# BIOLOGICAL CONTROL OF DOWNY MILDEW ON ONION PLANTS

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## ABSTRACT

Downy mildew is a potentially serious disease of onions (*Allium cepa* L.) particularly when onions grown under cool, moist, and humid conditions. The disease is caused by the air-borne fungus *Peronospora destructor* (Berk.) Casp. which is a common destructive disease of onion in Egypt. Numerous of microbial isolates (50) were isolated from healthy onion leaf surfaces. These isolated microorganisms comprised bacteria (70%), fungi (25%), actinomycetes and yeasts (5%). The initial screening of these isolates which were carried out under greenhouse conditions resulted the selection of 4 different bacterial isolates and one fungal isolate exhibited potency against *P. destructor*. The microscopic examination of downy mildew on treated leaves using light microscopy revealed dead and lysis of sporangia (conidiospores) and sporangiophores of the tested fungal pathogen. The predominant effective bacterial isolates belonged to Bacilli and Pseudomonads. They were identified as *Bacillus subtilis* and *Pseudomonas* sp. and the fungal isolate was identified as *Trichoderma harzianum*. Bioassays were conducted under greenhouse conditions at the experimental greenhouse, Fac. of Agric., Kafr El-Sheikh Univ., Egypt. Open field experiments using two onion cultivars, Balady and Giza 20 were carried out to test the efficacy of antagonists applied to protect onion plants from downy mildew disease. Performance of *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas* sp. in a single or bacterial mixture in controlling downy mildew disease of onion plants under greenhouse and field conditions was evaluated. Spraying onion plants under greenhouse and field conditions with previously mentioned antagonists as protective and curative treatments gave satisfactory control to downy mildew disease either sprayed weekly or every two weeks. The obtained results showed that, all bioagents treatments led to significant decrease in the disease incidence and disease severity compared with control treatment. The highest

inhibition of the disease incidence was obtained when a mixture of 4 different bacterial isolates (Isolates No.23,41 and 67 of *Bacillus subtilis* and isolate No. 14 of *Pseudomonas* sp.) were used. The effects were similar to those of Ridomil MZ 72 wp fungicide. Similar results were obtained when isolate No. 14 of *Pseudomonas* sp. was used spraying on onion plants alone and when mixed with isolates No.23,41 and 67 of *Bacillus subtilis*. Results also showed that onion cv. Giza 20 was less susceptible with downey mildew than Balady cv. In general results of this study suggest that the use of the tested bacterial bioagents could be used in controlling downy mildew disease of onion alternative to existing chemical products especially when sprayed weekly.

**Keywords:** *Allium cepa* L., downy mildew, Biological control, Bioagents

## INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important crops in many countries of the world including Egypt. Green onion is ranked nowadays as an economic vegetable crop for exportation. Onion plants are subjected to infection with many diseases that affect crop quantity and quality. Downy mildew disease caused by *Peronospora destructor* (Berk.) Casp. is the most destructive one ( Agrios, 2005 ).

Fungicides are still the principle means to control fungal pathogens, but there is becoming more and more controversial. Many investigators have indicated potentially undesirable environmental effects of the fungicides on humans, plants and other beneficial organisms. In addition, upsetting the biological balance by toxic chemicals may lead to severe outbreak of diseases as well as appearance of new races (Manon, 1998). Biological control could be considered as an alternative mean of chemical control. It depends on the potential of beneficial antagonistic microorganisms. Successful biological control of foliar diseases such as blights, blast, powdery and downy mildews has been achieved by a number of researchers under greenhouse and field trials using fungal and bacterial antagonists (Vozenilkova *et al.*, 1992, Bettiol *et al.*, 1997, Belanger and A· is, 1998, Umesha *et al.*, 1999 , Singh *et al.*, 2000, Abd El-Moneim, 2001, Mosa, 2002, McGrath, 2004 and Hussein *et al.*, 2007).

The present work aimed to study the ability of certain antagonistic microorganisms to control downy mildew disease of onion plants under greenhouse and field conditions.

