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BIOCONTROL OF WHEAT POWDERY MILDEW DISEASE UNDER FIELD CONDITIONS IN EGYPT

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ABSTRACT: Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, is an important disease of bread wheat (*Triticum aestivum* L.) in many countries worldwide, including Egypt. Powdery mildew infects all parts of wheat plants that negatively affect grain yield and therefore caused economic loss. Some microorganisms *i.e. Bacillus subtilis*, *B. chitinospora, B. pumilus* and yeast (*Rhodotarula s*p.) were used in the current study as biocontrol agents (BCAs) for controlling powdery mildew in wheat variety (Sakha93), under field conditions, compared to the recommended synthetic fungicide; Tilt. The superior and best treatments decreasing infection type (IT), disease severity (%), and increasing 1000 kernel weight and grain yield per plot. Application of *B. subtilis* and *B. chitinospora*, were compared to the other treatments and the tested fungicide. Scanning electron microscopy studies confirmed the superiority of the aforementioned two biocontrol agents (BCAs), as application of each, minimized suppressed mycelial growth and collapsed the spores of the causal pathogen. Application of *B. chitinosporain,* particular, suppressed the conidial germination, caused a rupture, and deformation of germ tube. Also, both mycelia and conidiospores became shrinking on the surface of the treated wheat leaves. Extracts of the four microorganisms used in this study interacted directly with *Blumeria graminis*, and this antifungal effect was responsible for the observed disease protection. The obtained results in this investigation gave an evidence to the possible use of such microorganisms as safe and effective alternatives for controlling powdery mildew disease of wheat under field conditions.

Key words: Wheat, *Blumeria graminis*, biological control, scanning electron microscopy, *Bacillus*, *Rhodotarula*.

INTRODUCTION

Bread wheat cultivars (*Triticum aestivum* L.) in Egypt are liable to severely attack by some major diseases *e*.g. rusts, powdery mildew, and loose smut, as well as other diseases of a minor importance. In cool and humid areas allover the world, powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Marchal, became one of the most devastating diseases of wheat (**Benett, 1984).** Wheat powdery mildew could incite yield losses up to 45%, if the initial infection occurred early and the environmental conditions remain favorable for disease incidence and development during the growing season **(Leath and Heun, 1990; Griffey *et al.,* 1993; Tomas and Solis, 2000; Hong *et al*., 2018)**. The disease was noticed and recorded in some governorates of both the Delta region and Upper Egypt, as it was annually occurred in the following localities: Bahteem, Gemmeiza, Giza, Sakha, El-Serow, Etay El-Barood, Sids, El-Mataana, Shandaweel, and Kom Ombo. However, some of the new released wheat varieties and lines, as well as most of the commercial wheat cultivars (both bread and durum wheats), showed different levels of resistance and/or susceptibility to powdery mildew (**El-Salamony, 2002; Esmail Samar, 2009; Boulot *et al*., 2015).**

Biological control agents (BCAs), as the effective alternatives or the vital control biocides are attracting more attention in some

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numbers of the previous investigations. However, most of the previous studies as well as different applications of BCAs, have mainly focused on the powdery mildew of vegetables and ornamentals under greenhouses conditions (**Paulitz and Belanger**, **2001; Giotis *et al*., 2012)**. While, few or little studies have been conducted on the control of wheat powdery mildew using BCAs (**Li *et al*., 2013)**.

Several microorganisms, for example *Ampelomyces quisqualis*, have been found to be effective as a successful biocontrol agent that have a strong suppression activities against various powdery mildew fungi**.** Also, early studies on the biocontrol of *Blumeria graminis* f. sp. *tritici,* the causal fungus of powdery mildew in wheat, clearly demonstrated that *Pseudomonas fluorescents* can reduce about 50% of the total number of colonies of the causal pathogen on the inoculated leaves (**Elad *et al*., 1996; Romero *et al*., 2004)**.

Previously identified *Bacillus subtilis* strain EIR-j, that have a broad spectrum biocide effects against the majority of plant pathogens (**Liu *et al*., 2009).** The author and his co-workers also reported, in particular, that strain EIR-j have the potential to control take- all in wheat, as it exhibited high antifungal activity to *Gaeumannomyces graminis* var. *tritici in vitro* and *in vivo*. In addition, EIR-j significantly reduced disease index, suppressed the conidial germination and caused rupture and deformation in germ tubes of conidiaspores, on the surface of wheat leaves. Also, both of mycelia and conidiophores became shrinking. Similar results for this strain was reported by **Xiaoning *et al*. (2015**). While, **Li *et al*. (2013)** determined that endophytic *Bacillus subtilis* strain; EIR-j had an inhibitory effect against *Puccinia striiformis,* and can successfully controlled wheat stripe rust disease in both greenhouse and field trials.

On the other hand, **Koitabashi and Tsushima (2007)** screened some microorganisms that isolated from wheat leaf surface for controlling wheat powdery mildew disease. They reported that among all the tested microorganisms, a fungal strain designated as Kyu-W63 had an especially strong inhibitory effect on the growth of the wheat powdery mildew fungus.

The present study was Therefore, carried out as an attempt to evaluate the biocontrol effects and/or antifungal activities of three bacterial isolates. and one yeast extract against the causal pathogen of powdery mildew in wheat (*Blumeria graminis* f. sp. *tritici*), under filed conditions. An additional objective of this study was to confirm and explain the inhibitory effects of the tested bioagents, by using scanning electron microscopy (SEM) examinations.

MATERIALS AND METHODS

Field experiment was carried out at Sakha Agricultural Research Station, during the two successive growing seasons *i.e*. 2017/18 and 2018/19. A completely randomized block design, with three replicates was adopted in this experiment. Five treatments were tested; three treatments with the three bacterial isolates (*B. subtilis*, *B. pumilus* and *B. chitinospora*) and one treatment with yeast extract (*Rhodotarula* sp). The tested microorganisms were kindly supported by: Plant Pathology and Biotechnology Lab., Agric. Botany Dept., Fac. Agric., Kafrelsheikh University, Egypt.

Positive control treatment with the synthetic fungicide, Tilt, compared to the check or control treatment (inoculated and untreated wheat plants). Sakha 93, as a highly susceptible wheat cultivar to powdery mildew disease was the target of the evaluation of biocontrol agents, under study.

