African Journal of Microbiology Research Vol. 6(1), pp. 190-196, 9 January, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1299
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Biocontrol of *Fusarium udum* diseases for some wheat cultivars by *Streptomyces spororaveus*

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Accepted 9 December, 2011

This work was designed to explore the potential of microbial antagonism in the control of wheat *Fusarium* disease. The *in vitro* studies showed that a 70% concentration of the culture filtrate of *Streptomyces spororaveus* significantly inhibited spore germination, mycelial growth and sporulation of *Fusarium udum*. The *in vivo* studies involved different treatments. The seed coating treatment was the most effective in controlling *F. udum* at all cultivation periods in all the three-wheat cultivars FFR 502W, FFR 518 and FFR 525W. The former cultivar showed the highest growth response compared with the other two cultivars. Soil pre-inoculation was less effective whereas seed-soaking treatment was the least effective in this respect.

Key words: Actinomycetes, antifungal activities, plant fungal diseases.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most strategically important crop globally. Root and crown rot of wheat occurs throughout the growing season of wheat; it is a disease problem in many producing areas of the world. Common root and crown rot is caused by several different soil borne pathogens, but it is demonstrated that the most important cause of the common root and crown rot complex is known as the fungus, *Bipolaris sorokiniana* (Mathre et al., 1993). The telemorph stage is rarely found under natural condition, and was first found in Zambia where two different mating types must appear together (Kumar et al., 2002; Raemarkers, 1988) and usually does not develop in culture unless in the presence of opposite mating types (Mathre et al., 1993; Kumar et al., 2002). It is said that in addition to *Crocus sativus*, several species of *Fusarium* are associated in the common root and crown rot complex (Windels and Holen, 1989; Wiese, 1987; Sallans, 1965).

Fusarium is one of the most ubiquitous abundant and important genera of soil microflora (Nirenberg, 1990; Nelson et al., 1987; Booth, 1971). Several *Fusarium* species are widespread pathogen causal agent of brown foot rot of cereals, especially, in both temperate and semitropical areas (Windels and Holen, 1989; Wiese, 1987; Sallans, 1965). Take-all of wheat is caused by the fungus, *Gaeumannomyces graminis* var. tritici. It is the most deadly root disease of wheat worldwide; it also affects triticale, barley and ray, but to a lesser extent (Freeman and Ward, 2004), causes stunting and nutrient-deficiency symptoms in the tops, and progresses upward into the bases of the stems. Here, it disrupts the flow of water to the tops and causes premature death of the plant (Cook, 2003).

Common root and crown rot of wheat is widely documented in different Iranian wheat belt (Darvishnia et al., 2006). *Fusarium* head blight is a fungal disease of wheat found in both temperate and semi-tropical regions. A number of species of *Fusarium* may be responsible but generally *Fusarium graminearum* and *Fusarium culmorum* predominant. Both fungi cause root rot, food rot, crown rot, stem rot and head blight in wheat. Head blight causes reduced kernel set and kernel weight, destruction of starch granules and storage proteins and seed infection.

The fungus first infects the extruded anthers and then ramifies throughout the developing caryopsis, floral bracts, and rachis. The severity of infection is correlated

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with the percentage of retained anthers.

Wheat is generally most susceptible to infection at the flowering stage, and susceptibility declines at later stages of caryopsis development. Some cultivars, however, may be most susceptible at the milk or soft dough stages. Head Infection in nature can occur anytime after beginning of flowering, when temperature and moisture are favorable (Bai and Shaner, 1994).

The aim of this research was to evaluate the potential of *Streptomyces spororaveus* for biological control of *Fusarium udum*.

MATERIALS AND METHODS

Microorganisms and culture conditions

F. udum was isolated from diseased wheat roots and maintained on the artificial medium according to Johnson and Curl (1972). *S. spororaveus* was isolated from soil of Riyadh city and shown to be potent antagonists to some pathogenic fungi was maintained on starch nitrate agar medium (Waksman and Lechevalier, 1961).

Host plant

Seeds of wheat (*T. aestivum* L.) cultivars FFR 502W, FFR 518 and FFR 525W were obtained from the middle area of Saudi Arabia. These were surface-sterilized with 0.1% $HgCl_2$ for 30 s, then thoroughly washed with sterile distilled water.

