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Biological control of some garlic diseases using antagonistic fungi and bacteria

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Abstract

Eight *Trichoderma* isolates which isolated from rhizospher of garlic plant and one isolte *T. asperellum* was obtained on PDA medium, from the commercial product (Biocontrol T34). Also eleven isolates of rhizobacteria namly; *B. subtilis*, two isolates (Bs1 and Bs2), *B.megaterium* two isolates (Bm1and Bm2), *P. fluorescens* two isolates (Pf1 and Pf2), four isolates *A. chroococcum* (Az1, Az2, Az3 and Az4) and *Penibacillus polymyxa* one isolate were tested in vitro to study thir ability against *S. ceprivorum* , *F. oxysporum* f. sp. cepae and *P. terrestris* which caused white rot, basal rot and pink rot of garlic plants, respectively. The results showed that *Trichoderma* isolate number (T3) gave the highest reduction on maycelial growth of three pathogenic fungi, which adentified as *Trichoderma harzianum*, followed *T. asperellum* (T34), then isolate (T5) and isolate (T7). which adentified as *Trichoderma harzianum* and *Trichoderma hamatum*, respectively. *Pseudomonas. fluorescens* isolate (Pf1), followed by *P. fluorescens* (Pf2), *B. subtilis* (Bs2), *A. chroococcum* (Az4) and *B. subtilis* (Bs1), then *A. chroococcum* (Az2), *B. megaterium* (Bm2) and *Penibacillus polymyxa* gave highly antagonistic effect was clear against the tested fungi respectively. A pot experiment was crried out under greenhouse conditions to evaluate the efficacy of commercial biofungicides biozeid , Bio-Arc, Plant Guard, T34 biocontrol and Rhizo-N, and biofertilizers Nitrobien, phosphoren, Biogen, Potassiumag, Ascobein and carialin were evaluated individually against garlic white rot, basal rot and pink rot diseases. Data showed that treated soil with biofungicides and biofertilizers reduced white rot, basal rot and pink rot diseases compared with the control. Treated soil with Rhizo-N, T34 biocontrol, Phosphoren and Nitrobien gave the best reduction of disease severity throughout two successive growing seasons.

Keywords: garlic, rhizobacteria, biofungicides, biofertilizers, *Trichoderma*.

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1. Introduction

Garlic (*Allium sativum* L.) is one of the most important bulb vegetable crops and is next to onion (*Allium cepa)* in importance (Hamma et al., 2013). It is commonly used as a spice or in the medicinal purposes. In Egypt, it has been generally cultivated for both local consumption and export. Garlic is affected by several fungal diseases, with the most important being white rot (*Sclerotium ceprivorum*), Fusarium basal rot (*Fusarium oxysporum* f.sp. cepae), pink root rot (*Pyrenocheta terrestris*), rust (*Puccinia allii*) and leaf blight (*Stemphylium* spp.) (Laura et al., 2017). White rot of garlic and onion, caused by the soilborne fungi *Sclerotium cepivorum* Berk, is a continuing concern for worldwide garlic production. An inoculum density with few soil sclerotia in a litter of field soil can result in great crop losses (Davis et al. 2007). White rot pathogen survives in the soil in the absence of *Allium* crops as a dormant small, round and poppy-seed-sized black sclerotia on plant debris for more than 20 years (Entwistle, 1990). *Sclerotium cepivorum* is widely distributed in Egypt causing white rot of onions and garlic (Embaby, 2003). According to the pathogen properties control of white rot disease is difficult (Crowe et al., 1993). Among these, basal rot caused by *Fusarium oxysporum* f. sp. *cepae* Snyder and Hansen is an economically important and widespread disease in onion, garlic and a number of other Allium species, such as chive and shallot and causes significant yield losses in all the growing areas of the world (Behrani *et al.* 2015; Coskuntuna and Ozer 2008). Pink root rot is one of the most important diseases that attack cultivated *Allium* spp. Pink root rot disease caused by *Pyrenochaeta terrestris* has been reported as a serious disease to garlic in Egypt (Shalaby et al., 2002). Pink root pathogen is soil-borne fungus, which remains viable in the soil for many years (Rengwalska and Simon, 1986). Roots infected by *P. terrestris* turn pink initially and then become brittle and die. Although *P. terrestris* can be present in roots, it does not invade the basal plate or stem of the bulb (Coleman and Ellerbrock, 1997). Crop rotation, solarization, fumigation and chemical fungicides are the most common methods for controlling soil-borne diseases (Porter et al., 1989). Despite the fact that the utilization of fungicides gave satisfactory control against plant diseases, they could accumulate hazardous toxic compounds which pose threat to human life and the surrounding environment. Pathogens are additionally found to develop resistance against several fungicides (Deising et al., 2008). The other suggested control methods are including application of biological control. Several fungal and bacterial antagonists have proved to control different plant pathogenic fungi (Blaszczyk, 2014). The best results to control white rot of onion and garlic recorded by using *Trichoderma harzianum, T. Koningii, T. asperellum, Talaromyces flavus* and *Bacillus subtilis* (Mahdizadehnaraghi *et al.,* 2015; Shalaby *et al.* 2013). El-Meneisy Afaf (2019) found that *Bacillus subtilis, Bacillus amyloliquefacienes* and *Bacillus velezensis* were the most effective isolates to reduce *S. cepivorum* growth *in vitro* and significant reduction of disease index in pots experiment. Biological control of Fusarium basal rot by inoculation of antagonistic fungi and bacteria such as *Trichoderma* and *Pseudomonas* has been considered as an alternative approach to chemical control (Coskuntuna and Ozer, 2008; Rajendran, 1996). *Pseudomonas fluorescens and T. viride* exhibited the highest disease reduction, i.e. 69.5 and 61.8%, followed by 53.8, and 53.7% induced by *B. subtilis*

