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# BIOLOGICAL CONTROL OF WHEAT LEAF RUST USING *PSEUDOMONAS FLUORESCENS* AND *BACILLUS* SPP

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**ABSTRACT**: **The effect of *Pseudomonas fluorescens*, *Bacillus polymyxa*, *Bacillus circulance* and their mixture were evaluated as a bioagents on leaf rust disease of Giza-139 wheat cultivar caused by *Puccinia triticina* f.sp. *tritici*. In greenhouse the bioagents were used as suspension for soaking wheat grains (24 h before sowing) and for spraying seedlings (seven days old) with each bioagent singly or mixture of two of them. Experiments were carried out on both seedling and adult stages. In greenhouse experiments, the incubation period was increased from 8.0 days (in control plants) to 10.33 days when wheat seedlings were sprayed with *Pseudomonas fluorescens*, 72 hour after inoculation. Biocontrol agents used in this study caused significant decrease in No. of pustules/leaf. The inhibition effect of bioagents in reducing disease components extended to the significant reduction in infection type, compared with the control. The highest percentage of disease reduction (54.02%) was obtained by soaking seeds in *Bacillus circulans* followed by *Pseudomonas fluorescens* (48.0%). However, in field experiments, *Pseudomonas fluorescence* significantly reduced rust severity leading to an increase in weight of 1000 kernel as well as yield plot, compared with the control.**

**Key words: *Puccinia triticina* f.sp. *tritici*, wheat, biological control.**

## INTRODUCTION

Wheat in Egypt is liable to attack by many diseases such as rust, smut, mildew and some other minor diseases. Till now wheat rusts are still the main biotic stress, that

limited the productivity of most of wheat cultivars, under the Egyptian environmental conditions.

Biological control of plants using microorganisms is a very promising alternative to the extended use of fungicides, which are often expensive and accumulate in plants, having adverse effects on humans. Several *Bacillus* spp. are antagonistic to plant pathogenic fungi and bacteria. *Bacillus* spp. produced at least 66 different antibiotic compounds (Ferreira *et al.*, 1991). Many plant species stimulate and support populations of rhizosphere bacterial (rhizobacteria) as a first line of defense against soil borne plant pathogens (Cook *et al.* 1995).

*Pseudomonas aureofaciens* 63-28 is antagonistic to several plant pathogenic fungi, including *Pythium* spp. The bacterium produced at least four antifungal metabolites active against *Pythium ultimum* and *Phytophthora cryptogea*. Two of these compounds were identified as the novel butyllactones (Z)-4 hydroxy-4- methyl-2- (1-hexenyl)-2-butenolide and (Z)-4-hydroxy-methyl-2-(1-hexenyl)-2-butenolide by using nuclear magnetic resonance (NMR) technique and mass spectroscopy (MAS). All compounds were different from other antibiotics produced by *Pseudomonas* spp. including pyoluteorin, pyrrolritrin and 2,4-diacetylphloroglucinol as determined by high performance liquid chromatography (HPLC) technique (Gamard *et al.*, 1997).

Several mechanisms of endophyta activity of plant pathogen are known, including production of antifungal compounds, siderophore production, nutrient competition, and the induction of systemic resistance (Chen *et al.*, 1995). The induction of systemic resistance was the main mechanisms of activity on plant. Phytoalexin are formed in plant tissues which are important in resistance to diseases and pests (Ramamoorthy *et al.*, 2001).

Phloroglucinols (PG) are phenolic, secondary metabolites produced by plants, algae and bacteria (Ishiguro *et al.*, 1998; Bangera & Thomashow, 1999; and Bokesch *et al.*, 1999). More than 60 PG derivatives has been described and were reported to have anti-viral, anti-microbial, insect and mammal antifedant, phytotoxic, antioxidant, cytotoxic, antitumer plant growth regulating activities (Debabrata and Naik, 2000).