Isolation of actinomycetes from soil

Isolation of actinomycetes from soil had been carried out using dilution method according to Johnson and Curl (1972).

Identification of actinomycete isolate

Genus identification of the selected isolate was identified by 16S rRNA sequencing. 16S rRNA was amplified in a thermocycler (Perkin Elmer Cetus Model 480) by using universal primers of 27f (5'-AGA GTT TGA TCC TGG CTC AG -3') and 1525r (5'-AAG GAG GTG ATC CAG CC-3') under the following condition: 94°C for 5 min, 35 cycles of 94°C for 60 s, 55 for 60 s, 72°C f or 90 s and final extension at 72°C for 5 min. The product was directl y sequenced by a BigDye terminator cycle sequencing kit (PE Applied Biosystems USA) on an ABI 310 automated DNA sequencer (Applied Biosystems, USA). Homology of the 16S rRNA sequence of isolate was analyzed by using BLAST program from Genbank database (Prapagdee et al., 2008).

Effect of culture filtrates of *Streptomyces spororaveus* on growth activities of *Fusarium udum* (*in vitro*)

S. spororaveus was grown on starch nitrate agar for 10 days at 29°C on plates. The sporulating aerial hyphae were r emoved, and then 10 ml sterile distilled water was added to the substrate mycelium. The mixture was homogenized and filtered through Whattman No. 1 paper and the filtrate was filter-sterilized through 0.45 µm filter. Different concentrations of the filtrate (30, 50, 70 and 90%, v/v) were prepared with sterile starch nitrate broth. Germination of fungal spores and lengths of germ tubes were studied employing microscopic slides according to (El-Abyad et al., 1983). Radial growth was determined by mixing aliquots of filtrates aseptically with sarcina agar medium to give concentrations ranging from 30 to 90% (v/v), and poured in Petri dishes. The pathogenic fungus *F. udum* was grown on tap water agar (1.5%, w/v) at 28°C for 7 days. Disks (5 mm) were cut from the margins of colonies and transferred to the sarcina agar plates amended with filtrate for a period of 7 days at 28°C. Control without filtrate was prepared. Sporulation was studied by inoculating plates containing sarcina-amended agar medium with a 5-mm disk of tap water agar bearing fungal mycelium, and subsequently incubating them at 28°C for 10 days. The density of spores/ml was counted using a haemocytometer as described by El-Abyad et al. (1983). A control sarcina agar plate without filtrate was prepared.

Preparation of inocula for biological control treatments

For the seed-coating treatment, *S. spororaveus* grown at 28°C for 7 days on starch nitrate agar. The hyphae and spores of three plates were suspended in 15 ml sterile distilled water. For soil pre-inoculation treatment, the *S. spororaveus* grown on starch nitrate broth on a rotatory shaker (200 rpm) at 28°C. The hypha e were harvested by centrifugation at 3000 g for 20 min and the pellets resuspended in 10 ml of sterile distilled water. The treatment with seeds soaked in the filtrate of antagonistic *S. spororaveus* was done with filtrates as described in the *in vitro* experiments. The pathogenic fungus *F. udum* was grown on sarcina agar medium for 7 days. Agar disks (5 mm) bearing mycelium were inoculated into sarcina broth in Erlenmeyer flasks and incubated at 28°C for 3 days. The cultures were blended for 30 s, and 5 ml of each was used to inoculate fresh sarcina broth (45 ml). After incubation at 28°C for 3 days the mycelia were harvested on Whattm an No. 1 paper. These were weighed, resuspended in sterile distilled water and blended for 30 s, after which the appropriate volume for the soil treatment was obtained.

Twenty seeds of wheat were coated with 15 ml of aqueous suspension of hyphae and spores of *S. spororaveus* as described by Singh and Mehrotra (1980). Both pathogen inoculation and treated seeds were done simultaneously. Treated seed were air-dried for 30 min before sowing. The wheat pathogen *F. udum* was inoculated into the soil at a rate of 0.2 g/Kg soil (Rothrock and Gottlieb, 1984). In the soil pre-inoculation treatment, the *S. spororaveus* was inoculated into the soil 7 days prior to the inoculation of pathogen and seed sowing at a rate of 0.6 g/Kg soil. In the third treatment, wheat seeds were soaked in the filtrates of *S. spororaveus* or distilled water as control for 30 min prior to the sowing and infestation of soil. Control treatments included seeds soaked in sterile distilled water. The pots were kept in the greenhouse for 60 days. After 2, 4, 6 and 8 weeks from planting, the plants were removed, washed with tap water and the following measurements were made % emergence, % infection, root depth, root dry weight, number of spikes, shoot dry weight of plants.

