SVU-International Journal of Agricultural Sciences
Volume 6 Issue (4) pp.: 12-23, 2024
**Print ISSN 2636-3801 | Online ISSN 2636-381X**
Doi: 10.21608/svuijas.2024.319563.1393
**RESEARCH ARTICLE**

# Biological control of garlic white and basal rot pathogens *in vitro* and under field conditions

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## Abstract

Garlic white and basal rot are two types of soil-borne diseases fungi harmful to the cultivation of *Allium sativum* in all over the world and Egypt, caused by *Sclerotium cepivorum*, *Fusarium oxysporum* f. *Sp. cepae*, and *Fusarium solani*. The effective of *Bacillus subtilis*, *Penicillium janthinellum* bioagents fungi and bacteria isolated from healthy garlic plants samples compared with Tebuconazole fungicide *in* vitro and under field conditions for their ability to inhibit the garlic white and basal rot pathogens radial growth and diseases severity were tested using two varieties of garlic named Baladi and Sids 40. The results showed that *Bacillus subtilis* and *Penicillium janthinellum* had antagonistic effect against the pathogens. Whereas, *Penicillium janthinellum* was the most effective isolated bioagents suppressed the pathogens mycelial growth *in vitro*, followed by *Bacillus subtilis*, while used different concentration of Tebuconazole fungicide affected the pathogens growth *in* vitro as well as showed the highest efficiency of reducing the disease severity under field conditions and significantly increased the length of treated plants

**Keywords:** *Allium sativum; Fusarium oxysporum fsp Cepae; Sclerotium cepivorum; Penicillium janthinellum; Bacillus subtilis*; Tebuconazole.

## 1. Introduction

The world's most important horticultural crop, garlic (*Allium sativum*), is prized for both its culinary and medicinal qualities. It is ranked second in significance among bulb vegetables worldwide, after onions (*Allium cepa*) (Hamma *et al.*, 2013). Due to its lipid-lowering, anti-inflammatory, and antioxidant characteristics, its secondary metabolites have demonstrated outstanding health-promoting and disease-preventing activities (Ansary *et al.*, 2020). Tropical and temperate regions produced more than 28 million tonnes of garlic plants in 2020 (Chrétien *et al.*, 2021). From an economic and social perspective, garlic is a strategically important crop for many locations (Spagnoli, 2014). It is commonly grown in Egypt for both domestic and export markets, with uses ranging from the production of anticancer and antibiotic components to the treatment of respiratory, cardiovascular, and metabolic illnesses (Najda *et al.*, 2016). It is also grown for domestic use as a spice. Garlic plants are susceptible to diseases, which can lead to decreased yields, poor bulb quality, and greater susceptibility to secondary infections like foliar, bulb, and root pathogens (Anum *et al.*, 2024). *Sclerotium cepivorum*, the disease that results in white rot, is one of the most common diseases worldwide, with yield losses reaching 100%. The fungus forms long-lived survival structures called sclerotia, which can

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*Received: September 9, 2024; Accepted: October 6, 2024; Published online: October 12, 2024.*

survive in the soil for more than 20 years without needing a host plant (Ahmed and Ahmed, 2015). According to Cooke *et al.* (2006) and Davis *et al.* (2007), the sclerotia infect onion plants by penetrating them and causing white rot diseases. *Fusarium oxysporum* f. sp. *Cepae* (FOC), a soil-borne fungus, is the cause of basal rot, which accounts for 60% of yield losses in seed and bulb crops globally (Durrant and Dong ,2004; Jones and Dangl, 2006; Chand *et al.*, 2017). Garlic's roots and basal plates are infected by the pathogens, which result in symptoms across the whole plant life cycle, from damping off and delayed seedling emergence to bulb rot during the pre- and post-harvest stages (Durrant and Dong, 2004). Chemical fungicides have been the main means of controlling this disease (Lourenço Jr et al., 2018). Due to differences in the resistance response during the stages of seedling and bulb development, attempts at host resistance breeding of garlic against FOC have shown to be incredibly unsuccessful. In addition, FOC creates Chlamydospores, which are extremely difficult to control since they can linger in the soil for a long time (Durrant and Dong, 2004).

