Quality enhancement of fruits conserved with essential oils prolonging storage

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Description of the subject. Raspberries and strawberries are highly perishable due to postharvest fungal development. Numerous studies have shown that essential oils can be promising biopesticides for maintaining the quality of fresh fruit during postharvest storage.

Objectives. This work aimed to evaluate the potential of *Ismelia carinata* and *Cladanthus arabicus* (Asteraceae) essential oils (EOs) to extend the shelf life of raspberries and strawberries.

Method. The chemical composition of EOs from *I. carinata* and *C. arabicus* was analyzed using GC/MS. *In vitro* antifungal effects of EOs using agar dilution and disk diffusion methods were tested against *Botrytis cinerea*. The *I. carinata* and *C. arabicus* EOs were used as bioactive agents to preserve the quality of berries during 15 days in cold storage. Also, pH, titratable acidity, total soluble solids, and maturity index of berries treated with 400 mg·l^{-1} of *I. carinata* EO are analyzed.

Results. The GC/MS analysis revealed that the major compounds were tau-cadinol (65.93%) and tau-muurolol (24.65%) for *I. carinata*, β-pinene (23.58%), and tau-cadinol (9.54%) for *C. arabicus*. Both EOs tested showed best inhibition percentages against *B. cinerea* using the agar dilution method. *Ismelia carinata* EO was an efficient preservative agent for raspberries and strawberries during storage at 4 °C. The maturity index of both berries was significant compared to untreated controls ($p \leq 0.05$).

Conclusions. *Ismelia carinata* EO holds promise for extending fruit quality in cold storage, with subsequent benefit to fruit marketing supply chains.

Keywords. Keeping quality, postharvest decay, antifungal properties, food preservation, *Botrytis cinerea*.

Amélioration de la qualité des fruits conservés avec des huiles essentielles prolongeant le stockage

Description du sujet. Les framboises et les fraises sont très périssables à cause du développement fongique après récolte. De nombreuses études ont démontré que les huiles essentielles peuvent être des biopesticides prometteurs pour maintenir la qualité des fruits frais pendant le stockage post-récolte.

Objectifs. Ce travail visait à évaluer le potentiel des huiles essentielles (HEs) d'*Ismelia carinata* et de *Cladanthus arabicus* (Asteraceae) à prolonger la durée de conservation des framboises et des fraises.

Méthode. La composition chimique des huiles essentielles d'*I. carinata* et de *C. arabicus* a été analysée par CG/SM. Les effets antifongiques *in vitro* des HEs ont été testés contre *Botrytis cinerea* par les méthodes de dilution en gélose et de diffusion

sur disque. Les HEs d'*I. carinata* et de *C. arabicus* ont été utilisées comme agents bioactifs pour préserver la qualité des baies pendant 15 jours en chambre froide. Le pH, l'acidité titrable, les solides solubles totaux et l'indice de maturité des baies traitées avec 400 mg·l^{-1} d'HE d'*I. carinata* ont également été analysés.

Résultats. L'analyse par CG/SM a révélé que les principaux composés d'*I. carinata* étaient le tau-cadinol (65,93 %) et le tau-muurolol (24,65 %) et ceux de *C. arabicus* étaient le β-pinène (23,58 %) et le tau-cadinol (9,54 %). Les deux HE testées ont montré les meilleurs pourcentages d'inhibition contre *B. cinerea* en utilisant la méthode de dilution en gélose. L'HE d'*I. carinata* était un agent de conservation efficace pour les framboises et les fraises pendant le stockage à 4 °C. L'indice de maturité des deux baies était significatif par rapport aux témoins non traités ($p \leq 0,05$).

Conclusions. L'HE d'*I. carinata* est prometteuse pour l'amélioration de la qualité des fruits en chambre froide, avec un bénéfice ultérieur pour les chaines d'approvisionnement en fruits.

Mots-clés. Aptitude à la conservation, maladie postrécolte, propriété antifongique, préservation des aliments, *Botrytis cinerea*.

1. INTRODUCTION

It is estimated that approximately one-third of horticultural products are lost after harvest reaching 1.3 billion tons of food worldwide, costing the global economy around 750 billion US dollars (Gastavsson et al., 2011; Prusky, 2011). Strawberries and raspberries are among the most rapidly perishable fruits after harvest because of their susceptibility to mechanical injury, physiological deterioration, water loss, and postharvest decay. A postharvest attack is the factor causing the most drastic economic losses, especially in the fruit marketing chain (Prusky, 2011). *Botrytis cinerea*, the cause of gray mold, is one of the most common postharvest decays of fresh fruit and vegetables (Romanazzi & Feliziani, 2014). Based on scientific and economic importance, this fungus ranked second into the world Top 10 fungal plant pathogens list (Dean et al., 2012). Research has shown that *B. cinerea* is one of the most prevalent fungi on strawberries and raspberries, reducing their shelf life after harvest (Tournas & Katsoudas, 2005; Farzaneh et al., 2015). Therefore, handling these berries from harvest to consumer represents a real challenge.

To increase the marketability of fresh berries, the use of postharvest technologies of cold storage and modified atmosphere packaging results in maintaining the postharvest quality of raspberry and strawberry fruit for 10-12 days. However, the application of an efficient treatment significantly reducing the postharvest incidence can imply a considerable change in fruit sweetness and palatability (Agar & Streif, 1997; Zheng et al., 2007).

Essential oils (EOs) are plant extracts composed of aromatic and lipophilic volatile compounds that plants synthesize as secondary metabolites to protect themselves from abiotic and biotic stress (Bakkali et al., 2008). Research has focused on their use as potential biopesticides to sustain fresh fruit quality during postharvest periods. Many EO treatments showed promising results in controlling gray mold of table grapes (Abdollahi et al., 2012) and apples (Lopez- Reyes et al., 2010), and their effectiveness

against several other postharvest decays (Suradeep & Proshanta, 2018). Also, consumers accept EOs more readily because aromatic plants have wide use in general culinary practices (Sivakumar & Bautista-Baños, 2014; Sarkhosh et al., 2017).

