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STUDIES ON SOIL BORNE FUNGI OF MANGO ROOTSTOCKS IN EGYPT

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ABSTRACT: Disease survey study indicated that, root-rot and/or wilt diseases of mango rootstocks were distributed in all examined localities of six different governorates of Egypt. The lowest percentage of natural infection seedling was recorded in El-Giza governorate while, the lowest percentage of natural infection tree was recorded in Aswan governorate. On the other hand, the highest percentage in both natural infection of seedlings and trees was detected at El-Sharkia governorate. Fourteen fungal genera were isolated from naturally infected seedlings and/or trees of mango rootstocks. The most frequently isolated fungi were *Botryodiplodia theobromae* Pat. followed by *Fusarium solani* (Mart) Sacc. while *Ganoderma* sp. was the lowest one. *B. theobromae* and *F. solani* were the most pathogenic fungi compared with *Ganoderma* sp. which caused the lowest percentage of infection. Under greenhouse conditions all the tested rootstocks varieties were susceptible at different degrees to be infect with the pathogenic fungi. The most resistance rootstock variety for all tested fungi was SH 1 followed by Sediek while, G3 was the lowest one.

Key wards: Soil borne fungi, mango rootstocks, root-rot, wilt diseases.

INTRODUCTION

Mango (*Mangifera indica* L.) is considered one of the most important national crop in Egypt and a major item within the "National Food Basket". The mango is known to suffer from a number of diseases caused by different organisms, which affect different parts of trees, at all stages of growth and during development. Among them root rot is prevalent

in almost orchards, capable of causing significant damage to seedlings, nurseries, stocks and mature trees. Although soil-borne diseases of mango are relatively less important than the foliar and floral diseases, they are still capable of causing significant damage to seedlings, stocks and mature trees (Kore and Mane, 1992; Tsao *et al.*, 1994; Ploetz and Prakash, 1997; and Abd- El. Ghany, 2001). Rots of mango stocks are considered one of the most important soil-borne diseases affecting mango production and causing great losses in the nurseries. Several fungi including *Botryodiblodia theobromae* Pat., *Fusarium solani* (Mart) Sacc., *Rhizoctonia solani* Kühn, *Macrophomina phaseolina* (Tassi) Goid., *Pestalotia* spp. and *Phytophthora* sp. were frequently isolated from mango rootstock suffering from root-rot disease in different parts in the world (Kore and Patil, 1985; Saxena and Rawal, 1989; Verma *et al.*, 1991; Tsao *et al.*, 1994 and Al-Adawi *et al.*, 2002). In case of *Pestalotia* sp. Das (1993) isolated *Pestalotia sapotae* from wilted guava trees. Thus this investigation was conducted to survey mango root rot and/or wilt disease on different mango rootstocks cultivars in governorates, as well as isolation, purification and identification the fungal causal organisms. The pathogenicity test and rootstocks varietal reactions as well as differences obtained between *B. theobromae* isolates was also studied.

MATERIALS AND METHODS

1. Survey Studies

Mango root rot and/or wilt diseases survey was carried out during 1999 and 2000 growing seasons on different mango rootstocks cultivars, grow in different soil types and irrigation systems at different districts belonging to six governorates namely, El-Sharkia {Belbies, Ramsis Company and Inshas (El-Saraia Orchared)}; El-Ismaelia {El-Asher Company, 5 group}; Giza {Horticulture Research

Inst.}; Aswan {Aswan Storage, Horti. Serves Orchards}; Sohag {Shandawel}; and El-Behera {El-Nobaria, Cairo-Alexandria Desert Road}. The survey was conducted by calculating the percentage of the disease incidence (root rot and/or wilt). This was carried out through counting the number of infected and non infected mango (seedlings or trees) in each governorate. In each orchard and/or nursery, randomly choices of 100 tree and/or seedling were randomly chosen and percentage of disease incidence, was calculated.

2. Isolation, Purification and Identification of Fungi Associated with Mango Root Rot and/or Wilt Diseases

Roots from mango showing symptoms of root rot or wilt were collected during survey in different governorates. Roots were carefully washed with tap water before cutting into small pieces then surface sterilized using 3% sodium hypochlorite solution for 3 mines. Pieces were rinsed several times in sterilized distilled water, dried between two folds of sterilized filter papers, and transferred, under aseptic conditions to sterilized Petri dishes containing water agar medium and incubated at 25 °C for one week. All the isolated fungi were purified using either single spore and/or hyphal tip techniques (Dhingra and Sinclair, 1985) then sub-cultured on PDA medium. The isolated fungi were identified by the Survey and Taxonomy of Fungi Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. The frequencies of the isolated fungi were calculated separately for each collected sample. Identification was based on morphological and cultural characters according to Nelson *et al.*, (1983) and Barnett and Hunter (1987).

3. Pathogenicity Test and Rootstocks Varietal Reactions

3.1 Plants

Five mango rootstock varieties, six month old, obtained kindly from Agri-El-Salheia Company, were used in this study. These varieties and origin were tabulated in Table 1.

