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Original Paper

Occurrence of fungal species associated with grapevine dieback in western of Algeria (case of the Tlemcen region)

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Abstract

Introduction: The present study carried out in the Tlemcen region (In four different farms) between 2021 and 2022, focuses on the isolation, identification and characterization of phytopathogenic fungi responsible for fungal diseases of the vine (*Vitis vinifera* L.).
Methods: Out of a total of 60 samples, the isolation of fungi from the aerial part and underground of symptomatic trees revealed the presence of significant fungal biodiversity on the culture medium.
Results: Sixteen fungal species of different genera have been isolated and identified, namely: *Aspergilus fumigatus*, *Aspergilus niger, Alternaria alternata, Penicillium sp., Penicillium communeon*, *Penicillium solitum, Fusarium sp., Fusarium solani, Fusarium culmorum, Pythium sp., Pythium ramorum, Diplodia corticola, Botrytis cinerea, Mucor racemosus*, *Rhiropus stolonifer* and *Monilia fructicola.* The two species *Diplodia corticola* and *Botrytis cinerea* are strongly present in the vascular tissues of the collar and trunk, with 9 and 12%, respectively.
Conclusion: As well as species of the *Fusarium* genus with 18% in the rhizosphere of symptomatic trees.

Keywords: *Vitis finifera* L. – fungi - plant pathogen - Tlemcen.

Introduction

The vine (*Vitis vinifera* L.) is a plant that has been cultivated by man for a long time, so much so that the history of viticulture is intertwined with the history of man. It has great adaptability to soil and climate conditions where it is cultivated in hot regions and in relatively cold climates (Reynier 1989; Galet 1998).
In Algeria, as in all wine-growing countries, the vine occupies an important place economically. Indeed, currently the Algerian vineyard occupies an area of 97,000 hectares, or 12% of the agricultural area (Amarni 2009). The vine, is subject to many attacks by pests and diseases, particularly those caused by pathogenic fungi (Galet 2000). These fungi can attack all parts of the plant, partially destroying crops and are the cause of many economic problems.
During field surveys, several symptoms were observed on the trees of the vines: Blackening of the fine roots, canker at the crown, yellowing of the leaves, …etc. Which led us to wonder about the reason leading to the appearance of these symptoms, and by comparing them with previous studies we assumed that they could be at the origin of these fungi.
In order to deepen our knowledge on the fungal species associated with the mortality of vine subjects in the Tlemcen region, a study was carried out, which aims to: (i) Inventory the fungal mycoflora associated with decline and mortality of the vines in the wilaya of Tlemcen; (ii) Identify and characterize isolated fungal species.

Material and methods

Description of prospected sites

In this study, the four farms chosen all belong to the wilaya of Tlemcen. It is: Mansourah farm: this farm is based on the cultivation of a wide variety of plants, including vines. It is characterized by an arid climate.

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They have been using old methods of growing vines for 20 years. Irrigation relies on well water and uses sulfur to fight against phytopathogenic diseases and various insect attacks. It is the only chemical means used by the managers of this farm.
Hamadouche farm: it is the only pilot farm in the commune of Chetouane. It is characterized by a semi-arid climate. They use as chemical products; Comac, baton and rophosate for all cryptogamic diseases.
The farm has different types of wine grape varieties, including the introduced Italian grape varieties known as noble, Valenci and Beirut date palm.
El-Fhoul farm: It is characterized by a temperate climate and persistent drought. The farm produces several varieties of grapes including the Beirut date palm and Valencia.
Koreib farm: It is is a pilot farm in the municipality of Sabra, wilaya of Tlemcen. The farm has a total area of 1034 ha. Farmers began cultivating vines at the beginning of 2001, with a drip irrigation system.
The most common phytopathogenic diseases on the farm are due to fungi, namely: powdery mildew, and as treatments, farmers use: Anti-mold, kadi paint, anti-powdery mildew, etc.
The choice of these stations is based on the health state of the vineyard on these farms, as well as on the presence of symptoms of infection by phytopathogenic fungi such as those described by several authors in the different Mediterranean countries (Arkam et al. 2021; Linaldeddu et al. 2015 and Ammad et al. 2014).

