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Research Article

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Study of *Pestalotiopsis palmarum* pathogenicity on *Washingtonia robusta* (Mexican palm)

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ABSTRACT

The pathogenicity study of Pestalotiopsis palmarum was carried out on the leaves of Washingtonia robusta, using two artificial inoculation methods: mycelial discs and conidial suspension. P. palmarum was isolated from the leaves of W. robusta showing chlorosis symptoms during a study conducted on the mycoflora associated with this ornamental palm.

In vitro, artificial inoculation of healthy leaves of W. robusta showed that P.palmarum can induce significant symptoms after 7 days of inoculation with the mycelial discs, pathogenicity was also significant with a percentage of 40.16% infection when the leaves were inoculated with the conidial suspension, which was higher compared to inoculation with mycelial discs with an infection percentage of 25.66%. All the developed lesions were sporulating, therefore able to provide a source of a secondary inoculum.

Keywords: *Washingtonia robusta, Pestalotiopsis palmarum, pathogenicity, inoculation, sporulation, lesions.*

INTRODUCTION

Ornamental plants are important elements in the modern life because it gives a lot of attraction to our environment; whether it's in our house, our company, leisure or our workplace[1] among these plants we find the ornamental palms, which are very popular in the ornamental crop. They grow well under semi-arid climates, but have also proven to be very adaptable to different types of climates[2] they prefer both warmth and drought[3].

W. robusta is one of these ornamental palms, belongs to the Arecaceae family, it is native to the Southeastern United States, and Mexico[4] specifically, mountain valleys and canyons of the Sonora desert and Baja Mexico. It is a popular landscape plant in Florida, California and Arizona, and in areas where it is hardy in the world. It is a slender palm, haughty, flexible, can exceed 30 m in length[5].It can be recognized from its trunk swollen at the base and its leaves with rounded blade. Petioles, covered with thorns over its entire length, tough enough[3]. they are severely armed with dark brown notes, sometimes bifid, where the petiole is attached to the leaf, the underside of the limbus bears a scales task woolly white. The flowers are hermaphrodite, white, clustered in long panicles spadix[4]. Fruit oblong, black, about 1 cm long or less, fibrous roots, shallow, flower clusters develop in the lower crown of leaves. 3 petals, 3 calyx lobes[5].

Mexican palm prefers a moderately rich, well-drained soil, it grows in light conditions in sunny land, drought resistant and grows faster if moisture is adequate[6]. It prefers a subtropical climate[7] and it is widely cultivated and naturalized in warm regions of the world[8].

W. robusta encounters various pest problems, which qualitatively affect its aesthetic appeal such as: *Paysandisia archon* that devours the heart of the palm, leading to their inevitable death, *Rhynchophorus palmarum*[9]. *Chalara paradoxa* causes rots of bud, meristem and weevils[10].

Generally, the *Washingtonia* is attacked more often by *Cercospora sp. Colletotrichum sp. Cylindrocladium macrospora, Phomas palmicola* and *Gliocladium vermoeseni* causing canker disease and bud rot[11]. Other fungal diseases are known to *Washingtonia robusta*, such as the Fusariosis induced by *Fusarium oxysporum*[12]. Eyespot, caused by *Cylindrocladium pteridis*, False coal, caused by species of Graphiola, the burn of petiole induced by *Cocoicola californica*[13] and bud rot caused by *Phytophthora palmivora* [14].

The genus Pestalotiopsis is pathogenic, but considered endophyte and weakly parasite, its species also infect many economically important plants such as Mango (*Pestalotiopsis mangiferae*), rice (*Pestalotiopsis versicolor*) Tea (*Pestalotiopsis theae*)[15] and Washingtonia (*Pestalotiopsis palmarum*). *Pestalotiopsis palmarum* also attacks many palm grow slowly on the palms, the symptoms are usually observed on the older leaves and infection requires injury and facilitates the pathogens penetration[16].

In Morocco, until now, the species *Pestalotiopsis palmarum* has never been reported or described on *Washingtonia robusta* and its pathogenicity has never been shown on this plant. The objective of this work was to isolate *Pestalotiopsis palmarum* from *W. robusta* and to study its pathogenicity on detached leaves.

