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Morphological and Molecular Characterization of *Alternaria* sect. *Ulocladioides*/*Ulocladium* Isolated from Citrus Fruits in Upper Egypt

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Authors' contributions

This work was carried out in collaboration among all authors. Author YAG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TAM and KEA managed the analyses of the study. Author MAH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Citrus is the most important crop in Upper Egypt. 150 infected samples were collected from citrus samples (Navel orange, Tangerine and Lemon) in Upper Egypt, 50 samples from each fruit. Twenty-two isolates representing three species of *Alternaria* belong to *A.* sect. *Ulocladioides* and *A.* sect. *Ulocladium* were isolated on dichloran chloramphenicol- peptone agar (DCPA) medium at 27°C. Tangerine samples were more contaminated with *Alternaria* followed by Navel orange and no *Alternaria* species appeared from Lemon samples. Based on the *Alt a1* the phylogenetic analysis identified the isolates as *Alternaria atra*, *Alternaria botrytis* and *Alternaria oudemansii*. The pathogenicity of the isolates was tested by inoculation of healthy navel orange, the resulted data showed that all tested isolates were pathogenic to healthy navel orange with different degrees ranged from 31.5±1 - 20±1 mm and *A. oudemansii* had a low virulent effect. The mycotoxins ability of tested isolates indicated that about 83% of the isolates were TeA toxin producers with amount ranged from 1.54 - 18.47 ug/ml.

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1. INTRODUCTION

Citrus fruits widely used as edible fruits belonging to citrus and related genera of the family Rutaceae (orange family) [1]. It serves as the main source of vitamins, minerals elements and sugar; hence, it controls the building process of human bodies [2]. The citrus fruit is attacked by a number of pathogens from bloom to harvesting stage and subsequently by post-harvest pathogens that affect fruit yield and considerably deteriorate the fruit quality [3]. The common postharvest fungi of fruits include *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., and *Penicillium* sp. [4].

Alternaria species cause four different diseases of citrus: *Alternaria* brown spot of tangerines (ABS), *Alternaria* leaf spot of rough lemon, *Alternaria* black rot of many citrus fruits, and Mancha foliar on Mexican lime. *Alternaria* brown spot affects many tangerines and their hybrids and produces lesions on and abscission of immature fruit and leaves, and corky lesions on mature fruit [5]. Some species of *Ulocladium* (synonymy: *Alternaria*) were plant pathogens, Leaf blight caused by *U. atrum* is an important disease on potato, causing considerable damage worldwide [6,7,8]. In addition, conidia of *U. atrum* were able to survive on the surface of grapevine bark, leaves, inflorescences and berries [9]. Coviello et al. [10] reported that the major fruit disease affecting fig and smut in dried fruits caused by *Alternaria* rot [*A. alternata, A. atra* (synonymy: *Ulocladium atrum*) and other *Alternaria* spp.

Ulocladium species closely resemble some of the saprotrophic *Alternaria* species based on extensive morphological and phylogenetic studies [11]. Phylogenetic studies place *Ulocladium* convincingly within *Alternaria*, suggesting that the latter is the correct classification for these species [11]. Nineteen former *Ulocladium* species have been distributed among three main sections within the enlarged genus *Alternaria* based on phylogenetic data: *A*. sect. *Pseudoulocladium*, *A*. sect. *Ulocladioides*, and *A*. sect. *Ulocladium* [12]. For *Alternaria* species, the *Alt a 1* sequence supported the separation of groups of *Alternaria* spp. and related taxa into several species-groups [13]. Although of *Alt a1* in fungal biology remains unclear, some evidence has suggested that the role of *Alt a 1* can be related to virulence and fungal infection pathogenicity [14]. Lately, sequencing of alternative regions has been explored, such as a segment of an endopolygalacturonase (endoPG) gene the *Alternaria* major allergen 1 (*Alt a1*) gene and two anonymous noncoding regions, OPA10-2 and OPA1-3 [15,16].

