Journal of Cereals and Oilseeds Vol. 2(6), pp. 77-87, November 2011
Available online at http://www.academicjournals.org/JCO
DOI: 10.5897/JCO11.029
ISSN-2141-6590©2011 Academic Journals

Full Length Research Paper

Effect of bio-control agents on yield, yield components and root rot control in two wheat cultivars at New Valley region, Egypt

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Accepted 24 October, 2011

The main aim of this study is to evaluate efficiency of four bioagents, *Bacillus subtilis* (isolate BSM1), *Bacillus megaterium* (isolate BMM5), *Trichoderma viride* (isolate TVM2) and *Trichoderma harzianum* (isolate THM4) for the control of rot pathogens *Fusarium graminearum, Drechslera halodes and Rhizoctonia solani* on two wheat cultivars (Sakha 93 and Bani Suif 5) under greenhouse conditions. Moreover, their effect on wheat growth and yield were also studied under field conditions. *In vitro*, all tested bioagents significantly reduced redial growth of the pathogenic fungi. *T. viride* was active more than the other tested bioagents followed by *T. harzianum*, while *B. subtilis* was the least ones. Under greenhouse conditions, all tested bioagents were able to reduce significantly damping-off and root rot caused by the tested pathogens compared with control and increased fresh and dry weight of the survival plants when applied as soil or grain treatments however, there was variation among bioagent isolates effect on reduction of disease severity both application methods. *T. viride* and *B. megaterium* were recorded the highest effective in this respect compared with other tested bioagents. Under field conditions, analysis of variance and mean performance were estimated for four characters: grain yield 4.8 m^{-2}, No. of spikes m^{-2}, No. of kernels $spike^{-1}$ and 1000-kernel weight. Significant mean squares were obtained for all studied characters between the seasons (S), methods (M), (S) × (M), treatments (T), (S) × (T), (M) (T) and interaction (S) × (M) × (T) for Sakha 93 cultivar and Bani Suif 5 cultivars except application methods and (S) × (M) for number of kernels $spike^{-1}$ and 1000-kernel weight, respectively. While the treatments *T. harzianum* and *B. megaterium* were best treatments to increase grain yield, the treatments *B. subtilis* and *T. harzianum* were best treatments to increase number of spikes and the treatments *B. subtilis* and *B. megaterium* were best treatments to increase number of kernels for soil and grain application methods, respectively.

Key words: Bio-agents, root rot disease, wheat growth, yield and yield components.

INTRODUCTION

Wheat is one of the most important feeding crops in Egypt and many other countries in the world. It is primarily grown as a food crop but the straw is also used for industrial products as feed for livestock. Wheat is subjected to relatively large number of diseases during its growing season which attack all plant parts causing serious losses in crop productivity (Bakr, 1997).

Root-rot diseases caused by soil-borne fungi are the most important wheat diseases. Several fungi were recorded as causal pathogens of root-rot diseases such as *Fusarium graminearum, Fusarium equiseti, Fusarium solani, Drechslera halodes and Rhizoctonia solani* (Hashem and Hamada, 2002; Atef, 2008; Asran and Eraky, 2011). These diseases remain prevalent because of the trends toward a higher frequency of cereals in the rotation, including, commonly, continuous cereals, and the use of less or no tillage.

Agricultural practices for management of soil borne pathogens in the field includes cultural practices, crop rotation, fungicide applications, methyl bromide fumigation,