Table 1: Varieties and their origin used in this study

Variety	13-1	G_3	SH-1	Sokary	Sediek
Origin	Egypt and other countries	Southern America	Local collection	Egypt	Egypt

3.2 Soil infestation

Soil infestation was carried out using corn meal medium inoculated with 3 mm. disc each of the isolated fungi taken from 7 days old cultures. Pots of 25 cm diameter were sterilized with 5% formalin solution and left for a week for formalin evaporation then were filled with autoclaved sand and clay soil (1:1 v/v). The soil was infested with each fungus separately at the rate of 5% of soil weight. The inoculum was thoroughly mixed with the upper surface of the soil and irrigated regularly 7 days before planting to ensure the distribution of the fungus. Control treatment was applied using fungus free corn meal medium. Three mango seedlings from each variety (six month old) were cultivated in each pot and five pots were used for each treatment. Plants were examined for root rot and/or wilt disease after 60 and 90 days from inoculation.

4. Differences Between *Botryodiplodia theobromae* Isolates

Experiments were conducted according to Krupinsky (1983) and Leavitt and Munnecke (1987) to determine the differences between 4 representative isolates of *Botryodiplodia theobromae* Pat. collected from different governorates as it also proved to be the highest pathogenic fungus. Differentiation depending on morphological characteristic, pigment production abilities, growth on 36 °C and molecular characterization.

The Code No. of tested isolates and origin were tabulated in Table 2.

4.1 Morphological characters

Spores of *B. theobromae* Pat. obtained from individual pycnidia as well as pycnidia were compared with each other using light and scanning electron microscope (SEM). Differences in length and

width between spores and pycnidia of isolates, were determine

Table 2: Code No. of tested isolates and their origin

Code. No.	1	2	3	4
Origin	Giza	El-Sharkia	Sohag	Ismaelia

4.2 Pigment production ability and growth rate at 36 °C

The ability of the tested *B. theobromae* Pat. isolates to produce red pigments as well as growth rate at 36 °C were studied. The inoculated plates with the tested isolates were incubated at 36 °C in ultra water bath for 7 days. The tested isolates were examined for the presence of visible red pigments. The linear growth was determined daily for each isolate by measuring the two dimensions of growth in each plate and the mean was estimated from three replicates. This experiment was terminated whenever; the mycelial growth covered the plate surface of any treatment.

4.3 Molecular characterization of different isolates of the pathogens

The extraction of DNA from *Botryodiplodia theobromae* isolates was preformed according to the following procedure

4.3.1 DNA extraction

Fifty mg of the fungal powder were used to extract DNA according to Dellaporta *et al.* (1983).

4.3.2 Random amplified polymorphic DNA (RAPD -PCR)

4.3.2.1 Amplification reaction mixture

RAPD reactions were preformed in a total volume of 25 ul containing a 1X polymerase chain reaction (PCR). Reaction mixture (Promega); 2.5 mM Mg Cl_2; 50 uM of each dNTP (Promega); 0.3 uM of primer; 50 ng of genomic DNA; and 5 units of *Taq* polymerase. In negative control distilled deionized water was used instead of the target DNA included to test for contamination. Amplification conditions of the thermocycler MJ research model PTC-200 were as shown in Table 3 for each used primer.

4.3.2.2 Electrophoresis and data analysis

The amplified products and the DNA marker- DNA molecular weight marker XIV, BOHRINGER MANNHEIM Cat. No. 1 721 933- (10 ul per tested sample) were loaded on 1.5% agarose gels, separated by electrophoresis in, 0.5 x TBE buffer (45 mM Tris-borat and 1 mM EDTA), stained with Ethidium bromide and photographed under UV light using Herolab gel documentation system model Mitsubishi.

Table 3: Primer No., Sequence and PCR program

Primer No.	Sequence	PCR program
P_3	^{5}, GTAGACCCGT, ^{3}	95 °C 5 min
		45 cycles of:
P_4	5 AAGAGCCCGT 3	95 °C 1 min
		36 °C 1min
		72 °C 2min
		Final cycle 72 °C for 5 min

Dendrograms were calculated using computer soft ware Phoretix ID advanced (Nonlinear Dynamics limited- UK).

RESULTS AND DISCUSSION

1.Survey of the Disease Incidence Under Natural Infection

Results showed clearly that, typical rootstock rot disease symptoms were observed in mango rootstocks examined in the sixth governorates. Data in Table 4 show that, percentage of rootstock rot and/or wilt in seedlings and trees were higher in 1999 growing season than in 2000. Percentage of disease incidence for seedlings in 1999 season, ranged from 2% in El-Giza governorate to 26.16% in El-Sharkia governorate, while ranged from 1.66% to 24% in 2000 growing seasons. On the other hand, data also revealed that, rootstock rot disease incidence was higher in seedlings than in tress of both tested growing season. It is also clear that, the highest percentage of disease incidence in trees was recorded in El-Sharkia

Table 4: Percentage of disease incidence during 1999 and 2000 growing seasons under natural infection

