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Research Article
Studies on *Botrytis* spp. Infected Ornamental Plants and Emergence of Resistant Isolates Against Fenhexamid in Egypt

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Abstract

Background and Objective: *Botrytis* is a serious fungal pathogen which infects most ornamental plants and its resistance to effective fungicide is a continuous challenge facing disease management. The current research aimed to identify resistant *Botrytis* spp. from different ornamental plants against fenhexamid. **Materials and Methods:** About 64 single-spore isolates were collected from different symptomatic and asymptomatic ornamental plants using the modified Kerssies medium, m1KERS. Phenotypic variability was shown in some features including growth texture, sclerotia pattern and conidial dimensions. Pathogenicity and mycelial growth rate tests were statistically carried out using one way analysis of variance (ANOVA). Molecular characterisation was demonstrated by transposable elements (TEs) analysis and fenhexamid resistance was revealed using mycelial growth assay. **Results:** All isolates were identified as *Botrytis* spp. according to their morphological and molecular characteristics. Based on phylogenetic analysis, BCLi1 isolate seemed to be distantly related to *B. cinerea* and other *Botrytis* species. Significant variation in aggressiveness among isolates was observed on detached lettuce leaves. Four TE genotypes, *transposa*, *boty*, *flipper* and *vacuma*, were detected in *Botrytis* populations with TE distribution reached 50, 29.7, 10.9 and 9.4%, respectively. The low virulence level of some *Botrytis* spp. isolates seemed to be related to *vacuma* isolates suggesting a possible correlation between virulence and TE genotype. Moreover, a low to moderate resistance of *Botrytis* spp. isolates was revealed towards the hydroxyanilide fungicide, fenhexamid, in three *transposa* isolates, BCT6, BCGL2 and BCGL5, with a mean EC_{50} value of 0.2, 0.3 and 0.5 μg mL^{-1}, respectively. **Conclusion:** The results demonstrated the first observation of the emergence of resistant isolates of *Botrytis* from ornamental plants against the highly effective fungicide in Egypt, providing a serious attention towards *Botrytis* management.

Key words: Asymptomatic plants, *Botrytis* spp., fungicide resistance, molecular divergence, ornamental plants, transposable elements

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Botrytis spp. and *B. cinerea* Pers.: Fr. (teleomorph, *Botryotinia fuckeliana* (de Bary) Whetzel) are necrotrophic pathogens that attack flowers at any stage, especially new tender and senescent tissues which are exceptionally susceptible[1] at any time proper conditions prevail in greenhouses, fields or during shipping. *B. cinerea* has the widest host range and can infect more than 1000 plant species[2]. This pathogen is found worldwide and causes disease in many fruit, flower and leafy crops[1,3]. One of the molecular variation factors implicated in fungal genomic development is the transposable elements (TEs) which were characterized by their ability to transpose within the genome resulting in a huge impact on genome function and evolution[4,5]. Based on the presence of TEs, *flipper* and *boty*, *Botrytis* sp. isolates have been classified to four TEs genotypes, namely, *transposa*, *boty*, *flipper* and *vacuma*[6-8]. For *Botrytis* management, growers use many fungicides such as fenhexamid (a non-systemic fungicide with both protective and curative activity), chlorothalonil and iprodione to which *Botrytis* spp. strains have shown various degrees of resistance. Previously, no resistant isolate of *Botrytis* to the hydroxyanilide fungicide, fenhexamid was observed on various plants in Egypt[6,8]. As *Botrytis* spp. attack a broad range of plant species in various geographic conditions, we should study different characterization aspects especially on those isolated from ornamental plants which were not, in our knowledge, well studied in Egypt. Additionally, *Botrytis* spp. known to make adaptation towards many fungicides including fenhexamid as it was highly efficient, leading to emergence of new resistant isolates. Naturel resistance to fenhexamid was also evident in *Botrytis* isolates due to their genetic plasticity. Despite this fact, low reduction of fenhexamid efficacy had been recorded in various countries for many years after its registration. At the same time, the other toxic fungicides, such as dicarboximides are nearly banned in many countries as high frequency of resistant *Botrytis* isolates had arisen[3]. In addition, a widespread resistance to the benzimidazole fungicides has been reported[9] and therefore they are no longer suggested for use in *Botrytis* management. The aims of the present investigation were to identify and characterise *Botrytis* spp. isolated from symptomatic and asymptomatic ornamental plants and to determine the baseline sensitivity of *Botrytis* isolates to fenhexamid.

MATERIALS AND METHODS

Plant and fungal sample collection: Samples from ornamental plants were collected from rose (*Rose* sp.), begonia (*Begonia rex*), narcissus (*Narcissus tazetta*), gladiolus (*Gladiolus communis*), lily (*Lilium* sp.) and tulip (*Tulipa gesneriana*) grown in different Egyptian governorates (Table 1) during 2015-2016. These samples were either symptomatic, showing typical symptoms of *Botrytis* blight or asymptomatic, with no symptoms whatsoever. The symptomatic and asymptomatic plant samples were cut into small pieces (0.5 cm), dipped separately in sterilised water for 5 min, dried on sterilised filter paper, then plated onto the modified Kerssies medium, m1KERS[10] and incubated at 23°C for 3-21days.

