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Effect of seed treatment using *Inula viscosa* essential oil in controlling seed-borne fungi of chickpea

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ABSTRACT

Three concentrations (250, 500, 1000 ppm) of essential oil extracted from Inula viscosa were tested for their efficacy in controlling seed-borne fungi associated with chickpea under laboratory conditions. Obtained results show that the treatments with the concentration 1000 ppm have demonstrated good effect in reducing seed-borne infection. The treatments with this concentration have also good effect on seed germination in comparing with the untreated seed. The seed to seedling transmission were also reduced by using these treatments in comparing with control.

Key words: Chickpea, seed borne fungi, treatment, *Inula viscosa* essential oil.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a highly nutritious legume crop cultivated throughout the world and is placed third in the importance list of the food legumes. The production of chickpea is limited by many factors; the fungal diseases are the important ones. Many plant pathogens are seed-borne transmitted, which can cause enormous crop losses (Ahmed et *al.*, 1993, Dawar et *al.*, 2007). Among the different practices used, seed treatment is one of the cheapest and safest methods of direct control of seed-borne diseases by eliminating seed-borne inocula **(**Masum et *al.*, 2009). The use of plant extracts has long been recognized as an area of investigation against various fungal diseases. Application of plant extract which are easily available for controlling plant diseases are non-pollutive, cost effective non hazardous and do not disturb ecological balance. Investigations are progress to test the efficacy of these extracts in the field application **(Babu et *al.*, 2008).**

Inula viscosa (L.) Aiton (=*Drittrichia viscosa* (L) W. Greuter); (Tribe Inulae, Asteraceae) is a herbaceous perennial medicinal plant, widespread in the Mediterranean area. Previous researches on this plant haverevealed in this specie the presence of flavonoids **(Wollenweber et *al.*, 1991; Benayache et *al.*, 1991),** sesquiterpenes **(Ceccherelli et *al.*, 1985),** triterpenes **(Oksuz, 1976)** and essential oil **(De Laurentis, 2002).** Extract from *Inula viscosa* especially essential oil have been demonstrated to possess different biological activities **(Cafarchia et *al.*¸2001; Omezzine et *al.*, 2011).**

The aim of this research is to evaluate the effect of the essential oil extracted from *Inula viscosa* on the seed borne fungi associated with chickpea as a biological treatment applied specifically to the seed surface which can provide an effective alternative to chemical fungicides.

EXPERIMENTAL SECTION

Isolation, quantification and identification of fungi associated with chickpea seed's

Three varieties of chickpea seed originally from the Technical Institute of Field Crops in Saïda, (North West of Algeria) were used in this study. The germination rate, seed-borne infection by fungi was studied by using agar test and blotter test (**Dawar et *al.*, 2007)**, seed were disinfected using sodium hypochlorite solution at 3% for 3min then washed twice with sterile distilled water and dried by using sterilized filter paper. After sterilization, seed were placed into Petri dish containing PDA medium (agar test), and sterilized filter paper as described by blotter method. After 10 day of incubation at 24±2°C, cultures of fungi growing on the seeds were isolated, quantified, purified, and identified on the basis of their cultural and morphological characteristics and confirmed with standard literature (**Dawar et *al.*, 2007).**

- The frequency of infection was calculatedby using the formula.

Infected Seed (%) =Number of the infected seed /total Number of the analyzed seed x100

- The frequency of isolation or dominance (%) of each of fungi corresponded to the number of isolation of the considered fungi in comparing to the total number of fungi isolation.

Fungi isolation (%) =Number of repetition of the considered fungi */total number of the fungi isolation x100.*

- The transmission (%) of each one of fungi corresponded to the number of isolation of the considered fungi on to the total number of analyzed seed.

Fungi transmission (%) =Number of repetition of the considered fungi */total number of the analyzed seed x100.*

Germination test

Seed germination (%) was performed by using blotter test wheredisinfected seeds were placed into sterile 180 mm Petri dish (plate) containing sterilized filter paper as described by blotter method as 16-18 seed/Petri dish and the result of 50 seeds (in three plate) represent a replicate. Seed germination (%) was determined after 10 days of incubation at 24±2°C.

