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MORPHOLOGICAL, PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATIONS OF EGYPTIAN *Pestalotiopsis* ISOLATES

El-Badawy, Noha F. ; Maggie E. M. Hassan ; Sahar Sh. Abd Allah and M. A. M. Kamhawy
Plant Pathol. Res. Inst., Agric. Res. Cent., Giza, Egypt.

ABSTRACT

Morphological characteristics of authentic cultures of *Pestalotiopsis* isolates using scanning electron microscop (SEM) revealed that, no presence of pycnidia for all examined *Pestalotiopsis* isolates, that produced acervuli only . Acervuli size ranged between 350 – 800 µ in length to 270 – 500 µ in width. Isolates varied in its ability to utilize different carbon sources. There were significant differences in the growth of *Pestalotiopsis* isolates in different carbon sources containing medium. Maltose gave maximum mean dry weight of mycelial growth. Ammonium nitrate recorded the maximum growth of *Pestalotiopsis isolates* as the nitrogen source, followed by potassium nitrate. On the other hand, the minimum mycelia dry weight of the fungus was in Aspartic acid containing media. Sporulation was dense where sodium nitrate was used as the nitrogen source, and deprived when asparagine was used. The genetic variation between fifteen isolates of *Pestalotiopsis* spp. were studied using four ITS primers (1&2, 4&5)) and SSR 1,2,4,5 and protein electrophoresis.

Keywords: Pestalotiopsis, SEM, carbon sources, nitrogen source, SSR primers, ITS primers, protein electrophoreses.

INTRODUCTION

Many species names of *Pestalotiopsis* refer to the host species from which they were recovered (Jeewon *et al*.,2004) ; Some investigations have shown that *Pestalotiopsis* species are not highly host-specific. A large number of *Pestalotiopsis* species have been recorded on single host, that does not mean they are host-specific (Kamhawy *et al*.,2011). La Rue and Bartlett (1921) isolated strains of *Pestalotiopsis guepini* from different hosts and cited that these strains did not appear to be confined to particular hosts. Suto and Kobayashi (1993) examined herbarium specimens infested with *Pestalotiopsis* species and reported synonymous of several species based on morphology. They stated that difference of host plant should not be used as a criterion for distinguishing species. Recently, Hopkins and McQuilken (2000) assessed the pathogenicity of 18 isolates of *Pestalotiopsis sydowiana*. They demonstrated that the isolates were not host-specific and infected other species of hardy ornamentals other than those were originally isolated .There is a little direct evidence to support host-specificity of *Pestalotiopsis* species needed for additional work to verify host range of these species. The concept of naming *Pestalotiopsis* species, especially those which are weakly parasitic, based on host and disease symptoms, is probably an inaccurate means of naming a particular species. However, genetic differences among morphologically indistinguishable species from different hosts assumed to be host-specific reflect an evolutionary adaptation to different hosts remains to

be investigated. The objective of this work designed to find out the source of carbon and nitrogen which can be most efficiently utilized by our *Pestalotiopsis* isolates for its growth and sporulation and its possible use in differential studies, scanning electron microscopy view for spores and asexual fruiting structure. In the present study, attempts were made to investigate the morphological, physiological variations among *Pestalotiopsis* isolates collected from different hosts and locations as well as genetic diversity. This work is complementary to the first part, morphological and phylogenetic characterization of *pestalotiopsis* in relation to host association (Kamhawy *et al*, 2011).

MATERIALS AND METHODS

1-Sources of *Pestalotiopsis* isolates

Fifteen monosporic cultures of *Pestalotiopsis* spp. from six different hosts showing different symptoms from various governorates of Egypt were obtained in pure culture and maintained on potato dextrose agar medium. These isolates were obtained from Research Department of Fruit and Woody Trees Disease, Plant Pathology Research Institute, A.R.C. details of the obtained isolates are shown in Table 1**.** (Kamhawy *et al*, 2011).

