



Efficacy of *Inula viscosa* extracts on seed germination and fungal infection of three varieties of wheat (*Triticum aestivum* L.)

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Abstract

The aim of the present investigation was to look for bioactive substances which may decrease the use of chemical pesticides to control seed borne fungi and thus protect the environment from an excessive chemicalization. In this study three concentrations (500, 1000 and 1500 ppm) of *Inula viscosa* extracts were tested for their efficacy in controlling seed-borne fungi associated with wheat (*Triticum aestivum* L.) under laboratory conditions. Obtained results revealed that both extracts (methanolic and aqueous) were active in reducing seed-borne infection in a concentration dependent manner. Seed treatment with 1500ppm of methanolic extract was the most effective, where it caused a highly inhibition of fungal seeds infection (69.25 to 83.86%). The plant extracts tested have also positive and a significant impact on seed germination in comparing with the untreated seed. The easy seed treatment process and the accessibility of the plant used in the present study could lead to high adoption of the use of the plant extracts as seed treatments to prevent bio deterioration of seeds in an eco-friendly way.

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Introduction

Wheat is considered as one of the most important cereal crops in the world that provides on average 21% of calories and 20% of protein to more than 4.5 billion people in 94 developing countries (Braun *et al.*, 2010). Algeria is a major consumer of wheat that is a basic staple food for very large segments of the population (Riba *et al.*, 2008).

Fungal deterioration of stored seeds and grains is a major problem in Algeria. Harvested grains are colonized by various fungal species and during storage grains undergo quantitative and qualitative losses due to mycotoxin production (Riba *et al.*, 2010). Seedborne fungi are responsible for both pre and post-emergence death of grains, affect seedling vigor, and thus cause some reduction in germination and also variation in plant morphology (Van Du *et al.*, 2001; Rajput *et al.*, 2005; Niaz and Dawar, 2009).

Furthermore, infection rate of seeds depending on some environmental conditions such as high relative humidity, suitable temperature and also high level of moisture content in seed. Yield losses caused by seed-borne pathogens to wheat are reported between 15 to 90% of untreated seeds grown in fields (Mathre, 1997).

Seed treatment is the safest and the cheapest way of control of seed-borne fungal diseases and to prevent bio deterioration of grains (Chandler, 2005; Bagga and Sharma, 2006). A large number of fungicides are being used in the form of dusting, slurry and soaking treatment. Even though effective and efficient control of seed-borne fungi can be achieved by the use synthetic chemical fungicides. However, many fungicidal agents available in the market are toxic and have undesirable effects on other organisms present in the environment. Some synthetic fungicides are non-biodegradable, and hence can accumulate in the soil, plants and water, and consequently effect the humans through the food chain (Athukoralage, 2001). The development of resistance of pathogenic fungi towards the synthetic fungicides is of great concern (Boungab *et al.*, 2012). For that reason, the interest is

increasing in the use of natural substances produced on the basis of different plant species. Biologically active substances obtained from them may be active against bacteria, fungi and insects (Kavitha and Satish, 2011; Boungab *et al.*, 2015; Righi *et al.*, 2014). It is also important that the dressing substances should stimulate the healthiness and vitality of the plants (Sas-Piotrowska and Piotrowski, 2010).

In the present study, seed health testing was carried out to evaluate fungi associated with seeds of three Algerian wheat cultivars, then testing the potential of applying *Inula viscosa* extracts as seed treatments for controlling fungal infection in seeds as well as stimulating seed germination.

Materials and methods

Preparation of plant extracts

For the study, fresh leaves of *Inula viscosa* in the flowering stage were collected from the area of Mascara (north western of Algeria). They were washed with tap water to remove debris and dust particles and then rinsed with sterile distilled water. The plant material were dried in the laboratory at room temperature then grounded into powder form. Twenty grams of this dried powder were added to 100 ml of sterile distilled water or alcohol in 250 ml beaker then extracted by cold maceration for 48h. After filtration through Whatman filter paper No.1, the resulting extracts were evaporated at 45°C and transferred into sterile bottles and kept in refrigerator until used.

Isolation and identification of seed-borne fungi

Seeds of three cultivars of wheat originally from the Technical Institute of Field Crops in Saïda, (North West of Algeria) were used for this study (Ain abid, Anza and Hiddab). Isolation and identification of seed-borne fungi were conducted according to standard tests described by the International Seed Testing Association (ISTA). Three replications of 100 seeds were placed on Potato Dextrose Agar (PDA) medium and SBM (Standard blotter method) at the rate of 10 seeds per plate without surface sterilization. The plates were incubated at 22±2°C for seven days

under alternating cycles of 12 h light and 12h darkness. After the incubation period, the seeds were examined under microscope in order to record the incidence of different seed borne fungi on seeds. Seed infection (%) was evaluated as: (number of seeds (samples) with occurrence of fungi/total number of evaluated seeds) × 100.

