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Rose Stem Canker Caused by *Coniothyrium fuckelii* Sacc. in Egypt

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One hundred and fifteen fungal isolates representing nine fungal species were isolated from six rose cultivars grown in both outdoor and greenhouses at Giza and Qalubiya governorates during April, 2009. The occurrence of each fungus varied from cultivar to another. Isolation trials showed that *Coniothyrium fuckelii* and *Alternaria alternata* were the most dominant ones, followed by *Nigrospora* sp. Meanwhile, *Cercospora* sp. showed the lowest frequency.

Pathogenicity test showed that *C. fuckelii* was the only pathogenic fungus and was able to cause the rose stem canker. Symptoms are usually begun as small yellow to red spots on the bark and gradually expand. The centres of the cankers become light brown and the margin a darker brown. Cankers may girdle the stem causing wilting and death of the plant parts above the canker. Small pycnidia are sometimes produced on the canker.

The causal fungus was identified as *C. fuckelii* Sacc., the anamorph of *Leptosphaeria coniothyrium* (Fuckel) Sacc., according to its morphological features and pathogenic potentially. As the authors are far aware, rose stem canker and *C. fuckelii*, the causal fungus are described herein for the first time in A.R. Egypt.

Reaction of five rose cultivars was tested against four isolates of *C. fuckelii*. All the tested isolates were pathogenic to cvs. African Dawn, First Red, Mercedes, Sentrix and Vercellia Rose. No correlation was found between virulence of isolates and their origin. Cv. Vercellia Rose was the highly susceptible, while Sentrix was the least susceptible one.

The reaction of nine plant species belonging to different families to the four tested isolates revealed that *C. fuckelii* isolates failed to infect any plant species tested, except for *Syngonium podophyllum* which showed slight infection with isolate No. 1 and isolate No. 4 isolated from cvs. African Dawn and Vercellia Rose, respectively.

Differentiation among the four tested isolates of *C. fuckelii* was assessed by the use of Random Amplified Polymorphism DNA technique (RAPD), which showed high similarity levels (85.56%) with primer OPC5 as compared to (76.73%) with primer OPG 14. The two primers showed obvious variation in isolate No. 1 isolated from cv. African Dawn, where the results of genetic analysis showed two DNA bands with primer OPC5 and three bands with primer OPG14 compared to eight bands in rest isolates.

Keywords: *Coniothyrium fuckelii*, Electron microscope, host range, RAPD-PCR, rose stem canker and rose varieties.

Rose (*Rosa* spp.) has a very wide geographical distribution and is a popular ornamental plant. It was grown long before history was written and roses are indeed among the most ancient ornamentals, especially in the orient where they were grown for their beauty and production of rose oil. Some of the wild species also supply us with food, cosmetics and even medicine.

Rose, like most other plants, is subjected to a number of diseases, which may impair the beauty of the flowers or may even kill the plants. Stem canker disease caused by *Coniothyrium* spp. is among the most common and destructive diseases, especially on the canes, where infection may approach 100 percent if no control is practiced (Pataky, 1990).

Rose stem canker caused by *C. fuckelii* was first reported by Saccardo (1884) and later shown to be related to the stem canker of apple by O'Gara (1911). As described by Alfieri (1969); Horst (1983) and Pataky (1990) rose stem canker is probably widespread in occurrence in Europe and the United States on both outdoor and glasshouse-grown roses. The same authors gave good accounts about the characteristic symptoms of the disease and the causal organism.

During the last few years, rose growers in most areas, where rose is grown in Egypt, claimed that high numbers of their plants are frequently died as a result of infection by a disease begins on the stem and extends to the branches causing serious economic losses and has been a major problem for field grown roses, roses grown in gardens and greenhouses elsewhere. The occurrence of rose canker disease in Egypt has not been reported, nor has the importance of the causal fungi been well documented (Melchers, 1931 and Ali *et al.*, 1972). Accordingly, the present work was carried out to throw some light on the disease with respect to its prevalence. Moreover, the work was expanded to identify and explain the behaviour of the causal pathogen(s). Also, pathogenic capabilities of some *C. fuckelii* isolates, their host range, morphological features and molecular variations were carefully studied.

Materials and Methods

Isolation, purification and identification of the associated fungi:

Samples of naturally infected rose plants representing six cultivars, *i.e.* African Dawn, First Red, Mercedes, Galica, Sentrix and Vercellia, showing the typical symptoms of stem canker disease were collected from several greenhouses, nurseries and gardens located at Giza and Qalubiya governorates during 2009. Diseased plant samples were cut, placed in plastic bags and kept in a cool container during transportation. Plant samples were kept in a refrigerator at 5°C for further studies.

For isolation, infected stems were washed thoroughly with tap water and cut into small fragments (0.5-1.0 cm), superficially sterilized with 0.1 % sodium hypochlorite for 3 min. Then, the fragments were rinsed several times in sterilized water, blotted to dry between folds of sterilized filter paper. Small pieces were transferred onto PDA plates and incubated at 26±2°C for 7 days. Observations were daily carried out and any emerged fungus was picked up and cultured on PDA slants, and the frequency of the obtained fungi was calculated.

The fungal growth was examined microscopically and purified using the single spore and/or the hyphal tip techniques (Dhingra and Sinclair, 1985).

