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First report of *Botrytis cinerea* and *Alternaria alternata* on *Pelargonium grandiflorum* in Iran

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Abstract: Large flowered pelargonium (*Pelargonium grandiflorum*) is a perspective flowering crop which is distributed around the world. The area of its application is quite wide: from room floriculture to design the gardens and parks. Observation of leaf spot symptoms on this plant, which was collected from Alborz province (Karaj) motivated us to find the causal agent(s) of the disease. So, the symptomatic parts were cultured on the PDA medium after surface sterilization. Two fungal colonies were appeared on the culture medium. They were identified as *Alternaria alternata* and *Botrytis cinerea* according to the morphological characterizations. Molecular study using the *gapdh* for *Alternaria* and ITS regions and *rpb2* gene for *Botrytis* confirmed the result of the morphological identification. In the pathogenicity tests, the same spots on inoculated plants with *Alternaria* and the same spots plus gray mold symptoms and fungal body on leaves, buds and stems of the inoculated plants with *Botrytis* were another confirmation. Based on our knowledge, this is the first report of these two fungal species on the *Pelargonium grandiflorum* in Iran.

Keywords: Disease, fungi, phylogeny, pathogenicity, Pelargonium

INTRODUCTION

These days, population density and the need to the agricultural products around the world is becoming larger and larger. So, anything that affect the agricultural products can be so important for people. Plant diseases can be the most devastative threats for the agricultural systems. They can harm the products and decrease their consumer acceptance. The worst situation is the destroying the agricultural products completely (up to 100%) (Fekrikohan et al. 2021).

Large flowered pelargonium (*Pelargonium grandiflorum* Willd.) is a perspective flowering crop which is distributed around the world. It also be known as regal plargonium. The area of its application is quite wide: from room floriculture to design the gardens and parks. This plant, as well as the majority of flowering plants, are damaged by the numerous diseases (Polyakov and Korchagina 2016). Regal plants have bacterial blight (*Xanthomonas campestris* pv. *pelargonii*), which can devastate geraniums. Leaf spot (*Alternaria alternata*), grey mold (*Botrytis cinerea*), black stem rot (*Pythium splendens*), verticillium wilt (*Verticillium albo-atrum* and *V. dahliae*), Rhizoctonia and viruses also affect regal plants. Crown gall (*Agrobacterium tumefaciens*) and leafy gall (*Corynebacterium fascians*) occur on occasion. Geranium rust (*Puccinia pelargonii*) can also be a serious problem in geraniums (Loehrlein and Creig 2001). On *Pelargonium grandiflorum*, there are some studies that report some diseases such as wilt and gray mold. Nirenberg et al. (2009) have reported *Phytophthora* ×*pelgrandis* as the causal agent of crown rot disease in Germany. Miclea et al. (2012) have reported the causal agent of gray mold disease as *Botrytis cinerea* from some geranium plants such as *Pelargonium grandiflorum* in Romania. Four years later, symptoms of Verticillium wilt (*Verticillium nonalfalfae*) were reported from this plant in Italy (Bertetti et al. 2016, Garibaldi et al. 2016). Bertetti et al. (2019) have reported the susceptibility of different pelargonium plants to *Verticillium nonalfalfae* in Italy. They might have different symptoms. One of the symptoms is brown spot with the yellow margin. Many plant pathogens can cause this symptom. *Botrytis* and *Alternaria* are the major casual agents of the leaf spots. Despite of the numerous reports about these two fungi from different hosts in Iran, there is no report of *Alternaria alternata* on other pelargonium plants. There are just two reports about the isolation and identification of the *Botrytis cinerea* from horseshoe

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pelargonium (*Pelargonium zonale*) in Iran (Ershad 2022, Nabizadeh et al. 2021).

Botrytis is a fungus that belongs to the Sclerotiniaceae (Helotiales, Ascomycota), which is commonly known as grey mold fungus. The fungus destroys the tissue of the living plants, and can grow and continue to live on the host plant even after the tissue destruction. The fungus can survive as mycelium or conidia for a long time and provide a source of subsequent infections and can live in both parasitic and saprophytic forms (Fekrikohan et al. 2021). Microconidia are mononuclear and rarely germinate *in vitro*, and play as the male gametes in sexual reproduction (Dowling 2018). The optimum temperature for growth of the fungus is 20-25˚C, and the maximum growth temperature is 40˚C. However, the growth of the pathogen is possible in 0-10˚C in the storage (Zhou et al. 2020). The genus *Alternaria* was first described in 1816 with *A. tenuis* as the type species (Nees, 1817). This fungus is associated with a wide variety of substrates including the seeds, plants, agricultural products, soil and also as the emerging human pathogens.