The two different media *i.e.,* nutrient broth (NB) medium and potato dextrose broth (PDB) medium were selected and prepared according to the methods of **Daingra and Sinclair (1995).**

Liquid medium required to be obtained in flask of 500 ml. capacity containing 200 ml medium. Inculcated cultures by BCAs were incubated at 28ºC for 10 days, in shaking incubator (140 r/min.). Cultures were paper filtered and hand homogenized and counts of yeast and bacterial cells were adjusted to 10^7/ml. Rate of use was 1:1 liter water mixed before spraying.

Field Experiment Inoculation

Wheat leaves exhibited powdery mildew symptoms were collected from naturally infected fields. Infected samples were microscopically examined and single powdery mildew colony was selected to produced the experiment inoculum cultivar. The resulted powdery mildew pathogen (*Blumeria graminis* f. sp. *tritici*) was used to propagate under greenhouse condition.

The resulted powdery mildew inoculum was propagate under greenhouse conditions and used to infect the experiment border and wheat plants. The border consists of the more susceptible wheat verities for powdery mildew.

Disease Assessment

Tested samples of wheat leaves (ten days after inoculation with *Blumeria graminis* f. sp. *tritici*) were treated with isolates of *Bacillus subtilis, B. chitinosporus, B. pumils* and yeast extract of *Rhodotrual* sp. as bio agents, in addition to the fungicide; Tilt, compared to the untreated control treatment. Infection type (IT) (Table 1) and disease severity (%) of powdery mildew for each treatment was estimated. Infection type was recorded as disease rate at 0-9 scale, according to the method of **Saari and Prescott (1975)**. While powdery mildew disease severity (%) was estimated for all the tested treatments, using the method adopted by **Leath and Heun (1990).**

Assessment of Yield Components and Determine the Efficacy of the Tested Bio-Control Agents

The two yield components *i.e.*1000-kernel weight (g) and plot grain's weight (kg), were estimated at Wheat Diseases Research Department at Sakha Agricultural Research Station. Experiment plot (6 × 7 m = 42 m^2) was prepared in field grain experiment. Each plant was sown with 20 of wheat variety; Sakah93.

Increase over the control (%) in the two yield components due to the application of each of the tested treatments, was determined according to an equation proposed by **Calpouszos *et al.* (1976),** as follows:

Increase over the control (%) = (Yh/Yd-1) × 100

Where: Yh = Yield of healthy (treated) plants, and Yd = Yield of diseased plants (untreated control).

The efficacy (%) of the tested biocontrol agents for controlling powdery mildew, under filed conditions, was determined according to the method adopted by **Rewal and Jhooty (1985)**:

Efficacy (%) = Severity (%) of the control - Severity (%) of the treatment/Severity (%) of the control ×100

Scanning Electron Microscopy (SEM) Studies

Samples were taken from wheat leaf cuts (4 mm²) from each infected and treated leaves. These samples were immediately fixed in glutraldhyde (2.5%) for 24 hr., at 4ºC, then post-fixed in osmium tetra oxide (1% $OS0_4$) for one hour at room temperature (**Harley and Ferguson, 1990**).

Samples were dehydrated with passing through ascending concentration of acetone (30-100%). Each sample was dried till the critical point. Finally, leaf was sputter coated with gold. The examination and photographing were done through a Jeol Scanning Electron Microscopy (T.330 A). Studies were carried out in Electron microscopy laboratory, Plant Pathology Department, Faculty of Agriculture, Ein Shams University.

Statistical Analyses

The obtained data were statistically computed by MSTAT-C program (**Anonymous, 1991),** and comparison of means was accomplished by Duncan's Multiple Range Test (**Gomez and Gomez, 1984)**. Correlation and regression analyses were carried out using "SPSS Regression Modeling". Comparison of the means was calculated by LSD test ($P < 0.01$ and $P < 0.05$).

RESULTS

Effect of Different BCA Treatments and Tilt Fungicide on Wheat Powdery Mildew Disease Reaction, Under Field Conditions

Four biocontrol agents (BCAs) *i.e. Bacillus subtilis, B. chitinosporus, B. pumilus* and *Rhodotrula* sp., as well as a synthetic fungicide; Tilt was evaluated for controlling wheat powdery mildew infection in terms of disease severity (%) and infection type (IT), expressed on the highly susceptible wheat variety; Sakha93.

Table 1. Wheat powdery mildew infection type (IT) according to a (0-9) scale. (Saari and Prescott, 1975)

IT	Host response	Symptoms
0	Immune (I)	No visible signs or symptoms.
1	Highly resistant (HR)	Small spots only.
2	Resistant (R)	Chlorosis spots evident.
3	Moderately resistant (MR)	Large spots with chlorosis and necrosis
4	Low intermediate (LI)	Mycelium and conidia detectable.
5	Intermediate (I)	Small to moderate sized colony and conidia present.
6	High intermediate (HI)	Predominance of moderate sized colony and conidia present.
7	Moderately susceptible (MS)	At the least 50% of the large colony and conidia are visual.
8	Susceptible (S)	75 to 80% of the leaf segment was covered with large colony and conidia.
9	Highly susceptible(HS)	100% of the leaf segment covered with large colony and conidia.

Analysis of variance (ANOVA) for the combined data of the two growing seasons under study indicated that the interaction between treatments (T) and years(Y) (T × Y), for the disease severity (%) and infection type (IT), were highly significant (Table 2). Therefore, LSD estimates were used to compare between mean effects of the tested bioagents within each year of the study.

In general, all the biocontrol agents (BCAs) under evaluation and the synthetic fungicide have the potentiality to decrease both the disease severity (%) and infection type (IT), but with different or variant where, degrees (Table 3).