Twenty strains of *Pseudomonas fluorescens* were evaluated for their potential in promoting plant growth

and in the control of late leaf spot disease caused by *Cercosporidium personatum* in ground nut, under greenhouse conditions. Seed treatment with *P. fluorescens* strain Pf1 significantly controlled leaf spot disease of ground nut and increased plot yield. When the treated seeds were sown in soil, the antagonist colonized well in the ground nut rhizosphere *P. fluorescens* Pf1 showed the maximum production of indole acetic acid *in vitro*. (Zhang *et al.*, 2006). *Pseudomons chlororaphis* PA23, *Pseudomonas* spp. strain DF41 and *Bacillus amyloliquefaciens* B56 conseistently inhibit infection of canola petels by *Sclerotinia sclerotiorum* in both greenhouse and field experiments. *Pseudomonas chlororaphis* contains biosynthetic genes for phnazine-1-carboxylic acid and pyrrolnitrin (Zhang *et al.*, 2006).

Therefore other safe methods in plant protection are required. Among these methods, the use of biological control is very effective (Abd Allah *et al.* 1997; Sallam, Minaas, 1997, 2001 and Krzysztofal *et al.*, 2007).

This work was undertaken to investigate the effect of fluorescent *Pseudomonas*, *Bacillus polymxa* and *Bacillus circulance* on process associated with the development of leaf rust of wheat caused by *Puccinia triticina* f.sp. *tritici,* in both seedling and adult stages.

## MATERIALS AND METHODS

The most susceptible wheat cultivar namely Giza-139 was used in these experiments. The cultivar grains were kindly obtained from Crops Research Institute, ARC, Giza, Egypt.

*Bacillus* sp. and *Pseudomonas fluorescens* were grown on nutrient glucose broth (NGB) medium for 48 h. The bacterial suspension (1 : 1) was adjusted to be contain 30 x $10^6$ cfu/ml by using a mechanical shaker. Mixture of bacteria was also adjusted to be contain 30 x $10^6$ cfu/ml.

### Seedling Stage Experiments

Freshly collected urediospores of *Puccinia triticina* f.sp. *tritici* (race 77) were kindly provided by Wheat Dis. Res. Dept., Pl. Pathol. Res. Inst., ARC (the virulent strain) were used as inoculum.

Seven days old wheat seedlings were divided into two groups. The first group was sprayed with *Pseudomonas fluorescens*, *Bacillus polymyxa*, *Bacillus circulance* and a mixture including the previous three strains, 24, 48 and 72 hours before

inoculation. While, the second group was sprayed with the previously illustrated bacterial strains, 24, 48 and 72 hours after inoculation.

Time from inoculation to commencement of sporulation, incubation period (IP) was recorded according to Katsuya and Green (1967). Plants were kept under daily observation till 50% of the pustules were erupted to estimate the latent period (LP) according to Parlevliet (1975). Infection types and number of pustules/cm$^2$ were determined by the method reported by Stakman *et al.* (1962).

Wheat grains were soaked for 24 hrs. in the tested solutions of bioagents then sown in pots. Grains soaked in tape water for the same period served as check treatments. Seven days old seedlings were inoculated with race (77) of *Puccinia triticina* f.sp. *tritici*. The above mentioned disease components were estimated.

## Field Experiments

Field experiments were carried out at Etai El-Baroud Res. in two successive growing seasons (2006-2007 and 2007-2008). Area of each experiment was divided into plots (3 x 2 m) containing 5 rows of 3.0 m long with 30 cm between rows. Each row was sown by 5 g wheat seeds (Giza 139 susceptible wheat cultivar).

Artificial inoculation was carried out in boating stage (70 days old) as mentioned by Large (1954). Plants dusted with urediospores talk mixture (1:20) using a baby cyclone (Tervet and Cassel, 1951). Giza 139 wheat cultivar was sprayed with *Pseudomonas*, *Baccilus polymyxa* and their mixture. The tested strains were sprayed before and after inoculation by 24 hours. Three plots were used as replicates for each one. Untreated plants were sown in three plots and used as infected control plants (diseased plants). However, three plots treated with sumi-8 (0.35 ml/l) fungicide were used in this experiment to serve as control treatment (healthy plants).

