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Antifungal activity and evaluation of phenolics contents of dill *Anethum graveolens* L. extracts original from Algeria

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The present study aims to evaluate the antifungal activity of dill *Anethum graveolens* L. against two fungus pathogens of cereals in Algeria, where quantification of the secondary metabolite was investigated in different plant part (whole plant, seeds) and two soil conditions, one is non-salty and second salty soil.The polyphenols, flavonoids, and tannins ranged from 6.25±0.74 mg/g dried weight for the tannins to 167±9.47 mg/g dried weight for polyphenols. The antifungal activity of extracts was tested against *Rhynchosporium secalis* and *Pyrenophora tritici-repentis*. The mycelial growth of the tested fungi was also determined to evaluate the antifungal activity. The ideal concentration showed a significant reduction of the two tested fungi was found to be 10 mg/mL, and the mycelial growth inhibition was determined after 7 days of incubation. Observations on the mycelial growth after extracts treatment decreased growth diameters. The effect of the hexanoic extract on inhibition of *P. tritici-repentis* was 91.17% and the methanolic extract inhibited *R. secalis* at 93.42% as the highest inhibition rate. Thus, the extract of dill could be used to control fungus pathogens as a potential source of eco-friend fungicide.

Keywords: Anethum graveolens L., Antifungal activity, Pyrenophora tritici-repentis, Rhynchosporium secalis, Secondary metabolite.

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Introduction

Wheat and barley are staple food in Algeria and many other countries. In Algeria, they are susceptible to attack by numerous diseases such as leaf scald and tan spot caused by *Rhynchosporium secalis* in barley[1] and *Pyrenophora tritici-repentis* in wheat[2] respectively. The losses outcome of these two fungi attack is economically significant[3].The use of fungicides to control the fungal attack of the crop has been practised for very long time ago but in recent years the fungicides are used with a small amount reported with a negative impact on the non-target organisms and consumers[4]. However, several environmental and health problems were investigated due to harmful effects (carcinogenicity), severe toxicity, and persistence of the synthetic molecules[5] where the use of ecofriendly pesticides became a critical board for the world leaders.

Plant extracts aid as antifungal agent[6] against a wide range of fungi[7]. *Anethum graveolens* L., an aromatic herb[8] belonging to the family Apiaceae ex Umbelliferae[9] is known for the bioactive compounds it contain[10]. The quinones seem to be the key substance to impede some fungi[11].

Dill has been reported to be used in traditional medicine and its antibacterial activity has been reported in earlier study[12]. The antihyperlipidemic and antihypercholesterolemic properties have also been studied earlier[13]. From the beneficial virtues of this plant, heightening of milk production in nursing women and countersigns menstruation in females have been observed[14]. Studies have been conducted to study the antifungal activity of dill seed oil against spoilage fungi[15-18]. In addition, studies have reported high antifungal activity of the dill essential oil[19-21].

Due to the lack of studies about the antifungal activity of the crude extract of *A. graveolens*, the present research was undertaken to evaluate the antifungal activity of different dill plant parts which were grown in two soil conditions (salty soil, normal soil) against two fungi strain pathogens that infect wheat and barley.

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Material and Methods

Plant material

The plant (stem, leaf, and root) at flowering stage and seeds of *A. graveolens* were harvested from the Technical Institute of Vegetable and Industrial Crops (ITCMI) in two different soil conditions, at sidi bel abbes where the soil is normal (non-saline) and Mohammadia with saline soil. Samples were washed, dried at room temperature, ground and stored for future use.

Extraction procedure

The extraction was performed following two methods, by fractionation for the quantification of secondary metabolites and by Soxhlet for the antifungal activity.

Fractionation

The crude methanolic extract was obtained by the method of Benhammou *et al.*[22], after evaporation, the residue of each sample was weighed and stored for next step. The methanolic crude extracts were dissolved in boiled water, the flavonoids were extracted by ethyl acetate and n-butanol following the technique of Bekkara *et al*.[23]. The tannins extracted by the process described by Zhang *et al.*[24], the ethyl acetate extract is used after evaporation.

Soxhlet extraction

The direct Soxhlet extraction was performed by the method of Harboune[25], with some modifications, 10 g of each sample was extracted by methanol, ethanol and hexane separately for 3 hours, the extracts were evaporated under vacuum using Büchi R-200 and the residue pondered and stored for further use.The aqueous extracts were obtained by mixing 10 g of powder with distillate water under reflux for 2 hours[26].

