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The Dieback of *Citrus* Trees in Tahrir District

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A SURVEY covering 3150 *Citrus* trees including grapefruit, lemon, lime, mandarin, orange and sweet lime grafted on different *Citrus* rootstocks has been carried out in Tahrir district during 2000 and 2001 seasons. It is indicated that the dieback incidence and its severity ranging from 23.9 to 58.8% and 10.7 to 28.2%, respectively. Lemon trees were the highest *Citrus* species affected with dieback, while grapefruit was the lowest one. *Botryodiplodia* sp., *Fusarium* sp., *Alternaria* sp., *Penicillium* sp., *Aspergillus niger, Aspergillus flavus, Derscherlla* sp. and *Trichodermia* sp. were isolated from *Citrus* branches which showed dieback symptoms. Pathogenicity revealed that *Botryodiplodia* sp. was the pathogenic fungus on the tested *Citrus* species, *i.e.* orange and mandarin.

Citrus crop is one of the most important subtropical fruits in the world as well as in Egypt. Several diseases affected the growth and yield of *Citrus* orchards during the growing season. Dieback disease was very severe in mandarin trees, in addition to gummosis, sooty mould, scab, powdery mildow and canker were recorded in *Citrus* orchards (Mukhopadhyay, 1985 and Gupta, 1994). The incidence of decline (dieback) of Nagpur mandarin trees was 19% (Kolte & Diware, 1989), while it was 14.2 to 58.8% in the mandarin trees in Darjeeling district.

Twig dieback can be caused by several fungi, as well as by other factors (Raychaudhuri & Khurana, 1998). Fungal infection often occurs following a freeze, mechanical or chemical injury. Affected young branches showed dieback 1 inch or more from the tip; sometimes producing gum exudation. Wood is discolored underneath the brak.

The causal agent of dieback disease in *Citrus* was studied by many workers. Mukhopadhyay (1985), Davis *et al.* (1987), Suarez (1990) and Gupta (1994) reported that the dieback of branches caused by *Diplodia natalensis (Botryodiplodia theobromae), Colletotrichum gloeosporioides (Glomerella cingulata)* and *Fusarium* sp. which often penetrated through injuries made by the insects *Solenoposis geminata* and *Wasmannia auropunctata.* Mourishon (1994) and Paez & Castano (1998) reported that the *Ceratosystis fimbriata* was the causal agent of dieback of *Citrus* trees. Traiceveski (1991) found that the fungus *Phytophthora nicotianae* var. *parasitica* was the a causal agent of mandarin tree dieback, while Forster & Adaskaveg (1999) mentioned that the fungus of *Colletotrichum acutatum* was isolated from *Citrus* branches showing dieback symptoms.

This work aimed to determine the disease incidence and severity of dieback under natural infection conditions on different *Citrus* species, *i.e.* grapefruit (Red Blush and Star Ruby cvs.); lemon (Corona Foothall, Frost, Limoneira, Monroe and Prios cvs.); lime (Bears); mandarin (Dancy, Fremont, Kinnow, Minneola and Orlando cvs.); orange (Gulette and Hamlin cvs.); and sweet lime. Isolation and identification of the causal pathogen has been carried out.

Material and Methods

Survey of disease incidence

Incidence and severity of the dieback disease on *Citrus* species, *i.e.* grapefruit (*Citrus paradisi*); lemon (*Citrus Limonia*); lime (*Citrus aurantifolia*); orange (*Citrus sinensis*); mandarin (*Citrus nobilis*) and sweet lime (*Citrus Limetia*) grafted on different rootstocks (Table 1) were recorded under natural infection condition in Tahrir distract at experimental farm, Desert Development Center, American University in Cairo Behiera governorate during 2000 and 2001 seasons. The following scale was used to study the dieback incidence and severity : 3 = severe infection (51% or more of branches); 2 = moderate infection (26 to 50% of branches); 1 = light infection (1 to 25% of branches) and o = no symptoms. The percentage of incidence of dieback disease was calculated as follows :

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected tree}}{\text{Total number of tree}} \times 100$$

The disease severity index was calculated by the formula (Liu *et al.* 1995) as follows :

$$\text{Disease index (severity \%)} = \frac{\sum (\text{rating No. x No. of trees in the rating})}{\text{Total No. of trees x highest rating}} \times 100$$

TABLE 1. Citrus cultivars grafted on different rootstocks.

