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Diversity of Postharvest Pathogenic Fungi of Green Bean Pods in Egypt

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In Egypt, rot disease of green bean pods (*Phaseolus vulgaris.* L) is the major problem during storage and marketing. *Sclerotinia sclerotiorum* and *Botrytis cinerea* were most dominant fungi followed by *Alternaria* sp., *R. solani* and *Fusarium* sp. *Sclerotinia sclerotiorum* was the most dominate fungi isolated from rotted pods collected from different markets in Egypt. All the tested fungi were able to infect pods of green bean Valintino Cultivare. The highest records of disease infection (80 and 75.6 %) on green bean pods were found with two isolates of *S. sclrotiorum* (Sc 11 and 13). But the two isolates of *Botrytis cinerea* isolate 2, 1 and 3 show moderate disease incidence .Meanwhile, other isolated fungi cause the least records of rot disease incidence on faba bean pods. Physiological studies Cleary show that there is a variation among tested isolates of *S. sclerotiorum* according to pathogenic ability, linear growth, the number and size of sclerotia .

Keywords: Green bean pods-Postharvest disease- *Sclerotinia sclrotiorum* - Pathogenicity test

Introduction

Bean (*Phaseolus vulgaris* L.) is one of the most important economic vegetable crop in Egypt for both local consumption and exportation for Europe and other countries. Snap bean pods could be attacked with several diseases caused by fungi during the growth in the fields, harvest, storage, marketing or exporting. Many phytopathogenic postharvest fungi such as *Sclerotinia sclerotiorum* , *Botrytis cinerea*, *Alternaria alternate* , *Rihzctonia solani* , *Pythium aphanidermatum* and *Fusarium solani* cause great losses in quantity and quality of the snap bean pods Snowdon (1992) . The most serious postharvest diseases were gray mould (*Botrytis cinerea*) and white mould (*Sclerotinia sclerotiorum)* Suslow and Cantwell (1998).

The aim of this work was to determine the most isolated fungi from rotted snap bean pods and to investigate the variation among isolates of *Sclerotinia sclerotiorum*.

Materials and Methods

Survey of green bean pod rots:

The pods of green bean were collected from green bean cultivar Valentino recently harvested from different Governorates, *i.e.*, Behaira, Kafer El-shakh, Ismailia, Giza, Gharbia, qualubia, Beni Sueif and Sohage during 2014 and 2015 seasons. Other samples were collected from wholesale, retail markets, some farms and refrigerators from the same locations mentioned above. On hundred pods from each location were kept in incubator for seven days at 20±1 ℃, then were examined for disease incidence and disease severity percentages were calculated according to Spalding and Reeder (1974) by following equation.

$$\text{Infection \%} = \frac{Number\ of\ diseased\ pods}{Total\ number\ of\ pods} \times 100$$

$$\textbf{Severity (\%) =} \frac{\text{Weight of diseased Pods}}{\text{Total weight of pods in treatment}} \times 100$$

Isolation of the casual organisms:

Samples of diseased pods from green bean was carefully washed with sterilized water, cut into small pieces, surface sterilized in 70% ethanol for one minute, washed several times with sterilized distilled water (SDW) and dried between two sterilized absorbent paper then transferred to Potato dextrose agar (PDA) plates medium and incubated at 20±2 ℃ for seven days. Developed fungi from diseased specimens were purified by single spore technique described by Fang *et al.* (1983) and by hyphal tip transfers method mentioned by Hawker (1950) and Howard (1981). Stock cultures of the obtained fungi were maintained onto PDA slants and stored in a refrigerator for further studies. Identification of isolated fungi was carried out in Plant Pathology Laboratory, Plant Pathology Department, National Research Center. Isolated fungi were identified according to Gilman (1957), Ellis (1971) and Barnett and Hunter (1972).While *Sclerotinia sclerotiorum* identification was achieved according to its morphological characteristics of mycelium and sclerotia (Abuel -Ela, 1993). Purified fungi were maintained on PDA slant under refrigerator conditions at 5˚C as stock cultures for further studies.

Pathogenicity tests:

The purified isolated of fungi were tested for their pathogenicity on apparently healthy green bean pods Valentino cultivar.Healthy green bean pods Valentino cv., obtained from Behaira, were washed by sterilized distilled water and surface sterilized by dipping in 70% ethanol for one minute and left to dry at sterilized room condition.

