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Biological Control of Onion White Rot Disease using *Bacillus* spp. Isolated from Egyptian Soil

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The antagonistic ability of eleven bacterial isolates against *Sclerotium cepivorum*, causal agent of onion white rot disease (OWR), was determined *in vitro* and the best five isolates were identified as *Bacillus pumilus* (BP_1 and BP_2) and *B. marinus* (BM_1, BM_2 and BM_3). The *in vitro* antagonistic efficacy ranged between 11.1 to 68.9% depending on the concentration of the bacterial cell suspension and number of applied sprays under greenhouse and field conditions. It was found that using double sprays with *B. pumilus* (BP_2) in the greenhouse experiment gave the highest efficacy in reducing OWR (93.8%) at the concentrations of 10 and 20 ml/l. In case of field experiment there was no significant deference between number of sprays or the tested bacteria in reducing infection percentage of OWR. The significant difference was reported only between the concentrations of the bacterial suspension during the two growing seasons 2008/2009 – 2009/2010. In case of using one spray during season 2008/2009, the most effective bacteria in reducing OWR was *B. pumilus* (BP_2) giving 64.7 and 61.6% efficacy when used at the concentrations 20ml/l and 10 ml/l, respectively, followed by *B. marinus* (BM_3) which gave 58.2% at the concentration 20ml/l. On the other hand, when two sprays of bacterial suspension were used *B. marinus* (BM_1) and *B. pumilus* (BP_1) gave the highest efficacy in reducing OWR reached 63.7 and 63.4%, respectively. During season 2009/2010 *B. pumilus* (BP_2) was the most effective bacteria reached 80.0% efficacy followed by *B. marinus* (BM_3) recorded 76.9% when both used at the concentration 20ml/l for one spray. When two sprays were used, *B. marinus* (BM_2) gave the highest percentage of efficacy in reducing OWR (87.3%). Data showed that applying the tested bacteria at any concentration, either once or twice, significantly increased onion bulb yield compared with control treatment during the two growing seasons.

Keywords: *Bacillus marinus, B. pumilus*, Biological control and white rot.

Onion (*Allium cepa* L.) is an important crop in Egypt for local consumption and exportation. According to the Statistical Department of Ministry of Agriculture and Land Reclamation, the total bulb crop acreage was 125397 feddan yielded 1731824 tons in 2009/2010 (Anonymous, 2010).

Onion white rot (OWR) disease, caused by *Sclerotium cepivorum* Berk., is a serious disease of onion and other *Allium* spp. in Egypt (Abd El-Moity, 1981 and Satour *et al*., 1992) as well as many other onion growing areas of the world (Crowe *et al.*, 1980). Onion white rot disease affects the genus *Allium* and threatens Allium production in several parts of the world. For example, between 1965 and 1982, white rot disease caused a 65% reduction in the production area of winter onions in Egypt, and a 90% decrease in exports. The disease attacks the root system of host plants, resulting in either death before harvest or postharvest decay (Entwistle, 1990a). The pathogen survives in the soil as sclerotia and may remain dormant in this state in the absence of the host for 20 years (Coley-Smith, 1990).

A number of control methods have been researched including fungicide application (Stewart *et al.*, 1994b and Khaled *et al*., 1997), soil fumigants (Entwistle, 1990b), soil solarization (Porter and Merriman, 1983, Melero-Vara *et al*., 2000) and use of biocontrol agents (Stewart *et al*., 1994a; Gevlagh *et al*., 1996 and Abd El-Magid and Abd El- Momen, 2008).

The continuous use of chemicals at high concentrations has developed pathogen fungicidal resistance and impacted nontarget species and the environment with potential public health concerns (De Weger *et al.,* 1995 and Zhang *et al.*, 2009). There is a growing need for alternative control measures that are environmental friendly for control of OWR disease. The use of microorganisms to control plant diseases is a promising strategy and has potential for managing OWR disease. Compared with chemical control, using microbial agents to control plant pathogens can be eco-friendly and cost-effective component of an integrated pest management (Mao *et al.*, 1997).

