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Control of *Alternaria* rot disease of pear fruits using essential oil of *Viola odorata*

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Abstract

The economic losses of fruits due to the post-harvest diseases exceeded 50% of the total production. Although control of post-harvest pathogens still relies mainly on fungicides, but the emergence of fungicide-resistant strains and environmental problems stimulated the search for ecofriendly alternatives. In this study, three isolates of *Alternaria alternata* were isolated from pear fruits, naturally have symptoms of *Alternaria* rot disease. The pathogenicity test confirmed that *A. alternata* AUMC11410 was the most aggressive isolate causing the highest rotted area on the pear fruits. Herein, four tested essential oils of *Ocimum basilicum*, *Eucalyptus globulus*, *Rosmarinus officinalis* and *Viola odorata* exhibited antifungal impacts against *A. alternata* AUMC11410. *Viola odorata* had the highest fungicidal effect on the mycelia growth of the pathogen producing reduction up to 92.50%. By evaluation of the minimum inhibitory concentration (MIC), *V. odorata* oil showed MIC value at 0.4 µl/ml. Subsequently, application of *V. odorata* oil (0.4 µl/ml) reduced the percentage of *Alternaria* rot disease by 75.0 and 62.5% both before and after the pathogen inoculation. The GC-MS analysis of the *V. odorata* oil revealed that, it was rich in bioactive ingredients such as benzyl benzoate (8.0% of total ingredients), β-ionone (5.04%), α-hexyl cinnamaldehyde (2.93%), 6-methyl γ-ionone (2.29%) and β-linalool (1.16%). Furthermore, it had some monoterpenoids and their derivatives, namely; p-cymene, dihydro-α-terpineol, p-menth-3-en-9-ol, 1,4-cineole, p-menth-6-en-2-one, citronellyl formate, β-citronellol, linalyl acetate and isobornyl acetate that collectively amounted 5.53% of the total ingredients. In conclusion, *V. odorata* oil included bioactive compounds that may be responsible for this fungicidal effect against the pathogen. Therefore, application of *V. odorata* oil may be considered as a promising ecofriendly precautionary measure for controlling the post-harvest diseases of pear fruits.

Key words: *Alternaria alternata*, essential oils, pear fruits, postharvest diseases, *Viola odorata*.

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Introduction

Pear (*Pyrus communis* L; Family: Rosaceae) is the second important fruit crop, after apple, in the temperate regions of the world (Yakovin et al., 2011). In Egypt, the production of pear fruits reached approximately 66403 tons in 2013 (FAOSTAT, 2013). Pear fruits, associated with nutritional value and attractive taste, are high in vitamin C, potassium, iodine, and fibers. Pears are low in calories, stimulate digestion and bowel peristaltic movement, affect blood pressure, and exhibit diuretic, antipyretic, and antitussive activities (Reiland & Slavin, 2015; Konarska, 2013). In the developing countries, the substantial economic losses of fruits due to pests and diseases during storage, as well as in transit and commercialization exceeded 50% of the total production, because of the lack of adequate storage facilities (Eckert & Ogawa, 1985). Post-harvest diseases in pear are mainly caused by *Penicillium expansum* (blue mould), *Botrytis cinerea* (grey mould), *Mucor piriformis* (Mucor rot), *Neofabraea malicorticis* ex *Pezicula malicorticis* (bull's-eye rot) and *Alternaria* spp. (*Alternaria* rot) (Wan & Tian, 2005; Pierson et al., 1971). In a survey conducted by Lennox and Spotts (2003) on the diseases of pear fruits, *Botrytis cinerea* caused 55% of the total decay, while *Penicillium* spp. and *Mucor piriformis* were responsible for 24 and 8% decay, respectively. The remaining 13% was ascribed to other pathogens, including *Alternaria* and *Pezicula* spp. Control of post-harvest pathogens still relies mainly on the use of synthetic fungicides, but the development of fungicide-resistant strains, the public demands to reduce the use of pesticides, as well as the appearance of diseases which are increased in their severity by the use of a specific chemical product (iatrogenic diseases) has stimulated the search for ecofriendly alternative control strategies (Mari et al., 2003). The post-harvest phase is appropriate to the application of biological control methods (Mari & Guizzardi, 1998). In a controlled storage environment, parameters such as temperature, relative humidity and gas composition are stable, and the direct contact between the biological agent and pathogen improves biocontrol (Wilson & Pusey, 1985). Application of antagonists, plant-derived natural substances and genetically changing resistance responses in harvested crops are a quite promising for controlling post-harvest diseases (Janisiewicz & Korsten, 2002). In recent years, numerous studies demonstrated that the essential oils of aromatic plants, due to their biocidal activities, exhibited a significant reduction of the post-harvest diseases of fruit and vegetables (Gilles et al., 2010; Alim et al., 2009). Although, *Alternaria alternata* is less aggressive pathogen (causing *Alternaria* rot), but it's become a serious problem in fruit stored for longer periods (Pierson et al., 1971), as well as *A. alternata* is not sensitive to the most commonly used post-harvest fungicide thiabendazole (Benbow & Sugar, 1999). This study aimed to evaluate the ability of some essential oils for controlling of *Alternaria* rot disease of pear fruits.