Rust severity was recorded using modified Cobb's scale (Peterson *et al.*, 1948) during the course of the disease cycle. Also, area under disease progress curve (AUDPC) was calculated using a simple formula adopted by Pandey *et al.* (1989) as follows;

$$\mathbf{AUDPC = D\ [1/2\ (Y_1 + Y_k) + (\ Y_2 + Y_3 + \ldots\ldots\ Y_{k-1})]}.$$

where; D = time intervals

$Y_1 + Y_k$ = sum. of the first and last disease scores

$Y_2 + Y_3 + Y_{k-1}$ = sum. of all in between disease scores

Statistical analysis was carried out using the procedures "ANOVA" (Snedecor and Cochran 1980).

## RESULTS AND DISCUSSION

The effectiveness of using different bioagents on components of wheat leaf rust disease caused by *P. triticinia* f.sp. *tritici* was evaluated under greenhouse and filed conditions.

### Greenhouse Experiments

Foliar spraying of wheat seedlings (before and after inoculation) and seed soaking experiments (24 hour before cultivation) were carried out under greenhouse conditions.

Data in Table 1 indicated that, all studied bioagents led to significant increase in incubation period when sprayed onto wheat seedlings. Also, spraying wheat seedlings with different bioagents gave a significant differences when the time of spraying (before or after inoculation) was taken in consideration. This might be due to that treatment with biopreparation induce systemic resistance as the main mechanism of activity on a plant (Urszula *et al.*, 2004; Ramamoorthy *et al.*, 2001). The best result was obtained when wheat seedlings were sprayed with *P. fluorescens* (72 hour after inoculation) where the incubation period was increased from 8.0 days (in control plants) to 10.33 days. This effect might be due to *P. fluorescens* produce different types of antibiotics including active 2,4 diacetyl-phloroglucinole (2,4 DAPB) which control diseases (Lenda *et al.*, 2003 and Desuza *et al.*, 2003).

Data in Table 2 revealed that, the most prolonged latent period (13.67 days) was obtained by spraying wheat seedlings with *P. fluorescent* (72 hour after inoculation), compared with control treatments (10 days). This might be due to that *P. fluorescens* has several methods to control the disease such as production of antifungal compounds including siderophre production, nutrient competition and the induction of systemic resistance (Chen *et al.*, 1995; Ramamoorthy *et al.*, 2001 and Urszula *et al.*, 2004).

**Table 1. Effect of spraying wheat seedlings (Giza-139) with different bioagents and their mixture, before and after inoculation with *Puccinia triticina* f.sp. *tritici* race (77), on the incubation period**

| Treatments | Before inoculation (h.) | | | | After inoculation (h.) | | | | Total Mean |
|---|---|---|---|---|---|---|---|---|---|
| | 24 | 48 | 72 | Mean | 24 | 48 | 72 | Mean | |
| Control | 7 | 7 | 7 | 7 | 8 | 8 | 8 | 8 | 7.5 |
| 1. *Pseudomonas flourescens* | 8 | 8 | 8 | 8 | 8 | 9 | 10.33 | 9.11 | 8.56 |
| 2. *Bacillus polymexa* | 8 | 8 | 8 | 8 | 8 | 8.66 | 8 | 8.22 | 8.11 |
| 3. *Bacillus circulance* | 8 | 8 | 8 | 8 | 8 | 8.33 | 8.33 | 8.22 | 8.11 |
| Mixture (1+2+3) | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Mean | 7.8 | 7.8 | 7.8 | | 8 | 8.53 | 8.53 | | |
| Mean | 7.80 | | | | 8.31 | | | | |

| L.S.D. at 5%: | | |
|---|---|---|
| For Inoculation time | (I) | 0.281 |
| Period | (P) | n.s. |
| Treatment | (T) | 0.444 |
| (I × P) | | n.s. |
| (I ×T) | | 1.777 |
| (P × T) | | n.s. |
| (I × P × T) | | n.s. |

