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The Ability of Some Antagonistic Bacteria on Control of Onion Pink Root Rot Compared to Fungicide Efficiency

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ABSTRACT

Biological control considered one of the alternatives to fungicides, which have raised serious concerns of food contamination and environmental pollution, beside it is eco-friendly, safe and may provide long-term protection to the crop and this due to its ability to suppress the activities and populations of the plant pathogens. In line with this trend, thirteen bacterial isolates were used to study their antagonistic effects on onion pink root rot caused by *Setophoma* (*Pyrenochaeta*) *terrestris*. *In vitro* evaluation only eight isolates caused moderate to strong inhibition to the three tested isolates of *Setophoma terrestris*. *Bacillus subtilis* (Bs.2) gave the high significant antagonistic effect against the three tested isolates followed by *Bacillus subtilis* (Bs.1), and *Pseudomonas fluorescens* (Pf.5). In greenhouse and field experiments, the most effective isolates in reducing pink root rot disease were *Bacillus subtilis* (Bs.2) followed by *Bacillus subtilis* (Bs1) and *Pseudomonas fluorescens* (Pf.5). Also the highest onion bulb yield in the two seasons (2018/2019 and 2019/2020) were obtained by *Bacillus subtilis* (Bs2) followed by *Bacillus subtilis* (Bs.1). The results confirmed the ability of some tested bioagents to be near to the efficacy of commercial fungicide in reducing onion pink root rot disease. In this respect, in greenhouse and field trials *Bacillus subtilis* (Bs2) effect was the nearest one to fungicide effect in minimizing of pink root rot and also superior to the biocide treatment followed by *Bacillus subtilis* (Bs1) in the two seasons (2018/2019 and 2019/2020). The presented study confirmed the ability of some tested bioaegents to be alternative to the fungicides in reducing onion pink root rot diseases as well as increasing the onion bulb yield.

Keywords: *Setophoma* (*Pyrenochaeta*) *terrestris*, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Brevibacterium carei*, *Bacillus amyloliquefcciens*, bioagent, Biological control, and Fungicides efficiency

1. Introduction

Onion (*Allium cepa* L.) is a biennial crop plant which plays a key role in human life both as food or a cash crop. Onions are grown at least in 175 countries and are used to some extent as medicinal plants; and rank commercially second after tomatoes in world production (Patel and Patel, 2013). Egypt is one of the main onion producing countries with a cultivated area of 173498 fed. and a production of 2509322 ton as mentioned by the yearly book 2019 of Economics and Statistics of the Economic Affairs Sectors, Agriculture Ministry in Egypt. However, it is susceptible to many diseases, including pink root rot.

Pink root rot disease caused by *Setophoma* (*Pyrenochaeta*) *terrestris* Hansen is one of the most devastating diseases of onions grown in warm environmental conditions. Plants infected by *S. terrestris* appear stunted as a result of the damaged root system, leading to significant losses of yield and marketable quality of onions (Sumner, 1995). The causal organism is soil-borne and remains viable in the soil for many years (Ahmed *et al.,* 1991 and Kim *et al.,* 2003).

Because the negative effects of synthetic chemicals compounds (fungicides) on the environment and human health the biological comes as alternatives for plant disease control, beside that in organic

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agriculture, there are restrictions on the use of more pesticides. Therefore, the application of biological control in the prevention of phytopathogen infection has gradually gained attention because it is environment-friendly (Labuschagne, 2010, Alvarez *et al.,* 2019 and Zhansheng *et al.,* 2019). Bacteria, especially plant growth-promoting rhizobacteria (PGPR), can suppress a variety of root and vascular disease caused by soil-borne pathogens. *Bacillus, Pseudomonas* and *Brevibacterium* were considered as important genera of these bacteria (Meena *et al.*, 2001, Collins, 2006, Faisal and Hasnain, 2006 Ibrahim *et al.,* 2008 and Mishra *et al.,* 2013).

