Plant extract control of the fungi associated with different Egyptian wheat cultivars grains

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Abstract: Grain samples of 14 Egyptian wheat cultivars were tested for seed-borne fungi. The deep freezing method was used. Five seed-borne fungi *viz*., *Aspergillus flavus, A. niger, Curvularia lunata, Fusarium moniliforme* and *Penicillium chrysogenum* were isolated from the wheat cultivars *viz*., Bani Suef 4, Bani Suef 5, Gemmiza 7, Gemmiza 9, Gemmiza 10, Giza 168, Misr 1, Misr 2, Sakha 93, Sakha 94, Shandaweel 1, Sids 1, Sids 2 and Sids 3. *A. flavus, A. niger* and *F. moniliforme* were the most prevalent fungal species. Their incidence ranged from 21.0–53.5%, 16.0–37.5%, and 12.0–31.0%, respectively. The antifungal potential of water extracts from aerial parts of five wild medicinal plants (*Asclepias sinaica, Farsetia aegyptia, Hypericum sinaicum, Phagnalon sinaicum*, and *Salvia aegyptiaca*) were collected from the Sinai Peninsula, Egypt. The antifungal potential of water extracts from the aerial parts of these five plants were tested in the laboratory against the dominant fungi isolated from the wheat cultivars. All the aqueous plant extracts significantly ($p \leq 0.05$) reduced the incidence of the tested seed-borne fungi. But the extract of *Asclepias sinaica* exhibited the most antifungal activity on tested fungi at all concentrations used when compared with other plant extracts. Maximum infested grain germination was observed in Giza 168 and minimum in Bani Suef 5. Treating grains with plant extract of *A. sinaica* (10%) enhanced the percentage of grain germination of all cultivars in both laboratory and pot experiments. Maximum root and shoot length of seedlings was recorded in Bani Suef 4 during fungal infestation or treatment by plant extract. For one hour before sowing or storage, the aqueous extract of *A. sinaica* can be used to treat wheat grains, to reduce the fungal incidence. Aqueous extracts of the aerial parts of selected medicinal plants, particularly *A. sinaica*, are promising for protecting Egyptian wheat grain cultivars against major seed-borne fungi. The aqueous extracts are expected to improve crops.

Key words: antifungal activity, Egypt, plant extracts, seed-borne fungi, wheat cultivars

Introduction

Wheat (*Triticum aestivum* L.) has served as a staple food source for mankind since times immemorial (Satish *et al*. 2010). It is considered as a major agricultural commodity and dietary component across the world and it is one of the most important cereals in view of nutritional value (Abd El-Baky 2009).

In Egypt, wheat is considered a first place strategic food crop (Ouda 2006). Because of its importance in the Egyptian diet, wheat is a strategic commodity in the country. It provides more than one-third of the daily caloric intake of Egyptian consumers and 45% of the total daily protein consumption by Egyptians (Ali and Adams 1996).

Infested wheat grains are the carry pathogens which can have long-distance dissemination. The major impact of seed-borne diseases in wheat is not only yield reduction but also the deteriorate of marketable grain quality. Early detection of pathogens is a crucial step in the diagnosis as well as for the management programs in wheat (Majumder *et al*. 2013).

Seed-borne fungi of wheat were reported by many authors in the world. These fungi included *Acremonium* spp., *Alternaria* spp., *Aspergillus* spp., *Bipolaris* spp., *Drechslera* spp. *Cladosporium* sp., *Curvularia* spp., *Fusarium* spp., *Mucor* spp., *Penicillium* spp., *Rhizopus* spp., *Stemphylium* spp. and *Trichoderma* spp. (Bhatti and Bhutta 2002; Rajput *et al*. 2005; Fakhrunnisa *et al*. 2006; Singh *et al*. 2011; Hajihasani *et al.* 2012; Majumder *et al*. 2013; Pathak and Zaidi 2013; Zrari 2013).

