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New pathogens associated with cantaloupe vine decline in Egypt and their chemical control under greenhouse conditions

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Cantaloupe (*Cucumis melo* L.) is severely attacked by many soil-borne pathogens through different growth stages, which usually result in vine decline and considerable losses in fruit yield. The genus *Fusarium* is considered one of the most important genera that include serious plant pathogens. The present study aimed to isolate and identify the fungal isolates of cantaloupe vine decline disease from fields located in different agroecological zones: Al Ayyat (Giza Governorate), Seds (Beni Suef Governorate), Wadi El Natrun (Beheira Governorate), Mut (New Valley Governorate), and Al Hammam (Matrouh Governorate). Identification of the associated fungi was carried out using both morphological and microscopic characteristics, besides molecular techniques using the internal transcribed spacer (ITS) region. Four fungal isolates of *Fusarium* were identified as *Fusarium inflexum*, *F. oxysporum*, *F. nygamai*, and *F. ambrosium*, and one isolate was identified as *Paecilomyces dactylethromorphus*. *F. inflexum* was the most frequent fungus, while the lowest frequency percentage was recorded for *P. dactylethromorphus*. ITS sequences of the isolates F04 and F05 showed 100% identity to *Paecilomyces dactylethromorphus and Fusarium ambrosium,* respectively. To our knowledge, this is the first record of *Paecilomyces dactylethromorphus and Fusarium ambrosium* causing cantaloupe vine decline disease in Egypt. *In vitro* studies clearly showed the inhibitory effect of the three tested fungicides, namely, uniform SC 39%, Maxim XL 3.5%, and double WP 56%, with four concentrations against the growth of the five tested pathogenic fungi. It was found that all the tested fungicides significantly decreased the development of the tested fungi. Moreover, applying fungicides under greenhouse conditions reduced the root rot incidence and decreased the disease severity.

Keywords: Cantaloupe, Chemical control, Fungal vine decline, *Fusarium ambrosium,* Greenhouse conditions, ITS sequences, *Paecilomyces dactylethromorphus,* Soil-borne pathogens

INTRODUCTION

Cucumis melo L. is a crop of enormous economic importance worldwide, belongs to the Cucurbitaceae family, and is well known for its culinary and medicinal properties. Its high fruit consumption produces a large quantity of waste materials, such as peels and seeds, which are still rich in molecules like polyphenols, carotenoids, and other biologically active components that influence human health and wellness .It is well known that the nutritional components of cantaloupe, including vitamins, minerals, antioxidants, and dietary fiber, contribute to improving immunity, hydration, and protection against chronic diseases. Plant diseases cause significant losses in vegetable production and pose a threat to crop production, resulting in losses of 40–60% annually (Ali et al., 2023; Silva et al., 2024; Tripathi et al., 2024). Vegetable crops are comparatively more susceptible to various types of diseases caused by fungi, bacteria, viruses, viroids, phytoplasma, and nematodes. *Alternaria*, *Ascochyta*, *Colletotrichum*, *Didymella*, *Phoma*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Sclerotinia*, *Sclerotium*, *Fusarium*, and *Plectosphaerella melonis* are among the main genera, which include the causals of fungal diseases that have significantly expanded their prevalence on vegetable crops (Monica et al., 2019; Mostafa et al., 2019; Tripathi et al., 2024). The basic management strategy involves disruption of one or more of the disease components at any stage of disease development, which aims to effectively reduce the amount of disease while causing the least amount of environmental damage possible. A complex disease results from soil-borne pathogens that often survive in soil for many years as they may reproduce in diverse host plants (Jay & Vittorio, 2015; Katan, 2017).

Melons are subjected to invasion by many soil-borne pathogens through their different growth stages, which results in vine decline and considerable losses in fruit yield. Fungal root rot of melon crops is considered the major disease responsible for vine collapse and deterioration (Davis & Gordon, 1995; Bruton & Miller, 1997; EI-Desouky & El-Wakil, 2003; Fita et al., 2007; Mostafa et al., 2019; Paudel et al., 2023). Cantaloupe plants affected by sudden wilt exhibited yellowing, death of the crown leaves, and a gradual decline of the vine as the plant approached maturity, resulting in canopy collapse as well as necrosis of the root system, with numerous discrete lesions and lack of most of the secondary and tertiary feeder roots (Boughaleb et al., 2009). Sometimes root vascular tissue is discolored, but this rarely extends into the stem. Vine collapse typically occurs just prior to harvest, resulting in premature fruit ripening and low sugar accumulation (Aegerter et al., 2000). *Fusarium* is considered to be one of the most important genera of plant pathogens, which are known to cause serious diseases in several economically valuable plants, including cantaloupes, cucumbers, watermelons, and tomatoes (Ajmal et al., 2022; Oyedeji et al., 2022;). Fungicides are crucial parts of crop protection and are important for controlling a number of destructive crop diseases and achieving the highest possible crop yields (Thind, 2017**)**.

The present study aimed to investigate the disease occurrence of fungal pathogens that are causing cantaloupe vine decline in the fields of Al Ayyat (Giza Governorate), Seds (Beni Suef Governorate), Wadi El Natrun (Beheira Governorate), Mut (New Valley Governorate), and Al Hammam (Matrouh Governorate); isolation and identification of the causal agents were carried out using their morphological and microscopical characteristics, beside molecular techniques using the sequencing of the internal transcribed spacer (ITS) region. Pathogenicity tests of the isolated fungi were evaluated under the greenhouse conditions located at Faculty of Agriculture, Cairo University. Some growth parameters of cantaloupe plants, i.e., stem length (cm), plant fresh weight, and plant dry weight (gm), were taken also into consideration. Chemical control using three different fungicides to control cantaloupe root rot and lower the disease severity was also carried out.