Crop rotation, solarization, fumigation, and chemical fungicides are the most widely used methods for both the prevention and treatment of soil-borne diseases. Fungicide use gave plant diseases acceptable control, but it also carried a risk of accumulating toxic compounds that could harm human health, the environment, and pathogen resistance (Deising *et al.*, 2008).

More effective, alternative, and safer control agents are highly desired. Therefore, the effect of using *Penicillium janthinellum* and *Bacillus subtilis* isolated from healthy garlic plants as biocontrol against of garlic white rot and basal rot pathogens were evaluated in vitro and under field conditions compared with Tebuconazole fungicide.

## 2. Materials and methods

### *2.1. Samples collection, Pathogens isolation and identification*

Diseased garlic plants showing up white rot and basal rot symptoms were gathered from different fields grown in different areas in Luxor and Qena Governorates. According to per Clarkson *et al*. (2002), sclerotia particles were extracted from an infected garlic bulb and mycelia development was removed from diseased garlic. The hyphal tip and single spore methods were used to purify the fungal pathogen colonies (Brown, 1924) The obtained isolates were stored for future research at 5 °C in a refrigerator on PDA slants for microscopical observation and molecular identification.

### *2.2. Isolation of the bioagents*

The isolation of the antagonism bioagents was carried out by collecting healthy roots samples of garlic plants grown in Luxor and Qena governorate. To get rid of the soil particles that stuck to the healthy roots of the garlic plants, tap water was gently used to wash them. After being cleaned, the roots were split into two groups and sliced into tiny pieces. In order to isolate the bacterial bioagent organisms, the first method involved immersing the root pieces in 1% Na-hypochlorite solution for five minutes. After that, the roots were repeatedly washed in sterilised distilled water and transferred to the surface of potato dextrose agar (PDA) amended with rose bengal (0.003%) and streptomycin sulphate (0.01%) in Petri dishes. while the second one was left without antibiotic in order to isolate the bacterial bioagents organisms. Every plate was incubated for five days at 27°C. Individually, the fungi or bacteria that were growing were placed on PDA or Nutrient agar medium (NA), then identified morphological and microscopical as described by Nelson *et al.* (1983) and Leslie and Summerell (2006), and their identity was confirmed through polymerase chain reaction

(PCR) at the Animal Health Research Institute in Dokki, Giza, Egypt.

### *2.3. In vitro antagonistic*

This study was aimed to evaluate the effectiveness of *Penicillium janthinellum* and *Bacillus subtilis* against the mycelial growth of *Sclerotium cepivorum(SC)*, *Fusarium oxysporum (FO)* and *Fusarium solani (FS)*. On PDA, an antagonistic fungus was cultured for seven days at 22–27 °C. One disc (10 mm in diameter) of the antagonist bioagents facing one disc of the pathogen on the PDA medium poured in sterilized Petri dishes. When using bacterial bioagents, one disc of the pathogen should be faced streaked with *Bacillus subtilis* on the other side of PDA surface, keeping them somewhat close to the plate's edge. Growing a single disc of the pathogenic fungus without an antagonistic disc served as the control treatment. There were three copies of each treatment. For seven days, plates of *F. oxysporum*, *F. solanum*, and *S. cepivorum* were incubated at 27 °C and 20 °C, respectively. When the pathogen isolates' growth completely covered the petri dishes surface in the control treatment, the percentage of inhibition was computed using the following equation according to (Tiru *et al.*, 2013) as follows: I %=[(C2-C1) / C2] × 100 with C2: Mean diameter of the control colony and C1: Mean pathogen colony diameter in the presence of the antagonistic bioagents. The experiment was repeated twice.

### *2.4. Antagonistic activity of Tebuconazole*

The liquefied autoclaving PDA medium was mixed with the tested fungicide (Tebuconazole) to create concentrations of 0.5, 1, and 1.5/100ml. Poured into sterile Petri dishes, untreated PDA was used as a control. Each isolate was replicated three times, and 10 mm discs from a 7-day-old culture of the pathogens were used to inoculate at plates center (Kay and Stewart, 1994). Incubation of the plates took place at 27 ºC. In comparison to the untreated control. Colony diameters were measured at two perpendicular points and the mean was determined. The mycelial growth inhibition percentage was calculated according to (Tiru *et al.*, 2013) as follows: R %=[(C2-C1) / C2] × 100 with C2: Mean diameter of the control colony and C1: Mean pathogen colony diameter in the presence of the tested compound. The experiment was repeated twice.