Many Bacillus strains are known to suppress the *in vitro* fungal growth by the production of one or more antifungal antibiotics (Katz and Demain, 1977). Some of these antibiotics producing strains were also shown to suppress *in vivo* fungal plant diseases (Fravel, 1988).

Bacillus pumilus MA111M4a showed a strong inhibitory activity against the fungi *Rhizoctonia solani, Pythium aphandermatum* and *Sclerotium rolfsii* (Fálvia *et al.*, 2009). Also, many authors investigated the ability of *B. pumilus* isolates to control other plant pathogenic fungi such as *Rhizoctonia solani* on rice (Padaria and Aqbal-Singh, 2009) root-rot disease complex of chickpea (Sayed and Siddiqui, 2008) as well as pathogenic bacterium *Pseudomonas syringae* pv. *tomato* on tomato (Silva *et al.*, 2008). In other researches, an antifungal compound that is active against Mucoracea and *Aspergillus* species have been reported (Edward and Richard, 2003).

Several workers suggested *B. marinus* as a biocontrol agent. Odile *et al.* (2003) used it to control cucumber damping-off, also Gao *et al*. (2010) observed that tomato gray mould and early blight could be controlled using *B. marinus*.

The objective of this work is to determine the ability of two species of *Bacillus*, *i.e. B. pumilus* and *B. marinus*, to control onion white rot under greenhouse and field conditions.

Materials and Methods

1- The pathogen:

An isolate of *Sclerotium cepivorum* used in this study was isolated from onion plants exhibiting typical symptoms of white rot disease. Infected samples were collected from Mallawi Agricultural Research Station, El-Minia Governorate. Isolated pathogen was purified and preserved, on potato dextrose agar (PDA), for subsequent studies.

2- Isolation and identification of the biocontrol agents:

Eleven bacterial isolates were collected from leaves of 90-day-old healthy onion plants (cv. Giza) grown in naturally field at Nobaria, Behera Governorate. The bacteria were isolated on nutrient agar (NA) medium and incubated at 27^{o}C. Cultures were kept under glycerol oil for farther studies. Identification of antagonistic bacterial isolates was made based on morphological, physiological and biochemical characteristics according to (Fahy and Persely, 1983; Lelliot and Stead, 1987 and Schaad, 1988).

3- Evaluation of antagonistic potential of bacteria against S. cepivorum:

Bacterial isolates were streaked on one side of PDA plates and incubated at 27^{o} C for 24 hr. Discs (0.5 cm) of (4-day-old) *S. cepivorum* cultures were aseptically transferred into the bacterial plates, and incubated at 25^{o}C±2 for 5 days. Plates of the tested fungus only were kept as check (control) treatment. Three plates were served as replicates for each treatment. The percentage of reduction in the radius growth of the fungus was calculated according to Fokkema (1973) as follows:

Reduction in linear growth (%)= $(R_1 - R_2) \diagup R_1 \times 100$

Whereas: $R_{1=}$ The radius of normal (control) growth .

$R_{2=}$ The radius of inhibited growth.

4- Greenhouse evaluation of the tested bacterial antagonists:

Greenhouse experiment was conducted to evaluate the ability of *Bacillus pumilus* and *B. marinus* to control OWR. *Sclerotium cepivorum* inoculum was prepared by growing pure culture of the pathogen on sorghum - washed sand (1:1 v/v) for 15 days at 27^{o} C, then used to infest sterilized soil at the rate 2% (w/w) seven days before planting. Onion seedlings (cv. Giza 6) were dipped for 15 minutes in 25 ml of the cell suspension ($2x10^7$ cfu) of each tested bacteria, amended with (0.5 ml/l) Arabic gum as adhesive material, before planting in pots (25 cm) containing the infested soil. Five seedlings were transplanted in each infested pots. Tested treatments were carried out as the following: A) one spray with each of the tested bacterial cell suspensions after 6 weeks from transplanting at the rate 5, 10 and 20 ml/l. B) two sprays with the bacterial cell suspensions after 6 and 12 weeks from transplanting with the same former rates. Four pots served as replicates for each treatment as well as four untreated pots served as control. Disease incidence and infection percentage were calculated at the end of the growing season.

