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## Occurrence of Some Fungal Diseases on Date Palm Trees in Upper Egypt and its Control

<sup>1</sup>Eman S.H. Farrag and <sup>2</sup>Kamal A. Abo-Elyousr

<sup>1</sup>Department of Agricultural Botany (Plant Pathology), Faculty of Agriculture,  
South Valley University, Qena, Egypt

<sup>2</sup>Department of Plant Pathology, Faculty of Agriculture, Assiut Univeristy, 71526 Assiut, Egypt

**Abstract:** In 2007, date palm trees surveyed for different symptoms on leaves and fruits in Upper Egypt. The samples were collected from symptomatic rachis, fronds and fruits in four localities (El-Minia, Assiut, Sohag and Qena Governorates). The major diseases noticed were leaf spots, off-shoot decline, black scorch, leaf basal rot and fruit spots. Seven different fungal species were isolated from date palm exhibited different symptoms. The results of pathogenicity test proved the ability of *Thielaviopsis paradoxa*, *Alternaria alternata* and *Negrospora oryza* to infect date palm rachis, fronts and fruits, respectively. *Helminthosporium* sp. and *Epicocum nigrum* were associated with the leaf spots. *E. nigrum* was associated with off-shoot decline, black scorch and leaf basal rot, while *Fusarium proliferatum* and *Aspergillus niger* were associated with fruit spots. Antagonistic effect of *E. nigrum* against *A. alternata* and *T. paradoxa* was examined by dual culture technique which leads to clear inhibition zone. Calcium chloride (CaCl<sub>2</sub>) and *E. nigrum* filtrates effectively controlled the leaf spot (as major disease) when applied at suitable time (when disease severity was low).

**Key words:** Date palm, fungal diseases, *Thielaviopsis paradoxa*, *Alternaria alternata*, *Negrospora oryza*

### INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is considered one of the most important fruit crops in Egypt. The cultivated area is about 14 million trees (FAO, 2009). It is cultivated all over the country especially in New Valley, Aswan, Sinai, Sharkiya, Beheira, Kafr El-Sheikh and Giza Governorates. There are various economically important diseases infecting date palm trees in all date palm growing countries (Anonymous, 1993; Saadi, 1992; Zaid *et al.*, 2002). Among these diseases, bayoud caused by *Fusarium oxysporum* f. sp. *albedinis* (Chakroune *et al.*, 2008), leaf spot caused by *Alternaria* spp. and *Mycosphaerella tassiana* (Abdalla *et al.*, 2001; Livingston *et al.*, 2002) inflorescence rot caused by *Mauginiella scaetae* (Michael and Sabet, 1970) or *T. paradoxa* (Abdullah *et al.*, 2005), black scorch caused by *Thielaviopsis paradoxa* (Mubarak *et al.*, 1994; Suleman *et al.*, 2001; Al-sharidy and Molan, 2008), leaf blight caused by *Glomerella cingulata* (Al-Rokibah, 1991) and off-shoot decline caused by *Chalara paradoxa* (Molan *et al.*, 2004). According to available literatures, no previous data was published on date palm fungal diseases in

Upper Egypt. So, this study was carried out to evaluate the distribution and incidence of various fungal diseases on date palm major growing areas of Upper Egypt. On the other hand, the study was conducted to evaluate of *E. nigrum* filtrates and CaCl<sub>2</sub> in control leaf spot disease.

### MATERIALS AND METHODS

**Survey of diseases incidence:** Surveys were conducted on date palm trees of all ages in El-Minia, Assiut, Sohag and Qena Governorates in 2007 season. Each site was inspected once a month from March to October. Percentage of infection with leaf spot, black scorch, leaf basal rot, fruit rot and off-shoot decline was recorded. Percentage of infection was calculated according to the following formula:

$$\text{Infection percentage (\%)} = \frac{\text{Number of infected date palm off-shoots}}{\text{Total number of date palm off-shoots}} \times 100$$