Several *Alternaria* species were exhibited virulence activities against different crops [17,18]. Abass [19] showed that *Ulocladium* (synonyomy *Alternaria*) succeeded to invade wounded and unwounded date palm fruits. Species of *Alternaria* are well known for the production of a variety of about 70 toxic secondary metabolites [20], some of them recognized as mycotoxins, such as alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), altenuene (ALT), and altertoxins I, II, III (ATX-I, -II, -III) [21,22]. *Alternaria* mycotoxins have been frequently isolated and reported in fruits and vegetables, such as tomatoes, citrus fruits, Japanese pears, carrots, barley, oats, olives, mandarins, melons, peppers, apples, raspberries, cranberries, grape, wheat, and other grains [23]. Tenuazonic acid (TA) is one of the major mycotoxins produced by some *Alternaria* species [24]. TeA has been reported to be acutely toxic for several animals such as mice, chickens, and dogs, and it has been associated with human haematological disorders like Onyalai and in various *Alternaria*-contaminated crops, fruits, and vegetables [25].

This work focused on characterization of *Alternaria* species from infected citrus samples with attention to pathogenicity and Tenuazonic acid activities.

2. MATERIALS AND METHODS

2.1 Collection of Samples

A total of one hundred and fifty samples of infected citrus (Navel orange and tangerine) were collected from five Governorates in Upper Egypt (Aswan, Luxor, Qena, Sohag and Assuit), Thirty samples from each. Each sample was put in a sterile polyethylene bag sealed and transferred to the mycological laboratory. Samples were kept in a cool place during storage 5°C till fungal analysis.

2.2 Isolation and Morphological Identification of *Alternaria* spp.

The dilution plate method was used for isolation the fungal strains from navel orange, tangerine and lemon as described by Christensen [26]. A defined weight of each infected citrus samples placed in a sterile conical flask containing 100 ml of sterile distilled water. Flasks were shaken by hand in a rotating motion for 10 minutes. One m1 of desired dilution will be transferred aseptically into each of sterile Petri-dishes and followed by addition of about 15 to 20 m1 of liquefied Dichloran chloramphenicol- peptone agar (DCPA) medium. The dishes were rotated by hand in abroad swirling motion to ensure uniform distribution of homogenates. Three replicates were prepared for each sample and cultures were incubated at 27°C for 5 - 7 days. Morphological identification based on length of primary, secondary conidiophores, shapes, sizes and colours of conidia was done for each isolate grown on Synthetic Nutrient Poor Agar plates at 22°C for 7 days according to Woudenberg et al. [11]. Further criteria also including colony texture, colour and diameter of fungal colony were estimated on Potato Dextrose Agar (PDA) plates at 25°C for 7 days.

2.3 Molecular Identification of *Alternaria* Isolates

2.3.1 DNA extraction

For DNA extraction, fungal isolates were cultured in 250 ml flasks containing 50 ml Potato Dextrose Broth (PDB) for 2-3 days using a rotary shaker for 25°C at 120-150 rpm. The mycelium was harvested by filtration, frozen at -80°C. Mycelium was ground in liquid nitrogen using sterile mortar to obtain homogeneous fine powder. Afterwards, DNA extracted from 50 mg of mycelium powder using acetyl trimethylammonium bromide (CTAB) according to Mohammadi et al. [27]. The resulted DNA pellet dissolved in 1 ml of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) as described by Moeller et al. [28]. The DNA quantity and quality checked by electrophoresis on a 0.8% agarose gel revealed with ethidium bromide and visualized by UV trans-illumination.

