جامعة عين شمس
Faculty of Women for, Arts, Science, and Education

Scientific Publishing Unit

# Journal of Scientific Research in Science

Biological Sciences

Volume 38, Issue 1, 2021

ISSN 2356-8372 (Online) \ ISSN 2356-8364 (print)

Journal of Scientific Research in Science 2021, 38, (1): 83-115

Contents lists available at EKB

Journal of Scientific Research in Science

Journal homepage: https://jsrs.journals.ekb.eg/

# Systemic Resistance in Chickpea (*Cicer arietinum* L.) Elicited by Some Biotic Inducers Against Root Diseases

**Ahmed k. A. Mawad[a,*], Ehab A. D. Sarhan[b] , Hoda H. Abo-Ghalia[a] , Zeinab M. H. kheiralla[a]**

[a]Botany Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Egypt.

[b]Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.

## Abstract

The effect of seed treatment of chickpea (*Cicer arietinum* L.) with biotic inducers such as *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* in contrast to the fungicide Rizolex-T, were evaluated in the greenhouse and under field conditions during the 2017/2018 season to control the plant disease caused by *Fusarium oxysporum*, *Rhizoctonia solani*, or *Sclerotinia sclerotiorum*, at Giza Agriculture Research Station, Agricultural Research Center, Giza Governorate, Egypt. The tested strains significantly inhibit the mycelial growth of the three tested fungi for pathogenic growth. Compared to the untreated control under greenhouse and field conditions, all the biotic inducer treatments tested significantly decreased the percentages of damping-off, root rot, stem rot and/or wilt diseases. It was noticed that Rizolex-T and (*Trichoderma viride* + *Trichoderma harzianum*) have reached the highest percentage of surviving plants followed by (*Pseudomonas fluorescens* + *Bacillus subtilis*), *Trichoderma viride*, *Trichoderma harzianum*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Serratia marcescens*, respectively. As well as all the treatments of the checked biocontrol agents increased the growth and yield parameters of chickpea significantly, i.e., plant hight, branches number per plant, pods number per plant, seeds number per plant, seeds weight per plant, 100 seeds weight, and chickpea yield ton/fed. In the presence of the three studied pathogens, defense-related enzyme activities (β-1,3 glucanase, peroxidase, and polyphenoloxidase) have also been determined in all chickpea plants treated with tested biotic inducers compared to untreated infested and non-infested control. The treatment of (*Trichoderma harzianum* + *Trichoderma viride*) showed the highest increase in phenol content and the activities of defense-related enzymes.

**Keywords:** Biotic inducers, chickpea, pathogenic fungi, plant diseases

## 1. Introduction

Chickpea (*Cicer arietinum* L.) is a highly nutritious grain legume crop, including adecent carbohydrate and protein sources, and consider the one of the most

***Corresponding author:** Ahmed k. A. Mawad**,** Botany Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Egypt.
**E-mail**: kemoadwia@yahoo.com

essential pulse crops grown in over fifty nations. It also helps to increase soil fertility by biological fixation $N_2$. About 14.78 million metric tons of chickpeas are globally grown [1,2].

Chickpea is attacked with several soil-borne fungi, i.e., *Fusarium oxysporum, F. solani, Fusarium eumartii, Fusarium spp., Rhizoctonia solani, Sclerotinia sclerotiorum, Sclerotinia trifoliorum, Pythium ultimum, Sclerotium rolfsii, Macrophomina phaseolina,* and *Verticillium alboatrum* causing damping-off, root rot and/or stem rot and wilt diseases, can have significantly negative effects on the growth and yield production of chickpea plants [3,4,5,6,7].

Plant diseases are conventionally controlled by chemical fungicides. However, because of the obvious harmful impacts on the environment due to utilization of fungicide, biological control is an important alternative strategy to reduce the use of fungicides in plant disease management that have achieved great success in plant disease control and decrease the severe side effects of chemical plant disease control [8, 9].

Plant Growth-Promoting Rhizobacteria (PGPR) plays an essential role in agricultural development. Those significant effects of PGPR have a directly or indirectly effect on plants, direct motivation of growth via the producing of metabolites that enhance plant growth, but indirect motivation of growth through the elimination of pathogens via the producing of secondary metabolites [10, 11].

Biotic and abiotic inducer applications have the potential to control plant diseases [12]. An significant means of suppressing plant diseases is considered to be induced systemic defense reaction in plants using plant growth-promoting rhizobacteria (PGPR) as it can motivate plant defense in the host plants in response to microbial infection, including defense-related enzymes and pathogenesis-related proteins such as β-1,3-glucanase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, indoleacetic acid (IAA), lignin synthesis, accumulation of phenolic compounds and specific flavonoids [13,14,15,16]. Meanwhile, a potential approach to disease management due to soil-borne pathogens [17, 11, 18]. The defense-related enzymes β-1,3 glucanase, peroxidase (PO), phenylalanine and polyphenol oxidase (PPO) were already recognized as induced systemic resistance (ISR) elicitors in plants associated with disease control [19,20,21]. These enzymes contribute to the

phenylpropanoid pathway, causing the synthesis of a variety of plant metabolites, such as phenolic compounds, flavonoids, tannins, and lignin [17, 14]. These metabolites can be used in plant protection toward pathogenic attacks [22].

Several studies have shown that greater phenolic accumulation can protect against plant disease due to increased defense-related enzyme activity [23, 24, 18, 25].

This study aimed to assess the capability of certain biotic inducer treatments to increase resistance toward damping-off, root rot, stem rot and/or wilt diseases in chickpea plants under greenhouse and field conditions and to examine their effectiveness in inducing defense-related enzymes and phenolic compound accumulation. The relation between resistance and biochemical modifications in induced plants, resistance to pathogen infection and its effects on growth and yield parameters have been determined.

## 2. Materials and Methods

### 2.1. Source of fungal pathogens:

Naturally infected chickpea plants displaying typical symptoms of damping-off and root rot, stem rot and/or wilt diseases gathered from different districts of six Governorates, i.e., Kafrelsheikh, Beheira, Gharbia, Giza, Beni-Suef, and Assiut, were isolated and tagged for identification. The purification process involves washing the infected roots thoroughly with tap water and cut each root into small parts (1 cm) and disinfect for two min with 2% sodium hypochlorite. The pieces were then washed multiple times with sterilized water, dried between folds of sterilized filter paper, and put in Petri dishes contain Potato Dextrose Agar (PDA) medium supplemented with streptomycin sulfate (100 μg mL-1). Petri dishes were incubated at 25 ± 1 ° C then scanned daily for fungal growth for five days.

Isolated fungi were purified by single conidial spores or hyphal tip techniques and identified based on morphoogical characters as described by [26, 27, 28]. The isolates were preserved for short-term storage in corn meal agar. However, for long-term storage, the pathogen was on grown Corn Meal Agar (CMA) slants and preserved under sterilized paraffin oil.

### 2.2. Molecular Characteristics of the tested Pathogens:

**DNA extraction:**

DNA isolation was performed according to the method of Lee and Taylor [65]. The mycelia from 14 days old cultures of each isolate were harvested from a fresh colony growing on PDA by scraping with a sterile scalpel and ground in liquid nitrogen. The samples were suspended in 500 µl. of extraction buffer (50 mM Tris–HCl pH: 8, 150 mM NaCl, 100 mM EDTA, % 2 SDS) and incubated for 30 min at 65°C. DNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1) twice and precipitated by the addition of one volume of isopropanol. DNA pellets were washed with ethanol, dissolved double-distilled water (ddH2O), and stored at −20°C.

#### 2.2.1. PCR Conditions:

The internal transcript spacer (ITS) region of rDNA was amplified using ITS 1 (5′ TCC GTA GGT GAA CCT GCGG 3′) and ITS 4 (5′TCC TCC GCT TAT TGA TATGC 3′) primers [66]. Thermocycler program for amplification of the ITS region was: 95°C for 3 min followed by 40 cycles of 95°C for 30 s, 68°C for 45 s, 72°C for 90 s. A final extension was made at 72°C for 8 min. PCR reactions were performed in (Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.).

#### 2.2.2 Sequencing

DNA sequences were generated from sequencing the amplified PCR products using the ABI Prism 3130*xl* Genetic analyzer, in both directions using the same primers ITS 1 (5′ TCC GTA GGT GAA CCT GCGG 3′) and ITS 4 (5′TCC TCC GCT TAT TGA TATGC 3′) [66], sequencing were performed in (Macrogen Corp., Korea). DNA sequences have been deposited in the NCBI GenBank.

### 2.3. Preparation of pathogen inoculation:

The plant pathogens, *F. oxysporum, R. solani,* or *S. sclerotiorum* were inoculated with equal five fungal disks (0.5 cm in diameter) of seven days old culture from the fungal isolates and grown in 500-ml glass bottles containing 100 g sterilized sorghum grains medium at 25°C±1 for two weeks. Inoculated bottles were vigorously shaken daily to encourage more rapid colonization of the sorghum grains and ensure uniform distribution of the fungal growth. The colonized sorghum grains were removed from the bottles and air-dried at room temperature and was grounded in a

mill then sieved through 60 mesh (0.25 mm) sieve, thin were kept in a polythene bag and treated as the fungal inoculum within one week [29].

