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FIG DIE-BACK DISEASE IN EGYPT

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ABSTRACT

Several locations belonging to three Egyptian governorates, i.e. Matrouh, Alexandria and North Sinai were surveyed for die-back disease incidence on fig trees. The highest record of fig die-back disease incidence was found at Marssa Matrouh location (45.0%), while the lowest was record at El-Sheikh Zewaied location (23.0%). The disease occurrence was more prevailing during the season 2009 than 2008. Pathogenicity tests proved that *Botryodiplodia theobromae, Fusarium solani* and *Alternaria alternata* are the causal pathogens of fig die-back disease. The fig cultivars cultivated in Egypt were differed in their reactions against the three causal pathogens. They were descendingly arranged as Sultani, Adssi, Gizi and Aboudi. The host range of Fig die-back pathogens were inclusive apricot, mango and grapevine trees. Saline stress has an important role in increasing the disease incidence. At 3000 ppm NaCl in irrigation water, the disease incidence on nurseling of cv. Sultani was 66.8%, while it reached 28.9% at 1000 ppm NaCl. Histopathological studies revealed that after 7 days from artificial inoculation, the pathogenic fungus *B. theobromae* caused plasmolysis and disorganization with a dark brown discoloration of epidermal and cortical cells, but after 21 days, the disease had rapidly progressed and therefore necrotic areas were formed in both xylem parenchyma and xylem vessels and colonized by hyphae, tylosis and by dark inclusions. This study is thoughout to be the first in this attitude in Egypt.

Keywords: *A. alternate, B. theobromae*, Fig, die-back, *F. solani*, saline stress.

INTRODUCTION

Common fig (*Ficus carica*, Fam. *Moraceae*) is considered one of the most favorable fruit crops to cultivate under the new reclaimed areas. The fig area is still small in Egypt, although, it has high nutrient value. This cultivated area is representing about 3.78% of the total fruit cultivated area in Egypt (Anonymous, 2005). Fig tree is facing problem of several diseases (Morton and Julia, 1987) and die-back is considered one of them (Barthelet and Vinot, 1944). Die-back symptoms appear at different stages of growth, kills twig, shoot, branch and trunk wood causing great reduction in yield of many fruitful trees (Ragab *et al*., 1971). Field observations state that there are several causes for Fig die-back

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disease such as frost, low temperature, drought conditions and some biotic agents. Apple (2004) stated that fig trees in Texas (USA) are often injured by frost that kills younger twigs. Although their death is not related directly to loss in production, they may serve as a site for secondary fungi such as Botrytis, Diplodia and Corticium.

The present study was designed to find out the distribution and occurrence of the disease in Egypt to study some environmental factors affecting disease incidence such as water salinity, varietal reaction, host range and to investigate the histological changes caused by the causal organisms.

MATERIALS AND METHODS

Disease survey

Die-back disease was surveyed during two successive seasons of 2008 and 2009 at the main growing area of fig in Egypt. Several locations at three governorates of fig cutivation were examined, i.e. Marssa Matrouh, El-Hamam and El-Dabaa (Matrouh governorate), El-Agami, Abo Tallat and Borg El-Arab (Alexandria governorate) and Al-Arish, Rafah and El-Shiekh Zoowaied (North Sinai governorate). Disease incidence was determined as infection percentage according to Korra (1989). On the other hand, disease severity was calculated according to the modified scale of Wicks and Davies (1999) as follows:

D.S. = Σ_n x V ÷ 5 N were:

n = The number of diseased branches in each infected category.
V= Numerical value of the grade as follows: 0 = No infection, 1 = 1.0 - 10 % infection, 2 = 10.1 - 24 % infection, 3 = 24.1 – 50 % infection, 4 = 50.1 – 75 % infection, 5 = 75.1 – 100 infection.
N = Total number of the branches inspected.
5 = Maximum disease severity grade.

During disease survey, the infected samples were collected from each examined location and brought to the laboratory to carry out the different studies.