Bacillus subtilis one from the most commonly bacteria that used in the biocontrol which have many of direct and indirect mechanisms include nutrient competition, and broad range of antibiosis (Khan *et al.*, 2018; Abbas *et al*., 2019). Many researches showed ability of *B. subtilis* in control a variety of root and vascular disease caused by soil-borne pathogens fungi caused by *Fusarium* spp, *Rhizoctonia solani* and *Macrophomina phaseolina* (Mishra *et al.,* 2013, Wang *et al*., 2018 and Khan *et al*., 2018).

The study conducted by Albarracín *et al.,* 2016 showed that, the isolates bacteria of *Bacillus subtilis* ALBA01 a strain isolated from the rhizosphere of onion is capable of strongly inhibit the growth of *S. terrestris in vitro*. Moreover, the antifungal inhibition activity is by secreting extracellular diffusible factors. It is noteworthy that this behavior appeared to be specifically provoked by the interaction with *S. terrestris* since no antagonistic activity from *Bacillus subtilis* ALBA01 against two other onion pathogens, *Fusarium oxysporum* and *F. proliferatum.* In another study onion plants (susceptible onion cultivar) which inoculated with *Bacillus subtilis* BsA01 to evaluated their biocontrol effect against *S. terrestris*, under greenhouse and field conditions when compared with plants inoculated only with *S. terrestris* showed significantly reducing of pink root rot and biocontrol levels was above 50% as well as led to improve plant growth (Sayago *et al.,* 2020).

Pseudomonas fluorescens is considered as an important antagonistic bacterium where it was effective against several soil-borne pathogens in field and greenhouse (Karunanithi *et al.,* 2000 and Jayashree *et al.,* 2000). While on onion *Pseudomonas* sp. (Pf 12) gave a highest reduction of mycelial growth of *F. oxysporum* f. *sp. cepae* (75%) which caused basal rots (Malathi, 2015).

Antifungal activity of *P. fluorescens*, due to some mechanisms include production of antibiotics, hydrogen cyanide (HCN), competition for iron mediated by siderophores, competition for carbon, and induced systemic resistance (Weller *et al.* 2012). Moreover, certain strains of *Pseudomonas* can produce several siderophores such as pyoverdine (pseudobactin) pyochelin, pyrollnitrin (antibiotic), salicylic acid, and lytic enzymes (Bhimeshwari *et al.,* 2018).

This work was carried out to study the effect of some bacterial isolates in reducing of onion pink root rot disease under greenhouse and field conditions.

2. Materials and Methods

2.1. The fungal isolates

Isolates of *Setophoma terrestris* were obtained from Beheira (El-Nobaria), Sharkia and Fayoum Governorates. The fungus was isolated from field-grown onion roots with pink-root symptoms. Infected onion plants with prominent pink-root disease symptoms were selected and their roots were first washed in running tap water for 20 min, then cut into small pieces (0.5–1cm), soaked in 0.5% sodium hypochlorite (10% Chlorox) for surface sterilization, rewashed in sterilized water and dried between two pieces of sterilized filter paper. Three root pieces were inoculated equidistantly, in each Petri dish containing potato Dextrose Agar medium (PDA). The inoculated plates were incubated at 24ºC for 4 to 7 days.Watson, (1961) technique was used for identification of *S. terrestris*.

2.2. Preparation of fungal inoculum:

The Inoculum of *S. terrestris* was prepared using sorghum - coarse sand- water (2:1:2 v/v) medium. The ingredients were mixed, bottled and autoclaved at 121˚C for 60 minutes. Each bottle containing sterilized medium was inoculated with 5 mm fungal growth disc obtained from the periphery of 7-day-old culture of the fungus. The inoculated media were incubated at 24°C for 25 days before used for soil infestation.

2.3. Soil infestation:

Fungal propagules of *S. terrestris* (Nobaria isolate) were mixed thoroughly with the surface soil of each pot, at the rate of 0.1% w/w, and then covered with a thin layer of sterilized soil. Pots containing infested soil were irrigated and kept for 10 days until sown for establishment of fungal inoculum in the soil.