Materials and methods

Fungal isolation, purification and

identification: The samples of pear fruits (*Pyrus communis* L.) showing *Alternaria* rot disease were collected from markets at Assiut Governorate, Egypt. The samples were packed into sterilized polyethylene bags and immediately transferred to the Mycological Laboratory. Potato dextrose agar (PDA) medium containing 66.7 mg/L rose bengal and 250 mg/L streptomycin was used for fungal isolation. Fungal pathogen was isolated through surface sterilization technique described by Abo-Elyousr et al. (2014). Under a laminar air flow chamber, infected lesions were detached from fruits, washed by sterilized distilled water, surface sterilized (by immersing in 75% ethanol for 1 min, 1% sodium hypochlorite for 5 min and again in 75% ethanol for 30s) and dried using a sterilized paper towel. Segments were placed on the surface of PDA medium and then Petri plates were incubated at 25°C for 3 days. Fungi emerging out of the plant tissues were purified using single spore isolation technique (Choi et al., 1999). The pathogen was firstly identified according to culture and microscopic characteristics (Ellis, 1971). Simultaneously, the identification was confirmed at Assiut University Mycological Center (AUMC).

Pathogenicity test: Inoculum of the pathogen was prepared by growing it in Petri dishes (9 cm. diameter) containing PDA medium for 7 days at 25°C, then 10 ml of sterilized distilled water were added to each plate and the conidia were carefully scraped with a sterilized needle and the resulting conidial suspension used for inoculation. The concentration of conidia (12 x 10^6 conidia /mL) in the applied propagules was measured using a haemocytometer. Healthy and uniform pear fruits, without physical injuries or disease infection, were sterilized for 3 min. by 96% ethanol, rinsed with sterilized distilled water and drained at room temperature. Two wounds (5 mm diameter and 3 mm deep) were made at opposite equatorial lines of each fruit using the tip of a sterile cork-borer. 50 µl of conidial suspension of *Alternaria alternata*, containing 12 x 10^6 conidia /mL, were placed on each wound. Pear fruits having artificial wounds with 50 µl of sterilized distilled water were used as a control. Three fruits were used for each treatment and then the fruits were air dried and placed in plastic boxes (with wetted sterilized cotton pieces to maintain high humidity). After 7 days, the virulence of the tested isolates was detected by observing the development of rot disease on infected pear fruits (Mohamed & Saad, 2009). The virulence was determined depending on the decay lesion diameter that expressed as the means of the width and length of the decay area on the fruits.

***In vitro*, fungicidal effect of essential oils on pathogen growth:** Four essential oils namely; basil (*Ocimum basilicum* L.), blue Gum (*Eucalyptus globulus* Labill), rosemary (*Rosmarinus officinalis* L.) and sweet viola (*Viola odorata* L.) were purchased from the pharmacy at Assiut Governorate, Egypt. Preliminary, fungicidal activities of the selected essential oils were tested using the dry weight method (Kumar & Prasad, 1992). Potato dextrose (PD) broth medium containing 5 % essential oils was inoculated with *Alternaria alternata* AUMC11410 (disc, 1 cm^2). The control

was kept in parallel to the treatment sets without essential oil. Three flasks as replicates were used for each treatment or control. All flasks were incubated under shaking at an agitation speed of 120 rpm for five days at 25 °C. Thereafter, the fungal pellets were collected by filtration using a pre-weighed filter paper and then the fungal biomass was determined after drying at 80 °C for 24 h. The percentage of fungal growth inhibition was assessed using the following formula:

%Growth inhibition= [(C-T)/C] ×100

Where, C and T were the fungal dry weight at control and treated with essential oil, respectively.

