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BIOLOGICAL CONTROL OF ROOT-ROT DISEASE OF WHEAT

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Abstract

The isolation of microorganisms from rhizosphere of healthy wheat plants was carried out to select the most antagonistic one to be used in biological control of root-rot disease of wheat caused by *Fusarium graminearum, Helminthosporium sativum* and *Rhizoctonia solani. Trichoderma harzainum, T. hamatum, Streptomyces griseus* and *Bacillus subtilis* were the most frequently isolated microorganisms. According to *in vitro* testing, *T. harzianum* gave the highest significnt reduction in mycelial gowth of the pathogenic fungi followed by *T. hamatum.* Antagonistic fungi (*T. harzainum* and *T. hamatum*) completely inhibited sclerotial formation in *R. solani.* The antagonism between the pathogen and the bioagent could be affected by the type of media. Slide techinque was used to study the mode of action of bioagents on pathogenic fungi. *T. harzianum* caused malformation in the mycelium of pathogenic fungi and grew over the host mycelium followed by more close contact interaction and invasion. *B. subtilis* also caused malformation and decaying for pathogenic mycelia.

In vivo test was carried out to evaluate the efficacy of adding different bioagents in different forms, dates and rates to the soil infested with pathogenic fungi. Adding the antagonistic fungi as powder gave better result in controlling root-rot disease. While adding bacteria and streptomyces as suspenison was the most effective. One week before planting time was the most suitable date for adding bioagents to the infested soil to control root-rot disease. While 5 and 10 g/Kg soil were the most effective rate of bioagents which led to reduction in disease incidence of wheat root-rot.

INTRODUCTION

Wheat root-rot caused by *Fusarium graminearum, helminthosporium sativum* and *Rhizoctonia solani,* is considered one of the serious soil-borne problem in Egyptian dry land (EL-Nashar *et al.* 1999).

Man has greatly decreased his options by polluting his environment by accumulating, in the soil and water, chemical residues harmful to himself, to his crops and soil

microorganisms. These highly toxic chemicals led to develop new aggressive races of different pests and to the appearance of new pests due to the reduction of their natural enemies.

Biological control when effective as stated by Abd-EL-Moity (1981) usually is more enduring with no toxic residue in natural food chains, cheaper and safer for application. Research workers made attempts to find out another method to solve the enormous problems with different pests. These problems stimulate our effort to find out biological method(s) to control this disease using friendly and safe microorganisms.

Therefore, the objective of this study was to evaluate *in vitro* and *in vivo* antagonstic effect of the most frequent microorganisms isolated from wheat root rhizosphere on pathogenic fungi of root-rot disease of wheat.

MATERIALS AND METHODS

The tested pathogenic fungi were previously isolated from diseased roots of wheat plants grown at different locations (EL-Nashar *et al.*, 1999) and were identified as *Fusarium gaminearum, Helminathosporium sativum* and *Rhizoctonia solani.*

I- Isolation and identification of the antagonistic microorganisms from wheat rhizosphere

Isolation was carried out from rhizosphere of healthy wheat plants, grown in heavily infested fields with root-rot pathogens. The method described by Abd-El-Moity (1976) was used in this study. One gram of wheat plant rhizosphere, on dry weight basis, was added to flask containing 99 ml sterilized water, each flask was shaked periodically for approximately three hours. Such suspension was subjected to a serial dilutions of 10^{-2} to 10^{-6}. Different dilutions and media were used for isolation of different groups of microorganisms as shown in Table (1).

Table 1. Dilutions and media

Microorganisms	Dilutions	Media used
Fungi	10^{-4}	Peptone-Dextrose- Agar + Rose Bengal and Streptomycin (Johnson *et al.* , 1960).
Actinomycetes	10^{-5}	Jensen's agar medium (Johnson *et al.*, 1960
Bacteria	10^{-6}	Soil extract- Agar (Raper and Fennell. 1965)

One ml of each dilution was added to sterilized Petri dishes which were supplemented with about 10ml of melted, cooled specific agar medium and incubated at

25±ºC. Four plates were used as replicates for each dilution. After 2-6 days, plates were examined and separate colonies of either bacterium, actinomycetes or fungi were transferred to gliotoxin fermentation agar (GFA) (Brain and Hemming, 1945) or nutrient glucose agar (NGA) (Dowson, 1957) for purification and identification.

The isolated fungi and actinomycetes were purified using single spore or hyphal tip techniques. Bacteria were purified using dilution plate technique.

Identification of the selected isolated microorganisms was carried out according to their cultural morphological and physiological characters (Waksman and Henrici, 1943 and Bergey's Manual of Systemic Bacteriology, 1984). Identification was confirmed through both, Mycology Department and Bacterial diseases and Biological Control Dept., Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt.