5- Field experiment:

Field experiment was carried out at Mallawi Research Station Farm, during 2008-2009 and 2009-2010 seasons, to evaluate the efficiency of the antagonistic

bacterial isolates to control onion white rot (OWR) under naturally infested field conditions with *S. cepivorum* was chosen in this study. Split plot design was used where onion transplants were planted in 3.5 x 3 m experimental plots. Two hundred onion seedlings were dipped in 100ml of the cell suspension of each of the tested antagonistic bacteria containing $2x10^7$ cfu for 15 minutes and sprayed with the cell suspension of each of the antagonistic bacteria as mentioned before in the greenhouse experiment with same two treatments. Each treatment was replicated three times and three control plots were planted and left without bacterial treatment.

After 150 days from transplanting, percentage of infection with white rot disease as well as the yield of onion bulbs were estimated for each plot.

Statistical analysis:

In all experiments the least significance difference (LSD) at 0.05 confidences was determined according to Gomez and Gomez (1984).

Results

1- In vitro evaluation of bacterial antagonists:

Eleven isolates of the tested bacteria were used to investigate their ability to inhibit the mycelial growth of *Sclerotium cepivorum*. Data in Table (1) show that all the tested bacterial isolates significantly decreased the radial growth of the fungus compared with control treatment. The efficacy of antagonistic bacteria ranged between 11.1% to 68.9%. The most antagonistic bacteria was the isolate (D) with efficacy 68.9% followed by isolates (B), (C) and (F) with 65.6, 64.1 and 64.1% efficacy, respectively.

Table 1. Antagonistic effect of some bacteria on the radial growth of *Sclerotium cepivorum*

Tested bacteria	*S. cepivorum* mycelial growth (cm)	Growth reduction (%)
A	3.93	56.3
B	3.10	65.6
C	3.23	64.1
D	2.80	68.9
E	3.67	59.2
F	3.23	64.1
G	8.00	11.1
H	3.60	60.0
I	5.90	34.4
J	5.10	43.3
K	8.00	11.1
Control	9.00	0.0
L.S.D at 1%	1.7	

2- Identification of the isolated biocontrol agents:

The most effective bacterial isolates in inhibiting *S. cepivorum* mycelial growth were identified according to cultural properties, morphological, physiological and biochemical characteristics. From identification tests it was revealed that isolates (B and F) belonged to *Bacillus pumilus* isolates BP_1 and BP_2, respectively. Meanwhile, isolates (C, D and H) belonged to *B. marinus* isolates BM_1, BM_2 and BM_3, respectively.

3- Greenhouse experiment:

Data in Table (2) indicate that all the tested antagonistic bacteria significantly decreased the infection percentage of onion white rot (OWR) disease under greenhouse conditions. *Bacillus pumilus* (BP1) and *B. marinus* (BM_3) were the most effective ones since they recorded 68.8% efficacy of reducing OWR when one spray of 5 ml/l ($2x10^7$ cfu/ml) dose was used. It was found that using two sprays with the bacterial cell suspension was more efficient in reducing OWR disease compared with one spray application.