Secondary metabolites quantification

Total phenols

The total phenolic contents of tannins ethyl acetate extracts and flavonoids n-butanol, ethyl acetate extracts of dill plant (non-salt, salt) and seeds (non-salt, salt) were determined using the Folin-Ciocalteu according to the method described by Singleton and Rossi[27].

Total flavonoids

The total flavonoids of the n-butanol and ethyl acetate extracts were determined by the Aluminum chloride assay using a method described by Zhishen *et al.*[28].

Total condensed tannins

Vanillin assay was used[29] to determine the tannins contents in tannin ethyl acetate extracts of dill plant of Mohamadia and Sidi bel abbes as well as seeds.

Antifungal activity

Fungal strain

P. tritici-repentis and *R. secalis* were isolated from the host crop and purified through repeated planting method in Potato Dextrose Agar (PDA) media, the strains were identified according to the morphological characteristics of the colony in the National Institute of Agronomic Research of Algeria (INRAA). The fungal strain cultures were maintained in PDA media at 4 °C.

Antifungal assay principal

Exactly 20 mL of Potato Dextrose Agar (PDA) medium was placed in each Petri Dishes, to which a necessary quantity of the *A. graveolens* extract was added to obtain 1.25, 2.5, 5, and 10 mg/mL concentrations of the ethanolic, methanolic, hexanoic and aqueous extract.The effect on the mycelial growth of the tested fungi was determined following the method of Brewer[30] and Leach[31]. The mycelial growth was determined with the following equation:

$$L=D-d/2 \quad \ldots \text{Eq (1)}$$

where L mycelian growth, D: colony diameter and d the explant diameter.

Sample without any treatment was considered as control. The Petri dishes were then incubated for 7 days at 28 °C. The mycelial growth was calculated regularly from the first day after incubation until the last day of incubation.

The inhibition rate of the fungus colony is calculated according to the method of Kordali *et al.*[32].

$$IR\%= [(Dt-D)/Dt]x100] \quad \ldots \text{Eq (2)}$$

where IR inhibition rate of the mycelial growth, Dt diameter of mycelial growth of the control and D diameter of mycelial growth of the extract.

Statistical analysis

All the measurements were replicated in a triple for each treatment, and the data reported as mean±standard deviations. Significant differences between the mean values were determined at (P <0.01), following MANOVA. The statistical analysis was performed using SPSS V20.

Results and Discussion

Total polyphenols, flavonoids, and tannins

The phenolic contents are represented in Table 1, the results were ranked from 62.79±5.98 mg gallic acid equivalent (GAE)/g dry weight to 212.3±4.2 mg/g dry weight for the polyphenols, from 6.11±0.29 to 23.99±0.86 mg catechin equivalent (CE)/g dry weight for the flavonoids and from 6.25±0.74 to 6.73±0.75 mg gallic acid equivalent (GAE)/g dry weight for tannins.Phenols comprise a great variety of compounds, such as polyphenols, flavonoids, condensed tannins[33]. The result showed that the ethyl acetate obtained from fractionation of flavonoids contains the highest quantity of phenolics compound followed by the ethyl acetate of tannins, whereas the n-butanol had the lowest value which may be referred to the polarity of the solvent.

Comparing the contents on polyphenols and flavonoids of the plant part revealed that the seeds are significantly richer than the whole plant (stems, flower, and root), however, the study of Yung-Shin Shyu[34], showed that dill flower contains more polyphenols and flavonoids than the leaf, seeds and root[35] respectively, the flower contents is 196.65 mg/g dried extract, 67.10 mg/g dried extract polyphenols and flavonoids respectively. The fresh herb comprises more flavonoids than the dry samples used in this study, where the HPLC analysis showed that the flavonoid content up to 100 mg/g fresh weight[36]. The use of bio-fertilizers on dill affect the flavonoids contents[37] which confirm that the stress induces more production of bioactive nevertheless the plant and seed coming from salty soil are significantly containing more polyphenols and flavonoids than the plant and seed in normal soil condition and is important to note that the result obtained in this study showed that *A. graveolens* original from Algeria contains more polyphenols and flavonoids than wild dill[38]. For the tannins, there is no significance between the samples, where the plant, seeds, non-salt and salt conditions contain almost the same quantity of tannins.

