



ASSOCIATION OF *PLECTOSPHAERELLA MELONIS* WITH CANTALOUPE DECLINE FOR THE FIRST TIME IN EGYPT

Mostafa M.A.¹, Attia M.F.¹, Merghany M.M.² and Salama R.M.¹

¹Plant Pathology Department, Faculty of Agriculture, Cairo University, Egypt.

²Vegetable Department, Faculty of Agriculture Cairo University, Egypt.

Abstract

Cantaloupe (*Cucumis melo* var. *cantalupensis*) is a member of the family Cucurbitaceae. Under Egyptian condition, this crop is cultivated under protected plastic tunnels to produce an off-season crop for mainly exportation, as well as local consumption during the Autumn and Winter seasons. During harvesting time of cantaloupe (October, 2017) grown at Wadi El Natron Farm, Faculty of Agriculture, Cairo Univ., a collapse of cantaloupe plants was noticed as yellowing of older leaves, great reduction in green growth, corky and decayed areas on roots, after 70 days of transplanting. Isolation trials from naturally infected cantaloupe roots resulted in the presence of 6 different fungi. These fungi were identified as *Plectosphaerella melonis*, *Sclerotium bataticola*, *Rhizoctonia solani*, *Botryodiplodia* sp., *Monosporascus cannonballus* and *Fusarium solani*. The most dominant fungus in Behera governorats was *Plectosphaerella melonis*. Through light microscopy of the isolated fungus the conidia formed on phyalides were observed. The chlamydo-spores were also obvious. Scanning electron microscopy of the fungal colonies of *Plectosphaerella melonis* clearly revealed the intensive formation of the characteristic chlamydo-spores. Pathogenicity test carried out by using the six testes fungi either singly or in different double combination under greenhouse condition in pots and in plots near the greenhouse revealed that these fungi had the ability to cause the typical symptoms of cantaloupe decline (root rot with different disease severity degrees) in Gal 290 cv. This was accompanied with great reduction in some plant growth parameters tested. On the basis of these data, it is considered that *Plectosphaerella melonis* may cause cantaloupe decline under Egyptian condition during winter plantation and this is the first record of the fungus in Egypt.

Key words : *Cucumis melo*, *Plectosphaerella melonis*, cantaloupe plants.

Introduction

The soil complex conditions including soil type, texture, pH, moisture content, temperature and nutrient levels strongly influence not only the root growth, but also the diversity and activity of soil organisms. Soil stresses including soil borne diseases become particularly difficult to predict, detect and overcome. Pathogens responsible about soil borne diseases cause severe damages to many crops; they include seedling, vascular and root rot diseases. Soil borne diseases control depends undoubtedly on a thorough knowledge of the pathogen, the host plant, and the environmental conditions that support the infection. Therefore, they are strongly influenced by soil's abiotic and biotic components, as well as by agricultural practices which are applied to the soil, such as irrigation, tillage, manure application and fertilization. Basic management strategy involves

disruption of one or more of the disease components, at any stage of disease development, to achieve an economic reduction in disease with minimal disturbance to the environment. A complex disease is resulting from soil borne pathogens which often survive in soil for many years as they may reproduce in diverse host plants (Koike *et al.*, 2003, Jay and Vittorio 2015 and Katan, 2017).

Cantaloupe is considered one of the major summer and nili vegetable crops in commercial fields and under low protected cultivation tunnels during winter in Egypt. Melons in hot, arid and semiarid regions of the world are subjected to invasion through their different growth stages by many soil borne pathogens which resulted in vine decline and considerable losses in fruits yield. Fungal root rot of melon crops is considered the major disease responsible about vine decay or collapse (Davis and Gordon, 1995; Bruton and Miller, 1997 and El-Desouky

and El-Wakil, 2003 and Ana Fita *et al.*, 2007). A root rot and associated vine decline have been continuing problems on many types of melons, including cantaloupe, various mixed melons, and watermelon (*Citrullus lanatus*). Aboveground symptoms include yellowing and death of the crown leaves. Sometimes, only scant or no evidence of root rot is apparent but, in most cases, root symptoms include a rot of secondary and feeder roots and reddish or corky lesions on the taproot. Although root vascular tissue is sometimes discolored, this rarely extends into the stem. Vine collapse typically occurs just prior to harvest, resulting in premature fruit ripening, low sugar accumulation, and exposure of fruit to the sun (Aegerter, *et al.*, 2000). Several fungi have been isolated from root, collar and fruit of melon plants with symptoms of vine decline or collapse. The main causes have been attributed to *Macrophomina phaseolina* (Bruton and Jeger, 1987), *Rhizoctonia solani* and *Fusarium solani* (Champaco *et al.*, 1988 and Pivonia *et al.*, 1997), *Monosporascus cannonballus* (Martyn and Miller, 1996, Aegerter *et al.*, 2000 and El-Kolaly and Abdel-Sattar 2013), *Pythium ultimum*, *P. aphanidermatum*, (Amann, 1989 and Aegerter, *et al.*, 2000), and recently *P. cucumerina* and *P. melonis* (Carlucci *et al.*, 2012).

In 1995, Palm *et al.*, found that there was a considerable morphological variation between isolates of *P. cucumerina* associated with root and collar rots of horticultural crops in southern Italy. They indicated that this could be due to a complex of species. Asexual states of *Plectosphaerella* are differentiated based on the proportion of septate conidia (Pitt *et al.*, 2004), presence or absence of chlamydospores (Pitt *et al.*, 2004), conidial shape (Antignani *et al.*, 2008) and conidial dimensions (Duc *et al.*, 2009). Carlucci *et al.*, (2012) gave full description of *Pa. melonis* the causal of sudden decline of melons.