Citrus species	Cultivars	Rootstocks				
		Carimno	Citrumelo	Rangpur	Sour orange	Volkam-eriana
Lemon	Corona Foothall	+[1]	+	+	+	-
	Frost	+	-	+	-	-
	Limoneria	+	+	+	+	-
	Monroe	+	+	+	+	-
	Prios	+	+	+	+	-
Mandarin	Dancy	+	-	+	+	-
	Fremont	+	-	+	+	+
	Kinnow	-	-	+	+	+
	Minneola	+	-	+	+	+
	Orlando	+	-	+	+	+
Grape fruit	Red Blush	-	+	+	+	+
	Star Ruby	-	+	+	+	+
Orange	Hamlin	-	+	+	+	-
	Gulette	-	+	+	+	-
Lime	Bears	-	-	+	+	-
	Sweet Lime	-	-	-	-	-

[1] - = No rested + = tested.

Isolation and identification of the causative fungi

Samples of naturally infected branches and twigs of different *Citrus* species

grafted on different rootstocks (Table 1) showing typical symptoms of dieback disease were collected from Tahrir district at Behiera governorate. Isolation was carried out from these samples on PDA medium according to the method described by Michailides (1991). The growing fungi were purified by haphal tip technique and sub-cultured on PDA medium. Pure cultures were identified according, to morphological and cultural characters (Gilman, 1957 and Barnett & Hunter, 1972).

Pathogenicity tests

Pathogenicity test of all isolated fungi were carried out using *Citrus* transplants, *i.e.* orange (Hamlin and Sour orange) and mandarin (Orlando and Fremont) grown under greenhouse conditions at Tahrir district, Behiera governorate. Ability of all isolated fungi to invade the *Citrus* twigs tissue was tested. Inoculum of each isolated fungus was grown singly on PDA medium for 7 days at 25°C, then the growth (mycelium and conidia or mycelium alone) was gently collected by aid of sterilized brush in 20ml of sterilized water per Petri dish just before inoculation. Three twigs on each transplant were chosen, surface-sterilized and wounded with a sterile scalpel (Korra, 1989). Twenty ml of the spore suspension of isolated fungus (5×10^6 spore/ml) were sprayed onto the wounded twigs of each treatment using a sterile hand atomizer. Control treatments were similarly treated with sterilized water was used. Then, inoculated transplants were kept in humid champer for 24 hr and after that placed out under greenhouse conditions. The dieback symptoms were recorded.

Pectolytic and cellulolytic enzymes activity

Pectolytic and cellulolytic enzymes activity of *Botryodipolodia* sp. fungus were estimated in culture filtrates according to the method described by Matta & Dimond (1963) and Tolboys & Busch (1970). The production of pectic enzymes, *i.e.* polygalacturonase (PG) and pectin methylestrase (PME) were carried out using the basal synthetic medium (Tolboys & Busch, 1970) containing 4.6 g of citrus pectin. Flasks containing 100 ml of the medium, were inoculated with disc of fungus growth. After incubation at 28°C for 7, 14 and 21 days, the cultures were filtrated through filter paper Watman No. 1, and filtrates (contain crude enzyme preparation) were centrifuged at 3000 rpm for 5 min, were used for enzymatic activity. PG enzyme activity was assayed by estimating the loss in

viscosity of 1.2% citrus pectin. While PME enzyme activity was determined by the titration method of Matta & Dimond (1963).

Also, the basal synthetic medium (Tolboys & Busch, 1970). supplemented with 4.6g carboxymethyl cellulose (CMC) instead of pectin was used for the production of cellulolytic (Cx) enzymes. The cultures received the same treatments as that of pectic enzymes production. Cx enzyme activity was determined by measuring the loss in viscosity of 1.2% CMC solution (Matta & Dimond, 1963). Boiled crude enzyme preparations were used as control.