**Table 2. Effect of spraying wheat seedlings (Giza-139) with different bioagents and their mixture, before and after inoculation with *Puccinia triticina* f.sp. *tritici* race (77), on the latent period**

| Treatments | Before inoculation (h.) | | | | After inoculation (h.) | | | | Total Mean |
|---|---|---|---|---|---|---|---|---|---|
| | 24 | 48 | 72 | Mean | 24 | 48 | 72 | Mean | |
| Control | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| 1. *Pseudomonas flourescens* | 10.17 | 10.00 | 10.00 | 10.06 | 10.00 | 10.00 | 13.67 | 10.22 | 10.64 |
| 2. *Bacillus polymexa* | 10.67 | 10.33 | 10.00 | 10.33 | 11.33 | 10.33 | 10.67 | 10.77 | 10.56 |
| 3. *Bacillus circulance* | 10.33 | 10.00 | 10.00 | 10.11 | 10.67 | 10.67 | 11.00 | 10.78 | 10.45 |
| Mixture (1+2+3) | 10.00 | 10.67 | 11.00 | 10.56 | 11.00 | 10.67 | 10.33 | 10.67 | 10.61 |
| Mean | 10.23 | 10.20 | 10.20 | | 10.60 | 10.33 | 10.13 | | |
| Mean | 10.21 | | | | 10.35 | | | | |

| L.S.D. at 5%: | | |
|---|---|---|
| For Inoculation time | (I) | 0.079 |
| Period | (P) | 0.096 |
| Treatment | (T) | 0.124 |
| (I × P) | | 0.136 |
| (I ×T) | | 0.176 |
| (P × T) | | 0.215 |
| (I × P × T) | | 0.305 |

It is important to note that, the biocontrol agents caused a significant decrease in No. of pustules/leaf compared with control treatments (Table 3). The significant reduction in No. of pustules/leaf was obtained by spraying seedlings with *P. fluorescens* at 24 hour after or before inoculation treatments. This high potentiality in antagonism might be due to that *P. fluorescens* acts through different mechanisms including production of antifungal substances (Gamard *et al.*, 1997 and Zhang *et al.*, 2006), induction of systemic resistance in the plant and stimulate the plant to form phytoalexin (Ramamoorthy *et al.*, 2001 and Urszula *et al.*, 2004).

The inhibition effect of bioagents in reducing disease components extended to the significant reduction in infection type, compared with control (Table 4). Data in such table revealed also that, there are no significant differences regarding the time of spraying. However, regarding the relation between treatments, significant differences were obtained compared with control.

**Table 3. Effect of spraying wheat seedlings (Giza-139) with different bioagents and their mixture, before and after inoculation with *Puccinia triticina* f.sp. *tritici* race (77), on the No. of pustules/leaf**

| Treatments | Before inoculation (h.) | | | | After inoculation (h.) | | | | Total mean |
|---|---|---|---|---|---|---|---|---|---|
| | 24 | 48 | 72 | Mean | 24 | 48 | 72 | Mean | |
| **Control** | **106.33** | **106.33** | **106.33** | **106.33** | **44.44** | **44.44** | **44.44** | **44.44** | **75.39** |
| *Pseudomonas flourescens* | 2.78 | 20.33 | 42.11 | **21.74** | 1.52 | 5.66 | 4.55 | **3.91** | **12.83** |
| *Bacillus polymexa* | 5.88 | 23.11 | 36.11 | **21.70** | 9.89 | 4.11 | 4.67 | **6.22** | **13.96** |
| *Bacillus circulance* | 19.67 | 4.88 | 28.22 | **17.59** | 9.22 | 2.89 | 3.22 | **5.11** | **11.35** |
| **Mixture (1+2+3)** | 6.67 | 37.67 | 9.89 | **18.08** | 12.22 | 5.33 | 3.89 | **7.15** | **12.62** |
| **Mean** | **28.27** | **38.46** | **44.53** | | **15.46** | **4.49** | **4.15** | | |
| **Mean** | | | **37.09** | | | | **8.03** | | |

| L.S.D. at 5%: | | | |
|---|---|---|---|
| For | Inoculation time | (I) | 0.430 |
| | Period | (P) | 0.527 |
| | Treatment (T) | | 0.681 |
| | (I × P) | | 0.746 |
| | (I ×T) | | 0.962 |
| | (P × T) | | 1.179 |
| | (I × P × T) | | 1.668 |