Isolation and identification of the associated organisms

Fig twigs showing the identical symptoms of die-back disease collected from the different examined locations were brought to the laboratories of Department of Botany, Faculty of Agriculture, Al-Azhar University, Nasr City, Egypt. Samples were washed thoroughly with tap water then cut into small portions (0.5cm.), sterilized by dipping in (0.1%) sodium hypochlorite for 3 min., followed by rinsed several times in sterilized distilled water and dried between two pieces of sterilized filter paper. Sterilized portions were placed in Petri dishes containing 10–15 cc of potato agar medium. Petri dishes were incubated at 25°C for seven

days. Hyphal tip and single spore techniques were used to purify the associated organism on PDA slants.

Purified organisms obtained were identified at Laboratory of Plant Pathology, Department of Botany, Faculty of Agriculture, Al-Azhar University, Nasr City, Egypt, according to Alexopoulos and Beneke (1979); Jacobs and Rehner (1998) and Simons (2007). It is not enough for identification. Stock cultures were maintained on PDA slants and kept in a refrigerator at 5-10 °C and routinely subcultured on fresh slants every three months.

Pathogenicity test

Pathogenicity test was carried out by superficially wounds about 1 cm in long 10 cm under the terminal bud. Two twigs on each use plant were chosen to test the pathogenesis capability of each isolated fungus. Discs (0.5 cm in diameter) from each test fungal organism taken from 7-day-old cultures were used separately for wounds inoculation. Inoculated shoots were covered with plastic bags to maintain the relative humidity around the inoculated twigs. Twenty grown into black polyethylene bags fig nurslings 1-year-old, cv. Soltani x 5 replicates were used for each treatment. The same numbers of bags cultivated with non inoculated fig plants were left as check treatment. All bags were kept under greenhouse conditions. Data were recorded 30 days after inoculation according to Munkvold *et al.* (1994) as percentage of disease incidence and severity.

Varietal reaction

Six fig varieties, i.e. Soltani, Gizi, Kadota, Aboudi, Black Meshun and Adssi were tested for their ability to infection. *Botryodiplodia theobromae, Fusarium solani* and *Alternaria alternata* were the test pathogens. One-year-old fig nurslings were individually inoculated with test pathogens as mentioned before. Twenty nurslings (3 fungi + Control = 4 x 5 replicates) were used for each variety. The same number of non-inoculated nurslings were left to serve as control. All nurslings were kept under greenhouse conditions and watered as needed. Infection percentage was calculated 30 days after inoculation.

Host range

The host ranges of the causal pathogens of fig die-back disease were investigated under greenhouse conditions. Nurslings of different fruit crops, *i.e.* apricot (cv. Hammawy), mango (cv. Zebdia), and grapevine (cv. Thompson) were collected and inoculated with the test pathogens as mentioned before. Twenty nurslings of each test plant (3 fungi + Control = 4 x 5 replicates) were used for each plant.

All plants were kept under greenhouse conditions. Die-back disease severity was recorded after 7, 14 and 21 days from twigs inoculation.

Histopathological study

Histological studies were carried out to investigate the disease development into plant tissues. Inoculation of fig twigs (cv. Soltani) was carried out using tooth pick method according to Young (1943) to avoid tissue wounding. The inoculated twigs were covered with polyethylene bags to provide high humidity for 2 days at 25°C around the infected twigs. After 7 and 21 days from inoculation, the infected twigs were washed in three changes of sterilized water and dried between folds of sterilized paper towels. The infected and non-infected tissues were cut into small portions (5–10 mm long), fixed in formalin-acetic acid-alcohol solution (FAA) according to Sassm (1961). Specimens were then dehydrated and cleared in N butyl alcohol series and embedded in wax according to Johansen (1940). Sections were cut at 15 and 20 µ thick, then stained with safranin light green combination and mounted in Canada balsam. The stained sections were examined by classic microscope.

