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## Dieback and Sooty Canker of *Ficus* Trees in Egypt and its Control

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**Abstract:** This study was designed to throw lights on dieback and canker disease on urban trees of *Ficus* sp. in Egypt, its causal pathogens and disease control. Diseased samples were collected from five locations. Pathogenicity test was done on one year old of three different healthy seedlings of *Ficus* trees (*Ficus benghalensis*, *Ficus nitida* and *Ficus hawaii*). *Lasiodiplodia theobromae* and *Phomopsis* sp. were consistently isolated from infected tissues and were pathogenic. The fungicides Antracol Combi and Topsin M 70 provided effective control of the infection. Accordingly, protecting ficus trees from diseases threatening is considered a major goal to attain their benefits.

**Key words:** *Ficus*, sooty canker, *Lasiodiplodia*, *Phomopsis*, chemical control, host range

### INTRODUCTION

Urban trees are considered the first line of defense against undesirable meteorological conditions such as windy and sandy storms especially in the new cities established in Egyptian desert. Beside their important role in air purification, they are used to beautify the landscape. *Ficus* often grown is an attractive ornamental tree found mostly outdoors and is frequently encountered along city streets lining parkways, medians and sidewalks. It finds use in parks and other large, open spaces but is, perhaps, most familiar as a street tree and some species of *Ficus* are commonly used as interior ornamentals. Under Egyptian conditions, ficus trees have special importance in parks and in the newly established cities to reduce the impact of the desert environment.

*Ficus* trees are usually affected by several diseases particularly in warm and moist conditions where the disease can lead to heavy defoliation; trunk and branch canker (El Atta and Aref, 2013). In 2012, we noticed that Individual *Ficus* trees (*Ficus nitida* and *Ficus hawaii*) lining many of the streets are losing their leaves and undergoing canopy dieback. Trees cankers were investigated by several plant pathologists (Grove, 1935; Anderson and Hartman, 1983; Ellett, 1979; Hudler, 1979).

Hampson (1981) isolated *Phomopsis cinerescens* and *Botryodiplodia* sp. as pure cultures from cankers on *Ficus benjamina*. He suggested that fungus penetrated the tree through bark wounds. *Phomopsis cinerescens* Trav. was identified to be the causal agent of fig branch and twig canker on *Ficus* sp. (Anderson and Hartman, 1983; Ellett, 1979; Hudler, 1979).

Other fungal specie (*Neoscytalidium dimidiatum* and *Scytalidium dimidiatum*) has been identified as the causal pathogens of *Ficus* dieback and dying in Southern

California and Sudan (Elshafie and Ba-Omar, 2002; Elshafie and Ali, 2005; Crous *et al.*, 2006).

*Botryodiplodia theobromae* and *Phomopsis* sp. have been reported as responsible for causing branch canker and dieback of trees in the tropics and subtropics trees and threat to shade and other trees of economic value in Egypt (Mansour, 1986; Attia and Saber, 1995; Atia *et al.*, 2003; Haggag and Nofal, 2006; Rashed *et al.*, 2006; Haggag, 2010; Kamhawy, 2011; Ismail *et al.*, 2012).

This study was undertaken with the aim determining the nature of the disease and its causal agent on some *Ficus* sp. Trees and to draw attention to the causal agent in Egypt. Part of this research was conducted to evaluate some of the available fungicides against pathogenic fungi to control the disease through *in vivo* applications.

### MATERIALS AND METHODS

**Survey and plant materials:** In the present study surveys were carried out during 2011-2012. Out of them five sites including: Sixth of October City, Tenth of Ramadan City, Sheikh Zayed City, Giza Zoo and Park Al Orman Gizawere selected by random to study the diversity of disease incidence on park and roadside trees. The tree communities were recorded on three hosts including *Ficus benghalensis*, *Ficus nitida* and *Ficus hawaii*.

Diseased samples of infected tissues comprise chlorosis, necrosis, wilt of foliage, dieback on twigs or branches were collected separately in polythene bags for examination and symptoms were recorded. Samples of canker and black sooty layer of stem bark was peeled off from the infected tree.

