post inoculation | | | Days post inoculation | | | PO | | | PPO | | |
| | | | | | | | Days post inoculation | | | Days post inoculation | | |
| | 0 | 3 | 5 | 0 | 3 | 5 | 0 | 3 | 5 | 0 | 3 | 5 |
| DS1 | 3.6 | 4.4 | 6.5 | 0.8 | 1.1 | 1.5 | 328.6 | 65.2 | 73.7 | 23.8 | 62.7 | 26.1 |
| DS2 | 4.4 | 5.3 | 9.6 | 1.2 | 2.0 | 3.0 | 428.6 | 100.0 | 156.7 | 93.7 | 192.5 | 160.0 |
| DS3 | 5.0 | 7.6 | 10.9 | 1.3 | 2.3 | 3.6 | 498.8 | 186.4 | 192.7 | 100.0 | 241.8 | 214.8 |
| *B. subtilis* | 4.1 | 5.2 | 8.9 | 1.2 | 1.4 | 2.5 | 392.9 | 95.5 | 138.2 | 84.1 | 114.9 | 114.8 |
| *Ps. fluorescens* | 2.6 | 4.6 | 7.6 | 1.0 | 1.2 | 1.7 | 214.3 | 72.7 | 105.1 | 57.1 | 80.6 | 49.6 |
| *A. quisqualis* | 4.5 | 5.7 | 10.3 | 1.3 | 2.1 | 3.4 | 433.3 | 115.5 | 176.3 | 101.6 | 219.4 | 197.4 |
| *T. album* | 3.1 | 3.6 | 5.1 | 0.8 | 1.1 | 1.6 | 264.3 | 36.4 | 36.0 | 33.3 | 61.2 | 36.5 |
| *T. harzianum* | 5.2 | 8.1 | 12.2 | 1.3 | 2.7 | 4.1 | 520.2 | 205.3 | 229.0 | 112.7 | 297.0 | 258.3 |
| *T. viride* | 3.7 | 4.8 | 8.2 | 1.0 | 1.2 | 2.5 | 342.9 | 79.9 | 119.4 | 61.9 | 85.1 | 113.0 |
| Control | 0.8 | 2.6 | 3.7 | 0.6 | 0.7 | 1.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Table (6): Changes in chitinase and β-1,3-glucanase enzymes activity in treated faba bean plants with the tested bio-inducers.

| Treatment | Chitinase | | | β-1,3-glucanase | | | Efficacy (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days post inoculation | | | Days post inoculation | | | Chitinase | | | β-1,3-glucanase | | |
| | | | | | | | Days post noculation | | | Days post inoculation | | |
| | 0 | 3 | 5 | 0 | 3 | 5 | 0 | 3 | 5 | 0 | 3 | 5 |
| DS1 | 1.4 | 2.7 | 5.8 | 2.2 | 4.3 | 7.9 | 77.8 | 28.7 | 161.4 | 307.4 | 733.3 | 92.9 |
| DS2 | 2.4 | 3.8 | 8.8 | 3.5 | 7.3 | 12.4 | 190.1 | 83.3 | 299.1 | 546.3 | 1333.3 | 202.7 |
| DS3 | 3.0 | 4.0 | 9.7 | 4.1 | 10.5 | 12.5 | 274.1 | 90.0 | 339.1 | 653.7 | 1964.7 | 207.4 |
| *B. subtilis* | 2.1 | 3.4 | 7.8 | 3.0 | 5.4 | 9.2 | 159.3 | 63.2 | 255.5 | 463.0 | 951.0 | 125.5 |
| *Ps. fluorescens* | 1.2 | 2.2 | 5.5 | 1.1 | 2.4 | 5.7 | 44.4 | 3.4 | 151.8 | 111.1 | 368.6 | 39.5 |
| *A. quisqualis* | 2.1 | 3.1 | 7.4 | 1.6 | 4.0 | 7.7 | 154.3 | 48.3 | 234.1 | 203.7 | 680.4 | 89.2 |
| *T. album* | 2.7 | 3.8 | 9.4 | 3.2 | 6.2 | 11.9 | 227.2 | 83.7 | 327.3 | 487.0 | 1111.8 | 190.7 |
| *T. harzianum* | 4.6 | 6.0 | 15.1 | 4.0 | 12.1 | 21.5 | 463.0 | 184.7 | 587.3 | 642.6 | 2262.8 | 425.7 |
| *T. viride* | 2.2 | 3.6 | 8.6 | 3.2 | 5.7 | 10.5 | 166.7 | 72.7 | 289.6 | 485.2 | 1023.5 | 157.4 |
| Control | 0.8 | 2.1 | 2.2 | 0.5 | 0.5 | 4.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