The aim of the present work is follow up the occurrence of cantaloupe decline in fields of El-Ayaat country (Giza gov.), Seds (Beni Suef gov.), Wadi Elnatron (Behera gov.) and Alhamam (Matruh gov.); isolation of the associated fungi and to test their pathogenicity under greenhouse conditions. Investigation was also focused on *Pa. melonis* as a new pathogen of cantaloupe causing vine decline by light and scanning microscopy.

Materials and Methods

Isolation and identification of the associated fungi

Isolation trials were carried out using samples of cantaloupe plants grown under low protected tunnels located at Giza (El- Ayaat), Beni Suef (Seds), Behera (Wadi El Natrown) and Marsa Matruh (Alhamam)

governorates in Marsh, 2017. The collected samples showed typical symptoms of vine decline, *i.e.* yellowing and death of the older leaves and corky reddish brown root rot. Twenty plants per field were taken from three fields in each site. The symptomatic plants were uprooted, put in polyethylene bags (60×40 cm) then transferred into ice box at 4°C to the lab. The root system of each of the collected plants was thoroughly washed several times under running tap water to remove the adhering soil particles. The roots were cut into small pieces (0.5 cm long), surface sterilized in 0.3% sodium hypochlorite for two minutes then passed through four changes of sterilized distilled water to remove the rest of sodium hypochlorite. The pieces were then dried between folds of sterilized filter paper. The surface sterilized root pieces were plated onto Potato Dextrose Agar (PDA) medium amended with 100 ppm streptomycin sulfate and incubated at 24±2 °C with daily observation until the formation of the fungal colonies. The total number of fungal colonies and the frequency of each fungus were determined.

The emerged fungi were separately picked and purified using the hyphal tip and/or single spore techniques adopted by Dhingra and Sinclair (1985). Identification of the purified fungi was carried out in Pl. Pathol. Dept. Fac. of Agric. Cairo Univ., based on the morphological and microscopical characteristics of the recovered fungi according to keys given by Barnett (1960), Pollack and Uecker (1974); Nelson *et al.*, (1983) and Carlucci *et al.*, (2012).

Morphology of *P. melonis* isolated

Colony characters of *P. melonis* were determined using cultures grown on PDA plates incubated in the dark at temperature 25°C for 14 days. Microscopic characters were determined from slide cultures prepared according to the method described by Palm *et al.*, (1995). For observations of conidiogenesis, a small block of the agar (about 2-3 mm) from a young fungal colony was placed in the centre of clean and sterile glass microscope slide, which was kept in a moist chamber consisting of a sterile Petri plate lined with filter-paper soaked in distilled water. After 7–10 d of incubation at 25 ± 2°C in the dark, the block of agar was removed and mycelium, conidiogenous hyphae and conidia were mounted in 100 % lactic acid. Dimensions of conidiogenous cells, hyphal coils and conidia were measured from images recorded on a Leica ADM 500 digital camera on a Leica ICC50W microscope fitted with differential interference contrast optics. From measurements of at least 25 conidia, the mean was calculated. Dimensions of other structures are given as the range of at least 20 measurements. *P. melonis* isolate was checked using scanning electron microscopy

(SEM). Colonies formed after 14 days of incubation at $25 \pm 1^\circ\text{C}$ on the surface of the solid medium (PDA) were prepared for this purpose following the method described by Samson *et al.*, (1979). Agar blocks with 3×7 mm (in dimension) bearing growths of *P. melonis* were cut out and fixed in 6% aqueous glutaraldehyde over night (16-18) hr at 4°C , washed twice in 2-methoxyethanol for 20 minutes. Blocks were then washed two times in absolute acetone then dried in CO_2 in a critical point drying apparatus, mounted on stubs with double sided sticking tape, and coated with gold in a polaron sputter coater. The specimens were examined with JoeSM-5200.

Pathogenicity test

Six highly frequent fungal species recovered from isolation and identified trials were tested for their pathogenicity in a separated mean, in different double combinations and a mixture of all of these fungi against cantaloupe (cv. Galia 290) seedlings (25 d-old) in pot experiments under greenhouse conditions and in plots near the greenhouse. In all greenhouse experiments, the soil temperature ranged from 23°C at day time to 11°C at night during December 5th, 2017 to March, 10th 2018 and 21°C at day time to 11°C at night during the period between December, 10th 2018 to March, 15th 2019. Soil temperature was recorded using logger thermometer placed 15 cm depth of soil in 3 pots as well as plots. Daily records of soil temperature were carried out at mid-day and 6 pm and average of soil temperature per day was transformed to weekly average and consequently to the monthly average per month.

Preparation of fungal inoculum

The fungi tested were grown on cornmeal-sand medium (CMS medium). A mixture of 75 g grinded corn meal and 25g fine sand previously washed with distilled water were transferred into a 250 ml glass bottle. Thereafter, each bottle received 50 ml of distilled water and plugged with a cotton stopper and autoclaved. The tested fungi were grown on PDA and incubated for 8 days at 24°C . Two discs (5-mm) of agar bearing mycelium growth taken from 8 days old culture of each fungus tested were transferred onto the surface of the bottled medium. All bottles were incubated at 24°C in an incubator. When the hyphae had grown out 2 to 3 cm, the bottles were shaken to distribute the fungal growth. Colonization of the substrate was completed in 15 days.

Soil and pots disinfestation technique

Nile silt soil with 7.2 pH was disinfested using 5% formalin solution then covered with polyethylene sheet for 10 days. After the elapse of the this period the sheet cover was removed and the soil homogenized together

to remove the formalin odor. Plastic pots (25 cm in diam.) were sterilized by dipping in formalin (5%) then converted and left to air dry for 2 days.

