

## **Leaf Blight of Date Palm Caused by *Glomerella cingulata* in Al-Qassim Region**

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**Abstract.** This is the first report on the occurrence of leaf blight disease of date palm caused by *Glomerella cingulata* in Saudi Arabia. The disease was found in several date palm farms in Al-Qassim region. The fungus was isolated from infected plants and its pathogenicity to Aum Al-Khashab, Shagra and Helwa date palm cultivars was tested under controlled environment. The wounded leaves of all cultivars were susceptible to the tested fungus while non-wounded tissues were either not infected or slightly affected. The growth of the fungus on PDA was optimum at 30°C. The toxicity of Shafy, Bavistin, Benlate, Dithane M-45, Topsin and Impact fungicides to *G. cingulata* was tested on agar plates. Shafy was the most toxic while Dithane M-45 was the least toxic to *G. cingulata* growth.

### **Introduction**

Date palm (*Phoenix dactylifera* L.) is the most important fruit tree in the Kingdom of Saudi Arabia. About 11.1 million date palm trees are grown in the Kingdom and about one million of which are grown in Al-Qassim region, Central Saudi Arabia [1].

Several disease problems of date palm trees have been reported in many date producing countries including Beyond disease caused by *Fusarium oxysporum* var. *albedinis* which is the most serious date palm disease in Morocco and Algeria [2,3]. Inflorescence rot (Khamiedj) caused by *Mauginiella scaettae* is a serious disease of date palm in Iraq, Libya, Morocco, Tunisia and Saudi Arabia [4-6]. Leaf spots caused by *Helminthosporium* spp. and *Alternaria* spp. were reported in Al-Qassim region, Saudi Arabia [7]. *Glomerella cingulata* (Ston.) Spa and V. Sch. (Imperfect stage, *Collectotrichum gloeosporioides* CIK) is a serious pathogen to several fruit trees causing leaf rot of coconut palm [3] and Bitter rot of apple (*Malus sylvestris* Mill) [8]. The imperfect stage of the fungus, *C. gloeosporioides*, has been shown to cause disease in citrus [9], avocado [10], papaya [11] and blueberry [12].

In recent years, leaf blight symptoms similar to those described on coconut palm were observed on young trees and off shoots of date palm in several farms in Al-Qassim region.

The purposes of this study were to examine the leaf rot disease of date palm in Al-Qassim region and to identify its causal agent. The susceptibility of several date palm cultivars, toxicity of several fungicides to the pathogen and effect of temperature on its growth were also investigated.

### Materials and Methods

Twenty trees from each of five date palm farms scattered in 100 km<sup>2</sup> area were examined for the occurrence of leaf blight symptoms. Infected and healthy leaf samples were collected in plastic bags and transferred under cooled conditions to the laboratory. Infected leaf tissues were surface sterilized by dipping in 1% NaCl for 1 min then rinsed in sterilized distilled water and plotted dry on sterilized filter paper. Pieces (3 × 3mm) of the surface sterilized tissues were placed on potato dextrose agar (PDA) plates and incubated at 25°C for one week. The infected tissues were colonized with a fungus that was indentified as *Glomerella cingulata*. Pure cultures of the pathogen were obtained by hyphal tip technique. The isolation experiments were made from 30 leaf samples and represent different date palm cultivars and farms.

#### Pathogenicity test

Young (4-6 months old) and old leaves (1-2 years old) of Aum Al-Khashab, Shagra and Helwa date palm cultivars were tested for their tolerance to the isolated fungus. The Leaflets were detached, surface sterilized as described above and placed upright in beakers with distilled water that cover the lower 2 cm of the leaflets. The fungus was maintained on PDA slant agar at 4°C. Inocula were prepared by shaking 5 ml of sterile distilled water in each culture tube and spreading of 0.1 ml spore suspension evenly over the surface of freshly prepared PDA plates. The cultures were incubated at 25°C in plastic bags in the dark [8]. Sparse growth and abundant sporulation occurred in 5 days. A conidial suspension was prepared by flooding plates with sterile distilled water, gently rubbing the agar surface with a sterile bent glass rod, and filtering the suspension through two layers of cheesecloth. Hemocytometer counts were used to adjust spore concentrations to  $1 \times 10^4$  conidia per milliliter. Leaflets inoculation was accomplished by spraying the spore suspension using a high pressure atomizer. Check treatments were sprayed with sterilized water. In other treatments leaflet tissues were wounded with sterile needle before inoculation. Inoculated and noninoculated leaflets were covered with plastic bags for 48 h while incubated at 30°C for 20 days in a growth chamber. The severity of infection was determined according to an arbitrary key; 0 = No visible symptoms 1 = germination of fungal spores on inoculated tissues, 2 = small lesion (1-3 mm in diameter), 3 = medium size lesions (4-6 mm), 4 = large lesion (1 cm), 5 = severe leaf blight symptoms.