As, these six treatments showed, in general, noticeable antifungal action ties, as they significantly reduced the wheat powdery mildew severity (%) and minimized infection type (IT), compared to the untreated control treatment, in both seasons. The four biocontrol agents (BCAs), in particular, activated and enhanced host resistance in the wheat plants of the susceptible cultivar; Sakha 93, against subsequent powdery mildew infection (Table 3). The enhancement and/or activation of this resistance, as a result of the pre-treatment with the four tested bioagents (BCAs) was evident as a significant decrease in powdery mildew disease severity (%) and infection type (IT) in both seasons, with an obvious superiority in the application with the two bioagents; *B. subtilis* and *B. chitinosporus*. The obtained results in Table 3, also reveal that there are relatively wide variation between the effects of the tested bioagents on powdery mildew reaction; infection type (IT) and disease severity (%), during the two growing seasons of the study. Among the five treatments under evaluation, only the two bioagents *i.e. B. subtilis* and *B. chitionosporus*, as well as the Tilt fungicide exhibited the highest antifungal effects activities for controlling wheat powdery mildew disease in the treated wheat plants of the susceptible wheat variet; Sakha93, during the two growing seasons. As they recorded the lowest percentages of disease severity (5%, 20%, and 0.33% in the first season; 2017/18, respectively). Display also, they showed similar results the least as they showed values of disease severity; (5%, 20% and 0.33%) in the second season; 2018/19, respectively (Table 3).

As for the effect of the tested treatments on powdery mildew infection type expressed on the susceptible wheat variety; Sakha93, results in Table 3 run in a parallel line with those previously obtained, for disease severity (%). Since, the two BCAs *i.e. B. subtilis* and, *B. chitionosporus* and Tilt fungicide were in the first rank, as they showed the highest capacity or

Table 2. Combined analysis of variance for the effects of the tested four bioagents and Tilt fungicide on disease severity (%) and infection type (IT) of wheat powdery mildew, under field conditions during the two growing seasons

SOV	df	Disease Severity (%)		Infection type (IT)	
		MS	P Value	MS	P Value
Replications (R)	2	139.95	0.002	0.08	0.004
Years (Y)	1	48.04**	0.001	9.01**	0.003
Treatments (T)	5	6091.11**	0.000	60.96**	0.000
Interaction (Y × T)	5	25**	0.000	28.03**	0.006
Error		47		0.85	

Table 3. Effect of the application of the four biocontrol agents (BCAs) and a fungicide (Tilt) for controlling powdery mildew disease on wheat the susceptible variety; Sakha93 in terms of disease severity (%) and infection type (IT), during 2017/18 and 2018/19 growing seasons

No.	Treatment	2017/2018				2018/2019			
		Disease severity (%)	Efficacy (%)	Infection type (IT)	Efficacy (%)	Disease severity (%)	Efficacy (%)	Infection type (IT)	Efficacy (%)
1	*B. subtilis*	5	95.0	1	88.88	5	94.44	1	88.88
2	*B. chitinosporus*	20	80.00	2	88.88	10	88.88	2	77.77
3	*B. pumilus*	60	40.00	5	44.44	60	33.33	4	55.55
4	*Rhodotrula sp.*	50	50.00	٤	55.55	50	44.44	4	55.55
5	Tilt	0.33	97.44	0.66	94.66	0.33	97.44	0.66	94.66
6	Control (untreated)	100	0.00	9	0.00	90	0.00	9	0.00
LSD 0.05 for	Year (Y)	14.57		1.68		14.57		1.68	
	Treatment (T)	8.26		1.09		8.26		1.09	
	Interaction (Y × T)	11.69		1.49		11.69		1.49	

/biocide activity for controlling wheat powdery mildew, rather than the other two treatments under study.

The efficacy (%) of the four biocontrol agents (BCAs) as well as the chemical fungicide; Tilt in controlling wheat powdery mildew infection under the stress of the disease under field conditions, was also determined and results obtained were found in Table 3; in controlling powdery mildew infection under the stress of disease during the two growing seasons of the study. High efficacy (%) reached to 95.0% and 88.88% ware obtained in response to the application of the premising two BCAs; *B. subtilis* and *B. chitinosporus,* respectively. Also, foliar spray by the synthetic fungicide; Tilt as the positive check reaction displayed the highest efficacy (%); reached to 97.44%. While the other two treatments under study showed the lowest percentages (values) of efficacy (did not exceeded up to 55.55%) for controlling wheat powdery mildew disease under field conditions in both seasons of the study (Table 3).

Effect of Different BCA Treatments on the Two Grain Yield Components *i.e.* 1000 Kernel Weight (g.) and Plot Grain's Weight (kg.) Under Field Conditions

Combined analysis of variance for the obtained results during the two years of the study (Table 4), revealed that there was highly significant interaction between the tested treatments (T) and years (Y) for 1000 kernel weight, and plot grain's weight in both seasons. Thus, LSD values were used to compare between means of the tested bioagents within each year of the study.

The obtained results for the effect of the four biocontrol agents (BCAs), under evaluation as well as the synthetic fungicide (Tilt) on the two grain yield components *i.e.* 1000 kernel weight (g.) and plot grain's weight (kg.) of the wheat cultivar; Sakha 93 was found in Table 5. Results presented in this table show in general that there was a significant increase over the control (inoculated and untreated) treatment, in both yield parameters *i.e.* 1000 kernel weight (g.), and plot grain's weight (kg.) due to the application of the four bioagents under study, and the spraying of synthetic fungicide, Tilt (Table 5). Although, this detectable increment in the two yield components over the control, as a result of the application with all the five treatments, spraying wheat plants with Tilt fungicide and the two bioagents; *B. subtilis* and *B. chitinosporus* showed in particular an obvious superiority more than the other treatments under study during the two seasons 2017/18 and 2018/19 (Table 5). The highest 1000 kernel weight (g.) was recorded after spraying wheat plants (variety; Sakha93) with fungicide Tilt, (42.7g and 44.7 g) followed by the application of the two bioagents *i.e. B. subtilis* (40.3g and 43.3g) and *B. chitinosporus* (38.4g and 40.4g) in 2017/18 and 2018/19 seasons, respectively.

Also, high increments in 1000 kernel weight over the control (%), due to the application of Tilt fungicide, and the above two superiol BCAs; *B. subtilis* and *B. chitionosporus*, were also obtained during 2017/2018 and valued 39.54%, 31,69% and 25.49% respectively. While, in the second season (2018/19), the values of the increments in 1000 kernel weight over the control treatment reached to 37.11%, 32.82% and 23.92% after the application of Tilt fungicide, *B. subtilis* and *B. chitionosporus*, respectively (Table 5).