The purified fungi were identified according to their morphological features, either to the generic or to the species level using the descriptions of Booth and Waterston (1964) and Barnett and Hunter (1972). Identification of the isolated fungi was carried out at the Plant Pathology Dept., Fac. of Agric. Cairo Univ. Moreover, identification of *Coniothyrium fuckelii* Sacc. was confirmed by the Mycol. Centre, Assiut Univ. and also at the Mycol. and Dis. Survey Dept., Plant Pathol. Res. Instit., ARC., Giza, Egypt.

Stock cultures were maintained on PDA slants under paraffin oil in a refrigerator at 5-10 °C and were sub-cultured onto fresh medium every 6-8 weeks.

Pathogenicity tests:

Pathogenicity tests were conducted on First Red Rose cultivar for the nine isolated fungi namely, *Alternaria alternata, Botrytis cinerea*, *Cercospora* sp., *Chaetomium* sp., *Coniothyrium fuckelii*, *Dicoccum asperum*, *Fusarium semitectum*, *Nigrospora* sp. and *Trichothecium roseum* in order to investigate their pathogenic capabilities. All the experiments were carried out in pots kept in the greenhouse of the Plant Pathol. Dept., Fac. Agric., Cairo Univ.

Sowing medium (clay soil) as well as the pots used in the greenhouse experiments were treated with 5% formalin solution. The soil was covered with plastic sheets for 3 days to retain the gas. The soil was not planted until the odor of formaldehyde had disappeared.

Inoculum preparation:

The inoculum used in the foregoing studies consisted of uniform discs of 4 mm. in diameter fungal growth of any of the tested fungi grown on PDA medium for 15 days at 25°C.

Artificial inoculation technique:

Inoculation of rose stems and branches was carried out according to the method described by Siriphong (1967). Three months old plants growing in No. 25 pots were used for these experiments. For each rose cultivar, three transplants were transplanted in each pot and each pot was replicated 7 times. The stem was wounded with a sterilized cutter, three wounds (4x4 mm.). A disc of PDA medium (4mm.) bearing the fungal growth was placed on the wound with the mycelium toward the stem. When pressed lightly against the stem, the agar held the inoculum firmly in place. As a rule, inoculation was made on the lower part of the stems about two inches above the crown. Control plants were divided into two groups, the first one was wounded and treated with only agar disc without inoculum and the second group was unwounded and similarly treated but with agar disc with inoculum.

The inoculated transplants were covered with plastic bags for 24 hours to provide high level of humidity. Observations were daily carried out and percentage of infection was counted twenty-one days after inoculation.

Description of C. fuckelii using light and electron microscope:

Procedures described by Johansen (1940) were followed to prepare microtome sections through pycnidia of *C. fuckelii*. Discs measuring 4 mm. in diameter taken from the edges of 15-day-old PDA culture were killed and fixed in F.A.A for 48 hr., the vials containing samples were subjected to mild vacuum for 15 min. then dehydrated using N-butyl alcohol. Samples were embedded in paraffin wax of 56° C. melting point. Sections 10 µ were cut using a rotary microtome. Preparations were stained by Safranin-light green. Canada balsam was used as a mounting medium. Sections were examined by light microscope model M-200M.

For electron microscope, discs measuring 4 mm. in diameter taken from the edges of 15-day-old PDA culture were transferred in a separate vial to be fixed in 2.5% Glutaraldehyde with 0.1 M sodium phosphate buffer (pH 7.2) and rotate overnight. After removing the fixative solution, the samples were washed in sodium phosphate buffer three times for 30 min each. After washing, the buffer was pulled out and 1% of osmium-tetroxide (OsO4) was added to the tube and allowed to sit overnight at 4° C. After removing the fixative solution the samples were dehydrated in an ethanol series of 15, 30, 50, 70, 80 and 95%, before exposing to 100% for 15 minutes for every step. The last step was repeated twice according to the methodology described by Timothy and Kristen (2000) and Rocchetta *et al.* (2007). Infiltrate with Spur's epoxy resin, one large drop into the sample tube every 15 minutes, until at 75% resin and allow to rotate overnight. Samples were put into 100% resin, rotate for at least a day, then placed into flat or BEEM capsule moulds, before hardening the resin overnight in an oven at 60°C. Resin blocks were removed from oven, then sectioned (90 µm thick) with ultra-microtome (Leica model EM-UC6, Japan), sections were stained with uranyl acetate and lead citrate, then examined by camera Lica ICC50 HD. Samples were then mounted on copper grids (400 mesh) and examined by transmission electron microscope JEOL (JEM-1400 TEM, Japan) at the candidate magnification. Images were captured using CCD camera model AMT, optronics camera with 1632x1632 pixel format as side mount configuration.

Host range:

Nine plant species belonging to different families, *i.e. Rosa fountain, Rosa huddly, Malus domestica, Prunus persica, Fragaria* sp., *Morus nigra*, *Syngonium podophyllum*, *Dieffenbachia amoena* and *Schefflera actinophylla* were inoculated by the four *C. fuckelii* isolates. The experiment was conducted in sterilized pots No. 25 filled with sterilized clay soil during November, 2010. One year old transplants were allowed to grow for three months before inoculation. Three new vigorous shoots of each plant host were selected to conduct the inoculation trials and repeated 7 times. The general procedures of inoculation were the same as described under the Pathogenicity test, except for *Fragaria* sp. where plants were wounded and sprayed with the spore suspension ($30x10^{5}$ spore/ml) of any of the tested isolate. Tween 20 (ca 0.02% v/v) was added as a wetting agent. Immediately after inoculation, plants were kept at high humidity in polyethylene bags for 24 hours. Control plants were similarly treated with only distilled water. Five plants were used for each treatment. Disease reactions were determined 21 days after inoculation as follows: no infection (-), slight infection (+), moderate infection (++) and severe infection (+++).