Germination test

This investigation was assessed in laboratory conditions by using blotter test. Hundred seeds for each cultivar were tested. Seeds were placed in Petri dishes on moist filter paper in four replicates, then incubated at 24 ± 2 °C under natural room light for ten days. The germinated seeds were counted, then percentages of germination was calculated.

$$\% \text{ germination} = \frac{\text{No. of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Antifungal Assay

Aqueous and methanolic plant extracts were screened for their antifungal activity against seed borne fungi of wheat. Different concentrations of each extract (500, 1000 and 1500 ppm) were used for assay.

In all experiments, hundred seeds of tested varieties were immersed at different concentration of *Inula viscosa* extracts. The treated seeds were dried for 20h at ambient temperature (Sas-Piotrowska and Piotrowski, 2010). Seeds soaked in sterile distilled water served as control. Treated seeds and control

were subjected to agar test and germination test as described previously. All tests were performed in triplicate.

The percentages of inhibition of seed infection were obtained based on the comparison with the control.

Statistical analysis

All results were expressed as the mean ± standard error (SE). For all experiments three replicates were realized per treatment and concentration. The statistical significance was evaluated by analysis of variance (ANOVA) and Duncan's multiple range test procedures using SPSS software version 20. Differences were considered statistically significant at the $P < 0.05$ level.

Results

Prevalence of seedborne fungi

Seed borne mycoflora associated with three varieties of wheat (Ain abid, Anza and Hiddab) was investigated through standard blotter paper and agar plate method. At least nine fungal genera were recovered from seeds, including *Alternaria alternata*, *Aspergillus* sp, *Fusarium* sp, *Penicillium* sp, *Rhizopus* sp, *Cladosporium* sp, *Mucor* sp, *Bipolaris sorokiniana* and *Metarrhizium* sp.

The majority of these fungal genera were isolated through both standard blotter paper and agar plate method, However, with a higher incidence in agar plate method (Table 1).

Table 1. Seed borne fungi associated with three wheat cultivars.

Seed mycoflora	Seed infection (%)					
	Anza cultivar		Ain Abid cultivar		Hiddab cultivar	
	Agar test	Blotter test	Agar test	Blotter test	Agar test	Blotter test
<i>Alternaria alternata</i>	19.32	13.56	23.45	13.22	30.25	24.86
<i>Aspergillus</i> sp	13.03	8.33	16.86	10.80	07.14	03.05
<i>Fusarium</i> sp	07.24	05.16	08.22	14.06	08.09	12.15
<i>Bipolaris sorokiniana</i>	—	—	—	—	04.22	05.45
<i>Penicillium</i> sp	09.06	09.25	11.59	08.12	07.16	04.66
<i>Rhizopus</i> sp	8.66	10.86	10.14	05.01	06.32	04.50
<i>Cladosporium</i> sp	13.45	12.06	06.76	06.87	15.95	12.15
<i>Mucor</i> sp	10.78	08.11	04.05	03.45	14.01	09.67
<i>Metarrhizium</i> sp	04.02	—	—	—	—	—

(-) Absence of fungus.

The frequency of associated fungi of wheat seeds was influenced by the tested cultivars. The highest frequency of seeds fungi was observed on the wheat cultivar Hiddab (76.49% to 93.14%), followed by Anza (67.33% to 85.56%).

The lowest frequency was recorded for the seeds of Ain Abid wheat cultivar (61.53% to 81.07%) (Fig. 1).

Seven fungal genera (*Alternaria sp*, *Aspergillus sp*, *Fusarium sp*, *Penicillium sp*, *Rhizopus sp*, *Cladosporium sp*, and *Mucor sp*) were isolated from all cultivars; while *Bipolaris sorokiniana* and *Metarrhizium sp* were recorded at a low frequency in one wheat cultivars only.

Of the isolated fungi, *Alternaria sp* was the most predominant fungus, with incidence ranged from 13.22 to 30.25%, followed by *Cladosporium sp* (6.76

to 15.95%), *Aspergillus sp* (03.05 to 16.86%), *Fusarium sp* (05.16 to 14.06%), *Mucor sp* (3.45 to 14.01%), *Penicillium sp* (4.66 to 11.59%) and *Rhizopus sp* (4.50 to 10.86%), however, *Bipolaris sorokiniana* and *Metarrhizium sp* were less frequently isolated. Table 1 summarizes the infection rates and species spectrum for fungi detected on Blotter and PDA medium among wheat seeds used in the experiments.