MATERIALS AND METHODS

Isolation and identification of the fungi associated with declined cantaloupe plants

Isolation trials were carried out from cantaloupe samples collected from plants grown under low protected tunnels and/or grown under field conditions located in Al Ayyat (Giza Governorate), Seds (Beni Suef Governorate), Wadi El Natrun (Beheira Governorate), Mut (New Valley Governorate), and Al Hammam (Matrouh Governorate). The collected samples showed typical symptoms of vine decline, i.e., yellowing and death of the older leaves and corky reddish-brown root rots. Each site was represented by three fields and 20 plants. The symptomatic plants were gently uprooted, put in polyethylene bags (25×20cm), and then transferred to the lab in an icebox at 4°C. 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 (0.5cm long), surface- sterilized in 0.3% sodium hypochlorite for two minutes, and then passed through four successive changes of sterilized distilled water to remove the remaining sodium hypochlorite. The pieces were then dried between two folds of sterilized filter paper. The surface-sterilized root pieces were plated onto potato dextrose agar (PDA) medium amended with 100 mg/L streptomycin sulfate in Petri dishes and incubated at 24±2°C with daily observation for the formation of the fungal colonies. The emerged fungi were separately picked up and purified using the hyphal tip and/or single spore techniques adopted by Dhingra & Sinclair (1985). Fungi were identified according to their morphological and microscopic characteristics following the key given by Barnett (1960), Nelson et al. (1983), and Carlucci et al. (2012). The total number of fungal colonies and the frequency (%) of each fungus were determined according to the formula:

% Frequency= Number of counted colonies of fungi/ total number of the counted colonies of fungi × 100.

Molecular identification of the isolated fungi

DNA extraction from fungal cultures was performed according to Brandfass & Karlovsky (2008). The internal transcribed spacer (ITS) regions of extracted DNA were amplified using primers ITS1 (5′-CTTGGTCATTTAGAGGAAGTA-3′) and ITS2 (5′-GACGCTTCTCCAGACTACAAT-3′). The PCR reaction mixture contained 12.5µL of 2X PCR Master Mix, 1 µL each of ITS1 and ITS2 primers (10µM), 1µL of template DNA (10–50ng), and nuclease-free water. The PCR conditions were as follows: initial denaturation at 94°C for 5min; 35 cycles of denaturation at 94°C for 30sec, annealing at 55°C for 30sec, and extension at 72°C for 1min; and final extension at 72°C for 10min. PCR products were verified on a 1.5% agarose gel stained with ethidium bromide. PCR products were purified using a PCR purification kit, followed by Sanger sequencing. The resulting sequences were trimmed and analyzed using the DNASTAR Lasergene (version 17.6.1) and the Basic Local Sequence Alignment (BLAST) tool provided by the National Center for Biotechnology Information (NCBI) database. The phylogenetic analysis of the identified strains was performed using the neighbor-joining method of Saitou & Nei (1987). The evolutionary distances were computed using the Kimura 2-parameter method reported by Kimura (1980) and are in the units of the number of base substitutions per site. This analysis involved 37 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). The phylogeny was tested through 1000 bootstrap replicates. A phylogenetic tree was constructed in Molecular Evolutionary Genetics Analysis version 11 (MEGA11) (Stecher et al., 2020; Tamura et al., 2021). The resulting tree was visualized using Itol v6 (Stecher et al., 2020).

Pathogenicity test

Five fungal species, *i.e*., *F. inflexum*, *F. oxysporum*, *F. nygamai*, *F. ambrosium*, and *P. dactylethromorphus*, recovered from isolation were tested for their pathogenicity against cantaloupe (cv. Galia 290) 25-day-old seedlings in pot experiments under greenhouse conditions at Faculty of Agriculture, Cairo University.

Preparation of fungal inocula

The tested fungi were grown on cornmeal-sand medium (CMS medium) in glass bottles (500mL). A mixture of 75 g ground cornmeal and 25g fine sand previously washed with distilled water was transferred into a glass bottle. Thereafter, each bottle received 75 mL of distilled water, was plugged with a cotton stopper, and was autoclaved at 121°C and 1.05kg/cm^2 for 20min. The tested fungi were grown on PDA medium in Petri dishes (9cm in diameter) for 8 days at 25ºC. Two discs (5mm) of agar bearing mycelium growth taken from the periphery of an 8-day-old culture of each tested fungus were transferred to inoculate the medium in the bottle. All bottles were incubated at 25ºC in an incubator for 15 days (Dhingra & Sinclair, 1985).

Soil and pots disinfestation technique

Nile silt soil of pH 7.2 was disinfested using 5% formalin solution and then covered with polyethylene sheet for 10 days. After the elapse of the period, the sheet cover was removed and the soil was homogenized to remove the formalin odor. Plastic pots (25cm in diameter) were sterilized by dipping in formalin (5%), then converted, and left to air-dry for 2 days.

Greenhouse pathogenicity test

Pathogenicity tests of five fungal species, *F. inflexum*, *F. oxysporum*, *F. nygamai*, *F. ambrosium*, and *P. dactylethromorphus*, were evaluated under greenhouse conditions .Cantaloupe plants were grown under ideal greenhouse conditions (soil temperature ranged from 21 to 29ºC and relative humidity ranged from 65 to 75%) for three months, beginning from March to May (Gilbert et al., 1997).