Table 1. Isolates of *Pestalotiopsis* **used in this study, their hosts, symptoms and samples origin**. **(Kamhawy *et al* , 2011)**

Code No. of isolates	Hosts	Symptoms	Governorate
***Pestalotiopsis psidii* (P1)**	"Guava"(*Psidium guajava* L.)	Guava Leaf Spot	Menofia
***Pestalotiopsis psidii* (P2)**	"Guava"(*Psidium guajava* L.)	Guava Canker	Beheira
***Pestalotiopsis psidii* (P3)**	"Guava"(*Psidium guajava* L.)	Root Rot	Beheira
***Pestalotiopsis* sp. (P4)**	"Pomegranate"(*Punic granatum* L.)	Leaf and Flower Spot	Assyout
***Pestalotiopsis* sp. (P5)**	"Pomegranate"(*Punica granatum* L.)	Leaf and Flower Spot	Assyout
***Pestalotiopsis palmarum*(P6)**	"Avocado"(*Persea americana* L.)	Leaf Spot and Fruit canker	Qalyubia
***Pestalotiopsis palmarum*(P7)**	"Avocado"(*Persea Americana* L.)	Leaf Spot and Fruit canker	Qalyubia
***Pestalotiopsis mangiferae* (P8)**	"Mango"(*Mangifera indica* L.)	Root Rot	Sharqia
***Pestalotiopsis mangiferae*. (P9)**	"Mango"(*Mangifera indica* L.)	Root Rot	Beheira
***Pestalotiopsis mangiferae*.(P10)**	"Mango"(*Mangifera indica* L.)	Leaf Spot	Gharbia
***Pestalotiopsis mangiferae*. (P11)**	"Mango"(*Mangifera indica* L.)	Leaf Spot	Beheira
***Pestalotiopsis palmicola*. (P12)**	"Date palm"(*Phoenix dactylifera* L.)	Leaf Spot and Blight	Giza
***Pestalotiopsis palmicola*. (P13)**	"Date palm"*(Phoenix dactylifera* L.)	Leaf Spot and Blight	Giza
***Pestalotiopsis* sp.(P14)**	"Apple"(*Malus domestica* Borth)	Root Rot	Mnofia
***Pestalotiopsis* sp.(P15)**	"Apple"(*Malus domestica* Borth)	Root Rot	Mnofia

Scanning electron microscopy (SEM) view of *Pestalotiopsis* isolates.

SEM was performed using a method described by Palhano *et al*. (2004), with some modifications. Pieces of *Pestalotiopsis* isolates growth on PDA medium were excised with agar using a sterile scalpel and fixed with 2 % (v/v) glutaraldehyde (Merck) in 0.1 M potassium phosphate buffer (pH 7.0) for 3 h. After fixing, tissues were briefly rinsed in 0.1 M potassium phosphate buffer (pH 7.0) and sequentially dehydrated in a graded ethanol series (10, 30, 50, 70 and 90 % for 15 min with a double change) and in 100 % (v/v) ethanol (Merck) for 1 h. The ethanol was removed from the tissues by critical point drying (HITACHI, HCP-2 Critical point Dryer). Tissues were mounted on the stubs using graphite and allowed to air-dry, covered with carbon and coated with gold, and observations were made with a JEOL, JSM-840 instrument operated at 15 kV.

Effect of different carbon and nitrogen sources on mycelial growth and sporulation of tested *Pestalotiopsis* isolates.

This experiment was conducted to find out the source of carbon and nitrogen (Tandon and Bharcava1961). which can be preferably utilized by Monosporic culture of *Pestalotiopsis* isolates for its growth expressed as mycelial dry weight(mg) and sporulation expressed as a number of acervulus. Asthana and Hawker,s basal medium (liquid and solid) consist of (Glucose 5.0g, KNO_3 3.5g KH_2PO_3 1.75g and MgSO4- 7H2O 0.75. and distilled water 1000ml.) was taken as the basal medium. The carbon and nitrogen nutrition was studied by replacing the glucose and potassium nitrate in the basal medium with various carbon and nitrogen compounds.

