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Characteristics and Control of *Colletotrichum gloeosporioides* Isolates in Behera Governorate, Egypt

Eman El-Argawy
Plant Pathol. Dept., Fac. of Agric., Damanhour Univ.

Eight isolates of *Colletotrichum gloeosporioides* were recovered from guava and banana fruits showed anthracnose symptoms collected from different orchards in Behera Governorate. Isolates were characterized according to types of colony colour, colony diameter, conidium shape and size, appressorium shape and size and the effect of different temperature degrees on isolates radial growth. The isolates showed mostly gray colony phenotype while the olive-gray and the dark-gray phenotypes were also existed in four out of the investigated eight isolates. Diameter of the developed colonies ranged between 80 and 85 mm. Conidia of the developed eight isolates were mostly monomorphic and exhibited cylindrical, hyaline conidia with size ranged between 14.5 and 19.1 µm for length and 4.4 and 6.5 µm for width. Clavate appressoria were recorded in all isolates. Irregular appressoria were also recorded accompanied with the clavate ones in five of the eight investigated isolates. The optimal temperature for radial growth was 25°C with rate of 8.0 - 8.37 cm, five days after inoculation. All isolates were pathogenic on different host species Guava cv. Balady, Apple cv. Golden Delicious, Banana cvs. Balady and Grantidin, Mango cv. Ewasy and Pepper cv. Balady. Apple fruits were the most susceptible for the infection by *C. gloeosporioides* isolates while pepper was the most tolerant for the infection. Moreover RAPD-PCR analysis using five random oligonucleotide primers revealed DNA fingerprints and considerable variations were revealed with primers tested. However, the RAPD-PCR phylogenetic analysis did not reveal known genetic entities for isolates from each guava and banana, since it did revealed two clusters for the highly virulent isolates and the moderately virulent isolates in the dendrogram of the tested *C. gloeosporioides* isolates. Anthracnose control of fruits by chitosan as a natural product was tested. The *in vitro* 2.5 % chitosan application significantly inhibited the growth of *C. gloeosporioides* isolates tested by 96.6 % on agar plates. The *in vivo* tests on fruits of different species, *i.e.* banana, mango, apple and guava confirmed the *in vitro* results. The chitosan treatment to artificially infected fruits reduced the development of symptoms, at the different chitosan concentrations, *i.e.* 1.5%, 2% and 2.5%. The 2.5% chitosan was the most affective concentration for anthracnose control in the different fruit species. The study supported the view that chitosan offers a safe alternative to synthetic fungicides in postharvest anthracnose diseases control and could be considered as a potential agrochemical of low environment impact.

Keywords: Anthracnose, chitosan and *Colletotrichum gloeosporioides*.

Colletotrichum spp. are economically important pathogens that cause anthracnose in a wide range of woody and herbaceous crops. They cause significant economic losses to crops in tropical, subtropical and temperate regions. Cereals, legumes, ornamentals, vegetables and fruit trees may be seriously affected by the pathogen (Bailey and Jeger, 1992). Many pathogenic strains in the genus have been frequently recognized as *C. gloeosporioides* or *Colletotrichum* sp. (Promputtha *et al*., 2002). *Colletotrichum gloeosporioides* has been associated with quiescent infections and post-harvest diseases on several fruits such as avocado, mango, papaya, guava, banana, citrus, apple and grapes (Alahakoon *et al*., 1994 and Timmer *et al*., 1998).

Banana (*Musa sapientum* L.) is one of the most popular fruits. *Colletotrichum* sp. can infect banana fruits at any time during the growing season in the field (Simmonds and Mitchell, 1940). Banana anthracnose usually starts as quiescent infections on green fruit in the field. Therefore, symptoms generally can be seen only in overripe fruits. Anthracnose becomes a serious problem when bananas are shipped as bunches for a long time and ripened under high temperature (Prusky and Plumbley, 1992).

Also, guava (*Psidium guajava*) a vitamin C enrich. It is an important fruit in many parts of the world where the climate is suitable for its production (Samson, 1986). The anthracnose has become a serious obstacle to guava cultivation (Rahman *et al*., 2003 and Amusa *et al*., 2005).

Colletotrichum gloeosporioides was commonly isolated from a range of host species (Bussaban *et al*., 2001 and Photita *et al*., 2001). Strains of *C. gloeosporioides* and *C. musae* were also detected in banana (Jeger *et al*., 1995). Differentiation between *Colletotrichum* spp. based on host rang or host origin may not be reliable, since taxa such as *C. acutatum, C. gloeosporioides, C. graminicola* and others infect a broad range of host plants (Sutton, 1992). Traditional identification and characterization of *Colletotrichum* spp. species has relied primarily on differences in morphological features such as colony colour, size and shape of conidia and appressoria, optimal temperature for growth, growth rate and presence or absence of setae (Smith and Black, 1990; Gunnell and Gubler, 1992; Sutton, 1992 and Photita *et al*., 2005). Recently, a variety of molecular approaches have been used to discriminate various *Colletotrichum* spp. (Denoyes-Rothan *et al*., 2003) or to study the genetic diversity within *Colletotrichum* spp. (Freeman *et al*., 2001). Random amplified polymorphic DNA (RAPD) or arbitrary primer – PCR or (ap-PCR) has been used extensively for identification and characterization of isolates in *C. gloeosporioides* (Mills *et al*., 1992; Alahakoon *et al*., 1994; Freeman *et al*., 1996; Freeman *et al*., 1998; Weeds *et al*., 2003; Xiao *et al*., 2004; Photita *et al*., 2005; Shampatkumar *et al.*, 2007 and Gupta *et al*., 2010).

Traditionally, the use of synthetic fungicides has been the preferred post-harvest treatment to control this microorganism (Aked *et al*., 2001). However, over time the reported use of fungicides has resulted in serious problems; the pathogens have developed resistance and residue levels have considerably increased (Mari *et al*., 2003). Chitosan is a naturally occurring polysaccharide derived from chitin that has

exhibited potential to control several post-harvest plant diseases and to extend the shelf life of fruits and vegetables (Meng *et al*., 2008 and Badawy & Rabea, 2009). Several reports have shown that chitosan has antimicrobial activity and can interfere with spore germination and mycelial growth of phytopathogenic fungi (Rabea *et al*., 2003 and Muňoz *et al*., 2009). It was reported that chitosan confers protection against *Botrytis cinerea* in *Vitis vinifera* and controlled grey mould in cucumber plants (Romanazzi *et al*., 2006 and Nascimento *et al*., 2007). Tomato seeds were also, protected against *Fusarium oxysporum* after immersion into a chitosan solution (Borges *et al*., 2000).

The objectives of the present study were to 1) identify the casual agent of anthracnose on guava and banana fruits collected in Behera Governorate, 2) to reveal its morphological characteristics, genetic variability and pathogenicity on different host species and 3) to investigate the potential of chitosan treatment to control post-harvest anthracnose on banana, guava, mango and apple.

