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Morphological Differentiation and Factors Affecting the Growth and Pathogenicity of Some *Colletotrichum* spp.

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Anthracnose is a major disease of different commercial crops. *Colletotrichum* spp. isolates were obtained from different fruit and vegetable plants showing anthracnose symptoms. *Colletotrichum* spp. isolates which were isolated from guava (*Psidium guajava*), mango (*Mangifera indica*), strawberry (*Fragaria ananassa*), pepper (*Capsicum* sp.) and tomato (*Solanum lycopersicum*) were used to carry out this study. Based on cultural and morphological characteristics there are large differences among fungal species of *Colletotrichum* spp. isolates in addition to their pathogenic effects. *Colletotrichum* spp. differed and distinguished according to conidial morphology. Effect of temperature degrees on mycelial growth of *Colletotrichum* spp. isolates from mango, guava and pepper indicate that optimal growth rates were recorded at 30ºC. However, 25ºC was the optimum temperature for strawberry fungal isolates. Host range studies reveal that mango *Colletotrichum* spp. isolate causing severe or moderately rot for apple, peach, mango and strawberry fruits, but appeared less symptoms on tomato and lemon fruits. This study has brought to light a wide variation in the pathogenicity, morphological physiological characters of different *Colletotrichum* spp. isolates.

Keywords: Anthracnose, *Colletotrichum* spp., guava, mango, pepper, strawberry and tomato.

Genus *Colletotrichum* is one of the most important genera of plant pathogens. It has a worldwide distribution mainly in subtropical and tropical regions. *Colletotrichum* spp. cause significant economically losses of plants (generally as anthracnose disease) that affect cereals, grasses, legumes, vegetables, and perennial crops, including fruit trees (Bailey and Jeger, 1992). Great economic losses are due to postharvest disease of fruits such as tomato, pepper, strawberry, mango and guava. *Colletotrichum* spp. are also found on decayed fruits (Tang *et al*., 2003). Morphological characteristics and host range have traditionally been used to define the species. This may be partially due to the wide host range of *Colletotrichum* spp. and the fact that several species may be associated with a single host (Freeman *et al*., 1998).

Colletotrichum acutatum and *C. gloeosporioides* are two members of the genus that are most commonly associated with fruit rots in the literature. *C. gloeosporioides* is considered cumulative species and is found on a wide variety of fruits, including almond, apple, avocado, citrus, mango, olive, and strawberry (Freeman *et al*., 1998; Martín and García-Figueres, 1999; Arauz, 2000 and Timmer and Brown, 2000).

Some *Colletotrichum* spp. have been successfully separated based on a number of characteristics including culture morphology, conidia shape and size, and host range (Smith and Black, 1990; Sutton, 1992 and Förster and Adaskaveg, 1999). On the other hand, differentiation between *Colletotrichum* spp. based on host range or host origin may not be reliable, since taxa such as *C. acutatum*, *C. gloeosporioides* and others infect a broad range of host plants. In separate study, Freeman *et al*. (2001) pointed that Colletotrichum pathogen causes black spot on fruit, root necrosis, and crown rot resulting in mortality of transplants in the field.

The present study aimed to investigate the diversity of genus *Colletotrichum* isolated from some crops in Egypt by studying its morphological characters supported by scanning electron microscope and the host range for each species.

Materials and Methods

Isolation of the pathogens:

Isolates of *Colletotrichum* spp. were obtained from anthracnose lesions of strawberry, pepper, tomato and mango fruits. A small section of the lesion from the leading edge was cut. Five pieces (5×5 mm each), were surface sterilized by dipping in 0.5% sodium hypochlorite for 3-5 minutes and washed in 2 or 3 series of sterile water and then placed on the surface of potato dextrose agar medium (PDA). Plates were incubated at 28±2°C and examined periodically. The growing edges of any fungal hyphae developing from the infected tissues were then transferred aseptically to PDA slants. The fungi were identified following sporulation. The colonies of the fungus were identified according to the Colletotrichum description reported by Sutton (1992) and kindly confirmed by the Assiut Mycological Centre, Fac. of Sci., Assiut Univ. (AUMC). Pure cultures were stored at 4°C on PDA slants.