Results presented in the same Table 5 regarding to the effect of the tested BCAs and the synthetic fungicide Tilt on grain yield per plot (kg.) indicated that the spraying wheat plants (variety; Sakha 93) with the four (BCAs) and fungicide used, showed significant increase over the untreated control treatment spraying with water only). The highest grain yield per plot was recorded as a result of spraying wheat plants with the synthetic fungicide; Tilt (21.0 kg. and 21.2 kg.), followed by the application of the two bioagents; *B. subtilis* (19.2 kg. and 19.5 kg.) and *B. chitinosporus* (18.6 kg. and 18.5 kg.) during 2017/18 and 2018/19 growing seasons, respectively. While the treatment with the other two BCAs resulted in the lowest grain yield per plot (Table 5).

On the other hand, increases over the control in response to foliar spraying with the Tilt fungicide, and the two bioagents *B. subtilis*and *B. chitionosporus*, during the first season 2017/ 2018 were 36.36%, 24.67% and 20.77%, respectively. While in the second season; 2018/ 19, the highest increase in grain yield per plot over the control of treatment reached to 35.89%, 25.00% and 18.58% due to the application of the positive check fungicide, Tilt, as well as the two superior BCAs, *B. subtilis* and *B. chitionosporus*, respectively (Table 5).

Correlation Between Powdery Mildew Disease Parameters and Yield Components

Relationship between the two disease parameters; infection type (IT) and disease severity (%) and each of the two yield components *i.e.* 1000 kernel weight and grain yield per plot, was determined and carried out during 2017/18 and 2018/19 growing seasons, using regression analysis, (Table 6 and Figs., 1 and 2). Significant negative correlation coefficient was obtained between powdery mildew disease severity (%) and 1000 kernel weight (-0.870 and -0.866) during 2017/18 and 2018/19 growing seasons, respectively. Also correlation between disease severity (%) and grain yield per plot, revealed high significant negative R values (R= -0.926 and -0.858) in two season) overall the two growing seasons of the study (Table 6).

Table 4. Combined analysis of variance for the mean effects of the four biocontrol agents (BCAs) and Tilt fungicide, on grain yield components, under field conditions in 2017/18 and 2018/19 growing seasons

SOV	df	1000 kernel weight (g)		Grain yield/plot (kg)	
		MS	P Value	MS	P Value
Replication(R)	2	0.03	0.002	0.02	0.004
Year (Y)	1	10.37**	0.001	0.2	0.003
Treatment (T)	5	116.18**	0.000	23.07**	0.000
Interaction (Y ×T)	5	4.79**	0.000	0.03	0.000
Error		0.03		0.03	

Table 5. Effect of the application of four biocontrol agents (BCAs) and one fungicide (Tilt) on the two yield components; 1000 kernel weight (g.) and grain yield/plot of the susceptible wheat variety; Sakha93, under field conditions, during 2017/18 and 2018/19 growing seasons

Treatment	2017/2018				2018/2019			
	1000 Kernel weight	Increase over control (%)	Grain yield/ plot (kg)	Increase over control	1000 Kernel weight	Increase over control	Grain yield/plot (kg)	Increase over control
B. subtilis	40.3	31.69	19.2	24.67	43.3	32.82	19.5	25.00
B. chitinosporus	38.4	25.49	18.6	20.77	40.4	23.92	18.5	18.58
B. pumilus	37.7	23.20	17.13	11.03	38.7	19.01	17.3	10.89
Rhodotrula sp.	34.7	13.39	17.21	11.3	35.8	9.81	17.2	10.25
Tilt	42.7	39.54	21.0	36.36	44.7	37.11	21.2	35.89
Control (untreated)	30.6	0.0	15.4	0.0	32.6	0.0	15.6	0.0
LSD 0.05 for Years (Y)	0.35		0.30		0.35		0.29	
LSD 0.05 for Treatment (T)	0.17		0.20		0.17		0.20	
LSD 0.05 for Interaction (Y × T)	0.23		0.27		0.23		0.27	

Table 6. Correlation among the two disease parameters disease severity (%) and infection type (IT) and the two yield components; 1000 kernel weight (g) and grains yield/plot under field conditions during 2017/18 and 2018/19 growing seasons

Disease parameter components	Disease parameter and yield components			
	2017/18 growing season			
	Severity	Infection type	1000 Kernel weight	Grains yield/plot (kg)
Severity (%)	-	0.974**	-0.943**	-0.954**
Infection type (IT)	-	-	-0.947**	-0.918**
1000 Kernel weight (g)	-	-		0.958**
	2018/19 growing season			
Severity (%)	-	0.965**	-0.949**	-0.954**
Infection type (IT)		-	-0.947**	-0.922**
1000 Kernel weight (g)	-	-	-	0.957**