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) and irrigated till saturation for a few days before the sowing date. 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 vermiculite (v/v) for 25 days, were carefully uprooted and separately transferred to transplant in pots at the rate of three transplants per 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 the average percentage of infection with root rot 2 months after transplanting according to the following formula:

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

At the same time, roots of diseased plants were gently uprooted and 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, more than 0-1= slight or limited area of discoloration, more than 1-2= general discoloration (one or two lesions), more than 2-3= general discoloration (rot of some tissues), and more than 3-4= rot of the entire tap and feeder roots. In addition, some growth parameters, *i.e.*, stem length and plant fresh and dry weights, were also taken into consideration at harvest time.

Disease severity index (DSI %) was calculated according to the following formula of Song et al. (2004):

$$\text{DSI\%} = \sum \frac{\text{d X(number of plants in the grade)}}{\text{(d max X number of plants in that grade) X n}} \text{ X } 100,$$

where, d= The disease rating in grade, d max= The maximum disease rating, and n= Total number of plants in each treatment.

Percentages of healthy survived plants were also calculated after 2 months of transplanting according to the formula:

%Healthy survived plants= Number of healthy plants/total number of the cultivated plants X 100.

Chemical control
***In vitro* study**

Three commercial fungicides, namely, uniform SC 39%, Maxim XL 3.5%, and double WP 56%, were used in this study. Effects of any of the three tested fungicides on the linear growth of the tested fungi, i.e., *F. inflexum*, *F. oxysporum*, *F. nygamai*, *F. ambrosium*, and *P. dactylethromorphus*, were determined *in vitro* at concentrations of 100, 200, 300, and 400 ppm. Petri dishes (9cm in diameter) containing 15mL of fungicide-modified potato dextrose agar (PDA) medium were used. Petri dishes containing 15mL free fungicide PDA medium were used as a control. Three replicates of Petri dishes were used for each treatment. Each Petri dish was inoculated with 1 cm disc of 7-day-old fungal culture in the center. All inoculated plates were incubated at 25 ±2°C, until the mycelia growth reached the edge of the control plate. Percentages of reduction in the linear growth of the pathogenic fungi were calculated using the following formula:

Fungal mycelial growth reduction (%)= (C-T)/Cx100,

where, C= Mycelial diameter in the control and T= Mycelial diameter in the treatment.

***In vivo* study**

Based on the *in vitro* studies, the most effective fungicides, *i.e.*, uniform SC 39% (400 ppm), Maxim XL 3.5% (400 ppm), and double WP 56% (300 ppm), were evaluated to test their efficacy under greenhouse conditions, located at the Faculty of Agriculture, Cairo University, against the infection by any of the five tested fungi, *i.e.*, *F. inflexum*, *F. oxysporum*, *F. nygamai*, *F. ambrosium*, and *P. dactylethromorphus.* Preparation of inoculum and soil infestation by any of the tested fungi were carried out as mentioned before under the pathogenicity test.

Galia hybrid transplants (3 weeks old) grown under greenhouse in peat moss in trays were uprooted and treated by dipping their roots before transplanting in Maxim XL 3.5% with a concentration of 400 ppm for ten minutes. Three treated transplants were sown in each pot and six replicate pots were used for each treatment. All pots were irrigated when necessary. After 15 days of planting, transplants were sprayed with a mixture of uniform SC 39% and double WP 56% with a concentration of 400 and 300 ppm, respectively. Spraying with a mixture of the two previously mentioned fungicides was repeated every 15 days for two months. All agricultural procedures were performed according to normal practices. Disease assessments were calculated as the percentage of infection with root rot and disease severity after 2 months of transplanting. Some growth parameters included stem length (cm) and plant fresh and dry weights (gm) were also determined at the same time.

Statistical analysis

A randomized complete block design with one factor was used for the analysis of data, with three replications for each parameter. The treatment means were compared by the least significant difference (LSD) test as given by Snedecor & Cochran (1994) using the Assistant program.

RESULTS

Isolation and identification of the fungi associated with vine-declined cantaloupe plants

The results of isolation from samples of cantaloupe plants exhibiting vine decline grown under field conditions in Giza (Al Ayyat), Beni Suef (Seds), Beheira (Wadi El Natrun), Matrouh (Al Hammam), and New Valley (Mut) governorates indicated the presence of five fungal species. These fungi were identified according to morphological and microscopical characteristics as *F. oxysporum* (Snyder & Hansen, 1940), *F. ambrosium* (Gadd & Loos, 1947), *P. dactylethromorphus* (Bat & Maia, 1957), *F. inflexum* (Schneider & Snyder, 1975), and *F. nygamai* (Burgess & Trimboli, 1986). It is clear from the data in Table 1 that the highest number of fungal colonies was obtained from the diseased samples collected from Giza governorate (Al Ayyat), being 14 colonies. Meanwhile, samples from Matrouh (Al Hammam) and Beni Suef (Seds) governorates yielded the same number of fungal colonies, being 11 colonies. In contrast, samples from Beheira (Wadi El Natrun) and New Valley (Mut) governorates gave the lowest number of fungal colonies, being 7.

The data (Table 1) also indicate that, among the fungi isolated from five governorates, *F. inflexum* recorded the highest number of fungal colonies followed by *F. nygamai*, *F. ambrosium*, and *F. oxysporum* with frequencies 28, 24, 22, and 20, respectively. On the other hand, *P. dactylethromorphus* recorded the lowest number colonies (3 colonies). It is worthy to note that isolation trials from diseased samples collected from New Valley governorate yielded only *F. ambrosium* (Table 1).