**Table 4. Effect of spraying wheat seedlings (Giza-139) with different bioagents and their mixture, before and after inoculation with *Puccinia triticina* f.sp. *tritici* race (77), on the infection type**

| Treatments | Before inoculation (h.) | | | | After inoculation (h.) | | | | Total mean |
|---|---|---|---|---|---|---|---|---|---|
| | 24 | 48 | 72 | Mean | 24 | 48 | 72 | Mean | |
| **Control** | **4.00** | **4.00** | **4.00** | **4.00** | **4.00** | **4.00** | **4.00** | **4.00** | **4.00** |
| ***Pseudomonas flourescens*** | 3.33 | 2.67 | 1.67 | **2.56** | 1.67 | 1.67 | 3.00 | **2.11** | **2.33** |
| ***Bacillus polymexa*** | 3.00 | 2.67 | 2.00 | **2.56** | 1.67 | 2.00 | 1.67 | **1.78** | **2.17** |
| ***Bacillus circulance*** | 2.67 | 2.00 | 2.33 | **2.33** | 2.67 | 2.00 | 2.33 | **2.33** | **2.33** |
| **Mixture (1+2+3)** | 1.67 | 2.00 | 1.00 | **1.56** | 3.67 | 2.67 | 2.33 | **2.67** | **2.11** |
| **Mean** | **2.93** | **2.67** | **2.20** | | **2.60** | **2.47** | **2.67** | | |
| **Mean** | | | **2.60** | | | | **2.58** | | |

| L.S.D. at 5%: | | |
|---|---|---|
| For Inoculation time | (I) | n.s. |
| Period | (P) | n.s. |
| Treatment (T) | | 0.399 |
| (I × P) | | 2.820 |
| (I × T) | | 0.365 |
| (P × T) | | n.s. |
| (I × P × T) | | 0.894 |

Data in Table 5 summarized the influence of the used bioagents on No. of pustules/leaf and infection type. Results in such table revealed that, there are significant differences between treatments, in No. of pustules/leaf. On the other hand, non significant differences were recorded, regarding the infection type treatments.

Seed soaking in biocontrol agents yields significant reduction in infection type, latent period and No. of pustules/leaf (Table 6). While, non significant reduction in incubation period was observed. In general, data in Table 6 revealed that, the highest percentage of disease reduction (54.02 %) was obtained by soaking seeds in *Bacillus circulance* followed by *Pseudomonas fluorescens* (48.0%). This reduction might be due to that either *Bacillus circulance* or *Pseudomonas fluorescens* produce antifungal substances. *Bacillus* sp. produces at least 66 different antibiotic compounds (Ferreira *et al.*, 1991) while *Pseudomonas fluorescent* produces pyoluteorin, pyrrolritrin and 2,4 diacetyl phloroglucinol (Duffy and Defego, 1997; Gamard *et al.*, 1997 and Sharifi *et al.*, 1998). These antifungal material inhibit growth of pathogenic fungi consequently reduce the disease symptoms.

**Table 5. Relation between bioagents (*Pseudomonas fluorescent, Bacillus polymexa, Bacillus circulance* and their mixture) and time of spraying (24, 48 and 72 hrs.).**

| Treatments | No. pustules/leaf | | | Infection type | | |
|---|---|---|---|---|---|---|
| | 24 hr. | 48 hr. | 72 hr. | 24 hr. | 48 hr. | 72 hr. |
| Control | 75.39 | 75.39 | 75.39 | 4.00 | 4.00 | 4.00 |
| 1. *Pseudomonas flourescent* | 2.15 | 12.99 | 23.33 | 2.50 | 2.17 | 2.33 |
| 2. *Bacillus polymexa* | 7.89 | 13.61 | 20.39 | 2.33 | 2.33 | 1.83 |
| 3. *Bacillus circulance* | 14.45 | 3.89 | 15.72 | 2.67 | 2.00 | 2.33 |
| Mixture (1+2+3) | 9.45 | 21.50 | 6.89 | 2.33 | 2.33 | 1.67 |
| Mean | 21.86 | 25.48 | 28.34 | 2.77 | 2.57 | 2.43 |
| L.S.D. at 5%: | | | | | | |
| ( P × B) | | 1.179 | | | n.s. | |