**Isolation and identification of causal fungi:** Random samples of diseased rachis, fronds and fruits were collected from date palm off-shoots in surveyed area for

isolation. Collected samples were washed with tap water then tissues from the margins of lesions on rachis, fruits and frond were surface sterilized with 2% sodium hypochlorite solution for 3 min, rinsed in sterile distilled water, allowed to dry and plated on Potato Dextrose Agar (PDA) at 30°C for 8 days. The developed fungal colonies were purified using single spore isolation technique according to Baudion (1988). The purified fungi were identified according to fungal morphological characteristics as described by Barnett and Hunter (1998) and Moubasher (1993) and then confirmed in Plant Pathology Department, National Research Centre (NRC), Dokki, Egypt. Frequencies of the isolated fungi from date palm parts were calculated according to the following formula:

$$\text{Frequency (\%)} = \frac{\text{Number of isolates of each fungus}}{\text{Total number of all isolates}} \times 100$$

**Pathogenicity test:** Inoculation was carried out by techniques used by Hooker (1957) on date palm (Saidy var.). A 8 mm in diameter and 5 mm deep wound (hole) was made at the center of off-shoot rachis by using sharp flamed needle and inoculated with a PDA disc (8 mm in diameter) which was cut from a 10 days old culture plate of *Helminthosporium* sp., *T. paradoxa*, *E. nigrum*, *F. proliferatum*, *N. oryza* and *A. niger*. Agar discs which were containing conidia, chlamydospores and mycelial fragments, were inserted into the whole cuts, covered with small pieces of wet, sterile cotton and covered with polyethylene bags to maintain high relative humidity necessary for infection according to Sultan and El-Hussieni (1999). Fruits and fronds were pin prick and sprayed with the fungal suspensions. Rachis, fronds and fruits free from fungi were used for control test and ten replicates were used in each test. One month after inoculation, the disease severity index (damage necrotic or dark-brown area around the point of infection) was calculated using a scale (0-5), where 0: No apparent infection, 1: 1-10% damage necrotic or dark-brown area around the point of infection, 2: 11-25%, 3: 26-50%, 4: 51-75% and 5: 76-100% according to Baudion (1988). Re-isolation was carried out from infected tissues and the isolated fungi were compared with the original cultures used.

#### Control of leaf spot caused pathogen and disease

**Antagonistic effect of *E. nigrum*, in vitro:** The antagonistic effect of *E. nigrum* isolate against *A. alternata* and *T. paradoxa* (as main pathogens) was tested by dual culture method according to Hozarika and Das (1998). *E. nigrum* was inoculated 48 h earlier before the fungi tested. After 5 days of

inoculation at 28±2°C on PDA medium, the radial growth of the fungi tested and inhibition zones were measured.

#### Effect of CaCl<sub>2</sub> and *E. nigrum* exudates in controlling leaf spot disease under field conditions:

The experiment was conducted in date palm trees (5 to 8 years old) off-shoot (Saidy var.) growing on privet farms located at Qena Governorate. All palm trees used in this study showed high disease severity (as a nature infection) at the start of the experiment. The 3rd whorl leaves from the bottom of tested trees were selected and labeled. Three trees were used as a replicates for each treatment. *E. nigrum* culture filtrate (diluted to 50%) and CaCl<sub>2</sub> (0.5 g L<sup>-1</sup>) were used. Treatments were sprayed once a month starting from March to October 2009. The disease severity ranged from 3.1-6.7 at the initiation of spray. At starting November 2009, another set of leaves, the 6th whorl from the bottom was selected and labeled for observation. Less than 2.6 disease severity was observed at the start of spray which continued until October 2010. Five leaflets from each labeled leaf were selected at equidistant and disease severity was calculated using the following formula (James, 1971):

$$\text{Disease severity} = \frac{\text{Mean area of plant tissue affected}}{\text{Mean total area of the leaflet}} \times 100$$

## RESULTS

**Survey:** The main symptoms noticed during survey were leaf spots, black scorch, leaf basal rot, off-shoot decline and fruit spots. Leaf and fruit spots had the highest disease incidence, compared with the other disease symptoms in most regions (Fig. 1). Black scorch was found in Assiut and Sohag Governorates (ranged from 1.8-2.3%). Fruit spots found to be the highest symptoms in three over four surveyed Governorates (ranged from 3.2-6.4%). El-Minia regions showed lowest symptom incidence, whereas, leaf spots only was noticed 2.2%.

#### Field symptoms

**Leaf spots:** Symptoms appeared as small scattered, irregular, brown to black (about 3-7 mm). The spots appeared on the upper and lower surface of rachis and fronds (Fig. 2a).