2.3.2 PCR amplification and sequencing

Amplification of *Alt a 1* gene was conducted using primer pairs; Alt-for (5-ATGCAGTTCACCACCATCGC-3) and Alt-rev (5 ATGCAGTTCACCACCATCGC-3 [13]. Each PCR reaction was performed in a 25 µL mixture that contained 5 µL of the master mix (buffer, dNTP, Taq DNA polymerase, 2 mM MgCl2), 1µL of the template DNA, 0.5µL of both forward and reverse primers and the volume was completed to 25 µL with PCR water. Amplification was performed in a thermal cycler (Flexigene, Techne, Cambridge, UK). PCR) cycles for *Alt a1* gene were using the following cycling protocol: initial denaturation at 94°C for 1 min, 35 amplification cycles of 94 °C for 30 s, 57°C for 30 s and 72°C for 1 min, and a final extension at 72°C for 10 min. PCR product was observed in a 1.4% agarose gel, stained with ethidium bromide and visualized with UV transilluminator. Amplified products were purified, quantified and sequencing in Macrogen (South Korea).

2.3.3 Phylogenetic analysis

Sequencing data were edited using Chromas Lite, aligned and clustered by Mega 6.0 [29]. The *Stemphyllium* genus is closely related to *Alternaria*. Therefore, phylogenetic tree was rooted with *Stempphyllium callistephi* (AY563276) as out groups. The phylogenetic reconstruction was done using the neighbor joining (NJ) algorithm, with bootstrap values calculated from 1,000 replicate runs, using the software routines included in the MEGA software.

2.4 Pathogenicity Test of the Selected Strains

Pathogenicity test was carried out as described by Baiyewu et al. [30] and Chukwuka et al. [31]. The selected healthy fruits of mature "Washington" Navel orange was surface sterilized by 1% Sodium hypochlorite solution for 5 min, then washed by rinsing these fruits in sterilized distilled water for three times and dried with sterilized filter paper. Inoculation procedures were performed under sterile conditions by inserting 5 mm agar disc of the tested fungus into holes (5mm diameter and 5mm depth) made in surface of fruit using a sterilized cork borer. Agar discs of each tested fungi (about 5 mm diameter) were taken from the margin of 10 days old PDA-cultures of the tested fungi and placed into the wound. After inoculation, the holes were plugged with the removed pieces of the peel. Three replicates, each of three fruits, were used for each tested fungi and control treatment was carried out by inoculated a plug of sterile PDA. The inoculated fruits were placed in black

polyethylene bags, and then incubated in the dark at 25°C for 3 weeks. Following incubation, fruit were cut in half and the fungal growth was determined by measuring the diameter of lesion produced in mm.

2.5 Mycotoxin Production

The strains were cultured on autoclaved rice at 40% moisture. The rice was inoculated with a 5mm disk of 1 week old (PDA) and incubated at 25°C for 3 weeks in the dark [32]. The cultural material was homogenized with 30 ml of methanol and filtered through a Whatman filter paper (no.1). The filtrate was clarified with 60 ml of 20% ammonium sulphate. Twenty ml was adjusted to pH 2 with 6 N HCl and extracted twice for TA with 15 ml of chloroform. TA was then partitioned into 10 ml of 5% sodium bicarbonate, acidified to pH 2 again, and extracted twice with 10 ml of chloroform. The chloroform extracts were combined, washed with 7.5 ml of water, and evaporated to dryness. The residue was made up to 1 ml with methanol and analyzed for TeA by HPLC combined with UV-detection at 280 nm [32].

2.6 Statistical Analysis

Results data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System. Means were separated by Duncan's multiple range test at $P < 0.05$ level.

3. RESULTS

3.1 Morphological and Molecular Characteristics of *Alternaria* spp.

Twenty-two isolates representing three *Alternaria* spp. belong to *A.* sect. *Ulocladioides* and *A.* sect. *Ulocladium* were isolated from 150 samples of citrus fruits on plates of DCPA media at 27 °C. *Alternaria botrytis* recovered from 10 and 8% of the samples comprising 75 and 28.57% of *Alternaria* from Navel orange and Tangerine samples, respectively. *A. oudemansii* was isolated from Tangerine samples only constituting 64.24% of total *Alternaria*. In the last place come *A. atra* which isolated from 2% of Navel orange and Tangerine samples comprising 25 and 7.14% of *Alternaria*, respectively (Table 1 and Fig. 1).