Effect of water salinity

This experiment was conducted to investigate saline stress on the occurrence of fig die-back disease. Shoot infection was carried out with each test pathogen as mentioned before. One -year-old fig nurslings (cv. Soltani x 5 replicates) were used for each fungal treatment. Sodium chloride was dissolved in a tap water to prepare different concentrations of saline solution, *i.e*. 1000, 1200, 1400, 2000 and 3000 ppm. The same number of non-inoculated nurslings were cultivated, irrigated with tap water instead of saline solution and used as a control treatment. All plants were kept under greenhouse conditions. Percentage of disease incidence was recorded 30 days after inoculation.

RESULTS AND DISCUSSION

Disease survey

Fig die-back disease was found in all examined locations during the two successive growing seasons 2008 and 2009 (Table 1). Disease incidence was differed from one location to another. The highest percentage of the disease incidence was recorded at Marssa Matrouh location (45.0% in medium), followed by El-Hammam location (42.0%), while the lowest was observed at El-Shiekh Zowaied location (23.0%). On the other hand, Matrouh governorate was revealed the highest infection percentage followed by Alexandria and North Sinai governorates. At the same time, there are no significant differences obtained between the two tested seasons. Also, the same trend of disease incidence was observed in respect to disease severity. Matrouh showed the highest disease severity percentage (17.43%), followed by Alexandria (12.0%), while North Sinai gave the lesser disease severity (2.71%). The higher disease severity of tested locations was recorded in Marssa Matrouh (19.36%), followed by El-Hammam (18.8%), while the lowest one was recorded in Rafah (2.56%) and El-Shiekh Zowaied (1.67%).

Table 1. Disease incidence [*] and disease severity [**] of fig die-back during the two successive seasons 2008 and 2009 in different examined locations.

Governorate		Matrouh				Alexandria				North Sinai			
Disease record	Season	Marssa	Hamma m	Dabaa	Mean	Agami	Talat	B.Arab	Mean	Arish	Rafah	Zewied	Mean
D.I.[*]	2008	44	40	36	40	40	36	32	36	24	28	20	24
	2009	46	44	37	42	42	40	33	38	28	34	26	29
	Mean	45	42	36	41	41	38	32	37	26	31	23	26
D.S.[**]	2008	19	17	14	16	13	11	10	11	4	2	1	2
	2009	20	18	15	17	13	12	10	11	4	3	2	3
	Mean	19	18	14	17	13	11	10	12	4	3	1	2

L.S.D. at 5 % for D.I., Years= 6.71, Govers. =3.05, Locations= 3 = 2.67.
For D.S., Years= 2.35, Govers. =3.15 , Locations= 3 = 1.55.

Isolation and identification of the associated organisms

The most prevailing fungi on diseased fig shoots were identified as *Botryodiplodia theobromae, Fusarium solani* and *Alternaria alternata*. The frequencies of these fungi obtained were slightly higher at 2009 season than at 2008 (Table 2).

Table 2. Frequencies of fungal isolates obtained from disease samples of fig. collected from different surveyed governorates.

Pathogen	Frequency	
	Season 2008	Season 2009
Botryodiplodia theobromae	30	35
Fusarium solani	26	27
Alternaria alternata	20	21
Penecillium italicum	8	9
Aspergillus niger	5	6
Trichoderma harzianum	3	5
Rhizopus nigricans	2	3

These fungi were recorded as the causal organisms of die-back disease on different crops, i.e. *Botryodiplodia* on sycamore, grapevine, rose and rubber (Filer, 1969; El-Goorani and El-Melegi, 1972; Glaser *et al*., 1981; Chattopahay and Hajra, 1982), *Fusarium* on Fig, currants and carnation (Barthelet and Vinot, 1944; Bestango, 1959); *Alternaria* on carnation and doddor (Bestango, 1959 and Rudakov, 1961).

Pathogenicity test

The three tested fungi differed in their ability to induce fig die-back infection. *B. theobromae* showed high percentage of disease incidence (95%) followed by *F. solani* (80%) and *A. alternata* (65%). The same trend was observed in relation to disease severity (Table 3).