Cladanthus arabicus Cass. and *Ismelia carinata* Schousb. are Anthemideae species from the Compositae family (Benabid, 2000). *Cladanthus arabicus* is an annual plant with many erected stems and yellow flowers, growing in southern Spain and North Africa (Tutin et al., 1964). Previous studies have investigated the chemical composition of *C. arabicus* EO and some of its biological activities (El Hanbali et al., 2005; Aghraz et al., 2017). *Ismelia carinata* is a spring-flowering plant native to Morocco (El Oualidi et al., 2012). This bushy plant has fleshy-light green and lacinate leaves and its flowers are characterized by variable colors that form different concentric rings. *Ismelia carinata* is also known as *I. versicolor* Cass. or *Chrysanthemum carinatum* Schousb. and it represents the only species of the *Ismelia* genus (Turland, 2004). These species of plants are naturalized outside their native geographical sites (Buist, 1846; Soreng & Cope, 1991).

The purpose of this study is to examine the chemical composition of EOs of two Anthemideae species and to evaluate their *in vitro* antifungal effect against *Botrytis cinerea* using two different screening assays. Also, the EOs will be tested for their ability to preserve fresh raspberries and strawberries and assessed in terms of consequences on fruit sweetness after storage.

2. MATERIALS AND METHODS

2.1. Plant materials and essential oils extraction

Aerial parts of two plants were collected between March and May 2017, from wild populations in different regions of Morocco: *Ismelia carinata* in Tiznit, Tifnit (geographic coordinates 30°12'0"N, 9°37'48"W) and *Cladanthus arabicus* in the Agadir region (geographic coordinates 30°25'12"N, 9°35'53"W). Plants were identified at the Scientific Institute of Rabat (Morocco).

A voucher specimen of each plant was deposited (*C. arabicus* No. CA-17 and *I. carinata* No. IC-17) in the herbarium of the Laboratory of Biotechnology at the National School of Applied Sciences, Ibn Zohr University, Agadir, Morocco. The plant materials were air-dried at shade and ambient temperature (25 °C) for about 4 weeks. Essential oils were obtained by hydrodistillation of the air-dried plants for 3 h using a Clevenger type apparatus as recommended by European Pharmacopoeia (Council of Europe, 1997). Essential oils were dried over anhydrous sodium and kept in the dark at 4 °C until use.

2.2. Gas chromatography-mass spectrometry analysis

The chemical composition of essential oils was investigated by gas chromatography/mass spectrometry (GC/MS) using a Shimadzu system. A VB5 capillary column was used (30 m x 0.25 mm inner diameter, 0.25 μm film thickness), and Helium was the carrier gas at 1 ml·min^{-1}. The GC oven temperature was conserved at 50 °C for 1 min and programmed to 280 °C at a rate of 10 °C·min^{-1}, then kept constant at 280 °C for 2 min. The split flow was used; the injector temperature was 250 °C. The mass spectrometer was operating at ionization energy of 70 eV and 300 °C. The mass spectrometer range was from 20 to 350 m/z, and the interface was at 300 °C. A library search was composed using the "Wiley GC/MS Library" Nist147 database LIB (Adams, 2007).

2.3. Fungal culture of *Botrytis cinerea*

Botrytis cinerea was isolated directly from naturally decayed strawberries in three replicates using potato dextrose agar medium (PDA), amended with gentamycin (10 mg·l^{-1}) to stop the growth of bacteria. Plates were incubated in the dark for five days at 25 ± 2 °C. The isolate collected was repeatedly purified before its identification. A pure culture of *B. cinerea* was maintained on PDA and stored at 4 °C.

2.4. *In vitro* antifungal assays

The agar dilution (Chebli et al., 2003) and disk diffusion (Znini et al., 2013) methods were adopted to evaluate the *in vitro* antifungal effect of *I. carinata* and *C. arabicus* EOs against mycelial growth of *B. cinerea*. Six concentrations of *I. carinata* and *C. arabicus* EOs, 0.125, 0.25, 0.5, 1, 2, and 4 μl·ml^{-1} were tested in both antifungal assays.

In the agar dilution method, a stock solution of EOs was prepared by dissolving pure EOs in a sterile solution of Tween 80 (2 ml·l^{-1}). To obtain the desired concentrations, appropriate amounts of a stock solution were mixed with autoclaved potato dextrose agar medium (PDA) maintained at 45 °C just before pouring into Petri dishes. A mycelial plug, 6 mm in diameter, was excised from a pure culture of *B. cinerea* and placed at the center of each Petri dish. Controls consisted of un-amended PDA. The plates were sealed with parafilm and incubated at 25 °C.

In the disk diffusion assay, Petri dishes were filled with sterile PDA medium and then inoculated with mycelial plugs, cut from a pure culture of the fungus. After solidification, Petri dishes were inverted, and according to each concentration, pure EOs were deposited in sterile filter paper disks, which then were placed on the inner surface of Petri dish lids. The plates were sealed with parafilm and incubated upside-down at 25 °C. The control consisted of sterile distilled water-soaked filter paper.

Tests were stopped when control Petri dishes were covered by mycelial culture. Three replicates were used for each concentration. The percentage inhibition of fungal growth was determined on the growth in the test plate compared to that of the control plate according to the equation:

$$\text{Inhibition}\% = \frac{(C-T)}{C} \times 100$$

where C was the diameter of fungal growth on the control, and T was the diameter on the test plate.

2.5. Evaluation of EOs on the shelf life of raspberries and strawberries

The experiment was performed using Vu et al. (2011) method with slight modifications. Two concentrations (200 and 400 mg·l^{-1}) of the EOs from *I. carinata* (IC200; IC400) and *C. arabicus* (CA200; CA400) were prepared as aqueous emulsions using a sterile solution of Tween 80 (4 ml·l^{-1}) and distilled water under vigorous stirring. Fresh raspberry and strawberry fruits were harvested from orchards using standard culture practices in the Agadir region. The berries were commercially mature and showed no spoilage signs.