The suppressive activity and the minimum inhibitory concentration (MIC) of the most potent essential oil was determined using the agar dilution method. The essential oil was dissolved in 0.5% Tween 80, sterilized by filtration through a 0.45 µm membrane filter and then serial dilution were prepared (0.5, 0.4, 0.3, 0.25, 0.2, 0.1 µl/ml). One ml of each diluted solution was mixed with 9.0 ml of PDA and was poured into the Petri plate. The plates were inoculated with 5 mm disc of A. alternata. PDA plates containing 0.5% Tween 80 without oil were used as a control. All inoculated plates were incubated at 25°C for 48 h. MIC was determined as the lowest concentration of oil causing the visible inhibition of the pathogen growth.

Chemical analysis (GC-MS) of the essential oil: the most potent essential oil was chemically analyzed using GC-MS at the Analytical Chemistry Unit (ACAL), Faculty of Science, Assiut University. The mass spectrometer was Agilent Model 6890 N/ 5975 B (Agilent Technologies, Palo Alto, CA, USA) with DB 5MS (30 mm, 0.25 mm, 0.25 mm) capillary column. The components of the essential oil were identified by comparing their mass spectra with those of Wiley GC-MS 275 libraries.

***In vivo*, management of *Alternaria* rot by sweet viola oil:** Inoculum of *Alternaria* was prepared by growing it in Petri dishes (9 cm. diameter) containing PDA medium for 7 days at 25°C, then 10 ml of sterilized distilled water were added to each plate and the conidia were carefully scraped with a sterilized needle and the resulting conidial suspension used for inoculation. The mycelial suspension was filtered through a piece of sterile cheese cloth. The concentration of the conidial suspension was adjusted to 12 x 10^6 conidia/mL using a haemocytometer. Pear fruits were surface sterilized with 96% ethanol for 3 min., then washed with sterilized distilled water and air-dried. Two wounds were carried out for each fruit using a sterilized cork-borer (5 mm diameter and 3 mm deep). Twelve pear fruits were subjected to the oil (0.4 µl/ml) and sterilized distilled water (as a control) by using sprayer and left to air-dry for 1 h. Thereafter, wounded fruits were inoculated with a spore suspension of *Alternaria alternata* (50 µl) and left again to air-dry. Application of sweet viola oil was achieved after and before 48 hours of pathogen inoculation on the wounds. All fruits were transferred into a plastic container containing wetted sterilized cotton pieces to maintain a high relative humidity. After 7 days of incubation at 15°C, the disease severity

was measured depending on decay lesion diameter that expressed as the means of the width and length of the decay area on the fruits. On the other hand, there were wounded fruits, inoculated with neither pathogen, nor any treatments, act as a healthy control. The experiments were repeated twice and each treatment was replicated three times.

Statistical analysis: The data were subjected to one-way ANOVA using the SPSS 10.0 (SPSS, Chicago, IL, USA) software program. Mean and standard errors were calculated for three replicate values. Means were compared by the Duncan's multiple tests, and statistical significance was determined at the 5% level.

