

Original Research Article

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**First Record of *Alternaria alternata* (Fr.) Keissler on
Rosa damascena Mill in Iraq**

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The field survey had done to isolate and diagnose the fungus *Alternaria alternata* on *Rosa damascena* that Collected from three nurseries in Maysan province, as well as pathogenicity for these isolates done. The results showed that the highest rate of injury in the nursery Alzhor was 97.2 %. Then after the nursery Alsalam percentage of injury was 96.3 % and Significant differences compared to the nursery Altor rate of injury was 70.9%. It has been isolated and diagnosed four isolates of the fungus *A. alternata* from the plant leaves *Rosa damascena* that showed symptoms of disease leaves spotted and diagnosed isolates by comparing the phenotypic characteristics of the fungus pathogen on PDA medium, the results showed the presence of different degrees of pathogenicity according to the isolation, Isolation A2 were more pathogenesis ability was 79.5% followed by isolates (A1, A3, A4) were (72.2, 68.2 and 63.7%) respectively. Significant differences were observed for all isolates compared to the control treatment. This is the first record of the fungus *Alternaria alternata* on this plant in Iraq.

Introduction

Rosa damascena mill, commonly known as Damask rose. It is one of the most important species of Rosaceae family. Rosaceae are well-known ornamental plants and have been referred to as the king of flowers (Evans *et al.*, 1996). *R. damascena* Mill which grow in some part of Europe and Asia particularly in Middle East (Ghahreman, 2001). Apart from the use of *R. damascena* as ornamental plants in parks, gardens, and houses, they are principally cultivated for using in perfume, medicine and food industry (Widrlechner, 1981). The flowers are renowned for their fine fragrance, and

commercially harvested for rose oil and to make rose water (Baydar and Baydar, 2005). Damascus rose oil also has therapeutic properties that sooth the mind and helps with depression, nervous tension and stress, and believed to assist conditions like frigidity, chronic bronchitis, asthma, skin disease, cancer, ulcers, wounds, wrinkles, infections, as well as constipation (Mirheydar,1993; Boskabady *et al.*, 2011).

The genus *Alternaria* belongs to the Phylum: Ascomycota, Subdivision: Pezizomycotina, Class: Dothediomycetes, Order:

Pleosporales and Family: Pleosporaceae. *Alternaria* belongs to the division Deuteromycota with several species (Mamgain,2013). *Alternaria alternata* has an important place among species of this genus, because of wide range of hosts including garden plants, field crops, vegetables, and ornamentals (Kohmoto *et al.*, 1993).

The fungal pathogen, *A. alternata* mostly affects the foliar parts causing light brown to dark brown, roundish-oval to irregular spots of 1 to 2 mm in diameter in initial stage, while later expanded, often coalesced and produced 'Shot hole' during severe infection. The disease severity on foliage was more common during humid weather and symptoms were most pronounced on nutrient deficient leaves (Nagrle, 2007 and Nagrle *et al.*, 2012).

This fungus is characterized by its ability to produce a number of enzymes able to injury many types of plant families (Fanta *et al.*,2003; Fawzi *et al.*,2009). It also features produced black pigment melanin, which is often believed to have an important role in pathogenicity and resistance to harsh Living situation of drought, heat and UV irradiation and other melanin is produced by the metabolism of a secondary fungus (Jacobson, 2000).

When studying the pathogenesis fungus on that a breach cannot happen through the stomata, although the tube germination grow near or above in some cases be a member of sticking near them, while penetration directly happens in the case of a wound in plant tissue and without be appressorium (Gupta, 1998), or the occurrence of penetration through stomata and wounds and be without appresoria (Allen *et al.*, 1983). The appearance of symptoms of the leaves, the occurrence of yellowing of plant tissue

followed by the death of this tissue are shown symptoms of body concentric overlapping circles, as spotted at the ends apical Exchange shows (Securities corners) where the fabric is characterized as dead as a result of a complete decomposition of the tissues in these areas (Grogan, 1975).

Materials and Methods

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

Field survey

Three nurseries in Maysan were included in random survey. They are Altor, and Alsalam and Alzhor nurseries, checked Rosa damascene plants appeared symptoms of spotted leaves were selected to conduct the study which calculated the preparation of plant in each nursery and the rate of injury 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.

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.

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 40C in a refrigerator for further use.

