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The antifungal activity of *Artemisia herba-alba* aqueous extract and essential oil against storage fungus *Alternaria* spp and *Fusarium* spp

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Abstract

The purpose of this study was to evaluate the antifungal activity of aqueous extract and essential oil of *Artemisia herba-alba* on some specific storage fungi. This plant has been selected on the basis of its ethnobotanical uses. Different concentration of aqueous extract (20, 25 and 30%) and essential oil (0.15, 0.175, 0.200 and 0.250%) were evaluated *in vitro* for their antifungal activity against *Fusarium* spp and *Alternaria spp*. The concentration of 30% of extracts inhibits the growth of *Alternaria* while 0,025 % of essential oil recorded a good antifungal activity. On the other hand, the aqueous extracts showed better efficacy than essential oil on *Fusarium* spp and *Alternaria* spp. Therefore the *Artemisia herba-alba* aqueous extract and its richness of secondary metabolites (flavonoids, alkaloids, tannins, saponins and steroids) could be considered as a potential source of treatment of plant diseases, this study presents the different results obtained.

Keywords: *Artemisia herba-alba.* Aqueous extract. Essential oils. *Alternaria. Fusarium.* Antifungal activity.

INTRODUCTION

Crop protection plays a significant role in ensuring human food security and the quality of their food. Plant pathogens are probably the biggest constraint of agricultural production and crop yields [1]. Cereals, a strategic crop with high stakes in the nationwide food security acquisition process, can be contaminated by many fungal pathogens like *Aspergillus, Alternaria, Penicillium* and *Fusarium*. Their spores are scattered in the air. It can come from the fields or the dust existing in the infrastructures of storage.

The deterioration of stored cereals is the subject of many studies which have shown that fungal contamination of cereal seeds is one of the main causes, engendering considerable losses and a variation in the technological factors of cereals grain [2,3].

Many molds present in seed lots or in the impurities that accompany them are capable during their development to produce toxic substance like the mycotoxins, it's the metabolites synthesized by the fungi themselves. They can be produced during the entire phenological cycle of the plant but also at the harvest time. The storage conditions are often implicated in the deterioration of the grain quality by mold development [4].

They estimated that globally, 23 million kg of synthetic fungicides are used annually. However, the use of chemical fungicides can lead in the medium and long term to several problems such as environmental pollution, ecotoxicity phenomena whereas the recurrent use may cause the appearance of resistant pathogens [5]. Hence it is necessary to develop new alternative "green" methods for crop protection [5]. These components naturally are in medicinal plants which give them a therapeutic activity thanks to the presence of chemicals such as essential oils, saponins, flavonoids and alkaloids. These substances possess therapeutic virtues whose fields of application are very varied, particularly in the food industry and agriculture.

Currently, Great efforts have been directed at alternative or complementary sources to synthetic fungicides. [6] These non-toxic substances are easily biodegradable and safe for the environment [7].

In this context, this study aims to test the effectiveness of aqueous extract and essential oil of *Artemisia herba-alba* on both the inhibition of the growth and the antifungal activity of some fungi responsible of the cereals grains deterioration in post-harvest period (*Alternaria* spp and *Fusarium* spp) aims to develop natural substances alternatives to chemical control used in agriculture.

METHODOLOGY

Plant material

The aerial part of *Artemisia herba-alba* at the vegetative stage was harvested in the region of Illizi (southeastern Algeria) (Fig. 1). The plant material was washed in the laboratory, then dried in the dark in a cool well-ventilated place.The dry plant material was powdered, Using a mechanical grinder after that placed in hermetically sealed glass containers until use.

Photo 1: Aerial and roots parts of *Artemisia herba-alba* Asso (Asteraceae)

Preparation of aqueous extracts

First, to prepare the aqueous extract of the plant *Artemisia herba-alba* Asso by maceration method**,** 10 g of powder of *Artemisia herba-alba* aerial part was soaked in 100 ml of distilled water (w /v) with stirring for 2h at a temperature of 25 °C [8]. Then the mixture was filtered through Wattman # 1 filter paper and then centrifuged at 3600 rpm for 15 min at 4 °C. The filtrate was sterilized by microfilters with 0.22 µm. In the end, the extract obtained was recovered in a sterile bottle and stored at 4 °C away from light until it is used.

Extraction of essential oil

The hydrodistillation of fresh aerial parts (100 g) of *Artemisia herba-alba* Asso for 3 hours, in a Clevenger-type apparatus according to the British Pharmacopoeia.