Fungal identification: Single spore of each isolate was grown on a PDA medium and the resulting culture was preserved under sterilised paraffin oil at 4°C. *Botrytis* isolates were identified based on morphological and cultural characteristics according to previous studies[6,8].

Molecular identification of *Botrytis* isolates using PCR: Genomic DNA was extracted and purified from harvested mycelia using the mini preparation procedure according to Moller *et al.*[11]. *Botrytis* identification was carried out using specific primers[12] (Table 2) and then DNA sequence was revealed using ITS1/ITS4 primers[13] (Table 2). The PCR programme was performed in a total volume of 25 µL consisting of 2 µL genomic DNA (50 ng μL^{-1}), 0.5 µL of each primer "Bio-search Technologies" (10 µM), 12.5 µL Red PCR master mix (Bio-line) and 9.5 µL H_2O. Amplification was conducted in a thermocycler (Techne-Progene) as follows: Initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55-64°C for 1 min, extension at 72°C for 1 min and a final extension step at 72°C for 10 min.

Evolutionary analysis by maximum likelihood method: The maximum likelihood method and Tamura-Nei model were used according to Tamura and Nei[14] to produce evolutionary analyses which were conducted by MEGA X[15]. From 1000 replicates, a bootstrap consensus tree was produced to represent the evolutionary taxa analysed[16]. Initial tree consisting of 21 nucleotide sequences was obtained using Neighbor-Join and BIONJ algorithms.

Table 1: Collection of *Botrytis* isolates from symptomatic and asymptomatic ornamental plants

Host plants	Plant organs	Location	Isolates	Number of isolates
Rose	Petal	Ismailia	BCR1:BCR9	24
		EL-Beheira- Idko	BCR10:BCR15	
		Giza	BCR16:BCR21	
		Alexandria	BCR22:BCR24	
Tulip	Bulb	Alexandria	BCT1:BCT10	
		Cairo	BCT11:BCT14	14
Gladiolus	Petal	Cairo	BCGL1:BCGL9	9
Begonia	Leaf	Alexandria	BCB1:BCB8	8
Narcissus	Bulb	Alexandria	BCN1:BCN5	5
Lily	Bulb	Alexandria	BCLi1:BCLi4	4

Table 2: Molecular identification and characterisation of *Botrytis* isolates collected from ornamental plants using different primers

Primers	Primer sequence, 5'→3'	Expected amplicon length (bp)	References
C729F	CTGCAATGTTCTGCGTGGAA	700	Rigotti *et al.*[12]
C729R	AGCTCGAGAGAGATCTCTGA		
ITS1	TCC GTA GGT GAA CCT GCG G	550	White *et al.*[13]
ITS4	TCC TCC GCT TAT TGA TAT GC		
BotyF4	CAG CTG CAG TAT ACT GGG GGA	510	Diolez *et al.*[17]
BotyR4	GGT GCT CAA AGT GTT ACG GGA G		
Flipper F300	GCA CAA AAC CTA CAG AAG A	1250	Levis *et al.*[18]
Flipper F1550	ATT CGT TTC TTG GAC TGT A		

Detection of transposable elements (TEs): The PCR amplification of the two transposable elements, *boty* and *flipper*, was done using their specific primers[17,18] (Table 2). The PCR preparation and programme were performed according to Abdel Wahab[6].

Pathogenicity assay using detached lettuce leaves: Pathogenicity of *Botrytis* isolates was carried out using the detached lettuce (*Lactuca sativa* L. cv. 'Baladi') leaf technique[6-8]. Isolate aggressiveness was determined by measuring the lesion diameter of each inoculated leaf.

Determination of EC_{50} of fenhexamid for *Botrytis* isolates: Thirty isolates of *Botrytis* collected from the current ornamental samples: BCN1, BCN2, BCN4, BCLi1, BCLi2, BCLi3, BCLi4, BCGL1, BCGL2, BCGL3, BCGL4, BCGL5, BCGL6, BCGL7, BCGL8, BCGL9, BCT1, BCT2, BCT3, BCT4, BCT5, BCT6, BCT7, BCT8, BCT9, BCT10, BCT11, BCT12, BCT13 and BCT14 were tested for their sensitivity to the hydroxyanilide fungicide, fenhexamid (Teldor SC, 500 g L^{-1}, Bayer) using PDA medium amended with 2 concentrations of fenhexamid (0.1 and 1 μg mL^{-1}). Each isolate was represented by 3 replicate plates and the entire experiment was conducted 2 times. The diameters of the resulting colonies were measured in 2 perpendicular directions after 3 days of incubation at 23°C. Isolates that were able to grow on PDA containing >0.2 μg mL^{-1} fenhexamid were putatively considered fenhexamid resistant, while those that became inhibited were classified as fenhexamid sensitive. The 50% effective concentration (EC_{50}), 50% mycelial growth inhibition, should be determined by regressing the relative growth rate (% control) against the log of the fungicide concentration.

Statistical analysis: Results were statistically tested using one way analysis of variance (ANOVA) to determine the significant difference. Data means were compared at $p = 0.05$ level using the least significant difference test.

