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Biological control assay against date palm diseases by using an aqueous extract of *Zygophyllum album* in the southeast of Algeria

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Abstract
The fungicidal activity of an indigenous plant extracts from the southeastern Algeria *Zygophyllum album* L. was evaluated for its efficiency on date palm phytopathogenic fungi, *Alternaria* sp., *Fusarium* sp., *Phytophtora* sp., *Cladosporium* sp., *Aspergillus niger* and *Pinicilium* sp, the causative agents of date palm diseases. In the aqueous extract, maximum inhibition was observed in *Penicillium* sp. and recorded 86,25% and 81,20% inhibition at 100% and 50%, respectively, followed by *Cladosporium* sp.(D1: 69,16%; D2: 65,90%), *Phytophthora*. (D1: 67, 63%; D2: 65, 03%). On the other hand, the powder of this plant which was mixed with PDA medium inhabited mycelia growth of all the phytopathogenic fungi while *Pénicillium* sp. (71,25%), *Alternaria* sp. (62,66%) and *Phytophthora* sp. (55,93%) had the maximum inhibition rates. These effective plant extracts may contribute to development of potentially effective and environmentally safer alternative fungicide to control date palm diseases caused by the se phytopathogenic fungi.

Keywords: Date palm, *Zygophyllum album*, Biological control, Antifungal activity, Algeria

1. Introduction
The date palm (*Phoenix dactylifera* L.) is considered a symbol of life in the desert, because it tolerates high temperatures, drought and salinity more than many other fruit crop plant species. It is one of the oldest trees from which man has derived benefit, and it has been cultivated since ancient times. It the only indigenous wild desert plant definitely domesticated in its native harsh environments appears to be the date palm [1]. Date palms can be subject to many diseases or complexes of diseases, among which some, which are serious such as *Diplodia phoenicum, Thielaviopsis paradoxa, Phytophthora*sp., *Helminthosporium* sp., *Stemphyium botryosum, Alternaria* sp., *Cladosporium* sp., *Penicillium, Stemphylium* [2].
In order to look for other alternatives of biological control against aggressive pathogenic fungi of date palm, several studies regarding the action of plant extracts against some phytopathogenic fungi have been performed. Most of the plants contain several compounds with antifungal properties for protection against pathogenic agents. *Zygophyllum album* is one of these plants which is rich by antifungal compounds and used traditionally in Algeria for the treatment of different types of diseases [3].
The aim of the study was to evaluate the antifungal activity of an aqueous extract of a native plant used in traditional medicine in the southeast of Algeriaagainst deferent phytopathogenic fungi of date palm.

2. Material and methods
2.1. Study area
This study was conducted in the region of Sidi Mehdi, Which is an area located in the Southeastern part of Algeria (Fig. 1). It is a saharian region with one dry period throughout the year (Fig. 2). This very low region is located at an altitude of 69 m at 06°4’ E and 33°7’N. This area is approximately 07 km of Touggourt on the road leading to the airport [4].