Materials and Methods

1. Isolation and identification of the causal fungus:

Fruit samples showing anthracnose symptoms were collected from different guava and banana orchards in Behera Governorate. Pieces of tissue were surface-disinfested by 1.5% sodium hypochlorite for 7-10 min, rinsed three times in sterile distilled water, air – dried and plated on to potato dextrose agar (PDA) amended with streptomycin (100 µg/ml) and lactic acid (25 µg/ml). Plates were incubated at 25ºC for five days and then purified using the hyphal tip technique on new PDA medium; the developed colonies were identified according to Mordue (1971). The isolates were sub-cultured on PDA slants and kept at 4ºC for preservation and further use.

2. Characteristics of the recovered isolates:

Colony colour and diameter were recorded for the recovered isolates grown on (PDA) six days after inoculation at 25ºC in darkness according to Peres *et al*. (2002). The shape and size of conidia of the isolates were, also investigated under the light microscope 10 days after incubation at 25ºC, in 25 conidia. Appressoria were produced using a slide culture technique, in which 10 mm^2 plugs of PDA were placed in an empty Petri dish. The edge of the agar is inoculated with spores taken from a sporulating culture and a sterile cover slip is placed over the inoculated agar, after 7 days the shape and size of the appressoria formed on the underside of the cover slip were recorded (John-ston and Jones, 1997). Also, a set of four PDA plates were inoculated at the centre with 5mm diameter mycelial plugs excised from the margin of a 7-day-old PDA culture. The plates were incubated in darkness at a range of temperatures, 5, 10, 15, 20, 25, 30 and 35ºC, diameters of the developed colonies and the radial growth rate were recorded 5 days after inoculation.

3. Pathogenicity and reaction of host species:

Pathogenicity and inter reaction of host species of the recovered isolates were tested by inoculating the physiologically mature banana cvs. Balady and Grantidin, guava cv. Balady; mango cv. Ewasy; apple cv. Golden Delicious, and pepper

(cv. Balady), were kindly provided by EL-Maghraby Agricultural Farms. The fruits were first surface disinfested by immersion in 10% NaOCl for 4 min, then rinsed in sterile distilled water and air dried. Four fruits for each cultivar were placed in plastic chambers and inoculated with discs (5 mm diameter) of PDA culture of the tested isolates according to Lim *et al*. (2002). Controls were inoculated with PDA plain discs. Moistened paper towels were placed in the plastic chambers containing the inoculated fruits and incubated at 25ºC for 5 days and then the disease severity was evaluated.

4. Molecular characterization of the recovered C. gloeosporioides isolates:

The eight *C. gloeosporioides* isolates recorded from banana and guava fruits, showed anthracnose, collected from different orchards in Behera Governorate were analyzed for the DNA banding pattern.

4.1- Genomic DNA extraction:

Isolates were grown on potato dextrose broth for 10 days at 28±2ºC in darkness. Mycelial mats were harvested by filtration using filter paper No. 1. Then, DNA was extracted using the hexadecyltrimethyl ammonium bromide method according to Murray and Thompson (1980). Concentration and purity of the obtained DNA were determined and adjusted using the standard methodology of Sambrook *et al*. (1989).

4.2- Random amplified polymorphic DNA (RAPD):

DNA from *C. gloeosporioides* isolates was amplified by the RAPD methods (Williams *et al*., 1990) using five random oligonucleotide primers shown in (Table 1). Amplification was conducted in a Thermocycler (Eppendorf, Germany) programmed for 35 cycles. The entire reaction mixtures were loaded on 1.5% Agarose gel and amplified DNA fragments were resolved by electrophoresis and stained by ethidium bromide and photographed under UV light (320 nm) according to Jana *et al*. (2003). All chemicals were Bio-Rad products.

Table 1. Nucleotide sequences of 5 primers used to screen the polymorphism of *C. gloeosporioides* isolates recovered in the present study

Primer code	Nucleotide sequence (5′ to 3′)
BAR	CCA GGC AAT TTC ATC AAG CC
BAQ	GGT CTT GAA GTC GAG CGC AG
18	CGC ATA GGA CCC GAT GCG AG
A9B4	GGT GAC GCA GGG GTA ACG CC
A9B10	GGA CTG GAG GTG GAT CGC AG

4.3- Phylogenetic analysis:

Band patterns of DNA developed in the RAPD-PCR analysis were scored visually for each tested isolate. Dendrogram of the phylogenetic relationship was produced using the software program "Statistical Version 5.0" according to Rholf (2000).

5. Control studies:

5.1- In vitro effect of different concentrations of chitosan on the mycelium growth of C. gloeosporioides isolates:

The chitosan solution was prepared by dissolving chitosan 2.5% deacetylated in 0.25 N HCl with continuous stirring at 50ºC. Insoluble material was removed by centrifugation and chitosan was precipitated by neutralization with 1 N NaOH, washed three times with deionised water and air dried (El-Ghaouth *et al*., 1991) . For incorporation into the PDA, purified chitosan was dissolved by stirring in 0.25 N HCl and adjusting the pH to 5.6 using 1 N NaOH. Chitosan solution was added to the PDA medium to obtain final concentrations of 0, 1.5, 2 and 2.5% after autoclaving before pouring into Petri dishes. A 5 mm diameter disc from the margin of an actively growing PDA culture of the tested isolate was placed side down at the centre of each Petri dish. Four replicates were used for each chitosan concentration. The inoculated plates were incubated at 25ºC for 5 days in the dark. Mycelial growth was determined by measuring colony diameter. Two isolates exhibited the highest aggressiveness in the pathogenicity test were used in the control studies.

5.2- Effect of chitosan to control anthracnose on different host species:

Mature fruits were selected based on uniformity of size and absence of visible symptoms. Fruits were surface sterilized in 10% NaOCl for 5 min, rinsed in sterile water and then air dried. Superficial wounds in the epidermis, made by a sterile scalpel (0.5-1.0 cm deep), were treated with 15 µl of the chitosan concentrations (1.5, 2 and 2.5%). For inoculation with the fungus, a 4 mm diameter disc of PDA was removed from the margin of an actively growing PDA culture and placed mycelium side - upside down on the wound. Four fruits were placed in plastic chambers. Moistened paper towels were placed in the plastic chambers containing the inoculated fruits and incubated at 25ºC. Lesion diameters on the treated fruits were measured 5 days after inoculation.

6. Statistical analysis:

The obtained data were statistically analyzed, whenever needed, using the American SAS/STAT Software, version 6 and means were compared by the least significant difference test (LSD), (Anonymous, 2000).