2.4. Disease assessment:

a) The number of plants having typical pink rot symptoms was counted after two and four month from planting and their percentage were calculated according to Hovius and Goldman (2004) as follows:

$$\%\ Pink\ root\ rot = \frac{Number\ of\ plants\ infested\ with\ pink\ rot}{Total\ No.\ of\ plants} \times 100$$

$$\%\ Healthy\ plants = \frac{Number\ of\ survived\ healthy\ plants}{Total\ No.\ of\ plants} \times 100$$

b) Percent efficacy of treatment in reducing the diseases infection was calculated.

$$\%\ Treatment\ efficiency = \frac{Control - Treatment}{Control} \times 100$$

$$\%\ Bioagents\ efficiency\ to\ fungicides\ efficacy = \frac{Bioagents\ efficiency}{Fungicides\ efficiency} \times 100$$

2.5. Source of antagonistic bacteria:

Thirteen known bacterial isolates including five isolates of *Pseudomonas fluorescens*; (Pf1 & Pf2 & Pf3 & Pf4 & Pf5), one isolate of *Pseudomonas. putida*; PP, three isolates of *Bacillus. subtilis* (Bs1&Bs2&Bs3), two isolates of *Brevibacterium carei* (Bc1 & Bc2) and two isolates of *Bacillus amyloliquefcciens* (Ba1&Ba2) were obtained from cculture ccollection of Department of Onion, Garlic and Oil Crops Diseases, Plant Pathol. Res. Inst., Agric. Res. Center (ARC), Giza, Egypt and were identified by using Biolog technique (Carbon and amino acid utilization profile of microorganisms) Biolog TM micro-plates (Biolog, Inc., 3938 Trust way, Hayward, CA94545, USA) at the Unit of Identification of Microorganisms Plant Pathol. Res. Inst., Agric. Res. Center (ARC), Giza, Egypt (Mahmoud, 2018).

2.6. Evaluation of antagonists *in vitro*:

All bacterial isolates were tested by streaking the bacteria in the center of culture plate containing PDA medium, and then incubated for 48 hours at 25°C. Plates were inoculated with the pathogen isolates by placing two 5-mm-disks, from five days old culture, 3 cm. apart from both sides of bacterial growth. Plates were incubated at 24 °C for 5 days and fungal colony diameter in the presence or the absence of bacteria was measured. The inhibition zone between bacteria and the pathogen was measured as described by Maurhofer *et al.,* (1995).

2.7. Preparation of bacterial inoculum:-

Bacterial suspensions (1 x 10^6 cfu / ml) were prepared by dilution plate assay as described by **Callan *et al.,* (1990).**

2.8. Methods of application:

The antagonistic bacteria isolates (10^6 cfu / ml) were applied as transplants treatment by soaking for 2 hours before transplanting in a suspension of bio-agent plus 0.1 % gum Arabic then onion transplants planted in pots and field. While fungicide Topsin M (Thiophanate-methyl WP 70%) were applied as dipping of onion transplants at the rate of 1g/L of water and the commercial biocides Rhizo-

N (*Bacillus subtillis* 3 x 10^6 c.f.u/ml) at the rate 5g/L of water. Also, all treatments were applied as soil drench at the 7 and 15 days from transplanting.

2.9. Greenhouse experiments

Pots (50 cm-diam) were sterilized by 5.0% formalin solution for 15 minutes, left to dry for two days to get rid of formalin residues, then filled with soil previously sterilized by formalin solution (5.0%) for 15 days and left for 15 days. Fungal propagules (Nobaria isolate) were added to the potted soil as mentioned before.

Apparently healthy onion transplants of Giza 20 cultivar were soaked for 2 hours in bacterial suspensions, fungicides and commercial biocide as mentioned before, then planted rate of 10 transplants per pot. Also, were applied as a soil drench at the 7 and 15 days from planting (100 ml/pot). Experiment was replicated for four times. Disease assessment was recorded as previously mentioned. Also, fresh weight of onion plants from each pot of different treatments were recorded directly after harvest as g/pot and dried in an oven at about 70°C for two days to obtain the dry weights.