Inoculum preparation*:*

To prepare standard inoculum; the pure fungal isolates of *Botrytis cinerea, Alternaria alternata, Fusarium solani* and *Rihzcotina solani* were grown separately at 25±2 ℃ for seven days on PDA plates. Spore suspension, from each fungus was obtained by brushing the surface of the culture in the presence of 10 ml sterilized distilled water of by each plate, then the spore suspension were filtered through muslin. The concentration of spore suspension was adjusted to about $4x10^6$ spores/ml using a haemocytometer. Inoculum of *S. sclerotiorum* was prepared as a mycelial suspension. The fungus was grown on PDB (Potato Dextrose Broth) medium at 20˚C for 10 days, and then mass of growth was blended with sterilized distilled water to get mycelial fragment (Soltan, 1993). Inoculum concentration of *S. sclerotiorum* was prepared as mycelial suspension and adjusted to about $4x10^6$cfu/ml as recommended by the American Public Health Association (1960).

Inoculation of green bean pods.

Apparently healthy green bean pods cv. Valentino obtained from EL-Behera Governorate, were surface sterilized by dipping in 70 % ethyl alcohol for one minute and washed several times with sterilized distilled water then dried at room temperature. Sterilized bean pods were artificially wounded (by tooth brush), inoculated with prepared inoculum using an atomizer for each fungus at the rate of 100 ml spore suspension ($4x10^6$ spores/ml). While, inoculum of *S. sclerotiorum* was used as mycelial fragments at the same rate ($4x10^6$cfu/ml). Healthy wounded green bean pods was sprayed with the same amount of sterilized distilled water served as control. Fifty pods of the inoculated and un- inoculated green bean pods were kept in foam tray (23×12×4cm), and inserted in polyethylene bags to increase the relative humidity. Three replicates were used for each treatment. Inoculated and un- inoculated pods were stored at 23- 25˚C, and pods inoculated with *S. sclerotiorum* were stored at 20±1˚C. After seven days pods of all treatments were examined. Disease incidence was measured as the percentage of number of infected pods to the total number of pods in the treatment, while, disease severity was estimate by two methods, the first by determine the weight percentage (gm) of infected pods compared to the total weight of the treatment according to Spalding and Reeder (1974) as follows:

$$\textbf{Severity (\%)} = \frac{\text{Weight of infected pods} \times 100}{\text{Total weight of the treatment}}$$

The second methods by knowing the length as the percentage of the summation of the infected parts of pods to the total length of pods. according to Khalil (2010) as follows:

$$\textbf{Severity (\%)} = \frac{\sum \text{length of infected parts of pods} \times 100}{\text{Total length of pods}}$$

Pathogenicity tests of pod inoculation:

Healthy pods of bean were surface sterilized using ethyl alcohol **(**70%) and wounded by a sterile scalpel. PDA disk bearing mycelium (5 mm in diameter) taken from the edge of a 3 days old colony of the tested isolates. Fourteen isolates of *Sclerotinia sclerotinia* were used for artificial inoculation and placed under aseptic conditions on each wound. Control samples were treated with agar disks free of the pathogen. Ten samples were used for each treatment. All inoculated samples were kept in sterile plastic boxes supplied with water-wetted pieces of cotton and incubated at 20˚C rot length were measured (cm) after 3, 5 and 7 days. Number of sclerotia per pod was counted after 10 days., each isolate was tested in four replicates, each included 10 pods of similar approximately the same size. Disease severity was determined by two different methoed as mentioned befor.

Physiological Variation among isolates of Sclerotinia sclerotiorum:

Physiological variation among fourteen isolates of *Sclerotiorum sclerotiorum* from different locations in Egypt were chosen for this study. Discs (5 mm in diameter) bearing fungal mycelium were taken from 7 days old cultures, was placed in the center of 9 cm plates containing potato dextrose agar (PDA) medium (Morrall et al., 1972). Plates were incubated at 20±2˚C in four replicates, each of 3 dishes, from each isolate. Linear growth measured as soon the mycelial growth reached the edge of any Petri dish, number of formed sclerotia per plate and sclerotia size (mm) were determined after 14 days.

Statistical Analysis:

Effects of treatments were analyzed by ANOVA and significance of differences among means was tested applying the LSD test at the 5 % level of probability according to Steel *et al*. (1997) using the SAS Statistical package ver. 9.00 (SAS Institute, Cary, USA).

