Egypt. J. Phytopathol., Vol. 32, No. 1-2, pp. 119-128 (2004)

Cladode and Fruit Rots of Prickly Pear (*Opuntia ficus-indica* L. Mill.) in Egypt

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Cladode and fruit rots of prickly pear cactus (*Opuntia ficus-indica* L. Mill.) were observed in some commercial orchards located at the major producing areas of four governorates in Egypt. Symptoms initially begin as brown to dark spots on the affected cladode and fruit tissues, rot and discolouration of internal tissues with exudates oozing from the affected tissues. The survey proved that the disease was found in the inspected orchards at various degrees according to the locality. *Alternaria alternata*, *Botryodiplodia theobromae* and *Fusarium solani* were consistently isolated from infected tissues and were pathogenic to both cladodes and fruits of prickly pear. This is the first report of the later two fungi on prickly pear in Egypt. The study on the effect of different fungicides on the growth of the three pathogens revealed that Topsin M70, Bellis and Tecto were the best treatments against the fungi. The same fungicides were used to control cladode rot disease on detached cladodes. Tecto, Bellis and Topsin M70 were the most effective to control the disease.

Key words: Cladode rot, *Opuntia ficus-indica* and prickly pear cactus.

Prickly pear (*Opuntia ficus-indica* (L.) Mill) is an important crop in arid and semiarid regions of the world (Swart *et al.*, 2003). It is usually grown in sandy soil of Egypt for many decades. (Abo-El-Ela *et al.*, 2001). Prickly pear fruits are harvested from various species of the prickly pear cactus, Genus *Opuntia* of the cactus Family (Cactaceae). Fruits are also called cactus pears or cactus fruits, although these names can result in confusion with fruits from other Cactus species. Native Cactus species are tolerant to desert conditions and trouble-frees and they are a native of regions with very hot and dry climates (Swart *et al.*, 2003). However, there are numerous fungal diseases that can cause a lot of problems on prickly pear during its different growth stages and can badly affect fruit yield. Among the most important diseases (fungal and bacterial as well as other agents) are cactus anthracnose, charcoal spot, dry rot, foot rot, scorch or sunscald, soft rot, root rot and stem rot are of great economic importance (Raabe and Alcon, 1968; Caciola and Magnano, 1988; Varvaro *et al.*, 1993; Carballo *et al.*, 2000; Granata and Sidoti, 1997 and 2000and Swart and Kriel, 2002).

Cladode and fruit rots are claimed to be caused by different agents (Raabe and Alcon, 1968).In the recent years, plants in some orchards of the major producing areas have been suffered from infection with this disease. Until the commencement of the present work *Alternaria alternata* was the only causal pathogen recoded in Egypt (Abo-El-Ela *et al.*, 2001). Although, in other countries, different investigators attributed the disease to different pathogens (Raabe and Alcon, 1968; Granata and Sidoti., 1997and Swart *et al.*, 2003).

There is always infection by rot on prickly pear throughout the cultivated areas. Harvest damage to the peel and stem-end of cactus fruit may lead to attack by numerous microorganisms and result in fruit decay. The disease is not of epidemic nature in the country. However, if the infection of cladodes with rot occurred in landscape, it can threaten valued plants. The objectives of this study were to isolate and identify the causal agents and their pathogenic capabilities on prickly pear and to find out a promising method for their control.

Materials and Methods

Isolation and identification:

Diseased samples including cladodes (stems) and fruits were collected from prickly pear trees showing the symptoms of rot from some plantations of five governorates, *i.e.* Giza (Imbaba), Menofiya (Khatatba), Ismailia (Kasassin), Sharkiya (Abo-Hammad) and Beheira (Nobaria). In all inspected areas, the visible symptoms were described as possible and percentage of affected trees were calculated. Tissue pieces were cut with a sterile scalpel from the advancing margins of the infected portions of the cladodes and fruits, sterilized superficially with sodium hypochlorite (1%), washed in sterilized water and plated onto ready potato-dextrose agar (PDA) plates. Plates were incubated at 25°C for 5 days. The emerged fungi were picked-off and purified. The isolated fungi were identified using the descriptions of Nelson *et al.* (1983) and Barnett and Hunter (1986).Identification of the isolated fungi was confirmed at the Mycol. and Dis. Survey Res. Dept., Agric. Res. Centre, Giza.