These fungi are associated with a heavy loss of grains rendering the grain unfit for human consumption. The fungi even produce mycotoxins affecting the total nutritive value of grains (Galvano *et al*. 2001). Many seed- borne fungi which cause severe damage to stored food commodities, were generally managed by synthetic chemicals. The chemicals were considered both efficient and effective. The continuous use of these synthetic fungicides, however, can cause non-biodegradability. Synthetic fungicides are known to have residual toxicity which causes pollution (Pimentel and Levitan 1986). Pesticide pollution of soil and water bodies is well documented (Nostro *et al.* 2000). Hence, in recent times, the application of plant metabolites for plant disease management has become an important viable component of Integrated Pest Man-

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agement (IPM). Plant metabolites are eco-friendly where botanicals play an important role (Sahayaraj *et al*. 2009).

In recent years much attention has been given to non-chemical systems for seed treatment to protect seeds against many plant pathogens (Begum *et al*. 2009; Szopińska *et al*. 2010; Perelló *et al*. 2013). Antifungal activity of different plant extracts have been reported earlier by several investigators against a number of plant pathogens (Hassan *et al*. 2005; Yang and Clausen 2007). Plant extracts have played a significant role in the inhibition of seed-borne pathogens and in the improvement of seed quality and emergence of seed embryo (Nwachukwu and Umechuruba 2001).

A review of the literature indicated that, several studies have been conducted in Egypt to survey seed-borne fungi of various plant seeds including cereal grains. However, few studies were conducted in Egypt with grains of wheat varieties such as Assawah and El-Arosi (1960), Moubasher *et al*. (1972), El-Kady *et al.* (1982) and Mazen *et al*. (1984).

Our present work was performed to identify the fungi on stored grains of different Egyptian wheat cultivars and to determine their effect on grain germination, plant emergence and vigor. Moreover, the capability of various plants extracts to control of isolated fungi was evaluated.

Materials and Methods

Grain samples of 14 Egyptian cultivars of *T. aestivum* were kindly provided by the Ministry of Agriculture, Egypt (Table 1). The provided samples were used for the isolation and detection of seed-borne fungi. The Deep Freezing Method (Limonard 1968) was recommended for the isolation of the fungi. Isolation was made from 200 seeds of each cultivar under aseptic conditions. Twenty five seeds per plate were placed on three layers of moistened blotters. The seeds were incubated for one day at 22°C followed by 24 h of freezing at –20°C. The plates were then placed for 4–5 days at 22±1°C. After incubation the growth characters as well as percentage of infection were recorded. The isolated *Fusarium* sp. was maintained on Spezieller Nahrastof Agar (SNA) medium (Hashmi and Thrane 1990) whereas other fungi were maintained on Potato Dextrose Agar (PDA) medium. The fungi were identified after reference to Booth (1971), Barnett and Hunter (1972), and Nelson *et al.* (1983).

Plant materials and extract preparation

Five wild medicinal plants were collected from different locations of the Sinai Peninsula, Egypt (Table 2). The aerial parts of these plants were extracted using water. For extraction, 100 g of each air-dried medicinal plant material were separately added to 1,000 ml of distilled water (1 : 10 w/v). Extraction then took place under cold conditions for 24 h (Rivillas-Acevedo and Soriano-Garcia 2007). Plant extracts were filtered through two pieces of cheese cloths. Aqueous extract at a concentration of 10% was used as an original concentration in the antifungal activity experiments.

Effect of plant extracts on linear mycelial growth

The seed-borne fungi (*Aspergillus flavus* Link ex Fries, *A. niger* van Tieghem, and *Fusarium moniliforme* Scheldon) were used for antifungal activity of water extracts from different selected plants because these fungi were the most prevalent species isolated from different wheat cultivars. PDA medium was used in the study. To every 15 ml of sterile PDA medium in Petri dishes, 5 ml of each of the aqueous extract concentrations (2.5%, 5%, 7.5%, and 10%) from each plant were added. The solution in each Petri dish was gently swirled and allowed to solidify. The extract-amended medium in the Petri dishes were inoculated each alone at the center with 5 mm inoculum-disc of each test fungus and incubated at 25±2°C for 14 days. The medium with the inoculum disc but without any extract served as the control.