Morphological and colonial studies:

Growth rate and colony characteristics were recorded from cultures grown on PDA plates. The cultures were incubated in darkness at 28±2°C, The diameter of the colony was recorded daily (three replicates, two measurements per replicate) for 7 days. In morphological characterization, shapes and sizes of the conidia were examined under a light microscope after incubation period. For each isolate spores were suspended in sterile water using a sterile needle. The length, width and length/width as well as spores shape were measured at 400X magnification by using bright field microscope (Ivanovic *et al*., 2007). Present or absent setae, acervuli and sclerotia were noticed. Cultural characteristics and colony growth were observed after 21 days on PDA in darkness such as texture, density, colour zonation, transparency aspect, nature of the growth margin, presence of conidial masses, and colour of the reverse side. Three replicates were prepared for each isolate. The description and determination of *Colletotrichum* spp. was done according to Sutton and Waterston (1970) and Kuramae-Izioka (1997).

Scanning electron microscopic studies:

Small specimens for scanning electron microscopy were taken from culture media inoculated with *Colletotrichum* spp. Samples were immediately fixed in glutaraldehyde (2.5%) for 24 h at 4°C, fixed in osmium tetroxide (1% OSO_4) for one

hour at room temperature (Harley and Ferguson, 1990) and then dehydrated through ascending concentrations of acetone and dried to the critical point. Finally, samples were sputter coated with gold. The examination and photographing were done through a Jeeol Scanning Electron Microscope (T.330A) in the Central Laboratory, Faculty of Agriculture, Ain Shams University.

Pathogenicity tests of Colletotrichum spp. and host rang studies:

Cultures of *Colletotrichum* spp. were flooded with sterile distilled water and filtered through four layers of cheesecloth to remove mycelia. Conidia concentration was determined using a haemocytometer and adjusted to 4×10^5 conidia ml^{-1} using serial dilution with distilled water. Conidia were obtained from 7-day-old cultures grown on PDA. Fruits of different hosts, *i.e.* apple, peach, strawberry, pepper, tomato, orange, lemon and guava, were thoroughly washed with detergent (liquid soap) to remove possible remained chemicals of postharvest application. The washed fruit were surface disinfected in 0.5% sodium hypochlorite for 2 min, rinsed twice in sterile distilled water, then air-dried in a laminar flow hood at 25°C. Fruits were then placed in humid plastic containers (30×20×10 cm) containing layers of sterilized filter papers impregnated with sterilized water for keeping humidity and incubated for 10 days at 25±2°C. Fruits were labelled with a permanent marker, and the point of inoculation was circled on each evaluated fruit. Wounds (2-mm-deep and 1×1×0.5 mm distances) were made using a sterile glass rod (Ivey *et al*., 2004). Twenty microliters of the conidial suspension was dispensed onto the fruit (over the wound for wounded fruit). A set of unwounded fruits were inoculated with the same amount of sterile distilled water and kept as control (check). Lesion diameters were measured after up to 10 days post inoculation. This test was repeated three times.

Results

In the present research, three species of genus *Colletotrichum*, *i.e*. *C. coccodes*, *C. acutatum* and *C. Gloeosporioides*, were isolated from deferent hosts in Egypt. Many isolates were obtained from different rotted fruits, *i.e.* mango, guava, strawberry, pepper and tomato, showing a wide range of morphological characters. These criteria were discussed and distinguished as following:

Colony morphology:

Colony characteristics of all tested isolates grown on PDA were compared (Table 1 and Figs. 1 & 2). Thus, cultures of isolated *Colletotrichum* sp. from mango and guava fruits represent typical characters of *C. gloeosporioides*. Mango isolates formed white aerial mycelium becoming gray with pink spore masses, salmon in colour, mainly at the centre of the colony and gray in reverse. Setae were abundant on host and on the media whereas sclerotia absent. Also, guava isolates formed colony contained white aerial mycelium becoming gray and forming a conidial masses salmon in colour and reverse to gray. These cultures lacked setae on media but developed on the host and not developed sclerotia. Concerning isolates taken from strawberry, colony colours were typical as *C. acutatum*, which usually forming white aerial mycelium during the first few days of growth then becoming orange to pink, or rose with pink conidial masses formation and the cultures were rose on reverse. Setae and sclerotia were absent developing. While, *Colletotrichum* sp.