**Off-shoot decline:** The symptoms appeared firstly on one or more of the inner leaves then appeared on the outside ones. It started on fronds as light brown blights with dark brown edges (Fig. 2b1, b2) then extended to the rachis midrib on both surface as a black color (Fig. 2b3) causing death of midrib followed by their drooping down.

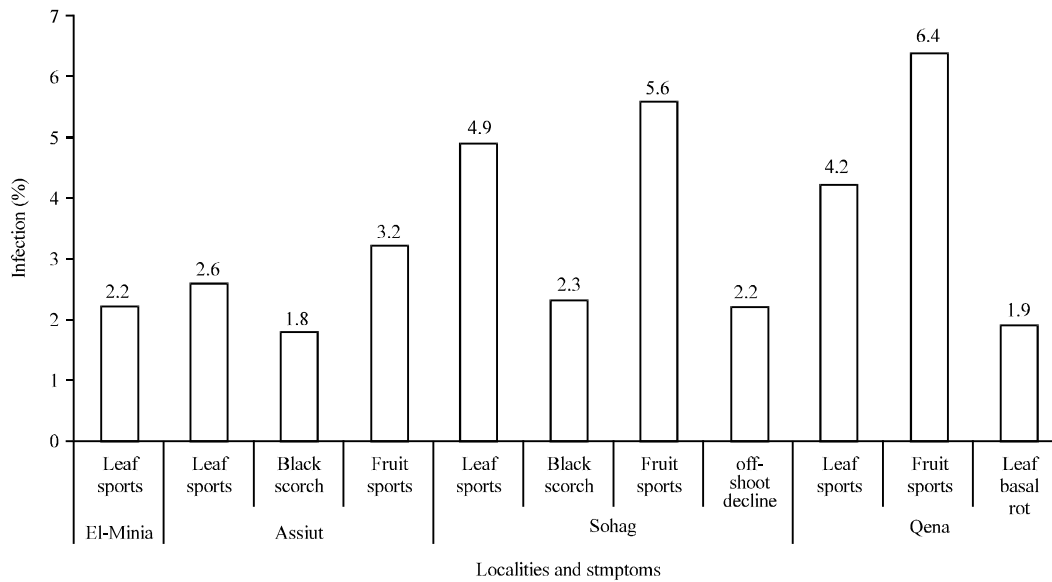


Fig. 1: Survey for symptoms and incidence of some diseases in date palm growing Governorates on Upper Egypt



Fig. 2(a-e): Different symptoms occurred in date palm trees as natural infection. (a) Leaf spots in rachis, (b1) Off-shoot decline in off-shoot, (b2) Fronds and (b3) Rachis, (c) Black scorch in leaf, (d1) Leaf basal rot at the base of leaves, (d2) Central leaf cluster die and (e) Fruit spots

**Black scorch:** Symptoms appeared firstly as grayish discoloration at leaves which turned into black discoloration followed by dwarfing and malformation (Fig. 2c).

**Leaf basal rot:** The symptoms appeared as brown to dark brown rot at the base of leaves (Fig. 2d1). The central leaf cluster might die before the older leaves and can easily detached (Fig. 2d2).

**Fruit spots:** Symptoms appeared as circular, black and stone spots in all parts of the fruits (Fig. 2e).

**Isolated fungi and pathogenicity:** Seven fungi associated with date palm disease symptoms were identified to species. The majority of isolates were obtained from leaves exhibited leaf spots, black scorch, off-shoot decline and fruit spots. The percentages of the predominance of *A. alternate* from samples exhibited leaf spot and basal rot (43.1-33.3%, respectively). *T. paradoxa* (Table 1, Fig. 3) was the most frequently isolated fungi from samples exhibited black scorch and off-shoot decline symptoms (52.7-37.4%, respectively). *N. oryza* was the most dominant fungi isolated from fruit spots (27.5%). Among seven fungal isolates obtained from date palm parts exhibited various disease symptoms, *A. alternate*, *T. paradoxa* and *N. oryzae* were pathogenic Table 1 and Fig. 3. The other isolates, *Helminthosporium* sp., *E. nigrum*, *F. proliferatum* and *A. niger* were non-pathogenic. *Helminthosporium* sp. and *E. nigrum* were associated with leaf basal rot and leaf spots, *E. nigrum* with black scorch and off-shoot decline, also *F. proliferatum* and *A. niger* with fruit spots.