### In vitro testing of fungicides

The fungicides, Dithane M 45 (Manganese zinc Ethylene bisdithiocarbamate), Topsin M (1,2- Bas (3-Methozycarbony-thioureido) benzene), Benlate (Methyl 1-(butyl carbamoyl)-2-benzimidazole carbamate), Shafy [RS] -2,4-difluro-a-(I-H-1,2,4, -triazol-I-Yl-Methyl) Benzhydryl Alcohol Flutriafol and Methyl-benimidazol-2 Yl-carbamate), Bavistin (Methyle 2-benzimidazol carbamate) and Impact (CRS) - 2,4-Difluro-a-(I-H-1,2,4-triazol-1 Yl-Methyl) (Benzhydryl Alcohol) were incorporated into PDA at concentrations of 10, 50 and 100 ppm active ingredients. The center of PDA-fungicide plates were inoculated with the fungus and incubated at 30°C. The toxicity of the fungicides to the fungus was determined according to the following equation: % toxicity =  $\frac{A-B}{A} \times 100$ , where A = diameter growth of untreated fungus and B = diameter of treated fungus [6].

### Effect of temperature on growth of *G. cingulata*

Mycelial plugs (5 mm in diameter) one week old cultures of a pathogenic isolate of *G. cingulata* were placed in center of PDA plates and incubated at 5, 15, 20, 25, 30 and 35°C. Ten plates were used for each treatment. The linear growth of the fungus was determined after one week.

## Results and Discussion

A single organism was associated with the symptoms of leaf blight regardless of date palm cultivars or farm location. The isolated organism was identified as *Glomerella cingulata* according to the taxonomical key given by Mordue [13]. The fungal conidia were colorless (10-21  $\mu\text{m}$  long  $\times$  4-6  $\mu\text{m}$  wide with 0-2 transverse septa).

The symptoms of leaf blight in the field appeared mostly on young leaves causing reddish brown spots with distinct dark edges up to 5 cm long and were mostly enlarged and coalesced (Fig. 1 a & b). The disease affected young leaves in the central spindle. The unfolded infected leaves became fan-like with rotted leaflet tips (Fig. 1-c).

In the artificially inoculated leaves, the leaf rot symptoms that developed on non-wounded leaflets of date palm cultivars were minor (severity 0-2) regardless of leaf age or date palm cultivar. Leaf spot symptoms similar to those found in the field were observed on wounded tissues of young leaflets of Aum Al-Khashab, Helwa and Shagra at severity 5, 3, and 3 respectively. The severity of infection in wounded, old leaves of the three date palm cultivar was 2 to 3.

No disease symptoms were observed on control plants. The leaf rot symptoms described above were similar to the leaf rot symptoms of coconut palm caused by *G. cingulata* as described by Cook [3].



Fig. 1. Symptoms of leaf blight of date palm observed in the field caused by *Glomerella cingulata* a, b, spots with dark edges 1-5 cm long on leaves c) fan-like leaflets with scorched tips.

The results of this study suggest that *G. cingulata* is primarily a wound parasite. Therefore, wounds induced by mechanical means or by insects in young leaves may contribute to the development of the disease. Non-wounded tissues may be infected but the progress of the infection is very slow.

The fungicide Shafy was the most toxic to *G. cingulata* at 10, 50 and 100 ppm (Fig. 2). Bavistin and Benlate were also very toxic causing over 90% reduction of the fungal growth. Dithane M-45 was the least effective fungicide against *G. cingulata*. Topsin and Impact showed high toxicity to the fungus at higher concentrations (Fig. 2). These results suggest that the control of leaf blight disease may be achieved by application of locally available fungicides i.e Shafy and Bavistin. However, further experiments should be conducted before making final recommendations on the use of a particular fungicide for control of the disease in the field. The growth of the fungus on PDA was greatest at 30°C (Fig. 3). Therefore, the inoculated leaflets were incubated at 30°C for reading the disease symptoms. This indicates that the *G. cingulata* isolate in Al-Qassim grows best at warm conditions which occur in most of the season.