Antifungal activity of plant extracts against seed borne fungi

Effect of *I. viscosa* extracts on seed borne fungi is shown in Table 2. Results indicated that all treatments were positively effective in reducing the seeds infection of all tested cultivars compared to the control. The reduction of wheat seeds mycoflora showed an increase when the concentration of the extracts was increased from 500 to 1500 ppm.

Table 2. Infection and germination rates recorded in wheat seeds treated with *Inula viscosa* extracts.

Cultivars	Treatment	Concentration (ppm)	Infection (%)	Inhibition (%)	Germination (%)	
Hiddab	aqueous extract	500	76,52 ± 8,54	17.84 ± 7,10	76,95 ± 1,76	
		1000	51,35 ± 3,40	44.86 ± 4,22	84,32 ± 4,32	
		1500	35.07 ± 4,17	62.34 ± 4,12	84,80 ± 0,36	
	methanolic extract	500	66,34 ± 11,93	28.77 ± 11,54	82,05 ± 2,06	
		1000	45,20 ± 7,57	51.47 ± 6,95	86,16 ± 5,42	
		1500	28,64 ± 5,25	69.25 ± 6,53	91,80 ± 2,12	
	Control			93.14 ± 2,13	00,00 ± 0,00	78,64 ± 1,24
	ANZA	aqueous extract	500	63,89 ± 4,82	25.32 ± 4,07	82,66 ± 3,05
			1000	35,56 ± 1,00	58.43 ± 0,74	89,15 ± 5,03
1500			31,05 ± 5,04	63.70 ± 4,82	94,33 ± 2,33	
methanolic extract		500	53,73 ± 3,15	37.20 ± 2,91	89,50 ± 1,75	
		1000	25,56 ± 13,05	70.12 ± 11,85	92,18 ± 3,66	
		1500	15,73 ± 4,82	81.61 ± 4,53	96,50 ± 1,24	
Control			85,56 ± 3,67	00,00 ± 00,00	84,33 ± 2,36	
Ain Abid		aqueous extract	500	58,78 ± 2,91	27.49 ± 3,05	92.66 ± 4,20
			1000	31,29 ± 4,23	61.40 ± 3,67	98,07 ± 1,15
	1500		24,21 ± 6,37	70.13 ± 6,33	98.95 ± 3,05	
	methanolic extract	500	35,62 ± 1,53	56.06 ± 1,15	94,33 ± 2,32	
		1000	21,30 ± 6,33	73.72 ± 5,85	96.33 ± 3,53	
		1500	13,08 ± 3,15	83.86 ± 3,47	98.66 ± 2,10	
	Control			81,07 ± 3,60	00,00 ± 0,00	90,05 ± 2,63

Values are means of three replicates ± standard error.

Generally, Seed treatment with methanolic extract was most effective in the reduction of seed-borne fungi. For the variety Hiddab, the treated seeds at the concentrations 1500, 1000 and 500 ppm have

presented infection of 28.64%, 45.20% and 66.34% respectively when the untreated seed of the same variety have demonstrated infection of 93.14%. The seed treatment with the concentrations 500, 1000

and 1500 ppm of methanolic extract have reduced the seed infection rate of the variety Anza to 53.73, 25.56 and 15.73% respectively with initial seed infection of

85.56% (control). An important reduction of infection is observed on the treated seeds of the variety Ain Abbid with the treatment 1000 and 1500 ppm.

Table 3. Average infection and germination rates across wheat seed as treated by *Inula viscosa* extracts.

Treatment	Infection (%)	Inhibition (%)	Germination (%)
Control	86,59 ± 3,36A	0±0,00C	84,34±2,33B
aqueous extract	45,31± 4,15B	47,95±4,66B	88,55±2,91AB
methanolic extract	33,91± 5,75C	61,34±6,03A	91,95±3,45A

Values are means of three replicates ± standard error.

Values in the same row followed by different letters are significantly different ($P \leq 0.05$).

In fact, aqueous extract of *I. viscosa* tested exhibited different degrees of antifungal activity against seed-borne fungi. The percent inhibition of seeds mycoflora was low than 50% at 500 ppm. However, the seed treatment with 1000 and 1500 ppm of aqueous extract, effectively controlled seed infection by all fungi compared to non-treated seeds.

Effect of plant extracts on wheat seed germination

The standard blotter method was used to find out the effect of seed borne fungi on seed germination. The maximum germination was recorded in Ain Abid (90,05%), followed by ANZA (84,33%), whereas, Hiddab showed minimum germination (78,64%). The effective results were recorded as regards to the highest germination reflected the lowest fungi incidence (Table 2).

