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ORIGINAL ARTICLE

Characterization of *Paramyrothecium roridum* (Basionym *Myrothecium roridum*) causing leaf spot of strawberry

Maali Shaker Soliman*

Mycology Research and Disease Survey Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

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*Corresponding address:
maali_soliman@yahoo.com

Abstract

Strawberry plants showing symptoms of leaf spots and petiole lesions were collected from El Qalubya governorate, which is one of the most famous areas that extensively grows strawberry in Egypt. The objectives of this study were to isolate and characterize the causal pathogen of the disease. The isolated pathogen was identified as *Paramyrothecium roridum* (formerly known as *Myrothecium roridum*) based on its morphological characteristics and sequencing the partial rDNA internal transcribed spacer (ITS). A pathogenicity test using detached leaf assay revealed that *P. roridum* is a potential pathogen of strawberry. Symptoms started as small necrotic areas which expanded rapidly to macerate whole leaflets and petioles. In advanced stages of infection, dark olive green sporodochia were clearly distinguished on the infected tissues. Six strawberry cultivars showed different levels of susceptibility to *P. roridum*. Florida was the most resistant cultivar while Beauty, Camarosa, Fortuna and Sweet Charlie were susceptible. Festival showed a moderate level of susceptibility. An *in vitro* assay on the effect of the liquid culture filtrate of *P. roridum* on strawberry leaves showed that the filtrate caused damage to tissues and clear necrotic symptoms were developed. High performance liquid chromatograph (HPLC) analysis on the filtrate of 10 day old *P. roridum* culture revealed the presence of various mycotoxins. The two major toxins detected were 8-alpha-hydroxyroridin H and myrothecin A in addition to other trichothecenes. Data also revealed the capability of *P. roridum* to produce polygalacturonase (PG) and cellulase (Cx) enzymes in liquid cultures. The activity of PG was found to be significantly correlated with the age of the growth culture. This is the first record of *P. roridum* on strawberry in Egypt.

Keywords: leaf spot, *Paramyrothecium roridum*, strawberry, trichothecenes

Introduction

The plant pathogenic fungus *Paramyrothecium roridum* (Tode) L. Lombard and Crous, formerly known as *Myrothecium roridum* Tode ex Fries, has a wide host range including over 300 plant species of vegetables, fruits, ornamental plants and field crops (Chase 1983; Fish *et al.* 2012; Lombard *et al.* 2016; Ben *et al.* 2017; Farr and Rossman 2019). It is a soil born fungus which causes diseases to the foliar parts of its host, usually leaf spots and stem lesions in addition to fruit rot in some hosts. However, *P. roridum* can also be transmitted by seeds of some plant species e.g. watermelon and yellow marsh cress *Rorippa islandica* (Nguyen *et al.* 1973; Tewari and Skoropad 1977; Bharath *et al.* 2006; Duvel *et al*. 2010). Similar to most leaf spot diseases, *P. roridum* affects plant health, vigor and productivity causing substantial losses especially in ornamental plants.

The leaf spots caused by *P. roridum* can vary slightly according to the host plant but generally they are brown at the beginning, look watery, and then they get

darker until black necrotic spot symptoms are seen. In advanced stages, small dark olive to black drops can be noticed on the spots presenting sporodochia formed by the pathogen (Han *et al.* 2014; Ben *et al.* 2017). In some plants, the centers of the spots can lyse giving a shot hole appearance (Tewari and Skoropad 1977). This pathogen favors warm temperatures and spreads widely in high humidity, therefore, it can cause severe losses in greenhouses (Han *et al.* 2014; Chen *et al.* 2016).

In 1975, *Myrothecium roridum* was reported in Egypt. It was isolated from the phyllosphere of *Gossypium barbadense* L. but was not reported to cause any disease symptoms to the plant (Wahab 1975). It was also isolated from gardenia leaves showing spot symptoms (Mostafa *et al.* 2013). Later, *M. roridum* was isolated from both the phyllospheres and phylloplanes of different plants collected from reclaimed Egyptian areas (Elkhateeb *et al.* 2016).

Pathological and biochemical studies of *P. roridum* revealed the ability of this pathogen to produce different mycotoxins, primarily trichothecenes (Kuti *et al.* 1989; Khisal *et al.* 2002; Talukdar and Dantre 2014). In addition to plants, trichothecenes can cause toxicity in animals and humans (McCormick *et al.* 2011). Toxins produced by *P. roridum* are key elements of the pathogenicity process of this pathogen (Kuti *et al.* 1985). In addition, *P. roridum* produces cellulytic enzymes that have been found to contribute significantly to its pathogenicity (Moreira *et al.* 2005; Okunowo *et al.* 2010; Talukdar and Dantre 2014).