MATERIALS AND METHODS

1) *P. palmarum* isolation

W. robusta leaves showing no symptoms were collected on March from three different locations of the Ibn Tofail Sciences Faculty garden's in Kenitra (North West of Morocco), samples were cleaned and placed in plastic bags, then brought to laboratory for the isolation tests.

Isolation of the fungal species associated with injured leaves was performed using two methods.

Buvard methods

Leaves were thoroughly washed with tap water, cut into pieces and placed in sterile Petri plates containing three filter paper discs (buvard), previously sterilized, and then moistened with sterile distilled water. The Petri dishes were then incubated at 22 ° C in the continuous light, or in the alternating light and dark. After 48 h, the fragments were examined under the optical microscope to observe the presence the fungal organs.

The isolated spores were transferred aseptically one by one under the microscope, using capillary glass tubing, previously sterilized to the flame and cooled on the media culture. They were then deposited and burst on the surface of an agar medium (15g agar agar, 1000 mL of distilled water) and then transferred using a sterile needle to the surface of the PSA medium (200 g of potato, 20 g of glucose, 15 g agar agar, 1000 mL distilled water).

Subculturing and microscopic observations allowed us to obtain pure fungal cultures.

Modified Buvard method

Leaves were cut into pieces, these pieces were incubated as the same manner as previously (Buvard methods), but after 3 days of incubation, these fragments were deposited on the PSA medium and again incubated in the dark at 28 ° C for 7 days. After incubation, colonies of the fungus appeared on the culture medium.

2) Inoculum production

P. palmarum was grown on PSA medium. The cultures were placed under continuous fluorescent light at room temperature (24 ^{o}C) for 10 days.

3) Mycelial discs and conidial suspension preparation

Mycelial discs of 5 mm diameter were removed from the front of growth of a young culture of the tested species to inoculate the leaves of *W. robusta* (using a sterile punch). Controls disks were removed from the PDA medium.

To prepare conidial suspension, the surface of each culture was aseptically scraped with a metal spatula; the mycelium was then suspended in sterile distilled water and then stirred for a few seconds in the vortex. Thus, the obtained suspension was filtered through muslin to separate the conidia mycelial fragments. After counting, using a Malassez cell, the conidia suspension was adjusted with sterile distilled water to obtain a final concentration of 10^5 conidia / mL and then added with 0.05% of Tween 20 and 0.5 of gelatin. For the control, the conidial suspension was replaced by distilled water supplemented with 0.05% of Tween20 and 5% of gelatin.

4) Inoculation

The *W. robusta* leaves apparently healthy, collected from the garden of the Science Faculty, Ibn Tofail University, Kenitra, were brought to the laboratory, washed and dried. Two methods were used for inoculation: inoculation with the mycelial disks and by spraying the leaves with the conidial suspension.

For the first method, artificially injury was performed sterilely on leaves, with a needle in the center and at the periphery, and then the pathogenic mycelial discs were deposited on the injury, control leaves were inoculated with discs of PDA medium.

For the second method, the inoculation was made on sterile conditions by spraying conidial suspension of the pathogen (10^5 spores / mL) tested on the studied plant leaves . The control leaves were sprayed with sterile distilled water supplemented with 0.05% of Tween 20 and 5% of gelatin. Three repetitions were performed for each lot of leaves for the two inoculations.

The inoculated leaves were kept on glass beads in the presence of sterile distilled water; the incubation was made at room temperature for 7 days.

5) Sporulation

After 7 days of incubation, inoculated leaves those showing lesions were cut into fragments of 1 cm^2. These fragments were placed in Petri plates containing 2 sterile filter paper discs humidified with sterile distilled water. The plates were then placed under continuous fluorescent light and at room temperature during 48h.

After the incubation period, sporulating lesions of each leaf were placed in the assay tubes containing 1 mL of sterile distilled water, the tubes were agitated using vortex for 1 minute to detach the conidia from the conidiophores and conidial suspension was then obtained and determined using a Malassez cell. (10 count per sample). The observation was carried out under magnification × 100.

6) Results notation

The taxonomic study of fungal species was accomplished by referring to the latest edition of the fungal dictionary[17]. The macroscopic description was based on the morphological characteristics of the colonies developed on different media cultures. The microscopic description was performed based on different key determination[18,19,20,21].