Table 1. Average total counts (ATC), number of cases of isolation (NCI, out of 50 samples) and occurrence remarks (OR) of *Alternaria* species recovered from 150 samples of navel orange and tangerine and citrus fruits on Dichloran chloramphenicol- Peptone Agar (DCPA) medium on at 27°C

Genera and species	Navel orange					Tangerine					Lemon
	ATC	%C	%F	NCI	OR	ATC	%C	%F	NCI	OR	-
Alternaria atra	300	25	2	1	R	150	7.14	2	1	R	-
Alternaria botrytis	900	75	10	5	L	600	28.57	8	4	L	-
Alternaria oudemansii	-	-	-	-	-	1350	64.28	12	6	L	-
Average total count	1200	100%				2100	100%				-

Occurrence remark: or (out of 50 samples): H = high occurrence from 25–50 cases, M = moderate occurrence from 12–24 cases, L = low occurrence from 3–6 cases and R = rare occurrence from 1–2 cases

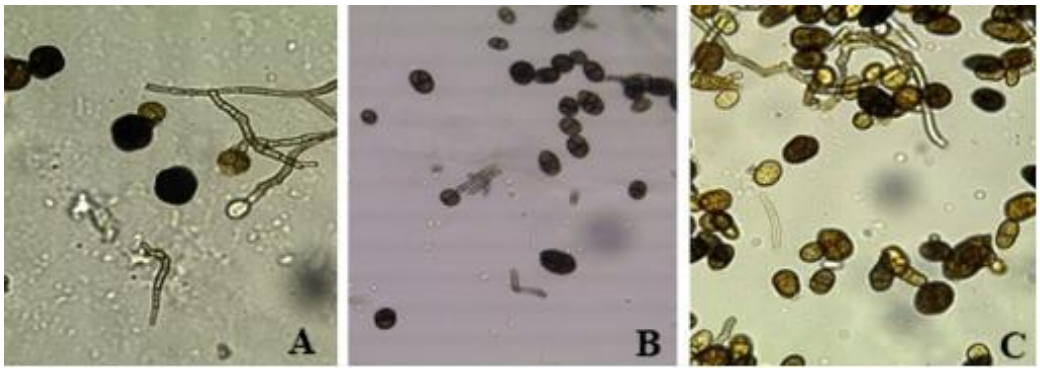

Fig. 1. Conidial shapes of different *Alternaria* species, *A. atra* (A), *A. botrytis* (B) and *A. oudemansii* (C)

3.2 Molecular Characterization of *Alternaria* Species

Alt a1 gene was successfully amplified from *Alternaria* strain recovered from citrus fruits samples. All the sequences for *Alt a1* gene were deposited in the GenBank and their accession numbers were indicated in Table 2. The highly resolution *Alt a1* gene dataset separated *Alternaria* species into three groups. First group contain *A. botrytis* (4 strain), second group contain *A. oudemansii* (4 strain) and third group contain *A. atra* (2 strain) (Fig. 2).

Phylogenetic analysis has been performed on the present 10 *Alternaria* (synonymy *Ulocladium* spp.) nucleotide sequences with the other *Ulocladium* spp. published in the GenBank and the results of this analysis are show in Fig (2). Phylogenetic tree was generated from 27 sequences including 5 *U. botrytis,* 5 *U. oudemansii*, 3 *U. atrum* and 13 *Ulocladium* spp. sequence in GenBank in addition to the out-group sequence *Stemphyllium vesicarium*. First group comprised 4 strain of *A. botrytis* (SVUAbo160, SVUAbo161, SVUAob162 and SVUAbo163) clustered together clustered with the *U. botrytis* AY563317 (synonymy: *A. botrytis*) obtained from GenBank with bootstrap (86%). Second group comprised 2 strain of *A. atra* (SVUAat158 and SVUAat159) clustered together clustered with the *Ulocladium atrum* AY563318 (synonymy: *A. atra*) obtained from GenBank with a bootstrap support (41%). Third group comprised 4 isolates (SVUAou164, SVUAou165, SVUAou166 and SVUAou167) of *A. oudemansii* clustered together clustered with *U. oudemansii* FJ266514 (synonymy: *A. oudemansii*) obtained from GenBank. The tree showed a well-supported relationship (76% bootstrap) between *U. oudemansii* (FJ266514) from GenBank and four isolates (SVUAou164, SVUAou165, SVUAou166 and SVUAou167) that based on morphological features were identified as *A. oudemansii*.