Strawberry (*Fragaria* × *ananassa*) is considered to be one of the most economically valuable crops. The crop is grown for its fruits, which are very nutritious, and particularly rich in antioxidants and vitamins. Strawberries have been grown in Egypt for decades and are considered to be very profitable since they are mainly grown for export. In 2017 the total Egyptian strawberry yield reached 400,000 tons (http://www.fao.org/faostat/en/#data/QC).

Strawberry plants are endangered by various plant diseases that can cause substantial damage and significantly affect plant productivity (Maas 1998). Disease management programs are adopted by strawberry growers to control losses caused by plant pathogens. However, the first step of effective management programs is to specify the causal agent of the disease and the means of spreading.

Symptoms of leaf spots and petiole lesions were observed on strawberry plants grown in El Qalyubia governorate, Egypt, and the diseased plants were collected for investigation. The aims of the present study were to isolate the pathogen causing such a disease and study its morphological, pathological and biochemical characteristics.

Materials and Methods

Isolation of the pathogen

Strawberry plants showing leaf spots and petiole lesions were collected from El Qalubya governorate, El Sohby area. The infected plants were washed with tap water and then left to dry at room temperature. Leaves and petioles showing symptoms were cut into small pieces (approximately 6 mm^2) and surface sterilized in 1.5% sodium hypochlorite for 2 min. They were then rinsed with sterilized distilled water and left to dry between two layers of sterilized filter paper. Plant segments were placed on Petri dishes containing potato dextrose agar medium (PDA), incubated at 25 ± 2°C and checked daily for any mycelial growth emerging from plant pieces. Mycelia from colonies that emerged from the pieces were transferred to PDA plates. Pure cultures were obtained by cutting hyphal tips of the grown colonies' margins. Cultures were maintained for further studies.

Morphological characterization

Morphological characteristics such as: colony growth patterns, conidial shape and color, conidiophores and conidiogenous cells, were examined carefully to identify the isolated fungus (Tulloch 1972; Domsch *et al.* 1980; Lombard *et al.* 2016).

Molecular identification

Molecular identification of the isolated fungus was carried out by sequencing the partial rDNA internal transcribed spacer (ITS). The internal transcribed region was amplified using the universal primer ITS1/ITS4 (White *et al.* 1990). For DNA extraction, the isolated fungus was grown on PDA at 25°C for 10 days. Mycelia were harvested and the extraction was carried out using DNeasy QIAGEN plant mini kit following the manufacturer's instructions.

The amplification was carried out in 25 µl reaction volume using 2 µl (50–100 ng) of genomic DNA, 5 µl 5X Gotaq buffer, 1.5 µl $MgCl_2$, 0.5 µl of 2.5 mM dNTPs mixture, 0.5 µl of 10 µM of each primer, 0.2 µl of 5 U · $µl^{-1}$ Taq DNA polymerase (Promega). PCR conditions used for amplification were: an initial cycle at 95°C for 10 min, 30 cycles at 95°C for 60 sec, 55°C for 60 sec and 72°C for 90 sec, then final extension at 72°C for 90 sec.

Phylogenetic analysis

The sequence of the partial rDNA internal transcribed spacer of the isolated fungus in addition to reference

sequences of 22 strains of different fungal species, were retrieved from the NCBI database. Sequences were aligned using MUSCLE and were manually trimmed to obtain identical ends. Phylogenetic analysis was carried out based on the neighbour-joining (NJ) method with maximum likelihood bootstrap of 1000 replicates (Kumar *et al.* 2018).

Pathogenicity studies

Detached leaf inoculation

Six strawberry cultivars were used to investigate the pathogenesis of *P. roridum* and the levels of their susceptibility to infection. Plantlets of strawberry cultivars, Beauty, Camarosa, Festival, Florida, Fortuna and Sweet Charlie were grown in the greenhouse of the Plant Pathology Research Institute, ARC, and young leaves were excised after 30 days. Leaves were surface sterilized by immersing in 1% sodium hypochlorite for 2 min and then they were rinsed by soaking in sterilized distilled water for 2 min. After complete dryness, each leaf was placed on the surface of two layers of sterilized filter paper placed on sterilized petri dishes. The leaves were inoculated using 4 mm in diameter mycelial plugs taken from the the margin of a 10 day old colony of *P. roridum* and placed in the middle of the upper side of each leaf after being gently scratched with a sterilized scalpel. The filter papers on each plate were soaked with sterilized distilled water and then the plates were covered with lids to ensure high humidity. Ten leaves of each strawberry cultivar were used as replicates. Surface sterilized leaves treated with PDA plugs as mentioned above were used as control. Observed symptoms were recorded and the disease severity on each leaflet was scored (Mansfield and Deverall 1974; Dhingra and Sinclair 1985).