After 7 days of inoculation, the developed symptoms have been described, the diameter of the lesions formed on leaves was measured in two perpendicular directions and the average of the two values was calculated. The percentage of diseased leaf area was estimated by summing the areas of the lesions measured for the entire observed leaf area (cm^2).

$$\%NLA = NLA / TLA \times 100$$

NLA: Necrotic leaf area

TLA: Total leaf area

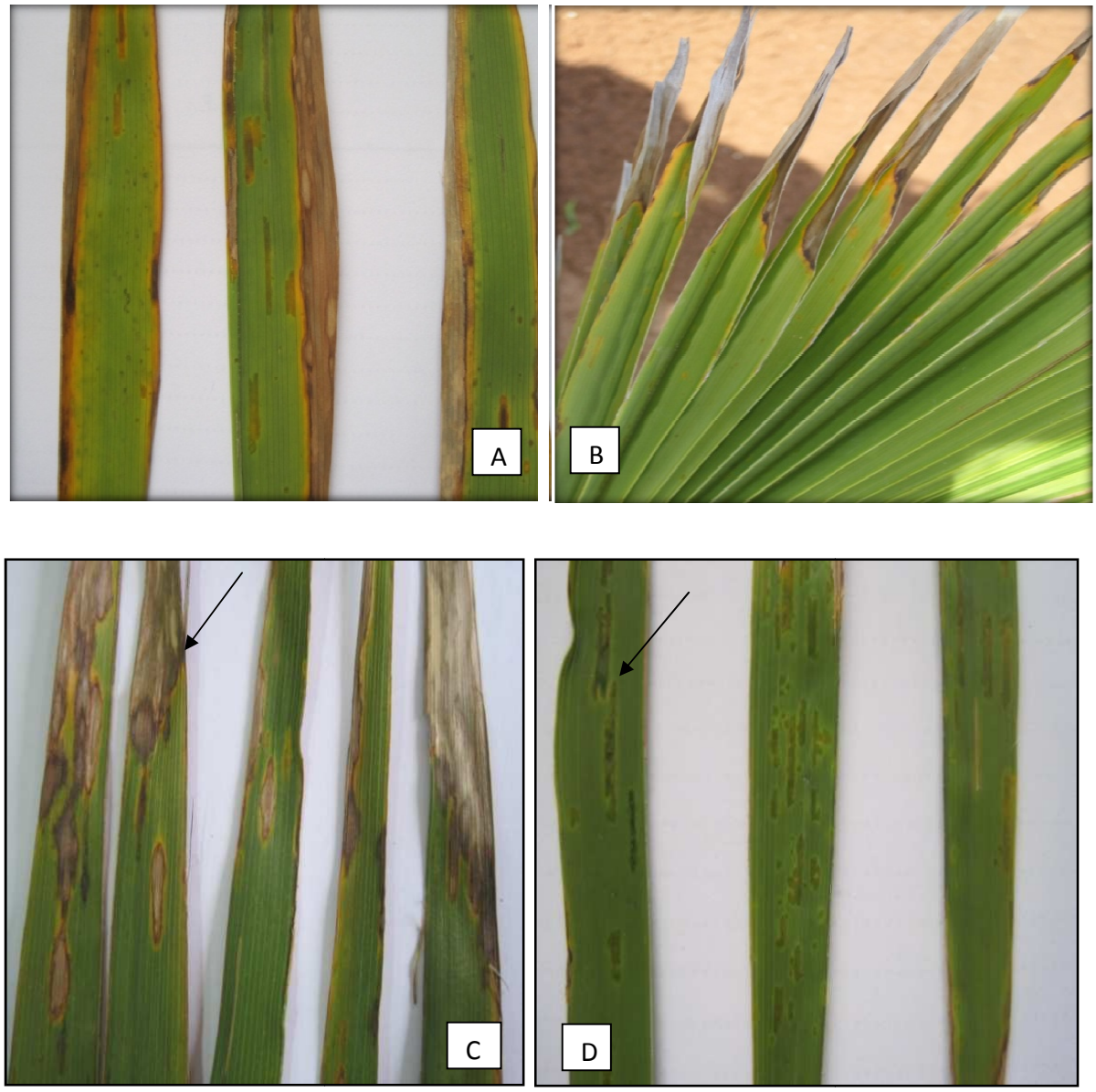
Sporulation on the host was evaluated following the procedure of Hill and Nelson (1983) by estimating the average number of conidia produced per unit surface of the injured leaves (Number of conidia / cm^2).