RESULTS

Characterisation of *Botrytis* isolates collected from ornamental plants: The formation of brown halos was observed around many *Botrytis* spp. colonies resulting from symptomatic and asymptomatic ornamental plant samples after 3 days of incubation at 23°C using m1KERS selective media indicating their infection with *Botrytis* spp. The fungal colonies which grew on such selective medium were sub-cultured on PDA (Fig. 1) and then identified microscopically as *Botrytis*. Data presented in Table 3 show the isolate characterisation after collection from rose, tulip, gladiolus, begonia, narcissus and lily. The results demonstrated that *Botrytis* isolates varied in growth texture which was categorised as follows: compact, heavy warty, light warty, watery and fluffy (Table 3). The conidial dimensions differed among isolates and ranged from 10.6-12.9 μm in length, 7.1-8.9 μm in width and 276.9-539.4 $μm^3$ in volume (Table 3). Conidia of all isolates were described as oval shape. The

Table 3: Phenotypic characteristics of *Botrytis* spp. isolates collected from ornamental plants

Isolates	Growth texture	Sclerotial shape	Distribution pattern of sclerotia	Number of sclerotia*	Conidial shape	Dimensions of conidia (μm)**			
						Length	Width	Length/ Width ratio	Conidial volume (μm³)
BCR1	Fluffy	Cerebriform	Large placed irregularly	33.3±8.1	Ovate	11.7±0.7	8.1±0.5	1.5±0.1	397.6±46.4
BCR2	Fluffy	Cerebriform	Large placed irregularly	23.3±7.0	Ovate	11.7±0.6	7.1±0.5	1.7±0.1	306.1±57.7
BCR3	Fluffy	Cerebriform	Large placed irregularly	34.7±8.5	Ovate	11.9±0.8	7.1±0.5	1.7±0.1	315.2±63.1
BCR4	Fluffy	Cerebriform	Large placed irregularly	30.7±11.5	Ovate	12.7±0.6	7.1±0.5	1.8±0.1	332.9±57.0
BCR5	Fluffy	Cerebriform	Large placed irregularly	22.0±7.4	Ovate	10.6±0.6	7.1±0.5	1.5±0.2	276.9±43.2
BCR6	Fluffy	Cerebriform	Large placed irregularly	19.3±7.3	Ovate	12.1±0.8	7.1±0.5	1.7±0.2	316.4±53.4
BCR7	Fluffy	Cerebriform	Large placed irregularly	24.0±9.0	Ovate	12.9±0.6	7.1±0.5	1.8±0.2	337.2±57.4
BCR8	Fluffy	Cerebriform	Large placed irregularly	26.3±6.8	Ovate	11.0±0.6	7.1±0.5	1.6±0.2	288.6±45.9
BCR9	Fluffy	Cerebriform	Large placed irregularly	24.3±7.2	Ovate	11.7±0.7	8.1±0.5	1.5±0.1	397.6±46.4
BCR10	Compact	Cerebriform	Numerous small and scattered	78.0±11.5	Ovate	11.7±0.6	7.1±0.5	1.7±0.1	306.1±57.7
BCR11	Compact	Cerebriform	Numerous small and scattered	83.7±9.0	Ovate	11.9±0.8	7.1±0.5	1.7±0.1	315.2±63.1
BCR12	Compact	Cerebriform	Numerous small and scattered	72.3±5.5	Ovate	12.7±0.6	7.1±0.5	1.8±0.1	332.9±57.0
BCR13	Compact	Cerebriform	Numerous small and scattered	73.7±9.7	Ovate	10.6±0.6	7.1±0.5	1.5±0.2	276.9±43.2
BCR14	Compact	Cerebriform	Numerous small and scattered	66.3±7.3	Ovate	12.1±0.8	7.1±0.5	1.7±0.2	316.4±53.4
BCR15	Compact	Cerebriform	Numerous small and scattered	73.7±6.6	Ovate	12.9±0.6	7.1±0.5	1.8±0.2	337.2±57.4
BCR16	Light warty	Cerebriform	Large placed irregularly	15.3±4.3	Ovate	11.0±0.6	7.1±0.5	1.6±0.2	288.6±45.9
BCR17	Light warty	Cerebriform	Large placed irregularly	21.3±5.6	Ovate	11.7±0.7	8.1±0.5	1.5±0.1	397.6±46.4
BCR18	Light warty	Cerebriform	Large placed irregularly	17.7±3.1	Ovate	11.7±0.6	7.1±0.5	1.7±0.1	306.1±57.7
BCR19	Light warty	Cerebriform	Large placed irregularly	25.3±9.2	Ovate	11.9±0.8	7.1±0.5	1.7±0.1	315.2±63.1
BCR20	Light warty	Cerebriform	Large placed irregularly	13.7±5.1	Ovate	12.7±0.6	7.1±0.5	1.8±0.1	332.9±57.0
BCR21	Light warty	Cerebriform	Large placed irregularly	16.3±5.1	Ovate	10.6±0.6	7.1±0.5	1.5±0.2	276.9±43.2
BCR22	Compact	Cerebriform	Large in circle	46.7±3.2	Ovate	12.1±0.8	7.1±0.5	1.7±0.2	316.4±53.4
BCR23	Fluffy	Cerebriform	Large in circle	48.7±5.8	Ovate	12.9±0.6	7.1±0.5	1.8±0.2	337.2±57.4
BCR24	Fluffy	Cerebriform	Large placed irregularly	33.3±8.4	Ovate	11.0±0.6	7.1±0.5	1.6±0.2	288.6±45.9
BCT1	Heavy warty	Cerebriform	Numerous small and scattered	78.7±9.5	Ovate	10.7±0.6	7.8±0.5	1.4±0.1	339.7±49.8
BCT2	Heavy warty	Cerebriform	Large placed irregularly	24.0±10.5	Ovate	11.1±0.5	8.1±0.7	1.4±0.1	379.2±68.4