Table 2. Effect of some bacterial bioagents and their rates of application on incidence of OWR disease under greenhouse conditions

Antagonistic bacteria	No. of applications	Infection (%) at			Efficacy (%)		
		5ml/l	10ml/l	20ml/l	5ml/l	10ml/l	20ml/l
B. marinus (BM_1)	1	65.0	60.0	70.0	18.8	25.0	12.5
	2	55.0	20.0	60.0	31.3	75.0	25.0
B. marinus (BM_2)	1	55.0	60.0	60.0	31.1	25.0	25.0
	2	30.0	45.0	50.0	62.5	43.8	37.5
B. marinus (BM_3)	1	25.0	60.0	75.0	68.8	25.0	6.3
	2	15.0	30.0	45.0	81.3	62.5	43.8
B. pumilus (BP_1)	1	25.0	35.0	65.0	68.8	56.3	18.8
	2	10.0	15.0	65.0	87.5	81.3	18.8
B. pumilus (BP_2)	1	60.0	45.0	40.0	25.0	43.8	50.0
	2	50.0	5.0	5.0	37.5	93.8	93.8
Control	0	80.0	80.0	80.0	0.0	0.0	0.0
LSD at 1% for: Spray (S)= 10.95; Bacteria (B)=8.77; Concentrations (C)= 9.1; S x B= 12.4; S x C= 12.87; B x C= 20.34; S x B x C= 28.77							

Data also revealed that *B. pumilus* (BP_2) gave the highest efficacy (93.8%) in reducing OWR when used as double spray at the concentrations 10 and 20 ml/l. It was followed by *B. pumilus* (BP_1) which gave 87.5% efficacy when used at the concentration of 5 ml/l. In this concern, *Bacillus marinus* (BM_3) recorded the lowest (6.3%) efficacy when used as one spray at the concentration 20 ml/l. Generally, in case of using one spray of bacterial cell suspension it was found that 5ml/l concentration was the most effective one and 10 ml/l concentration was the best one when used as two sprays.

4- Field experiment:

Five bacterial isolates were used to investigate their ability to control OWR disease with antagonistic potentials during two growing seasons (2008/2009 - 2009/2010). Data represented in Table (3) show the infection percentage of OWR and the efficiency of the tested bacteria to control it during the growing season 2008/2009. The tested bacteria significantly reduced the infection percentage of OWR compared with control treatment. There was no significant deference at the isolate levels between numbers of sprays but the significant difference was found between the concentrations of the bacterial cell suspension. In case of using one spray, the most effective bacteria in reducing OWR was *B. pumilus* (BP_2) giving 64.7 and 61.6% efficacy when used at the concentrations 20 ml/l and 10 ml/l, respectively, followed by *B. marinus* (BM_3) which gave 58.8% at the concentration 20ml/l. On the other hand, when two sprays of cell suspension were used *B. marinus* (BM_1) and *B. pumilus* (BP_1) gave the highest efficacy in reducing OWR when recorded 63.7 and 63.4%, respectively.

Table 3. Effect of number and rate of application of bioagent bacteria on infection percentage with onion white rot and efficacy under field conditions at Mallawi during 2008-2009

Antagonistic bacteria	No. of applications	Infection (%) at			Efficacy (%)		
		5ml/l	10ml/l	20ml/l	5ml/l	10ml/l	20ml/l
B. marinus (BM_1)	1	15.3	14.2	13.7	45.9	51.4	53.1
	2	12.3	11.7	10.7	58.3	60.3	63.7
B. marinus (BM_2)	1	15.8	15.3	14.7	45.9	47.6	49.7
	2	15.2	14.2	12.3	48.5	51.9	58.3
B. marinus (BM_3)	1	14.5	13.7	12.2	50.3	53.0	58.8
	2	14.0	13.3	13.2	52.5	54.9	55.3
B. pumilus (BP_1)	1	16.7	13.7	13.2	42.8	53.1	54.8
	2	13.8	11.7	10.8	53.2	60.3	63.4
B. pumilus (BP_2)	1	15.7	11.2	10.3	46.2	61.6	64.7
	2	12.7	12.3	11.8	56.9	58.3	60.0
Control	-	29.0	29.0	29.0	0.0	0.0	0.0
LSD at 5% for: Spray (S)= NS; Treatment (T)= NS; Concentration (C)= 1.47; S x T= 2.47; S x C= 2.07; T x C= 3.28; S x T x C= 4.64							