### 2.4. Pathogenicity tests:

Soil infestation was performed by mixing the previously prepared inoculum with the soil in each pot (rate of 3%) and pots were then irrigated. Sterilized uninoculated sorghum grains were put into the soil equal to the same rate and used as control. Seven days after soil infestation, five seeds of susceptible cultivar cv. 'Giza 3' [5] were cultivated in each pot and immediately irrigated. A randomized complete block pattern with five replicates were used.

#### 2.4.1. Disease assessment:

The disease incidence (DI) % was recorded as percentages of pre-, post-emergence damping-off and root-rot each 15, 30, and 90 days later after the sowing process, subsequently. The percentages of pre-, post-emergence damping-off and root-rot were calculated using the following formula [30]:

$$\text{Pre-emergence (\%)} = \frac{\text{No. of non-emerged seedlings}}{\text{Total No. of sown seeds}} \times 100$$

$$\text{Post-emergence (\%)} = \frac{\text{No. of dead seedlings}}{\text{Total No. of sown seeds}} \times 100$$

$$\text{Root-rot (\%)} = \frac{\text{No. of wilted plants}}{\text{Total No. of sown seeds}} \times 100$$

$$\text{Survived plants (\%)} = \frac{\text{No. of survived plants}}{\text{Total No. of sown seeds}} \times 100$$

Reduction or increasing % over the infected control was also calculated according to the following formula [31]:

$$\text{Reduction or Increasing (\%)} = \frac{\text{DI of Control - DI of treatment}}{\text{DI of Control}} \times 100$$

**2.5. Preparation of biocontrol agents:**

In this study, five Strains of biocontrol agents, *i.e*., *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Serratia marcescens* were kindly presented by the Department of Microbiology, Soil, Water and Environment Res. Inst., ARC, Giza. The fungal strains (*T. viride* and *T. harzianum*) were cultured for 7 days on Potato Dextrose Agar (PDA) medium individually. While *P. fluorescens*, *B. subtilis* and *S. marcescens* were separately grown in nutrient broth medium in 250-ml flasks and put on the shaker for 3 days at 150 rpm at 28°C, then a cell suspension from each strain was modified to give $10^9$ CFU/ml. utilizing a hemocytometer slide [32].

**2.6. In vitro antagonistic examination:**

Antagonistic activity of the tested bioagents strains were examined using dual inoculated culture plates on (PDA) media. Disk (5 mm in diameter) of actively growing mycelia of 7days old cultures from each strain of the antagonistic fungi were inoculated on PDA medium separately or mixed, i.e., *T. viride*, *T. harzianum or T. viride* + *T. harzianum* on one side of Petri plate, While each antagonistic bacterial strain was streaked on one side of Petri plate separately or mixed, i.e., *Ps. fluorescens*, *B. subtilis*, *Ps. Fluorescens* + *B. subtilis* and *S. marcescens* and the opposite side were inoculated by a disk (5 mm in diameter) of actively growing mycelia 7-day-old cultures from each pathogenic fungus inoculum [33]. Plates inoculated with the tested pathogens alone served as control. Plates were incubated at 25±1°C until the control plates reached full growth. The experiment was conducted once with five replicates. At the end of the experiment the average growth diameter was calculated. Mycelial growth inhibition was calculated by using the formula [34]:

$$\text{Mycelial growth inhibition (\%)} = 100\ (C\text{-}T/C)$$

Where C=growth of the pathogenic fungus in control plates and T=growth of the pathogenic fungus in treated plates.

**2.7. Preparation of fungal and bacterial bioagents:**

*T. viride* and *T. harzianum* were prepared by growing each fungus in glass bottles 500-ml containing 100 g sterilized sorghum grains medium at 25±1°C for two weeks. The bottles were inoculated with equal five disks 0.5 cm diameter fungal discs of seven days old of *T. viride* and *T. harzianum* culture. Inoculated bottles were vigorously shaken daily to encourage more rapid colonization of the sorghum grains and ensure uniform distribution of the fungal growth. The colonized sorghum grains were removed from the bottles an, air-dried at room temperature and grounded in a mill then sieved through 60 mesh (0.25 mm) sieve, then kept in a polythene bag and treated as the fungal inoculum within one week and kept in sterilized polyethylene bags at room temperature until used colony-forming units in all formulae of *T. viride* and *T. harzianum* were adjusted to $3 \times 10^7$ CFU/g. [29, 5]. In 250-ml flasks, *Ps. fluorescens*, *B. subtilis* and *S. marcescens* strains were grown separately in the nutrient broth medium and put at 120 rpm for 48 hrs at 28±1 °C on a rotary shaker. Then, each strain's cell suspension was modified to provide $10^9$ CFU/ml [32].

**2.8. The fungicide Rizolex-T 50%:**

**Common name** (Tolclofos-methyl & Thiram) – **Chemical name** (O-2,6-dichloro-p-tolyl O,O-dimethyl phosphorothioate & Tetramethylthiuram di sulfide).

**2.9. Plant material**

Chickpea (*Cicer arietinum* L.) seeds cultivar Giza 3 were obtained from Field Crop Institute, Agricultural Research Centre, Giza, Egypt.

**2.10. Greenhouse trials:**

This trial was carried out in the greenhouse Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. The aim of this experiment was the assessment of the tested bioagent strains efficiency, i.e., *T. viride*, *T. harzianum*, *Ps. fluorescens*, *B. subtilis* and *S. marcescens* in controlling damping-off and root and/or stem rot diseases caused by the fungal isolates of *F. oxysporum* (isolate F3), *R. solani* (isolate R3) and *S. sclerotiorum* (isolate S2) in chickpea plants. Fungal inoculums were processed as stated before in the pathogenicity experiment. Plastic pots (30 cm

in diameter) were filled with fungal inoculum-infested sterilized sandy clay soil at a rate of 3% (w/w), 7 days before sowing. The highly susceptible cultivar Giza 3 was used [5]. Chickpea seeds were surficial disinfested in 2% sodium hypochlorite for 3 minutes, washed three times in sterilized distilled water and dried between layers of sterilized filter paper before treatment with the tested bioagents. Seeds were treated at the time with a bacterial bioagents strains (10 mL of bacterial strain prepared suspension in 0.1 M $MgSO_4$ and 0.5% Carboxymethyl cellulose per 100 g of chickpea seeds) and fungal bioagents strains (10 g of *Trichoderma* prepared inocula and 10 mL of 0.5% Carboxymethyl cellulose per 100 gm chickpea seeds) as well as seeds treated with the fungicide Rizolex-T 50% at 3 g/kg seeds used as a control. Untreated chickpea seeds soaked in water were sown in both infested and non-infested soil served as untreated infested and untreated healthy control. 5 pots were utilized as replicates (5seeds/pot) for every treatment in addition to untreated infested and untreated healthy control checks. Five g of Rhizobium (*Mesorhizobium ciceri*) formula obtained from Biofertilizers Production Unit, Soils Water and Environment Res. Inst., Agric. Res. Centre (ARC), Giza, Egypt were mixed in each pot during sowing. The treatments with five replicates were arranged in randomized complete block design. The treatments were as follows: (1) *T. viride*, (2) *T. harzianum*, (3) *T. viride + T. harzianum*, (4) *P. fluorescens*, (5) *B. subtilis*, (6) *P. fluorescens + B. subtilis*, (7) *S. marcescens*, (8) Rizolex-T, (9) seeds soaking in water in infested soil served as infested control and (10) seeds soaked in water in healthy soil served as healthy control.

**2.10.1. Disease assessment:**

Disease assessment was recorded as percentages of pre-, post-emergence damping-off, and root-rot each 15, 30 and 90 days later subsequently. Percentages of pre-, post-emergence damping-off and root-rot were calculated as previously described in the pathogenicity test [30].

**2.11. Field trials:**

The field trials were conducted out during the growing seasons 2017-2018 at Giza Agricultural Research Station, Giza Governorate, Egypt, in fields naturally infested, with root-rot and damping-off diseases, to study the effect of the tested bioagents for management of damping-off and root-rot diseases. Chickpea seeds cv.