The examination conducted showed a diversified effect of *I. viscosa* extracts and the methods of their preparation on germination capacity and healthiness of the seeds for all wheat cultivars. Percent seed germination for all three wheat cultivars tested was more than 76%, and was greatly increased by either methanolic and aqueous treatments depending on the wheat cultivar tested (Table 2).

All the leaf extracts (methanolic and aqueous) significantly reduced the incidence of the seed-borne fungi, increased seed germination when compared with the untreated control seeds.

The germination capacity of the seeds was stimulated significantly most strongly by methanolic plant extracts. The range of his activity varied from 82,05% to 98.66% (Table 3).

Germination of wheat seeds increases gradually with the increase of doses. Seed treatment with 1500ppm of methanolic extract was the most effective, where it caused a highly seed germination (91,80 to 98.66%). Aqueous extract at the highest concentration also increased the germination at rates ranging between 84, 80 to 98.95 %. In general, the methanolic extract was more effective than the aqueous leaf extracts in reducing seed-borne fungi and in increasing seed germination.

Discussion

Seed Borne Mycoflora of wheat

Mycological analysis carried out revealed the presence of many fungal strains in the material tested. Generally, 9 fungal genera including both saprophytic as well as pathogenic were isolated and identified from the seed samples using the standard blotter test and agar plate method. The isolated fungi and infection rate indicated that *Alternaria alternata*, *Aspergillus sp*, *Cladosporium sp*, *Mucor sp*, *Rhizopus sp*, *Fusarium sp* and *Penicillium sp* were the predominant fungi, while, *Bipolaris sorokiniana* and *Metarrhizium sp* were of minor importance.

Many reports indicated the occurrence of many fungal genera in different wheat cultivars in other countries of the world. Included *Alternaria sp*,

Helminthosporium sp, *Fusarium sp*, *Stemphylium sp*, *Cladosporium sp*, *Aspergillus sp*, *Penicillium sp*, *Bipolaris sp*, *Mucor sp*, *Rhizopus sp*, and *Rhizoctonia sp* (Rajput *et al.*, 2005; Awad and Baka, 2014; Rahman *et al.*, 2015). Riba *et al.* (2008) observed that *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria* and *Mucor* were the most common genera in wheat grains in Algeria.

Alternaria alternata was the most frequently isolated fungus. This species reduce germination percentage and induce seedling blight (Karim, 2005). Özer

(2005) reported *Alternaria alternata* was the most dominant fungus isolated from black pointed seeds. Several species of *Aspergillus* and *Penicillium* 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).

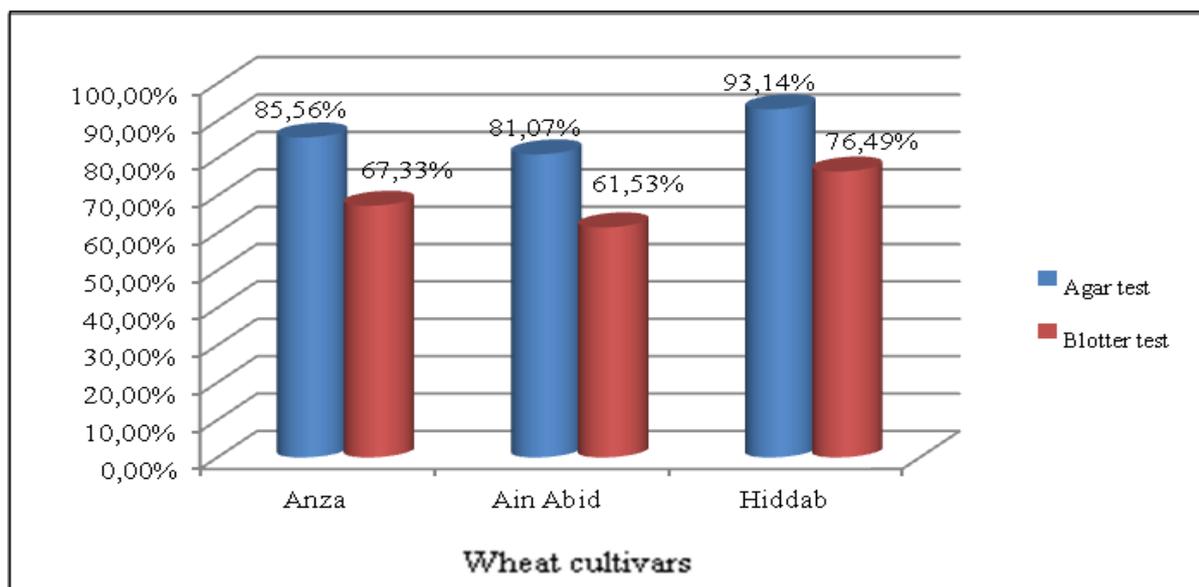


Fig. 1. Frequency (%) of seed associated fungi from wheat on Blotter and PDA medium.