### *2.5. In vivo experiment*

These experiments were carried out during 2019/2020 and 2020/2021 growing seasons at the experimental farm of faculty of agriculture, South valley university, Qena, Egypt, to compare the effect of biological control agents and chemical control to protect garlic plants from the infection with F. *oxysporum*, *F. solani* and *Sclerotium cepivorum* in Baladi and Sides40 variety under field conditions. The field experiment consisted of 12 Plots. Each plot consisted of 2 rows. Plot size: 13 m² (3 m × 4 m Width + 1 m² for the control) Garlic cloves were seeded in replicates of 10 in each row. Completely random block designs were used for the experiment. Cultivar garlic was planted by late November. The following method was used to prepare the inoculum for the two isolates of *F. solani* (FS) and *F. oxysporum* (FO) as well as the one isolate of *S. cepivorum* (SC)Ten discs of each pathogen were grown on autoclaved 250 ml flasks containing 200 g grain sorghum, and the inoculum was left in place for 14 days. Next, at a rate of 50 g per plant add to the sterilized soil.

#### *2.5.1. The antagonistic activity of Penecillium janthinellum*

The inoculum of *Penecillium janthinellum* bioagent was growing on grain sorghum medium as previously described. At a rate of 50 g/hole was added in the same day inoculated with the pathogens. Ninety days after planting, the disease's severity was noted.

#### *2.5.2. The antagonistic activity of Bacillus subtilis*

Bacterial strain was grown on nutrient agar then transferred to 250 ml flasks containing nutrient broth and grown aerobically in flasks on a rotary

shaker (95 rpm) for 48 hr at 30°C. The bacterial suspension was applied to the soil of garlic plants after being diluted in sterile distilled water to a final concentration of $1x10^8$ cfu/ml. Applications of bio-agents were carried out at the same date of adding the pathogens and added at rate of 50ml per plant. Before planting the garlic cloves, the bio-agent-treated soil was irrigated three times a week to guarantee that the pathogens and bio-agents were evenly distributed.

#### *2.5.3. The antagonistic activity of Tebuconazole*

Before planting, garlic cloves were soaked in tebuconazole fungicide at 1.5% concentration for one hours, and once the plants emerged (at the age of two to three true leaves), they were treated with a 1.5% concentration of the same fungicide once more (50ml per plant was added). The percentage of root rot every 15 days, starting with the 30th day of plant growth and continuing for 90 were noted. As negative controls, infested rows with the pathogens of FS, FO, and SC only. Positive control were non-treated garlic cloves (healthy). The following scale was used to determine the diseases severity percentage of basal root rot diseases in each experiment, Fakhouri *et al.* (2003).

0= indicates no symptoms.

1= 1–25% of the roots are slightly rotten.

2=26 - 50% of moderately decayed roots.

3 = 51–75 percent severely decayed roots.

4 = 76–100% of the plants died.

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

Where (A, B, C, and D) are the number of plants corresponding to the numerical grade, 1, 2,3, and 4 respectively and 4T is the total number of plants (T) have the maximum disease grade 4, where T=A+B+C+D (Shatla *et al.*, 1980; Rengwalska and Simon, 1986; Dilbo *et al.*, 2015).

#### *2.5.4. Effect on plant growth parameters*

The average of plant height was determined in relation to the disease severity percent at the end of the experiments.

### *2.6. Experimental design and statistical analyses*

All data were subjected to statistical analysis by Statistical Analysis System (SAS) software Version 6. One-way analysis of variance (ANOVA) was used to analyze experimental data, and the (LSD) at a probability level of 5.0% were used to separate means differences. (Gomez and Gomez 1984)

## 3. Results and discussion

### *3.1. Pathogen isolation*

Nineteen fungal isolates of *F. oxysporum* and *S. cepivorum* were isolated from infected garlic plants. *F. oxysporum* f. sp. *cepae* and *S. cepivorum* are the main casual agents for basal rot and white rot diseases of garlic under greenhouse and field conditions (Dilbo *et al.*, 2015; Rout *et al.*, 2016; Wang *et al.*, 2019; Akbari Oghaz *et al.*, 2021).