BCT3	Heavy warty	Cerebriform	Numerous small and scattered	85.3±11.9	Ovate	12.1±0.6	8.6±0.9	1.4±0.2	472.2±99.0
BCT4	Heavy warty	Cerebriform	Large placed irregularly	31.7±5.1	Ovate	12.7±0.6	8.5±0.5	1.5±0.1	481.3±60.5
BCT5	Heavy warty	Cerebriform	Numerous small and scattered	78.0±6.4	Ovate	10.7±0.5	8.5±0.5	1.3±0.1	406.3±56.8
BCT6	Heavy warty	Cerebriform	Large placed irregularly	19.0±4.6	Ovate	11.4±0.5	8.3±0.6	1.4±0.1	412.4±68.5
BCT7	Heavy warty	Cerebriform	Numerous small and scattered	80.7±4.5	Ovate	12.2±0.7	8.2±0.6	1.5±0.1	432.0±69.3
BCT8	Heavy warty	Cerebriform	Numerous small and scattered	69.0±9.6	Ovate	11.0±0.6	8.3±0.7	1.3±0.1	403.55±0.1
BCT9	Heavy warty	Cerebriform	Numerous small and scattered	76.0±7.5	Ovate	11.7±0.6	8.3±0.6	1.4±0.1	425.4±65.3
BCT10	Heavy warty	Cerebriform	Numerous small and scattered	78.0±4.0	Ovate	12.1±0.8	8.3±0.6	1.5±0.1	436.2±73.5
BCT11	Heavy warty	Cerebriform	Large placed irregularly	12.7±3.7	Ovate	11.5±0.5	8.4±0.6	1.4±0.1	422.8±47.5
BCT12	Heavy warty	Cerebriform	Large placed irregularly	15.7±4.7	Ovate	12.2±0.6	8.3±0.6	1.5±0.1	438.1±55.7
BCT13	Heavy warty	Cerebriform	Large placed irregularly	13.7±4.7	Ovate	10.7±0.6	7.9±0.9	1.4±0.1	352.5±86.4
BCT14	Heavy warty	Cerebriform	Large placed irregularly	28.3±8.5	Ovate	12.2±0.9	8.2±0.6	1.5±0.2	432.7±62.7
BCGL1	Heavy warty	Cerebriform	Large placed irregularly	37.0±6.2	Ovate	11.8±0.5	8.2±0.6	1.5±0.1	418.8±55.7
BCGL2	Heavy warty	Cerebriform	Large placed irregularly	21.7±4.6	Ovate	11.4±0.5	8.3±0.6	1.4±0.1	410.8±56.6
BCGL3	Heavy warty	Cerebriform	Large placed irregularly	31.7±9.4	Ovate	11.7±0.6	8.3±0.6	1.4±0.2	419.6±48.3
BCGL4	Light warty	Cerebriform	Large in circle	50.0±6.4	Ovate	12.2±0.6	8.3±0.6	1.5±0.1	442.8±62.0
BCGL5	Heavy warty	Cerebriform	Large placed irregularly	34.0±7.0	Ovate	10.7±0.6	8.2±0.6	1.3±0.1	378.7±60.1
BCGL6	Heavy warty	Cerebriform	Large placed irregularly	22.0±4.5	Ovate	11.8±0.7	8.5±0.5	1.4±0.1	448.9±67.0
BCGL7	Heavy warty	Cerebriform	Large placed irregularly	32.0±6.6	Ovate	12.2±0.8	8.7±0.9	1.4±0.2	480.2±92.9
BCGL8	Light warty	Cerebriform	Large in circle	59.0±9.8	Ovate	11.0±0.6	7.7±0.8	1.5±0.2	347.3±70.4
BCGL9	Heavy warty	Cerebriform	Large placed irregularly	26.7±7.5	Ovate	12.2±0.7	8.7±0.8	1.4±0.1	490.8±106.7
BCB1	Fluffy	Cerebriform	Numerous small and scattered	188.3±13.0	Ovate	11.5±0.5	8.3±0.6	1.4±0.1	412.6±55.1
BCB2	Fluffy	Cerebriform	Numerous small and scattered	190.0±10.5	Ovate	10.9±0.6	7.5±0.7	1.5±0.2	321.0±55.7
BCB3	Fluffy	Cerebriform	Numerous small and scattered	195.3±12.3	Ovate	11.8±0.5	8.2±0.6	1.4±0.1	418.8±55.7
BCB4	Fluffy	Cerebriform	Numerous small and scattered	181.0±16.6	Ovate	11.0±0.9	7.9±0.6	1.4±0.1	371.8±64.7
BCB5	Fluffy	Cerebriform	Numerous small and scattered	175.3±12.3	Ovate	11.8±0.5	8.3±0.6	1.4±0.1	425.2±64.4
BCB6	Fluffy	Cerebriform	Numerous small and scattered	188.3±14.2	Ovate	11.7±0.6	8.3±0.6	1.4±0.1	425.4±65.3
BCB7	Fluffy	Cerebriform	Numerous small and scattered	180.0±11.5	Ovate	12.5±0.7	8.5±0.5	1.4±0.1	481.9±66.5
BCB8	Fluffy	Cerebriform	Numerous small and scattered	196.7±11.0	Ovate	10.8±0.7	8.0±0.5	1.3±0.1	366.7±55.1
BCN1	Fluffy	Cerebriform	Large placed irregularly	16.0±3.4	Ovate	11.6±0.6	8.1±0.8	1.5±0.2	402.7±81.0
BCN2	Fluffy	Cerebriform	Large placed irregularly	30.3±6.4	Ovate	11.9±0.8	7.6±1.1	1.6±0.2	369.9±120.5
BCN3	Fluffy	Cerebriform	Large placed irregularly	20.7±3.7	Ovate	11.4±0.7	8.3±1.3	1.4±0.3	420.7±117.4
BCN4	Fluffy	Cerebriform	Large placed irregularly	17.0±5.2	Ovate	12.1±0.9	7.9±1.1	1.5±0.5	413.2±0.2
BCN5	Fluffy	Cerebriform	Large placed irregularly	22.7±7.2	Ovate	11.7±0.6	7.8±0.8	1.5±0.2	376.8±73.2
BCLi1	Watery	Cerebriform	Large in circle	79.3±9.9	Ovate	11.1±0.9	8.2±0.6	1.4±0.1	394.4±65.6
BCLi2	Watery	Cerebriform	Large in circle	68.0±9.5	Ovate	10.9±0.8	7.6±0.8	1.5±0.2	332.8±77.8
BCLi3	Watery	Cerebriform	Large in circle	83.0±7.2	Ovate	11.3±0.9	8.3±1.1	1.4±0.2	415.9±102.5
BCLi4	Watery	Cerebriform	Large in circle	71.0±7.2	Ovate	12.7±0.6	8.9±1.1	1.4±0.2	539.4±131.3