and T. harzianum, respectively of basal rot infection of onion (El-Mougy Nehal and Mokhtar, 2019). Four isolates of *Bacillus subtilis* exhibited the highest antagonistic effect against *F. oxysporum* f. sp. *cepae* during *in vitro* testing up to 71% and caused a significant suppression of garlic basal rot infection up to 58% (Dragana et al., 2018). Microbial antagonists as a bioagents are considered a suitable ecologically way to substitute chemical fungicides. This study aimed to use an effective and safe method for control white rot, basal rot and pink rot of garlic as an alternative method instead of using a harmful chemical fungicide.

Materials and methods

2.1 Isolation and identification of the causal pathogens

Samples of garlic plants showing white rot, basal rot and pink root rot symptoms were collected from different farms in Minia, Assiut, Sohag and Qena governorates, Egypt. *Sclerotium cepivorum* was isolated from sclerotia formed on the surface of garlic bulbs according to the methods of Clarkson et al. (2002). The sclerotia were disinfected in 70% ethyl alcohol for about two minutes, dried well using sterilized filter papers and transferred to Petri dishes containing potato dextrose agar (PDA) medium. The Petri dishes were kept at 20 ºC. After the germination and fungal growth, hyphal tip technique was used to purifying cultures (Brown, 1924). *Fusarium oxysporum* f.sp. *cepae* was isolated from rot symptomatic garlic bulbs by the tissue segment method (Rangaswami, 1958), basal plate tissues of diseased samples were cut into small pieces using a sterilized scalpel, surface sterilized with a 0.5% solution of sodium hypochlorite for two minutes and then washed several times in sterilized distilled water to remove any residual effect of Na-hypochlorite. The pieces were dried between two sterilized filter paper, then transfered on the Petri dishes containing sterilized potato dextrose agar (PDA) medium amended with streptomycin sulphate (200 mg/l) (Sigma-Aldrich, USA) and incubated at 25±2ºC, then examined daily for fungal growth. The fungal colonies were purified using single spore or hyphal tip techniques suggested by Booth (1985) and Rangaswami (1972). The cultures were identified according to their morphological and microscopical characters as described by Booth (1985), Barnett and Hunter (1972) and Leslie and Summerell (2006). *Pyrenochaeta terrestris* were isolated from garlic bulbs exhibiting symptoms of pink root rot (Mishra *et al.*, 2012; Netzer *et al*., 1985). The roots were cut in to 2-3 mm pieces and surface sterilized with a 70% ethyl alcohol for 30 seconds, immediately rinsed in sterile water and dried on sterile filter paper. The host tissue was plated on water agar (WA) medium and were allowed to grow for 4-5 days at 25ºC. Pinkish red colonies developed 4-5 days after the inoculation placed onto Potato Dextrose Agar (PDA) medium and were purified by using single spore technique. The pathogen was identified as *Pyrenochaeta terrestris* by using relevant literature (Schwartz and Mohan, 2008; Boerema *et al*., 2004). The obtained isolates of *S. cepivorum*, *F. oxysporum* f.sp. *cepa* and *P. terrestris* were maintained on PDA slants and kept in refrigerator at 5°C for further studies. The isolates were confirmed by

Mycological Research Center (AUMRC), Assiut University, Egypt.