Every tree showing a single and/or a combination of chlorosis, necrosis, wilt of foliage, twigs or branches,

canker and presence of the characteristic black sooty layer of conidia under the bark was counted as infected and occurrence of symptoms were estimated by the following formula:

$$\text{Occurrence} = \frac{\text{Sample plants infected}}{\text{Total No. of sample}} \times 100$$

**Isolation and identification:** Diseased parts (infected branches and bark) were washed with tap water and the outer bark (in case of cankers) was carefully removed with a scalpel to expose the layer underlying the discolored tissue. From the margin of healthy and discolored tissues, a few wood fragments were excised, surface-sterilized in 0.5% sodium hypochlorite for 1-3 min, rinsed with Sterile Distilled Water (SDW), placed on petri dishes containing Potato Dextrose Agar (PDA) and incubated at 25°C. After 1 week, fungal colonies appeared around each fragment. All cultures for further study were from single conidia and these were stored at 4°C.

The isolated and purified fungi were observed and identified according to morphological characteristics using taxonomic keys for imperfect fungi, using Barnett and Hunter (2006) and Sutton (1980) keys for genus and Punithalingam (1976) to identify *Lasiodiplodia theobromae*. Further authenticated were conducted at Mycology and Plant Diseases Survey Department, Plant Disease research Institute, ARC, Giza, Egypt.

**Pathogenicity tests:** An *in situ* pathogenicity test was done on one year old of different healthy seedling of a *Ficus benghalensis*, *Ficus nitida* and *Ficus hawaii* tree in a greenhouse of Fruit and Woody Trees Diseases Department, Plant Pathology Research Institute, Agricultural Research Center Giza-Egypt. Pathogenicity tests were performed on seedling using mycelial disks obtained from a seven-day-old culture (on PDA) of an isolated fungi recovered from a diseased tree.

Stem of different seedling species were wounded with a 5 mm diameter cork borer. A mycelial plug of a seven-day-old culture was inserted individually in each wound and wrapped with wetted cotton and covered with a polythene film to prevent desiccation of the inoculum. Similar seedlings were inoculated with only agar plugs that served as negative controls. Each treatment was replicate in triplets.

The evaluations were done every 7 days, watching symptoms and disease progression for five weeks after inoculation. Later, from the infected tissues of inoculated stems was isolated the fungus in pure culture and compared morphologically with the fungus inoculated to confirm Koch's postulates.

Table 1: Trade name, active ingredient and doses of five fungicides used to control

Trade name	Active ingredient	Formulation	Mode of action	Dosage (g/100 L)
Kopeix	Copper-Oxychloride	50 WP	Contact	250
Antracol combi	Cymoxanil+Propineb	76 WP	Systemic	200
Topsin M 70	Thiophanate Methyl	70 WP	Systemic	100
Kocide 2000	Copper Hydroxide	53.8 WP	Contact	250
Chaampion	Copper hydroxide	77 WP	Contact	250

**Host range study:** Healthy seedlings (one-year old) of the woody plants grapevine (*Vitis vinifera*), apple (*Malus domestica*), Mango (*Mangifera indica*), *Ficus benghalensis*, *Ficus nitida* and *Ficus hawaii* growing in sterile clay: sand (2:1) soil, were inoculated with *L. theobromae* and *Phomopsis* sp. using the method described above. Results were recorded after 35 days after inoculation.

**Chemical control:** Healthy seedlings (one-year old) of *Ficus nitida* growing in sterile clay: sand (2:1) soil was inoculated with *L. theobromae* and *Phomopsis* sp. using the method described above. The manufacturer recommended dose of systemic and contact fungicides mentioned in Table 1 were dissolved in water to get a final desired volume. The fungicides were used as foliar spray for the control of infection under greenhouse conditions. The experiment having nine seedlings in triplets as replicates for each treatment. The control treatment was kept unsprayed. The disease incidence and (%) efficiency were calculated at 21 days interval after fungicidal treatment. Disease incidence was evaluated as average of canker area (mm) after spray of each fungicide.

Efficiency (%) of fungicidal treatment were calculated according to the following equation suggested by Sunder *et al.* (1995):

$$Pv = \frac{Ic - Iv}{Ic} \times 100$$

whereas, Pv is % efficiency, Ic is disease incidence in control treatment and Iv is disease incidence in treatment.

**Statistical analysis:** When necessary, significance of mean differences between treatments and control were statistically compared using an Analysis of Variance (ANOVA) at the 5% probability level using factorial experiment design suggested by Snedecor and Cochran (1982).

## RESULTS

**Disease symptoms:** In recent years, ficus trees were observed the characteristic symptoms of regressive dieback and cankers presence mainly in weakened and/or stressed trees.