Effect of *P. roridum* culture filtrate on developing disease symptoms

An Elymenyer flask containing 100 ml potato dextrose broth (PDB) medium was inoculated with a mycelial disc of *P. roridum* and incubated for 10 days at 25°C. The culture was filtrated using filter paper to separate the mycelial mate, then it was filtrated through 0.45 µm Denville Syringe Filters to get rid of all spores and mycelia fragments. Young strawberry leaves of the Festival cultivar were excised from 30 day old plants and were surface sterilized as mentioned above. Leaflet surfaces were treated with droplets (~100 µl) of *P. roridum* culture filtrate. One drop was placed on each leaflet. Leaflets treated with sterilized distilled water drops served as control. Leaves were placed on the surface of two layers of filter paper placed on sterilized petri dishes. The filter papers were soaked with sterilized distilled water. The plates were incubated at room temperature (25–27°C) and checked daily to monitor the development of symptoms.

Biochemical studies

Characterization of toxins produced by *Paramyrothecium roridum*

An Elymenyer flask containing 100 ml of PDB was inoculated with 10 day old *P. roridum* mycelial disc. The flask was incubated at 25°C ± 2 for 10 days. A fungal mycelial pellet was removed from the flask and the culture filtrate was centrifuged at 4000 rpm for 15 min to precipitate the rest of the mycelia pieces and spores. The supernatant (culture filtrate) was then used to extract the secondary metabolites. The culture filtrate was extracted twice with two equal volumes (v/v) of ethyl acetate (EtOAc) and left to stir for 1 h at room temperature to extract the fungal secondary metabolites. The EtOAc layer was recovered from a separation funnel, dried over anhydrous Na_2SO_4 and evaporated to recover the crude extracts.

Crude extracts were subjected to silica gel chromatography (0.2 mm silica gel 60 F254 pre-coated alumina, Merck, Darmstadt, Germany), using stepwise different eluents (ex.$CHCl_3$, and $CHCl_3$–MeOH (9 : 1, 8 : 2, 1 : 1, v/v). The fractions were concentrated under vacuum, gently evaporated and dried under nitrogen gas stream. Forty microliters of crude metabolites were then purified and separated by reversed phase high performance liquid chromatograph (HPLC) and a photodiode array detector (DAD) (200–600 nm) was used to record their characteristic UV spectra.

Enzyme production

An *in vitro* experiment was carried out to investigate the production of cellulase (Cx) and polygalacturonases (PG) enzymes by *P. roridum*. Elymenyer flasks containing 100 ml of PDB were inoculated using 10 day old *P. roridum* mycelial discs. Flasks were incubated at 25°C ± 2 for 3, 7, 10, 14 and 22 days. Three flasks were set as replicates for each incubation period. After incubation, the culture filtrates were collected after incubation and the activity of PG and Cx produced by *P. roridum* was determined viscometrically (Mahadevan and Sridhar 1982).

Statistical analysis

The experimental design of the present laboratory experiment was a randomized complete block with nine replicates. Analysis of variance (ANOVA) of the data was performed with the (SPSS Inc., version 13.0, Chicago, IL, USA) statistical package. Least significant difference (LSD) was used to compare treatment means. Correlation and regression analyses were performed with the same statistical package.

Results

Identification and characterization of the isolated fungus

Morphological identification

Isolation resulted in some morphologically identical fungual colonies. These colonies, characterized on PDA by white to slightly buff floccose mycelia, generally looked quite wrinkled. Concentric dark olivaceous-black slimy drops were radially spread above the mycelia presenting sporodochia which gradually became dry and hardened when the culture aged. With microscopic examination, conidiophores were found to be hyaline and branched, and each branch beard a few (3–5) cylindrical shaped phialides. Conidia were aseptate and rod shaped with slightly rounded ends. They looked hyaline to light green especially in young cultures and became darker when cultures aged. Setae were found to be present (Fig. 1).