2.2 Pathogenicity tests

Pathogenicity tests of the isolated fungi were carried out under greenhouse conditions at Faculty of Agriculture, Al-Azhar University (Assiut Branch), Egypt. The plastic pots (25 cm diameter) were sterilized by immersing in 5% formalin solution for 15 minutes, then left for several days to get rid of the poisonous effect of the formalin. Thirteen isolates of *F. oxysporum* f.sp. *cepae*, nine isolates of *S. cepivorum* and seven isolates of *P. terrestris* were obtained from different locations. The fungi used throughout this experiment as well as the source of isolates are shown in Table (1). The inoculum which used in the foregoing studies consisted of uniform agar discs 5 mm. in diameter bearing 7 dayṡ old and grown in 500 ml. glass bottles containing the following substrate per bottle (25 g coarse sand, 75 g barley and 100 ml tap water to cover the mixture in bottles). The bottles were autoclaved for 30 minutes. The bottles were inoculated with the tested fungi, then incubated at 20±2ºC for two weeks to obtain sufficient growth of the fungi. The sterilized pots were filled with sterilized clay loam soil and infested with the fungal inoculum at the rat 2% (w/w) soil, then watered and lift for one week before sowing to ensure even distribution and growth of each particular fungus. Disinfested garlic cloves cultivar sides 40. were planted in the infested pots, each pot was planted with 5 cloves (25 cm in diameter). Four pots were used for each isolate, (which were considered as replicates). Pots containing sterile soil mixed with barley grains free of any fungus were sown similarly with disinfested garlic cloves at the same rate to be used as control treatment. Pots were kept under observation and irrigated as needed. Results were recorded after 90 days of planting for white rot and after 120 days for basal rot and pink rot. The infected plants of each replicate were removed from the soil after the end of inoculation period, washed thoroughly to remove soil debris. The white rot rating scale was as follows: 1 = healthy plants, 2= slightly severe (leaves yellowing, root system reduced), 3 = moderate severe (yellowing, die back of leaves and badly decayed root system), 4 = severe (completely yellowing plant, leaves die back, semi soft rot of cloves and roots) and 5 = highly severe (completely dead plants, extensive decayed roots and bulbs). The basal rot rating scale was as follows: 1 = without symptoms, 2 up to 10% rotted roots, 3 = 10-30% rotted roots with up to 10% rotted basal plates, 4 =completely rotted roots and 10-30 % rotted basal plates and *5* = completely rotted roots and more than 30% rotted basal plate. The pink root rot rating scale was as follows: 1 = without symptoms, 2 = less than 10% pink roots, 3 = 10-50% pink roots, 4 = more than 50% pink roots, and 5 = completely rotted roots. (DS%) was estimated as the following:

$$DS\ (\%) = \Sigma\ [\ (1A+2B+3C+4D+5E)\ /5T] \times 100$$

Where, A, B, C, D and E are the number of plants corresponding to the numerical grade, 1, 2,3,4 and 5 respectively and 5T is the total number of plants (T) multiplied by the maximum disease grade 5, where T=A+B+C+D +E. To detect the different degrees of disease,

plants were classified into five categories according to Chemeda *et al*. (2015), Rengwalska and Simon (1986) and Shatla *et al*. (1980).

2.3 Isolation of *Trichoderma* spp.

Soil samples were collected from rhizosphere of healthy garlic plants, In growing fields of Assiut Governorate. One hundred gram from rhizosphere soil were collected into each sterile plastic bag and kept in the refrigerator at Plant Pathology Laboratory, Faculty of Agriculture, Al-Azhar University (Assiut Branch), Egypt for further analysis. Isolation of antagonistic *Trichoderma* spp. from rhizosphere soil was made using serial dilution technique according to Waksman (1922), and Belete and Ahmed (2015). Eight *Trichoderma* isolates were identified according to Kubicek and Harman (2002) based on their conidial morphology, color and texture, and growth characteristics. The obtained isolates were confirmed by Mycological Research Center, Assiut University, Egypt as follow: two isolates *Trichoderma harzianum* (T3 and T5), three isolates *T. hamatum* (T2, T6 and T7), two isolates *T. koningii* (T1and T8) and one isolate *T. viride* (T4).

2.4 *In vitro* experiments

2.4.1 Evaluation of antagonistic activity of *Trichoderma* spp. against pathogenic fungi

Eight different species of *Trichoderma* isolated from rhizosphere of healthy garlic plants and one isolate of *T. asperellum* was obtained on PDA medium, from the commercial product (Biocontrol T34) provided by Shoura Agrochemicals Company were screened against the pathogenic fungi *in vitro*. The antagonistic effects of each *Trichoderma* sp. and *T. asperellum* against *F. oxysporum* f. sp. *cepae, S. cepivorum* and *P. terrestris* were tested using dual culture technique (El-Sheshtawi *et al.*, 2009; Coskuntuna and Ozer, 2008). The tested isolates of *Trichoderma* spp. were grown on potato dextrose agar (PDA) medium at 25°C, for 6 days and used as inocula. Discs from each isolate of *Trichoderma* sp. (5 mm in diameter) were transfered on PDA medium in one side of Petri plate and the opposite side was inoculated by pathogenic fungi. Four replicates were used for each treatment. Inoculated plates with pathogenic fungi only were used as the control. After 5 days incubation period at 22±2°C, the linear growth of the tested pathogen was recorded when the growth of the pathogen covered the plate surface in the control treatment. The percentage of mycelial growth inhibition was calculated according to the following formula:

$$\text{Mycelial growth inhibition (\%)} = [A-B/A] \times 100$$

Where: A = the length of the hyphal growth in the control, B = the length of hyphal growth of the tested fungus. The antagonistic *Trichoderma* isolates which gave a higher percentage of mycelia growth inhibition were identified as follow; *T. harzianum* (T3 and T5) and one isolate *T. hamatum* (T7) by Mycological Research Center, Assiut University, Egypt.