Greenhouse pathogenicity test

The pathogenicity of *Plectosphaerella melonis*, *Sclerotium bataticola*, *Rhizoctonia solani*, *Botryodiplodia* sp., *Monosporascus cannonballus* and *Fusarium solani*. was evaluated under the greenhouse conditions at Fac. of Agric. Cairo Univ. during the period between 5th December, 2017 to 10th March 2018 and 10th December, 2018 to 15th March, 2019).

I- Pot experiments

Sterilized Nile silt soil was thoroughly mixed with the inoculum of the desired fungal isolate grown on CMS medium at the rate of 30g/kg soil (3% inoculum level). Infested soil was equally distributed as 5kg / pot (25cm in diameter), few days before sowing and irrigated till saturation during this period. Three replicate plastic pots were used for each treatment. In check treatments pots were filled with soil mixed only with uninoculated substrate by the same ratio as mentioned before. Apparently healthy transplants of cantaloupe (cv. Galia 290) grown in seedling trays containing 209 cells of inverse pyramid shape, filled with a mixture of peat moss and vermiculate (v/v) for 25 days, were carefully uprooted and separately transferred to transplant in pots at the rate of two transplants /pot. Pots were fertilized once with 4 g of a slow-release fertilizer (Apex 21-5-6 plus micronutrients, J. R. Simplot Co., Boise, ID), and watered as needed. Each experiment was repeated twice. Disease assessment was calculated as average percentage of infection with root rot after 1 and 2 months of transplanting according to the following formula:

$$\% \text{ Root rot} = \frac{\text{Number of plants showing root rot symptoms}}{\text{Total number of the growing plants in each treatment}} \times 100$$

After 1 and 2 months of sowing, roots of diseased plants were recovered by gentle washing of the root ball after removed from the pot. Disease severity of root rot was rated based on the following scale: 0 = no symptoms, 1 = slight or limited area of discoloration, 2 = general discoloration (one or two lesions), 3 = general discoloration (rot of some tissues), and 4 = rot of entire tap and feeder roots. In addition, some growth parameters, *i.e.* length of the main shoot (cm) and shoot fresh and dry weights (gm), were also taken into consideration.

Disease severity index (DSI%) was calculated according to the formula given by Song *et al.*, (2004)

$$\text{DSI \%} = \frac{\sum d \times (\text{number of plants in the grade})}{(d \text{ max} \times \text{number of plants in that grade}) \times n}$$

Where, d= The disease rating in grade;
 d max = The maximum disease rating
 n = Total number of plants in each treatment

II- Plot experiments

Microplots (1×1×1 m), located near the greenhouse, Fac. of Agric. Cairo Univ., were filled with disinfested Nile silt soils with 7.2 pH. Different double combinations of any fungi tested and a mixture of all fungi tested were mixed into the upper 15 cm of the treated plots at the rate of 3% of soil weight, while the check treatments were plots filled with soil mixed only with uninoculated substrate by the same ratio as mentioned before. Two rows with 30 cm width were designed in each plot. Plots were irrigated three times with 2 days intervals thereafter, direct seeded with apparently healthy 25 day-old transplants of cantaloupe (cv. Galia 290) on the upper surface of the row with 30 cm apart. After one month of transplanting, plants were thinned to be four plants/plot. All plots were covered with finely meshed nylon (5 im in thickness) row covers to protect the young plants from virus vectors until flowering and vining. All plots were irrigated and fertilized as usual. Each inoculation treatment included three plots in a randomized complete block design. In all experiments, plants were harvested when the fruit was mature. Disease assessment was calculated as average percentage of infection during the two growing seasons (2017/2018 and 2018/2019) with cantaloupe root rot caused by different double combinations and a mixture of the fungi tested at harvest time (3 months after transplanting). Roots and lower stems of cantaloupe were gently removed from the plots, washed in 1% sodium metaphosphate, and rated for disease severity index as mentioned before. Also, some plant growth parameters, i.e. length of the main shoot (cm) and shoot fresh and dry weights (g) were recorded.

Statistical analyse

Data obtained were statistically analyzed as described by Snedecor and Cochran (1980). The mean values were compared to each other using Fisher's protected least significant difference ($P = 0.05$).

Results

Isolation and identification of the associated fungi

Isolation trials carried out from samples of cantaloupe plants suffering from vine decline grown under low protected tunnels located at Giza (El- Ayaat), Beni Suf (Seds), Behera (Wadi El Natrown) and Matruh (Alhamam) governorates in Marsh, 2017 yielded seven different species of fungi belonging to six genera (Table 1). These fungi were identified as *Plectosphaerella*

melonis, *Sclerotium bataticola*, *Rhizoctonia solani*, *Botryodiplodia* sp., *Monosporascus cannonballus*, *Fusarium solani* and *Fusarium oxysporum*.