Results

Isolation, identification and pathogenicity of *Alternaria alternata*: In this study, three isolates of *Alternaria alternata* were isolated from pear fruits showing natural symptoms of *Alternaria* rot disease (Fig. 1). These isolates were identified as *Alternaria alternata* depending on culture morphology and microscopic characteristics (Fig. 2). In details, the colony was an effuse, olivaceous black to brownish olive with black reverse and approximately 8 cm in diameter at 25 ± 2 °C on PDA medium after 7 days of incubation (Fig. 2A, B). The mycelium initially was hyaline that became to brownish, septate and abundant branched. Conidiophores were mild brown, straight or curved, geniculate, having terminal scars indicating the point of attachment of conidia. The conidiophores measured 27 - 35 μm in length and 3 - 4 μm in width. Conidia were born in chains on conidiophores. They were light olivaceous to dark brown in colour, varied in shape from obclavate to mostly ellipsoidal, having tapered apex with 1 to 3 longitudinal and 2-5 transverse septa. The muriform conidia including a beak measured 15-45 x 7-13 mm (Fig. 2C). Consecutively, the identification was confirmed at Assiut University Mycological Center (AUMC) and these isolates were deposited in the center obtaining accession numbers AUMC11410, AUMC11411 and AUMC11412. The pathogenicity test confirmed that the three isolates of *Alternaria alternata* have the ability to infect and colonize the fruits producing the typical symptoms of the disease such as dark brown to black, firm spots or decaying areas and the rot extending into the core of the fruits (Fig. 3). These isolates exhibited significant differences in their virulence, whereas *A. alternata* AUMC11410 was the most aggressive isolate causing the highest rotted area comprising 28.33 mm. On the other hand, *A. alternata* AUMC11411 and AUMC11412 caused molded areas contributing 23.5 and 15.0 mm (Fig. 4).

***In vitro*, fungicidal activity of the tested essential oil:** Preliminary application of 10% essential oil against *Alternaria alternata* AUMC11410 showed that there was a significant difference in their fungicidal effect against *A. alternata*. Among the tested essential oils, *Viola odorata* had the highest antifungal effect producing growth reduction up to 92.50%. Contrarily, essential oils of *Rosmarinus*

officinalis and *Ocimum basilicum* exhibited a moderate fungicidal effect, causing 72.33 and 63.67%, respectively. On the other hand, the essential oil of *Eucalyptus globulus* caused growth reduction contributing 86.67% (Table 1). In this study, MIC for *Viola odorata* against *A. alternata* was determined using serial dilution of 0.5, 0.4, 0.3, 0.25, 0.2, 0.1 µl/ml. The MIC value was 0.4 µl/ml that was selected for further application *in vivo*.

Figure 1: Naturally *Alternaria* rot disease on pear fruits collected from Assiut Governorate, Egypt.

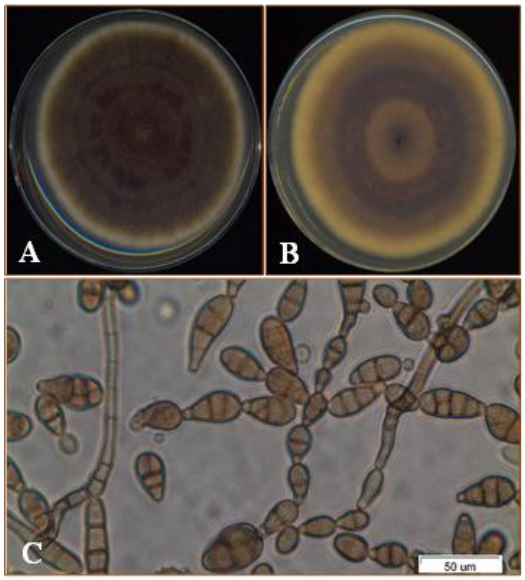

Figure 2: *Alternaria alternata* AUMC11410, the pathogen of *Alternaria* rot disease on pear fruits. **(A)** 8 days old culture at 25°C on PDA medium; **(B)** Culture reverse; **(C)** Microscopic features showing conidia and conidiophores.

Figure 3: Pathogenicity test of three isolates of *Alternaria alternata* showing the ability of these isolates to produce the symptoms of *Alternaria* rot disease. **(A)** *A. alternata* AUMC11412; **(B)** *A. alternata* AUMC11411; **(C)** *A. alternata* AUMC11410

