Comparative Efficacy of Some Safe Seed Treatments and Fungicide For Suppressing *Fusarium* Spp. on Some Crops

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ABSTRACT

Seeds of plants and types of soil may be carrying various types of pathogenic fungi, which play a significant role to reduce seed germination, increase the damping-off and wilt diseases caused by *Fusarium* spp. This pathogen identified as a major causal agent of seed or soil borne diseases. The ability of different seed treatments as save methods to suppress *Fusarium* spp., reduce the percentage of damping-off and increase the percentage of dry weight in plants were evaluated. Acetic acid (3%), ammonia solution (0.25%) and benomyl (3g/L) chemical agents and Halex (a mixture of three growth promoting nitrogen fixing rhizobacteria), *Bacillus polymyxa* and *Trichoderma harzianum* as antagonistic microorganisms were applied as a seed treatment. Results showed that all the tested treatments suppressed the growth of *Fusarium* spp. isolated from broad bean, pea, chickpea, barley and wheat seeds *in vitro*. *Trichoderma harzianum* was found to be the most effective one in reducing damping-off and increasing dry weight of pea, barley and wheat plants, followed by Halex, *Bacillus polymyxa* and benomyl fungicide. On the other hand, the use of acetic acid (3%) and ammonia solution (0.25%) affected the seed germination of the tested plants. It can be concluded that antagonistic microorganisms as seed treatments can be used to eradicate *Fusarium* spp. and reduce the incidence of damping-off caused by seed or soil borne *Fusarium* spp. in pea, barley and wheat plants without damaging seed germination.

INTRODUCTION

Seed health and freedom from seed borne diseases are constantly desired. Although fungicides are available for the control of such diseases, which may threat to human health and environment (Abbasi and Lazarovits, 2006). Pal *et al.*, (2001) found that there are no cultivars with complete resistance to soil borne fungal pathogen in some plants, and fungicides are not potent enough to protect the crop from infection by these pathogens and the development of biocontrol agents could be the best alternative to minimize the incidence of these diseases. Damping-off caused by soil borne pathogens, which affect the stand by reducing seedling establishment, removing feeder rootlets and thereby reducing productivity and killing plants weakened by other stresses (Hancock, 1985). An understanding of the origin and nature of seed borne fungi may be helpful in reducing losses and improving yields (Boughalleb and Mahjoub 2006). These early losses can be caused by soil or seed borne pathogens as *Fusarium, Pythium* and *Rhizoctonia* sp. (Pandey *et al.*, 2001, and Sheau *et al.*, 2006). *Fusarium* species are numerous and their damages vary according to the plant host, where they may be responsible of vascular wilt (*Fusarium oxysporum*) or root and collar rot of high number of plants (Champion, 1997). Many researchers have used non-fungicidal agents to control damping off in plants. Treatment of seeds with acetic acid (3%), ammonia (0.25%) and propionic acid (1%) for 60 minute were more effective to control seed borne fungi of pea *in vitro* (El-Sayed, 1992). Also, Du Toit and Hemandez, (2005) used chlorine or hot water as seed treatments to eradicate the *Cladosorium variabile, Stemphylium botryosum* and *Verticillium dahlias* which invade spinach without damaging germination. The plant growth promoting isolate of *Pseudomonas fluorescens* (EM 85) and two bacilli isolates (MR-11 and MRF) were found to have strongly antagonistic effect to *Fusarium moniliforms, F. graminearum* and *Macrophomina phaseolina*, causal agents of many diseases in maize (Pal *et al.*, 2001). Pandey *et al.*, (2001) found two strains of *Pseudomonas corrugate* which have capability to suppress disease severity caused by *Pythium ultimum, P. arrhenomanes* and *F. graminearum* in maize, and improve in various growth parameters (seed germination, plant height and fresh weight). Dandurand and Knudsen (1993) reported several biocontrol agents such as *Pseudomonas fluorescens, P. cepacia* and *Trichoderma harzianum* which antagonize seedling and root pathogens of pea. The objectives of this study were:(i) to isolate and identify *Fusarium* spp. from pea, chickpea, broad bean, barley and wheat seeds, (ii) to investigate the efficacy of a non-fungicidal chemicals and biocontrol agents as seed treatments to control Fusarium damping-off on pea, barley and wheat plants compared with a fungicides under growth room condition, and (iii) to determine the effects of these seed treatments for eradication of *Fusarium* spp. isolated from the tested seeds *in vitro*.