2.4.2 Evaluation of antagonistic activity of rhizobacteria against pathogenic fungi

Eleven isolates of rhizobacteria namly; *B. subtilis* two isolates, *B. megaterium* two isolates, *Penibacillus polymyxa* one isolate, *P. fluorescens* two isolates and four isolates of *A. chroococcum* were obtained from MERCIN, Faculty of Agriculture, Ain Shams University, Egypt. These isolates were tested against the pathogenic fungi *F. oxysporum* f.sp. *cepae*, *S. cepivorum*, and *P. terrestris* under vitro conditions. The tested isolates of bacteria were grown on Nutrient Sucrose Agar medium (NSA) (Peptone 5 g, beef extract 3 g, yeast extract 2g, sucrose 5 g, agar 20 g, and distilled water 1liter) and incubated at 28°C for one day and used as inocula (Sallam Nashwa et al., 2013). Petri plates (9 cm in diameter) containing potato dextrose agar (PDA) medium were inoculated in the middle by discs (5 mm in diameter) of pathogenic fungi, then inoculated with the tested bacterium on two opposite side of the tested pathogen. Four replicates were used for each treatment. Inoculated plates with the pathogenic fungi only were served as the control. After 5 days of incubation period at 22±2°C, the linear growth of the tested pathogens was recorded when the growth of the pathogens covered the plate surface in the control treatment. The percentage of mycelial growth inhibition were calculated according to the following formula:

$$\text{Mycelial growth inhibition (\%)} = [A-B/A] \times 100$$

where: A = the length of the hyphal growth in the control, B = the length of hyphal growth in the tested isolate.

2.5 Effect of commercial biofungicides and biofertilizers on controlling garlic white rot, basal rot and pink root rot diseases under greenhouse conditions

Five commercial biofungicides and six biofertilizers were tested against garlic white rot, basal rot and pink root rot under greenhouse conditions during 2019 and 2020 growing seasons. The commercial biofungicides were Biozeid (*Trichoderma album*), Plant Guard (*T. harzianum*), T34 biocontrol (*T. asperellum*), Bio-Arc (*Bacillus megaterium*) and Rhizo-N (*B. subtilis*). Each commercial biofungicide were tested at the rat 3 g /kg soil. While, commercial biofertilizers were Nitrobien (A*zotobacter* sp. and *Azospirillum* sp.), Biogen (*Azotobacter vinelauvii* and *A.chroococcum*),Potassiumag (*Bacillus circulans* and *Bacillus megaterium* var. *phosphaticum*), Phosphoren (As phosphorus solubilizing bacteria), Carialin (*Azospirillum* sp.) and Ascobein (As citric acid + ascorbic acid 38% and Organic plant growth stimulating materials 62%). These bio-fertilizers were obtained from bio-fertilization Unit, Agricultural Research Center Ministry of Agriculture, Giza, Egypt. Each tested commercial biofertilizers were used at the rat 4 g /kg soil, except Ascobien was used as foliar spraying at rat 1.3 g /L. Plastic pots (25cm in diameter) were filled with sterilized clay soil and mixed with the pathogenic fungi, *F. oxysporum* f.sp. *cepae*, *S. cepivorum* and *P. terrestris* at the rate 2% (w/w) one week before adding biofungicides, biofertilizers and planting. Pots were filled with sterilized soil and infested with the pathogenic fungi only served as a control. The commercial biofungicides and biofertilisers were added and

distributed in the infested soil at the time of planting. Four pots were used for each treatment as replicates, each pot was planted with 5 cloves. At the end of the experiment, garlic plants were uprooted, washed, rated for disease severity as mentioned before.

2.6 Greenhouse experiments

The effects of commercial biofungicides Biozeid , Bio-Arc, Plant Guard, T34 biocontrol and Rhizo-N, also biofertilizers Nitrobien, phosphoren, Biogen, Potassiumag, Carialin and Ascobein were evaluated individually against garlic white rot, basal rot and pink root rot diseases incited by *F. oxysporum* f. sp. *cepae*, *S. cepivorum* and *P. terrestris* under greenhouse conditions. This experiment was carried out during 2019 and 2020 growing seasons, under greenhouse conditions of Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt. Plastic pots were filled with sterilized clay soil and mixed with fungal inocula as described before at rate 2 % of clay soil (w/w), one week before planting. Biofungicides and biofertilizers were added to the infested soil at rat 3 g /kg soil and 4 g /kg soil at the time of planting. Each pot was sown with 5 disinfested cloves of garlic Sides 40 cv. Four pots were used for each treatment as replicates. The infested pots individually with pathogenic fungi only were sown with disinfested garlic cloves and served as control. At the end of the experiment plants were uprooted washed, rated for Disease severity. Disease severity was estimated as described before.

2.9 Statistical analysis

Analysis of variance of the data was carried out on the mean values of the tested treatments according to the procedures described by Gomez and Gomez (1984). The least significant difference (L.S.D) at 5% probability was used for testing the significance of the differences among the mean values of the tested treatments for each character.