R e s u l t s

1- Isolation of the recovered C. gloeosporioides isolates:

Six *Colletotrichum gloeosporioides* isolates from guava and two isolates from banana were isolated from fruits showed anthracnose in different orchards in Behera Governorate. Considerable variations were recorded among the recovered isolates as shown in Fig. (1) and Table (2).

2- Characteristics of the recovered C. gloeosporioides isolates:

2.1. Colony colour:

Colour of the developed colonies of the recovered *C. gloeosporioides* isolates on PDA ranged between plain gray, olive gray, and dark grey (Fig.1). However, three isolates out of eight recovered were of gray colonies. Also, three other isolates exhibited olive gray colonies, while, only two isolates showed dark-gray colonies.

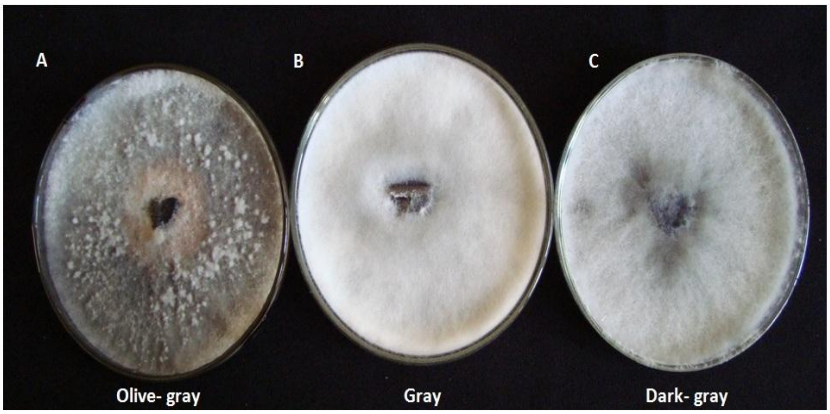

Fig. 1. Colony colour phenotypes of *C. gloeosporioides* isolates recovered from banana and guava fruits, showed anthracnose symptoms, collected from different orchards in Behera Governorate.

Table 2. Morphological characteristics of *Colletotrichum gloeosporioides* isolates recovered from banana and guava fruits, showed anthracnose, collected from Behera Governorate

Isolates code No.	Source	Colony		Conidium**			Appressorium***		
		Colour	Diameter (mm)*	Shape	Size (µm)		Shape	Size (µm)	
					Length	Width		Length	Width
CoG1	Guava	Gray	85 ±2.28	Cylindrical	17.4±2.0	4.5±1.5	Clavate-irregular	9.5±2.7	5.3±2.5
CoG2	Guava	Gray	85±4.43	Cylindrical	14.5±1.4	4.4±1.7	Clavate	9.8±1.7	6.2±2.6
CoG3	Guava	Dark-gray	85±4.2	Cylindrical	17.8±2.8	4.3±1.3	Clavate	9.9±2.7	6.7±2.6
CoG4	Guava	Olive-gray	85±4.26	Cylindrical	19.1±2.0	5.5±2.6	Clavate-irregular	11.5±2.5	6.5±1.5
CoG5	Guava	Dark-gray	80±3.34	Cylindrical	15.3±1.5	6.5±1.4	Clavate-irregular	10.4±2.1	6.8±1.8
CoG6	Guava	Olive-gray	85±4.4	Cylindrical	16.7±1.6	6.5±2.1	Clavate-irregular	10.2±1.8	6.6±2.1
CoB7	Banana	Gray	80±1.1	Cylindrical	14.7±2.6	5.1±2	Clavate	8.8±1.7	6.1±2.6
CoB8	Banana	Olive-gray	85±3.3	Cylindrical	18.2±2.6	5.5±1.5	Clavate-irregular	10.3±2.5	6.3±1.6

* Colony diameter was assessed five days after inoculation and incubation at 25 ºC, ± SD.
** Mean of 25 conidia.
*** Mean of 25 appressoria.

No linkage was revealed between source of the isolates, *i.e.* banana or guava and colour phenotype of the developed colonies (Table 2).

2.2. Colony diameter:

C. gloeosporioides isolates recovered exhibited colony diameters ranged between 80±1.1 mm and 85±4.4 mm recovered five days after inoculation. No indications for a distinct colony diameter linked to the source of the tested isolates, *i.e.* banana or guava origin (Table 2).

2.3. Conidia shape:

All the eight recovered isolates investigated of *C. gloeosporioides* showed typically cylindrical conidia with no effect for the isolate source (Fig. 2).

2.4. Conidia size:

Size of conidia of the recovered *C. gloeosporioides* isolates ranged between 14.5±1.4 µm and 19.1±2.0 µm for length and also between 4.4±1.7 µm and 6.5±2.1 µm for width. This was in a sample of 25 conidia for each isolate. No certain size for conidia was associated with source of the *C. gloeosporioides* isolates from banana or guava (Table 2).

2.5. Appressoria shape and size:

Shape of appressoria of the *C. gloeosporioides* isolates investigated was mostly clavate, size ranged between 8.8±1.7 µm and 11.5±2.5 µm in length and between 5.3±2.5 µm and 6.8±1.8 µm in width. However, irregular appressoria also appeared with clavate ones in five out of the eight investigated isolates. The two appressoria types were not confined to any of the two isolate sources, *i.e.* banana or guava (Fig. 2 and Table 2).

2.6. Mycelial growth rate at different degrees of temperature:

Data illustrated in (Fig. 3) revealed that the optimum temperature for *in vitro* mycelial growth of *C. gloeosporioides* isolates tested was 25-30ºC. Increasing this degree of temperature, mycelial growth considerably decreased while, decreasing this degree, the mycelium growth was obviously low particularly between 5 and 15ºC.

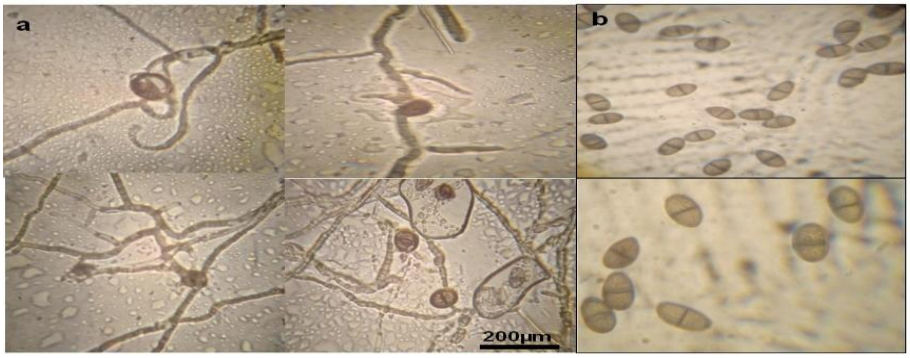

Fig. 2. Appressoria (a) and conidia (b) of *C. gloeosporioides* isolates recovered from banana and guava fruits collected from different orchards in Behera Governorate.