Giza 3 was handled in the same manner in a greenhouse experiment. Seeds were soaked in distilled water in the control treatment, as previously stated. The treated chickpea seeds were cultivated in the field on 1st November 2017. The field trial (27 plots) was designed in full blocks randomized with three replicates. Each plot had an area of 10.5 m2 consisting of five rows; each row was 3.5 m long and 0.6 m wide. All treatments on both sides of the row ridge were sown in hills 20 cm apart, with one seed per hill. Rhizobium (*Mesorhizobium ciceri*) formula was mixed with approximately 50 kg of moistened fine sandy soil and added to field soil into the seed furrow during sowing, at the rate of 800 g rhizobium formula/feddan. Following the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation, all other recommended agricultural practices have been implemented. The treatments were as follows: (1) *T. viride*, (2) *T. harzianum*, (3) *T. viride + T. harzianum*, (4) *P. fluorescens*, (5) *B. subtilis*, (6) *P. fluorescens + B. subtilis*, (7) *S. marcescens*, (8) Rizolex-T, and (9) seeds soaking in water served as untreated control. Data were recorded as pre-, post-emergence damping-off and root-rot when 15, 30 and 90 days later, subsequently, and were calculated as previously described. At the end of the experiment, parameters of growth and yield i.e., the height of the plant (cm), branches number per plant, pods number per plant, number of seeds per plant, seed yield/plant (gm), 100-weight of Seeds (gm) and chickpea seeds yield ton/feddan were also estimated.

**2.12. Biochemical changes associated with induced resistance:**

Te β-1,3-glucanase, peroxidase (PO), polyphenol oxidase (PPO), and total phenol content activities were analyzed in tissue extracts of chickpea plants that survived treatment with fungal and bacterial isolates *i.e., T. viride, T. harzianum, Ps. fluorescens, B. subtilis,* and *S. marcescens* and also non - treated seeds. These treatments were grown in soil infected with *F. oxysporum*, *R. solani* or *S. sclerotiorum* individually. Specimens of shoot chickpea seedlings for every treatment were gathered twelve days post-inoculation with the tested pathogenic fungi. Also, untreated infected and healthy seedlings have been used as control treatments. 1 gm of plant tissue, 10 mL of the ice-cold of 50 mM potassium phosphate buffer (pH 6.8) which includes 1M NaCl, 1% polyvinylpyrrolidone, 1 mM EDTA, and 10 mM β-mercaptoethanol were homogenized [35]. The homogenates were centrifuged at 8,000 rpm at 4°C for 25 min after filtration via cheesecloth. The supernatants (crude enzyme

extract) were kept at -20 °C or were directly used for β-1,3-glucanase, PO and PPO activity determination [36]. Each treatment consisted of three replicates (three plants/replicates) and two spectrophotometric readings were taken per replication using a Milton Roy 1201 Spectrophotometer for the determination of enzyme activities (PEMEDR, Denver, CO, USA).

**2.12.1. β-1, 3 glucanase assay:**

The activity of β-1,3 glucanase was calculated according to method of Abeles *et al*., [37]. The substrate used was laminarin, and the reagent was dinitrosalicylic acid. There was a reading of the optical density at 500 nm. The activity of β-1,3 glucanase was expressed as mM released glucose equivalents/g fresh weight tissue/60 min..

**2.12.2. Peroxidase (PO) assay:**

A spectrophotometric method was used to specifically assess peroxidase activity [38]. Guaiacol is used as a generic substrate. The reaction mixture consisted of a solution containing 1.40 mL guaiacol, hydrogen peroxide ($H_2O_2$) and sodium phosphate buffer (0.2 mL 1 percent guaiacol+0.2 mL 1 percent $H_2O_2$ +1 mL 10 mM potassium phosphate buffer) with 0.2 mL crude enzyme extract. The mixture was incubated for 5 min at 25°C and the initial rate of absorbance increase was calculated at 470 nm over 1 min. The activity was assessed as PO/mg protein units [39].

**2.12.3. Polyphenol oxidase (PPO) assay:**

Assay of PPO activity was calculated by adding 50 µL of crude extract to 3 mL of 100 mM potassium phosphate buffer solution, pH 6.5 and 25 mM pyrocatechol, respectively. The increase in absorption at 410 nm at 30°C for over 10 minutes was measured by [40]. At 410 nm per mg soluble protein per min, one PPO unit was expressed as the absorption variation.

**2.12.4. Phenolic compound determination:**

To determine the phenolic material, 1 g of fresh plant sample was homogenized to 80 percent methanol in 10 mL and agitated at 70 °C for 15 min. For 5 mL of distilled water and 250 µL of 1 N Folin-Ciocalteau reagent, one milliliter of the extract was applied and the solution was stored at 25 °C. The absorbance was measured at 725 nm using a spectrophotometer. As a standard, Catechol was used.

The quantity of phenolic content in mg/g fresh tissue was expressed in phenol equivalents [41].

### 2.13. Statistical Analysis

The obtained data were subjected to analysis of variance according to Fisher's statistics program (2002). Means were separated by fisher's protected least significant differences L.S.D at $p<0.05$ level [42].

## 3. Results

### 3.1. Isolation, identification of the causal fungal pathogens, and pathogenicity tests

Eighteen fungal isolates were obtained from naturally infected chickpea plants collected from six different governorates (Table 1). The isolated fungi were consisting of isolates belonging to the genera *Rhizoctonia*, *Fusarium* and *Sclerotinia* as shown by preliminary microscopic examination and the isolates were identified as *R. solani*, *F. oxysporum*, and *S. sclerotiorum*, respectively according to [26, 27, 28].

Data in Tab. 1 show that the highest percentage of pre-emergence damping-off was recorded by *F. oxysporum* isolate F3 (Gharbia) followed by isolate F2 (Beheira) then *F. oxysporum* isolate F4 (Giza) and F1 (Kafrelsheikh), F5 (Beni Suef) and F6 (Assiut), respectively. While the highest percentage of pre-emergence damping-off was recorded by *R. solani* isolate R1 (Kafrelsheikh) followed by isolate R3 (Gharbia), R6 (Assiut), R2 (Beheira), R4 (Giza) and R5 (Beni Suef), respectively. As well as the highest percentage of pre-emergence damping-off was recorded by *S. sclerotiorum* isolate S2 (Beheira) then *S. sclerotiorum* isolate S6 (Assiut), S4 (Giza), S3 (Gharbia), S5 (Beni Suef), and S1 (Kafrelsheikh)

**Table (1) Pathogenicity of *Fusarium oxysporum*, *Rhizoctonia solani*, or *Sclerotinia sclerotiorum* isolates obtained from naturally diseased chickpea plants collected from different locations, under field conditions.**

| Isolate No. | Damping off% | | % Dead plants [c] | Survival % |
|---|---|---|---|---|
| | Pre emergence [a] | Post emergence [b] | | |
| *Fusarium oxysporum* | | | | |
| (F1) Kafrelsheikh | 12[d] | 16 | 28 | 44 |
| (F2) Beheira | 16 | 16 | 32 | 36 |
| (F3) Gharbia | 20 | 20 | 36 | 24 |
| (F4) Giza | 12 | 20 | 28 | 40 |
| (F5) Beni Suef | 8 | 12 | 28 | 52 |
| (F6) Assiut | 12 | 12 | 20 | 56 |
| *Rhizoctonia solani* | | | | |
| (R1) Kafrelsheikh | 40 | 20 | 12 | 28 |
| (R2) Beheira | 28 | 16 | 12 | 44 |
| (R3) Gharbia | 32 | 16 | 16 | 36 |
| (R4) Giza | 20 | 16 | 16 | 48 |
| (R5) Beni Suef | 24 | 12 | 12 | 52 |
| (R6) Assiut | 32 | 16 | 16 | 36 |

| Isolate No. | Damping off% | | % Dead plants [c] | Survival % |
|---|---|---|---|---|
| | Pre emergence [a] | Post emergence [b] | | |
| *Sclerotinia sclerotiorum* | | | | |
| (S1) Kafrelsheikh | 20 | 8 | 16 | 56 |
| (S2) Beheira | 36 | 12 | 24 | 28 |
| (S3) Gharbia | 28 | 12 | 16 | 44 |
| (S4) Giza | 32 | 12 | 20 | 36 |
| (S5) Beni Suef | 24 | 8 | 16 | 52 |
| (S6) Assiut | 32 | 16 | 20 | 32 |
| LSD at 0.05 | | | | |
| Fungi F | 7.382 | 7.101 | 7.062 | 13.677 |
| Isolate I | 10.439 | 10.043 | 9.988 | 19.342 |
| (FxI) | 18.081 | 17.395 | 17.299 | 33.501 |

[a], [b], [c] Assessed 15, 30, 90 days after sawing, respectively; c Dead plants, % due to infection by root rot, stem-rot and/or wilt; d Values are means of 5 replicates.

### 3.2. Molecular Characteristics of the tested Pathogens:

BLAST analysis of the obtained ITS sequences to each of the selected three tested Isolates, *F. oxysporum* (F3), *R. solani* (R1) and *S. sclerotiorum* (S2) revealed 100% sequence homology with the other sequences in GenBank, as well as confirm the previous morphological Characteristics. The DNA sequences have been deposited in the NCBI GenBank by the NCBI accession numbers MW926317 for *Sclerotinia*

*sclerotiorum*, MW926318 for *Fusarium oxysporum* f. sp. *ciceris* and MW926319 for *Rhizoctonia solani*.

