Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 23, No. (1) : 2021 : 16-23

ISSN-0972-3005

ROLE OF SCLEROTIA IN THE AGGRESSIVENESS AND PATHOGENICITY OF *SCLEROTIUM CEPIVORUM*

YEHIA A.G. MAHMOUD*, REDA H. SAMMOUR, ABDEL-ZAHER M.A. MUSTAFA AND REEM A. ALHOZEIM

Tanta University, Faculty of Science, Botany and Microbiology Department, Tanta 31527, Egypt

(Received 6 November, 2020; Accepted 1 December, 2020)

***Key words**: Sclerotium cepivorum, Onion white rot, Sclerotia, Pathogenicity*

Abstract–Twenty-six Egyptian isolates of *Sclerotium cepivorum* Berk., were collected from eleven Egyptian governorates. Pathogenicity was tested against five onion cultivars grown in local (Giza 6, Giza 20), regional (Baladi from Saudi Arabia), and international (yellow onion from USA and Ailsa Craig from UK) areas. The study revealed that *S. cepivorum* isolates Kf11, As1, Is, Mf, So1, and So2 had high virulence against all five onion cultivars with ratings of 5, while isolates Kf1, Kf2, Kf3, and Kf4 were rated the lowest in virulence based on disease severity. *S. cepivorum* sclerotia with high and low pathogenicity of four strains were observed at high and low magnifications using electron microscope analysis to reflect the sclerotia surface and layers. A study of the most virulent *S. cepivorum* isolate (Is) using electron microscopy showed regular spherical sclerotia, with one pointed end at the germination point. The surface was observed to be rough with many cracks, and contained a thick, multilayered cell wall with small dark spherical precipitates. The inner components of the sclerotia included dense, sharply defined cytoplasmic organelles with a rough outer surface, and a large nucleus with dense chromatin. The avirulent *S. cepivorum* isolate Kf3 was observed to have irregular sclerotia, and a non-pustular surface with distinctive deep grooves. The Kf3 isolate's sclerotia contained less dense inner components; the cytoplasmic organelles were smooth and lacked an outer surface while the nucleus was small and granulated and had lighter chromatin than observed in the Is isolate. It can therefore be concluded that *S. cepivorum* aggressiveness may rely upon content of pigments such as melanin and thickness of cell wall.

INTRODUCTION

S. cepivorum Berk. is the causal agent of white rot disease in onions (*Allium cepa* L.), which is one of the most serious diseases of onion cultivation in many regions around the world (Eranshaw *et al*., 2000). Though *S. cepivorum* exists in soil as dynamic mycelium, it can also remain in field soil as durable, undeveloped structures known as sclerotia without a host plant for more than 20 years (Ahmed and Ahmed, 2015). Sclerotia are masses of compact mycelium, with thick dividers and a thinner stroma. They assume a significant role in dormancy and endurance in environmental extremes (Smith *et al*., 2014). Specifically, the pathogen's sclerotia remains in dormant conditions with fungistasis in the dirt, and interestingly, remains viable after a long period of time (Hyakumachi *et al*., 2014). The developed sclerotia of *Aspergillus niger*, a similar pathogen that affects various fruits, display morphological variations in size, shape and shading (Abu El-Souod *et al*., 2017).

Sclerotia begin to grow and invade Allium plants through root exudates, which initiates contamination of the species with white rot disease (Maude, 2006; Davis *et al*., 2007). High concentration of the pathogen in soil can result in yield reduction over many seasons, preventing further cultivation of onions, leek, and garlic (Crowe *et al*., 1980).

The endurance of sclerotium is due to its firm vegetative hyphae composition, which is known to prevent natural elimination and degradation (Butler, 1966). In its initial state, the sclerotium develops as a progression of side branches from the original hyphal strand (Gomez-Miranda and Leal, 1981). The cell mass of sclerotium is chemically composed of 64 % α-glucan and 15% β-glucan chitin complex (Gomez-Miranda and Leal, 1981). No known teleomorph of *S. cepivorum* exists except sclerotia and microconidia, so it is thought that the pathogen

may reproduce through these structures. It has also been proposed that sclerotia are simply the dormant structure, or a remaining vestige of sexual reproduction in species that have become asexual (Geiser *et al.,* 1994; Dyer and Kuck, 2017).

The aim of this study is to determine the relationship between *S. cepivorum* pathogenicity and the structure of its sclerotia. These discoveries may aid in developing a strategy to eliminate such pathogens.

MATERIALS AND METHODS

S. cepivorum isolation from different locations and cultivars

Twenty-six isolates of *S. cepivorum* were obtained from infected onion bulbs collected from 11 governorates in Egypt (Table 1).