Results and Discussion

Dieback has been observed in *Citrus* orchards grown in the desert, *e.g.* North and South of Tahrir. Observations and early studies revealed that symptoms started at the tip of growing branches, moved downward causing the death of inner tissues (turned to brown) and the defoliation of leaves, then followed by the death of infected branches.

Natural infection data in Tahrir district, during 2000 and 2001 seasons, showed some difference among *Citrus* species and cultivars to dieback disease incidence (Table 2 - 6). Field observation show that the disease incidence and severity on Citrus species were 43.3 and 19.8%, respectively. Data show that the light disease incidence was recorded on both sweet lime and grapefruit (14.8 and 23.9%, respectively), while on orange and lime disease incidence was moderate (35.7 and 37.5%, respectively). Natural infection data also indicated that the mandarin and lemon were severely attached, where a high percentage of disease incidence (45.2 and 58.8%, respectively) were recorded . It was clear that the disease severity of dieback on *Citrus* species illustrated some differences (Table 2- 6). Data show that the disease severity of sweet lime and grapefruit were 4.9 and 10.7%, respectively, while on orange and lime were 13.3 and 16.0%, respectively. Data also show that the highest disease severity was recorded with mandarin and lemon (21.2 and 28.6%, respectively). It can be concluded that the variability in disease severity and incidence was observed in *Citrus* species (Mukhopadhyay, 1985 and Kolte & Diware, 1989).

Citrus species (grapefruit, lemon, lime, mandarin, orange and sweet lime) grafted on different rootstocks, *i.e.* Carimno, Citrumelo, Rangpur, Sour orange

TABLE 2. Survey of dieback (%) incidence and severity of Lemon cultivars (Corona Foothall, Frost, Limoneira, Monroe and Prios) grafted on different citrus rootstocks under natural infection conditions.

Lemon cultivars	Different rootstocks	No. of Trees	Natural infection (%) of diaback				Mean	
			2000		2001			
			Incidence	Severity	Incidence	Severity	Incidence[x]	Severity %
Corona Foothall	Carimno	27	66.7	33.3	70.4	39.5	68.5 d	36.4
	Citrumelo	18	38.9	20.8	5.6	1.9	22.2 a	11.1
	Rangpur	27	66.7	33.3	48.2	29.6	57.4 cd	31.5
	Sour orange	26	50.0	21.8	50.0	19.2	50.0 bc	20.5
	Mean		57.1	27.9	46.9	24.5	52.0 A	26.2
Frost	Carimno	27	74.1	27.2	63.0	23.5	68.5 a	25.3
	Citrumelo	NT	NT	NT	NT	NT	NT	NT
	Rangpur	27	66.7	28.4	85.2	43.2	75.9 a	38.9
	Sour orange	NT	NT	NT	NT	NT	NT	NT
	Mean		70.4	32.1	74.1	33.3	72.2 C	30.6
Lemoneira	Carimno	27	85.2	48.2	92.6	58.0	88.9 b	53.1
	Citrumelo	18	33.3	13.0	38.9	13.0	36.1 a	13.0
	Rangpur	26	73.1	35.9	76.0	38.7	74.5 b	37.3
	Sour orange	27	40.7	24.7	22.2	7.4	38.9 a	18.5
	Mean		60.2	32.0	58.8	30.6	59.5 AB	31.3
Mónroe	Carimno	27	59.3	38.3	92.6	61.7	75.9 c	32.7
	Citrumelo	18	55.6	22.2	37.5	16.7	47.1 a	19.6
	Rangpur	27	66.7	24.7	63.0	25.9	64.8 b	25.3
	Sour orange	27	74.1	27.2	33.3	11.1	53.7 a	19.1
	Mean		64.7	28.6	58.8	30.2	61.7 B	29.4
Prios	Carimno	27	66.6	33.3	73.1	43.6	69.8 b	38.4
	Citrumelo	27	51.9	19.8	51.9	21.0	51.9 a	20.3
	Rangpur	27	33.3	11.1	55.6	29.6	44.4 a	20.3
	Sour orange	27	59.3	30.9	42.3	24.4	52.8 a	27.7
	Mean		53.7	23.8	55.7	29.6	54.7 AB	26.6
Mean			60.2	27.9	57.3	29.3		

NT = Not tested

X = Means followed by the capital or small alphabetical letters were not statistically significant according to Duncan's multiple range test

TABLE 3. Survey of dieback (%) incidence and severity of Mandarin cultivars (Dancy, Fremont, Kinnow, Minneola and Orlando) grafted on different citrus rootstocks under natural infection conditions.