## MATERIALS AND METHODS

### Laboratory studies

**A. Isolation of microorganisms from the phylloplane of onion plants:**

Healthy onion leaves were collected from severely affected plants by downy mildew growing in Kafr El-Sheikh and El-Gharbia Governorates, Egypt. Ten gm of leaf samples were added to 90ml nutrient or potato dextrose broth medium (PD). After thoroughly shaking for 30min, dilutions series were prepared in test tubes containing 9ml from the same previous media (nutrient or PD broth) up to $1:10^6$. One hundred µl from the three latter dilutions from the series were spreaded on PDA (Potato Dextrose Agar) for fungal isolation or Nutrient Agar and Kings B medium for bacterial isolation by using Drigalisky triangle and then the single colonies were selected and transferred to test tubes containing the same medium. After that pure cultures were kept in a refrigerator at 4°C for further studies.

**B. Screening of the isolated microorganisms for antagonism against onion downy mildew.**

The microorganisms which were previously isolated from onion leaves were recultured on potato dextrose broth for fungi and yeasts or nutrient broth for bacteria in flasks, 500ml, containing 200ml medium. Cultures were incubated at 28°C for 10 days in shaking incubator (140r/min.). Cultures were paper filtered and hand homogenized, counting of cells or spores in case of bacterial and yeast and fungi, respectively and adjusted at the rate of $10^7$ /ml. The prepared microbial cultures were sprayed on potted mature plants of onion, c.v. Balady, naturally infected with the downy mildew. Cultures were amended with calculated aliquots of an adhesive surfactant (New-Film 1265 registered by Ministry of Agric. and lands reclamation, Egypt) according the recommended dose (30ml/100L) and hand homogenized before fine spraying onto leaf surfaces of plants. Plants were sprayed with water or Ridomil MZ 72 wp as control. Three replicates (pots, 35cm in diameter) were used for each treatment, five seedlings were transplanted per pot. Data of disease incidence (mean number of spots/leaf) and severity (percent of surface infected area) were recorded before

complete sunrise and after 7days from the last spray according to the scale reported by Horsfall and Barratt (1945) and Biswas *et al.*(1992):

**C. Microscopic studies:**

Microscopic studies were carried out on the aforementioned treated onion leaves to determine the effect of different tested microorganisms on sporangiophore and sporangia (conidia) of the causal agent of onion downy mildew disease according to Falk et al.(1995). Using stereomicroscope, 50 x magnification power. The percentage of the sporangiophores which had collapsed were estimated by recording the number of erect sporangiophores present in different ten fields of the microscope. Sporulation was also assessed as the number of sporangia on ten erect sporangiophores and the percentage of inhibited sporulation were calculated. Each treatment was represented with different ten tested lesions taken randomly from 10 leaves on 10 plants.

**D. Identification:**

All previously isolated microorganisms from leaves of onion plants were grouped as fungi, yeasts, bacteria and actinomycets with preliminary description was generally done, moreover, accurate and precise identification to the probable genus and species was conducted especially for the effective isolates. The identification were achieved with the guidance, of Parry *et al.*, (1983) and Bergy's Manual of Systematic Bacteriology (1984) and Domsch *et al.*, (1980).

**E. Effect of temperature on growth of the antagonistic isolates:**

30ml in mineral medium supplemented with 20gm glucose/L for fungal isolate and 10gm/L for bacterial isolates were used to determine the optimum temperature for the antagonistic isolates. The growth was determined as dry weight of biomass (gm/30ml).

**F. Antagonism between the antagonistic isolates:**

This laboratory experiment was carried out to study the possibility of using combinations between the tested bacterial isolates and *T. harzianum*. Nutrient agar plates were streaked by inoculum, 48 h old culture, from each tested bacterial antagonistic isolate. The inhibition zone was measured between the isolates at

30°C after 3 days of incubation period. Three replicates were used for each isolate. On the other hand, PDA plates were inoculated at the center with a disc, 5 mm in diameter, at the margins of actively growing cultures, 7 days old cultures of *T. harzianum*. A loopful from each bacterial antagonistic isolate was put at the periphery of the plates. Plates were incubated at 30°C for 7 days and then inhibition zone was recorded. Three replicates were used.