Governorate	District	% Disease incidence during Season 1999			% Disease incidence during Season 2000		
		Rootstock rots	Trees wilt	Mean	Rootstock rots	Trees wilt	Mean
El-Sharkia	Belabies	27.33	23.33	25.33	23.00	20.00	21.50
	Inshas	25.00	20.66	22.83	25.00	18.00	21.50
	Mean	26.16	21.99	24.08	24.00	19.00	21.50
Ismaelia	El-Asher Company	15.00	9.33	12.16	14.00	9.33	11.66
El-Behera	El-Nobaria	8.00	5.66	6.83	5.66	3.00	4.33
El-Giza	Abo-Rowash	2.00	6.00	4.00	1.66	6.00	3.83
Sohag	Shandawel	*	7.00	3.50	*	6.33	3.16
Aswan	Aswan Storage	*	3.00	1.50	*	2.00	1.00
Mean		11.05	10.71		9.90	9.24	

* Nursery (Rootstocks) free localities.

governorate, being (21.99% and 19%, respectively) while, the lowest percentage of disease incidence were recorded in Aswan (3 and 2%, respectively) and the rest governorate were intermediate. Differences in disease incidence might be attributed to the prevailing factors in each inspected locality, i.e. soil properties (mechanical structure, water holding capacity, chemical components and microbial populations), the behavior of various pathogens and/or varieties reaction of cultivated rootstock. These results were in agreement with those obtained by (Abd El-Ghany, 2001 and Baioumy *et al.*, 2003).

2. Isolation, Purification and Identification of Fungi Associated with Mango Rootstock Rot and Wilt Diseases

Isolation from rotted roots of mango at different localities of Egypt, revealed the occurrence of several fungi i.e. *Alternaria solani* (Ellis & Martin) Sorauer.; *Alternaria* spp.; *Aspergillus* spp.; *Botryodiplodia theobromae* Pat.; *Fusarium solani* (Mart.)Sacc.; *Fusarium* spp.; *Ganoderma* sp.; *Macrophomina phaseolina* (Tassi) Goid.; *Penicillium* spp.; *Pestalotia* sp.; *Phytophthora* sp.; *Rhizoctonia solani* Kühn.; *Stemphylium* sp. and *Trichoderma* spp. (Table.5). Most of these fungal genera were previously reported to be associated with root rot and wilt diseases of mango in different countries (Muller, 1940; Nath, 1976; Kore and Patil 1985; Lim and Khoo, 1985; Rai, 1986; Verma *et al.* 1991; Kore and Mane, 1992; Tsao *et al.* 1994 and Al Adawi *et al.* 2002).

Data also showed that, the highest percentage of the frequently isolated fungi was *Botryodiplodia theobromae* followed by *Fusarium* spp. then *Fusarium solani* being 17.02; 16.11 and 13.8%, respectively. *Ganoderma* sp. recorded the lowest percentage (1.38%). The rest isolates were in between. These results were in line with those obtained by Abd El-Ghany 2001 and Baioumy *et al,* 2003. Differences between fungi

Table 5: Frequency percentage of the isolated fungi from naturally infected roots of different mango rootstocks collected from different governorates

Isolated fungi	Governorates											Total
	El-Sharkia		El-Ismaelia		El-Giza	El-Behera		Sohag	Aswan	Total		
	S.	Ts.	S.	Ts.	S.	S.	Ts.	Ts.	Ts.	S.	Ts.	
Alternaria solani	1.38	0.0	1.84	0.0	0.92	1.38	0.0	0.92	2.76	5.52	3.68	9.20
Alternaria spp.	0.92	0.0	0.46	0.0	0.46	1.38	0.0	0.92	1.84	3.22	2.76	5.98
Aspergillus spp.	0.0	0.0	0.0	0.0	0.46	1.84	0.0	1.38	0.0	2.3	1.38	3.68
Fusarium solani	1.38	1.38	1.38	0.92	3.22	0.0	1.84	1.38	2.30	5.98	7.82	13.80
Fusarium spp.	2.76	2.76	2.30	2.30	0.0	0.0	5.99	0.0	0.0	5.06	11.05	16.11
Botryodiplodia theobromae	6.44	2.30	1.38	2.30	1.38	0.0	0.0	3.22	0.0	9.2	7.82	17.02
Macrophomina phaseolina	0.0	0.0	0.0	0.0	0.0	0.0	3.68	0.0	0.0	0.0	3.68	3.68
Phytophthora sp.	0.0	2.30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.30	2.30
Pestalotia sp.	1.38	0.0	0.0	0.0	0.0	0.0	3.68	0.0	0.0	1.38	3.68	5.06
Rhizoctonia solani	0.0	3.22	0.0	1.38	0.0	0.0	2.30	0.0	0.0	0.0	6.9	6.90
Ganoderma sp.	0.0	1.38	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.38	1.38
Penicillium spp.	5.52	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.52	0.0	5.52
Trichoderma spp.	2.30	1.38	0.0	0.46	0.0	0.0	0.0	1.38	0.0	2.30	3.22	5.52
Stemphylium sp.	0.0	2.76	0.0	0.46	0.46	0.0	0.0	0.0	0.0	0.46	3.22	3.68
Mean	22.08	17.48	7.36	7.82	6.9	4.6	17.49	9.2	6.9	40.94	58.89	99.83
Total	39.56		15.18		6.9	22.09		9.2	6.9	99.83		

S: Seedlings
Ts: Tree

frequencies might be due to variation in soil structure and properties, susceptibility of rootstocks, root exudates and the prevailing environmental circumstances. The highest percentage of *Botryodiplodia theobromae* (6.44%) was detected at El-Sharkia governorate followed by Ismaelia and Giza governorates recorded 1.38% while, not recorded in El-Behera and Aswan governorates. Data in the same table revealed that, *Macrophomina phaseolina* was only recorded in samples collected from El- Behera similar to, *Phytophthora* sp. that was only recorded in El-Sharkia governorate.

