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# Diversity of the fungal flora associated with the white cochineal *Parlatoria blanchardi* Targ. (Himiptera-Diaspididae) in the palm groves of the Ouargla region, and determination of the pathogenicity of some fungal species

**Hayet Benameur-Saggou[1*], Amina Hassaine[2], Oum elkheir Abdelali[1], Chennouf Rekia[3], Keltoum Benbrahim[3], Samira Rebbouh[1]**

[1] *Phoeniculture Research Laboratory "Phoenix", Faculty of Natural and Life Sciences, University Kasdi-Merbah of Ouargla , Algeria*

[2] *Plant Biology and Environment Laboratory, Faculty of Sciences, Badji Mokhtar University of Annaba, Algeria*

[3] *Bioresources Laboratory, Faculty of Natural and Life Sciences, University Kasdi-Merbah of Ouargla, Algeria*

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## Abstract

The inventory of fungi associated with the white cochineal is an essential step in the possible microbiological control of this pest. A total of 16 fungal species belonging to 11 genera were inventoried on white cochineal infesting four date palm cultivars, namely Deglet-Nour and Ghars, Degla beidha and Hamraya. The infection rate of cochineal by these fungi differs from one cultivar to another; it is 38.42% in Deglet-Nour and 29.88% in Ghars. The *Aspergillus* is the most dominant genus on white cochineal, with three species present in the four cultivars studied. *Aspergillus niger* has a frequency of occurrence of 21.28% on Ghars. The factorial correspondence analysis (FCA) showed specific fungal species for white cochineal in each cultivar. The pathogenicity test of 05 fungal isolates showed that *Trichoderma sp*. caused the highest rate cochineal mortality (63% ±1.69), followed by *Aureobasidum pullulans* with 49%±1.65. These encouraging results open up new perspectives in the biological control of this very dangerous date palm pest.

*** Corresponding Author:** Hayet Benameur-Saggou ✉ hayetsaggou@gmail.com

## Introduction

The white cochineal *Parlatoria blanchardi* (Hemiptera- Diaspididae) is considered one of the most serious pests of date palm in Algeria and specifically in the palm groves of the South-East, notably the region of Ouargla (Allam, 2008; Achoura, 2013 and Benameur-Saggou, 2018). Great damages can be done by this scale insect by sucking the plant sap that gives low rates of photosynthesis and respiration, which leads to curling, yellowing, dropping to leaves. A characteristic symptom of infestation by *P. blanchardi* is the appearance and accumulation of its scales on attacked palm parts (El-Said, 2000 and El-Sherif *et al.*, 2001).

The control of cultural pests in Algeria generally involves the use of insecticides. But these treatments often prove to be insufficiently effective, with some species even developing races resistant to synthetic organic products (Saharaoui and Gourreau, 1998). In addition, the application of pesticides for controlling insects causes environmental pollution and affects natural enemies (Fan *et al.*, 2014).

Considering that palm grove are both fragile and complex ecosystems, any sudden and uncontrolled intervention can in the short term cause pathologies that are often irreversible in these environments. Biological control in palm groves could be an alternative to chemical control by combining it with other means of control that are not aggressive towards the environment (Idder, 2011). Our study, the first original approach, is oriented to the biological control of the white palm cochineal in the region of Ouargla through the use of entomopathogenic fungi associated with this insect. An inventory of the mycological community associated with this pest on different date palm cultivars is the first step, followed by pathogenicity tests in the laboratory to verify the effectiveness of these fungi in microbiological control of the white cochineal.

## Materials and methods

Fungi associated with the white cochineal are isolated from individuals of this insect collected from the leaflets of four date palm cultivars, namely Deglet-Nour, Ghars, Degla beidha and Hamraya. These cultivars belong to different date categories (soft, semi-soft and dry) and are located in three palm groves in the Ouargla region. The exploitation of the University of Ouargla (31°56'26" N; 05°17'38" E), the palm grove of Ksar (31° 58' 05,96″ N; 5° 19' 37,93 E) and the palm grove of Ain el Beidha (31° 56' 38,75″ N; 5° 23' 43,11" E).