Based on the above mentioned features the fungus was identified as *P. roridum* which was formerly known as *M. roridum*.

Molecular identification and phylogenetic analysis

To confirm the morphological identification of the isolated fungus, molecular identification was carried out by sequencing the partial rDNA internal transcribed spacer (ITS). The PCR products were sequenced and a GenBank search was carried out using BLASTn. The GenBank search revealed that the sequence was 99.49% identical to *P. roridum* (ITS accession No. JX867215). The consensus sequence of the strain was deposited in the Genbank as *P. roridum* with accession no. KX495189. This strain is preserved in the Mycology Research and Disease Survey Dept., Plant Pathology Research Institute, Agricultural Research Centre, Egypt, with the code MRDS19.

The partial sequences of the ITS region of *P. roridum* MRDS19 and other fungi species were utilized in phylogenetic analysis. The constructed phylogenetic tree elucidated the evolutionary relationship between *P. roridum*, other species of the genus *Paramyrothecium* and the closely related genus of the family Stachybotriaceae (Fig. 2). The phylogenetic tree revealed that *P. roridum* is most closely related to *P. parvum*. However, *P. acadiense*, *P. humi*cola, *P. nigrum* and *P. viridisporum* were distanced from *P. roridum* in the constructed tree. The most distanced members of Stachybotriaceae family from *P. roridum* were *Albifimbria* spp.

Pathogenicity test

Detached leaf inoculation

Disease severity and the aggressiveness of symptoms varied between the tested cultivars (Table 1). Symptoms

Table 1. Disease severity of leaf spot disease caused by *Paramyrothecium roridum* on six strawberry cultivars after 7 days of inoculation

Cultivar	Disease severity*	Class of reaction
Beauty	88.11	susceptible
Camarosa	92.63	susceptible
Festival	59.33	moderately susceptible
Florida	37.85	resistant
Fortuna	85.81	susceptible
Sweet Charlie	91.44	susceptible

LSD ($p < 0.05$) = 19.16

*disease severity was evaluated based on Mansfield and Deverall (1974)

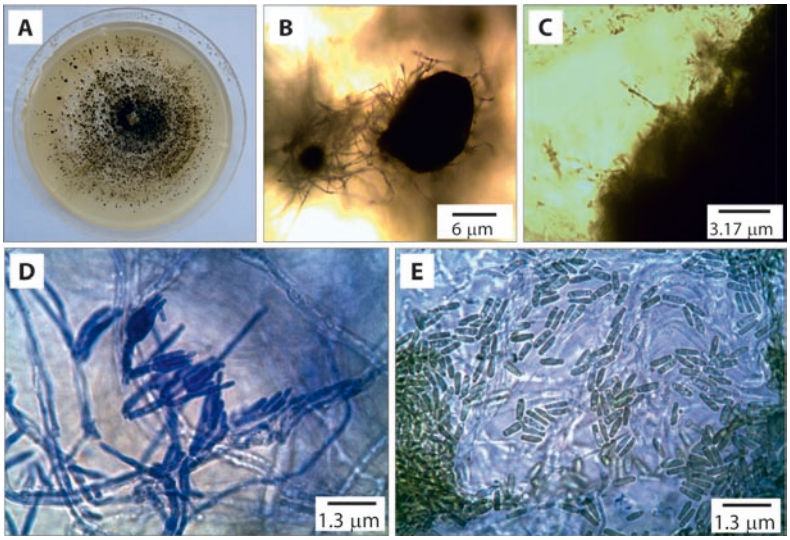

Fig. 1. *Paramyrothecium roridum* culture features. A – colony morphology on potato dextrose agar (PDA; 14 days old) showing white aerial mycelia with olivaceous-black sporodochia, B – sporodochia, C – setae, D – condiogenous cells, E – rod shaped conidia with slightly rounded ends. Scale bars: B = 6 µm, C = 3.17 µm, D–E = 1.3 µm