The highest number of fungal colonies was obtained from diseased samples collected from Behera governorate (Wadi El Natrown), being 35 followed by that from Beni Suf (Seds). Mean while samples of Matruh (Alhamam) and Giza (El- Ayaat) governorates gave the same number of fungal colonies, being 18. It is obvious from data in table 1 that *Plectosphaerella melonis* was only isolated from samples collected from Behera (Wadi El Natrown) governorate with frequency reached 57.14 % and the same is true in case of *F. oxysporum* from Giza. On the other hand, *Monosporascus cannonballus* was not isolated from samples collected from Giza (El- Ayaat) and Matruh (Alhamam) governorates. The same was also true in case of *Botryodiplodia* sp and *Sclerotium bataticola* which were not isolated from samples collected from Giza (El- Ayaat) and Behera (Wadi El Natrown) governorates, respectively (Table 1). Data also indicated that among the fungi isolated from four the governorates, *Rhizoctonia solani* recorded the highest frequency (23.40%), followed by *Plectosphaerella melonis* (21.28%), *Fusarium solani* (19.15%), *Sclerotium bataticola* (17.02%), *Monosporascus cannonballus* (9.57%) and *Botryodiplodia* sp (6.38%), respectively. Meanwhile, the lowest percentage of frequency was recorded for *F. oxysporum*, being 3.19% (Table 1).

Morphology of *P. melonis* isolated

The colonies of *Pa.melonis* formed on PDA medium were white, cottony aerial mycelium, reached a diameter of 9 cm after 14 days of incubation in dark at 25°C (Fig. 1A). Mycelium hyaline, branched, septate, occasionally forming loose hyphal coils (Fig. 1B). Conidiophores are solitary, sparingly branched, hyaline, smooth, thin-walled (Fig. 1C). Conidiogenous cells are phialidic, determinate, discrete, hyaline, smooth, thin-walled, with single basal septum, widest at base, straight, gradually tapering to the apex, phialide apex straight. Conidia aggregating in slimy heads, ellipsoid, tapering to rounded apex and base, hyaline, smooth, thin-walled, with a minute apiculus at either end, mostly aseptate with 5-6× 2-3.5 im, L/W. Chlamydospores intercalary (Fig.1), hyaline, thick-walled, 15-15.5 × 9-11.8im (Fig. 1D)

Greenhouse pathogenicity test

A-Pot experiments

Data presented in table 2 clearly indicate that all the fungi tested caused root rot to the grown cantaloupe plants grown in soil artificially infested with the tested fungi.

Table 1: Occurrence and frequency (%) of fungi associated with cantaloupe plants suffered from root rot or vine decline collected from Giza (El- Ayaat), Beni Suef (Seds), Behera (Wadi El Natrown) and Matruh (Alhamam) governorates in Marsh, 2017.

Fungi isolated	Giza (El-Ayaat)		Beni Suef (Seds)		Behera (Wadi El Natrown)		Matruh (Alhamam)		#	%
	#	%	#	%	#	%	#	%		
<i>Plectosphaerella melonis</i>	0	0.00	0	0.0	20	57.14	0	0.0	20	21.28
<i>Sclerotium bataticola</i>	4	22.22	7	30.43	0	0.00	5	27.78	16	17.02
<i>Rhizoctonia solani</i>	5	27.78	8	34.78	5	14.29	4	22.22	22	23.40
<i>Botrydiploia</i> sp	0	0.00	1	4.35	2	5.71	3	16.67	6	6.38
<i>Monosporascus cannonballus</i>	0	0.00	4	17.39	5	14.29	0	0.00	9	9.57
<i>Fusarium solani</i>	6	33.33	3	13.04	3	5.57	6	33.33	18	19.15
<i>Fusarium oxysporum</i>	3	16.67	0	0.00	0	0.00	0	0.00	3	3.19
Total	18	100	23	100	35	100	18	100	94	100