Pathogenicity tests:

The isolated fungi were used for pathogenicity tests. Four healthy-looking cladodes and fruits were washed and surface sterilized with ethanol 70%. A 5 mm. plug taken from the margin of each fungal actively growing on 7-day-old culture, was inserted into a hole (5-mm-diam.) made by a sterilized cork borer in the desired plant organ, then covered with the bark disk. Proper check treatments were inoculated with PDA only. The inoculated plant materials were kept in plastic moist containers with moistened cotton to maintain high humidity. Disease incidence was determined 30 and 7 days after inoculation for cladodes and fruits, respectively, by measuring the diameter of infected areas (Caciola and Magnano; 1988, Abo-El-Ela *et al.*, 2001 and Swart *et al.*, 2003).

Effect of different fungicides on fungal growth and disease control:

Seven different concentrations, *i.e.* 10, 50, 100, 200, 300, 400 and 500ppm of each fungicide (Bellis, Kocide 101, Sumisclex, Tecto and Topsin M70) were prepared in specified volume of autoclaved PDA medium in 250ml flasks. The prepared media were then poured into 9-cm Petri dishes. Plates containing only PDA medium were used as check. The plates were inoculated with one disk (5-mm-diameter) of 7-day-old culture of the desired fungus colony and incubated at 25°C for 7 days. Three replicates were used for each treatment. The linear growth was measured and the percentage of reduction in growth was estimated according to the formula described by Abo-El-Ela *et al.* (2001).

Chemical control of cladodes rot of prickly pear was carried out under laboratory conditions. Healthy cladodes were firstly inoculated with the desired pathogenic fungus in separate treatment using the same way described before. After 21 days, disease incidence in the inoculated cladodes, exhibited various degrees of rot symptoms, was determined by measuring the diameter of infected areas (Abo-El-Ela *et al.*, 2001). Each treatment was sprayed separately with the recommended dose of the desired fungicide. Check treatment was sprayed with distilled water. Three replicated cladodes were used for each fungicidal treatment. Disease incidence and decrease in disease incidence (Pv) were calculated 14 days after spraying according to the following equation suggested by Munkvold and Marios (1993):

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

whereas, **Pv**= decrease (%) in disease incidence,
Ic= Proportion of disease incidence in check treatment and
Iv= Proportion of disease incidence in each treatment.

Proportion of disease incidence was calculated as differences between before and after fungicidal treatment in each treatment. Obtained results were statistically analyzed, whenever needed, using factorial experiment design suggested by Snedecor and Cochran (1982).

Results

1. Symptoms on naturally infected trees:

Diseased plants showing different types of natural symptoms were collected from inspected governorates development according to the pathogen. *Alternaria alternata* and *Fusarium solani* caused top rot of cladodes. The infection initially begins on the top of cladodes on the surface of one side of cladode as brown to dark brown spots on the affected tissues. Spots at first are distinctly zonated, later enlarge slowly until it comprises both sides of cladodes which completely become rotted (Fig. 1) and finally cracked or die (Fig. 1a). Sometimes profuse gummy, amber-coloured exudate is oozed from the lesion surface (Fig. 1b).The infection also appears on the base of cladodes as light brown rot with dry amber-coloured exudates oozed from the rotted areas (Fig. 1c). When cladodes or fruits are infected, they entirely become rotted and dried, then take a shrunken mummy shape.

2. Survey, isolation and identification:

Disease incidence, in different localities representing the five governorates, shows that the disease is widespread. The percentages of natural infection greatly varied according to the governorate, thus the infection in Giza reached 30.0%. While in Sharkiya was 7.0% (Table 1). Four different fungi, *i.e. Alternaria alternata, Aspergillus niger, Botryodiplodia theobromae* and *Fusarium solani* were isolated from diseased samples of prickly pear collected from different localities. The frequency of the isolated fungi varied according to the governorate (Table 1). In general, the frequency in the different localities ranged from 4.0 to 16.0%. *Botryodiplodia theobromae* and *A. niger* represented the highest frequency ranging from 14.0 to 16.0% and 4.0 to 16.0%, respectively. While, *A. alternata* was less frequent as it ranged from 4.0 to 6.0%.