*Data are means of triplicate measurements±standard deviation (SD) at LSD 0.05, **All data are means of 20 conidial measurements±standard deviation (SD) at LSD 0.05

Fig. 1(a-c): Cultural characteristics of *Botrytis* isolates collected from ornamental plants (a) BCLi4, (b) BCN4 and (c) BCGL7

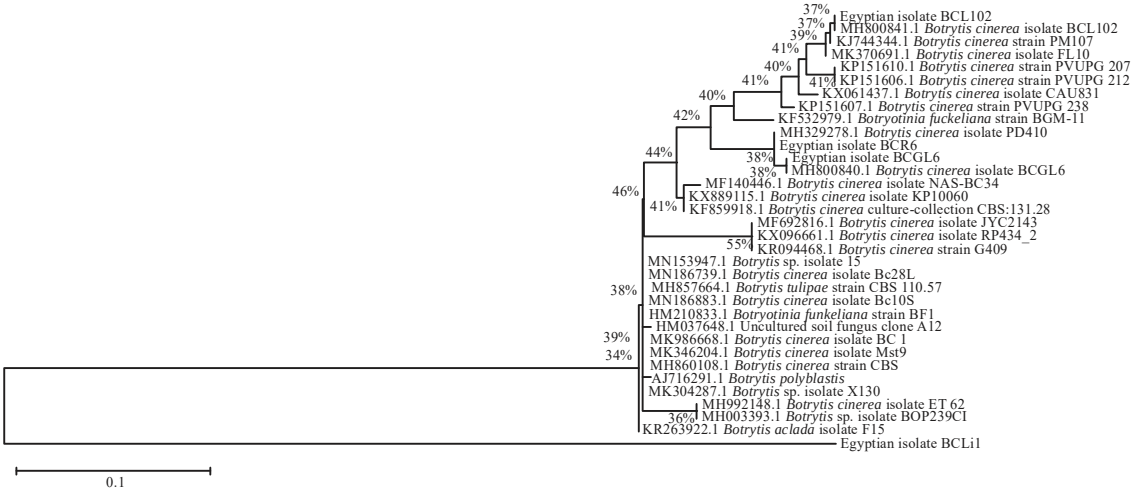

Fig. 2: Phylogenetic tree of the tested sequences based on maximum likelihood method, bootstrap support values are written for each clade