Table 1. Morphological characters of *Colletotrichum* spp. from different hosts

Character	*C. gloeosporioides*		*C. acutatum*	*C. coccodes*	
	Mango	Guava	Strawberry	Pepper	Tomato
Colony colour	White to gray	White to gray	White to pinkish	White	White
Conidia shape	Cylindinrical with narrowing in the centre	Oblong with obtuse end	Fusiform or tapering	Straight or fusiform	Straight or fusiform
Spore mass colour	Pink	Salmon	Salmon pink, or orange	Honey	Honey
Spore size (µm)	10.8-26.9 × 4.1-8.1	12-27.1 × 3.8-8.3	10.8-16.5 × 2.9-4	20.9-22.1 × 2.9-4.1	18-22.1 × 2.8-4.1
Acervuli colour	gray to black	salmon to gray	salmon to gray	black	black
Acervuli size (µm)	60-88 × 190-240	80-90 × 190-240	90-100 × 230-250	110-190 × 250-280	160-211 × 239-302
Setae on host	appear	appear	absent	appear	appear
Setae on media	appear	absent	absent	appear	appear
Sclerotia on host	absent	absent	absent	appear	appear
Sclerotia on media	absent	absent	absent	appear	appear

isolates from pepper and tomato were recognized by colony that similar and typical to *C. coccodes*, possessing white aerial mycelium, conidia masses honey and sclerotia and setae were abundant on both the media and the host (Table 1 and Fig. 1). Colony shape and colour of culture may vary considerably within and among species. For instance, most isolates of *C. acutatum* show a red-salmon pigmentation in reverse culture, whereas the colouration of *C. gloeosporioides* cultures varies from white to dark gray.

Conidia morphology and temperature relationships:

Isolates of *Colletotrichum* spp. can be distinguishable on the basis of conidial morphology which divided into four groups, *i.e.* cylindrical, oblong, fusiform and straight fusiform spores (Table 1 and Figs.1 & 2). All isolates of *Colletotrichum* spp. grew on the medium up to 7 days, sporulation on potato carrot agar medium was better than PDA for observing the main microscopic features of the tested fungi. All studied isolates of *C. coccodes*, *C. acutatum* and *C. gloeosporioides* produced conidiogenous cells directly on the agar surface. These species differ in having conidia which were cylindrical with obtuse end and narrowing in the centre.

