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First Record of Black Dot Disease Caused by *Colletotrichum coccodes* on Potato Plants in Egypt

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Black dot is an important disease of potato that affects all plant parts. The disease was observed for the first time at Salhiya and Abo Swair locations in potato fields irrigated with pivot system during 2009/2010 season. Therefore, this study was initiated to investigate the ability of black dot disease to develop and spread on potato plants grown under Egyptian field conditions. Six fungal isolates were identified as *Colletotrichum coccodes* ((Wallr.) S. Hughes), obtained from two locations in Ismailia Governorate. Pathogenicity tests with the six isolates indicate that isolate CCS-3 was the most virulent one, recorded 100 % wilt of potato plants, while isolate CCA-3 was the weakest one recorded 50.4%. The virulent isolate CCS-3 showed the highest number of sclerotia compared with other isolates. The optimum growth temperature for *C. coccodes* isolates was 30°C. At low temperatures (5 to 10 °C), the fungal growth was very limited after 8 to 14 days of incubation. Number of acervuli or sclerotia was observed during the first 8 days of incubations at 5, 10, 15 and 20°C. Abundant acervuli and sclerotia were formed during 20-30 days after incubation at 15 to 20 °C. The sensitivity of *C. coccodes* to eight fungicides *in vitro* revealed that the fungicides Octave WP, Euparen M50% and Switch M were the most effective ones as no fungal growth was observed at all tested concentrations. The survival percentage of *C. coccodes* sclerotia decreased above soil surface as compared to those burred below soil surface at 10 and 20 cm depth during June 2010 till June 2011. The obtained results indicate that sclerotium was able to survive in soil for at least one year. This is the first report of black dot disease caused by *C. coccodes* in Egypt.

Keywords: Black dot, *Colletotrichum coccodes*, chemical control, potato and sclerotia.

Black dot caused by *Colletotrichum coccodes*, is a common blemish disease on potato tubers (*Solanum tuberosum* L.). The disease occurred in different potato growing countries worldwide. Black dot disease has become an economically important problem in potato. The disease has become a serious disease recently in Tunisia (Daami-Remadi *et al.,* 2010).

Lees and Hilton (2003) found in a crop survey in England and Wales during 1999 and 2000 that the relative incidence of black dot varied from 100 and 70% in the south-east and midlands to 18% in the south-west and 8% in the north. Observations by the authors in Scotland suggest that incidence of black dot is low compared with that in the south-east of England. Incidence of *C. coccodes* in lots of certified seed tubers planted in Washington State, originating from nine western and Midwestern states in the United State and two provinces in Canada, ranged from 0 to 90% in 1994 and 0 to 53% in 1995 (Johnson *et al.,* 1997).

Black dot refers to the abundant, small, black sclerotia produced on infected tubers, stolons, roots and stems. Infection of potato tubers with *C. coccodes* resulted in the development of silvery lesions on the tuber surface, characterized by the production of black microsclerotia. Symptoms are commonly observed at the heel end of the tuber and the lesions can appear brown, with a poorly defined margin (Dillard, 1992). It has been demonstrated that potato early dying characterized by stunting, wilting, premature senescence and reduced yields (Powelson and Rowe, 1993). Symptoms of black dot disease on potato foliage are usually associated with wounds caused by wind-blown soil, particularly sand, and appear initially as water-soaked lesions that subsequently turn dark brown to black. Infected plants sometimes appear wilted, with the lower and middle leaves becoming chlorotic (Johnson, 1994).

During March 2009/2010, symptoms of black dot disease on potato plants grown in Salhiya and Abo-Swair Locations, under irrigation pivot system, were observed. The environmental conditions that favour disease development are still unclear and limited. Black dot disease is favoured by wet soil conditions (Hide *et al.,* 1994). The sclerotia serve as over wintering and survival structures for the fungus. Sclerotia can survive two years in the soil (Dillard, 1990).

This study was conducted to isolate the causal organism from naturally infected parts of potato plants and to report the results of the pathogenicity tests on potato seedlings inoculated by *C. coccodes*. Effect of different fungicidal concentrations and different temperature degrees on the *in vitro* radial growth of *C. coccodes*, was studied. Survival of *C. coccodes* in infected potato tissues above and below soil surface *in vivo* was also investigated.

Materials and Methods

Isolation and identification of the causal organism:

Plant samples of basal stems, daughter tubers, stolons and roots showing typical disease symptoms were collected from potato plants (cv. Desiree) grown in fields under Pivot irrigation system at Salhiya (isolates CCS-1, 2 &3), and Abo-Swair (isolates CCA-1, 2 & 3)**,** locations, Ismailia governorate during 2009 / 2010 season. Diseased samples were gently removed and carefully handled, then placed in polyethylene bags, to avoid their dryness and brought into the laboratory for isolation purposes. The diseased samples were cut at the advanced margin of lesions to small pieces (3 mm x 3 mm) and then surface sterilized with sodium hypochlorite 2% for 2 minutes, followed by washing in sterile distilled water and dried, then transferred on PDA medium in Petri dishes. Plates were incubated at 25 °C for 7-10 days. Percentages of developed *C. coccodes* isolates from different plant parts were recorded. The fungal colonies were identified according to the Colletotrichum description reported by Sutton (1992) and kindly confirmed in Assiut Internat. Mycol. Centre, Fac. of Sci., Assiut Univ. Pure cultures were stored on PDA slants at 4°C for further studies.

Pathogenicity tests:

Pathogenicity was confirmed by fulfilling Koch's postulates on potato tubers cv. Desiree.