3. Pathogenicity Test and Varietal Reactions

Data in Table 6 indicate that, *B. theobromae.; F. solani.; Macrophomina phaseolina.; R. solani.; Pestalotia* sp.; *Ganoderma* sp. and *Phytophthora* sp. were able to infect mango rootstock varieties causing different degrees of seedling disease incidence. *B. theobromae* and *F. solani* were the most pathogenic fungi to mango rootstocks varieties (54.71 and 48.55%, respectively) followed by *R. solani and M. phaseolina* being 37.29 and 36.80% while *Ganoderma* sp. caused the lowest percentage of infection being 4.76 %. The rest fungi were in between. These results are in the same line of those obtained by Nath (1976); Kore and Patil (1985); Lim and Khoo (1985); Verma *et al.* (1991); Kore and Mane (1992); Tsao *et al.* (1994); Abd El-Ghany (2001) and Al-Adawi *et al.* (2002). Increasing time after inoculation from 60 to 90 days caused significant increase in the percentages of infection with the tested fungi comparing with the control.

It is also clear that, all the tested rootstocks varieties were susceptible to be infect by the isolated fungi with different degree. Rootstock var. G_3 was the most susceptible one, (31.96%) followed by 13/1 (28.06%) while, Sediek proved to be the least affected exhibiting 23.10%. On the other hand, *F. solani* was the most pathogenic fungi against G_3 and Sediek varieties.

Table 6: Effect of isolated fungi on the percentage of disease incidence of some mango rootstock varieties

Isolated fungi	Mango rootstock															Means
	G_3			13/1			SH1			Sukary			Sediek			
	Infection percentage after (days)															
	60	90	Mean	60	90	Mean	60	90	Mean	60	90	Mean	60	90	Mean	
Fusarium solani	35.55	96.55	66.05	24.44	60.00	42.22	17.77	48.64	33.20	28.88	78.12	53.50	31.11	64.51	47.81	48.55
Botryodiplodia theobromae	26.66	75.75	51.20	28.88	84.37	56.62	28.88	68.75	48.80	46.66	91.66	69.16	42.22	53.33	47.77	54.71
Macrophomina phaseolina	20.00	55.55	37.77	33.33	86.66	59.99	26.66	72.72	49.69	11.11	20.00	15.55	17.77	24.32	21.04	36.80
***Phytophthora* sp.**	6.66	41.42	24.04	4.44	9.30	6.87	6.66	7.14	6.90	13.13	20.51	16.82	11.11	17.50	14.30	18.59
***Pestalotia* spp.**	13.33	25.64	19.48	13.33	28.20	20.76	13.33	25.64	19.48	11.11	25.00	18.05	8.88	29.26	19.07	19.36
Rhizoctonia solani	22.22	45.71	33.96	17.77	58.33	38.05	26.66	66.66	46.66	24.44	41.17	32.80	20.00	50.00	35.00	37.29
***Ganoderma* sp.**	2.22	45.45	23.83	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	4.76
Control	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Means	15.83	48.25	31.96	15.27	40.85	28.06	14.99	36.19	25.59	16.91	34.55	25.70	16.38	29.86	23.10	

LSD value for: at 5% level

Isolates (I) : 0.38 Varietes (V) : 0.30 Period (P) : 0.19

(I) (V) : 0.86 (I) (P) : 0.54 (V) (P) : 0.43

(I) (V) (P) : 1.22

M. phaseolina was the most pathogenic fungus against 13/1 and SH1 varieties. While, *B. theobromae* exhibit the same trend against Sukary rootstock variety.

Data also showed that, SH 1 variety was the most susceptible to be infect with *R. solani,* followed by 13/1 and Sediek, while Sukary variety was the least one.

Variety 13/1 was highly susceptible to *M. phaseolina* followed by SH 1 while, Sukary was the least one. Similar results were obtained by Abd El-Hafeez (1991); Abd El-Ghany (2001) and Baioumy *et al.,* (2003). Such differences in susceptibility of mango rootstoks to root rot and/or wilt disease might be due to differences in their genetic make up (Walker, 1975).

4. Differences Obtained Between *Botryodiplodia theobromae* Pat. Isolates

4.1 Morphological properties and microscopic differences

Data in Table 7 and Fig.1 reveal that, there are differences between isolate No. 2 and 4, 1 and 3 as well as 2 and 4 in length and width of pycnidia. Obtained results indicate also that, pycnidia of isolate No. 2 was the biggest in width and length followed by isolates No. 4 and No.1 while, isolate No. 3 was the least one. Data also indicate that, no correlation was detected between pycnidia size and its content from one or two-celled conidia.