Essential oil extraction

Aerial parts of wild *I. viscosa* in the flowering stage were collected in October 2010 from the North West of Algeria (area of Mascara) and used in this study. Essential oils are extracted from the leaves of *I. viscose* by water distillation procedure as El-Ajjouri*et al.*,(2008)procedure. The essential oil obtained was stored in dark at 4 °C.

Seed treatment with essential oil

Seed treatments were realized by using the modified method of **Nguefack et *al.* (2008)** with introduction of seeds into an emulsion of the extracted essential oils and 0.1% water agar solution for 20 min.The essential oil wasmixed at concentration 250, 500 and1000 ppm.Untreated seed soaked inwater agar solution were considered as control. Treated seeds and control were dried on blotter sheets and subjected to agar and blotter test as described previously.

Effect of treatments on seed infection

Mycoflora analysis of the treated chickpea seeds and control was performed after 10 days of incubation at 24±2°C from treatment on agar plates using PDA medium. The percent of the infected seed was noted for all treatments and control, then, calculated by using the same formula presented previously **(Nguefack et *al,* 2008)**.Fungi growing on the treated seeds and control were also isolated, quantified, purified, and identified.

Effect of treatmentson seed germination

The treated and control seed as described previously. For each variety, each of the concentrations treatments by using the essential oil and control, one hundred fifty (150)seeds were placed into sterile 180 mm Petri dish (plate) containing sterilized filter paper as described by blotter method as 16-18 seed/Petri dish and the result of 50 seed represent a replicate. Seed germination (%) was determined after 10 days of incubation at 24±2°C.

Seed to seedling transmission

The effect on the seed to seedling transmission was calculated as the percent difference of recovery between the non-treated and treated seedling **(Nguefack et *al.*, 2008)**. Treated and untreated seed as described previously were planted for 2 weeks in plastic pot containing 500 g of sterilized soil as 10 seeds / pot. Pots were then placed in laboratory conditions (T°22-24°C, RH 60%, Photoperiod D: L 12:12) and irrigated weekly. The 15 days old seedlings were carefully detached from soil, then surface sterilized in 1% sodium hypochlorite for 1 minute, cut under aseptic conditions into sections of about 5-10 mm and placed into Petri dish and incubated for 10 days at 24±2°C. The seed to seedling transmission was determined by using following formula.

Seed to seedling transmission (%) = Number of infected sections/total number of sections x 100.

Statistical analysis
All results were expressed as the mean ± standard error of mean (SEM). For all experiments threereplicates were realized per treatment and concentration. The statistical significance was evaluated using analysis of variance (ANOVA), followed by Newmen Keuls Test at 5% using STAT BOX.

RESULTS

Mycoflora analysis of seeds simples
Numerous diseases of chickpea are seed-borne so, we have analyzed the seed of chickpea as previously test to have initial known on seed infection and seed germination for each variety (**Table1**).Good correlation has been demonstrated **(Figure 1)** between the seed infection and the seed germination rate (R^2=0,80). The most infected variety FLIP 82-94c has demonstrated the lower germination rate and the variety which has demonstrated the lower infection has given themost important germination rate.

Results of quantification and identification of fungi isolated from each one of the three analyzed varieties are presented (**figure 2).**

- The variety FLIP82-94c has demonstrated the highest infection rate (**70.66%**) and the lowest germination rate **(10.7%).** Nine (9) genus of fungi were isolated from this variety *Paeicylomyces sp* (26.1%) *Aspergillus sp* (15.2 %.), *Penicillium sp* (13%) ,*Fusarium sp* (13.%), *Alternaria sp* (13.%), *Botrytis sp* (8.7%) ,*Actinomucor sp* (6.5%), *Rhizoctonia sp* (2.2%) and *Stemphylium sp* (2.2%).
- The variety ILC3279 with medially infection rate (**49.4%)** and germination (**68.7%)** has demonstrated the presence of *Aspergillus sp* (37.8%), *Penicillium sp* (24.5%), *Botrytis sp* (13.4%), *Fusarium sp* (6.7%), *Rhizoctonia sp* (6.7%), *Alternaria sp* (6.7%) and *Trichoderma sp* (4.5%).
- The variety ILC482 has demonstrated infection rate of 7.4 % and germination rate of 90.7%. Three (3) fungi, *Aspergillus sp,Trichoderma sp* and *Fusarium sp* were isolated from this variety with the frequency of isolation of 37.5%, 37.5% and 37.3 % respectively.