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soil solarization and use of resistant or tolerant varieties. At present, no single method provides adequate control of soil borne diseases (Hausbek and Lamour, 2004). Using of chemicals to control soil borne pathogens causes several negative effects, such as: i) development of pathogen resistance, ii) hazards to humans, iii) damage to beneficial organisms and iv) environmental pollution. Moreover, many chemicals will be banned in near future. However, for sustainable production, pathogens still need to be controlled in order to ensure healthy plant establishment and growth (Gerhardson, 2002). Therefore, developments of various beneficial micro-organisms or biological control agents' methods are urgently needed in order to provide an alternative to chemical control. Among different biological approaches, use of the microbial antagonists like yeasts, fungi and bacteria could be promised, effectively, safely and eco-friendly in controlling many of soil borne pathogens (Gravel et al., 2004). Many biological control agents such as *Trichoderma* spp. and *Bacillus* spp. could be effectively used in suppressing diseases caused by *Fusarium* spp. *Drechslera halodes* and *Rhizoctonia solani* as reported by many workers (Hashem and Hamada, 2002; Soleimani et al., 2005; Nourozian et al., 2006; Abdel- Monaim, 2010). Modes of action for beneficial micro-organisms include direct parasitism of plant pathogens, competition for space or nutrients, or production of antibiotics, enzymes or plant hormones (Lugtenberg et al., 2003). This led to promote plant growth during the growing season as reported by Mercier and Manker (2005). However, up to date, only a few antagonist microorganisms have been identified as potential, effective bio-control agents against soil borne pathogens (Spadaro and Gullino, 2005). Also, these bioagents increased significantly due to seed germination and increased of plant growth in wheat and other many crops (Riungu et al., 2007; Zafari et al., 2008; El-Mohamedy et al., 2011). The differences among genotypes, among methods and among treatments within all methods were significant for number of kernels spike^{-1}, 1000-kernel weight and grain yield on the wheat (Moubarak, 2007). There were significant differences among the genotypes (Mahdy et al., 1996 and Pawar et al., 1997).

The main objective of the present study was to evaluate the effectiveness of isolated micro-organisms from soil under New Valley governorate conditions as bio-control agents against the incidence of root rot disease caused by *Fusarium graminearum, Drechslera halodes* and *Rhizoctonia solani* and its impact on vegetative growth, yield and yield components of two wheat cultivars under field conditions.

MATERIALS AND METHODS

During 2008/2009 growing seasons, samples of wheat plants exhibiting root rot were collected from different fields in New Valley Governorate. Samples were washed thoroughly with tap water. Small portions of the root rot diseased samples were surface sterilized in 1% sodium hypochlorite solution for 5 min, rinsed in sterilized water and dried between folds of sterilized filter papers. The portions were placed on potato dextrose agar (PDA) medium and incubated at 25±1°C. The obtained fungal colonies were purified using single spore or hyphal tip techniques suggested by Dhingra and Sinclair (1984). Identification of the fungi detected was conducted according to Booth (1985) and Gilman (1998). Stock cultures were maintained on PDA slants and kept in a refrigerator at 5°C for further studies.

Pathogenicity tests

Groups of formalin-sterilized soil were separately infested with fungal inocula of four pathogenic fungi (15-days-old) grown on barley sand medium (3%, w/w), then slightly watered every other day for a week. Soil provided with the same amount of barley sand medium and free from fungal inocula were used as control. The infested and uninfested soils were packed in formalin-sterilized pots (30-cm-diam.). Wheat grain (cvs. Sakha 93 and Bani Suif 5) superficially sterilized with 1% sodium hypochlorite were sowed at the rate of 10 grains/pot. A set of 4 replicates were used for each fungus or check treatments. Percentages of damping-off determined after 30 days from planting. After 90 days from planting, wheat plants were removed from the soil, washed thoroughly to remove soil debris, root rot were recorded as follows:

0 = roots without discoloration (no infection), 1= 1-20%, 2= 21-40%, 3= 41-75%, 4= 75-100% discoloration root mass and 5= completely dead plants include pre-or post emergence damping-off or old plants for each replicate. The mean of disease index (DI) and disease severity index (DSI) for each replicate was calculated by the formula suggested by Liu et al. (1995) was calculates:

$$DSI = \frac{\Sigma d}{d\max \times n} \mathrm{X}100$$

Where as: d is the disease rating of each plant, d max is the maximum disease rating and n is the total number of plants examined for each replicate.

Efficacy of antagonistic bioagents against pathogenic fungi *in vitro*

Bacillus subtilis (isolate BSM1), *B. megaterium* (isolate BMM5), *Trichoderma viride* (isolate TVM2) and *T. harzianum* (isolate THM4), were used in these experiments as bioagents against root rot diseases on wheat were obtained from Plant Pathology Research Department, New Valley Agricultural Research Stations.