## Effect of some bio-inducers on chocolate spot disease under field conditions:

Data in Table (7) show that all tested bio-inducer treatments were significantly effective in reducing the disease severity % of faba bean chocolate spot disease comparing with control treatment when applied under field conditions during the two growing seasons (2012/13 and 2013/14). In this respect, DS3 followed by *B. subtilis* and DS2 were the best effective among the other tested bio-inducer treatments in reducing chocolate spot disease severity during the two growing seasons where, they recorded the least disease severity and higher reduction % respectively. On the other hand, the least affective bio-inducer treatment on faba bean chocolate spot disease was *Trichoderma viride* during the two growing seasons.

## Effect of some bio-inducers on some growth parameters and yield components of faba bean under field conditions.

Results in Table (8) reveal that all tested bio-inducer treatments affected significantly on the different growth parameters and yield components like plant height, number of pods and seed weight of treated faba bean plants with them under field conditions during seasons2012/13 and 2013/14. In this respect, the best parameters of plant height, number of pods and seed weight of plot or hectare were recorded with DS3 treatment. Moreover, DS3 was the best effective treatment than DS2 or DS1 while, *Bacillus subtilis* was the most effective treatment comparing to the other used antagonists as bio-inducers in improving faba bean plant growth and yield components. However, *Trichoderma viride* was the least effective one.

Table (7): Effect of some bio-inducers on chocolate spot disease under field conditions

| Treatment | 2012 / 13 | | 2013 / 14 | |
|---|---|---|---|---|
| | Disease severity % | % Reduction | Disease severity % | % Reduction |
| DS1 | 10.4 | 58.3 | 8.1 | 63.0 |
| DS2 | 6.7 | 73.2 | 3.7 | 83.0 |
| DS3 | 4.1 | 83.6 | 2.2 | 89.8 |
| *B. subtilis* | 6.3 | 74.7 | 3.0 | 86.4 |
| *Ps. fluorescens* | 9.1 | 63.5 | 7.3 | 66.4 |
| *A. quisqualis* | 8.3 | 66.5 | 6.3 | 71.2 |
| *T. album* | 7.0 | 71.7 | 4.9 | 77.8 |
| *T. harzianum* | 7.4 | 70.2 | 5.9 | 72.9 |
| *T. viride* | 11.1 | 55.2 | 9.6 | 55.9 |
| Control | 24.8 | 0.0 | 21.8 | 0.0 |
| L.S.D at 0.05 | 1.74 | | 1.71 | |

Table (8): Effect of some bio-inducers on some growth parameters and yield components of faba bean under field conditions