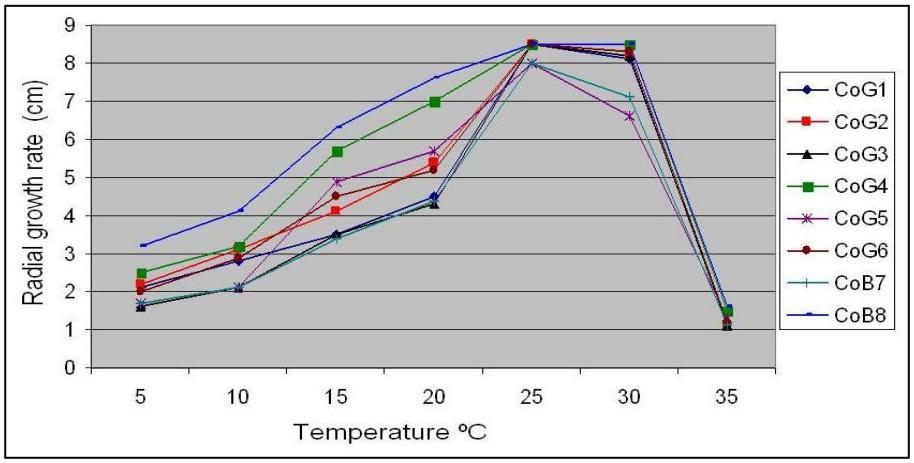

Fig. 3. The effect of temperature on radial growth rate of *C. gloeosporioides* isolates recovered from banana and guava fruits.

3. Pathogenicity and reaction of host species:

Fruits of banana (cvs. Balady and Grantidin), mango (cv. Ewasy), apple (cv. Golden Delicious), guava (cv. Balady) and pepper (cv. Balady) were individually inoculated with the eight isolates of *C. gloeosporioides*. All isolates were virulent to the different host species tested but varied in this respect. Isolate CoB8 (banana) was the most aggressive and showed a mean lesion diameter of 4.15 cm. This was followed by isolates CoG4 and CoG5 (guava) showed 3.76 cm and 3.16 cm mean lesion diameter, respectively. Other isolates, however, showed significantly lower lesion diameters of anthracnose. On the other hand, significant differences in mean lesion sizes were recorded on guava, banana, mango, apple and pepper fruits (Table 3 and Fig. 4). Apple fruits were the most susceptible for all isolates as exhibited mean anthracnose lesion diameter of 3.58 cm. This was followed by mango and guava where mean lesion diameter of anthracnose were 3.44 cm and 2.94 cm, respectively. Banana cv. Grantidin and guava cv. Balady exhibited more tolerance compared to other host species, while pepper was of the most tolerant and exhibited anthracnose lesions of 0.47 cm mean diameter.

4- Molecular characteristics of the recovered C. gloeosporioides isolates:

4.1. Random amplified polymorphic DNA (RAPD):

The use of five primers in RAPD-PCR showed clear differences among the eight tested of *C. gloeosporioides* isolates on basis of the amplified product band patterns revealed with each primer. The amplification profiles with the primers are showed in Fig. (5), they exhibited that primers tested succeeded to reveal polymorphic patterns and considerable variations among the isolates. Also, high similarity (88%) was observed between CoG1 isolate from guava and CoB7 isolate from banana but this was not the case between CoG1 and CoB8 as similarity was as low as 58%. The low similarity could indicate host specialization for the *C. gloeosporioides* isolates on guava and banana (Table 4).

Table 3: Pathogenicity and reaction of host species to *C. gloeosporioides* isolates recovered from banana and guava fruits collected from different orchards in Behera Governorate

Isolates code No.	Lesion diameter (cm)*						Mean
	Banana		Mango	Apple	Guava	Pepper	
	(cv.Balady)	(cv.Grantidin)	(cv.Ewasy)	(cv.Golden Delicious)	(cv.Balady)	(cv.Balady)	
CoG1	1.62**	1.83	2.78	2.7	2.05	0.5	1.91 ^{g}
CoG2	2.25	3.72	4.5	3.56	2.32	0.5	2.86 ^{d}
CoG3	2.35	2.66	3.22	3.66	3.65	0.7	2.67 ^{e}
CoG4	2.88	4.52	4.92	4.73	5.06	0.5	3.76 ^{b}
CoG5	1.75	2.36	4.83	5.66	3.9	0.5	3.16 ^{c}
CoG6	1.73	1.58	2.75	2.66	3.35	0.5	2.09 ^{f}
CoB7	1.66	1.5	2.36	3.75	1.76	0.6	1.97 ^{fg}
CoB8	4.73	4.78	5.03	5.5	4.42	0.5	4.15 ^{a}
Control	0	0	0	0	0	0	0 ^{h}
Mean	2.11 ^{e}	2.55 ^{d}	3.44 ^{b}	3.58 ^{a}	2.94 ^{c}	0.47 ^{f}	

* Diameters of lesions were estimated 5 days after inoculation.

** Data are means of 4 replicates.

Values followed by the same letter in the same column or row are not significantly different at P = 0.05.

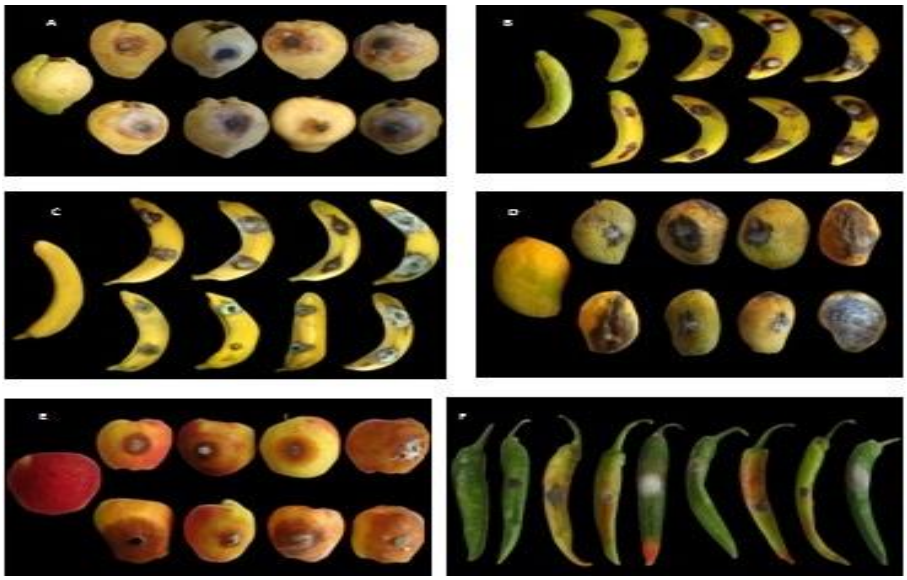

Fig. 4. Pathogenicity tests of *C. gloeosporioides* isolates recovered from banana and guava on different host species, from left to right, Control, CoG1, CoG2, CoG3, CoG4, CoG5, CoG6, CoB7 and CoB8. A: for guava (cv. Balady), B: banana (cv. Balady), C: banana (cv. Grantidin), D: mango (cv. Ewasy), E: apple (cv. Golden Delicious) and F: pepper (cv. Balady).