Antagonism of *Trichoderma viride, T. harzianum* on *Fusarium graminearum, Drechslera halodes and Rhizoctonia solani*, the causal pathogens of root rot on wheat plants, was studied by dual culture technique (Rama et al., 2000). A mycelial disc (7 mm diameter), obtained from the peripheral region of 5 days old cultures tested pathogenic and *bioagent* fungi were placed simultaneously on the periphery, about 1 cm from the edges of the Petri-dishes (9 cm diameter) content PDA medium at opposite sides. While in each isolate of *B. megaterium* and *B. subtilis* was streaked individually at one side on PDA medium in plates and incubated for 24 h, then one disc (7 mm in diameter) of any pathogenic isolate was placed on the opposite side (Kaur et al., 2007). The Petri dishes containing the PDA medium inoculated with the tested pathogens alone served as control. All plates were

Table 1. The name origin and pedigree of two wheat cultivars.

No.	Cultivars	Origin	Scientific name	Pedigree
1	Sakha 93	Egypt	*Triticum aestivum* L.	Sakha 92 / TR 810328.
2	Bani Suif 5	Egypt	*Triticum durum* L.	Dipper-2 / Bushen-3.

incubated at 28°C and measurements were taken after 5 days. At the end of incubation period, radial growth was measured. The percentage inhibition growth of tested pathogens in presence of bioagents was calculated. The growth inhibition was calculated by using the formula: Mycelial growth inhibition= 100 (C- T / C), Where C= growth in control and T = growth in treatment.

Efficacy of grain and soil treatments with some bioagents under greenhouse and field conditions

Production of bioagents inocula used for grain and soil treatments

Antagonistic bacterial inocula were produced as described by Landa et al. (2001). Bacterial concentration in the suspension was adjusted to proximately 5×10^8 cells ml $^{-1}$ by measuring absorbance at 600 nm in a spectrophotometer and using standard curves for each bacterial isolate. Inocula of antagonistic fungi were prepared as described by Sallam et al. (2008). The fungal suspension was adjusted to 3×10^7 cfu ml^{-1}, using haemocytometre for each isolate. The bacterial and fungal suspension was used for grain coating and soil drenching.

Under greenhouse conditions

Formalin-sterilized soil were inoculated with the highly pathogenic isolated fungi that is *R. solani*, *F. graminearum* and *D. halodes* individually at rate 3% then irrigated and left for 7 days. The infested pots were divided into two groups, the first group planted with wheat grains soaked in suspension of bioagent isolates for 6 h. before sowing and the second groups were drenching with 50 ml of bio agents isolates then planting with untreated wheat grains. Ten grains were sown in pots which were infested with the pathogens. Also untreated grains were sown in pots infested with the tested pathogens to serve as a control. All treatments were in 4 replicates and arranged in complete randomized design.

Under field conditions

The present study was carried out at the New Valley Agricultural Research Station by Wheat Research Department, during two successive growing seasons, 2009/2010 and 2010/2011. Two wheat (*Tritium* spp.) cultivars were used to study the bio-control agents.

The name and pedigree of these cultivars are presented in Table 1 In the growing seasons; the two wheat cultivars were grown for evaluation in a randomized complete blocks design (RCBD) with four replications. Each plot was planted in eight rows; four meter long, 20 cm. apart. The recommended cultural practices were applied from sowing to harvesting. Data for the studied characters recorded on six internal rows.

The studied characters were grain yield 4.8 m^{-2} (Kg.), number of spikes m^{-2}, number of kernels $spike^{-1}$ and 1000-kernel weight (g.). The obtained data were analyzed to estimate better methods and treatments of using bio-control agents.

Statistical analysis

The least significant differences (LSD.) test at 5% level of probability, according to Steel and Torrie (1980) was used to compare among means.