Results

Survey of green bean pods diseases

Survey of rots of green bean pods infection was carried out through eight different Governorates in Egypt *i.e.*, Behaira, Kafer El-Shakh, Ismailia, Giza, Gharbia, qualubia, Beni Sueif and Sohage during (autumn and winter) growing seasons. Results in Table 1 indicated that rot of green bean pods disease incidence and disease severity were recorded in all surveyed locations. Infection was higher in winter as compared with autumn growing season. The mean infection was (18.37 and 9.96%) for disease incidence and disease severity in the autumn (2014) and recorded (23.0 and 14.5%) for disease incidence and disease severity in winter November (2015) growing seasons, respectively. In concern of location, Behira governorate recorded the highest percentage of infection (34.0 and 20.07%) for disease incidence and disease severity respectively, while Sohage governorate recorded the lowest percentage of infection (14.0 and 8.0%) for disease incidence and disease severity respectively during winter growing seasons.

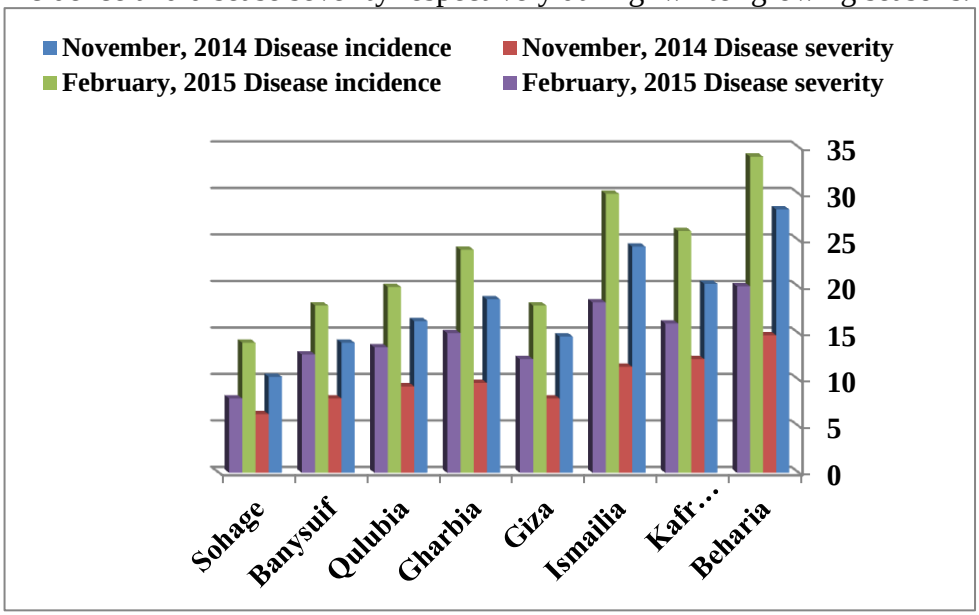

Fig.1. Survey of green bean pods diseases during 2014 and 2015 growing seasons in Egypt.

Concerning to the isolated fungi Table 2 showed that different fungi were isolated from green bean pods showing rot symptoms collected from Behaira, Kafer El-shakh, Ismailia, Giza, Gharbia, qualubia, BeniSueif and Sohage governorates during 2014 and 2015 growing seasons . Cultural characteristics and microscopic examination revealed that, the obtained isolates belonged to five different genera of fungi namely*; Sclerotinia sclerotiorum, Botrytis cinerea, Fusarium* sp., *Rihzcotina solana* and *Alternaria* sp . *Sclerotinia sclerotiorum* and *Botrytis cinerea* were most dominant fungi

followed by *Alternaria* sp., *R. solani* and *Fusarium* sp. *Sclerotinia sclerotiorum* recorded the highest percentage of frequency in the different governorates equal (58.4%), followed by *Botrytis cinerea* (14.6%), *Alternaria* sp. (9.97%), *R. solan*i (8.2%) Meanwhile, *Fusarium* sp. was the lowest frequency ratio (7.6%).

Table 2.Frequency (%) of isolated fungi from rotted green bean pods collected from different locations in Egypt.

