

## EFFECT OF SOME COMMERCIAL BIO-AGENTS ON YIELD AND DAMPING-OFF DISEASE OF WHEAT LINES (*Triticum aestivum vulgair* L.)

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### ABSTRACT

The current study was conducted to assess the possibility of using two bio-agents (BAs), *Trichoderma harzianum* T39 (Trichodex) and *Bacillus subtilis* (MBI 600) tested at 1, 3 and 5% concentration to control damping-off disease and also their ability to promote yield of wheat lines under field conditions. Seed inoculation with two BAs protected wheat seedlings and highly promoted seed germination. Also, inoculation with BAs significantly affected number of spikes/plant, biological yield/plant and grain yield/plant and there were statistically significant differences between bread wheat lines under field conditions. Moreover, the treatments at 5% of *T. harzianum* and *B. subtilis* were the best treatments to increase grain yield. Four lines, L11, L12, L27 and L38 were found to be more responsive to BAs treatments under field conditions.

**Keywords:** Wheat lines, Damping-off, Bio-agents, Control, Yield.

### INTRODUCTION

Wheat (*Triticum aestivum vulgair* L.) is one of the most important cereal crops in Egypt and many other countries in over the world. It 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). Among seedling diseases of wheat, damping-off and/or root rot caused by several soil-borne fungi *F.solani*, *F.oxysporium*, *F. graminearum*, *Pythium* sp. and *R. solani* that attack at seedling stages of crop and directly reduce plant population as well as yield of wheat (Hashem and Hamada, 2002; Abdelzaher, 2004; Atef, 2008; Ahmed *et al.*, 2009; Asran and Eraky, 2011 and Moubarak and Abdel-Monaim 2011). Control of damping-off mainly depends on fungicide treatment. As a fungicidal application causes hazards to human health and increases environmental pollution. Hence, alternatives and eco-friendly approaches for the control of plant diseases are needed. There have been many reports that bacterial and fungal isolates from soil or plant roots are able to control plant disease or directly stimulate crop growth (Ryder *et al.*, 1994 and Ogoshi *et al.*, 1997). In China, plant-associated *Bacillus*, mainly *B. cereus* Frankland and Frankland, collectively called yield-increasing bacteria (YIB), have been commercially produced and sold for

plant growth and yield promotion since the 1980s (Tang, 1994 and Chen *et al.*, 1996).

*Bacillus* spp. as a group offer several advantages over other bacteria for protection against root pathogens because of their ability to form endospores, and because of the broad-spectrum activity of their antibiotics (Cavaglieri *et al.*, 2005). *Trichoderma* spp. are most popular research tools as microbial inoculants which have been largely used against several plant pathogenic fungi causing soil borne, air borne and post-harvest diseases of plant through their high antagonistic and mycoparasitic potential in lab conditions. In recent years, they have become popular as plant growth promoter (Hermosa *et al.*, 2012; Yassin *et al.*, 2012 and Vermaa *et al.*, 2014). Some *Trichoderma* rhizosphere-competent strains have been shown to have direct effects on plants, increasing their growth potential and nutrient uptake, fertilizer use efficiency, percentage and rate of seed germination, and stimulation of plant defences against biotic and abiotic damage (Shoresh *et al.*, 2010). Studies have demonstrated that *Trichoderma* increases root development, crop yield, proliferation of secondary roots, seedling fresh weight and foliar area.

Among different biological approaches, use of the microbial antagonists like fungi and bacteria could be promised effectively in controlling many of soil-borne pathogens (Gravel *et al.*, 2004). Commercial products based on formulated antagonists of *Trichoderma* spp. and *Bacillus* spp. is widespread, available and marketable. Many of them like Trichodex and MBI 600 of *T. harzianum* T39 and *B. subtilis*, respectively, are potentially applied as bio-control agents or bio-fertilizers in agriculture. The importance of these products is not only for suppression the disease severity but also helps in sustenance and growth promotion of plant. Previous studies have emphasized that *Trichoderma* spp. and *Bacillus* spp. effectively could be used in suppressing soil-borne diseases of wheat caused by *Fusarium* spp., *Pythium* spp., and *R. solani* (Hashem and Hamada, 2002; Soleimani *et al.*, 2005; Nourozian *et al.*, 2006; Abdel- Monaim, 2010; Moubarak and Abdel-Monaim, 2011 and Perello *et al.*, 2013). 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), thus this led to increase significantly seed germination and promote plant growth of wheat and other crops through shoot and root systems as well as nutrient uptake properties during the growing season (El- Mohamedy *et al.*, 2001; Mercier and Manker, 2005; Riungu *et al.*, 2008 and Zafari *et al.*, 2008).

The objectives of this research were intended to (1) determine the level of resistance in several inbred lines of wheat against damping-off disease under naturally infested soil with the pathogenic fungi and (2) study the efficiency of seed treatments with some commercial bio-control products to promote yield of wheat lines under field conditions.

## MATERIALS AND METHODS

Fifty promising bread wheat lines (*Triticum aestivum vulgair* L.) were used in this study. These lines were derived from the two crosses as follow; 24 lines were derived from a cross between a high yielding local variety "Sids-4" with a drought tolerant variety "Tokwie" (South Africa) and 26 lines were derived from a cross between "Sids-4" and "Kasyon/glennson-81" (ICARDA). In addition, Giza-168 (local commercial variety) was also used in this study.

### **Used bio-agents and seed treatments:**

Commercial products MBI 600 and Trichodex of *B. subtilis* and *T. harzianum* T39, respectively, obtained from (Institute of Biological control of Julius Kühn Institute (JKI), Darmstadt, Germany) were used in this study. These bio-agents (BAs) were also re-isolated from their powder formulations by dilution plate method on nutrient agar (NA) and potato dextrose agar (PDA) medium for bacteria and fungus, respectively, where the colony forming unit  $g^{-1}$  (cfu) of powder formulation was determined to  $10^9$  and  $5 \times 10^6$ , respectively. Seed treatment with powder formulations of these BAs was used to coat wheat grains at 1, 3, and 5% concentrations.