RESULTS AND DISCUSSION

Isolation trials from diseased roots of wheat plants resulted in five pathogenic fungi, that is *Fusarium graminearum, F. equiseti, F. solani, Drechslera halodes and Rhizoctonia solani.*

Pathogenicity tests

Pathogenicity of isolated fungi was tested on two wheat cultivars (Sakha 93 and Bani Suif 5). Data in Table 2 indicate that all the tested fungi significantly caused damping-off and root rot in both wheat cultivars subsequently, decreased the percentages of healthy survival plants compared with the check treatment. *Rhizoctonia solani* was the most pathogenic fungi as they recorded percentage damping-off (60 and 50%) and 26.8 and 23% root rot in both cultivars, respectively followed by *D. halodes* where caused 50 and 40% damping-off and 25.8 and 20.4% On the contrary, *F. solani and F. equiseti* were the least pathogenic ones recording the lowest percentages of these criteria. It was also observed that the percentages of damping-off in all tested fungi were higher than those of root rot. These results are in harmony with those reported by Hashem and Hamada (2002), Atef (2008) and Asran and Eraky (2011).

Efficacy of antagonistic bioagents against pathogenic fungi *in vitro*

Bacillus subtilis, B. megaterium, T. viride and *T. harzianum,* strains were evaluated for antagonistic effect against *F. graminearum, D. halodes* and *R. solani* on Petri dishes containing PDA medium. Table 3 show that the bioagent strains succeeded in reducing the radial growth of the tested pathogenic fungi. *T. viride* was active more than the other tested bioagents for reducing the radial growth of fungi. The percent inhibition of radial growth of tested fungi viz., *F. graminearum* (67.7%), *D. halodes* (74.0%), and *R. solani* (59.5%) were reduced by *T. viride* which grown over the colonies of all three

Table 2. Pathogenic ability of four soil borne fungi on damping-off and root rot severity of wheat plants under greenhouse conditions.

Treatment	Cultivars					
	Sakha 93			Bani Suif 5		
	Damping-off (%)	Root rot (%)	Survival plants (%)	Damping-off (%)	Root rot (%)	Survival plants (%)
F. graminearum	40	30.0	30.0	35	22.4	42.6
F. equiseti	25	18.2	56.8	20	14.8	65.2
F. solani	20	15.4	64.6	20	10.0	70.0
D. halodes	50	25.8	24.2	40	20.4	39.6
R. solani	60	26.8	13.2	50	23.0	27 .0

LSD at 0.05	Damping-off	Root rot	Root rot	Survival plants
Treatments (A)	3.70	3.20	3.20	5.78
Cultivars (B)	2.33	2.03	2.03	3.65
Interaction (AxB)	5.23	ns	ns	ns

Table 3. Inhibitory effect of some bio-against isolates on mycelial growth of wheat pathogenic fungi.

Treatment	Mycelial growth inhibition (%)			
	F. graminearum	*D. halodes*	*R. solani*	Mean
B. subtilis	39.2	53.1	41.8	44.70
B. megaterium	52.0	58.5	48.1	52.87
T. viride	67.7	74.0	59.5	67.07
T. harzianum	60.2	66.7	50.9	59.27
Mean	54.77	63.07	50.07	-

LSD at 0.05 for: treatments (A) =3.55; pathogenic fungi (B) = 2.97; introduction (AxB) = 6.85.

pathogens. On the other hand Trichoderma isolates were effective more than Bacillus isolates in inhibition of redial growth of all the tested fungi and the greatest reduction occurring in *D. halodes* (63.07%) followed by *F. graminearum* (54.77%), while *R. solani* less affected ones (50.07%). The growth inhibition of wheat root rot fungi by dual culture in this study could be due to its fast growing nature, secretions of armful extra-cellular compounds like antibiotics, cell wall degrading enzymes such as β-1, 3 gluconase, endochitinase and chitinase enzymes which degrade the cell wall leading to lyses of mycelium of the pathogen and mycoparasitism in dual culture as found with other fungi (Lugtenberg et al., 2003; Riungu, 2008 and Rahman, et al., 2010).