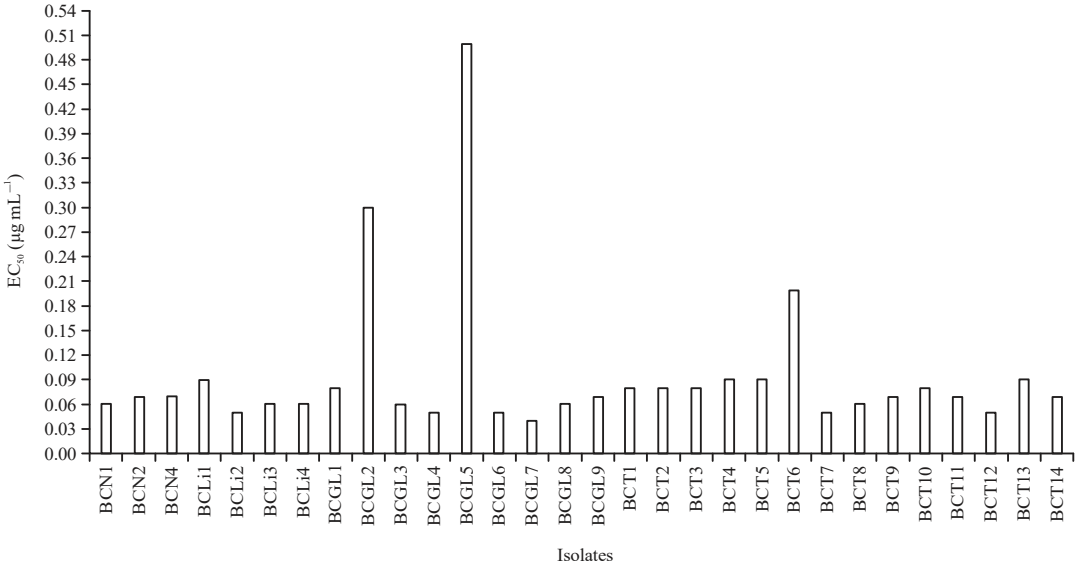

Fig. 3: Resistance of some *Botrytis* spp. isolates obtained from different ornamental plants to fenhexamid, expressed as EC_{50} (µg mL^{-1})

Table 4: Pathological, growth rate and TE patterns of *Botrytis* spp. collected from ornamental plants

Isolates	MGR (cm/day)*	Lesion diameter (cm)**	TE types***
BCR1	1.3±0.0^{hgf}	1.2±0.1^{h}	T
BCR2	0.9±0.4^{i}	1.2±0.1^{gh}	T
BCR3	2.6±0.7^{ab}	1.5±0.3^{gh}	T
BCR4	2.0±0.5^{bcdef}	2.5±0.2^{cd}	T
BCR5	1.7±0.5^{bcdefg}	3.4±0.1^{a}	T
BCR6	1.7±0.1^{ccdefgh}	2.4±0.1^{cd}	T
BCR7	1.6±0.3^{cdefgh}	0.3±0.1^{i}	B
BCR8	0.3±0.9^{i}	1.4±0.2^{gh}	T
BCR9	2.1±0.1^{bcde}	0.2±0.1^{i}	V
BCR10	1.9±0.4^{bcdef}	1.4±0.2^{gh}	B
BCR11	2.0±1.2^{bcdef}	2.4±0.2^{cde}	B
BCR12	2.4±0.1^{bc}	2.2±0.1^{de}	B
BCR13	2.1±0.3^{bcdef}	1.5±0.1^{g}	B
BCR14	2.3±0.2^{bcd}	2.5±0.2^{cd}	B
BCR15	2.6±0.2^{ab}	0.2±0.1^{i}	V
BCR16	1.7±0.2^{bcdefg}	0.2±0.1^{i}	B
BCR17	1.6±0.1^{cdefgh}	1.9±0.2^{f}	T
BCR18	1.5±0.7^{ehgf}	1.5±0.1^{gh}	B
BCR19	1.9±0.6^{bcdefg}	1.4±0.4^{hij}	F
BCR20	1.2±0.3^{hg}	4.7±0.3^{a}	T
BCR21	1.2±0.3^{hg}	2.6±0.4^{c}	F
BCR22	1.6±0.4^{cdefgh}	0.2±0.0^{i}	F
BCR23	3.4±0.3^{a}	2.6±0.0^{c}	B
BCR24	1.5±0.3^{ehgf}	2.1±0.1^{ef}	B
BCT1	2.1±0.3^{b}	2.4±0.2^{b}	T
BCT2	2.3±0.4^{b}	2.4±0.2^{b}	T
BCT3	2.0±0.2^{b}	1.4±0.2^{d}	F
BCT4	2.2±0.4^{b}	3.4±0.2^{a}	T
BCT5	1.9±0.2^{b}	0.3±0.0^{e}	T
BCT6	4.2±1.2^{a}	2.3±0.1^{b}	T
BCT7	3.1±0.1^{b}	1.5±0.1^{cd}	T
BCT8	2.3±0.3^{b}	1.5±0.1^{d}	F
BCT9	3.7±0.7^{b}	3.6±0.2^{a}	T
BCT10	1.7±0.2^{b}	1.8±0.3^{c}	B
BCT11	0.8±0.3^{b}	0.2±0.1^{e}	B
BCT12	2.0±0.2^{b}	0.4±0.2^{e}	T
BCT13	2.1±0.6^{b}	1.5±0.1^{d}	V
BCT14	2.0±0.3^{b}	2.5±0.1^{b}	T
BCGL1	2.9±0.9^{abc}	1.1±0.2^{f}	V
BCGL2	2.3±0.4^{cde}	2.5±0.5^{de}	T
BCGL3	2.1±0.2^{ed}	2.8±0.2^{d}	B
BCGL4	1.6±0.2^{e}	3.4±0.2^{b}	T
BCGL5	2.1±0.4^{ed}	2.3±0.1^{b}	T
BCGL6	2.5±0.3^{bcd}	3.1±0.2^{bc}	B
BCGL7	3.6±0.2^{a}	0.4±0.2^{g}	V
BCGL8	3.2±0.5^{ab}	1.4±0.1^{f}	F
BCGL9	3.7±0.3^{a}	4.1±0.3^{a}	T
BCB1	1.6±0.2^{abc}	2.4±0.1^{b}	T
BCB2	1.4±0.2^{bc}	1.3±0.1^{c}	T
BCB3	1.3±0.2^{c}	3.4±0.0^{a}	B
BCB4	1.5±0.2^{abc}	3.5±0.3^{a}	B
BCB5	1.9±0.1^{a}	2.5±0.3^{b}	T
BCB6	1.6±0.2^{abc}	1.4±0.2^{c}	T
BCB7	1.4±0.5^{bc}	0.3±0.1^{d}	V
BCB8	1.8±0.2^{ab}	3.4±0.2^{a}	B
BCN1	3.8±0.2^{a}	1.8±0.1	T
BCN2	3.3±0.1^{abc}	3.5±0.1	T
BCN3	3.7±0.1^{ab}	3.5±0.1	T
BCN4	2.9±0.5^{c}	0.5±0.1	B
BCN5	3.2±0.2^{c}	1.5±0.2	T
BCLi1	2.2±0.3^{a}	2.1±0.2	T
BCLi2	3.7±0.2^{ab}	4.1±0.3	T
BCLi3	2.9±0.5^{bc}	2.3±0.1	B
BCLi4	1.5±0.7^{c}	0.3±0.0	F