Effect of plant extracts on grains germination

Non-treated and plant extract-treated grains of wheat cultivars were used for laboratory and pot experiments. An efficacy of the water extract of *Asclepias sinaica* (Boiss.) Muschl. (the most effective for antifungal activity) was used. The grains were separately soaked in water extracts for one hour and then plated on moist blotters as well as in a sterilised soil mix in pots. The untreated grains were soaked in distilled water for one hour and plated on moist blotters and a sterilised and acted as the control. A total of 200 grains were soaked per extract Ten grains were plated on a blotter per Petri dish. The extract treated and untreated grains were incubated at 20±2°C for seven days. Grains plated on a blotter were examined for fungal growth and percentage grain germination, after 7 days of incubation. The percentage of seedling emergence was recorded in grains sown in the sterilised soil mix after 14 days. For the soil method, 20 grains were planted per pot equidistantly, at a depth of 2.0 cm and five pots were used per extract treatments. All tests were replicated eight times. The germination was counted when the first leaf of the seedling reached a length of 4.0 cm. The root and shoot lengths of germinated seedlings were also recorded.

Statistical analysis

Data were analysed using a Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks, combined with Dunn's method for pairwise comparison.

Results and Discussion

A total of five fungal species *viz*., *A. flavus, A. niger, F. moniliforme, Curvularia lunata* R.R. Nelson & Haasis, and *Penicillium chrysogenum* Thom were isolated from the grains of 14 wheat cultivars (Table 1). The frequency of associated fungi of wheat grains was influenced by the tested cultivars tested. The highest frequency of grain fungi was observed on the wheat cultivar Bani Suef 4 followed by Gemmiza 7, and Giza 168. The lowest frequency was recorded for the seeds of wheat cultivar Sakha 94, followed by Misr 2 and Gemmiza 10. Of the isolated fungi, *A. flavus* was the most pre-

Table 1. The occurrence of fungi *A. flavus, A. niger, C. lunata, F. moniliforme,* and *P. chrysogenum* on the grains of 14 wheat cultivars

Wheat cultivars	Fungi	Percentage of infested seeds	Wheat cultivars	Fungi	Percentage of infested seeds
Bani Suef 4	*A. flavus*	52.5	Misr 2	*A. flavus*	37.5
	A. niger	25.0		*A. niger*	18.5
	C. lunata	16.0		*C. lunata*	–
	F. moniliforme	29.5		*F. moniliforme*	12.0
	P. chrysogenum	10.5		*P. chrysogenum*	2.5
Bani Suef 5	*A. flavus*	41.0	Sakha 93	*A. flavus*	24.5
	A. niger	21.0		*A. niger*	27.0
	C. lunata	15.0		*C. lunata*	–
	F. moniliforme	31.0		*F. moniliforme*	17.5
	P. chrysogenum	0.45		*P. chrysogenum*	0.45
Gemmiza 7	*A. flavus*	48.0	Sakha 94	*A. flavus*	21.0
	A. niger	32.0		*A. niger*	28.0
	C. lunata	–		*C. lunata*	4.0
	F. moniliforme	27.5		*F. moniliforme*	17.5
	P. chrysogenum	6.0		*P. chrysogenum*	–
Gemmiza 9	*A. flavus*	39.0	Shandaweel 1	*A. flavus*	45.0
	A. niger	30.0		*A. niger*	27.5
	C. lunata	5.0		*C. lunata*	–
	F. moniliforme	29.0		*F. moniliforme*	29.5
	P. chrysogenum	–		*P. chrysogenum*	–
Gemmiza 10	*A. flavus*	22.5	Sids 1	*A. flavus*	37.5
	A. niger	19.0		*A. niger*	21.5
	C. lunata	7.5		*C. lunata*	–
	F. moniliforme	21.0		*F. moniliforme*	14.0
	P. chrysogenum	–		*P. chrysogenum*	–
Giza 168	*A. flavus*	53.5	Sids 2	*A. flavus*	27.5
	A. niger	34.5		*A. niger*	16.0
	C. lunata	–		*C. lunata*	4.5
	F. moniliforme	23.5		*F. moniliforme*	14.0
	P. chrysogenum	–		*P. chrysogenum*	–
Misr 1	*A. flavus*	38.0	Sids 3	*A. flavus*	41.5
	A. niger	16.0		*A. niger*	37.5
	C. lunata	4.0		*C. lunata*	–
	F. moniliforme	14.0		*F. moniliforme*	14.5
	P. chrysogenum	0.40		*P. chrysogenum*	3.5