MATERIAL AND METHODS

Isolation of *Fusarium* spp. :

All seed used in this study were fungicide free. Seeds of wheat (*Triticum aestivum* L.) cv. Yecora rojo, barley (*Hordeum vulgare* L.) cv. Giza 167, pea (*Pisum sativum* L.) cv. early onward, chickpea (*Cicer arietinum* L.) cv. PV 60 and broad bean (*Vicia faba*) cv. Giza 2 were used to isolate *Fusarium* seed borne. Three replicates of 50 seeds from each seed were surface disinfected by dipping for 2 minutes in 1% sodium hypochlorite, washed with sterile distilled water, placed directly on Komada' medium, specific for Fusarium isolation (Komada, 1975), and then incubated at 25 °C. Plates were daily monitored and the developing organisms were purified and identified.

Fusarium isolates identification:

Cultures on PDA (Potato Dextrose Agar) were incubated at 25 °C with an alternate of light and darkness of 12 h during 4 day. The diameter and coloration of the colonies and the aspect of the mycelia were recorded. The microscopic features were examined after 7 days of incubation. Identification of Fusarium colonies was based on the morphological criteria proposed by Booth (1971).

In vitro assay for treatments :

Disk diffusion assay was used in these experiments as described by Abd El-Moity *et al.*, 1993. Filter paper disks (Whatman No.1, 2 cm diameter) were carefully immersed in the following treatments: 1) acetic acid (3%); 2) ammonia solution (0.25%); 3) benomyl, 50% w.p. at the rate of 3 g/L); 4) Halex (a mixture of three growth promoting nitrogen fixing rhizobacteria, namely *Azospirillium barasilense, Azotobacter chrococcum,* and *Klebsiella pneumoneae*); 5) antagonistic bacteria, *Bacillus polymyxa*; and 6) spore suspension of antagonistic fungal *Trichoderma harzianum*. Then placed on a central PDA plate after inoculated with spore suspension of *Fusarium* spp. The inoculated plates were incubated at 25 °C, and the fungal growth of *Fusarium* spp. was followed daily for 7 days. The suppressive affects were visually assessed by comparing the treatments with their control (inoculated with *Fusarium* spp. only). The reactions were scored as (-) no suppression (no inhibition zone), (+) minimal, (++)

moderate, and (+++) high suppression (clear inhibition zone), according to the scale of Farfour (1995). Each treatment was replicated four times.

Pre-sowing seed treatments

Seeds of pea, barley and wheat were treated only with either of the six treatments mentioned above (acetic acid and ammonia solution for 60 minutes). *F. oxysporum* f. sp. *pisi* which isolated from pea seeds and *F. graminearum* which isolated from barley and wheat seeds were grown for 10 days on sterilized barley seeds as an inoculum. Pots 15 cm diameter filled with sterilized soil and mixed by the inoculum at rate of 5g inoculum/pot. The inoculated pots were kept for three days to secure establishment of the inoculated fungus, then sown with the tested treatment seeds (10 seeds/pot). In check, the non treated seeds were sown in infested soil with *Fusarium* spp. Four replicates were used for each treatment.

Seedling emergence assay :

Number of emerged seedling, damping-off plants, reduction percentages in damping-off and disease severity in all treatments were counted up to 21 days. Also, dry weight/10 plants were recorded and the increase in percentage of dry weight as a results of variance treatments were calculated.

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 Torri, 1980).

RESULTS AND DISCUSSION

The incubation of seeds on Komada' medium permit to isolate the seed borne Fusarium located at the seed surface . The results indicated that *F. oxysporum* f. sp. *pisi* and *F. oxysporum* f. sp. *ciceris* were the most frequent for the majority of pea and chickpea seeds respectively. *F. oxysporum* was the most majority of broad bean seeds, while *F.*