### 3.3. Antibiosis of PGPR towards pathogenic fungi:

The five tested bioagent strains were screened for their antagonistic activity against *F. oxysporum, R. solani* or *S. sclerotiorum.* Data in Table (2) illustrate that all the tested strains significantly suppress the mycelial growth of the three pathogenic fungi tested. The highest values of inhibition (74.07, 68.52 and 75.93 %) were observed with *F. oxysporum, R. solani* and *S. sclerotiorum* subsequently via *Ps. fluorescens + B. subtilis* followed by *Trichoderma viride, T. harzianum, T. viride + T. harzianum* respectively. Whereas the lowest inhibition values were recorded with *Serratia marcescens* (46.30, 53.70, and 55.56). *S. sclerotiorum* was the most sensitive fungus thereafter accompanied by *F. oxysporum* and *R. solani,* respectively.

**Table (2) in-*vitro* antagonism of biocontrol agents against *Fusarium oxysporum*, *Rhizoctonia solani*, or *Sclerotinia sclerotiorum*.**

| Treatments | Inhibition of linear growth (%) | | |
|---|---|---|---|
| | ***Fusarium oxysporum*** | ***Rhizoctonia solani*** | ***Sclerotinia sclerotiorum*** |
| *Trichoderma viride* | 70.37a | 57.41c | 70.37ab |
| *Trichoderma harzianum* | 64.81b | 59.26bc | 70.37ab |
| *T. viride + T. harzianum* | 62.96b | 68.52a | 75.93a |
| *Pseudomonas fluorescens* | 57.41c | 55.56c | 66.67b |
| *Bacillus subtilis* | 55.56c | 57.41c | 64.81b |
| *Ps. fluorescens + B. subtilis* | 74.07a | 64.81ab | 74.07a |
| *Serratia marcescens* | 46.30d | 53.70c | 55.56c |
| LSD at 0.05 | 5.43 | 6.99 | 4.971 |

Values in the column followed by different letters indicate significant differences among treatments according to LSD at 0.05.

### 3.4. Effect of biocontrol agents on the incidence of chickpea damping-off and, wilt, root and /or stem rot diseases caused by the tested pathogenic fungi under greenhouse conditions:

Results in Table (3) illustrate that all treatments induced a significant reduction in the percentages of pre- and post-emergence damping-off and/or wilt, root and /or stem rot caused by *F. oxysporum*, *R. solani* and *S. sclerotiorum* compared to untreated infected control. Rizolex-T, (*T. viride + T. harzianum*), and (*Ps. fluorescens + B. subtilis*) treatments gave the highest effect followed by *T. viride*, *B. subtilis*, *T. harzianum*, and *Ps. fluorescens*. While the lowest reduction effect was attributed to *S. marcescens* treatment.

### 3.5. Effect of biocontrol agents and chemical inducers on the incidence of chickpea root- rot diseases under field conditions:

Results in Table (4) exhibited that all the biocontrol separately and/or in combination as seed soaking against the incidence of damping-off, wilt, root rot and stem rot diseases of chickpea significantly reduced the percentages of pre-emergence damping-off of wilted chickpea plants as compared with untreated control. Rizolex-T and (*T. viride + T. harzianum*) gave the highest values in reducing diseases as well as increasing the survived chickpea plants compared with other treatments followed by *T. viride*, (*Ps. fluorescens + B. subtilis*), *T. harzianum*, *B. subtilis*, and *Ps. Fluorescens.* On the other hand, *Serratia marcescens* resulted in the lowest values even in decreasing diseases or increasing survival.

**Table (3): Effects of seed treatment with biocontrol agents on chickpea damping-off and, wilt, root and /or stem rot caused by *Fusarium oxysporum*, *Rhizoctonia solani*, or *Sclerotinia sclerotiorum* under greenhouse conditions.**

| Treatments | *Fusarium oxysporum* | | | *Rhizoctonia solani* | | | *Sclerotinia sclerotiorum* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pre-emergence | Post-emergence | Wilt | Pre-emergence | Post-emergence | Root rot | Pre-emergence | Post-emergence | stem rot |
| *Trichoderma viride* | 4b | 8a | 16b | 8b | 8bc | 4a | 8b | 4a | 8b |
| *Trichoderma harzianum* | 8b | 8a | 12b | 12b | 8bc | 8a | 12b | 4a | 4b |
| *T. viride + T. harzianum* | 0b | 4a | 4b | 4b | 0c | 4a | 8b | 4a | 4b |
| *Pseudomonas fluorescens* | 8b | 8a | 16b | 12b | 4bc | 4a | 8b | 8a | 8b |
| *Bacillus subtilis* | 4b | 8a | 16b | 8b | 8bc | 8a | 8b | 8a | 8b |
| *Ps. fluorescens + B. subtilis* | 4b | 4a | 12b | 4b | 4bc | 4a | 4b | 4a | 4b |
| *Serratia marcescens* | 8b | 12a | 16b | 16b | 12ab | 8a | 12b | 8a | 12b |
| Rizolex-T | 0b | 4a | 4b | 8b | 4bc | 4a | 8b | 0a | 4b |
| Control | 20a | 20a | 36a | 40a | 20a | 12a | 36a | 12a | 24a |
| LSD at 0.05 | 10.84 | NS | 15.39 | 14.28 | 11.12 | NS | 13.34 | NS | 11.60 |

Values in the column followed by different letters indicate significant differences among treatments according to LSD at 0.05.; N.S indicated P<0.05% not significant.

**Table (4): Effect of biocontrol agents on damping-off, wilt, and survival of chickpea plants naturally infected in field experiments.**

| Treatments | Pre-emergence | | Post-emergence | | % Dead plants | | survival % | Increasing % |
|---|---|---|---|---|---|---|---|---|
| | Incidence % | Reduction % | Incidence % | Reduction % | Incidence % | Reduction % | | |
| *Trichoderma viride* | 14.33cd | 56.57 | 6.33cd | 58.70 | 4.33bc | 68.30 | 75.00bc | 97.37 |
| *Trichoderma harzianum* | 16.33bcd | 50.51 | 6.33cd | 58.70 | 4.33bc | 68.30 | 73.00c | 92.11 |
| *T. viride + T. harzianum* | 12.00de | 63.64 | 5.67cd | 63.04 | 4.00bc | 70.73 | 78.33b | 106.14 |
| *Pseudomonas fluorescens* | 18.33bc | 44.45 | 8.00bc | 47.82 | 7.33b | 48.78 | 66.67d | 75.44 |
| *Bacillus subtilis* | 16.67bcd | 49.49 | 8.00bc | 47.82 | 7.00b | 46.35 | 68.00d | 78.95 |
| *Ps. fluorescens + B. subtilis* | 13.67cd | 58.58 | 6.67cd | 58.70 | 6.33b | 53.66 | 73.67c | 93.86 |
| *Serratia marcescens* | 20.33b | 38.38 | 10.00b | 34.78 | 8.00b | 41.46 | 61.67e | 62.28 |
| Rizolex T | 7.67e | 76.77 | 4.00d | 73.91 | 2.00c | 85.37 | 86.33a | 127.19 |
| Control | 33.00a | - | 15.33a | - | 13.67a | - | 38.00f | - |
| LSD at 0.05 | 5.16 | - | 3.65 | - | 4.11 | - | 3.86 | - |

Values in the column followed by different letters indicate significant differences among treatments according to LSD at 0.05.

### 3.6. Effect of biocontrol agents and chemical inducers on growth parameters and yield components of chickpea plants under field conditions:

Table (5) shows that under field conditions, the tested biocontrol agents' treatments significantly improved chickpea growth and yield parameters, *i.e.*, the height of the plant (cm), branches number per plant, pods number per plant, number of seed per plants, seed yield per plant (gm), 100 seeds weight (gm) and chickpea yield ton/fed during 2017/2018 growing season as compared with the untreated control. The highest significant increase in all parameters was recorded with the treatments Rizolex-T, and (*T. harzianum* + *T. viride*) followed by *Ps. fluorescens* + *B. subtilis*, *T. viride*, *T. harzianum*, *Ps. fluorescens* and *B. subtilis*, respectively. Whereas the lowest values were attributed to *Serratia marcescens* treatment.