Varietal resistance:

Five rose cultivars, *i.e.* African Dawn, First Red, Mercedes, Sentrix and Vercellia Rose, were used to evaluate their reaction to the four tested *C. fuckelii* isolates under greenhouse conditions. The aforementioned rose cultivars were obtained from Mahmoud Helmey Farm at Kirdasa, Giza, Egypt. Plants were inoculated by the tested fungi as mentioned under Pathogenicity test.

Random Amplified Polymorphism DNA technique (RAPD):

DNA extraction: Genomic DNA was isolated from each of the four *C. fuckelii* isolates using the CTAB method described in Rogers and Bendich (1985).

PCR analysis:

PCR reactions were performed in a total volume of 20 µl containing 10 ng DNA, 200µM dNTPs, 1 µM of five arbitrary 10-mer primers (Operon Technology, Inc., Alameda, CA, USA), 0.5 units of Red Hot Taq polymerase (AB gene House, UK) and 10-X Taq polymerase buffer (AB gene House, UK). For DNA amplification Biometra thermal cycler (2720) was programmed as follow: 94° C for 5 min. followed by 35 cycles 94° for 1 min., 35° C for 1 min., 72° C for 1 min. and 72° C for 7 min.

The amplification products were analyzed by electrophoresis in 1% agarose in TAE buffer, stained by ethidium bromide and photographed under UV light. The sequence of the tested primers was as follows:

Primers name	Sequence
OPB 5	5/ TGCGCCCTTC 3 /
OPG 14	5/ GGATGAGACC 3/

Gel analysis: The RAPD gel images were scanned using the Gel Doc 2000 Bio-Rad system and analyzed with Quantity One Software v 4.0.1 (Bio-Rad Laboratories, Hercules, Co. USA). The bands were sized and then binary coded by 1 or 0 for their presence or absence in each isolate. The systa (Ver. 7) computer program was used to calculate the pair wise differences matrix the dendogram among fungal isolates. Cluster analysis was based on similarity matrices obtained with the unweighted pair-group method (UPGMA) using the arithmetic average to estimate the dendogram.

Statistical analysis:

Most of the data were statistically evaluated according to Snedecor and Cochran (1967). Averages were compared at 5% level of probability using least significance differences (L.S.D) as mentioned by Fisher (1948).

Results

Isolation, purification and identification of the associated fungi:

Data in Table (1) indicate that 115 fungal isolates representing nine fungal species were isolated from the diseased stems of the investigated rose cultivars. According to their morphological features, the isolated fungi were identified as *Alternaria alternata* (Fr.) Keissler, *Botrytis cinerea* Person ex Fries, *Cercospora* sp., *Chaetomium* sp., *Coniothyrium fuckelii* Sacc., *Dicoccum asperum* (Corda) Sacc., *Fusarium semitectum* Berkeley et Ravenel, *Nigrospora* sp. and *Trichothecium roseum* Link ex Fries.

Coniothyrium fuckelii showed the highest number of isolates and mean of frequency was 33.9%, followed by *A. alternata*, being 24.4% on the average. *Nigrospora* sp. ranked third. Fungi belonging to other genera were isolated with low frequency (Table 1).

However, the occurrence and frequency of each of the isolated fungi varied from one rose cultivar to another (Table 1). Isolation trials from cv. African Dawn yielded 4 fungal species, *i.e. A. alternata, Chaetomium* sp., *C. fuckelii* and *T. roseum* and exhibited the lowest number of fungal isolates, being 17 isolates. Meanwhile, only 3 fungal species, *i.e. A. alternata*, *C. fuckelii* and *T. roseum,* were isolated from cv. Vercellia. The highest numbers of fungal species (7) were isolated from each of cvs. Mercedes and Sentrix followed by cv. First Red, being 5 species.

Table 1. Frequency (%) of fungi isolated from six rose cultivars grown in nurseries and greenhouses at Giza and Qalubiya governorates during 2009

Fungus	Frequency (%)												Mean
	African Dawn		First Red		Mercedes		Galica		Sentrix		Vercellia		
	No*	(%)**	No	%	No	%	No	%	No	%	No	%	
A. alternata	05.0	29.4	02.0	10.0	04.0	22.2	09.0	45.0	6.0	30.0	02.0	10.0	24.4
B. cinerea	00.0	00.0	04.0	20.0	01.0	05.6	00.0	00.0	02.0	10.0	00.0	00.0	05.9
Cercospora sp.	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	02.0	10.0	00.0	00.0	01.7
Chaetomium sp.	02.0	11.8	00.0	00.0	03.0	16.7	03.0	15.0	00.0	00.0	00.0	00.0	07.3
C. fuckelii.	07.0	41.2	11.0	55.0	04.0	22.2	00.0	00.0	00.0	00.0	17.0	85.0	33.9
D. asperum	00.0	00.0	00.0	00.0	1.0	5.6	03.0	15.0	01.0	05.0	00.0	00.0	04.3
F. semitectum	00.0	00.0	02.0	10.0	3.0	16.7	00.0	00.0	03.0	15.0	00.0	00.0	07.0
Nigrospora sp.	00.0	00.0	00.0	00.0	02.0	11.1	05.0	25.0	04.0	20.0	00.0	00.0	09.4
T. roseum	03.0	17.6	01.0	05.0	00.0	00.0	00.0	00.0	02.0	10.0	01.0	05.0	06.2
Total counts of fungal isolates	17.0	_	20.0	_	18.0	_	20.0	_	20.0	_	20.0	_	__

* Number of fungal colonies. ** Frequency (%) of fungi.