3. Results and Discussion

3.1 Isolation and identification of garlic white rot, basal rot and pink root rot the causal fungi

Twenty-nine fungal isolates were isolated from infected roots of garlic plants collected from different localities in Minia, Assiut, Sohag, and Qena Governorates, Egypt. Fungal isolates were identified by using the morphological features of mycelia and spores as described by Barnet and Hunter (1977) and Booth (1985) and confirmed by Mycological Research Center (AUMRC), Assiut University, Egypt. As shown in Table (1) that the isolated fungi were identified as thirteen isolates of *Fusarium oxysporum* f.sp. *cepae* Snyder and Hansen, seven isolates of *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker and Larson and nine isolates of *Sclerotium cepivorum* Berk.

Table 1: Source of 29 fungal isolates which isolated from four Egyptian governorates during 2017 growing season.

The isolated fungi	Isolate code	Governorate
F. oxysporum f.sp. *cepae*	F1	Sohag
	F2	Sohag
	F3	Assiut
	F4	Assiut
	F5	Minia
	F6	Qena
	F7	Qena
	F8	Sohag
	F9	Sohag
	F10	Minia
	F11	Minia
	F12	Assiut
	F13	Sohag
P. terrestris	P1	Minia
	P2	Sohag
	P3	Minia
	P4	Minia
	P5	Sohag
	P6	Qena
	P7	Assiut
S. cepivorum	S1	Minia
	S2	Minia
	S3	Sohag
	S4	Sohag
	S5	Sohag
	S6	Assiut
	S7	Minia
	S8	Assiut
	S9	Sohag

3.2 Pathogenicity tests

Twenty-nine fungal isolates were tested to study their pathogenic capbilities on garlic plants (Sides 40 cv.) under greenhouse conditions during 2018 growing season. Data in Table (2) exhibited that all tested fungal isolates of *S. cepivorum*, *F. oxysporum* f. sp. *cepae* and *P. terrestris* were able to infect garlic plants causing white rot, basal rot and pink root rot. All the tested isolates of *S. cepivorum* caused white rot disease compared with the control. In this respect, *S. cepivorum* (isolate No. 5) gave the highest disease severity, reached it 82.67 % followed by isolates No. 1, 2, 6 and 3 which reached 77.33, 70.67, 65.33 and 61.33 % respectively. While, isolates No7 and 4 gave moderate disease severity (57.33and 53.33 %). At the same time, isolates No. 8 and 9 came in the last 45.33 and 40.5 % disease severity. Data also showed that, all the tested isolates of *F. oxysporum* f.sp. *cepae* caused basal rot disease compared with the control. In this case, *Fusarium* isolate No. 7 exhibited the highest disease severity (74.66 %) followed by isolate No. 3 and 2 reached it 62.67 and 60.5 %, respectively. As regard, isolates No. 6, 4, 8, 13 and 10 gave the moderate disease severity (57.33, 50.66, 46.67, 48.0 and 40.0 %, relatively. On the other hand, *P. terrestris* with all tested isolates caused pink root rot disease compared with

uninfected plants. As shown in this table, isolate No. 3 recorded the highest disease incidence, followed by isolates No. 5, 6 and 4, relatively (73.30, 59.96, 46.63 and 44.40 %) relatively. As mean, isolates No 1 and No. 7 showed lower disease severity (37.73 and 33.30 %, while isolate No. 2 came in the last (28.83). Such results are in agreement with those obtained by Ellojita et al. (2016), Chemeda et al. (2015) and Shalaby et al. (2012), who found that *F. oxysporum* f. sp. *cepae* , *P. terrestris* and *S. cepivorum* caused basal rot, pink rot and white rot diseases of garlic under greenhouse and field conditions.

Table 2: Disease severity of garlic white rot, basal rot and pink root rot diseases caused by 29 fungal isolates under greenhouse conditions during 2017 growing season.

The tested isolates	Isolate code	Disease severity (%)		
		White rot	Basal rot	Pink root rot
S. cepivorum	Sc1	77.33	0	0
S. cepivorum	Sc2	70.67	0	0
S. cepivorum	Sc3	61.33	0	0
S. cepivorum	Sc4	53.33	0	0
S. cepivorum	Sc5	82.67	0	0
S. cepivorum	Sc6	65.33	0	0
S. cepivorum	Sc7	57.33	0	0
S. cepivorum	Sc8	45.33	0	0
S. cepivorum	Sc9	40	0	0
F. oxysporum f.sp. *cepae*	F1	0	33.33	0
F. oxysporum f.sp. *cepae*	F2	0	29.33	0
F. oxysporum f.sp. *cepae*	F3	0	62.67	0
F. oxysporum f.sp. *cepae*	F4	0	50.66	0
F. oxysporum f.sp. *cepae*	F5	0	38.67	0
F. oxysporum f.sp. *cepae*	F6	0	57.33	0
F. oxysporum f.sp. *cepae*	F7	0	74.66	0
F. oxysporum f.sp. *cepae*	F8	0	46.67	0
F. oxysporum f.sp. *cepae*	F9	0	22.66	0
F. oxysporum f.sp. *cepae*	F10	0	40	0
F. oxysporum f.sp. *cepae*	F11	0	28	0
F. oxysporum f.sp. *cepae*	F12	0	60	0
F. oxysporum f.sp. *cepae*	F13	0	48	0
P. terrestris	P1	0	0	37.73
P. terrestris	P2	0	0	28.83
P. terrestris	P3	0	0	73.3
P. terrestris	P4	0	0	44.4
P. terrestris	P5	0	0	59.96
P. terrestris	P6	0	0	46.63
P. terrestris	P7	0	0	33.3
Control		0	0	0
L.S.D at 5%		3.29	4.37	9.99