Diagnosis isolates fungus

The isolated fungi were identified based on morphological characteristics observed under a compound microscope following standard keys (Ellis, 1971; Watanaba, 2002).

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 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 *A. alternate* 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°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.*, 1996, : 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:

$$IS = (\sum nb / (N - 1) \times T) \times 100$$

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

Field survey

Field survey results (Figure 1) which included the nursery Altor, Alsalam and Alzhor showed that disease Leaf Spot is widespread on *R. damascena* in these the nurseries. The highest percentage of infection in Alzhor nursery was (97.2%),

followed by Alsalam nursery was (96.3%), and differ significantly compared with Altor nursery was (70.9%).

The reason may be due to the High percentage of infection to increased humidity and intensive farming converged for seedlings increases the spread of spores and the presence of spray irrigation water. The importance of fungi that cause disease Leaf Spot for being targeted at food-making processes the important growth Which The photosynthesis in the Leaf.

Isolation, Purification and Identification of Pathogen

The process of isolation resulted in four isolates of pathogen collected from *R.damascena* (Figure 2), by two isolates (A1 and A2) from Alzhor nursery, one isolate (A3) from Alsalam nursery and one isolate (A4) from Altor nursery. All the isolates were confirmed by morphological and cultural characters as isolates of *A. alternata*. Fungal colonies were olive green to sooty-black in colour and showed a minutely-densely turfy surface.

Table.1 Pathogenicity of *A.alternata* isolates

No. isolation	severity of injury%
A1	72.2
A2	79.5
A3	68.2
A4	63.7
control	0%
L.S.D	0.7