II- The interaction between isolated microorganisms and root-rot fungi

a- *In Vitro*

1. Effect of isolated microorganisms on linear growth of pathogenic fungi

Three types of media, i.e. REA (wheat root extract agar, Johnson *et al.*, 1960), GFA and NGA were used to evaluate the antagonism. Agar discs, 4mm in diameter, of each pathogen was placed in the middle of the Petri dish (9 cm.). Two equal discs (4 mm) of tested fungi or loopful of tested bacteria or actinomycetes were placed or streaked 3 cm apart from the pathogen. Four replicates were used for each treatment. Four plates were inoculated only with the pathogenic fungi as a control. Inoculated plates were incubated at 25± 1°C and observed daily. The percentage of reduction in mycelial growth of the pathogen was calculated by using the formula:

$$X = \left(\frac{G1 - G2}{G1}\right) X\ 100$$

Where X: percentage of mycelial growth reduction (PMGR).

G1: growth of pathogenic fungus in control plates (m m).

G2: growth of pathogenic fungus in treated plates (m m).

2. Mode of action of isolated microorganisms on pathogenic fungi

In this study, a thin film of GFA medium was spread on a microscope glass slide, then the antagonist was inoculated at one side, whereas the pathogen was inoculated at the other side (Abd-EL-Monieum, 1996). The pathogen was grown on a separate slide alone as control. Inoculated slides were then placed in sterilized plates containing filter paper saturated with sterilized water and incubated at 25± 1°C. Slides were examined periodically to observe different stages of antagonism using light microscope (X 400).

b. *In Vivo*

Three outdoor pot experiments were carried out to evaluate the effect of the form, date and rate of application of bioagents. Inocula of the pathogenic fungi were prepared by growing each fungus on autoclaved sand/barley medium (1:3 w/w) and incubated at 25±1°C for 21 days. One week before planting, soil was infested with fungal inoculum either singly or in combination at the rate of 5% of soil weight (Soliman *et al.*, 1993). Soil infested with the pathogen only (free from antagonists) served as control. Fifteen surface sterilized grains of susceptible wheat cultivar Sakha 69 were sown in each pot (20 cm in diam), watered and fertilized to maintain excellent growth conditions. Three replicates were used in each treatment. After 15 and 45 days (at seedling stage) from sowing, the percentage of pre-, post emergence damping-off and healthy survivals were calculated according to Abd-EL-Moity (1985). At maturity, the percentage of disease rating (DR %) was calculated using Mckinney's formula (Mckinney, 1923) slightly modified by Tinline and Hunter, (1982). Yield components *i.e.* number of tillers per plant (T/P) and weight of 1000 Kernels (1000 KW) were determined also at mature stage.

1. Application of antagonists in different forms

Different antagonists were grown in liquid media. The antagonistic fungi were grown on gliotoxin fermentation medium for 9 days, whereas nutrient glucose medium was used for bacteria and actinomycetes (4 days). All inoculated media were incubated at 25±1°C. Different cultures were formulated in two different preparations, *i.e.* powder and suspension.

Granules of antagonistic fungi were prepared according to Abd-EL-Moity (1986). Fungi as powder preparation was made by grinding dry granules, while bacteria and actinomycetes were prepared as powder by mixing culture with talc powder at the rate of

1:1 (v/w). Water suspension was prepared by suspending different antagonistic propagules in water. All antagonism preparations were adjusted to contain 3 x 10^7 propagules per ml or gram of final preparation. Different forms were added to the infested soil at the rate of 5 ml or 5 g/kg soil one week before sowing.

2. Application of antagonists at different dates

This experiment was conducted to determine the most favourable time for adding different antagonists to the infested soil and their antagonistic effect on disease incidence. Different antagonists were added (as a powder and at the rate of 5 g/kg soil) to the infested soil at three dates *i.e.* one week before planting, at planting time and one week after planting.

3. Application of antagonists at different rates

Different antagonists were added (one week before planting as a powder) to the infested soil at four different rates *i.e.* 1g, 3g, 5g and 10g/kg soil.

Data were computerized and analyzed at the special unit of CLDSA of Agricultural Research Centre (ARC) according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

I- Isolation and identification of the antagonistic microorganisms from wheat rhizosphere

Fungi, actinomycetes and bacteria in the rhizosphere of healthy plants were isolated, and the most antagonistic ones were selected. The selected antagonists were:

1.1 Fungi

a) *Trichoderma harzianum*

b) *Trichoderma hamatum*

1.2 Actinomycetes

Streptomyces griseus

1.3 Bacteria

Bacillus subtilis

II. The efficacy of different isolated antagonists against pathogenic fungi

a- *In vitro test*

1. Effect of isolated antagonists on linear growth of pathogenic fungi

Data in Table (2) reveal that all antagonists, significantly reduced the mycelial growth of pathogenic fungi. Different antagonists varied in their effects against pathogenic fungi, depending on the used medium. NGA was the most favourable medium for showing the antagonistic effect in most cases, while GFA was the least appropriate. This might be due to the effect of nutrient source on either the pathogen and/or antagonist. This result is in agreement with Turner (1971) and Abd-EL-Moity (1976). Data also indicated that *Trichoderma harzianum* gave the highest reduction in mycelial growth (average 47.73%) of pathogenic fungi followed by *T. hamatum* (average 46.98%). *Streptomyces griseus* showed less effect compared with *Trichoderma* spp., while *Bacillus subtilis* exhibited the least effect averaging over the three media. Moreover, *Trichoderma* spp. completely inhibited sclerotial formation of *Rhizoctonia solani,* while *B. subtilis* and *S. griseus* reduced only the number of sclerotia. The highest effect of *Trichoderma* spp. against pathogenic fungi can be explained according to the finding of Turner (1971) and Rifai (1969), who mentioned that this antagonist produces more than one antifungal substance which play an important role in reducing the mycelial growth of pathogens.

2. Mode of action of antagonists aganist pathogenic fungi

Mode of action of *T. harzianum* or *B. subtilis* on the mycelium of pathogenic fungi *i.e. Fusarium graminearum, Helminthosporium sativum* and *Rhizoctonia solani* was studied using the microscope slide technique. Toxic substances produced by *T. harzianum* led to malformation of the mycelium of pathogens. This result is in agreement with those obtained by EL-Assuity *et al.* (1986) on *Capholosporium acremonium.* Examination of slides also showed that *T. harzianum* hyphae grow over the host mycelium followed by closer contact and invasion and growth within it causing complete destruction for the invaded mycelium within 48 hours by the action of enzymes. These data are in harmony with those of Turner (1971), Abd-EL-Moity and Shatla (1981) and Paderes *et al.* (1992), who mentioned that *T. harzianum* produces chitinase and 1,3 glucanase which are responsible for dissolving the cell wall of the host fungus and facilitate penetration by *T. harzianum.* Pathogenic mycelium subjected to the effect of *B. subtilis* for

Table 2. Effect of different antagonists on the percent reduction in myelial growth of the pathogens on three different media.

Bioagents	Media used												
	Nutrient glucose agar medium				Root extract agar medium				Gliotoxin fermentation medium				Mean
	% of Reduction in mycelial growth of pathogenic fungi												
	F. gr. 1	F. gr. 2	H. sat.	R. sol.	F. gr. 1	F. gr. 2	H. sat.	R. sol.	F. gr. 1	F. gr. 2	H. sat.	R. sol.	
Trichoderma harzianum	65.44	46.95	56.51	61.28	22.22	58.34	58.34	60.28	17.92	21.33	16.75	54.45	47.73
Trichoderma hamatum	61.94	34.17	65.45	52.50	30.11	52.08	52.08	70.42	21.92	26.20	39.37	57.50	46.98
Bacillus subtilis	50.83	38.06	55.51	33.33	42.70	25.33	62.14	24.86	17.73	4.11	40.63	11.11	33.86
Streptomyces griseus	38.89	35.84	72.06	23.33	44.97	30.92	46.42	30.31	19.96	50.91	44.50	36.11	39.44
Mean	55.03	38.76	63.13	43.36	35.00	41.67	54.50	46.47	15.39	25.64	35.31	39.79	
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00

L.S.D. at 0.05 for	
Pathogen	2.540
Bioagents	2.540
Types of media	2.196
Pathogen x Bioagents	5.070
Pathogen x Types of media	4.390
Bioagents x Types of media	4.390
Pathogen x Bioagents x Types of media	8.780

Data were transformed to arcsine.

72 hours showed malformation and clear lysis. By increasing of period of incubation to 96 hours, pathogenic mycelium completely decayed and cell walls were digested and disappeared. These data can be explained in the light of the fact that *B. subtilis* can produce a group of enzymes which dissolve the cell wall of the pathogens, causing complete destruction for affected myclia (Gunsalus and Stanier, 1962).

b- *In Vivo* experiments

1- Effect of form of application on the efficacy of the antagonist

Data in Tables (3 and 4) show that different preparations (powder and suspension) of the same antagonist varied in their efficacy against the causal organisms of root-rot disease of wheat.

Adding the antagonistic fungi as powder signficantly reduced the perentage of disease incidence (in seeding stage) and disease rate (in adult stage) and significantly increased survival plants and yield components. This can be explained by the fact that using antagonist with food base powder, lead to increase in the stability and establishment of the antagonist in the infection court. This establishment causes reduction in the pathogenic propagules, consequently reduction in disease incidence (Abd-EL-Moity *et el.* 1991, Bicici *et al.*, 1991 and Kay and Stewart, 1994). Data in Tables (3 and 4) also show that adding bacteria or streptomyces as suspension was more effective than the powder form. This is due to the faster spread compared with powder preparation. These results are in agreement with those of Abd--EL-Monieum (1996).