Sclerotia and infected root parts obtained from infected onion bulbs were surface sterilized by immersion in 1% sodium hypochloride for 3 min, followed by three washes in sterile distilled water. Then small sections from the area between healthy and diseased tissue were cut and transferred onto potato dextrose agar (PDA) petri plates. Inoculated plates were then incubated at 20 °C and fungal growth was examined daily. Developed colonies of the sclerotia forming fungus (*S. cepivorum*) were purified using hyphal tip technique. *S. cepivorum* was grown on potato dextrose agar (PDA) petri plates for 10-14 days at 17-20 °C and the resultant sclerotia were harvested.

Pathogenicity of the collected isolates and evaluation using onion cultivars

Pathogenicity of the 26 collected isolates was tested using 5 onion cultivars of various origin: two local (Giza 6 and Giza 20), one regional (Baladi from Saudi Arabia), and two international (yellow onion from USA and Ailsa Craig from UK).

Five seeds of each cultivar were planted in pots (15 cm diameter) filled with a sterilized mixture of sandy soil and peat compost. Each pot was then inoculated with 30 sclerotia of each isolate and covered with 1 cm of soil (McLean and Stewart, 2000). Four pots were grown without inoculation of sclerotia as the control. Each treatment was replicated 4 times, and the pathogenicity was

Table 1. The collected *S. cepivorum* isolates and their location.

Code	Location	No. of isolate
Kf1	Kafr Al-Sheikh (Arround Kafr Al-Sheikh city)	1
Kf2	Kafr Al-Sheikh (Qulen)	2
Kf3	Kafr Al-Sheikh (Qulen)	3
Kf4	Kafr Al-Sheikh (Dessouk)	4
Kf5	Kafr Al-Sheikh (Dessouk)	5
Kf6	Kafr Al-Sheikh (Dessouk)	6
Kf7	Kafr Al-Sheikh (El-Shein)	7
Kf8	Kafr Al-Sheikh (El-Shein)	8
Kf9	Kafr Al-Sheikh (El-Shein)	9
Kf10	Kafr Al-Sheikh (Arround Kafr Al-Sheikh city)	10
Kf11	Kafr Al-Sheikh (Arround Kafr Al-Sheikh city)	11
As1	Assiut	12
As2	Assiut	13
Fa1	El-Fayom	14
Fa2	El-Fayom	15
Mn	Al-Minia	16
Gh1	Gharbia	17
Gh2	Gharbia	18
Da	Damietta	19
Gi	Giza	20
Qa1	Qaluobiya	21
Qa2	Qaluobiya	22
Is	Ismalia	23
Mf	El-Menofoya	24
So1	Sohage	25
So2	Sohage	26

repeated two times. Average of disease incidence was calculated ± standard deviation and recorded. Temperature in the green house was kept at 18-20 °C, with 12 hrs of light and 12 hrs of dark per day. The experiment lasted 6 weeks, with observations of plant growth, leaf yellowing, wilting, and death recorded every 7 days to determine the behavior of each isolate. Cultivated pots were watered as required. White rot severity was assayed using an arbitrary 0-5 scale, 0 indicating no symptoms, and 5 indicating completely dead plants and the disease severity was rated on 0-5 scale where 0=healthy; 1=bulb covered with mycelium but not rotted; 2=1-25% of the bulb rotted; 3=25-50% of the bulb rotted 4=50-75% of the bulb rotted and 5=75- 100% of the bulb rotted. White rot severity was calculated according to the methods of Liu *et al.* (1995) as follows. SDI= Σ d/(dmax. x N) x100 whereas: d is the disease rating possible and n is the total number of plants examined in each replicate. Furthermore, Disease incidence percentage (I = Σ X/N X 100) was the proportion of diseased plants, which consisted of the number of diseased plants (x) divided by the total number evaluated (N) multiplied by 100, (Groth *et al.*, 1999).

Symptoms of Onion White Rot Disease

The symptoms of white rot are unclear until the organism develops into the bulb of the onion. Indications within the foliage may include yellowing, leaf dieback, and wilting of older leaves, followed by stunted plant growth and death of all foliage (Chaput, 1995). Plants may suddenly die in large areas of the field if the soil is heavily infested. Leaf rot begins at the base, with older leaves being the first to fall, and the bulb may rot in a semi-watery fashion. As the disease advances, the mycelium becomes more compacted and less prominent, with sclerotia appearing as small circular dark bodies on the mycelial mat. These sclerotia, the dormant structures of the pathogen, are around the size of a pin head or poppy seed. Plants can become infected at any point during development.