Efficacy of grain and soil treatments with some bioagent isolates

Greenhouse experiments

In the seed coating and soil treatment tests, all the tested bioagents were able to reduce significantly damping-off and root rot caused by *F. graminearum, D. halodes* and *R. solani* compared with control, however, there was variation among bioagent isolates effect on reduction of disease severity both soil and seed application methods and both cultivates (Table 4A, B and C). *B. megaterium* and *T. viride* recorded the highest reduction in damping-off and root rot severity caused by the tested fungi, while soil and/or grain treatments with *B. subtilis* and *T. harzianum* gave the lowest reduction for these diseases in both cultivars.

Concerning the method of applications, it is cleared that there were a significant differences among method of applications. The highest significant values for suppressing fusarium, drechslera and rhizoctonia root rots incidence on wheat plants were recorded with soil treatment in all cases.

Also, there were significant variation among both cultivars; however cv. Sakha 93 was more susceptible than cv. Bani Suif 5 in all cases. On the other hand, the obtained data show that all of the soil and grain application treatments increased significantly the fresh and dry weight of the survival wheat plants compared with control, but there was difference among their effect

Table 4A. Effect of soil drenching, wheat grain treatment with different bioagents on damping-off and root rot diseases, fresh and dry weight plant^{-1} under artificial infection with *Fusarium graminearum* under greenhouse conditions.

Treatment	Application methods	Sakha 93				Bani Suif 5			
		Damping-off (%)	Root rot (%)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Damping-off (%)	Root rot (%)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})
B. subtilis	Soil	20	14.0	3.161	0.691	15	10.0	3.422	0.725
	Grain	30	16.4	2.481	0.524	25	12.8	2.235	0.473
B. megaterium	Soil	5	6.6	4.583	0.972	10	5.2	4.25	0.907
	Grain	10	8.0	4.028	0.872	15	6.8	3.657	0.796
T. viride	Soil	15	7.6	4.209	0.923	5	4.5	3.969	0.795
	Grain	15	9.4	3.412	0.731	10	6.0	3.414	0.699
T. harzianum	Soil	20	12.4	3.800	0.786	15	9.0	3.55	0.77
	Grain	25	13.6	3.078	0.622	25	10.6	2.914	0.621
Control		45	33.6	1.333	0.277	35	25.4	1.182	0.251

LSD at 0.05	Damping-off	Root rot	Fresh weight	Dry weight
Treatments (A)	1.71	1.20	0.177	0.057
Application methods (B)	*	*	*	*
Cultivars (C)	*	*	*	*
Interaction (AxBxC)	ns	2.55	ns	0.111

Table 4B. Effect of soil drenching, wheat grain treatment with different bioagents on damping-off and root rot diseases, fresh and dry weight plant^{-1} under artificial infection with *Drechslera halodes* under greenhouse conditions.

Treatment	Application methods	Sakha 93				Bani Suif 5			
		Damping-off (%)	Root rot (%)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Damping-off (%)	Root rot (%)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})
B. subtilis	Soil	20	12	3.142	0.674	20	10.8	2.916	0.634
	Grain	30	13.6	2.627	0.557	25	13.0	2.014	0.431
B. megaterium	Soil	5	6.8	5.092	1.088	5	5.8	4.071	0.833
	Grain	15	8.2	4.022	0.863	10	6.2	3.214	0.643
T. viride	Soil	5	10.6	3.613	0.738	5	7.6	3.208	0.628
	Grain	10	12.0	3.429	0.702	5	9.4	2.814	0.603

Table 4B contd.

T. harzianum	Soil	25	19.8	3.633	0.731	15	16.6	3.029	0.617
	Grain	35	22.2	3.240	0.672	25	19.8	2.492	0.541
Control		50	28.4	1.037	0.209	40	24.6	0.805	0.171

LSD at 0.05	Damping-off	Root rot	Fresh weight	Dry weight
Treatments (A)	2.45	1.28	0.177	0.048
Application methods (B)	*	*	*	*
Cultivars (C)	*	*	*	*
Interaction (AxBxC)	4.89	2.56	0.352	0.097

in this regard. The maximum fresh and dry weight obtained in *B. megaterium* followed by *T. viride*. While the lowest increased of fresh and dry weight of survival wheat plants were recorded in case of *B. subtilis* application.