Inoculum preparation:

Inoculums of six *Colletotrichum coccodes* isolates were prepared from PDA cultures grown for 10 days under continuous fluorescent light (Salazar *et al.*, 2007). Conidia were washed from cultures with distilled sterilized water. Conidial suspension was adjusted, using a haemocytometer slide, to concentration of $2x10^6$ conidia/ml.

The artificial inoculation:

Potato tubers were surface sterilized with sodium hypochlorite 2% for 2 minutes and sown in pots (4 pots for each isolate) during December 2010. Thirty days after sowing, potato plants were sprayed with spore suspension of each isolate and 15 ml of spore suspensions (2×10^6 spores/ml) of the same isolate were added to each pot. Plants grown in pots without fungal inoculation were served as control.

Disease assessment:

The amount of black dot on roots was recorded visually using 0 to 3 scale based on the coverage of roots with sclerotia as follows: 0 = no sclerotia, 1= 1 - 30% coverage of roots, 2 = 31- 60%, and 3 = > 60% (Nitzan *et al*., 2006). The amount of disease on the stems was assessed during 2009 and 2010. Stem segments (1cm) were removed aseptically with 2, 6, 10 and 14 cm away above ground. The stem segments were transferred onto modified PDA (Nitzan *et al*., 2010) and were incubated in the dark at 25 °C. Fungal growth was visible on the agar after 10 days, then, fungal presence was recorded according to Nitzan *et al.* (2009 and 2010) where 0 = absent, and 1 = present. A sum of outcome (0 or 1) multiplied by the height from which the segment was removed (2, 6, 10 or 14 cm), was produced. The sum was divided by 32, which was the maximum value the sum could attain, and was multiplied by 100 to transform the index into percentage (%) of disease severity. Hence, the disease severity index on the stem (stem DSI) was calculated as follow:

$$\{[2x(0 \text{ or } 1) + 6x(0 \text{ or } 1) + 10x(0 \text{ or } 1) + 14x(0 \text{ or } 1)]/ 32\} \times 100.$$

Effect of temperature degrees on growth of C. coccodes, in vitro:

Active growing culture of the highly pathogenic isolate (CCS-3) was established on PDA media from collections maintained in cool stored culture tubes for long term preservation. Fresh malt agar plates were inoculated with a 5 mm mycelial plug cut with a sterile cork borer from the margin of an 8-day-old colony of the isolate. Plates were placed in an incubator at 5, 10, 15, 20, 25 and 30 °C. Radial growth was measured in two perpendicular directions, 4, 8, 10, and 14 days after inoculations. Three plates were used as replicates and the whole experiment was performed twice.

Development of acervuli and sclerotia of C. coccodes isolate CCA-3, grown on PDA at different temperatures and incubation periods in days:

Spore suspensions of isolate CCS-3, were prepared from 10-days-old cultures grown at room temperature on PDA media. Conidia were harvested by flooding the plates with 10ml sterile distilled water and gently scraping the agar surface .The resulting suspension was filtrated through sterile cheesecloth to exclude sclerotia and its concentration adjusted to $2x10^6$ spores /ml after counting conidia with the aid of haemocytometer .Inoculums of isolate CCS-3 was spread on PDA in 9 cm Petri dishes, which were then incubated at 5, 10, 15 and 20°C. The production of acervuli

and sclerotia scored visually on a four-class scale (0, absent; 1, scarce; 2, abundant; 3, very abundant) according to Glais-Varlet *et al.* (2004), under a stereo dissecting microscope after 4, 8, 12, 20 and 30 days of incubation. Three dishes were used as replicates for each temperature.

In vitro effect of different fungicidal concentrations on C. coccodes radial growth:

Eight different concentrations (0.1, 1, 5, 10, 25, 50, 100, and 200 µg/ml) of each tested fungicide (Octave WP, Euparen M50%, Switch M, Maneb, Topsin M70, Tecto 500SC and Roveral 50WP) were tested to investigate their effect on mycelial growth of *C. coccodes* grown on PDA medium at 25 °C as described by Uribe and Loria (1994).

Survival of C. coccodes in infected potato tissues:

Samples of infected potato stems with abundant microsclerotia and acervuli were used to study the survival of *C. coccodes* above and below soil surface at 5, 10, and 20 cm depth from June 2010 to June 2011, *in vivo.* Small pieces about 1cm for each depth, kept in small net bags in nylon socks without soil were buried in them at 5, 10, 20 cm depth and on the soil surface under field conditions without shade (168 samples: 4 depths X 14 months X 3 replicates). These materials were removed at intervals of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13. Small parts of 3 samples from each depth (treatment) were plated on PDA monthly and incubated at 25°C for 10 days. Survival (%) were determined by counting growth from pieces every month and compared with the control at 0 times.

Statistical analysis:

Obtained data were statistically analysed by analysis of variance (ANOVA) using the fisher LSD method. Means were separated by fisher's protected least significant differences (LSD) at *P* 0.05 level (Gomez and Gomez, 1984).

Results

Symptomatology:

Black dot of potato (cv. Desiree) caused by *Colletotrichum coccodes* was characterized by development of small black sclerotia on senescent and dead plants. The pathogen infected roots, stems and hill end tubers (Fig. 1A, B, C & D) and causing stunting and wilting of potato plants. *Colletotrichum coccodes* was found to colonize all underground parts (daughter tubers, stolons, vascular tissues and roots) and above ground parts (basal stems and vascular tissues (Fig. 1F, G, H & I). Small black sclerotia were abundant in stem lesions (Fig. 1E) above and below ground level. Earlier in the season, foliage of infected plants showed yellowing and wilting. The disease decreased tuber yield weight due to early death of the plants and reduced tuber quality due to gray-brown blemishes on the epidermis of potato tubers. Potato tubers may be externally infected by *C. coccodes* sclerotia and/or internally in the vascular tissues near the stolon end by mycelial hyphae. The pathogen spreads into the aboveground stems and colonizes the plant. At the end of the season, sclerotia become visually evident on roots, stolons and stems. Percentage of basal stem lesions in the inspected potato plants grown under naturally infected field conditions at Salhiya location reached about 85% at the end of the season before harvesting (Fig.1A).