Symptoms initially begin with slightly discolored leaves appear as chlorosis or yellowing usually followed by rolling of leaves and dieback on *F. hawaii* and *F. nitida*. As disease progresses into the canopy, twig dieback is observed and eventually branch dieback will follow (Fig. 1). If the disease progresses into the trunk the tree will die (Fig. 1b). On *F. benghalensis*, symptoms first appear as brownish, moist areas on the bark of limbs. As the disease develops, these areas crack and split revealing a black, dusty mass of fungal spores (Fig. 2a, b). It is from this mass of spores that sooty canker derives its name.

Beneath the sootiness, the sapwood dies and stains gray to black. Usually, the whole tree eventually dies with increasingly the fungus infection (this may take two to three years or more). Sometimes the fungus occurs in *Ficus* trees without causing symptoms until the tree is weakened.

**Disease survey:** The information collected from survey study has shown the pattern of disease development at different locations. Figure 3 indicate that the percentage of disease incidence varied according to the locality. The percentage of disease incidence during 2011 ranged from 38. 5% in 10th Ramadan City to 18. 1% in Al Orman Park and during 2012 from 41. 5% at 10th Ramadan City to 19. 2% in Al Orman Park. In general, the highest number of infected tree species was found at 10th Ramadan City, on the contrary the least number of infected tree species was found at Al Orman Park.

**Isolated fungi:** There were isolated 298 fungal strains from diseased tissues. The genera isolated were described according to their morphological structures based on microscopic observations and literature guides. Data illustrated in Table 2 displayed fungi and their frequency (%) isolated from naturally infected samples of *Ficus*.

Opportunistic fungi that were found associated with the infection sites were *Lasio diplodiatheobromae*, *Phomopsis* sp., *Alternaria* sp. and *Nigrospora* sp. were isolated from diseased samples of *Ficus* trees collected from different localities. The frequency of the isolated fungi varied according to the locality (Table 2). In general, *L. theobromae* and *Phomopsis* sp. (Fig. 4a, b) was the most dominant fungi in survey locations and tree species record the highest frequency (46. 9 and 29. 9% on the average, respectively).

**Pathogenicity test:** At 14 days after inoculation were observed the first signs of disease in some seedlings (Table 3). Seedlings artificially inoculated with *L. theobromae* and *Phomopsis* sp. showed initially symptoms which developed into irregular necrotic areas around inoculation site after one week. The symptoms developed were similar to those generally associated with the natural infection in *Ficus* sp. First, the leaves became drooped and rolled over and then chlorosis developed in 25% of the inoculated seedlings. These symptoms were confined to stem inoculations. Canker area (mm) was measured four weeks later.



Fig. 1(a-b): Sooty canker progress on *F. Nitida* (a) The entire branch will die and (b) Tree is likely stressed and display reduced vigor, Notice affecting of the patchy nature of the tree which is conspicuous and dramatic

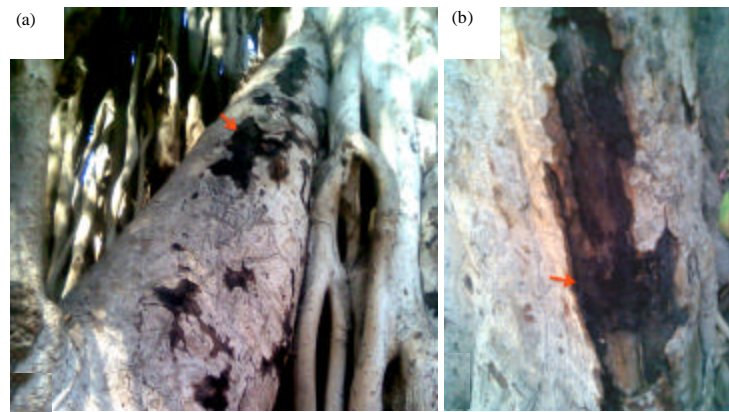


Fig. 2(a-b): Sooty canker symptoms on aerial prop roots grown into thick woody trunks of *F. Benghalensis* (a) Outer bark peeling and black sooty spores and (b) Sooty canker on *F. Nitida* trunk