On the other hand, soil application treatment improved the fresh and dry weights of wheat were more than grain application method in all cases.

Also, cv. Bani Suif 5 gave fresh and dry weights high than cv. Sakha 93 in most cases.

Generally, *B. megaterium* and *T. viride* recorded the highest reduction of damping-off, root rot and increased fresh and dry weight of survival wheat plants especially in soil application method. The obtained results are in good accordance with

previous studies which have been concluded that *Trichoderma* spp. and *Bacillus* spp. can effectively protect many plant species against root rot diseases (Hashem and Hamada, 2002; Soleimani et al., 2005; Nourozian, et al., 2006; Atef, 2008; Abdel- Monaim, 2010).

According to Harman (2001) natural factors limiting the number of soil borne pathogens occur through a combination of antagonism by other soil fungi and bacteria, natural release of antibiotics from other bacteria and fungi, and by competitive exclusion of habitat in the root zone or rhizosphere.

The mechanism of Trichoderma and Bacillus action on pathogens may be by attacking and binding the pathogenic organisms by sugar linkage and begins to secrete extracellular protease and lipase (Soleimani et al., 2005; Zaghloul et al., 2007), produce siderophores and hydrogen cyanide (Soleimani et al., 2005), production of secondary metabolites such as Phenazine -1-Carboxilic acid (PCA), 2,4-Pyrrolnitrin, Oomycin (Knudsen, 1995).

Under field conditions

Mean squares for all studied characters in (Table 5), showed significant differences between the seasons (S), methods (M), (S) x (M), treatments (T), (S) x (T), (M) x (T) and (S) x (M) x (T) for Sakha 93 cultivar, except methods and (S) x (M) for number of kernels spike^{-1}. These results are confirmed with those obtained by Mahdy et al. (1996), Pawar et al. (1997) and Moubarak (2007). Mean squares for all studied characters (Table 6), showed significant differences between the seasons (S), methods (M), (S) × (M), treatments (T), (S) × (T), (M) × (T) and (S) × (M) × (T) for Bani Suif 5 cultivar, except methods and (S) × (M) for 1000-kernel weight. These results are confirmed with those obtained by Mahdy et al. (1996), Pawar et al. (1997) and Moubarak (2007).

The results illustrated (Tables 7 and 8) showed that, in soil application method, the treatments *T. harzianum*, *B. subtilis*, *B. subtilis* and *B. megaterium* had highest values for grain yield, number of spikes m^{-2}, number of kernels spike^{-1} and 1000-kernel weight in the two seasons, respectively, while in grain application method, the treatments *B. megaterium*, *T. harzianum*, *B. megaterium* and *B. megaterium* had highest values for grain yield, number of spikes m^{-2}, number of kernels spike^{-1} and 1000-kernel weight in the two seasons, respectively. From the previous results, it could be concluded that, the treatments *T. harzianum* and *B. megaterium* were best treatments to increase grain yield, the treatments *B. subtilis* and *T. harzianum* were best treatments to increase number of spikes and the treatments *B. subtilis* and *B. megaterium* were best treatments to increase number of kernels for soil application method and grain application

Table 4C. Effect of soil drenching, wheat grain treatment with different bioagents on damping-off and root rot diseases, fresh and dry weight plant^{-1} under artificial infection with *Rhizoctonia solani* under greenhouse conditions.

Treatment	Application methods	Sakha 93				Bani Suif 5			
		Damping-off (%)	Root rot (%)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Damping-off (%)	Rootrot (%)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})
B. subtilis	Soil	20	14.2	2.345	0.512	15	10.0	2.267	0.501
	Grain	25	17.4	1.958	0.405	15	13.8	2.001	0.402
B. megaterium	Soil	10	7.6	3.900	0.809	10	6.0	3.100	0.668
	Grain	15	8.2	3.409	0.735	10	8.0	2.069	0.443
T. viride	Soil	5	5.6	2.967	0.602	5	4.6	2.933	0.625
	Grain	10	6.4	2.491	0.542	5	6.0	2.725	0.579
T. harzianum	Soil	25	16.4	2.817	0.600	20	13.6	2.521	0.512
	Grain	35	22.6	2.183	0.452	30	15.6	2.055	0.439
Control		55	30.4	1.038	0.219	45	23.2	1.058	0.229