Fig. 1. Natural symptoms of Black dot disease: wilted potato plants (A), microsclerotia on stem and in pith cells (B, C &D), magnified portion showing acervuli with setae on stem tissue (E), abundant sclerotia and acervuli on roots (F). Natural symptoms of microsclerotia on stolons (G), nick (H) and surface of potato tuber (I).

Isolation of C. coccodes from infected potato plants:

Data presented in Table (1) indicate that *C. coccodes* was the common associated fungus with black dot disease of potato plants. The fungus was isolated from any part of naturally infected potato plants (cv. Desiree), above or below soil surface grown at Abo-Swair and Salhiya locations. Potato stems below soil surface followed by stems above soil surface and vascular tissues recorded high percentages of *C. coccodes* occurrence in naturally infected plants as it recorded 94.32%, 91.97% and 92.32%, respectively. However, isolation from stolon, hill end tuber and roots recorded lower percentages of incidence as it reached 87.87, 86.82 and 82.71%, respectively. The occurrence of the pathogen differed in the two locations, the mean percentage of isolation from all infected parts recorded 94.58 and 84.04% at Salhiya and Abo-Swair locations, respectively.

Table1. Percentage of *C. coccodes* isolated from naturally infected parts of potato cv. Desiree grown at two locations, Ismailia Governorate during March 2009/2010 season

Potato organ	Isolation (%) from two locations		Mean
	Salhiya	Abo-Swair	
Stem (above soil surface)	98.6	85.4	91.9
Stem (blew soil surface)	99.1	89.5	94.3
Vascular tissues	97.2	87.3	92.2
Stolons	93.5	82.2	87.9
Hill end tuber	92.9	80.3	86.7
Roots	86.1	79.3	82.7
Mean	94.6	84.0	89.1

Identification of the causal pathogen:

Colletotrichum coccodes is an imperfect fungus belonging to the Melanoconiales (Davis and Johnson, 2002). All the fungal isolates obtained from potato were identified as *C. coccodes* (Wallr) Hughes, according to the classification of Sutton (1992). Colonies of *C. coccodes* grown on PDA were dominated by white and sparse aerial mycelium and consist of abundant black sclerotia that are distributed evenly over the agar surface (Fig. 2). Developed setae, with 1-8 septate, on the acervulus (Fig. 2C) or on the sclerotium, measured 34-246 x 4-5µm (average 154.0 CGAx4.5µm). Acervuli produced on culture as well as on stems, roots and neck tubers are almost rounded to elongate (diam. 150-300) with septate setae on the surface (Fig. 2 E & F) and normally in association with sclerotia which are black, globose to irregular and measure 155-300 x100-240µm on stem, hill tuber and root and those in PDA culture are black, globose (Fig.1C, D, G &H) and measure 90-740µm in diameter. Sclerotia of *C. coccodes* develop from acervuli differentiated from a stroma produced on infected plant tissue. Conidia are formed in honey, orange coloured masses and are 16-26 µm long by 3-4µm width (average 18 x 4µm), cylindrical, elongated fusiform, straight (Fig. 2 C, D, G, & H) and abruptly tapered to each end (Sutton, 1992).

Pathogenicity tests:

It is clear from data presented in Table (2) that there are highly significant differences among the six tested isolates of *C. coccodes.* Isolate CCS-1 caused the highest percentage of stem lesions, followed by isolates CCS-2, CCS-3 and CCA-1). However, isolates CCA-2 and CCA-3 were the weakest ones. In the same time, isolate CCS-3 was the virulent one; as it recorded 100% wilt of potato plants (Fig. 3A, C & E), followed by isolate CCS-1which showed 96% wilt, isolate CCA-2 exhibited 88.33% and isolate CCS-2 reached 74.66%. While isolates CCA-1 and CCA-3 were the weakest ones recording 66.66 and 50.36%, respectively. Stem infections of plants were observed 60 days after planting, on the surface of the stem and in vascular tissue. Concerning the formation of sclerotia, isolate CCS-3 significantly formed the highest number of sclerotia followed by CCS-1 and CCA-3, compared with the other isolates (Fig. 3C and E).