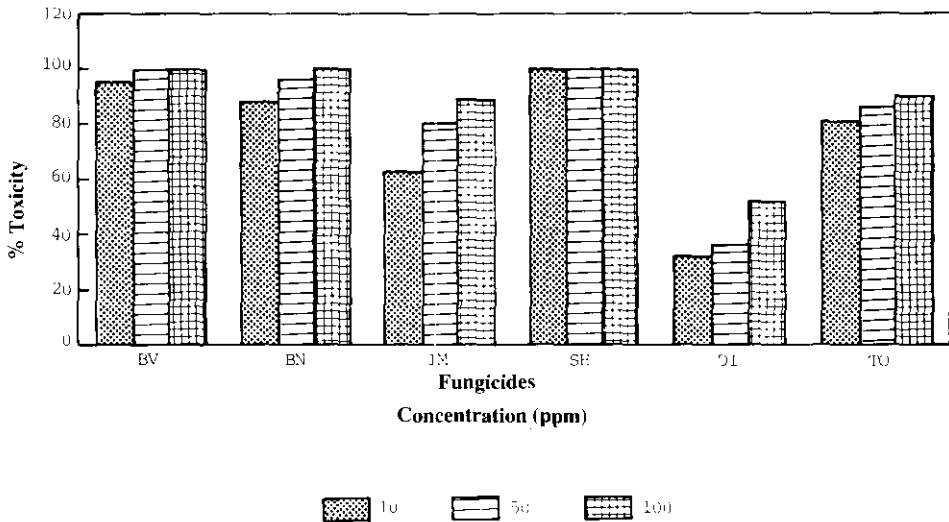
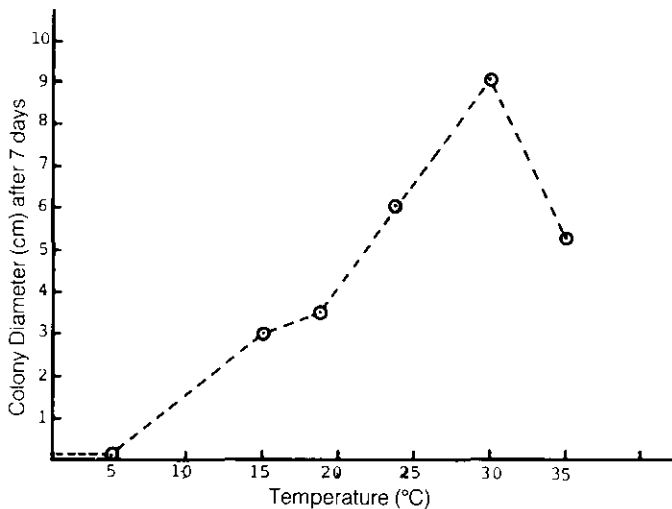


Fig. 2. Toxicity of different concentrations of six fungicides to *Glomerella cingulata* on PDA plantes



**Fig. 3. Effect of temperature on radial growth of *Gomerella cingulata* on potato dextrose agar (PDA) media.**

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## لفحة أوراق النخيل المتسببة عن الفطر *Glomerella cingulata*

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ملخص البحث. هذا هو التقرير الأول عن ظهور مرض لفحة أوراق نخيل البلح المتسبب عن الفطر جلوميريلا سنجيولاتا في المملكة العربية السعودية. فقد شوهد المرض في عدد من مزارع النخيل في منطقة القصيم. ثم عزل الفطر واختبرت قدرته المرضية على أصناف أم الخشب وشقرا وحلوا تحت ظروف بيئية محكمة وقد ظهرت إصابة شديدة على الأوراق المجروحة لهذه الأصناف، إلا أنه في حالة عدم تجريح الأوراق قبل العدوى كانت الإصابة معدومة أو قليلة. اختبرت سمية المبيدات الفطرية شافي، بافستين وبنليت ودياثين وتوبسين وإمباكت على نمو الفطر في أطباق الآجار وكان شافي أشدها سمية للفطر بينما كان دياثين أقلها تأثيرا عليه. كان أفضل نمو للفطر على بيئة البطاطس والدكستروز والآجار على درجة حرارة ٣٠م.