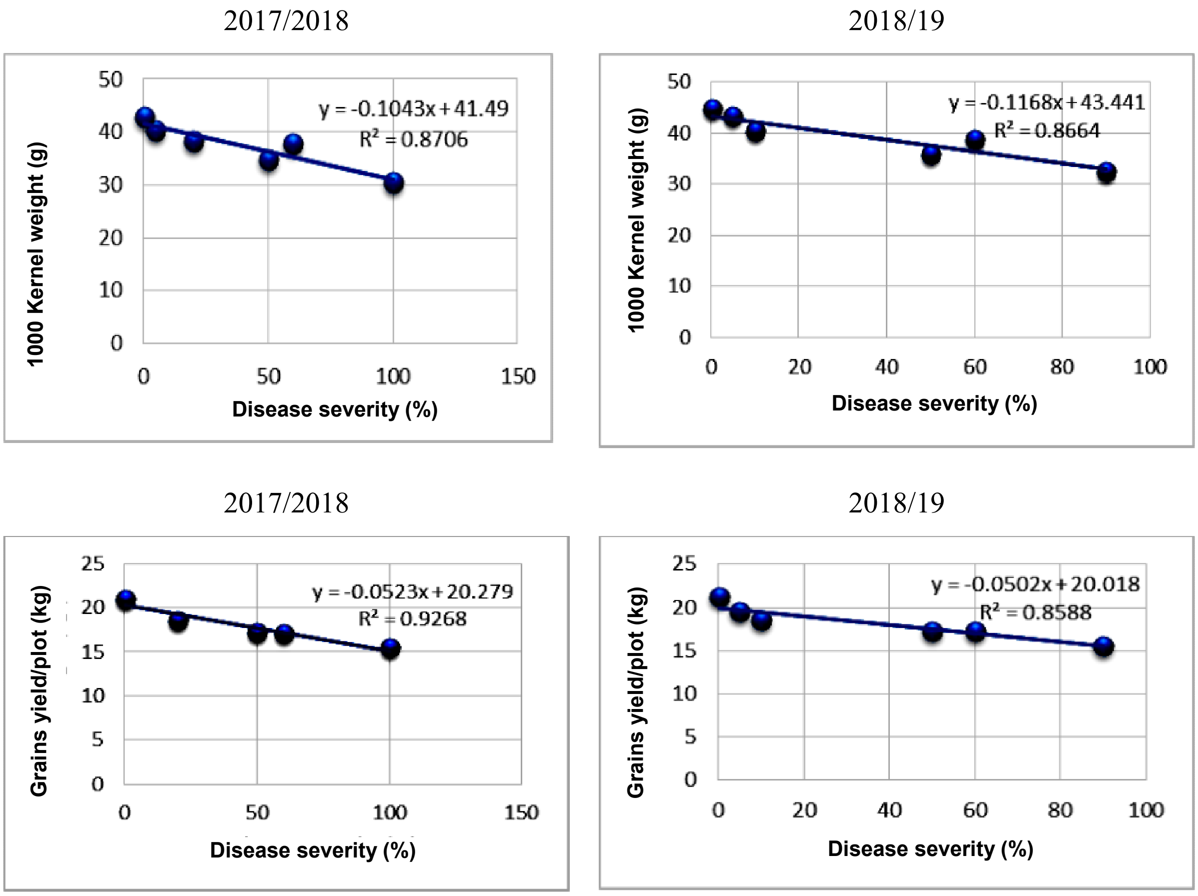


Fig. 1. Correlation between wheat powdery mildew severity (%) and of 1000 kernel weight (g) and grain yield per plot (kg) during; 2017/18 and 2018/19 growing seasons

On the other hand, association between powdery mildew infection type (IT) and each of the two yield components *i.e.* 1000 kernel weight and grain yield per plot, determined through regression analysis, revealed high significant negative correlation coefficient (R= -0.819 and -0.835), during the first season 2017/18, respectively. Similar results were obtained for the comparison between infection type (IT) and each of 1000 kernel weight and grain yield per plot through the second season of the study 2018/19. Since, the estimated values of the correlation coefficient (R) were -0.884 and -0.897) for the comparison between powdery mildew infection type and the above mentioned tow yield components, respectively (Table 6 and Fig. 2).

Scanning Electronic Microscope (SEM) Examination

Treatment with each of *B. chitinosporus* as promising bio-control agent (BCA) and Tilt fungicide showed an antifungal effect on conidiospores of wheat powdery mildew (*B. graminis* f. sp. *tritici*). Scanning electronic microscopy observations revealed that conidiospores became shrinking on the surface of the treated wheat leaves, compared with the untreated control leaves (Figs. 3, a, b and c). Also, conidia germinated normally on the surface of wheat leaves sprayed with water only (untreated control treatment). While, conidia were ruptured or remained non germinated and the germ tubes were damaged due to the application with the bacterial bioagent (*B. chitinosporus*), as well as the synthetic fungicide, Tilt, 10 days after infection (Figs. 4a, b and c). Microscopic examination of the two treatments showed in general collapsing of conidia, compared with the untreated control one. Results illustrated in (Figs. 4a,b,c) with regard to SEM examination of wheat leaves, also indicated that wheat leaves treated with each of the BCAs; *B. chitinosporus* or the synthetic fungicide, Tilt (10 days after infection), comparison with control treatment, exhibited morphological abnormally mycelium growth and displayed shrinking in conidiophore and hypha of the causal pathogen on the surface of the wheat treated leaves.

DISCUSSION

Wheat plants (*Triticum aestivum* L.) are liable to attack by many serious diseases which dramatically affect the annual crop production. This would in turn considered to be the cause of the occurrence and spread of severe epidemics and famines, all over the world.

During the last ten years, powdery mildew became one of the major diseases, as it has been occurred annually on most of the commercial wheat varieties, cultivated in many wheat growing areas in Egypt. Also, it is the main cause of yield losses (up to 32.74%) in the susceptible wheat varieties during the growing seasons in which environmental conditions favorable disease incidence and development, as well as when the initial infection occurs early in the field (**Esmail Samar, 2009; El-Shamy *et al*., 2012; Boulot *et al*., 2015)**.

Growing resistant cultivars and applying synthetic fungicides are commonly used as the two main strategies to successfully control powdery mildew in many countries, worldwide. Unfortunately, host genetic resistance is not sufficient and did not available in most of the commercial wheat cultivars in Egypt, due to the national breeding program for resistance to wheat diseases has mainly focused on breeding for only the three rust diseases. However, fungicide application is the currently available and the only recommended method for controlling wheat powdery mildew under the Egyptian conditions. Nevertheless, the wide application and complete dependence on the chemical fungicides carries high risks for farmers, economically cost and environmental disadvantage. Also, it resulted in the creation and evolution of new races of the causal pathogen that have the potentiality to tolerate and resist these synthetic fungicides (**Reis *et al*., 2013; Boulot *et al*., 2015)**

Biological control agents (BCAs) as an effective and viable alternative control materials, are attracting more attention for management of several plant diseases including wheat powdery mildew (**Cook, 1993**). The present investigation was, therefore, considered to be an attempt to evaluate and test the anti-fungal activity and biocide potentiality of the three bacterial isolates