Table 4. Similarity matrix, in percentage, among the analyzed *C. gloeosporioides* isolates based on RAPD band pattern analysis and Jaccard index

Isolate	COG1	COG2	COG3	COG4	COG5	COG6	COB7	COB8
COG1	100	77	57	77	50	81	88	58
COG2		100	81	81	64	57	64	76
COG3			100	71	83	62	60	62
COG4				100	69	71	69	76
COG5					100	69	57	55
COG6						100	74	62
COB7							100	74
COB8								100

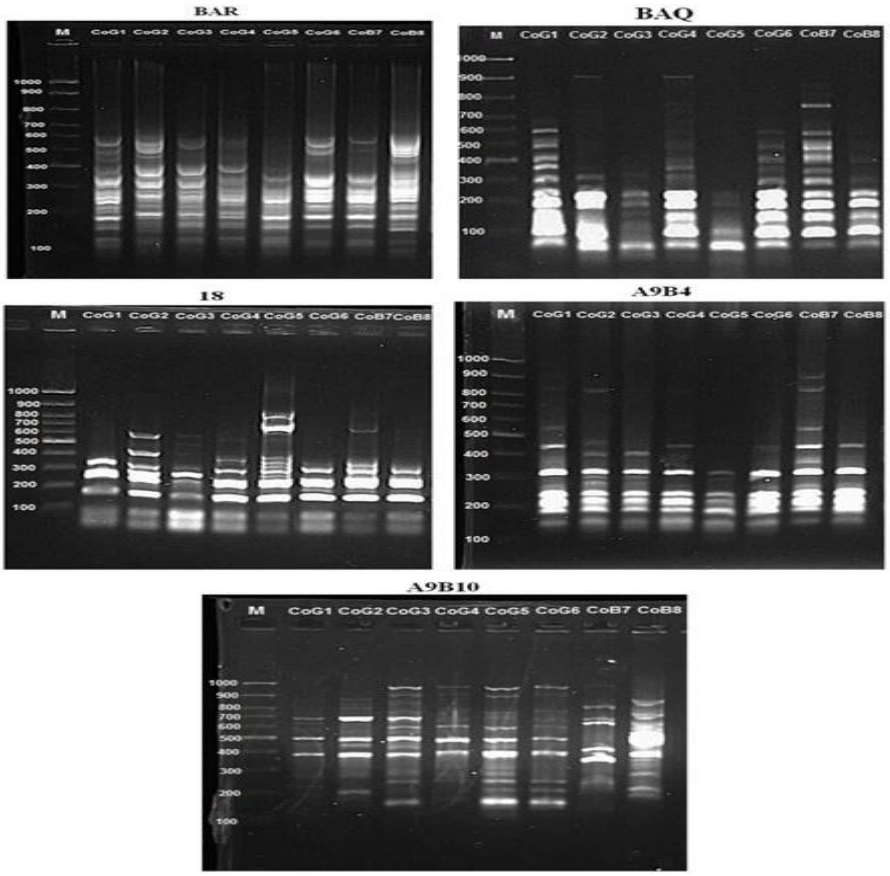

Fig.5. RAPD-PCR banding patterns of *C. gloeosporioides* isolates using five primers, *i.e.* BAR, BAQ, 18, A9B4 and A9B10. (M= DNA marker, Lanes from left to right are *C. gloeosporioides* COG1, COG2, COG3, COG4, COG5, COG6, COB7 and COB8.

4.2. Cluster analysis of RAPD results:

The RAPD band patterns were analyzed using UPGMA method to construct a dendrogram (Fig. 6) indicating the relationship between the eight analyzed *C. gloeosporioides* isolates. The presence or absence of any particular DNA bands was the only factor considered in the computer analysis.

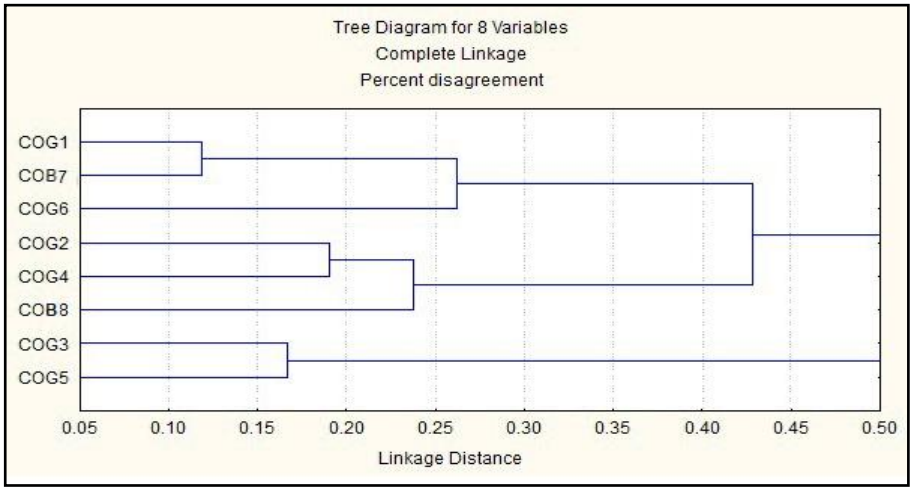

Fig. 6. Dendrogram obtained by clustering (UPGMA method) based on the band pattern obtained by the RAPD-PCR analysis for eight isolates.

The dendrogram obtained show linkage distance which indicated that the experimental isolates were classified into two main clusters. The first cluster A included two sub- clusters, sub-cluster A1 divided into two groups, group1 include isolate CoG1 from guava and CoB7 from banana, while group two included an isolate from guava. Sub-cluster A2 divided into two groups, group1 included isolates from guava while, group2 included single isolate from banana (CoB8 isolate). However, the second cluster (B) included isolate from guava (CoG5 isolate). Also, dendrogram show that the most highly virulent *C. gloeosporioides* isolates (CoB8 & CoG4) recognized in the pathogenicity test were grouped in the same sub- cluster. Meantime, analysis of isolates at the molecular level revealed considerable variations among *C. gloeosporioides* isolates investigated. The primers BAR, BAQ, 18, A9B4 and A9B10 were efficient to reveal each variation. However, the similarity matrix and the developed dendrogram did not support the assumption of host specialization of the *C. gloeosporioides* isolates. However, the most virulent *C. gloeosporioides* isolates, recorded in the pathogenicity tests were located in the same sub-cluster while the moderately virulent ones were grouped in several sub-clusters. The findings did not support the host specialization of *C. gloeosporioides* as the isolates derived from banana and isolates from guava showed the same phenotypes and were virulent on the both host species.

