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Biological and osmoprimed seed treatments for control damping-off on wheat and barley

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ABSTRACT

A greenhouse experiments was conducted to determine the efficacy of benomyl as a fungicide, bioagents (*Pesudomonas fluoresences*, *Pesudomonas cepacia* and *Bacillus subtilis*), osmopriming seed treatment and their combinations to control *Fusarium culmorum* damping-off on wheat and barley seeds. The results showed that all the tested bacteria had an inhibitory effect against the growth of *F. culmorum* on culture medium. In autoclaved soils, non of the tested treatments affected the percentages of germination of either wheat or barley seeds. On the other hand, all the tested treatments were significantly decreased the time required to attain 90 % of final emergence (T_{90}) by 20.6 - 36.5 % on wheat and 23.3 - 45.2 % on barley. Benomyl and the tested bacterial isolates either individually treated seeds or in combination with PEG gave more performance in reducing incidence of damping-off on wheat and barley. Therefore, the combination of osmoprimed seed and antagonistic bacteria can be used in integrated pest management programme.

INTRODUCTION

The increasing concern about the extensive use of agriculture pesticides is adding continuous pressure on scientists to develop safety alternatives to control pests. As a results, research on biological control of plant pathogens have received major impetus and attracted many investigators (Schisler *et al.*, 2002). A diverse group of antagonistic bacteria have been used widely as biological control agents against plant pathogens. In this concern, antagonistic bacteria such as *Pesudomonas fluoresences*, *Pesudomonas aureofaciens* and *Bacillus* sp. were used to control some cereal fungal diseases such as take all, foot, root and crown rot, and head blight (El-Meleigi, 1989 and Kim *et al.*, 1997). In addition, *P. aureofaciens* AB254 and *Pesudomonas* sp. AB842 were used for control *P. ultimum* and *P. oxalicum*, respectively on sweet corn (Mathre *et al.*, 1995). *B. subtilis* AS43.3 and *Cryptococcus* sp. OH 181.1 reduced the severity of Fusarium head blight on durum wheat (Schisler *et al.*, 2002). Other seed treatments such as osmoconditioning that enhance earlier and more uniform seedling

emergence (Bradford, 1986), may also represent another approach for controlling plant disease (Abdalla and El-Gizawy, 2000).

Wheat seedlings were found to be infected with more than 20 fungi (Jalaludin and Anwar, 1989) and the most predominant species is *Fusarium culmorum*. In Egypt, *F. culmorum* was found to be the most dangerous for wheat plants (Ibrahim, 1997), while *F. culmorum* and *F. graminearum* isolates were the most common species isolated from wheat and barley cultivated in New Zealand (Sayer and Lauren, 1991). Symptoms by *F. culmorum* were pre and post emergence damping-off of wheat seedling, crown and foot rot and head blight (Frank, 1985; Van Wyk *et al.*, 1986; Ibrahim, 1997 and Schisler *et al.*, 2002).

The objectives of this investigation were to evaluate the efficacy of different seed treatments such as benomyl, bioagents (*Pesudomonas fluoresences, Pesudomonas cepacia* and *Bacillus subtilis*), osmopriming seed treatment and their combinations to control *Fusarium culmorum* damping-off on wheat and barley seeds under greenhouse conditions.

MATERIALS AND METHODS

Strains: *Fusarium culmorum* culture was isolated from infected tissues of wheat plant with typical foot and root rot symptoms. *Bacillus subtilis* (Ehrenberg) Cohn culture and *Pseudomonas fluoresences* were originally isolated from soil of the Experimental Farm of the Sabahia Research Station, Alexandria, Egypt according to (Hassanein and El-Goorani, 1991). *Pseudomonas cepacia* (*Burkholderia cepacia* ex Burkholder) isolate was originally obtained from the Plant Pathology Dept., North Carolina State University, USA, Type culture collection (ATCC55344).

***In vitro* assay for antagonistic bacteria (Bioagents):** Bacteria isolates were screened *in vitro* for their antibiosis against *F. culmorum* as described by Abd El-Moity *et al.* (1993). Five plates were used for each treatment. The inoculated plates were incubated at 28 °C for five days and the inhibition zones were daily measured in the treated and non-treated control (inoculated with *F. culmorum* only).