Statistics

The data recorded, here in, are the means of at least three replicates and the results were analyzed according to Sokal and Rohlf (1981).

RESULTS

Analysis of the 16S rRNA gene sequences showed that the most potent actinomycete isolate which have highest

Table 1. Effect of different concentrations of cell-free extract of *S. spororaveus* on germination percentage, average length germ tubes, number of spores, dry weight and radial growth of *F. udum* at 28℃.

Filtrate conc. (%)		G (%)	Gt (µm)	No. of spores (× 10^5 ml^{-1})	Dry weight (mg)	Radial growth (mm)		
						2 days	4 days	6 days
Control		80.3	110.2	142.6	1107.7	23.5	55.6	85.5
30		66.7**	85**	104.8**	880.3**	20.2**	24.5**	35.5**
50		35.3**	32**	77.6**	220.7**	13.2**	15.3**	20.6**
70		0.0**	0.0**	39.2**	160.3**	8.0**	10.5**	15.8**
90		0.0**	0.0**	5.6**	90.7**	5.0**	5.0**	5.5**
LSD	5	3.9	3.5	4.9	9.3	1.1	2.0	2.4
	1	5.7	5.4	6.5	16.6	2.6	2.3	4.8

G (%) = germination percentage, Gt = average length germ tubes, ** = highly significant at 1 % LSD, and * = significant at 5 % LSD related to control.

antifungal activity and symbolized as RDS28 is identical to *S. spororaveus* (100% similarity) with GenBank database accession number (HQ834290) and then designated *S. spororaveus* RDS28.

The percentage of germination and average germ tube length of spores of *F. udum* decreased significantly by raising the concentration of culture filtrate of *S. spororaveus*. However, 70% filtrate concentration arrested (Table 1). The later also revealed that the number of *F. udum* spores significantly decreased with increasing the filtrate concentration of *S. spororaveus*. Table 1 involved the different concentrations of *S. spororaveus* filtrate that significantly inhibited the growth of *F. udum* at all incubation periods. The extent of inhibition increased with increased concentration and/or incubation period. Similarity the dry mass yield of the fungal pathogen significantly decreased with increasing the filtrate concentration.

Results in Table 2 showed that, under the different infestation treatments, the percentage emergence of FFR 502W and FFR 525W wheat seedlings significantly decreased whereas in FFR 518 seedlings significantly increased as compared with non-infested control. Soil pre-inoculation and seed coating treatments was significantly raised whereas seed soaking treatment non-significantly affected emergence as compared with the infested soil. The infection percentage significantly increased in infested control with progress of time, but decreased under soil pre-inoculation or seed soaking treatments, compared with infested soil. The infection percent also increased with progress of cultivation period. The infection percentage was nil in seed coating treatment in all three wheat cultivars.

Results in Tables 3, 4 and 5 showed that infestation of soil with *F. udum* alone, suppressed growth parameters of wheat cultivars FFR 502W, FFR 518 and FFR 525W, while application of different treatments with *S. spororaveus* nullified or even reversed the inhibitory effects of the pathogen. In the three wheat cultivars the antagonistic *S. spororaveus* when applied as seed coating is the best for controlling the fungal pathogen as well as, increasing the growth of the wheat cultivars.