The EO emulsions were sprayed onto fresh fruits at room temperature. The amounts sprayed were equal between treatments. After spraying, fresh fruits were allowed to dry for 15 min, and then were stored at 4 °C. Visual decay of raspberries and strawberries was evaluated for 15 days, and the contamination levels (percentage of berries showing decay signs) were observed daily during storage. Treatments and controls were performed in three replicates. To sum up, the experimental design was as follows: two concentrations of each essential oil (four modalities) are tested separately on strawberries and raspberries (two types of fruits) and three replicates of each

concentration were performed. This makes that: [(4 modalities x 3 replications) x 2 (types of fruits)] + 3 replicates of the strawberry control + 3 replicates of the raspberry control = 30 trays. Each replicate involved ten raspberries and six strawberries tested separately.

2.6. Physical and chemical qualities of berries treated with 400 mg·l⁻¹ of *I. carinata* essential oil

After 15 days of cold storage, each replicate of berries treated with 400 mg·l⁻¹ of *I. carinata* EO was subjected to physical and chemical quality analyses. The physical and chemical measurements were realized using the method of Gunness et al. (2009). In equal weights, deionized water and berries were mixed for 30 s and the pH of the puree was measured with a calibrated pH meter. The puree was centrifuged at 4,400 rpm for 30 min, and the supernatant was refined in glass fiber. A digital refractometer was used to gauge the refractive index of berries clear juice, and the reading was taken in Brix degrees (°Bx) that give the total percentage of solids content (TSS) in fruit. Titratable acidity (TA) was measured by diluting 5 g of clear juice in 100 ml distilled water and then titrating to pH 8.1 with a solution of NaOH (4 g of NaOH·l⁻¹ of distilled water). Citric acid is the main organic acid found in raspberry and strawberry fruit (Spanos, 1986; Watson et al., 2002); TA was expressed in g·l⁻¹ of citric acid equivalent in juice and was calculated using the formula:

$$\frac{\text{Volume NaOH (ml)} \times 0.1 \text{ M} \times 0.064 \times 100}{5 \text{ g juice}}$$

Also, the maturity index was calculated (TSS/TA). The physical and chemical qualities of controls were taken after 12 days of storage of untreated berries.

2.7. Statistical analysis

All data were subjected to analysis of variance (ANOVA) using SPSS version 16.0 software. Means comparison was evaluated using Newman Keuls tests. The results were expressed as mean values ± standard deviation with different letters, which are statistically significant at 5% level probability.

3. RESULTS

3.1. Chemical composition of essential oils

The average yield of *I. carinata* and *C. arabicus* EOs were 0.1 and 0.44% (w/w), respectively. Qualitative and quantitative analyses of the chemical composition of tested EOs are presented in **table 1**.

Ismelia carinata EO was dominated by the oxygenated sesquiterpenes, representing 90.58% of the total oil, which were tau-cadinol (65.93%) and tau-muurolol (24.65%). In *C. arabicus* EO, nine

Table 1. Chemical composition of essential oils from *Ismelia carinata* Schousb. and *Cladanthus arabicus* Cass. (Asteraceae) — *Composition chimique des huiles essentielles d'*Ismelia carinata *Schousb. et de* Cladanthus arabicus *Cass. (Asteraceae).*

Components	Retention index	*I. carinata*	*C. arabicus*
α-pinene	928	-	5.68 *
Sabinene	966	-	3.88
β-pinene	972	-	23.58
β-caryophyllene	1412	-	4.88
β-bisabolene	1502	-	2.42
Nerolidol	1557	-	2.90
Diethyl phthalate	1585	-	7.88
tau-cadinol	1634	65.93	9.54
tau-muurolol	1641	24.65	-
Chamazulene	1726	-	3.13
Total identified %		90.58	63.89
Monoterpenes hydrocarbons		-	33.14
Oxygenated monoterpenes		-	-
Sesquiterpenes hydrocarbons		-	7.3
Oxygenated sesquiterpenes		90.58	9.54

-: not detected — *non détecté*; *: peak area percent — *pourcentage de l'aire de pic*.

constituents were identified accounting for 63.89% of the total oil. It was dominated by hydrocarbon fraction (40.44%) followed by oxygenated sesquiterpenes (9.54%). Among which the major compounds were β-pinene (23.58%), tau-cadinol (9.54%), diethyl phthalate (7.88%), α-pinene (5.68%) and β-caryophyllene (4.88%).

3.2. *In vitro* antifungal assays

The inhibition percentages obtained in assays of antifungal activity at different concentrations of *I. carinata* and *C. arabicus* EOs using agar dilution and disk diffusion techniques are summarized in **table 2**.

In both techniques, the results showed that the inhibition of the mycelial growth of *B. cinerea* was significantly influenced by the EOs concentration ($p \leq 0.05$). In the agar dilution method, the EOs exhibited antifungal effect from 12.67 ± 0.57 to 86 ± 1.73% and from 7.50 ± 7.50 to 81.67 ± 1.44% for *I. carinata* and *C. arabicus*, respectively. In disk diffusion, at the highest concentration, the inhibition percentages were 35 ± 0.0% and 23.33 ± 25.54% for *I. carinata* and *C. arabicus* EOs, respectively. In the agar dilution technique, the IC_{50} values were 0.95 $\mu l \cdot ml^{-1}$ and 1.01 $\mu l \cdot ml^{-1}$ for *I. carinata* and *C. arabicus* EOs, respectively. However, in the disk diffusion method, the IC_{50} values were > 4 $\mu l \cdot ml^{-1}$. These results show that the inhibitory effect in the agar dilution technique was more prominent against *B. cinerea* than in the disk diffusion assay for the two EOs studied (**Figure 1**).