Mandarin cultivars	Different rootstocks	No. of Trees	Natural infection (%) of diaback				Mean	
			2000		2001			
			Incidence	Severity	Incidence	Severity	Incidence*	Severity %
Dancy	Carimno	27	92.6	60.5	44.4	18.5	74.1 b	39.5
	Rangpur	26	65.4	30.8	65.4	46.2	65.4 b	38.5
	Sour orange	26	69.2	41.0	40.7	25.9	54.7 a	33.3
	Volkameriana	NT	NT	NT	NT	NT	NT	NT
	Mean		76.0	44.3	50.0	30.0	62.9 C	37.1
Fremont	Carimno	27	48.2	16.1	14.8	4.9	31.5 c	10.5
	Rangpur	26	3.9	1.3	7.7	3.9	5.8 a	2.6
	Sour orange	26	65.4	29.5	8.3	2.8	38.0 c	16.7
	Volkameriana	27	7.4	2.5	29.6	9.9	18.5 b	6.2
	Mean		31.1	12.3	15.4	5.5	23.3 A	8.9
Konnow	Carimno	NT	NT	NT	NT	NT	NT	NT
	Rangpur	25	28.0	9.3	16.7	8.3	22.4 a	8.8
	Sour orange	15	80.0	35.6	30.8	10.3	57.1 c	23.8
	Volkameriana	25	28.0	10.7	56.0	28.0	42.0 b	18.0
	Mean		40.0	15.9	35.5	16.7	37.8 B	16.3
Minneola	Carimno	25	64.0	21.0	62.5	25.0	63.3 b	23.1
	Rangpur	27	48.2	16.1	63.0	33.3	55.6 b	24.7
	Sour orange	27	37.0	17.3	11.1	4.9	24.1 a	11.1
	Volkameriana	27	81.5	38.3	95.6	49.4	88.9 c	43.8
	Mean		58.5	23.3	57.1	27.3	57.8 C	25.8
Orlando	Carimno	27	48.2	24.7	28.0	9.3	38.5 a	17.3
	Rangpur	27	40.2	17.3	51.9	23.5	46.3 a	20.4
	Sour orange	26	73.1	34.6	32.0	10.7	52.9 ab	22.9
	Volkameriana	27	25.9	8.6	59.0	28.4	44.4 a	18.5
	Mean		46.7	21.2	44.2	18.3	45.5 B	19.8
Mean			49.9	22.8	40.4	19.5		

NT = Not tested

X = Means followed by the capital or small alphabetical letters were not statistically significant according to Duncan's multiple range test

TABLE 4. Survey of dieback (%) incidence and severity of Grape fruit cultivars (Red Blush and Star Ruby) grafted on different citrus rootstocks under natural infection conditions.

Grape fruit cultivars	Different rootstocks	No. of Trees	Natural infection (%) of diaback				Mean	
			2000		2001			
			Incidence	Severity	Incidence	Severity	Incidence[x]	Severity %
Red Blush	Citrumelo	27	37.0	14.8	18.5	6.2	27.8 b	10.5
	Rangpur	53	22.6	8.8	58.5	27.0	40.2 c	13.4
	Sour orange	27	0.0[(1)]	0.0	0.0	0.0	0.0 a	0.0
	Volkameriana	27	55.6	25.9	37.0	14.8	46.3 c	20.4
	Mean		27.6	11.7	34.6	17.0	31.1 B	13.4
Star Ruby	Citrumelo	27	7.4	2.5	29.2	16.1	18.5 ab	9.3
	Rangpur	54	1.9	0.6	38.9	14.8	20.4 b	8.6
	Sour orange	26	7.7	2.6	14.2	10.3	14.0 ab	8.0
	Volkameriana	27	3.7	1.2	18.5	9.9	11.1 a	5.6
	Mean		4.5	1.5	29.1	14.4	16.8 A	8.0
Mean			16.1	6.6	31.8	14.7		

1 = No infection

X = Means followed by the capital or small alphabetical letters were not statistically significant according to Duncan's multiple range test

TABLE 5. Survey of dieback (%) incidence and severity of Orange cultivars (Hamlin and Gulette) grafted on different citrus rootstocks under natural infection conditions.