**Table 6. Effect of soaking wheat seeds (Giza-139) in different bioagents on incubation period (IP), latent period (LP), No. of pustules/leaf and infection type (IT).**

| | IP | LP | No.P/leaf | IT | Reduction % |
|---|---|---|---|---|---|
| Control | 8.00 | 11.00 | 48.56 | 4.00 | 0.0 |
| 1. *Pseudomonas flourescens* | 8.00 | 10.67 | 25.25 | 2.33 | 48.00 |
| 2. *Bacillus polymexa* | 8.00 | 11.67 | 41.56 | 2.67 | 14.42 |
| 3. *Bacillus circulance* | 8.00 | 11.67 | 22.33 | 3.00 | 54.02 |
| Mixture (1+2+3) | 8.00 | 14.33 | 30.78 | 2.33 | 36.61 |
| L.S.D. at 5%: | n.s. | 1.61 | 2.74 | 0.035 | |

## Field Experiments

Data in Tables 7 and 8 represent the effect of bioagents application on two successive growing seasons (2006-2007 and 2007-2008), under field conditions. Data in such tables showed that, all treatments reduced rust severity. Application of *Pseudomonas fluorescens*, at all studied periods, led to the lowest rust severity, compared with the control. Data also indicated that AUDPC was reduced in all treatments, compared with control. These results might be due to several mechanisms of bioagents known on plant pathogen including production of antifungal compounds such as siderophores production, nutrient competition and the induction of systemic resistance (Ferrire *et al.*, 1991; Chen *et al.*, 1995; Pieterse *et al.*, 2001; Ramamoorthy *et al.*, 2001 and Urszula *et al.*, 2004).

Data given in Tables 9 and 10 represent the effect of applying different bioagents (*Pseudomonas fluorescens*, *Bacillus polymexa* and their mixture) on yield components including spike weight, No. of grains/spike, weight of grains/spike, weight of $10^3$ grains and weigh of plot, compared with untreated and fungicidal treated wheat plants, for 2006-2007 and 2007-2008 growing seasons, respectively. Data in such tables showed significant differences between treatments. Applying *P. fluorescens* led to the highest weight of $10^3$ grains and also the highest weight of yield/plot, compared with infected control plants. This increase of weight might be due to that *P. fluorescens* produces of indole acetic acid as a growth regulator (Meena *et al.*, 2006). *P. fluorescens* produces some antibiotic *i.e.* pyrrolnitrin, pyoluterin and 2,4 diacetyl phloroglucinol. These antibiotics suppress the disease and led to an increase in growth of plant (Sarnigute *et al.*, 1995).

Using biological control agents led to the reduction in the disease components and increased theweight of $10^3$ grains as well as weight of yield/plot.

**Table 7. Effect of bioagent application before and after pathogen inoculation, on rust severity and area under disease progress curve (AUDPC), under field conditions for 2006-2007 growing season.**

| Treatment | Rust severity | | | AUDPC | | |
|---|---|---|---|---|---|---|
| | Before inoculation | After inoculation | Mean | Before inoculation | After inoculation | Mean |
| Control (infected) | 90.00 | 90.00 | 90.00 | 694.25 | 694.25 | 694.25 |
| Control (healthy) | 10.00 | 5.00 | 7.50 | 80.50 | 53.67 | 67.09 |
| *Pseudomonas flourescens* | 50.00 | 50.00 | 50.00 | 425.8 | 425.8 | 425.8 |
| *Bacillus polymexa* | 53.33 | 53.33 | 53.33 | 420.0 | 388.5 | 415.9 |
| Mixture (1+2) | 56.67 | 63.33 | 60.00 | 397.8 | 411.8 | 404.8 |
| Mean | 51.33 | 51.67 | | 403.67 | 394.8 | |
| L.S.D. at 5%: | | | | | | |
| (I) | | 0.099 | | | 0.993 | |
| (T) | | 0.047 | | | 2.84 | |
| (I × T) | | 0.066 | | | 4.011 | |