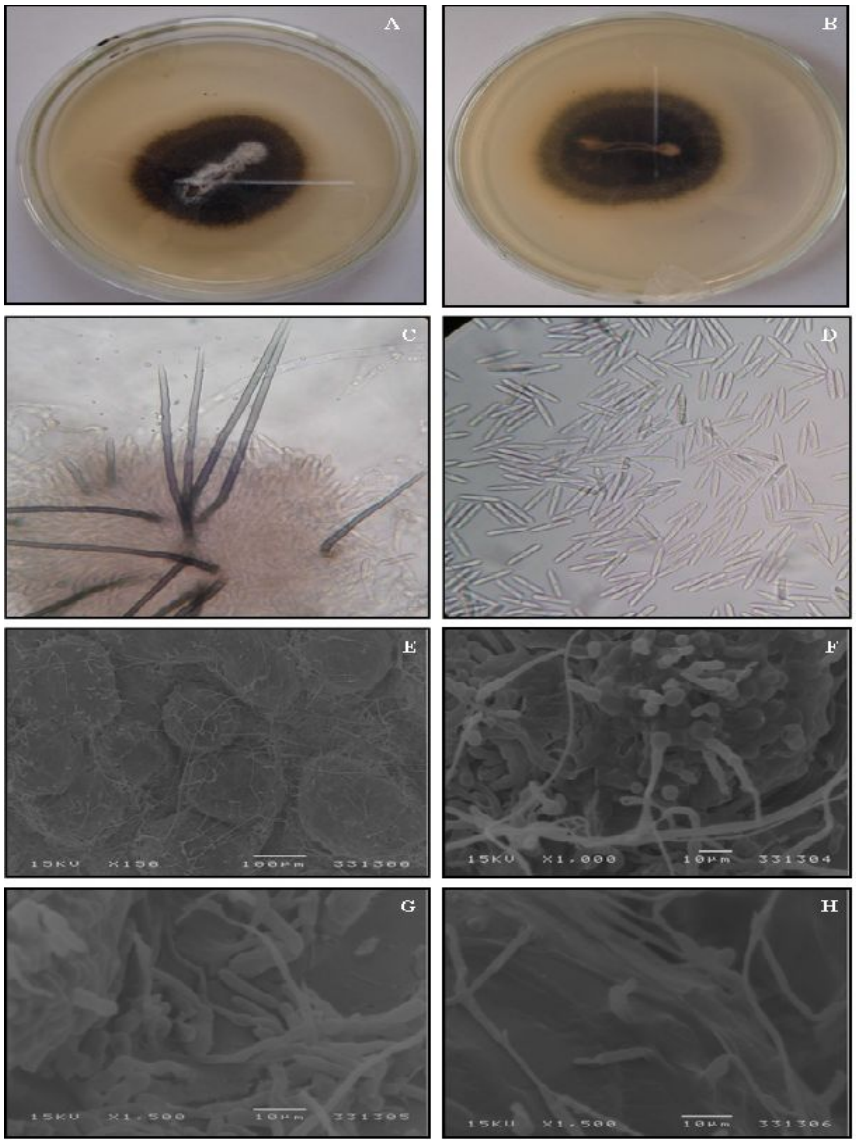

Fig. 2. Morphological characteristics of *C. coccodes* showing white aerial mycelium with black microsclerotia and pink spore masses during six days on PDA at 25°C (Upper surface side A, lower reverse side B). Acervuli with septate setae (C, E & F), conidia and conidia with appressoria (D, G & H). Figs. C & D were examined with light microscope, while E, F, G, & H were taken under scanning electron microscope.

Table 2. Percentage of disease severity index on stem, sclerotial occurrence on roots and wilt (%) of six isolates of *C. coccodes* 60 days after inoculation on potato plants under greenhouse condition during December 2010

Tested isolate from El-Salhiya Location, Pivot No.	Diseases severity		Wilt (%)
	Stem DSI (%)*	Sclerotia on root (0-3)**	
CCS-1	93.8	4.6	96
CCS-2	81.3	3.9	74.7
CCS-3	75.0	5.6	100.0
CCA-1	68.9	3.5	66.7
CCA-2	56.3	2.1	88.3
CCA-3	50.0	4.1	50.4
Control	0.0	0.0	0.0
L.S.D. at 5%	1.8	0.8	2.1

* DSI (%) = Diseases severity index on the aboveground stem where segment heights above the inoculation court were 2,6,10 and 14 cm; 0= none colonized segment and 1= colonized segment; and the maximum value the DSI could obtain was 32.

** Sclerotia on roots was recorded visually on 0 to 3 scale based on the coverage of roots with sclerotia as follows: 0= no sclerotia, 1= 1-30 coverage of roots, 2= 31-60% and 3= >60%.

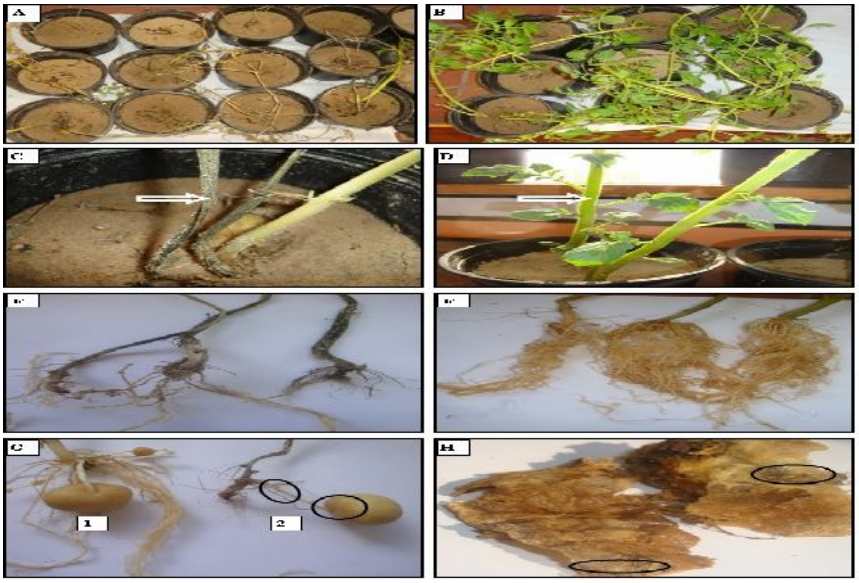

Fig. 3. Artificial inoculation with *C. coccodes* on susceptible potato (cv. Desiree), 60 days after inoculation under greenhouse conditions (A) as compared with healthy plant (B) and showing microsclerotia on stem and roots (C and E) compared with healthy (D&F), black dot on neck of potato tuber and stolon, artificially inoculated (G-2) compared with non inoculated (G-1) and the microsclerotia on tuber decayed skin, 60 days after planting (H).