Locations		**Frequency of the isolation fungi %**				
		S. sclerotiorum	*B. cinerea*	*F. solani*	*R. solana*	*A. alternata*
Behira	K.dwar	66.7	22.2	0.0	0.0	11.1
	K.dwar	62.5	12.5	12.5	0.0	12.5
	A.Homos	57.1	14.3	0.0	14.3	11.3
Kafer El-shikh	Dosouq	62.5	25.0	12.5	0.0	0.0
	K.shikh	55.6	22.2	11.1	0.0	11.1
	El.Riad	50.0	12.5	12.5	25.0	0.0
Ismailia	Ismailia	66.7	33.3	0.0	0.0	0.0
	El.Tall	50.0	16.7	16.7	16.7	0.0
Giza	Hiber	71.4	0.0	14.3	0.0	14.3
	Dokki	60.0	20.0	0.0	20.0	0.0
Gharbia	Tanta	62.5	25.0	12.5	0.0	0.0
Qualubia	Qualub	42.9	0.0	14.3	14.3	14.3
Banisouif	B.souif	60.0	0.0	0.0	0.0	40.0
Sohage	Sohage	50.0	0.0	0.0	25.0	25.0
Means		58.4	14.6	7.6	8.2	9.97

Pathogenicity test

The pathogenic capability of the isolated fungi from snap bean pods was carried out at plant pathology department National Research Center. Table (3) show a significant percentage of pathogenic capability to infect snap bean pods (valentino cv.) at ($20\pm1^{o}C$) and 90-95% relative humidity (R.H.) for 10 days. All isolates of *sclerotinia. Sclrotiorum* and *Botrytis cinerea* showed the highest level of both disease incidence and disease severity for snap bean pods which kept at $20\pm1^{o}C$ for 10 days. While , *Fusarium solani* showed the middle level of disease incidence and disease severity. No significant differences were recorded between *Alternaria alternate* and *Rihzoctonia solani*.

Table 3.Pathogenicity test of different isolated fungi on green bean pods Valentino cv. after incubation for ten days at 20 ±1°C.

Fungi	Isolate code	Disease incidence	Disease severity	
			Length	Weight
Sclerotnia sclerotiorum	11	90.67	80.00	80.67
	13	80.00	75.60	76.43
	12	59.33	45.00	45.50
Botrytis cinerea	7	52.67	35.90	36.30
	4	50.67	30.90	31.33
	10	53.33	29.50	29.73
Fusarium solani	5	22.7	13.70	14.17
	6	20.0	11.33	11.73
	8	16.0	10.50	10.93
Rihzoctonia solani	6	14.67	6.33	6.53
	2	11.33	5.40	5.73
	4	10	4.53	4.93
Alternaria alternata	7	18.70	8.13	8.17
	8	14.70	7.50	7.33
	2	11.33	5.40	5.60

Variation among isolates of S.sclerotioum

A- Pathogenic capability

Pathogenicity test using pod inoculation technique on bean Valentino cultivar show that all isolates were differed in their pathogenicity after 3 days of inoculation, data were recorded after 3,5 and 7 days (Table 4). Isolate Sc11 was the most virulent isolate and recorded disease severity ratio (14.63 and 18.0%) for length and weight respectively, followed by Sc12 Whereas isolates Sc3, Sc7, Sc4,Sc5 and Sc6 were the least virulent on pods, while other isolates gave moderate value. After 5 days, also isolates Sc11, was the most virulent isolate and recorded disease severity ratio (57.72 and 62.67%) for length and weight respectively. Followed by Sc8 and Sc 9 .

Whereas isolates Sc3 and Sc7 were the least virulent on pods, while other isolates gave moderate value. Also, after 7 days isolates Sc11 was the most virulent isolates recorded (81.30 and 85.0%) for length and weight respectively, followed by Sc12 (61.61 and 65.67%) and isolate Sc13 (58.68 and 63.50%). while isolate Sc1 was the least virulent isolate and recorded (30.0 and 38.45) for length and weight respectively. On the other hand, all

isolates produced sclerotia after 14 days isolate Sc11 was the most producing one, followed by Sc4, while isolate Sc3 was the lowest.

Table 4.Variation among *Sclerotinia sclerotiorum* isolates pods inoculation technique on Valentino cultivar.