Effect of carbon source:

Four different carbon sources viz., fructose, lactose, maltose and mannitol were incorporated into Asthana and Hawker,s basal medium and potassium nitrate was added as a source of nitrogen in all treatments. The various carbon were substituted to the basal medium as rate 2000 mg of carbon/ L. The pH of the various media was adjusted to 6.1. the percent of carbon was calculated using the following formula cited in web cite http://preparatorychemistry.com/Bishop_Percent_Element.htm

$$\%\ \text{element} = \frac{(\text{Number of atoms of element in compound formula})\ \text{atomic mass of element}}{\text{formula mass of compound}} \times 100$$

The methods were similar to those of Tandon and Bharcava(1961). Plates, each containing the tested carbon source in approximately 10ml of the basal medium, were used to count acervulus. Flasks, each containing Twenty-five of basal liquid medium were used to determine dry weights, both inoculated equal fungal disc separately. Four Petri dishes and flasks were used for each treatment. The inoculated plates and flasks were incubated for ten days at 25±1^{o}C .

Effect of nitrogen source:

Ammonium nitrate, Aspartic acid, L-Asparagine, Potassium nitrate and sodium nitrate were used as different nitrogen sources and incorporated into both liquid and agar Asthana and Hawker,s basal medium at 485 mg of

nitrogen per liter of the medium. glucose was used as source of carbon in all the treatments. percent of nitrogen was calculated using the formula mentioned before. Twenty-five of each medium was poured into 100ml flasks, plugged with non-absorbent cotton and autoclaved at 121^{o}C (1.5psi pressure) for 20 minutes. Each of the treatments was replicated four times. All the flasks and plates were aseptically inoculated with 5mm fungal discs from an actively growing zone of seven day old culture. Inoculated flasks and plates were incubated at room temperature (25±1^{o}C) for ten days. The fungal mycelial mat was filtered through Whatman No. 1 filter paper and the dry mycelial weight was recorded after drying it in hot air oven maintained at 60°C for 24 hours. The recorded data was statistically analyzed according to Snedcor and Cochran, 1982.

Total protein analysis

Three grams of fungal mycelium were ground in precooled mortar and pestle with liquid nitrogen to a fine powder then 0.7 ml of extraction buffer (0.6 ml 1 M Tris HCl pH 6.8, 5 ml 50% glycerol , 2 ml 10% SDS , 0.5 ml β-mercaptoethanol and 0.9 ml H_2O) was added and the resultants were centrifuged . (14000xg for 15 minutes under cooling). Supernatants containing soluble proteins fractions were transferred to clean tubes and stored at (- 20°C). Protein content was estimated according to the methods of Bradford (1976) using Bovine Serum Albumin (BSA) as a standard. Protein content was adjusted to 2 mg / ml per sample. SDS was added to the sample at the rate of 4 mg SDS / 1 mg protein, then 50 µl, ß- mercaptoethanol were added. The mixture was boiled at 100°C in a water bath for 3-5 min. Vertical slab (18x16 cm) gel electrophoresis apparatus was used as marketed by Hoofer (Hoofer SE 600 series Pharmacia). 20 µl of this crude protein solution were resolved on 11 % SDS – PAGE using molecular weight protein marker as a standard. Electrophoresis was carried out at 2 milliampere per sample at 10 °C for 3 hrs.Gels were stained by silver staining method for protein as described by Sammons *et al*. (1981). This method of staining is sensitive and detects as little as 2 ng of protein in a single band. Gels were scanned for estimation molecular weight by using gel documentation system (AAB Advanced American Biotechnology 1166 E. Valencia Dr. Unit 6C, Fullerton CA, USA 92631). The different molecular weights of bands were determined against protein standard (Peqlab) marker.

Molecular studies.

DNA was extracted from 50 mg of mycellium using Qiagen Kit for DNA extraction. The extracted DNA was dissolved in 100 ul of elution buffer. The concentration and purity of the obtained DNA was determined by using “Gen qunta” system-Pharmacia Bio-tech. The purity of the DNA for all samples was between 90-97% and the ratio between 1.7 – 1.8. Concentration was adjusted at 20 ng/ *ul* for all samples using TE buffer pH 8.0. PCR amplification was generally carried out using different primers ITS1 (5' -TCCGTAGGTGAACCTGCGG-3') &ITS2 (5' – GCTGCGTTCT TCATCGATGC-3') , ITS4 (5' - CAGGAGACTTGTACACGGTCCAG-3') &ITS5(5' - GGA AGT AAA AGT CGT AAC AAG G-3') and SSR1 (5' - CAACTCTCTCTTCCT-3),SSR2 (5'-TGTGTGTGTGTGTGTATATT-3') SSR4