Many species from the *Alternaria*, *Aspergillus*, and *Fusarium* genera, as well as some *Penicillium* species are known to produce mycotoxins which are receiving a considerable attention worldwide as they show a wide range of pathological effects such as carcinogenicity, teratogenicity and mutagenicity (Oueslati *et al.*, 2012).

Bipolaris sorokiniana (Sacc.) Shoem is a serious pathogen of wheat. It causes disease symptoms such as root rot, leaf blight, seedling blight and spot blotch. The pathogen affects seed germination and seedling emergence and causes considerable reduction in grain yield (Prashith Kekuda *et al.*, 2016).

The number of fungal colonies arising from agar plate method was larger than resulting from the blotter test method. These data confirms the results reported by (Kumari and Saxena, 2017), which they stated that Agar plate method had recorded more fungi than blotter method and dilution plate method.

Effect of Inula viscosa extracts on seed germination and fungal infection

Many reports have shown the importance of plant extracts for controlling seed-borne fungi of wheat grains (Hassan *et al.*, 2005; Perelló *et al.*, 2013; Awad and Baka, 2014).

The efficacy of *I. viscosa* extracts has been tested with three doses in laboratory against the seed mycoflora

of wheat. Both the extracts used (aqueous and methanolic) gave the best result with an optimum dose of 1500 ppm, and significantly reduce the mycoflora as compared to untreated seeds (control). The methanolic extract was found to be more effective than the aqueous extracts in reducing seed-borne fungi and in increasing seed 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 Hiddab. This confirms the association of seed-borne fungi with seed viability. It was reported by Awad and Baka, 2014, that seed borne fungi of wheat not only reduced the germination but also affected seedling vigor resulting in low yield.

Seed germination is the most important stage for the crop yield and it is also critical stage under stress condition (Akram *et al.* 2017). Treatment with *I. viscosa* extracts at different dose level had a positive effect on germination of wheat seeds. The percentage of germination proved to be dependent on the concentration and the type of extract tested.

These results are in agreement with those obtained by many authors (Shafique *et al.* 2007; Perelló *et al.* 2013; Akram *et al.* 2017) 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.

Several studies had shown that *I. viscosa* is an important source of bioactive compounds against different fungal species of medical or agronomic importance. Cafarchia *et al.*, (2002) reported that flower and leaf's extracts of *I. viscosa* obtained with different solvents showed an antifungal activity against *Candida* species and dermatophytes.

On the other hand, Wang *et al.*, (2004), showed that *I. viscosa* organic extracts were effective in

controlling late blight (*Phytophthora infestans*) in potato and tomato, downy mildew in cucumber (*Pseudoperonospora acubensis*), powdery mildew in wheat (*Blumeria graminis* f. sp. *tritici*), and rust in sunflower (*Puccinia heliathi*).

Seed-treatment trials with five plant extracts (*Anacyclus valentinus*, *Inula viscosa*, *Salvia officinalis*, *Rosmarinus officinalis* and *Tetraclinis articulata*), showed a significant reduction in seed borne inocula. Among the five plant species tested, aqueous extract of *I. viscosa* proved to be the most effective in inhibiting the barley seeds mycoflora (Boungab *et al.*, 2015).

Chemical analysis of extract from *I. viscosa* leaves have demonstrated the presence of sesquiterpene especially carboxy eudesmadienes (Cafarchia, 2002). However, Mahmoudi *et al.*, (2015), have identified 17 components with hydroxycinnamic acids, namely mono- and dicaffeoylquinic acids being the most prominent components. The presence of these phenolic compound conferred strong free radical scavenging and antifungal properties to *I. viscosa* extract.

In addition, Rhimi *et al.*, (2017), have detected the presence of condensed tannin (TCT), phenol (TPC), flavonoid (TFC), and caffeoylquinic acid.

Conclusion

In conclusion, an important finding in the present study is the demonstration that a aqueous and methanolic extracts of a local plant, *Inula viscosa* are capable of increasing efficaciously seed-borne fungi which make serious problems in seed production worldwide, as well as improving seed germination. This indicates that this plant may be useful for developing alternative approach to prevent biodeterioration of seeds in an ecofriendly way.

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