Molecular identification of the isolated fungi

The selected fungal isolates were identified through ITS sequencing. The amplified PCR products were visualized through gel electrophoresis showing the position of each ITS amplified PCR product of each fungal isolate (Figure 1). The variation in the sizes of PCR products on the gel indicates genetic diversity in the ITS regions among the fungal isolates, and they belong to distinct species. The resulting ITS sequences were analyzed using the NCBI BLAST tool and subsequently deposited in NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/). The accession numbers for the identified strains F01, F02, F03, F04, and F05 are PQ359447, PQ359448, PQ359449, PQ395853, and PQ359450, respectively. These fungal isolates were identified as *Fusarium inflexum* with 87% similarity level (F01), *F. oxysporum* with 100% similarity level (F02), *F. nygamai* with 100% similarity level (F03), *Paecilomyces dactylethromorphus* with 100% similarity level (F04), and *Fusarium ambrosium* with 100% similarity level (F05).

Additionally, Figure 2 shows the phylogenetic tree illustrating the relationship of the sequenced fungal strains to their closest relatives based on ITS sequence analysis.

Table 1. Occurrence and frequency (%) of fungi associated with cantaloupe plants suffering from root rot or vine decline collected from Giza (Al Ayyat), Beni Suef (Seds), Beheira (Wadi El Natrun), Matrouh (Al Hammam), and New Valley (Mut) governorates in March 2021

Isolated fungi	Giza (Al Ayyat)		Beni Suef (Seds)		Beheira (Wadi El Natrun)		Matrouh (Al Hammam)		New Valley (Mut)			
	#	%	#	%	#	%	#	%	#	%	#	%
F. iinflexum	8	57.14	6	54.54	0	0	0	0	0	0	14	28
F. oxysporum	6	42.86	4	36.36	0	0	0	0	0	0	10	20
*F. nygama*i	0	0	0	0	5	71.42	7	63.6	0	0	12	24
P. dactylethromorphus	0	0	1	9.10	2	28.57	0	0	0	0	3	6
F. ambrosium	0	0	0	0	0	0	4	36.36	7	100	11	22
Total no. of fungal colonies	14		11		7		11		7		50	

As row headings, # represents number of counted colonies and % represents frequency.

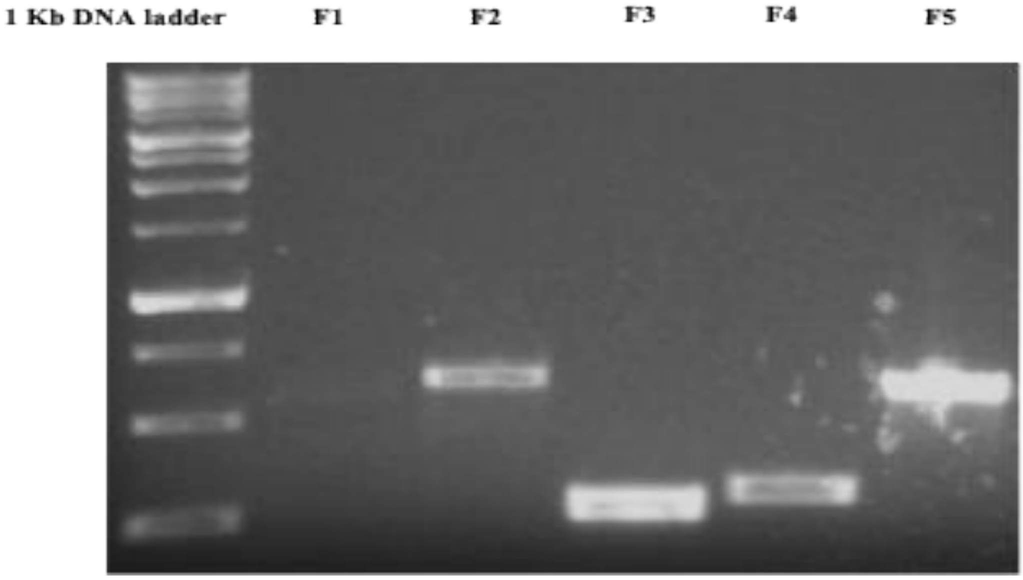

Figure 1. Agarose gel electrophoresis of amplified ITS PCR products from fungal isolates [The molecular weight ladder (leftmost lane) was used as a size reference, while distinct bands in the sample lanes represent ITS region amplification from each fungal isolate, showing variability in product sizes]

