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Date-Palm Leaves Infection With Spotting Fungi Isolated From Some Ornamental Palms.

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ABSTRACT

Forty eight fungal isolates attributed to twenty one plant pathogenic genera, *i.e.* *Asteromella*, *Bipolaris*, *Botryodiplodia*, *Cocoicola*, *Colletotrichum*, *Coniothyrium*, *Cuvularia*, *Cylindrocladium*, *Fusarium*, *Gliocladium*, *Gloeosporium*, *Microdiplodia*, *Pestalotiopsis*, *Phaeochoropsis*, *Phyllosticta*, *Phytophthora*, *Phoma*, *Phomopsis*, *Septoria*, *Serenomyces* and *Stigmina* were isolated from fallen leaves of ten ornamental palms grown in the public gardens at Cairo and Giza, Egypt, *i.e.* Azbakia, Orman and Zoo gardens, as a result of pruning process during 2012- 2013 seasons. Pathogenic ability of all isolates was tested on detached fronds of date palm (cv. Zaghlol) in Petri-plates. Different size spots were appeared during 10 days of treatment with spore suspensions of the seven isolates only identified as : *Bipolaris spicifera* (from Betel nut, Fiji Island fan , Fish-tail and Queen palms), *Botryodiplodia theobromae* (from Betel nut and Fiji Island fan palms), *Colletotrichum gloeosporioides* (from Betel nut, Cabbage, California fan, Mexican fan, Queen and Royal palms), *Gliocladium vermoeseni* (from Mexican fan, Palmyra and Queen palms), *Pestalotiopsis palmarum* (from Cabbage, Royal, Betel nut, Mexican fan, Mountain Serdang and Palmyra palms), *Phoma* sp. (from Fiji Island fan and Palmyra palms) and *Stigmina palmivora* (from Betel nut, Cabbage, Mexican fan, Palmyra and Royal palms). The infected date-palm fronds were more susceptible to Betel nut palm fungal isolates. Current results suggest susceptibility of date-palm leaves to infect with many spotting fungal isolates coming from ornamental palms. Bavistin 50 WP showed complete suppression of radial growth of all the tested pathogenic fungi at 1000 ppm.

Keywords: Date-palm spotting, cv. Zaghlol, Ornamental palm spotting, Mutual infection, Detached leaflet technique, Bavistin 50 WP.

INTRODUCTION

There are more than 2000 species of ornamental palms (25), are usually planted next to date-palm trees in public parks and other. All species of palm trees are subject to leaf blight and leaf spot diseases caused by a number of different fungal pathogens. For example, Betelnut palms are infect with *Colletotrichum capsici* and *Pestalotia palmarum* (24), Palmyra palms are infect with *Pestalotiopsis palmarum* and *Stigmia palmivora* (35), Fish-tail palms are infect with *Cylindrocladium pteridi*, *Exserohilum rostratum* and *Pestalotia palmarum* (31, 13 and 8), Mountain Serdang palms are infect with *Cocoicola livistoncola* and *Astrosphaeriella fronsicola* (37), Fiji Island fan palms are infect with *Botryosphaeria* sp. and *Curvularia ovoidea* (10 and 5), Royal palms are infect with *Asteromella* sp. and *Coniothyrium fukelii* (19), Cabbage palms are infect with *Myriangium sabaleos* and *Stigmia palmivora* (12 and 17), Queen palms are infect with *Cylindrocladium* sp. and *Cladosporium perangustum* (28 and 32), California fan palms are infect with *Serenomyces californica* and *Auerswaidia* sp. (10 and 16), Mexican fan palms are infect with *Cocoicola californica* and *Nalanthamala vermoesenii* (20 and 27).

Palm leaf spot symptoms are similar regardless of the specific fungus and initially display as tiny, water-logged lesions that take on a number of colors including black, brown, gray, reddish-brown and yellow. The lesion is typically surrounded by a circle of tissue that bears a color different than the spot itself. The stressed, injured and nutrient-deficient palms are susceptible to fungal diseases. Diseased tissue will eventually die (30).

Most of ornamental palms naturally shed their old fronds, dead leaves may have to be pulled or cut off manually. Other palms such as date - palm need to be pruned, where the removal leaves was only for sick or dead ones (33).

The goal of this work is to study the possibility of transmission of leaf spot fungi from leaves of ornamental palms to leaves of date-palms and evaluation the efficacy of the popular chemical control agent-Bavistin 50 WP against the spot fungi of palm leaves under laboratory conditions.