Samples

A total of 60 samples were taken from the different vine species, including 12 samples from the first zone, another 8 samples from the second, 18 from the third and 22 from the fourth farm.
For the aerial part: Sampling is done randomly, targeting only symptomatic subjects. A plant is considered diseased when it shows symptoms. Using a sterile scissor with 70% alcohol, samples of leaves, fruits, and trunk were taken.
For the underground part: The sampling procedure was based on the method described by (Scanu et al. 2013), slightly modified by Smahi (2019).
Using a spatula, a layer of the root part of the soil was dug (approximately 20 cm to 25 cm deep) and approximately 300g of soil was taken in opposite directions. Each sample was subsequently placed in a sterile plastic bag on which the sampling references were reported: Sample number, name of the farm, name of the host variety, part of the sample (Soil, roots , branch) and the sampling date. All samples taken were subsequently transferred to the laboratory, preferably analyzed immediately or stored in the refrigerator at 5°C until further use.

Isolation of pathogen

All samples were first disinfected before use. The leaves and stems, as well as the fruits of each sample were cut, using a sterile scalpel, into small pieces of 0.5 cm^2 to isolate the internal and external fungal flora. Then, it was removed, using forceps (regularly disinfected with 70% ethanol), and placed directly in a Petri dish (ϕ 90mm) containing the culture media (PDA or MEA). All plates were incubated in the dark at 25°C and examined after 4–7 days. After the incubation period, colonies with different appearances appear in the same Petri dish are subculture until pure strains are obtained.

Dilution method

For soil samples, the dilution method was based on the use of sterile physiological water: 1g from each sample of soil is added to 9 ml of physiological water, which corresponds to the 10^{-1} dilution. Then, 1 ml of this dilution is added to 9 ml of sterile water to have the 10^{-2} dilution until reaching the 10^{-5} dilution. After, 1 ml of each dilution was placed on PDA (Potatoes Dextrose Agar). The plates were incubated for 5-7 days at 23 ° C in the dark.

Identification of isolates

The different pure strains obtained were grouped according to their macroscopic characteristics to choose only representatives of each group for identification. The observation of macroscopic criteria is based on several aspects: The appearance of the colonies, relief, size, color...
Microscopic identification is based on microscopic criteria (the shape of the vesicle, the arrangement and shape of the spores and conidia, etc.). Guides used are: Jung et al. (2017), Smahi et al. (2017), Phillips et al. (2012) and (2013), Erwin and Ribeiro (1996), Botton et al. (1990) and Barnett and Hunter (1972).

Results and discussion

Symptomatology

During our field surveys, the first observation that was reported in the four study areas was that the vine crops which were more or less close together in space, displayed the same symptoms and which confirms the transport of spores and the spread of plant pathogenic diseases within the same farm. The most noted symptom is the wilting of the aerial part in certain trees. In addition, malformation, yellowing and necrosis of leaves were observed in the different trees surveyed. Young shoots were also infected, especially on the two farms: Mansourah and El Fhoule. On the different types of bunches, a brown coloring accompanied by rot which has a gray color tending towards black, has been noted. On the trunk and branches, scattered dark brown spots were observed in some trees. In longitudinal section, these spots progress over time, from the periphery towards the center to appear as dark cankers at the more advanced stage.
At the root level, rot and necrosis were observed. In some species, the absence of good roots has been noted (roots becoming brown-black with few rootlets). Then, dark brown necrotic root spots tending towards black which can move up the aerial part of the plant and invade the tissues of the root collar or the tissues of the stem and the bark (Fig 1).

Fig 1. The symptoms observed in the study areas: (top left) Yellowing and necrosis of the leaves, (top right) rot of the bunches, (bottom) necrosis at the level of the roots and trunk.