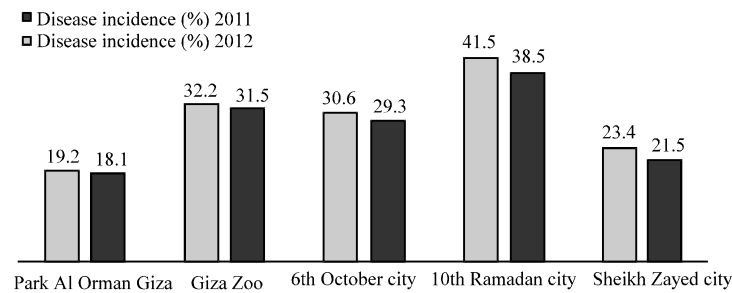


Fig. 3: Disease incidence (%) at different location of study during 2011, 2012 seasons

Table 2: Fungi and their frequency (%) isolated from naturally infected samples of ficus exhibited typical disease symptoms

Fungi and their frequency (%)					
Localities	<i>L. theobromae</i>	<i>Phomopsis</i> sp.	<i>Alternaria</i> sp.	<i>Nigrospora</i> sp.	Total
Sheikh Zayed city	12.3	5.7	3.4	3.4	24.8
10th Ramadan city	15.1	7.4	4.4	2.7	29.6
6th October city	10.1	6.1	4.0	2.7	22.9
Giza Zoo	6.4	4.7	2.7	1.3	15.1
Park Orman Giza	3.0	2.0	1.3	1.3	7.6
Total	46.9	25.9	15.8	11.4	100.0

Table 3: Pathogenicity of the isolated fungi on three different species of *Ficus*

Average of canker area (mm)					
<i>Ficus</i> sp.	<i>L. theobromae</i>	<i>Phomopsis</i> sp.	<i>Alternaria</i> sp.	<i>Nigrospora</i> sp.	Control
<i>F. benghalensis</i>	45.0	31.0	0.0	0.0	0.0
<i>F. nitida</i>	38.0	24.0	0.0	0.0	0.0
<i>F. hawaii</i>	36.0	24.0	0.0	0.0	0.0
LSD <sub>0.05</sub>	Fungi.: 1.11 Fic: N.S fungi and Fic: 1.62				

The pathogen that was re-isolated from diseased parts and cultured on PDA had similar culture and morphological characteristics as the original isolate. *Alternaria* sp. and *Nigrospora* sp. and the control treatment did not cause any outward symptoms.

The highest average of canker area observed in case of *L. theobromae* on *F. benghalensis*, *F. nitida* and *F. hawaii* (being 45, 38 and 36 mm, respectively). Meanwhile *Phomopsis* sp. record 31 and 24 mm, respectively.

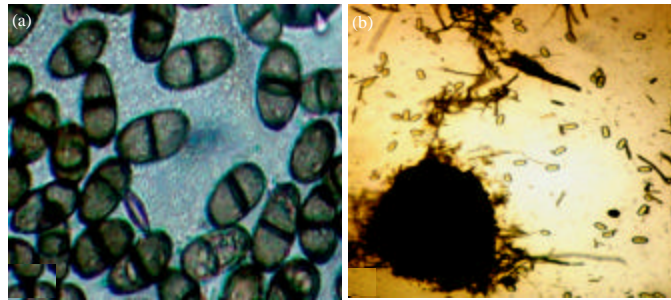


Fig. 4(a-b): (a) Two celled dark pycnidiospores of *Lasiodiplodia theobromae* and (b) Pycnidium and alpha conidia of *Phomopsis* sp. Alpha conidia are single celled, clear and oval to fusoid

Table 4: Host range of *Botryodiplodia theobromae* and *Phomopsis* sp.

Plant species	Family	Average of canker area (mm)	
		<i>L. theobromae</i>	<i>Phomopsis</i> sp.
<i>Vitis vinifera</i> (Grapes)	Vitaceae	32.0	36.00
<i>Mangifera indica</i> (Mango)	Anacardiaceae	41.0	50.00
<i>Malus domestica</i> (Apple)	Rosaceae	5.00	50.00
<i>Ficus benghalensis</i>	Moraceae	45.0	31.00
<i>Ficus nitida</i>	Moraceae	38.0	28.00
<i>Ficus hawaii</i>	Moraceae	36.0	28.00
LSD <sub>0.05</sub>		1.85	1.43

**Host range:** A study on the host range of *L. theobromae* and *Phomopsis* sp. causing dieback and cankers of *Ficus* sp. was conducted to identify possible hosts of these fungi. Six plant species belonging to four families were evaluated for their susceptibility to infection. Table 4 indicated that all tested plants showed positive reaction to both pathogenic fungi 35 days after inoculation but varied in their susceptibilities. These were: *Vitis vinifera* (Grapes), *Mangifera indica* (Mango), *Malus domestica* (Apple), *Ficus benghalensis* (ficus), *Ficus nitida* (ficus) and *Ficus hawaii* (ficus).