Antifungal activity

Mycelial growth

One day after the incubation of the fungus in PDA without extracts, *P. tritici-repentis* was grown 4 mm, whereas, the growth of *R. secalis* was relatively slow by 2 mm, the growth of two fungi in different extracts was shown in the Figs. (1–4).

The results indicated that;the mycelial growth was considerably reduced with increasing concentration of *A. graveolens* extracts while their growth increased with incubation time. From the Figs. (1,2) the mycelial growth of *P. tritici-repentis* by the plant extract was reduced at 5 mm for 10 mg/mL of hexanoic non-salt plant extract, followed by aqueous extract (7 mm), methanolic extract (9 mm) and

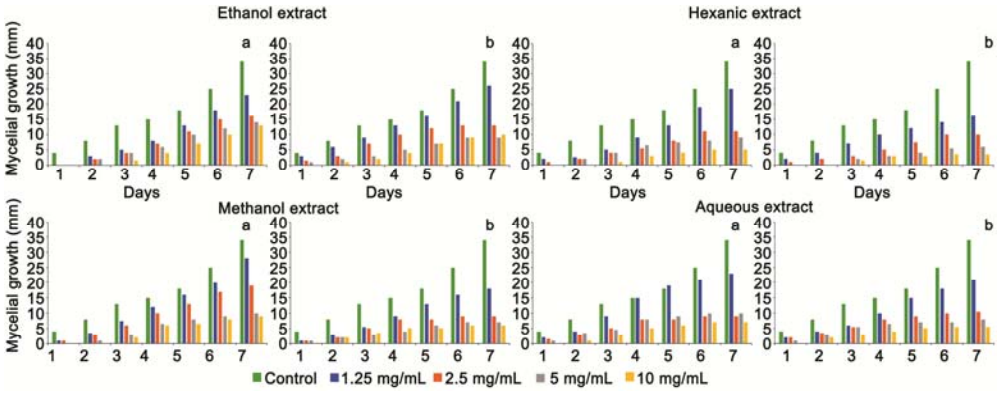

Fig. 1 — Mycelial growth of *Pyrenophora tritici-repentis* in dill, non-salt plant(a) and non-salt seed extracts (b).

Table 1 — Phenolics contents in dill plant and seed extracts, and various soluble fraction (mg/g)

	Polyphenols[T,F,E]			Flavonoids[T,F]		Tannins[E]
Extracts	T	F	E	T	F	E
Non-salt plant	66.66 ± 5.98	121.04 ± 4.31	78.40 ± 5.94	12.33 ± 0.41	8.25 ± 0.60	6.68 ± 0.70
Non-salt seed	142.19 ± 2.5	153.12 ± 6.43	62.79 ± 5.98	7.23 ± 1.17	6.11 ± 0.29	6.58 ± 0.38
Salt plant	76.01 ± 9.63	96.54 ± 9.63	87.79 ± 7.57	7.85 ± 0.12	23.99 ± 0.86	6.25 ± 0.74
Salt seed	212.30 ± 4.20	167.70 ± 9.47	90.56 ± 9.44	6.88 ± 0.18	12.63 ± 0.43	6.73 ± 0.75

T- tannins ethylacetate extract, F-flavonoids n-butanol extract, and E-ethylacetate extract

ethanolic extract (13 mm) respectively. For the salt plant extract the hexanoic extract reduced the mycelial growth at 3 mm by the concentration 10 mg/mL and by the same concentration the aqueous extract stopped the growth at 4 mm, the highest mycelial growth showed by the methanolic extract by 7 mm. the non-salt seed extracts showed the best mycelial reduction by 10 mg/mL of the hexanoic extract followed by the same concentration of aqueous extract, however, the salt seeds extract reduced the mycelial growth as well as the non-salt seeds extract for the hexanoic extract but reduced more for the ethanolic and methanolic extract (5 and 4.5mm) respectively.