### *3.2. Identification of the pathogenic fungi*

PCR results confirmed that isolates are *Fusarium solani (*FS), *Fusarium oxysporum* (FO), and *Sclerotium cepivorum* (SC). DNA-polymorphism amplified by arbitrary primer are useful as genetic markers (William *et al.*, 1990).

### *3.3. In vitro experiment*

#### *3.3.1. Effect of antagonistic fungi on mycelial growth*

Data present in table (1) and figure. (1) *P. janthinellum* showed high suppression of *Fusarium solani (*FS), *Fusarium oxysporum* (FO), and *Sclerotium cepivorum* (SC) mycelia growth. The inhibition percent were 79.3% for FS, 83.3% for FO and 84.4% for Sc respectively. *P. janthinellum* could limited the growth of *S. cepivorum* through secreting some enzymes such as peptides and endoglucanase (Mernitz *et al.*, 1996), production of some harmful chemicals and hydrolytic enzymes, such as proteases, which can break down other fungi's cell walls; mycoparasitism; competition for nutrients; and the synthesis of antibiotics (Harman *et al.*, 2004; El-Sheshtawi *et al.*, 2009).They can do this by

wrapping their hyphae around the parasite's and pathogen's hyphae, or by penetrating the FOL cytoplasm and then dissolving it (Howell, 2003; Özer and Köycü, 2004; Contreras-Cornejo *et al.*, 2016).

### *3.3.2. Effect of antagonistic bacteria on mycelial growth*

Results in Table (1) and figure. (1) demonstrated that *Bacillus subtilis* shows suppression effect against mycelial growth of *Fusarium* and *Sclerotium* isolates. The suppression of radial growth was (FS= 77%, FO=80% and SC=83%). (Chen & Dickman 2005; Basha *et al.*, 2006; Ahmadzadeh *et al.*, 2007), and according to Gaballa *et al.* (2008), *B. subtilis* inhibits the growth of *S. cepivorum* and *F. oxysporum's* mycelia. This significant antifungal activity was likely caused by the synthesis of antibiotic compounds, such as lipopeptides (Arima *et al.*, 1968), peptides (Banerjee and Hansen, 1988) and (Paik *et al.*, 1998), derivatives of phenylpropanol (Pinchuk *et al.*, 2002), and a new phospholipid molecule (Tamehiro *et al.*, 2002).

**Table 1.** The radial growth inhibition % of *Sclerotium cepivorum* (SC*)*, *Fusarium oxysporum* (FO*)* and *Fusarium solani*. (FS) *in vitro* agonistic by *Penicillium janthinellum, Bacillus subtilis* and (0.5, 1.00 and 1.5% concentration of fungicide Tebuconazole. Mean for three replicates

| Treatments | FS % | FO% | SC% | Mean |
|---|---|---|---|---|
| *Penicillium janthinellum* | 79.3 | 83.3 | 90.7 | 84.4 |
| *Bacillus subtilis* | 77.8 | 80 | 83 | 80.2 |
| (Tebuconazole) 0.5% Conc. | 73 | 67.4 | 70.4 | 70.2 |
| (Tebuconazole) 1% Conc. | 87 | 90.4 | 88.1 | 88.5 |
| (Tebuconazole) 1.5% Conc. | 96.3 | 98.9 | 100 | 98.4 |
| Control | 0 | 0 | 0 | 0 |
| Mean | 68.9 | 70 | 72 | |
| LSD | 1.7 | 2.4 | 4.2 | |


**Figure 1.** The effect of *P. janthinellum* and *B. subtilis* against *Sclerotium cepivorum* (SC*)*, *Fusarium oxysporum* (FO*)* and *Fusarium solani*. (FS) *in vitro* compared with untreated control (C).