| Treatment | 2012 /13 | | | | 2013 /14 | | | |
|---|---|---|---|---|---|---|---|---|
| | Plant height (cm) | Pod number | Seed weight/ Kg plot | Seed weight/ Kg hectare | Plant height (cm) | Pod number | Seed weight/ Kg plot | Seed weight/ Kg hectare |
| DS1 | 90.7 | 22.3 | 4.1 | 3391.7 | 106.3 | 24.7 | 4.7 | 3916.7 |
| DS2 | 95.7 | 26.7 | 4.6 | 3808.3 | 108.0 | 28.3 | 5.3 | 4416.7 |
| DS3 | 105.0 | 29.7 | 4.8 | 4000.0 | 118.7 | 31.3 | 5.5 | 4583.3 |
| *B. subtilis* | 97.7 | 28.7 | 4.6 | 3833.3 | 112.7 | 30.7 | 5.4 | 4500.0 |
| *Ps. fluorescens* | 82.0 | 23.3 | 4.2 | 3500.0 | 103.7 | 25.0 | 4.8 | 4000.0 |
| *A. quisqualis* | 94.3 | 24.7 | 4.4 | 3666.7 | 100.0 | 26.3 | 5.0 | 4166.7 |
| *T. album* | 89.7 | 25.3 | 4.5 | 3750.0 | 105.3 | 27.7 | 5.2 | 4333.3 |
| *T. harzianum* | 83.7 | 24.7 | 4.4 | 3666.7 | 98.3 | 27.3 | 5.1 | 4250.0 |
| *T. viride* | 78.0 | 20.3 | 3.9 | 3250.0 | 96.3 | 24.0 | 4.6 | 3858.3 |
| Control | 75.3 | 18.7 | 3.8 | 3166.7 | 78.7 | 22.3 | 4.3 | 3583.3 |
| L.S.D at 0.05 | 6.45 | 1.46 | 0.37 | | 4.39 | 1.68 | 0.43 | |

## 4 DISCUSSION

Chocolate spot disease caused by *Botrytis fabae* (Sardina) is the most important disease of faba bean in Egypt and worldwide. It is considered one of the most economically important diseases that damage the foliage, limit photosynthesis activity, and reduce faba bean production globally (Torres *et al*., 2004).

In this study, among six tested bio-inducers, all of them reduced significantly the growth of *B. fabae* comparing to control treatment *in vitro*. In this respect, the tested *Trichoderma* isolates were more effective than bacterial isolates. Also, *Trichoderma harzianum* and *Trichoderma viride* were the most effective among the six tested bioagents in inhibiting the growth of *B. fabae* whereas; *Pseudomonas fluorescens* was the least effective one. Under greenhouse conditions, spraying faba bean plants with any one of the tested bio-inducer treatments significantly decreased chocolate spot disease severity compared with control treatment. In this respect, DS3, *Bacillus subtilis* and DS2 were the most effective treatments respectively in reducing chocolate spot disease severity followed by *Trichoderma album, T. harzianum* and *Ampelomyces quisqualis*, The least determined disease severity % at 3 and 5 days post inoculation with *B. fabae* were recorded on faba bean plants treated with DS3 and *Bacillus subtilis* as bio-inducer treatments. On the other hand, *Trichoderma viride* was the least effective treatment in reducing chocolate spot disease severity caused by *B. fabae* compared with the other tested bio-inducers under greenhouse conditions. Also, all tested bio-inducer treatments were significantly effective in reducing the disease severity % of faba bean chocolate spot disease comparing with control treatment when applied under field conditions during 2012/13 and 2013/14 seasons. DS3 followed by *B. subtilis* and DS2 were the best effective among the other tested bio-inducer treatments in reducing chocolate spot disease severity during the two growing seasons while, the least affective bio-inducer treatment on faba bean chocolate spot disease was *Trichoderma viride* during the two growing seasons. Results indicated also that all tested bio-inducer treatments affected significantly the different growth parameters and yield components like plant height, number of pods and seed weight of treated faba bean plants with them under field conditions during seasons2012/13 and 2013/14. In this respect, the best parameters of plant height, number of pods and seed weight of plot or hectare were recorded