Alternaria alternata was isolated from all the six rose cultivars. Meanwhile, *C. fuckelii* was isolated from 4 rose cvs, but not from cvs. Galica and Sentrix. *Cercospora* sp. was isolated only from cv. Sentrix. Each of the other isolated fungal species was isolated from 3 rose cultivars (Table 1).

Pathogenicity tests:

Data presented in Table (2) cleared that the all tested fungi *i.e. Alternaria alternata, Botrytis cinerea, Cercospora* sp., *Chaetomium* sp., *Coniothyrium fuckelii* , *Dicoccum asperum, Fusarium semitectum, Nigrospora* sp. and *Trichothecium roseum* were not pathogenic to the tested rose cultivars, except the *C. fuckelii* isolate which caused the typical symptoms of the rose stem canker disease and lead to severe infection.

Table 2. Pathogenicity tests of the isolated fungi on cv. First Red Rose 21 days after inoculation under greenhouse conditions

Tested fungus	Infection *
Alternaria alternata	-
Botrytis cinerea	-
Cercospora sp	-
Chaetomium sp	-
Coniothyrium fuckelii	+
Dicoccum asperum	-
Fusarium semitectum	-
Nigrospora sp	-
Trichothecium roseum	-
Control (1)**	-
Control (2)***	-

* (-): No infection, (+): infection occurrence.
** Unwounded and inoculated rose plants.
*** Wounded un-inoculated rose plants.

Disease symptoms:

The symptoms of rose stem canker obtained due to the artificial inoculation by *C. fuckelii* are shown in Fig. (1). Cankers begin as small, pale yellow to reddish spots appear in the bark around wounds. The spots gradually expand and increase in size. The centre of the canker turns light brown with a dark brown margin. The epidermal tissue within the canker dries out and shrinks. The stem may ultimately be girdled, resulting in wilting and death of the plant parts above the canker. Large numbers of minute, black, small fruiting bodies (pycnidia) rupture the epidermis and are covered by sooty masses of blackish brown conidia (Fig. 1).

Fig.1. Symptoms of rose stem canker showing the epidermal tissue with the canker and wilting of the rose plant due to the artificial inoculation by *C. fuckelii.*

Description of C. fuckelii using light and electron microscope:

Depending upon its morphological features, *C. fuckelii* isolates grew well and produced abundant mycelium on potato dextrose agar. Colonies circular, smooth and radiating with regular margins. The aerial mycelium is white and fluffy at first but becomes darker as the cultures aged.

Conidia are borne on stalk-like projections (Fig. 2), within black, carbonaceous pycnidia with pseudoparenchymatous cells (Fig. 3). The inner most cells of the pycnidial wall are, to somewhat, small, and function as a sporogenous layer. There are no specialized conidiophores (Fig. 3).

Fig.2. Section through mature pycnidium of *C. fuckelii* under light microscope. 800x shows that the conidia are borne on stalk like projection and their accumulation in the pycnidium cavity.

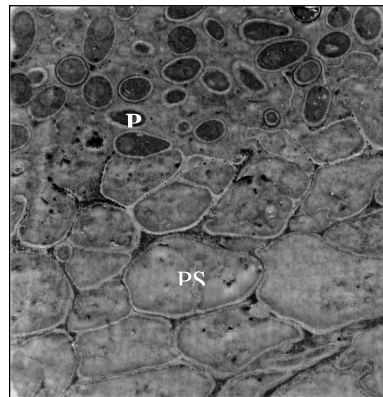

Fig.3. Section through mature pycnidium of *C. fuckelii* under electron microscope. 4000x. shows the inner most cells of pycnidial wall which function as a sporogenous layer. There are no specialized conidiophores. (P): Pycnidiospores; (PS): Pseudoparenche-matous cells of the pycnidium walls.

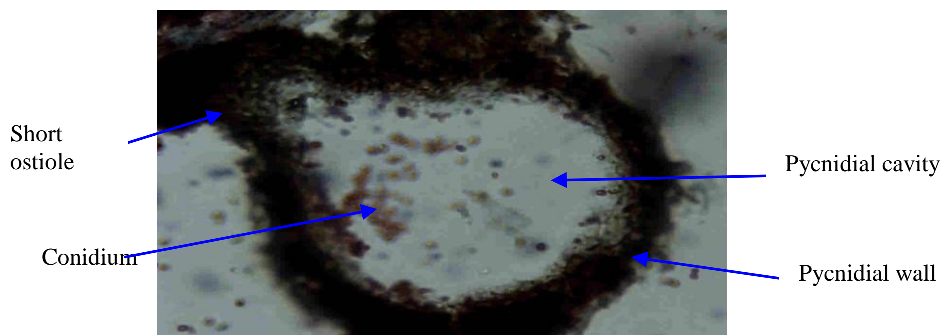

Fig. 4. Structure of *C. fuckelii* mature pycnidium under light microscope (800x).