Isolate	Disease severity after inoculation						Number sclerotia after 10 days
	3 Days		5 Days		7 Days		
	Length	Weight	Length	Weight	Length	Weight	
Sc 1	5.69 cd	8.33 cd	14.18 j	18.60 f	30.00 h	38.45 j	5.500 bcd
Sc 2	7.18 c	11.87 b	28.91 h	33.9 e	56.62 bc	61.50 cd	5.800 bcd
Sc 3	1.48 f	3.50 f	8.84 k	14.33 g	53.36 cd	57.67 de	3.333 e
Sc 4	3.92 de	5.17 ef	39.20 d	44.00 b	47.92 ef	52.45 fg	6.100 abc
Sc 5	4.24 de	8.30 cd	36.80 e	40.55 bc	43.92 fg	45.90 i	3.100 e
Sc 6	4.41 de	7.53 de	30.71 g	36.00 de	51.42 cde	56.00 ef	4.500 de
Sc 7	2.46 ef	4.27 f	15.15 j	19.50 f	48.41 def	52.33 fg	4.300 de
Sc 8	6.68 c	9.73 bcd	46.49 b	64.50 a	49.72 de	53.70 fg	0.3067 f
Sc 9	6.61 c	10.17 bc	41.28 c	61.67 a	46.37 efg	51.00 gh	3.333 e
Sc 10	7.55 c	11.50 b	38.97 d	41.45 bc	42.29 g	46.33 i	5.300 bcd
Sc 11	14.63 a	18.00 a	57.72 a	62.67 a	81.30 a	85.00 a	7.500 a
Sc 12	11.95 b	17.67 a	30.65 g	35.00 de	61.61 b	65.67 b	4.900 cd
Sc 13	7.69 c	10.18 bc	33.88 f	38.50 cd	58.68 b	63.50 bc	5.300 bcd
Sc 14	6.56 c	9.77 bcd	25.00 i	43.44 b	43.44 fg	47.50 hi	6.500 ab
L.S.D at 5 %	**2.120**	**2.579**	**1.672**	**4.191**	**5.247**	**3.836**	**1.515**

B- Physiological parameters:

Resuts in Table (5) show clear variation among isolates of *sclerotnia sclerotiorum* according to linear growth, number and size of sclerotia. Isolates, Sc3, Sc6, Sc9, Sc10, Sc11 and Sc12 were the fast growers followed by Sc14, Sc13, Sc1 and Sc2 while isolates Sc5, Sc7, Sc4 and Sc8 were the slow grower after incubation. On the other hand, isolate Sc11 followed by Sc6 recorded the highest number of sclerotia (28.9 and 25.5) respectively, while isolates Sc8 recorded the lowest number of sclerotia by (2.4) after 14 days from incubation .Also, the results showed that reverse relationships

between the numbers of sclerotia per plat and the size of sclerotia where isolate Sc11 gave 28.9 sclerotia/plate and the size of sclerotia was 1.68 cm. Also, isolate Sc6 gave 25.5 sclerotia/plate and the sclerotial size was 0.90 cm ,while isolate Sc8 gave 2.4 sclerotia/plate and the size of sclerotia was 0.8 cm. Also, isolate Sc13 gave 17.1 sclerotia/plate and the sclerotial size was 1.46cm.

Table 5.Variation among isolates of *Sclerotinia sclerotioeum* due to linear growth (mm), sclerotia formation and size of sclerotia (cm) on PDA medium at 20±1 ċ.

***Sclerotinia sclerotiorum* (Sc) Isolates**	**L.G mm**	**N.S after 14 days**	**S.Z cm**
Sc 1	80.67	18.6	0.50
Sc 2	80.17	11.2	0.98
Sc 3	90.00	20.1	0.48
Sc 4	70.83	9.6	0.74
Sc 5	60.33	11.5	0.62
Sc 6	90.00	25.5	0.90
Sc 7	70.50	15.9	0.80
Sc 8	70.83	2.4	0.80
Sc9	90.00	8.5	1.10
Sc 10	90.00	10.7	0.52
Sc 11	90.00	28.9	1.68
Sc 12	90.00	15.3	0.92
Sc 13	80.67	17.1	1.46
Sc 14	80.83	12.0	0.68

Sc = *Sclerotinasclerotiorum.* L.G = Linear growth.