The most effective bioagent in controlling root-rot disease was *Trichoderma harzianum* as powder followed by *Streptomyces griseus* as suspension and *T.hamatum* as powder, while the least effective one was *Bacillus subtilis* as powder (Tables 3 and 4).

2- Effect of time of application on the efficacy of the antagonist

The aim of this experiment, was to find out the most suitable date for applying different bioagents to infested soil.

Data presented in Tables (5 and 6) show that adding antagonist one week before sowing, at sowing time or one week after sowing led to reduced disease incidence, disease rate and increased yield components, comparing with control treatment. Adding bioagent one week before sowing gave, in most cases, the most effective results. Obtained data also revealed that *T. harzianum* was the most effective bioagent in controlling wheat root-rot, when added to infested soil at any of the tested dates. *T. hama-*

Table 3. Effect of adding bioagents in the form of powder or suspension to the infested soil on disease incidence in seedlings of wheat.

Bioagents	Form of application	Pathogens														
		F. graminearum			*H. sativum*			*R. solani*			Combination			Mean		
		Pre-	Post	Survival	Pre-	Post	Survival	Pre-	Post	Survival	Pre-	Post	Survival	Pre-	Post	Survival
T. harzianum	Powder	22.5	0.0	67.5	5.0	0.0	84.8	15.7	5.0	72.0	28.0	5.0	60.5	17.8	2.5	71.2
	Suspension	32.8	5.0	55.8	24.0	0.0	65.7	29.5	5.0	50.8	38.6	10.5	48.5	31.0	5.0	57.0
T. hamatum	Powder	29.5	0.0	60.5	12.8	0.0	77.0	10.5	5.0	77.0	32.8	10.0	54.0	21.0	3.9	67.0
	Suspension	38.6	10.5	48.5	29.5	5.0	59.0	29.0	5.0	59.0	42.9	15.7	42.9	35.0	9.0	52.0
B. subtilis	Powder	40.0	15.7	45.7	29.6	5.0	58.8	28.0	5.0	60.5	42.9	17.9	41.5	35.0	11.0	51.6
	Suspension	27.7	5.0	60.5	18.0	0.0	72.0	17.9	0.0	72.0	34.0	5.0	54.0	24.5	2.6	64.7
S. griseus	Powder	32.8	5.0	55.7	22.5	5.0	65.7	24.0	0.0	65.7	37.0	10.5	49.9	29.0	5.0	59.0
	Suspension	24.0	0.0	65.6	10.0	0.0	79.7	10.5	0.0	79.5	31.0	5.0	57.0	19.0	1.0	70.5
Control		47.0	20.0	35.6	41.5	15.7	44.0	32.8	10.0	54.0	50.0	24.0	29.0	42.8	17.7	40.9
L.S.D. at 0.05 for		Pre-	Post	Survival												
Pathogen		1.8	3.0	2.0												
Bioagents		1.8	NS	2.0												
Form of application		1.6	2.6	1.9												
Pathogen x Form of application		3.0	NS	3.8												
Bioagents x Form of application		3.0	5.0	3.8												
Pathogen x Bioagents x Form of application		NS	NS	NS												

Data were transformed to arcsine.

Table 4. Effect of adding bioagents in the form of powder or suspension to the infested soil on percentage of disease rating and wheat yield components in adult stage of cv. Sakha 69 wheat plants.