Similar trend was found in 2009/2010 (Table 4) since all the tested bacteria significantly reduced the infection percentages of OWR compared with the control treatment. Also, *B. pumilus* (BP_2) was the most effective bacteria giving 80.0% efficacy followed by *B. marinus* (BM_3) giving 76.9% when both used at the concentration 20ml/l for one spray. When two sprays were used *B. marinus* (BP_2) gave the highest percentage of efficacy in reducing OWR (87.3%) followed by *B. pumilus* (BP_2) and *B. marinus* (BM_3) giving 81.8 and 80.5%, respectively, when it applied as two sprays at the concentration 20ml/l.

Table 4. Effect of number and rate of application of bioagent bacteria on infection percentage with onion white rot and efficacy under field conditions at Mallawi Research Station during 2009-2010

Antagonistic bacteria	No. of applications	Infection (%) at			Efficacy (%)		
		5ml/l	10ml/l	20ml/l	5ml/l	10ml/l	20ml/l
B. marinus (BM_1)	1	18.0	16.7	13.3	67.7	69.6	75.8
	2	15.3	15.0	14.0	72.2	72.7	74.5
B. marinus (BM_2)	1	16.3	13.7	13.3	70.4	75.1	75.8
	2	14.3	9.3	7.0	74.0	83.1	87.3
B. marinus ($BM_{3)}$	1	15.3	15.0	12.7	72.2	72.7	76.9
	2	11.0	10.3	10.7	80.0	81.3	80.5
B. pumilus (BP_1)	1	18.7	15.3	14.7	66.0	72.2	73.3
	2	16.0	13.3	13.0	70.9	75.8	76.4
B. pumilus (BP_2)	1	17.3	14.0	11.0	68.5	74.5	80.0
	2	13.7	11.7	10.0	75.1	78.7	81.8
Control	0	55.0	55.0	55.0	0.0	0.0	0.0
LSD at 5% for: Spray (S)= NS; Treatment (T) = 0.72; Concentration (C) = 1.41; S x T= 1.01; S x C= 2.00; T x C= 3.16; S x T x C= 4.47							

In general, the tested bacteria were more effective in reducing OWR disease in the second growing season compared with the first one. Also, using the concentration 20ml/l gave the most efficacy wither it was sprayed once or twice.

5- Effect of the biocontrol bacteria on the yield:

Data represented in Table (5) indicate the effect of the tested bacteria, used in three concentrations and two application numbers, on onion yield during two growing seasons 2008-2009 and 2009-2010. Data show that applying the tested bacteria at any concentration either once or twice significantly increased onion bulb yield compared with control treatment during the two growing seasons. At the same time there were no significant difference between numbers of sprays or the tested bacteria or the reaction between sprays number but it was found between the concentrations. The most effective bacteria in increasing the bulbs yield in the growing season 2008/2009 was *B. pumilus* (BP2) giving 18.05 kg /plot when used at the concentration 20 ml/l as double sprays followed by *B. marinus* (BM2) giving 17.89 kg/plot at same concentration and number of sprays. On the other hand, in the growing season 2009/2010 although bulb yield was lesser than the former season (even in the control treatment),the same trend was observed since no significant difference between number of sprays or the tested bacteria was found but it was also found between the concentrations. However, applying the tested bacteria significantly increased bulb yield compared with nontreated plants. *B. marinus* (BM3) was the most effective in increasing bulb yield giving 15.54 kg/plot when used at 10 ml/l concentration for double sprays while giving 15.49 kg/plot when applied at 10 ml//l for one spray. *B. pumilus* (BP1) came secondly with 15.39 kg/plot when it was sprayed twice at 20 ml/l concentration.