3.3. Evaluation of EOs on the shelf life of raspberries and strawberries

The decay rates daily measured of EOs treated berries and their controls (untreated) during 15 days at cold storage are presented in **figure 2** (raspberry) and **figure 3** (strawberry).

For raspberries (**Figure 2**), from day 1 to 7 the decay rates (%) of treated raspberries with the two EOs were less than the control (50 ± 4.36%) ($p \leq 0.05$). During the eighth and ninth days, the decay levels of CA200 (40 ± 1.73%) and CA400 (53.33 ± 1.16%) treated raspberries were still lower than that of the control (56.67 ± 3.8%). On day 12, CA200 and CA400 treated raspberry fruits, and control samples were completely infected (100%). At the end-storage day (day 15), the decay rates of IC200 and IC400 treated raspberries were 16.67% and 13.33%, respectively.

For strawberries (**Figure 3**), from day 1 to day 7 the decay rates (%) of treated strawberries were lower than that of the control ($p \leq 0.05$). From day 8 to 12, the decay rates of CA200 and CA400 treated strawberries were statistically equal to that of untreated strawberry fruits and they were all completely (100%)

Table 2. Percent inhibition of *Ismelia carinata* and *Cladanthus arabicus* essential oils on mycelial growth of *Botrytis cinerea* — *Pourcentage d'inhibition des huiles essentielles d'Ismelia carinata et de Cladanthus arabicus sur la croissance mycélienne de Botrytis cinerea.*

Essential oils	Antifungal assays	Concentrations ($\mu l \cdot ml^{-1}$)						IC_{50} ($\mu l \cdot ml^{-1}$)
		0.125	0.25	0.5	1	2	4	
I. carinata	Agar dilution	12.67 ± 0.57[i]	12.67 ± 0.57[i]	15 ± 0.0[hi]	58.67 ± 1.15[c]	72.00 ± 1.73[b]	86.00 ± 1.73[a]	0.95
	Disk diffusion	17 ± 3.61[gh]	19.33 ± 4.04[g]	22.67 ± 2.52[f]	30 ± 0.0[e]	33 ± 0.0[de]	35 ± 0.0[d]	> 4
C. arabicus	Agar dilution	7.50 ± 7.50[ef]	26.67 ± 2.89[cde]	33.17 ± 7.08[cd]	45 ± 4.33[c]	62.50 ± 2.50[b]	81.67 ± 1.44[a]	1.01
	Disk diffusion	0 ± 0.0[f]	3.33 ± 2.89[ef]	8.33 ± 3.82[ef]	10 ± 4.33[def]	14.17 ± 15.88[def]	23.33 ± 25.54[def]	> 4

Mean value ± standard deviation — *valeur moyenne ± écart-type*; the small letters indicate significant differences according to the Newman-Keuls test ($p \leq 0.05$) — *les lettres minuscules indiquent des différences significatives selon le test de Newman-Keuls (p ≤ 0,05).*
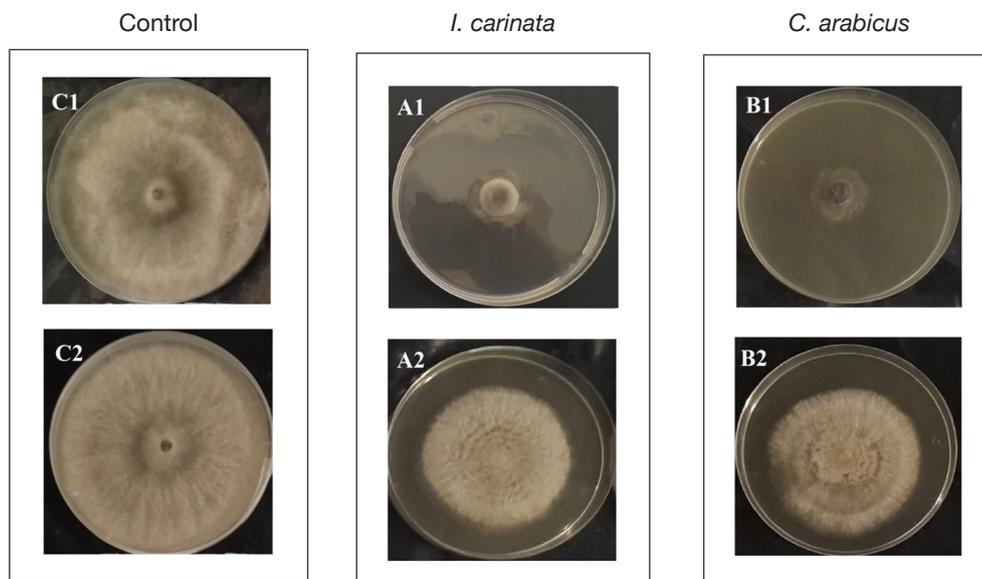
Figure 1. Antifungal effect of 4 μl·ml^{-1} of *Ismelia carinata* and *Cladanthus arabicus* essential oils in agar dilution (**A1**; **B1**) and disk diffusion (**A2**; **B2**) assays on mycelial growth of *Botrytis cinerea* compared to the control (**C1**; **C2**) — *Effet antifongique de 4 µl·ml^{-1} d'huile essentielle d'*Ismelia carinata *et de* Cladanthus arabicus *avec la dilution en gélose (**A1**; **B1**) et la diffusion sur disque (**A2**; **B2**) sur la croissance mycélienne de* Botrytis cinerea *par rapport au contrôle (**C1**; **C2**).*
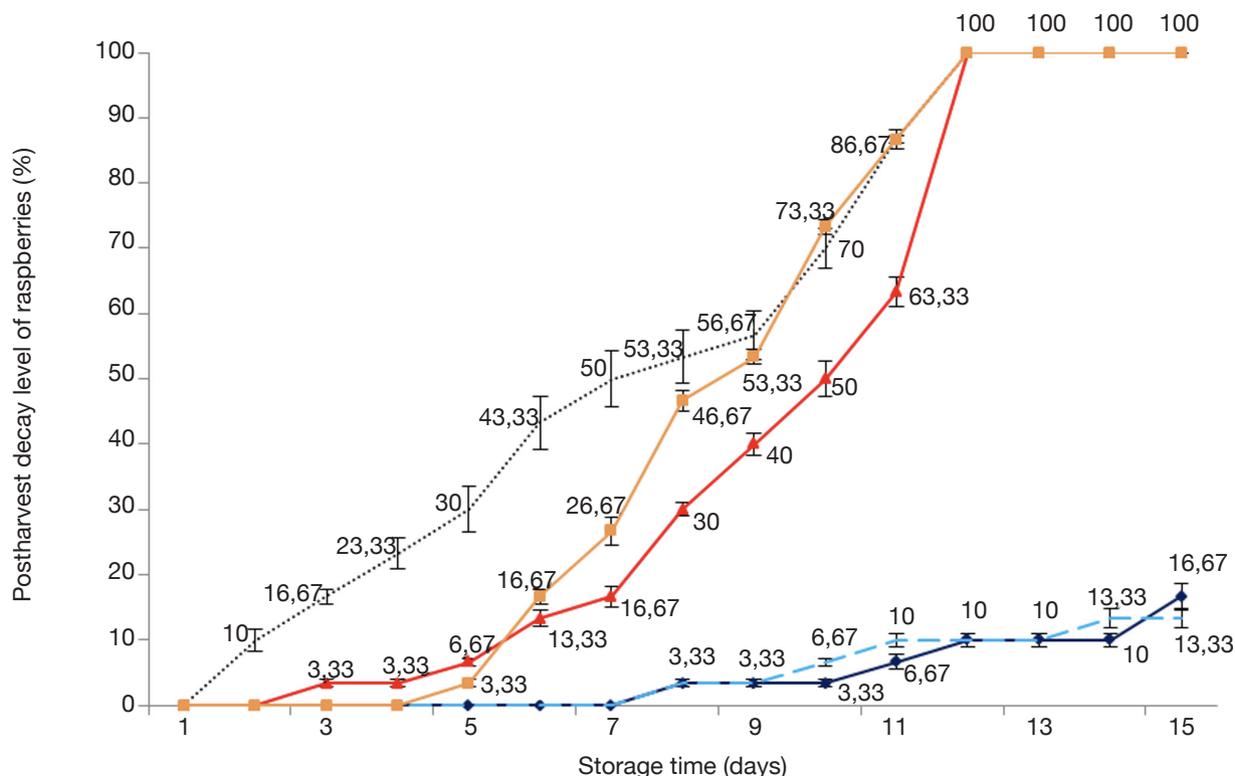