Bioagents	Form of application	Pathogens														
		F. graminearum			*H. sativum*			*R. solani*			Combination			Mean		
		*DR %	t/p	1000-KW g	DR %	t/p	1000-KW g	DR %	t/p	1000-KW g	DR %	t/p	1000-KW g	DR %	t/p	1000-KW g
T. harzianum	Powder	34.0	2.2	33.0	30.6	2.3	35.0	26.6	2.8	33.0	39.0	1.80	30.0	32.6	2.0	32.9
	Suspension	36.5	1.9	30.0	33.6	2.0	33.6	30.0	2.7	32.0	41.6	1.75	28.0	35.5	2.0	30.9
T. hamatum	Powder	38.8	2.1	30.0	34.0	2.1	33.0	31.5	2.8	35.0	43.9	1.70	26.0	37.0	2.0	31.0
	Suspension	40.0	1.8	28.0	35.7	2.0	31.5	33.0	2.6	33.0	45.0	1.70	26.0	38.6	2.0	29.7
B. subtilis	Powder	48.0	1.6	25.0	45.0	1.5	28.0	41.0	2.0	30.0	53.9	1.50	20.0	47.0	1.6	25.9
	Suspension	43.9	1.9	27.0	40.0	1.7	30.0	38.0	2.3	31.0	49.6	1.60	23.0	43.0	1.8	27.8
S. griseus	Powder	42.0	1.6	27.0	38.0	1.9	30.0	35.0	2.5	32.0	48.0	1.60	23.5	41.0	1.8	28.0
	Suspension	41.6	1.7	29.0	36.5	2.0	31.9	34.5	2.5	33.0	46.0	1.70	25.0	39.7	2.0	29.8
Control		63.0	1.0	19.15	54.7	1.0	23.0	45.5	1.0	26.0	63.0	1.00	14.5	55.0	1.3	20.8
L.S.D. at 0.05 for		DR %	t/p	1000-KW												
Pathogen		0.5	0.18	0.56												
Bioagents		0.5	0.18	0.56												
Form of application		0.4	0.16	0.50												
Pathogen x Bioagents		NS	NS	1.13												
Pathogen x Form of application		0.8	0.31	0.98												
Bioagents x Form of application		0.8	NS	0.98												
Pathogen x Bioagents x Form of application		NS	NS	1.95												

*Data were transformed to arcsine.

Table 5. Effect of date of adding bioagents to the infested soil on disease incidence in seedlings of cv. Sakha 69 wheat plants.

Bioagents	Date of application	Pathogens												
		F. graminearum			H. sativum			R. solani			Combination			Mean
		Pre- %	Post %	Survival %	Pre- %	Post %	Survival %	Pre- %	Post %	Survival %	Pre- %	Post %	Survival %	Survival %
T. harzianum	One week before	22.50	0.00	67.49	5.17	0.00	84.81	15.70	5.17	72.06	27.95	5.17	60.64	71.21
	One week after	36.32	5.37	52.19	33.23	5.17	55.30	22.80	0.00	67.19	39.18	15.70	46.57	55.31
	At planting date	27.95	0.00	62.04	22.50	0.00	67.49	10.53	0.00	79.45	34.31	5.17	54.22	65.80
T. hamatum	One week before	29.53	0.00	60.46	12.77	0.00	77.22	10.53	0.00	77.12	32.77	10.33	54.30	67.28
	One week after	39.18	5.37	49.32	33.23	5.37	55.22	28.33	0.00	61.66	43.52	14.81	40.96	51.79
	At planting date	34.31	5.17	54.22	22.50	0.00	67.49	15.70	0.00	74.29	40.04	5.17	48.59	61.15
B. subtilis	One week before	40.04	15.70	45.73	29.61	5.37	58.80	27.95	5.17	60.46	42.89	15.70	41.52	51.63
	One week after	45.00	20.27	38.10	41.52	15.70	44.26	31.37	10.33	55.68	49.24	24.38	30.21	42.06
	At planting date	41.40	15.10	42.86	37.24	10.33	51.36	22.50	5.17	65.70	47.10	20.27	35.69	48.90
S. griseus	One week before	32.77	5.37	55.68	22.50	5.17	65.70	24.29	0.00	65.70	37.24	10.53	49.87	59.24
	One week after	40.67	14.81	43.84	39.18	10.33	48.06	31.65	10.33	55.33	46.47	20.27	36.31	45.88
	At planting date	37.24	10.33	49.96	32.77	5.17	55.76	15.70	0.00	74.29	40.04	15.70	45.73	56.44
Control		47.10	20.27	35.63	41.52	15.70	47.03	32.77	10.33	54.30	49.96	24.38	29.23	
L.S.D. at 0.05 for		Pre-	Post	Survival										
Time		1.113	2.424	1.530										
Pathogen		1.290	2.800	1.764										
Bioagents		1.420	3.130	1.970										
Time x Pathogen		2.226	NS	3.050										
Time x Bioagents		2.489	NS	3.420										
Pathogen x Bioagents		2.870	NS	NS										
Time x Pathogen x Bioagents		4.980	NS	NS										

Data were transformed to arcsine.

Table 6. Effect of date of applying bioagents to the infested soil on percentage of disease rating and wheat yield components in adult stage of cv. Sakha 69 wheat plants.