(5'- ACACACACACTCTCTCTC-3'), SSR5 (5'- ACACACACACTCTCTCTC-3'). The amplification reaction include approximately 50 ng of fungal genomic DNA as template, 1 unit of *Taq* DNA polymerase, o.2 mM each dNTPase, 1x PCR buffer, 3mM $Mgcl_2$ and 10 pmol of primer. Thermal cycling parameters for ITS primers included an initial denaturation at 94 ˚C for 3 min, followed by 29 cycles of denaturation at 94 ˚C for 1 min, annealing at 54 ˚C for 50 sec and extension at 72˚C for 1 min followed by final extension at 72˚C for 10 min and hold at 4˚C. Where parameters for SSR primers included an initial denaturation at 95 ˚C for 10 min, followed by 40 cycles of denaturation at 95 ˚C for 1 min, annealing at 40 ˚C for 1 min. and extension at 72˚C for 2 min followed by final extension at 72˚C for 10 min and hold at 4˚C.

The amplified products were separated on 1.5% agarose gel to check for product size and purity, using 1X TBE buffer followed by staining in ethidium bromide solution (1ug/ ml), at 75 constant volt , and determine with UV transilluminator .The PCR product obtained by ITS primers was cleaved with EcoR1 (5'-G ∆ AATT-3') and DraI (5'- TTT ∆ AAA-3') restriction enzyme by adding 10 units of the restriction enzyme to the mixture and incubating it for 2 hours at 37°C in 1X buffer. The digestion product was separated by agarose gel electrophoresis and visualized on UV light after staining with ethidium bromide. All gels were scanned for band R_f using gel documentation system (AAB Advanced American Biotechnology 1166 E. Valencia Dr. Unit 6 C, Fullerton CA 92631). The different M.W. of bands were determined against DNA marker Ameresco (100 bp) by unweighted pair-group method based on arithmetic mean (UPGMA).

RESULTS AND DISCUSSION

Scanning electron microscopy (SEM) VIEW:

Morphological characteristics of typical cultures *Pestalotiopsis* isolates using SEM revealed that, no presence pycnidi for all examined *Pestalotiopsis* isolate, all examined isolates produce Acervuli only , as shown in Figure 1. Acervuli were black for all isolates examined. The colors became darker resulted releasing conidi mass as fungal age increased. Acervuli size ranged 350 – 800 µ in length to 270 – 500 µ in width. The conidia shape are ellipsoid or fusoid (football-shaped) multi-celled. (Fail and Langenheim, 1990 Kamhawy *et al*., 2011) demonstrated that the diagnostic feature is the two or more clear, whisker-like appendages arising from the end cell.

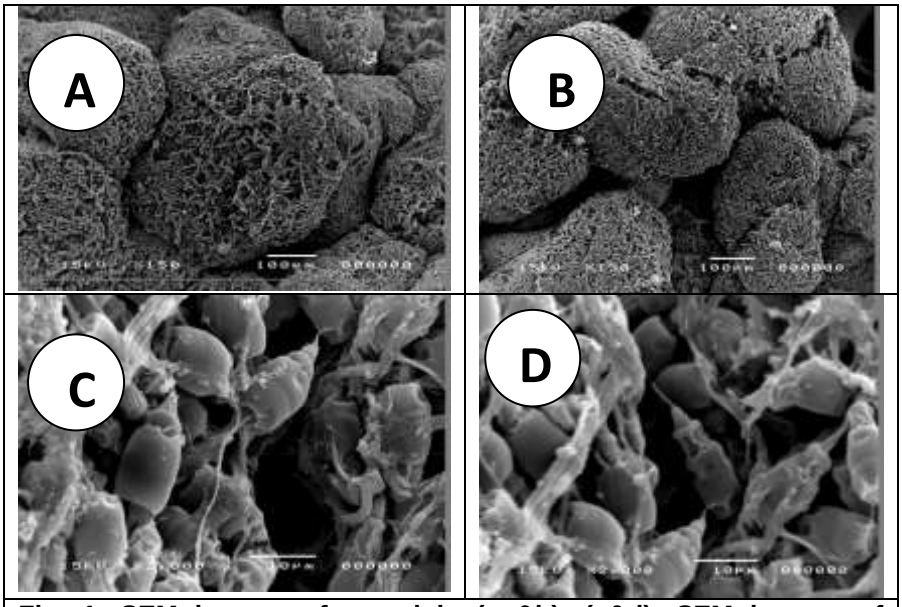

Fig. 1. SEM images of acerviula (a&b). (c&d), SEM images of appendages of conidia of the fungus P. *psidii*. Conidia of (c)&(d) The conidia were shape ellipsoid or fusoid (football-shaped)multi-celled. A diagnostic feature is the two or more clear, whisker-like appendages arising from the end cell.