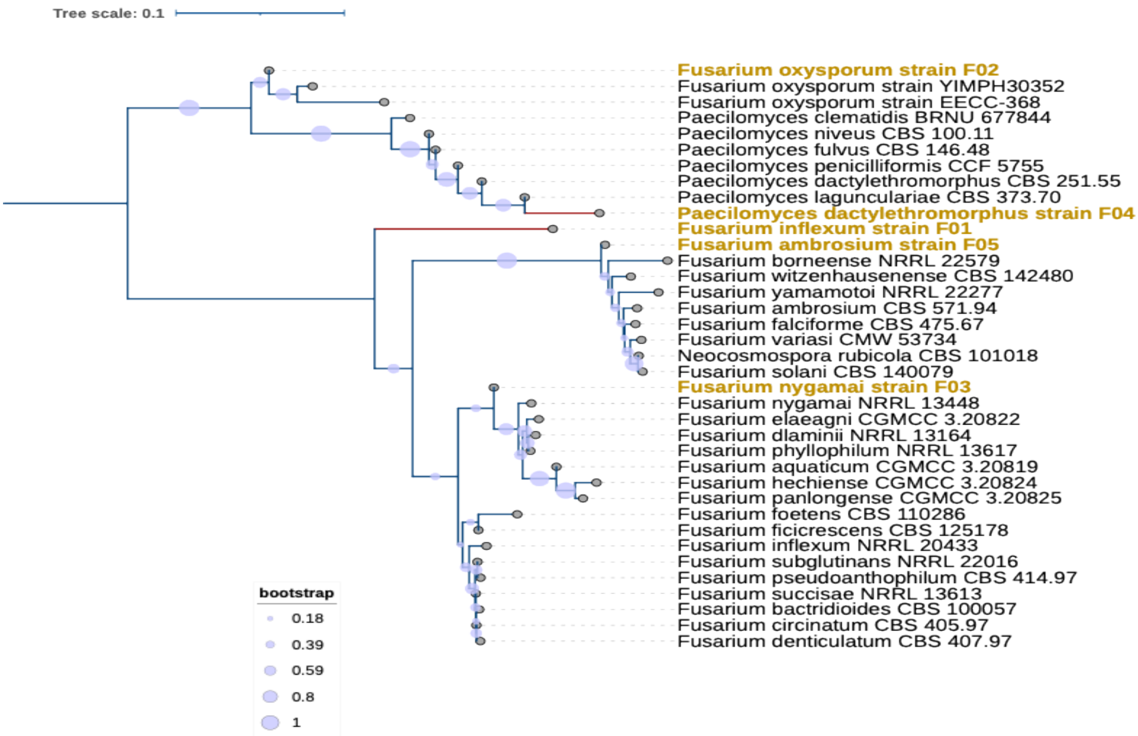

Figure 2. Phylogenetic analysis of the identified fungal strains in relation to other taxonomic groups based on the ITS regions [The phylogenetic relationships among closely related species are depicted using the maximum likelihood (ML) tree method based on the ITS sequence analysis. Bootstrap values greater than 75% (calculated from 1000 replicates) are indicated by bubbles on the branches, representing the confidence levels in the branching pattern]

Pathogenicity test

Pathogenicity tests carried out under greenhouse conditions indicated that all the five isolated fungi had the ability to cause root rot on cantaloupe plants cv. Galia 290 grown in artificially infested soil. Data presented in Table 2 indicate that the highest percentages of cantaloupe root rot were recorded due to infection by *F. nygamai*, being 77.8%, followed by *F. inflexum* and *F. ambrosium*, which gave the same percentage of cantaloupe root rot, being 66.7%.

In contrast, the lowest percentage of root rot was recorded from plants grown in artificially infested soil with *P. dactylethromorphus*, being 44.5% (Table 2).

Regarding the disease severity index (DSI), data in Table 2 show that the highest values were recorded due infection by *F. nygamai* (57.8%), followed by *F. inflexum* (55.6 %) and *F. ambrosium* (51.1%), without significant differences. In contrast, the lowest value of DSI was recorded for *F. oxysporum* followed by *P. dactylethromorphus*, being 35.6 and 40%, respectively, without significant differences. In respect to the percentages of surviving plants, data showed significant differences among the tested fungi (Table 2).

Data presented in Table 3 clearly show the effect of the isolated fungi on some cantaloupe growth parameters (2 months after transplanting), including main stem length (cm) and plant fresh and dry weights (g). Data indicated that all the tested fungi significantly reduced the determined growth parameters as compared with control plants. Also, according to data in Table 3, cantaloupe plants grown in soil artificially infested with *F. ambrosium* exhibited the highest reduction in main stem length (cm), being 44.7% followed by 40.4% and 36.3% for plants grown in a soil infested with *F. nygamai* and *F. oxysporum*, respectively.

The findings in Table 3 clearly show that cantaloupe plants grown in a soil artificially infested with *F. ambrosium* recorded the highest reduction in fresh and dry weights, followed by those grown in a soil infested with *F. inflexum*. The corresponding values were 58.7, 66, 54.9, and 52.6%, respectively. Conversely, cantaloupe plants grown in a soil artificially infested with *P. dactylethromorphus* had the lowest reduction values in plant fresh weight, while those grown in a soil infested with *F. inflexum* had the lowest reduction values in plant dry weight. The corresponding values were 37.6 and 52.6%, respectively.

Table 2. Pathogenicity tests of fungi isolated from naturally infected cantaloupe plants suffering from root rot and vine decline

Isolated fungus	Root rot incidence (%)	Disease severity index (DSI) (%)	Healthy plants that survived after 2 months (%)
F. inflexum	66.7	55.6	33.3
F. nygamai	77.8	57.8	22.2
F. oxysporum	55.6	35.6	44.5
P. dactylethromorphus	44.5	40	44 .5
F. ambrosium	66.7	51.1	33.3
LSD at 0.05	6.27	4.98	5.08

Table 3. Effect of soil infestation with different tested fungi on the average of stem length (cm) and shoot fresh and dry weights (g) of cantaloupe at harvest time