Species of *Alternaria* are known as the serious plant pathogens, causing major losses in a wide range of crops. The genus is distributed worldwide as saprophytes, endophytes and plant pathogens (Peever et al., 2004; Woudenberg et al., 2013). Infections by the *Alternaria* species typically cause the formation of necrotic lesions, which is sometimes have a target-like appearance surrounded by an un-invaded chlorotic halo. This halo is created by the diffusion of fungal metabolites, which act as the toxins (Agarwal et al., 1997; Tewari, 1983). One of the most common species of the genus *Alternaria* is *A. alternata* that was reported as destructive plant pathogens and affect the wide range of host plants, causing leaf spots, blights, blossom rots, and fruit rots. Some isolates of *A. alternata* cause severe diseases in different ornamental crops, including trees and shrubs (Matić et al., 2020).

Some leaf spot symptoms were seen on *Pelargonium regal* plant in Karaj during the sampling. Based on our knowledge, this is the first study on isolation and identification of these fungal pathogens of the *Pelargonium grandiflorum* in Iran.

MATHERIAL AND METHODS

Sample collection, isolation and morphological characterization

During sampling from *Pelargonium grandiflorum* plants (winter of 2020, by Abbas Atashi), in Alborz province, Iran, common occurrence of black spot symptoms was observed on the leaves. Specimens were collected and transferred to the mycology laboratory of Tehran University for further investigations. Samples were washed in tap water to remove the surface contaminations. Then, Small fragments from the area between the healthy and symptomatic tissue were cut and surface sterilized in 70% ethanol for 30 s, and then in 1% sodium hypochlorite for 3 min and finally rinsed in sterile distilled water 3 times (3 min each time). The surface-disinfected tissues were dried completely and cultured on PDA culture medium and incubated at 25°C for 21 days. Grown fungal isolates were purified by single spore method and the recovered fungal isolates were also deposited in the Iranian Research Institute of Plant Protection Culture Collection (IRAN). Obtained isolates were screened on the basis of the morphological (microscopic and macroscopic) features. In order to investigate the morphological features, PDA and PCA media were used. On the other hand, obtained isolates were cultured on 10 mm petri dishes containing PDA and PCA and were incubated in 25°C for 30 days under the fluorescent light with light periods of 8 hours light and 16 hours darkness.

DNA extraction

Purified fungal isolates were grown in Petri dishes containing PDA, and colonies were transferred to flasks containing 50 ml potato dextrose broth (PDB) and grown on an orbital shaker for two weeks at 100 rpm and 25˚C. Mycelium was submerged in liquid nitrogen and ground into a fine powder. Genomic DNA was extracted from the fine mycelial powder as described by Zhong & Steffenson (2001). DNA pellets were dissolved in 30µl of deionized sterile ddH2O and stored at -20°C.

Amplification and phylogenetic analysis

Total genomic DNA was extracted from seven days old fungal mycelium according to the Zhong & Steffenson (2001) method. The ITS–rDNA regions and *rpb2* gene (for *Botrytis*) and part of the gelyceraldehyde–3–phosphate dehydrogenase (*gpdh*) gene (for *Alternarira*) were amplified with the primer pairs of ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′), ITS4 (5′-TCCTCGCTTATTGATATGC-3′) (White et al., 1990), RPB2 for+ (5′-GATGATCGTGATCATT TCGG-3′), RPB2rev+ (5′-CCCATAGCTTGCTTAC CCAT-3′) (Staats et al. 2005) and gpd1 (5′-CA ACGGCTTCGGTCGCATTG-3′) and gpd2 (5′-GCCA AGCAGTTGGTTGTGC-3′) (Berbee et al., 1999) respectively. Conditions for PCR amplification of the ITS–rDNA regions, consisted of an initial denaturation for 4 min at 95 °C followed by 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 56 °C and 60 s extension at 72 °C followed by a final extension step for 6 min at 72 °C. For *gpdh* gene: initial denaturation for 5 min at 94 °C; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min; and then a final extension at 72°C for 10 min. The PCR products were analyzed in 1.5% agarose gel by electrophoresis with 1x Tris-Boric acid-EDTA buffer (TBE) and PCR products were sent to the Cardiogenetic Research Center (IRAN) for sequencing. Sequences were manually edited with Chromas 2.6.6 software (Technelysium, Australia) and the edited sequences were saved in FASTA format. The resulting sequences (479-585 bp for ITS and *gpdh* and 1083 bp for *rpb2*) was subjected to BLAST search (Altschul et al., 1990) to find the most identical sequences in the National Centre of Biological Information (NCBI). Twenty-