DISCUSSION

The *in vitro* studies showed that 70% of the culture filtrate of *S. spororaveus* completely inhibited spore germination and mycelial growth, but significantly decreased the sporulation of *F. udum*. Many microorganisms are capable of producing antibiotics following stimulation by the appropriate substrates (Takeuchi et al., 1988). Similar studies were done with different microorganisms antagonistic against various pathogens *Alternaria solani, Aspergillus niger, Curvularia pallescens* and *Helminthosporium oryzae* inhibited by the water soluble antibiotic produced by *Streptomyces galbus* (Paul and Banerjee, 1986). Wherever wheat is grown as well as, Moghan wheat belts, common root and crown rot is an important disease problem; it results in yield loss by damping off, reducing tiller number, head size and kernel yields (Backhouse et al., 2004). *F. culmorum* is a one of the principal causal agent of common root rot in wheat especially in many wheat growing regions as well as, several wheat growing fields in Iran such as Fars, Mazandaran, West Azerbaijan, Khorasan, Yazd, Golestan, Ilam, Boushehr, Kermanshah provinces (Darvishnia et al., 2006). Additionally, *F. graminearum* is the other important causal agents of wheat common crown and root rot, especially in warmer wheat-growing areas (Weise, 1987). In Iran, *F. graminearum* is reported from West Azerbaijan, Tehran, East Azerbaijan, Guilan, Mazandaran, Ilam, Ardabil, Hamadan, Golestan, Fars, Kerman, Markazi, Boushehr, Qom, Qazvin and Khorasan provinces (Darvishnia et al., 2006).

The results indicated that the control of the pathogen and growth of wheat cultivars differed according to the treatment, the wheat cultivar and the length of cultivation period. After 15 days of treatment, no infection symptoms have been detected in all wheat cultivars. The growth parameters of the three wheat cultivars have been significantly increased by different *Streptomyces*

Table 2. Effect of different treatments with *S. spororaveus* on the control of *F. udum* on wheat cultivars FFR 502W, FFR 518 and FFR 525W during 60 days post sowing.

Treatment		Emergence (%)	Infection (%)			
			15 day	30 day	45 day	60 day
1st cultivar FFR 502W						
Non-infested control		85.0	0.0	0.0	0.0	0.0
Infested control		73.5**	3.3**	19.4**	39.3**	70.5**
Soil pre-inoculation		80.0**++	0.0**++	0.0++	0.0++	12.5**++
Seed coating		80.0**++	0.0**	0.0++	0.0++	0.0++
Seed soaking		71.7**	0.0**+	6.4**++	12.1**++	41.9**++
LSD	1 %	3.3	0.0	6.4	6.1	1.9
	5 %	2.4	0.0	4.5	4.3	1.4
2nd cultivar FFR 518						
Non-infested control		76.7	0.0	0.0	0.0	0.0
Infested control		66.7**	5.6**	31.7**	65.8**	82.6**
Soil pre-inoculation		78.3++	0.0**++	0.0++	0.0++	19.2**++
Seed coating		78.3++	0.0**++	0.0++	0.0++	0.0++
Seed soaking		71.7**++	0.0**++	6.4**++	8.8**++	55.8**++
LSD	1 %	3.3	0.0	6.4	0.5	2.7
	5 %	2.4	0.0	4.5	0.4	1.9
3rd cultivar FFR 525W						
Non-infested control		80.0	0.0	0.0	0.0	0.0
Infested control		66.7**	13.4**	35**	48.9**	77.5**
Soil pre-inoculation		73.3**++	0.0**++	0.0++	0.0**++	20.5**++
Seed coating		78.3++	0.0**++	0.0++	0.0**++	0.0++
Seed soaking		73.3**++	0.0**++	6.4**++	9.1**++	52.2**++
LSD	1 %	3.3	0.0	6.4	0.0	2.2
	5 %	2.4	0.0	4.5	0.0	1.6

** = highly significant at 1 % LSD, * = significant at 5 % LSD related to non-infested control, ++ = highly significant at 1 % LSD, + = significant at 5 % LSD related to infested control.

treatments. After 30, 45 and 60 days from sowing, the best treatment for controlling the pathogen by the selected antagonistic *S. spororaveus* was the seed coating with spores of the experimental *S. spororaveus*. This may be due to the spores of the antagonistic strain in contact with seed of wheat, which continuously supplied the seed with the antimicrobial compounds. Similar results were obtained by other workers in the control of maize root rot (Singh and Mehrotra, 1980).