In general, *Botryodiplodia theobromae* gave the highest average of canker area (mm) on all tested plant species than *Phomopsis* sp. Apple (*Malus domestica*) was the least susceptible one (recorded 5 mm canker area in both fungi) while, Mango (*Mangifera indica*) were least susceptible plant to infection by *Phomopsis* sp.

**Chemical control:** Five commercial fungicides were evaluated under greenhouse conditions for their effect on trunk and stem canker incidence of *Ficus nitida*. In chronological order, the fungal infection in treated *Ficus* plants gradually reduced with fungicidal sprays. It was accompanied with a gradual reduction in the disease incidence in treated plants as compared to untreated control. Table, 5 indicated that, fungicides used varied in their efficacy on reducing average of canker area.

Table 5: Chemical control of trunk and stem canker of *Ficus* using different fungicides

Fungicides	Fungi	Average of canker area (mm)		Efficiency (%)	
		2011	2012	2011	2012
Kopeix	<i>L. theobromae</i>	25	22	48.9	42.1
	<i>Phomopsis</i> sp.	19	21	47.2	40.0
Antracol combi	<i>L. theobromae</i>	10	12	79.6	68.4
	<i>Phomopsis</i> sp.	11	15	69.4	57.1
Topsin M 70	<i>L. theobromae</i>	12	13	75.5	65.8
	<i>Phomopsis</i> sp.	9	11	75.0	68.6
Kocide 2000	<i>L. theobromae</i>	21	19	57.1	50.0
	<i>Phomopsis</i> sp.	19	20	47.2	42.9
Chaampion	<i>L. theobromae</i>	20	19	59.2	50.0
	<i>Phomopsis</i> sp.	17	19	52.8	45.7
Control	<i>L. theobromae</i>	49	38		
	<i>Phomopsis</i> sp.	36	35		
LSD <sub>0.05</sub>	<i>L. theobromae</i>	1.66	1.51		
	<i>Phomopsis</i> sp.	1.37	1.44		

Antracol Combi proved to be the highly effective fungicide for the control of disease followed by Topsin M 70.

Antracol combi gave the best efficiency to reducing the average of canker area by 79.6 and 69.4% in case of *L. theobromae* and *Phomopsis* sp., respectively followed by Topsin M 70, being 75.5 and 75.0% in season 2011. Kopeix, Kocide 2000 and Champion gave intermediate effects. All fungicide treatments have the trend in 2012 season.

## DISCUSSION

New cities in Egypt are considered one of the most important solutions to chronic housing crisis that currently exist. Started in the establishment of these cities of more than twenty years and has set up all in the desert around Greater Cairo to be the magic solution to the housing crisis in Egypt. Therefore cultivation of shade trees and other plants take an important place in these cities to the ability of these trees to change the desert

environment to attract environment for the establishment of population in these cities. *Ficus* often grown is an attractive ornamental tree found mostly outdoors and is frequently encountered along city streets lining parkways, medians and sidewalks. It finds use in parks and other large, open spaces but is, perhaps, most familiar as a street tree.

In recent years dieback and sooty canker, a fungal disease characterized by branch dieback and tree death, has ravaged street plantings and roadside *Ficus* trees in the new cities and some public parks in Giza, as well as in the zoo. In the present study surveys were carried out in five various regions during 2011-2012 on three different *Ficus* species i.e., *F. benghalensis*, *F. nitida* and *F. awaii*. The information collected from survey study has shown the pattern of disease development at different locations. In general, the highest percent of disease incidence recorded at 10th Ramadan City while the lowest percent was record in Al OrmanPark during two seasons. Analysis of the growing conditions suggested the trees were being subject to stress induced through transport, mechanical injury, medium and environmental factors, it is highly likely that the trees were predisposed. It could also be attributed to existence of variability in the pathogen. These may be attributed to differential build up of inoculums which may be affected by various environmental conditions that affect natural infection in orchards (Jayalakshmi, 2010).