*Fungal isolation*

White cochineal with external mycelial growth of fungi was disinfected with 75% alcohol for 1 min and rinsed with sterile distilled water 03 times, then dried with sterile paper towels. Sterile cochineal insects are placed on a potato-dextrose agar (PDA) with ten insects on each plate. Petri dishes were incubated at 35± 1 C° for 5 to 7 days.

Three replicates were prepared from each cultivar sample and a total of 30 cochineals were used per cultivar, i.e., a total of 120 cochineal insects. Purification of the strains is done by a series of subculturing onto a potato-dextrose agar (PDA) plate. Identification is based on both macroscopic and microscopic aspects according to the keys of Botton *et al.*, 1990 and Chabasse *et al.*, 2002.

*Fungi ecological indices*

Two ecological indices are used to exploit the results obtained. The total richness S is applied to the fungal species contacted. It corresponds to the total number of species in the stand considered in a given ecosystem. The occurrence frequencies (F) of each species were calculated by the formula of Ramade (2003) as follows:

$$\text{Frequency of occurrence} = \frac{\text{Number of repetitions of each species}}{\text{Total number of species recorded}} \times 100$$

*Pathogenicity test*

These tests were carried out to verify the pathogenicity of five fungal isolates, namely *Aspergillus niger*, *Aureobasidum pullulans*, *Curvularia sp.*, *Fusarium moniliforme* and

*Trichoderma sp.* on the mortality of white cochineal infesting two date palm cultivars, Deglet-Nour and Ghars.

Fragments of sterilized leaflets of the white cochineal-infested cultivars Deglet-Nour and Ghars were placed in sterile Petrie dishes on cotton wool soaked with sterile distilled water (5ml) after counting the live white cochineal. A 5 ml spray of the spore suspension with a concentration of $1X10^6$ spores/ml was carried out on the scale insects (Al Doussari *et al.*, 2013; Fan *et al.*, 2014 and Hallouti *et al.*, 2020).

The test was carried out with three replicates for each cultivar and each fungal isolate. The control was treated with distilled water containing 0.1% Tween 80. The plates were incubated at a temperature of 37°C. After 24 hours, a second count was made under a binocular magnifying glass to calculate the mortality rate according to the following formula:

$$\text{Mortality rate} = \frac{\text{Number of dead white cochineal}}{\text{Number of total white cochineal}} \times 100$$

*Statistical analysis*

The frequencies of occurrence of fungi on white cochineal according to the cultivars studied are treated using factorial correspondence analysis (FCA). One-way ANOVA and Fisher LSD test were used to compare mortality rates of white cochineal treated with sporal suspensions. Results were considered statistically significant when p-values were <0.05. The software used is XLSTAT 2021.3.1.

## Results and discussion

*Fungal species inventory*

The isolation of fungi associated with the white cochineal allowed us to observe the presence of 16 fungal isolates. These fungal species are grouped into two divisions, 05 classes, 07 orders and 07 families (Table 1).

**Table 1.** Inventory of fungal species associated with white cochineal of four date palm cultivars Deglet-Nour, Ghars, Degla-beidha and Hamraya in the Ouargla region

| Division | Class | Order | Family | Genus | Species |
|---|---|---|---|---|---|
| Ascomycota | Eurotiomycetes | Eurotiales | Trichomaceae | *Aspergillus* | *Aspergillus nidulans* |
| | | | | | *Aspergillus niger* |
| | | | | | *Aspergillus ustus* |
| | | | | | *Aspergillus terreus* |
| | | | | | *Aspergillus sp.* |
| | | | | *Penicillium* | *Penicillium notatum* |
| | | | | | *Penicillium sp.* |
| | *Dothideomycetes* | *Dothideales* | Dothioraceae | *Aureobasidum* | *Aureobasidum pullulans* |
| | | *Pleosporales* | Pleosporaceae | *Curvularia* | *Curvularia sp.* |
| | | | | *Alternaria* | *Alternaria alternata* |
| | | *Capnodiales* | Davidiellaceae | *Cladosporium* | *Cladosporium cladosporioides* |
| | Sordariomycetes | Hypocreales | Nectriaceae | *Fusarium* | *Fusarium moniliforme* |
| | | | Hypocreaceae | *Trichoderma* | *Trichoderma sp.* |
| | *Saccharomycetes* | *Saccharomycetales* | *Dipodascaceae* | *Geotrichum* | *Geotrichum candidum* |
| Zygomycota | Mucoromycetes | Mucorales | Rhizopodaceae | *Rhizopus* | *Rhizopus stolonifer* |
| | Phycomycetes | | Mucoraceae | *Mucor* | *Mucor sp.* |