with DS3 treatment followed by *Bacillus subtilis* in this respect. The obtained data could be discussed in light the findings of EL-Gammal (2005) who showed that spraying faba bean plants with suspension of un-viable heated spores of *B. fabae* spores scored a remarkable depression in chocolate spot disease severity comparing with unsprayed one which being 2.07 and 8.87%, respectively after 7 days from inoculation. He added also that, *T. harzianum*-II, *T. hamatum*-II, *T. hamatum*-I and *Bacillus subtilis*-I were effective antagonists in reducing infection of chocolate spot disease severity after 7 days post inoculation. While, Eisa *et al.,* (2006) recorded that *T. harzianum* and *T. koningii* were effective in reducing the growth of *B. fabae in vitro*. Also, El-Sayed, (2006) reported that application of antagonistic isolates reduced significantly disease severity of chocolate spot disease caused by *B. fabae* compared to control during two seasons under field conditions and gave a significant effect on plant height, fresh weight, dry weight, number of pods, pods weight/plant and 100 seed weight. Saber *et al*., (2009*)* reported that, foliar spraying of grown faba bean with several *Trichoderma* isolates significantly reduced chocolate spot disease severity caused by *B. fabae* as compared to control. Also, El-Banoby *et al.,* (2013) found that, *Trichoderma harzianum, Bacillus subtilis* and *Ampelomyces quisqualis* caused the highest reduction of *Botrytis fabae* growth while, *Pseudomonas fluorescens* showed moderate effect in reducing the fungal growth area. They added also that *B. subtilis, P. fluorescens* and *T. harzianum* gave the highest seed yield / plot, the highest hundred seed weight (g) and the highest seed yield ton / fed. as compared with the control treatment. The use of *P. fluorescens* isolates could increase the faba bean growth and yield performance. These isolates can be used as potential bio-fertilizers and plant growth promoter (Fekadu and Tesfaye, 2015).

As for changes in chlorophyll, phenols and flavonoids contents in treated faba bean plants with the tested bio-inducers, all bio-inducer treatments increased chlorophyll content in treated faba bean plants compared with control treatment at 0, 3 and 5 days post inoculation. The highest increase in chlorophyll content among was recorded in case of treating faba bean plants with DS3 at 0, 3 and 5 days post inoculation with *B. fabae* respectively, followed by *Ampelomyces quisqualis* treatment at 0 time and DS2 treatment at 0, 3 and 5 days post inoculation. Also, the tested bio-inducers increased the total phenols content comparing with control treatment. The highest increase in the total phenols was recorded in treated faba bean plants with DS3 followed by DS2 and *Bacillus subtilis* at 0, 3 and 5 days post inoculation with *B. fabae*. On the other hand, the least increase of total phenols content was recorded with *Ampelomyces quisqualis* treatment at 0, 3 and 5 days post inoculation. The highest increase in flavonoids content was scored in case of treating faba bean plants with DS3 followed by DS2 and *Bacillus subtilis* respectively at 0, 3 and 5 days post inoculation with *B. fabae*. However, the least increase in the flavonoids content was recorded with *Trichoderma harzianum* treatment at all tested inoculation times. These results could be discussed in light the findings of Hahlbrock and Scheel, (1989) who cleared that phenols are essential for biosynthesis of lignin, which is considered an important structural component of plant cell walls. Also, Lamba *et al.,* (2008) who explained that the first step of the defense mechanism in plants involves a rapid accumulation of phenols at the infection site, which restricts or slows the growth of the pathogen because of its action as antioxidant, antimicrobial and photoreceptor. While, Mohamed *et al.,* (2012) mentioned that the defense strategy of plants consists of two stages. The first stage is assumed to involve the rapid accumulation of phenols at the infection site, which function to slow the growth rate of the pathogen and to allow for the activation of "secondary" strategies that will more thoroughly restrict the pathogen. The secondary responses involve the activation of specific defense mechanisms, such as the synthesis of molecules related to pathogen stress. Also the obtained results could be supporting with those obtained by Saber *et al.,* (2009) who reported that foliar spraying of faba bean with *Trichoderma viride* (tag3 and tag4) and *T. harzianum* tag7 reduced chocolate spot disease and enhanced the physiological activities (photosynthetic pigments, total phenol and polyphenoloxidase). However, Fekadu and Tesfaye**,** **(**2013) reported that applying *Pseudomonas fluorescens* 9 and 10 by bio-primed seed of faba bean treatment enhanced the accumulation of total phenols and flavonoids compared to untreated infected and uninfected untreated faba bean. However, El-Rahman and Mohamed, (2014) found that application of *Trichoderma harzianum* as foliar treatment significantly reduced the severity of chocolate spot disease as compared with untreated infected plants and also, inducers markedly increased total chlorophyll content in treated infected plants as compared with untreated. However, Fekadu and Tesfaye**,** **(**2013) reported that applying *Pseudomonas fluorescens* 9 and 10 by bio-primed seed of faba bean treatment enhanced the accumulation of total phenols and flavonoids compared to untreated infected and uninfected untreated faba bean.