Bioagents	Date of application	Pathogens														
		F. graminearum			*H. sativum*			*R. solani*			Combination			Mean		
		DR %	t/p	1000-KW	DR %	t/p	1000-KW	DR %	t/p	1000-KW	DR %	t/p	1000-KW	DR %	t/p	1000-KW
T. harzianum	One week before	34.03	2.17	33.19	30.64	2.32	35.18	26.56	2.76	33.11	39.23	1.82	30.17	32.62	2.27	32.91
	One week after	45.38	1.00	26.08	41.54	1.08	29.00	38.44	1.35	30.03	50.76	1.00	25.24	44.03	1.11	27.59
	At planting date	40.78	1.89	30.13	36.47	1.57	32.96	23.04	2.93	35.35	45.00	1.17	28.98	36.32	1.89	31.86
T. hamatum	One week before	38.84	2.10	30.05	34.02	2.13	33.09	31.52	2.79	35.11	43.85	1.71	26.22	37.06	2.18	31.12
	One week after	53.93	1.30	24.06	45.76	1.32	27.05	40.78	1.25	28.73	55.54	1.00	20.51	19.00	1.22	25.09
	At planting date	43.85	1.91	26.02	39.23	1.07	30.00	26.56	2.87	34.42	48.06	1.53	32.03	39.43	2.07	30.62
B. subtilis	One week before	48.44	1.58	25.22	45.00	1.47	28.08	41.16	2.09	30.06	53.93	1.48	20.22	47.13	1.56	25.90
	One week after	57.34	1.00	19.73	54.73	1.00	32.18	45.47	1.00	26.06	63.43	1.00	15.02	55.24	1.00	21.00
	At planting date	53.93	1.17	19.90	49.60	1.32	23.98	33.21	2.34	31.65	59.64	1.00	18.03	49.10	1.46	23.39
S. griseus	One week before	42.32	1.62	27.18	38.05	1.90	30.18	35.26	2.51	32.27	48.44	1.60	23.45	41.02	1.91	28.27
	One week after	54.73	1.00	20.51	51.55	1.00	24.06	43.85	1.18	26.00	60.38	1.00	18.18	52.63	1.05	22.19
	At planting date	45.16	1.30	22.02	43.08	1.36	27.00	29.77	2.63	33.62	54.23	1.33	25.00	43.46	1.61	26.91
Control		57.34	1.00	19.15	54.73	1.33	23.22	45.47	1.37	26.33	63.43	1.00	14.46	55.24	1.14	20.79
L.S.D. at 0.05 for		DR %	t/p	1000-KW												
Time		0.366	0.049	0.316												
Pathogen		0.422	0.051	0.365												
Bioagents		0.472	0.057	0.408												
Time x Pathogen		0.731	0.089	0.333												
Time x Bioagents		0.818	0.099	0.707												
Pathogen x Bioagents		0.964	0.115	0.817												
Time x Pathogen x Bioagents		1.636	0.199	1.415												

Data were transformed to arcsine.

Table 7. Effect of different rates of different bioagents added to the infested soil on percentage of the disease incidence in seedling stage of cv. Sakha 69 wheat plants.

Bioagents	Rate of application g/kg soil	Pathogens												
		F. graminearum			*H. sativum*			*R. solani*			Combination			Mean
		Pre-	Post	Survival	Pre-	Post	Survival	Pre-	Post	Survival	Pre-	Post	Survival	Survival
T. harzianum	1	45.70	20.27	37.00	37.0	15.7	48.47	32.6	10.3	54.26	47.0	24.4	32.57	43.17
	3	32.80	15.70	52.80	26.0	5.4	62.00	28.0	10.3	58.88	37.0	17.9	47.0	55.19
	5	22.50	0.00	67.49	5.0	0.0	84.81	15.7	5.2	72.00	28.0	5.2	60.46	71.21
	10	18.00	0.00	71.96	0.0	0.0	89.97	5.0	5.2	79.45	22.0	0.0	67.85	77.31
T. hamatum	1	45.70	20.20	37.24	38.7	15.7	47.00	32.7	10.3	54.36	48.5	23.9	31.11	42.43
	3	35.69	17.94	48.59	29.6	5.2	58.80	29.5	10.3	57.42	41.5	20.3	41.40	51.55
	5	29.50	0.00	60.46	12.8	0.0	77.22	10.5	5.4	77.12	32.8	10.3	54.30	67.28
	10	26.00	0.00	63.91	10.0	0.0	79.65	5.0	5.2	79.45	25.9	0.0	64.12	71.78
B. subtilis	1	47.00	19.90	35.43	41.5	15.7	44.26	32.8	10.3	54.30	49.9	29.5	42.80	40.88
	3	44.00	15.00	40.00	35.8	10.5	51.27	31.0	10.3	56.00	45.6	35.6	39.20	45.73
	5	40.00	15.70	45.73	29.6	5.4	85.83	28.0	5.2	60.46	42.9	41.5	35.10	51.64
	10	34.00	10.50	52.75	27.8	5.2	60.38	24.0	5.2	64.12	38.6	48.5	31.30	56.44
S. griseus	1	47.00	20.27	35.63	41.5	15.7	44.26	32.6	10.3	54.56	49.9	23.9	29.53	41.00
	3	40.00	15.70	45.73	32.8	10.3	54.30	27.8	10.3	58.80	44.0	20.3	38.64	49.37
	5	32.77	5.40	55.68	22.5	5.2	65.70	24.0	0.0	65.70	37.0	10.5	49.87	59.24
	10	29.60	0.00	60.38	20.0	0.0	69.72	20.0	0.0	69.72	34.0	0.0	55.76	63.90
Control		47.00	20.27	35.63	41.5	15.7	44.26	32.8	10.3	54.30	50.0	24.4	29.32	40.85
L.S.D. at 0.05 for		Pre-	Post	Survival										
Rate		1.33	2.37	1.59										
Pathogen		1.33	2.37	1.59										
Bioagents		1.48	2.65	1.78										
Rate x Pathogen		2.65	NS	NS										
Rate x Bioagents		2.97	5.30	3.56										
Pathogen x Bioagents		2.97	NS	3.56										
Time x Pathogen x Bioagents		NS	NS	NS										