*MGR: Mycelial growth rate expressed as means of triplicate measurements±standard deviation (SD) at LSD 0.05, **data for lesion diameter were means of triplicate measurements±standard deviation (SD) at LSD 0.05 after 72 h of infection, ***Transposable element type, B: *Boty*, F: *Flipper*, T: *Transposa*, V: *Vacuma*, means followed by the same letter are not significantly different (p = 0.05)

sclerotia which were cerebriform in all tested isolates showed varied characteristics in their number which ranged from 12.7-196.7 sclerotia/plate (9 cm).

Molecular identification of *Botrytis* spp.: PCR produced a single specific band of 700 bp long (data not shown) using a pair of specific primers corresponding to the expected amplicon length. Sequence analysis of the amplified product revealed that the three isolates tested, BCR6, BCLi1 and BCGL6 were *Botrytis* spp. based on the BLAST identity.

Pathological, MGR and TE patterns of *Botrytis* spp.: Virulence of 64 isolates of *Botrytis* spp. collected from the ornamental plants subjected to the present study was tested on detached lettuce leaves. Isolates showed significant differences in their aggressiveness (Table 4). Lesion diameter ranged from 0.2-4.7 cm and could be divided into 3 categories, namely highly, moderately and low virulent with a lesion diameter >2.5, >1-2.5 and 0.2-1 cm, respectively (Table 4). As to mycelial growth rate, it ranged from 0.3-4.2 cm/day (Table 4). TE typing of *Botrytis* isolates used in this study was achieved using the specific primers quoted in the material and methods section for separately amplifying *boty* and *flipper* elements and four TEs genotypes could be recognised (Table 4). PCR generated 510 bp and/or 1250 bp long products for *boty* and *flipper* elements, respectively. About 32 out of the 64 isolates tested demonstrated the co-presence of the two TEs, *boty* and *flipper* (*transposa* type). While, 19 isolates showed the presence of only *boty* element (*boty* type), 7 revealed the presence of only *flipper* element (*flipper* type) and 6 have neither *boty* nor *flipper* element (*vacuma* type) (Table 4).

Phylogenetic analysis of *Botrytis* spp. isolates: The result of the amplified region (ITS1-5.8s-ITS4) revealed that, the tested isolates were *Botrytis* spp. according to the percentage of sequence identity imported from GenBank using BLAST search. Considerable variation was observed using a multiple sequence alignment of amplified region. Phylogenetic tree branches which corresponded to partitions produced in less than 50% bootstrap replicates were collapsed. The replicate tree percentage was put next to tree branches in which taxa clustered together in the bootstrap test (1000 replicates)[16]. Sequence analysis of the amplified products revealed that the isolate BCLi1 was clearly distinguished from other *Botrytis* isolates deposited in the GenBank and those under study (Fig. 2).

Fenhexamid resistance of some *Botrytis* spp. isolates: Thirty isolates under study: BCN1, BCN2, BCN4, BCLi1, BCLi2, BCLi3, BCLi4, BCGL1, BCGL2, BCGL3, BCGL4, BCGL5, BCGL6, BCGL7, BCGL8, BCGL9, BCT1, BCT2, BCT3, BCT4, BCT5, BCT6, BCT7, BCT8, BCT9, BCT10, BCT11, BCT12, BCT13 and BCT14 were tested for resistance to fenhexamid. The EC_{50} of all sensitive isolates was <1 μg mL^{-1}. The EC_{50} values ranged from 0.06-0.07, 0.05-0.09, 0.05-0.2 and 0.04-0.5 μg fenhexamid mL^{-1} for BCN, BCLi, BCT and BCGL isolates, respectively (Fig. 3). The highest sensitive isolates were BCGL7, BCLi2 and BCT7, having had EC_{50} values at 0.04, 0.05 and 0.05 μg fenhexamid mL^{-1}, respectively and the highest resistant isolates were BCGL5, BCGL2 and BCT6, having expressed EC_{50} value at 0.5, 0.3 and 0.2 μg fenhexamid mL^{-1}, respectively (Fig. 3).