Orange cultivars	Different rootstocks	No. of Trees	Natural infection (%) of diaback				Mean	
			2000		2001			
			Incidence	Severity	Incidence	Severity	Incidence[x]	Severity %
Hamlin	Citrumelo	54	29.6	12.4	13.7	4.6	21.9 a	8.6
	Rangpur	54	11.1	3.7	48.2	17.9	32.0 b	10.8
	Sour orange	26	57.7	25.6	15.4	5.1	36.5 b	15.4
	Mean		27.6	11.4	28.2	10.2	27.9 A	10.8
Gulette	Citrumelo	54	68.5	22.8	50.0	22.2	59.3 b	20.4
	Rangpur	51	23.5	7.8	40.8	14.3	32.0 a	11.0
	Sour orange	27	33.3	11.1	37.0	14.8	35.2 a	13.0
	Mean		43.9	14.7	43.1	16.9	43.5 B	15.8
Mean			35.7	13.0	35.6	13.5		

X = Means followed by the capital or small alphabetical letters were not statistically significant according to Duncan's multiple range test

TABLE 6. Survey of dieback (%) incidence and severity of Lime and Sweet lime cultivars grafted on different citrus rootstocks under natural infection conditions.

Lime cultivars	Different rootstocks	No. of Trees	Natural infection (%) of diaback				Mean	
			2000		2001			
			Incidence	Severity	Incidence	Severity	Incidence	Severity%
Lime (Bears)	Rangpur	27	48.2	18.5	74.1	35.8	61.1	20.4
	Sour orange	77	35.1	14.7	40.1	17.1	37.3	15.9
Sweet lime	-	27	14.8	4.9	14.8	4.9	14.8	4.9
Mean			33.6	13.5	41.5	18.5		

and Volkameriana showed a variant reaction to disease incidence (Prates *et al.*, 1987 and Ochoa *et al.*, 1988). Lemon cultivars, *i.e.* Corona Foothall, Frost, Limoneira, Monroe and Prois grafted on Citrus rootstocks of Carimno, Citrumelo, Rangpur, and Sour orange showed a variant dieback incidence (Table 2). It was clear that the Corona Foothall X Citrumelo; Limoneira X Citrumelo; Limonara X Sour orange, Monroe X Citrumelo; Monroe X Sour orange and Prios X Rangpur showed less infection with dieback disease than others.

Fremont mandarin cultivar showed a slight infection while the severe infection was recorded with Dancy cv. (Table 3). Data also (Table 3) show that the Fremont X Rungpur; Fremont X Volkameriana; Kinnow X Rangpur and Minneola X Sour orange show slight disease incidence ranging from 5.8 to 24.1%. The grapefruit cultivar Red Blush X Sour orange showed no dieback disease symptoms, while Star Ruby X Volkameriana and Star Ruby X Sour orange showed reaction with dieback [incidence 11.1 and 14.0%, respectively (Table 4)]. Also, Hamlin orange X Citrumlo showed a less disease incidence (21.9%) than other one's (Table 5). It is important that the rootstocks Citrumelo, Sour orange, Rangpur and Valkmeriana were the most effective in reducing disease incidence on some Citrus species cultivars (Beretta, *et al.*, 1988, Ochoa *et al.*, 1988, Matheron & Matejka, 1989, Laviola *et al.*, 1990 and Paez & Castano, 1998).

A total number of 8 fungi were obtained during isolation trials from all *Citrus* species are shown in Table 7. Identification studies showed that the isolated fungi belong to 7 genera namely, *Botryodiplodia, Fusarium, Alternaira, Trichoderma, Aspergillus, Pencillium,* and *Derscherella*. Among the isolated fungi, *Botryodipolodia* fungus showed higher frequency than others followed by *Fusarium*. It is obvious that the occurrence and frequency of the aforementioned fungi varied according to *Citrus* species. *Trichoderma* fungus was isolated from grapefruit, orange and lime, where a less dieback symptoms according to natural infection survey.

