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Isolating and diagnose of the fungus *Pestalotia spp* that causes spotted leaves for four plants collected from some nurseries of the province of Maysan / Iraq

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Abstract:

The field survey had done to asolate and diagnose the fungus pestalotia spp on Ficus carica L. (Moraceae), Citrus sinensis, Cestrum nocturnum and Gardenia jasminoides that Collected from three nurseries in Maysan province, as well as pathogenicity for therse isolates done. The results showed that the highest rate of injury was to the F. carica (36.6%) in the nursery Altor, while the lowest percentage of injury was to Gardenia (10.0%) in the nursery Altor also. Significant differences were found at 0.05 P≥ = 8.07 between the plants.

Twelve isolates of the fungus were obtained their pathogenicity to these plants were tested. The results showed the presence of different degrees of pathogenicity according to the isolation and the type of plant. The index is estimated the seriousness of the disease on the different plant leaves as the highest severity was injured on the C. sinensis and the isolation (B2) was (79.50%), while it was less severe injury on the cestrum t in the isolation (C1) was (26.00%). This is the first record of the fungus Pestalotia spp on these plants in Iraq.

Key words: *Pestalotia spp*, Leaf spot, *Ficus carica* L, *Citrus sinensis, Cestrum nocturnum*, *Gardenia jasminoides*.

INTRODUCTION

Nurseries occupies an important position in the agricultural life they supplies farmers with seedlings and fruit trees and place produce agricultural seedlings and ornamental trees. There are many plants that are grown in nurseries such as *Ficus carica* L. (Moraceae) is native to southwest Asia and the eastern Mediterranean Sea, one of the first plants that have been cultivated by human and is valid for dry and fresh fruit consumption and are used to treat various ailments, such as stomach problems , inflammation and cancer (Mawa et al,2013), *Citrus sinensis* (L. Osbeck) (Rutaceae) or sweet orange originated from south East Asia, but is consumed all over the world as an excellent source of vitamin C, a powerful natural antioxidant that builds the body immune system. Important phytochemicals like liminoids, synephrine, hesperidin flavonoid, polyphenols, pectin, and sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium are also present. These biologically active compounds prevent arteriosclerosis, cancer, kidney stones, stomach ulcers and reduction in cholesterol level and high blood which promote human health. However, the impact of diverse diseases caused fungi (sweet orange scab, citrus black spot, powdery mildew) (Etebu and Nwauzoma, 2014), *Cestrum nocturnum* L. (Solanaceae) commonly known as night-blooming Jessamine is native to warm subtropical and tropical areas of America , (Li et al., 1988) The leaves of *C. nocturnum* have pharmacological significance in Chinese folk medicine and have been used for the treatment of burns and swellings (Xiao, 1989). The leaves of the plant have shown significant analgesic and bactericidal activity (Chatterjee and Bhattacharjee, 2007) and *Gardenia jasminoides* Ellis This genus is belonging to Rubiaceae (Coffee) family is native to china (Mostafa et al., 2013), The richly scented Gardenia jasminoides Ellis is suffering from several diseases such as leaf spot (Zheng-shiweii and Lao-chong, 2004).

The genus Pestalotia is one of the most common cause of the spotted leaves disease on the plants which cause many diseases during its life cycle (Watanabe et al,2010) . Leaf Spot disease that attacks the gardens, fields and reduces the growth and development of plants (Islam et al, 2004). The fungus has many species that cause spotted leaves disease on many trees and ornamental plants (Dube and Bilgrami, ***2005*** ., Monica, 2009), the fungus also affects the leaves , fruits and plant stems (Tandon,1955., Mouden, 2014), the first record of this fungus was by De Notaris in (1839) , and this anamorphic genus has been studied extensively by Steyaert (1949) while Guba (1961) adopt broader generic concept (Dube and Bilgrami,1965.,We and Tong,2004 and Kamil et al.,2012).

Pestalotia fungi are anamorphic forms in the family Amphisphaeriaceae (Xylariales) are coelomycetous genera with saprobic, endophytic or plant pathogenic life styles residing (Kang et al.,1999 and Arzanlou et al.,2012).

The optimum temperature for the growth of *Pestalotia was* 20^0C, 25^0C and 30^0C

The growth of *Pestalotia* were not affected by the light condition(Adeniyi et al.,2011) and Best(PH) for the growth of fungus *Pestalotia psidii* is 6.5(Younis et al.,2004).