Scanning electron microscope (SEM) studies indicate that, there are variations in spore size (width and length) as well as pycnidia between *Botryodiplodia theobromae* isolates. Stromatic pycnidia include isolate No.1 (El-Giza isolate) were embedded in stromatic tissues. On the other hand, isolate No.2 (El-Sharkia isolate) produced ostioled pycnidia. The pycnidia associated together in groups. Such results were also in harmony with those obtained by Hassan (1980) who found that, *B. theobromae* isolates obtained from different hosts varied in the formation of pycnidia, pycnidiospores and stroma. Aly *et al.* (2002) obtained similar results of *B. theobromae* on grapevine.

Table 7: Morphological and microscopical differences between *Botryodiplodia theobromae* isolates in pycnidia and conidia width and length measured as (µ)

B. theobromae isolates	Pycnidia		Conidia			
			One-celled conidia		Two-celled conidia	
	Width (u)	Length (u)	Width (u)	Length (u)	Width (u)	Length (u)
Isolate (1)	193.0-287.5	200.0-325.0	8-12	12-16	8-12	12-18
Average	214.9	265.0	10	14.63	10.27	16.63
Isolate (2)	250.0-375.0	287.5-400.0	8-12	14-16	10-12	17-20
Average	286.0	339.2	10.92	15.23	11.47	16.43
Isolate (3)	178.0-226.0	147.0-287.0	8-12	12-16	10-12	16-20
Average	201.6	231.0	10.71	15.30	10.38	17.63
Isolate (4)	262.5-350.0	275.0-350.0	8-10	12-18	10-12	16-20
Average	284.94	295.0	9.10	16.57	10.29	18.29

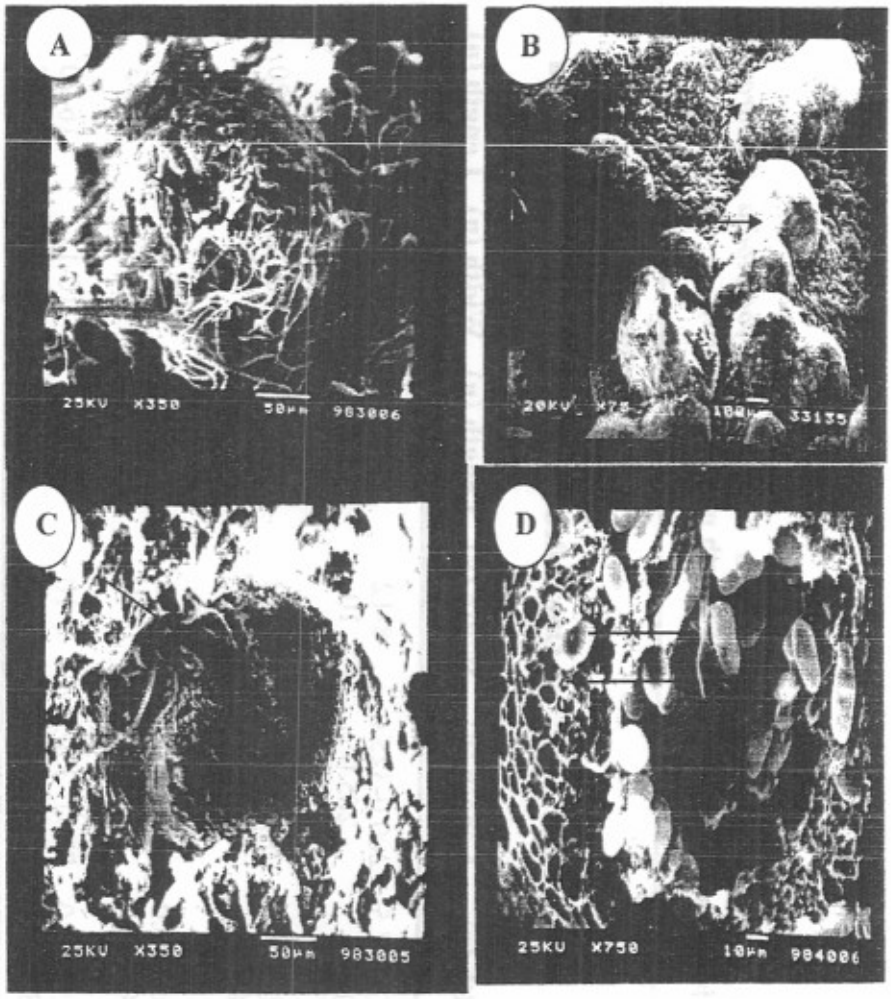

Fig. 1: Scanning Electron Microscope (SEM) view of *Botryodiplodia theobromae* pycnidia

A. Immature pycnidium, note: ostiole (indicated by arrow).

B. Mature pycnidium of isolate No.2 note, ostioles of pycnidia and each two pycnidia associated together in one group

C. Undistigushed ostiole and pycnidia formed in stromata of isolate No. 1 indicated by arrow.

D. Cross section in mature pycnidium, note: conidia (c) and conidiophore (cp).

According to the morphological properties, obtained isolates of *B. theobromae* were categorized under two main groups divided into two subgroups i.e subgroup 1 include single and grouped pycnidia and subgroup 2 include pycnidia in groups. These information could be used as a particle properties in determining taxonomic relationships among biotypes.