Figure 2. The daily evolution of the decay rate of raspberries treated with *Ismelia carinata* (IC) and *Cladanthus arabicus* (CA) essential oils during 15 days at 4 °C with IC200 mg·l^{-1} (-♦-), IC400 mg·l^{-1} (–·–), CA200 mg·l^{-1} (-▲-), CA400 mg·l^{-1} (-■-). The sample of untreated raspberries was used as control (···). Mean of three replicates (mean [number] ± SD [vertical bar]) — *L'évolution journalière du taux de pourriture des framboises traitées aux huiles essentielles d'*Ismelia carinata *(IC) et de* Cladanthus arabicus *(CA) pendant 15 jours à 4 °C avec IC200 mg·l^{-1} (-♦-), IC400 mg·l^{-1} (–·–), CA200 mg·l^{-1} (-▲-), CA400 mg·l^{-1} (-■-). L'échantillon de framboises non traitées a servi de témoin (···). Moyenne de trois répétitions (moyenne [nombre]) ± écart-type [barre verticale].*
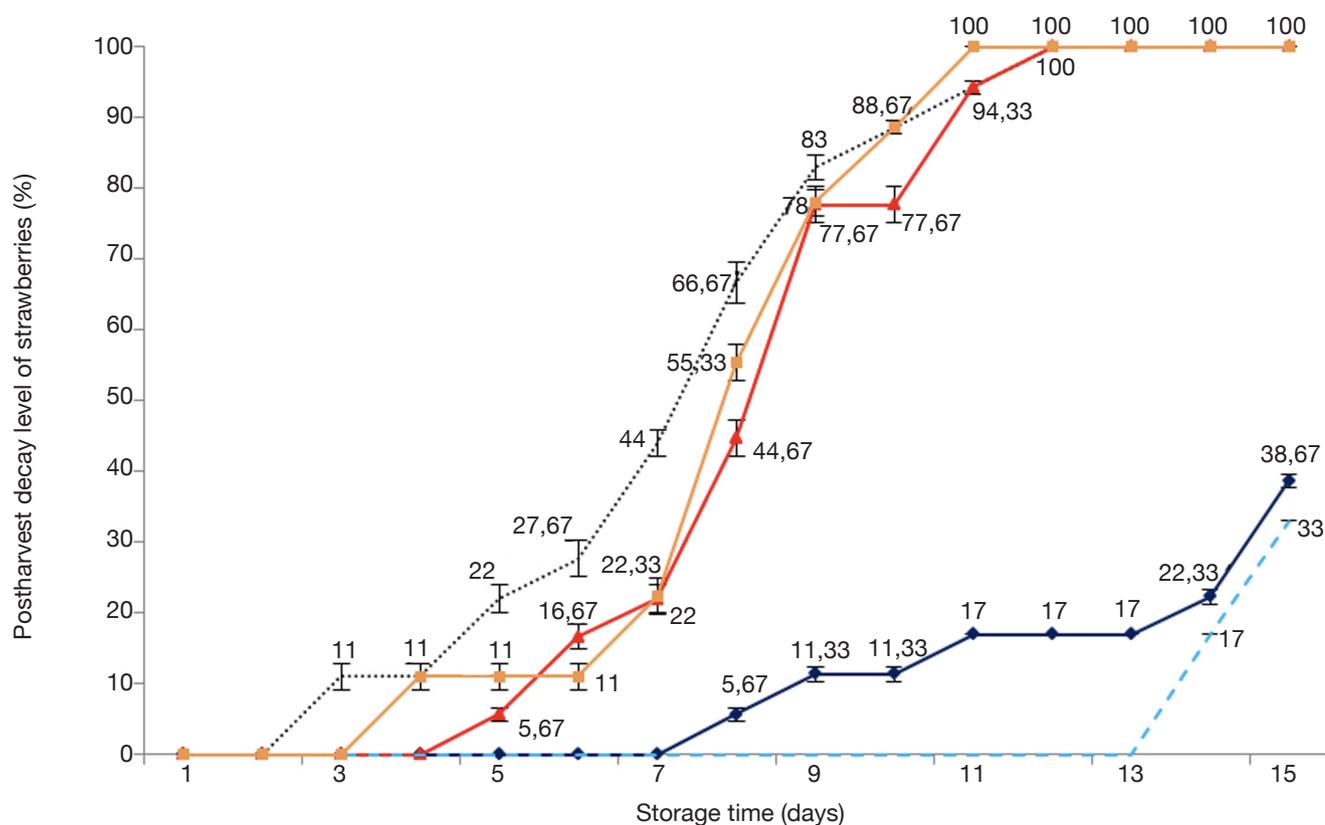
Figure 3. The daily evolution of the decay rate of strawberries treated with *Ismelia carinata* (IC) and *Cladanthus arabicus* (CA) essential oils during 15 days at 4 °C with IC200 mg·l⁻¹ (-♦-), IC400 mg·l⁻¹ (–·–), CA200 mg·l⁻¹ (-▲-), CA400 mg·l⁻¹ (-■-). The sample of untreated strawberries was used as control (···). Mean of three replicates (mean [number] ± SD [vertical bar]) — *L'évolution journalière du taux de pourriture des fraises traitées aux huiles essentielles d'*Ismelia carinata *(IC) et de* Cladanthus arabicus *(CA) pendant 15 jours à 4 °C avec IC200 mg·l⁻¹ (-♦-), IC400 mg·l⁻¹ (–·–), CA200 mg·l⁻¹ (-▲-), CA400 mg·l⁻¹ (-■-). L'échantillon de fraises non traitées a servi de témoin (···). Moyenne de trois répétitions (moyenne [nombre]) ± écart-type [barre verticale]).*