The fungus was isolated Jerbera and Chrysanthemum in Turkey (Sezgin et al.,2006).The fungus *Pestalotia langloissii* infects gardenia plant and cause a Leaf Spot (Mostafa et al.,2013) *Pestalotia longisetula* reported for the first time on strawberry in Morocco (Mouden et al.,2014) ,and the first report of *Pestalotia fici* causing leaf chlorosis and fruit rot on olive in Morocco (Chliyeh et al.,2014), The recording of *Pestalotia sp*.as true pathogen on Date Palm leaf was the first time in Iraq (**Abass** et al., 2007)

MATERIALS AND METHODS:

The experiment was conducted in the laboratory of plant protection department / Faculty of Agriculture / Maysan University in 2015/2016.

1) Field survey:

Three nurseries in Maysan were included in random survey. They are Altor, and Alsalam and Alzhor nurseries, several plants were checked. Four plants appeared symptoms of spotted leaves they are *F. carica*, *C. sinensis, C. nocturnum* and *G. jasminoides were selected* to conduct the study which calculated the preparation of each plant in each nursery and the proportion of infection calculated to them according to the law following:

Percentage of infection = number of infected plants / Number of plants tested × 100

Samples from the infected leaves or apparent symptoms were taken and put in a Polyethylene bags with record information such as the name of the nursery and the location, date and transported to the laboratory for pathogens isolating.

2) Pathogen Isolation

The fungi were isolated from the samples following the "Tissue Planting method". The specimens were cut into small pieces (2 mm × 2 mm) and surface sterilized by dipping in 10% chlorox for 3-5 minutes followed by rinsing in sterilized water. Surface sterilized plant pieces were placed on PDA medium (Tuite 1969). Then samples were dried on filter paper Type Watman-No4 Then (4) pieces were transferred to a sterile Petri dish diameter (9 cm) were taken and placed on solidified PDA (Potato Sucrose Agar: 200 g potato, sucrose: 20 g, Agar-agar: 20 g, distilled water: 1000 ml) and add anti biotic Chloramphenicol

at a rate of (250 mg / L) in Petri dishes at 4 pieces per plate. The plates were incubated for 5-7 days at 25±1°C.

3) Purification and preservation:

To obtain pure culture of pathogen, the hyphal tips were transferred aseptically onto PDA plate by using the flame sterilized tip of an inoculation needle. The plate was incubated at room temperature for seven days. Advance hyphae were collected and transferred in to the test tube slants containing PDA and incubated at room temperature for seven days .After incubation ,the slant were carefully checked for contamination and then preserved at 4^0C in a refrigerator for further use.

4) Diagnosis isolates fungus

The isolated fungi were identified based on morphological characteristics observed under a compound microscope following standard keys (Steyaert, 1949, Guba, 1961, Watanaba, 2002)

5) Pathogenicity tests

The isolates were proved for their pathogenicity according to the modified technique of phong, et al.(2014) in the laboratory. A sterilized filter paper was placed in a sterilized 9 cm-diameter Petri dish. Taking (15) leaves of each plant and each isolation, The leaves were wounded by a sterilized needle before placed on the filter paper in the Petri dish. 0.5 cm diameter sterilized cork borer was used to remove agar plugs from the actively growing edge of the cultures of the *Pestalotia spp*. and placed onto the wounded position of the leaf surface. The filter paper in the Petri dish was moistened by sterilized distilled water. The non-inoculated leaves were treated with 0.5 cm sterilized agar plug served as control. All Petri dishes were incubated at room temperature (27-30^{o}C) for 10 days before data collection.

The diseased leaf area was scored after 15 days of inoculation using the scale of Stover modified by Gauhl et *al*.,11 : **0**= No symptoms ; **1** = -0.5% of the limbus with symptoms ; **2** = 0.6 to 5% of the limbus with symptoms ; **3** = 6 to 15% of the limbus with symptoms ; **4** = 16 to 30% of the limbus with symptoms; **5** = 31 to 50% of the limbus with symptoms ; **6** = 51 to 80% of the limbus with symptoms ; **7** : 81 to 100% of the limbus with symptoms.

The severity index (IS) of disease was calculated using the formula:

$$\mathbf{IS=(\Sigma nb/(N-1)\ x\ T)\times 100}$$

n= Number of leaves for each degree of the scale.
b= Degree of the scale.
N= Number of the degrees used in the scale.
T= Total number of the scored leaves.

