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A Brief Study on Sclerotinia Sclerotiorum, the Cause of Post-Harvest White Mold on Some Vegetable in Libya

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ABSTRACT
Sclerotinia rot is one of the most devastating postharvest diseases on many crops. laboratory studies were done to evaluate the mycelial growth and formation of sclerotia on different media and apothecia development of *Sclerotinia sclerotiorum.* Varied in mycelium growth, size and number of produced sclerotial was recorded. Potato Sucrose Agar was found significantly superior in mycelial growth and sclerotia formation among the others media under study. Germination of overwintered sclerotia release apothecia discs.
Keyword: Sclerotinia sclerotiorum, Growth, Carpogenic germination, Postharvest disease, Libya.

INTRODUCTION
Sclerotinia sclerotiorum are serious pathogens of several important crops in Libya and regularly cause major losses in crops such as cucumber [1] pea [2] and bean [3]. The soil-borne fungi *S. sclerotiorum* (Lib.) de Bary has worldwide distribution in temperate and subtropical climates and cause significant losses to horticultural and ornamental crops [4]. The genus *Sclerotinia* produces sclerotia that remain in the soil under adverse climatic conditions for several years [5]. The sclerotia of *S. sclerotiorum* germinate by forming abundant mycelium; however, only *S. sclerotiorum* can germinate carpogenically by producing apothecia which carry on ascospores that represent the main source for newly initiating infection in plant tissues [6-8]. This may provide ecological advantaged to this fungus for rapidly colonizing and infecting new hosts. The objective of this study was to identify the causal agent associated with postharvest *Sclerotinia* rot disease, based on culture characteristics including Mycelium growth (MLG), size and number of sclerotia and carpogenic germination.

2. Materials and Methods
2.1. Samples Collection and Pathogen Isolation
Naturally infected vegetable fruits were purchased from local markets in El-Beida city, Libya. Samples were brought to the laboratory in separate sterilized polythene bags, examined critically with respect to symptomatology. The pathogen was isolated, purified from plant samples rotted-infected tissues, and activated in potato-dextrose-agar (PDA). The cultures were kept at room temperature (~20 °C) in the dark for 15 days until sclerotia production. The fungus that appeared was primarily identified up to species level using cultural and morphological features [9, 10].

2.2. Growth Characteristics at Different Media
To determine the growth of this fungus at different media, mycelial discs (6.5 mm in diameter) were collected from areas of active growth near the edges of 5-days-old cultures, transferred to petri dish contain five different media: Potato Sucrose Agar (PSA), Potato Carrot Agar (PCA), Corn Extract Agar (CEA), Malt Extract Agar (MEA) and Sabroud Dextrose Agar (SDA), and incubated at 20°C in the dark for 5 days. Three replicates were prepared. The diameters of colonies on all plates were measured using the criss-cross method [11]. After 2 and 5 of culture, the colony diameters, Number of sclerotia and measured the dimensions of sclerotia By Digital Caliper at different media were recorded.

2.3. Sclerotia Germination
Sclerotia were scraped off the PSA medium, air-dried for 2–3 days and surface sterilization by ethanol 70% before being transferred to deep Petri dishes (96mm×25 mm) containing 10mL of sterile water. Sclerotia were incubated for 6–8 weeks in the dark at 4◦C and 15◦C for 4–8 weeks. When stipes formed, usually within 4–8 weeks, the Petri dishes were transferred to another incubator and illuminated under daylight fluorescent tubes until apothecial discs were formed.

2.4. Statistical Analysis
Data were analyzed by ANOVA (Analysis of Variance) using CO-Stat program and mean values were compared using Duncan's LSD multiple range test (P 0.05).

3. RESULTS
3.1. Symptoms on Fruits
The signs observed were soaked area on the infected tissues. Specifically, white and fuzzy mycelial growth was seen on the tissues. The entire fruit surface is rapidly covered with the mycilum, which is easily spread if the fruit is handled or exposed to air currents (Fig. 1). After 10 days showed small sclerotia that were produced on the infected tissues (at arrow). These sclerotia were black in color and globose, cylindrical, or irregular in shape.