In vitro effect of different temperature degrees on growth of C. coccodes:

Data in Table (3) indicate that temperature had significant effect on *C. coccodes* growth. At low temperature (5 to 10°C) the fungal growth was very limited after 8 to 14 days of incubation. It appears that the optimum temperature for the fungal growth ranged from 25 to 30°C. The maximum average temperature for the fungal growth was 30 °C, and the growth rate increased by increasing the incubation period from 4 to 14 days. The *in vitro* growth experiments showed that the optimum growth temperature was 30°C for the tested isolate (CCS-3).

Table 3. Growth of *C. coccodes* isolate (CCS-3) on PDA at different temperature degrees

Incubation time (days)	Radial growth (mm) at different Temperatures (°C)					
	5	10	15	20	25	30
4	0	0	10	22	36	52
8	0	5	20	35	58	70
10	3	10	35	55	76	85
14	6	20	45	65	80	90

L.S.D. at 5% for: Time= 0.78 and Temperature= 0.78.

Development of acervuli and sclerotia of C. coccodes isolate (CCS-3) grown on PDA at different temperature degrees:

Data in Table (4) show that acervuli and sclerotia were detectable in PDA culture of *C. coccodes* grown at different temperature degrees till 30 days of incubations. No acervuli or sclerotia were detected or observed during the first 8 days of incubations at 5, 10, 15 and 20°C. It was also found that abundant acervuli and sclerotia were formed 20-30 days after incubation at 15 to 20°C. It is also clear that sclerotia were absent 12 days after incubation at 5, 10 and 15°C. However, scarce sclerotia were observed at the same period at 20°C. Generally, incubation at 20°C. greatly favoured the development of acervuli and sclerotia as compared with tested low temperatures.

Table 4. Development of acervuli and sclerotia of *C. coccodes* isolate (CCS-3) grown on PDA at different temperature degrees and incubation periods

Incubation temperature (°C)	Scoring date (days after inoculation)									
	4		8		12		20		30	
	A*	S*	A	S	A	S	A	S	A	S
5	0	0	0	0	1	0	2	0	2	2
10	0	0	0	0	1	0	2	1	3	3
15	0	0	0	0	1	0	3	2	3	3
20	0	0	1	0	2	1	3	3	3	3

* A= Acervulus and S= Sclerotia.

- Semi quantitative scale: 0, absent; 1, scarce; 2, abundant; 3, very abundant.

Effect of different fungicides on growth of C. coccodes in vitro:

Data in Table (5) show the effect of different concentrations of 7 tested fungicides on radial growth of *C. coccodes* grown on PDA medium at 25 °C. The fungicides Octave WP, Euparen M 50% and Switch M were significantly the most effective, as no fungal growth was observed at all concentrations tested. Maneb over 10 mg/ml and Topsin M70% over 25 mg/ml completely inhibited the fungal growth. However, Tecto 500SC (Thiabendzol) and Roveral 50 % WP had the least inhibition effect on the radial growth of *C. coccodes*. It is also clear that the increase in fungicides concentration had an obvious decrease in the linear growth of the tested fungus. The obtained results indicate also that seed tubers are an important source of inoculums, since *C. coccodes* produces large numbers of microsclerotia in periderm lesions. The plants grown from infected seed tubers will be infected earlier, resulting in an increase in disease incidence and severity. So, treating seed tubers with Octave WP or Euparen M50% or Switch M before planting could reduce the quantity of *C. coccodes* inoculums introduced into fields and therefore reduce infection by this fungus.

Table 5. Radial growth of *C. coccodes* isolate (CCS-3) grown on PDA at different concentrations of fungicides, 10 days after incubation at 25°C.

Tested fungicide	Radial growth at 10 days (mm) in response to different * fungicide concentrations (mg/ml)							
	0.1	1	5	10	25	50	100	200
Octave WP	0	0	0	0	0	0	0	0
Euparen M 50%	0	0	0	0	0	0	0	0
Switch M	0	0	0	0	0	0	0	0
Maneb	25	21	11	11	0	0	0	0
Topsin-M70%	30	26	20	11	5	0	0	0
Tecto 500SC	80	76	63	42	30	25	18	13
Roveral50% WP	82	78	70	62	55	46	34	26
Control	90	90	90	90	90	90	90	90
L.S.D at 5% for: Fungicide (F)= 0.71, Concentration (C)= 0.73, F x C= 2.01								

* Data were recorded when one plate in the treatment was completely covered with the fungal growth.

Survival of C. coccodes in infected potato tissues:

Data presented in Table (6) indicate that the survival percentage of *C. coccodes* sclerotia significantly decreased above soil surface as compared with those burred below soil surface at 10 and 20 cm depth during June 2010 till June 2011. Survival of *C. coccodes* was less on the soil surface than at 10 or 20cm deep. In the same time, the fungal survival significantly recorded lower percentage in samples of infected stems and roots left above soil surface which recorded 41.33, compared with those burred below soil surface at 5, 10 and 20cm depth which reached 46, 48.33 and 55.58, respectively. The fungal lesions on the stems and roots lost their viability completely when stored above soil surface, 11 months after storage. However, the fungus still survives at the same period on infected stems and roots

Table 6. Survival (%) of *C. coccodes* in infected potato tissues above and below soil surface at 5, 10 and 20cm depth during 13 month