nine reference *gpdh* sequences of *Alternaria*, *Chalastospora*, species and *Stemphylium solani* as the out-group taxon were selected for phylogenetic analyses (Table 1). Also, twenty-three ITS–rDNA and *rpb2* reference sequences of *Botrytis*, *Sclerotinia*, *Monilinia* and *Sordaria fimicola* as the out-group taxon were selected for phylogenetic analyses (Table 1). Then, the sequences were aligned with Clustal W (Thompson et al., 1994). Maximum likelihood (ML) analysis (Felsenstein 1973) was performed by heuristic search with Mega X (Kumar et al., 2018). Bootstrap analysis (Felsenstein 1985) of the ML tree was performed with 1000 replicates (Fig. 3 and 4). The newly generated sequences of ITS and *gpdh* in this study were submitted to GenBank (Table 1).

Pathogenicity tests

14 days old isolates of obtained fungi (grown in 25°C and 12-12 photoperiod) were used to investigate the pathogenicity on the leaf of the *Pelargonium grandiflorum* plants. Pathogenicity tests were done based on the method provided by Capdeville et al. (2004). In this method, inoculated plants, which was sprayed with 1×10^5 spore suspensions, were covered with plastic bag, and are kept in greenhouse condition (20–27° C, normal daylight) and started to analyze for symptoms from second to seventh day. The causal agents were re-isolate from symptomatic plants to fulfill the Koch's postulates.

RESULTS

Fungal characterization

Based on the morphological and molecular characterization, *Botrytis cinerea* and *Alternaria alternata* were identified as the causal agents of the brown spot symptoms observed on the leaves. Culture characteristics, such as colony color, conidial morphology (length and width, transverse and longitudinal septa) and sporulation patterns were observed using an Olympus BH2 light microscope (Olympus, Japan) respectively. These species are described as follow:

***Alternaria alternata* (Fr.) Keissl., Beih. Bot. Centralbl., Abt. 2, 29: 434. (1912)**

Under the *in vitro* condition, colony on PCA reached to 62 mm in diameter after seven days incubation at 25 ± 2°C, under the fluorescent light with light periods of 8 hours light and 16 hours dark. Colony was initially white with aerial mycelium, which is turning to olivaceous green to dark green, with an entire smooth edge. Sporulation was abundant mostly from the superficial hyphae. Hyphae were pale brown, septate, branched and 4–6 µm in wide. The conidiophores were branched, smooth, straight, golden brown in colour. measuring up to 65 µm long and 3–4 µm thick. Conidia were borne in long chains (often branched), golden brown to dark brown, ellipsoidal to obpyriform, and 20–45 × 5–11 µm in size, with 2 to 7 transverse septa and 1 to 3 longitudinal septa (n = 50). These characteristics matched well with the description of *Alternaria alternata* (Ellis, 1971; Simmons 1990). Sexual morph was not observed (Fig. 1C and 2D-F)

***Botrytis cinerea* Pers. (1794)**

Under *in vitro* condition, colony on PDA reached to 100 mm in diameter after seven days incubation at 25 ± 2°C, under the fluorescent light with light periods of 12 hours light and 12 hours dark. Colony had the cottony appearance, which is turned to light grey with age. The young hyphae were thin, light brown and 7–15.5 µm wide, which is became brown and septate with age. The conidiophores were branched, smooth, straight, golden brown in colour and swollen at the end, measuring up to 58 µm long and 2–4 µm thick. Light brown, smooth, ellipsoidal or globose conidia, which form as a cluster, measured 5–11× 7–19 µm (n = 50). They often had a slightly protuberant hilum and unicellular. Sclerotia were not observed on the PDA culture medium (Fig. 1A-B and 2A-C).