In this investigation inoculation of soil with the antagonist 7 days prior to sowing was less effective in controlling the wheat pathogen than the seed coating treatment. No symptoms were observed in soil pre-inoculation treatment with *S. spororaveus* at 15, 30 and 45 days post sowing. On the other hand, the increase in the percentage infection by *F. udum* at the late stage of cultivation in the soil pre-inoculation treatment may indicate the *F. udum* was more tolerant to the environmental conditions. This may be also due to the decline of the antagonistic actinomycete population resulting in a decreased production of antimicrobial substances. At the early stages of growth, the decrease of infection may be due to the high population of *S. spororaveus*. Similar studies were applied for the control of root rot of pea seedlings (Rothrock and Gottlieb, 1984), damping-off of cauliflower (Kundu and Nandi, 1993). In this work the significant improvement in wheat growth via the seed coating treatment may be due to the increased availability to the wheat seeds of the growth regulators produced by the antagonistic actinomycete together with their continuous supply to the developing plants as a result of the intimate contact between the seeds and the antagonist. Similar results were observed by (El-Abyad et al., 1996).

This investigation also showed that the seed soaking treatment was least effective in controlling *F. udum* compared with the other treatments. This may be attributed to the decreased absorptive capacity of wheat seeds for the antagonistic compounds due to their hard coat and hence low-level accumulation in the germinating seeds. Our

Table 3. Effect of different treatments with *S. spororaveus* on the growth parameters of the wheat cultivar FFR 502W during 60 days post sowing.

Treatment	Root system								Shoot system							
	Root depth (mm)				Dry wt (g)				No. of spikes				Dry wt (g)			
	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
Non-infested control	30	45	80	100	0.9	1.3	2	2.7	3	5	8	12	2	3.2	5.2	8.1
Infested control	25**	42*	63**	83**	0.7**	1.2**	1.8**	2.4**	2**	4**	7**	11**	1.6**	3**	4.9**	7**
Soil pre-inoculation	29^{++}	48**	77^{++}	101^{++}	0.8*$^{+}$	1.4**$^{++}$	2.1*$^{++}$	2.8*$^{++}$	2**$^{++}$	5^{++}	8^{++}	13**$^{++}$	1.8**$^{++}$	3.4**$^{++}$	5.8**$^{++}$	8.1^{++}
Seed coating	33**$^{++}$	55**$^{++}$	86*$^{++}$	114**$^{++}$	0.9^{++}	1.5**$^{++}$	2.4**$^{++}$	3.1**$^{++}$	3**$^{++}$	5^{++}	9**$^{++}$	14**$^{++}$	2.1*$^{++}$	3.6**$^{++}$	6.4**$^{++}$	9**$^{++}$
Seed soaking	27**$^{++}$	45*	72**$^{++}$	95^{++}	0.8^{++}	1.2**$^{++}$	1.9^{++}	2.5**$^{++}$	2**+$^{+}$	4**	8^{++}	12^{++}	1.8**$^{++}$	3**	5.2^{++}	7.3**$^{++}$
LSD 5%	1.4	3.4	4.8	5.8	0.1	0.08	0.1	0.1	0	0	0.5	0.5	0.1	0	0.08	0.8
LSD 1%	2	4.8	6.8	8.2	0.2	0.1	0.2	0.2	0	0	0.7	0.7	0.2	0	0.1	0.1

** = highly significant at 1% LSD, * = significant at 5 % LSD related to non-infested control, $^{++}$ = highly significant at 1% LSD, $^{+}$ = significant at 5% LSD related to infested control.

Table 4. Effect of different treatments with *S. spororaveus* on the growth parameters of the wheat cultivar FFR 518 during 60 days post sowing.