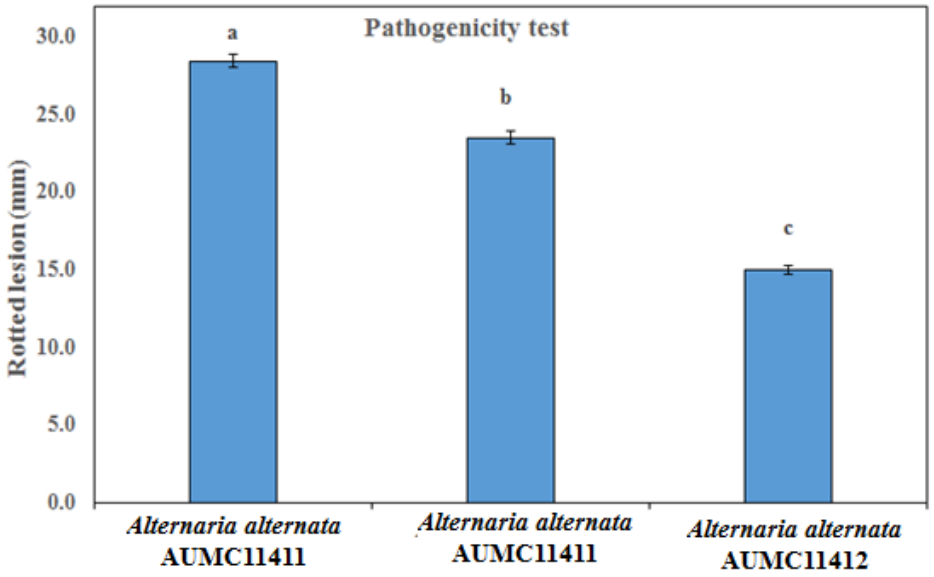

Figure 4: Pathogenicity test of three isolates of *Alternaria alternata* showing the significant differences.

GC-MS analysis of the essential oil: In the present study, the chemical analysis of *Viola odorata* oil revealed that 20 components were identified representing 74.62% of the total ingredients. The component 2-(2-methoxypropoxy) propan-1-ol represented the major compound in the oil formalizing 46.88% of the total ingredients. While, benzyl benzoate was ranked as a second compound comprising 8.00% of the total ingredients. Also, the essential oil of *Viola odorata* included β-ionone, 3-(3-hydroxybutan-2-yloxy) butan-2-ol, α-hexyl cinnamaldehyde, 6-Methyl γ-ionone, β-linalool at the rate of 5.04%, 3.60%, 2.93%, 2.29% and 1.16% of the total ingredients, respectively (Table 2). On the other hand, some ingredients such as p-Cymene, Dihydro-α-terpineol, p-Menth-3-en-9-ol, 1,4-cineole, p-Menth-6-en-2-one, Citronellyl formate, O-Trifluoroacetyl dihydrocinnamyl alcohol, β-Citronellol, Linalyl acetate and Isobornyl acetate were less than 1.00% of the total ingredients (Table 2).

Table 1: *In vitro*, evaluation of antifungal activity of 5% essential oil of aromatic plants against the mycelial growth of *Alternaria alternata*.

Number of tested oils	Scientific name	Common name	Family	% of growth reduction
1	*Eucalyptus globulus* Labill	Blue Gum	Myrtaceae	86.67 ± 0.5^{b}
2	*Ocimum basilicum* L.	Basil	Lamiaceae	63.67 ± 0.3^{d}
3	*Rosmarinus officinalis* L.	Rosemary	Lamiaceae	72.33 ± 0.5^{c}
4	*Viola odorata* L.	Sweet viola	Violaceae	92.50 ± 0.2^{a}

Numbers within columns are means of three replicates. Values within the same column that are associated with different letters indicate significant differences ($P \leq 0.05$) based on one-way ANOVA.

Table 2: The chemical analysis of the essential oil of *Viola odorata* using Gas chromatography mass spectrometry (GC/MS).

	Ingredients	Value (%)	Retention time (min.)
1	2-(2-methoxypropoxy)propan-1-ol	46.88	15.220
2	3-(3-hydroxybutan-2-yloxy)butan-2-ol	3.60	15.432
3	β-Citronellol	0.29	16.749
4	Linalyl acetate	0.22	17.016
6	Citronellyl formate	0.46	17.356
9	Isobornyl acetate	0.20	17.665
10	p-Menth-6-en-2-one	0.49	18.376
11	1,4-cineole	0.61	18.445
12	p-Cymene	0.76	19.367
13	6-Methyl gamma ionone	2.29	21.225
14	β-ionone	5.04	21.331
15	β-linalool	1.16	21.925
16	Dihydro-alpha-terpineol	0.69	22.437
17	p-Menth-3-en-9-ol	0.65	23.512
18	α-hexyl cinnamaldehyde	2.93	25.86
19	Benzyl benzoate	8.00	26.316
20	O-Trifluoroacetyl dihydrocinnamyl alcohol	0.35	27.779
	Total ingredients	74.62	

Application of the essential oil and evaluation of disease reduction: *In vivo*, *Viola odorata* oil (0.4 µl/ml) was applied to reduce *Alternaria* rot disease of pear fruits. By applying the oil to pear fruits before the pathogen inoculation, the disease was reduced by 75.0 %. In details, the rotted lesion decreased from 32 mm (infected control) to 8 mm (treated fruits). On the other hand, the disease was reduced by 62.5% by applying the oil after the pathogen inoculation, whereas the molded lesions were decreased to 12 mm (Table 3).