RESULTS

The leaf symptoms appeared first on the tips of the leaves leading to the dry out and to the burns at the ends and the to the whithering toward the center. Symptoms were variable, elliptical or rectangular tasks reddish brown to dark brown (3.5 ± 5.5 mm in diameter).

Sometimes tasks are circular or elliptical with gray centers and dark brown to black outline around (6.5 ± 8.2 mm in diameter). Others are smaller tasks elongated dark green to black 2.2 ± 3.6 mm in diameter (Figure 1).

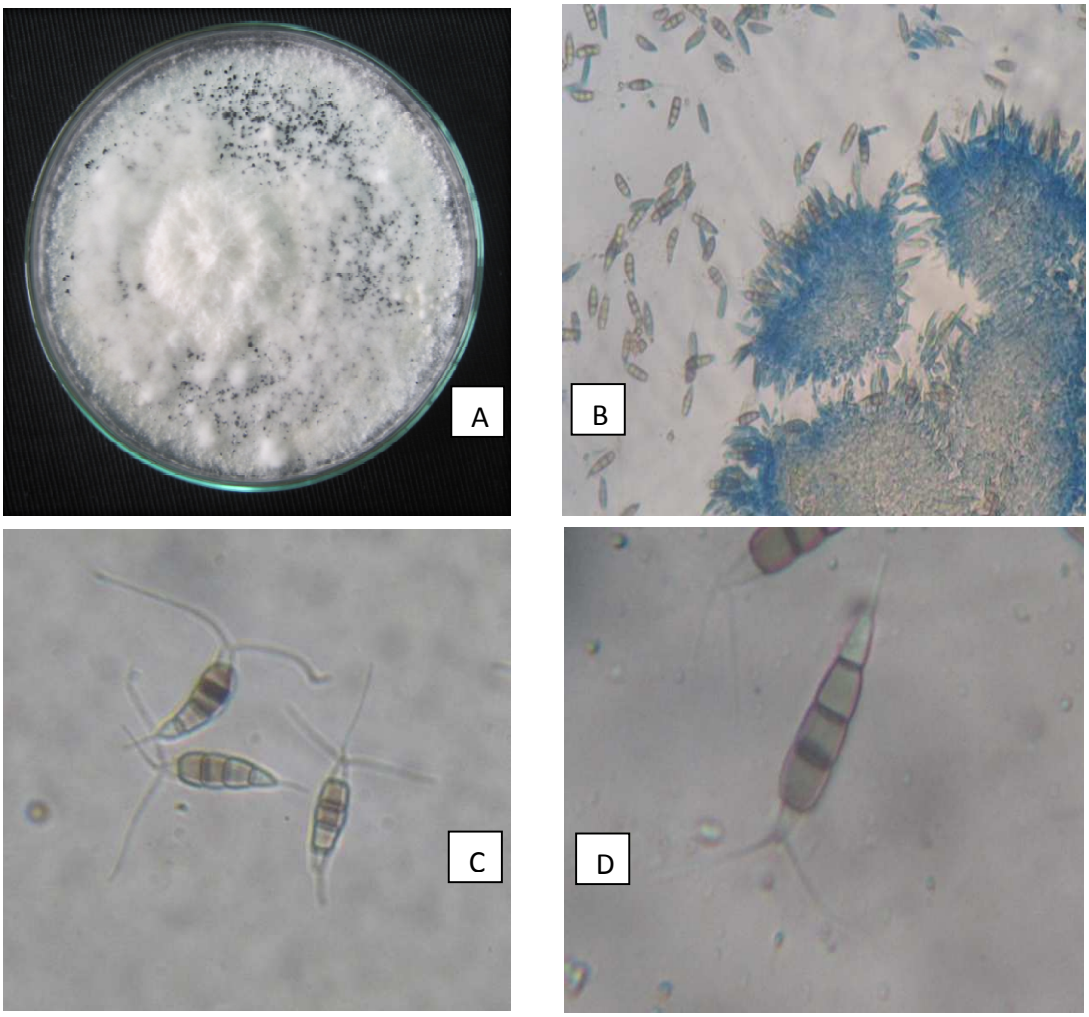
Fig.1: Types of foliar symptoms developed on the leaves of *Washingtonia robusta* (A, B, C, D).