RESULTS AND DISCUSSION:

1) Field survey:

Field survey results (table 1) which included the nursery Altor , Alsalam and Alzhor showed that disease Leaf Spot is widespread on all studied plants in these the nurseries , also observed symptoms of infection in the form of spots on the leaves light brown color with different sizes in diameter (0.5 -2 cm) oval or irregularly at first, then gradually expand and combine with each other to be large gray patches to the color brown with dark brown edges Then gradually expand and combine with each other to be large gray to brown patches with dark brown edges then the leaves dried and die because of the infection with *Pestalotia spp*. The highest percentage of infection to plant *F. carica* were (36.6%) in the Altor nursery, followed by Alzhor nursery (32.0%), while the lowest percentage of infection to *G*. jasminoides which was (10.0%) in the Altor nursery

Table (1) the percentage of infection to *F. carica*, *C. sinensis*, *C. nocturnum* and *G. jasminoides with l*eaf Spot disease

Nurseries	percentage of infection			
	F. carica	*C. sinensis*	*C. nocturnum*	*jasminoides.G*
altor	*36.6*	*30.0*	*16.6*	*10.0*
alsalam	*26.6*	*23.3*	*13.3*	*24.0*
alzhor	*32.0*	*28.0*	*20.0*	*16.0*

Less significant difference at the level of probability of 0.05 P≥ = 8.07

2) Isolate and diagnose fungus *Pestalotia spp*

Twelve (12) isolates of the fungus *Pestalotia spp were obtained* (Figure 2) by four isolates (A_1,A_2,A_3,A_4) from *F. carica* and three isolates (B_1,B_2,B_3) from *C. sinensis* and three isolates (C_1,C_2,C_3) from *C. nocturnum* and two isolate (D_1,D_2) from *G.* jasminoides . Fungal isolates were classified by comparing the phenotypic characteristics. The fungal colony was colorless, turning cottony white with abundant scattered acervuli containing black, slimy spore masses colonies attained 5.2 cm in diameter on PDA after 5 days at 25°C and produced abundant, white, aerial mycelium. Conidiophores short, simple. Conidia spindle-shaped or ellipsoidal, 4- to 5-celled with 2–3 central, pigmented cells (especially darker in 2 cells), with (appendages (setula) in apical cells and 1 short appendage (pedicel 4-2) in basal cells. conidia (excluding apical and basal appendages) 14–18 × 5–6 µm: pigmented cells 10–14 µm long: apical appendages 26–44 × 0.8–1 µm (Figure 2) This corresponds with (Guba, 1961) and is it the first record of the fungus *Pestalotia spp* on these plants in Iraq.

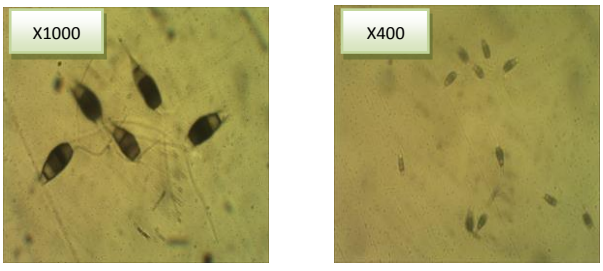


Figure 1: conidia of *pestalotia spp*

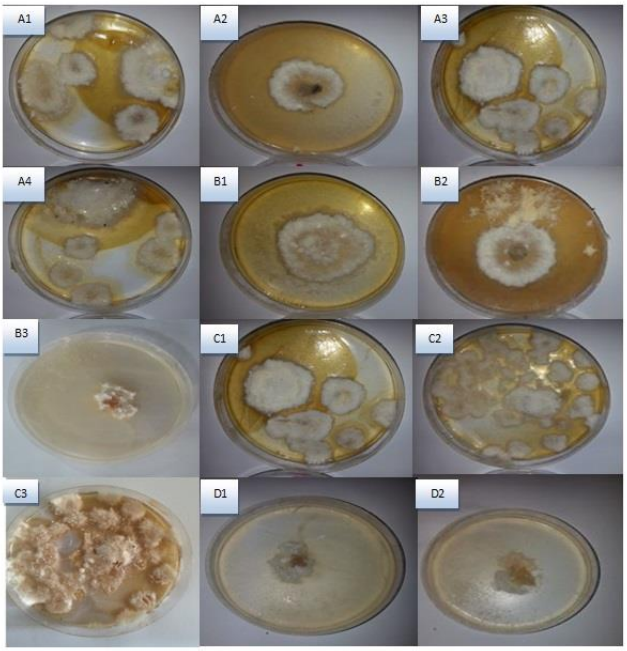


Figure 2: isolates fungus *Pestalotia spp* isolates (A1, A2, A3, A4) from the plant *F. carica* and isolates (B1, B2, B3) of *C. sinensis* and isolates (C1, C2, C3) from the *C. nocturnum* isolates (D1, D2) from the *G. jasminoides*.