Fig.1 the percentage of infection to *R. damascena* with leaf Spot disease

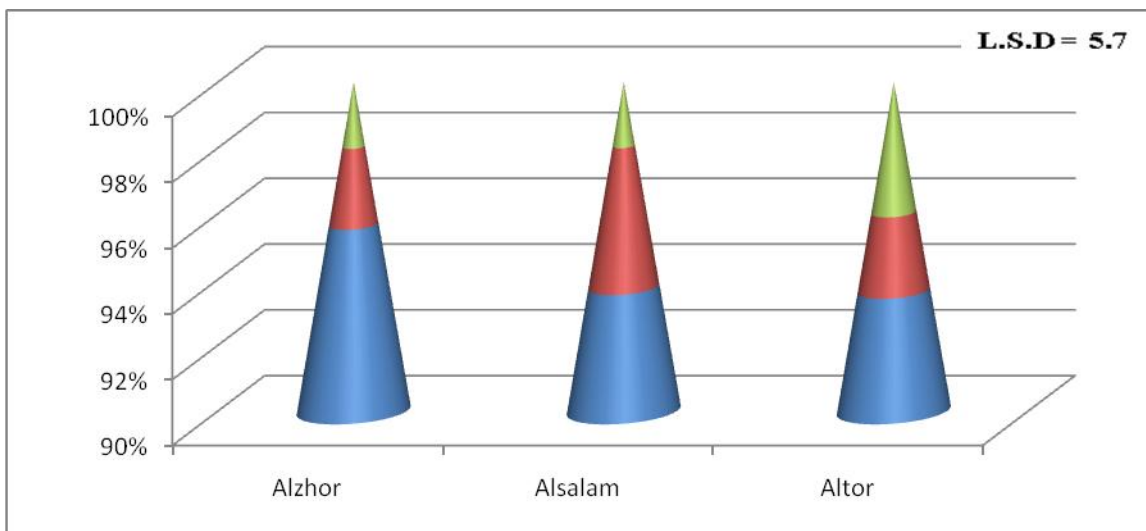


Fig.2 Isolates fungus *A. alternata* from *R.damascena* on PDA

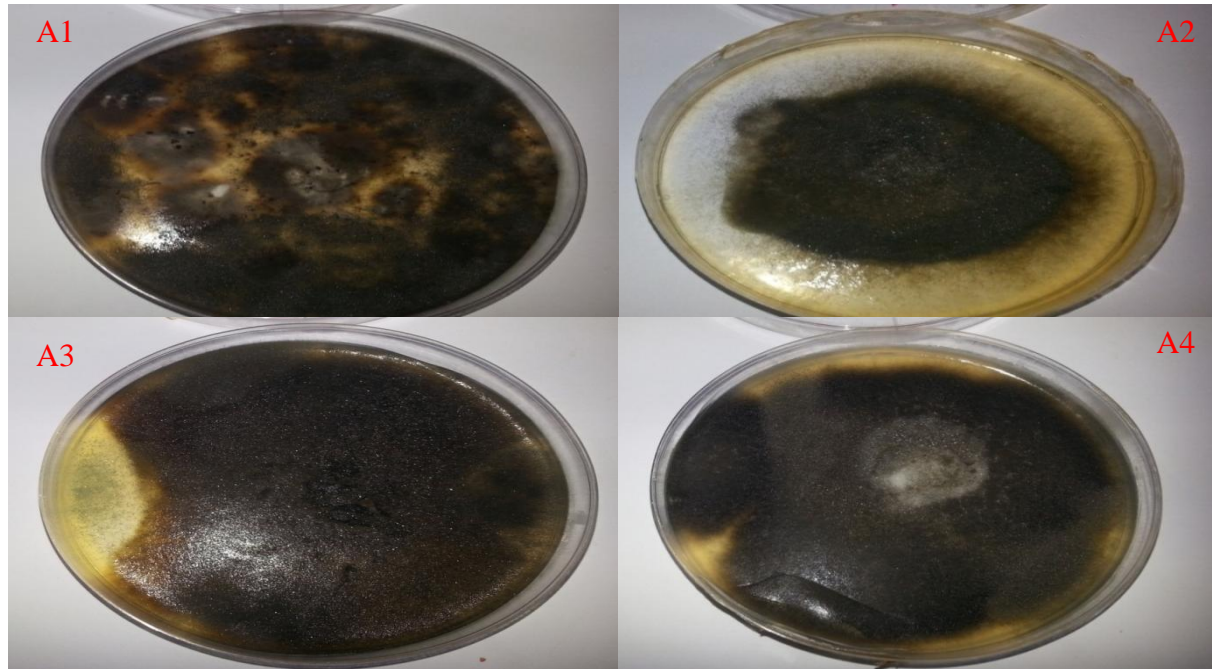


Fig.3 conidia of *A.alternata*

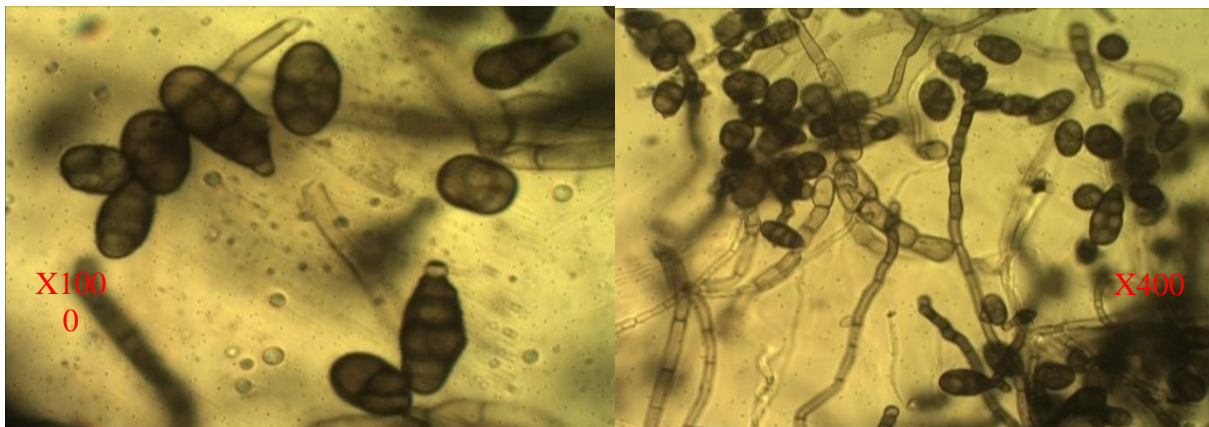
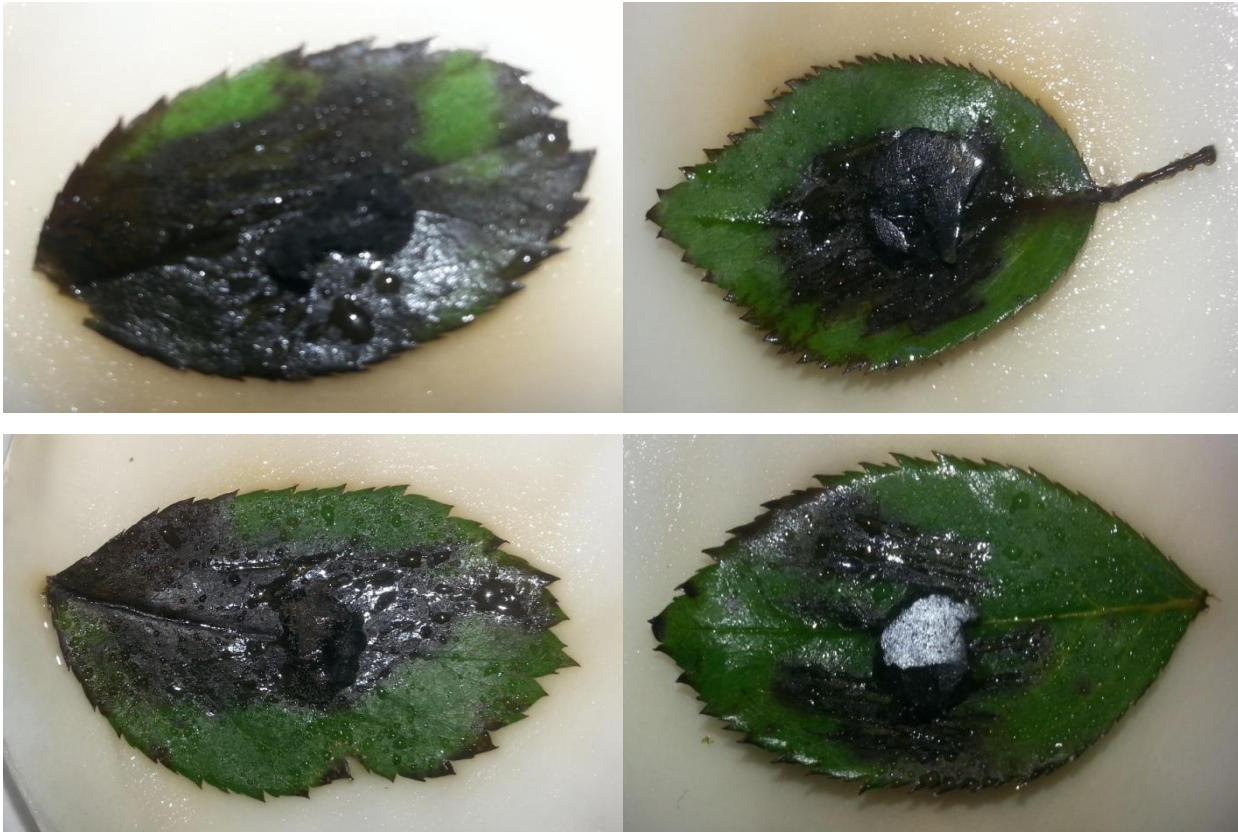


Fig.4 Pathogenesis *A.alternata* isolates on *R.damascena*



During the initial colony growth, a white margin of mycelia was observed that progressively changed to olive green, and then to grey-black. Single suberect conidiophores arose on aerial mycelia and produced clusters of small conidia in branched chains. Conidia were yellowish to golden brown with longitudinal and transverse septa and a short beak (Figure 3). This Results are consistent with Hubballi *et al.*, 2011. It the first record of *A. alternata* on *R.damascena* in Iraq.

The pathogenicity of *A.alternata* isolates

Disease severity appeared at different degrees according to the isolates and. 10 days after inoculation, the estimated disease severity index on *R.damascena* (Table 11 Figure 4), The isolation A2 were more pathogenesis ability as the severity index

recorded 79.5% Followed isolates (A1, A3, A4), which amounted to (72.2, 68.2 and 63.7%) respectively, and significant differences for all isolates were compared with the control treatment. The reason for the difference may be due to the estimated pathogenicity of different isolates to the production of extracellular enzymes such as cellulase and lipase and protease, etc. (Aba Alkhail, 2005; Fawzi *et al.*, 2009), Or the difference may be due to the secretion of toxic compounds such as Alternariol and Alternic acid Which has an important role in demonstrating the severity of injury (Harven and Pero, 1984).

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