Presowing seed treatments: Seeds of wheat (*Triticum aestivum* L.) cv. Sakha 8 and barley (*Hordeum vulgare* L.) cv. Giza 119 were treated only with either benomyl, *P. fluoresences, P. cepacia and B. subtilis* or combined

with polyethylene glycol 6000 (PEG). Seeds osmoconditioned or osmoprimed in aerated solution of -1.2MPa of PEG (30.2 g / 100 ml H_2O) for 3 days at 15 °C. Treated seeds were rinsed with distilled water and then dried. All the tested bacteria were grown on potato dextrose agar (PDA) for 48 hrs, then the bacterial matrix from four plates of each isolates was scraped with a sterile glass rod into 5 ml of 1.5 % methyl cellulose in distilled water (4,000 centipoises, Sigma Chemical Co., St. Louis, Mo., USA). Twenty milliliter of this suspension was added to 350 seeds of either wheat or barley, agitated very well to insure uniform coating of seeds surface and then air-dried at room temperature before planting. Seeds were treated with benomyl (Benlate, 50 % WP, Du Pont, France) at the rate of 0.5 g a.i. / Kg seeds as recommended by the manufacturer.

Seedling emergence assay: The seedling emergence rate and final percentage emergence of wheat and barley seeds exposed to the previously mentioned presowing treatments as well as non-treated control were assayed in autoclaved soil under the greenhouse conditions (at temperature of 25 ± 3 °C and 16 hrs illumination, 200 $uEm^{-2}.sec^{-1}$). Wheat and barley seeds were planted at the rate of 10 seeds / pot (5 replicates for each treatment), kept in the greenhouse and watered daily. Seedlings were considered have emerged when the plumules protruded above the soil surface. Seedling emergence rate was expressed at T_{90} (time required for 90 % emergence to occur). Final percentage emergence and the reduction in percentage of time to attain 90 % final emergence in all treatments were recorded 21 days after planting.

Disease control assays: *F.culmorum* was isolated from wheat plant with typically foot and root rot symptoms and then grown for 10 days on sterilized wheat seeds. Pots were infested with *F. culmorum* at the rate of 0.5 % of the soil dry weight. Number of emerged seedlings, damping-off plants and reduction percentages in damping-off in all treatments were counted daily up to 21 days. Damping-off seedlings were collected and assayed on PDA medium in order to verify that *F. culmorum* was responsible for the disease. Growth inhibition (I) percentages were calculated using the following equation:

I %= (growth in control – growth in treatment / growth in control) x 100

Statistical analysis: The data were calculated as mean ± S.D and analyzed using analysis of variance technique (ANOVA). Probability of 0.05 or less

was considered significant according to Duncan's multiple range (Steel and Torrie, 1980).

RESULTS

Antibiosis: The *in vitro* inhibitory effects of the antagonistic bacteria on *F. culmorum* are illustrated in Table (1). The results revealed that all the tested bacteria were significantly affected the growth of *F. culmorum*. *P. fluorescences* was the most inhibitory one followed by *B. subtilis* and *P. cepacia*. The corresponding growth inhibition were 70.4, 51.9 and 48.2 %, respectively.

Table (1): *In vitro* effect of the antagonistic bacteria on the growth of *Fusarium culmorum*

Antagonistic bacteria	Growth (cm)	Inhibition %
Control	2.8 ± 0.1^{d}	0.0
P. fluorescences	0.8 ± 0.0^{a}	70.4
P. cepacia	1.4 ± 0.1^{b}	48.2
B. subtilis	1.3 ± 0.1^{b}	51.9

Data are expressed as mean ± S.D (n=5).
Means followed by the same letter are not significantly different from each other according to Duncan's multiple range test ($p \leq 0.05$).

Seedling emergence assay: The effects of different treatments on the seedling emergence and T_{90} values of wheat and barley are presented in Table (2). No significant differences between treated and non-treated seeds of either wheat or barley regarding the percentages of seedling emergence were detected. On the other hand, significant differences were recorded in the T_{90} values between treated and non-treated seeds of either wheat or barley. Also, the data showed that PEG was the most potent to reduce T_{90} value in wheat seeds followed by PEG + benomyl ≅ PEG + *P. cepacia* ≅ PEG + *B. subtilis* ≅ PEG + *P. fluorescences* and then benomyl ≅ *P. cepacia* ≅ *B. subtilis* ≅ *P. fluorescences*. PEG reduced the T_{90} value in wheat seeds by 36.5 %, while the percentages of reduction were 25.4 % when PEG combined with either benomyl or the tested bacteria. When wheat seeds treated only with either benomyl or the tested bacteria, T_{90} values were

Table (2): Effect of different treatments on the seedling emergence and T_{90} of wheat and barley.