**Table (5): Effect of biocontrol agents on the growth and yield parameters of chickpea seeds grown under field conditions.**

| Treatments | Plant Height (cm) | Branches Number /plant | No. of pods /plant | No. of seed/ plant | Seed yield/ plant (g) | 100-seed weight (gm) | Yield ton/fed |
|---|---|---|---|---|---|---|---|
| *Trichoderma viride* | 64bc | 5.0bc | 111.4bc | 120.0c | 20.6cd | 17.13cde | 2.12cd |
| *Trichoderma harzianum* | 63cd | 4.3cde | 110.3bc | 121.8c | 20.8c | 17.07de | 2.09d |
| *T. viride* + *T. harzianum* | 66b | 5.3b | 113.6b | 124.1b | 21.6b | 17.37bcd | 2.35b |
| *Pseudomonas fluorescens* | 60e | 4.3cde | 104.4d | 111.1e | 20.0d | 18.00ab | 1.88e |
| *Bacillus subtilis* | 61de | 4.0de | 106.1d | 110.9e | 19.0e | 17.14cde | 1.81e |
| *Ps. fluorescens* + *B. subtilis* | 65bc | 4.7bcd | 109.4c | 117.6d | 21.0bc | 17.87abc | 2.18c |

| Treatments | Plant Height (cm) | Branches Number /plant | No. of pods /plant | No. of seed/ plant | Seed yield/ plant (g) | 100-seed weight (gm) | Yield ton/fed |
|---|---|---|---|---|---|---|---|
| *Serratia marcescens* | 57f | 3.7e | 90.0e | 102.0f | 16.8f | 16.45ef | 1.47f |
| Rizolex T | 72a | 7.3a | 120.0a | 130.6a | 24.0a | 18.38a | 2.88a |
| Control | 48g | 2.3f | 68.3f | 65.0g | 10.6g | 16.24f | 0.78g |
| LSD at 0.05 | 2.311 | 0.873 | 3.306 | 2.045 | 0.620 | 0.756 | 0.072 |

Values in the column followed by different letters indicate significant differences among treatments according to LSD at 0.05.

### 3.7. Effect of chickpea seed treatments with different bioagents on the phenol content and the activity of oxidative enzymes in chickpea plants grown in infested soil by *F. oxysporum, R. solani, or S. sclerotiorum* under greenhouse conditions.

#### 3.7.1. Phenol content:

Data in Table (6) indicate that phenolic compounds, *i.e.*, total, free and conjugated phenols were significantly higher in chickpea plants treated with the tested bioagents than those of untreated infected and untreated healthy control plants in the presence of the three tested pathogens ( *F. oxysporum*, *R. solani* or *S. sclerotiorum*). The maximum increase in the content of total phenolic compounds was recorded with (*T. harzianum* + *T. viride*) treatment followed by *T. viride*, (*Ps. fluorescens* + *B. subtilis*), *T. harzianum, Ps. fluorescens* and *B. subtilis*, compared with untreated control. The content of free phenols was similar with the trend of data of conjugated phenols, where (*T. harzianum* + *T. viride*) gave the highest increase over untreated control treatment followed by *T. viride*, *T. harzianum*, (*Ps. fluorescens* + *B. subtilis*), *Ps. fluorescens* and *B. subtilis*, respectively. With respect to conjugated phenols, one can noticed that *B. subtilis, Ps. fluorescens* and (*Ps. fluorescens* + *B. subtilis*) treatments gave the highest increase over untreated control followed by *T. viride*, (*T. harzianum* + *T. viride*) and *T. harzianum*. Whereas, the lowest values were recognized in the total, free and conjugated phenols when *S. marcescens* was applied. Moreover,

the least values in total, free and conjugated phenols were recorded in the healthy control treatment.

**Table (6): Effect of some bioagents as seed treatments on levels of phenolic compounds in chickpea plants grown in artificially infested soil by *F. oxysporum*, *R. solani* or *S. sclerotiorum* under greenhouse conditions.**

| Treatments | Phenolic contents (mg/g fresh weight) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total phenols contents | | | Free phenols | | | Conjugated phenols | | |
| | *Fuo | Rs | Ss | Fuo | Rs | Ss | Fuo | Rs | Ss |
| *Trichoderma viride* | 7.22b | 7.70b | 7.42b | 5.65b | 5.95b | 5.71b | 1.57cd | 1.75b | 1.71a |
| *Trichoderma harzianum* | 6.91c | 7.44c | 7.35b | 5.54c | 5.93b | 5.64b | 1.37e | 1.51cd | 1.71a |
| *T. viride + T. harzianum* | 7.74a | 7.95a | 7.65a | 6.19a | 6.23a | 5.90a | 1.56d | 1.72bc | 1.75a |
| *Pseudomonas fluorescens* | 6.26e | 6.49f | 6.83d | 4.51e | 4.85d | 5.33c | 1.76ab | 1.64bcd | 1.49b |
| *Bacillus subtilis* | 6.30e | 6.65e | 6.94d | 4.39f | 4.48e | 5.18d | 1.92a | 2.17a | 1.75a |
| *Ps. fluorescens + B. subtilis* | 6.66d | 7.10d | 7.18c | 4.95d | 5.44c | 5.36c | 1.72bc | 1.66bcd | 1.82a |
| *Serratia marcescens* | 5.58f | 6.13g | 6.20e | 4.39f | 4.64de | 4.38e | 1.19f | 1.493d | 1.82a |
| Control (infected) | 4.31g | 4.50h | 4.28f | 3.25g | 2.96f | 2.77f | 1.06fg | 1.54bcd | 1.51b |
| Control (healthy) | 3.06h | 3.06i | 3.06g | 2.12h | 2.12g | 2.12g | 0.94g | 0.937e | 0.94c |
| LSD at 0.05 | 0.098 | 0.123 | 0.135 | 0.112 | 0.225 | 0.109 | 0.154 | 0.210 | 0.162 |

* Fuo, *F. oxysporum*; *Rs, R. solani* and *Sc, S. sclerotiorum*

Values in the column followed by different letters indicate significant differences among treatments according to LSD at 0.05.

### 3.7.2. Activity of oxidative enzymes:

Activities of β-1,3 glucanase (Table 7), peroxidase (Table 8) and polyphenol oxidase (Table 9) enzymes of chickpea plants were evaluated with the different bioagents treatments in the presence of *F. oxysporum*, *R. solani* or *S. sclerotiorum* under greenhouse conditions. Results showed that all treatments were effective in increasing enzyme activities. The highest increase of β-1,3 glucanase, peroxidase and polyphenol oxidase activities as compared to the untreated control was achieved with (*T. viride* + *T. harzianum*) treatment either in the presence of *F. oxysporum*, *R. solani* or *S. sclerotiorum*. Meantime, the *T. viride*, *T. harzianum*, (*Ps. fluorescens* + *B. subtilis*), and *Ps. fluorescens* treatments showed a considerable increase in the activity of the three enzymes. Whereas the lowest activity of the enzymes was obtained when *S. marcescens* and *B. subtilis* were applied. However, Results showed that clear higher values of β-1,3 glucanase activity than peroxidase and polyphenoloxidase in all treatments in the presence of the three pathogens. Meanwhile, it has to notice that infestation with any of the three fungal pathogens in the absence of the tested bioagents, clearly increased the activity of the enzymes than that recorded in healthy untreated plants as blank of all treatments. In addition, chickpea plants inoculated with *F. oxysporum* recorded a high level of β-1,3 glucanase, peroxidase and polyphenoloxidase enzymes more than plants inoculated with *S. sclerotiorum* or *R. solani* in treated chickpea plants.

**Table (7): Effect of some bioagents as seed treatments on β-1,3 glucanase activity in chickpea plants grown in artificially infested soil by *F. oxysporum*, *R. solani* or *S. sclerotiorum* under greenhouse conditions.**

| Treatments | β-1,3 glucanase activity (Enzyme activity as µM of glucose released / ml /hr.) | | |
|---|---|---|---|
| | ***Fusarium oxysporum*** | ***Rhizoctonia solani*** | ***Sclerotinia sclerotiorum*** |
| *Trichoderma viride* | 74.67b | 76.33b | 71.87bc |
| *Trichoderma harzianum* | 71.00b | 74.33b | 68.13c |
| *T. viride + T. harzianum* | 93.00a | 92.33a | 86.03a |
| *Pseudomonas fluorescens* | 92.00a | 89.00a | 83.68ab |
| *Bacillus subtilis* | 75.33b | 79.00b | 75.81abc |
| *Ps. fluorescens + B. subtilis* | 76.00b | 79.33b | 75.67abc |
| *Serratia marcescens* | 79.00b | 76.33b | 84.32ab |
| Control (infected) | 41.00c | 36.67c | 39.67d |
| Control (healthy) | 35.00c | 35.00c | 35.00d |
| LSD at 0.05 | 8.01 | 9.29 | 13.56 |

Values in the column followed by different letters indicate significant differences among treatments according to LSD at 0.05.

**Table (8): Effect of some bioagents as seed treatments on Peroxidase activity in chickpea plants grown in artificially infested soil by *F. oxysporum*, *R. solani*, or *S. sclerotiorum* under greenhouse conditions.**

| Treatments | Peroxidase activity (enzyme unite/mg protein /min) | | |
|---|---|---|---|
| | ***Fusarium oxysporum*** | ***Rhizoctonia solani*** | ***Sclerotinia sclerotiorum*** |
| *Trichoderma viride* | 2.17b | 1.91abc | 1.93b |
| *Trichoderma harzianum* | 2.10bc | 1.88bcd | 1.86b |
| *T. viride + T. harzianum* | 2.42a | 2.05a | 2.53a |
| *Pseudomonas fluorescens* | 1.98bcd | 1.89bcd | 1.98b |
| *Bacillus subtilis* | 1.87d | 1.76d | 1.84b |
| *Ps. fluorescens + B. subtilis* | 2.07bcd | 1.97ab | 1.92b |
| *Serratia marcescens* | 1.88cd | 1.82cd | 1.86b |
| Control (infected) | 0.93e | 0.84e | 0.96c |
| Control (healthy) | 0.72e | 0.72e | 0.72c |
| LSD at 0.05 | 0.224 | 0.15 | 0.23 |

Values in the column followed by different letters indicate significant differences among treatments according to LSD at 0.05.