#### Control of leaf spot disease

**Dual culture antagonism:** The antagonistic effect of *E. nigrum* against the most frequent fungi (*A. alternate*, *T. paradoxa*) was studied. The obtained results demonstrated that the radial growth of the two fungal species was significantly inhibited by

*E. nigrum*. The mean inhibition zone was 0.82 and 0.66 for *A. alternate* and *T. paradoxa*, respectively.

**Application of CaCl<sub>2</sub> and *E. nigrum* exudates against date palm leaf spot disease:** When the spray was initiated in March 2009, the disease severity on the 3rd whorl leaves ranged from 3.1-6.7. Although, the *E. nigrum* exudates and CaCl<sub>2</sub> were sprayed every month until October 2009, the disease didn't decrease but increased gradually (Fig. 4). The spray was continued and new disease that developed on the 6th whorl leaves was recorded from November 2009 to October 2010. The disease severity on the 6th leaves ranged from 1.2-2.6% at time of the first spray. In two treatments, the disease severity decreased gradually. The disease severity didn't vary significantly between two treatments, although, the differences between the treatments and control were highly significant (Fig. 5-6).

Table 1: Frequency and pathogenicity of fungal pathogens isolated from date palm

Symptoms	Isolated fungi	Frequent (%)	Disease severity
Leaf spots	<i>Helminthosporium</i> sp.	4.9	2.1
	<i>A. alternata</i>	43.1	36.7
	<i>E. nigrum</i>	14.8	2.5
Black scorch	<i>T. paradoxa</i>	52.7	65.8
	<i>E. nigrum</i>	14.6	1.2
Off-shoot decline	<i>T. paradoxa</i>	37.4	57.3
	<i>E. nigrum</i>	16.2	1.4
Leaf basal rot	<i>A. alternata</i>	33.3	44.5
	<i>E. nigrum</i>	9.1	2.8
Fruit spots	<i>F. proliferatum</i>	8.5	2.1
	<i>A. niger</i>	3.7	2.0
	<i>N. oryza</i>	27.5	36.4

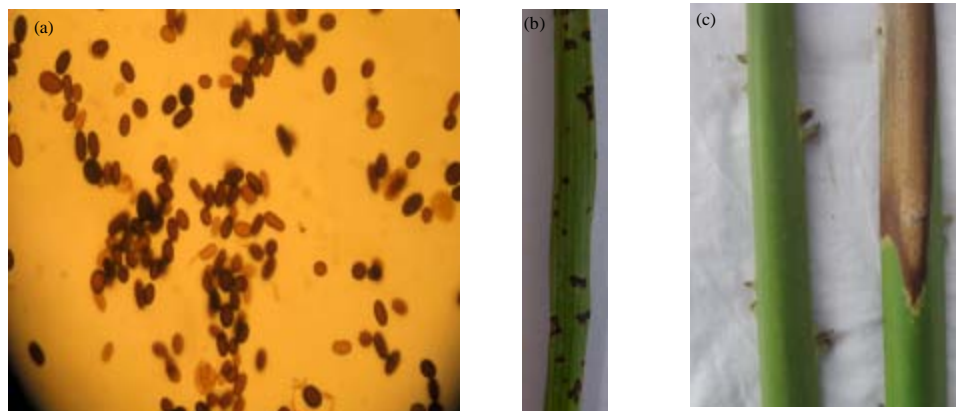


Fig. 3(a-c): (a) Microscopic photograph of *T. paradoxa* isolate showing microconidia: which were hyaline and cylindrical form, also, chlamydospores: have dark brown with thick walls, (b) Pathogenicity test for *A. alternate* on fronds and (c) *T. paradoxa* on rachis