Fig 1: Study area

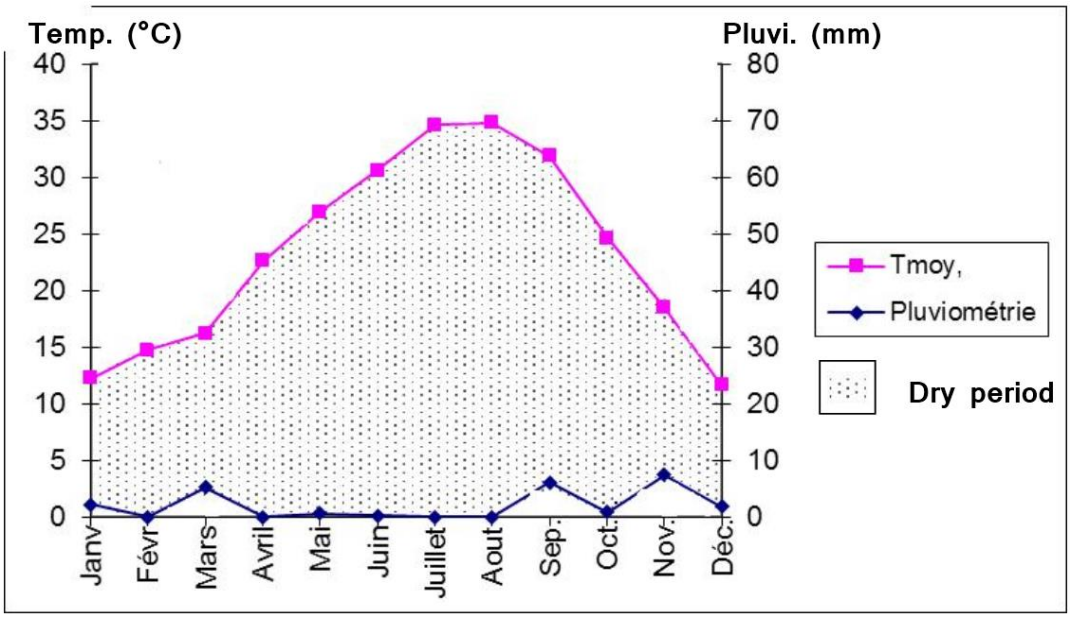

Fig 2: Ombrothermic diagram of Gaussen in the region of Touggourt region in 2014

2.2. Isolation of pathogen fungi

Infected vascular tissues from stem, leaflet and root regions of date palm cultivar showing symptoms were collected separately from farmer's field in the regions of Megarine and Beldet Omar which are situated in the southeastern of Algeria (Fig. 03).

Fig 3: Symptoms of date palm diseases

Samples were bringing to the plant pathology laboratory in the INRAA station for the seeding and the purification of date palm fungi. Fragments of reached parts from 5 to 10 mm presenting of the typical symptoms are cut out then planted in a suitable culture medium after disinfection, rinsing with sterile distilled water, and then drying (Fig. 04). Incubation takes place at a temperature between 24-26 °C.

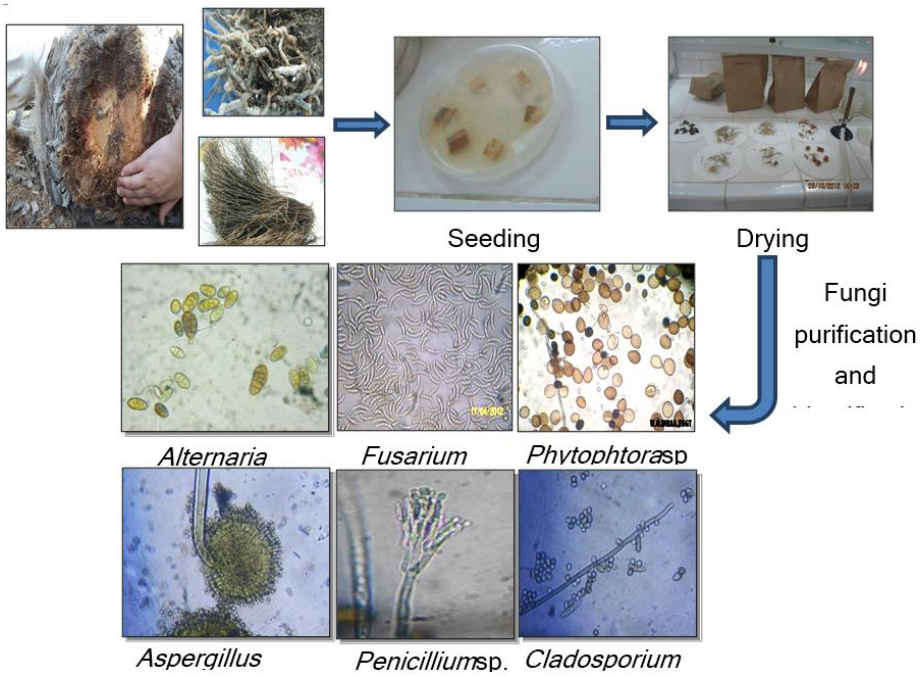

Fig 4: Fungi seeding and purification

2.2.1. The isolated fungi

- ***Phytophthora* sp.:** Belâat disease is caused by *Phytophtora* sp. similar to *P. palmivora* [5]. This disease causes a terminal bud decay (heart rot), but initial symptoms are the discoloration of the spear leaf (youngest leaf). Spear leaf will rot and is easily removed [6].
- ***Fusarium* sp.:** It's a soil-borne fungus; a vascular wilt disease of date which can cause discoloration of the vascular tissues in the palm rachis [7].
- ***Aspergillus niger*, *Penicillium* sp., *Cladosporium* sp. and *Alternaria* sp.:** They are saprophytes such as fruit rots, causing a calyx-endrot in the late khalal or early tamar stages [8].