= No of counted colonies % = Frequency

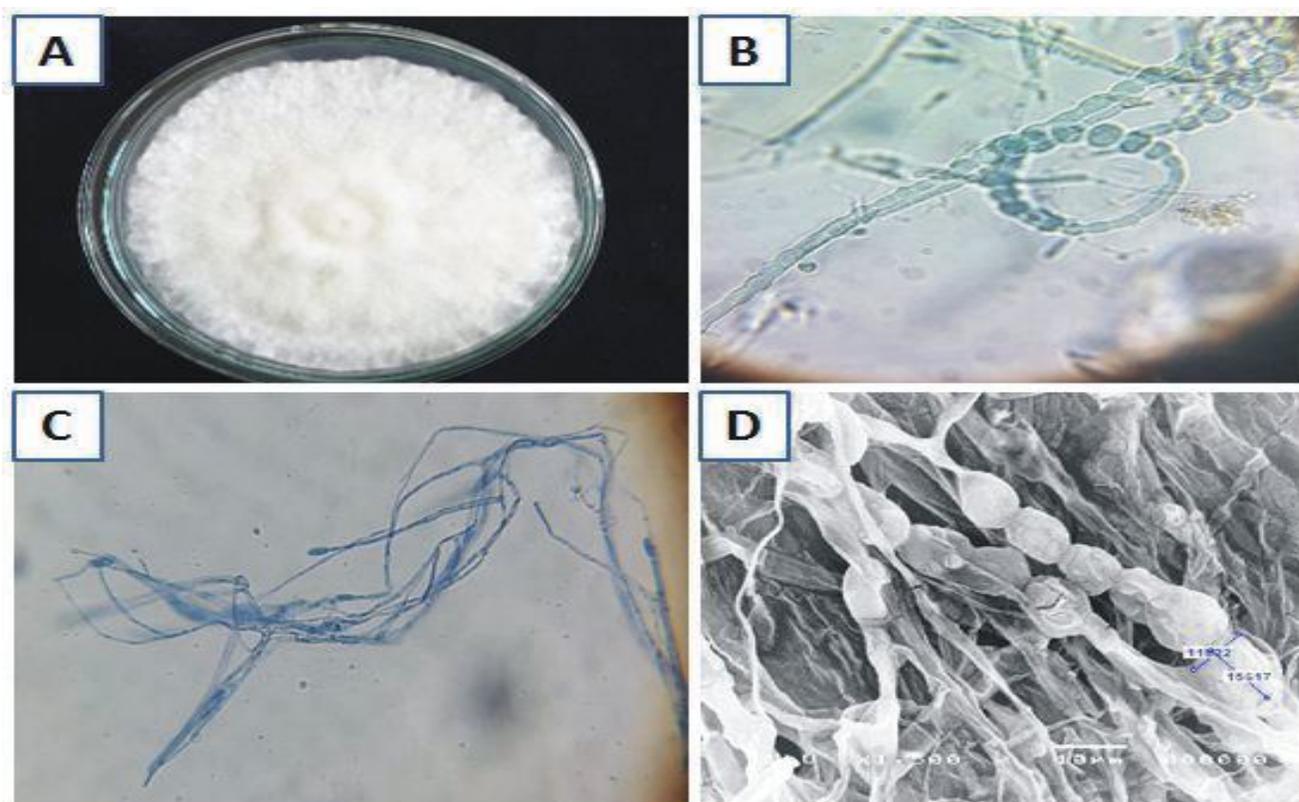


Fig.1: Shows (A). *Plectosphaerella melonis* colony on PDA 14 days of incubation at 25 °C (B). chlamydoconidia intercalary by light microscope (400x) (C). Conidiophores and conidia by light microscope (D). Scanning electron micrograph indicating the formation of chlamydoconidia.

The highest percentages of cantaloupe root rot were recorded from *F. solani* treatment even after one or two months post transplanting, being 50 and 100%, respectively. On the other hand, both *M. cannonballus* and *P. melonis* were the second in this respect. The lowest percentage of root rot after one month post transplanting was recorded from pots infested with each of *R. solani* and *Botrydiploia* sp., being, 16.65%.

Regarding to the disease severity index, data table 2

show that the highest value (80.00%).

Was recorded from infection by either *M. cannonballus* and *Pa. melonis* after 2 months post transplanting followed by that incited by *F. solani*, *R. solani* and *S. bataticola*, respectively. The lowest value of DSI was recorded from plants infected by *Botrydiploia* sp., being 20% after 2 months.

B. Plot experiment

Effect of different combinations of the fungi tested in addition to a mixture of all fungi on the average

Table 2 : Pathogenicity test of six different fungi isolated from naturally infected cantaloupe plants suffering from root rot and vine decline (pot experiments).

Fungi tested	Root rot % after (month)		Disease severity index(DSI) % after (month)		Survived plants % After 3 months
	1	2	1	2	
<i>S. bataticola</i>	22.20	66.6	15.02	31.25	33.4
<i>Botryodiplodia</i> sp.	16.65	66.6	9.50	20.00	33.4
<i>F. solani</i>	50.00	100,0	33,0	60.30	0.0
<i>M. cannonballus</i>	33.30	66.6	45.55	80.00	33.4
<i>R. solani</i>	16.65	66.6	22.80	46.60	33.4
<i>P. melonis</i>	33.30	66.6	45.00	80.00	33.4

percentage of infection with cantaloupe root rot during the two growing seasons (2017/2018 and 2018/2019) was carried out in the microplots (1×1×1 m). The highest average percentage of cantaloupe root rot (66.67%) and index of disease severity (14.58%) were obtained from plants growth in soil artificially infested with all the tested fungi (Table 3). On the other hand, among the combinations tested, a combination of *F. solani* + *Pa. melonis* gave the highest average percentages of both of cantaloupe root rot and index of disease severity, followed by combinations of *S. bataticola* + *F. solani* and *S. bataticola* + *Pa. melonis*, respectively. The corresponding mean values were 50.00 and 16.67% ; 41.46% and 11.46% and 41.46 and 11.46%, respectively. Meanwhile the lowest percentages of the cantaloupe root rot and index of disease severity were obtained from soil artificially infested by the combination of *S. bataticola* + *Botryodiplodia* sp., being 16.67% and 6.25%, respectively (Table 3). Results indicating the effect of soil infestation by different combinations and the mixture of the fungi tested on some growth parameters of cantaloupe[main shoot length (cm), shoot fresh and dry weights (g)] during two growing seasons (2017/2018 and 2018/2019) at harvest time (3 months after transplanting) are shown in table 4. Data clearly indicate that all the combinations and a the mixture of fungi used in soil infestation significantly reduced all the growth parameters recorded in comparison with growth parameters for plants grown in check treatment (Table 4).

It's also obvious from data in table 4 that the highest reduction in main shoot length, shoot fresh and dry weights was obtained from cantaloupe plants grown in soil artificially infested with a mixture of all fungi tested. The corresponding values were 61.48, 30.55 and 65.39%, respectively. On the other hand, the combination of *F. solani* + *Pa. melonis* used in soil infestation gave the great reduction in the recoded main shoot length (57.90%) and shoot fresh weight (25.82%) in comparison with other

combinations tested. Cantaloupe plants grown in soil artificially infested with different combinations of fungi were varied in their dry weight of the main shoot. The highest reduction recorded in this respect was from plants grown in artificially infested soil with the combination of *Botryodiplodia* sp.+ *F. solani* followed by the combination of *S. bataticola* + *P. melonis*. The corresponding values were 23.72 and 23.52%, respectively (Table 5).