4.2 Red pigments production and growth rate at 36 °C

Red pigments and growth rate production of *B. theobromae* during growth at 36 °C was one of the most interesting characters that used to differentiate between isolates. Pigments are produced during hyphae growth and varied from white to almost black. Isolates No. 1 and 2 produced black mycelium and isolate No. 3 produced grey mycelium while isolate No. 4 white mycelium.

Data in Table 8 indicated that both isolates No. 3 and 4 are able to produce red soluble pigment in media and/or mycelium at 36°C. Data also indicate that, isolate No. 2 recorded the highest growth at 36°C being 50.54 mm while the lowest growth was recorded with isolate No. 3 and the rest isolates were intermediate. Results showed differences in morphological characteristics among isolates of *B. theobromae* isolated from different governorates of Egypt. Variation between isolates was detected in, rate of mycelial growth at 36°C, mycelial color and red pigment production. Leavitt and Munnecke (1987) and Aly *et al.*, (2002) classified *B. theobromae* isolated from grapevine into two biotypes, biotype 1 rapidly grow at 36°C, producing red pigment and the other biotypes growing slowly at the same temperature without producing red pigment. Accordingly Egyptian isolates of *B. theobromae* are classified into two groups or biotypes. Biotype I include isolate No. 1 (El-Giza isolate), and isolate No. 2 (El-Sharkia isolate) produced black mycelium. Biotype II include isolate No. 3 (Sohag isolate) that produced grey mycelium while isolate No. 4 (El-Ismaelia isolate) produced white mycelium. Both

Table 8 : Effect of incubation at 36 °C on the linear growth (mm), mycelium colour, red pigments production and pycnidia formation

Tested isolates	Linear growth (mm) Intervals (days)								Mycelium colour	Red pigment production		Pycnidia formation		
	1	2	3	4	5	6	7	Mean		In the media	On the mycelium tip	In the media	On the surface	Together
Isolate No. 1	00.00	15.60	33.30	41.00	48.60	65.30	81.30	40.72	Black	-	-		*	
Isolate No. 2	00.00	14.00	34.60	54.30	74.60	86.30	90.00	50.54	Black	-	+			*
Isolate No. 3	00.00	11.60	18.30	24.60	33.00	42.60	51.60	25.95	Gray	-	+	*		
Isolate No. 4	00.00	10.00	23.30	29.00	34.60	44.00	63.6	29.21	white	+	+	*		

\+ presence of visible red pigment.
\- No visible red pigment.
* Presence of pycnidia.

L S D value at 5.% level for:
Isolate (I): 2.56
Periods (P): 3.38
(I) × (P) : 6.76

isolates No. 3 and 4 (Sohag and Ismaelia isolates) were able to produce red pigments in both medium and/or mycelium at 36 °C. Also, biotype I recorded the highest growth while biotype II recorded the lowest growth. These results were in harmony with those recorded by Douglas Barde and Hewitt (1965). They found that some isolates of *B. theobromae* on PDA produce pigment in the agar and on mycelium tip and it opposites with them in a high temperature response to red pigment production.

4.3 PCR studies

RAPD-PCR primers for each tested *Botryodiblodia theobromae* isolates are shown in Figure 2. These Figs. reveal that, both used primers gave different banding pattern between the tested isolates of each tested genera.

As shown in Fig.2A primer P3 has the highest ability to flank the DNA sequences of the four pathogenic *B. theobromae* tested isolates than primer P4.

The phylogenic relationship of *B. theobromae* isolates based on the combined data from Fig.2B showed that, the four isolates are divided into three groups, the first group involved isolate No. 4 and the second group involved isolate No. 2 while, the third group is divided into two subgroups the first involved isolate No. 1 and the second involved isolate No. 3.

The geographic isolation of each biotype of *B. theobromae* might be contributed to that genetic diversity revealed by RAPD analysis. This obtained result concerning classification of *B. theobromae* into its biotypes agree with those obtained by Tuskan *et al.*, (1990); Wostemeyer *et al.*, (1991) and Guthrie *et al.*, (1992) and Aly *et al.*, (2002). Accordingly, obtained isolates of *B. theobromae* were categorized under 4 genotypes directly related to geographical origin using RAPD method. These results are agreement with those obtained by Moller *et al.*, (1999). According to these results RAPD-PCR analysis is a simple and fast technique for isolates differentiation (Alvarez *et al.*, 1997 and Huang *et al.*, 1997)