**Table (9): Effect of some bioagents as seed treatments on Polyphenoloxidase activity in chickpea plants grown in artificially infested soil by *F. oxysporum*, *R. solani* or *S. sclerotiorum* under greenhouse conditions.**

| Treatments | Polyphenoloxidase (enzyme unite/mg protein /min) | | |
|---|---|---|---|
| | ***Fusarium oxysporum*** | ***Rhizoctonia solani*** | ***Sclerotinia sclerotiorum*** |
| *Trichoderma viride* | 0.62a | 0.58b | 0.59a |
| *Trichoderma harzianum* | 0.60ab | 0.54b | 0.55a |
| *T. viride + T. harzianum* | 0.63a | 0.65a | 0.65a |
| *Pseudomonas fluorescens* | 0.53c | 0.50c | 0.58a |
| *Bacillus subtilis* | 0.54bc | 0.49c | 0.44b |
| *Ps. fluorescens + B. subtilis* | 0.57abc | 0.54b | 0.58a |
| *Serratia marcescens* | 0.37d | 0.31d | 0.40b |
| Control (infected) | 0.20e | 0.17e | 0.22c |
| Control (healthy) | 0.13e | 0.13f | 0.13c |
| LSD at 0.05 | 0.068 | 0.038 | 0.10 |

Values in the column followed by different letters indicate significant differences among treatments according to LSD at 0.05.

## 4 . Discussion

Chickpea (*Cicer arietinum* L.) is a legume crop belonging to the Fabaceae family and is globally consumed, especially in Afro-Asian countries. Chickpea is a pulse crop and the third in production in the world after dry beans and field beans [43].

Damping-off and root-rot are major diseases of pathogenic fungi caused by chickpea (*Cicer arietinum* L.) in Egypt, i.e., *Fusarium oxysporum, Sclerotinia sclerotiorum and Rhizoctonia solani* [5, 44].

Induced systemic resistance against soil-borne disease is one of the safe alternative approaches decrease the use of fungicides [49, 50,10]. Induced systemic resistance has a broad spectrum against many pathogens and prove promising management of diseases, reach to 85% disease control [45, 46, 47, 48].

The goal of this study was to reduce the amount of chemical pesticides required to protect plants from pathogen attack by substitution with beneficial rhizosphere microorganisms (biocontrol agents).

In the present work, evaluation of the efficacy of some biotic inducers *(T. harzianum*, *T. viride*, *B. subtilis*, *Ps. fluorescens*, and *S. marcescens*) in management root diseases of chickpea plants was dependent on inducing systemic resistance as the major action mechanism and as demonstrated by reducing the disease incidence and severity under greenhouse and field conditions

The results illustrated that the application of the bio-agents tested and the Rizolex-T fungicide, as seed treatment significantly reduced the severity of root diseases on chickpea plants infected with *R. solani*, *F. oxysporum* or *Sclerotinia sclerotiorum* under greenhouse and field conditions in comparison to the untreated control. Moreover, all the tested bioagents improved crop components of chickpea plants. These results are consistent with those stated by [53, 54, 55].

The present study showed that antagonistic five tested bioagent strains inhibited the growth of the pathogenic tested fungi with different degrees of inhibition. Similar results were also obtained by many investigators who noticed that many *Trichoderma* spp., *Bacillus* spp. and *P. fluorescence* able to inhibit growth of the pathogenic fungi [56, 55, 57, 58, 25].

Under greenhouse conditions, both *T. viride* and *T. harzianum* recorded higher results. Sallam *et al.*, [57] revealed that *Trichoderma* spp. is effective for bio-controlling *R. solani, F. oxysporum and Sclerotinia sclerotiorum* [59,25]. In the present work, (*T. viride* + *T. harzianum*) treatment was the most successful treatment in controlling the diseases among all bioagent treatments compared to the untreated control.

Under field conditions, reduction of disease severity was reflected on increasing in crop yield, particularly in using both *T. viride, T. harzianum* against the mentioned diseases according to [5,60,25,61]. In this research, (*T. viride* + *T. harzianum*) treatment recorded the maximum results between all bioagents compared to the untreated control.

The application of biotic and abiotic inducers has a good potential in controlling plant diseases. They elicit processes that lead to various defence reactions in host plants in response to microbial infection, including the accumulation of pathogenesis-related PR-proteins, defence- related enzymes, lignin synthesis, accumulation of phenolic compounds and specific flavonoids [45,8,18,25]

The activity of defense-related enzymes, *i.e.*, polyphenol oxidase, peroxidase, and β-1,3 glucanase is known to be induced via systemic resistance of many infected plants with fungal pathogens [51]. These products can be used in plants to protect against pathogenic attacks [52, 45, 44]. These enzymes act as elicitors of the phenylpropanoid activity, causing the biosynthesis of a diverse variety of plant metabolites such as phenolic compounds, flavonoids, tannins, and lignin. Many previous studies indicated a greater accumulation of phenolics because of increasing the activities of these oxidative enzymes which could offer protection against plant diseases [8, 51, 25, 44].In this study, all treatments exhibited rising in enzyme activities and (*T. viride* + *T. harzianum*) treatment showed the highest increase of β-1,3 glucanase, polyphenol oxidase (PPO)and peroxidase (PO)enzymes, in addition to the total phenol content as compared to the untreated control in the presence of *F. oxysporum, R. solani* or *S. sclerotiorum* .

The biotic inducers as seed treatments led to an increase in the phenolic compounds content compared with the untreated control. In this respect, the role of phenolic compounds in disease resistance was postulated by many authors like [23,

60, 25, 61] which indicated that phenols are oxidized to quinones or semi-quinones which are more toxic and play a great role as antimicrobial substances on the invaded pathogen. In addition, phenolic compounds may impede pathogen infection by increasing the mechanical strength of the host cell wall [63]. It was reported that, there is a link between the accumulation of phenolic compounds at infection sites and the restriction of pathogen development, as such compounds are toxic substances to pathogens. The resistance can also be increased by changing the pH of plant cell cytoplasm because of an increase in the content of phenolic acid, causing the inhibition of pathogen development. [64, 25, 61].

### 4.1. Conclusion

The current study indicated that the applying of biotic inducers *i.e.*, *Trichoderma* spp., *Bacillus* spp. as well as S. *marcescens* could play a significant protective role against white soil-borne diseases of chickpea plants as reduced the disease incidence and severity under greenhouse and field conditions, mainly by the induced systemic resistance via increasing the activities of peroxidase (PO), polyphenol oxidase (PPO) and β-1,3-glucanase and improving the phenolic contents. Besides, promote plant growth and parameters of chickpea plants. Our results provide a basis for a better understanding of this interaction and the theoretical basis for biotic inducers on the field scale. Also, the used treatments could replace conventional pesticides with three main advantages, as they have a broad spectrum of action, a low environmental impact and the absence of any risk regarding the selection of pathogen-resistant strains.

## 5. References

[1] Jukanti, A. K.; Gaur, P.M.; Gowda, C.L.L. and Chibbar, R..N. Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. British Journal of Nutrition, 108: (2012) (S1) S11-S26.

[2] FAOSTAT. Data base result from Food and Agriculture Organization of the United Nations, Rome (2017). http://fao.org/

[3] Singh S.; Gumber R.K.; Joshi N. and Singh, K. Introgression from wild *Cicer reticulatumto* cultivated chickpea for productivity and disease resistance. Plant Breeding, 124: (2005) 477-480.

[4] Njambere, E.N.; Chen, W.; Frate, C.; Wu, B.M.; Temple, S.R. and Muehlbauer, F.J. Stem and crown rot of chickpea in California caused by *Sclerotinia trifoliorum*. Plant Disease, 92: (2008) 917-922.

[5] Abdel-Monaim, M.F. Integrated management of damping-off, root and/or stem rot diseases of chickpea and efficacy of the suggested Formula. Notulae Scientia Biologicae. 3:(2011) 80-88.

[6] Jiménez-Díaz, R.M.; Castillo, P.; Jiménez-Gasco, M.M.; Landa, B.B. and Navas-Cortés J.A. *Fusarium* wilt of chickpeas: Biology, ecology, and management. Crop Protection, 73: (2015) 16-27.

[7] Baturo-Ciesniewska, A.; Groves, C.L.; Albrecht, K.A.; Grau, C.R.; Willis, D.K. and Smith, D.L. Molecular identification of *Sclerotinia trifoliorum* and *Sclerotinia sclerotiorum* isolates from the United States and Poland. Plant Disease 101: (2016) 192-199.