Table 2. Wild medicinal plants used in the present study, collected from different locations of the Sinai Peninsula, Egypt, and their bioactive compounds of the plants

Scientific name	Family	Bioactive compounds	References
Asclepias sinaica (Boiss.) Muschl.	Asclepiadaceae	imidacloprid, levamisole, pyrantel	Semida *et al*. 2006
Farsetia aegyptia Turra	Brassicaceae	flavonoid kaempferol-7,8-diglucoside kaempferol triosole	Atta *et al*. 2013
Hypericum sinaicum Hochst ex Boiss	Hypericaceae	hypercin, protohypericin, pseudohypericin, hyperforin and proto pseudohypericin	Alali *et al*. 2009
Phagnalon sinaicum Bornm. & Kneucker	Asteraceae	thymol, dammadienyl acetate, squalene, phytol	El-Dahmy *et al*. 1994
Salvia aegyptiaca L.	Lamiaceae	6-methyleryptoacetalide, aegyptinones A and B, 6-methyl-epicryptoacetalide, 6-methylcryptotanshinone	Al-Yousuf *et al*. 2002

dominant fungus (21.0–53.0%) followed by *A. niger* (16.0–37.5%), *F. moniliforme* (12.0–31.0%), *C. lunata* (4.0–16.0%) and *P. chrysogenum* (2.5–10.5%) (Table 1). *A. flavus, A. niger* and *F. moniliforme* were isolated from all cultivars, while *C. lunata* and *P. chrysogenum* from seven wheat cultivars only.

Assawah and El-Arosi (1960), El-Kady *et al*. (1982) and Mazen *et al*. (1984) observed that *Aspergillus*, *Fusarium*, *Penicillium*, and *Rhizopus* were the most common genera in wheat grains in Egypt. Many reports indicated the occurrence of many fungal genera in different wheat cultivars in other countries of the world. Included are *Alternaria, Helminthosporium, Fusarium, Curvularia, Stemphylium, Cladosporium, Aspergillus, Penicillium, Microdochium, Bipolari, Mucor, Botrytis, Rhizopus, Aureobasidium, Dreschslera,* and *Rhizoctonia* (Bhutta and Hussain 1999; Rajput *et al*. 2005; Hassan *et al*. 2005; Javaid and Anjum 2006; Fakhrunnisa *et al*. 2006; Singh *et al*. 2011; Hajihasani *et al*. 2012; Hussain *et al*. 2013; Jalal and Zrari 2013; Majumder *et al*. 2013; Pathak and Zaidi 2013). Ghosh and Nandi (1986) reported that several species of *Aspergillus* and *Penicilium* are responsible for the deterioration of wheat grains during storage. Pre and post-harvest biodeterioration and spoilage of grains due to infestation by microorganisms may cause losses of up to 100% (Satish *et al*. 2010). The species of *Aspergillus* has been reported to cause a significant loss in the seed quality and nutritional value of grains (Koirala *et al*. 2005).