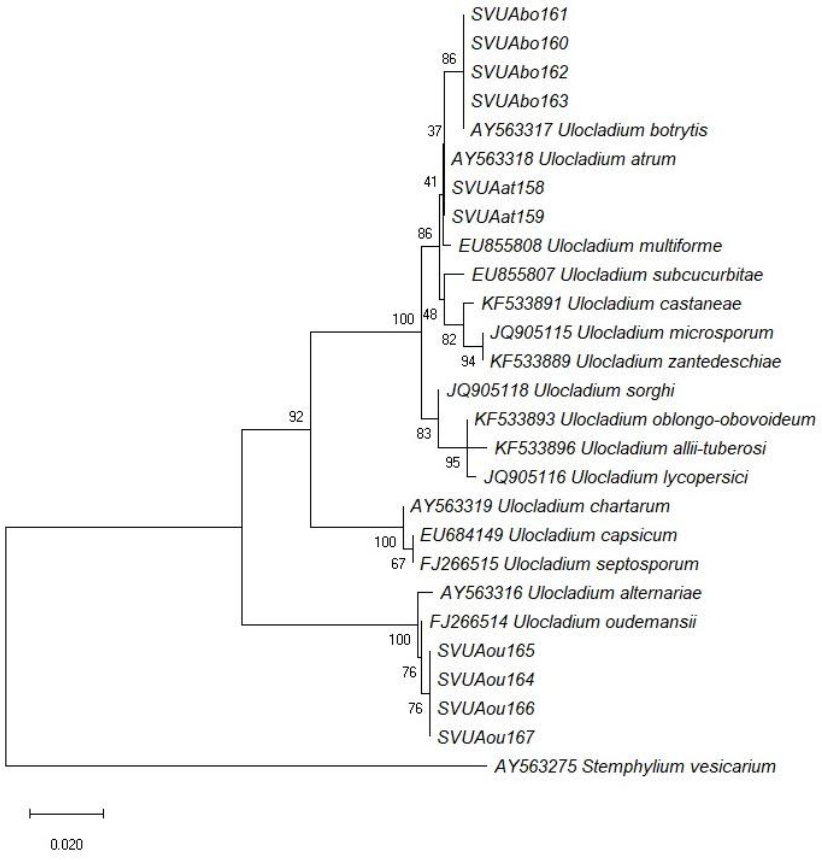

Fig. 2. Phylogenetic tree of *Alternaria* strains isolated from citrus fruits based on *Alt a1* gene sequence data. The numbers above branches indicate bootstrap values

3.3 Pathogenicity of the *Alternaria* Species

Ten of *Alternaria* isolates were evaluated for their pathogenicity on healthy Navel orange citrus fruits. The obtained results of the pathogenicity test revealed that all isolates caused black rot symptoms of the oranges with average lesion size ranged from 20 to 31.5 mm. Among the tested isolates, the highest average lesion size was 31.5 mm which achieved by *A. botrytis* (SVUAob161). The other isolates including *A. atra* (SVUAat158 and SVUAat159), *A. botrytis* (SVUAbo160, SVUAbo162 and SVUAbo163) exhibited virulent capacity with lesion size ranged 30 to 31 mm. Whereas the lowest lesion size was produced by *A. oudemansii* isolates (SVUAou164, SVUAou165, SVUAou166 and SVUAou167) with lesion size ranged from 20-25.5 mm (Table 2 and Fig. 3).