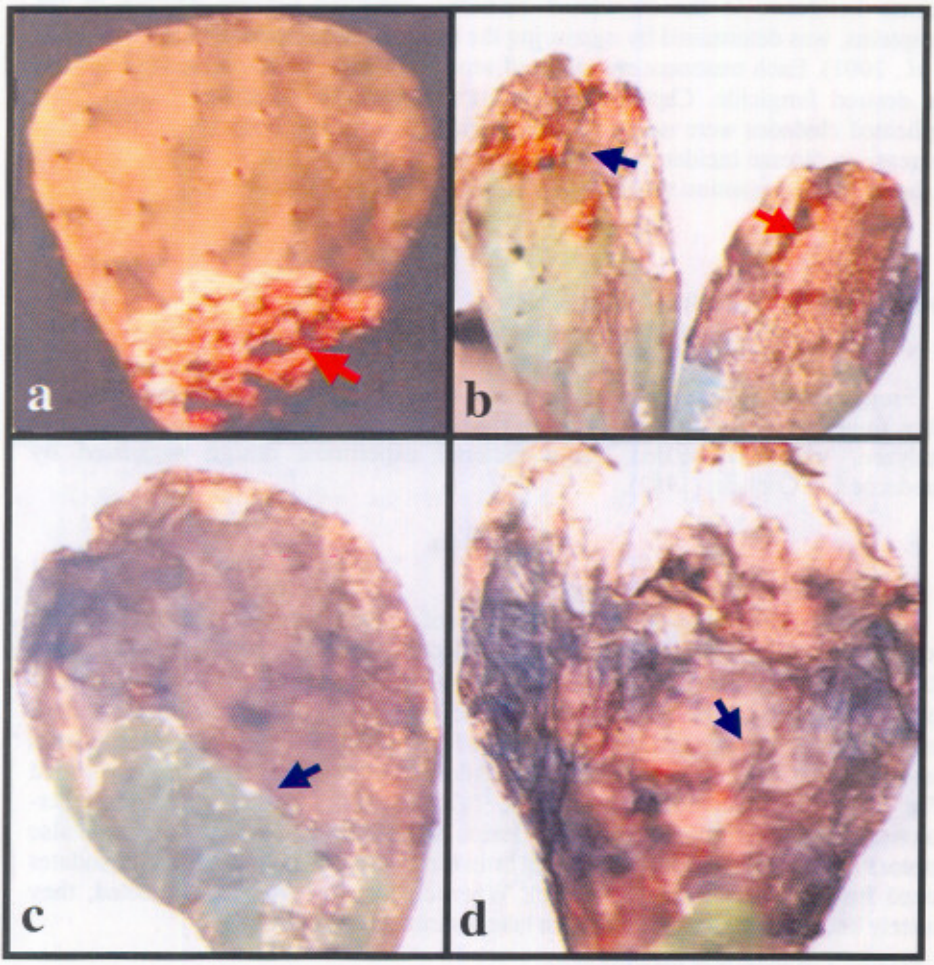

Fig. 1. The most characteristic symptoms on cladodes of prickly pear cactus under natural infection conditions. The basal dry rot of cladodes, showing the amber-coloured exudates (a). Top rot of cladodes with gummy ooze on the rotted surface, the rotted cladodes show a shrunken mummy shape (b, c and d).