3) The pathogenicity of *pestalotia spp* isolates

Disease severity appeared at different degrees according to the isolates and the studied plant kinds. 10 days after inoculation, the estimated disease severity index on *F. carica* Isolates ((A_1,A_2 ,A_3, A_4) was (71.25,72.00,68.25 and 63.75) respectively, as for as *C. sinensis* was the highest severity index recorded by isolation (B_2) (79.50) the isolates (B_1, B_3) were estimated severity index respectively (44.00,46.00) respectively, as for as *C. nocturnum* the isolates (c_1,c_2,c_3) estimated severity index (26.00,38.5 and 29.500) respectively ,and for *G. jasminoides* the isolates (D_1,D_2) estimated severity index (36.00 and 38.50) respectively (Figure3A,B) .

The fungus *Pestalotia spp* produced conidia on the leaves of plants when with the mycelium fungus from *F.*

carica (3.3 , 2.7, 3.2 and 2.9 × 10^5 conidia /cm^2) respectively and isolates from *C. sinensis* recorded (3.4 , 3.1 and 2.9 × 10^5 conidia / cm ^{2}) respectively, as well as isolates from the *C. nocturnum* recorded (2.2 , 2.3 and 2.7 × 10^5 conidia / cm ^{2}) respectively, and isolates from *G. jasminoides* recorded (2.9 and 2.8 × 10^5 conidia / cm ^{2}) respectively (Figure 3C) .The results showed that there are no significant differences between the isolates of the fungus *Pestalotia spp* ,these findings are consistent with Mouden et al., (2014), which tested the pathogenicity of the fungus isolates *Pestalotia longisetula* on strawberry leaves.

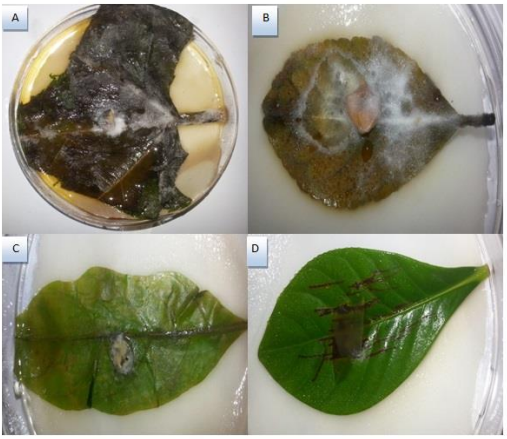


Figure 3A: Lesions developed on plants leaves after artificial inoculation with *Pestalotia spp.* A) *F. carica B) C. sinensis C) C. nocturnum D) G. jasminoides*

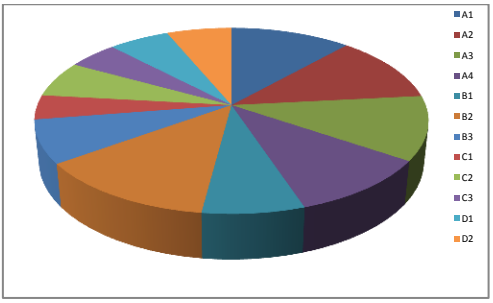
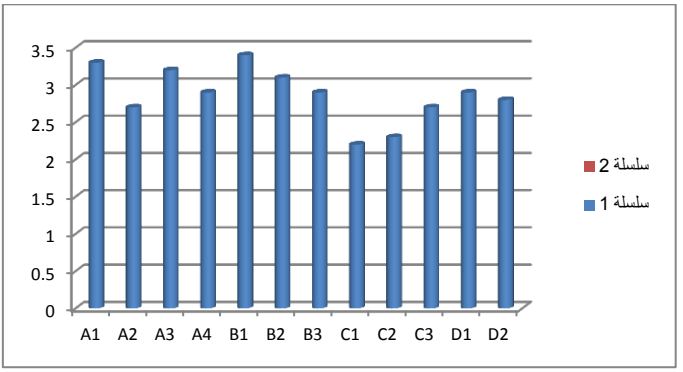


Figure 3B: Disease severity on leaves of *F. carica, C. sinensis, C. nocturnum and G. jasminoides* after inoculation with mycelial disc *pestalotia spp*



Values followed by the same letter do not differ significantly at 5%.

Figure 3C: Conidia production of *Pestalotia spp* on the leaf surface of *F. carica, C. sinensis, C. nocturnum and G. jasminoides* after inoculation with mycelial disc *pestalotia spp* .

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