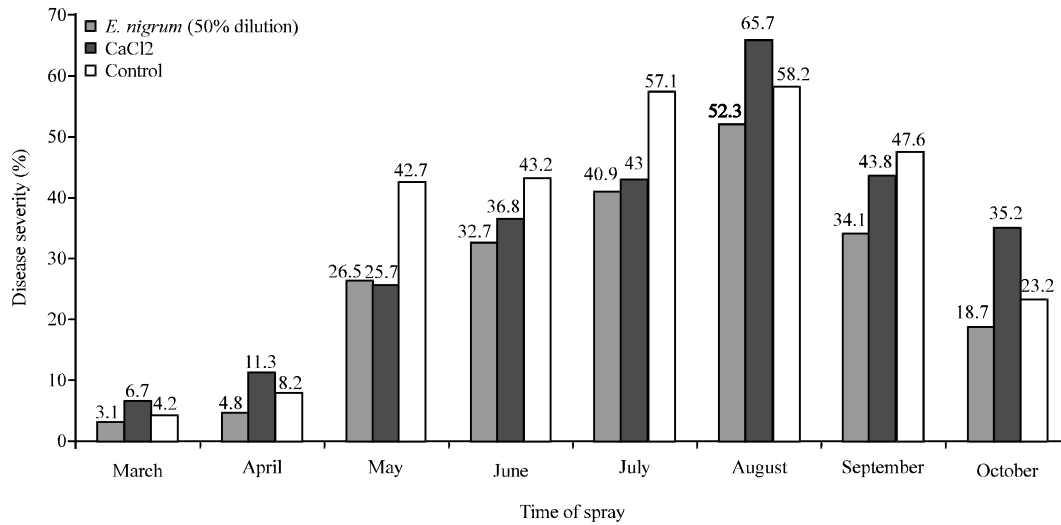


Fig. 4: Disease severity of leaf spot disease on the 3rd whorl leaves of date palm from March to October 2009 after CaCl<sub>2</sub> and *E. nigrum* exudates spraying

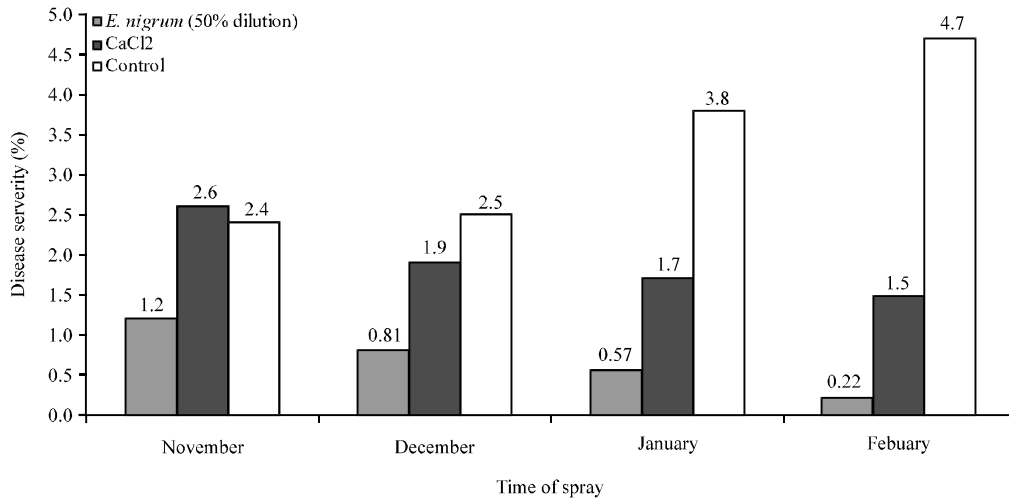


Fig. 5: Disease severity of leaf spot disease on the 6th whorl leaves of date palm from November 2009 to February 2010 after CaCl<sub>2</sub> and *E. nigrum* exudates spraying

## DISCUSSION

Fungi are known as the most pathogens on date palm trees (Zaid *et al.*, 2002). The present study showed limited spread of fungal diseases on date palm trees in the surveyed area because of the dry conditions and high temperature in surveyed regions. The results indicated that leaf and fruit spots are widespread. High incidence of two diseases may be due to the absence of control measures in Upper Egypt. Diseases are common on similar conditions in Saudi Arabia (Al-sharidy and Molan, 2008), Kuwait

(Mubarak *et al.*, 1994), Sultanate of Oman (Anonymous, 1993) and Iraq (Djerbi, 1983; Sarhan, 2001). Despite the widespread of leaf spot disease, the author noticed absence of Graphiola leaf spot, this may be due to the dry climate. Black scorch and leaf basal rot are low spread. *A. alternate*, *T. paradoxa* and *N. oryza* were the most predominant fungi isolated from date palm trees showed visible symptoms. It was pathogenic 13-19 days after inoculation, confirming Koch's postulates. Similar results were reported by El-Meleigi *et al.* (1986), Sarhan (2001), Al-sharidy and Molan (2008) and Abdullah *et al.* (2009).