Table 1. Occurrence and frequency of fungi associated with rotted cladodes and fruits of prickly pear collected from different governorates during 2003 grown season

Governorate	Locality	Plant organ	Isolated fungi	Frequency (%)	Infection (%)
Giza	Imbaba	Cladodes & Fruits	*A. alternata*	4.0	30.0
			A. niger	12.0	
		Cladodes	*F. solani*	8.0	
Menofiya	Khatatba	Cladodes	*F. solani*	12.0	18.2
			A. niger	4.0	
Ismailia	Kasassin	Cladodes& Fruits	*B. theobromae*	16.0	13.0
			Alternaria sp.	4.0	
Sharkiya	Abo-Hammad	Cladodes& Fruits	*B. theobromae*	14.0	7.0
			Alternaria sp.	6.0	
Beheira	Nobaria	Cladodes	*Alternaria* sp.	4.0	15.0
			A. niger	16.0	

3. Pathogenicity tests:

In artificial inoculation of detached cladodes and fruits in the laboratory, *A. alternata*, *B. theobromae* and *F. solani* were able to induce disease symptoms identical to those observed in nature in the commercial orchards, except the formation of gummy exudates from lesions surface. Symptoms became visible in most inoculation sites on cladodes, 21-30 days after inoculation while in fruits were after 7 days. The other tested fungi and the check treatments showed very little necrosis on either cladodes or on fruits (Table 2).

Virulence of the three fungal pathogens was differed from each other. Generally, *B. theobromae* was the most virulent on either detached cladodes or fruits being 7.0 and 4.5cm, respectively, followed by *A. alternata* which recorded 4.9 cm on cladodes. *F. solani* caused smaller lesions in diameter on the detached cladodes (being 2.5 cm) meanwhile, it was more virulent in fruit inoculations than that caused by *A. alternata*.

4. Effect of different fungicides on fungal growth and disease control:

Variation was found in growth according to the fungicide used and the tested fungus. The results illustrated in Table (3) indicate that Topsin M 70 completely inhibited the growth of *B. theobromae* (100% Toxicity) at all concentrations used and at 50 and 500 ppm for *F. solani* and *A. alternata*, respectively. Growth of *A. alternata* was inhibited at 300 ppm of Bellis and Sumisclex. In particular, *B. theobromae* was more sensitive to both Tecto and Bellis at 300 and 400 ppm, respectively. On the other hand, Kocide 101 and Sumisclex at all concentrations used showed no visible effect. Generally, toxicity was more evident with the increase in the concentration of the tested fungicides. Growth of *F. solani* was completely inhibited using Tecto at 400 ppm and Sumisclex at 500 ppm.

Table 2. Pathogenicity of the isolated fungi on detached cladodes and fruits of prickly pear plants

Fungus	Average diameter (cm) of rotted areas on		Mean
	Cladodes	Fruits	
A. alternata	4.9	1.75	3.33
B. theobromae	7.0	4.5	5.75
F. solani	2.5	2.2	2.35
A. niger	0.0	0.0	0.0
Check	0.0	0.0	0.0
Mean	2.88	1.69	--
L.S.D. at 5% for: Fungi (F) = 0.43 Plant organs (P)= 0.28 F X P = 0.27			

Table 3. Effect of different concentrations of five fungicides on the growth of the pathogenic fungi

Fungicide	Fungus	Toxicity (%)* in different concentrations (ppm)							Mean
		10	50	100	200	300	400	500	
Kocide 101	*A. alternata*	6.7	66.7	71.1	74.4	80.0	81.1	100	68.7
	B. theobromae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	F. solani	0.0	28.9	46.7	60.0	60.0	74.4	77.9	49.7
Mean		2.2	31.9	39.3	44.8	46.7	51.9	59.3	39.5
Bellis	*A. alternata*	62.2	64.4	76.7	85.6	100	100	100	84.1
	B. theobromae	44.4	70.0	75.6	81.1	83.3	100	100	79.2
	F. solani	18.9	26.7	33.3	35.6	45.6	62.2	83.3	43.7
Mean		41.5	53.7	61.9	67.4	76.3	87.4	94.4	69.0
Sumisclex	*A. alternata*	70.0	83.3	87.8	87.8	100	100	100	89.8
	B. theobromae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	F. solani	0.0	37.8	46.7	53.3	56.7	66.7	100	51.6
Mean		23.3	40.4	44.3	47.1	52.2	55.6	66.7	47.1
Tecto	*A. alternata*	0.0	0.0	18.9	33.3	42.2	62.2	83.3	34.3
	B. theobromae	0.0	15.6	42.2	72.2	100	100	100	61.4
	F. solani	0.0	26.7	28.9	37.8	42.2	100	100	47.9
Mean		0.0	14.1	30.0	47.8	61.5	87.4	94.4	47.9
Topsin M70	*A. alternata*	0.0	22.2	41.1	44.4	53.3	83.3	100	49.2
	B. theobromae	100	100	100	100	100	100	100	100
	F. solani	62.2	100	100	100	100	100	100	94.6
Mean		54.1	74.1	80.4	81.5	84.4	94.4	100	81.3
L.S.D. at 5% for: Fungicides= 0.87; Fungi= 0.61; Concentration= 0.94; Fungicides x Conc.= 2.31; Conc. x Fungi= 1.64; Fungicides x fungi x Conc.= 4.01.									