Effect of the essential oil treatment on seed infection
The seed treatments with the essential oil extracted from *Inula viscosa* leaves have affected significantly the seed infection(*p=0.00*) **(table 2)** by using the concentrations 500 and 1000 ppm.

For the variety FLIP 82-94 c, the treated seeds at the concentrations 1000, 500 and 250 ppm have presented infection of 6.3%, 21.9% and 66.7% respectively when the untreated seed of the same variety have demonstrated infection of 71.6%.

The seed treatment with the concentrations 250, 500 and 1000 ppm of essential oil have reduced the seed infection rate of the variety ILC3279to 30.5, 22.3 and 4.2% respectively with initial seed infection of 46.9 % (control).

Total reduction of infection is observed on the treated seeds of the variety ILC 482 with the treatment 500 and 1000 ppm.

For each one of the three varieties of chickpea used, each concentration of the essential oil treatments and control, the isolation of each fungus was quantified in percent. The result obtained **(Table 3)** show that the different treatment have reduced significantly(*p=0.00008*) fungi apparition on the treated seed in comparing with control of the same variety (variety FLIP82-94c, *p=0.00008*), (variety ILC 3279, *p=0.00*), (variety ILC482, *p=0.04*).

On the variety FLIP82-94c a total reduction of apparition *Stemphylium sp.* and *Rhizoctonia sp.* was demonstrated with the three concentrations of essential oil. We have also observed that the seed treatments of the variety ILC 3279 have reduced 100% of *Rhizoctonia sp.* And *Fusarium sp.* A total reduction of *Fusarium sp* and *Trichoderma sp.* was showed with these treatments of the variety ILC482.

Effect of the essential oil treatment on seed germination
The seed germination rates were also affected (*p=0.009*) with these treatments by using the same concentrations in comparing with the untreated seed of the same variety **(Table 2)**.

We have also to signaled that using the concentration 1000 ppm of *Inula viscosa* essential oil, on the variety FLIP82-94c which have initial the germination rate of 10.19%, the infection was reduced to 27.5% but the seed

germination rate was 12.6%, so we think that in this case it is probably that the germ of theses seed are dead previously, and fungi may be located in the germs.

In this study, the essential oil exhibited antifungal activity which varies with varied degrees of concentrations; this antifungal action was represented by reducing the percent of seed borne infection, and the highly percent of germination seed.

Effect of the essential oil treatment on seed to seedling transmission

For all the tested varieties and the concentrations used, the seed to seedling transmission are reduced in comparing with the control **(Table 2)**.

For all the treatment at the different concentrations, an important antifungal activity on the seed borne fungi associated with chickpea was observed in comparing with the control of the same variety. However at higher concentration 1000 ppm, a considerable reduce in the incidence of the fungi isolation from seed and plants.

DISCUSSION

Seed is a common carrier of plant pathogens, it may be acted as the primary source many diseases. Most of the major diseases of chickpea are seed-borne. **Dawar et *al.* (2007)** have detected different fungi in Pakistan in chickpea seeds, a total number of 21 species belonging to 13 genera of fungi were isolated. **Ahmed et *al.*, (1993)** have been reported from chickpea seed's many fungal species including into genus of *Alternaria, Aspergillus, Botrytis, Cladosporium, Curvularia, Fusarium, Macrophomina, Myrothecium, Penicillium, Rhizoctonia,* and *Rhizopus***.** Many authors proved such seed infection and its effect on whole plants leading to great economic loss (**Burgess et *al.*,1997).** These fungal diseases can kill chickpea crops and is difficult to remove once it sets in **(Agarwal and Sinclair, 1997; Agarwal et *al.*, 2011).**

These results are clear indication for the potential of plant extract in controlling seed-borne fungi of chickpea. In this study, the usedessential oil exhibited anti-fungal activity which is represented by reducing the seed infection rate and the seed to seedling transmission percent which varied with the different concentrations.