### **Isolation of the causal pathogen:**

Samples of wheat seedlings exhibiting damping-off symptoms at 1-3 weeks-old were collected from the Experimental Farm, Fac. of Agric., Sohag Univerity, El-Kawther, Sohag during 2010/2011 growing season to isolate causal pathogens. Diseased samples (basal part of stem and root) were washed thoroughly with tap water, cut into small pieces (0.5-1 cm) and surface sterilized by immersing in 1% sodium hypochlorite solution (SH) for 3 min, then immediately rinsed for several times with sterile water. Disinfested pieces were dried between folds of sterile filter papers, placed on potato dextrose agar (PDA) medium containing 40 mg streptomycin sulphate per 100 ml medium, and then incubated at  $25 \pm 5$  C° for two days. The resulted fungi were purified using single spore or hyphal tip techniques. Identification of all isolated fungi was conducted according to Domsch et al. (1980), Plaats-Niterink (1980), Nirenberg (1981), Booth (1985) and Gilman (1998). Stuck cultures of isolated fungi were maintained on PDA slants and kept at 5C° for further studies.

### **Pathogenicity tests:**

To investigate the pathogenic capability of all isolated fungi, greenhouse trials were conducting in 2010/2011 growing season. Groups of formalin-sterilized soil (F-SS) were separately infested with each inoculums of tested fungi (15-days-old) grown on barley sand medium (3%, w/w), then slightly irrigated every other day for a week. Otherwise, soil provided with 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 in diameter). Wheat grains of Giza 168 cv. superficially sterilized with 1% SH were sowed at the rate of 10 grains per each pot. Three pots were used for each fungus of check treatments. Percentages of pre- and post- emergence damping-off (%) of infected seedlings were determined after

10 and 21 days from planting, respectively. The main pathogen was consistently re-isolated from infected seedlings.

**Field trials under naturally infested soil with damping-off pathogens:**

Field experiments were conducted in the successive growing seasons of 2011/12 and 2012/13 at the Expert. Farm, Fac. of Agric., Sohag Univ., to screen bread wheat lines against damping-off and test also the efficacy of seed treatment with commercial bio-agents on disease control and yield. Field soil, naturally infested with previously referred fungi causing damping-off was sandy-loam with organic matter (2.3%), sand (73.2%), silt (16.4%), clay (8.5%) and a pH of 7.7. The experiment was carried out as factorial based on randomized complete block design (RCBD). Wheat seeds of each tested genotype or variety were sterilized with 1% sodium hypochlorite solution, treated with each tested BAs and then sown in plots in with three replicates. Plots were two rows, each 3 m long with 20 cm width and 10 cm plant spacing. All cultural practices were applied as recommended for wheat production. Number of spikes/plant, biological yield/plant (g) and grain yield/plant (g) were measured for the plot area.

Damping-off (%) of infected seedlings were determined after 21 days from planting, as above mentioned. Any genotype/variety/inbred line with damping-off 0% was graded as immune (I), with 0.1-5% as highly resistant (HR), with 5.1-15% as resistant (R), with 15.1- 30% as susceptible (S) and with 30.1% or more as highly susceptible (HS) modified scales after (Ahmed et. al., 2009).

**Statistical analysis:**

The combined analysis of the two season's data was conducted according to Gomez and Gomez (1994). The means of genotypes were compared using the Revised Least Significant Difference (RLSD) method at 5% and 1% of probability. Statistical analysis was performed using "MSTATC" microcomputer program (MSTATC 1990).

## RESULTS

Isolation trials from diseased wheat plants showing damping-off symptoms resulted in five fungi, that were identified as *F. solani*, *F. oxysporium*, *F. graminearum*, *Pythium* sp. and *R. solani*. Pathogenicity tests of these isolated fungi were tested on wheat Giza 168 cv under greenhouse conditions. Data in Table 1 indicate that all isolated fungi were significantly pathogenic on wheat plants, decreased the percent of germinated grains and caused both pre- and post- emergence damping-off of wheat seedlings as compared with check control. *Rizoctonia solani* was the most pathogenic fungi followed by *F. oxysporium* and *F. graminearum* with highly decreased germination to 63.33, 66.67 and 70.00% and recorded percentages of pre- and post- emergence damping-off (33.33 and 3.34%), (16.67 and 16.67%) and (23.33 and 6.67%), respectively. Otherwise, *Pythium* sp. and *F. solani* were the least pathogenic ones.

**Table 1: Pathogenic ability of five soil-borne isolated fungi on germination and damping-off of wheat plants of Giza 168 cv under greenhouse conditions in 2010/2011 growing season.**

Fungi	Germination (%)	Damping-off (%)		
		Pre-emergence	Post-emergence	Total
<i>F. solani</i>	76.67	13.33	10.00	23.33
<i>F. oxysporium</i>	66.67	16.67	16.67	33.33
<i>F. graminearum</i>	70.00	23.33	6.67	30.00
<i>Pythium</i> sp.	73.33	16.67	10.00	26.67
<i>R. solani</i>	63.33	33.33	3.34	36.67
Control	100.00	0.00	0.00	0.00
LSD at 0.05	8.79	10.33	6.36	

**Screening of wheat inbred lines to incidence with damping-off under naturally infested soil:**

In field trials of naturally infested soil with damping-off pathogens, during 2011/2012 and 2012/2013 growing seasons all tested inbred lines varied significantly to incidence with damping-off with a range of means from 15.00 to 50.00% (Table 2). Out of 50 inbred lines of wheat including Giza 168 cv (check), 25 line were graded as highly susceptible (HS) for seedlings damping-off, 21 lines were susceptible (S), and remaining 4 lines were resistant (R). Moreover, no highly resistant (HR) lines were recorded.