3.4 Mycotoxin Production

Six strains were chosen for tenuazonic acid toxin production. The HPLC analysis of the standard metabolites was done for characterization and quantitative determination of mycotoxins recovered from culture filtrates of different strains. About 83% of the isolates showed the ability to produce TeA toxin and only one strains of *Alternaria oudemansii* (SVUAou164) failed to give any detectable amount of toxin. The tested isolates exhibited significant TeA activities and the detected amounts of TeA toxin ranged between 1.45 to 18 ug/ml. The maximum concentration of TA toxin (18 ug/ml) was obtained from *A. botrytis* (SVUAbo161) followed by *A. botrytis* (SVUAbo160) with amount 15.01 ug/ml. The detected TeA amount from *A. atra* (SVUAat158) and (SVUAat159) isolates were 8.75 and 15.01 ug/ml, respectively. The minimum TeA amount was showed by *A. oudemansii* (SVUAou165) isolate (1.45 ug/ml) (Table 3 and Fig. 4).

4. DISCUSSION

This study is the comprehensive research for identification and genetic diversity of *A.* sect. *Ulocadioides* and *A.* sect. *Ulocladium* species, affecting the citrus fruits in Upper Egypt. In this study, three *Alternaria* species identified as *A. atra, A. botrytis and A. oudemansii* were

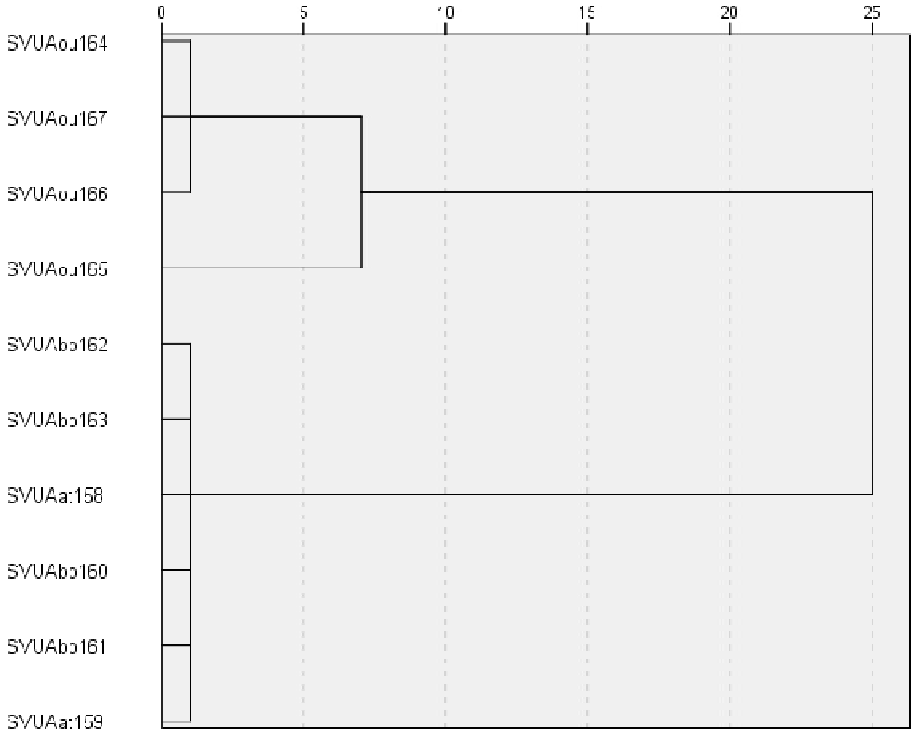

Fig. 3. Dendrogram showing relationships among 10 isolates of *Alternaria* spp. based on pathogenicity test

Table 2. Origin of sample collection, Code, accession numbers, name of the pathogen identified and measurement of lesion expansion and rank of the Navel oranges condition 21 days after incubation from each sample