The drop of mycelial growth of *R. secalis*is seen in Figs. 3,4. The growth stopped at 3.5 mm for the ethanolic extract and 4.5 mm for the hexanoic and aqueous extract of non-salt plant while it was reduced at 2.5 mm for the methanolic salt plant extract with the same concentration of 10 mg/mL, the non-salt

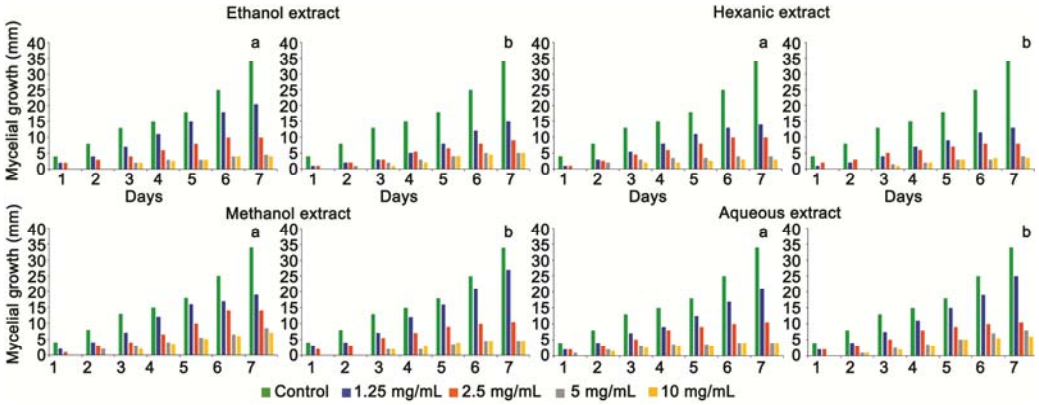

Fig. 2 — Mycelial growth of *Pyrenophora tritici-repentis* in dill, salt plant (a) and salt seed extracts (b).

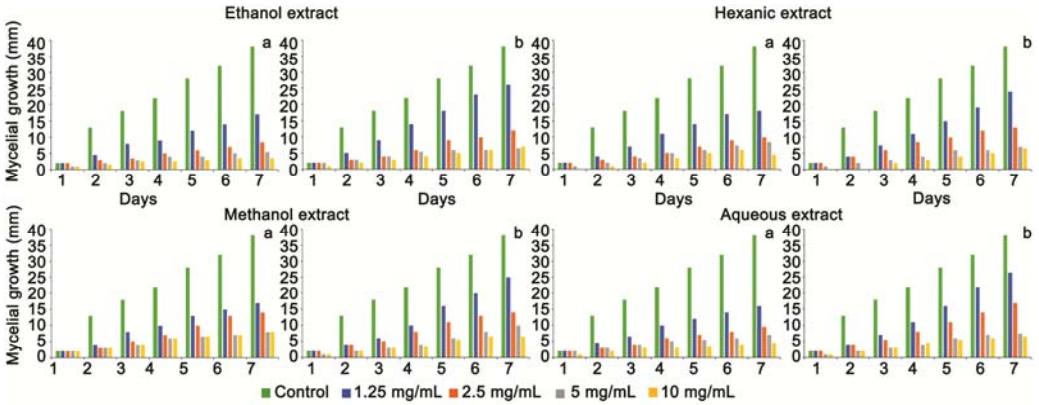

Fig. 3 — Mycelial growth of *Rhynchosporium secalis* in dill, non-salt plant (a) and non-salt seed extracts (b).

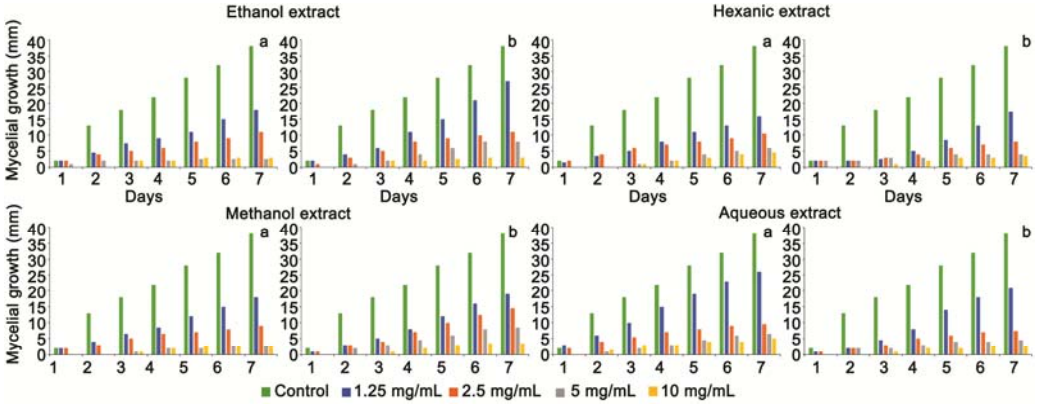

Fig. 4 — Mycelial growth of *Rhynchosporium secalis*in dill, salt plant (a) and salt seed extracts (b).