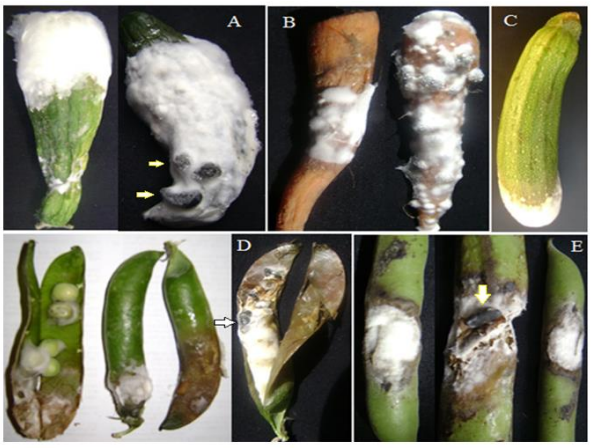

Figure-1. Postharvest white mold on some of vegetables caused by *Sclerotinia sclerotiorum*, (A) Typical symptoms of sclerotinia rot on cucumber, (B) carrot, (C) squash, (D) pea and (E) broad bean.

3.2. Growth Characteristics

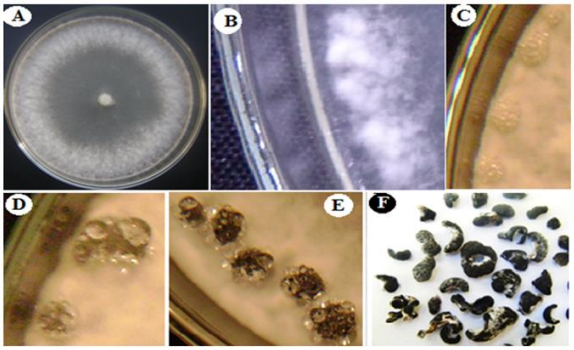

Figure-2. Macroscopic growth and development of *S. sclerotiorum* sclerotia. A. three days old colony of fungus. B. Development. C. Secretion of a mucilage-like substance. D. Curling of hyphae and formation of localized crystalline structures. E. Crystalline structures and hyphal fusion. F. Maturation of black sclerotia.

After isolation, the fungal culture was purified by adopting hyphal tip method. Uniformly one type of fungus colony with whitish growth started appearing after 24 hours. Later on, the growth of fungus was very fast which covered the entire Petri plate within 72 hours (Fig. 2- A). After 5 days of growth, small mycelial tufts started to develop at the periphery of the Petri plates and later such growth covered the entire Petri plate (Fig. 2- B). Shiny water droplets were seen frequently in culture plates around the mycelial tufts (Fig. 2-C,D). Later on, these mycelial tufts were converted into hard black coloured sclerotia. Individual sclerotium was seen embaded in white mycelium net. Semispherical to round or irregular shape sclerotia measured 2-10 mm x 13-15 mm in size (Fig. 2- E,F). Based on the symptoms, cultural and morphological description the fungus was identified as *Sclerotinia sclerotiorum*.

3.3. Growth and Sclerotia Development on Different Media

To find out a suitable medium for mycelial growth of *S. sclrotiorum*, five different media were tested. Perusal of data (Table 1) revealed that potato sucrose agar medium was significantly superior in supporting maximum mycelial growth (8 cm). This was followed by malt extract agar medium (6.2 cm) and corn extract agar (5.2 cm). Mycelial growth of the fungus was slowly on potato carrot agar and sabroud dextrose agar media.

Table-1. Effect of different solid media on mycelial growth and sclerotia formation of *S. sclerotiorum* at 20 °C

Medium	Mycelial growth (cm)*		Initiation of Sclerotia formation (DAI)	Number of Sclerotia**	Dimensions of Sclerotia (cm)***
	2 DAI	5 DAI			
PSA	3.7	8	5 b	18.33 a	0.9 a
PCA	1	3	8 a	12.66 ab	0.4 bc
CEA	1.6	5.2	7 a	2.00 c	0.1 c
MEA	3.4	6.2	8 a	8.33 bc	0.6 b
SDA	0	3	8 a	17.0 a	0.5 b
LSD at 0.05	Media: 0.24 Day: 0.19 M × D: 0.40				

*: Average of three replication
**: After 10 days
***: Mean of ten sclerotia DAI: Days after incubation.
Numbers followed by the same letter(s) are not significantly different at P= 0.05

Concerning to the time of sclerotia formation was faster on PSA medium than the others medium. It recorded to beginning after 5 days whereas it to take 7-8 days on others media. Maximum number of sclerotia formation on solid media was more on PSA (18.33). Good numbers of sclerotia formation on solid media was more on PSA (18.33) followed by SDA (17) and PCA (12.66). CEA was no suitable for sclerotia formation. Maximum sclerotia dimensions (0.9 cm) was recorded on PSA medium.