5. Control studies:

5.1- The in vitro effect of different concentrations of chitosan on the mycelium growth of C. gloeosporioides isolates:

Radial growth of tested *C. gloeosporioides* isolates significantly inhibited with increasing concentration of chitosan from 1.5 to 2.5%, 5 days after inoculation (Table 5). Chitosan concentrations of 1.5, 2 and 2.5% inhibited the mycelium radial growth by 78.11, 91.76 and 96.6%, respectively, compared to the untreated control.

Table 5. Colony diameter of *C. gloeosporioides* isolates on PDA amended with different concentrations of chitosan

Treatment	Colony diameter (cm) of different isolates			
	CoG 4	CoB 8	Mean	Inhibition (%)
Unamended control 0%	8.5	8.5	8.5[a]	0.0
chitosan 1.5%	1.73	2.0	1.86[b]	78.11
chitosan 2%	0.54	0.87	0.70[c]	91.76
chitosan 2.5%	0.21	0.36	0.28[d]	96.6

- Values are colony diameters in cm determined 5 days after inoculation.
- Data are means of 4 replicates.

5.2- Effect of chitosan on controlling anthracnose of different host species:

Diameter of anthracnose lesions developed on Banana cvs. Balady & Grantidin, guava cv. Balady, mango cv. Ewasy and apple cv. Golden Delicious fruits inoculated with *C. gloeosporioides* isolates (CoG4 & CoB8) significantly decreased with chitosan treatment at 1.5%, 2% and 2.5% chitosan solutions compared to the untreated control (Table 6). However, the highest effect of chitosan was obtained with the 2.5% solution on the different fruit species. Meantime, the effect was more pronounced (>60% inhibition) on mango cv. Ewasy, banana cv. Balady and apple cv. Golden Delicious, while, on guava cv. Balady the inhibition effect was the less than 50% (Table 6 and Figs. 7 & 8).

Table 6. Lesion diameter on detached fruits of host species inoculated with *C. gloeosporioides* isolates (CoG4 & CoB8) and treated with chitosan in different concentrations.

Treatment	Lesion diameter (cm) of different isolates *														
	Guava (cv. Balady)			Banana (cv. Balady)			Banana (cv. Grantidin)			Mango (cv. Ewasy)			Apple (cv. Golden Delicious)		
	CoG4	CoB8	Mean	CoG4	CoB8	Mean	CoG4	CoB8	Mean	CoG4	CoB8	Mean	CoG4	CoB8	Mean
Chitosan 0%	5.1	4.7	4.90	2.9	4.7	3.80	4.5	4.8	4.65	4.9	5.0	4.95	4.7	5.5	5.10
Chitosan 15%	2.8	3.0	2.90	1.5	1.9	1.70	1.7	1.9	1.80	2.0	2.3	2.15	1.6	1.9	1.75
Chitosan 2%	2.5	2.8	2.65	1.5	1.7	1.60	1.6	1.7	1.65	1.6	2.0	1.80	1.5	1.7	1.60
Chitosan 2.5%	2.2	2.6	2.40	1.5	1.5	1.50	1.6	1.6	1.60	1.5	1.5	1.50	1.5	1.6	1.55
Mean	3.15	3.28	3.21	1.85	2.45	2.15	2.35	2.50	2.43	2.50	2.70	2.60	2.33	2.68	2.49

* Lesion diameters were determined 5 days after treatment. Each figure represents the mean of 4 replicates.

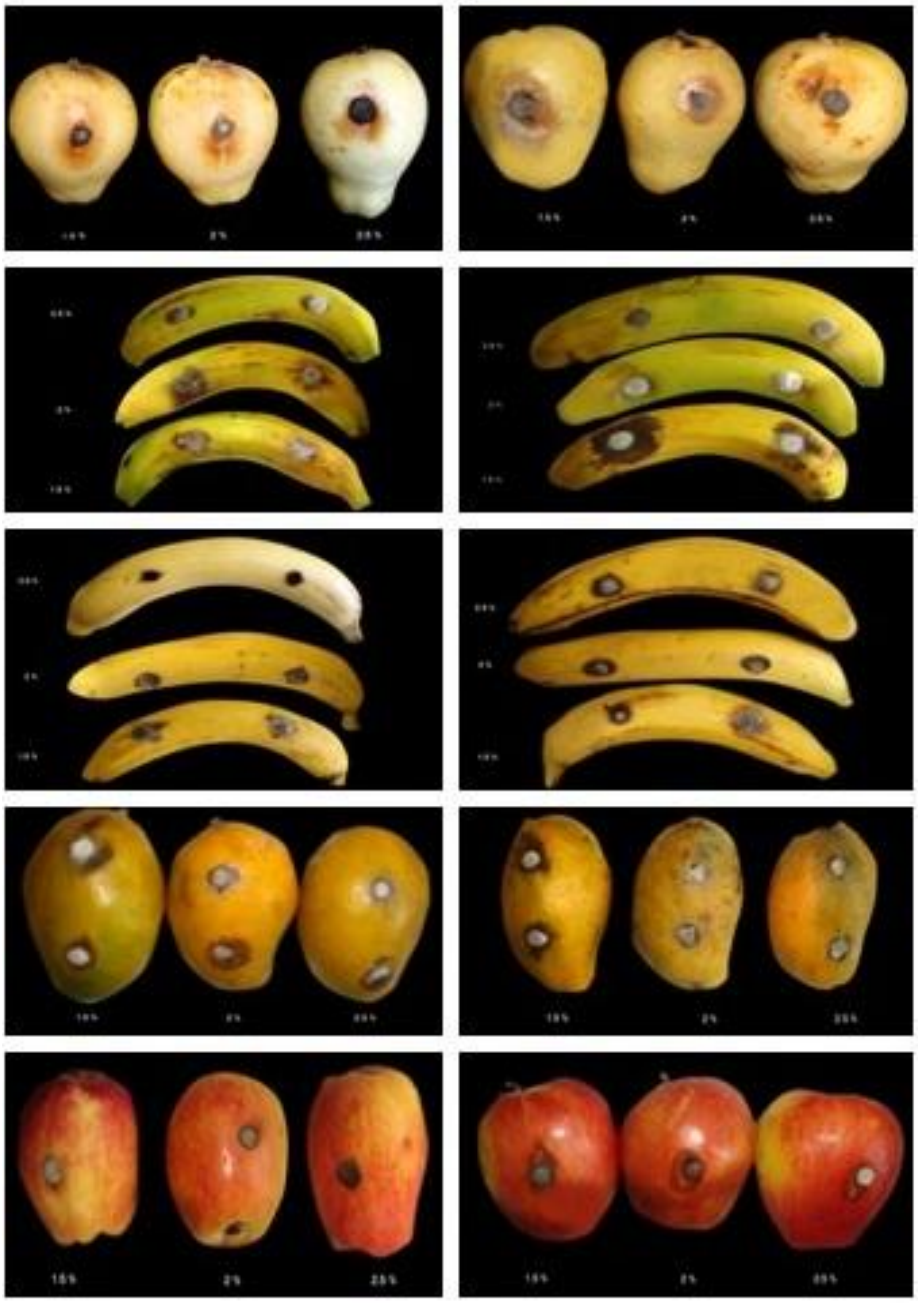

Fig. 7. Lesion diameter on detached fruits of host species inoculated with *C. gloeosporioides* isolates (CoG4 left & CoB8 right) and treated with different concentrations chitosan.