graminearum was the most majority of barley and wheat seeds. The results of *in vitro* suppressing effect of the chemicals and antagonistic microorganisms on *Fusarium* spp. isolated from seeds are illustrated in Table 1. All the tested treatments suppressed the growth of *Fusarium* spp. compared with control treatment. *Trichoderma harzianum* demonstrated the highest suppressing effect on the growth of all the tested *Fusarium* spp. followed by Halex (mixture of rhizobacteria) and then benomyl fungicide. On the other hand, the other treatments tested were ranged from moderate to minimal suppressing effect. Similar effects were reported by many investigators. Dandurand and Knuden, (1993) found that the application of *T. harzianum* to pea seeds reduced root rot diseases and increased plant top weight compared with non coated seeds. Ibrahim (2001) found that Halex had potent suppressing effect on the growth of *F. oxysporum* f. sp. *ciceris*. Also, Ali *et al*., (1982) used benomyl 50 w.p. (3g/L) as a seed treatment for controlling seed borne fungi of pea. Richardson, (1990) mentioned that seed treatment of chickpea with 30% benomyl + 30% thiram eradicated seed borne pathogens. Also, the used of some chemicals were reduced the incidence of a number of seed bone fungi. The incidence of *Cladosporium variabile* was reduced from 49.0 to o.3% after chlorine treatment for 10 minutes (Du Toit and Derie 2005). While, phosphonate seed treatment provided more than 80% control of *Pythium* damping-off of cucumber seedling (Abbasi and Lazarovis 2006).

In the present investigation, *Fusarium* spp. reduced the yield of the tested plants (pea, barley and wheat), by increasing disease severity, damping-off and decreasing dry weight. Table 2 and 3 showed that there were significant differences in disease severity among seed treatments and non-treated seeds. The data in Table 2 showed that *Trichoderma harzianum* and *Bacillus polymyxa* were the most effective treatments in reducing disease severity of pea from (4.66 to 1.0). Ammonia solution and benomyl were less effective in reducing disease severity compared with the other seed treatments, on the other hand, non-treated seeds (control) gave the highest disease severity (4.66). Also, data in Table 3 showed that *Trichoderma harzianum* and *Bacillus polymyxa* were the most effective treatments in reducing disease severity of barley from (4.0 to 0.8) and in wheat from (5.0 to 0.8). Similar results were reported by Khot *et al*., (1996) who found that rhizobacteria reduced the wilt of chickpea caused by *F.*

oxysporum f. sp. *ciceris* under field condition. Also, Ibrahim, (2001) found three strains of rhizobacteria, which reduced disease severity from 4.0 to 1.4. Pandey *et al.* (2001) found two strains of *Pseudomonas corrugate,* which decreased disease severity as well as growth promotion when treated grains of maize against seed borne and soil borne diseases.

The effect of different seed treatments to reduce damping-off and increase dry weight in pea, barley and wheat are presented in Tables 2 and 3. There were a significant differences in the damping-off incidence in the tested plants when seeds coated with *Trichoderma harzianum*, Halex , *Bacillus polymyxa*, benomyl fungicide and seeds coated with either acetic acid or ammonia solution. *T. harzianum* proved to have strong act in reducing the incidence of damping-off in pea, barley and wheat (10.23, 6.9, and 16.7% respectively), followed by Halex, *B. polymyxa*, and benomyl. The corresponding percentages of reduction were 70.6, 29.5 and 17.5% in pea; 69.2, 61.5 and 61.5% in barley and 58.8, 52.9 and 58.8% in wheat respectively. There were no significant differences between non-treated seeds and acetic acid, ammonia solution in the damping-off incidence of pea, barley and wheat. Johnson *et al.*, (1998) found that the application of *Pseudomonas* and *Bacillus* spp. controlled seedling blight, root rots and stalk rots of cereal borne diseases. Salchpour *et al.*, (2005) mentioned that *T. viride, T. harzianum* increased plant height, fresh and dry weight of roots and shoot of wheat. De and Chandhary (1996) found significant decrease in chickpea wilt and increase in dry weight when seed were coated with growth promoting rhizobacteria.

The highest dry weight of the tested plants was found in the seed treated with Halex and *Trichoderma harzianum.* No significant differences were found in dry weight of the pea and wheat plants when seeds coated with acetic acid 3% or ammonia solution 0.25%, compared with control. This result could be due to the phytotoxicity of these chemicals. Abbasi and Lazarovits, (2006) mentioned that the tests for potential phytotoxicity in the greenhouse showed that radish and bok choy germination was reduced by phosphonate treatment but corn, cucumber, soybean, sugar beet and tomato were not affected. Also, Neeti and Karan (1985) reported that 2% and 5% ammonia eliminated the mycoflora of pea seed, but retarded seed germination. Also, the data show that *T. harzianum* and Halex were more effective to increase the dry weight of either pea or wheat, while *T. harzianum* and benomyl had the highest effect to increase dry weight in barley. It can be concluded that antagonistic microorganisms as seed treatments can be used to eradicate *Fusarium* spp. and reduce the incidence of damping-off which caused by seed or soil borne *Fusarium* spp. in pea, barley and wheat plants without damaging seed germination.