**Table 8. Effect of bioagent application before and after pathogen inoculation, on rust severity and area under disease progress curve (AUDPC), under field conditions for 2007-2008 growing season.**

| Treatment | Rust severity | | | AUDPC | | |
|---|---|---|---|---|---|---|
| | Before inoculation | After inoculation | Mean | Before inoculation | After inoculation | Mean |
| Control (infected) | 63.33 | 63.33 | 63.33 | 713.33 | 713.33 | 713.33 |
| Control (healthy) | 8.33 | 8.33 | 8.33 | 86.67 | 86.67 | 86.67 |
| 1. *Pseudomonas flourescens* | 16.67 | 20.0 | 18.33 | 133.33 | 175.00 | 154.17 |
| 2. *Bacillus polymexa* | 30.0 | 8.67 | 19.33 | 295.67 | 86.67 | 191.17 |
| Mixture (1+2) | 33.33 | 30.0 | 31.67 | 340.00 | 276.67 | 308.33 |
| Mean | 30.33 | 26.07 | | 313.80 | 267.67 | |
| L.S.D. at 5%: | | | | | | |
| (I) | | n.s. | | | n.s. | |
| (T) | | 7.94 | | | 41.66 | |
| (I × T) | | 11.24 | | | 59.23 | |

**Table 9. Effect of spraying wheat plants with different bioagents before and after pathogen inoculation on yield components, under field conditions, for 2006-2007 growing season.**

| Treatments | Spike wt. (g) | | | No. grains/spike | | | wt. of grains/spike (g) | | | $10^3$ grains wt. (g) | | | Wt. of yield/plot (g) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before | After | Mean | Before | After | Mean | Before | After | Mean | Before | After | Mean | Before | After | Mean |
| Control (infected) | 2.00 | 2.00 | **2.00** | 48.00 | 48.00 | **48.00** | 1.45 | 1.45 | **1.45** | 39.00 | 39.00 | **39.00** | 1840.0 | 1840.0 | **1840.0** |
| Control (healthy) | 2.78 | 2.09 | **2.43** | 44.66 | 49.66 | **47.16** | 1.92 | 2.18 | **2.05** | 41.56 | 43.21 | **42.39** | 1885.0 | 1643.33 | **1764.17** |
| . *P. fluorescens* | 2.88 | 2.78 | **2.83** | 53.66 | 54.00 | **53.83** | 2.12 | 2.07 | **2.09** | 43.33 | 43.70 | **43.52** | 2181.67 | 2100.0 | **2140.83** |
| ?. *B. polymexa* | 3.69 | 2.95 | **3.32** | 49.00 | 50.66 | **49.83** | 2.10 | 1.72 | **1.91** | 42.35 | 36.60 | **39.48** | 2160.0 | 1543.33 | **1851.67** |
| Mix. (1 + 2) | 3.52 | 3.15 | **3.33** | 50.33 | 51.00 | **50.67** | 2.06 | 2.18 | **2.12** | 40.75 | 39.82 | **40.29** | 1730.0 | 2080.0 | **1905.0** |
| **Mean** | **2.97** | **2.59** | | **49.13** | **50.66** | | **1.93** | **1.92** | | **41.39** | **40.47** | | **1959.33** | **1841.33** | |
| **L.S.D at 5%:** | | | | | | | | | | | | | | | |
| **(I)** | | **0.266** | | | **0.990** | | | **0.005** | | | **0.014** | | | **n.s.** | |
| **(T)** | | **0.203** | | | **0.863** | | | **0.002** | | | **0.534** | | | **114.75** | |
| **(I × T)** | | **0.286** | | | **1.220** | | | **0.031** | | | **0.755** | | | **163.82** | |