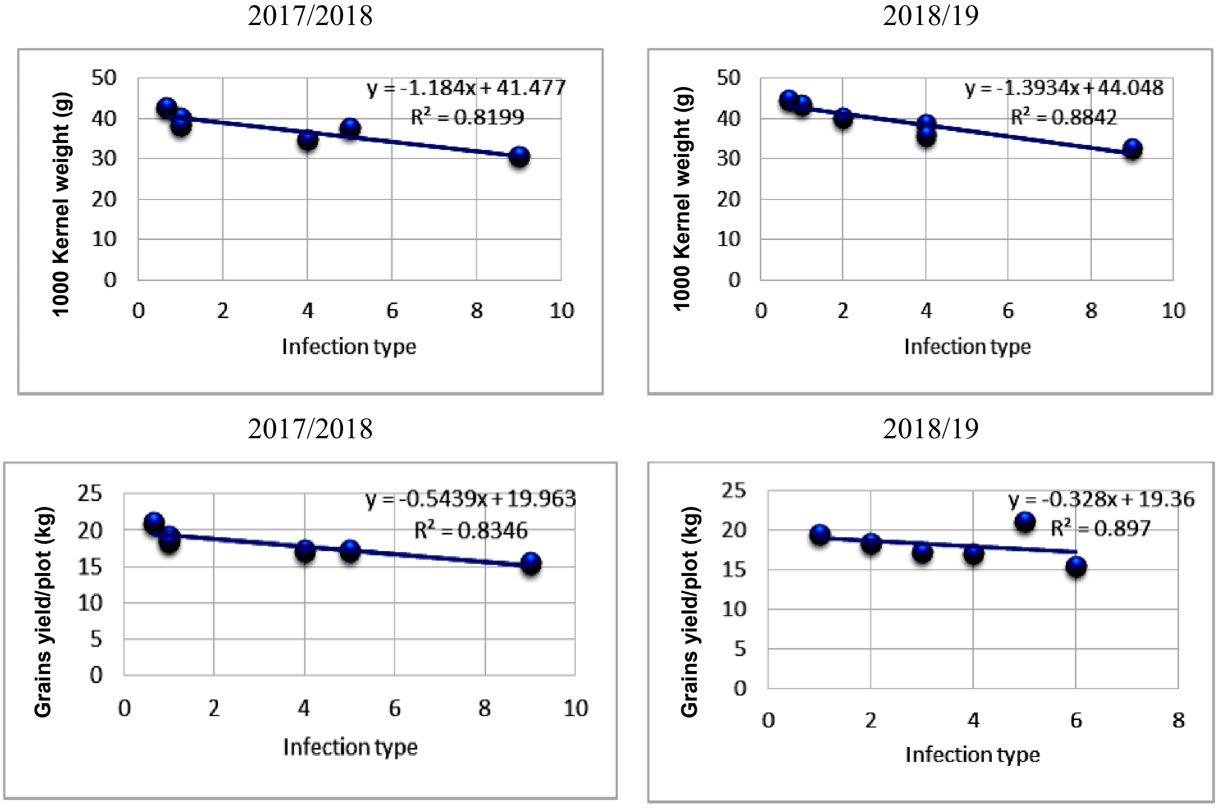


Fig. 2. Correlation between wheat powdery mildew Infection type (IT) and the of 1000 kernel weight and grain yield per plot during the two growing seasons; 2017/18 and 2018/19

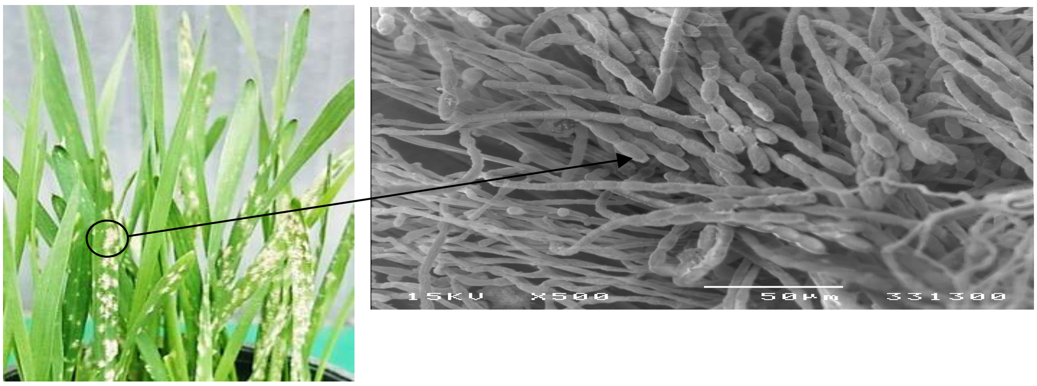


Fig. 3a. Scanning electron microscopy examination of wheat leaves (variety; Sakha93) untreated and infected with *Blumeria graminis* f sp. *tritici* (10 days after inoculation). Arrow indicate the normal growth of the pathogen fungus on wheat leaves

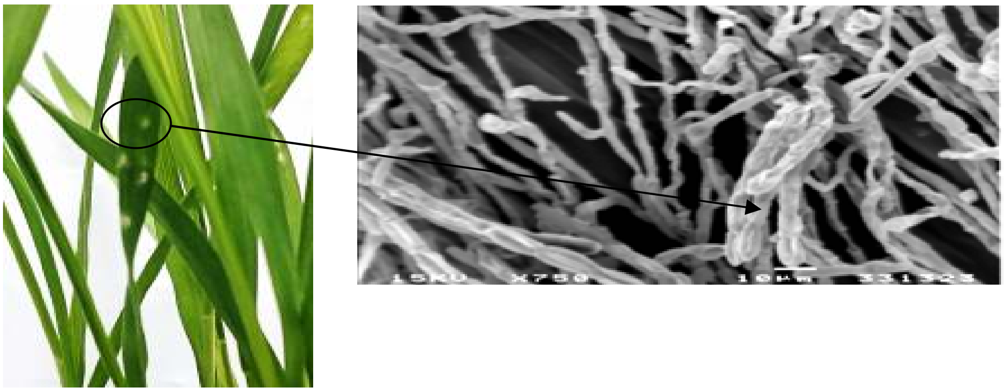


Fig. 3b. Scanning electron microscopy examination of wheat leaves infected with *Blumeria graminis* f sp. *tritici* (10 days after inoculation. and 24 hours after treatment) with bioagent (*Bacillus chitinosporus*). Arrow indicate that conidiospores and hypha of the causal pathogen became shrinking on the surface of the treated wheat leaves.