MATERIALS AND METHODES

Palm leaf sample collection

Leaf samples of ten ornamental palms (Table 1) grown in Azbakia, Orman and Zoo gardens at Cairo and Giza, Egypt were collected during pruning process of the two seasons (2012 and 2013). Samples were put in plastic bags and transferred under cooled conditions to the laboratory.

Table (1): Common and scientific names of the target ornamental palms that grows in three famous Egyptian gardens at Cairo and Giza.

Target ornamental palm	Scientific name
Betel nut	<i>Areca catechu</i>
Cabbage	<i>Sabal palmetto</i>
California fan	<i>Washingtonia filifera</i>
Fish-tail	<i>Caryota mitis</i>
Fiji Island fan	<i>Pritchardia pacifica</i>
Mexican fan	<i>Washingtonia robusta</i>
Mountain Serdang	<i>Livistona speciosa</i>
Palmyra	<i>Borassus flabellifer</i>
Queen	<i>Syagrus romanzoffiana</i>
Royal	<i>Roystonea elata</i> (<i>R.regia</i>)

Isolation and genera identification of palm leaf spot fungi

Infected leaf tissues of the palms were surface sterilize by dipping in 0.5 % sodium hypochlorite solution for 2 min., then rinsed in sterilized distilled water two times and plotted dry on sterilized filter paper. Pieces (3×3 mm) of the surface sterilized tissues were placed on potato dextrose agar (PDA) plates and incubated at 25°C for one week. Fungal pure cultures were obtained by hyphal tip technique. The isolation

procedure was made from 10 infected leaf samples of each kind of palm. Fungi were identified by the staff of Plant Pathology Department, National Research Centre, Dokki, Egypt. Pure culture on PDA slants were kept at low temperature (5°C) for further studies.

Inoculation of date-palm fronds with the isolated spotting fungi

Fungal 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. After 5 days incubation period, 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 1×10^4 conidia / ml.

Young fronds (4-6 months old) of Zaghlol date-palm were detached, surface sterilized as described above, wounded by a sterile needle before inoculation with the spore suspension using a high pressure atomizer and placed upright onto large Petri-dishes (20 Cm in diameter) containing two moistened paper towels. Four plates (each contain two fronds) were used for each fungal isolate. Check treatments were sprayed with distilled water. All plates were incubated in the dark at $27 \pm 2^\circ\text{C}$ for 10 days. The severity of infection was determined according to an arbitrary key as follows:

0 = No symptoms, 1 = Small size lesion (1-3 mm in diameter),
2 = Medium size lesion (4-6 mm) and 3 = Large size lesion (1 Cm).

Identification of the aggressive isolates toward date-palm fronds

The aggressive isolates toward date-palm fronds were identified to species level by the same staff of Plant Pathology Department, National Research Centre, Dokki, Egypt according to the fungal descriptions of Chase and Broschat (9) and Elliott and Uchida (19).

Effect of Bavistin fungicide on radial growth of some palm leaf spot pathogens

Poison plate technique was employed to test the effect of the popular systemic fungicide- Bavistin 50 WP (a.i. carbendazim = methyl benzimidazol-2-yl carbamate against radial growth of the isolated fungal pathogens. To prepare medium plate incorporated with Bavistin, the fungicide was dispensed in sterilized distilled water at concentrations of 1000 ppm. 1 ml of this preparation was homogeneously mixed with 9 ml of medium and poured on 90 mm Petri plate. Similarly, media plates with 500, 250, 100 and 50 ppm of the fungicide were prepared (34 and 15). These fungicidal concentrations were mixed separately in the sterilized PDA medium in such a way to get the final desired concentrations. A disc (5 mm diam.) obtained from the periphery of 7 days old fungal colony of the pathogens grown on PDA medium was placed in the center of the Petri plate. When the control plate showed nearly full growth (8.8 mm), the radial growth of the pathogens in each plate was measured and percent of inhibition was calculated by using the following formula : $I = 100 (C - T) / C$, where, I = percent inhibition over control; C = growth of pathogen in control; T = growth of pathogen in treatment. The data were analyzed by Simple Factorial Design (36).

RESULTS AND DISCUSSION

Isolation and genera identification of palm leaf spot fungi

Data presented in Table (2) show that forty eight fungal isolates attributed to twenty.

One plant pathogenic genera were isolated from fallen leaves of ten ornamental palms. Some of them isolated from 5-7 hosts, i.e. *Colletotrichum*, *Pestalotiopsis* and *Stigmata*, while *Bipolaris*, *Gliocladium* and *Phoma* were isolated from 3-4 hosts. The others were isolated from 1-2 hosts only, i.e. *Asteromella*, *Botryodiplodia*, *Coccoloba*, *Coniothyrium*, *Cucurbitaria*, *Cylindrocladium*, *Fusarium*, *Gloeosporium*, *Microdiplodia*, *Phaeochoropsis*, *Phyllosticta*, *Phytophthora*, *Phomopsis*, *Septoria*, *Serenomyces*.