**Efficacy of seed treatment with commercial BAs on:-**

**A- Damping-off control**

Results in Table 2 indicate that the two BAs at all tested concentrations were able to reduce significantly damping-off infection compared with untreated control, however, there was variation among concentrations of the two tested BAs on reduction of infection with damping-off of all tested wheat lines. Infection with damping-off of most tested lines decreased with increasing bio-agent concentration up to 5%. Finally, *T. harzianum* (Trichodex) at all tested concentrations was better than *B. subtilis* (MBI 600) and caused highly reduction of infection of all tested lines.

**B- Agronomic traits of bread wheat lines under field conditions**

**Analysis of variance**

The combined analysis of variance for number of spikes/plant, biological yield/plant and grain yield/plant in Table 3 revealed that highly significant differences between years (Y), lines (L), (Y) x (L), treatments (T), (Y) x (T), (L) x (T) and (Y) x (L) x (T). These results showed that inbred wheat lines responded differently when they were grown under treatments.

**Mean performances**

**Number of spikes/plant**

Comparing the different treatments of BAs (*T. harzianum*, 1%, 3% and 5% and *B. subtilis*, 1%, 3% and 5%) under field conditions, it was observed that highly significant differences existed among the different treatments of BAs on number of spikes/plant (Table 5). The highest mean values (12.05 and 11.46 spikes/plant) were obtained with *T. harzianum* 5%

and *B. subtilis* 5%, respectively, in both seasons. *T. harzianum*, 3% and *B. subtilis*, 3% were ranked secondly in the two seasons. While, the lowest value (8.21 spikes/plant) was obtained at untreated seed (control) in both seasons. The results indicate that increasing bio-agent's concentration from 1% to 3% and 5% caused significant increasing in number of spikes/plant.

**Table 2: Effect of seed treatments of wheat lines with different bioagents on damping-off disease under natural infection with pathogenic fungi.**

Inbred lines	Bio-agents									Mean	
	Control (non treated grains)		<i>B. subtilis</i> (MBI 600)			Mean	<i>T. harizantum</i> (Trichodex)				Mean
			1%	3%	5%		1%	3%	5%		
1	45.0 <sup>a</sup>	HS <sup>b</sup>	25.0 <sup>a</sup>	20.0 <sup>a</sup>	15.0 <sup>a</sup>	20.00 <sup>a</sup>	25.00 <sup>a</sup>	20.0 <sup>a</sup>	10.0 <sup>a</sup>	18.33 <sup>a</sup>	
2	20.0	S	20.0	15.0	5.0	13.33	15.0	15.0	5.0	11.67	
3	25.0	S	20.0	20.0	10.0	16.67	15.0	15.0	10.0	13.33	
4	45.0	HS	20.0	10.0	10.0	13.33	20.0	10.0	10.0	13.33	
5	45.0	HS	30.0	25.0	20.0	25.00	30.0	30.0	25.0	28.33	
6	35.0	HS	30.0	25.0	25.0	26.67	25.0	25.0	20.0	23.33	
7	40.0	HS	30.0	20.0	10.0	20.00	25.0	15.0	0.0	13.33	
8	35.0	HS	30.0	20.0	20.0	23.33	25.0	20.0	20.0	21.67	
9	40.0	HS	25.0	20.0	20.0	21.67	25.0	15.0	5.0	15.00	
10	35.0	HS	35.0	35.0	20.0	30.00	30.0	30.0	20	26.67	
11	30.0	HS	20.0	20.0	15.0	18.33	25.0	25.0	20.0	23.33	
12	15.0	R	15.0	15.0	10.0	13.33	10.0	10.0	0.0	6.67	
13	30.0	S	25.5	25.0	20.0	23.50	25.0	20.0	20.0	21.67	
14	30.0	S	30.0	30.0	20.0	26.67	30.0	15.0	15.0	20.00	
15	40.0	HS	30.0	25.0	20.0	25.00	25.0	25.0	20.0	23.33	
16	35.0	HS	25.0	20.0	20.0	21.67	30.0	25.0	25.0	26.67	
17	30.0	S	20.0	20.0	15.0	18.33	20.0	10.0	10.0	13.33	
18	25.0	S	15.0	15.0	10.0	13.33	15.0	10.0	0.0	8.33	
19	30.0	S	20.0	20.0	20.0	20.00	20.0	20.0	10.0	16.67	
20	25.0	S	25.0	25.0	15.0	21.67	20.0	15.0	0.0	11.67	
21	45.0	HS	35.0	25.0	25.0	28.33	35.0	25.0	20.0	26.67	
22	30.0	S	30.0	25.0	15.0	23.33	20.0	20.0	20.0	20.00	
23	45.0	HS	25.0	25.0	20.0	23.33	30.0	10.0	5.0	15.00	
24	30.0	S	25.0	15.0	5.0	15.00	20.0	15.0	0.0	11.67	
25	40.0	HS	25.0	25.0	15.0	21.67	20.0	10.0	0.0	10.00	
26	15.0	R	5.0	5.0	0.0	3.33	10.0	5.0	5.0	6.67	
27	30.0	S	25.0	20.0	20.0	21.67	20.0	20.0	15.0	18.33	
28	45.0	HS	30.0	30.0	25.0	28.33	35.0	15.0	15.0	21.67	
29	50.0	HS	35.0	30.0	30.0	31.67	35.0	30.0	25.0	30.00	
30	40.0	HS	20.0	20.0	10.0	16.67	15.0	5.0	5.0	8.33	
31	45.0	HS	35.0	35.0	30.0	33.33	35.0	35.0	20.0	30.00	
32	35.0	HS	15.0	15.0	10.0	13.33	20.0	10.0	10.0	13.33	
33	20.0	S	20.0	15.0	10.0	15.00	15.0	10.0	0.0	8.33	
34	40.0	HS	20.0	20.0	10.0	16.67	20.0	15.0	15.0	16.67	
35	25.0	S	20.0	20.0	15.0	18.33	20.0	15.0	5.0	13.33	
36	30.0	S	30.0	20.0	15.0	21.67	20.0	0.0	0.0	6.67	
37	50.0	HS	40.0	30.0	25.0	31.67	35.0	35.0	15.0	28.33	
38	30.0	S	25.0	15.0	15.0	18.33	20.0	20.0	10.0	16.67	
39	15.0	R	25.0	20.0	20.0	21.67	15.0	10.0	0.0	8.33	
40	30.0	S	20.0	20.0	15.0	18.33	10.0	10.0	5.0	8.33	
41	45.0	HS	40.0	40.0	25.0	35.00	40.0	15.0	15.0	23.33	
42	30.0	S	25.0	25.0	15.0	21.67	20.0	20.0	15.0	18.33	
43	30.0	S	20.0	20.0	15.0	18.33	30.0	25.0	20.0	25.00	
44	35.0	HS	25.0	15.0	5.0	15.00	20.0	10.0	10.0	13.33	