Discussion

Pears are highly perishable products, especially during the storage, when considerable losses can occur. In long-term storage, secondary spread of decay of infected fruit to adjacent, previously uninfected fruit poses a significant decay risk (Lennox & Spotts, 2003). Rotting and decaying of the pear fruits, especially during the post-harvest phase, are the most serious problem associated with tremendous losses in commercial pear industries (Benbow & Sugar, 1999). The post-harvest losses, mainly are attributed to pathogenic fungi, especially those cause the following diseases; gray mold (*Botrytis cinerea*), Mucor rot (*Mucor piriformis*), blue mold (*Penicillium expansum*), bull's-eye rot (*Pezicula* spp.), *Alternaria* rot (*Alternaria alternata*) and side rot (*Phialophora malorum*) (Tian et al., 2006).

Table 3: Reduction of *Alternaria* rot disease in pear fruits by application of *Viola odorata* oil before and after 48 hours of *Alternaria alternata* inoculation.

Treatments	Treatment before pathogen inoculation		Treatment after pathogen inoculation	
	Lesion diameter (mm)	Disease reduction (%)	Lesion diameter (mm)	Disease reduction (%)
Infected control	32 [a]	-	32 [a]	-
Viola odorata oil	8 [b]	75	12 [b]	62.5

Numbers within columns are means of three replicates. Values within columns that associated with different letters indicate significant differences (P≤0.05) based on One-way ANOVA analysis.

In the current study, three isolates of *Alternaria alternata* were isolated from pear fruits, naturally have symptoms of *Alternaria* rot disease. Thereafter, these isolates were identified as *Alternaria alternata* obtaining accession numbers AUMC11410, AUMC11411 and AUMC11412.The pathogenicity test confirmed that *A. alternata* AUMC11410 was the most aggressive isolate causing the highest rotted area comprising 28.33 mm. In this respect, many studies reported that *Alternaria alternata* could be regarded as the most frequent *Alternaria* species resembled 96% responsible for *Alternaria* rot in many fruits (Mohsan et al., 2011; Peever et al., 2005). On the other side, Pierson et al. (1971) reported that *Alternaria alternata* is less aggressive pathogen, but it causes a critical problem in pear fruits stored for longer periods. Additionally, many studies confirmed that, *A. alternata* has the ability to produce mycotoxins in fruit tissues, particularly patulin (Laidou et al. 2001) and AK-Toxin I (Shimizu et al., 2006) that may play a role in pathogenicity. These mycotoxins are embryotoxic, hepatoxic, nephrotoxic (Scott, 2001) and possibly carcinogenic (Kiessling, 1986). Interestingly, Benbow and Sugar (1999) proved that *Alternaria alternata*, the pathogen of *Alternaria* rot disease of pear fruits, is not sensitive to the most commonly used post-harvest fungicide thiabendazole. Therefore, the search for ecofriendly alternative control methods is an exigent requirement. During the last years, numerous investigations confirmed that essential oils are a rich source of biologically active compounds that possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties (Kordali et al., 2005). The biocidal effect of the essential oils is a key for applying them in food preservation (Ait-Ouazzou et al., 2011), crop protection, plant disease management (Sivakumar & Bautista-Banos, 2014), pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin & Deans, 1997). Herein, the four tested essential oils (*Ocimum basilicum*, *Eucalyptus globulus*, *Rosmarinus officinalis* and *Viola odorata*) exhibited fungicidal impacts against *A. alternata*. *Viola odorata* had the highest antifungal effect on the mycelia growth of the pathogen, especially at MIC value of 0.4 µl/ml. Connectedly, Hammami et al. (2011) reported that the essential oil of *Viola odorata* showed a strong antifungal activity against *Botrytis cinerea*, the pathogen of gray mold disease of tomato. In this matter, recent investigations proved that the essential oil of *V. odorata* had a significant antioxidant,