Most the isolated fungi were previously recorded on ornamental palms by several researchers such as Hodel (22), Hyde (23), Elliott (18) and Ligoxigakis *et al.* (27).

Table (2): The fungal genera isolated from leaf samples of ten ornamental palms grown in Azbakia, Orman and Zoo gardens at Cairo and Giza, Egypt ,during pruning process of the two seasons (2012 an 2013).

Fungal genera of leaf spotting	Host ornamental palm	Number of pathotype
<i>Asteromella</i>	Royal palm	1
<i>Bipolaris</i>	Betel nut, Fiji Island fan, Fish-tail and Queen palms	4
<i>Botryodiplodia</i>	Betel nut and Fiji Island fan palms	2
<i>Cocoicola</i>	Mexican fan palm	1
<i>Colletotrichum</i>	Betel nut, Cabbage, California fan, Mexican fan, Queen and Royal palms	6
<i>Coniothyrium</i>	Royal palm and Mountain Serdang palms	2
<i>Curvularia</i>	Betel nut and Fiji Island fan	2
<i>Cylindrocladium</i>	Betel nut and Royal palms	2
<i>Fusarium</i>	Cabbage palm	1
<i>Gliocladium</i>	Mexican fan, Palmyra and Queen palms	3
<i>Gloeosporium</i>	Cabbage palm	1
<i>Microdiplodia</i>	Palmyra palm	1
<i>Pestalotiopsis</i>	Betel nut, Cabbage, Mexican fan, Mountain Serdang, Palmyra and Royal palms	6
<i>Phaeochoropsis</i>	Mexican fan palm	1
<i>Phoma</i>	Fiji Island fan, Palmyra and Royal palms	3
<i>Phomopsis</i>	Mountain Serdang palms	1
<i>Phyllosticta</i>	Betel nut and Mexican fan palm	2
<i>Phytophthora</i>	Cabbage and California fan palms	2
<i>Septoria</i>	Queen palm	1
<i>Serenomyces</i>	California fan palm	1
<i>Stigmia</i>	Betel nut ,Cabbage, Mexican fan, Palmyra and Royal palms	5
Total	-	48

Inoculation of date-palm fronds with the isolated spotting fungi

Different size spots were appeared on some detached fronds of date-palm cv. Zaghlol during 10 days incubation period after spraying with spore suspensions of the fungi isolated from leaves of the target ornamental palms. Seven fungi only (Table3) were pathogenic to detached date-palm fronds, *i.e.*:

- *Bipolaris* (from Betel nut, Fiji Island fan, Fish-tail and Queen palms). These spots begin as tiny, water-soaked, becoming dark-brown in color, may be chlorotic halo appear around the lesions.
- *Botryodiplodia* (from Betel nut and Fiji Island fan palms). This fungus cause yellowish-brown spots, turn to brown.
- *Colletotrichum* (from Betel nut, Cabbage, California fan, Mexican fan, Queen and Royal palms). This infection cause reddish brown spots with distinct dark edges.
- *Gliocladium* (from Mexican fan, Palmyra and Queen palms). This fungus produce orange pink spores, then turn to brown.
- *Pestalotiopsis* (from Betel nut, Cabbage, Mexican fan, Mountain Serdang, Palmyra and Royal palms). This infection starts as small tiny black spots, which gradually enlarge in size.
- *Phoma* (from Fiji Island fan, Palmyra and Royal palms). The lesion begin as a black spot with white center, then became white with black edges.

Table (3): Av. disease severity on detached date-palm fronds inoculated with the isolated fungal pathotypes after incubation in the dark at 27± 2°C for 10 days.