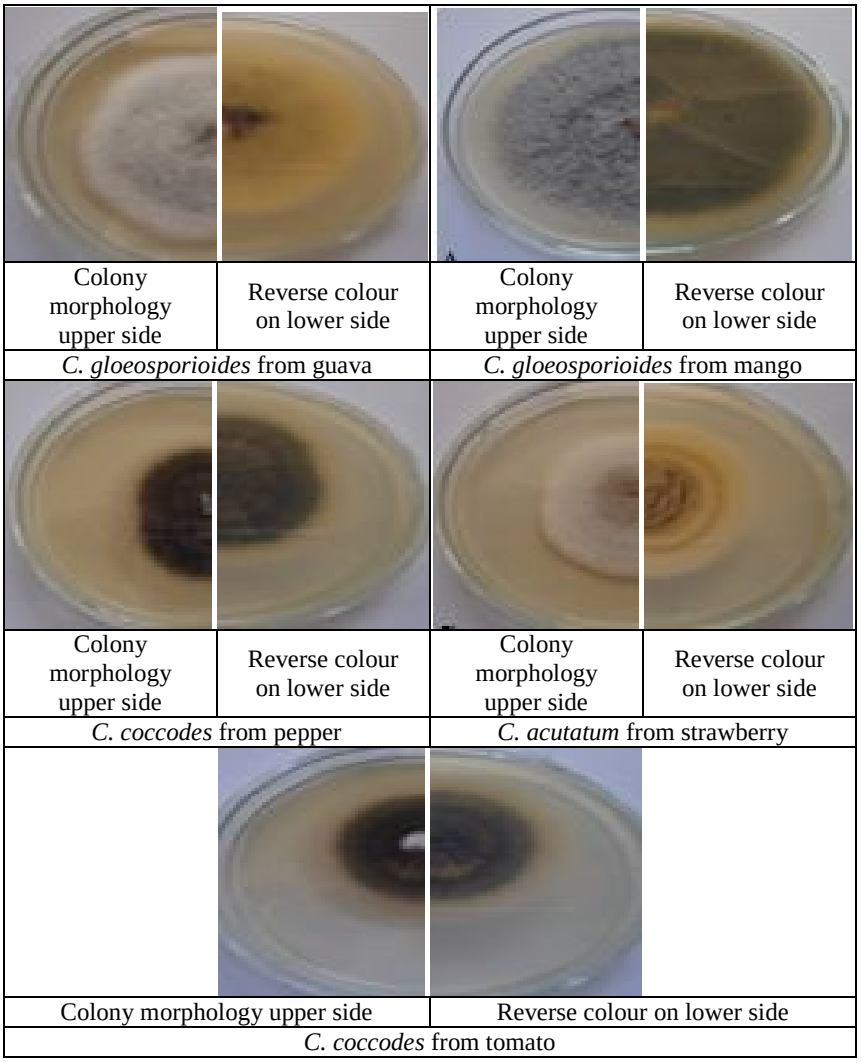
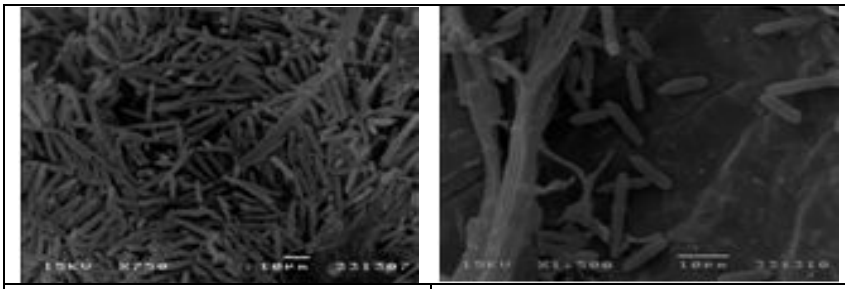
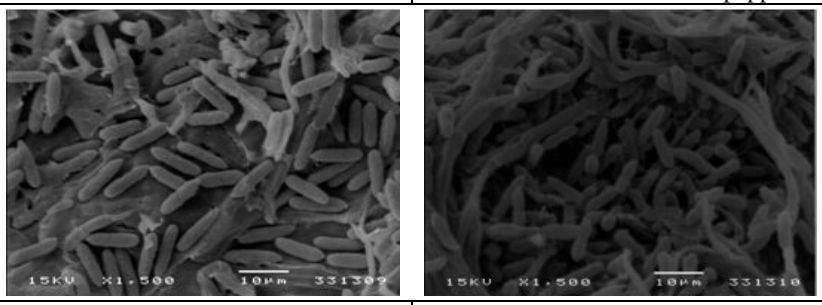


Fig. 1. Colonies morphology of different *Colletotrichum* isolates on PDA.

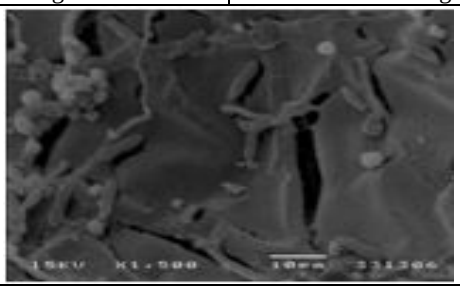
According to the obtained results in Table (1), mango isolates formed peanut-pod shape with pink spore masses and salmon in colour mainly at the centre of the colony and produced gray to black acervuli with 60-88×190-240 µm in diam. On the other hand, sclerotia were absent in both host and artificial media. Meanwhile, guava isolates showed oblong with obtuse ends, the conidial masses were salmon and colony reveres gray in addition, salmon to gray acervuli with 80-90×190-240 µm in diam. Setae appeared in the host and were absent in the artificial media. Also, sclerotia were absent in the host or artificial media.



C. coccodes isolated from tomato | *C. coccodes* isolated from pepper



C. gloeosporioides isolated from mango | *C. gloeosporioides* isolated from guava



C. acutatum isolated from strawberry

Fig. 2. Scanning electron micrograph details of different *Colletotrichum* isolates.