Regarding changes in activities of oxidative and catalyzed enzymes in treated faba bean plants with the tested bio-inducers, all bio-inducer treatments increased peroxidase (PO) and polyphenoloxidase (PPO) activity compared with control treatment at all days post inoculation. The highest activities of PO and PPO enzymes were recorded with *Trichoderma harzianum*, DS3 and *Ampelomyces quisqualis* treatments at 0, 3 and 5 days post inoculation. Also, *Trichoderma harzianum*, DS3 and *Trichoderma album* respectively, were the most effective bio-inducers in increasing chitinase activities at 0, 3 and 5 days post inoculation of faba bean plants with *B. fabae*. On the other hand, *Pseudomonas fluorescens* was the least effective bio-inducer treatment on chitinase activity. While, *Trichoderma harzianum*, DS3 and DS2 respectively were the most effective bio-inducer treatments in increasing the activities of β-1,3-glucanase, *Pseudomonas fluorescens* was the least effective treatment on β-1,3-glucanase activity at the three times post inoculation.

Thus, the increments in chitinase and β-1,3 glucanase activity in leaves of faba bean indicate that plants are ready to act against chocolate spot pathogen by directly degrading the pathogen cell wall and in turn protecting the plant. Hence, the high activities of PO, PPO, Chitinase and β-1, 3- glucanase in plant leaves might have a great role in induced resistance against chocolate spot disease. These results could be interpreting in light the findings of Abeles *et al*., (1970) who reported that β-1,3 glucan and chitin, polymer of N-acetylglucosamine (NAG) are major cell wall components of many fungi. Since β-1,3 glucanase and chitinases have been shown to be capable of attacking cell wall of fungal pathogens, these enzymes have been proposed as direct defense enzymes of plants. While, Vance *et al*., (1980) and Fry, (1982) stated that peroxidase is known to be involved in the oxidation of polymerization of hydroxycinnamyl alcohols to yield lignin and crosslinking isodityrosine bridges in cell wall, Ride, (1983) and Tarrad *et al*., (1993), reported that the increase in peroxidase activity enhance lignification in response to chocolate spot infection which may restrict the fungal penetration. These findings indicate a positive relationship between resistance and peroxidase activity.

Peroxidase also produces free radicals and hydrogen peroxide which are toxic to many microorganisms (Pena and Kuc, 1992). Induced resistance is defined as an enhancement of the plants defensive capacity against a broad spectrum of pathogens and pests that is acquired after appropriate stimulation. Pre-treatment of plants with avirulent pathogens (bio-inducers) or chemical compounds (abio-inducers) can enhance resistance to subsequent attack not only at the site of treatment, but also in tissues distant from the initial infection sites. Typically, this inducible resistance system known as systemic acquired resistance (SAR) is effective against diverse pathogens including viruses, bacteria and fungi (Ryals *et al*., 1996). Also, Nawar and Kuti, (2003) stated that an increase in peroxidase activity is considered as a preliminary indicator for resistance of broad beans to chocolate spot disease. These compounds act as barriers against pathogen invasion. These results are also in agreement with those obtained by Emeran *et al*., (2006) who reported that treating faba bean plants with *Bacillus subtilis* and *Trichoderma harzianum*, incited significant increment in the activity of peroxidase compared with the untreated plants.

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