### *3.3.3. Effect of Tebuconazole on mycelial growth*

Data in Table (1) and figure. (2) show that Tebuconazole has high inhibition of fungal growth, which reduced the mycelial growth at the concentrate of 0.5% gave inhibition percent as follow (FS=73%, FO=67% and SC=70%), at 1% concentrate gave (FS=87%, FO=90% and SC=88%), while, 1.5 % concentrate showed the highest inhibition occurred for the tested fungi (FS=96%, FO=98% and SC=100%). When compared with untreated control. These results were confirmed by (Pung *et al.,* 2007) and (El-Sheshtawi *et al.,* 2009) They found that reduction in mycelial growth of onion white rot pathogens achieved by dicarboximides such as vinclozolin and iprodione fungicides *in* vitro.

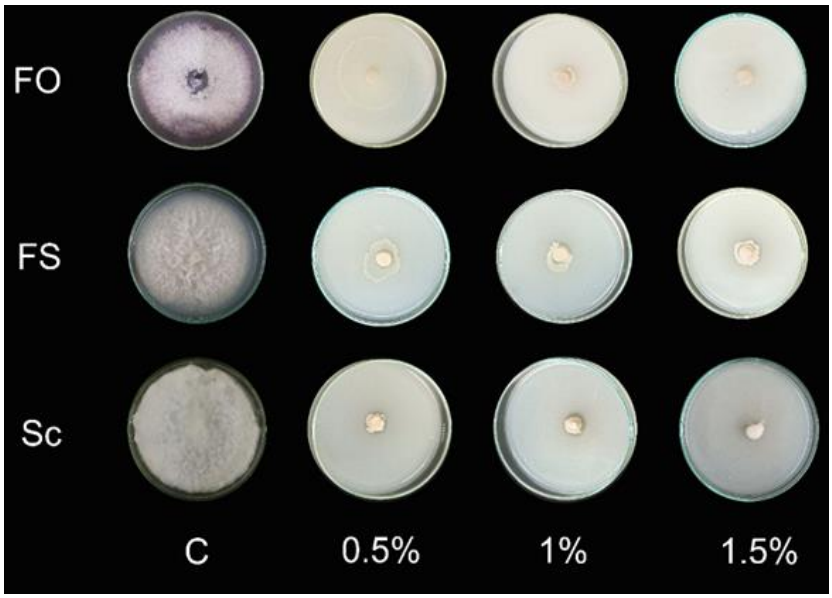


**Figure 2.** Effect of using different concentration of Tebuconazole on *Sclerotium cepivorum* (SC*)*, *Fusarium oxysporum* (FO*)* and *Fusarium solani*. (FS) growth *in vitro* compared with untreated control (C).

## *3.4. Field experiment*

### *3.4.1. Effect of the Bioagents against garlic root rot and basal rot pathogens*

Data in Table (2) and figures (3 and 4) presented that the treated soil with *P. janthinellum and Bacillus subtilis* significantly reduced the disease severity of garlic plants compared with the control. The two bio-agents gave high disease severity reduction. (Niknejad Kazempour *et al.,* 2000), (Osuinde *et al.,* 2002), (Ozbay and Newman, 2004), (EL-blasy, 2006) mention that the pathogen *Fusarium* spp. may be competing with the bio-agents for carbon and nitrogen sources in the plant rhizosphere, which is thought to be a necessary compound for the germination of chlamydospores and sclerotia. By preventing the pathogen from this compound, it may be possible to stop the spores from germinating and to produce certain toxic compounds or extracellular enzymes that break down the pathogen's cell wall, such as chitinases, viridian, gliotoxin, and chaetomin (Brimner *et al.,* 2003). (Küçük and Kivanç, 2003; Anitha and Rabeeth, 2009). These bioagents fungi can produce systemic resistance in plants against infection with many pathogens and enhance the growth of treated plants (Niknejad Kazempour *et al.,* 2000). They can also coil around disease hyphae, penetrate, and parasitize (Howell, 2003). Therefore, plant height in all the treatments was significantly increased compared with infected control, especially when applied tebuconazole at tested concentration (table.3). Soil treated with tebuconazole significantly reduced the disease severity of garlic disease and increase the plants

height compared with the infected control in (Baladi cv) and (Sides 40 cv), (Melero-Vara *et al.*, 2000) found that tebuconazole was effective in reducing the incidence and progress of the garlic white rot disease and increasing the yield when applied as alcove treatment. Tebuconazole is the most effective fungicide in preventing outbreaks of onion white rot disease and provides a superior yield advantage when compared with captan and mancozeb (El-Sheshtawi *et al.*, 2009, Zeray *et al.*, 2013).