**Biological control of onion downy mildew under greenhouse conditions:**

Greenhouse experiment was used efficient bioagents in controlling this disease on plants of two onion cvs., Balady and Giza 20. Onion plants were transplanted in pots(35 cm in diameter) at 15th November during season 2004/2005. Different bioagents preparations ($10^8$ cfu/ml) were used on onion plants grown under naturally infected with downy mildew disease under greenhouse conditions. Cultures were amended with calculated aliquots of an adhesive surfactant (New-Film 1265 registered by Ministry of Agric. and lands Reclamation, Egypt) as recommended (30ml/100L) and hand homogenized before fine spraying on the experimental plants (Abd El-Moneim, 2001). Plants were sprayed with each of the aforementioned treatments after approximately two months from transplanting, since the first signs of the symptoms were observed. For control treatments, plants were sprayed with water and another treatments with Ridomil MZ 72 wp as fungicide. Three replicates (pots) were used for each treatment, Pots were transplanted by using five seedlings per pot. Disease incidence and severity were determined before complete sunrise and after 7days from the last spray according to the scale adopted by Horsfall and Barrett (1945) and Biswas *et al.*, (1992) as mentioned before.

**Biological control of onion downy mildew under field conditions:**

The efficacy of the efficient biocontrol agents [*Trichoderma harzianum, Bacillus subtilis* (three isolates) and *Pseudomonas* sp.(one isolate)] were evaluated for controlling downy mildew on two onion plants of cultivar, Balady and Giza 20 under field conditions during two seasons, 2005/2006-2006/2007. Mixture of these bacterial isolates was used at ratio 1:1:1:1 v/v from the original concentration ($10^8$ cfu/ml) because these isolates did not exhibit any antagonism between them. On the other hand, *T.*

*harzianum* was not mixed with the bacterial isolates because they showed antagonism to it. Plots of 2m long and 1m width were transplanted at 15th November with fifty seedlings/plot. Each treatment was represented by 3 plots. The biocontrol agents and the chemical fungicide Ridomil MZ 72 wp were applied in the same manner conducted in pot experiments and also disease incidence and severity were determined seven days after the last spray following the same procedures used for the greenhouse experiment.

**Effect of time and number of applications with different preparations on onion downy mildew**

To determine the effect of time in weeks and number of applications on the incidence and severity of downy mildew on the two used onion cvs., either under greenhouse or field conditions, onion plants were classified into three groups. The first group received one spray per week, the second group received one spray every two weeks and the third group was used as control ( sprayed with water ).

***Statistical analysis:*** The obtained data were subjected to the proper statistical procedures for analysis of variance according to Duncan (1954) and Gomez and Gomez (1984).

## RESULTS

**Laboratory studies**

**A. Isolation from the phylloplane of onion plants**

Isolation of microorganisms was carried out using healthy onion leaves, were collected from onion fields suffering from infection by downy mildew in Kafr El-Sheikh and El-Gharbia Governorates, Egypt. Isolation trials resulted various microbial isolates comprising fungi, yeasts, general bacteria (positive and negative gram bacteria) and actinomycetes. The predominant groups were bacteria (70%), followed by fungi (25%), and the lowest percentage was for either the yeast and actinomyecetes (5%).

**B. Screening and identification of the isolated microorganisms for antagonism against onion downy mildew.**

The preliminary screening of the obtained microorganisms to inhibit downy mildew disease on potted onion plants showed variable effect. The most effective fungal isolate was the isolate No. 36 which was identified as *Trichoderma harzianum*(isolate No.8). It inhibited disease severity by 27%. The other isolated fungi which didn't inhibit downy mildew on onion plants were identified according to their

morphological characteristics as *Penicillium* sp., *Alternaria* sp., *Stemphyllum* sp., *Nigrospora* sp., *Aspergillus* sp., *Cladosporium* sp., *Botrytis* sp. *Mucor* sp. and *Rhizopus* sp. Similarly, all isolated yeasts didn't inhibit downy mildew on onion plants. Concerning the bacterial isolates, four bacterial isolates identified as *Bacillus subtilis,* isolates No. 23, 41 and 67 (Table,1) and *Pseudomonas* sp., isolate No. 14 (Table,2). These isolates were inhibit disease incidence by 32, 31, 31 and 37%, respectively compared with check treatment (treated with tape water). On the other hand, no antagonistic activities were detected by the rest bacterial isolates as well as actinomycetes against downy mildew disease on onion plants.