Studying the ultra-structure of *S. cepivorum* sclerotia using Electron Microscopy

Sclerotia of *S. cepivorum* isolates Kf3 and Is, were determined to have the lowest (2) and highest (5) disease severity for Onion Giza20 cultivar respectively, were collected from PDA grown culture and subjected to TEM examination**. Also,** Sclerotia of *S. cepivorum* IS isolate with high disease severity of 5 (highest) and *S. cepivorum* Kf4 of disease severity of 2 (lowest) were examined with SEM and TEM, in case of yellow onion (USA) cultivar.

Preparation of *S. cepivorum* sclerotia for Scanning Electron Microscope study (SEM)

Mature sclerotia obtained from each isolate that was previously grown on PDA medium were incubated in vials containing 2% gluteraldehyde in 0.1 M phosphate buffer, pH 7.3 for 2 hrs at 4 °C. After three rinses with cold buffer (10 mins per rinse), the material was incubated with 2% OsO_4 for 4 hrs in the same buffer. Then, the sclerotia were dehydrated in a graded ethanol series followed by acetone and subsequently placed in a critical point dryer. Some sclerotia were then divided in half with a razor blade under a binocular microscope and then carefully applied to specimen stubs coated with a thin layer of gold (Sputtering Device, Union) and examined with a JEOL JSM-35C scanning electron microscope.

Preparation of *S. cepivorum* sclerotia for Transmission Electron Microscope study (TEM)

Sclerotia were incubated for 2 hrs in 5% gluteraldehyde in 0.1 M cacodylate buffer, pH 7.0, at 4 °C, and washed with the buffer, and then incubated in 2% OsO_4 in 0.1 M phosphate buffer, pH 7.2, for 4 hrs. Washing with buffer was repeated, and then sclerotia was dehydrated in a graded ethanol series. The material was embedded in Spurr's resin (Spurr, 1969) and cut with a glass knife in a LKB Ultratome ^{222}. Sclerotia were stained with uranyl acetate followed by lead citrate and viewed in a JEOL JEM-100 CX electron microscope.

RESULTS

Pathogenicity of the causal agent of the Allium white rot disease on 5 onion cultivars

Six weeks after planting seeds of the 5 different onion cultivars in soil artificially inoculated with 26 isolates of *S. cepivorum* under controlled greenhouse conditions, the disease severity rate and incidence on Giza 6 cultivar were determined (Table 2).

White rot severity was assayed using a 1-5 scale, where 1 represents asymptomatic plants and 5 represents completely dead plants. *S. cepivorum* isolates were tested on Giza 6 cultivar, with As1, As2, Gh1, Gh2, Gi, Qa2, Is and So2 showing 100%

white rot incidence, and isolate Kf5 showing the lowest incidence rate of 29% (Table 2). Isolates were then divided into three groups based on severity. The first and most pathogenic group with severity ratings of 5 contained isolates Kf3, Kf6, Kf8, Kf9, Kf10, Kf11, As2, As2, Mn, Gh1, Gh2, Da, Gi, Qa2, Is, Mf, So1 and So2. The second group with a moderate disease severity of 4 contained isolates Kf2, Kf4, Kf7, Fa1, Fa2 and Qa1. The third group with the lowest severity of 3 contained isolates Kf1 and Kf5 (Table 2).

Isolates were then tested on Giza 20 cultivar: Is and So1 performed with the highest disease incidence of 93%, and isolate Kf3 was the least pathogenic with a 22% incidence rate (Table 2). Isolates were divided into four different groups according to disease severity (Table 2). The first group with severity ratings of 5 contained isolates Kf11, As1, Is, Mf, So1 and So2. The second group included isolates Kf9, As2, Mn, Gh1, Gh2, Da, Gi and Qa1, which had severity ratings of 4. The third group with a severity of 3 contained isolates Kf1, Kf2, Kf4, Kf5, Kf6, Kf7, Kf8, Kf10, Fa1, Fa2 and Qa2. The fourth and last group with a disease severity of 2 contained isolate Kf3 only. Again, it can be concluded that groups with larger disease severity on Giza 20 is more pathogenic than groups with moderate to low severity ratings.

On the Baladi (KSA) cultivar, the collected data indicates that almost all isolates performed with 100% disease incidence, save for isolates Kf3 and Kf6 which performed with the lowest incidence of 50% (Table 2). No germination was observed with isolates Kf5, Kf8, As2, Fa2, Gh1, and So1, indicating a severity of 5. The isolates were divided into two

Table 2. Percentage of white rot disease incidence and severity (mean ± Standard deviation) caused by 26 isolates of *S. cepivorum* on five onion cultivars after six weeks from planting in infested soil.