Effect of different carbon and nitrogen sources on mycelial growth of tested *Pestalotiopsis* isolates.

Carbon is the most important and an essential structural component of framework of the fungal cell. Study the effect of different carbon sources on growth (in mgs) and sporulation of *Pestalotiopsis* isolates heterotrophy were clarified in (Table 2) .Obtaining their carbon requirement mainly from various organic sources, and the nature of the organism largely determines the range of substrates demonstrated by Steinberg,1950 , Bilgrami (1956) and Bilgrami and Verma, (1978). Its requirement (and utilization by *Pestalotiopsis* isolates were studied with five different carbon sources using Asthana and Hawker's broth basal medium broth as the basal medium (Table, 2).. There were significant differences in the growth of *Pestalotiopsis* isolates in various carbon sources. Maltose gave maximum mean dry mycelial weight of the fungus (100.8mg) which was followed by glucose (96.5mg) and fructose (82.8mg). The sporulation was heavy in the treatment where maltose was used as the carbon source among all tested isolates. Data also indicated that, Fructose and lactose treatments showed acerviula ranging from dense to scarce in production . There was no sporulation in mannitol treatment with exception isolate P2 and P5 Poor acerviula production in manitol treatment. Blank and Talley (1941) reported that the behavior of maltose in the process of acerviula production may be connected with the availability of active

glucose during decomposition of the substance by *Pestalotia banksiana and P. citri*. they suggested that impurities of the chemical might have been responsible for this behavior. Variations in the growth of different Isolates on various source carbon media may be due to variations in the nutritional requirements for these fungi Younis *et al*(2004). Tandon and Bilgrami (1958). Found that Maltose was the best sugar for the organisms. This fungus converted maltose by oligosaccharide (malto – trios) with simultaneous liberation of glucose. Nitrogen is an important component required for protein synthesis and other vital functions. Its requirement by *Pestalotiopsis isolates* was studied using different sources and the results are presented in Table 3. The study revealed that maximum growth of *Pestalotiopsis* isolates were in Asthana and Hawker,s basal medium where ammonium nitrate (120.53 mg) was used as the nitrogen source followed by potassium nitrate (110.66 mg), sodium nitrate (106.8 mg) and asparagine (97.33 mg). The minimum dry mycelial weight of fungus was in aspartic acid (80 mg). Data in Table (3) also indicate that sporulation was heavy where sodium nitrate was used as the nitrogen source, while sporulation was not observed where asparagine was used as the nitrogen source (Tandon and Mitra 1970)

Table 2: Effect of different carbon sources on growth (in mgs) and sporulation of *Pestalotiopsis* isolates .

Code no. of tested isolates	source of carbon									
	Fructose		Glucose		Lactose		Maltose		Mannitol	
	dry weight /mg	Acerviula	dry weight /mg	acerviula	dry weight /mg	Acerviula	dry weight /mg	Acerviula	dry weight / mg	acerviula
P(1)	86	++	96	++	80	++	107	+++	69	_
P(2)	80	++	90	+++	80	++	103	+++	74	+
P(3)	90	++	100	+++	80	++	100	+++	74	_
P(4)	78	+++	86	+++	80	++	98	+++	72	_
P(5)	80	++	100	+	83	++	98	+++	77	+
P(6)	88	++	98	+++	79	++	98	+++	75	_
P(7)	80	++	95	++	80	++	100	+++	70	_
P(8)	81	+	96	+++	77	_	104	+++	70	_
P(9)	77	+	98	+	77	_	96	+++	76	_
P(10)	88	+	98	+	84	+	98	+++	70	_
P(11)	88	++	100	+++	79	++	98	+++	70	_
P(12)	90	++	100	+	83	++	108	+++	68	_
P(13)	79	++	99	+++	83	++	104	+++	72	_
P(14)	79	++	95	+	80	_	100	+++	66	_
P(15)	79	++	97	++	80	_	100	+++	66	_
Means	82.8		96.5		80.3		100.8		71.2	
LSD at 5%: 1.88										