Isolated fungus (T)	Stem length (cm)	% Reduction*	Plant fresh weight (g)	% Reduction*	Plant dry weight (g)	% Reduction*
F. inflexum	101.5	35.8	41.3	54.9	17.3	52.6
F. nygamai	94.3	40.4	42.1	54	15.2	58.3
F. oxysporum	100.8	36.3	44.2	51.7	168.	53.9
P. dactylethromorphus	114.1	27.9	57.2	37.6	16.9	53.6
F. ambrosium	87.6	44.7	37.8	58.7	12.4	66
Control (C)	158.3	0	91.7	0	36.5	0
LSD at 0.05	5.08	6.85	5.81	6.73	2.06	4.88

*%Reduction = C-T/C x100

Chemical control
In vitro study

In vitro, the inhibitory effect of three fungicides on the growth of the tested fungi was determined using a PDA-poisoned medium with each of uniform SC 39%, Maxim XL 3.5%, and double WP 56%. Four concentrations of each fungicide were used, namely, 100, 200, 300, and 400 ppm/L (Table 4). The growth reductions were recorded after incubation for 7 days at 25°C±2.

It is evident from data in Table 4 that all the tested fungicides significantly decreased the growth of the tested fungi. Moreover, the fungicide double WP 56% completely inhibited the growth of *F. inflexum* at any of the concentrations tested, while it caused complete inhibition to the growth of *F. oxysporum*, *F. nygamai*, and *P. dactylethromorphus* at a concentration of 400 ppm/L .Meanwhile, it was found that the fungicide uniform SC 39% completely inhibited the growth of *F. inflexum* and *F. oxysporum* when used at a concentration of 400 ppm/L (Table 4). Meanwhile, growth of *F. nygamai* and *P. dactylethromorphus* was also strongly affected when the two fungi were grown on a medium treated by the fungicide uniform SC 39% as the reduction in mycelial growth reached 73.4 and 82.2%, respectively, at a concentration of 400 ppm/L .Also, the fungicide Maxim XL 3.5% completely inhibited the growth of F. *nygamai* at a concentration of 400 ppm/L. This fungicide also gave very significant decreases in the growth of *F. Inflexum* and *P. dactylethromorphus* which reached 70 and 48.9 %, respectively, at a concentration of 400 ppm/L. In contrast, there was no effect on mycelial growth of *F. ambrosium* at any concentrations tested.

In vivo study

The fungicides, namely, uniform SC 39%, Maxim XL 3.5%, and double WP 56%, were evaluated for their efficacy under greenhouse conditions in a pot experiment against cantaloupe root rot caused by any of the tested fungi, *i.e.*, *F. inflexum*, *F. oxysporum*, *F. nygamai*, *F. ambrosium*, and *P. dactylethromorphus*.

Data in Table 5 clearly indicate that all tested fungicides significantly decreased root rot incidence and disease severity values. Application of fungicides strongly reduced infection by *F. oxysporum* from 77.8% in control to 33.4%, and in the case of *F. inflexum* and *F. ambrosium*, the infection was reduced from 66.7% to 33.4 and 44.5% in the treatments, respectively. Meanwhile, infection by root rot caused by *P. dactylethromorphus* and *F. nygamai* was slightly affected by fungicides. The corresponding percentages of cantaloupe root rot incidence were 33.4 and 44.5%, respectively, in comparison to the control (untreated plants grown in infested soil) treatments, which gave 44.5 and 55.6%, respectively.

Table 4. Effect of four concentrations (ppm/L) of three fungicides tested (double, uniform, and Maxim XL) on the growth of five pathogenic fungi tested (expressed as a percentage of growth reduction (%)) after incubation for 7 days at 25ºC.

Fungicides	Conc. (ppm/L)	*F. inflexum*	*F. oxysporum*	*F. nygamai*	*F. ambrosium*	*P. dactylethromorphus*
Double	100	100	75.6	45.6	53.4	37.8
	200	100	77.8	48.9	63.4	51.1
	300	100	83.4	74.5	64.5	86.8
	400	100	100	100	78.9	100
	Mean	100	84.2	67.3	67.6	68.9
Uniform	100	52.2	67.8	58.9	32.2	51.1
	200	61.1	82.2	61.1	43.4	62.2
	300	74.5	100	67.8	56.7	70
	400	100	100	73.4	68.8	82.2
	Mean	71.9	87.5	65.3	50.3	66.5
Maxim XL	100	0.0	0.0	21.1	0.0	25.6
	200	25.6	8.9	57.8	0.0	33.3
	300	44.5	13.4	76.7	0.0	38.9
	400	70	24.5	100	0.0	48.9
	Mean	35.1	11.7	36.7	0.0	36.7
LSD at 0.05		4.11	2.83	3.50	3.03	4.07

Table 5. Effect of dipping roots of cantaloupe transplants in Maxim XL 3.5% (400 ppm/L) and spraying plants 15 days after transplanting with a mixture of uniform SC 39% and double WP 56% (400 and 300 ppm, respectively) and repeating this procedure at 15-day intervals for two months on root rot incidence (%) and disease severity in cantaloupe plants grown in soil artificially infested with any of the tested fungi under greenhouse conditions

Isolated fungi	Root rot incidence (%)	Disease severity index (DSI) (%)
F. inflexum	33.4	20
Control	66.7	55.6
F. oxysporum	33.4	15.6
Control	77.8	57.8
F. nygamai	44.5	22.2
Control	55.6	35.6
P. dactylethromorphus	33.4	17.8
Control	44.5	40
F. ambrosium	44.5	22.2
Control	66.7	51.1
LSD at 0.05	5.48	4.44

Regarding the disease severity index, data (Table 5) show that the lowest value (15.6%) was recorded due to infection by *F. oxysporum*, followed by *P. dactylethromorphus* and *F. inflexum* with values of 17.8 and 20%, respectively, in comparison to the control treatments, which gave 57.8, 40, and 55.6%, respectively. It was also noticed (Table 5) that both *F. ambrosium and F. nygamai* gave the same value of the disease severity index, being 22.2%, in comparison to the control treatments, being 51.1 and 35.6%, respectively.