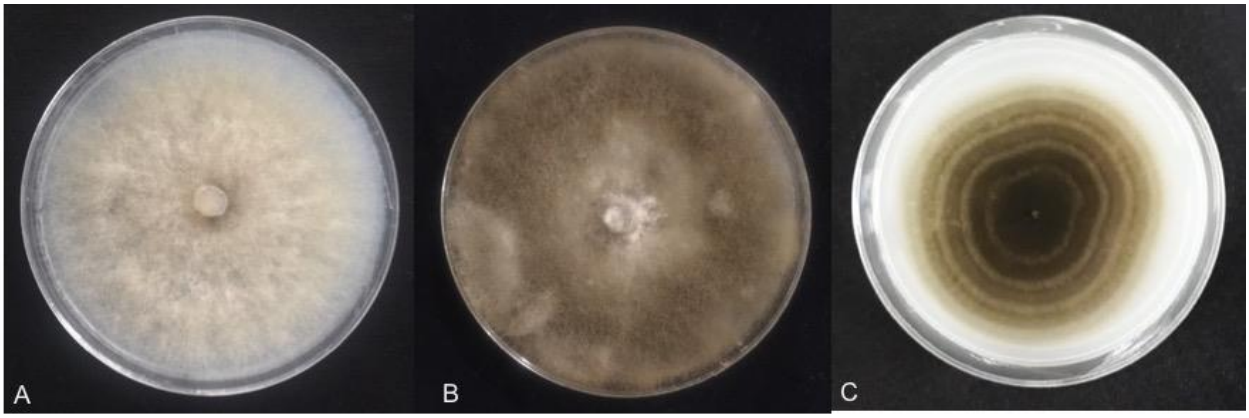

Fig. 1. Surface of A) 7-days old *Botrytis cinerea* isolate UTS1, B) 14-days old *Botrytis cinerea* and C) 14-days old *Alternaria alternata* isolate UTS1 colony on PDA.

Table 1. Used sequences for phylogenetic analyses. Newly generated sequence is in boldface.

Species	Isolate	Accession number			Reference
		gapdh	ITS	*rpb2*	
Alternaria solani	SAs14[1]	MG525472.1	-	-	Ding et al. (2019)[1]
	SAs13[1]	MG525471.1	-	-	Ding et al. (2019)[1]
A. accalyphicola	-[1]	JQ646355.1	-	-	Lawrence et al. (2013)[1]
A. vulgaris	YZU161235[1]	MW579309.1	-	-	He et al. (2021)[1]
	YZU161234[1]	MW579308.1	-	-	He et al. (2021)[1]
A. alstroemeriae	CBS 118809[1]	KP124154.1	-	-	Woudenberg et al. (2015)[1]
A. aspera	CBS 115269[1]	KC584166.1	-	-	Woudenberg et al. (2015)[1]
A. gaisen	CBS 632.93[1]	KC584116.1	-	-	Woudenberg et al. (2015)[1]
A. gossypina	CBS 104.32[1]	JQ646312.1	-	-	Lawrence et al. (2013)[1]
A. longipes	CBS 540.94[1]	AY278811.1	-	-	Pryor & Bigelow (2003)[1]
A. jacinthicola	CBS 133751[1]	KP124287.1	-	-	Woudenberg et al. (2015)[1]
A iridiaustralis	CBS 118486[1]	KP124284.1	-	-	Woudenberg et al. (2015)[1]
Alternaria alternata	**UTS1**[1]	**ON623893**	-	-	**This study**
	-[1]	OL601993	-	-	Yan et al. (2022)[1]
	ZHX2[1]	MT559264.1	-	-	Zhang et al. (2021b)[1]
A. telliensis	NB667[1]	MK904523.1	-	-	Bessadat et al. (2021)[1]
	DA44[1]	MK904522.1	-	-	Bessadat et al. (2021)[1]
A. brassicicola	434/2[1]	KR051389.1	-	-	Blagojević et al. (2020)[1]
	-	AY278813.1	-	-	Pryor et al. (2003)[1]
A. graminicola	NB569[1]	MK904514.1	-	-	Bessadat et al. (2021)[1]
	EGS 41-139[1]	JQ646291.1	-	-	Lawrense et al. (2013)[1]
A. rosae	EGS 41-130[1]	JQ646279.1	-	-	Lawrense et al. (2013)[1]
Chalastospora cetera	CBS 110898[1]	FJ214813.1	-	-	Andersen et al. (2009)[1]
	BMP 0033[1]	AY562398.1	-	-	Hong et al. (2005)[1]
A, malorum	CBS 135.31[1]	JQ646278.1	-	-	Lawrense et al. (2013)[1]
	CBS 540.75[1]	FJ214848.1	-	-	Andersen et al. (2009)[1]
Stemphylium solani	LT3[1]	KC796686.1	-	-	Nasehi et al. (2012)[1]
Botrytis cinerea	UTS1[2] UTS1[3]	-	ON598604	OP225392	This study This study
	HNSMJ4[2] MS05[3]	-	MW820601.1	MZ541963.1	Guo et al. (2021)[2] Zhang et al. (2021)[3]
	HNSMJ-4[2] HA08[3]	-	MW831622	MZ541963.1	Guo et al. (2021)[2] Zhang et al. (2021)[3]
	HNSMJ-4[2] MHT4[3]	-	MW831630	MZ541963.1	-Guo et al. (2021)[2] Zhang et al. (2021)[3]
B. eucalypti	CERC 7160[2] 7208[3]	-	KX301014.1	KX301029.1	-Liu et al. (2016)[2] Liu et al. (2016)[3]
	SA6[2] 7170[3]	-	MF996367.1	KX301028.1	-Sahab (2018)[2] Liu et al. (2016)[3]
B. tulipae	CBS 298.68[2] Bt9601[3]	-	MH859132.1	AM231330.1	-Staats et al. (2007)[2] Staats et al. (2007)[3]
	CBS 130.37[2] Bt9806[3]	-	MH855853.1	AM231328.1	Staats et al. (2007)[2] Staats et al. (2007)[3]