[8] Reddy, M. S.; Ilao, R. I and Faylon, P. S. (Eds). Recent Advances in Biofertilizers and Biofungicides (PGPR) for Sustainable Agriculture. Cambridge Scholars Publishing. (2014*)* http://oar.icrisat.org/id/eprint/8441.

[9] Rais, A.; Jabeen, Z.; Shair, F.; Hafeez, F.Y and Hassan, M.N. *Bacillus* spp., a bio-control agent enhances the activity of antioxidant defense enzymes in rice against *Pyricularia oryzae*. PLoS One 12: (2017) https://doi.org/10.1371/journal.pone.0187412

[10] Spadaro, D and Gullino, M.L. Improving the efficacy of biocontrol against soilborne pathogens. Crop Prot. 24: (2005) 601-613. https://doi.org/10.1016/j.cropro.2004.11.003

[11] Sarhan, E.A.D. and Shehata, H.S. Potential plant growth-promoting activity of *Pseudomonas* spp. and *Bacillus* spp. as biocontrol agents against damping-off in Alfalfa. Plant Pathol. J., 13: (2014) 8-17.

[12] Simonetti, E.; Carmona, M.A.; Scandiani, M.M.; Garcı´a, A.F.; Luque, A.G.; Correa, O.S. and Balestrasse, K.B. Evaluation of indigenous bacterial strains for biocontrol of the frogeye leaf spot of Soybean caused by *Cercospora sojina*. Letters in Applied Microbiology, 55: (2012) 170-173.

[13] Basha, S.A.; Sarma, B.K.; Singh, D.P.; Annapurna, K and Singh, U.P. Differential Methods of Inoculation of Plant Growth-Promoting Rhizobacteria Induce Synthesis of Phenylalanine Ammonia-Lyase and Phenolic Compounds Differentially in Chickpea. Folia Microbiol.51: (2006) 463-468.

[14] Abd El-Rahman, S.; Mazen, M.M.; Mohamed, H.I.; Mahmoud, N.M. Induction of defence related enzymes and phenolic compounds in lupin (*Lupinus albus* L.) and their effects on host resistance against *Fusarium* wilt. Eur J Plant Pathol., 134: (2012)105–116.

[15] Prasad, R.M.; Sagar, B.V.; Devi, G.U.; Triveni, S.; Rao, S.R.K. and Chari, D.K. Isolation and screening of bacterial and fungal isolates for plant growth promoting properties from tomato (*Lycopersicon esculentum* Mill.). Int. J. Curr. Microbiol. App. Sci., 6: (2017) 753-761.

[16] Yassin, S.; Aly, A.; Abdel-Kader, D.; Morsy, K. and Atallah, O. Antagonistic potential of rhizospheric biocontrol agents against soybean root rot-wilt disease complex syndrome. ZagazigJ.Agric.Res. 46: (2019) 1395-1418.

[17] Govindappa, M.; Lokesh, S.; Rai, V.R.; Nail, V.R and Raju, S.G. Induction of systemic resistance and management of safflower *Macrophomina phaseolina* root rot disease by biocontrol agents. Archives of Phytopathology and Plant Protection, 43: (2010) 26-40.

[18] Hussein, M.M.A.; Kamal, A.M.; Abo-Elyousr, K.A.M.; Hassan, M.A.H.; Hashem, M.; Hassan, E.A and Alamri, S.A.M. Induction of defense mechanisms involved in disease resistance of onion blight disease caused by *Botrytis allii*. Egyptian Journal of Biological Pest Control 28: (2018) 80.

[19] Yasmin, S.; Zaka, A.; Imran, A.; Zahid, M.A.; Yousaf, S.; Rasul, G.; Arif, M and Mirza, M.S. Plant growth promotion and suppression of bacterial leaf blight in rice by inoculated bacteria. PLoS One 11:(2016). https://doi.org/10.1371/journal.pone.0160688

[20] Sarhan, E.A.D.; El-Far, E.M.M. and Ebrahiem, A.M.Y. Systemic resistance in snap bean (*Phaseolus vulgaris* L.) elicited by some chemicals and biotic inducers against white mold disease caused by (*Sclerotinia sclerotiorum*). Egyptian Journal of Phytopathology, 46: (2018) 61-84.

[21] Elsisi, A.A. Evaluation of biological control agents for managing squash powdery mildew under greenhouse conditions. Egyptian Journal of Biological Pest Control, 29: (2019) 89.

[22] Hahlbrock, K. and Scheel, D. Physiology and molecular biology of phenylpropanoid metabolism. Annu. Rev. Plant Physiol. Plant Mol. Biol., 40: (1989) 347-369.

[23] Nicholson, R.L. and Hammerschmid, T.R. Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology, 30: (1992) 369-389.

[24] Seleim, M.A.; Abo-Elyousr, K.A.M.; Mohamed, A.A.A. and Al-Marzoky, H.A. Peroxidase and polyphenoloxidase activities as biochemical markers for biocontrol efficacy in the control of tomato bacterial wilt. Plant Physiol. Pathol. 2: (2014) 2-8.

[25] Sarhan, E.A.D. Induction of induced systemic resistance in fodder beet (*Beta vulgaris* L.) to Cercospora leaf spot caused by (*Cercospora beticola* Sacc.). Egyptian Journal of Phytopathology, 46: (2018) 39-59.

[26] Sinclair, J. and Dhingra, O. Basic Plant Pathology Methods. 2nd Edition Boca Raton: CRC Press, (1995) 448.

[27] Barnett, H.L. and Hunter, B.B. Illustrated Genera of Imperfect Fungi. 4th Edition, APS Press, St. Paul, (1998) 218.

[28] Leslie, J.F. and Summerell, B.A. The *Fusarium* laboratory manual. blackwell publishing professional. 2121 State Avenue, Ames, Iowa 50014. USA. (2006) 399.

[29] Tewari, L. and Bhanu, C. Evaluation of agro-industrial wastes for conidia-based inoculum production of biocontrol agent: *Trichoderma harzianum*. Journal of Scientific and Industrial Research (JSIR). 63: (2004) 807-812.

[30] Sarhan, E.A.D. Effectiveness of certain biocides and essential oils in controlling Damping-Off and Root-Rot diseases of Soybean (Glycine max (L.) Merr.). Journal of Plant Protection and Pathology, 11: (2020) 79-87.

[31] Atwa, M. Combination of Biocontrol agents for controlling soybean damping-off caused by *Rhizoctonia solani*. Egyptian Journal of Phytopathology, 46: (2018) 15-38.

[32] Hafez, Y.M.; El-Nagar, A.S.; Elzaawely, A.A.; Kamel. S. and Maswada, H.F. Biological control of *Podosphaera xanthii* the causal agent of squash powdery mildew disease by up regulation of defense-related enzymes. Egypt. J. Biol. Pest. Control. 28: (2018) 57.

[33] Larkin, R.P. and Fravel, D.R. Efficacy of various fungal and bacterial biocontrol organisms for control of *Fusarium* wilt of tomato. Plant Disease, 82: (1998) 1022-1028.

[34] Fokemma, N.J. The role of saprophytic fungi in antagonism against *Derchslera sorokaniana* (*Helminthosporium sativum*) on agar plates and on rye leaves with pollen. Physiol Mol Plant Pathol., 3: (1973) 195-205.

[35] Biles, C.L. and Martyn, R.D. Peroxidase, polyphenoloxidase, and shikimate dehydrogenase isozymes in relation to tissue type, maturity and pathogen induction of watermelon seedlings. Plant Physiol. Biochem., 31: (1993) 499-506.

[36] Soltis, D.E. and Soltis, P.S. Isozymes in Plant Biology. Dioscorides press; Portland; Oregon (1990) p259.

[37] Abeles, F.B. and Forrence, L.E. Temporal and hormonal control of β-1,3 glucanase in *Phaseolus vulgaris* L. Plant Physiol., 45: (1970) 395-400.

[38] Hammerschmidt, R.; Nuckles EM and Kuc, J. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to Colletotrichum lagenarium. Physiol. Plant Pathol., 20: (1982) 73-82.

[39] Urbanek, H., E. Kuzniak-Gebarowska, and K. Herka. Elicitation of defence responses in bean leaves by Botrytis cinerea polygalacturonase. *Acta Physiologiae Plantarum* (Poland) (1991). doi: 10.1017/S0007114512000797.

[40] Gauillard F.; Richard-Forget, F. and Nicolas, J. New spectrophotometric assay for polyphenol oxidase activity. Anal. Biochem., 215: (1993) 59-65.

[41] Velioglu, Y.S.; Mazza, G.; Gao, L. and Oomah, B.D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry, 46: (1998) 4113-4117.

[42] Gomez, K. and Gomez, A. Statistical procedures for agricultural research, 2nd ed. Wiley, New York, (1984) 680.

[43] Sofi, S. A.; Muzaffar, K.; Ashraf, S.; Gupta, I., and Mir, S. A. Chickpea. In Pulses (2020) 55-76. Springer, Cham. https://doi.org/10.1007/978-3-030-41376-7_4.