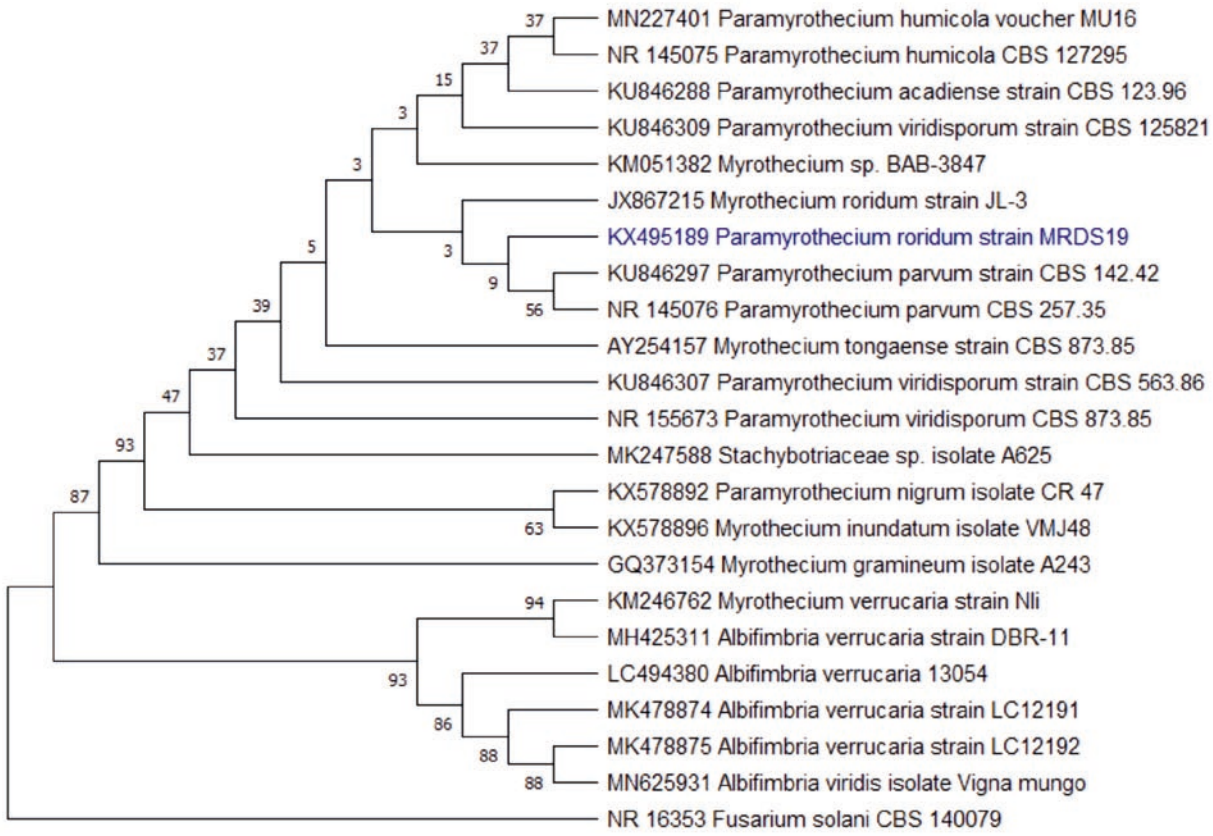

Fig. 2. Phylogenetic tree constructed using the neighbour-joining method and inferred from internal transcribed spacer (ITS) region partial sequence. Bootstrap test (1000 replicates) is shown next to the branches. The analysis involved 23 nucleotide sequences. *Paramyrothecium roridum* of this study is in blue. The tree was rooted to *Fusarium solani* (CBS 140079)

generally started as very small dark spots in the area of inoculation which then merged together forming irregular black areas of dead tissues and were readily observed in most susceptible cultivars. A yellow halo appeared in some leaves at the beginning of infection. In advanced stages, deep green sporodochia were formed which looked like droplets above the dead tissues (Fig. 3). In susceptible cultivars, symptoms spread to the petioles and leaves turned dark black.

Tested strawberry cultivars varied in the level of resistance to infection with *P. roridum*. The Florida cultivar showed the highest level of resistance (severity 37.85), while Sweet Charlie and Camarosa were highly susceptible, with degrees of severity of 91.44 and 92.63, respectively.

The influence of *Paramyrothecium roridum* culture filtrate on developing disease symptoms

Symptoms on strawberry leaves started to develop 3 days after treatment with *P. roridum* culture filtrate droplets. Small black dots were noticed in the area of the droplets. These dots became more distinct after a few days with some yellowing in the surrounding tissues (Fig. 4).