-: no acerviula production
+: Poor acerviula production: less than 10 acerviula
++: Moderate acerviulla production 11- 50 acerviula
+++: Good acerviula production overer 50 acerviula

Table 3: Effect on growth and sporulation of *Pestalotiopsis* isolates on different nitrogen sources

Code no. of tested isolates	Source of nitrogen									
	Ammonium nitrate		Asparagine		Aspartic acid		Potassium nitrate		Sodium nitrate	
	dry weight /mg	acerviula	dry weight/ mg	Acerviula	dry weight /mg	Acerviula	dry weight /mg	acerviula	dry weight /mg	Acerviula
P(1)	127	+	98	_	96	+	114	++	111	+++
P(2)	123	+	98	_	90	++	110	++	105	+++
P(3)	118	+	97	_	84	+	110	+++	107	+++
P(4)	119	+	101	_	86	++	110	+++	107	+++
P(5)	120	+	98	_	84	+	107	++	104	+++
P(6)	120	+	98	_	80	+	112	++	112	+++
P(7)	118	+	100	_	83	++	117	+++	111	+++
P(8)	114	+	99	_	83	++	112	+++	109	+++
P(9)	122	+	100	_	86	++	111	+	100	+++
P(10)	127	+	100	_	83	+	105	+	102	+++
P(11)	122	+	94	_	80	++	106	++	103	+++
P(12)	119	+	97	_	80	++	110	++	107	+++
P(13)	125	+	95	_	85	++	110	++	104	+++
P(14)	117	+	95	_	80	_	112	++	110	+++
P(15)	117	+	90	_	80	_	114	++	110	+++
means	120.53		97.33		84		110.66		106.8	
LSD at 5%: 2.11										

--: no acerviula production
+: Poor acerviula production: less than 10 acerviula
++: Moderate acerviula production 11- 50 acerviula
+++: Good acerviula production overer 50 acerviulla

Molecular studies.

Two different sets of primers were used in order to amplify universal fragments of *Pestalotiopsis* genotype. Four ITS primers (1&2, 4&5) were used to study the genetic variation between fifteen isolates from *Pestalotiopsis* fungus collected from different hosts and regions in Egypt. Polymerase chain reaction amplification products spanning approximately 600 bp of the ITS 1&2 and 550 bp of the ITS 4&5 as shown in Fig. (2&3), The amplified product from ITS4 & ITS5 was digested with Eco R1, DraI enzyme. The obtained results after digestion were shown in Fig (4&5) respectively, the pattern in Fig.4 showed that Isolate no.1 can be grouped with isolates no.7 with 94.13% similarity, although these isolates from different hosts and different governorates, they cause the same symptoms. This result is agreement with the result from protein pattern. On the other hand isolate no.3 is located with the isolate no.4 in the same group with 98.46% similarity. Isolate no.6 and isolate no.11 which pattern showed high similarity between all isolates ranged from 99.36% between isolate no.6 & 7, which were isolated from Avocado plants, to 90.77% between all isolates.

Fig. (2): 1.5% Agarose gel electrophoresis showing the amplified PCR products of *Pestalotiopsis* DNA using ITS1&ITS2 primers. M: DNA Molecular weight Marker

Fig.(3) : 1.5% Agarose gel electrophoresis showing the amplified PCR products of Pestalotiopsis DNA using ITS4&ITS5 primers. M: DNA molecular weight.