The pycnidia produce short papillae that rarely extend above the agar surface (Fig. 4). The width of pycnidia varied from 127.5-135.0 to 211.5-214.5 µ and averaged 174.15-176.98 µ; the length ranged from 136.5-145.5 to 234.0 µ and averaged 188.1-191.1µ. (Table 3). Conidia are typically unicellular, oval and hyaline. When matured, the conidia are released into the pycnidial cavity and accumulate there. Subsequently such masses of conidia move out through the ostiole. The conidia are very uniform in size; length varied from 2.38-3.03 to 5.20-5.20 µ and averaged 3.90 to 4.42 µ., width varied from 2.00-2.17 to 3.46-3.68 µ and averaged 2.62 to 2.86 µ. (Table 3).

Table 3. Measurements of pycnidia and conidia of the four tested *C. fuckelii* isolates grown on PDA medium for 15 days

Fungal isolate	Measurement of pycnidia (µ)*				Measurement of conidia (µ)*			
	Length		Width		Length		Width	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
1	136.5-234.0	188.1	127.5-211.5	174.15	2.38-5.20	3.90	2.00-3.46	2.67
2	145.5-234.0	190.8	135.0-208.5	175.5	3.03-5.20	4.42	2.17-3.68	2.86
3	141.0-234.0	191.1	127.5-211.5	175.18	2.38-5.20	3.84	2.00-3.46	2.62
4	142.5-234.0	190.65	130.5-214.5	176.98	2.60-5.20	4.01	2.17-3.68	2.73
LSD at 5%	-	n.s	-	n.s	-	n.s	-	n.s

* Each value is the average of 30 pycnidium and 100 pycnidiospore, 1: isolate No.1 isolated from cv. African Dawn Rose, 2: isolate No.2 isolated from cv. First Red Rose, 3: isolate No.3 isolated from cv. Mercedes Rose, 4: isolate No.4 isolated from cv. Vercellia Rose.

Varietals resistance:

Data in Table (4) indicate that all the tested rose cultivars were susceptible to infection by *C. fuckelii*. Cv. Vercellia showed the highest infection, followed by cv. First Red, being 59.5 and 54.0% on the average, respectively. While, cv. Sentrix was the lowest susceptible one followed by cvs. Mercedes and African Dawn, being 28.6, 34.1 and 42.1% on the average, respectively. No correlation was found between virulence of isolates and their origin. In all cases isolate No. 1 was the most virulent being, 72.4% on the average, while, isolate No. 3 was the least aggressive being, 62.9% on the average without significant differences among the isolates (Table 4).

Table 4. Susceptibility of five rose cultivars to infection by four *C. fuckelii* isolates, 21 days after inoculation under greenhouse conditions

Tested isolate*	Cultivar infection (%)					Mean
	African Dawn	First Red	Mercedes	Sentrix	Vercellia	
1	66.7	100	52.4	42.9	100.0	72.4
2	71.4	81.0	52.4	38.1	85.7	65.7
3	52.4	100	47.6	42.9	71.4	62.9
4	61.9	85.7	52.4	47.6	100.0	69.5
Control (1) **	00.0	00.0	00.0	00.0	00.0	00.0
Control (2) ***	00.0	00.0	00.0	00.0	00.0	00.0
Mean	42.1	54.0	34.1	28.6	59.5	---
L.S.D. at 5% for: Isolates (I)= 12.8 ; Cultivars (C)= 14.4 and I X P = 28.8						

- Data (%) were transformed into arcsine angles before carrying out the analysis of variance.
* Isolates 1, 2, 3 and 4: As described in footnote of Table (3).
** Unwounded and inoculated rose plants without wounds.
***Wounded and un-inoculated rose plants.

Host range:

Nine different plant species were used to study their reaction to four *C. fuckelii* isolates. Data in Table (5) show that no disease symptoms were noticed on each of *Malus domestica*, *Prunus persica*, *Fragaria* spp., *Morus nigra*, *Dieffenbachia amoena* and *Schefflera actinophylla.* Meanwhile, slight infection was noticed on *Syngonium podophyllum* when it was inoculated by each of isolates No.1 and No.4.

Table 5. Host range of four *C. fuckelii* isolates, 21 days after inoculation under greenhouse conditions

Tested plant species	Family name	Common name	Severity of the disease*			
			1**	2	3	4
Rosa fountain	Rosaceae	Fountain	+++	++	++	++
Rosa huddly	Rosaceae	Huddly	+	+	+	+
Malus domestica	Rosaceae	Apple	-	-	-	-
Prunus persica	Rosaceae	Peach	-	-	-	-
Fragaria spp.	Rosaceae	Strawberry	-	-	-	-
Morus nigra	Rosaceae	Mulberry	-	-	-	-
Syngonium podophyllum	Araceae	Syngonium	+	-	-	+
Dieffenbachia amoena	Araceae	Dieffenbachia	-	-	-	-
Schefflera actinophylla	Euphorbiaceae	Schefflera	-	-	-	-

* (+): slight infection, (++): moderate infection, (+++): severe infection and (-): No infection.
** Isolates 1, 2, 3 and 4: As described in footnote of Table (3).