2.3. Plant material

Fresh healthy plant parts of a native plant in Southeast Algeria, *Zygophyllum album* (Fig. 05), were collected from the experimental station of the National Institute of Agronomic Research (INRAA) in Sidi Mehdi region. This plant is one of the medicinal plants which is rich by antifungal compounds and used traditionally in Algeria for the treatment of different types of diseases such as skin diseases, its aqueous extracts are used in the treatment of diarrhea and diabetes; *Z. album* is carminative, anti-septic and stimulant [3].

Fig 5: *Zygophyllum album*

2.4. Preparation of aqueous plant extract and powder

Plant parts were washed with tap water and were surface sterilized by dipping them in 0.1% sodium hypochlorite solution for one minute. After that plant parts were washed with distilled water and were dried at room temperature (25 °C) for about seven days on a laboratory desk. Samples were covered with clean sheets of paper to avoid any deposition of dust. The dried material was ground to a fine powder using a grinding mill and stored in airtight bottles in the dark until extraction was done. According to Sasanelliand Divitto [9], the preparation of plant extract the powdered plant material (25 g of each) was extracted with 150 ml distilled water (Fig. 06). Different concentrations *i.e.*, 100% and 50% of the aqueous extracts were added in the PDA medium.

The dried material was used like it is; by 2g in 180 ml of PDA medium [10].

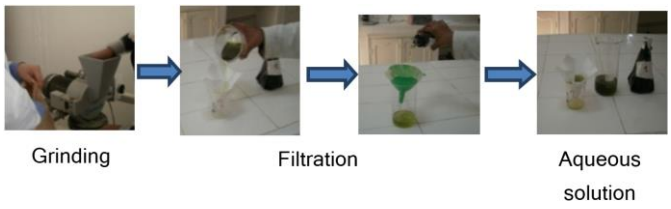

Fig 6: Preparationof the aqueous extract

2.5. *In vitro* bioassay of the antifungal activity

The assay was conducted in the laboratory of plant protection in the experimental station of INRAA; six species of fungi: *Alternaria* sp., *Fusarium* sp., *Phytophtora* sp., *Cladosporium* sp., *Aspergillus niger* and *Piniciliums*p. were isolated from date palm trees and were used as test fungi for antifungal activity assay.

The aqueous extract of *Z. album* was evaluated for its activity against pathogenic fungi of date palm; Disc of any fungi (5 mm) was remove from the edge colonies of active cultures and placed on the center of Petri dishes containing the aqueous extract rolled out with Potato Dextrose Agar (PDA) medium(Fig. 07). Similar dishes of each pathogenic fungus isolates grown in the same manner were placed on different Petri plates and made five replicates and media without plant extract served as control. Cultures were observed daily and recorded for antifungal activity of *Z. album* against pathogenic fungi. Incubation was continued for 7 days in a temperature of 28 °C.

The operation and replications were done also for the powder test; with the mixture of powder into the Potato Dextrose Agar (PDA) medium (Fig. 08).