Table 5. Effect of number of sprays and cell suspensions concentrations of antagonistic bacteria on weight of onion bulbs at 2008-2009 and 2009-2010 seasons

Antagonistic bacteria	No. of applications	Weight of onion bulbs (kg/plot) at seasons					
		2008 - 2009			2008 - 2009		
		5ml/l	10ml/l	20ml/l	5ml/l	10ml/l	20ml/l
B. marinus (BM_1)	1	15.82	16.64	16.67	12.22	12.85	14.00
	2	16.40	17.53	17.88	12.67	13.11	13.56
B. marinus (BM_2)	1	16.31	16.65	16.98	12.14	12.91	14.18
	2	16.80	16.89	17.89	13.48	13.56	13.82
B. marinus (BM_3)	1	16.96	17.13	17.36	12.49	13.24	15.49
	2	16.57	17.77	17.79	13.13	15.54	15.05
B. pumilus (BP_1)	1	16.54	17.33	17.77	12.00	12.15	14.84
	2	16.18	16.70	17.15	12.67	14.79	15.39
B. pumilus (BP_2)	1	16.60	17.70	17.92	12.74	13.67	14.24
	2	16.73	17.10	18.05	13.58	13.91	14.33
Control	0	11.83	11.83	11.83	9.67	9.67	9.67
LSD at 5% for		Spray (S)= NS Bacteria (B)=NS Concentration (C)=0.38 S x B = NS S x C = 0.54 B x C = 0.85 S x B x C = 1.2			(S) = NS (B)=NS (C)= 0.86 S x B = NS S x C = 1.22 B x C = 1.93 S x B x C = 2.72		

Discussion

Onion white rot (OWR) is responsible for partially or completely destruction of the bulb yield of *Allium* crops in Egypt and all over the world (Khaled *et al.,*1997). The long term use of broad spectrum chemical pesticides has been identified as one of the major causes of environmental pollution and contributes to the deterioration of agricultural land and ecosystem.

Because of biocontrol agents are usually isolated from natural fields, the risk of environmental contamination may be limited but also must be looked upon with great concern. In addition, the bioagents isolated from root zones or rhizosphere soil may colonize the root system and multiply in these rhizospheres (Chao *et al.*, 1986 and Mao *et al.*, 1997), providing long-lasting and long-term effect in minimizing root rots and ensuring healthy plants of the current and the subsequent crops.

The antagonistic ability of eleven bacterial isolates against *Sclerotium cepivorum*, the causal agent of OWR, was *in vitro* investigated. The best five isolates were identified according to the cultural properties, morphological, physiological and biochemical characteristics. Results from dual-culture experiment demonstrated that some isolates of *Bacillus pumilus* and *B. marinus* significantly reduced the mycelial growth of the pathogen compared with control.

The antagonistic potential of bacteria ranged from 11.1 to 69.9%. The area of inhibition has in most cases been used as major criteria in the selection of biocontrol agents and has been used as evidence on the production of antifungal secondary metabolites by bacterial strains (Jakson *et al*.,1991 and Crawford *et al*., 1993). The reduction in mycelial growth by *Bacillus pumilus* and *B. marinus*, selected in this study, might suggest that these bacteria have produced some diffusible inhibitory substances in the medium. *Bacillus* spp. have been reported to produce a large number of peptide antibiotics and antifungal secondary metabolites representing at least 25 different chemical structures that inhibit mycelial growth of various pathogens by diffusion in culture medium (Mutaz and Hasanain, 2006).

This study demonstrated that 5 Bacillus isolates, *i.e. B. pumilus* isolate $BP_1$and BP_2 as well as *B. marinus* isolates BM_1, BM_2 and BM_3, out of 11 tested bacterial isolates provided significant and protection of onion plants against *Sclerotium cepivorum* under greenhouse and field conditions. The disease was significantly reduced by these bacterial strains with significant increase in bulb yield. In addition, the greenhouse and field tests indicated that the concentration of the bacterial cell suspensions and the number of sprays could affect the biological control results of these bacteria to protect onion plants against *S. cepivorum*. Based on OWR reduction and increase of bulb yield, the 20 ml/l dose with double sprays seems to be the most effective method of application.