Phytochemical tests

Phytochemical tests were techniques facilitate to determine the different chemical families contained in a plant organ. Photochemical groups are numerous, but the main phytochemical groups were detected by simple qualitative methods including alkaloids [9], polyphenols (flavonoids, anthocyanins, tannins) [10], the saponins, steroids, terpenes [11].

Antifungal test

To evaluate the antifungal activity, Potato Dextrose Agar medium (PDA) was used which contains in one liter: Potato 200g, Glucose 20g, Agar 15g and PH 5.6 ± 0.2. The funguses used in this study were *Alternaria* spp and *Fusarium* spp. The strains were obtained from the collection of the Saharan bio-resources laboratory, Faculty of Natural and Life Sciences (University of Ouargla). The tested strains were incubated at 25 °C in Petri dishes (9 cm diameter) on PDA for 7 days. (Photo 2).

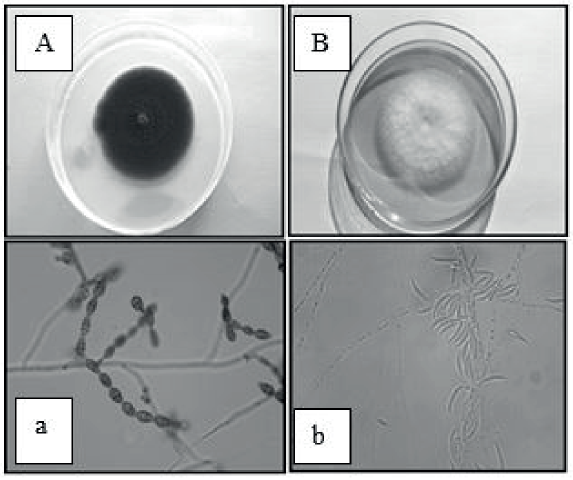

Photo 2: The macro and microscopic aspect of the isolated strains from durum wheat seeds (Left: *Alternaria infectoria*. Right: *Fusarium* spp) (x400).

The direct contact method was applied to test the sensitivity of fungal strains to the aqueous extract or essential oils. To create multiple extract concentration, liquid PDA at a temperature of 56°C was mixed with different volumes of the aqueous extract to final concentration 20, 25 and 30%. As for the essential oil, different volumes of the oil and 0.5% of Tween20 were added to the PDA to final concentrations 0.15, 0.175, 0.20 and 0.25%. To homogenate, extract/PDA mixture was soaked for a few minutes. In Petri dishes (60 mm Ø), 7ml from each concentration was poured in triplicate and left to solidify.

A mycelial disc of 6 mm in diameter was deposited aseptically on the surface of the extract/PDA medium in the center of the dish. In parallel, the control test was performed in PDA medium without any extract. Finely the incubation was carried out in an oven at a temperature of 20°C for 72 hours. The observations and radial growth measurements in each colony were made daily. The inhibition percentage [12] and the mycelial growth speed [13] of each concentration are determined by the following formulas.

Inhibition Percentage (IP) :

$$IP (\%) = (Cd-Td) / Cd \times 100$$

Cd: Colony diameter of the control (mm).
Td: Colony diameter at the test plate (mm)

Mycelial Growth Speed (MGS) :

$$MGS = [D1/T1] + [(D2-D1)/T2] + [(D3-D2)/T3] + \ldots + [(Dn-Dn-1)/Tn]$$

D: diameter of the daily growth zone.
T: incubation time.

Statistical analysis

Analysis of variance (ANOVA) was used for data analysis using CoStat-Statistics Software version 6.4. The significance of the differences among treated samples was evaluated using the least significant difference (LSD) test for multiple comparisons of the means of the growth diameter of mycelia. Each experiment has three replicates. Three determinations were conducted and the significance level for all measurements was considered at .P <0.05.

RESULTS

Antifungal test

The inhibition percentage results and the mycelial growth speed under the effect of the aqueous extract and the essential oils of *Artemisia herba-alba* are shown in Table 1

Table 1: The effect of aqueous extract and essential oils of *Artemisia herba-alba* on the inhibition percentage (IP) and the mycelium growth Seeped (MGS) of *Alternaria* spp. and *Fusarium* spp.