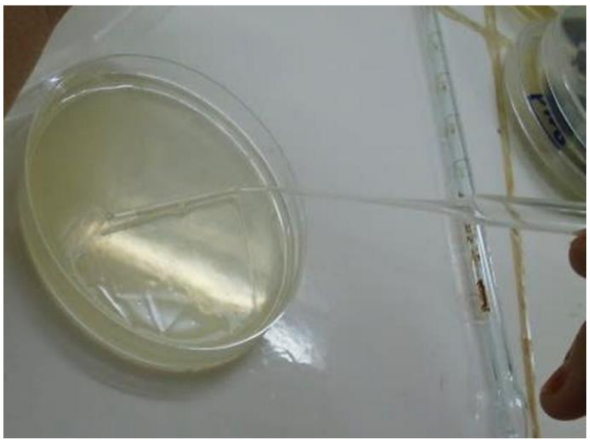

Fig 7: Mixing the aqueous extract

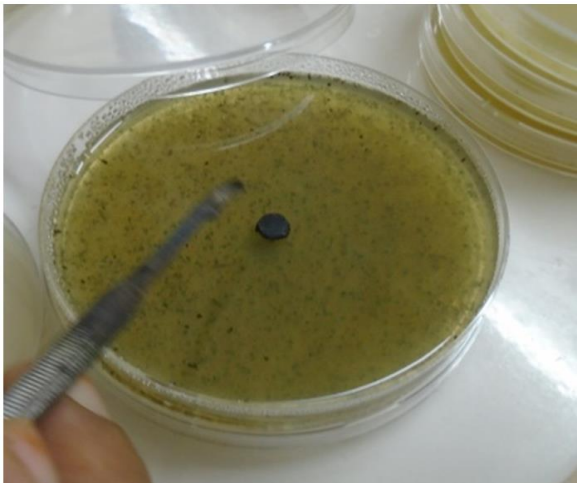

Fig 8: Mixing the powder

The evolution of mycelia growth is performed every 24 hours by measuring the diameter of the colony of the pathogen. The valuation of inhibition by the aqueous extract is estimated by calculating the percentage inhibition of mycelia growth by the following formula:

$$I\,\% = \left[\frac{Cn}{Co}\right] x\ 100$$

Cn: average diameter of colonies of pathogen in the presence of extract.

Co: average diameter of colonies of control.

3. Results

3.1. Antifungal activity

The present study tested the antifungal activity of medicinal plant (*Zygophyllum album*) extracts and its respective dilutions against six pathogenic fungi have isolated from date palm trees (*Alternaria* sp., *Fusarium* sp., *Phytophtora* sp., *Cladosporium* sp., *Aspergillus niger* and *Pinicilium* sp.), this medicinal plant was chosen based on traditional usage.

According to the data showed in the table below, all the fungus were inhibited by this plant extract and powder.

Table 1: Inhibition percentages of *Z. album* against the isolates fungi from date palm trees

Fungi	Extract		Powder
	Dose 1	Dose 2	
Fusarium sp.	53,03%	50,69%	42,21%
Phytophthora sp.	67,63%	65,03%	55,93%
Cladosporium sp.	69,16%	65,90%	34,48%
Aspergillus niger	44,43%	40,55%	40,98%
Penicillium sp.	86,25%	81,20%	71,25%
Alternaria sp.	53,33%	50,33%	62,66%

The inhibitory activities of extracts of *Z. album* on the fungal species tested are shown in the last table. Generally, the inhibition percentage increased with increase in concentration of extract. Throughout the experimentation, we have observed that all the Petri dishes which contain the aqueous extract have released an oily substance that increased the development of all the fungi.

The aqueous extract of *Z. album* inhibited the mycelium growth of *Fusarium* sp. and let this fungus changed its aspect and color from pinkish white to canary yellow; after six days of incubation we had an inhibition percentage 53,03% for the first dose (100%) but for the second dose (50%) the inhibition increased to 50,69%, all the Petri dishes of this test showed a high difference in the development of *Fusarium* sp. with the deformation of spores and changed its color (Fig. 9C). In the powder test, we noted also an inhibition percentage of 42,21% against this fungus.

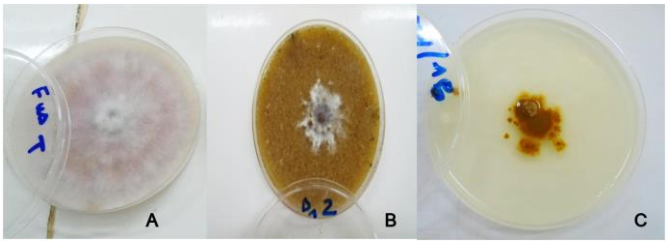

Fig 9: Antifungal activity of *Z. album* against *Fusarium* sp. (A: control; B: with powder; C: with extract)

For *Phytophthora* sp.it was inhibited by the aqueous extract of *Z. album* in the two concentrations and the powder of it (Fig. 10). The crud of this extract exhibited a percentage of 67,63% and the concentration of (50%) showed a rate of 65,03%, and the powder of this plant; represented an inhibition percentage about 55,95%.