Treatment	Wheat			Barley		
	Seedling emergence	T_{90}	Reduction (%)	Seedling emergence	T_{90}	Reduction (%)
Non-treated	93.2±5.8^{a}	6.3±0.58^{a}	0.0	96.7±5.8^{a}	7.3±0.58^{a}	0.0
PEG	100±0.0^{a}	4.0±0.0^{c}	36.5	100±0.0^{a}	4.0±0.0^{d}	45.2
Benomyl	93.2±5.8^{a}	5.0±0.0^{b}	20.6	100±0.0^{a}	5.0±0.58^{bc}	31.5
P. cepacia	96.7±5.8^{a}	5.0±0.0^{b}	20.6	96.7±5.8^{a}	5.0±0.58^{bc}	31.5
B. subtilis	96.7±5.8^{a}	5.0±0.0^{b}	20.6	100±0.0^{a}	5.6±0.58^{b}	23.3
P. fluorescences	100± 0.0^{a}	5.0±0.0^{b}	20.6	100±0.0^{a}	5.6±0.58^{b}	23.3
PEG+Benomyl	96.7±5.8^{a}	4.7±0.58^{bc}	25.4	100±0.0^{a}	4.0±0.58^{d}	45.2
PEG+ *P. cepacia*	100±0.0^{a}	4.7±0.58^{bc}	25.4	100±0.0^{a}	4.7±0.58^{cd}	35.6
PEG+ *B. subtilis*	100±0.0^{a}	4.7±5.8^{bc}	25.4	100±0.0^{a}	4.7±0.58^{cd}	35.6
PEG+*P. fluorescences*	96.7±5.7^{a}	4.7±5.8^{bc}	25.4	100±0.0^{a}	4.7±0.58^{cd}	35.6

Data are expressed as mean ±S.D (n=5).
Means within the same column and followed by the same letter are not significantly different from each other according to Duncan's multiple range test ($p \leq 0.05$).
PEG = Polyethylene glycol

reduced by 20.6 %. On the other hand, when barley seeds treated with PEG alone or combined with benomyl, the T_{90} values were found to be equals (45.2 %), followed by the combination of PEG with any of the tested bacteria (35.6 %), benomyl ≅ *P. cepacia* (31.5 %) and then *B. subtilis* ≅ *P. fluoresences* (23.3 %).

Disease control assay: When soil was artificially infested with *F. culmorum*, all the tested treatments resulted in significant increase in the number of healthy seedling of either wheat or barley compared with non-treated seeds (Table 3). In addition, the data showed that PEG reduced damping-off by 31.1 and 52.4 % on wheat and barley, respectively. Damping-off was significantly suppressed on wheat and barley by all the binary mixtures with percentage reduction ranged from 62.5 - 81.2 and 38.1 - 61.9 % as compared with control, respectively. Moreover, highest reduction in damping-off was recorded with PEG + *P. cepacia* and PEG + *B. subtilis* for wheat and barley, respectively. Benomyl and the tested bacteria alone or combined with PEG had more potencies to reduce damping-off on wheat than barley.

DISCUSSION

Osmotic conditioning has been recommended as an effective method to obtain earlier and more uniform seedling emergence (Khan, *et al.*, 1983 and Bradford, 1986) as well as reduce seed susceptibility to damping-off by several pathogens for many crops (Rush, 1992 and Mathre *et al.*, 1995). The present data support these findings, where osmopriming wheat and barley seeds have markedly improved the germination rate of seeds and significantly reduced damping-off caused by *F. culmorum*. In addition, several studies proposed the use of the antagonistic bacteria to control damping-off caused by several pathogens (Mathre *et al.*, 1995 and Abdalla *et al.*, 1999). In this concern, the present data showed that the antagonistic bacteria inhibited the growth of *F. culmorum* by 48.2 - 70.4 % and reduced damping off by 43.7 - 49.9 on wheat. Antagonistic bacteria, which produce antibiotics, are known to reduce the severity of some plant diseases (Anuratha and Gnanamnickam, 1990 and Aoki *et al.*, 1990). *B. subtilis* strain was found to reduce disease severity of *F. graminearum* by 90 % (Schisler *et al.*, 2002), while *P. cepacia* reduce damping-off (Aoki *et al.*, 1990). Another important aspect is that, the combined seed treatments could lead to enhancement of disease control. In this respect, the present

Table (3): Effect of different treatments on the incidence of *Fusarium culmorum* damping-off of wheat and barley seeds.