DISCUSSION

Cantaloupe root rot disease is one of the most destructive diseases affecting cantaloupe cultivation worldwide**.** In the present study, isolation was carried out from samples of cantaloupe plants exhibiting vine decline grown under field conditions in Giza (Al Ayyat), Beni Suef (Seds), Beheira (Wadi El Natrun), Matrouh (Al Hammam), and New Valley (Mut) governorates. The isolated fungi were identified according to their morphological features as *F. oxysporum* (Snyder & Hansen, 1940), *F. ambrosium* (Gadd & Loos, 1947), *P. dactylethromorphus* (Bat & Maia, 1957), *Fusarium inflexum* (Schneider & Snyder, 1975), and *F. nygamai* (Burgess & Trimboli, 1986). Moreover, ITS sequences of the isolates F04 and F05 showed 100% identity to *P. dactylethromorphus* and *F. ambrosium*, respectively. To the best of our knowledge, this is the first record of *P. dactylethromorphus* and *F. ambrosium* associated with cantaloupe samples to have exhibited root rot in Egypt. *Paecilomyces* is a universal fungus that is mainly known for its nematophagous capacity, but it has also been reported as an insect parasite and a biological control agent of several plant pathogenic fungi and phytopathogenic bacteria through different mechanisms of action (Amaresan et al., 2020). In addition, species belonging to this genus have recently been described as biostimulants of both plant growth and crop yield (Alejandro et al., 2020; Fernando et al., 2020; El-Habashy et al., 2021). The change in the behavior of the fungus might be due to a change in the soil conditions in which the cantaloupe plants grow, which led to the generation of high numbers of *Paecilomyces* spores that attacked the roots of the growing plants causing root rot. On the other hand, there were not any published papers dealing with *F. ambrosium* or *P. dactylethromorphus* as causals of plant diseases of any crop.

The highest number of fungal colonies isolated from diseased plants was obtained from samples collected from Giza governorate (Al Ayyat). Meanwhile, samples from Matrouh (Al Hammam) and Beni Suef (Seds) governorates yielded the same number of fungal colonies. In contrast, cantaloupe samples from Beheira (Wadi El Natrun) and New Valley (Mut), governorates gave the lowest numbers of fungal colonies. Data also revealed that, among the fungi isolated from the five governorates, *F. inflexum* had the highest number of fungal colonies, followed by *F. nygamai*, *F. ambrosium*, and *F. oxysporum*, respectively. On the other hand, *P. dactylethromorphus* recorded the lowest number of colonies. It is clear from the obtained data that isolation trials from diseased samples collected from New Valley governorate yielded only *F. ambrosium*. The high frequency of *Fusarium* may be due to cosmopolitan soil-borne fungus with both saprophytic and pathogenic members. The differences in the number of *Fusarium* colonies might be due to the type of the previous crop, as most of the fungi belonging to *Fusarium* are not specialized and have a wide host range, except for some species and forma specials. Also, soil conditions, including moisture content, temperature, availability of nutrients, and the relationships that arise between groups of microorganisms living in the soil, also play a major role in this area (Zhou et al., 2010; Martyn, 2014; Pathak & Stoddard, 2018; Mostafa et al., 2019; Zhang et al., 2024).

Regarding isolation of *F. ambrosium* alone from rotted roots of cantaloupe samples for the first time in Egypt, we believe that this happened as a result of soil contamination from imported peat moss which was used to grow cantaloupe seedlings.

During the progress of the present study, pathogenicity tests were carried out to throw light on pathogenic capabilities of the tested fungi. Data revealed that the highest percentages of cantaloupe infection by root rot were recorded for *F. nygamai* followed by *F. inflexum* and *F. ambrosium*, which gave the same percentage. On the other hand, the lowest percentage of root rot was recorded for *P. dactylethromorphus*. Regarding the disease severity index, it was found that the highest percentages of cantaloupe DSI were recorded due to using *F. nygamai* followed by *F. inflexum*. In contrast, the lowest value of DSI was recorded for *F. oxysporum* followed by *P. dactylethromorphus*. Additionally, the impact of the cantaloupe infection with the tested fungi on some growth parameters varied. This demonstrates that the assessment of pathogenicity depends on the analyzed isolates, the cultivar, and the interaction of both with the environmental conditions. Root rot diseases remain a major global threat to the productivity of agricultural crops. They are usually caused by more than one type of pathogen and are thus often referred to as a root rot complex. Fungal pathogens become the most serious microorganisms responsible for root rots in plants. These fungi have the ability to degrade root cell wall throughout cell wall-degrading enzymes, thus facilitating penetration and tissue colonization, but they are also virulence determinants responsible for symptom development once growth of the fungi has been initiated (Bruce et al., 2021; Lorrai & Ferrari, 2021).