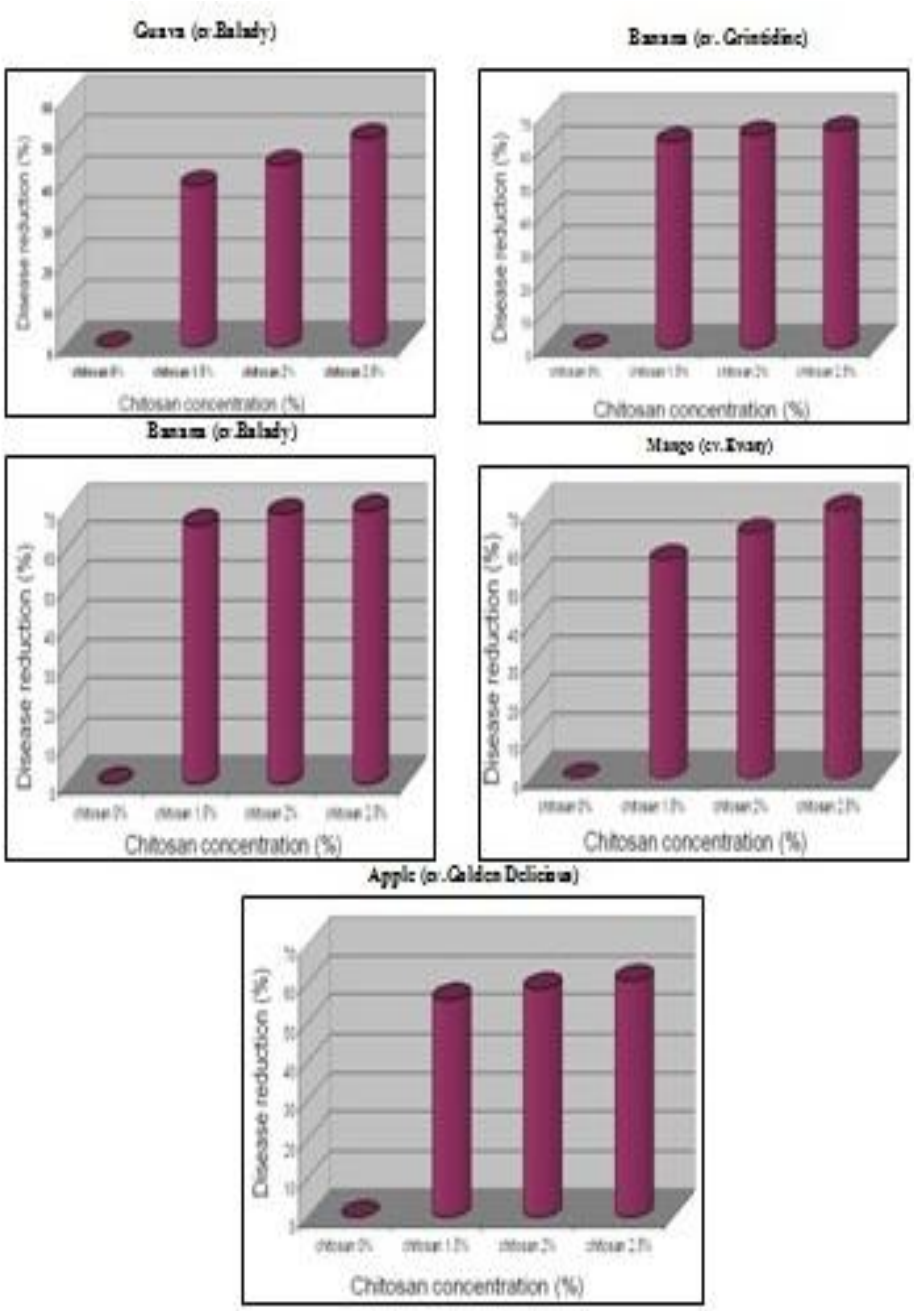

Fig.8. Effect of different concentrations of chitosan on anthracnose development on fruits of different species inoculated with *C. gloeosporioides* isolates, 5 days after treatment and inoculation.

Discussion

Several species belonging to the genus *Colletotrichum* cause important diseases in a wide range of crops. *Colletotrichum gloeosporioides* was reported on a wide variety of fruits, including apple, almond, avocado, guava, mango, banana and strawberry. Eight isolates of *C. gloeosporioides* were recovered from banana and guava fruits, showed anthracnoses, collected from different orchards in Behera Governorate. The isolates showed mostly gray colony phenotype while the olive-gray and the dark-gray phenotypes were also existed in four out of the investigated eight isolates. Diameter of the developed colonies ranged between 80 and 85 mm, six days after inoculation, with radial growth rate 8.0-8.37 cm and 25°C optimal temperature. No linkage was recognized between source of the isolates, *i.e.* banana or guava, and any of the colony-colour phenotypes identified. Conidia of the developed eight isolates were mostly monomorphic and exhibited cylindrical, hyaline conidia with size ranged between 14.5 µm and 19.1 µm for length and 4.4 and 6.5 µm for width. Clavate appressoria were recorded in all isolates. Irregular appressoria were also recorded correlated with the clavate ones in five of the eight investigated isolates. These findings were in harmony with several investigations (Photita *et al.,* 2005; Nguyen *et al.,* 2010 and Pria *et al.,* 2010). Meantime, analysis of isolates at the molecular level revealed considerable variations among *C. gloeosporioides* isolates investigated. The primers BAR, BAQ, 18, A9B4 and A9B10 were efficient to reveal each variation. However, the similarity matrix and the developed dendrogram did not support the assumption of host specialization (Freeman *et al.,* 1998; Gupta *et al.,* 2010 and Sangdee *et al.,* 2011) of the *C. gloeosporioides* isolates. However, the most virulent *C. gloeosporioides* isolates, recorded in the pathogenicity tests were located in the same sub-cluster while the moderately virulent ones were grouped in several sub-clusters. The findings did not support the host specialization of *C. gloeosporioides* as the isolates derived from banana and isolates from guava showed the same phenotypes and were virulent on the both host species (Freeman, 2000; Hong *et al*., 2008; Zakaria *et al*., 2009 and Pria *et al*., 2010). All the recovered isolates were virulent to a range of host species, *i.e.* banana, guava, apple, mango and pepper to different degrees. However, apple fruits cv. Golden Delicious were the most susceptible followed by mango cv. Ewasy, guava cv. Balady, banana cvs. Grantidin and Balady, while pepper cv. Balady was of the highest tolerance according to the size of the anthracnose lesions initiated on the different fruits. These results were in agreement with reports in different parts of the world (Timmer and Brown, 2000; Peres *et al*., 2002; Afanador *et al*., 2003; Sera *et al*., 2007 and Gonçalves-Vidigal *et al*., 2008).