Cont.2

Inbred lines	Bio-agents									
	Control (non treated grains)		<i>B. subtilis</i> (MBI 600)			Mean	<i>T. harizianum</i> (Trichodex)			Mean
			1%	3%	5%		1%	3%	5%	
45	15.0	R	10.0	10.0	0.0	6.67	5.0	0.0	0.0	1.67
46	30.0	S	25.0	25.0	15.0	21.67	25.0	20.0	20.0	21.67
47	40.0	HS	25.0	20.0	10.0	18.33	20.0	15.0	15.0	16.67
48	30.0	S	25.0	25.0	15.0	21.67	25.0	20.0	20.0	21.67
49	30.0	S	20.0	20.0	10.0	16.67	15.0	15.0	0.0	10.00
50	40.0	HS	30.0	15.0	10.0	18.33	25.0	25.0	20.0	23.33
Giza 168	50.0	HS	25.0	25.0	20.0	23.33	30.0	25.0	25.0	26.67

LSD at 0.05

Inbred lines (L) = 5.89

Bio-agent concentration (B) = 3.74

L × B = No significant

<sup>a</sup> values are the means over the two years 2011/2012 and 2012/2013.

<sup>b</sup> Grades ; Immune = (0%), Highly resistant (HR)= 0.0 – 5.0%, Resistant (R)= 6.0 – 15.0%, Susceptible (S) = 16.0 – 30.0% and Highly susceptible (HS) = 31.0% or more damping-off.

Table 3: The range and mean values for all studied traits under two treatments.

Treatments	Traits	No. of spikes/plant		Biological Yield/plant (g)		Grain yield/plant (g)	
		Range	Means±S.E	Range	Means±S.E	Range	Means±S.E
Normal	0%	5.72 – 11.66	8.21±0.14	32.74 – 69.08	48.44±0.67	9.96 – 22.80	15.67±0.23
	1%	6.60 – 12.88	9.56±0.17	36.65 – 85.29	57.23±0.84	12.33 – 28.48	18.73±0.24
Trichodex	3%	7.59 – 14.38	10.80±0.18	40.72 – 88.69	64.44±0.88	13.26 – 30.80	21.29±0.27
	5%	7.93 – 17.63	12.05±0.17	45.37 – 120.10	74.08±0.89	14.80 – 38.75	24.25±0.29
MBI 600	1%	6.56 – 13.88	9.31±0.15	37.90 – 83.47	55.09±0.75	12.67 – 25.24	17.93±0.26
	3%	7.30 – 14.38	10.41±0.17	41.72 – 97.15	62.28±0.80	13.35 – 32.13	20.28±0.28
	5%	8.33 – 14.92	11.46±0.21	44.45 – 101.26	69.57±0.95	14.39 – 32.60	22.70±0.36

The data in Table 4 showed a highly significant difference among lines in number of spikes/plant under the different treatments of BAs in both seasons. Seven lines, L2, L3, L4, L15, L17, L27 and L42 were superiority in mean value of number of spikes/plant under *T. harizianum* treatments in both seasons, while the highest values were obtained by lines L2, L3, L23, L26, L27 and L38 under *B. subtilis* treatments in the two seasons.

**Table 4: The combined analysis of variance for all studied traits.**

S.o.v	D.F	Mean Squares		
		No. spikes/plant	Biological Yield/plant	Grain yield/plant
Year (Y)	1	1064.16**	127595.92**	14855.55**
Lines (L)	49	115.62**	4649.16**	536.29**
Y x L	49	19.54**	608.10**	19.84**
Error a	200	0.783	12.249	2.023
Treatments (T)	6	526.03**	23122.10**	2582.87**
Y x T	6	8.51**	345.73**	37.09**
L x T	294	3.15**	253.49**	26.31**
Y x L x T	294	1.92**	67.02**	6.12**
Error	1200	0.509	0.824	0.911

\* & \*\*Significant at 5 % and 1 % levels of probability, respectively.