In this connection, isolates of *C. acutatum* isolated from strawberry produced fusiform or tapering, acute end and straight or fusiform conidia. Conidial mass showed salmon pink or orange colour and acervuli were salmon to gray 90-100×230-250 µm in diameter. Setae and sclerotia were absent in both host and artificial media. On the other hand, isolates of *C. coccodes* from pepper and tomato produced straight or fusiform spores with honey colour in conidia masses. Setae and sclerotia appeared in both host and artificial media. Neither conidiogenous cells nor setae were useful characters to add differentiation among tested species. Useful characters were the presence or absence of sclerotia and the morphology of conidia and appressoria.

Concerning the effect of temperature on the *in vitro* mycelial growth, results in Table (2) show that *Colletotrichum* spp. isolated from mango, guava and pepper, had optimal growth rates at 30ºC. Meanwhile, *C. acutatum* isolated from strawberry plants and *C. coccodes* isolated from tomato fruits had similar temperature responses with optimal growth rates at 25ºC. Seven days after inoculation, all isolates grew at 20 and 35ºC but no growth was observed at 40ºC.

Table 2. Effect of different temperature degrees on mycelial growth of *Colletotrichum* spp.

Fungal isolate	Mycelia growth (cm.) at different temperatures (^{o}C)			
	20	25	30	35
C. gloeosporioides (mango)	6.4	8.7	8.9	5.0
C. gloeosporioides (guava)	7.3	6.9	7.6	2.7
C. acutatum (strawberry)	5.6	6.6	3.9	2.9
C. coccodes (pepper)	5.7	5.6	7.5	4.8
C. coccodes (tomato)	6.9	7.0	6.8	4.8
L.S.D at 5%	0.3	0.2	0.4	0.3

Host Range:

Pathogenicity of the obtained *Colletotrichum* spp. isolates was tested on mature fruits of different hosts, *i.e.* apple, lemon, mango, orange, peach, pepper, strawberry and tomato. Results presented in Table (3) reveal that mango *Colletotrichum* isolate was capable to cause severe or moderately on apple, peach and mango fruits followed by strawberry, tomato and lemon where it was less virulent. However, the fungus produced essentially rot on orange fruits. In this regard, guava *Colletotrichum* isolate showed approximately the same trend of mango isolate. Guava isolate caused severe rot on peach, apple, and tomato fruits followed by moderately sever rot on mango, strawberry and lemon. On the other hand, guava isolate was less virulent on pepper fruits but not pathogenic to orange summer fruits. Strawberry isolate yielded typical symptoms of anthracnose disease on all tested fruits except orange within 8-10 days after inoculation. Also, peach fruits were highly susceptible to strawberry *Colletotrichum* isolate. Meanwhile, pepper and potato *Colletotrichum* isolates were pathogenic to tomato and pepper fruits. In the meantime, both isolates were not pathogenic to apple, strawberry, mango, peach, orange and lemon. It is clear that all *Colletotrichum* isolates were not or less pathogenic to summer orange.

Table 3. Virulence of different isolates of *Colletotrichum* spp. on different hosts

Isolate	Host range of different *Colletotrichum* isolates							
	M*	L	A	O	P	Pe	S	T
Mango	+++**	++	+++	+	+++	++	++	++
Guava	++	++	+++	-	+++	+	++	+++
Strawberry	++	++	++	+	+++	++	++	++
Pepper	-	-	-	-	-	+++	-	++
Tomato	-	-	-	-	-	++	-	+++

* M= Mango, L= Lemon, A= Apple, O= Orange, P= Peach, Pe= Pepper, S= Strawberry, T= Tomato.
** Sever rot (+++), Moderately Sever (++), Essentially rot (+), Non Pathogenic (-).