Effect of water extracts on the linear mycelial growth of tested fungi (*A. flavus, A. niger*, and *F. moniliforme*) is shown in table 3. Results indicated that all treatments were positively effective in reducing the mycelial growth of all fungi tested, compared to the control. The reduction of mycelial growth showed an increase when the concentration of the extracts was increased from 2.5 to 10.0%. The highest inhibition of mycelial growth was indicated by the water extracts of *A. sinaica* followed by *H. sinaicum, P. sinaicum, F. aegyptia*, and *S. aegyptiaca* for all the tested fungi (Table 3). Many reports have shown the importance of plant extracts for controlling seed-borne fungi of wheat grains (Hassan *et al*. 2005; Shafique *et al*. 2007; Perelló *et al*. 2013). The inhibitory effect of the tested extracts might be due to natural bioactive materials present in these extracts (Atta *et al*. 2013). Baka (2014) reported that aqueous extract of *A. sinaica* gave the highest antifungal activity against the phytopathogen, *Phytophthora infestans*. Such activity may be due to the presence of milky latex containing bitter and toxic alkaloids (Elbanna 2007). Many authors believe that latex produced from the members of Asclepiadaceae contains many bioactive components which can be used as antimicrobials (Neenah 2013).

Germination of wheat grains was low in pots (11.9–20.3%) as compared to laboratory tests (20.8–30.2%) (Table 4). In both experiments, the maximum germination was recorded in Sakha 94, followed by Misr 2, and Gemmiza 10, whereas, Bani Suef 4, followed by Gemmiza 7, and Giza 168, showed minimum germination. It was evident from these results that high infestation led to minimum germination since the highest frequency of fungi was recovered from the variety Bani Suef 4. It was reported by Oppitz and Hoesser (1979) that seed borne fungi of wheat not only reduced the germination but also affected seedling vigor resulting in low yield. Sulaiman and Husain (1984) observed that *A. flavus* reduced 90% of wheat grain germination of as compared to healthy grains. The wheat grains treated with the aqueous extract of *A. sinaica* enhanced the germination in pots (19.8–75.8%) and in the laboratory (35.5–80.5%).

The maximum root and shoot lengths were obtained in Bani Suef 4 (4.2 and 9.1 cm) followed by Bani Suef 5 (3.8 and 8.9 cm) and Gemmiza 7 (3.6 and 8.7 cm), respectively (Table 5). Treatment of grains with the aqueous *A. sinaica* extract also increased also seedling vigor. These results are in agreement with those obtained by many authors (Hassan *et al*. 2005; Shafique *et al*. 2007; Perelló *et al*. 2013) who reported that plant extracts can be used to enhance the germination of wheat grains and seedling vigor. The ability of the extracts to increase grain germination and seedling emergence could be attributed to the suppression of the incidence of the seed borne fungi that could have killed the embryo of the grains.

Table 3. Inhibition of mycelial growth of tested fungi by water plant extracts at different concentrations

Scientific name	[a]Mean ±SE[b] of inhibition of linear mycelial growth [mm]											
	2.5%			5%			7.5%			10%		
	AF[c]	AN[d]	FM[e]	AF	AN	FM	AF	AN	FM	AF	AN	FM
A. sinaica	09.2 ±0.4	10.2 ±0.3	10.6 ±0.5	08.5 ±0.3	09.7 ±0.3	09.1 ±0.5	06.3 ±0.3	08.4 ±0.3	07.2 ±0.6	05.3 ±0.4	06.5 ±0.2	05.2 ±0.1
F. aegyptia	38.2 ±0.4	37.2 ±0.3	37.6 ±0.5	35.5 ±0.3	34.7 ±0.3	35.1 ±0.5	32.3 ±0.3	33.4 ±0.3	32.2 ±0.6	29.3 ±0.4	30.5 ±0.2	30.2 ±0.1
H. sinaicum	20.4 ±0.4	19.2 ±0.3	20.6 ±0.5	18.5 ±0.3	17.7 ±0.3	18.1 ±0.5	16.3 ±0.3	16.4 ±0.3	17.2 ±0.6	14.3 ±0.4	14.5 ±0.2	15.2 ±0.1
P. sinaicum	25.2 ±0.4	24.2 ±0.3	24.6 ±0.5	23.5 ±0.3	23.7 ±0.3	22.1 ±0.5	21.3 ±0.3	22.4 ±0.3	20.2 ±0.6	19.3 ±0.4	20.5 ±0.2	18.2 ±0.1
S. aegyptiaca	40.1 ±0.4	42.2 ±0.3	44.6 ±0.5	37.5 ±0.3	40.7 ±0.3	45.1 ±0.5	35.3 ±0.3	38.4 ±0.3	42.2 ±0.6	32.3 ±0.4	35.5 ±0.2	40.2 ±0.1
The control	46.3	48.2	47.5	46.3	48.2	47.5	46.3	48.2	47.5	46.3	48.2	47.5