Isolates	code	Incidence, % on onion cvs					Severity on onion cvs				
		Giza 6	Giza 20	Balady	Alisa	USA	Giza 6	Giza 20	Balady	Alisa	USA
Kafr Al-Sheikh 1	Kf1	50±4.5	35±3.0	100±9.3	80±7.0	50±4.2	3±0.1	3±0.1	5±0.1	5±0.2	3±0.1
Kafr Al-Sheikh 2	Kf2	75±6.0	40±4.0	100±10.0	75±6.2	50±3.0	4±0.2	3±0.1	5±0.2	4±0.1	3±0.1
Kafr Al-Sheikh 3	Kf3	85±8.0	20±1.3	50±4.2	100±10.0	30±2.3	5±0.2	2±0.2	4±0.1	5±0.2	3±0.1
Kafr Al-Sheikh 4	Kf4	70±6.0	45±3.0	100±9.5	90±8.0	25±2.0	4±0.2	3±0.1	5±0.2	5±0.1	2±0.1
Kafr Al-Sheikh 5	Kf5	30±2.3	50±3.0	100±5.6	90±8.5	70±6.0	3±0.1	3±0.1	5±0.1	5±0.2	4±0.2
Kafr Al-Sheikh 6	Kf6	85±7.0	40±3.0	50±4.5	85±4.5	60±5.3	5±0.3	3±0.1	4±0.1	5±0.1	4±0.1
Kafr Al-Sheikh 7	Kf7	75±6.0	40±4.0	100±10.0	60±5.0	60±5.0	4±0.2	3±0.1	5±0.2	4±0.2	4±0.3
Kafr Al-Sheikh 8	Kf8	90±7.0	50±2.4	100±9.3	100±5.0	100±9.5	5±0.2	3±0.2	5±0.1	5±0.3	5±0.1
Kafr Al-Sheikh 9	Kf9	90±8.0	60±5.0	100±9.5	80±6.5	90±8.5	5±0.2	4±0.1	5±0.1	5±0.2	5±0.2
Kafr Al-Sheikh 10	Kf10	90±9.0	40±2.5	100±8.5	100±7.5	100±9.5	5±0.3	3±0.2	5±0.1	5±0.1	5±0.1
Kafr Al-Sheikh 11	Kf11	80±7.2	80±7.3	100±9.0	90±8.0	80±7.0	5±0.3	5±0.1	5±0.2	5±0.2	5±0.3
Assiut 1	As1	100±10.0	80±7.0	100±5.7	100±9.0	100±9.3	5±0.2	5±0.2	5±0.2	5±0.1	5±0.1
Assiut 2	As2	100±9.2	75±6.0	100±10.0	100±10.0	100±6.7	5±0.2	4±0.2	5±0.2	5±0.2	5±0.1

Table 2 (continued)

Isolates	Code	Incidence, % on onion cvs					Severity on onion cvs				
		Giza 6	Giza 20	Balady	Alisa	USA	Giza 6	Giza 20	Balady	Alisa	USA
El-Fayom 1	Fa1	60±5.3	40±3.0	100±10.0	80±7.5	80±8.0	4±0.2	3±0.1	5±0.1	5±0.2	5±0.1
El-Fayom 2	Fa2	75±6.0	50±4.5	100±5.3	90±8.5	90±6.5	4±0.1	3±0.2	5±0.1	5±0.2	5±0.1
Al-Minia	Mn	80±7.5	60±5.6	100±8.5	90±9.0	80±7.0	5±0.2	4±0.1	5±0.2	5±0.1	5±0.2
Gharbia 1	Gh1	100±10.0	60±5.0	100±8.5	100±10.0	100±10.0	5±0.1	4±0.1	5±0.2	5±0.1	5±0.2
Gharbia 2	Gh2	100±9.5	65±5.0	100±9.0	100±5.6	100±9.5	5±0.2	4±0.3	5±0.1	5±0.3	5±0.2
Damietta	Da	90±6.5	60±5.3	100±10.0	100±10.0	100±9.4	5±0.1	4±0.1	5±0.3	5±0.1	5±0.1
Giza	Gi	100±8.5	75±7.0	100±9.0	100±5.6	100±10.0	5±0.2	4±0.1	5±0.1	5±0.1	5±0.2
Qaluobiya 1	Qa1	75±7.0	65±6.0	100±10.2	90±8.0	100±9.0	4±0.3	4±0.2	5±0.2	5±0.2	5±0.3
Qaluobiya 2	Qa2	100±9.5	50±4.0	100±9.5	85±5.7	100±9.0	5±0.1	3±0.1	5±0.1	5±0.2	5±0.2
Ismalia	Is	100±5.3	90±8.0	100±9.2	100±10.0	100±8.5	5±0.2	5±0.2	5±0.2	5±0.2	5±0.1
El-Menofoya	Mf	100±10.0	90±5.3	100±5.6	100±9.5	100±10.0	5±0.1	5±0.2	5±0.1	5±0.2	5±0.2
Sohage 1	So1	80±77.0	90±8.2	100±10.0	100±10.0	100±6.7	5±0.2	5±0.1	5±0.1	5±0.2	5±0.2
Sohage 2	So2	90±8.2	80±7.0	100±9.5	100±9.5	100±7.8	5±0.1	5±0.2	5±0.2	5±0.1	5±0.1
Control	C	00±	00±	00±	00±	00±	00±	00±	00±	00±	00±