Table 1 (continued)

Species	Isolate	Accession number			Reference
		gapdh	ITS	*rpb2*	
	CBS 130.37[2] Bt9806[3]	-	MH855853.1	AM231328.1	Staats et al. (2007)[2] Staats et al. (2007)[3]
B. elliptica	HGUP196008[2] Be9623[3]	-	MZ541963.1	AM231323.1	-Zhang et al. (2021)[2] Staats et al. (2007)[3]
	HGUP196005[2] Be9732[3]	-	MZ541962.1	AM231321.1	Zhang et al. (2021)[2] Staats et al. (2007)[3]
B. sinoallii	OnionBC-59[2] OnionBC-59[3]	-	FJ169664.2	FJ169678.1	Zhang et al. (2010b)[2] Zhang et al. (2010b)[3]
	OnionBC-23[2] LeekBC-18[3]	-	EU519203.1	FJ169679	-Zhang et al. (2010b)[2] Zhang et al. (2010b)[3]
B. sinoviticola	GBC-3-3c[2] GBC-3-3c[3]	-	JN692384.1	JN692426.1	Zhou et al. (2014)[2] Zhou et al. (2014)[3]
	GBC-5[2] GBC-5[3]	-	JN692386.1	JN692427.1	Zhou et al. (2014)[2] Zhou et al. (2014)[3]
B. californica	X1348[2] X655[3]	-	KJ937041.1	MZ541963.1	Saito et al. (2016)[2] Zhang et al. (2021)[3]
	X503[2] X766[3]	-	KJ937038.1	KJ937050.1	Saito et al. (2016)[2] Saito et al. (2016)[3]
	OnionBC-15[2] OnionBC-18[3]	-	EU093077.2	FJ169674.1	-Zhang et al. (2010b)[2] Zhang et al. (2021)[3]
Monilinia fructigena	MFSRB-3[2] 9201[3]	-	KP279667.1	AJ745715	Vasic et al. (2016)[2] Staats et al. (2005)[3]
Sordaria fimicola	S3_ITS_1[2] ----[3]	-	KX990277.1	AY780194.1	Liu et al. (2016)[2] Miller and Huhndorf (2005)[3]
Botrytis fabiopsis	Broadbeanbcs30[2] EU514473.1[3]	-	EU563126.1	EU514473.1	Zhang et al. (2010a)[2] Zhang et al. (2010a)[3]

1: Isolate name and reference related to *gapdh* gene ;2: Isolate name and reference related to ITS ;3: Isolate name and reference related to *rpb2* gene