2.10. Field experiments:

Field experiments were carried out during onion sowing seasons, 2018/2019 and 2019/2020 under naturally infested soil with pink rot root pathogen, at El-Nobaria, Behira Governorate Egypt.. Randomized complete block design with four replicates was used and the plot was 3.0 x 3.5 m^2 (10.5 m^2 = 1/400 fed.). Each plot included 6 rows (each 3.0 m length and 50 cm width). Sixty day-old transplants of onion cultivar Giza 20 were dipped for 2 hours in in bacterial suspensions, fungicides and biocides as mentioned before, and then planted per each plot at the recommended spacing 10 cm x 10 cm, within each row. Also, all treatments were applied as a soil drench at the 7 and 15 days from planting. Onion transplants were planted on the first week of December, approximately 90 plants/ row. The recommended agricultural practices and irrigation for onion crop were followed. The experiment was arranged in completely randomized block design with four replicates. Disease assessment recorded as previously mentioned and onion bulb yield was estimated as Kg/plot

2.11. Statistical analysis

The data were statistically analyzed by analysis of variance (ANOVA) using the Statistical Analysis software "COStat 6.4" (CoStat, 2005). Means were separated by the Duncan test at $P \leq 0.05$.

3. Results

3.1. Screening of bacterial antagonists, *in vitro*:

Thirteen known isolates of *P. fluorescens*; (Pf1&Pf2&Pf3&Pf4&Pf5) *P. putida*; PP, *B. subtilis* (Bs1&Bs2&Bs3), *Brevibacterium carei* (Bc1&Bc2) and *Bacillus amyloliquefcciens* (Ba1&Ba2) were evaluated *in vitro* for their antagonistic effect against three isolates of *S. terrestris* on PDA medium (Table 1). Only eight isolates caused moderate to strong inhibition to the three tested isolates.

Table 1: Antagonistic effect of various bacterial isolates against three tested isolates of *S. terrestris*.

Bacterial isolates	***S. terrestris* isolates**		
	Sharkia	**Fayoum**	**Nobaria**
***P. fluorescens* (Pf. 1)**	30 e	31 c	22 c
***P. fluorescens* (Pf. 2)**	22 f	3 m	0 g
***P. fluorescens* (Pf. 3)**	15 k	5 l	0 g
***P. fluorescens* (Pf.4)**	18 j	8 k	0 g
***P. fluorescens* (Pf. 5)**	32 c	32 d	23 c
***P.Putida* (PP)**	30 d	29 e	21cd
***B. subtilis* (Bs.1)**	32 b	33 b	25 b
***B. subtilis* (Bs.2)**	34 a	38 a	28 a
***B. subtilis* (Bs.3)**	21g	23 f	20 d
***Brevibacterium carei* (Bc1)**	20 i	18 h	16 f
***Brevibacterium carei* (Bc2)**	10 m	8 j	0 g
***Bacillus amyloliquefcciens* (Ba.1)**	12 l	13 i	0 g
***Bacillus amyloliquefcciens* (Ba.2)**	20 h	20 g	18 e

B. subtilis (Bs.2) gave the high significant antagonistic effect against the three tested isolates on PDA medium followed by *B. subtilis* (Bs.1) and *P. fluorescens* (Pf.5) then, *P. fluorescens* (Pf.1) and *P. putida* (PP) (Table 1). Meanwhile *B. subtilis* (Bs.3), *B. amyloliquefcciens* (Ba.2) and *Brevibacterium carei* (Bc1) gave moderate effect in their inhibition of three tested isolates growth. While *P. fluorescens* (Pf2 & Pf3 & Pf4), *Brevibacterium carei* (Bc2) and *B. amyloliquefcciens* (Ba.1) do not showed obvious antagonistic effect against all tested isolates specially Nobaria isolate.

3.2. Evaluation of antagonistic bacteria under soil infestation with *S. terrestris*:

3.2.1. On onion pink root rot incidence:-

Eight selected bacterial isolates beside check standard consisting of Rhizo-N (biocide) and Topsin M (fungicide) were evaluated under soil infestation with *S. terrestris* (Table 2).

Results in Table (2) show that, all tested bioagents have significant effect in reducing onion pink root rot compared to control. In this respect, the most effective isolates were *B. subtilis* (Bs.2) followed by *B. subtilis* (Bs.1) and *P. fluorescens* (Pf.5) which gave the highest effect in reducing onion pink root rot compared to other tested bioagents. While, *B. amyloliquefcciens* (Ba.2) isolate gave the lowest effect in reducing onion pink root rot disease compared with the other tested bioagents.