Discussion

Morphological and cultural characters were used to distinguish between different *Colletotrichum* spp. Colony characteristics of all tested isolates which grown on PDA were recorded. *Colletotrichum* isolates from mango and guava were morphologically similar in most morphological characters, *i.e.* aerial mycelium colour, setae and sclerotia, to the description of *C. gloeosporioides*. These results are in agreement with Sutton (1992). In addition, the morphological characters of strawberry *Colletotrichum* isolate such as cultural colour conidial masses, absent setae and sclerotia were typical characters for *C. acutatum*. Our results are in agreement with Sutton (1992). Also, *Colletotrichum* isolates from pepper and tomato were recognized by colony, similar and typical to *C. coccodes*, possessing white aerial mycelium, conidia masses honey and sclerotia and setae were abounded on both media and host. Similar results were found by Sutton (1992).

Numerous reports suggested that isolates of *C. acutatum* grew at a slower rate than isolates of *C. gloeosporioides* (Bernstein *et al*., 1995, Shi *et al*., 1996 and Smith and Black, 1990). Colony shape and colour of culture may vary considerably within and among species. For instance, most isolates of *C. acutatum* show a red-salmon pigmentation in reverse culture, whereas the colouration of *C. gloeosporioides* cultures varies from white to dark gray. Isolates of *Colletotrichum* spp. can be distinguishable on the basis of conidial morphology. Our results are in accordance with Cano *et al*. (2004). Also, sclerotia was absent in the host or artificial media. Similar findings were obtained by Photita *et al*. (2005) and Latiffah *et al*. (2009).

In this connection, isolates of *C. acutatum* which isolated from strawberry was produced fusiform or tapering, acute end and straight. Colour of conidial mass and acervulus and production setae and sclerotia are in agreement with other researchers such as Afanador-Kafuri *et al*. (2003). On the other hand, isolates of *C. coccodes* which isolated from pepper and tomato produced straight or fusiform spores with honey colour in conidia masses. Setae and sclerotia were appearing in both host and artificial media. Similar results were recorded by Lees and Hilton (2003) and Cano *et al*. (2004). As well as, neither conidiogenous cells nor setae were useful characters to add differentiation among tested species. Other studies have shown that the characterisation of fungi isolated from affected plants by anthracnose is complex (Cano *et al.*, 2004). Also, Ivey *et al*. (2004) found that the characteristics such as the colony morphology, conidial shape and the presence or absence of setae and sclerotia, could be used for differentiation of the genus *Colletotrichum*.

The effect of temperature on mycelial growth were studied and discussed. Sangeetha and Rawal (2008) observed good growth of *C. gloeosporioides* isolates at 30°C. Such observations were also carried out by Quimio (1974) for *C. gloeosporioides* from mango, Lii (1972) for *C. gloeosporioides* from guava in addition Yee and Sariah (1993) for *C. gloeosporioides* from cocoa. In these connections, *C. acutatum* which isolated from strawberry plants showed a moderate required temperature. According to the above results, Vinod and Benagi (2009) confirmed that temperature requirement of the fungus was 30°C where good growth was observed. In addition, Adaskaveg and Förster (2000) noted that 25 and 30°C

were the optimum temperature for *C. acutatum* and *C. gloeosporioides*, respectively. Traditional methods for identifying *Colletotrichum* spp. have relied primarily on morphological differences such as colony colour, size and shape of conidia, optimal temperature, growth rate, and presence or absence of setae (Gunnell and Gubler, 1992 and Sutton 1992).