Parameters	IP (%)		MGS (mm/h)	
Fungal strains / Traitement (%)	*Alternaria* spp	*Fusarium* spp	*Alternaria spp*	*Fusarium spp*
Control	--	--	0.65^a ± 0,01	0,65^a ±0,008
Aqueous extract				
20	94,32^a ± 4.65	57,44^c ± 1,22	0.03^b ± 0.01	0,18^b ±0,003
25	97,16^a ± 2,84	62,40^b ±0,70	0.006^b ± 0 .001	0,14^c ± 0,01
30	100^a ± 0	71,27^a ± 0,62	0^b	0,13^c ± 0,005
Essential oil				
0,150	62,40^c ± 3,94	73,04^b ± 2,55	0.14^b ± 0.01	0.10^b ± 0.008
0,175	68,08^{bc} ± 2,45	68,08^b ± 3,25	0.10^{bc} ± 0.008	0.10^b ± 0.008
0,200	74,46^{ab} ± 3,68	73,75^{ab} ±6,05	0.08^c ± 0.01	0.07^b ± 0.02
0,250	76,59^a ± 1,22	82,97^a ± 1,22	0.07^c ± 0.005	0.06^b ± 0.01

The data represented as mean ± SEM of three replicates. Different letters (a,b,c . indicate significant differences (LSD test; P <0,05)

The result shows that the mycelial growth of *Alternaria* spp was affected by the *Artemisia herba-alba* aqueous extracts had not a significance. The concentration used at the 30 % we observed that the growth was completely inhibited. at 25 and 20 % we notice a partially inhibited with 79.16 and 94.32 respectively.

On the other hand, *Fusariu m* spp also was significantly affected by the different treatment of *Artemisia herba-alba* Extracts, the inhibition increased with the concentration of aqueous extract. At the concentration 30% of the aqueous extract *Fusarium* was partially inhibited with inhibition value 71.27%, whereas in the concentration 20% of aqueous extract the IP 57.44% (Table I).

Moreover, the effect of essential oil was recorded that the IP of *Alternaria* and *Fusarium* increased significantly with extract concentration. The values of inhibition percentage were 76,59 and 82,97% at the highest concentration (0.25%) respectively but with the lowest concentration (0.15%) the inhibition percentage was 62.40 and 73.04% at *Alternaria* and *Fusarium* respectively (Table I).

It is important to note that, the two strains tested showed different degrees of sensitivity to the extracts; in particular, the *Fusarium* shows a certain resistance compared to the *Alternaria* was the most sensitive at aqueous extract. As for the essential oil, the two species have the same response.

the mycelial growth speed was decreased with the increasing aqueous extract and essential oil concentration; at 30 % aqueous extract concentration showed that the most values of speed decrease to 0.13mm /h at *Fusarium*. For essential oil, it was noted that the speed decline with percentages of 89.23and 90.76% in *Alternaria* and *Fusarium* respectively.

Phytochemical tests

The phytochemical tests of *Artemisia herba-alba* aqueous extract were carried out qualitatively using standard procedures to identify the major phytochemical constituents.

The result of phytochemicals screening was summarized in Photo 3. Actually, six groups of bioactive compounds were detected: flavonoids, alkaloids, tannins, saponins, steroids and anthocyanins.

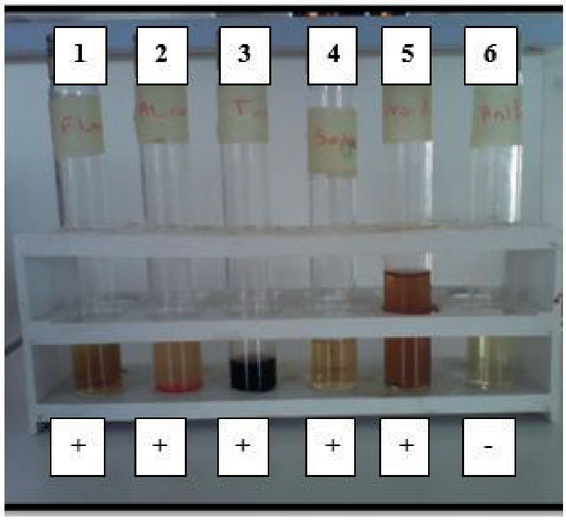

Photo 3: qualitative test for detected the presence and absence of major secondary metabolite groups for the *Artemisia herba alba* aqueous extract (1: Flavonoids, 2: Alkaloids, 3: Tannins, 4: Saponins, 5: Steroids, 6: Anthocyanin. + = Present and – = Absent).

The results noted the presence of flavonoids by the coloration of the aqueous extract with dark yellow, and yellow precipitation which indicates the presence of the alkaloids, while the gallic tannins were stained by a black-blue. On the other hand, the presence of the saponins are indicated by the appearance of foam and steroids by a change of color in purple and turns to blue then green *whereas* we noted the absence of anthocyanin in the aqueous extract.