Table 3. Pathogenicity test of fungal isolates to induce die-back disease of fig. nurslings twigs cv. Soltani under greenhouse conditions during season 2008.

Pathogen	Infected twig %	Severity	Mortality (mm)
B. theobromae	95	60	51
F. solani	80	52	42
A. alternata	65	40	20
P. italicum	-	-	-
A. niger	-	-	-
T. harzianum	-	-	-
R. nigricans	-	-	-

L.S.D. at 5 % for infected twigs = 12.6, Mortality = 11.8.

The observed variation of the recorded pathogenicity of the three organisms on fig die-back incidence percentage may be due to the differences of the chemical components of their toxin secretion and enzymes influences on cell walls and the host plant reaction. This conclusion was in harmony with Ferrari *et al*. (1996); Chakraborty *et al.* (2004); Rakholiya *et al.* (2004).

Varietal reaction

Among the fig cultivars tested against die-back pathogen appeared as cv. Soltani was the highly susceptible fig cultivar, followed by cvs. Gizi, Abboudi and Adssi. Black Mession and Kadota cultivars were the less susceptible showing 15 and 10 % disease incidence, respectively. The same trend was observed with respect to disease severity.

Table 4. Effect of the causal pathogens on fig die-back disease incidence* and severity** under greenhouse conditions.

Pathogen	Die-back record	Disease incidence % for fig varieties						Mean
		Soltani	Gizi	Aboudi	Adssi	Black Mession	Kadota	
B. theobromae	D.I.* D.S.**	70 50	60 35	55 45	50 35	15 06	10 02	43
F. solani	D.I. D.S.	35 43	30 25	30 25	50 40	00 00	00 00	24
A. alternata	D.I. D.S.	20 21	00 00	00 00	15 28	00 00	00 00	6
Mean	D.I. D.S.	31 29	23 15	21 8	29 26	4 2	3 1	-
Check	00.0	00.0	00.0	00.0	00.0	00	00	-

L.S.D. at 5 % for pathogen (P) = 2.67, Varity (V) = 3.86, PxV = N.S.

Similarly, Kosack and Jones (2000) reported that morphological and anatomical characteristics of host varieties, caused various types of disease reactions.

Host range

The data presented in Table 5 showed that all the tested pathogens affected all the tested hosts, *i.e.* apricot (cv. Hammawy), mango (cv. Zebdia) and grapevine (cv. Thopson). *B. theobromae* was found to be the most virulent pathogen followed by *F. solani* and *A. alternate*. Mean of infection area was increased by increasing of incubation period. Several authors have studied the former relation, such as Korra (1989) on apricot; Arl and Oz (1990) on grapevine and Sharma and Gupta (1994) on mango.

Table 5. Die-back disease severity at 7, 14 and 21 days from infection on some fruit trees by using the pathogenic fungi of fig twigs.

Pathogen	Apricot			Mango			Grapevine		
	7 d.	14 d.	21 d.	7 d.	14 d.	21 d.	7 d.	14 d.	21 d.
B. theobromae	80	100	100	50	90	100	60	80	95
F. solani	60	50	90	40	60	85	00	00	00
A. alternata	30	50	80	00	00	00	30	45	60

L.S.D. at 5 % for isolates (I) = 11.23, Cultivars (C) = 9.87, I x C = 13.10.

Histopathological study

Cross sections in the fig twigs inoculated with *B. theobromae*, after 7 days from artificial infection clearly show that plasmolysis and disorganization with dark brown discoloration of epidermal and cortical cells, most of phloem and cambial cells are destroyed. It is also seemed that the xylem rays were suitable pass ways for rapid and easy extension of hyphae and the intercellular spaces are filled with fungal hyphae.

After 21 days from the artificial inoculation, transverse sections revealed that the disease had rapidly progressed. Necrotic area in both xylem parenchyma and xylem vessels colonized by hyphae and dark inclusions inside the xylem as well as abundant production of tylosis. The pathogen spread to all various tissues causing complete break down.