The Ascomycota are the most abundant with 87.5% and a predominance of the class Eurotiomycetes (43.75%), the order Eurotiales (43.7%) and the family Trichomacae (43.75%). The division Ascomycota is the most common in most of the fungi isolated from insects. However, hemipterans are usually associated with fungi from the Ophiocordycypitaceae family (Kirk *et al.*, 2008 and Szklarzewicz *et al.*, 2021). This is not the case for the white cochineal in our region. The number of fungal species inventoried by our work is considerable (16 fungal species) compared to the inventory of the mycological flora associated with white cochineal made by Al Doussari (2013) on Iraqi cultivars, which identified 09 fungal species including *Alternaria alternata*, and the two genera *Fusarium* and *Cladosporium* are in common with our isolated fungal species. According to Evans and Prior (1997) in Fan *et al.* (2014), approximately 35 species of pathogenic fungi have been recorded from armored scale insects (Diaspididae).

**Table 2.** Frequency of occurrence of fungi on white cochineal infesting cultivars.

| | Frequency of occurrence of fungi on white cochineal infesting cultivars (%) | | | |
|---|---|---|---|---|
| Species of isolated fungi | Deglet-Nour | Ghars | Degla beidha | Hamraya |
| *Aspergillus niger* | 12.90 | 21.28 | 14 | 09 |
| *Aspergillus ustus* | 9.68 | 2.13 | 14 | 4 |
| *Aspergillus terreus* | 0 | 8.51 | 7 | 13 |
| *Aspergillus nidulans* | 4.84 | 4.26 | 0 | 0 |
| *Aspergillus sp.* | 0 | 0 | 0 | 9 |
| *Rhizopus stolonifer* | 12.90 | 6.38 | 10 | 0 |
| *Mucor sp.* | 8,06 | 8.51 | 0 | 17 |
| *Penicillium notatum* | 11.29 | 6.38 | 17 | 17 |
| *Penicillium sp.* | 4.84 | 0 | 7 | 9 |
| *Cladosporium cladosporioides* | 3.23 | 14.89 | 10 | 22 |
| *Alternaria alternata* | 12.90 | 0 | 4 | 4 |
| *Geotrichum candidum* | 6.45 | 6.38 | 17 | 0 |
| *Curvularia sp.* | 3.23 | 6.38 | 0 | 0 |
| *Fusarium moniliforme* | 1.61 | 2.13 | 0 | 0 |
| *Trichoderma sp.* | 3.23 | 4.26 | 0 | 0 |
| *Aureobasidum pullulans* | 4.84 | 8.51 | 0 | 0 |

*White cochineal infection rate by fungi according to date palm cultivars*

The white cochineal infesting of the four date palm cultivars studied showed different rates of infection by fungal species (Fig. 1).

White cochineal infesting the cultivar Deglet-Nour is the most associated by fungal species with a rate of 38.42%, followed by the cultivar Ghars with 29.88%.

Mohamed Mahmoud (2017), in the region of Adrar in Algeria, announces the presence of 460 endophytic fungi in the date palm tree affiliated to 10 taxa. According to Vega (2008), various genera of fungal entomopathogens have been isolated as endophytes in several different plants. Studies on fungi (pathogenic or non-pathogenic) associated with date palm in the Ouargla region show that the cultivar Deglet-Nour is the most affected with percentages varying from 52% to 45%, followed by the cultivar Ghars (from 47% to 34%) and in the last position the cultivar Degla-beidha (Benzeghmane, 2011; Chacha, 2016). It should be noted that Deglet-Nour is still more infested by the white cochineal than other cultivars (Bousaid and Maache, 2000; Boughezala-Hamad, 2011; Allam, 2013 and Benameur-Saggou, 2018). A plant attacked by fungi implies its weakening and therefore, an installation of the insect is facilitated (Sauvion *et al.*, 2013). However, from observations on the morphology of the white cochineal insects, we found only those infesting Deglet-Nour to be fragile and pale in appearance compared to the cochineal insects infesting other cultivars such as Ghars.