**Table 5: Mean performance of no. of spikes/plant over the two seasons.**

Treats	Control		Trichodex		MBI 600		
	0%	1%	3%	5%	1%	3%	5%
1	9.46	10.73	11.34	11.59	10.11	11.02	11.66
2	10.16	12.46	11.36	11.73	11.54	12.63	14.92
3	11.66	12.88	11.38	11.92	13.88	14.21	14.84
4	11.19	12.63	11.17	11.94	12.59	13.28	13.50
5	6.90	8.06	8.44	11.06	7.47	8.26	8.77
6	7.46	8.47	9.69	11.10	9.19	11.01	11.74
7	8.52	9.60	11.44	11.71	9.89	11.58	13.12
8	8.60	9.15	11.22	11.97	9.32	10.04	11.88
9	7.39	8.31	9.70	11.92	7.55	9.20	9.36
10	5.92	6.61	8.26	8.32	6.56	8.73	9.21
11	8.61	10.15	11.69	11.33	8.80	9.56	11.87
12	9.29	10.71	11.78	11.80	11.30	12.04	12.97
13	7.42	8.80	11.98	11.11	7.92	10.64	10.51
14	8.54	10.09	11.81	11.36	10.29	12.77	13.32
15	9.91	11.69	11.46	11.87	10.06	11.39	11.60
16	6.83	7.96	9.74	11.14	8.07	9.20	11.05
17	11.66	12.45	11.70	11.80	11.83	12.47	13.54
18	7.22	10.24	11.00	11.70	8.27	9.92	10.61
19	6.56	7.62	8.89	11.78	7.85	8.80	10.20
20	7.40	7.99	11.41	11.90	8.38	8.65	10.32
21	6.68	8.24	8.89	11.92	7.81	8.46	9.00
22	6.95	8.07	9.09	11.04	7.60	9.26	10.78
23	9.57	11.71	11.73	11.96	11.54	14.38	14.39
24	7.25	9.13	11.01	11.16	8.86	9.97	10.82
25	9.43	9.78	11.49	11.02	10.28	11.15	12.36
26	10.43	11.01	11.49	11.87	10.29	11.52	13.61
27	10.59	12.20	11.78	11.80	10.45	11.28	14.20
28	6.79	7.94	9.82	11.74	9.33	9.67	9.57
29	7.18	7.52	8.71	11.14	8.19	9.84	9.92
30	7.19	8.65	9.78	11.78	10.24	12.02	12.18
31	7.86	9.30	11.17	11.01	8.31	9.94	10.05
32	5.93	6.60	7.09	7.93	6.77	7.30	8.33
33	6.84	7.68	8.26	9.39	8.19	8.40	9.05
34	6.50	9.19	11.00	11.28	7.02	8.74	11.56
35	9.27	11.90	11.99	11.73	9.34	9.34	11.20
36	10.77	11.07	11.91	11.39	8.47	9.37	10.24
37	7.90	8.99	11.43	11.73	9.05	10.61	11.96
38	10.17	11.37	11.06	11.78	10.17	11.56	13.67
39	6.56	7.30	8.77	11.43	7.28	8.41	10.28
40	7.51	9.58	11.71	11.82	9.56	10.28	12.62
41	11.24	11.92	11.99	11.28	10.95	11.42	12.44
42	9.96	12.20	11.00	11.96	11.34	11.20	13.49
43	6.01	7.67	8.01	8.03	7.31	7.59	8.34
44	9.32	10.46	11.08	11.28	13.27	13.73	12.79
45	7.03	8.46	11.00	11.32	8.12	10.38	11.90
46	6.66	9.08	9.99	11.80	10.40	11.07	9.61
47	5.72	8.08	8.70	11.00	8.29	8.63	10.47
48	6.77	7.21	7.89	8.31	7.35	8.05	8.54
49	7.26	9.42	11.48	11.32	8.92	10.57	11.50
50	8.67	9.83	11.07	11.73	10.13	11.15	12.43
Mean	8.21	9.06	11.00	11.00	9.31	10.41	11.46
Giza 168	9.50	10.60	10.90	11.15	9.95	10.25	11.50
RLSD <sub>at 0.05</sub>				1.26			
0.01				1.62			



**Biological yield/plant (g)**

The data in Table 6 showed that the highest mean values of biological yield/plant (74.08 and 69.57 g) were obtained with *T. harzianum*, 5% and *B. subtilis*, 5% treatments, respectively, in two seasons. While the lowest value (48.44 g) was obtained at untreated seeds (control) in both seasons. The results indicate that increasing agent's concentration from 1% to 3% and 5% cause significant increasing in biological yield/plant.