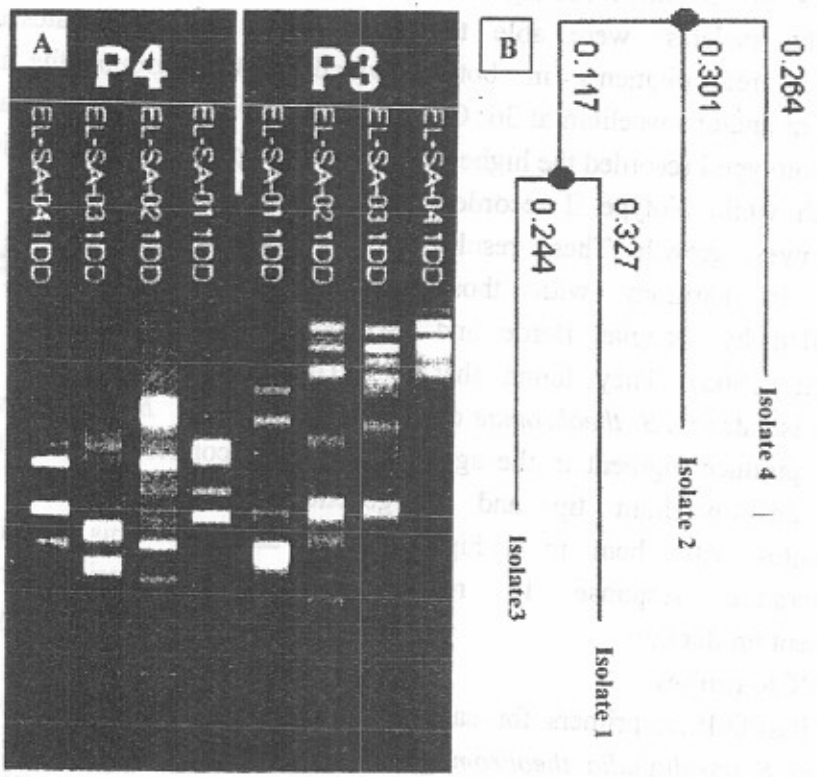

Fig. 2. A. Random amplified polymorphic DNA patterns obtained by amplification of DNA from *Botryodiplodia theobromae* isolates by the random primers P3 and P4

B. Linkage dendrogram for *Botryodiplodia theobromae* isolates on the basis of combined data of RAPD-PCR products from the two tested primers

and might played an important role for studying genetic variation within fungal species that differed in their geographic distribution and virulence (Guthrie *et al.*, 1992).

REFERENCES

Abd-El-Ghany, K. M. 2001. Studies on root rot of mango seedlings. M.Sc. Thesis, Fac. Agric., Cairo Univ., Egypt, 131 pp.

Abd-El-Hafeez, N. E. 1991. Studies on root rot of mango seedlings. Ph.D. Thesis, Fac. Agric., Menofiya Univ.,129 pp.

Al-Adawi, A. O., M. L. Deadman, A. K. Al-Rawahi; A. J. Khan and Y. M. Al- Maqbali. 2002. *Diplodia theobromae* associated with sudden decline of mango in the Sultanate of Oman. Vol. 6: August 2002 - January 2003. Agricultural and Marine Sciences, Sultan Qaboos University.

Alvarez, E., M.I. Chacon, J.B. Loke and P.L. Gomez. 1997. Pathogenic and molecular characterization of *Thielaviopsis paradoxa* strains by RAPD and PCR amplification. Phytopathology, 87: 6, S3.

Aly, A.Z., M.R.A., Tohamy, M.M.M., Atia, H.A. El-Shimy and M.A.M. Kamhawy. 2002. Grapevine twigs tip die back disease in Egypt. Egypt J. Phytopathology, 30 (1): 45-56

Baiuomy, M.A.M., M.A.M. Kamhawy, H.A. El-Shimy and Z.M.M. Mustafa. 2003. Root-rot of guava and its control in Egypt. Zagazig J. Agric. Res., 30 (3): 801-817.

Barnett, H. L. and B. B. Hunter. 1987. Illustrated genera of imperfect fungi. Mac Millan Publishing Company, London, 218 pp.

Das, S. 1993. Notes on plant pathogenic fungi on fruit trees, not recorded in Orissa. Orissa J. Hort, 21 (1-2): 89-94.

Dellaporta, S. L., J. Wood, and J. B. Hicks. 1983. A plant DNA mini preparation. Verson III. Plant Mol. Biol., 1: 19-21.

Dhingra, D. D and J. B. Sinclair. 1985. Basic plant pathology methods. CRC press. Inc. Boca, Florida.

Douglas Barde, G. and W.B. Hewitt. 1965. The principle fungus in the summer bunch rot of grapes. Phytopathology, 55: 815-816.

Gowily, Ahlam, M. 1987. Effect of various soil and irrigation on damping-off disease in some leguminous plants. Ph.D. Thesis, Fac. Agric., Zagazig University.

Guthrie, P.A.L., C.W. Mogill, A.P. Frederikson and G.N. Odvody. 1992. Random amplified polymorphic DNA markers: A system for identifying and differentiating isolates of *Colletotrichum graminicola.* Phytopathology, 82: 832-835.

Hassan, M.M.M. 1980. Taxonomical and pathological studies on Diplodia and Diplodia-like fungi. Ph. D., Thesis, Fac. Agric., Cairo Univ. 158 pp.

Huang, R., M. Galperin, Y. Levy and R. Perl-Treves. 1997. Genetic of *Fusarium moniliforme* detected by vegetative compatibility groups Random Amplified Polymorphic DNA markers. Plant Pathol., 46: 871-881.

Kore, S. S. and A. V. Mane. 1992. Dry root -rot of Kagzilime seedlings caused by *Fusarium solani.* Journal of Maharashtra Agricultural Universities, 17 (2): 276-278. (c.f CAB abstracts 1993-1994).

Kore, S. S. and D. S. Patil 1985. Dry rot disease of mango seedlings caused by *Fusarium solani.* Indian. J. of Mycol., and Plant Pathol.,15 (3): 287-288. (c.f. Rev. Pl. Pathol., 67(1): 301).