Pathogenicity tests revealed that the fungus *Botryodiplodia* was pathogenic to different *Citrus* transplants species and cultivars, *i.e.* orange (Hamlin, Gulette and Sour orange) and mandarin (Orlando and Fremont) where a typical dieback symptoms of branches were occurred (Sauarez, 1990).

TABLE 7. Frequency (1) (%) of isolate fungi from citrus twigs showing dieback symptoms.

Isolated fungi	Frequency of isolated fungi (%) in twigs of				
	Lemon	Mandarin	Grape fruit	Orange	Lime
Botryodiplodia sp.	60.0	37.5	37.5	18.2	33.3
Alternaria sp.	20.0	37.5	-	9.1	-
Trichodermia sp.	-	-	25.0	18.2	16.7
Aspergillus niger	-	-	-	18.2	16.7
Aspergillus flavus	-	-	-	-	16.7
Penicillium sp.	-	-	-	9.1	-
Derscherella sp.	-	-	-	9.1	-
Fusarium sp.	20.0	25.0	37.5	18.2	16.6

(1) Four speciments were used for isolation.

The activity of pectolytic and celluloytic enzymes produced by *Botryodiplodia* sp. *in vitro* are shown in Table 8. Data show that *Botryodiplodia* sp. has the ability to produce pectinase and cellulase. The highest reduction in viscosity of PG and Cx enzymes for tested fungus were noticed after 14 days as 20.2 and 61.2 %, respectively. Also pectin methylestrase (PME) activity of

TABLE 8. Pectolytic and cellulolytic enzymes activities of *Botryodiplodia* sp. fungus.

Enzymes	Enzymatic activities in culture age (days)		
	7	14	21
Polygalecturonase (PG)(1)	14.6	20.2	20.1
Pectin-methylestrase (PME)(2)	1.1	2.3	2.1
Cellulose (Cx)(3)	46.8	61.2	60.0

(1) Average percentage of relative loss in viscosity of 1.2% citrus pectin solution (pH, 5.6) after 3h incubation with crude enzyme.

(2) Average milliters of 0.01 N NaOH required to neutralize the carboxylic group produced from 1.5 % citrus pectin solution after 24h incubation with crude enzymes.

(3) Average of relative loss in viscosity of 1.2% CMC solution after 3hr incubation with crude enzyme.

Botryodiplodia sp. was higher after 14 days, where average of millititers of 0.01 N NaOH required to netralize the carboxlic group produced from 1.5% citrus pectin solution was 2.3 ml. It was obvious that *Botryodiplodia* sp. are active producers of PG, PME and Cx enzymes. This result show that the secretion of pectolytic and cellulolytic enzymes may play an important role in pathogenesis.

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الموت الرجعى لأشجار الموالح فى منطقة التحرير

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فى دراسة حصرية لمرض الموت الرجعى تحت ظروف العدوى الطبيعية شملت ٣١٥٠ شجرة موالح من أنواع الجريب فروت والليمون واليوسفى والبرتقال والمطعمومة على أصول موالح مختلفة فى منطقة التحرير أثناء موسمى ٢٠٠٠ و ٢٠٠١ . أتضح أن وبائية هذا المرض تتراوح من ٩ر٣٣ إلى ٨ر٥٨٪ بينما شدة المرض تتراوح من ٧ر١٠ إلى ٢ر٢٨٪ . كانت أشجار الليمون أكثر الأنواع إصابة بينما كانت الجريب فروت الأقل إصابة . عزلت الفطريات البوتريودبوبلوديا والفيوزاريوم والأسبرجللس والديلشليرى والألترنازيا والبنسيليوم والترايكودرما من أفرع الموالح والتى أوضحت أعراض الإصابة بهذا المرض . أوضحت اختبارات القدرة المرضية قدرة الفطر البوتريودبلوديا فقط على إحداث أعراض مرض الموت الرجعى لأنواع الموالح المختبرة مثل البرتقال واليوسفى .