**Table 10. Effect of spraying wheat plants with different bioagents before and after pathogen inoculation on yield components, under field conditions, for 2007-2008 growing season.**

| Treatments | Spike wt. (g) | | | No. grains/spike | | | wt. of grains/spike (g) | | | $10^3$ grains wt. (g) | | | Wt. of yield/plot (g) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before | After | Mean | Before | After | Mean | Before | After | Mean | Before | After | Mean | Before | After | Mean |
| Control (infected) | 2.80 | 2.80 | **2.80** | 48.0 | 48.0 | **48.0** | 2.02 | 2.02 | **2.02** | 39.33 | 39.33 | **39.33** | 1195.0 | 1195.0 | **1195.0** |
| Control (healthy) | 2.97 | 2.97 | **2.97** | 56.67 | 56.67 | **56.67** | 3.07 | 3.07 | **3.07** | 45.04 | 45.04 | **45.04** | 2544.67 | 2544.67 | **2544.67** |
| . *P. fluorescens* | 3.43 | 3.61 | **3.52** | 58.25 | 57.35 | **57.80** | 2.59 | 2.79 | **2.69** | 45.05 | 45.32 | **45.18** | 3650.0 | 1506.0 | **2578.0** |
| ?. *B. polymexa* | 3.56 | 3.79 | **3.67** | 60.93 | 61.56 | **61.25** | 2.61 | 2.82 | **2.72** | 43.33 | 46.60 | **44.97** | 2596.67 | 2088.0 | **2342.33** |
| Mix. ( 1 + 2 ) | 3.27 | 3.13 | **3.20** | 50.0 | 50.67 | **50.33** | 2.20 | 2.06 | **2.13** | 47.67 | 39.67 | **40.67** | 1650.0 | 1550.0 | **1600.0** |
| **Mean** | **3.21** | **3.26** | | **54.77** | **54.85** | | **2.49** | **2.55** | | **42.89** | **43.19** | | **2327.27** | **1776.73** | |
| **L.S.D at 5%:** | | | | | | | | | | | | | | | |
| **( I )** | | **n.s.** | | | **n.s.** | | | **n.s.** | | | **n.s.** | | | **383.05** | |
| **( T )** | | **0.361** | | | **0..311** | | | **0.301** | | | **2.88** | | | **300.78** | |
| **( I × T )** | | **n.s.** | | | **n.s.** | | | **n.s.** | | | **n.s.** | | | **425.37** | |

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# المقاومة الحيويـــة لمرض صدأ الأوراق في القمح باستخدام بكتيريا سيدوموناس فلوروسنس وأنواع من بكتيريا الباسيلس

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تم دراسة المقاومة الحيوية لمرض صدأ الأوراق على صنف القمـــح جيـــزة – ١٣٩ والذي يسببه الفطر بكسينيا تريتيسينا تحت نوع تريتيساي، وذلك باستخدام أنواع من بكتيريا مختلفة مثل سيدوموناس فلوروسنس وباسيلس بوليميكسا والباسيلس سركيولانس ومخلوط من هذه الأنواع البكتيرية. تم إجراء التجارب في طوري البادرة والنباتات البالغة. لـــوحظ أن استخدام هذه البكتيريا في تجارب الصوبة على البادرات أدى إلى اختزال عدد البثـــرات لكـــل ورقة نبات وبالتالي اختزال المكونات المرضية. عند غمر البذور في معلق بكتيريا الباسيلس سركيولانس تم الحصول على أعلى نسبة مئوية لاختزال المرض (٥٤,٠٢ %) يليها الغمـــر في معلق بكتيريا بسيدوموناس فلوروسنس (٤٨,٠ %) . بينما في تجارب الحقل لـــوحظ أن استخدام بكتيريا بسيدوموناس فلوروسنس يؤدي إلى اختزال واضح في شدة الإصابة والذي بدوره يؤدي إلى زيادة وزن الألف حبة وزيادة محصول الحوض.