**Table 6: Mean performance of biological yield/plant (g) over two seasons.**

Treats Genotypes	Control	Trichodex			MBI 600		
	0%	1%	3%	5%	1%	3%	5%
1	59.95	67.87	٧٧.٤٢	٨٥.٠٠	63.52	69.22	73.21
2	55.50	73.98	٨٨.٦٩	١٢٠.١٠	61.33	67.11	79.55
3	65.41	75.79	٨٣.١٤	٨٧.١٨	78.58	80.31	85.60
4	65.31	73.24	٨١.١٧	٨٦.٣١	72.55	76.55	77.79
5	34.64	40.76	٤٢.٠٨	٥٠.٩٧	37.90	41.86	44.45
6	32.74	36.65	٤١.٢٧	٥٢.٧٧	40.05	52.32	55.50
7	43.29	46.90	٥٧.٨١	٦٤.٧١	48.54	56.81	69.26
8	46.93	51.96	٥٧.٤٤	٧٤.٤٣	53.00	57.06	70.37
9	50.59	55.73	٦٤.١٣	٧٢.٥٢	50.10	61.05	62.12
10	41.82	46.73	٥٧.٧٧	٥٨.٨٥	46.29	61.56	64.98
11	54.15	62.81	٧١.٦٧	٨٨.٣٨	54.12	58.79	73.02
12	61.01	70.00	٧٨.٤٣	٩٧.٠٥	77.12	78.74	84.78
13	41.71	49.15	٦٣.٤٧	٦١.٩١	44.11	59.30	58.55
14	44.38	49.75	٦٤.٨٨	٦٨.٢٣	50.68	70.30	83.01
15	51.41	66.62	٦٩.٨٧	٩٤.٣٠	66.32	73.06	74.16
16	47.46	53.32	٦٧.٩٦	٧٧.٧٥	53.79	61.34	73.66
17	51.90	58.60	٦٤.٢١	٧٦.٩٧	60.32	60.98	72.10
18	48.89	71.12	٧٩.١٤	٨٠.٧٠	54.22	65.01	69.56
19	45.89	52.74	٦٠.٧٠	٧٣.٨٨	54.42	61.05	70.73
20	49.86	54.50	٧٠.٥٣	٨١.٦٥	57.26	59.07	70.46
21	43.12	50.79	٥٤.١٤	٦١.٧٤	48.13	52.12	57.96
22	40.07	43.20	٥١.٢٤	٥٦.٩٧	43.66	53.15	61.87
23	52.16	67.48	٦٤.٣٢	٦١.٨٨	60.99	87.15	89.30
24	44.14	53.37	٥٧.٩٢	٦٥.٤٣	51.54	58.04	71.56
25	41.57	43.05	٤٧.٧٨	٥٢.٩٦	45.29	49.11	63.26
26	51.01	53.93	٦٠.٧٦	٧٠.٣٧	50.11	56.28	66.48
27	61.94	78.00	٨٣.٧٥	٩٠.١٦	60.54	65.97	88.85
28	40.97	47.10	٥٧.٥٨	٧٥.٦٤	54.64	56.60	56.02
29	51.65	53.47	٦٤.٠٦	٧٥.٦١	58.19	69.91	84.70
30	48.34	59.26	٦٢.٧٠	٨٣.٠٠	66.59	78.15	85.72
31	43.90	50.23	٥٣.٩١	٦٩.٩٤	44.83	53.62	54.24
32	33.68	38.11	٤٢.٩٧	٤٥.٤٧	39.05	42.14	48.10
33	35.80	38.44	٤٠.٧٢	٤٦.٨٢	40.68	41.72	44.96
34	42.26	67.76	٦٩.٤٤	٨٠.٩٦	44.35	55.17	73.16
35	47.46	64.42	٧١.٧٢	٧٥.٥٥	46.62	46.61	55.94
36	69.08	65.65	٦٩.٩٦	٦٨.١٥	51.40	56.91	61.67
37	44.03	51.56	٦٤.٨٩	٨٧.٢٥	51.84	60.77	68.52
38	66.68	75.81	٨٣.٠٧	١٠٨.١٣	68.06	77.37	101.26
39	42.88	46.93	٥٤.٨٧	٦٧.٠٢	46.86	54.17	66.17
40	47.10	71.50	٧٨.١٠	٨٨.٩٩	61.45	66.07	81.14
41	53.19	56.78	٦١.١٢	٧٢.٦١	51.97	58.92	63.78
42	58.92	70.72	٨٠.٥٣	٩١.٩٥	65.07	64.22	77.35
43	48.70	61.01	٥٩.٧٨	٦٣.٧٢	58.42	60.64	66.61
44	53.72	55.24	٥٧.٨٥	٥٩.٦٢	83.47	83.28	70.34
45	44.59	52.63	٧٨.١٦	٧٣.٤٢	50.59	71.09	80.61
46	52.45	85.29	٨٧.٨٢	١٠٦.٣٥	80.18	85.28	92.81
47	36.92	48.10	٥٤.٩٤	٥٩.٧٩	49.70	51.76	62.69
48	36.42	39.05	٤٢.٣٥	٤٥.٣٧	42.80	46.83	49.70
49	48.99	60.01	٦٦.١٦	٧٥.٣٥	56.84	67.29	73.28
50	47.27	54.43	٥٧.٧٦	٦٩.٩٩	56.44	62.19	69.47
Mean	48.44	٥٧.٢٣	٦٤.٤٤	٧٤.٠٨	55.09	62.28	69.57
Giza 168	56.17	64.93	67.79	75.81	53.23	59.41	66.34
RLSD <sub>at 0.05</sub>				4.97			
0.01				6.43			

The data in Table 4 showed also clearly that a highly significant difference among lines in biological yield/plant under the different treatments of BAs in both seasons. Seven lines, (L2, L12, L15, L27, L38, L42 and L46)

gave the highest mean values of biological yield/plant under *T. harzianum* treatments in the two seasons, while the highest values were obtained by lines L3, L23, L27, L30, L38 and L46 under *B. subtilis* treatments in the two seasons.

**Grain yield/plant (g)**

Comparing different treatments of BAs under field conditions, it was observed that the highest mean values of grain yield/plant (24.25 and 22.70 g) were obtained with *T. harzianum*, 5% and *B. subtilis*, 5% treatments, respectively, in the two seasons (Table 7).