different groups of disease severity, the first group being isolates Kf3 and Kf6 with a severity of 3, and the second group being other isolates with disease severity of 5 (Table 2). The data indicates that the Baladi onion cultivar was susceptible to white rot by almost all tested isolates of *S. cepivorum*.

Severity of white rot was also observed on the Ailsa Craig (UK) cultivar (Table 2). According to the severity scale, isolates were divided into two different groups (Table 2). The first group with a disease severity of 4 contained isolates Kf2 and Kf7. The second group with a severity scale of 5 contained the remaining isolates. White rot incidence and severity caused by group two isolates in Ailsa Craig UK cultivar, was more pathogenic than those in group one.

On the yellow onion (USA), severity of observed white rot indicates that isolates Kf8, Kf10, As1, As2, Gh1, Gh2, Da, Gi, Qa1, Qa2, Is, Mf, So1 and So2 performed at 100% incidence, while isolate Kf4 had 25% disease incidence (Table 2). Isolates were divided into four different groups of severity (Table 2). The first group had a severity of 5 with isolates Kf8, Kf9, Kf10, Kf11, As1, As2, Fa1, Fa2, Mn, Gh1, Gh2, Da, Gi, Qa1, Qa2, Is, Mf, So1 and So2. The second group with a severity of 4 contained isolates Kf5, Kf6 and Kf7. The third group, with a severity of 3, contained isolates Kf1, Kf2, and Kf3. The fourth group with a disease severity of 2 contained only the Kf4 isolates. Group one's isolates could be observed as stronger than the severity of isolates in other groups.

Overall, after the five cultivars of onion were infected with 26 isolates of *Sclerotium cepivorum*, disease incidence ranged from 22% to 100%, indicating partial resistance to the pathogen. Considerable differences in resistance were found between cultivars, with the highest in Giza 20 and Giza 6.

Studying the ultrastructure of *S. cepivorum* sclerotia

Sclerotia of *S. cepivorum* IS isolate with high disease severity of 5 (highest) and S. cepivorum Kf4 of disease severity of 2 (lowest) were examined with SEM and TEM to determine if the structure of pathogen sclerotia has a relationship with its incidence and severity in case of yellow onion (USA) cultivar (data not shown). We got a similar data, by examining the sclerotia of *S. cepivorum* isolates Kf3 and Is, that were determined to have the lowest disease severity (2) and highest (5) disease severity for Onion Giza 20 cultivar respectively (Figures 1A and 1B). Therefore, *S. cepivorum* sclerotia with high and low pathogenicity were observed at high and low magnifications using electron microscope analysis to reflect the sclerotia surface and layers. We have examined and observed four strains of each

Table 3. *S. cepivorum* sclerotia microscopic observation for high and low pathogenic isolates

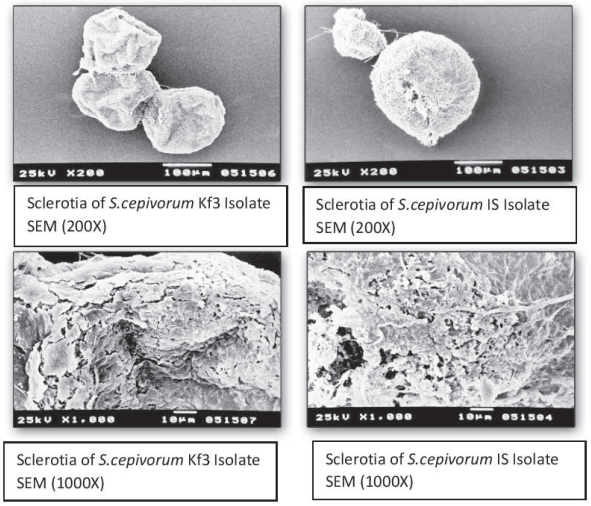
Sclerotia features	*S. cepivorum* strains (Low pathogenic)	*S.cepivorum* strains (High pathogenic)
Sclerotia shape	Irregular	Regular spherical shape, with one pointed end at the germination point
Sclerotia surface (200x)	Sclerotia, with a smooth, non-pustular surface and deep grooves	Sclerotia with a rough surface with many cracks was observed, along with germinating hyphae and small spherical precipitated bodies
Sclerotia cross sections: (20000x)	Cross section Kf3 sclerotia appeared with thin walls and lighter pigment precipitates was observed (Figure 1A).	Sclerotia sections appeared dense, dark precipitates within a multi-layered wall (Figure 1B), indicated that the inner components of the sclerotia are more dense, with sharply defined cytoplasmic organelles and a rough outer surface.
(25000x)	Sclerotia appeared with less dense inner components, and smooth cytoplasmic organelles with no outer surface	The cross section was examined at 25000X, and found to have a large sclerotia nucleus with dense chromatin that occupied most of the protoplasm
(40000x)	Sclerotia showed a less dense protoplasm with a small, granulated nucleus containing lighter chromatin	