N.S = Number sclerotia / plate. S.Z = Sclerotia size

Discussion

Snap bean is one of the most important leguminous crops cultivated in Egypt where the seeds and pods are rich in calcium, some vitamins, proteins, mineral salts, some amino acids especially lysine. It is characterized by its well growth in the moderate regions.The pre- and postharvest losses in world crops due to fungal disease may amount to more than 12% in developing countries (Agrios 1997).White rot (*Sclerotina sclerotorium*) reduces the shelf life and market values of food commodities and renders them unfit for human consumption and cause undesirable effect on human health (Sharif, *et al.,* 2010, Williams, *et al.*, 2004). Snap bean pods (*Phaseolus vulgaris* L.*)* decay during the growth at field, storage, transport, marketing or export by a variety of fungi mainly: *Sclerotinia sclerotiorum, Botrytis cinerea* and *Pythium aphanidermatum*, which are pathogens on more than 200 species of vegetables during storage (Catherin *et al*, 2000, Siviero and Motton, 2000, Naffa and Rabie, 2006).

Different fungi were isolated from naturally infected snap bean pods which were collected from different localities of some Egyptian Governorates. The highest number of isolated fungi was recorded on bean pods samples collected from Behera while, the least number of isolated fungi was recorded on samples collected from sohag . Infection was higher in winter as compared with autumn growing season

As for the isolated fungi, five different genera of fungi namely*; Sclerotinia sclerotiorum, Botrytis cinerea, Fusarium* sp, *Rihzcotina Solana* and *Alternaria* sp. were isolated from naturally infected snap bean pods which collected from the different Egyptian Governorates. This was agree with Ahmed (2010) and Fahiem (2010), who found that the seven isolated fungi i.e., *Alternaria spp., Botrytis cinerea, Fusarium* sp., *Mucor* sp., *Pythium aphanidermatum, Rhizoctonia solani* and *Sclerotinia sclerotiorum* from naturally infected snap bean pods which collected from the different Egyptian Governorates were able to infect the wounded and un-wounded bean pods of cv. Paulista. *Sclerotinia sclerotiorum* and *Botrytis cinerea* were most dominant followed by *Alternaria* spp., *Rihzctonia solani* and *Fusarium* spp. Isolated fungi from decayed pods were identified according to Gilman (1957), Ellis (1971) and Barnett and Hunter (1972). While *Sclerotinia sclerotiorum* identification was achieved according to its morphological characteristics of mycelium and sclerotia (Abuel-Ela, 1993).

Concerning the main isolated fungi which recorded the highest isolation number and frequency, *Sclerotinia sclerotiorum* recorded the highest isolation number on bean pods samples collected from Behera, Ismailiya and Kafer El-shikh. Meanwhile, the least numbers of *Sclerotinia sclerotiorum* isolates were recorded in Sohag. These results could be discussed in light of the findings of Shah and Dillard (2007) who mentioned that *Sclerotinia sclerotiorum* caused the white mold of snap bean is one of

the most destructive diseases of snap bean where it caused 2.2% infection to pods out of 40% to the whole bean plants). While, Fath El-bab (2013) isolated and identificate *Fusarium solani, Rhizoctonia solani* and *Fusarium oxysporum* from green been collected from different Governorates in Egypt. *Macrophomina phaseolinae, Pythium* spp and *Sclerotium rolfsii* show less frequent. All tested fungi showed some pathogenic capability to infect snap bean pods (valentino, cv.). All isolates of*sclerotinia.sclrotiorum*and *Botrytis cinerea* showed the highest level of both disease incidence and disease severity for snap bean pods which kept at $20\pm1^{o}C$ for 10 days. While*Fusarium solani* showed the middle level of disease incidence and disease severity. No significant differences were recorded between *Alternaria alternate* and *Rihzoctonia solani*. This was agree with Hatamleh *et al.* (2013) who studied the pathogenicity of *Scleritinia sclerotiorum*to 11 different bean cultivars and they observed significant differences between different cultivars.

Regarding to the variation among isolates of *S.sclerotioum*, the Obtained results showed clear variation among isolates of *sclerotnia sclerotiorum* according to linear growth, number and size of sclerotia. Isolates, Sc3, Sc6, Sc9, Sc10, Sc11 and Sc12 were the fast growers followed by Sc14, Sc13, Sc1 and Sc2 while isolates Sc5, Sc7, Sc4 and Sc8 were the slow grower after incubation. On the other hand ,isolate Sc11 followed by Sc6 recorded the highest number of sclerotia.

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