On the other hand, *B. subtilis* (Bs.2) was the nearest one to fungicide efficacy (Topsin M) in reduction of onion root rot (83.33%); it was also superior to the biocide treatment (Rhizo-N), followed by the *B. subtilis* (Bs.2), which was equal to the biocide treatment in its efficiency (75%) for the fungicide efficacy (Topsin M) followed by *P. fluorescens* (Pf. 5) (Table 2).

Table 2: Effect of antagonistic bacterial isolates on onion pink root rot incidence (%) under soil infestation with *S. terrestris*.

Bacterial isolates	Infection (%)	Survival (%)	Efficacy (%)	Efficacy to fungicides
***P. fluorescens* (Pf. 1)**	32.50 ef	67.50 de	35.0	58.33
***P. fluorescens* (Pf. 5)**	30.00 fg	70.00 cd	40.0	66.67
***P.putida* (PP)**	35.00 de	65.00 e	30.0	50.00
***B. subtilis* (Bs.1)**	27.50 gh	72.50 bc	45.0	75.00
***B. subtilis* (Bs.2)**	25.00 h	75.00 b	50.0	83.33
***B. subtilis* (Bs.3)**	37.50 cd	62.50 fg	25.0	41.67
***B. carei* (Bc1)**	40.00 c	60.00 g	20.0	33.33
***B. amyloliquefcciens* (Ba.2)**	45.00 b	55.00 h	10.0	16.67
Rhizo-N	27.50 gh	72.50 bc	45.0	75.00
Topsin M	20.00 i	80.00 a	60.0	100.00
Control (without bacteria)	50.00 a	50.00 i	0.0	0.00

3.2.2. On onion fresh and dry weight:

Results illustrated in Table (3) revealed that, all tested bioagents significantly increased onion fresh and dry weight except with *B. amyloliquefcciens* in Fresh weight compared to untreated control. In this respect, the highest values of onion fresh and dry weight were recorded by *B. subtilis* (Bs.2) followed by *B. subtilis* (Bs.1) and *B. subtilis* (Bs.3) while, *B. amyloliquefcciens* (Ba.2) gives the lowest value compared to all tested bioagents and untreated control.

However, compared to check standard Rhizo-N (biocide) and Topsin M (fungicide) data also showed that, *B. subtilis* (Bs.2), *B. subtilis* (Bs.1), *B. subtilis* (Bs.3) and *P. fluorescens* (Pf.5) superior on Rhizo-N in fresh and dry weight values moreover *B. subtilis* (Bs.2), didn't gave significant differences with Topsin M in their effect on onion fresh weight (Table 3).

4. Evaluation of antagonistic bacteria under field conditions:

4.1. On onion pink root rot incidence:-

Six selected bacterial isolates beside standard consisting Rhizo-N (biocide) and Topsin M (fungicide) were evaluated under field conditions during two successive seasons (2018/2019 and 2019/2020). Data in Table (4) indicate that, all tested bioagents have significant effect in reducing of onion pink root rot compared with the control during the two successive seasons. *B. subtilis* (Bs.2) followed by *B. subtilis* (Bs.1) and *P. fluorescens* (Pf.5) were the most effective isolates in reducing onion pink root rot during the two seasons 2018/2019 and 2019/2020 (Table 4).

In the same time the effect of *B. subtilis* (Bs.2) was the nearest one to fungicide effect in reduction of onion pink root rot, followed by *B. subtilis* (Bs.1) and *B. subtills* (Bs1) compared to the other tested bioagents, and also superior to the biocide treatment (Rhizo-N) during the two successive seasons 2018/2019 and 2019/2020 (Table 4).

Table 3: Impact of antagonistic bacterial isolates on onion fresh and dry weight under soil infestation with *S. terrestris*.