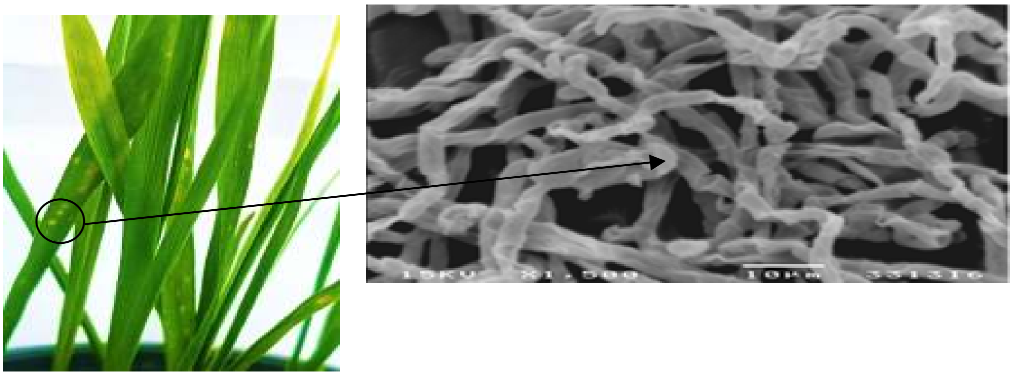
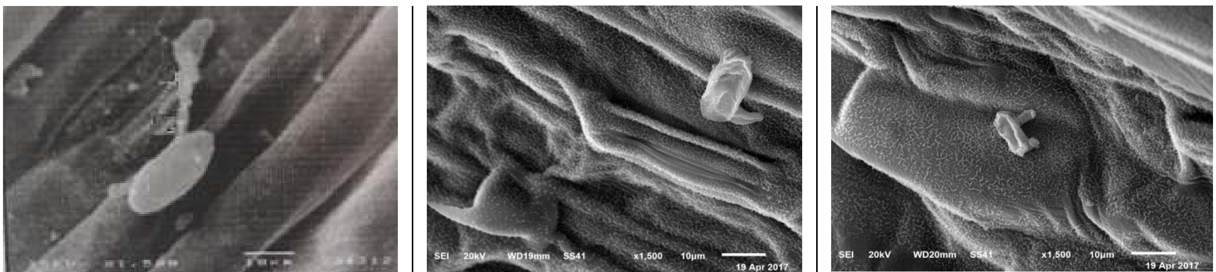


Fig. 3c. Scanning electron microscopy of wheat (*Triticum aestivum* L.) infected with *Blumeria graminis* f. sp. *tritici* 10 days after inoculation. 24 hour after treatment with fungicide (Tilt). Arrow indicate that morphological abnormally mycelium growth and displayed shrinking in conidiophore and hypha on the wheat treated leaves.



(a) Untreated (control treatment) | (b) Treatment with biocontoal | (c) Treatment with Tilt fungicide

Figs. 4, a,b,c. Germination of *B. graminis* f. sp *tritici* (*Bgt*) conidiospores on leaves of wheat cultivar; Sakha 93, 24 hours after inoculation examined by scanning electron microscope (SEM). (a) Untreated (control treatment), (b) Treatment with biocontoal agent, *Bacillus chitinosporus* (c) Treatment with Tilt fungicide

(*i.e. Bacillus subtilis, B. chetinosporus, B. pumilus*, and one yeast extract; *Rhodoturula* sp. as the promising bio-control agents (BCAs) against the causal pathogen of wheat powdery mildew; *Blumeria graminis* f. sp. *tritici*, under field conditions during 2017/18 and 2018/19 growing seasons.

The obtained results in this study revealed, in general, that use or the application of the four bio-control agents (BCAs) enhanced the host resistance in the susceptible wheat cultivar; Sakha 93 against powdery mildew infection. However, high significant difference could be detected among the bio-control effects for each of the four bioagents, under study *i.e. Bacillus subtilis, B. chetinosporus, B. pumilus* and *Rhodoturula* sp. and the systemic fungicide; Tilt (Propiconazole) in comparison with the untreated control treatment. However the four bioagents were effective in reducing both the two disease parameters *i.e.* disease severity (%) and infection type (IT). Also, they have high potentiality for controlling powdery mildew disease under field conditions in both seasons. These results also revealed that the application of the chemical fungicide as the positive check treatment overcome the tested bioagents.

Similar results were previously reported by **Anith *et al.* (1999)**, where they succeeded in reducing powdery mildew by 50% by the use of *Pseudomonas fluorescens*. On the other hand, strain E1R-j was previously identified as *Bacillus subtilis* strain that had a broad spectrum effect against the important plant pathogens (**Liu *et al.,* 2009)**. They reported that E1R-j exhibited high antifungal activity to *Gaeumanomyces graminis* var. *tritici in vitro* and in *vivo*. Also, **Li *et al.* (2013)**, determined that E1R-j strain, had the inhibitory effect on wheat stripe rust in both greenhouse and field trials. As, it displayed a protective mode in reducing disease severity compared to the un-treated plots in the field experiments conducted in 2007 and 2008 growing seasons. Furthermore, **Vitich *et al.* (1998)**, succeeded in controlling barley powdery mildew *in vitro* and *in vivo,* as well as an enhancement of plant growth of barley, using the BCA fungus *Cheatomium* spp. Also, **Velazquez-Sepulveda *et al.* (2012**) isolated some strains of *Bacillus* species from wheat rhizosphere, that produce antagonistic activities against phytopathogenic fungi. They found that the cells of single bacterial strains; BLB369, BLB277, or BLB267, or their combinations allowed better wheat grain germination, and lower wheat stem and leaf rust disease indexes (disease incidence), than bacterial culture media in the presence of *F. graminearum*. They suggested that a concluded such specific compatibilities between host plants and microorganisms could be associated with a symbiosis (**Zalila-Kolsi *et al.,* 2016**).

The obtained results in the current study in connection with effect of the different treatments on the grain yield components under field conditions, showed in addition, that the application of the tested BCAs, not only can strongly reduce the severity (%) and infection type (IT) of wheat powdery mildew fungus, but also it can significantly improve these two grain yield components over the untreated control wheat plants in both seasons. In general, significant increase over the control (inoculated and untreated treatment) was found in both 1000 kernel weight (g), grain yield/plot weight (kg) due to the application of the four bioagents under study, and the synthetic fungicide.