Our results agree with those reported by several reports mentioned that the plant extracts and especially essential oil extracted from medicinal species play an important role in controlling seed-borne fungi of rise **(Miah et *al.*,1990; Nguefack et *al*,. 2008; Thobunluepop, 2009),** cowpea **(Akinbode and Ikotun, 2008)**, wheat **(Enikuomehin, 1994 ;Khaleduzzaman, 1996)**, sorghum **(Masum et *al*, 2009; Bonzi et *al.*, 2012).**

Inula viscosa leaf extract especially essential oilmight be a substantial alternative of chemical pesticides in controlling plant diseases. It possess broad-spectrum activity against plant diseases of crop plants foliar caused by pathogens belonging to the families Oomycetes, Ascomycetes, and Basidiomycetes **(Ziv, 1996; Wang et *al.*, 2004)**. Late blight of potato or tomato, powdery mildew of wheat, rust of sunflower, downy mildew of cucumber, downy mildew caused by *Plasmopara viticola*in grapevines **(Cohen et *al.*, 2006)** and *Fusarium* wilt of lentil **(Singh and Tripathi ; Belabid et *al.*, 2010)**.

The inhibitory effect of *I. viscose* extracts might be attributed to the presence of antifungal compounds.The characterization of the essential oil of *I. viscosa* has been the subject of many works.The composition of essential oils of *I. viscosa* growing in different countries of the Mediterranean area has been investigated **(De Laurentis et *al.*, 2002).**

Quantitative and qualitative analysis of extract from *I. viscosa* leaves have demonstrated the presence of sesquiterpene especially carboxyeudesmadienes **(Cafarchia, 2002).** However, **Abu Zarga et *al* (1998, 2002)** have detected 6 new sesquiterpenic type eudesmane: 3b-hydroxyilicique acid, 3a-hydroxy-epiilicic acid, 2a-hydroxyilicic acid, 9b-hydroxy-2-oxoisocosticacid, 1b- hydroxyilicic acid and 2b-hydroxyilicic acid from essential oil of *Inula viscosa* growing in Jordan. **Berhail et *al.*, (2012)**, have detected the presence of the major components namely, isocostic acid, costic acid, nerolidol, linoleic acid, *neo*-intermedeol and fokienol from *Inula viscosa* essential oil.

The application of plant extracts in the control of seed borne fungi diseases is gradually gaining ground especially under the nascent organic agriculture. Our finding shows that the treatment with the essential oil of *Inula viscosa* have a good potential for antifungal activity and the control of the chickpea's seed fungi, biological treatment applied specifically to the seed surface could provide an effective alternative to fungicides. Further studies on control of the plant diseases with plant extracts and essential oil are recommended.

Table1. Characteristics of Chickpeas' seed used

Variety of chickpea	Infected seed	Seed germination
ILC 482	*7.33± 4.16C*	90.66± 5.03A
ILC 3279	*49.33± 1.15B*	68.66± 3.05B
FLIP82-94c	70.66± 4.16A	10.66± 1.15C

Numbers in the same column followed by the same letters are not significantly different according to NewmenKeuls Test at 5%

Table2: Effect of the essential oil treatment on seed infection, seed germination and to seedling transmission.

Treatment	Seed infection	Seed germination	Seed to seedling transmission
Variety FLIP82-94c			
Control	71.63 ± 10.02A	9.92 ± 3.18 F	100 ± 0.00A
250 ppm	66.66 ± 12.24A	19.97 ± 3.12 E	57.96 ± 1.48B
500 ppm	21.93 ± 2.75C	25.85 ± 6.15E	24.01 ± 0.85E
1000 ppm	6.26 ± 0.39D	24.01 ± 0.85E	6.00 ± 0.21FG
Variety ILC 3279			
Control	46.93 ± 3.12B	65.92 ± 4.17D	46.07 ± 4.49C
250 ppm	30.51 ± 5.18C	75.98 ± 0.85C	29.88 ± 5.14D
500 ppm	22.30 ± 6.15C	77.94 ± 3.88C	19.97 ± 3.12E
1000 ppm	4.16 ± 3.60D	83.94 ± 3.75B	7.96 ± 3.29F
Variety ILC 482			
Control	7.84 ± 3.40D	96.07 ± 3.40A	7.94 ± 3.1F
250 ppm	4.04 ± 3.50D	100 ± 0.00A	3.72 ± 3.22FG
500 ppm	0.00 ± 0.00D	100 ± 0.00A	0.00 ± 0.00G
1000 ppm	0.00 ± 0.00D	100 ± 0.00A	0.00 ± 0.00G