**Table (1) Morphological and physiological characteristics of the bacterial antagonistic isolates (No. 23,41 and 67)**

| Test | Result (Isolates Nos. 32,41 and 67) |
|---|---|
| Shape of cell | Rods |
| Sporulation, Spore shape | + |
| Motility | Motile |
| Gram reaction | + |
| Anaerobic growth | - |
| Lecithinase reaction (Lv reaction) | - |
| Citrate utilization | + |
| V.P. reaction | + |
| Nitrate reduction | + |
| Indole production | - |
| Growth in 7%NaCl | + |
| Starch hydrolysis | + |
| Casein hydrolysis | + |
| Gelatin hydrolysis | + |
| Catalase reaction | + |
| Urease activity | + |

\+ positive - negative

**Table (2) Morphological and physiological characteristics of the bacterial antagonistic isolate (No. 14)**

| Test | Result (Isolate No. 14) |
|---|---|
| Shape of cell | Short rods |
| Sporulation | Non – Sporformer |
| Motility | Motile |
| Gram reaction | - |
| Anaerobic growth | - |
| Pigment production | + |
| Oxidase test | + |

\+ positive - negative

**C. Microscopic studies**

Onion downy mildew caused by *Peronospora destructor* on treated leaves with the efficient used biocontrol agents as well as those treated with Ridomil were examined using light microscope. Downy mildew signs were observed by unaided eye as fade colonies with lack appearance of sporangia. In the same time, microscopic examination revealed dead, lysis of sporangiophores and sporangia of the tested fungal pathogen.

**D. Effect of temperature on growth of the antagonistic isolates**

The effect of different Temperatures on growth of the antagonistic bacterial and fungal isolates ( in mineral medium supplemented with glucose) is shown in Fig.(1). A temperature of 30°C appears to be the optimum for all isolates. All isolates grew at 18°C but all isolates did not grew at 45°C. this indicates that, all isolates comprising the fungal and bacterial isolates are mesophilic microorganisms and in the same time they can producing their metabolites under these conditions. On the other hand, they can grow under psycrophilic conditions and this allow to protect the onion plants under the either condition by producing their metabolites.

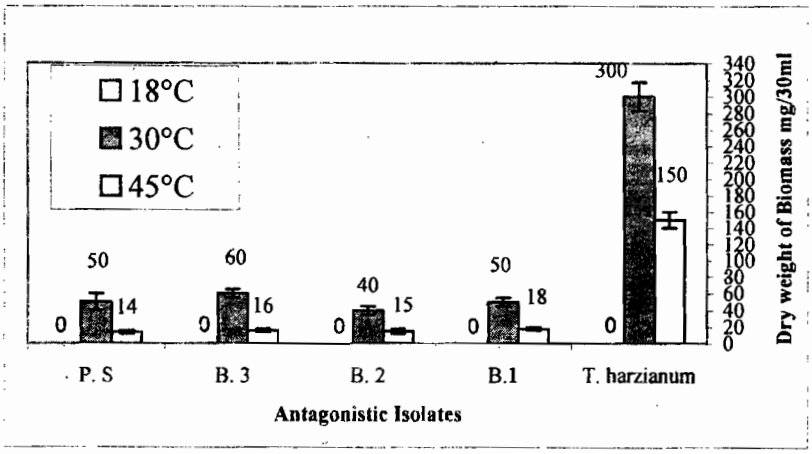


**Fig (1). Effect of Temperature on growth of the antagonistic bacterial and fungal isolates**

**P.s** = *Pseudomonas* sp.(isolate No.14), **B.3** = *Bacillus subtilis* (isolate No.23), **B.2**= *Bacillus subtilis* (isolate No.41) and **B.1**= *Bacillus subtilis* (isolate No.67),

**E. Antagonism between the antagonistic isolates**

It was not observed any inhibition zone between the used antagonistic bacterial isolates. This finding allow to use these bacterial

isolates in a single or a mixture to control downy mildew on onion plants. It can also explain that metabolites of these antagonistic bacterial isolates have not effects on the bacterial organisms. On the other hand, the results in Table ( 3 ), show that the aforementioned used 4 antagonistic bacterial isolates inhibited growth of *T. harzianum.* Therefore, it can not be use the antagonistic bacterial isolates and *T. harzianum* in a mixture to control downy mildew disease of onion.