Increasing yield of the pretreated wheat plants over the untreated control ones is, in fact, directly correlated with the reduction of disease severity (%) and infection type (IT) and due to the improvement of plant growth. Several BCAs that have been previously identified and well known as the producers of various secondary metabolites that enhancement and exhibit some important biological functions, of the treated plants, which led to an improve of the plant growth and its yield potentiality **Xiaoning *et al.* 2015)**. However, the previous studies revealed, in general, that the application of different BCAs improved the plant growth through the production of various secondary metabolites, such as phytohormones *i.e.* indole acetic acid, cytokinin, ethylene **(Awal *et al*., 2017)**. Also, due to an increment of nutrient uptake, as well as an obvious increase of NPK content in the treated plants, over the untreated control plants (**Al-Askar *et al*., 2016)**. Plant pathogens are antagonized by the presence and activities of other microorganisms that they encounter. However, there are two main bio-control mechanisms described above are likely to be operating, to some extent, in natural and managed ecosystems *i.e.* direct antagonism and indirect antagonism. Direct antagonism results from physical contact and/or a high-degree of selectivity for the pathogen by the mechanism(s) expressed by the biocontrol active microorganisms. While, indirect antagonism can be resulted from the activities that do not involve or targeting a pathogen by a bio-control active microorganism. Improvement, stimulation and induction of plant host defense mechanism by non-pathogenic microorganisms and/or by the bio-control agents is the most indirect form of antagonism (**Silva *et al*., 2004**).

Characterization of different mechanisms occurring in response to the application of different BCAs, particularly use of *Bacillus* spp. Strains, that previously demonstrated for controlling plant diseases included the production of some volatiles metabolites with antimicrobial activities. Many microbes (BCAs) produce and secrete one or more compounds with antibiotic activities such as mycocins, phenazines or phloroglucinols, azalomycine complex gliovirin, pyrones, khatoniamycin, viridian and peptaibols. These antibiotics are microbial toxins that can, at low concentrations, poison or kill other microorganisms and are particularly effective against plant pathogens (**Al-Askar *et al*., 2016**; **Fiddaman and Rossall, 1993**; **Li *et al*., 2012**).

The previous studies suggested that *Bacillus subtilis* exerts its inhibitory activity through production of an antifungal protein (**Liu *et al*., 2007; Liu *et al*., 2010)**.

Results previously obtained in the first part of this study relative to antifungal activity of the tested bioagents against wheat powdery mildew disease, under field conditions, were in consistent with microscopic examination. Scanning electron microscopic (SEM) observations in this study showed, in general, an abnormality in the morphology of *B. graminis tritici* mycelium growth, damaging and malformation of conidiospores on the surface of wheat leaves pretreated with the biocontrol agent, *Bacillus chitinosporus,* and Tilt fungicide, compared to those sprayed only with water (untreated control treatment). These results are in accordance with the findings of the study carried previously by **Xiaoning *et al*. (2015)**. During that study and on the basis of scanning electron microscopic observations it was noticed that the percentage of conidial germination and appressorial formation was significantly decreased due to the spraying of endophytic *Bacillus subtilis* strain EIR-j (24h before inoculation), as a promising biocontrol agent for wheat powdery mildew. Also, the number of haustoria significantly reduced, and the speed or rate of mycelial extension was slowed down in the treated wheat leaves. Furthermore, scanning electron microscopy observations revealed, in addition that, EIR-j significantly suppressed the conidial germination and caused rupture and deformation in germ tubes of condiospores. Also, mycelia and conidiophores became shrinking on the surface of wheat leaves treated with the promising biocontrol agent; *B. subtilis* strain EIR-j **Xiaoning *et al*. (2015).**

Further investigations are needed or required to confirm the obtained results in this investigation, and to determine if such BCAs may be effective under conditions that are varied from those which prevailed in the experiments of the current study. However, until now it is not possible to be unequivocal about recommendation for using or applying the tested BCAs for the successful control of powdery mildew disease in wheat, under field conditions. Anyhow, biocontrol of powdery mildew and other diseases as an active control method that could be extensively utilized in the near future rather than other methods of disease control.

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المكافحــة الحيـويــة لمـرض البيــاض الدقيقــي فــي القمــح تحــت الظــروف الحقليــة في مصــر

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مرض البياض الدقيقي المتسبب عن فطر باليميرا جرامينيس تريتيساى مرضا من الأمراض المهمة التي تصيب أقماح الخبز (*Triticum aestivum*) في العديد من بلدان العالم، بما في ذلك مصر، حيث يصيب الفطر جميع أجزاء نبات القمح مما يؤثر سلبًا على محصول الحبوب الناتج من النباتات المصابة وتسبب بالتالي خسائر اقتصادية كبيرة، وقد تم في هذه الدراسة استخدام بعض الكائنات الحية الدقيقة مثل *Bacillus subtilis* و *B. chitinospora* و *B. pumilus* ومستخلص الخميرة (*Rhodotarula* sp.) كعوامل مكافحة حيوية للسيطرة على الفطر المسبب للبياض على القمح وتم استخدام صنف حساس للإصابة (سخا ٩٣) تحت ظروف الحقل، مقارنة بمبيد فطرى (التليت)، كانت أفضل النتائج في تقليل مستوى العدوى (IT)، والشدة المرضيه (%)، وزيادة وزن ١٠٠٠ حبه وكذلك إنتاجية الحبوب لكل قطعة تجريبية، هي المعاملة *B. subtilis* و *B. chitinospora* ، مقارنة بالمعاملات الأخرى، كما أكدت دراسات الفحص المجهري باستخدام الميكرسكوب الالكتروني (SEM) تفوق اثنين من طرق المكافحة الحيوية (BCAs) المذكورة سابقاً ، حيث أن إضافة كل منها أدى إلى تثبيط وتقييد النمو الميسليومى للفطر وتشوهات وانهيار لجراثيم الفطر المسبب للمرض، وباستخدام أو تطبيق *B. chitinospora* خاصة تسبب ذلك في تهتك ، وتشوه الجراثيم، حيث أصبحت كل من جراثيم الكونيدية والميسليوم للفطريتقلص على سطح أوراق القمح المعاملة بالبكتيريا مقارنه بالنباتات غير المعاملة، وفي النهاية أعطت النتائج التي تم الحصول عليها في هذا الدراسة دليلًا على الاستخدام المحتمل لهذه الكائنات الحية الدقيقة كبدائل آمنة وفعالة لمكافحة مرض البياض الدقيقي على القمح تحت ظروف الحقل.

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