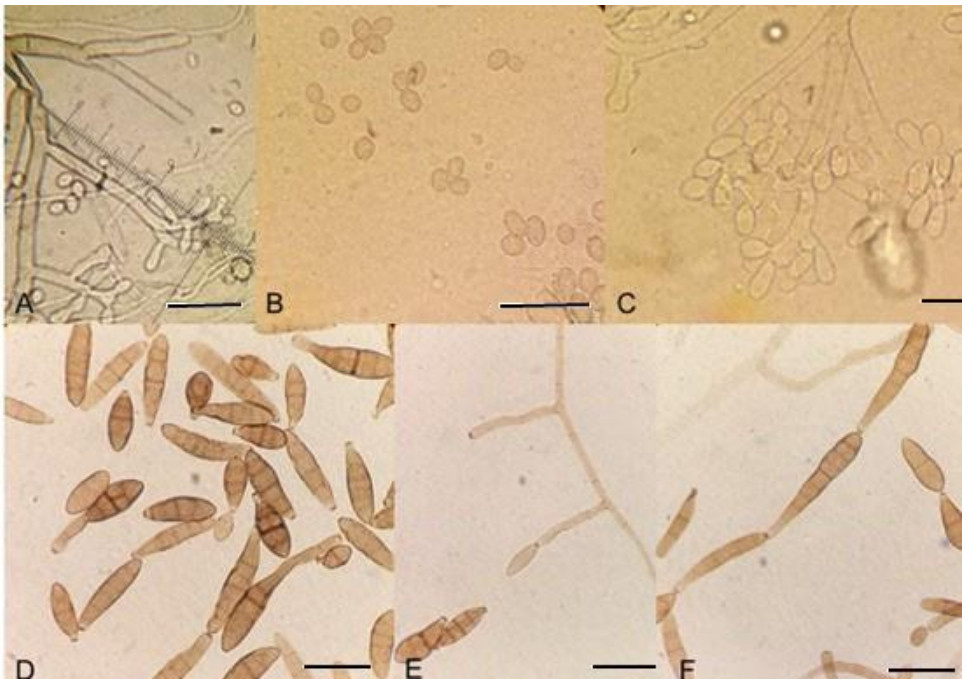

Fig. 2. A) conidiophore, B) conidia and C) conidia on conidiophores of *Botrytis cinerea* isolate UTS1 on PCA and D) Conidia, E) Conidiophores and F) conidial chain of *Alternaria alternata* isolate UTS1 on PDA (scale bare = 20 µm).

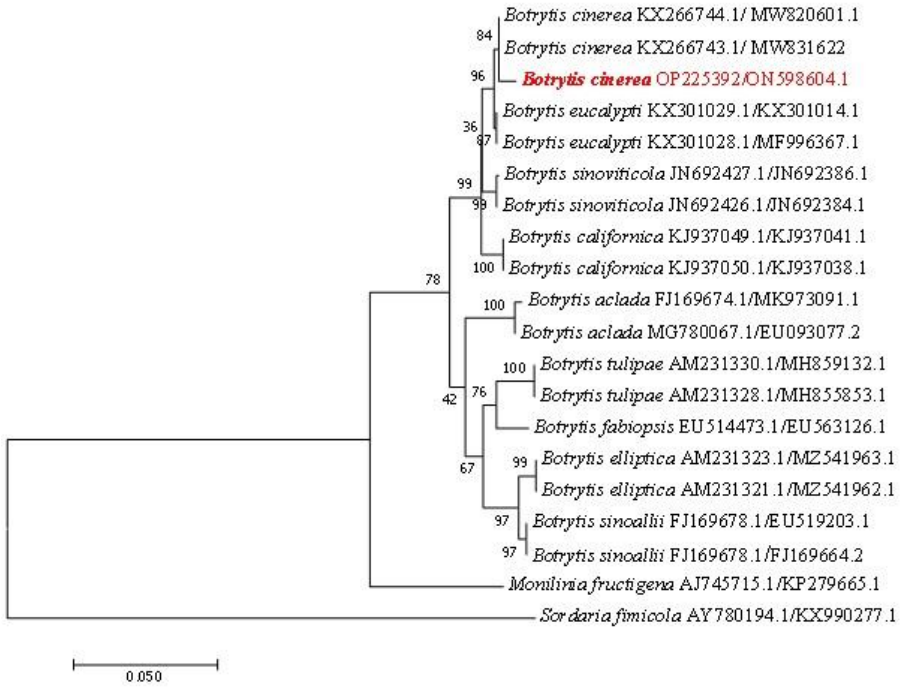

Fig. 3. Maximum likelihood phylogenetic tree based on the ITS and *rpb2* sequences of *Botrytis cinerea* isolate obtained in this study and some other sequences of the *Botrytis* species from the GenBank (NCBI). Numbers at the nodes are the bootstrap values obtained form 1000 replicates.