Discussion

In the present study, fungal pathogens including *Plectosphaerella melonis*, *Sclerotium bataticola*, *Rhizoctonia solani*, *Botryodiplodia* sp., *Monosporascus cannonballus*, *Fusarium solani* and *fusarium oxysporium* were isolated from collected cantaloupe samples showing typical symptoms of vine decline. The collected samples exhibited symptoms of yellowing, death of the older leaves and corky reddish brown root rot. The frequencies of all of the fungi isolated and identified in the present study demonstrated that *R. solani* recorded the highest frequency (23.40%), followed by *Pa. melonis* (21.28%), *F. solani* (19.15%), *S. bataticola* (17.02%), *M. cannonballus* (9.57%) and *Botryodiplodia* sp (6.38%), respectively. Meanwhile, the lowest percentage of frequency was recorded for *F. oxysporum*, being 3.19%. *P. melonis* was only isolated from samples collected from Behera (Wadi El Natrown) governorate with frequency reached 57.14 %. The association of *P. melonis* with diseased cantaloupe plants in Behera (Wadi El Natrown) governorate with high frequency may be due to the soil condition in this governorate especially soil temperature and soil moisture content during harvesting. Carlucci *et al.*, (2012) indicated that the minimum temperature for *Pa melonis* growth on PDA medium was 9°C whereas, optimum was 25°C and maximum was 31°C. It's worthy to know that host plant infections by *Plectosphaerella* spp. are generally not well defined, as they are sometimes reported as either causal agents of wilt disease (Xu *et al.*, 2014), or root rot disease (Carrieri *et al.*, 2014 and Maria Luisa and Antonia, 2018). The fluctuation in the frequency percentages of isolated fungi in the four governorates may probably influenced by different variables such as climatic conditions, microbial competition close to the roots of the plants and soil texture in these governorates. It is also possible that all of the isolated fungi almost act

Table 3: Average percentage of infection during two growing seasons (2017/2018 and 2018/2019) with cantaloupe root rot caused by different combinations and a mixture of the fungi tested at harvest time (3 months after transplanting) and their effects on the root disease severity index (plots experiment).

Fungi tested	Root rot %	DSI %
<i>S. bataticola</i> + <i>Botryodiplodia</i> sp.	16.67	6.25
<i>S. bataticola</i> + <i>F. solani</i>	41.67	11.46
<i>S. bataticola</i> + <i>M. cannonballus</i>	33.33	9.38
<i>S. bataticola</i> + <i>R. solani</i>	33.33	10.42
<i>S. bataticola</i> + <i>P. melonis</i>	41.46	11.46
<i>Botryodiplodia</i> sp.+ <i>F. solani</i>	16.67	6.25
<i>Botryodiplodia</i> sp.+ <i>M. cannonballus</i>	33.33	3.13
<i>Botryodiplodia</i> sp.+ <i>R. solani</i>	25.00	6.25
<i>Botryodiplodia</i> sp.+ <i>P. melonis</i>	25.00	7.29
<i>F. solani</i> + <i>M. cannonballus</i>	33.33	12.50
<i>F. solani</i> + <i>R. solani</i>	33.33	8.33
<i>F. solani</i> + <i>P. melonis</i>	50.00	16.67
<i>M. cannonballus</i> + <i>R. solani</i>	33.33	11.46
<i>M. cannonballus</i> + <i>P. melonis</i>	41.67	13.54
A mixture of all fungi tested	66.67	14.58
LSD at 5%	2.97	2.14

similarly by suppressing the root system, finally affecting the water balance, especially during the period of fruit maturation. Abiotic stresses, such as high or low temperature, may further aggravate the stress situation (Miller *et al.*, 1995; Bruton and Miller, 1997; El-Desouky and El-Wakil, 2003 and Ana Fita *et al.*, 2007). Light and scanning electron microscopy of the colonies of the newly recorded fungus gave identical characters of *Pa.melonis* as described by Carlucci *et al.*, (2012). The pathogenicity of *P. melonis*, *S. bataticola*, *R. solani*, *Botryodiplodia* sp., *M. cannonballus* and *Fusarium solani*. was evaluated under the greenhouse conditions Fac. of Agric. Cairo Univ. during the period between 5th December, 2017 to 10th Marsh 2018 and 10th December, 2018 to 15th Marsh, 2019). In pot experiments it was found that the great percentages of cantaloupe root rot were recorded from soil infested with *F. solani* even after one or two months' post transplanting, being 50 and 100% respectively. On the other hand, both *M. cannonballus* and *P. melonis* were the second in this respect. The highest value of DSI (80.00%) was recorded from cantaloupe plants grown in infested soil with any of *M. cannonballus* or *Pa.melonis* after 2 months post transplanting followed by that incited in

Table 4: Effect of soil infestation with different combinations and a mixture of the fungi tested on the average of main shoot length (cm) and shoot fresh and dry weights (g) of cantaloupe at harvest time. (plot experiment).