DISCUSSION

Certainly, the biological control through the use of a natural alternative has given a lot of interest. Many researchers have noted the possibility of using the plant extract as an effective natural alternative.

The direct contact technique unsure the contact between the microorganism and the natural extract and the result are expressed by the microorganism growth inhibition. The aqueous extract of *Artemisia herba-alba* has antifungal activity against the fungi *Fusarium* and *Alternaria*. The mycelium growth was decreased as the concentration of the aqueous extract was raised and the total inhibition for *Alternaria* was achieved in 30% of the aqueous extract.

In the study of Fawzi et al., [14], the results show that the aqueous extract of plants *Cymbopogon Proximus*, *Zeylanicum Cinnamomum*, *Laurus nobilis*, *Persea americana* and *Zingiber officinal* antifungal effect on *Alternaria alternata* was increasing with the concentration of the extract, which indicates the importance of the extract concentration on the fungal inhibition.

Among the phytochemicals, the presence of flavonoids, alkaloids, steroids, tannins, and saponins was noticed. Our results were confirmed by other studies showing that the phytochemical screening of aqueous extracts of *Artemisia herba-alba* is an important source of polyphenols [15]. This class mainly includes tannins, flavonoids with the absence of anthocyanins. Tannins isolated from medicinal plants proved to be effective fungicide [16]. Saponins are a special class of glycosides which have both a soapy characteristic and a very good antifungal activity [17]. The antifungal activity of aqueous extract can be explained by the synergistic effect between the different extract compounds. Indeed, the majority of compounds are often responsible for the antifungal activity of this extract [18].

The study of the inhibitory effect of *Artemisia herba-alba* essential oil on *Alternaria* and *Fusarium* shows that the mycelium growth speed was decreased whereas the concentration increases to 72.56 and 86, 87% for *Alternaria* and *Fusarium* at concentration 0.25% of essential oil.

This result was confirmed by many experiments, that the antifungal activity of *Artemisia herba-alba* essential oil on the species of *Fusarium moniliforme*, *Fusarium solani*, *Fusarium oxysporum* is revealed by the absence or presence of mycelial growth. The increase of the mycelial growth was signed in the concentrations which correspond to the absence of essential oil (control).

On the other hand, the mycelial growth decrease whereas the concentration of essential oil increases until the total inhibition with the concentration 0.75% [19].

The essential oil of *Cistus* has been tested against seven molds: *Rhizopus*, *Mucor, Alternaria*, *Fusarium, Penicillium, Trichoderma* and *Aspergillus*. The oil has been very active on all strains. However, this activity depends on the concentration of oil and mold [20]. The essential oil has shown good antifungal activity. The molds showed an increased sensitivity to the increase of the concentration of the essential oil *in vitro,* where the diameter of the colony was reduced each time the dose of the essential oil was increased to an inhibition where no growth is observed. the degree of antifungal activity was proportional to the concentration of essential oil [21].

The advantage of essential oils of plants has a characteristic that makes them attractive for the protection of stored products such as cereal seeds against the fungi and even the blockage of their ecotoxigenesis [22].

In general, the results obtained with the different concentrations of aqueous extract and essential oil reveal that the antifungal activity increases with the concentration increases.

CONCLUSION

In the course of our study, we test the antifungal activity of *Artemisia herba-alba* aqueous extract and essential oil on some storage fungal, as *Alternaria spp.* and *Fusarium spp.* For this objective, the method of direct contact permitted to use the antifungal activity of the extracts about the strains tested. Certainly, the antifungal activity of *Artemisia herba-alba* aqueous extracts was mostly active on both strains, especially at the concentration 30% of *Artemisia herba-alba* aqueous extract. Moreover, *Alternaria* spp presented a sensitive to the aqueous extract with a total inhibition.

For phytochemicals test, the results showed the richness of the *Artemisia herba-alba* aqueous extract in secondary metabolites compound like alkaloids, steroids, tannins, flavonoids, saponins and the absence of anthocyanins. These substances play a determining role in antifungal activity

The antifungal activity of *Artemisia herba-alba* essential oil against *Fusarium* spp. and *Alternaria* spp. can be attributed through the chemical composition of essential oil.

The antifungal activity progress with the improve of concentration of essential oil which induced the regression of mycelial growth. it has been observed by a decrease in diameters.

Finally, our results indicate the *Artemisia herba-alba* aqueous extract and essential oil which presents a good antifungal activity that can be considered as a very promising preservative for the food industry with the reduction of the mycelial growth which is responsible for the alteration of foods.

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