Fig. (4): 1.5% Agarose gel electrophoresis showing the digestion of amplified PCR products of ITS region using EcoR1 enzyme. M: DNA Molecular weight Marker

Fig. (5): 1.5% Agarose gel electrophoresis showing the digestion of ITS region using Dra 1 enzyme. M: DNA Molecular weight Marker

caused the same symptoms and infected different hosts appeared high similarity with 97.31%. So this previous results clearly showed that phylogenitic analysis don't support a close relationship between species isolated from the same host. And this agreement with (Jeewon, *et al*., 2004) who found a close relationship among morphologically related species of pestalotia rather than association with host, and hence provide new insights to the concept of host-based nomenoclasture in *Pestalotiopsis* species.When PCR products were digested by Dra1enzyme, the

New set of primers (SSR, primers) were used, it was noticeable that SSR primers amplified polymorphic regions differed according to the used primer. The obtained amplified bands by SSR1 primer showed high similarity between all isolates reached to 95.24% approximately except the isolate no.13 which appeared 47.34% similarity to all isolates as shown in figure 6

Fig. (6): 1.5% Agarose gel electrophoresis showing the digestion of amplified PCR productsof SSR region using SSR1 primer and the cluster analysis to thephoto. M: DNA Molecular weight Marke

Figure (7) presents DNA polymorphism of fifteen isolates *Pestalotiopsis* isolates using SSR2 primer The obtained amplified bands exhibited different relationships between isolates with 54.81% similarity between all subgroups , where this primer classified the fifteen isolates into several sub groups. Isolate no.1 & 12 were located in one group with 95.54% similarity , isolate no 14 & 15 presents 98.45% similarity, isolate no.6 &8 presents 96.75% similarity , isolate no.10 & 13 presents 98.12% similarity and isolate no.2 &5 presents 94.03% similarity.

Fig. (7): 1.5% Agarose gel electrophoresis showing the digestion of amplified PCR products of SSR region using SSR2 primer and the cluster analysis to the photo. M: DNA Molecular weight Marker

When we use SSR 4 primer another classification appeared, it classified the fifteen isolates into five subgroubs. Subgroub no.1 include isolates no. 10 & 11 which were isolated from mango plants and both of them causes the same symptoms , with 99.13 % similarity. And these two isolates present 98.49% similarity with the isolate no. 1 which was isolated from Guava plants, and also cause leaf spot symptoms.

The subgroub no.2 includes isolate no.2 & 3 which were isolated from Guava plants , but they cause canker and root rot respectively with 99.72% similarity. And isolate no.6 & 7 which were isolated from Avocado plants and cause leaf spot and fruit canker with 99.58% similarity. And subgroup no. 5 includes isolates no 9 & 8 which were isolated from mango plants with 99.73% similarity. Furthermore we found the five subgroubs appears high similarity with 92.86% between all of them. This results similar to (Kamhawy *et. al*., 2011) who study the phylogenetic characterization of *Pestalotiopsis* isolates by RAPD-PCR.