According to the same author, tripartite plant-insect-fungus interactions can be beneficial to the host plant, which can manipulate pathogenic fungi to play the role of bodyguard and reduce the insect's performance by decreasing its growth fecundity and survival.

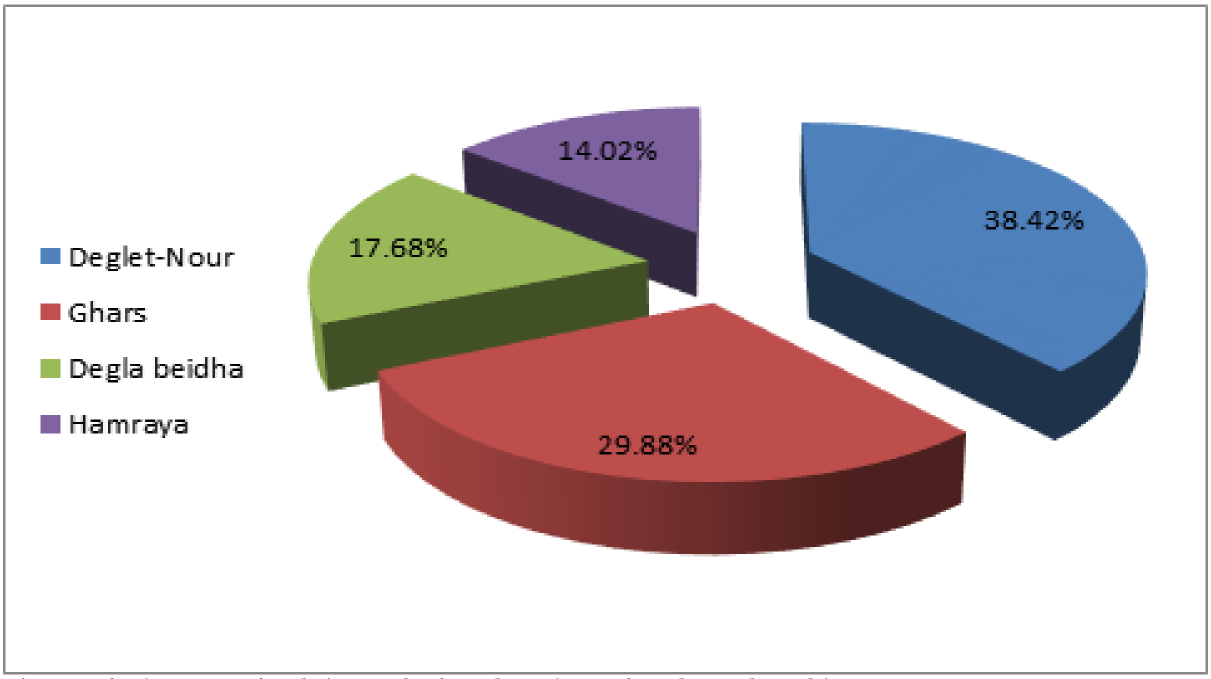


**Fig. 1.** Infection rates of scale insects by fungal species on four date palm cultivars.

*Occurrence frequencies of fungal species on the white cochineal of the cultivars studied*

The fungal species inventoried show different frequencies of occurrence on cochineal infesting date palm cultivars (Table 2).

From Table 2, we found that the 16 fungal species are distributed differently on the cochineal infesting of the cultivars studied. The species *Aspergillus niger* has the highest frequency of occurrence on the cultivars Ghars and Deglet-Nour; it is respectively 21.28% and 12.90%. It is also reported that other fungal species are present with the same frequency on Deglet-Nour, namely *Alternaria alternata* and *Rhizopus stolonifer,* which are phytopathogenic species.