**Table (3) Inhibition of *T. harzianum* by antagonistic bacterial isolates**

| Antagonistic bacterial isolates | Inhibition zone for *T. harzianum* (mm) |
|---|---|
| *Bacillus subtilis* (No.23) | 25 d |
| *Bacillus subtilis* (No.41) | 22 c |
| *Bacillus subtilis* (No.67) | 16 b |
| *Pseudomonas sp.* (No.14) | 8 a |

In the same column, means followed by the same letter are not significantly different according to DMRT

**Biological control of onion downy mildew under greenhouse conditions**

In this study three isolates of *B. subtilis*, one isolate of *Pseudomonas* sp. and *T. harzianum* were used to control downy mildew on two onion cultivars, Balady and Giza 20 under greenhouse conditions.

Data presented in Tables 4 and 5 indicated that, all tested biocontrol agents significantly decreased disease incidence and disease severity on the two used onion cultivars under greenhouse conditions. Data also show that the highest reduction of disease incidence and severity was observed when mixture of isolates no.23,41 and 67 of *Bacillus subtilis* and isolate no.14 of *Pseudomonas sp.* was sprayed on onion plants every week after approximately two months from transplanting, since the first signs of the symptoms were observed. This treatment significantly decreased disease incidence and severity on Balady cv. from 5.2 – 1.7, 42.1 – 4.0% when sprayed weekly and from 5.2 – 2.6%, 42.1 – 7.0% when sprayed every two weeks respectively (Table, 4 ). Similar effect was recorded on Giza 20 cv., (Table, 5). These results were similar more or less to those obtained from treatment with the fungicide Ridomil MZ 72 wp. However *T. harzianum* was the least effective bioagents in this respect. Results also showed that onion cv., Giza 20 was less susceptible to downy mildew disease than Balady cv., since the incidence and severity on Giza 20 were 4.5, 27.0% respectively compared with 5.2, 42.1% on Balady cv.

**Table (4) Effect of the tested antagonistic microorganisms on downy mildew disease incidence and severity on onion Balady cv. under greenhouse conditions during season 2004/2005**

| Isolated microorganisms | *Disease incidence | | **Disease severity | |
|---|---|---|---|---|
| | Sprayed weekly | Sprayed every two weeks | Sprayed weekly | Sprayed every two weeks |
| *T. harzianum* (No.8) | 3.1 d | 3.5 c | 10.2 f | 13.4 f |
| *Bacillus subtilis* (No.23) | 2.5 b | 2.9 ab | 6.4 c | 8.8 d |
| *Bacillus subtilis* (No.41) | 2.7 c | 3.0 b | 7.6 e | 9.4 e |
| *Bacillus subtilis* (No.67) | 2.6 bc | 3.0 b | 7.0 d | 9.4 e |
| *Pseudomonas* sp. (No.14) | 2.2 b | 2.8 a | 5.4 b | 8.2 c |
| Mix. of isolates nos.23,41,67 and 14 | 1.7 a | 2.6 a | 4.0 a | 7.0 a |
| Fungicide (Ridomil) | 1.8 a | 2.7 a | 4.2 a | 7.6 b |
| Water(Control) | 5.2 e | 5.2 d | 42.1 g | 42.1 g |

In the same column, means followed by the same letter are not significantly different according to DMRT

*Disease incidence = Mean number of downy mildew spots/onion leaf

**Disease severity = percent of surface infected area

**Table ( 5 ) Effect of the tested antagonistic microorganisms on downy mildew disease incidence and severity on onion Giza 20 cv. under greenhouse conditions during season 2004/2005**

| Treatments | *Disease incidence | | **Disease severity | |
|---|---|---|---|---|
| | Sprayed weekly | Sprayed every two weeks | Sprayed weekly | Sprayed every two weeks |
| *T. harzianum* (No.8) | 2.1 c | 2.7 d | 5.0 d | 7.6 f |
| *Bacillus subtilis* (No.23) | 1.8 b | 2.2 b | 4.2 b | 5.4 c |
| *Bacillus subtilis* (No.41) | 1.9 b | 2.3 c | 4.4 c | 5.7 d |
| *Bacillus subtilis* (No.67) | 2.1 c | 2.1 b | 5.0 d | 5.0 b |
| *Pseudomonas* sp. (No.14) | 1.5 a | 2.5 c | 3.5 a | 6.4 e |
| Mix. of isolates nos.23,41,67 and 14 | 1.4 a | 1.8 a | 3.3 a | 4.2 a |
| Fungicide (Ridomil) | 1.8 b | 1.9 a | 4.2 b | 4.4 a |
| Water(Control) | 4.5 d | 4.5 e | 27.0 e | 27.0 g |