3.3 Preliminary tests for antagonistic capability of fungi and bacteria against the growth of pathogenic fungi *in vitro*

It was shown in Table (3) that the antagonistic fungal isolates (*Trichoderma* spp.) were able to inhibit mycelial growth of the tested pathogenic fungi (*S. cepivorum* (Sc5), *F. oxysporum f.sp. cepae* (F7) and *P. terrestris* (P3)

compared with the control. *Trichoderma harzianum* (T3) gave the greatest reduction of mycelial growth of the tested pathogens followed by isolate *T. asperellum* (T34), then isolate *T. harzianum* (T5) and isolate *T. hamatum* (T7). The other tested antagonistic showed moderate inhibition against the tested pathogenic fungi. While, the least reduction of mycelial growth was obtained by *Trichoderma koningii* (T1) followed by (T8). These results are in agreement with those recorded by El-Sheshtawi et al. (2009) and Malathi (2015). Antagonistic potential of different *Trichoderma* species arranges of mechanisms have to be considered one in Production of antibiotic, volatile and nonvolatile chemicals. These substances influence the permeability of cell membranes and result in anefflux of the cytoplasm (Howell, 1998). The antifungal enzyme system of *Trichoderma* spp. plays an important role for detection and destroying the pathogenic cell wall. Also, the mechanism depends on competition, Competitiveness is based on rapid growth and the production of various asexual generated conidia and chlamydospores (Shi et al., 2012; Chet et al., 1998; Chet, 1990). The direct influence of *Trichoderma* spp. against pathogens through colining their hyphae around the hyphae of the pathogens to prevent their continued growth. Bettucci et al. (1996) reported that the secondary metabolites trichozianins obtained from *T. harzianum* inhibited mycelial growth of *S. cepivorum.*

Table 3: Effect of antagonistic *Trichoderma* mycelial growth inhibition of the tested fungi *in vitro*.

Antagonistic *Trichoderma*	Mycelial growth inhibition (%)			Mean
	S. cepivorum (Sc5)	*F. oxysporum* f.sp. *cepae* (F7)	*P. terrestris* (P3)	
T. koningii (T1)	26.6	28.1	32.6	29.1
T. hamatum (T2)	31.1	34.4	35.5	33.6
T. harzianum (T3)	74.4	69.5	86.6	76.8
T. viride (T4)	22.2	31.8	39.9	31.3
T. harzianum (T5)	77.7	58.1	84.4	73.4
T. hamatum (T6)	20.33	17.7	25.5	21.17
T. hamatum (T 7)	67.7	50.7	75.6	64.66
T. koningii (T8)	13.3	23.3	18.8	18.46
T. asperellum (T34)	78.8	57.3	85.5	73.86
Control	0	0	0	-
LSD at 5%	1.92	3.03	2.03	-

The same experiment was carried out by using antagonistic bacteria. It was clear from data present in Table (4) that all the tested antagonistic rhizobacteria (PGPR) inhibited growth of the tested pathogenic fungi. The highest reduction of the mycelial growth was acheived by *P. fluorescens* isolate (Pf1), followed by *P. fluorescens* (Pf2), *B. subtilis* (Bs2), *A. chroococcum* (Az4) and *B. subtilis* (Bs1), whenever the mycelial growth it 71.9, 67.9, 64.46, 62.16, and 61.6 %, respectively. At the same time, Az2, Az1, *B. megaterium* (Bm2) and *Penibacillus polymyxa* (Bp) showed moderate reduction of mycelia growth of the tested pathogenic fungi. While, the lowest inhibition of mycelial growth of

the tested fungi was obtained with *B. megaterium* (Bm1) and *A. chroococcum* (Az3), except in case of *F. oxysporum* f.sp. *cepae* non-antagonistic was observed. These results are in line with those obtained by Manoj et al. (2014), Haggag-Karima et al. (2015) and El-Meneisy-Afaf et al. (2019).

Table 4: Effect of certain antagonistic rhizobacteria on mycelial growth inhibition of the tested fungi *in vitro*.