On the other hand, all tested isolates were able to infect *Rosa fountain* and *R. huddly* at different levels. Isolate No.1 showed severe infection on *R. fountain*, while the other isolates showed moderate infection in this respect. Meanwhile, all isolates showed slight infection on *R. huddly.*

Differentiation among the C. fuckelii isolates by Random (RAPD-PCR):

The random amplified polymorphism DNA technique (RAPD) was used for fast differentiation among four *C. fuckelii* isolates. The differences among isolates were detected by using two different DNA primers, *i.e.* (OPB5 and OPG14) and illustrated by photographs (Figs. 5A and 6A) and cluster analysis (Figs. 5B and 6B).

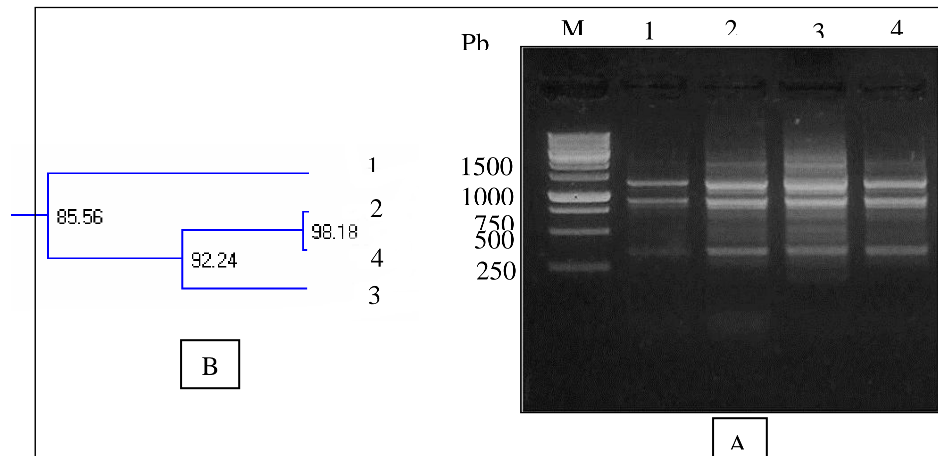

Fig. 5. Photograph (A) and cluster analysis (B) of DNA bands separated from *C. fuckelii* isolates using DNA primer OPB5.
M: Marker. Isolates 1, 2, 3 and 4: As described in footnote of Table (3).

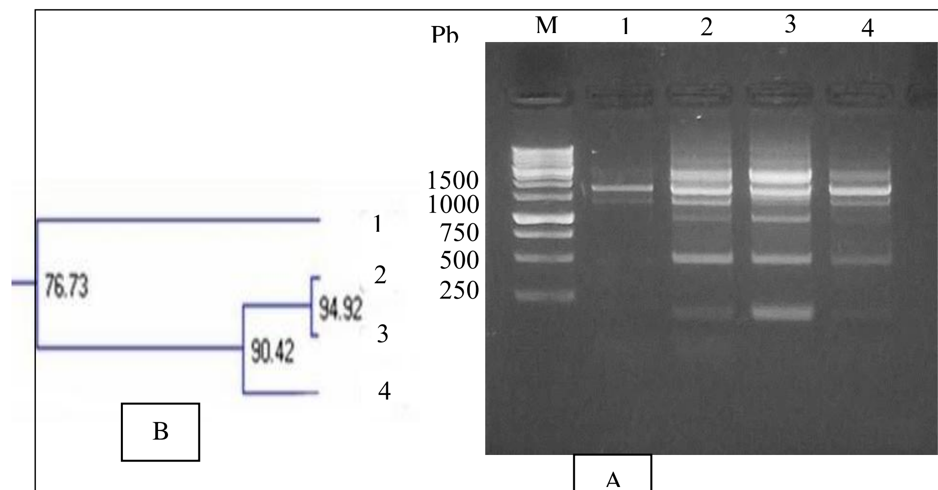

Fig. 6. Photograph (A) and cluster analysis (B) of DNA bands separated from *C. fuckelii* isolates using DNA primer OPG14.
M: Marker. Isolates 1, 2, 3 and 4: As described in footnote of Table (3).

The two different DNA primers showed high similarity levels among the four *C. fuckelii* isolates. It is clear (Figs. 5A and 6A) that the two DNA primers resulted in separation of several bands. Cluster analysis of DNA illustrated in Figs. (5B and 6B) show that the four isolates gave high similarity levels (85.56 %) with primer OPB5 as compared to (76.73%) similarity with primer OPG 14.

Both primers formed one main cluster included two sub-clusters. The first one contained isolate No.1, which was isolated from cv. African Dawn rose, while other sub-clusters showed similarity level of 92.24% for primer OPB5 and 90.42% similarity for primer OPG14 among isolates No. 1, 2 and 3 isolated from cvs. First Red, Mercedes and Vercellia Rose, respectively.

Discussion

Rose stem canker caused by *Coniothyrium fuckelii* was first reported in 1884 by Saccardo and later shown to be related to the stem canker of apple by O'Gara (1911). As described by Alfieri (1969); Horst (1983) and Pataky (1990) rose stem canker is probably widespread in occurrence in Europe and the United States on both outdoor and glasshouse-grown roses. The same authors gave good accounts about the characteristic symptoms of the disease and the causal organism. According to the available literature, rose stem canker caused by *C. fuckelii* is reported here for the first time in Egypt (Melchers, 1931 and Ali *et al*, 1972).