Concerning the control of anthracnoses, there is an increasing interest for the use of the natural products, such as chitosan, in plant diseases control. In the present study, chitosan significantly inhibited the *in vitro* fungal growth of *C. gloeosporioides* isolates at all concentrations (1.5%, 2%, 2.5%) compared to the control. The *in vitro* studies conducted on banana, guava, mango and apple confirmed the *in vitro* results where the 2.5% chitosan solution was the most effective for anthracnose control. These results are in harmony with those of Romanazzi *et al*. (2001), No *et al*. (2007), Muňoz *et al*. (2009) and Reglinski *et al*. (2010). Previous studies demonstrated that the induction of systemic resistance in

plants with natural compounds, including chitosan, was a promising approach for plant diseases control (Gozzo, 2003). Chitosan is an exogenous elicitor whose activity is due to its polycationic structure and its receptor is a 78 kDa binding protein (Chen and Xu, 2005). Faoro *et al*. (2008) and Coqueiro *et al*. (2011), however, showed that the activity of chitosan was attributed to the accumulation of hydrogen peroxidase in treated tissues, which induces a hypersensitive reaction as a consequence of oxidative microburst and phenolic compound deposition. Meantime, Howe (2005) indicated that chitosan activated jasmonic acid synthesis in treated hosts, while, Aziz *et al*. (2006) indicated that chitosan induced the accumulation of phytoalexins in grape vine leaves, which reduced *Botrytis cinerea* and *Plasmopara viticola* infection. Also, there is evidence that the response may be initiated by a chitosan-induced depolarization of the plasma membrane and this event leads to the activation of inducible host defences (Amborabé *et al*., 2008). The experimental data in this study demonstrates that the antimicrobial characteristics of this substance make it a potential, and moreover, a naturally occurring, food coating material. Besides it is non-toxic for humans and has a low environmental impact (Shahidi *et al*., 1999) and was used as a food additive in Korea and Japan and recognized by the International Commission on Natural Health Products as a natural product for the 21st century (No *et al.,* 2007). Results confirmed that chitosan offers a safe alternative to synthetic fungicides in postharvest anthracnose diseases control and could be considered as a potential agrochemical of low environment impact.

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خصائص ومقاومة عزلات *كوليتوتريكم جلويوسبوريويدز* في محافظة البحيره ، مصر

إيمان العرجاوي
قسم أمراض النبات - كلية الزراعة - جامعة دمنهور.

تم الحصول علي ثمانية عزلات من الفطر كوليتوتريكم جليوسبوريويدس وذلك من ثمار الجوافة والموز التي ظهرت عليها أعراض مرض الأنثراكنوز والتي تم الحصول عليها من بساتين مختلفة من محافظة البحيرة.

وقد تم توصيف هذة العزلات طبقا للون المستعمرة وقطر المستعمرة وشكل الكونيديات وحجمها وشكل عضو الألتصاق وحجمة وكذلك تأثير درجات الحرارة المختلفه علي قطر النمو الفطري.

وقد أظهرت معظم العزلات لون رمادي للمستعمرات بينما اللونين الزيتوني – الرمادي والرمادي الغامق ظهرا في أربعة عزلات من أصل الثمانية عزلات المختبرة. تراوح قطر النمو للمستعمرات من ٨٠ – ٨٥ مم . كما أظهرت الثمانية عزلات المختبرة كونيديات متماثلة في الشكل وكانت أسطوانيه شفافة بحجم تراوح من ١٤،٥ ميكرومتر و ١٩،١ ميكرومتر للطول وكذلك ٤،٤ ميكرومتر و ٦،٥ ميكرومتر للعرض. وقد تم ملاحظة أن شكل عضو الألتصاق كان خطافي في معظم العزلات بينما تم تسجيل عضو الألتصاق غير المنتظم مصاحبا للشكل الخطافي في خمسة عزلات من أصل الثمانية عزلات المختبرة. كما أوضحت النتائج أن درجة حرارة ٢٥°م كانت أفضل درجة حرارة للنمو القطري بمعدل تراوح من ٨- ٨،٣٧ سم.

وبأختبار المقدرة المرضية للعزلات أظهرت كل العزلات قدرة علي أحداث الأصابة علي العوائل النباتية الأتية : الجوافة صنف بلدي والتفاح صنف جولدن دليشيس والموز صنفي بلدي وجرنتدين والمانجو صنف عيسوي والفلفل صنف بلدي وكان التفاح هو أكثر الأصناف قابلية للأصابة بعزلات الفطر بينما كان الفلفل أكثرهم تحملا للأصابة.

بأستخدام تقنية تفاعل تسلسل البلمره العشوائي RAPD-PCR لدراسة الاختلافات الوراثيه أظهرت النتائج بأنه ليس هناك أختلاف بين العزلات المتحصل عليها من الجوافة أو الموز وراثيا ولكنها أظهرت أختلافات بين العزلات العاليه في المقدرة المرضية والمتوسطة في المقدرة المرضية.

وقد تم أختبار مقدرة الشيتوزان كمستحضر طبيعي لمقاومة الأنثراكنوز وقد أظهرت النتائج في المعمل أن تركيز ٢،٥ % شيتوزان قد ثبطت نمو الفطر كوليتوتريكم جليوسبوريويدس بصوره معنوية علي أطباق الأجار وكذلك بمعاملة الشيتوزان علي ثمار الأصناف المختلفة والمعداة بصوره صناعيه أدي الي تقليل تطور الأعراض علي الثمار المعداة وذلك بتركيزات ١،٥% و ٢% و تركيز٢،٥% والذي كان أفضل التركيزات لمقاومة الأنثراكنوز علي الثمار المختلفة.

وتهدف الدراسه الي التوصيه بأستخدام الشيتوزان لمقاومة أمراض الانثراكنوز ما بعد الحصاد وكبديل أمن لأستخدام المبيدات الفطرية.