

**CURVULARIA LUNATA, FOLIAR PARASITE OF POPULUS NIGRA
IN MOROCCO**

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ABSTRACT

A survey was done in the main streets of Kenitra city (north-western Morocco) in the spring of 2007 and 2008 revealed that 75% of the alignment trees showed foliar symptoms. 50% of the leaves of *Populus nigra* had shown lesions. The morphological and cultural characters indicated that the isolated fungus was *Curvularia lunata*. The Koch's postulate was verified by inoculating healthy leaves of *Populus nigra* with main fungus using two techniques of inoculation and similar symptoms were observed on all inoculated leaves. Infection started at the periphery, spreaded and invaded 75 % to 80% of the leaves surface. *C. lunata* produced conidia abundantly on the inoculated leaves of *P. nigra* both by the conidial suspension (4.96×10^5 conidia/cm²) and mycelial disks (3.61×10^5 conidia/cm²)

Keywords : *Morocco, populus nigra, curvularia lunata, leaf spot, koch's postulate*

RÉSUMÉ

Curvularia lunata, un parasite foliaire de populus nigra au Maroc

Des enquêtes, menées dans les principales *rues et artères* de la ville de Kénitra (Nord-ouest du Maroc) au printemps de 2007 et 2008, ont révélé que 75% des arbres d'alignement présentent des symptômes foliaires. Cinquante pour cent des feuilles de *Populus nigra* montrent des lésions. Les caractères morphologiques et microscopiques indiquent que le champignon isolé est *Curvularia lunata*. Deux techniques d'inoculation des feuilles de *P. nigra* ont été utilisées pour vérifier le postulat de Koch. Les symptômes induits par *C. lunata* sur les feuilles sont identiques à ceux observés sur les arbres.

L'infection commence à la périphérie puis envahie 75 à 80% de la surface des feuilles. Pour les deux techniques d'inoculation, *C. lunata* sporule abondamment sur les lésions des feuilles inoculées de *P. nigra* ($4,96 \times 10^5$ et $3,61 \times 10^5$ conidies / cm²)

Mots-clés : Maroc, *populus nigra*, *curvularia lunata*, lésions foliaires, postulat de koch.

I - INTRODUCTION

In Morocco, the *Populus* genus is represented by three natural species, *Populus nigra* L., *Populus alba* L. and *Populus euphratica* Oliv. with some sub species and varieties [1]. Poplar trees are mainly localized in a valley close to water, as well in plain that in mountain. The Poplar was imported from Spain, Italy, France, Iran and Syria. Surface of Poplar plantations is about 2600 ha, however surface of natural stands can be appraised to 2500 ha, this woody plant show an advanced decline due to a complex causes as drought, diseases and human actions etc... [2]. *P. nigra* L. ssp. *italica* (Duroi) Asch. & Gr. is introduced in Morocco as an ornamental tree. The crown of this tree is pyramidal, the bark is black and fissured, and the leaves are alternates with dentate margins [1]. A survey was done in the main streets of Kenitra city (north-western Morocco) in the spring of 2007 and 2008 revealed that 75% of the alignment trees showed foliar symptoms. 50% of the leaves of *P. nigra* had shown lesions; the brown spot essentially peripheral 8 to 12 mm in length, diffuse, also central, rounded 3 to 5 mm of diameter (**Figure 1**).The objective of this work was to achieve isolation of the pathogenic fungus from the diseased leaves of *Populus nigra* and to verify the Koch's postulate



Figure 1 : Infected leaves of *Populus nigra* with *Curvularia lunata*

II – MATERIAL AND METHODS

Infected leaves were collected and washed with tap water. Surfaces were disinfected with alcohol, rinsed with sterile distilled water and placed in Petri dishes on filter paper moistened with sterile distilled water. Half of the Petri dishes were incubated for 48h at 28°C in the dark, the other under continuous light (White fluorescent tubes) at 23°C. Lesions were microscopically observed, the conidia were taken with a capillary tube and placed on PDA medium (Potato Dextrose Agar) in the dark at 28°C.

To complete Koch's postulates, healthy leaves of *P. nigra* were disinfected with alcohol, rinsed with sterile distilled water and inoculated with *C. lunata* using two techniques. 10 leaves were wounded at periphery and at centre; a mycelial disk was applied to each wound. 10 leaves were inoculated by the conidial suspension adjusted to a final concentration of 10^5 conidial ml^{-1} with sterile distilled water containing 0.05% Tween 20 and 0.5% gelatin. All inoculated leaves were placed in 90 mm Petri dishes containing small glass beads and sterile distilled water. Inoculated leaves were kept in the laboratory at 22°C under continuous light.