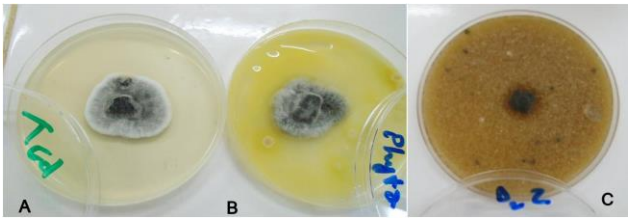

Fig 10: Antifungal activity of *Z. album* against *Phytophthora*sp. (A: control; B: with extract; C: with powder)

For the *Cladosporium* sp., the 100% concentration of *Z. album* extract has inhibited the growth of *Cladosporium* sp. with a percentage of 69,16% and the concentration of (50%) showed a rate of 65,90%, not like the powder of this plant; which represent an inhibition percentage less than the extract with 34,48% (Fig. 11).

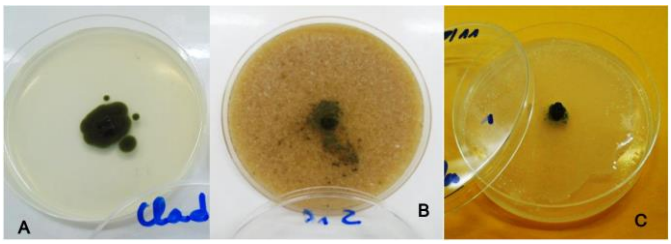

Fig 11: Antifungal activity of *Z. album* against *Cladosporium* sp. (**A:** control; **B:** with powder; **C:** with extract)

The aqueous extract of *Z. album* inhibited the mycelium growth of *Aspergillus niger* after six days of incubation with inhibition percentage 44,43% for the first dose (100%) but for the second dose (50%) the inhibition increased to 40,55%. In the powder test, we noted also an inhibition percentage of 40,98% against this fungus (Fig. 12).

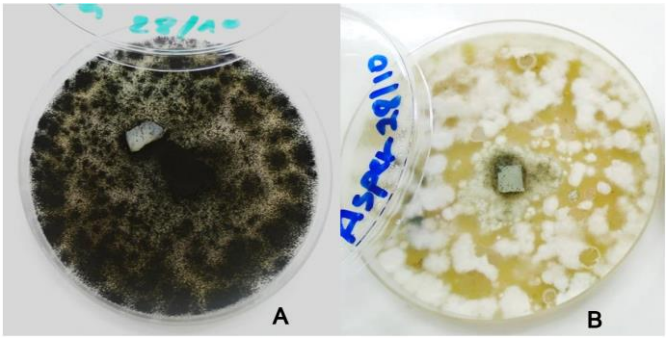

Fig 12: Antifungal activity of *Z. album* against *Aspergillus niger* (A: control; B: with powder)

However, the significant inhibition was observed in the Petri dishes of *Penicillium* sp. with the aqueous extract and the powder of *Z. album*. *Penicillium* sp. was inhibited by the aqueous extract of *Z. album* in the two concentrations and the powder of it (Fig. 13). The crud of this extract exhibited a percentage of 86,25% and the concentration of (50%) showed a rate of 81,20%, and the powder of this plant; represented an inhibition percentage about 71,25%.

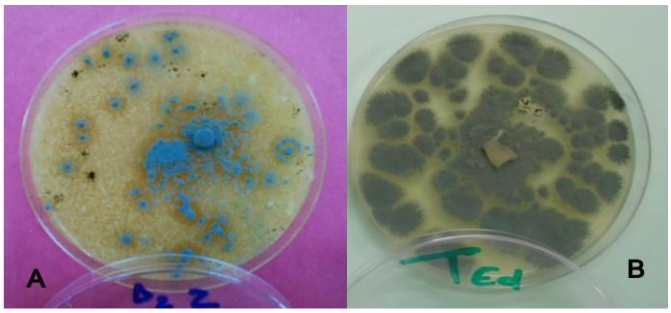

Fig 13: Antifungal activity of *Z. album* against *Penicilliumsp.* (A: with powder; B: control)

At last, we have the inhibition of *Alternaria* sp. by the aqueous extract and the powder of *Zygophyllum album* (Fig. 14). This extract inhibited the mycelia growth of *Alternaria* sp. in the first concentration (100%) with a rate of 53,33% and the second concentration (50%) with 50,33%. Unlike, the powder effect was noted higher than the extract with an inhibition percentage of 62,66%.