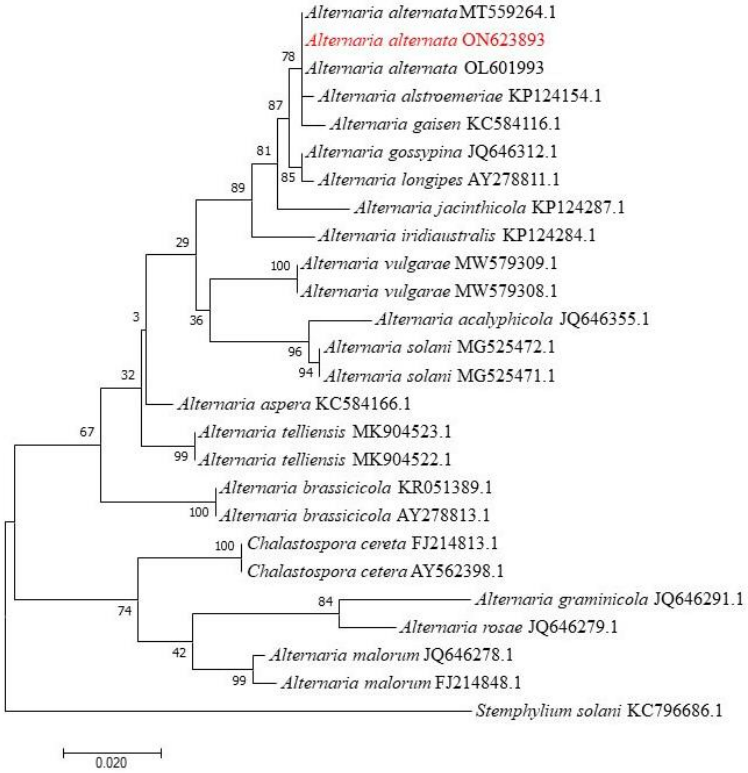

Fig. 4. Maximum likelihood phylogenetic tree based on the *gapdh* sequences of *Alternaria alternata* isolate obtained in this study and some other sequences of the *Alternaria* species from the GenBank (NCBI). Numbers at the nodes are the bootstrap values obtained form 1000 replicates.

The inoculated plants were observed daily (after covering by plastic bags for 2 days) to explore the progress of the disease symptoms. In plant that sprayed with *Botrytis cinerea* suspension, the first symptoms (small light-yellow spots) were observed in second day (immediately after open the plastic bag). The same symptom on inoculated plant with *Alternaria alternata* was observed after 5 days from inoculation. This symptom went darker and more in number daily. Finally, typical and big Brown spots with yellow margin were observed on the inoculated leaves with both *Botrytis* and *Alternaria* isolates obtained in this study after 12 days. Stem and bud rot with fungal bodies on leaves, buds and stems were other symptoms on the plant that was inoculated by *Botrytis cinerea*. These symptoms were stated in fifth day after the inoculation. Symptoms are available in figure 5.

Discussion

Pelargonium grandiflorum is a commercial plant that can be infected by different pathogenes (Bacteria, fungi and viruses) (Loehrlein and Creig 2001). In recent years, some studies (Miclea et al. 2012; Bertetti et al. 2016; Garibaldi et al. 2016; Bertetti et al. 2019) have stated that fungi were the most important pathogens on *Pelargonium grandiflorum*. On the other hand, *Botrytis* and *Alternaria* are two kinds of most destructive plant pathogens with a wide host range that can cause different symptoms in the host plants all around the world.

As leaf spots, which be observed on the leaves of our plant, is attributed to *Alternaria* most of the time and according to some research that mentioned *Botrytis cinerea* as the causal agent of leaf spot, the symptomatic parts were cultured on the PDA medium after surface sterilization in order to find the exact causal agent.

In this study, two different fungi from the brown spots of the leaves of *Pelargonium grandiflorum* were isolated. These two isolates, based on the morphological characterization (macroscopic and microscopic features), were identified as *Alternaria alternata* and *Botrytis cinerea*.