Biochemical studies

Characterization of toxins produced by *Paramyrothecium roridum*

HPLC analysis revealed eight known natural compounds (Fig. 5) from two main fractions of the crude

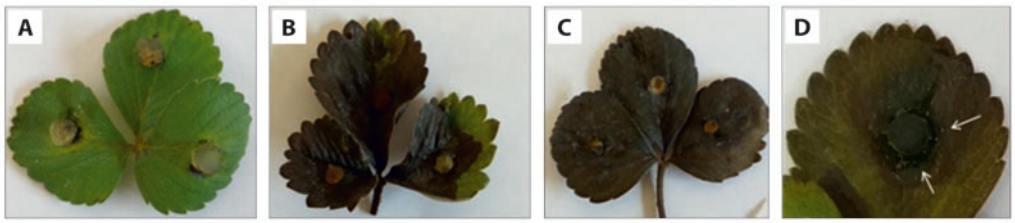

Fig. 3. Different reaction levels of strawberry cultivars to *Paramyrothecium roridum* 7 days post inoculation using detached leaf assay. A – Florida, B – Fortuna, C – Camarosa, D – sporodochia formed in advanced stages of infection

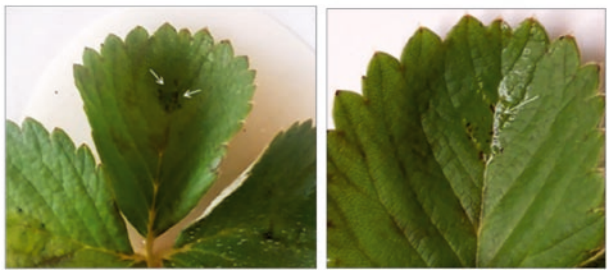

Fig. 4. Strawberry leaflets of the Festival cultivar treated with 10 day old *Paramyrothecium roridum* culture filtrate. Obvious black dots were readily distinguished in areas where the droplets had scattered 3 days post inoculation with some yellowing in the tissues around treated areas

extract of *P. roridum*. Fraction 1 had five compounds namely 8-alpha-hydroxyroridin H, myrothecin A, 8-beta-acetoxy-roridin H, isororidin E, verrucarin A. Fraction 2 had three compounds, namely, verrucarin J, verrucarin L and 8a-acetoxy verrucarin L. The two major compounds detected were 8a-hydroxyroridin H and myrothecin A.

Enzyme production

Data revealed that *P. roridum* is able to produce PG and Cx enzymes in culture medium and the activity of the produced enzymes was affected by the age of the culture. The regression equations shown in Table 2 and Figures 6 and 7 indicated a significant relationship between the activity of PG and the age of *P. roridum* cultures. According to this relationship, younger cultures showed higher PG enzyme activity which decreased with aging. On the other hand, the relationship between Cx and the age of the *P. roridum* cultures was non-significant ($R = 0.782$, $P = 0.12$).

Discussion

Clear symptoms of spots and lesions on strawberry leaves and petioles were observed in one of Egypt's major strawberry growing areas. *Paramyrothecium roridum* (Tode) L. Lombard and Crous, 2016 (formerly known as *M. roridum*) was isolated from diseased tissues and identification was carried out according

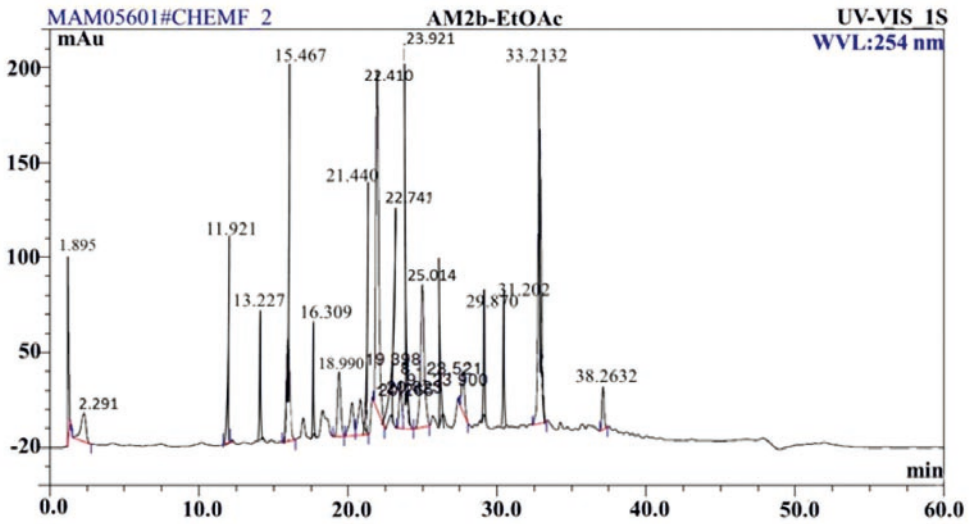

Fig. 5. High performance liquid chromatograph (HPLC) of 10 day old culture filtrate of *Paramyrothecium roridum*