On Degla beidha, *Penicillium notatum* and *Geotrichum candidum* are the most dominant, with a frequency of occurrence of 17% for each. While on Hamraya, the most important frequency is that of *Cladosporium cladosporioides* (22%). In the second position comes the species *Mucor sp*. with 17%.

For the correspondence factor analysis AFC (Fig. 2), axes 1 and 2 represent a high percentage of inertia (83.79%). The graphical representation of axes 1 and 2 shows the existence of groupings (A, B, C and D) distributed among the four cultivars of date palm.

Group A includes the fungal species that most affect the white cochineal of the Ghars cultivar, namely *Aureobasidum pullulans*, *Curvularia sp*., *Trichoderma sp*., *Alternaria alternata* and *Fusarium moniliforme*. Twelve (12) species of *Fusarium* that parasitize scale insects have been recorded up to now (Fan *et al*., 2014). 04 fungal species form group B.

These are the most frequent fungal species on cochineal insects of the cultivar Hamraya. Citing *Cladosporium cladosporioides*, *Aspergillus terreus*, *Aspergillus sp*. and *Mucor sp*. The genus *Cladosporium* usually does little harm to insects but can contribute to their mortality (Toumanoff, 1965).

It should also be remembered that certain fungi said to be occasionally entomopathogenic, such as the genus *Mucor* can only infect the insect through accidental lesions or wounds of the integument (Ferron, 1975). Group C includes the most common fungal species on Degla beidha white cochineal. These are *Geotrichum candidum*, *Aspergillus ustus*, *Penicillium notatum* and *Penicillium sp*. The genus *Geotrichum* is found in different habitats such as plants, soil and water. This mold is a production base of enzymes that degrade xylem and cellulose (Pottier *et al*., 2008; Pavlov, 2018). The genera *Penicillium* and *Aspergillus* have been reported on date palm as endophytic fungi with large proportions (Mohamed Mahmoud, 2017). Group D is specific for fungal species associated with cochineal insects infesting the cultivar Deglet-Nour. These are *Rhizopus stolonifer* and *Alternaria alternata*. These two species of fungi are reported to be phytopathogenic fungi of several crops, including date palm (Benlamoudi, 2021; Chacha, 2016; Benzeghmane, 2011).