Treatment	Root system								Shoot system							
	Root depth (mm)				Dry wt (g)				No. of spikes				Dry wt (g)			
	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
Non-infested control	20	40	80	102	0.9	1.2	2	2.3	2	4	7	10	0.9	2.8	4.1	6.2
Infested control	10**	25**	59**	74**	0.3**	1**	1.5**$^{++}$	2**	1**	3*	6**	8**	0.7*	2.6**	3.9**	5.8**
Soil pre-inoculation	8**$^{++}$	47**$^{++}$	79^{++}	99**$^{++}$	0.2**$^{++}$	1.1**$^{++}$	1.7**$^{++}$	2.2*$^{++}$	3**$^{++}$	4^{+}	7^{++}	9**$^{++}$	0.8	2.7**$^{++}$	4.7**$^{++}$	6.4**$^{++}$
Seed coating	16**$^{++}$	50**$^{++}$	88*$^{++}$	110**$^{++}$	0.5**$^{++}$	1.3**$^{++}$	2.3**$^{++}$	2.9**$^{++}$	4**$^{++}$	6**$^{++}$	8**$^{++}$	12**$^{++}$	1.2**$^{++}$	3.3**$^{++}$	6.3**$^{++}$	8.4**
Seed soaking	10**	39*$^{++}$	67*$^{++}$	83**$^{++}$	0.3**$^{++}$	0.9**$^{++}$	1.6**$^{++}$	2**$^{++}$	1**	2*$^{++}$	4**$^{++}$	6**$^{++}$	0.7*	2.3**$^{++}$	4.2*$^{++}$	5.8**$^{++}$
LSD 5%	0.8	1.4	6.2	4.1	0	0.09	0.04	0.1	0.5	0.8	0.5	0.5	0.2	0	0.1	0
LSD 1%	1.2	2	8.8	5.8	0	0.1	0.06	0.2	0.7	1.2	0.7	0.7	0.3	0	0.2	0

** = highly significant at 1% LSD, * = significant at 5% LSD related to non-infested control, $^{++}$ = highly significant at 1% LSD, $^{+}$ = significant at 5% LSD related to infested control.

Table 5. Effect of different treatments with *Streptomyces spororaveus* on the growth parameters of the wheat cultivar FFR 525W during 60 days post sowing.

Treatment	Root system								Shoot system							
	Root depth (mm)				Dry wt (g)				No. of spikes				Dry wt (g)			
	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
Non-infested control	23	43	67	90	0.7	1.8	2.3	3.1	2	4	9	13	2	3.5	6.1	8.7
Infested control	21**	38**	63**	81**	0.6*	1.7	1.6**	2.2**	2	3*	7**	10**	1.4**	2.4**	4.4**	6.4**
Soil pre-inoculation	25**$^{++}$	45^{++}	73**$^{++}$	97**$^{++}$	0.7^{++}	1.9^{++}	2.7**$^{++}$	3.5*$^{++}$	3**$^{++}$	5*$^{+}$	8**$^{++}$	11*$^{++}$	1.6**$^{++}$	3.8**$^{++}$	6.4**$^{++}$	7.8**$^{++}$

Table 5. Contd.

Seed coating		30**$^{++}$	51**$^{++}$	82**$^{++}$	108**$^{++}$	0.9**$^{++}$	2*$^{++}$	3.3**$^{++}$	4.3**$^{++}$	3**$^{++}$	6**$^{++}$	10**$^{++}$	14**$^{++}$	2.5**$^{++}$	5.9**$^{++}$	8.7**$^{++}$	12**$^{++}$
Seed soaking		24*$^{++}$	43^{++}	70*$^{++}$	93^{++}	0.7^{++}	1.2**$^{++}$	2^{*++}	2.7^{++}	2	4*	9^{++}	12*$^{++}$	1.6**$^{++}$	3.1**$^{++}$	5.5**$^{++}$	7.1**$^{++}$
LSD	5%	1.4	2.9	2.2	4	0.1	0.2	0.3	0.4	0.5	0.8	0.5	0.8	0.08	0	0	0.1
	1%	2	4.1	3.1	5.7	0.2	0.3	0.4	0.6	0.7	1.2	0.7	1.2	0.1	0	0	0.2

** = highly significant at 1% LSD,* = significant at 5% LSD related to non-infested control, $^{++}$ = highly significant at 1% LSD, $^{+}$ = significant at 5% LSD related to infested control.

findings are confirmed to some extent by the results obtained by other workers in controlling blight of pepper and tomato (Turhan, 1981), with of soybean and French bean (Khalid, 1987), root disease of cucumbers (Tahvonen, 1988), damping-off of sugarbeet (Rath and Wolf, 1992), wilt of tomato (El-Abyad et al., 1993a).

In conclusion, this investigation has demonstrated the potential of *S. spororaveus* in the biological control of some wheat diseases. Coating wheat seeds with spores of an antagonist prior to sowing were found to be the most effective treatment in affecting the disease incidence by *F. udum*.

ACKNOWLEDGEMENT

The authors acknowledge King Saud University represented in Research Center of Teachers College which funded this research so that researchers can get the desired and promising results.

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