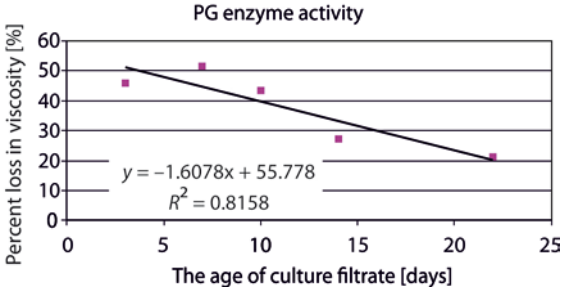

Fig. 6. The activity of polygalacturonase (PG) enzymes in 3, 7, 10, 14 and 22 day old culture filtrate of *Paramyrothecium roridum*

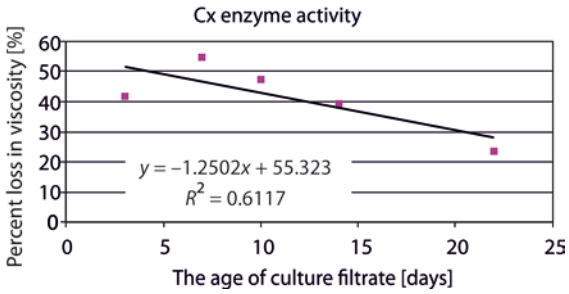

Fig. 7. The activity of cellulase (Cx) enzyme in 3, 7, 10, 14 and 22 day old culture filtrate of *Paramyrothecium roridum*

Table 2. Equations that describe the effect of *Paramyrothecium roridum* culture age (x) on the activity of polygalacturonase PG (y_1) and cellulase Cx (y_2) enzymes

Enzyme	Regression equation	F value	$P > F$	R	R^2
PG	$y_1 = -1.6078x + 55.778$	13.286	0.036	0.903	0.816
Cx	$y_2 = -1.2502x + 55.323$	4.727	0.118	0.782	0.612

Sample size (n) = 5

to morphological characteristics and confirmed with molecular characterization (Lombard *et al.* 2016).

The plant pathogenic fungus, *P. roridum* (Basionym *M. roridum*) has a broad host range and is known to cause diseases to more than 300 host plants (Farr and Rossman 2019). *Paramyrothecium roridum* was previously reported to infect gardenia plants in Egyptian nurseries, and was also isolated from the phyllosphere of different plants. To the best of our knowledge this is the first report of *P. roridum* on strawberry in Egypt (Mostafa *et al.* 2013; Elkhateeb *et al.* 2016; Farr and Rossman 2019).

The genus *Myrothecium* has been of great interest for many taxonomists since 1790 when it was first described by Tode (Tulloch 1972; Lombard *et al.* 2016). In 1972, Margaret Tulloch revised the genus *Myrothecium* and provided a detailed morphological key of that genus with a comprehensive description of 13 species including *M. roridum*. Morphological features such as sporodochia and spore shape, the presence or absence of setae and the development of synnema were fundamental in the differentiation between species. However, Lombard *et al.* (2016) provided a detailed phylogenetic and morphological study of the family Stachybotriaceae in which they exploited DNA sequences of different molecular markers and accordingly reshaped this family. The genus *Paramyrothecium* was added to the family and *M. roridum* was replaced by *P. roridum*.

In the study, Koch's postulates were fulfilled by performing a pathogenicity test using a detached leaf method on different strawberry cultivars. A pathogenicity test revealed that the isolated *P. roridum* strain is capable of infecting strawberry plants and developing clear symptoms. Detached leaf assay is considered to be a valid method that has been widely used to study the effect of a broad range of plant pathogenic fungi causing leaf spot diseases (Dhingra and Sinclair 1985; Jia *et al.* 2003). Miller-Butler *et al.* (2018) used detached leaf assay in studying the effect of the anthracnose pathogen, *Colletotrichum* spp., on strawberry leaves. Furthermore, this technique was previously used in pathogenicity studies of *P. roridum* on different plant hosts, i.e. garden hydrangea and soybean (Mmbaga *et al.* 2010; Haudenshield *et al.* 2018).