Bacterial isolates	Fresh (weight /g)	Increase (%)	Dry (weight /g)	Increase (%)
P. fluorescens **(Pf. 1)**	168.82 f	49.41	132.52 g	58.51
P. fluorescens **(Pf. 5)**	176.27 d	56.00	138.44 e	65.60
P.putida **(PP)**	165.13 g	46.15	131.83 h	57.69
B. subtilis **(Bs.1)**	181.50 b	60.64	145.20 c	73.69
B. subtilis **(Bs.2)**	186.55 a	65.10	149.92 b	79.33
B. subtilis **(Bs.3)**	179.17 c	58.57	141.64 d	69.43
B. carei **(Bc1)**	156.57 h	38.57	128.83 i	54.11
B. amyloliquefcciens **(Ba.2)**	138.64 i	22.70	122.42 j	46.43
Rhizo-N	172.34 e	52.53	134.88 f	61.34
Topsin M	187.59 a	66.02	151.30 a	80.98
Control (without bacteria)	112.99 i	0.00	83.60 k	0.00

Table 4: Influence of antagonistic bacterial isolates on onion pink root rot incidence (%) under field conditions, during 2018/2019 and 2019/2020 seasons.

Bacterial isolates	Season 2018/2019			
	Infection %	**Survival %**	**Efficacy %**	**Efficacy to fungicides**
P. fluorescens **(Pf. 1)**	30.55cd	69.45ef	34.02	57.48
P. fluorescens **(Pf. 5)**	28.85de	71.15de	37.69	63.69
P.putida **(PP)**	33.65bc	66.35fg	27.32	46.17
B. subtilis **(Bs.1)**	24.35fg	75.65bc	47.41	80.11
B. subtilis **(Bs.2)**	22.31g	77.69b	51.81	87.54
B. subtilis **(Bs.3)**	34.86b	65.14g	24.70	41.74
Rhizo-N	25.90fe	74.10cd	44.06	74.45
Topsin M	18.90h	81.10a	59.18	100.00
Control	46.30a	53.70h	0.00	0.00
	Season 2019/2020			
P. fluorescens **(Pf. 1)**	28.00c	72.00d	36.87	60.34
P. fluorescens **(Pf. 5)**	26.90c	73.10d	39.35	64.40
P.putida **(PP)**	31.60b	68.40e	28.75	47.05
B. subtilis **(Bs.1)**	23.80d	76.20c	45.89	75.10
B. subtilis **(Bs.2)**	19.96e	80.04b	54.99	90.00
B. subtilis **(Bs.3)**	32.36b	67.64e	27.03	44.24
Rhizo-N	24.00d	76.00c	46.11	75.47
Topsin M	17.25f	82.75a	61.10	100.00
Control	44.35a	55.65f	0.00	0.00

4.2. On onion bulb yield under field conditions:

Data presented in Table (5) demonstrate that, all tested bioagents caused significant increases in onion bulb than the control. Percentage of increases however reached (8.29-50.60) and (8.34-52.43) in the first and second seasons for bioagents treatments respectively. The highest onion bulb yield in the two seasons obtained with *B. subtills* (Bs.2), followed by *P. fluorescens* (Pf.5) and *B. subtills* (Bs.1). While *P. fluorescens* (Pf.1) gave the lowest onion bulb yield and the lowest effect on increase yield in the two successive seasons 2018/2019 and 2019/2020 compared with the other bioagents.

On the other hand, the effect of *B. subtilis* (Bs.2) was the nearest one to fungicide effect in increase of onion bulb yield compared to other tested bioagents, and also superior to the biocide treatment (Rhizo-N) during the two successive seasons 2018/2019 and 2019/2020 (Table 5).

Table 5: Effect of antagonistic bacterial isolates on onion bulb yield and loss of yield under field conditions during two seasons 2018/2019 and 2019/2020.