**Table 2.** The result of applying *Penicillium janthinellum, Bacillus subtilis* and 1.5% Tebuconazole for controlling garlic white rot and basal rot disease under field conditions (diseases severity percent) on Baladi and Sids 40. Values are mean of two experiments with three replicates each. Mean values followed by the same letter are not significantly different according to LSD at P ¼ 0.05. *Sclerotium cepivorum* (SC*)*, *Fusarium oxysporum* (FO*)* and *Fusarium solani*. (FS).

| Cultivars | Treatments | Rotten root (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Season 1 | | | Season 2 | | |
| | | FS | FO | SC | FS | FO | SC |
| Baladi | *P. janthinellum* | 27.5 B-E | 25 B-F | 25 B-F | 25 C-E | 22.500 D-F | 30 CD |
| | *B. subtilis* | 20 D-F | 25 B-F | 27.5 B-E | 25 C-E | 27.5 C-E | 20 E-G |
| | Tebu. 1.5% | 12.5 FG | 15 EF | 15 EF | 7.5 H-J | 10 HI | 7.5 H-J |
| | Control (infected) | 72.5 A | 75 A | 77 A | 72.5 B | 72.5 B | 77.5 AB |
| | Control (untreated) | 0 G | 0 G | 0 G | 0 J | 0 J | 0 J |
| Sids 40 | *P. janthinellum* | 22.5 C-F | 27.5B-E | 27.5 B-E | 22.5 D-F | 25 C-E | 22.5 D-F |
| | *B. subtilis* | 30 B-D | 37.5B | 35 BC | 25 C-E | 25 C-E | 32.5 C |
| | Tebu. 1.5% | 17.5 D-F | 17.5 D-F | 15 EF | 12.5 G-I | 15 F-H | 5 IJ |
| | Control (infected) | 82.5 A | 85 A | 75 A | 82.5 A | 80 AB | 82.5 A |
| | Control (untreated) | 0G | 0 G | 0 G | 0 J | 0 J | 0 J |


**Figure 3.** Effect of using *P. janthinellum, B. subtilis* and Tebuconazole fungicide on diseases severity caused by *Sclerotium cepivorum* (SC*)*, *Fusarium oxysporum* (FO*)* and *Fusarium solani*. (FS) under field conditions on Baladi cultivars compared with (P.C), untreated garlic plants, (N.C), inoculated garlic plants with the pathogen only.