Fungi tested	Shoot length (cm)	% Reduction*	Shoot fresh weight (g)	% Reduction*	Shoot dry weight (g)	% Reduction*
<i>S. bataticola</i> + <i>Botryodiplodia</i> sp.	89.1	42.99	188.20	10.17	56.64	9.88
<i>S. bataticola</i> + <i>F. solani</i>	81.2	48.05	171.20	15.42	54.78	12.84
<i>S. bataticola</i> + <i>M. cannonballus</i>	83.2	46.77	169.00	19.33	50.70	19.33
<i>S. bataticola</i> + <i>R. solani</i>	80.1	48.75	166.50	20.52	48.15	23.39
<i>S. bataticola</i> + <i>P. melonis</i>	77.2	50.61	160.20	23.53	48.06	23.53
<i>Botryodiplodia</i> sp.+ <i>F. solani</i>	80.6	48.43	159.80	23.72	47.94	23.72
<i>Botryodiplodia</i> sp.+ <i>M. cannonballus</i>	81.4	47.92	163.20	22.10	49.56	21.15
<i>Botryodiplodia</i> sp.+ <i>R. solani</i>	80.1	48.75	168.2	19.71	50.46	19.71
<i>Botryodiplodia</i> sp.+ <i>Pa. melonis</i>	78.7	49.65	163.00	22.20	53.79	14.41
<i>F. solani</i> + <i>M. cannonballus</i>	77.0	50.73	161.30	23.01	51.62	17.87
<i>F. solani</i> + <i>R. solani</i>	72.4	52.40	159.20	24.01	52.54	16.40
<i>F. solani</i> + <i>Pa. melonis</i>	65.8	57.90	155.40	25.82	49.73	20.87
<i>M. cannonballus</i> + <i>R. solani</i>	81.0	48.18	166.60	20.48	51.65	17.82
<i>M. cannonballus</i> + <i>P. melonis</i>	71.2	54.44	165.32	20.86	52.9	15.83
A mixture of all fungi tested	60.2	61.48	146.50	30.55	43.95	65.39
Check	156.3	209.50	62.85
LSD at 5%	1.68		4.32		1.47	

$$* \% \text{Reduction} = \frac{\text{check} - \text{treatment}}{\text{check}} \times 100$$

soil infested with any of *F. solani*, *R. solani* and *S. bataticola* respectively. The lowest value of DSI was recorded from plants grown in soil infested with *Botrydiplodia* sp., being 20% after 2 months. Our results from the greenhouse studies (pot experiments) confirm the previous reports of the susceptibility of cantaloupe to infection by *P. melonis*, *S. bataticola*, *R. solani*, *Botrydiplodia* sp., *M. cannonballus* and *F. solani* (Bruton and Jeger, 1987; Champaco *et al.*, 1988; Pivonia *et al.*, 1997; Aegerter *et al.*, 2000; Carlucci *et al.*, 2012 and El-Kolaly and Abdel-Sattar 2013). In microplots experiment using different combinations of the fungi tested in addition to a mixture of all fungi, the effect on percentage of infection with cantaloupe root rot and on some growth parameters was varied. The highest average percentage of cantaloupe root rot (66.67%) and index of disease severity (14.58%) were obtained from soil artificially infested with all the tested fungi. On the other hand, among the combinations tested the combination of *F. solani* + *P. melonis* gave the highest average percentages of both the cantaloupe root rot and index of disease severity, followed by combinations of *S. bataticola* + *F. solani* and *S. bataticola* + *P. melonis*, respectively. This was a combined with great reduction in plant growth parameters tested. Plant pathology has focused predominantly on single host-single disease interactions. Whilst this simplification has proved useful, plants in nature interact with multiple pathogen species/genotypes (Katan, 2017, Kozanitas *et al.*, 2017 and Tollenaere *et al.*, 2017). Some pathogenic infections can be detrimental to the defense systems predisposing the plant to subsequent secondary infections. This complex interaction is known as co-infection. In co-infection systems, pathogen interactions include: (i) Competition, in which competing pathogens develop physical barriers or utilize toxins to exclude competitors from resource-rich niches; (ii) Cooperation, whereby pathogens beneficially interact, by providing mutual biochemical signals essential for pathogenesis, or through functional complementation via the exchange of resources necessary for survival; (iii) Coexistence, whereby pathogens can stably coexist through niche specialization. Furthermore, hosts are also able to, actively or passively, modulate niche competition through defense responses that target at least one pathogen. Typically, however, virulent pathogens subvert host defenses to facilitate infection, and responses elicited by one pathogen may be modified in the presence of another pathogen. In contrast, pathogen–pathogen and host-multiple-pathogen interactions can lead to various results: antagonism, synergism, coexistence, mutualism, or cooperation (Araz

et al., 2017).

References

- Aegerter, B.J., T.R. Gordon and R.M. Davis (2000). Occurrence and pathogenicity of fungi associated with melon root rot and vine decline in California. *Plant Dis.*, **84**:224-230.
- Amann, A.B. (1989). The etiology of Pythium sudden wilt of cantaloupe in the Imperial Valley of California. MS thesis. University of California, Davis.
- Antignani, V., W. Gams and F. Marziano (2008). *Plectosporium detsorboi* nov. sp., a pathogen of Curcuma, Zingiberaceae. *Nova Hedwigia*, **86**: 209-214.
- Araz, S.A., S.M. Caroline, J.L. Francisco, R.G. Mark, H. John and Z. Ayalsew (2017). Host-Multi-Pathogen Warfare: Pathogen Interactions in Co-infected Plants. *Plants. Front. Plant Sci.*, **8**:1806. doi: 10.3389/fpls.2017.01806
- Barnett, H.J. (1960). “*Illustrated Genera of Imperfect fungi*”. Burgess Minneapolis. USA, 225.
- Bruton, B.D. and M.J. Jenger (1987). Microphomina phaseolina infection and vine decline in cantaloupe in relation to planting date, soil environment and plant maturation. *Plant Disease*, **71**:259-263.
- Bruton, B.D. and M.E. Miller (1997). Occurrence of vine decline of melons in Honduras. *Plant Disease*, **81**: 696.
- Carlucci, A., M.L. Raimondo, J. Santos and A.J.L. Phillips (2012). *Plectosphaerella* species associated with root and collar rots of horticultural crops in southern Italy. *Persoonia.*, **28**: 34-48.
- Carrieri, R., G. Pizzolongo, G. Carotenuto, P. Tarantino and E. Lahoz (2014). First report of necrotic leaf spot caused by *Plectosphaerella cucumerina* on lamb’s lettuce in southern Italy. *Plant Disease*, **97**: 998.
- Champaco, E.R., R.D. Martyn, L.W. Barnes, M.E. Miller, J.M. Amador and A. Perez (1988). Root rot, a new disease of muskmelon in South Texas. (Abstr.) *Phytopathology*, **78**: 626.
- Davis, R.M. and T.R. Gordon (1995). Occurrence of *Acremonium cucurbitacearum* sp. and *Monosporascus cannonballus* in the major cantaloupe and watermelon growing areas of California. *Plant Disease*, **79**: 754.
- Dhingra, O.D. and J.B. Sinclair (1985). “*Basic Plant Pathology Methods*”. 2nd ed. CRC, Boca Raton, Florida, USA, 434.
- Duc, P.M., K. Hatai, O. Kurata, K. Tensha, Y. Uchida, T. Yaguchi and S.I. Udagawa (2009). Fungal infection of mantis shrimp (*Oratosquilla oratoria*) caused by two anamorphic fungi found in Japan. *Mycopathologia*, **167**: 229-247
- El-Desouky, S.M. and A.A. El-Wakil (2003). Occurrence of *Monosporascus* root rot and vine decline of cantaloupe and watermelon in Egypt. *Egyptian Journal of Phytopathology*, **31**: 141-150.
- El-Kolaly, A.A. Ghada and M.A. Abdel-Sattar (2013). The Etiology of Sudden wilt disease Syndrome on Melon in Egypt. *Nature and Science*, **11**(11).