groups. *S.cepivorum* features and layers were listed in Table 3.

The most pronounced sclerotia structures was observed with *S. cepivorum* isolates Kf3 and Is as the lowest and highest pathogenicity isolates respectively. They were examined by electron microscopy to determine if there was an observable relationship between the pathogenicity and constituents within the sclerotia. *S. cepivorum* isolate Kf3 with a scanning electron microscope at 200X and 1000X, was found to have an irregularly shaped sclerotia, with a smooth, non-pustular surface and deep grooves (Figure 1A).

Observation of isolate Is under a scanning electron microscope at 200X indicated that the sclerotia was a regular, spherical shape, with one pointed end at the germination point (Figure 1A).

Examination of the *S. cepivorum* Is isolate at 10000X with TEM showed dense, dark precipitates within a multi-layered wall (Figure 1B). When examination of isolate Kf3's cross section was performed at 20000X, a smooth outer surface of sclerotia with thin walls and lighter pigment precipitates was observed (Figure 1B).



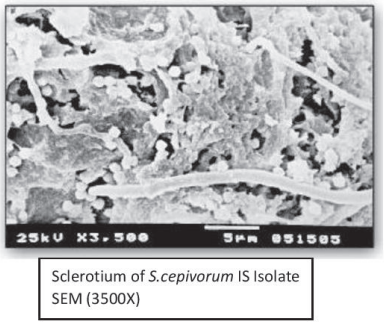


Fig. (1a). Scanning electron micrography of Sclerotia of *S. cepivorum* isolates Kf3 and IS

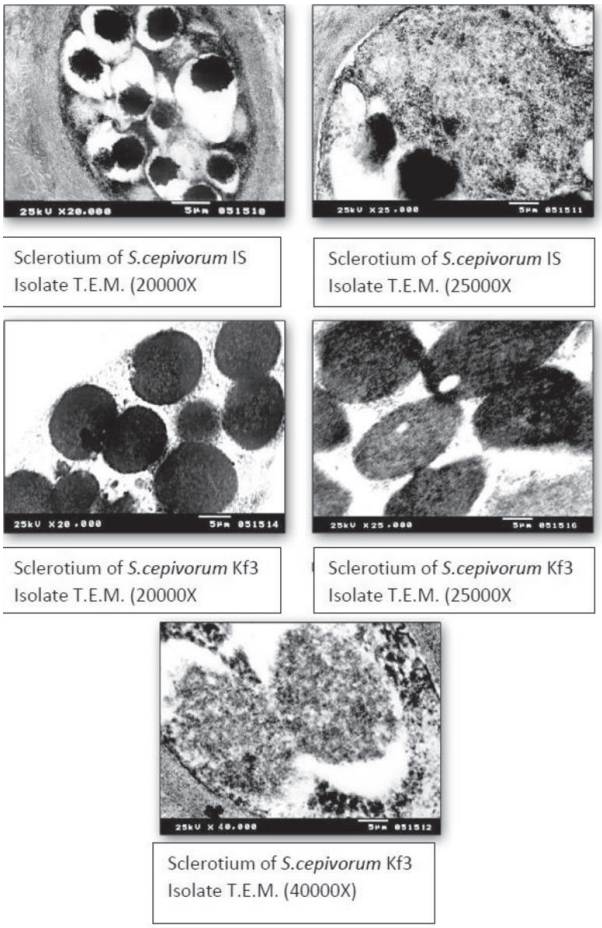


Fig. (1b). Transmission electron micrography of Sclerotia of *S. cepivorum* isolates Kf3 and S.

DISCUSSION

Sclerotium formation in fungi progresses through the three stages of initiation, development, and maturation (Zarani and Christias, 1997). The firm aggregates of vegetative hyphae within the sclerotia allow for resistance to biological degradation and higher survival (Butler, 1966). It has been found through light microscopy that *Aspergillus alliacens* sclerotia first propagate as a series of side branches from hyphal strands, and when matured, only two components remain: an expansive and pigmented skin with extremely thick walls, and the colorless, thick-walled medullary components (Rudolph, 1962). Rather than a hazy pigmentation as observed in components found in other fungi species such as *Alliaceus*, the cells of the sclerotium-like structures in *A. niger* were found to be whitish even following

reintroduction of the fwnA polyketide synthase (Frisvad *et al.,* 2019; Jørgensen *et al.,* 2020).