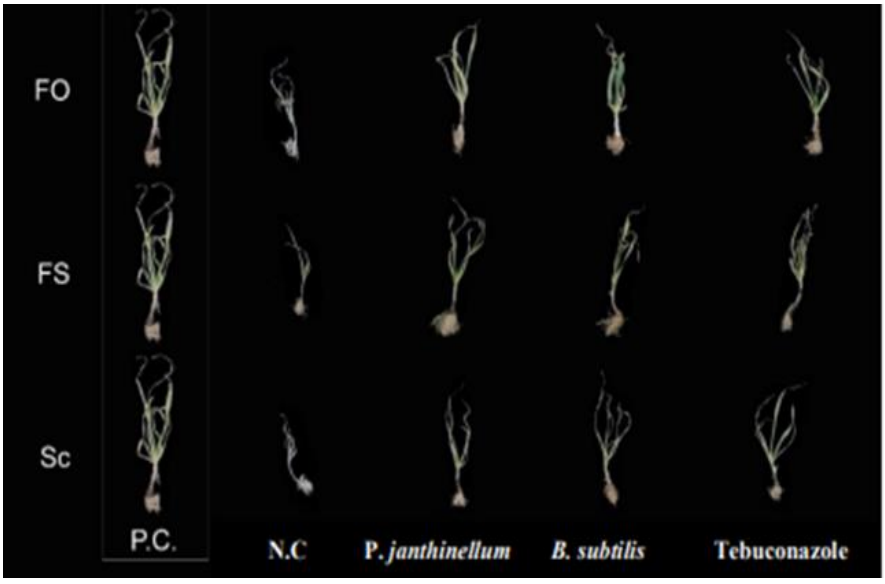


**Figure 4.** Effect of using *P. janthinellum, B. subtilis* and Tebuconazole fungicide on diseases severity caused by *Sclerotium cepivorum* (SC*), Fusarium oxysporum* (FO*)* and *Fusarium solani*. (FS) under field conditions on Sids 40 cultivars compared with (P.C), untreated garlic plants, (N.C), inoculated garlic plants with the pathogen only.

**Table 3.** Garlic plant height with centimeters(cm) resulted of applying *Penicillium janthinellum*, *Bacillus subtilis* and1.5% Tebuconazole fungicide for controlling garlic white rot and basal rot disease for two season cultivated under green-house conditions on Baladi and Sids40 cultivars. Values are mean of two experiments with three replicates each. Mean values followed by the same letter are not significantly different according to LSD at P ¼ 0.05. *Sclerotium cepivorum* (SC*), Fusarium oxysporum* (FO*)* and *Fusarium solani*. (FS)

| Plant height (cm) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cultivars | Treatments | Season 1 | | | Season 2 | | |
| | | FS | FO | SC | FS | FO | SC |
| Baladi | *P. janthinellum* | 33.2 C | 31.1D-F | 30.3 FG | 34 D-F | 33.1 E-G | 33.1E-G |
| | *B. subtilis* | 31.1 D-F | 31.5 C-F | 31.6 C-F | 37 BC | 35.4 C-E | 36.1 CD |
| | Tebu. 1.5% | 33.2 C | 32.5 CD | 32.4 C-E | 38.5 B | 36.9 BC | 38.8B |
| | Control (infected) | 16.2 K | 16.4 K | 15.2 K | 24.8 J | 22.9 J | 23 J |
| | Control (untreated) | 38.1 A | 38.3 A | 37.9 A | 41.5 A | 41.5 A | 41.5A |
| Sids 40 | *P. janthinellum* | 30.6 E-G | 28.8 GH | 27.9 HI | 30.6 HI | 30.4 HI | 31.2 G-I |
| | *B. subtilis* | 28.3 H | 28.1 HI | 30.3 FG | 30.1 I | 30.1 I | 30.7 HI |
| | Tebu. 1.5% | 26.3 IJ | 25.5 J | 25.2 J | 32.7 F-H | 31.5 G-I | 34 D-F |
| | Control (infected) | 12.3 L | 13.3 L | 11.5 L | 20.2 K | 19 K | 20.2 K |
| | Control (untreated) | 35.2 B | 35.2 B | 35.1 B | 34.1 D-F | 34.1 D-F | 34.1 D-F |

## 4. Conclusion

*Sclerotium cepivorum, Fusarium oxysporum f. Sp. cepae*, and *Fusarium solani*, which cause white rot and base rot diseases were isolated from infected garlic plants samples collected from different areas in Qena and Luxor governorates, as well as biocontrol agents isolated from healthy samples. The ability of isolated bioagents as *Bacillus subtilis, Penicillium janthinellum* compared with Tebuconazole fungicide *in* vitro and under field conditions to suppress the

pathogens growth, reduce diseases severity and increased plant height were investigated. *Penicillium janthinellum* was the most effective bio-agent control of mycelial growth inhibition *in vitro* followed by *Bacillus subtilis*, while Tebuconazole fungicides highly significantly reduced the disease severity *in vitro, in vivo*, and highly increased garlic plant length under field conditions.

**Authours Declarations**

**Ethics approval and consent to participate**

*Not Applicable*

*This manuscript is in accordance with the guide for authors available on the journal's website. Also, this work has not been published previously and is approved by all authors and host authorities*

**Consent for publication**

*Not applicable*

**Availability of data and material**

*Not applicable*

**Competing interests**

*No potential conflict of interest was reported by the authors.*

**Funding**

*Not applicable*

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