Antagonistic rhizobacteria	Mycelial growth inhibition (%)			Mean
	P. terrestris (P3)	*S. cepivorum* (Sc5)	*F. oxysporum* f.sp. *cepae* (F7)	
P. fluorescens (Pf 1)	64.7	80.3	70.7	71.9
P. fluorescens (Pf 2)	70.3	58.4	74.03	67.6
Penib. polymyxa (Bp)	61.1	20.3	42.5	41.3
B. megaterium (Bm1)	57.3	24.4	37.3	39.6
B. megaterium (Bm2)	60.7	26.9	42.5	43.36
B. subtilis (Bs1)	69.6	50.3	65.1	61.6
B. subtilis (Bs2)	74.7	51.4	67.3	64.46
A. chroococcum (Az1)	58.8	18.1	61.1	46
A. chroococcum (Az2)	60.5	20.7	68.8	50
A. chroococcum (Az3)	61.1	37.3	0	32.8
A. chroococcum (Az4)	61.4	54.4	70.7	62.16
Control	0	0	0	-
LSD at 5%	2.62	2.61	2.57	-

Bacterial bioagents showed antifungal potential against the tested fungi, which could be attribute to mechanism of diffusible antagonistic substances and volatile metabolites depending on the bacterium and the pathogen combination. The diffusible substances include antibiotics (pyrrolnitrin) and siderophores (enterobactin and aerobactin) and volatilic metabolites include hydrogen cyanide and acetoin (Rakh et al., 2011). Results from bioassays suggest that production of antifungal substances by these bacteria may be responsible for the inhibition of fungal growth, where no direct contact between bacterial colonies and mycelium of pathogenic fungi, so that the fungal growth inhibition was caused by diffusion of substances into the agar medium. On the other hand, most of bacteria that used as a biocontrol agent like *Bacillus* spp. produce antibiotics responsible for their antifungal activities (Bhattacharjee and Dey, 2014).

3.4 Greenhouse experiments

3.4.1 Effect of commercial bio-fungicides on controlling garlic white rot, basal rot and pink root rot diseases under greenhouse conditions

The influence of soil treatment with different biofungicides Rhizo-N, Bio-Arc, Plant-guard, Biozeid and T-34 (Biocontrol) on incidence of white rot, basal rot and pink root rot diseases of Sides 40 garlic cv. was carried out under greenhouse conditions during 2019 and 2020 growing seasons. The obtained data in Table (5) illustrated that the treated soil with biofungicides significantly reduced the disease severity of white rot, basal rot and pink root rot diseases compared with the control. The effect of biofungicides against *S. cepivorum*, data revealed that all biofungicides exhibited slight decrease in disease severity caused with *S. cepivorum*. However, T34 biocontrol was the most effective ones in

controlling garlic white rot disease, followed by Plant- guard, whereas the disease severity reached 27.9 and 30%, respectively. Whatever the other treatment gave moderate effective compared with the control. At the same time, Rizo- N followed by T-34 and Plant-guard were the most effective in reducing basal rot caused with *F. oxysporum* f.sp. *cepae* as compared with Bio-Arc and Biozeid as well as the control, whereas the disease severity was 15.3, 19.9 and 21.9 %, respectively. Concerning the tested biofungicides decreased the disease severity of pink root rot disease compared with the check treatment. In this respect, all the tested biofungicides gave promising results in controlling pink rot caused with *P. terrestris*. In addition, the tested biofungicides Rizo- N, followed by T-34 and Plant-guard gave the best results in controlling *P. terrestris,* whereas the disease severity was 11.9, 18.3 and 21.3%, respectively. While, Bio-Arc and Biozeid gave last effect in controlling the disease. The efficacy of certain biofungicides was observed in case of infested soil with *P. terrestris* and *F. oxysporum* f.sp. *cepae*, however, Rhizo-N and T34 were effective biofungicides in controlling garlic pink root rot and basal rot diseases, which showed clear effect in decreasing disease severity.

Table 5: Effect of different biofungicides on controlling garlic white rot, basal rot and pink root rot diseases under greenhouse conditions during 2019 and 2020 growing seasons.

Biofungicides	Disease severity (%)								
	S. cepivorum (Sc5)			*F. oxysporum* f.sp. *cepae* (F7)			*P. terrestris* (P3)		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Bio-Arc	36	38.6	37.3	28	32	30	25.3	24	24.6
Plant-guard	28	32	30	21.3	22.6	21.9	20	22.6	21.3
T-34 Biocontrol	29.3	26.6	27.9	18.6	21.3	19.9	18.6	20	18.3
Biozeid	37.3	40	38.6	33.3	29.3	31.3	32	34.6	33.3
Rhizo-N	34.6	37.3	35.9	13.3	17.3	15.3	10.6	13.3	11.9
Control	81.3	85.3	83.3	73.3	74.6	73.9	70.6	73.3	71.9
LSD at 5%	3.75	4.44		5.56	3.75		5.30	3.36	