Fungal isolate	Host of pathotype	Av. disease Severity*
<i>Asteromella</i>	Royal palm	0
<i>Bipolaris</i>	Betel nut	3
	Fiji Island fan	1
	Fish-tail	2
	Queen	2
<i>Botryodiplodia</i>	Betel nut	3
	Fiji Island fan	1
<i>Cocoicola</i>	Mexican fan	0
<i>Colletotrichum</i>	Betel nut	3
	Cabbage	2
	California fan	1
	Fish-tail	2
	Mexican fan	1
	Queen	2
	Royal	2
<i>Coniothyrium</i>	Royal	0
	Mountain Serdang	0
<i>Curvularia</i>	Betel nut	0
	Fiji Island fan	0
<i>Cylindrocladium</i>	Betel nut	0
	Royal	0
<i>Fusarium</i>	Cabbage	0
<i>Gliocladium</i>	Mexican fan	1
	Palmyra	2
	Queen	1
<i>Gloeosporium</i>	Cabbage	0
<i>Microdiplodia</i>	Palmyra	0
<i>Pestalotiopsis</i>	Betel nut	3
	Cabbage	2
	Mexican fan	1
	Mountain Serdang,	1
	Palmyra	1
	Royal	2
<i>Phaeochoropsis</i>	Mexican fan	0
<i>Phoma</i>	Fiji Island fan	1
	Palmyra	2
	Royal	2
<i>Phomopsis</i>	Mountain Serdang	0
<i>Phyllosticta</i>	Betel nut	0
<i>Phytophthora</i>	Cabbage	0
	California fan	0
<i>Septoria</i>	Queen	0
<i>Serenomyces</i>	California fan	0
<i>Stigmina</i>	Cabbage	2
	Fish-tail	2
	Mexican fan	1
	Palmyra	1
	Royal	2

*The key of disease severity:

0 = No symptoms, 1 = Small size lesion (1-3 mm in diameter),
2 = Medium size lesion (4-6 mm) and 3 = Large size lesion (1 Cm).

-*Stigmina*, (from Cabbage, Mexican fan, Palmyra, Queen and Royal palms). A tan band of necrotic tissue develops around the original leaf spot. In turn, these necrotic area are usually surrounded by a thin ring of dark water soaked tissues.

The mentioned symptoms on date-palm leaves were confirmed with the findings of **Reynolds (35), Lopez-Jimenez and Lorca (29), Fayad and Mania (21)** and **Al-Rokibah (4)**.

The portion of date-palm fronds affected by the pathogenic fungi was associated with wounding. This result was in agreement with **Chase and Broschat (9)**. The infected date-palm fronds were more susceptible (showed large size lesion) to Betel palm fungal isolates. The results suggest susceptibility of date-palm leaves to infect with many spotting fungal isolates coming from ornamental palms.

Identification of the aggressive isolates toward date-palm fronds

The seven pathogenic fungi to date-palm fronds were identified as *Bipolaris setariae*, *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Gliocladium vermoeseni*, *Pestalotiopsis palmarum*, *Phoma* sp. and *Stigmina palmivora*.

Effect of Bavistin fungicide on radial growth of some palm leaf spot pathogens

Data presented in Table (4) show that Bavistin 50 WP showed complete suppression of radial growth of all the tested pathogenic fungi at 1000 ppm. *Colletotrichum* and *Pestalotiopsis* were more sensitive to Bavistin at the concentration 500 ppm.

Table (4): Effect of Bavistin fungicide on radial growth of some palm leaf spot pathogens:

Bavistin Con.(ppm)	<i>Bipolaris</i>		<i>Botryodiplodia</i>		<i>Colletotrichum</i>		<i>Gliocladium</i>		<i>Pestalotiopsis</i>		<i>Phoma</i>		<i>Stigmina</i>	
	G	%	G	%	G	%	G	%	G	%	G	%	G	%
50	44.4	49.5	39.6	55.0	18.3	79.2	28.4	67.7	16.1	81.7	37.6	57.2	62.6	28.9
100	42.3	51.9	32.2	63.4	11.0	87.5	17.7	79.9	10.9	87.6	27.5	68.7	57.0	42.1
250	40.2	54.3	16.5	81.2	2.7	96.9	10.6	88.0	8.7	90.1	24.6	72.1	34.8	60.5
500	30.0	65.9	6.7	92.4	0.0	100	6.8	92.3	0.0	0.0	17.6	80.1	26.9	76.3
1000	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100
Control	88.0	-	88.0	-	88.0	-	88.0	-	88.0	-	88.0	-	88.0	-
LSD at 5%	2.0	2.1	6.5	7.3	2.5	2.9	3.5	3.7	2.0	2.0	2.5	2.9	5.0	11.2

G= Colony growth diameter (mm) when the control plate showed nearly full growth (8.8 mm),
 % = Inhibition percent of the growth after treated with different Bavistin concentrations.

These results were confirmed with the findings of **Ahmed et al. (2), Aggarwal et al. (1), Bhanumathi (7) , Das et al. (14), Aktar et al. (3)** and **Bhadra et al.(6)**.

Aggarwal et al. (1) reported that carbendazim works by inhibiting the development of fungi probably by interfering with spindle formation at mitosis (cell division). Using Bavistin at the concentration 1000 ppm may be effective to control spotting pathogens of palm leaves.

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