In the same column, means followed by the same letter are not significantly different according to DMRT

*Disease incidence = Mean number of downy mildew spots/onion leaf

**Disease severity = Percent of surface infected area

**Biological control of onion downy mildew under field conditions**

Under field conditions the tested bacterial and fungal bioagents were used for controlling onions downy mildew on the

two cultivars (Balady and Giza 20) during two seasons 2005/2006-2006/2007.

Data presented in Tables (6 and 7) show similar results to those obtained under greenhouse conditions. Since the least disease incidence and severity were recorded on the two used onion cultivars when onion plants sprayed with the bacterial mixture either sprayed weekly or every two weeks during the two successive seasons. Also, *T. harzianum* proved to be the least effective bioagent. However, Balady cv., was more susceptible to infection with the disease than Giza 20 under field conditions. In the same time disease incidence and severity were higher on the two used onion cultivars under field conditions than under greenhouse conditions.

**Table ( 6 ) Effect of the tested antagonistic microorganisms on downy mildew disease incidence and severity on onion Balady cv. under field conditions during two seasons, 2005/2006-2006/2007**

| Treatments | *Disease incidence | | | | **Disease severity | | | |
|---|---|---|---|---|---|---|---|---|
| | Sprayed weekly | | Sprayed every two weeks | | Sprayed weekly | | Sprayed every two weeks | |
| | 1st Season | 2ND Season | 1st Season | 2ND Season | 1st Season | 2ND Season | 1st Season | 2ND Season |
| *T. harzianum* (No.8) | 4.3c | 4.2c | 4.6c | 4.5c | 23.7e | 22.0e | 29.1d | 27.0e |
| *B.subtilis* (No.23) | 3.4a | 3.6a | 3.6a | 3.5a | 12.6c | 11.8c | 14.5b | 13.4c |
| *B.subtilis* (No.41) | 3.4a | 3.5a | 3.5a | 3.4a | 12.6c | 11.0b | 13.4a | 12.6b |
| *B.subtilis* (No.67) | 3.5b | 3.6a | 3.6a | 3.7b | 13.4d | 12.6d | 14.5b | 15.5d |
| *Pseudomonas* sp. (No.14) | 3.3a | 3.7b | 3.7b | 3.5a | 11.8b | 11.0b | 15.5c | 13.4c |
| Mix. of 23,41 , 67 and 14 | 3.2a | 3.6a | 3.6a | 3.4a | 11.0a | 10.2a | 14.5b | 12.6b |
| Fungicide (Ridomil) | 3.4a | 3.5a | 3.5a | 3.3a | 12.6c | 10.2a | 13.4a | 11.8a |
| Water(Control) | 6.6d | 6.4d | 6.6d | 6.4d | 74.7f | 70.9f | 74.7e | 70.9f |

In the same column, means followed by the same letter are not significantly different according to DMRT

*Disease incidence = Mean number of downy mildew spots/onion leaf

**Disease severity = Percent of surface infected area

**Table (7) Effect of the tested antagonistic microorganisms on downy mildew disease incidence and severity on onion Giza20 cv. under field conditions during two seasons, 2005/2006-2006/2007**