No. of isolate	Code of isolate	Molecular identification	Substrate	Origin	Measurement (mm)	Pathogenicity/ virulence	Accession number
0	-	Control	Navel orange	Assuit	7.5	A	-
1	SVUAat158	*A. atra*	Tangerine	Aswan	30±1	B	MT711112
2	SVUAat159	*A. atra*	Tangerine	Sohag	31±1	B	MT711113
3	SVUAbo160	*A botrytis*	Tangerine	Qena	31±1	B	MT711114
4	SVUAbo161	*A. botrytis*	Navel orange	Qena	31.5±1	B	MT711115
5	SVUAbo162	*A. botrytis*	Navel orange	Assuit	30±1	B	MT711116
6	SVUAbo163	*A. botrytis*	Tangerine	Aswan	30±1	B	MT711117
7	SVUAou164	*A. oudemansii*	Tangerine	Luxor	20±1	C	MT711118
8	SVUAou165	*A. oudemansii*	Tangerine	Aswan	25.5±1	C	MT711119
9	SVUAou166	*A. oudemansii*	Tangerine	Aswan	21±1	C	MT711120
10	SVUAou167	*A. oudemansii*	Navel orange	Assuit	20±1	C	MT711121

Means with different letters are significantly different from control ($P < 0.05$)
A: healthy, no visible symptoms (nonvirulent), B (high virulent) and C (moderate virulent)

Table 3. Teuanzonic acid toxin production by *Alternaria* strains

Code of isolate	Fungal species	Tenuazonic acid concentration (ug/ml)
SVUAat158	*Alternaria atra*	8.75
SVUAat159	*Alternaria atra*	10.87
SVUAbo160	*Alternaria botrytis*	15.01
SVUAbo161	*Alternaria botrytis*	18.47
SVUAou164	*Alternaria oudemansii*	0.00
SVUAou165	*Alternaria oudemansii*	1.54

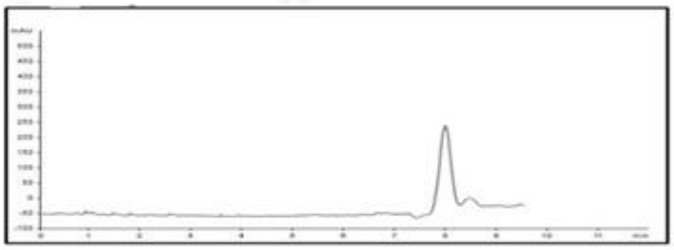

Fig. 4. TeA level from *A. botrytis*

associated with citrus fruits collected from the markets in Upper Egypt. Our results were identical with several literatures [33,34]. *Alternaria* sp. causes black rot and massive deterioration–of citrus fruits [35,36]. Uzuegbu and Emifoniye [37] in their work of postharvest fungal spoilage of some Nigerian fruits and vegetables isolated *Alternaria* in 40% of the total samples. Zora [38], who was recorded four species *Ulocladium* species (synonymy *Alternaria*) including *U. atrum*, *U. botrytis*, *U. charatum* and *U. chlamydosporum*) from soils and palm fields in Iraq. Our results indicated that *Alternaria* species belonging to *A.* sect. *Ulocadioides* and *A.* sect. *Ulocladium* groups was weakly appeared and this in agreement with [13] who reported that *Ulocladium atrum* (synonymy *A. atra*) and *Ulocladium botrytis* (synonymy *A. botrytis*) were poorly supported.