* Toxicity (%)= Reduction (%) in mycelial growth.

The same fungicides were used to control cladodes rots of prickly pear. In chronological order, the fungicides Tecto, Bellis and Topsin M 70 were the best in detached cladodes trials (Table 4). The highest decrease percentage in disease incidence was obtained in case of using any of the above mentioned three fungicides and ranged from 94.44 to 100.0%, 35.29 to 100.0% and 100.0 in case of *A. alternata*, *B. theobromae* and *F. solani*, respectively. Whereas, Kocide 101 recorded the highest decrease in infection in case of *F. solani* and *B. theobromae* (100.0% and 88.89%, respectively) and gave intermediate effects against *A. alternata* (being 41.18%). In contrast, Sumisclex was the least effective treatment against the rot caused by *B. theobromae* and *A. alternata*, but it gave 100.0% decrease in disease incidence in case of *F. solani*.

Table 4. Chemical control of cladodes rot of prickly pear on detached cladodes using different fungicides

Fungicide	Fungus	Disease incidence (%) *		Decrease (%) in disease incidence
		Before fungicidal treatment	14 days after fungicidal treatment	
Kocide 101	*A. alternata*	1.0	2.0	41.18
	B. theobromae	1.0	1.4	88.89
	F. solani	0.9	0.9	100.0
Bellis	*A. alternata*	0.9	0.9	100.0
	B. theobromae	0.85	0.85	100.0
	F. solani	1.0	1.75	37.50
Sumisclex	*A. alternata*	1.05	2.5	14.71
	B. theobromae	0.75	2.25	11.77
	F. solani	0.8	0.8	100.0
Tecto	*A. alternata*	1.0	1.0	100.0
	B. theobromae	0.8	1.0	94.44
	F. solani	0.9	0.9	100.0
Topsin M70	*A. alternata*	0.65	1.25	35.29
	B. theobromae	0.65	0.65	100.0
	F. solani	0.85	0.85	100.0
Check	*A. alternata*	1.3	3.0	--
	B. theobromae	0.9	4.5	--
	F. solani	1.0	2.2	--
L.S.D. at 5% for: Fungicides= 0.30; Fungi= 0.22; Time of treatment= 0.19; Fungicides x fungi= 0.49; Fungicides x Time= 0.40; Fungi x Time= 0.31; Fungicides x fungi x Time= 0.70.				

* Diameter (cm) of infected areas.