infected on day 12, while no decayed strawberry fruits were obtained when *I. carinata* EO was applied at 400 mg·l⁻¹ and for IC200 treated strawberries, only 17% of decay rate was obtained. On the end-storage day, the decay rates of IC200 and IC400 treated strawberries were 38.67% and 33%, respectively.

For both treated berries no significant difference was observed between 200 and 400 mg·l⁻¹ of *I. carinata* EO ($p \leq 0.05$). The pictures of treated berries with *I. carinata* EO and controls (untreated berries) are presented in **figure 4**. It can be observed that untreated berries (A1; A2) are largely postharvest decayed at day 12, while IC200 and IC400 treated berries (B1; B2; C1; C2) maintain good red color and aspect. Furthermore, the berries treated with IC400 (D1; D2) showed good appearance even at day 18.

3.4. Physical and chemical qualities of berries treated with 400 mg·l⁻¹ of *I. carinata* essential oil

The results of physical and chemical analyses of berries treated with 400 mg·l⁻¹ of *I. carinata* EO after storage for 15 days at 4 °C compared to the control (untreated berries) are presented in **table 3a** (raspberries) and **table 3b** (strawberries).

For raspberries, pH and total soluble solids did not differ whereas titrable acidity and maturity index were significantly different between control and treated samples ($p \leq 0.05$). For strawberries, the difference between control and treated samples is significant in all quality factors ($p \leq 0.05$). On both berries, the EO treatment maintained high titratable acidity compared to the control, which reduced the change in maturity index of treated berries compared to the controls.

4. DISCUSSION

To the best of our knowledge, it is the first report of the chemical composition of the *I. carinata* EO. The EO of *I. carinata* contained two oxygenated sesquiterpenes as main compounds. This phytochemical profile is different from that of the other species belonging to the *Chrysanthemum* genus to which *I. carinata* was earlier
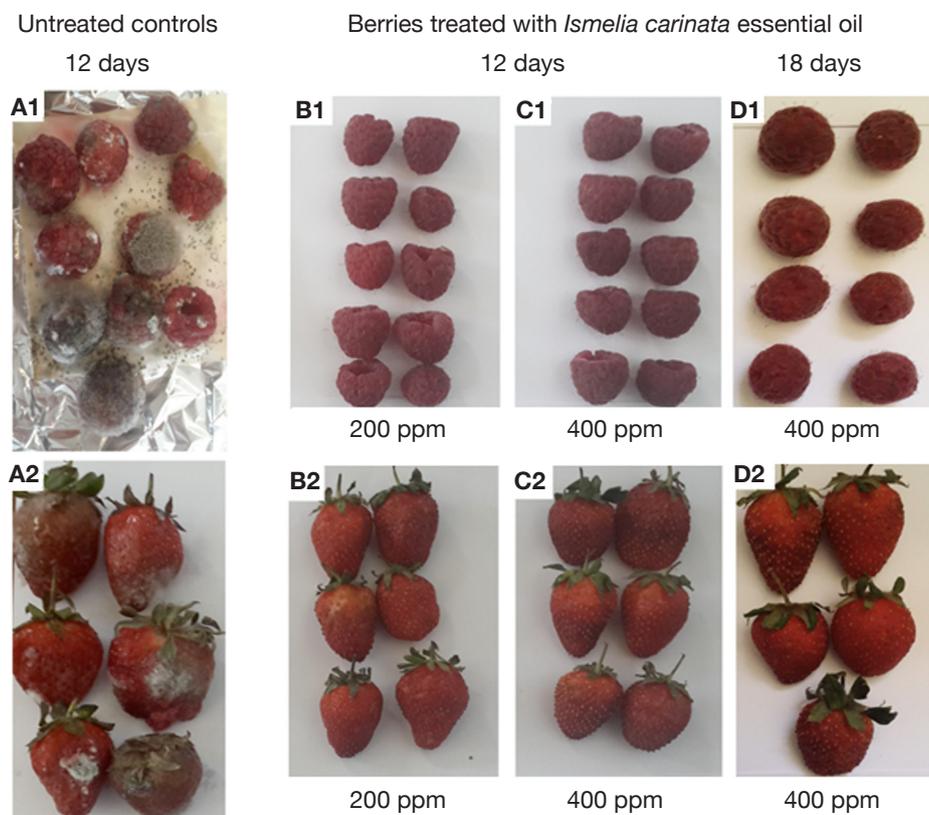
Untreated controls
12 days