| Treatments | *Disease incidence | | | | **Disease severity | | | |
|---|---|---|---|---|---|---|---|---|
| | Sprayed weekly | | Sprayed every two weeks | | Sprayed weekly | | Sprayed every two weeks | |
| | 1st Season | 2ND Season | 1st Season | 2ND Season | 1st Season | 2ND Season | 1st Season | 2ND Season |
| *T. harzianum* (No.8) | 4.2e | 4.1f | 4.3c | 4.4d | 22.0g | 20.4g | 23.7f | 25.4f |
| *B.subtilis* (No.23) | 2.8c | 2.7d | 2.9a | 3.0b | 8.2d | 7.6d | 8.8b | 9.4c |
| *B.subtilis* (No.41) | 3.0c | 2.9e | 3.1b | 3.3c | 9.4e | 8.8e | 10.2d | 11.8d |
| *B.subtilis* (No.67) | 3.1d | 3.0e | 3.3b | 3.4 | 10.2f | 9.4f | 11.8e | 12.6e |
| *Pseudomonas* sp. (No.14) | 2.5b | 2.4c | 3.0a | 3.0b | 6.4c | 6.0c | 9.4c | 9.4c |
| Mix. of 23,41 , 67 and 14 | 2.0a | 2.1b | 2.9a | 2.8a | 4.7b | 5.0b | 8.8b | 8.2b |
| Fungicide (Ridomil) | 1.9a | 1.8a | 2.8a | 2.7a | 4.4a | 4.2a | 8.2a | 7.6a |
| Water(Control) | 5.6f | 5.7g | 5.6d | 5.7e | 51.7h | 54.4h | 51.7g | 54.4g |

In the same column, means followed by the same letter are not significantly different according to DMRT

*Disease incidence = Mean number of downy mildew spots/onion leaf

**Disease severity = Percent of surface infected area

**Effect of time and number of applications with different preparations on onion downy mildew**

The effect of time in weeks between two applications either under greenhouse or field conditions on the efficacy of used biocontrol agents was studied.

In this study data represented in tables 4,5,6 and 7 indicate that there were negative correlation between length of interval period and efficacy of all used antagonists on disease under study. Since all antagonists showed the highest effect when treatments repeated every week.

## DISCUSSION

Recently, man realized that using highly toxic substances in agriculture led to great disturbance in biological ecosystem and led to accumulation of toxic chemicals in human food chain (Manon, 1998). The present work was planned to reduce using of toxic

chemicals in agriculture processes and find out the most suitable non chemical method to protect onion plants against downy mildew disease.

In this study, data obtained showed that all used biocontrol agents significantly reduced both disease incidence and severity on onion plants. Mixture of isolates no.23,41and 67 of *B. subtilis* and isolate no. 14 of *Pseudomonas* sp. were the most effective since they significantly reduced downy mildew compared with the other treatments. This effect may be due to that *Pseudomonas* sp. can inhibit the disease through more than one mode of action, for example it cleats iron and prevents other organisms to utilize this element. As a result of iron starvation the pathogen cannot grow, penetrate and cause disease (Loper 1988). Also, *Pseudomonas* sp. produces some antifungal substances, i.e. pyrrolnitrin, pyoluteorin and 2,4 diacetyploroglucinol (Duffy and Defago, 1997 and Sharifi *et al.,* 1998). It is suggested that the principal mechanism of *B. subtilis* isolates is inhibition of the fungus by production of antibiotics. Kowall *et al.*, (1998) reported that the antibiotics surfactin, fengycin, mycosubtilin and bacillomycin are produced by *B. subtilis*. These compounds are amphilic, membrane active surfactants and peptide antibiotics with specific antimicrobial potential. Surfactin and pumilacidin are bioactive cyclic peptides produced by some Bacillus isolates (Morilkawa *et al.,* 1992, Peltola *et al.,* 2001 and McGrath,2004). The synergistic effect of mixture might be due to complementary effect between different isolates included in the mixture (Abd El-Moity, 1985). Although the least effective treatment was *T. harzianum*, it significantly reduced disease incidence and severity compared to control treatments. This can be explained by Abd El-Moity (1981), who stated that *T. harzianum* works through different mechanisms, i.e. production of gliotoxin, mycoparasitism and grows very fast and acts as barrier between susceptible plant tissues and virulent pathogens.

Length of intervals between two applications has a significant effect on the plant health. Results showed that, onion plants received one spray every week were more healthy compared to plants received the same treatment every two weeks. This might be due to some of these antagonists when sprayed on plant surface, prior to infection led to stimulate plant resistance and enforce treated plants to produce some metabolites which depress the pathogen (Bolar *et al.,* 2000 and Howell *et al.,* 2000)

In this study, the two tested onion cultivars varied in their reaction to downy mildew disease. Giza 20 was less susceptible to downy mildew than balady cv.. This might be due to that some physiological factors encourages and permits pathogen to invade and causes high percentage of disease severity (Neykov and Dobrev, 1988). Variation in susceptibility or resistance of hosts was reported by many authors (Thomas, 1986 and El-Shenawy *et al.*, 1987). Source of variation might be due to morphological and /or physiological differences (Cill and Mandapuri, 1978).