These findings are in agreement with those previously obtained by Salama et al. (2008) and El-Naggar et al. (2018). Biofungicides may be microorganism such as bacteria, fungi or yeasts based on product like secondary metabolite. Biocontrol bacteria such as Rhizo-N and Bio-Arc suppressed the different growth by the producing secondary metabolites like antibiotic, cell wall degrading enzymes and hydrogen cyanide (Bakker and Schippers, 1987). Multiple mechanisms seem to be used by *Bacillus* spp. in the biocontrol such as: (i) activate the defense mechanisms of plant (ii) competition for iron through production of siderophores (iii) competition to establish an ecological site and metabolize root exudates (iiii) degradation of pathogenicity factors such as toxins (Castillo et al., 2013). Also, many studies showed that *Trichoderma* spp. have antagonistic effect against a wide range of the soil borne pathogens. The inhibitory effect of Plant Guard is related to the antagonistic action exerted by *T. harzianum*. No single mechanism of how *T. harzianum* is able to inhibit the growth of fungal plant pathogen is known. The competition, antibiosis and

mycoparasitsm are all important depending on which plant-pathogen situation is considered (Chet, 1987). Metabolites produced by *T. harzianum* may also play a role in mycoparasitism of the hyphae or the sclerotia produced by *S. cepivorum*. The mycoparasitism and penetration may be followed by the release of antibiotics that permeate the perforated hyphae and prevent resynthesis of the host cell wall (Lorito et al., 1996). In addition, *T. asperellum* has been recently shown to induce systemic resistance in plants through a mechanism that employs jasmonic acid and ethylene signal-transduction pathways (Zlata, 2008).

3.4.2 Effect of commercial biofertilizers on controlling garlic white rot, basal rot and pink root rot diseases under greenhouse conditions

The treated soil with different biofertilizers Nitrobien, Phosphoren, Biogen, Potassiumag and Carialin, as well as treated plants with Ascobein as foliar spraying were evaluated for their effectiveness to control pink root rot, basal rot and white rot diseases of Sides40 garlic cv. under greenhouse conditions during 2019 and 2020 growing seasons. It was clear from data presented in Table (6) that Phosphoren has significantly decreased pink root rot disease incidence (18.6%), followed by Nitrobien (19.9%) and Biogen (35.3%) compared with the control (71.9%). While, treated plants with Ascobien as foliar spraying gave the highest percentage of pink root rot disease. Nitrobien followed by Phosphoren were the most effective in reducing basal rot caused with *F. oxysporum* f.sp. *cepae* if compared with other treatments and also with the control. Whereas the disease severity was 20.6 and 30 %, respectively. In this respect Ascobien and Biogen gave the lowest effect in controlling basal rot disease. Data also revealed that all biofertilizers exhibited slight decrease of disease severity caused with *S. cepivorum*. However, Nitrobien was the most effective ones in controlling garlic white rot disease, followed by Phosphoren, whereas the disease severity reached 35.3 and 36.6%, respectively. While, the other treatment gave the lowest effect in controlling white rot disease caused with *S. cepivorum.*

Table 6: Effect of different biofertilizers on controlling garlic white rot, basal rot and pink root rot diseases under greenhouse conditions during 2019 and 2020 growing seasons.

Biofertilizers	Disease severity (%)								
	P. terrestris (P3)			*F. oxysporum* f.sp. *cepae* (F7)			*S. cepivorum* (Sc5)		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Nitrobien	18.6	21.3	19.9	18.6	22.6	20.6	34.6	36	35.3
Phosphoren	28	29.3	18.6	28	32	30	36	37.3	36.6
Biogen	33.3	37.3	35.3	38.6	42	40.3	45.3	48	46.6
Potassiumag	34.6	37.3	35.9	29.3	33.3	31.3	38.6	40	39.3
Sarialein	36	40	38	32	34.6	33.3	42.6	41.3	41.9
Ascobein	41.3	45.3	43.3	40	41.3	40.6	52	54.6	53.3
Control	70.6	73.3	71.9	73.3	74.6	73.9	81.3	85.3	83.3
LSD at 5%	4.32	4.58	-	5.51	4.58	-	3.74	3.05	-

Generally, Nitrobien followed by Phosphoren gave the best results in controlling pink root rot, basal rot and white rot diseases in both seasons. These

results are in line with those recorded by El-Naggar et al. (2018). Bio-fertilization was recently recommended to be effective mean controlling soil-borne fungal diseases on the ornamental plants as reported by Abo El-Ela (2003) who mentioned that, the benefit of bio-fertilization might due to its cumulative effects such as supplying the plant with nitrogen in addition to growth promoting substances produced by microorganisms. Dhir (2017) stated that, *Azotobacter brasilensis* and *A. chroococcum* were very effective against the infestation with *R.solani* and *F. oxysporum*. This effect was attributed to the decrease of population density in the Rhizosphere. Also, Brown (2012) observed that *Azotobacter* besides the N-fixation was able to produce growth substances and fungal antibiotics, and the response of the crops to the inoculation could be attributed to the substances produced by the organisms. Also, Chung and Wu (2000) recorded the efficiency of *Bacillus megaterium* var. *phosphaaticum* to control root-rot caused by *R.solani* also, Potassiumag containing *Bacillus cerculanes* was suppressive compared with the control. These findings could be interpreted in light that *Bacillus* increase the plant P uptake, water status inside the plant tissues and hence increases the plant amino acids and activate its rates and enhance the action of succinic and lactic acids which induce the root growth.

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