Date of isolation/month	Survival (%) / month							
	Sclerotia in vascular tissues				Infected stems and roots			
	Above	Below			Above	Below		
	0.0	5 cm	10 cm	20 cm	0.0	5 cm	10 cm	20 cm
Jun 2010 (Zero time)	100	100	100	100	96	96	96	96
Jul 2010	94	95	97	100	90	92	94	96
Aug 2010	89	90	93	96	80	84	86	94
Sep 2010	84	88	90	94	72	78	82	90
Oct 2010	82	86	88	92	68	72	79	90
Nov 2010	82	84	87	92	58	62	70	81
Dec 2010	80	84	86	90	47	55	62	74
Jan 2011	77	80	84	86	38	50	53	58
Feb 2011	75	78	82	83	26	32	38	44
Mar 2011	71	78	80	82	12	16	18	21
Apr 2011	65	73	75	78	5	9	12	14
May2011	56	62	64	70	0.0	2	4	5
Jun 2011	49	53	57	66	0.0	0.0	0.0	0.0
Main	75.3	72.8	81.9	85.8	41.3	46	48.3	55.6
L.S.D. 5%	2.94	4.6	3.0	2.85	2.37	2.69	3.15	4.22

burred below soil surface at 5, 10 and 20cm depth. On the other hand, 12 months after storage above or below soil surface, the fungal lesions on the basal of stems and roots lost its viability completely. In both samples stored above or below soil surface still survive sclerotia in vascular tissues for a year and still viable as survival remained over 50%, either above or below soil surfaces at 5, 10 and 20cm depth.

Generally, survival remained over 50% for a year of study at the 10 and 20 cm depths, but was reduced at the soil surface. Potato roots are a hidden source of conidia and sclerotia of *C. coccodes.* The fungus produces sclerotia on infected plant tissues, and these structures provide long-term survival ability. It is interesting to note that *C.* coccodes was isolated from potato plants in three fields (3 locations A, B and C, each location include 150 fadden) under Pivot sprinklers irrigation systems in sandy soil at Salhiya location. These locations cultivated several years with potato plants.

D i s c u s s i o n

Black dot has become a serious disease that causes decrease tuber yield weight due to early death of the plants and can reduce tuber quality due to gray-brown blemishes on the epidermis of potato tubers (Lees and Hilton, 2003 and Daami-Remadi *et al.,* 2010). The results of this study clearly demonstrate the ability of *Colletotrichum coccodes* to colonize all underground parts (daughter tubers, stolons, vascular tissues and roots) and above ground parts (basal stems and vascular

tissues). Small black sclerotia were abundant in stem lesions above and below ground level. Earlier in the season, foliage of infected plants showed yellowing and wilting as reported by Alexander and Eric (1992).

This fungus has been isolated from infected potato plants showing symptoms of black dot disease in different countries (Johnson, 1994; Johnson *et al.,* 1997; Leah *et al.,* 1999 and Glais-Varlet *et al.,* 2004). However, inoculums of *C. coccodes* can be spread by both seed tuber and soil.

In the present study, *C. coccodes* was detected on several potato organs, *i.e.* stems, roots stolons and tubers. Dillard (1992) isolated *C. coccodes* from infected potato tubers, daughter tubers, hill end tubers, stolons, root lesions and affected stems appeared prematurely wilted, and abundant sclerotia present on their base.

All the fungal isolates obtained from potato were identified as *C. coccodes* (Wallr) Hughes, according to the classification of previous workers (Mordue, 1967; Mcintyre and Rusanowski, 1975 and Sutton, 1992).

Pathogenicity tests indicate that there are highly significant differences among the six tested isolates of *C. coccodes.* Isolate CCS-3 was the virulent one; as it recorded 100 % wilt of potato plants. While, isolates CCA-1 and CCA-3 were the weakest ones recording 66.66 and 50.36 % respectively. Similar conclusions were found by Leah *et al.* (1999) who reported that the response of potato cultivars to *C. coccodes* differed in artificially inoculated fields.

Effect of temperature degrees on growth of *C. coccodes*, *in vitro* indicate that the fungal growth was very limited at low temperature 5 to 10°C, while the optimum temperature for the fungal growth ranged from 25 to 30°C. These results confirming previous reports on single isolate (Dillard, 1988). Glais-Varlet *et al.* (2004) found that the first visible growth of *C. coccodes* colonies at 5°C was noticed after 10 days, and further growth was very slow at this temperature. Incubation at 20°C favoured greatly the development of acervuli and sclerotia as compared with low temperature. These results are in agreement with those reported by Glais-Varlet *et al.* (2004) as they found that 21days after inoculation, large numbers of acervuli and sclerotia were observed in the plates incubated at 10 and 15°C. They also reported that no sclerotia were observed 6 days after deposition of inoculums on the plates. The fungicides Octave WP, Euparen M50% and Switch M were significantly the most effective, as no fungal growth was observed at all concentrations tested. Seed tubers are an important source of inoculums, since *C. coccodes* produces large numbers of microsclerotia in periderm lesions. The plants grown from infected seed tubers will be infected earlier, resulting in an increase in disease incidence and severity. So, treating seed tubers with Octave WP or Euparen M50% or Switch M before planting could reduce the quantity of *C. coccodes* inoculums introduced into fields and therefore reduce infection by this fungus. Eleonora and Rosemary (1994) reported that four fungicides (Maneb, Thiabendazole, Imazalil and CGA a phenylpyrrole) are currently used or are being tested for use as potato seed treatments in the United States. They found also that isolates of *C. coccodes* differed in their sensitivity to Maneb, Thiabendazole and Imazalil in radial growth on Petri plates containing fungicide-amended media.