Seven days after inoculation with *F. solani*, transverse sections appear microscopically that all cells without any visible change in their histological structure as the control samples. After 21 days from the artificial inoculation, transverse sections revealed that epidermal cells and cortex are destroyed. It shows that also mycelial fragments of the fungus were present in xylem vessels. The fungus spread intra and intercellular in all tissues and the vessels colonized by hyphae and plugged with gum as well as abundant production of tylosis.

Cross sections in the fig twigs inoculated with *A. alternata*, after 7 days from artificial infection show that the tissues appear similar to the control samples without any visible change in their histological structure. After 21 days from inoculation notice that the fungal mycelium present intra and intercellular in all tissues of the twigs, particularly in the cortex tissues, which were destroyed and the wood was brownish and vessels particularly metaxylem were plugged with gum as well as tylosis. Transverse sections of healthy fig twig microscopically show that epidermis are in one row of healthy, tightly, and undiminished cells. Cortex cells are healthy and narrow with satiety pressure; vessels rows are clear and empty from gum as well as tylosis. All various tissues are free from hyphae and looks quite health. These results are in agreement with those obtained by Giorbdize (1984) for *Phomopsis citri* on citrus, Biggs and Britton, 1988 for *B. theobromae* on peach and Atia *et al.* (2003) for *B. theobromae* on grapevine, who stated that fungal hyphae and tylosis might bring out a disfunction of the xylem element with restriction in the flow of water.

Effect of water salinity

Water salinity significantly affected the disease incidence. The infection percentage was significantly increased by increasing water salinity concentration (Table 6).

Table 6. Effect of water salinity on the disease incidence of fig die-back disease.

Pathogen	Water salinity cos. (ppm)					Mean
	1000	1200	1400	2000	3000	
B .theobromae	43.8	60.5	61.6	85.5	86.0	67.5
F. solani	36.5	43.7	60.8	77.2	85.0	60.6
A. alternata	35.4	52.2	51.9	68.9	77.2	54.1
Mean	28.9	39.1	43.6	61.5	66.8	-
Check	00.0	00.0	00.0	12.6	18.9	6.3

L.S.D. at 5 % for pathogen, (P) = 12.8, Concentration, (C) = 14.3, PxC = N.S.

The highest infection percentage was found at 3000 ppm of water salinity, while the lowest ones were at 1000 ppm. In this regard, *B. theobromae* proved to be more virulent, since it caused the highest infection percentage. These results are in harmony with findings obtained by MacDonald *et al*. (1984) and Rasmussen and Stanghellini (1988).

It may be concluded from the present finding that die-back disease of fig. trees in Egypt is widespread throughout Matrouh, Alexandria and North Sinai governorates. The incidence and severity of the disease increase as salinity of irrigation water is increased. This fact must be taken in consideration especially fig trees plantations growing in cultivated areas depending mainly on rainfall or ground water for irrigation which has higher salinity concentration compare with fresh water. The items studied in the present work thought to be the first in this attitude in Egypt.

CONCLUSION

It could be concluded from the studies carried out first time in Egypt that fig. die-back disease incidence ranged from 230 to 45.0% in Egypt. The disease occurrence was more prevailing during the season 2009 than 2008 one. Pathogenicity tests proved that *Botryodiplodia theobromae, Fusarium solani* and *Alternaria alternata* are the causal pathogens. The Fig cultivars cultivated in Egypt were differed in their reactions against the three causal pathogens were descendingly arranged as Sultani, Adssi, Gizi and Aboudi. The host range of Fig die-back pathogens were inclusive apricot, mango and grapevine trees. Saline stress has an important role in increasing the disease incidence. At 3000 ppm NaCl in irrigation water, the disease incidence on nurseling of cv. Sultani was 66.8%, while it reached 28.9% at 1000 ppm NaCl. The pathogenic fungus *B. theobromae* caused plasmolysis and disorganization with a dark brown discoloration of epidermal and cortical cells, necrotic areas were formed in both xylem parenchyma and xylem vessels and colonized by hyphae, tylosis and by dark inclusions.

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