During the progress of the present work, 115 fungal isolates representing nine fungal species were isolated from six rose cultivars. According to their morphological features, the isolated fungi were identified as *Alternaria alternata* (Fr.) Keissler, *Botrytis cinerea* Person ex Fries, *Cercospora* sp., *Chaetomium* sp., *Coniothyrium fuckelii* Sacc., *Dicoccum asperum* (Corda) Sacc., *Fusarium semitectum* Berkeley et Ravenel, *Nigrospora* sp. and *Trichothecium roseum* Link ex Fries. Rose stem canker disease caused by *C. fuckelii* was reported also by Siriphong (1967) and Schwer (2006). During the present investigation, *C. fuckelii* showed the highest number of isolates.

Pathogenicity test revealed that all the isolated fungi were not able to infect the inoculated rose plants, except *C. fuckelii* which caused the symptoms of the rose stem canker. The symptoms are similar to those described by Alfieri (1969) and Pataky (1990).

Genus: *Coniothyrium* Corda represents one of the anamorph genera associated with *genus: Leptosphaeria* Ces. & De Not. (Leptosphaeriaceae) and *Paraphaeospheria* O.E. Erikss. (Phaeosphaeriaceae) in the Dothideales sensu (Hawksworth *et al*., 1995) or the *Pleosporales sensu* (Barr , 1987). This genus has a widespread distribution and approximately 28 species are described (Hawksworth *et al.*, 1995 and Swart *et al.,* 1998), although in the Index of Fungi and the Index of Saccardo nearly 1100 species are described (Reed and Farr, 1993). *Coniothyrium fuckelii* is the anamorphic phase of *Leptosphaeria coniothyrium* (Fuckel) Sacc. It has been found that there are different synonyms for this fungus (Anonymous, 2012) as follows:

Melanomma coniothyrium (Fuckel) Holm.
Sphaeria coniothyrium Fuckel.
Diapleella coniothyrium (Fuckel) Barr.
Kalmusia coniothyrium (Fuckel) Huhndorf.
Clisosporium fuckelii (Sacc.) Knutze.
Microsphaeropsis fuckelii (Sacc.) Boerema.

The present work showed that conidia of *C. fuckelii* are produced within black, carbonaceous pycnidia with pseudoparenchymatous cells. The pycnidia produce short papillae that rarely extend above the agar surface. The inner most cells of the pycnidial wall are, to somewhat, small, and function as a sporogenous layer. There are no specialized conidiophores. Conidia are typically unicellular, oval and brown, and borne singly on stalk-like projections. When matured, the conidia are released into the pycnidial cavity and accumulate there. Subsequently such masses of conidia move out through the ostiole. The obtained results are in agreement with those obtained by Sutton (1980); Siriphong (1967) and Camara *et al.* (2001), who gave detailed descriptions to the anamorph of genus *Coniothyrium* and found that the most distinctive feature is the conidiogenous cells, which are annellidic, *i.e*. percurrently proliferating after the secession of each conidium. According to Sutton (1980) conidia of genus *Coniothyrium* are thick-walled and verruculose, with a truncate base and sometimes a basal frill.

The width of pycnidia varied from 127.5-135.0 to 211.5-214.5 μ and averaged 174.15-176.98 μ; the length ranged from 136.5-145.5 to 234.0 μ and averaged 188.1-191.1μ. These measurements are, to somewhat, respond with measurements obtained by Massee (1915) who mentioned that the diameters of pycnidia averaged 180 μ to 200 μ. The conidia are very uniform in size; length varied from 2.38-3.03 to 5.20-5.20 μ and averaged 3.90 to 4.42 μ, meanwhile width varied from 2.00-2.17 to 3.46 - 3.68 μ and averaged 2.62 to 2.86 μ. The obtained measurements are differed, to somewhat, from those obtained by Saccardo (1884) who recorded that spores size were 2.4-5.0 x2.0-3.5μ. The description of *C. fuckelii* given by O'Gara (1911) corresponds with that given by Saccardo, except for the spore measurements, which he gave as 2 μ. to 4.5 μ. by 2 μ. to 3.5 μ. The difference in the measurements given for spore length might be explained as the result of an accidental transposition of the decimal point.

Verkley *et. al*. (2004) mentioned that many hundreds of species have been described on the basis of material found on plants, and most of these species have never been critically re-examined or studied in culture. Their morphology is relatively simple and provides few diagnostic characters, and the taxonomy has been primarily based on the host. This point of view was confirmed by Cortinas *et. al.* (2006) who reported that morphological characteristics of the genus *Coniothyrium* have been shown to be insufficient to differentiate among species where various features overlap.

The reaction of five rose cultivars to infection by each of the four *C. fuckelii* isolates indicated that all the tested isolates were pathogenic to the tested rose cultivars, *i.e.* African Dawn, First Red, Mercedes, Sentrix and Vercellia.

No correlation between the isolate and its origin was found. African Dawn isolate was the most virulent on the tested cultivars, while Mercedes isolate was the least aggressive one, however the differences were not significant. Ellis *et. al*. (1984) reported that among eight different fungi namely *Fusarium* spp., *Alternaria* spp., *Epicoccum purpurescens*, *Cytospora* sp., *Pestalotiopsis* sp., *Leptosphaeria coniothyrium*, *Gnomonia rubi* and *Botryosphaeria obtuse* isolated from cane cankers on thornless blackberry in Ohio, only *L. coniothyrium, G. rubi* and *B. obtuse* were pathogenic. Variation in these plants susceptibility to the pathogen infection could be attributed to differences in their genetic makeup and to different host pathogen interactions.