antimicrobial, insecticidal and anti-inflammatory impacts (Sheikh, 2014; Akhbari et al., 2012; Koochek et al., 2003). Subsequently, the results of this study indicated that, the application of *Viola odorata* oil (0.4 µl/ml) reduced the percentage of *Alternaria* rot disease by 75.0 and 62.5% both before and after the pathogen inoculation, respectively. Therefore, the application of *V. odorata* oil at the beginning of the storage period of the fruits is considered as a promising precautionary measure for controlling the postharvest disease. It is worth mentioning that, the significant reduction of *Alternaria* rot disease caused by using of *V. odorata* oil before pathogen inoculation may attributed to the wound-invading necrotrophic fungi such as *A. alternata* require nutrients for germination and initiation of the pathogenic process. Therefore, application of the potent bioactive compounds such as *V. odorata* oil prevents the pathogen to establish and complete the infection and colonization processes. The results of this study confirmed that, the application of essential oils as a protective procedure has been frequently encountered. Similarly, Hammami et al. (2011) found out that by the application of *V. odorata* oil to tomatoes before seven days of *Botrytis cinerea* inoculation in storage conditions, the decay of fruits caused by the pathogen could be prevented completely. With regarding to this, Benbow and Sugar (1999) reported that among the mechanisms used by bio-agents to management necrotrophic pathogen, they are able to colonize wound sites and produced secondary metabolites that reduce pathogen establishment. The GC-MS study of the *Viola odorata* essential oil revealed that it was rich in bioactive components such as benzyl benzoate (8.0% of total ingredients), β-ionone (5.04%), α-hexyl cinnamaldehyde (2.93%), 6-methyl γ-ionone (2.29%) and β-linalool (1.16%). Furthermore, it had some monoterpenoids and their derivatives, namely; p-cymene, dihydro-α-terpineol, p-menth-3-en-9-ol, 1,4-cineole, p-menth-6-en-2-one, citronellyl formate, β-citronellol, linalyl acetate and isobornyl acetate that collectively amounted 5.53% of the total ingredients. Correspondingly, other investigations found out that the antifungal and antibacterial activities of *V. odorata* oil were mainly attributed to the occurrence of high proportions of bioactive compounds, particularly monoterpenes and sesquiterpenes (Kedia et al., 2014; Akhbari et al., 2012; Hammami et al., 2011). Interestingly, numerous studies reported that β-ionone and α-ionone have operative biocidal effects and these components were the major ingredients in the volatile substances of the essential oils of the genus *Viola* (Chandra et al., 2015), particularly *V. odorata* (Svangard et al., 2003; Cu et al., 1992), *V. tricolor* (Svangard et al., 2004) and *V. arvensis* (Anca et al., 2009). Moreover, previous investigations have indicated that the essential oils, such as cassia oil, showing high levels of benzyl benzoate exhibited high biocidal impacts (Pawar & Thaker, 2006; Jantan et al., 2005). In this connection, cinnamaldehyde was the main component in cinnamon oil, which showed the strongest antifungal activity (Choi et al., 2016; Shan et al., 2007). In agreement with this study, Linalool and terpinen-4-ol were found to be responsible for the antifungal activity of

some essential oil of medicinal plants (Pawar & Thaker, 2006; Cakir et al., 2004). On the other hand, the following components were isolated from the essential oils of higher plants and documented to play an important role as effective antifungal agents; β-citronellol (Shafei et al., 2011), cineole, p-cymene (Van Vuuren & Viljoen, 2007). This study confirmed that the essential oil of *Viola odorata*, due to its fungicidal effect, may be considered as an environmental friendly alternative to toxic chemical fungicides for controlling the post-harvest diseases. Moreover, *V. odorata* oil is rich in bioactive ingredients e.g. monoterpenoids, benzyl benzoate, β-ionone, cinnamaldehyde, γ-ionone and β-linalool which may be responsible for the fungicidal impact. This study recommends using the essential oil of *V. odorata*, as a green approach in the food safety and industry, to manage post-harvest diseases of pear fruits.

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