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Table 3 : Effect of different treatments on the incidence of *F. graminearum* damping-off barley and wheat seeds.

Treatments	Barley					Wheat				
	Disease severity (0- 5)	Damping -off (%)	Reduction of damping-off (%)	*Dry weight (g)	Increase over the control (%)	Disease severity (0- 5)	Damping -off (%)	Reduction of damping-off (%)	Dry weight (g)	Increase over the control (%)
Acetic acid 3%	1.33ab	36.7bc	15.4	1.2ab	-9.4	1.7ab	50..0 b	11.8	1.0 a	- 3.6
Ammonia solu. 0.25%	1.33ab	33.3bc	23.1	1.0a	-21.9	1.3ab	30.0 a	46.9	1.1ab	0.0
Benomyl (3g/L)	1.0ab	16.7ab	61.5	1.5d	21.9	1.7ab	23.3 a	58.8	1.3 b	11.6
Halex (rhizo-bacteria)	1.0ab	13.3ab	69.2	1.4cd	9.4	2.0b	23.3 a	58.8	1.6 c	41.1
Bacillus polymyxa	0.8 a	16.7ab	61.5	1.4cd	7.0	0.8a	26.7 a	52.9	1.3 b	12.5
Trichoderma harzianum	0.8 a	6.9 a	84.1	1.5d	17.9	0.8a	16.7 a	70.6	1.6 c	36.6
Control (non-treated)	4.0 c	43.3 c	0.0	1.3bc	0.0	5.0c	56.7 b	0.0	1.1ab	0.0

* Dry weight/ 10 plants after 3 weeks from sowing.
- Means within the same column followed by a common letter are not significantly different according to Duncan's multiple range test (p≤ 0.05).

الملخص العربي

تقييم كفاءة بعض الطرق الآمنة لتقليل ضرر الأمراض التي تصيب بذور بعض المحاصيل

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يوجد العديد من الفطريات الممرضة في بذور النباتات أو التربة وهذه الفطريات تلعب دور هام في خفض نسبة إنبات هذه البذور وزيادة نسبة موت البادرات وذبول النباتات . وتعتبر أنواع من فطر الفيوزاريوم من أهم الفطريات التي يمكن أن تتواجد في بذور النباتات أو التربة الملوثة. وقد تم اختبار مقدرة عدد من المعاملات لتقيم كفاءتها كمعاملة لبذور بعض النباتات حيث تم استخدام حامض الخليك (٣%) ومحلول الامونيا (٠,٢٥%) ومبيد البنوميل (٣جم/لتر) كمواد كيميائية وأيضا استخدمت بعض الأنواع من البكتيريا والفطريات كعوامل مضادة لنمو الفطريات الممرضة وقد اتضح من النتائج أن جميع هذه المعاملات كان لها تأثير على إيقاف الأنواع المختلفة من فطر الفيوزاريوم الذي تم عزلة من بذور نباتات الفول البلدي والبسلة والحمص والقمح والشعير معمليا, وكانت معاملة البذور بالفطر *Trichoderma harzianum* أكثر المعاملات تأثيرا في تقليل موت البادرات وزيادة نمو نباتات البسلة والقمح والشعير, يليه معاملة البذور بمخلوط هاليكس (مخلوط من ثلاث عزلات بكتيرية جذرية مثبتة للنيتروجين), يليه معاملة البذور بالبكتيريا *Bacillus polymyxa* ثم معاملة البذور بالمبيد الفطري البنوميل. وعلى الجانب الأخر وجد أن معاملة البذور بحمض الخليك بتركيز (٣%) وكذلك بمحلول الامونيا بتركيز (٠,٢٥%) قد اثر على نسبة إنبات كل من بذور البسلة والقمح والشعير . وتوصي الدراسة الحالية بإمكانية استخدام بعض من فطريات وبكتيريا التضاد الحيوي بدلا من المبيدات أو المواد الكيميائية التي يمكن أن يكون لها تأثير سلبي على نسبة إنبات البذور.