[44] Sarhan, E.A.D.; El-Sayed, S.A.; Abdelmaksoud, H.M. and Elmarsafawy, T.S. Influence of biofumigation with mustard or canola seed meal in controlling soil-borne pathogenic fungi of chickpea. Egyptian Journal of Agricultural Research, 98: (2020) 40-51.

[45] Walters, D.R.; Ratsep, J. and Havis, N.D. Controlling crop diseases using induced resistance: challenges for the future. J. Exp. Bot., 64: (2013) 1263-1280.

[46] Burketova L.; Trda L.; Ott, P.G. and Valentova, O. Bio-based resistance inducers for sustainable plant protection against pathogens 33: (2015) 994-1004.

[47] Hartman G.L., Pawlowski, M.L.; Chang H.X. And Hill C.B. Successful technologies and approaches used to develop and manage resistance against crop diseases and pests. (2016) 43-66 In Emerging technologies for promotin food Security. https://doi.org/10.1016/B978-1-78242-335-5.00003-2

[48] Kannojia P., Choudhary K.K., Srivastava A.K. and Singh A.K. PGPR bio elicitors: induced systemic resistance (ISR) and proteomic perspective on biocontrol. (2019) 67-84 In PGPR Amelioration in Sustainable Agriculture https://doi.org/10.1016/B978-0-12-815879-1.00004-5.

[49] Da Rocha, A.B. and Hammerschmidt, R. History and perspectives on the use of disease resistance inducers in horticultural crops. Hort. Technology, 15: (2005) 518-529.

[50] Walters, D.; Walsh, D.; Newton, A. and Lyon, G. Induced resistance for plant disease control: maximizing the efficacy of resistance elicitors. Phytopathology, 95 :( 2005) 1368-1373.

[51] Prasannath, K. Plant defense-related enzymes against pathogens: a review. AGRIEAST: J. of Agric. Sci., 11: (2017) 38-48.

[52] Mayer, A.M. Polyphenoloxidases in plants and fungi: going places? A review. Phytochemistry, 67: (2006) 2318-2331.

[53] Kau, R.; Singh, R.S and Alabouvette, C. Antagonistic activity of selected isolates of *fluorescent Pseudomonas against Fusarium oxysporum* f. sp. ciceri. Asian J of Plant Sci 6: (2007) 446-454.

[54] Khalil, M.S.M. Studies on Some Chickpea (*Cicer arietinum* L.) Fungal Diseases. (2007) M.Sc. Thesis, Fac. Agric. Minia Univ. Egypt.

[55] Siddiqui, Z.A and Akhtar, M.S. Biocontrol of a chickpea root rot disease complex with phosphate- solubilizing microorganisms. J. of Plant Pathology 9: (2007) 67-77.

[56] Prasad, R.D.; Rangeshwaran, R.; Anuroop, C.P. and Rashni, H.J. Biological control of wilt and root rot of chickpea under field conditions. Ann. Pl. Prot. Sci., 10: (2002) 72-75.

[57] Sallam N.M.A.; Abo-Elyousr, K.A.M. and Hassan, M.A.E. Evaluation of *Trichoderma* species as biocontrol agent for damping-off and wilt diseases of *Phaseolus vulgaris* L. and efficacy of suggested formula. Egyptian Journal of Phytopathology, 36: (2008) 81-93.

[58] Alareny, G. A.; H. A. Mahran.; Heidi. I.G.; Abo-Elnaga. and M.S. Mohamed. Biological Control of Chickpea Damping-off and Root Rot Diseases. Assiut J. Agric. Sci., 44: (2013) 22-38.

[59] Pandey, P.; Kumar, R. and Mishra, P. Integrated approach for the management of *Sclerotinia sclerotiorum* (Lib.) de Bary, causing stem rot of chickpea. Indian Phytopathology, 64: (2011) 37-40.

[60] Pandey, R. N.; Gohel, N. M. and Jaisani, P. Management of Wilt and Root Rot of Chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* and *Macrophomina phaseolina* through Seed Biopriming and Soil Application of Bio-Agents. Int. J. Curr. Microbiol. Appl. Sci, 6: (2017) 2516-2522.

[61] Atwa, M. A.; Sarhan, E. and Zian, A. Effect of different inducers on controlling damping-off and wilt diseases of lupine. Arab Universities Journal of Agricultural Sciences, 27: (2019) 1967-1983.

[62] Youssef, S.A.; Tartoura, K.A and Greash, A.G. *Serratia proteamaculans* mediated alteration of tomato defense system and growth parameters in response to early blight pathogen *Alternaria solani* infection. Physiological and Molecular Plant Pathology, 103: (2018) 16-22.

[63] Benhamou, N.; Gagné, S.; Le-Quéré, D. and Dehbi, L. Bacterial-mediated induced resistance in cucumber: beneficial effect of the endophytic bacterium Serratia plymuthica on the protection against infection by Pythium ultimum. Phytopathology, 90: (2000) 45-56.

[64] Khaledi, N.; Taheri, P. and Tarighi, S. Antifungal activity of various essential oils against *Rhizoctonia solani* and *Macrophomina phaseolina* as major bean pathogens. J. Appl. Microbiol., 118: (2015): 704-717.

[65] Lee, S.B. and Taylor, J.W. Isolation of DNA from fungal mycelia and single spores. In: Innis MA, Gelfand DH, Sninsky, J.J. and White, T.J. (eds) PCR protocols: a guide to methods and applications. (1990) 282–287 Academic Press, New York, http://dx.doi.org/10.1016/b978-0-12-372180-8.50038-x.

[66] White, T.J.; Bruns, T.; Lee, S. and Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications 18: (1990) 315–322.

## الملخص العربى

### المقاومة الجهازية فى الحمص (*Cicer arietinum* L.) المحفزه ببعض المستحثات الحيوية ضد أمراض الجذور

**أحمد كمال أحمد معوض[أ] , إيهاب على ضياء سرحان[ب] ,هدى حسن أبو غالية[أ] , زينب محمد حسن خيرالله[أ]**

أ- قسم علم النبات - كليه البنات لالداب و العلوم و التربيه- جامعه عين شمس

ب- معهد بحوث أمراض النباتات-مركز البحوث الزراعية-الجيزة- مصر

**الملخص**

تم دراسة تأثير معاملة بذور الحمص (*Cicer arietinum* L.) بالمستحثات الحيوية *Trichoderma viride,. Trichoderma harzianum Pseudomonas fluorescens, Bacillus subtilis Serratia marcescens* مقارنة بالمبيد الفطرى Rizolex-T تحت ظروف الصوبة والحقل في الموسم 2018/2017 فى مركز البحوث الزراعية بالجيزة لمكافحة أمراض الجذور المتسببة عن الفطريات *Fusarium oxysporum, Rhizoctonia solani,* او*Sclerotinia sclerotiorum* . ثبطت السلالات المختبره النمو الفطري للفطريات الثلاث المختبره بشكل معنوي. كما ادت المعامله بكل السلالات الحيوية المختبرة تحت كلاً من ظروف الصوبة والحقل الي اختزال معنوي في نسبة موت البادرات ، اعفان الجذور ، عفن الساق وأمراض الذبول مقارنه بالعينة الغير معجالة. وقد لوحظ أن أعلي نسبة للنباتات الباقية علي قيد الحياه هى المعاملة بالمبيد ريزولكس-تي و (*Trichoderma viride* + *Trichoderma harzianum*) يليها (*Pseudomonas fluorescens* + *Bacillus subtilis*), *Trichoderma viride, Trichoderma harzianum, Bacillus subtilis, Pseudomonas Fluorescens* و *Serratia marcescens* علي التوالي. كذلك فإن كل المستحثات الحيويه المختبرة قد شجعت معنويا مقاييس النمو والانتاج لنباتات الحمص المعامله ، ممثلة في اطوال النباتات ، عدد الفروع للنبات ، عدد القرون للنبات ، عدد البذور للنبات ، وزن البذور من النبات ، وزن ال 100 بذره ،و الانتاج المحصولي للحمص (طن/فدان). كذلك تم تقدير محتوي الفينولات الكليه والحرة والمرتبطة وايضا نشاط الانزيمات الدفاعية البولي جلوكانيز والبيروكسيديز والبولي فينول أوكسيديز في نباتات الحمص المعاملة بالمستحثات الحيوية المختبرة ومقارنتها بالمعاملات المعالجةوالغير معالجة بالمسببات المرضية *Fusarium oxysporum, Rhizoctonia solani,* or *Sclerotinia sclerotiorum*. وقد أظهرت النباتات المعاملة (*Trichoderma harzianum* + *Trichoderma viride*) اعلي زيادة في المحتوي الكلي للفينولات والفينولات الحرة والمرتبطه وكذلك في نشاط الانزيمات الدفاعيه المرتبطه بالمقاومة وقد لوحظ أن أقل زيادة فى محتوي النباتات من الفينولات والأنزيمات الدفاعية بفطر*Serratia marcescens*.