Data were transformed to arcsine.

Table 8. Effect of different rates of bioagents on percentage of the disease rate and wheat yield components in adult stage of cv. Sakha 69 wheat plants.

Bioagents	Rate of application g/kg soil	Pathogens														
		F. graminearum			*H. sativum*			*R. solani*			Combination			Mean		
		DR %	t/p	1000-KW g	DR %	t/p	1000-KW g	DR %	t/p	1000-KW g	DR %	t/p	1000-KW g	DR %	t/p	1000-KW g
T. harzianum	1	50.00	1.00	23.12	52.00	1.00	26.21	45.00	1.37	28.32	60.38	1.00	18.00	51.89	1.10	23.92
	3	43.00	1.47	28.13	39.87	1.63	31.14	35.64	1.81	30.10	48.19	1.22	24.13	41.70	1.53	28.38
	5	34.00	2.17	33.19	30.64	2.32	35.18	26.56	2.76	33.11	39.23	1.82	30.17	32.62	2.27	32.91
	10	31.90	2.54	35.10	27.00	2.73	36.11	24.59	3.10	35.00	36.47	2.68	32.10	30.00	2.76	34.57
T. hamatum	1	52.00	1.00	21.10	53.39	1.00	24.10	45.47	1.37	27.10	62.66	1.00	17.00	53.40	1.10	22.33
	3	45.63	1.56	25.11	42.45	1.54	29.12	37.91	1.92	32.21	51.42	1.13	20.23	44.35	1.54	26.67
	5	38.84	2.00	30.11	34.00	2.13	33.10	31.52	2.79	35.11	43.85	1.71	26.22	37.00	2.18	31.12
	10	34.85	2.55	33.11	29.77	2.61	35.19	27.00	2.72	36.00	42.32	2.64	30.00	33.49	2.63	33.59
B. subtilis	1	56.81	1.00	19.10	54.73	1.00	23.10	45.00	1.37	26.00	63.43	1.00	15.10	55.00	1.10	20.81
	3	53.40	1.29	21.13	48.19	1.28	25.50	42.45	1.33	28.18	58.93	1.00	16.26	50.74	1.22	22.77
	5	48.44	1.58	25.22	45.00	1.47	28.10	41.17	2.10	30.10	53.93	1.48	20.22	47.13	1.64	25.90
	10	43.00	1.64	28.30	38.84	1.80	30.13	37.66	2.14	33.13	51.16	1.83	25.18	42.68	1.85	29.18
S. griseus	1	54.73	1.00	20.38	54.73	1.00	23.34	45.47	1.37	26.35	63.43	1.00	16.00	54.59	1.10	21.52
	3	48.83	1.39	23.35	45.00	1.37	27.32	39.87	1.85	30.44	54.73	1.00	18.24	47.11	1.40	24.84
	5	42.32	1.62	27.18	38.00	1.90	30.18	35.26	2.51	32.27	48.44	1.60	23.45	41.00	1.91	28.27
	10	29.23	1.89	31.07	36.00	2.10	33.95	31.95	2.89	33.15	46.52	2.10	28.20	38.44	2.23	31.60
Control		57.34	1.00	19.15	54.73	1.00	23.22	45.47	1.37	26.33	63.43	1.00	14.46	55.24	1.13	20.79

DR %: Disease rating, t/p: tillers/plant 1000 Kernel weight

L.S.D. at 0.05 for	DR %	t/p	1000-KW
Rate	0.415	0.048	0.265
Pathogen	0.415	0.048	0.265
Bioagents	0.460	0.050	0.296
Rate x Pathogen	NS	0.095	0.530
Rate x Bioagents	0.930	0.107	0.592
Rate x Bioagents	0.930	0.107	0.592
Rate x Pathogen x Bioagents	NS	0.213	1.185

Data were transformed to arcsine.

tum and *S. griseus* occupied the second rank, while *B. subtilis* was the least effective. Adding the antagonist one week before sowing provides a chance to affect pathogenic structures found in soil and prevent them from infecting roots of the host plants (Abd-EL-Moity *et al.*, 1990). Other investigators (Moody and Gindrat, 1977 Soliman *et al.*, Andreson *et al.* 1994 and Worasatit *et al.*, 1994) found that adding antagonist one week or more before sowing to soil infested with the pathogens was most appropriate for increased efficacy of biological treatments.