An *in vitro* study using three fungicides (uniform SC 39%, Maxim XL 3.5%, and double WP 56%) to evaluate their effects on growth of the tested fungi on a PDA-poisoned medium was carried out. Four concentrations of each fungicide were used, namely, 100, 200, 300, and 400 ppm/L. It was found that all the tested fungicides strongly decreased the growth of the tested fungi. Moreover, the fungicide double WP 56% completely inhibited the growth of *F. inflexum* at any of the concentrations tested while it caused complete inhibition to the growth of *F. oxysporum*, *F. nygamai*, and *P. dactylethromorphus* at a concentration of 400 ppm/L. Moreover, it was found that the fungicide uniform SC 39% completely inhibited the growth of *F. inflexum* and *F. oxysporum* when used at a concentration of 400 ppm/L. *F. nygamai* and *P. dactylethromorphus* growth levels were also strongly affected by poisoning the growing medium with the fungicide uniform SC 39% which completely inhibited the growth of *F. inflexum* and *F. oxysporum* when used at the concentration of 400 ppm/L. *F. nygamai* and *P. dactylethromorphus* growth levels were also strongly affected by poisoning the growing medium with fungicide uniform SC 39% at a concentration of 400 ppm/L.

Also, the fungicide Maxim XL 3.5% completely inhibited the growth of *F. nygamai* at concentrations of 400 mg/L. This fungicide also showed a significant decrease in growth of *F. Inflexum* at a concentration of 400 ppm/L. In contrast, there was no effect on mycelial growth of *F. ambrosium* at any concentration. For Maxim XL 3.5% (Fludioxonil 2.5% Metalaxyl-M 1%), Fludioxonil is a phenyl pyrrole, a group L fungicide which inhibits RNA synthesis, preventing spore production and mycelial growth. Its mode of action is to inhibit transport-associated phosphorylation of glucose, which reduces mycelial growth rate. Fludioxonil is used against fungi belonging to the genera *Fusarium*, *Rhizoctonia*, *Alternaria*, *Botrytis cinerea*, and *Stromatinia cepivora*. Metalaxyl is a phenyl amide, a group D fungicide that inhibits the protein kinase PK-III, which is involved in the osmosensing transduction pathway. It is systemic with protective and curative action (Chao et al., 2011; Abdul Khaliq et al., 2022; Akhtar et al., 2024).

Meanwhile, the action of uniform SC 39% (Azoxystrobin- 28.2-Metalaxyl-M10.8) is due to the mode of action of azoxystrobin, which prevents the respiration of fungi, resulting in disruption of the electron transport chain, preventing ATP synthesis. Meanwhile, Metalaxyl is a systemic phenylamide fungicide that acts by disrupting fungal nucleic acid synthesis—RNA polymerase, suppressing sporangial formation, mycelial growth, and the establishment of new infections. It demonstrated a very broad spectrum of activity against fungal pathogens from all four taxonomic groups (Robert & Joseph, 1999; Paiva et al., 2022; Akhtar et al., 2024).

Also, in double WP 56%, Hymexazol is used worldwide as a systemic soil and seed fungicide for controlling diseases caused by fungi belonging to the genera *Fusarium*, *Aphanomyces*, *Pythium*, and *Corticium* spp. in rice, sugar beet, fodder beet, vegetables, cucurbits, and ornamentals. Hymexazol is a systemic fungicide that inhibits fungal growth by inhibiting nucleic acid synthesis (DNA/RNA synthesis). This effect is mainly due to its rapid translocation into plants and rapid transformation into glucosides. The *O*-glucoside has a fungitoxic activity, whereas the N-glucoside has been reported as a stimulant of lateral root hair development in seedlings through certain plant growth-promoting effects (Chao et al., 2011; Francisca et al., 2023).

Under greenhouse conditions, the fungicides uniform SC 39%, Maxim XL 3.5%, and double WP 56% were chosen to evaluate their efficacy against cantaloupe root rot caused by any of the tested fungi. It was found that all tested fungicides greatly decreased root rot incidence and disease severity index. The application of fungicides strongly reduced infection by *F. oxysporum*, followed by infection caused by *F. inflexum* and *F. ambrosium*, respectively. Meanwhile, infection by root rot caused by any of *P. dactylethromorphus* and *F. nygamai* was slightly affected by fungicides. Regarding the disease severity index, it was also noticed that the lowest value of DSI was recorded from plants infected by *F. oxysporum*, followed by infection by *P. dactylethromorphus* and *F. inflexum,* respectively, in comparison with the control treatments. It was found that cantaloupe plants infected by both *F. ambrosium* and *F. nygamai* gave the same value of the disease severity index, being 22.2%, in comparison with the control treatments. In this concern, the results of the greenhouse experiment are following the previous studies given by some investigators (El-Mougy et al., 2012; El-Kolaly et al., 2013; Breno et al., 2024; Cynthia et al., 2024).

Conclusion

Five fungi associated with cantaloupe vine decline disease were isolated and identified using both morphological and microscopic characteristics, besides the internal transcribed spacer (ITS) region. To the best of our knowledge, this is the first record of *Paecilomyces dactylethromorphus* and *Fusarium ambrosium* causing cantaloupe vine decline disease in Egypt. Additionally, the results indicated that the used fungicides, namely, uniform SC 39%, Maxim XL 3.5%, and double WP 56%, were able to reduce the cantaloupe root rot incidence and the disease severity index caused by the tested fungi under greenhouse conditions.

Conflict of interest: Authors declare that no conflict of interest.

Authors' contributions: M.A.M.: Conceptualization, methodology, writing and reviewing; M.F.A.: Conceptualization, methodology, writing and reviewing; R.MS.: Conceptualization, methodology, writing and reviewing; M.N.A: Molecular analysis, writing and reviewing and M.M.Y.: Molecular analysis, methodology, writing and reviewing. The authors read and approved the final manuscript.

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