Survival of *C. coccodes* in infected potato tissues indicate that the survival percentage of *C. coccodes* sclerotia significantly decreased above soil surface as compared with those burred below soil surface at 10 and 20 cm depth during June 2010 till June 2011. Survival of *C. coccodes* was less on the soil surface than at 10 or 20 cm deep, which likely was due to greater fluctuation in temperature and moisture at the soil surface. In the same time, the fungal survival significantly recorded lower percentage in samples of infected stems and roots left above soil surface. The higher temperature during summer might be played a role on decreasing the survival percent than that below soil surface. It is also might be due to, the sun rays which include infrared and ultraviolet had an effect on decreasing the fungal survival above soil surface. The fungal lesions on the stems and roots lost their viability completely when stored above soil surface. However, the fungus still survives, 11 months after storage on infected stems and roots burred below soil surface at 5, 10 and 20 cm depth. On the other hand, 12 months after storage above or below soil surface, the fungal lesions on the basal of stems and roots lost its viability completely. It could be concluded that the sclerotia and acervuli beside the lesions on the stems and roots of potato black dots caused by *C. coccodes* are one of the source of inoculums and the fungus can survive from season to another in infected potato tissues.

Similar results in previous studies reported that *C. coccodes* is able to survive at least 2 years in soil (Dillard, 1990 and Dillard and Cobb, 1993). From the previous results, it can be expected that the soil will be contaminated with *C. coccodes* sclerotia. The obtained results also indicated that *C. coccodes* sclerotia survive in soil for at least one year. Treating seed tubers and spraying the foliage during growth with a specific fungicides such as Octave WP, Euparen M50 or Switch-M which were the most effective against *C. coccodes* as no fungal growth was observed at all concentrations tested during the present study. It is also might reduce disease levels. In the same time, it can prevent secondary foliar infection of adjacent fields, and consequently minimize soil infestation. Thus in the short term, it might prevented yield losses, and in the long term, it might minimize soil infestation.

Generally, this study confirms that *C. coccodes* is distributed among potato-production areas at Salhiya region within seed tubers and soil where multiple primary infections may occur from sclerotia in the soil. The present study support the practice of multiple-year rotations with non-host crops before black dot become problems in order to prevent an increase of *C. coccodes* sclerotia in soil.

The disease may add additional losses to the potato production in Egypt, particularly to the export market. Therefore, control strategies should be considered within the frame of integrated disease management of potato diseases employed in Egypt.

References

Alexander, D.P. and Eric, D.K. 1992. Black dot of potato caused by *Colletotrichum coccodes* in Nebraska. *Plant Dis.*, **76**: 1077.

Daami-Remadi M.; Bouallegue, R.; Jabnoun-Khiareddine, Hayfa and El-Mahjoub, M. 2010. Comparative aggressiveness of Tunisian *Colletotrichum coccodes* isolates on potato assessed via black dot severity, plant growth and yield loss. *Pest Technol*., **4**(1): 45-53.

Davis, J.R. and Johnson, D.A. 2002. Diseases caused by fungi-black dot. Pages: 16-8. In: *Compendium Potato Diseases*. Stevenson W.R.; Loria, R.; Franc, G.D. and Weingarnter D.P. (eds.). APS Press, St Paul, MN, USA.

Dillard, H.R. 1988. Influence of temperature, pH, osmotic potential, and fungicide sensitivity on germination of conidia and growth from sclerotia of *Colletotrichum coccodes*. *Phytopathology*, **78**: 1357-1361.

Dillard, H.R. 1990. Survival of *Colletotrichum coccodes* in New York. *Phytopathology*, **80**: 1026.

Dillard, H.R. 1992. *Colletotrichum coccodes*, the pathogen and its hosts. Pages: 225-236. In: *Colletotrichum*: *Biology, Pathology and Control*. Bailey, J. and Jeger, M. (eds.). CAB International, Wallingford, UK.

Dillard, H.R. and Cobb, A.C. 1993. Persistence of *Colletotrichum coccodes* on tomato roots and in soil. *Phytopathology*, **83**: 1345.

Eleonora, U. and Rosemary, L. 1994. Response of *Colletotrichum coccodes* to fungicides *in vitro*. *American Potato J*., **71**: 455-463.

Glais-Varlet, T.; Bouchek-Mechiche, K. and Andrivon, D. 2004. Growth *in vitro* and infectivity of *Colletotrichum coccodes* on potato tubers at different temperatures. *Plant Pathol.*, **53**: 398-404.

Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedures of Agricultural Research*. 2nd Ed. John Wiley and Sons Ltd. New York, USA. 680 pp.

Hide, G.A.; Boorer, K.J. and Hall, S.M. 1994. Effects of watering potato plants before harvest and of curing conditions on development of tuber diseases during storage. *Potato Res.*, **37**: 169-72.

Johnson, D.A. 1994. Effect of foliar infection caused by *Colletotrichum coccodes* on yield of Russett Burbank potato. *Plant Dis*., **78**: 1075-8.

Johnson, D.A.; Rowe, R.C. and Cummings, T.F. 1997. Incidence of *Colletotrichum coccodes* in certified potato seed tubers planted in Washington State. *Plant Dis.*, **81**: 1199-1202.

Leah, T.; Orly, E. and Marina, H. 1999. Effect of *Colletotrichum coccodes* on potato yield, tuber quality and stem colonization during spring and autumn. *Plant Dis*., **83**(6): 561-565.

Lees, A.K. and Hilton A.J. 2003. Black dot (*Colletotrichum coccodes*): an increasingly important disease of potato. *Plant Pathol.*, **52**: 3-12.

Mcintyre, G.A. and Rusanowski, C. 1975. Scanning electron microscope observation of the development of sporophores of *Colletotrichum atremenatium* on infected potato periderm. *Amer. Potato J*., **52**: 269-75.