On the other hand, the reaction of nine plant species belonging to different families to the four tested isolates of *C. fuckelii* revealed that there is great specialization in this respect. *C. fuckelii* isolates failed to infect any plant species tested, except for *S. podophyllum.* These results are not in line with those obtained by different authors, who found that in beside rose plants; *C. fuckelii* could infect apple, European dewberry, willow, raspberry, spindle tree, *Euonymus* spp., greenbrier, *Smilax* spp.*, Fragaria* spp., *Malus sylvestris*, blackberry, raspberry, *Rubus* spp. and other hosts (Protsenko, 1959; Alfieri, 1969 and Thaung, 2008). Moreover in Kazakhstan, Visova *et al.* (1968) mentioned that the host range of this pathogen included many species belonging to genera *Berberis, Robinia, Rubus, Rosa, Citrus, Vitis, Pathenocissus* and *Helianthemum.*

Random amplified polymorphism DNA technique (RAPD) by using two different primers showed high similarity levels among the four *C. fuckelii* isolates. However, the use of both primers showed a difference in one of the isolates, which could be related to the difference in pathogenicity was noticed. Further studies should be conducted on such genetic variations for more precise conclusions.

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تقرح ساق الورد المتسبب عن الفطر *Coniothyrium fuckelii* Sacc. فى مصر

عفت عبد المجيد زاهر و محمد فاروق عطية
و نورالهدى عبد التواب رياض
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تم عزل ١١٥ عزلة فطرية تمثل تسعة أنواع فطرية مختلفة من ستة أصناف مختلفة من نباتات الورد والتى تم جمعها من العديد من الصوب والمشاتل بمحافظتى الجيزة والقليوبية وذلك خلال شهر أبريل من عام ٢٠٠٩. اختلف تواجد كل فطر من صنف الى آخر وكانت المسببات المرضية كونيوثيريم فكالياى والترناريا الترناتا هما الأكثر تكرارا فى العزل يليهم فطر من الجنس نيجروسبورا بينما كان الفطر من النوع سيركوسبورا أقلها تكرارا.

أظهرت اختبارات القدرة المرضية أن الفطر *Coniothyrium fuckelii* هو الممرض الوحيد والذى تمكن من إصابة النباتات بتقرح ساق الورد.
تبدأ الأعراض كبقع صغيرة ذات لون أصفر أو أحمر وذلك على القلف وتتسع تدريجيا ، ويسير لون التقرحات بنى فاتح فى مركز البقعة بينما تكون الحافة ذات لون بنى داكن. قد يحدث تحليق بسبب التقرحات الموجودة على الساق مسببة ذبول وموت النباتات فوق أماكن التقرحات. تظهر عادة الأوعية البكنيدية على الأماكن المتقرحة.

عرف الفطر المسبب للمرض بأنه الفطر كونيوثيريم فكالياي ، الطور الناقص للفطر ليبتوسفيريا كونيوثيريم وذلك طبقا لخصائصه المورفولوجية وقدرته المرضية. وعلى حد علم المؤلفين فإن تقرح ساق الورد والفطر المسبب له والمعروف بكونيوثيريم فكالياى وصف فى هذه الدراسة لأول مرة فى جمهورية مصر العربية.

ولقد اختبر رد فعل خمسة أصناف نباتية من الورد ضد أربعة عزلات من الفطر كونيوثيريم فكالياى. أظهرت النتائج أن جميع العزلات المختبرة كانت ممرضة للأصناف النباتية المختبره وهى أفريكان داون ، فيرست رد، ميرسيدس ، سنتريكس ، وفيرسيليا. لم يكن هناك ارتباط بين الشدة المرضية للعزلات ومصدرها. وكان الصنف فيرسيليا هو الأكثر قابلية للإصابة فى حين كان الصنف سنتريكس أقلهم فى ذلك.

وباختبار المدى العوائلى للعزلات الاربعة باستخدام تسعة أنواع نباتية تنتمى لفصائل مختلفة ، تبين وجود نوع من التخصص فى اصابة أصناف الورد دون غيرها من النباتات الأخرى المختبره ، حيث اقتصر التفاعل الايجابى للعزلات المختبره على أصناف الورد باستثناء نباتات النوع سنجونيم بودوفيللم التى أظهرت تفاعل إيجابى مع العزلتين الفطريتين رقم ١ و ٢ المعزولتين من صنفى الورد أفريكان داون وفيرسيليا على التوالى.

تم التفريق بين العزلات الأربعة باستخدام Random amplified polymorphism DNA technique بواسطة بادئين مختلفين وأظهرت نتائج التحليل الوراثى تماثل بنسبة ٨٥,٥٦% مع البادئ OPC5 مقارنة بنسبة ٧٦,٧٣% مع البادئ OPG14 وتمكن هذان البادئان من اظهار فرق معنوى فى العزلة Conio.1 المعزولة من الصنف African Dawn تمثل فى وجود حزمتين من الدنا مع البادئ OPC5 وثلاثة حزم مع البادئ OPG14 مقابل الحصول على ثمانية حزم لباقى العزلات.