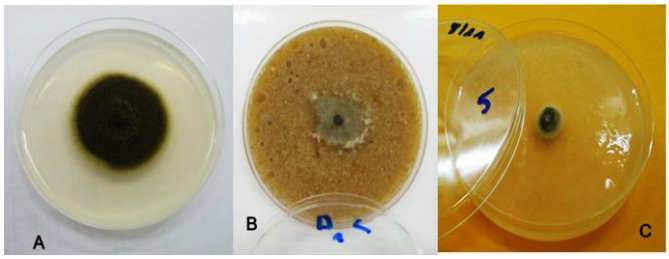

Fig 14: Antifungal activity of *Z. album* against *Alternaria* sp. (A: control; B: with powder; C: with extract)

Comparison of the growth inhibition of the crude extracts and their respective dilutions shows a strongdependent effect on extract concentrations. In general, the antifungal activity of extract dilutions is weaker compared to crude extract. Same remarks were obtained in the powder test against these fungi. These results revealed that antifungal activity of the crude extracts was enhanced by increasing the concentration of the extracts, in effect; the inhibition activity of the extracts was concentration dependent.

The aqueous extract of *Z. album* showed a fort inhibition against these fungi which were isolated from date palm trees. The greatest inhibition percentage was *Penicillium*sp. (D1: 86,25%, D2: 81,20%) followed by *Cladosporium*sp. (D1: 69, 16%, D2: 65,90%) and *Phytophthora*sp. (D1:67,63%, D2: 65,03%). The powder of this plant, on the other hand, *Penicillium* sp. was also the biggest one (71,25%), then we have *Alternaria* sp. (62,66%) followed by *Phytophthora* sp. (55,93%). No inhibition was observed in the negative control. In conclusion, the fungal species used in this study showed variable sensitivity to extracts of *Z. album*.

After few days of treatment, the visual observations showed us a big curb in all the Petri dishes; with microscopic observations, all spores of fungi were destroyed from this extract which is very clear in the photos below.

For *Alternaria* sp., after three days of mycelia growth following, we have observed that the extract curbed its development with microscopic observation; we have noted a destruction of *Alternaria* sp. spores (Fig. 15).

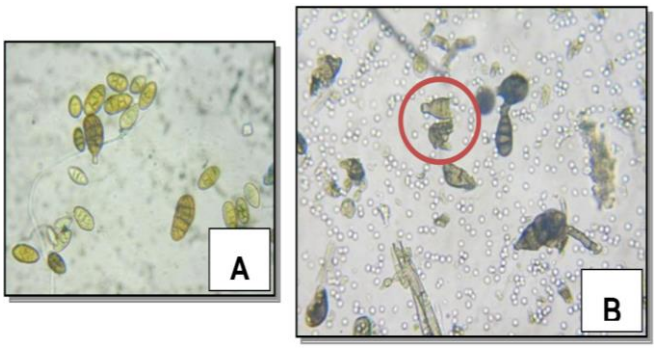

Fig 15: Microscopic aspect of *Alternaria* sp. (A: control; B: destruction of spores)

For *Phytophthora* sp., after three days of mycelia growth following, we observed that the extract inhibited its development. According to microscopic observation, we have noted a deformation and destruction of *Phytophthora* sp. spores (Fig. 16).

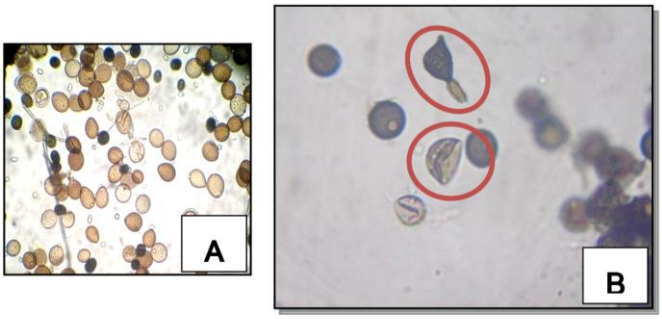

Fig 16: Microscopic aspect of *Phytophthora* sp. (A: control; B: deformation and destruction of spores)

For *Fusarium* sp., after three days of mycelia growth following, we have observed that the extract stopped its development. Among microscopic observation, we have noted a deformation of *Fusarium* sp. spores with formation of chlamydospores (conservation form) (Fig. 17).