Mordue, J.E.M. 1967. *Colletotrichum coccodes*. CMI Descriptions of Pathogenic Fungi and Bacteria, No. 131. Commonwealth Agricultural Bureau, Kew, UK.

Nitzan, N.; Evans, M.A.; Cummings, T.F.; Johnson, D.A.; Batchelor, D.L.; Olsen, C.; Haynes, K.G. and Brown, C.R. 2009. Field resistance to potato stem colonization by the black dot pathogen *Colletotrichum coccodes*. *Plant Dis*., **93**: 1116-1122.

Nitzan, N.; Evans, M.A. and Johnson, D.A. 2006. Colonization of potato plants after aerial infection by *Colletotrichum coccodes* causal agent of potato black dot. *Plant Dis*., **90**: 999-1003.

Nitzan, N.; Quick, R.A.; Hutson, W.D.; Brown, C.R. 2010. Partial resistance to potato black dot, caused by *Colletotrichum coccodes* in *Solanum tuberosum* Group Andigena. *Amer. J. Potato Res*., **87**: 502-508.

Powelson, M.L. and Rowe, R.C. 1993. Biology and management of early dying of potatoes. *Ann. Rev. Phytopathol.*, **31**: 111-26.

Salazar, S.M.; Castagnaro, A.P.; Arias, M.E.; Chalfoun, N.; Tonello, U. and Daz Ricci, J.C. 2007. Induction of a defence response in strawberry mediated by an avirulent strain of *Colletotrichum*. *Eur. J. Plant Pathol*., **117**: 109-122.

Sutton, B.C. 1992. The genus *Glomerella* and its anamorph *Colletotrichum*. Pages: 1-26. In: *Colletotrichum: Biology, Pathology and Control*. Bailey, J.A. and Jeger, M.J. (eds.). CAB International, Wallingford, UK.

Uribe, E. and Loria, R. 1994. Response of *Colletotrichum coccodes* to fungicides *in vitro*. *Amer. Potato J*., **71**: 455-465.

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التسجيل الأول لمرض النقط السوداء المتسبب عن الفطر *Colletotrichum coccodes* على نباتات البطاطس فى مصر

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يُعتبر مرض النقط السوداء فى البطاطس المتسبب عن الفطر *Colletotrichum coccodes* أحد أمراض البطاطس التى انتشرت حديثاً فى عدة دول. ففى عامي ٢٠٠٩ و٢٠١٠سجلت أعراض المرض لأول مرة فى مصر على نباتات البطاطس المنزرعة بالحقول المكشوفة تحت ظروف الري المحوري. تم عزل ٦ عزلات من منطقة الصالحية و أبوصوير والتى تم تعريفها على أنها الفطر *Colletotrichum coccodes*. أثبتت تجارب العدوى الصناعية بالعزلات المستنبتة أن العزلة CCS-3 من الصالحية كانت أقوى العزلات على احداث المرض حيث بلغت نسبة الأصابة بالذبول ١٠٠% من نباتات البطاطس المحقونة بينما كانت العزلة CCA-3 من أبو صوير اقل العزلات مقدرة على احداث الأصابة حيث بلغت نسبة الذبول ٥٠%. أوضحت النتائج ان العزلة القوية ايضا CCS-3 الصالحية أظهرت اكبر عدد من الاجسام الحجرية مقارنة بالعزلات الاخرى. أوضحت التجارب المعملية ان درجة الحرارة المثلى لنمو الفطر كانت عند ٣٠ درجة مئوية للعزلة المختبرة CCS-3. كما أثبتت الدراسة ايضا ان نمو الفطر كان محدودا بعد ٨-١٤ يوم من التحضين على درجات الحرارة المنخفضة ٥-١٠ درجة مئوية للعزلة المختبر CCS-3. لم يتم تكوين أو ملاحظة اجسام حجرية او اسرفيولس خلال ٨ ايام من التحضين على درجات حرارة ٥ و١٠ و١٥ و٢٠ درجة مئوية. تكونت كميات وفيرة من الاسيرفيولس والاجسام الحجرية بعد ٢٠-٣٠ يوم من التحضين على ١٥-٢٠ درجة مئوية. بينما كانت الاجسام الحجرية غائبة بعد ١٢ يوم من التحضين. أوضحت الدراسة تحت ظروف المعمل ان المبيدات الفطرية اوكتاف القابل للبلل– الايوبارين (م ٥٠) والسويتش (أم) كانت أكثر المبيدات كفاءة حيث لم يلاحظ اى نمو للفطر على كل من التركيزات المختبرة.

تناقصت النسبة المئوية لبقاء الاجسام الحجرية للفطر كوليتوتريكم كوكيدى فوق سطح التربة وذلك مقارنة بتلك التى تم دفنها تحت سطح التربة على عمق ١٠ و٢٠سم اثناء موسم ٢٠١٠ و٢٠١١.

أوضحت النتائج السابقة أن الأجسام الحجرية للفطر يمكن أن تبقى حية على الأقل لمدة عام. ولن معاملة الدرنات قبل الزراعة ورش المجموع الخضرى بالمطهرات المتخصصة لمكافحة الفطر المسبب لمرض النقط السوداء يمكن ان يمنع الاصابة الثانوية لنباتات البطاطس التى يمكن ان تنتقل عن طريق الحقول المجاورة و من ثم نقل الاصابة للتربة. وعلية فإنه على المدى القصير يمكن ان نحمى المحصول من الاصابة و على المدى الطويل نحد من تلوث التربة ولا نحتاج بعد ذلك لمعاملات التربة المختلفة.