The conidia production (conidia cm^{-2}) of *Curvularia lunata* on the inoculated popular leaves was estimated according to the technic described in [15]. Fifteen days after inoculation, the leaves those had shown lesions were cut into pieces of 1 cm^2 and placed in 90 mm Petri dishes on three filter paper discs moistened with sterile distilled water. The dishes were incubated from 48 hours under continuous fluorescent lighting. Then each fragment was placed in a test tube containing 1 ml of sterile distilled water and agitated by a vortex mixer for 2 min. The conidia of the pathogen were counted using a Malassez slide under an optical microscope at magnification of $\times 100$ with 10 counting of each sample.

III – RESULTS AND DISCUSSION

After seven days, the mycelium of the growing colonies was septate, hyaline, color vary from grey to black (**Figure 2**). The conidiophores length was $89.24 \mu\text{m}$, erect. The conidia were smooth walled, tri-septate, predominantly curved, straight also, size: small broad to wards the apical tip and mid-or dark brown, average dimensions $21.88 \mu\text{m} \times 9.51 \mu\text{m}$ (**Figure 2**). The morphological and cultural characters indicated that the isolated fungus was *Curvularia lunata* (Wakker) Boedijn 1933 [5]. Opportunistic fungi associated with the lesions were *Aspergillus* sp., *Alternaria* sp. and *Penicillium* sp.

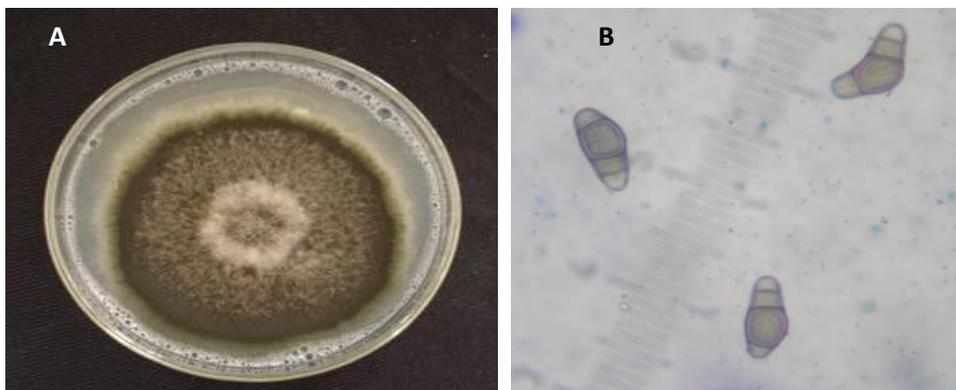


Figure 2 : *Mycelial growth of Curvularia lunata on PDA medium after 7 days in the dark at 28°C (A); Conidia at magnification of $\times 400$ (B).*

The similar lesions to tree symptoms were observed on all inoculated leaves. Symptom development on the inoculated leaves with the conidial suspension appeared after 7 days of inoculation. The developed lesions on the upper and the lower surface were brown, rounded and measured 2 to 3 mm diameter (**Figure 3a**). The inoculated leaves with mycelial disks showed the symptoms after 10 to 15 days, the same type of lesions occurred (3 to 5 mm diameter) **Figure 5a**. Infection started at the periphery, spreaded and invaded 75 % to 80% of the leaves surface in the both tests (**Figure 3b and Figure 4b**).



Figure 3 : *Symptoms on leaves of Populus nigra artificially inoculated by conidial suspension of Curvularia lunata*



Figure 4 : Symptoms on leaves of *Populus nigra* artificially inoculated by mycelial disks of *Curvularia lunata*.

C. lunata produced conidia abundantly on the inoculated leaves of *P. nigra* both by the conidial suspension (4.96×10^5 conidia/cm²) and mycelial disks (3.61×10^5 conidia/cm²). Artificially infected portions of leaves incubated in moist chambers consistently yielded *C. lunata* and Koch's postulate were performed. In this study, *C. lunata* showed a high pathogenicity. To our Knowledge this is the first report of this parasite on Poplar foliar in Morocco.

IV - CONCLUSION

Curvularia lunata is a causal agent of leaf spots on *Zea mays* [4, 15], *Gladiolus* sp. [8, 18], *Allium cepa* [6], *Alnus rubra* [16], *Ananas comosus* [20, 9], *Zoysia japonica* and *Zoysia matrella* [15], *Cocos nucifera* [11], Carpetgrass [14]. It is also a causal agent of stem blight on Cassava [13 - 12]. In Morocco *C. lunata* was isolated from the seeds [2] and leaves [7] of *Oryza sativa*. This fungus is also found on leaves of *Hibiscus rosa-sinensis* [10] and *Sorghum bicolor* [3].

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