Examination using electron microscopy of the aggressive *S. cepivorum* isolate Is indicated the presence of dark nucleic materials, a multilayered cell wall, and dark pigments in the cytoplasm and cell wall. Therefore, it can be proposed that a darker pigmentation plays a role in pathogenic resistance against defensive enzymes located in the onion plant. Although the exact role of melanin phenoloxidase in pathogen virulence is unknown, several hypotheses have suggested that pigmented deposits in the cell wall act as a shield against the host's defense mechanisms, since there is no known enzyme that digests melanin (Bloomfield and Alexander, 1966). In fungi, those with melanin in its wall will survive microbial or enzymatic lyses better than those lacking pigment (Bloomfield and Alexander, 1966; Kuo and Alexander, 1967).

A study of the sclerotia structure produced under low levels of O_2 using TEM and SEM showed smaller masses (Paster, 1982). These sclerotia exhibited an external layer of thickened pigmented cells and a medullary district comprised of various cells, indicating that these bodies are organized in a predictable pattern (Willetts, 1978).

Extracts of sclerotia were found to contain phenols and polyphenols, which are likely concentrated in the rind cells, thus indicating the presence of melanin-like pigments (Chet *et al*., 1967). It was found that *Rhizobium phaseoli* strain 1233 easily affects the appressoria in albino mutants and wild type isolates, which were exposed to melanin synthesis inhibitors and thus lack the inner electron-dense layer. This unique pattern is thought to be essential for direct penetration of the host tissue (Kubo and Fweusawa, 1986). During the development stage, the sclerotia surface becomes pigmented, as melanin creation is favored for its role in protection of the organism against UV radiation and attack from microorganisms (Bolton *et al*., 2006; Erental *et al*., 2008).

CONCLUSION

White rot disease incidence was ranged from 22% to 100%, after the five cultivars of onion were infected with 26 isolates of *Sclerotium cepivorum*, indicating partial resistance to the pathogen. Considerable differences in resistance were found between cultivars, with the highest in Giza 20 and Giza 6. It can be concluded that the dark pigment contained in sclerotia might play an important role in the aggressiveness of the *S. cepivorum* strain. Melanin and other related compounds may allow for severe virulence and survival of the pathogen in soil for extended periods of time.

ACKNOWLEDGEMENTS

The authors would like to thank Elgazzar MA of Tennessee State University, USA for critically reading the manuscript.

Compliance with ethical standards

Conflict of interest - The authors declare no conflict of interest.

REFERENCES

Abu El-Souod, S.M., Awadalla, O.A., Assawah, S.M.W., Mahmoud, Y.A. and El-Debaiky, S.A. 2017. Studies on the sclerotia of some species in the genus *Aspergillus. Egypt J Bot*. 57 : 395-404. doi: 10.21608/ejbo.2017.683.1036

Ahmed, H.A.M. and Ahmed, N.G. 2015. Management of white rot of onion using composts and *Trichoderma harzianum*. *Current Life Sciences.* 1 (2): 63-69.

Bloomfield, B.J. and Alexander, M. 1966. Melanin and resistance of fungitolysis. *J Bacteriology.* 93 : 1276-1280.

Bolton, M.D., Thomma, B. and Nelson, B.D. 2006. *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. *Mol Plant Pathol.* 7 (1): 1-16.

Butler, G.M. 1966. The multicellular condition, Vegetative structures. In: Ainsworth GC and Sussman AS, ed. *The fungi*, Vol 2. pp. 83-112. New York, Academic Press.

Chaput, J. 1995. Identification of diseases and disorders of onions. Factsheet no 95-063.

Ministry of Agriculture, Food and Rural Affairs, Ontario.

Chet, I., Henis, Y. and Mitchell, R. 1967. Chemical composition of hyphal and sclerotia walls of *Sclerotium rolfsii* Sacc. *Can Microbiol.* 13 : 137-141

Crowe, F.J., Hall, D.H., Greathead, A.S. and Baghott, K.G. 1980. Inoculum density of *Sclerotium cepivorum* and the incidence of white rot of onion and garlic. *Phytopathol*. 70 : 64-69.

Davis, R.M., Hao, J.J., Romberg, M.K., Nunez, J.J. and Smith, R.F. 2007. Efficacy of germination stimulants of sclerotia of Sclerotium cepivorum for management of white rot of garlic. *Plant Dis.* 91 (2) : 204-208.