Treatment	Wheat			Barley		
	Healthy seedling stand	Damping-off (%)	Reduction (%)	Healthy seedling stand	Damping-off (%)	Reduction (%)
Non-treated	46.7±5.8^{f}	53.3±5.8^{a}	0.0	30.0±0.0^{d}	70.0±0.0^{a}	0.0
PEG	63.3±5.8^{e}	36.7±5.8^{b}	31.1	66.7±5.8^{ab}	33.3±5.8^{cd}	52.4
Benomyl	86.7±5.8^{ab}	13.3±5.8^{ef}	75	50.0±0.0^{c}	50.0±0.0^{b}	28.6
P. cepacia	73.3±5.8^{cd}	26.7±5.8^{cd}	49.9	36.7±5.8^{d}	63.3±5.8^{a}	9.6
B. subtilis	73.3±5.8^{cd}	26.7±5.8^{cd}	49.9	36.7±5.8^{d}	63.3±5.8^{a}	9.6
P. fluorescences	70.0±0.0^{de}	30.0±0.0^{bc}	43.7	36.7±5.8^{d}	63.3±5.8^{a}	9.6
PEG+Benomyl	80.0±5.8^{bc}	20.0±0.0^{de}	62.5	66.7±5.8^{ab}	33.3±5.8c^{d}	52.4
PEG+ *P. cepacia*	90.0±0.0^{a}	10.0±0.0^{f}	81.2	56.7±5.8^{bc}	43.3±5.8^{bc}	38.1
PEG+ *B. subtilis*	80.0±0.0^{bc}	20.0±0.0^{de}	62.5	73.3±5.8^{a}	26.7±5.8^{d}	61.9
PEG+*P. fluorescences*	83.3±5.8^{ab}	16.7±0.0^{ef}	68.7	63.3±5.8^{ab}	36.7±5.8c^{d}	47.6

Data are expressed as mean ±S.D (n=5).
Means within the same column and followed by the same letter are not significantly different from each other according to Duncan's multiple range test ($p \leq 0.05$).

investigation showed that the highest reduction in damping-off was obtained when PEG was combined with either the antagonistic bacteria or benomyl on both wheat and barley. Superior control of *Rhizoctonia solani* on pea were obtained when *B. subtilis* combined with carboxin or thiram compared with either chemical or biological seeds treatment only (Hwang and Chakravarty, 1992). Also, damping-off on cotton caused by *Pythium ultimum* was reduced by 100 - fold when metalaxyl combined with *P. fluorescences* (Becker and Schwinn, 1993).

It could be concluded that the use of antagonistic bacteria with osmopriming wheat or barley seeds resulted in markedly reduced incidence of damping-off, in addition to its effect on seed germination rates, providing further reasons for exploring the use of antagonistic bacteria with osmopriming in integrated pest management programme (IPM).

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خفض الإصابة بمرض موت البادرات الفيوزاريومي في القمح والشعير بواسطة المعاملةالبيولوجية والكيميائية للبذور

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تم دراسة كفاءة معاملة بذور كل من القمح و الشعير بمحلول الإثيلين جليكول PEG، بكتريا التضاد الحيوي (*Pseudomonas fluorescences, P. cepacia* and *Bacillus subtilis*) و مبيد البنوميل أو معاملتها بخليط من مادة PEG مع البكتريا أو بنوميل على الإصابة بمرض موت البادرات المتسبب عن فطر *Fusarium culmorum* وذلك عند الزراعة تحت ظروف الصوبة الزجاجية. أوضحت نتائج التجارب المعملية أن بكتريا التضاد الحيوي لديها المقدرة على تثبيط نمو الفطر *F. culmorum* على البيئات المغذية. من ناحية أخرى أظهرت النتائج أنه لم يكن هناك أي تأثيراً معنوياً لأي من المعاملات على النسبةالمئوية للإنبات عند زراعة النباتات في التربة المعقمة، ولكن هناك زيادة معنوية في سرعة نمو البادرات في كل المعاملات عند مقارنتها بالبذور غير المعاملة حيث بلغت الزيادة في سرعة النمو بما يساوي ٢٠,٦- ٣٦,٥% في القمح و ٢٣,٣- ٤٥,٢% في الشعير. أما عند زراعة البذور في التربة المعداة صناعياً بالفطر *F culmorum* فوجد أن خلط مادة PEG مع البنوميل أو البكتريا أدى إلى خفض الإصابة بمرض موت البادرات بما يعادل ٦٢,٥- ٨١,٢ % في القمح و ٣٨,١- ٦١,٩ % في الشعير.

وتوصي الدراسة الحالية بإمكانية مكافحة مرض موت البادرات في القمح والشعير بواسطة معاملة البذور بخليط من مادة PEG و بكتريا التضاد الحيوي كوسيلة من وسائل برنامج المكافحة المتكاملة للآفات.