Bacterial isolates	Season 2018/2019		Season 2019/2020	
	Yield (Kg/plot)	*Increases (%)	Yield (Kg/plot)	*Increases (%)
***P. fluorescens* (Pf. 1)**	18.14 g	10.25	20.11 g	8.88
***P. fluorescens* (Pf. 5)**	21.43 d	30.25	23.39 d	26.62
***P.putida* (PP)**	18.79 f	14.23	21.60 f	16.95
***B. subtilis* (Bs.1)**	20.40 e	24.02	22.25 e	20.44
***B. subtilis* (Bs.2)**	24.77 b	50.60	28.15 b	52.43
***B. subtilis* (Bs.3)**	17.81 h	8.29	20.01 g	8.34
Rhizo-N	22.99 c	39.74	25.92 c	40.33
Topsin M	28.55 a	73.56	32.18 a	74.21
Control	16.45 i	---	18.47 h	--

4. Discussion

Chemical control is not technically or economically feasible mainly due to the risk of degradation of the products in the soil, the potential presence of residuals over edible parts of the plant and environmental. Biocontrol is a reliable alternative to chemical fungicides, which have raised serious concerns of food contamination and environmental pollution.The presented study, indicated that, Most of all tested bacterial bioagents have significant effect in reducing onion pink root rot disease. In this respect the most effective isolates were *Bacillus subtilis* (Bs2 & Bs1) followed by *Pseudomona fluorescens* (Pf5). This result are in agreement with Mishra *et al.,* (2013), Wang *et al*., (2018), and Khan *et al*., (2018), who stated that, certain strains of *Bacillus* appear to be most effective as a biological control agent through inhibiting the mycelial growth of plant pathogenic fungi. Also *P. fluorescens* was found to be the most effective bio-control agent against various soil-borne diseases caused by *F. oxysporum, R. solani, P. ultimum, M. phaseolina* and others (Jayashree *et al.,* 2000, Hussien, 2011 and Malathi, 2015). Mahmoud *et al.,* (2006) and Ibrahim *et al.,* (2008) found that, *B. subtilis* (BS1) and *P. fluorescens* (Pf5), caused strong inhibition on the mycelium growths of the four tested pathogens (*R. solani, S. rolfsii, F. solani* and *M. phaseolina*).

Many studies in this respect showed that certain *B. subtilis* and *P. fluorescens* isolates were effective rhizobacteria for inhibition to fungi hyphal growths *in vitro.* This suggested that their biocontrol activity had been associated with the production of certain substance such as enzymes, phenazines, pyrrole type antibiotics, pyo-compounds, indole derivatives peptide antibiotic, moenomycins, difficidins, bacillomycins and bacillaenes as reported by Battu1 and Reddy, (2009) and Awais *et al.,* (2010).

The ability of antagonistic isolates to inhibit growth of soil-borne pathogens, *in vitro* and to produce certain secondary metabolites has been claimed to be important for biological control (Defago and Hass 1990 and Maurhofer *et al.,* 1995). Antibiosis is well documented for *P. fluorescens* (Pf5) against soil borne pathogens (Howell and Stipanovic 1979). Moreover, certain strains of *Pseudomonas* can produce several siderophores such as pyoverdine (pseudobactin) pyochelin, pyrollnitrin (antibiotic), salicylic acid, HCN and lytic enzymes (Leeman *et al.,* 1996; De Meyer and Hofte, 1997; Karunanithi *et al.,* 2000, and Meena *et al*., 2001). Moreover, *P. fluorescens* strain (Pf1) showed the maximum of antagonistic effect by produced *in vitro* HCN, salicylic acid siderophore and beta–1,3 gluconase (Meena *et al*., 2001, Shanmugam *et al*., 2002 & 2003).

On the other hand *B. subtilis* can induce resistance by stimulation of phytoalexins production and increasing the activity of lytic enzymes (Sailaja *et al.,* 1998). Also, Albarracín *et al.,* 2016 showed that, the isolates bacteria of *Bacillus subtilis* (strain ALBA01) which isolated from the rhizosphere of onion is capable of strongly inhibit the growth of *S. terrestris in vitro* by inhibition their activity through secreting extracellular diffusible factors. In another study onion plants (susceptible onion cultivar) which inoculated with the same isolates (strain ALBA01) showed significantly reducing of pink root rot and as well as led to improve onion plant growth under greenhouse and field conditions (Sayago *et al.,* 2020).

The presented study confirmed the ability of some tested bioaegents to be near the fungicides efficiency in reducing onion pink root rot disease as will as increasing the onion bulb yield.

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