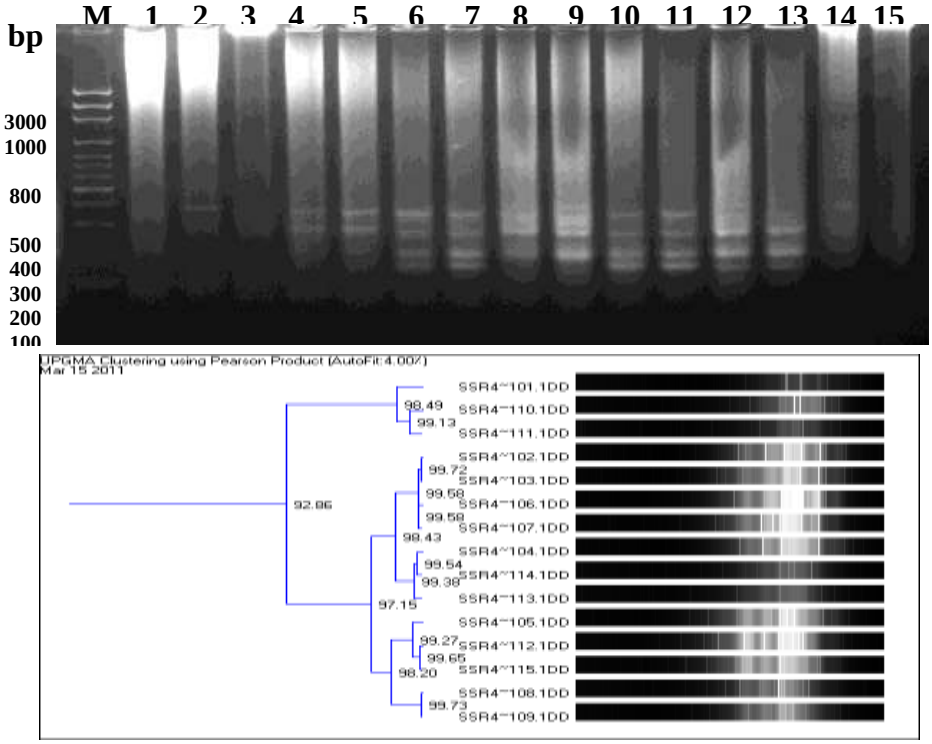

Fig. (8): 1.5% Agarose gel electrophoresis showing the digestion of amplified PCR products of SSR region using SSR4 primer and the cluster analysis to the photo. M: DNA Molecular weight Marker

SSR 5 primer presents different degree of similarity between all isolates. This degree ranged from 97.26 % between isolates no. 2 & 3 To 47.38% between all isolates.

Fig. (9): 1.5% Agarose gel electrophoresis showing the digestion of amplified PCR products of SSR region using SSR5 primer and the cluster analysis to the photo. M: DNA Molecular weight Marker

Total protein analysis:

The total protein pattern is presented in Fig. (10). The cluster of the isolates, revealed that *Pestalotiopsis* isolates can be grouped into three of subclusters. Isolate no.1 can be grouped with isolates no.7 with 82.28% similarity, although these isolates from different hosts and different governorates, they cause the same symptoms. On the other hand there is 92.8% similarity between isolate no.9 and isolate no.11 which were isolated from mango, from Beheira governorate, but they cause different symptoms on the plant, where isolate no. 9 cause root rot meanwhile isolate no. 11 cause leaf spot symptom. Another interesting example, isolates 12 & 13 which were isolated from Date Palm showed 89.5 % similarity, they isolated from the same host, the same governorate and cause the same symptoms.

In addition isolates no.3 and 5 can be grouped together with 96.35 % similarity, both isolates were isolated from guava and pomegranate respectively, they isolated from different governorates and cause different symptoms. So it is clearly showed that phylogenitic analysis don't support a close relationship between species isolated from the same host.

Fig.(10): SDS – PAGE polyacrylamide gel electrophoresis showing the protein patterns purified from 15 pestolotiopsis isolates from different hosts . M mid range molecular weight marker.

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الصفات المورفولوجية والفسيولوجية والتوصيف الجزيئى لعزلات مصرية من فطر بستالوشيوبسيس

نهى فريد البدوى ، ماجى السيد محمد حسن ، سحر شرقاوى عبدالله و محمود أحمد محمود قمحاوى

معهد بحوث أمراض النباتات – مركز البحوث الزراعية – جيزة – مصر .

دراسة الصفات المورفولوجية لعزلات فطر بستالوشيوبسيس باستخدام الميكوسكوب الإلكترونى أوضحت أن جميع العزلات المختبرة لا تكون أوعية بكنيدية فى حين اثبت الدراسة وجود اسيرفيولا فقط . يتراوح حجم الأسيرفيولا مابين 350 – 800 ميكرون للطول و مابين 270 – 500 ميكرون فى العرض . كما اوضحت الدراسة وجود اختلافات معنوية بين العزلات فى قدرتها على الاستفادة من مصادر الكربون المختلفة. سجل المالتوز الحد الأقصى كوزن الجاف للفطريات تحت الدراسة . استخدام نترات الأمونيوم كمصدر النيتروجين سجل الحد الأقصى كوزن الجاف للفطريات يليه نترات البوتاسيوم . من ناحية اخرى - اوضحت الدراسة ان استخدام حمض الأسبارتيك اعطى الحد الأدنى للوزن الجاف . كما ان تكوين الجراثيم بكثافة تم فى وجود صوديوم نتريت كمصدر للنتروجين . تم دراسة التباين الوراثي بين الخمسة عشرة عزلة باستخدام اربعة بوادىء من التتابع التكرراى البسيط ارقام 1,2,4,5 و بوادئ نسخ الفاصل الداخلى ارقام (5 ,4 & 1,2) وأنماط البروتين .

قام بتحكيم البحث

أ.د / محمد الششتاوى عبد ربه — كلية الزراعة – جامعة المنصورة

أ.د / نبيل صبحى فرج — مركز البحوث الزراعية