Numbers in the same column followed by the same letters are not significantly different according to NewmenKeuls Test at 5%

Table3: Fungi isolation (%) from treated seed with essential oil of *Inula viscosa.*

Fungi isolation from seed (%)	Seed treatment	Variety FLIP82-94c	Variety ILC3279	Variety ILC482
Alternaria sp.	Control	12.01 ± 0.43 BC	12.01 ± 0.43CD	/
	250ppm	1.96 ± 3.39 D	3.92 ± 3.39FG	/
	500ppm	0.00 ± 0.00 D	0.00 ± 0.00G	/
	1000ppm	0.00 ± 0.00 D	0.00 ± 0.00G	/
Actinomucor sp	Control	6.00 ± 0.21CD	/	/
	250ppm	1.96 ± 3.39 D	/	/
	500ppm	0.00 ± 0.00 D	/	/
	1000ppm	0.00 ± 0.00 D	/	/
Aspergillus sp	Control	13.96 ± 3.20B	31.98 ± 3.01A	3.92 ± 3.39A
	250ppm	5.88 ± 5.88 CD	15.93 ± 2.96C	1.96 ± 3.39B
	500ppm	1.96 ± 3.39 D	6.00 ± 0.21EF	0.00 ± 0.00B
	1000ppm	0.00 ± 0.00 D	0.00 ± 0.00G	0.00 ± 0.00B
Botrytis sp.	Control	7.84 ± 3.39 BCD	12.01 ± 0.43CD	/
	250ppm	6.00 ± 0.21 CD	6.00 ± 0.21EF	/
	500ppm	4.04 ± 3.50 D	7.96 ± 3.39DEF	/
	1000ppm	4.04 ± 3.50 D	0.00 ± 0.00G	/
Cladosporium sp.	Control	/	/	/
	250ppm	/	/	/
	500ppm	/	/	/
	1000ppm	/	/	/
Fusarium sp.	Control	12.01 ± 0.43 BC	12.01± 0.43CD	1.96 ± 3.39B
	250ppm	7.96 ± 3.29 BCD	0.00 ± 0.00G	0.00 ± 0.00B
	500ppm	6.00 ± 0.21 CD	0.00 ± 0.00G	0.00 ± 0.00B
	1000ppm	4.04 ± 3.50 D	0.00 ± 0.00G	0.00 ± 0.00B
Paeicylomyces	Control	23.89 ± 5. 34A	/	/
	250ppm	13.96 ± 3.20B	/	/
	500ppm	7.96 ± 3.29 BCD	/	/
	1000ppm	4.04 ± 3.50 D	/	/
Penicillium	Control	12.00 ± 0.42BC	19.97 ± 3.12B	/
	250ppm	4.04 ± 3.50D	9.92 ± 3.18DE	/
	500ppm	1.96 ± 3.39 D	10.04 ± 3.62DE	/
	1000ppm	0.00 ± 0.00 D	3.92 ± 3.39FG	/
Rhizoctonia sp.	Control	1.96 ± 3.39 D	12.01 ± 0.43CD	/
	250ppm	0.00 ± 0.00 D	0.00 ± 0.00G	/
	500ppm	0.00 ± 0.00 D	0.00 ± 0.00G	/
	1000ppm	0.00 ± 0.00 D	0.00 ± 0.00G	/
Stemphylium sp	Control	2.08 ±3.06 D	/	/
	250ppm	0.00 ± 0.00 D	/	/
	500ppm	0.00 ± 0.00 D	/	/
	1000ppm	0.00 ± 0.00 D	/	/
Trichoderma sp	Control	/	7.96 ± 3.29DEF	4.04 ± 3.50A
	250ppm	/	3.92 ± 3.39FG	0.00 ± 0.00B
	500ppm	/	0.00 ± 0.00G	0.00 ± 0.00B
	1000ppm	/	0.00 ± 0.00G	0.00 ± 0.00B

Numbers in the same column followed by the same letters are not significantly different according to NewmenKeuls Test at 5%.