LSD at 0.05	Damping-off	Root rot	Fresh weight	Dry weight
Treatments (A)	2.00	1.50	0.229	0.051
Application methods (B)	*	*	*	*
Cultivars (C)	*	*	*	*
Interaction (AxBxC)	3.98	Ns	0.460	ns

Table 5. Mean square analysis of four characters for Sakha 93 bread wheat cultivar using bio-control agents by soil and grain methods in 2009/2010 and 2010/2011 seasons.

		Mean squares			
S.O.V	d.f.	Grain yield 4.8m^{-2}	No. of spikes m^{-2}	No. of kernels spike^{-1}	1000-kernel weight
Replications	3	0.004	34.533	13.933	0.635
Seasons (S)	1	9.716*	12152.450*	2121.800*	1570.878*
Methods (M)	1	0.021*	6265.800*	0.200	24.753*
SM	1	0.036*	2184.050 *	7.200	110.215*
Treatments (T)	4	0.388*	4976.513*	122.856*	298.386*
ST	4	0.120*	892.825*	42.581*	11.924*
MT	4	0.201*	5423.737*	308.606*	40.884*
SMT	4	0.023*	926.800*	14.231*	17.766*
Error	57	0.004	46.130	3.065	2.101

* Significant at 5% probability.

Table 6. Mean square analysis of four characters for Bani Suif 5 durum wheat cultivar using bio-control agents by soil and grain methods in 2009/2010 and 2010/2011 seasons.

		Mean squares			
S.O.V	**d.f.**	**Grain yield 4.8 m^{-2}**	**No. of spikes m^{-2}**	**No. of kernels spike^{-1}**	**1000-kernel weight**
Replications	3	0.007	41.233	1.512	2.204
Seasons (S)	1	3.113*	9159.200*	1453.513*	1553.203*
Methods (M)	1	0.021*	8946.450*	32.513*	0.001
SM	1	0.031*	530.450*	35.112*	0.435
Treatments (T)	4	0.520*	4649.019*	371.294*	119.963*
ST	4	0.032*	1000.544*	15.919*	7.977*
MT	4	0.315*	5239.794*	383.106*	3.414*
SMT	4	0.018*	1127.294*	64.206*	23.208*
Error	57	0.004	45.996	3.153	1.616

* Significant at 5% probability.

Table 7. Effect of bio-control against on grain yield (Kg 4.8 m^{-2}), No. of spikes m^{-2}, kernels $spikes^{-1}$ and 1000-kernel weight for Sakha 93 bread wheat cultivar in 2009/2010 and 2010/2011 seasons.

Season	Treatment	Application methods	Grain yield (Kg 4.8 m^{-2})	No. of spikes m^{-2}	No. of kernels spike^{-1}	1000-kernel weight
2009/ 2010	*B. subtilis*	Soil	1.73	349.50	62.25	50.63
		Grain	1.66	330.50	56.25	45.08
	B. megaterium	Soil	1.62	237.50	46.50	57.10
		Grain	1.89	347.75	61.00	49.45
	T. viride	Soil	1.68	309.00	55.75	42.93
		Grain	1.70	316.25	48.25	45.95
	T. harzianum	Soil	1.86	326.00	57.75	53.53
		Grain	1.69	368.25	54.25	46.40
	Control		1.59	305.00	59.25	44.95
2010/ 2011	*B. subtilis*	Soil	2.44	359.00	74.25	56.88
		Grain	2.12	336.75	67.50	55.15
	B. megaterium	Soil	2.46	322.75	60.25	64.03
		Grain	2.86	358.75	76.25	64.03
	T. viride	Soil	2.41	342.50	63.25	48.30
		Grain	2.23	337.50	63.25	53.08
	T. harzianum	Soil	2.76	351.25	66.75	57.85
		Grain	2.49	378.75	61.00	60.98
	Control		2.10	327.00	65.50	54.65