Importance of isolated fungal species

A fairly significant fungal diversity was observed after carrying out a mycological analysis of our samples on the different types of culture medium. On the basis of cultural and microscopic characteristics sixteen (16) species have been identified, mostly are ascomycetes namely: *Aspergilus fumigatus*, *Aspergilus niger, Alternaria alternata, Penicillium sp., Penicillium communeon*, *Penicillium solitum, Fusarium sp., Fusarium solani, Fusarium culmorum, Diplodia corticola, Botrytis cinerea, Monilia fructicola.* However, others are oomycetes like *Pythium sp., Pythium ramorum, and zygomycetes as Mucor racemosus*, and *Rhiropus stolonifer* (Fig 2).

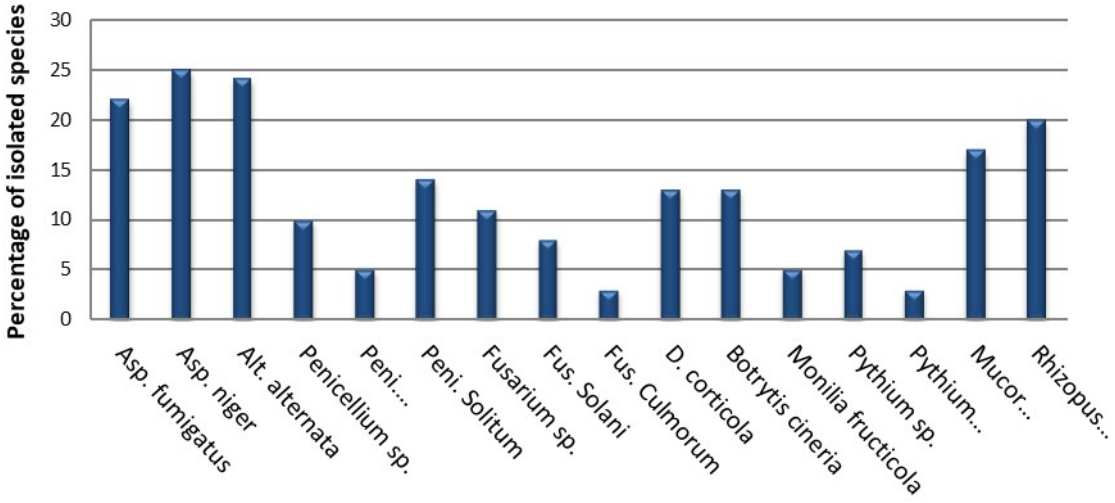

Fig 2. Importance of isolated fungal species.

Fungi such as *Aspergillus niger, Asp. fumigatus* and *Alternaria alternata* were the most present with a frequency varying between 12 and 13%. Both species of the genus *Aspergillus* grow in the soil and on diseased plants that have been damaged by insect bites or other fungi. According to Scheidegger and Payne (2003), they are both opportunistic species that only attack weak subjects.
Parasitic species of the Botryosphaeriacea family, such as Diplodia corticola and Botrytis cinerea, are strongly present, with a percentage of 9 and 12%, respectively. These fungi have already proven their pathogenicity on various fruit, forest or ornamental species (Smahi et al. 2017), causing significant damage to the vascular tissues of trees. On the other hand, two other species of the genus Fusarium were also isolated from the crowns of symptomatic trees.

Conclusion

This work consists of inventorying the telluric, vascular and foliar fungi present on the vineyards of four farms representative of the wilaya of Tlemcen. Most of the isolated species have already confirmed their pathogenicity on fruit trees such as vines in several previous studies.
Knowledge of the species having a relationship with the symptoms observed during field surveys allowed us to better control the health status of vine trees against these cryptogamic diseases in the future. Finally, our study like any other study is only an initiation, which requires other more in-depth ones.

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Conflict of interest:

The author states that he has no conficts of interest

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