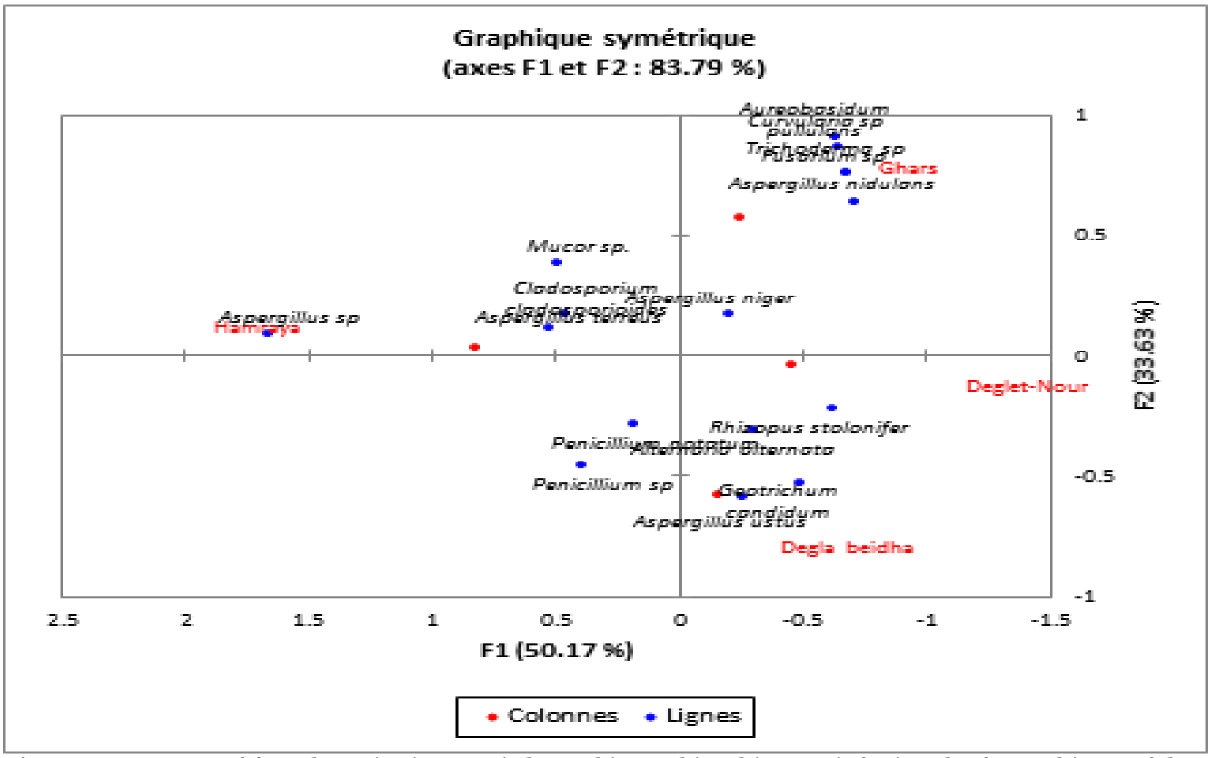


**Fig. 2.** Factor map of fungal species inventoried on white cochineal insects infesting the four cultivars of date palm (AFC).

*Pathogenicity test of isolates on white cochineal*

To verify pathogenicity, virulence tests of 05 fungal species isolated from white cochineal insects were performed (Fig. 3). Mortality rates of white cochineal individuals treated with spore suspensions of five strains show that each species causes a different mortality rate depending on its pathogenicity. The one-way analysis of variance (mortality rate) shows that there is a highly significant difference with $p$>0.006 between the mortality rates of white cochineal on the cultivar Deglet-Nour. Fisher's LSD test at the 5% level indicates that *Trichoderma sp.* is the most virulent causing a mortality rate of 63% ±1.69. Entomopathogenic Hypocreales are phylogenetically closely related to plant pathogens and endophytes. *Trichoderma* sp. exhibits an ability to utilize a wide range of substrates of natural or chemical origin (Hu *et al*., 2014; Ben Amira, 2018). The species of this genus are known as biological control agents against phytopathogens and as a source of enzymes and metabolites of industrial interest (Sadfi-Zouaoui *et al*., 2008).

On the same cultivar, the spore suspension of *Aureobasidum pullulans* came second with a mortality rate of 49%±1.65. This species seems to have good potential for use as a biological control agent due to its ability to colonise a wide host range (Webb and Mundt, 1978 in Al Hamouchi, 2011).

The ANOVA also showed that there was a non-significant difference ($p$>0.059) between the mortality rates of white cochineal treated with sporal suspensions on the cultivar Ghars.