P. palmarum belongs to Sordariomycete class, Sub Class Xylariomycetidae, Order of Xylariales, Family Amphisphaeriaceae.

On PDA media, colonies are white with black spots scattered which represent Acervuli each containing a mass of spores (Figure 2). Conidia have 5 cells, 3 intermediate colorblocking, brown, yellow, olive ridley, and 2 hyaline at the two extremities apical and basal, where the not knobbed appendages are attached. They are about 16-22 microns in length, 5-7 microns wide, the appendages are often in the number of three, with filiform around 16µm in length.

Fig.2: Colonies conidia and acervuli of *P. palmarum* isolated from lesions leaves on *W. robusta*



A: Macroscopic appearance of the fungus on PSA medium; (B, C and D): Microscopic appearance of fungus (G × 400); Mounting liquid: tap water.

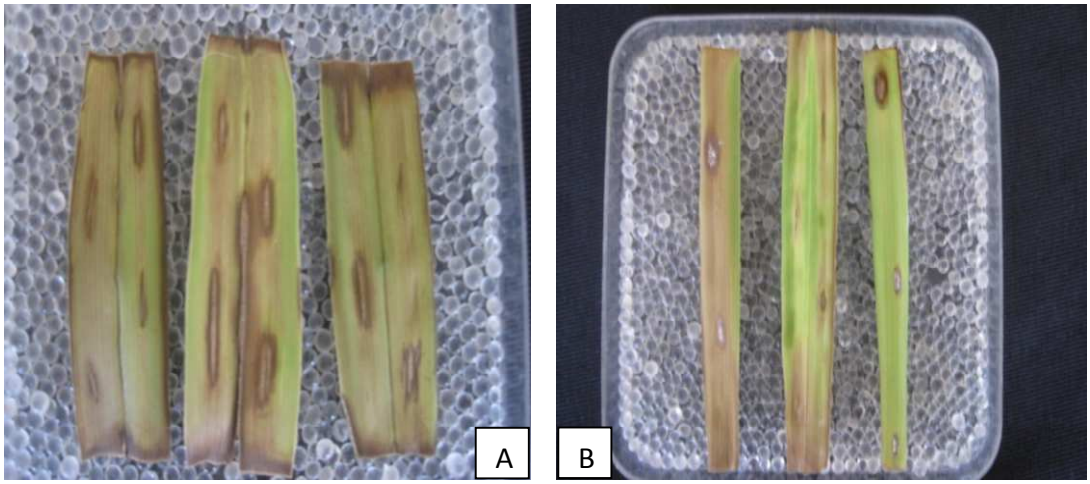
Symptoms appeared on the leaves of *W. robusta* artificially inoculated with *P. palmarum*. The leaves inoculated with conidial suspension are the first who developed symptoms, lesions are greater compared to those observed in leaves inoculated with mycelial discs. White mycelium appeared three days after on the leaves inoculated with the mycelial disks. Under this mycelium, small tasks are developed, they were elongated and dark brown to brownish color in the rib as well as in the periphery. These tasks are developed with time on both sides of the inoculated leaf.

On the inoculated leaves, lesions have varying diameters. The lesions are highly developed on the leaves inoculated with the conidial suspension; the diameter of lesions sometimes exceeds 7.08 mm. In those inoculated with mycelial disks, lesions diameter is in the order of 4.40 mm.

The necrotic area percentage after the artificial inoculation with *P. palmarum* has mounted that this species is able to induce a high infection percentage in leaves inoculated with the two used inoculation techniques. It is higher, in leaves inoculated with conidial suspension in the order of 40.16% and 25.66% in those inoculated with mycelial discs.