Krupinsky, J. M. 1983. Variation in virulence of *Botryodiplodia hypodermia* to *Ulmus pumila.* Phytopathology, 73: 108-110.

Leavitt, G. M. and D. E. Munnecke 1987. The occurrence and distribution of *Botryodiplodia theobromae* on grapes (*Vitis vinifera* L.) in California. Phytopathology, 77: 1690. (Abstr.)

Lim, T. K. and K. C. Khoo. 1985. Diseases and disorders of mango in Malaysia. Tropical Press, Kuola Lumpur.

Moller, E.M., J. Chelkowski and H.H. Geiger. 1999. Species-specific PCR assay for the fungal pathogens *Fusarium moniliforme* and *Fusarium subglutinans* and their application to diagnose maize ear rot disease. J. Phytopath., 147 (9): 497-508.

Muller, H. R. A. 1940. Survey of the principal mango diseases in the Dutch East Indies. Meded. Alg . Proef, Landb., Batavia, 40: 9 pp. (c.f. Rev. Appl, Mycol., XXII: 215- 216. 1943).

Nath, R. M. 1976. Guava wilt in India. A/R Review Prog. Hort., 8 (2): 41-48.

Nelson, P. E., T. A. Toussoun and W. F. O. Marasas. 1983. *Fusarium* species on illustrated manual for identification. Pennsylvania State University. Press, University Park, 193 pp.

Ploetz, R.C. and O. Prakash. 1997. Foliar, Floral and Soil-borne Diseases. Pp 281-325. In: The Mango: Botany Production and Uses. Litz, R.E, ed. CAB International, Oxon, UK.

Rai, M. K. 1986. New host records of Pestalotiopsis from India. Indian Botanical Reporter, 5(1): 98. (c.f. Rev. Pl. Path., 66, 6, 2244).

Rushdi, M.H.K. and A. Sirry. 1960. Effect of soil factors on behaviour of *Rhizoctonia* sp. in soil. Effect of soil texture, moisture, temperature and pH. Annals of Agric. Sci., Fac. Agric., Ain Shams Univ. Cairo, Egypt, Vol. 5, No. 2.

Saxena, A. K. and R. D. Rawal. 1989. Wilt of mango a new disease. Plant Disease Research, 4 (1): 89. (c. f. CAB Abstracts 1990-1991).

Tsao, P. H. , P. B. Luzaran, A. B. Santos, L. A. Portales, A. M. Gochangco, L. C. Gruber and De-Los-Santos. 1994. Phytophthora crown and root rot of mango detected in Philippine nurseries. Plant Disease, 78 (1): 100.

Tuskan, G.A., J.A. Wella and J.E. Lundquist. 1990. Genetic-geographic variation in Peridermium. Phytopathology, 80, 857. (Abst.)

Verma, K.S., S.S. Cheema, M. S. Kong and A. K. Sharma. 1991. Hitherto unrecorded disease problems of mango from Punjab. Plant Disease Research, 6 (2): 141-142.

Walker, J. C. 1975. Plant Pathology. third Ed. Mc Graw-Hill Book Co., Inc., New York, Toronto and London.

Wostemeyer, J., C. Schafer, M. Kellner and M. Wisfeld. 1991. DNA polymorphisms detected by random primer dependent PCR as a powerful tool for molecular diagnostics of plant pathogenic fungi. Page 306-312 in Advances in Molecular Genetics Hutig-Verlag, Heidelberg, Germany.

دراسات على الفطريات المحمولة بالتربة لأصول المانجو في مصر

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ينتشر مرض عفن جذور أو ذبول أصول المانجو فى جميع المحافظات الست التى تم اجراء الحصر بها خلال موسم ١٩٩٩-٢٠٠٠ وقد اختلفت هذه النسب من محافظه الى أخرى.أثبتت الدراسه أن أقل نسبة اصابه طبيعيه للشتلات في محافظة الجيزه بينما كانت أقل نسبة اصابه للأشجار في محافظة أسوان، في حين كانت أعلى نسبة اصابه سواء للأشجار أو الشتلات في محافظة الشرقيه. تم عزل ١٤ جنس من الفطريات المصاحبه لهذا المرض من مناطق الدراسه وقد تم تعريفها وتقدير نسبة ظهورها. وكانت أعلى الفطريات المعزوله تكرارا سواء من الأشجار أو الشتلات هو فطر بترورديبلوديا ثيوبرومى يليه فطر فيوزاريوم سولانى، في حين كان فطر جانودرما هو الأقل تكرارا. كان الفطر بترورديبلوديا ثيوبرومى والفطر فيوزاريوم سولانى أكثر الفطريات قدرة مرضيه في حين كان فطر الجانودرما هو أقل الفطريات قدره على احداث المرض. كانت كل أصناف الأصول الجذريه المستخدمه قابله للاصابه بالفطريات المرضيه المختبره بدرجات مختلفه. حيث كان أقل الأصناف اصابة هو الأصل الجذرى SH 1 (شرقيه ١) يتبعه الأصل الجذرى صديق بينما كان الأصل الجذرى G3 هو أكثر الأصناف قابلية للإصابة.