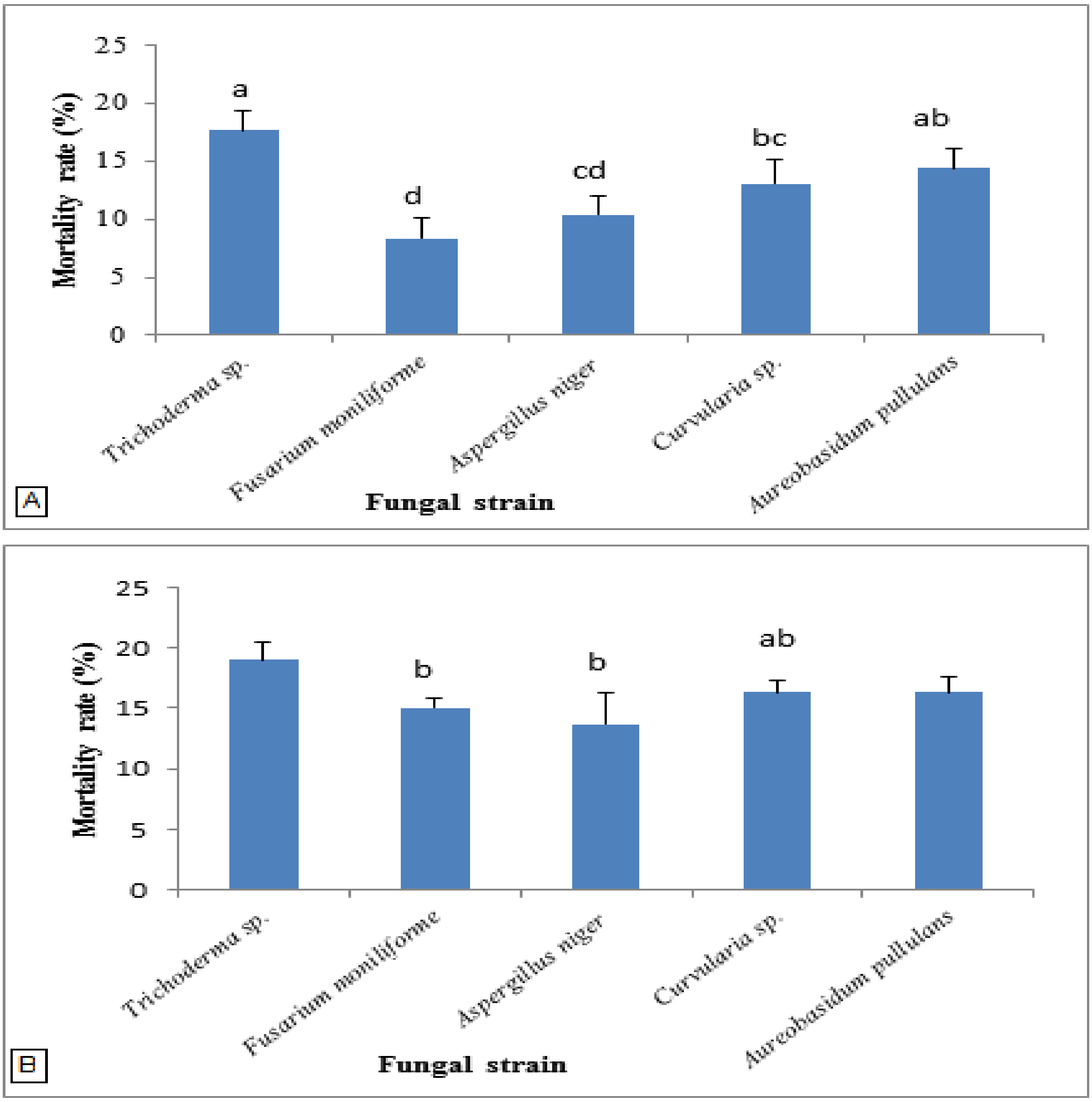


**Fig. 3.** Mortality rates of white cochineal related to the fugal strains (A: Deglet-Nour, B: Ghars). Mean (± SE) of mortality, Means followed by different letters differ significantly (comparison were performed using Fisher's LSD test, P < 0.05), Treatments sharing the same letter are not significantly different.

**The mortality rates of white cochineal at the control level are very low for Deglet-Nour and Ghars. For this reason, we do not need to make corrections to the mortality rates according to Abbott's formula.*

The lowest mortality rates are recorded with *Fusarium moniliforme* and *Aspergillus niger* with 25% ±1.63 and 28% ±1.45 respectively on Deglet-Nour and 20%± 0.83 and 27%± 2.62 on Ghars. Although the Fusarium are pathogenic to both insects and plants and they present great potential as a biological control agent against the *cochineal* insect, no Fusarium species have been reported as a pathogen of the brown soft scale until now (Fan *et al.*, 2014). This is the case for the white *cochineal*. The same applies to species of the genus *Aspergillus* which have proven to be nematicidal (Jin *et al.*, 2019).

## Conclusion

The inventory of the mycological flora associated with the white cochineal has shown that this pest is a host to a considerable number of fungal species. Some species have very important biocidal capacities, and they have already been used in the microbiological control of several crop pests. Pathogenicity tests using these species against the white cochineal have given very encouraging results. This opens up new prospects for expanding the list of biological agents for this type of control, and we are moving towards microbiological control.

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