3. Effect of rate of application on the efficacy of the antagonist

Data in Tables (7 and 8) show that adding different antagonists at different rates to soil infested with the pathogenic fungi led to reduction in disease incidence. Increased rates of bioagent led to an increase in the efficacy of the treatment. It was found that the best rates of bioagent were 5g and 10g/kg soil. These rates caused sharp and significant reduction in disease incidence and disease rate and led to significant increases in healthy survival plants and yield components. Obtained data also proved that the most effective bioagent was *T. harzianum* followed by *T. hamatum* and *S. griseus,* while *B. subtilis* was the least effective. These results are in agreement with those obtained by Hadar *et al.*, (1979), who reported that an inverse relationship between amount of *T. haraianum* applied to soil infested with *R. solani* at rates from 2g to 10g/kg soil and incidence of diseased plants. These results might be due to that increasing the amount of *T. harzianum* added to infested soil led to increased amount of toxic metabolites in soil and consequently more inhibition of mycelial growth of the pathogens leading to a decrease in the incidence of diseased plants (Benson and Baker, 1970, Lui and Baker, 1980 and Abd-EL-Moity *et al.*, 1991).

These results denote the importance of biological control. Using bioagents year after year will lead to reduction in resting structure and inoculum density of the pathogens in infested soil. Number of pathogen propagules will be reduced by using active antagonists, while the number of antagonists will be increased in soil till reaching what we call suppressive soil, which prevents the pathogens from causing any considerable crop losses (Cook and Baker, 1983).

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المكافحة الحيوية لمرض عفن الجذور في القمح

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في هذه الدراسة تم عزل وتعريف كائنات دقيقة مختلفة (فطريات - بكتريا - اكتينومايسيتس) من رايزوسفير نباتات قمح سليمة لإستخدامها في المكافحة الحيوية لمرض عفن الجذور في القمح والمتسبب عن الفطريات فيوزاريوم جرامينيرم وهلمنثوسبوريم ساتيفم ورايزوكتونيا سولاني. ولقد وجد أن أكثر الكائنات المضادة تواجداً هي فطر ترايكودرما هارزيانم وترايكودرما هاماتم والبكتريا ستربتومايسيس جريسيس وباسيلس ساتلس. ولقد أثبتت الدراسة المعملية أن الفطر ترايكودرما هارزيانم أعطى أعلى تأثير تثبيطي لنمو عزلات الفطريات الممرضة يليه ستربتومايسيس جريسيس بينما أعطت البكتريا باسيلس ساتلس أقل تأثير تثبيطي لنمو هذه الفطريات. لم تسمح الفطريات ترايكودرما هارزيانم وترايكودرما هاماتم بتكوين أي أجسام حجرية للفطر رايزوكتونيا سولاني في البيئة. ولقد ثبت من الدراسة المعملية أن نوع البيئة قد يؤثر على التضاد بين الكائن المضاد والكائن الممرض. باستخدام طريقة الشريحة الميكروسكوبية تم دراسة تأثير الفطر ترايكودرما هارزيانم وكيفية تطفله وأختراقه للغزل الفطري الممرض والنمو داخله حيث استطاع القضاء على الفطر الممرض بعد ٤٨ ساعة من المعاملة في حين أظهرت بكتريا بسيلس ساتلس القدرة على تحليل الغزل الفطري للفطر الممرض بعد ٩٦ ساعة من المعاملة.

أجريت كذلك دراسة على النباتات الحية في الأصص لتقدير تأثير شكل وميعاد وكمية الإضافة للكائن المضاد إلى التربة الملوثة بالفطريات الممرضة على حدوث مرض عفن الجذور في القمح. ولقد وجد أن استخدام فطريات التضاد في شكل مسحوق واستخدام البكتريا في شكل معلق أعطت أفضل النتائج في مقاومة مرض تعفن الجذور. ولقد ثبت أيضاً أن إضافة كائنات التضاد قبل اسبوع من الزراعة إلى تربة ملوثة بالفطريات الممرضة أدت إلى انخفاض معنوي في نسبة حدوث المرض. ولقد وجد أيضاً أن إضافة كائنات التضاد بمعدلات ٥، ١٠ جم/كجم تربة أدى إلى انخفاض في نسبة حدوث المرض بدرجة كبيرة.