Data showed that applying the tested bacteria at any concentration either once or twice significantly increased bulb yield compared with control treatment during the two growing seasons under field conditions. Generally, it was found that the tested bacteria were more effective in reducing OWR disease in the second growing season compared with the first one. This may be due to differential colonization on onion roots by the tested bacteria and on conditions in the field since survival of the bacteria and the production of antifungal components by these bacteria are greatly affected by soil conditions such as soil type and pH (Schmidt *et al*., 2004). Previous studies in biocontrol of root rot diseases have demonstrated that the colonization of biocontrol agents at target sites on root system is a prerequisite for suppression of plant pathogen infection (Weller, 1988 and Bull *et al*., 1991).

In this regard, Edward and Richard (2003) stated that *B. pumilus* produce an antifungal metabolite that inhibits the growth of Mucoracea and some *Aspergillus* species including *A. flavus*, *A. fumigatus* and *A. terreus*. Jetiyanon (2007) attributed the efficiency of mixture of *Bacillus* strains (*B. amyloliquefaciens* strain IN937a and *B. pumilus* strain IN937b) to provide systemic protection against multiple diseases in various crops to induction of defense-related enzyme responses. The author found that before inoculation tomato plants with *S. rolfsii*, higher levels of superoxide dismutase and peroxidase activities were observed in plants treated with a mixture of the IN937a and IN937b compared with non-challenged healthy and nonbacterized pathogen controls. Lakesh *et al*. (2007) indicated that three isolates of *B. pumilus* (INR-7, SE-34 and T-4) enhanced seed germination and seedling vigor of watermelon to the greatest extent and suppressed seedling diseases caused by broad range of fungal species. Padaria and Aqbal-Singh (2009) reported that *B. pumilus* MTCC7615 has been identified as a potent isolate against *Rhizoctonia solani*, the

cause of sheath blight in rice. They attributed the fungal antagonism of *B. pumilus* towards *R. solani* to the existence of 23 kb size pJCP07plasmid which found to carry some of the genes involved in the production of a fungal antagonistic compound against *Rhizoctonia solani*. Sari *et al*. (2006) verified that induced resistance was another mechanism through which *B. pumilus* 7km can suppress wheat take-all disease caused by *Gaeumanomyces graminis*. Also, this study revealed that *B. pumilus* 7km has plant –promotion activity that affect disease severity .Wheat plants treated with *B. pumilus* 7km showed increased presence of soluble peroxidase, cell-wall bound peroxidase, beta-1,3 glucanase, beta -1,4- glucanase and phenolic compounds in bacterized roots challenged with the pathogen. The results suggest that the inhibitory effect of *B. pumilus* 7km on take-all disease may be related to its ability to enhance defense response in the wheat roots.

On the other hand, Odile *et al.* (2003) found that *B. marinus* was effective in controlling cucumber damping-off caused by *Pythium ultimum*. Chunmel *et al.* (2008) isolated a new 24-membere macrolide macrolactin T (1) and a new polyne δ-lactone macrolactin U (2) along with macrolactins A, B, D, O and S from the cultured broth of the bacterium *B. marinus.* They found an inhibitory activity of macrolactins T, B, and D against *Alternaria solani* and *Pyricularia oryzae*. The ability of *B. marinus* to control OWR could be attributed to the highest induction activities of phenylalanine ammonialyase (PAL), polyphenol oxidase (PPO) and superoxidase dismutase (SOD). Also, Gao *et al.* (2010) observed that the fermentation broth of *B. marinus* B-9987 had the highest activity in both fungitoxicity and controlling tomato gray mold and early blight.