- Fita, A., P. Belen and N. Fernando (2007). Melon roots under stress: Melon Vine Decline. *Plant Stress*, **1(1)**: 93-104 Global Science Books.
- Jay, R.L. and V. Vittorio (2015). Synergisms between microbial pathogens in plant disease complexes: a growing trend. *Front. Plant Sci.*, **6**: 385.
- Katan, J. (2017). Diseases caused by soilborne pathogens: biology, management and challenges. *Journal of Plant Pathology*, **99(2)**: 305-315.
- Koike, S.T., K.V. Subbarao, R.M. Davis and T.A. Turini (2003). Vegetable diseases caused by soilborne pathogens. Pub 8099 UC Davis, 12.
- Kozanitas, M., T.W. Osmundson, R. Linzer and M. Garbelotto (2017). Interspecific interactions between the Sudden Oak Death pathogen *Phytophthora ramorum* and two sympatric *Phytophthora* species in varying ecological conditions. *Fungal Ecol.*, **28**: 86-96. doi: 10.1016/j.funeco.2017.04.006.
- Maria Luisa, R. and C. Antonia (2018). Characterization and pathogenicity of *Plectosphaerella* spp. Collected from basil and parsley in Italy. *Phytopathologia Mediterranea*, **57(2)**: 284-295.
- Martyn, R.D. and M.E. Miller (1996). *Monosporascus* root rot and vine decline: an emerging disease of melons worldwide. *Plant Dis.*, **80**: 716-725.
- Miller, M.E., R.D. Martyn, B.R. Lovic and B.D. Bruton (1995). An overview of vine decline diseases in melons. Pages 31-35 in: Proc. Cucurbitaceae 94: Evaluation and Enhancement of Cucurbit Germplasm. G. E. Lester and J. R. Dunlap, eds. Gateway Printing, Edinburg, TX.
- Nelson, P.E., T.A. Toussoun and W.F.O. Marasas (1983). “*Fusarium species: An illustrated manual for identification*”. Pennsylvania State University Press, University Park, 205.
- Palm, M.E., W. Gams and H.I. Nirenberg (1995). *Plectosporium*, a new genus for *Fusarium tabacinum*, the anamorph of *Plectosphaerella cucumerina*. *Mycologia*, **87**: 397-406.
- Pitt, W.M., S.B. Goodwin, G.J. Ash, N.J. Cother and E.J. Cother (2004). *Plectosporium alismatis* comb. nov., a new placement for the Alismataceae pathogen *Rhynchosporium alismatis*. *Mycological Research*, **108**: 775-780.
- Pivonia, S., R. Cohen, U. Kafkafi, I.S. Ben Ze'ev and J. Katan (1997). Sudden wilt of melons in southern Israel: Fungal agents and relationship with plant development. *Pl. Dis.*, **81**: 1264-1268.
- Pollack, F.G. and F.A. Uecker (1974). *Monosporascus cannonballus* an unusual Ascomycete in cantaloupe roots. *Mycologia*, **66**: 346-349.
- Samson, R.A., J.A. Stalpers and W. Verkerke (1979). A simplified technique to prepare fungal specimens for scanning electron microscopy. *Cytobios.*, **24(93)**: 7-11.
- Snedecor, G.W. and W.G. Cochran (1980). “Statistical Methods” Iowa State College Press, Ames.
- Song, W., L. Zhou, C. Yang, X. Cao, L. Zhang and X. Liu (2004). Tomato *Fusarium* wilt and its chemical control strategies in a hydroponic system. *Crop Protection*, **23(3)**: 243-247.
- Tollenaere, C., S. Lacombe, I. Wonni, M. Barro, C. Ndougounga and F. Gnacko *et al.*, (2017). Virus-bacteria rice co-infection in Africa: field estimation, reciprocal effects, molecular mechanisms, and evolutionary implications. *Front. Plant Sci.*, **8**: 645. doi: 10.3389/fpls.2017.00645
- Xu, J., X.D. Xu, Y.Y. Cao and W.M. Zhang (2014). First report of greenhouse tomato wilt caused by *Plectosphaerella cucumerina* in China. *Plant Disease*, **98**: 158.