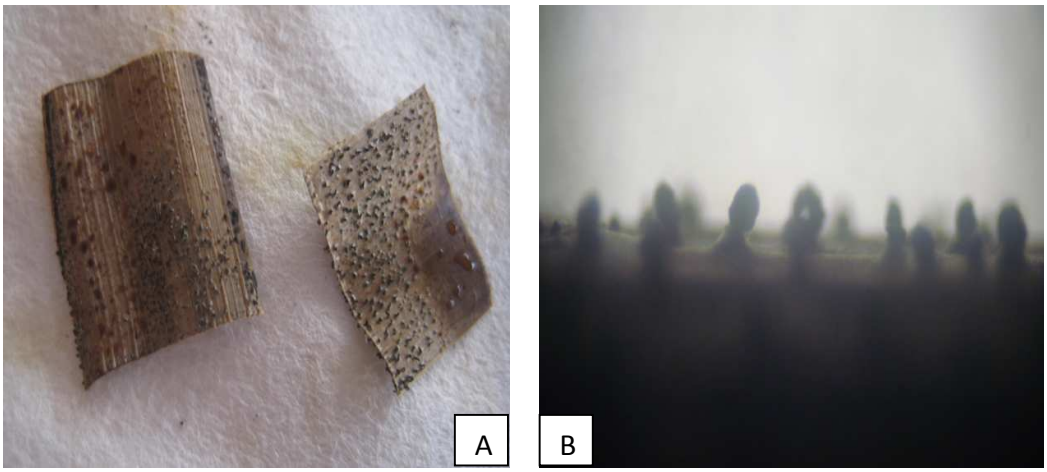
P. palmarum has shown its ability of sporulation on the leaves of *W. robusta* and this regardless of the type of inoculation. The conidia number of the lesions formed on the leaves inoculated with conidial suspension is slightly greater than those formed on the lesions induced by the inoculation with mycelial disks, respectively 2.49 and 2.30 conidia / cm^2. The monosporal isolation from the lesions developed on inoculated leaves only allowed to isolate the species.

Fig.3: Symptoms developed on *W. robusta* leaves inoculated with *P.palmarum*



(A): inoculation by conidial suspension (B): Mycelial disc inoculation

Fig.4: Fruiting (Acervuli) developed on the leaves of *W. robusta* inoculated with *P. palmarum* in a humid chamber



(A): View to the naked eye; (B): observed with the optical microscope G × 400

DISCUSSION AND CONCLUSION

In general, species of the genus *Pestalotiopsis* are widely distributed in the world, thus developing on a wide range of substrates[22]. Most of them are plant pathogens[23], but some are soil saprophytes[24] and plant debris[25].

Pestalotiopsis palmarum (Cooke) Steyaert was isolated from Washington palm leaf lesions, the fungus pathogenicity was proven on leaves of this ornamental plant[14] reported that many palm leaves diseases are reported to be associated with the species of the genus *Pestalotiopsis*, they induce tasks, more or less frequent, gray leaf burns and disease tissue.

P. palmarum does not exhibit host specificity, several plant species can be infected with this pathogen: *Washingtonia*, *Areca catechu*, *Borassus flabellifer L*., *Chamaerops humilis* and *Elaeis guineensis*, *Hevea brasiliensis*, *Manilokara hexandra* and *Musa sp.*[26]. Symptoms formed on diseased leaves are brown yellow tasks, white to gray, with a dark brown outline, oval, elongated and parallel to the ribs. Dark Acervuli can be observed on the upper surface at the center leaf.

P. palmarum is widespread in the tropics; the pathogen induces the formation of severe tasks[27] and gray leaf blights *Coconut*[28,29.] Similarly, the *P. palmarum* disease develops slowly on the palms of several palm tree species, the symptoms are usually observed on older leaves and infection requires injury and other

pathogens penetration[16]. In case of *cocos romanzoffiana*, the fungus penetrates so deep in tissues, causing brown coloration and grayish-brown spots that develop on the leaf blade, then reply to form large brown spots[30].

The pathogenicity study of *P. palmarum* on the *W. robusta* foliage showed that this fungal species is able to induce lesions with various shapes, and sporulating on lesions seven days after inoculation. The leaves are responsive to this pathogen, and the symptoms are in the form of small tasks elongated dark brown to brownish in the ribs and peripheries, then move slowly because the inoculation is performed at the center in case of mycelial disks inoculation. By cons, when inoculating with conidial suspension lesion progression is rapid, and the injured area is more extensive as the spores are dispersed over the entire leaf surface.

Developed symptoms in the inoculated leaves of *W. robusta* are not truly specific, it is assumed that the conditions of development are different than the conditions prevailing *in situ.*

Artificial inoculation tests performed in this study have helped to define *in vivo* pathogenicity of the species tested and the ability of *P. palmarum*, to induce sporulating lesions on diseased leaves in survival *W. robusta*.

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