Berries treated with *Ismelia carinata* essential oil
12 days   18 days

A1   **B1**   **C1**   **D1**

200 ppm   400 ppm   400 ppm

A2   **B2**   **C2**   **D2**

200 ppm   400 ppm   400 ppm

Figure 4. Pictures of berries treated with *Ismelia carinata* essential oil (**B1**, **B2**, **C1**, **C2**, **D1**, **D2**) and untreated controls (**A1**, **A2**) — *Photographies des baies traitées (**B1**, **B2**, **C1**, **C2**, **D1**, **D2**) à l'huile essentielle d'*Ismelia carinata *et des témoins non traités (**A1**, **A2**).*

Table 3a. Results of physical and chemical analyses of raspberries treated with 400 mg·l⁻¹ of *Ismelia carinata* essential oil after a storage during 15 days at 4 °C — *Résultats des analyses physico-chimiques des framboises traitées avec 400 mg·l⁻¹ d'huile essentielle d'*Ismelia carinata *après un stockage de 15 jours à 4 °C.*

Variables	At harvest	Untreated control	Treated and stored berries
pH	3.23 ± 0.031^a	3.23 ± 0.031^a	3.2 ± 0.012^a
TSS (%)	4.36 ± 0.04^a	4.44 ± 0.02^a	4.4 ± 0.02^a
Titratable acidity (g·l⁻¹)	0.83 ± 0.01^a	0.47 ± 0.01^c	0.65 ± 0.02^b
Maturity index	5.23 ± 0.03^a	9.45 ± 0.18^c	6.73 ± 0.12^b

Mean value ± standard deviation — *valeur moyenne ± écart-type*; the small letters indicate significant differences according to the Newman-Keuls test ($p \leq 0.05$) — *les lettres minuscules indiquent des différences significatives selon le test de de Newman-Keuls (p ≤ 0,05).*

Table 3b. Results of physical and chemical analyses of strawberries treated with 400 mg·l⁻¹ of *Ismelia carinata* essential oil after storage during 15 days at 4 °C — *Résultats des analyses physico-chimiques des fraises traitées avec 400 mg·l⁻¹ d'huile essentielle d'*Ismelia carinata *après un stockage de 15 jours à 4 °C.*

Variables	At harvest	Untreated control	Treated and stored berries
pH	3.62 ± 0.03^a	3.42 ± 0.04^c	3.51 ± 0.02^b
TSS (%)	3.94 ± 0.02^a	3.5 ± 0.05^c	3.6 ± 0.06^b
Titratable acidity (g·l⁻¹)	0.36 ± 0.01^a	0.3 ± 0.01^b	0.35 ± 0.01^a
Maturity index	10.95 ± 0.25^a	11.8 ± 0.29^c	10.19 ± 0.33^b

Mean value ± standard deviation — *valeur moyenne ± écart-type*; the small letters indicate significant differences according to the Newman-Keuls test ($p \leq 0.05$) — *les lettres minuscules indiquent des différences significatives selon le test de de Newman-Keuls (p ≤ 0,05).*

grouped (Hosni et al., 2013). These results support a previous study indicating the chemotaxonomic difference of *I. carinata*, based on flavonoids analysis (Williams et al., 2001).

The results of the chemical composition of *C. arabicus* EO showed qualitative and quantitative dissimilarities compared to previous studies using GC/MS to ascertain the composition of *C. arabicus* EO collected during the flowering stage in Ouarzazate and Marrakech Moroccan regions (El Hanbali et al., 2005; Aghraz et al., 2017). El Hanbali et al. (2005) have identified 36 constituents while Aghraz et al. (2017) have investigated 67 compounds. These two studies cited sabinene, β-pinene, α-pinene, myrcene as major compounds but in different fractions. The current work and the prior studies found α and β-pinene among the major constituents and highly dominance of monoterpenes hydrocarbons in the chemical composition of *C. arabicus* EO. Diethyl phthalate in the chemical composition of *C. arabicus* is considered an environmental pollutant. The mechanism of production of this compound by plants is not yet confirmed to conclude that it is a biogenic compound (Romeh, 2013). It is suggested that the plant may possess an ability to accumulate phthalate diethyl from its environment (Saeidnia & Abdollahi, 2013).

The variations recorded in the chemical composition of *C. arabicus* EO are postulated to be due to various factors related to geographical settings, plant genotype, seasonality, and physiological harvesting age (Shanjani et al., 2010; Aboukhalid et al., 2016; Angioni et al., 2006).

In the *in vitro* antifungal tests, the agar dilution method presented the best inhibiting effect of EOs against *Botrytis cinerea*. It has been proposed that Tween 80 enables better distribution of components through the agar. Also, Alvarez-Castellanos et al. (2001) tested the fungicide effect of *Chrysanthemum coronarium* EO. They concluded that the volatile activity of *C. coronarium* EO was less persistent against fast-growing fungi as *B. cinerea*.