Dyer, P.S. and Kück, U. 2017. Sex and the imperfect fungi. *Microbiology Spectrum.* 5: 3. doi: 10.1128/microbiolspec. FUNK-0043-2017.

Eranshaw, D.M., McDonald, M.R. and Boland. G.J. 2000. Interaction among isolates and mycelial compatibility groups of *Sclerotium cepivorum* and cultivars of onion (*Allium cepa*). *Can J Plant Pathol.*

22 : 387-391.

Erentala, A., Dickmanb, M.B. and Yarden, O. 2008. Sclerotial development in *Sclerotinia sclerotiorum*: awakening molecular analysis of a "Dormant" structure. *Fungal Biol Rev.* 22 : 6-16.

Frisvad, J.C., Hubka, V., Ezekiel, C.N., Hong, S.B., Nováková, A., Chen, A.J., Arzanlou, M., Larsen, T.O., Sklenáø, F., Mahakarnchanakul, W., Samson, R.A. and Houbraken J. 2019. Taxonomy of *Aspergillus* section Flavi and their production of aflatoxins, ochratoxins and other mycotoxins. *Stud Myco.* l93: 1-63. doi: 10.1016/j.simyco.2018.06.001.

Geiser, D.M., Arnold, M.L. and Timberlake, W.E. 1994. Sexual origins of British *Aspergillus nidulans* isolates. *Proceedings of the National Academy of Sciences of the United States.* 91 : 2349-2352.

Gomez-Miranda, B. and Leal, J.A. 1981. Extracellular and cell wall polysaccharides of *Aspergillus alliaceus*. *Transactions of the British Mycological Society.* 76 : 249-253.

Groth, J.V., Ozmon, E.A. and Busch, R.H. 1999. Repeatability and relationship of incidence and severity measures of scab of wheat caused by *Fusarium graminearum* in inoculated nurseries. *Plant Dis.* 83 :1033–1038.

Hyakumachi, M., Mondal, S.N., Elsharkawy, M.M. and Hassan, N. 2014. Carbon loss by sclerotia of *Sclerotium rolfsii* under the influence of soil pH, temperature and matric potential and its effect on sclerotial germination and virulence. *Appl Soil Ecol.* 77 : 34–41

Jørgensen, T.R., Burggraaf, A.M., Arentshorst, M., Schutze, T., Lamers, G., Niu, J., Jin Kwon, M., Park, J., Frisvad, J.C., Nielsen, K.F., Meyer, V., van den Hondel, C.A.M, Dyer, P.S. and Ram, A.F.J. 2020. Dentification of SclB, a Zn (II)2Cys6 transcription factor involved in sclerotium formation in *Aspergillus niger*. *Fungal Genet Biol*. 138, in Press.

Kubo, Y. and Fweusawa, I. 1986. Localization of melanin in appressoria of *Colletotrichum lagenarium*. *Can J Microbiol*. 32 : 182-184.

Kuo, M.J. and Alexander, M. 1967. Inhibition of the lysis of fungi by melanins. *J Bacteriol.* 94 : 624-629.

Liu, L., Kloepper, J.W. and Tuzun, S. 1995. Induction of systemic resistance in cucumber against bacterial angular leaf spot by plant growth-promoting rhizobacteria. *Phytopathol*. 85 : 843-847.

Maude, R.B. 2006. Onion Diseases. In: *The Epidemiology of Plant Diseases Cooke*, BM, Jones DG, Kaye B, (Eds.), Vol 19, Springer, USA.

Mclean, K.L. and Stewart, A. 2000. Infection sites of *Sclerotium cepivorum* on onion roots. *N. Z. Plant Prot.* 53 : 118-121.

Paster, N. 1982. Influence of controlled atmospheres on formation and ultrastructure of *Aspergillus ochraceus* sclerotia. *Transactions of the British Mycological Society*. 78: 315-322.

Rudolph, E.D. 1962. The effect of some physiological and environmental factors on sclerotial Aspergilli. *Am J Bot.* 49: 71-78.

Smith, E.M., Henkel, T.W. and Rollins, J.A. 2014. How many fungi make sclerotia? *Fungal Ecology.* xx x: 1 -1 0.

Spurr, A.R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J Ultrastruct Res.* 26 : 31-34.

Willetts, H.J. 1978. Sclerotium formation. In: J.E. Smith and D.B. Berry ed. *The Filamentous Fungi*, vol. 3. London: Edward Arlond. pp. 197-213.

Zarani, F. and Christias, C. 1997. Sclerotial biogenesis in the *basidiomycete Sclerotium rolfsii*. A scanning electron microscopy study. *Mycologia.* 89 (4): 598-602