Host range of the *Colletotrichum* isolates was studied on mature fruits of different hosts, *i.e.* apple, lemon, mango, orange, peach, pepper, strawberry and tomato. *Colletotrichum* mango isolate was capable of causing severe, moderately or less severe on all tested hosts. Strawberry isolate caused typical symptoms of anthracnose disease on all tested fruits except orange fruits. These results are in harmony with those of Smith (1998) who found that three *Colletotrichum* spp. were responsible for strawberry anthracnose. Moreover, *C. gloeosporioides* and *C. fragariae* usually cause petiole and stolon lesions and crown rot on strawberry but may also produce fruit rot symptoms. *Colletotrichum acutatum* is predominantly on flowers and fruits inducing rotting, and causing the most yield reduction worldwide. Also, Ivey *et al.* (2004) noted that *C. acutatum* caused symptoms on both strawberries and wounded or non wounded green tomato fruit. In the meantime, both pepper and potato *Colletotrichum* isolates were not pathogenic to apple, strawberry, mango, peach, orange and lemon. It is clear that, all *Colletotrichum* isolates were not pathogenic or less pathogenic to summer orange. The reasons for this result could be, that the using inoculation technique was not suitable for orange fruits and that penetration of inoculum into the peel was not sufficient to result in infection. Other investigators confirmed that, species of *Colletotrichum* that have been reported to cause anthracnose on pepper include *C. capsici* (Kousik *et al*., 1998), *C. gloeosporioides* (Kim *et al*., 1986, Park *et al*., 1989 and Alexander and Waldenmaier, 2003) and *C. piperatum* (Lambe *et al*., 1984). Such results indicate that the tested hosts showed differ in their susceptibility to the five *Colletotrichum* isolates, based on the external rotted areas in proportion to the total areas of the fruits. Because of its economic importance as a plant pathogen, *Colletotrichum* has received a lot of attention from numerous authors to detect it in plant tissue. Differentiation between *Colletotrichum* spp. based on host range or host origin may not be reliable, since taxa such as *C. acutatum*, *C. gloeosporioides* and others, infect a broad range of host plants (Gunnell and Gubler, 1992 and Sutton, 1992). The difference between responsible for anthracnose diseases for all isolates of *Colletotrichum* spp. of various fruits may be due to the enzymatic activity of the all tested species which can degrade the cell walls of the fruits, their nutrient components and the genetic structure of hosts. These differences could possibly be ascribed to adaptation of the pathogen to a less susceptible host, thereby becoming more virulent to overcome the host defence mechanisms (Alahakoon *et al*., 1994). *Colletotrichum graminicola* from sorghum could be grouped into three distinct groups, viz. most aggressive, intermediate and least aggressive (Pande *et al*., 1991).

Conclusion

Results pointed out that different *Colletotrichum* spp. were isolated from guava, mango, strawberry, pepper and tomato which showed anthracnose symptoms. Wide

variations among fungal species of *Colletotrichum* isolates according to morphological characteristics were recorded. The species were differed and distinguished based on conidial morphology. The isolates had optimal growth rates at 30ºC which isolated from mango, guava and pepper. Whereas, the optimum temperature of strawberry isolates was recorded at 25ºC. Mango isolate was capable of causing severe or moderately severe rot on a wide host range. This study has brought to light a wide variation in the pathogenicity, morphological physiological characters of different *Colletotrichum* isolates.

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الاختلافات الظاهرية والعوامل المؤثرة على نمو ومرضية بعض انواع الجنس *Colletotrichum*

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مرض الانثراكنوز من أهم الأمراض التى تصيب المحاصيل الاقتصادية. تم الحصول على عزلات الفطر *Colletotrichum* من ثمار خضر وفاكهة مختلفة يظهر عليها أعراض الانثراكنوز. حيث استخدمت فى هذه الدراسة عزلات عديدة من الفطر *Colletotrichum* مأخودة من ثمار كل من الجوافة (*Psidium guajava*) والمانجو (*Mangifera indica*) والفراولة (*Fragaria ananassa*) والفلفل (*Capsicum* sp.) والطماطم (*Solanum lycopersicum*). وقد أوضحت نتائج هذه الدراسة ان هناك مدى واسع من الاختلافات بين عزلات الفطر *Colletotrichum* وذلك وفقا للصفات الموفولوجية والفسيولوجية والمدى العوائلى. ووفقا للصفات المزرعية والظاهرية والمرضية فقد اظهرت عزلات الفطر *Colletotrichum* اختلافا واضحا فيما بينها. وكان الاختلاف بناءً على الشكل الظاهرى للجراثيم الكونيدية. وكانت درجة الحرارة المثلى لنمو الميسليوم الفطـــرى تتراوح ما بين ٣٠°م للعزلات المأخوذة من ثمار المانجو والجوافة والفلفل. بينما كانت درجة الحرارة المثلى للعزلة الخاصة بثمار الفراولة هى ٢٥°م. اظهرت دراسة المدى العوائلى ان العزلة المأخودة من ثمار المانجو كانت قادرة على الاصابة بدرجة شديدة الى متوسطة لثمار التفاح والكمثرى و المانجو يليها الفراولة بينما كانت قليلة المرضية لثمار كلا من الطماطم والليمون.