Symptoms developed by infection with *P. roridum* (*M. roridum*) vary according to plant hosts. Mulberry plants infected with *Myrothecium* leaf spots exhibited circular and irregular spots which looked tan with dark edges and then turned to dark brown necrotic areas (Takahashi *et al.* 1994). The pathogenicity test carried out in our investigation revealed that strawberry cultivars may vary in the level of susceptibility to *P. roridum*. The Florida cultivar was more resistant than the other tested strawberry cultivars, while Camarosa and Sweet Charlie showed the highest levels of susceptibility and Festival was moderately susceptible. Therefore, these results suggest that the level of resistance of the cultivar is an important element especially when plants are grown in areas endangered with pathogens.

In this study, the culture filtrate of *P. roridum* was found to affect leaf tissues and develop clear symptoms of necrotic spots which started small and distinct and expanded rapidly with some chlorotic appearance of the affected areas. Consequently, a characterization of the culture filtrate of *P. roridum* was carried out to identify the major components that may play a potential role in the pathogenesis of that fungus. The toxic effects of fungal pathogens culture filtrates on plant hosts have been widely investigated using both attached and detached leaf techniques (Kuti *et al.* 1989; Kapat *et al.* 1998; Murakami *et al.* 1999; Talukdar and Dantre 2014).

In our investigation we carried out a HPLC analysis for the culture filtrate of *P. roridum* to identify the major toxins that can be produced by this pathogen. Eight different toxins were identified with 8-alpha-hydroxyroridin H and myrothecin A being the two major trichothecenes present in the culture filtrate. The extract also contained different roridin and verrucarin toxins. Many studies have been carried out aimed at characterizing different secondary metabolites produced by *P. roridum* and different *Myrothecium* species and how these metabolites can contribute to the pathogenesis of these pathogens. Most of these investigations identified large groups of trichothecenes that were produced by this group of fungi (Jarvis *et al.* 1985; Murakami *et al.* 1999; Abbas *et al.* 2001; McCormick *et al.* 2011). Generally, trichothecenes are mycotoxins that can be produced by a large number of fungal genera including *Myrothecium*, *Fusarium*, *Trichoderma*, *Paramyrothecium Trichothecium*, and *Cylindrocarpon* (McCormick *et al.* 2011). Trichothecenes are well known for their potent toxic effects not only for plants

but also for humans and animals (Lakornwong *et al.* 2019). The toxic effects of trichothecenes for plants can be exhibited by necrotic and chlorotic lesions of affected plant leaves (McCormick *et al.* 2011). Murakami *et al.* (1999) isolated myrothecin B from the culture of *M. roridum* and treated mulberry leaves with the toxin. They concluded that myrothecin B plays a major role in the pathogenesis process of this pathogen.

Like most plant pathogens, *P. roridum* is able to produce various kinds of extracellular hydrolytic enzymes that the pathogen employs in the invasion and pathogenesis process of its host (Okunowo *et al.* 2010). Polygalacturonase (PG) and cellulases (Cx) are hydrolytic enzymes produced by a large number of plant pathogens targeting pectins and cellulose in plants (Bellincampi *et al.* 2014).

In our study, an in vitro experiment was carried out to investigate the capability of *P. roridum* to produce PG and Cx enzymes and to what extent the age of the growth culture can affect the activity of these enzymes. Our results indicated that *P. roridum* produces both PG and Cx in cultures during growth. Moreover, the activity of PG was significantly correlated with the age of the growth culture since the activity of the enzyme in young cultures was higher than in old cultures. Previous studies revealed that different elements, i.e. culture age, type of culture and pH, may affect the activity of different hydrolytic enzymes produced by plant pathogens (Onuh and Ohanzurike 2008). A study carried out by Gautam *et al.* (2010) revealed that cellulases produced by *Trichoderma viride* were greatly affected by the age of the culture.

Conclusions

Paramyrothecium roridum was isolated from strawberry plants showing leaf spot symptoms. A pathogenicity test using detached leaf assay confirmed *P. roridum* to be pathogenic for strawberry, however tested cultivars showed different levels of resistance. The fungus culture filtrate was found to affect leaf tissues and develop symptoms. Biochemical studies revealed that this pathogen deploys different toxins and enzymes to cause disease to host plants and develop symptoms. To our knowledge this is the first report of leaf spot disease on strawberry plants caused by *P. roridum*.

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