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## الملخص العربي

# المقاومة الحيوية للبياض الزغبى على البصل

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يعتبر مرض البياض الزغبى من أخطر الأمراض التى تصيب نباتات البصل وخاصة عندما تزرع تحت ظروف الجو البارد الرطب ويتسبب المرض عن الفطر بيرونوسبورا دستراكتور *Peronospora destructor*.

تم عزل حوالى ٧٢ عزلة من الكائنات الدقيقة من على سطح أوراق نباتات بصل سليمة نامية خلال حقول بصل مصابة بالبياض الزغبى فى محافظتى كفرالشيخ والغربية ووجد أن ٧٠% منها كانت بكتيريا و٢٥% فطريات و٥% خمائر.

أشارت النتائج الأولية لاختبار كفاءة هذة الميكروبات في مقاومة المسبب المرضى للبياض الزغبى على البصل والتي أجريت تحت ظروف الصوبة الزجاجية أن هناك أربعة بكتيريات وفطر واحد فقط قد أظهرا كفاءة في مقاومة المسبب المرضى موضع الدراسة. وقد أوضحت الدراسات الميكروسكوبية باستخدام الميكروسكوب الضوئي أن رش أوراق البصل المصابة بالبياض الزغبى بالميكروبات المضادة سواء بكتيريا أو فطر قد أدى إلى تحلل وموت للحوامل الاسبورانجية والحوافظ الجرثومية الفطر الممرض .

أثبتت اختبارات التعريف للعزلات البكتيرية والتي أظهرت كفاءة في التأثير على الفطر الممرض أنها تنتمي إلى الجنس باسيللس *Bacillus subtilis* (ثلاث عزلات أرقام ٢٣و ٤١و٦٧) والجنس سيدوموناس *Pseudomonas* sp. (عزلة واحدة رقم رقم ١٤) فى حين وجد أن عزلة الفطر رقم ٨ عبارة عن تريكودرما هاريزيانم *Trichoderma harizianum*.

تمت الاختبارات على مستوى الصوبة والحقل بصوبة ومزرعة كلية الزراعة جامعة كفر الشيخ باستخدام صنفين من البصل والمنزرعين فى المنطقة وهما البلدي وجيزة ٢٠ لاختبار مدى كفاءة العزلات المضادة سواء للوقاية من أو مقاومة مسبب البياض الزغبى على البصل. وقد أجرى الرش بمعلق جراثيم الفطر تريكودرما هارزيانم أو بمعلق خلايا البكتيريا المضادة إما على صورة عزلات فردية أو على صورة مخلوط من عزلات البكتيريا الأربعة أسبوعيا أو كل أسبوعين بمجرد ظهور أول عرض للبياض الزغبى على نباتات البصل فى حين استخدم مبيد الريدوميل للمقارنة.

أثبتت النتائج سواء على مستوى الصوبة أو الحقل أن رش نباتات البصل بالعزلات السابقة قد أدى الى خفض معنوي كبير فى عدد تبقعات البياض الزغبى على النباتات المصابة وكذلك فى المساحة المصابة من الأوراق وكانت أفضل النتائج عندما استخدم مخلوط البكتيريات ( ثلاث عزلات من الباسيللس وعزلة من السيدوموناس) وكانت النتائج مشابهة تقريبا للمتحصل عليها نتيجة الرش بمبيد الريدوميل كما أن هذة النتيجة كانت قريبة أو مشابهة للمتحصل عليها من المعاملة بعزلة البكتيريا سيدوموناس بصورة مفردة. وكانت أفضل النتائج عندما كان الرش أسبوعيا عنة عندما كان كل أسبوعين. وقد أشارت النتائج كذلك أن الصنف جيزة٢٠ كان أقل قابلية للإصابة بالمرض عن الصنف بلدي.

وبصفة عامة فان النتائج تشير الى إمكانية استخدام العزلات البكتيرية المستخدمة فى الدراسة سواء فى صورة فردية أو على صورة مخلوط فى مقاومة مرض البياض الزغبى على البصل كوسائل آمنة و بديلة للمبيدات وخاصة عندما ترش أسبوعيا على النباتات.