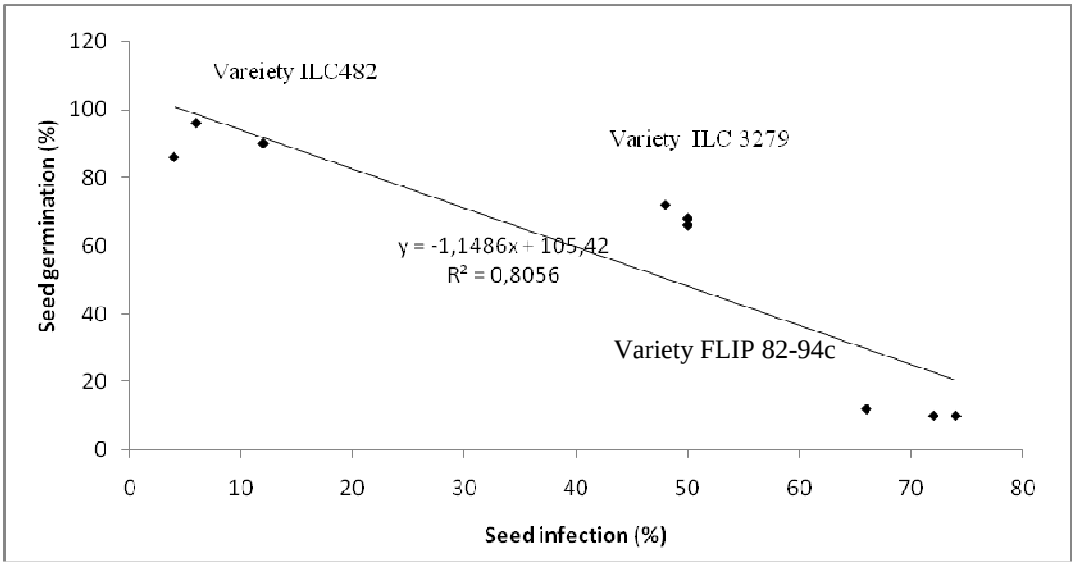

Figure1: Relationship between fungi infection and germination rate of three varieties of chickpea simple used (each point represent the result of 50 seed).

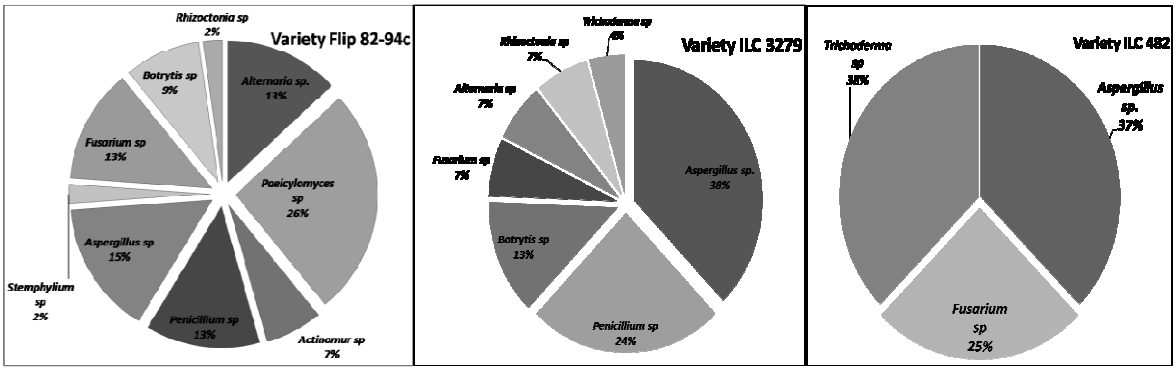

Figure 2: Frequency of fungi isolation from chickpea seed samples

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