DISCUSSION

The current study provides informative results about the morphological, cultural, molecular and fungicide-resistance features of *Botrytis* spp. infecting a number of ornamental plants in Egypt. The results showed a phenotypic and pathological diversity among isolates regardless their host plant and location as previously reported[19]. Similar observations had been documented in numerous isolates obtained from a number of host plants from California[20] and Tunisia[21]. All conidial and sclerotial morphology demonstrated the same shape (oval and cerebriform, respectively) as was previously recorded for vegetable and grape[8] while other study results on strawberry isolates had showed sclerotia with a flat shape[8]. Molecular characterisation showed different frequencies of the four TE genotypes with *transposa* having been represented by 50%, *boty* by 29.7%, *flipper* by 10.9% and *vacuma* by 9.4% in accordance with those reported in previous studies[6,8,22-25] but contradictory to others[26,27]. Interestingly, this evidence also showed the predominance of *transposa* in the *Botrytis* population investigated here and this was consistent with data reported elsewhere[6,8,20,22,23,26,28,29-32], followed by *boty*, *flipper* and *vacuma* type. These findings are also compatible with those reported in previous studies[24,25]. Furthermore, the existence of the four TEs genotypes in the isolates investigated here was demonstrated[20-22,33-37] in group 2 of *Botrytis* population. No correlation was found between isolate phenotype and TE type as the conidial dimensions of all genotypes did not significantly vary between *transposa* and *vacuma* isolates and this was consistent with those obtained in other studies conducted on grape and strawberry isolates[8]. While, a significant variation in virulence was observed on detached

lettuce leaves as a lower virulence level of some *Botrytis* isolates seemed to be correlated with *vacuma* type as previously reported by many other studies[6,8,26-28,30,36,38]. Moreover, the results determined a low to moderate resistance towards the hydroxyanilide fungicide, fenhexamid in the three *transposa* isolates, BCT6, BCGL2 and BCGL5 with average EC_{50} values of 0.2, 0.3 and 0.5 μg mL^{-1}, respectively. No correlation was found between sensitivity to fenhexamid and the TE type and this was consistent with that reported by other studies[28,39] as we did not find any resistant *vacuma* isolate. In addition, the high resistance to the fungicide fenhexamid was not detected in our current nor previous studies[8] indicating that group 1 isolates may not be widely existing at least in Egypt. In fact, *Botrytis* population of group 1 may exist at low frequency level of 0.7-15%[30,31,35,40] or may not exist at all[21,32,34]. Similar results derived from 33 isolates from Germany[41], 55 isolates from Chile, South Asia and Australia[32,34] along with 99.3% of many isolates in other studies[40] demonstrated that they all belonged to group 2. These findings may confirm that group 1 isolates do exist but in a small number[42,43]. Although a number of studies reported that *Botrytis* spp. belonging to group 1 differed from *B. pseudocinerea* in morphology and phylogeny[40,44], BLAST analysis, in the present study, showed a high sequence similarity (98-100%) with that of the GenBank for *Botrytis* spp. The low frequency of *vacuma* genotype did not permit to conclude whether the absence of group 1 (*B. pseudocinerea*) was due to the strategy of sampling used or to the intrinsic absence of *vacuma* isolates in Egypt. Further study on more *vacuma* isolates remains to be needed to elucidate this observation. Moreover, phylogenetic analysis revealed that BCLi1 isolate was distantly related to *B. cinerea* and other *Botrytis* species. Further molecular analysis of the current isolates is still needed to investigate their phylogenetic relationship and the effect of *transposa*/*vacuma* types on their infection severity, through the host-pathogen interaction and fenhexamid resistance of *Botrytis* isolates, both *in vitro* and *in vivo*.

CONCLUSION

In order to investigate whether resistant isolates of *Botrytis* against fenhexamid started to emerge in Egypt, different symptomatic and symptomless plant samples were collected from various ornamental plants and locations. Based on morphological and molecular characteristics, the pathogen isolates were identified as *Botrytis* spp. Significant variation in aggressiveness among *Botrytis* isolates was observed and correlated to *vacuma* type suggesting a possible effect of TE genotype on *Botrytis* virulence. The results also revealed a low to moderate resistance of some *Botrytis* isolates towards fenhexamid using the mycelial growth assay. Such resistance was not found in any *vacuma* population under study suggesting that the current *Botrytis* population does not belong to group 1.

SIGNIFICANCE STATEMENT

The current study showed a new emergence of resistant isolates of *Botrytis* spp. against fenhexamid in Egypt. This is a serious alarm for gray mold control. The results will help the researchers to make attention with fenhexamid application using alternative measures in order to decrease resistance probability to such fungicide.

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