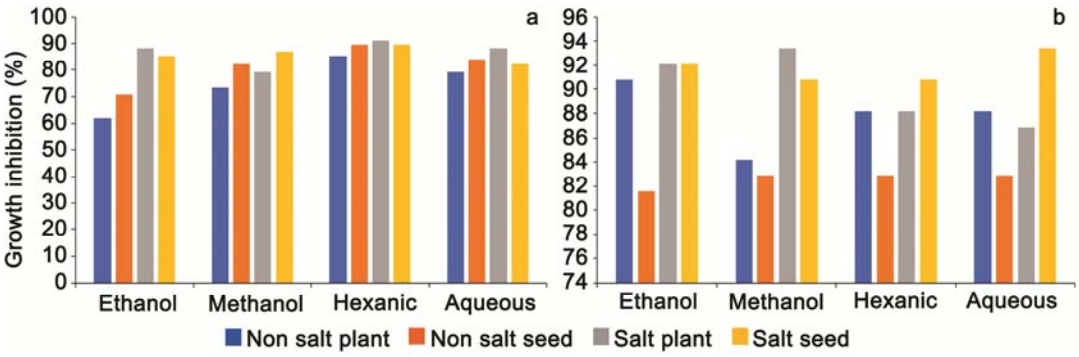

Fig. 5 — Inhibition rate of the fungi in different dill extracts of salt and non-salt plant and seeds, *Pyrenophora tritici-repentis* (a) and *Rhynchosporium secalis* (b).

seeds extract showed equal mycelial growth reduction at 10 mg/mL of methanolic, hexanoic and aqueous extract (6.5 mm) while the salt seeds extracts at the same concentration reduce the growth at 2.5 mm for the aqueous extract and at 3 mm for the ethanolic extract.

Inhibition rate

The inhibition rate of *P. tritici-repentis* (Fig. 5a) ranged between 61.76 to 91.17% for the plant samples of different extracts and between 70.58 to 89.70% for seed extracts. Fig. 5b shows the inhibition rate of dill extract on *R. secalis* which fluctuated between 84.21 to 93.10% for the Plant extracts; also, it was recorded between 81.57 to 93.42% for seed extracts.

Recently, world demand for effective, safe, and natural products without synthetic chemicals to control food has been high. Aromatic volatile products of plant secondary metabolism have formed the basis of many applications in food flavouring and preservation industries[18,39]. In our study, the different extract of *A. graveolens* showed pronounced antifungal efficacy against all the tested fungi. Mycelium growth was recorded to decrease with increasing concentrations of the extract. However, at 10 mg/mL, the fungal colony of *P. tritici-repentis* was significantly reduced by the hexanoic extract of all the samples which confirm the sensibility of the fungus to the essential oil of dill[18]. This study reported that; dill seed oil is found to be highly effective for controlling the growth of *A. niger* and *A. flavus* at doses of 6 mL by poison food techniques. On the other hand, *R. secalis* was more affected by the ethanolic extract.The plant and seeds obtained from salty soil condition showed significant antifungal activity on both fungi than the samples from non-salt conditions.

In vitro studies on *A. graveolens* illustrate its potential as an ideal antifungal agent against fungi. Therefore, the present results clearly demonstrate that the different extract of *A. graveolens* significantly reduces the development of two fungus pathogens for wheat and barley which are a very important crop worldwide. The *in vivo* experiment shows that contact of produce to the vapours may persuade inferior confrontation to fungal challenge[40]. Nevertheless, higher plant extract concentrations reported necessary in the field than in laboratory[41], which may be available for favourable conditions to fungus development.

Conclusion

Wheat and barley are the main food crop in Algeria and many other countries. These crops are susceptible to attack by leaf scald and tan spot caused by *Rhynchosporium secalis* in barley and *Pyrenophora tritici-repentis* in wheat, respectively. The present study showed that dill original from Algeria has antifungal activity against cereals fungus pathogens, however, the saline stress has significantly increased this activity. *Anethum graveolens*, is a rich aromatic plant with the phenolics contents, where the seeds are advised to be more explored, whereas the soil salinity increase significantly the phenolic contents in the plant and seeds. Dill could be one of the best eco-friendly fungicides. Furthermore, a study of the impact of different level of salinity on the production of a secondary metabolite is highly needed.

Conflict of interest

The authors have no conflict to declare.

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