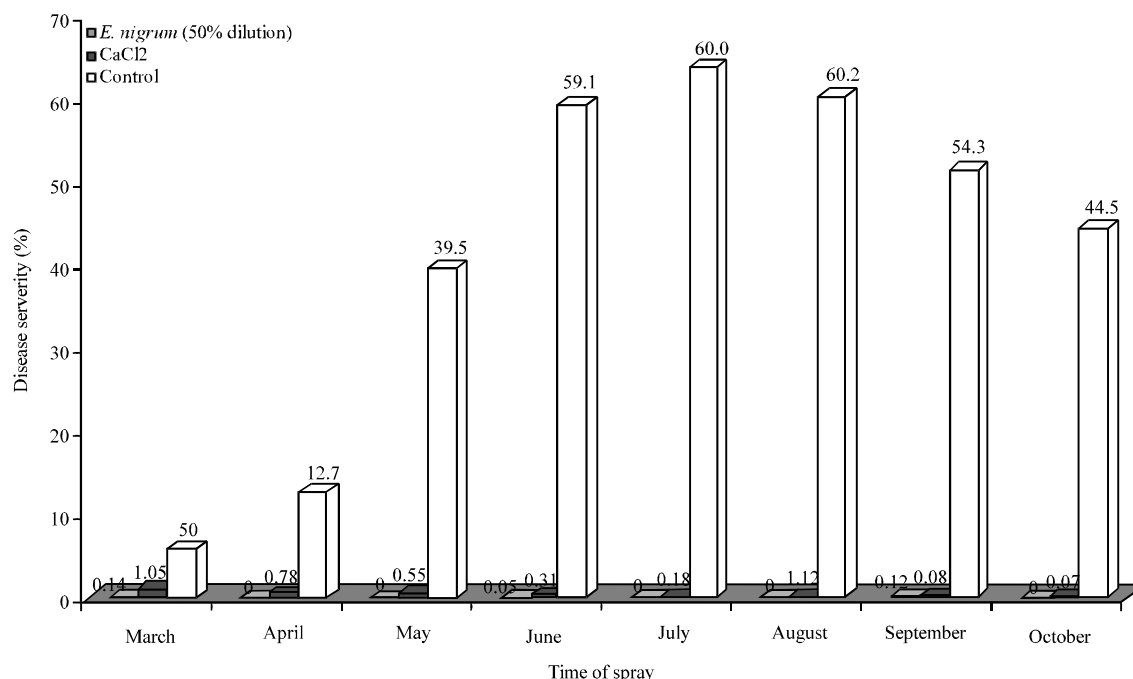


Fig. 6: Disease severity of leaf spot disease on the 6th whorl leaves of date palm from March 2010 to October 2010 after CaCl<sub>2</sub> and *E. nigrum* exudatis spraying

There has been evidence in recent years of the role calcium in plant nutrition relative to lessening the impact of *Pythium*, *Sclerotium*, *Botrytis* and *Fusarium* at many crops. Calcium has important roles in the integrity of cell walls which infers a resistance mechanism to reduce penetration of fungal pathogens. Among the fungi that we isolated, *E. nigrum* selected because of ability to inhibit growth *T. paradoxa* and *A. alternate* on PDA through production of diffusible metabolites. The inhibition of *T. paradoxa* and *A. alternate* by *E. nigrum* is similar to the inhibition growth of *Phytophthora erythroseptica* and *T. paradoxa* by *Trichoderma virens* and *T. longibrachiatum*, respectively (Etebarian *et al.*, 2000; Sanchez *et al.*, 2007).

Although, the *E. nigrum* exudates and CaCl<sub>2</sub> were sprayed every month until October 2009, the disease didn't decrease but increased gradually. This implies that treatments used were not effective in controlling the disease when the disease severity was high (above 3.1) at the initial time of application. From the results obtained, it can be concluded that spraying *E. nigrum* exudates or CaCl<sub>2</sub> before the initiation of the symptoms can reduce the incidence of leaf spot disease.

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