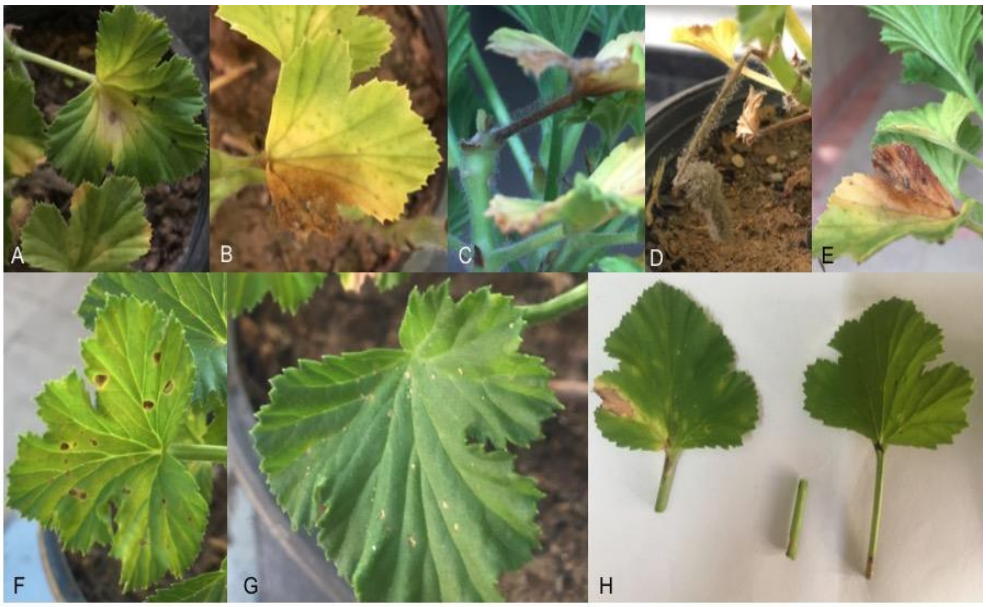

Fig. 5. Symptoms on leaves, stems and buds. A) Leaf spots by *Botrytis cinerea*, B and E) Leaf blight by *Botrytis cinerea* with mycelium, conidia and sclerotia of the causal fungus, C) Stem rot by *Botrytis cinerea* (aerial mycelium are visible on the stem), D) Bud rot by *Botrytis cinerea* (the bud is covered by conidia) and F-H different range of leaf spot by *Alternaria alternata*.

Phylogenetic analysis of the obtained isolates of the *Alternaria* and *Botrytis* based on the *gpdh* (for *Alternaria*), ITS and *rpb2* sequences (for *Botrytis*) along with the same spots in the inoculated plant with *Alternaria* and the same spots plus gray mold symptoms and fungal body (mycelia, conidia and sclerotia) on leaves, buds and stems of inoculated plant with *Botrytis* isolate during the pathogenicity tests (started 5 days after inoculation), confirmed the results of morphological studies. It should be noticed that although our *Alternaria* isolate stay on the side of other *Alternaria alternata* isolates, it place in one group with *A. alsteroemeriae* and *A. gaisen*. So, it is better to use different genes in order to separate these species from each other. The sequences of *Alternaria alternata* (ON623893) and *Botrytis cinerea* (ON598604 and OP225392, based on ITS and *rpb2* data respectively) were submitted in NCBI gene bank.

Based on previous study, there are not much reports from fungal diseases on *Pelargonium grandiflorum* all around the world. Although there are some studies report different diseases (like leaf spot and gray mold) on *Pelargonium grandiflorum* (Loehrlein and Creig 2001, Miclea et al. 2012, Bertetti et al. 2016, Garibaldi et al. 2016, Bertetti et al. 2019) all around the world and based on Ershad 2009 and Nabizadeh et al. 2021, which report horseshoe pelargonium (*Pelargonium zonale* (L.) L'Hér. ex Ait.) as a host of *Botrytis cinerea* in Iran, to our knowledge, it is the first report of these two fungi on this host plant in Iran. Also, before our study, there was no report from *Alternaria alternata* on any kind of pelargoniums in Iran.

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اولین گزارش از *Botrytis cinerea* و *Alternaria alternata* از شمعدانی اژدر در ایران

سپیده فکری‌کهن[۱] ✉ ، عباس آتشی[۲] ، خلیل بِردی فتوحی فر[۲] و بهرام شریف نبی[۱]
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چکیده: شمعدانی اژدر (*Pelargonium grandiflorum*) گیاهی گلدار و چشم‌نواز با توسعهٔ جهانی است. دامنهٔ کاربرد آن بسیار وسیع است: از تزئین اتاق گرفته تا طراحی باغ‌ها و پارک‌ها. مشاهدهٔ علائم لکه‌برگی بر روی این گیاه، که از کرج (استان البرز) جمع‌آوری شده بود، انگیزهٔ یافتن عامل (عوامل) بیماری را در ما ایجاد کرد. به این منظور قسمت‌های دارای علائم جدا و پس از ضدعفونی سطحی، بر روی محیط کشت PDA کشت داده شد. دو قارچ بر روی محیط کشت ظاهر شدند. آنها، با توجه به ویژگی‌های ریخت‌شناختی، به عنوان *Alternaria alternata* و *Botrytis cinerea* شناسایی شدند. بررسی مولکولی با استفاده از *gpdh* برای قارچ *Alternaria* و نواحی ITS و ژن *rpb2* برای قارچ *Botrytis* نتایج حاصل از شناسایی ریخت‌شناختی را تأیید کرد. لکه‌های مشابه بر روی گیاهان تلقیح‌شده با *Alternaria alternata* در کنار لکه‌های مشابه همراه با علائم کپک خاکستری و اندام‌های قارچی بر روی برگ، جوانه و ساقهٔ گیاهان تلقیح‌شده با *Botrytis cinerea* در آزمایشات بیماریزایی، تأیید دیگری بودند. بر اساس اطلاعات ما، این اولین گزارش از این دو گونهٔ قارچی بر روی *Pelargonium grandiflorum* در ایران است.

کلمات کلیدی: بیماری، قارچ، فیلوژنی، بیماریزایی، *Pelargonium*

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