**Table 7: Mean performance of grain yield/plant (g) over two seasons.**

Treats Genotypes	Control	Trichodex			MBI 600		
	0%	1%	3%	5%	1%	3%	5%
1	21.11	24.06	22.78	22.10	22.47	24.49	25.90
2	17.40	23.88	22.83	23.70	19.78	21.64	25.63
3	19.22	22.71	20.10	22.12	23.54	24.06	25.04
4	21.60	24.50	22.21	23.82	24.20	25.53	25.95
5	14.89	17.39	18.22	16.11	16.11	17.79	18.89
6	13.17	14.93	12.27	12.22	16.24	21.21	22.50
7	15.37	17.29	12.22	12.82	17.86	20.91	25.49
8	14.21	15.14	12.22	12.22	15.42	16.60	20.48
9	17.68	19.96	22.22	22.22	18.01	21.95	22.33
10	12.97	14.53	14.22	14.22	14.36	19.01	20.13
11	21.91	25.93	22.22	22.22	22.33	24.25	30.13
12	19.85	22.88	22.22	22.22	25.24	25.77	27.75
13	14.67	17.44	22.22	22.22	15.64	21.03	20.76
14	12.51	14.79	12.22	12.22	15.07	20.91	24.67
15	16.01	21.27	22.22	22.22	21.12	23.27	23.62
16	15.74	18.39	22.22	22.22	18.56	21.17	25.42
17	17.85	19.06	22.22	22.22	19.56	19.78	23.39
18	15.19	22.64	22.22	22.22	17.36	20.82	22.28
19	12.04	13.97	12.22	12.22	14.39	16.15	18.71
20	14.92	16.11	22.22	22.22	16.92	17.45	20.82
21	13.02	16.07	12.22	12.22	15.24	16.51	18.32
22	12.02	12.98	12.22	12.22	13.16	16.01	18.64
23	16.75	22.25	22.22	22.22	20.17	32.13	29.54
24	13.14	16.58	12.22	12.22	16.03	18.05	22.26
25	14.48	15.02	12.22	12.22	15.78	17.12	22.04
26	19.22	20.29	22.22	22.22	18.87	21.18	25.02
27	22.77	28.48	22.22	22.22	22.30	24.20	32.60
28	12.41	14.62	12.22	12.22	16.97	17.58	17.40
29	14.88	15.62	12.22	12.22	16.97	20.41	24.82
30	12.58	16.03	12.22	12.22	17.89	21.00	23.03
31	14.05	16.66	12.22	12.22	14.87	17.79	18.00
32	11.11	12.36	12.22	12.22	12.67	13.68	15.61
33	10.92	12.33	12.22	12.22	13.02	13.35	14.39
34	14.18	23.38	22.22	22.22	15.25	18.95	25.19
35	16.51	23.05	22.22	22.22	16.48	16.37	19.90
36	19.74	18.68	22.22	22.22	14.55	16.10	17.48
37	16.45	18.75	22.22	22.22	18.81	22.05	24.86
38	19.60	21.86	22.22	22.22	19.62	22.30	29.20
39	14.07	15.62	12.22	12.22	15.99	18.03	22.02
40	14.97	22.09	22.22	22.22	19.05	20.48	25.15
41	22.80	24.21	22.22	22.22	22.20	25.17	27.24
42	19.34	23.83	22.22	22.22	21.91	21.62	26.04
43	15.70	20.02	12.22	12.22	19.10	19.84	21.80
44	13.77	15.45	12.22	12.22	23.32	23.27	19.65
45	13.35	16.05	22.22	22.22	15.41	21.61	24.51
46	14.35	23.87	22.22	22.22	22.41	23.84	20.70
47	9.96	14.03	12.22	12.22	14.43	15.03	18.21
48	12.97	13.04	12.22	12.22	14.08	15.35	16.37
49	13.21	17.15	12.22	12.22	16.23	19.18	20.95
50	10.94	19.04	22.22	22.22	19.78	21.79	24.36
<b>Mean</b>	<b>15.67</b>	<b>18.22</b>	<b>21.29</b>	<b>22.20</b>	<b>17.93</b>	<b>20.28</b>	<b>22.70</b>
<b>Giza 168</b>	<b>18.90</b>	<b>21.85</b>	<b>24.14</b>	<b>25.14</b>	<b>20.70</b>	<b>22.87</b>	<b>24.31</b>
<b>RLSD</b> <small>at 0.05 0.01</small>				<b>2.02 2.61</b>			

The average of *T. harzianum* 3% and *B. subtilis* 3% treatments (21.29 and 20.28 g) were ranked secondly in two seasons. While the lowest mean value (15.67 g) was obtained at untreated seeds (control) in the two seasons. The results indicate that increasing bio-agent's concentration from 1% to 3% to 5% caused significant increasing in grain yield/plant.

The data in Table 7 indicated a highly significant difference among lines in grain yield/plant under the different treatments of BAs in the two seasons. Six lines L2, L11, L12, L27, L37 and L38 gave the highest mean values of grain yield/plant under *T. harzianum* treatments in the two seasons, while the highest values of grain yield/plant were obtained by lines L3, L23, L27, L30, L38 and L46 under *B. subtilis* treatments in the two seasons.

## DISCUSSION

In wheat, soil-borne fungal diseases play a major role in the yield losses, among which is damping-off caused by several pathogenic fungi, a serious disease in almost all major growing areas of wheat. Damping-off diseases remain prevalent because of the trends toward a higher frequency of cereals in the rotation, including continuous cereals, and use of less or no tillage. In the current study, five fungi; *F. solani*, *F. oxysporium*, *F. graminearum*, *Pythium* sp. and *R. solani* were obtained from diseased wheat plants and they were pathogenic to Giza 168 cv causing damping-off. These results are in accordance with those reported by (Hashem and Hamada, Amal, 2002; Abdelzaher, 2004; Atef, 2008; Ahmed *et al.*, 2009; Asran and Eraky, Amal, 2011 and Moubarak and Abdel-Monaim, 2011).

Host plant resistance is considered the best strategy to control diseases in agriculture therefore it is seemed highly desirable to explore the resistant genotypes of wheat to damping-off disease. In this study we have tested 50 inbred lines of wheat in field trials of naturally infested soil with referred damping-off pathogens, results of study revealed that all inbred lines varied significantly to incidence and 37, 2, and 12 inbred lines were HS, S, and R to infection, respectively. Similarly, other wheat varieties or genotypes or lines in different parts of the world exhibited different levels of resistance to seedlings diseases including damping-off were documented (Kulkarni *et al.*, 1978; Anaso *et al.*, 1984; Corrazza *et al.*, 1987; Jalaluddin and Anwar, 1989; Ahmed and Bakar, 1991; Mishra *et al.*, 1992; Harlapur *et al.*, 1993 and Bhandari and Shrestha, 2004). However, the present knowledge about the resistant inbred lines of wheat would be useful in breeding programs for resistance in future studies.