It is concluded from this study that using *B. pumilus* and *B. marinus* could be a promising treatment to control OWR disease but it should be pointed out that further studies is needed to investigate the main reasons behind the ability of *B. pumilus* and *B. marinus* to control OWR disease .

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المكافحة الحيوية لمرض العفن الأبيض في البصل باستخدام البكتريا باسيلس المعزولة من التربة المصرية

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تم تقييم كفاءة القدرة التضادية لاحدي عشرة عزلة بكتيرية ضد الفطر سكليروشيم سيبيفورم مسبب مرض العفن الابيض في البصل تحت ظروف المعمل. وتم تعريف اكفأ ٥ عزلات وقد وجدت عزلتين تتبع النوع باسلس بيوميلس (BP_1 , BP_2) ثلاث عزلات تتبع باسيلس مارينس (BM_1, BM_2 , BM_3) و تراوحت الكفاءة التضادية بين ١١.١-٦٨.٩%. تم دراسة تاثير تركيز معلق الخلايا البكتيرية المختبرة وكذلك عدد الرشات المضافة علي الاصابة بالمرض تحت ظروف الصوبة والحقل. وقد وجد عند اضافة رشتان كانت العزلة (BP_2) من البكتيريا باسلس بيوميلس في تجربة الصوبة أكثر كفاءة في خفض المرض عند اضافتها بتركيز ١٠ و٢٠ مل/لتر. وفي تجارب الحقل لم يكن هناك فرق معنوي في خفض نسبة الإصابة بمرض العفن الابيض بين عدد الرشات المضافة اوالبكتريا المختبر لكن وجد فرق معنوي بين تركيز معلق خلايا البكتريا المختبرة وذلك في موسمي الزراعة ٢٠٠٨/٢٠٠٩ و ٢٠٠٩/٢٠١٠. في حال اضافة رشة واحدة من معلق الخلايا البكتيرية خلال موسم الزراعة ٢٠٠٨ / ٢٠٠٩كانت البكتيريا باسيلس بيوميلس العزلة(BP_2) الاكثر كفاءة في تخفيض نسبة الاصابة بمرض العفن الابيض حيث اعطت ٦٤.٧ ، ٦١.٦% كفاءة عند اضافتها بتركيز ٢٠ مل/لتر و ١٠ مل/لتر علي التوالي يليها البكتيريا باسلس مارينس العزلة (BM_3) والتي اعطت ٥٨.٢% كفاءة عند رشها بتركيز ٢٠مل/لتر. من جهة أخري وجد انه عند إضافة رشتين من معلق الخلايا البكتيرية المختبرة كانت العزلة (BM_1) من البكتيريا باسلس مارينس والعزلة (BP_1) من البكتريا باسلس بيوميلس الاعلي كفاءة في تخفيض نسبة الاصابة بالمرض بنسبة ٦٣.٧ و ٦٣.٤% علي التوالي. خلال موسم الزراعة ٢٠٠٩/٢٠١٠ كانت العزلة (BP_2) من البكتريا باسلس بيوميلس الاكثر كفاءة بنسبة ٨٠% يليها العزلة (BM_3) من البكتريا باسلس مارينس والتي اعطت ٧٦.٩% عند رشهما بتركيز ٢٠مل/لتر مرة واحدة وعند رش معلق الخلايا البكتيرية مرتين كانت العزلة (BM_2) من البكتريا باسلس مارينس الاعلي كفاءة في خفض نسبة الاصابة بالمرض بنسبة ٨٧.٣%. وقد اوضحت النتائج ان المعاملة بالبكتريا المختبرة بأي من التركيزين المختبرين اما رشة واحدة أو رشتين أعطي زيادة معنوية في محصول الابصال مقارنة بالكونترول خلال موسمي الزراعة. وقد وجد ان استخدام رشتين من البكتريا باسيلس بيوميلس (BP2) في تجارب الصوبة اعطي اعلي كفاءة في تخفيض الاصابة بالعفن الابيض بنسبة ٩٣.٨%.