3.4. Sclerotia Germination

On carpogenic germination (Fig. 3) these sclerotia give rise to 2-5 columnar structures (stipes). The exposed portion of stipe was brown coloured. Apothecia were produced after 50 days. Apothecia were cup-shaped with disc concave (at arrow), light yellowish brown in colour and varied from 2-10 mm (average being 5-6 mm) in diameter Apothecium is formed on a slender stalk of 20-50 mm in length called stipe.

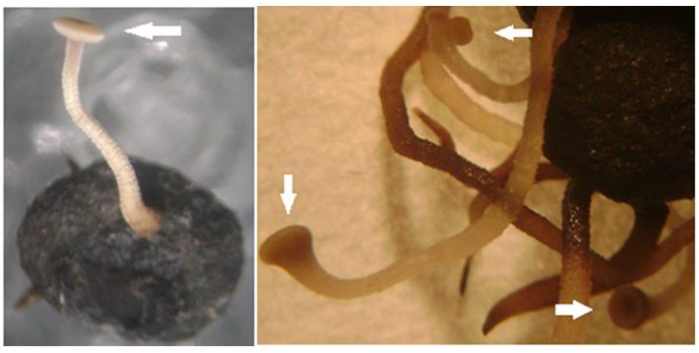

Figure-3. Apothecia formation in vitro

4. DISCUSSION

The crops after harvesting suffers from number of rots diseases caused by phytopathogenic fungi. Amongst all the rots of vegetables, cottony rot incited by *Sclerotinia sclerotiorum*, is one of the important disease and becoming a big obstacle in successful commerce crops. Typical symptoms appeared initially as the watered area. The infected tissues completely covered with white mycelium grows on the plant surface. Isolations were made from

infected portions of plant. The culture of *S. sclerotiorum* was purified and cultural characters i.e. growth of the aerial mycelium and sclerotial production on PSA. El-Gali [1] also isolated *S. sclerotiorum* on some vegetables.

The fungus was capable of growing on different media, with variation in growth and amount of sclerotial formation was observed on five solid media. It was found that potato sucrose agar medium gave good growth and sclerotial formation. Inability of a pathogen to grow and sporulate equally on all media suggests their preference and choice for certain constituents responsible for differences among species and even isolates within species which are inherent and genetic in character [12]. Several study was used PSA culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi [13-15].

The fungus produced white to gray colonies on PSA medium incubated at 20 °C for 5 days, which after 5 days showed small sclerotia that were produced on the peripheries of the plates. These sclerotia were black in color and globose, cylindrical, or irregular in shape.

Overall, the proliferation of vegetative mycelium by *S. sclerotiorum* is the previous fungal stage that initiates the formation of sclerotia and, during this growth period (5 days under our experimental conditions), the mycelium can absorb essential nutrients required by the fungus for further development, as described by Fernando et al. (2004). Thus, structuraland reserve components already incorporated into mycelial tissue are subjected to degradation processes mediated by the activity of specific enzymes (e.g. arylesterase or acid phosphatase) to provide energy and release of those nutrients required during sclerotia development [16]. Nevertheless, it should be borne in mind that the development of sclerotia, as well as the majority of fungal structures, depends on the growth and incubation conditions.

For sclerotia germination, Apothecia were obtained from germinating sclerotia after 4-8 week incubation. The results are in conformity with those observed by Kim, et al. [7] observed that Apothecia were obtained from germinating sclerotia after 5~6 week incubation.

The present study of *S. sclerotiorum* provides information on the symptoms cottony rot, growth characters and initiation of the sclerotia formation that plays a key role in the biological cycle of this important plant pathogen.

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