Previous works have tested the fungicidal activity of EOs from the Asteraceae family species against *B. cinerea*. The reported activities depend on EO composition and especially on the main antifungal component. Liu et al. (2016) demonstrated that the vapors of *Solidago canadensis* L. EO rich in α–pinene (59.5%) inhibited by 42% the mycelial growth of the fungus. It has been indicated that 1.85 μl·ml^{-1} of *Tagetes patula* EO composed of nearly 50% of monoterpenes ketones, reduced by 47.4% the mycelial growth in liquid phases (Romagnoli et al., 2005). Rahman et al. (2010) showed that *Erigeron ramosus* EO containing 38.5% of sesquiterpenes hydrocarbons was not able to produce any growth inhibition at 1 μl·

ml^{-1}. As a result of the current study, it is suggested that liquid phases of the EOs tested have a great *in vitro* antifungal potential against *B. cinerea*. Also, the antifungal effects of *I. carinata* and *C. arabicus* EOs are related to their main components, which were reported as effective antifungal agents against phytopathogenic fungi (Hammer et al., 2003; Chang et al., 2008). Inhibitory potential of EOs depends on plant taxonomy and the types of chemical molecules in EOs (Pinto et al., 2006 ; Bajpai et al., 2013). Chang et al. (2008) noted that sesquiterpenes exhibited stronger antifungal effect than monoterpenes. Also, EOs have been suggested to act on cell membrane integrity of pathogens (Bajpai et al., 2013; Liu et al., 2016). Further, the minor components of EOs may interact in synergy with other active compounds linked to cellular signaling and transduction mechanisms (Znini et al., 2013).

In the berry test, the efficiency of *C. arabicus* EO has not been visualized, while *I. carinata* EO minimized the postharvest decay of raspberries and strawberries during 15 days of storage at 4 °C. Many mechanisms of essential oils and their components to deactivate fungi were described (Freiesleben & Jager, 2014). The difference of mode of action between the main compounds of the two EOs could explain the *in vivo* efficiency difference (Andrade et al., 2018; dos Santos et al., 2021). The components of *C. arabicus* EO may be more volatile than those of *I. carinata* EO, as Hay & Waterman (1993) noted that sesquiterpenes are less volatile than other terpenes. This may afford for EO long-term stability under *in vivo* conditions. It could also be suggested that some components in *C. arabicus* EO had detrimental effects on sensitive berries and counteracted the fungicidal effect of other major constituents. For example, diethyl phthalate representing 7.88% of the total oil in the investigated chemical composition of *C. arabicus* EO should be used in limited doses to avoid its toxicological effects (Api, 2001). It is likely that a solubility problem of *C. arabicus* EO occurred (Demirci et al., 2008) or that the *in vivo* results were retained from the interactions of phenolic compounds with the food matrix (Yooussef et al., 2016). Previous works reported similar results on *Lavandula angustifolia* and *Origanum compactum* EOs, whose antifungal effect under *in vivo* conditions was not as strong as that *in vitro* (Hadian et al., 2008; Vu et al., 2011). Further, Lopez-Reyes et al. (2010) concluded that the choice of EOs for delaying postharvest decay should take into consideration the characteristics of the fruit and feasible storage time.

Other studies have shown the efficiency of EOs, and/or their components against raspberry and strawberry postharvest decays. Allyl isothiocyanate (5 μl·l^{-1}) and methyl jasmonate (22.4 μl·l^{-1}) preserved fresh raspberries without fungal rot deterioration

during a storage of 10 days at 10 °C (Wang, 2003; Chanjirakul et al., 2006). Carvacrol and methyl cinnamate - enriched films inhibited by 57% the less postharvest decay in treated strawberries than the control (Peretto et al., 2014). Also, strawberries exposed to EOs vapors of *Eucalyptus globules* and *Cinnamomum zeylanicum* showed 57 to 63% less spoilage than unexposed fruits (Tzortzakis, 2007). Alikhani & Daraei Garmakhany (2012) noted that microencapsulated *Rosmarinus officinalis* and *Thymus vulgaris* EOs were able to reduce the decay development on strawberry fruits stored at 5 °C for 9 days. Liu et al. (2016) reported the effectiveness of *Solidago canadensis* EO vapor to maintain the apparent quality of fresh strawberries stored at 20 °C. Although appearing as a costly option, the high commercial value of treated strawberries and raspberries would compensate for their postharvest treatment with EOs.

As raspberries and strawberries are non-climacteric, slight changes in TSS and organic acids occurred. However, it was reported that several factors such as cultivar, storage, and fruit maturity at picking could incur fruit quality changes during the postharvest life of these berries (Shin et al., 2008). In the current study, it was observed that the postharvest treatment with 400 mg·l^{-1} of *I. carinata* EO reduced changes in physical and chemical qualities of berries compared to the control.

The results of this work are consistent with previous works carried out on raspberries and strawberries treated with EOs on postharvest and revealing changes in fruit quality (Wang, 2003; Alikhani & Daraei Garmakhany, 2012).

5. CONCLUSIONS

In this paper, the EOs of *I. carinata* and *C. arabicus* were evaluated for their chemical compositions and *in vitro* antifungal potential using two different screening assays. Furthermore, the study assessed the efficiency of EOs to preserve visual acceptance and fruit sweetness of raspberries and strawberries during cold storage. *Ismelia carinata* and *C. arabicus* EOs showed the best *in vitro* antifungal effect in liquid phases. *Ismelia carinata* EO minimized postharvest decays of both berries. The results of the current study suggest that *I. carinata* EO is a promising alternative in preserving fruit quality during fresh raspberries and strawberries storage and transit. Further work on other quality properties after storage of treated raspberries and strawberries is required. *Cladanthus arabicus* EO should be tested on other fruits as a postharvest treatment to clarify its antifungal potential under *in vivo* conditions.

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