[a] mean of five replicates; [b] standard error of mean; [c] *A. flavus*; [d] *A. niger*; [e] *F. moniliforme*

Table 4. The effect of *A. sinaica* extract (at 10% concentration) on seeds germination and plant emergence of 14 wheat cultivars

Cultivars	Non-treated		Plant extract-treated	
	grain germination [%]	plant emergence [%]	grain germination [%]	plant emergence [%]
Bani Suef 4	20.8	12.3	38.2	19.8
Bani Suef 5	27.2	17.6	45.3	32.8
Gemmiza 7	22.8	11.9	38.5	28.1
Gemmiza 9	27.5	18.3	35.5	24.8
Gemmiza 10	28.2	20.0	48.4	41.2
Giza 168	23.0	13.2	42.6	25.2
Misr 1	25.9	14.3	45.4	28.1
Misr 2	29.1	20.1	55.7	40.6
Sakha 93	25.0	16.8	48.9	35.3
Sakha 94	30.2	20.3	80.5	75.8
Shandaweel 1	23.6	14.5	40.7	26.9
Sids 1	23.2	13.9	38.4	27.2
Sids 2	25.5	15.1	40.2	32.7
Sids 3	27.2	17.4	44.2	25.6

Table 5. The effect of *A. sinaica* extract (at 10% concentration) on root and shoot length of 14 wheat cultivars seedlings

Cultivars	Non-treated		Plant extract-treated	
	root length [cm]	shoot length [cm]	root length [cm]	shoot length [cm]
Bani Suef 4	4.2 a	9.1 a	6.8 a	14.9 a
Bani Suef 5	3.8 b	8.9 a	6.5 a	12.7 b
Gemmiza 7	3.6 bc	8.7 b	5.9 b	12.5 b
Gemmiza 9	3.6 bc	7.8 bc	5.9 bc	12.1 b
Gemmiza 10	3.5 bc	7.2 bc	4.9 cd	11.7 b
Giza 168	3.4 bc	7.1 bc	4.7 cd	11.3 c
Misr 1	3.2 cd	7.0 c	4.5 cd	10.9 c
Misr 2	3.1 de	6.2 c	4.0 adf	10.3 c
Sakha 93	3.0 de	6.2 c	3.8 def	10.1 c
Sakha 94	2.9 ef	3.2 d	3.7 def	7.6 d
Shandaweel 1	2.6 ef	3.1 d	3.6 ef	6.5 e
Sids 1	2.5 ef	2.8 de	2.9 ef	5.9 e
Sids 2	2.5 f	2.6 de	2.8 ef	5.5 f
Sids 3	2.4 f	2.1 f	2.8 f	4.9 f

Different letters indicated significant differences ($p < 0.05$)

In conclusion, from the present investigation, it is clear that seed-borne fungi are a threat to the health of wheat grains. Due attention should be paid to the health status of wheat grains prior to sowing. Grain treatment by plant extracts, particularly medicinal plants, may be a quick technique. Such a technique reduces or eliminates seed-borne fungi and also increase grain germination and seedling vigor. Grain treatment with plant extracts is an eco-friendly measure for controlling seed-borne pathogens. Grain treatment with aqueous extract of *A. sinaica* for one hour before sowing or storage, can be used to treat wheat grains to reduce fungal incidence.

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