Bio-control of plant diseases using various beneficial microorganisms are urgently needed to provide an alternative to chemical control. For this propose, a variety of microorganisms have isolated from rhizosphere of cultivated plants and they have demonstrated antagonistic activity against soil-borne fungal pathogens. A range of these microorganisms are commercially formulated and many of them are potentially applied as bio-control agents or biofertilizers in agriculture. Earlier studies have emphasized that *Trichoderma* spp. and *Bacillus* spp. are specific, play important roles of

agriculture in control soil-borne fungal diseases and promotion of plant growth (Kennedy and Smith, 1995 and Shivanna *et al.*, 1996) upon root colonization and increase minerals uptake as well as crop production (Cakmakci *et al.*, 2005; Han and Lee, 2006; Rajankar *et al.*, 2007; Ha *et al.*, 2008; Pandey and Saraf, 2010; Srivastava *et al.*, 2010 and Moharam and Negim 2012). Therefore, the present work was started to evaluate the efficacy of seed treatments of wheat inbred lines with two commercial BAs *T. harzianum* (Trichodex) and *B. subtilis* (MBI 600) at 1, 3, and 5% conc. in field trails under naturally infested soil with damping-off pathogens for controlling the disease and enhancing wheat production. Our results emphasized that the two BAs at all tested concentrations were able to reduce significantly damping-off infection, however *T. harzianum* (Trichodex) at all applied concentrations was better than *B. subtilis* (MBI 600) and caused highly reduction of infection of all tested lines. Our results are in a good accordance with previous studies which have been concluded that *Trichoderma* spp. and *Bacillus* spp. can effectively protect wheat plants against damping-off and/or root rot diseases caused by same or other pathogens (Hashem and Hamada, Amal, 2002; Abdelzaher, 2004; Nourozian *et al.*, 2006; Atef, 2008; Moubarak and Abdel-Monaim, 2011 and Abo-Elnaga 2012). On the other hand, application of tested bio-agents with increasing also their concentrations up to 5% caused significant increase in grain yield/plant. These findings could be attributed to the positively effect of BAs on yield components such as number of spikes/plant and biological yield/plant which reflected on the grain yield/plant. The increase in yield of wheat was found similar to the findings of Sallam *et al.* (2008) and Sharma *et al.* (2012) where the formulation of *Trichoderma* spp. treatments enhanced green yield of bean plants compared to infected control. In this experiment the increase in yield can also be attributed to the application of *Trichoderma harzianum* (Th3) bio-formulation along with the Farm yard manure which helped increasing the colonies by providing nutrient to *Trichoderma* thereby increasing the plant growth and yield of wheat. Also, many investigators obtained similar results such as Hashem and Hamada, Amal, (2002), Abdelzaher (2004), Harman (2006), Atef (2008), Ahmed *et al.*, (2009), Asran and Eraky (2011), Moubarak and Abdel-Monaim (2011) and Sharma *et al.* (2011). It is evidence from the obtained results that the inbred wheat lines responded differently when they were grown under seed treatments and our results could be confirmed with those previously reported (Mahdy *et al.*, 1996; Pawar *et al.*, 1997; Moubarak, 2007 and Moubarak and Abdel-Monaim, 2011). Finally, seed treatment with Tricodex of *T. harzianum* T39 was better than MBI 600 of *B. subtilis* for protecting wheat against damping-off, in promoting growth and crop production. Generally, fungi have been reported to possess greater ability to solubilize phosphate than bacteria, viz., *B. subtilis* and *B. megatarium* (Rajankar *et al.*, 2007), this may explain the greater effectiveness of *T. harzianum* than *B. subtilis* in growth promotion and crop production. Thus, use of these specific bio-agents in agriculture against damping-off not only suppressed the disease but also helped in sustenance and growth promotion of plants.

## CONCLUSION

It can be concluded that the commercial bio-agents (BAs) at all tested concentrations were able to reduce significantly damping-off infection and caused significant increase in wheat production. Moreover, the inbred lines L11, L12, L27 and L38 may represent the best lines having a high yield potential when seed inoculation with BAs than the others.

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## تأثير بعض العوامل الحيوية التجارية علي مرض موت البادرات و المحصول في سلالات قمح خبز

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أجريت هذه الدراسة في تجربتان حقليتان خلال موسمي ٢٠١٢/٢٠١١ و ٢٠١٣/٢٠١٢ بمزرعة بحوث الكوثر - كلية الزراعة - جامعة سوهاج وذلك بهدف تقييم إمكانية استخدام اثنين من المستحضرات الحيوية التجارية وهما الترياكودكس للفطر ترياكوديرما هارزيانوم و أم بي أي 600 للبيكتريا باسلس ساتلس عند تركيز و 3 و 5% لمقاومة مرض موت البادرات في القمح ، وكذلك قدرتهما على تحسين نمو وأنتاجيه نباتات القمح تحت ظروف الحقل.

أظهرت النتائج ان معاملة حبوب القمح بالمستحضرين قبل الزراعة ادى الى وقايه بادرات القمح النامي من موت البادرات و ايضا زياده نسبه إنبات الحبوب. وكذلك اثرت المعاملة ايضا بشكل كبير بزياده عدد السنابل /النبات والمحصول البيولوجي /نبات ومحصول الحبوب /النبات، وكانت هناك فروق معنويه بين التراكيب الوراثية المختبره من القمح تحت ظروف الحقل. وعلاوة على ذلك ، كانت المعاملة بمستحضرى الترياكودكس و ال أم بي أي 600 عند 5% من أفضل المعاملات لزيادة محصول الحبوب. أيضا وجدت أربعة سلالات من القمح أكثر استجابته للمعاملة بالمستحضرات الحيوية تحت ظروف الحقل هي L11 ، L12 ، L27 ، و L38.

وبناء على نتائج هذه التجربة يمكن القول ان تلقيح حبوب القمح بالمستحضرات الحيوية التجارية قبل الزراعة يقلل من الاصابة بموت البادرات في الحقل وكذلك يزيد من الانتاجية.