In our study, fungi associated to *Ficus* sp. were morphologically and identified and incidence of diseases induced by these fungal species was determined using pathogenicity tests. The results showed that *Lasiodiplodia theobromae* and *Phomopsis* sp. were the most dominant fungi in survey locations and tree species and was responsible for the causing branch canker and dieback disease in Egypt. The association of *Lasiodiplodia* and *Phomopsis* sp. Is an interesting one since a similar association was demonstrated in the etiology of rose stem desiccations in Brazil (Pitta and Teranishi, 1973). The *L. theobromae* and *Phomopsis* sp. produced heavy masses of loose spores under the bark of infected trees, which can readily spread the infection. It is probable that certain cultural practices might have enhanced the outbreak of the disease.

The fungus may introduced with the trees, penetrated through bark wounds and incubated under conditions stressful to the well-being of the tree. *Phomopsis cinerescens* has been reported on *Ficus benjamina* in Canada (Hampson, 1981) and has been isolated from *Ficus carica* in Europe where it is responsible for fig branch and twig canker (Celiker and Michailides, 2012).

*Phomopsis* sp. is wound invaders (Forsberg, 1975; Morehart *et al.*, 1980). Since *P. cinerescens* is an introduced species and the hosts appear to have suffered laceration damage, it is suggested the fungus entered through wounds and developed under a stress syndrome. Forsberg (1975) stated that the past various physiological parameters such as acclimatization treatments and maintenance practices including the supply of water have also been implicated in the leaf loss phenomenon in *F. benjamina* indoors. Plants receiving minimum light levels show a higher incidence of twig die-back caused by *P. cinerescens*. These observations are consistent with the fact that *P. cinerescens* is a weak pathogen and invades only stressed plants (Morehart *et al.*, 1980).

Banihashemi and Javadi (2009) suggested that wounds and bark killed by frost and sunburn were the chief avenues of infection, the pathogen invades host branches mainly through pruning cuts but sunburn and frost probably also enable infection and that leaf scars were only of minor importance.

Trees that are weaker are more likely to become infected. Trees might be compromised by any combination of unfavorable growing conditions such as poor soil, unsuitable climate, or having a major portion root zone covered with pavement. Cultural practices impacting tree health such as inadequate irrigation, frequent or excessive canopy pruning, or root pruning to repair pavement.

Results proved that the two pathogenic fungi *L. theobromae* and *Phomopsis* sp. can infect six plant species belonging to four families i.e., *V. vinifera*, *M. indica*, *M. domestica*, *F. benghalensis*, *F. nitida* and *F. hawaii*. Extremely wide host range of both fungi increase chance of disease dissemination. These results are matching with Mansour (1986), Attia and Saber (1995), Haggag (2010), Atia *et al.* (2003), Rashed *et al.* (2006), Haggag and Nofal (2006) and Ismail *et al.* (2012). *Lasiodiplodia theobromae* is a cosmopolitan soil-borne fungus causing both field and storage diseases on different plant species including crops, fruits and plantation trees (Khurana and Singh, 1972; Talukder, 1974; Ilag and Marfil, 1977; Singh *et al.*, 1977; Domsch *et al.*, 1980). Also, both fungi produces similar symptoms in many other economic plants such as *Ficus carica* (Banihashemi and Javadi, 2009 and Celiker and Michailides, 2012); on *F. benjamina* (Hampson, 1981; On *F. nitida* and *P. macrophyllus* (Benschop *et al.*, 1984); on mango (Khanzada *et al.*, 2005) and on *F. religiosa* and *F. bengalensis* (Maheswari and Rajagopal, 2011).

Five commercial fungicides were evaluated under greenhouse conditions for their effect on trunk and stem



canker incidence of *F. nitida*. Antracol Combi and Topsin M 70 provided effective control of the infection meanwhile, Kopeix, Kocide 2000 and Champion fungicides were moderately effective. Present results are in accordance with Lonsdale and Kotze (1993), Li *et al.* (1995), Rawal (1998) and Mahmood *et al.* (2002) who recorded that foliar spray of Topsin-M (Thiophanate-methyl) and broad-spectrum systemic fungicides reduced the infestation of *L. theobromae*.

High effectiveness of systemic fungicides results from their ability of translocation into the plant tissues and destruction of the pathogens during the time of infection and incubation, it affects mitosis and cell division of fungal pathogens. Systemic fungicides form a protective barrier on the plant, permeate into the plant, move upward in the plant's xylem and move downward in the plant's phloem. These fungicides have protective activity including new growth and have good curative activity. These results agree with the findings of Mansour (1986), Haggag (2010), Attia and Saber (1995), Atia *et al.* (2003), Rashed *et al.* (2006), Haggag and Nofal (2006) and Ismail *et al.* (2012).

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