Discussion

Prickly pear trees are subjected to infection by different diseases in the cultivated regions of the world (Raabe and Alcon, 1968; Granata and Sidoti., 1997and Swart *et al.*, 2003). In Egypt, little is known about prickly pear diseases and their distribution (Abo-El-Ela *et al.*, 2001).The need for research on the diseases of *O. ficus-indica* in Egypt has become very important because the growers claimed that the diseases caused great losses. Cladode and fruit rots are of the most important diseases. The observed symptoms during the disease survey were the same as mentioned by other workers (Varvaro *et al.*, 1993; Granata and Sidoti, 1997 & 2000; Abo-El-Ela *et al.*, 2001and Swart and Kriel, 2002). Field survey revealed that, the disease was found in all inspected governorates but percentage of disease incidence varied from one location to another. Many fungi were isolated with differences in frequency. Differences in disease incidence and fungi isolated from different governorates may probably be due to varietal differentiation and/or variation in climatic conditions (Turner, 1981). Pathogenicity tests indicated that *B. theobromae, A. alternata* and *F. solani* were pathogenic to both cladodes and fruits of prickly pear reproducing typical symptoms of the disease. The former two fungi were the most aggressive. These results are in harmony with those obtained by many workers (Varvaro *et al.*, 1993; Granata and Sidoti, 1997 and 2000and Swart and Kriel, 2002). As far as the authors are aware, only one fungal pathogen, *i.e. A. alternata,* was reported on *O. ficus-indica* in Egypt (Abo-El-Ela *et al.*, 2001). According to the available literature, this is the first report of *B. theobromae* and *F. solani* on prickly pear in Egypt. In other countries, different investigators attributed the disease to different pathogens. Swart and Kriel (2002), in South Africa, concluded that *Alternaria tenuissima, Fusarium sporotrichoides* and *Lasiodiplodia theobromae* were associated with necrosis of cactus pear cladodes. Also, similar symptoms were found on both cladodes and fruits caused by *Phialocephala virens, Lasiodiplodia theobromae* and *Fusarium* spp. (Swart *et al.*, 2003). The study on the effect of different fungicides on the growth revealed that there were highly significant differences among the tested fungicides and reaction of each pathogen was also different from a fungicide to another at a particular concentration. The variations obtained among the different fungicides could be attributed to one or more of the following factors :a) degree of permeability of cell wall and/or plasmalemma of fungi for uptake and passage of the fungicide into the fungal cell. (Giffin, 1981 and Warrd and Ragsdale, 1977). b) Mode or degree of the resistant action of the fungal cell to a specific fungicide (Watkins *et al.*, 1977). c) chemical composition of fungicides (Carnegie *et al.*, 1990). These results are in line with those obtained by Abo-El-Ela *et al.* (2001). Application of the same fungicides for control of cladodes rot of prickly pear revealed that Tecto, Bellis and Topsin M 70 were the best fungicides for decreasing disease incidence. Successful efficacy of using fungicides against fungal spot disease on cacti was recommended by Chase(1992) and Abo-El-Ela *et al.* (2001).They found that the best control is to avoid wounds, remove and destroy diseased specimens and treat broken surfaces right away with a copper fungicide.

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(Received 28/06/2004;
in revised form 02/09/2004)

عفن الـــواح وثمـــار التين الشـــوكـــى فـــى مصـــر

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معهد بحـوث أمراض النباتات- مـــركز البحوث الزراعية-الجيزة- مصـــر.

لوحظت أعراض الإصابة بعفن ألواح وثمار التين الشوكى فـــى بعض المزارع بالمناطق الرئيسية للإنتاج بأربع محافظات فـــى مصـــر. تظهر الأعراض الأولية على هيئة بقع بنية إلى داكنة اللون على الألواح والثمار المصابة مع حدوث تعفن وتحلل للأنسجة الداخلية وظهور إفراز صمغى من السيقان المصابة. أثبتت دراسات الحصر المرضى أن المرض موجود بدرجات متفاوتة تبعاً للمنطقة. تم عزل العديد من الفطريات والتى من أهمها الترناريا الترناتا وبوتريوديبلوديا ثيوبرومى وفيوزاريم سولانىمن الأنسجة المصابة وقد كانت قادرة على إحداث الأصابة لكل من الواح وثمار التين الشوكى. يعتبر هذا أول تسجيل للفطرين الأخيرين على التين الشوكى فى مصـــر. بدراسة تأثير المبيدات المختلفة على نمو المسببات المرضية الثلاثة وجد أن مبيدات التوبسن م ٧٠ والبليبز والتكتو كانت أفضل المبيدات تأثيراً على النمو وكانت الأكثر فعالية فـــى مقاومة الأصابة عند إستخدامها فى مقاومة عفن الألواح.