A phylogenetic analysis of small-spored, citrus-associated *Alternaria* isolates was recently completed and included the 10 morphospecies [39]. Using morphological characters in *Alternaria* identification is not enough to discriminate among common small-spored species [16,40]. Therefore, the DNA analysis is needed for accurate identification and characterization of the species targeting the *Alt a 1* gene has been developed for the rapid detection of DNA by PCR amplification for phylogenetic analysis of *Alternaria* and related genera [41,42]. *Alt a 1* is expressed by both *Alternaria* and other members of the Pleosporaceae family, including the allergenic species (*Stemphylium*, *Ulocladium*, *Nimbya* and *Embellisia*) [13,43,44]. The phylogenetic analysis of our isolates with other strain from Genebank illustrated that our isolates grouped in three species namely *A. atra* (synonymy *U. atrum*), *A. botrytis* (*U. botrytis*) and *A. oudemansii* (*U. oudemansii*). The obtained result are in agreement with the past study by Hong et al. [13] indicated that bootstrap support for *Ulocladium* group was low <50% and *Ulocladium* group was divided into two monophyletic groups, one of which was composed of *A. cheiranthi*, *E. indefessa* and *U. chartarum* and the other was composed of *U. cucurbitae*, *U. botrytis*, and *U. atrum.* The analysis of 13 species of *Ulocaldium* (synonymy *Alternaria*) using *Alt a1* and *Gpd* sequences, Wang et al. [45] showed that *Ulocladium* species is divided into two distinct monophyletic clades with high bootstrap values. Clade 1 included nine *Ulocladium* species, while clade 2 includes four species of *Ulocladium*. Also, Runa et al. [46] described the phylogenetic relationship of 13 species of *Ulocladium* with other related species of *Alternaria*, *Embellisia*, and *Stemphylium* based on sequences of *Alt a1* and *Gpd* and they revealed that ten species of *Ulocladium* clustered into a core *Ulocladium* group but *Ulocladium alternariae* and *U. oudemansii* clustered together in a second clade sister to and immediately basal to the primary *Ulocladium* clade.

The obtained results of the pathogenicity test showed that all of the *Alternaria* isolates caused

significant black rot on the healthy Navel orange, some isolates are highly pathogenic on Navel orange, for example, *A. botrytis* (SVUAbo161) had a mean lesion size of 31.5 mm. The current results are in a good agreement with many other published researches showed the virulence of *Alternaria* species and their association with fruit rot on different plant hosts such as date palm, pistachio and citrus fruits [47,48,49]. Our results are in agreement with Abass [19] indicated that the results of pathogenicity test on date palm fruits of Barheee cultivar revealed the as lesion sizes of *Ulocladium* (synonymy *Alternaria*) were 1.97 and 1.75 mm in wounded and unwounded treatments, respectively. Peever et al. [50] reported that 92% of *Alternaria* isolates recovered from citrus were pathogenic to detached Minneola leaves. Our findings also revealed high variability in virulence among the *Alternaria* isolates. Several workers have already reported pathogenic variability among isolates of *Alternaria* spp. [51,52]. Bukar et al. [35] who reported that different spoilage types were observed when the healthy oranges were re-inoculated with the pure isolates of the pathogens. Mojerlou and Safaie [53] claimed that all *Alternaria* isolates caused black rot to Navel and Valencia oranges cultivars and significant differences were observed between cultivars and among isolates. Nemsa et al. [54] studies the pathogenicity of nine isolates of *Alternaria* against Fortune mandarin. He demonstrated that 6 isolates were considered as pathogens on unwounded fruits with different degrees of aggressiveness as low (33.3% of isolates), medium (33.3%) and high (33.3%).

In this study 83% of tested *Alternaria* species were tenuazonic acid producers with different concentrations. The genus *Alternaria* is well known for its ability to secrete a wide range of toxins, which can be either specific for the host or common for several hosts. TeA is considered to be having highest toxicity amongst the mycotoxins produced by *Alternaria* [55,56]. Several studies demonstrated that various isolates of *Alternaria* species isolated from different sources were TeA producers [25,57,58, 59,60]. Wei et al. [61] found TeA as the most recurrent toxin in all dried fruits with concentration in the range of 6.9–5665.3 µg/kg. Davis et al. [62] and Kinoshita et al. [63] screened 185 strains of *Alternaria* species and found the wide-spread occurrence of tenuazonic acid (TA). Sixty-five percent of feed samples were contaminated with TeA up to 1983 µg/kg [64,65,66]. Previous studies suggest that strains may differ in their mycotoxin secretion [67,68, 69].

5. CONCLUSION

Alternaria atra, A. botrytis and *A. oudemansii* were recovered from citrus samples in Upper Egypt and Tangerine samples were more contaminated with *Alternaria* spp. All tested isolates were pathogenic to healthy navel orange and *A. oudemansii* was less virulent, about 83% of *Alternaria* isolates were TeA toxin producers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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