LSD at 0.05	Grain yield 4.8 m^{-2}	No. of spikes m^{-2}	No. of kernels spike^{-1}	1000-kernel weight
Seasons (S) and methods (M)	0.057	6.075	1.566	1.297
(S) (M)	0.08	8.591	2.215	1.833
Treatments (T)	0.057	6.075	1.566	1.296
(S) (T) and (M) (T)	0.081	8.592	2.215	1.833
(S) (M) (T)	0.114	12.15	3.132	2.593

method, respectively. The obtained results are in harmony with that obtained by Riungu et al. (2007). Zafari et al., 2008 on wheat**.** All these workers reported that using beneficial micro-organisms as biocontrol agents led to enhancement of plant growth parameters.

Such enhancement may be due to induce plant resistance (De Meyer et al., 1998), produce extracellular enzymes and antifungal or antibiotics, which decrease biotic stress on plant, and produce growth promoters substances (Szczech and Shoda, 2004).

In addition, Egamberdiyeva (2007) hypothesized that there are several mechanisms by which rhizosphere bacteria may stimulate plant growth, such as production of plant growth substances, nitrogen fixation, phytohormones, vitamins, solublizing minerals besides, their role in

Table 8. Effect of bio-control against on grain yield (Kg 4.8 m^{-2}), No. of spikes m^{-2}, kernels $spikes^{-1}$ and 1000-kernel weight for Bani Suif 5 durum wheat cultivar in 2009/2010 and 2010/2011 seasons.

Season	Treatments	Application methods	Grain yield (Kg 4.8 m^{-2})	No. of spikes m^{-2}	No. of kernels spike^{-1}	1000-kernel weight
2009/ 2010	*B. subtilis*	Soil	2.99	325.50	62.25	53.23
		Grain	2.83	287.75	62.00	52.60
	B. megaterium	Soil	2.81	289.75	47.25	60.20
		Grain	3.08	280.00	67.50	58.43
	T. viride	Soil	2.88	310.50	59.00	51.00
		Grain	2.77	269.00	49.25	53.88
	T. harzianum	Soil	3.34	286.00	65.75	55.68
		Grain	2.99	295.00	55.25	55.90
	Control		2.72	250.50	50.25	56.65
2010/ 2011	*B. subtilis*	Soil	3.28	350.00	73.25	61.15
		Grain	3.10	256.25	69.25	64.60
	B. megaterium	Soil	3.18	287.50	59.25	65.83
		Grain	3.76	311.75	75.25	67.30
	T. viride	Soil	3.26	357.50	62.25	62.88
		Grain	3.19	288.75	67.25	57.65
	T. harzianum	Soil	3.72	317.50	68.25	64.48
		Grain	3.42	324.25	64.25	64.00
	Control		3.08	282.50	57.50	67.23

LSD at 0.05	Grain yield 4.8m^{-2}	No. of spikes m^{-2}	No. of kernels spike^{-1}	1000-kernel weight
Seasons (S) and methods (M)	0.054	6.066	1.588	1.137
(S) (M)	0.077	8.579	2.246	1.608
Treatments (T)	0.054	6.066	1.588	1.137
(S) (T) and (M) (T)	0.077	8.579	2.246	1.608
(S) (M) (T)	0.108	12.133	3.177	2.274

direct inhibition of pathogen growth and suppression of diseases caused micro-organisms and increased of plant growth and yield.

Conclusion

Specific rhizobacterial and rhizofungal agents can influence disease suppression and could be considered as part of disease control strategy under an integrated pest management which offers a successful approach for the deployment of both agro-chemicals and biocontrol agents.

Also, this study suggest that simultaneous screening of rhizobacteria and rhizofungi for growth and yield promotion under field experiment is a good tool to select effective rhizobacteria and rhizofungi for biofertilizer development biotechnology.

The treatments *T. harzianum* and *B. megaterium* were best treatments to increase grain yield, the treatments *B. subtilis* and *T. harzianum* were best treatments to increase number of spikes and the treatments *B. subtilis* and *B. megaterium* were best treatments to increase number of kernels for soil application method and grain application method, respectively.

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