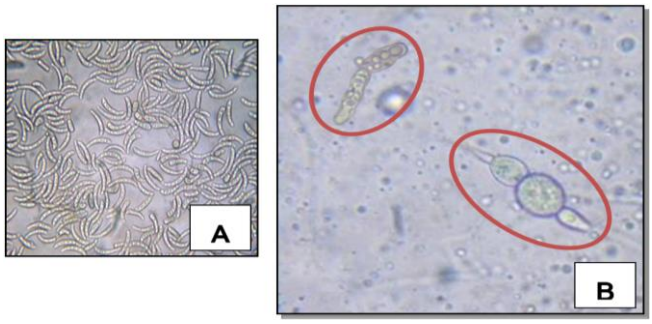

Fig 17: Microscopic aspect of *Fusarium* sp. (A: control; B: deformation of spores with formation of Chlamydospores)

4. Discussion

Most of plants contain several compounds with antifungal properties for protection against aggressor agents, especially microorganisms. As a biological control, several plant extracts were testified in antifungal activities against many pathogenic fungi of different cultures and many species belonging to the genus *Zygophyllum* which have been used in traditional medicine for many centuries in several regions of the world like: *Zygophyllum fabago* [11]; *Zygophyllum album* [12]; *Zygophyllum cornutum* [13, 14]. No reports are available on using of *Z. album* plantand in controlling fungi; but [12] mentioned that the leaves of *Z. album* contain about 296.83cmg GA/g DW of Phenolic content, 216.41c mg Q/g DW of Flavonoïds contents, 5.65cIC50 ìg/ml of DPPH· scavenging activity and 220c IC_{50} ìg/ml of Metal chelating activity. So we recommend utility this plant extracts as effective eco-friendly agents for an antifungal control.

The obtained data from our study showed that the aqueous extracts and the powder of *Z. album* were potent and exhibited an antifungal activity against these pathogenic fungi that are isolated from date palm.

According to several studies of the antifungal activity of different plant extracts against pathogenic fungi of numerous cultures around the world, we find a significant reduction in growth of *A. niger* which was observed with extracts of five medicinal plants and the extracts showed significant differences in their mode of action [15].

The antifungal activity of ethanol extract of *Lowsonia inermis* and *Psidium guajava* against Fusarium wilt in tomato was assessed. All the extracts inhibited mycelia growth at various levels. Among them the superior inhibition (100%) was found in 15% concentration [16].

The results of [17] revealed that plants extracts *Azadirachta indica* and *Jatropha curcas* had a strong antifungal activity with significant inhibition on the growth of the all tested fungi (*Aspergillus flavus, Alternaria alternate, Fusarium oxisporium, Rhizopus stolinifier* and *Cladosporium herbaru*). Extracts of *Azadirachta indica* and *Jatropha curca*s were the most effective to inhibit the growth of the tested fungi.
According to [18], the garlic (*Allium sativum*) extracts with different concentrations have exhibited a significant antifungal activity in the protection of potato plant against *Phytophthora infestans.*

5. Conclusion
The *in vitro* study of antifungal activity with an indigenous plant in the southeastern Algeria (*Zygophyllum album* L.) against the fungi of date palm revealed that the species *Z. album* has a significant antifungal activity by recording an inhibitory percentage exceeded 40% until 86%. However, the powder of this plant showed also a significant effect on these fungi because it presented an inhibition rate exceeded 40% until 71%.
Because the aqueous extracts and the powder of *Zygophyllum album* L., is found effective against the mycelia growth of date palm fungi (*Alternaria* sp., *Fusarium* sp., *Phytophtora* sp., *Cladosporium* sp., *Aspergillus niger* and *Pinicilium* sp.). Therefore, this study suggests that aqueous extracts of these species would be helpful in treating diseases in date palm trees caused by these fungi. In conclusion, the findings of this experiment confirmed that plant extracts and powder canbe used as natural fungitoxicant to control the growth of date palm pathogenic fungi and thus reduce the dependence on the synthetic fungicides.

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