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Vapour or dipping applications of methyl jasmonate, vinegar and sage oil for pepper fruit sanitation towards grey mould

Nikos Tzortzakis[a,*], Antonios Chrysargyris[a], Dharini Sivakumar[b], Kostas Loulakakis[c]

[a] Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, 3036 Limassol, Cyprus
[b] Department of Crop Science, Tshwane University of Technology, Pretoria Campus, South Africa
[c] Department of Agriculture, School of Agriculture Food and Nutrition, Technological Educational Institute of Crete, Stavromenos, 71004 Heraklion, Greece

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ABSTRACT

Grey mould (*Botrytis cinerea*) development *in vitro* or on pepper (*Capsicum annuum*) fruit was evaluated after treatment with chlorine (CHL), methyl jasmonate (MJ), vinegar (VIN), or sage oil (SAG) and storage at 11 °C and 95% relative humidity following sanitary exposure (volatiles or dipping). Fruit treated (up to 12 days) with MJ and SAG vapours reduced lesion development and spore production while no differences were found for spore germination. The benefits associated with volatile enrichment was maintained in fruit pre-exposed to MJ and SAG oil vapours, resulting in suppression of lesion growth, while fungal reproduction decreased only in SAG pre-exposed fruit. Studies performed on fungi grown on Potato Dextrose Agar (PDA) revealed colony growth suppression and spore production for direct SAG vapour application or PDA pre-exposed to SAG following *B. cinerea* inoculation, implying that suppression of pathogen development was mainly due to the impact of volatiles on fruit-pathogen interactions and/or residual effects on fruit tissue and/or medium culture. *In vitro*, fungal biomass was examined under different concentrations (10-50-100-500-5000 $\mu L\,L^{-1}$) and was accelerated in high SAG concentrations, while spore production decreased (including MJ and VIN) on fungi grown in Potato Dextrose Broth (PDB), implying the effects to be concentration dependent. Moreover, sanitary dips proved to be less effective in fruit sanitation compared to vapour application. The results of this study indicate that SAG-, followed by MJ volatiles may be considered as alternatives to the traditional postharvest sanitising techniques. Each commodity needs to be individually assessed, and the volatile concentration and sanitising technique optimised, before commercialisation.

1. Introduction

Nowadays, growing health and environmental concerns have emerged over present sanitation techniques due to their inadequacy on a wide spectrum of micro-organisms as well as potentially carcinogenic residues (Spotts and Peters, 1980). Various pre- and postharvest technologies have been used to control decay, but the postharvest application of chemicals as fungicides is restricted in most countries. Additionally, there is an increasing interest in the possible use of natural compounds to prevent microbial growth in fresh commodities, thus answering to consumer pressure to minimise chemical additives and pesticide residues in foods. Thus, new preservation technologies are necessary, which have to be considered as safe to consumers and environmentally friendly. Among these technologies, natural products from higher plants, including methyl jasmonate (MJ), vinegar (VIN) and essential oils (EOs) are relatively broad-spectrum, bio-effective, environmental safe and attractive to scientists globally as alternative candidates for use as agro-chemicals.

Several volatile compounds have shown promise in reducing postharvest diseases and disorders in horticultural crops. Natural volatile compounds, including MJ, VIN and EOs, have been widely investigated and demonstrated to have antibacterial and antifungal activities (Moline et al., 1997; Wang, 1998; Droby et al., 1999; Wang, 2003; Aguilar-González et al., 2015). Methyl jasmonate, a methyl ester of jasmonic acid, is a phytohormone with ubiquitous distribution among plants, with vital roles as endogenous signal molecules in plant development (*i.e.* skin colour development by promoting β-carotene synthesis and chlorophyll degradation) (Li et al., 2001; Turner et al., 2002) and in regulating stress responses to numerous biotic and abiotic stresses (such as wounding,

* Corresponding author.
E-mail address: nikolaos.tzortzakis@cut.ac.cy (N. Tzortzakis).

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pathogen attack, and desiccation) by triggering a transcriptional reprogramming that allows cells to cope with pathogens and stress factors (Li et al., 2001). Subsequent investigations of the effects of MJ treatments as a vapour or an emulsion dip during low temperature storage of several fruits and vegetables showed a decrease in fresh produce deterioration with prolonged commodity storage. Thus, MJ reduced chilling injury and extended storage period of fresh cut celery and pepper by decreasing the rate of quality deterioration and suppressing microbial growth (Buta and Moline, 1998; Gonzalez-Aguilar et al., 2003).

Acetic acid is a metabolic intermediate that occurs in nature in many fruit while its inhibitory effect on micro-organisms is greater than that due to pH alone, causing toxic effects on microbial cells following penetration (Tripathi and Dubey, 2004). Acetic acid or VIN vapour were efficient in preventing conidial germination of brown rot (*Monilinia fructicola*), grey mould (*Botrytis cinerea*), blue mould (*Penicillium italicum*), anthracnose rot (*Colletotrichum coccodes*) and subsequent decay of stone fruit, strawberries, apples and tomatoes (Sholberg et al., 2000; Tzortzakis, 2010; Aguilar-González et al., 2015). Moreover, acetic acid vapour reduced postharvest brown rot of apricots and plums (Liu et al., 2002). The use of VIN is even safer and still effective (Sholberg et al., 2000). However, the low acetic acid content, that makes VIN safe, also limits its efficacy.

Essential oils and their constituents have often been used as biological control agents because of their curative activity and their toxicity to fungi, bacteria and insects (Delespaul et al., 2000). The advantage of EOs is their bioactivity in the vapour phase, a characteristic that makes them appropriate as potential fumigants for stored fresh produce conservation (Serrano et al., 2005). Essential oils are thought to play a role in plant defense mechanisms against phytopathogenic micro-organisms, while various reports highlighting the efficacy of EO on fresh produce preservation, such as sweet cherry, pear, avocado, banana, kiwi, grape, strawberry, tomato and eggplant (Thanassoulopoulos and Yanna, 1997; Pesis et al., 1998; Ju et al., 2000; Anthony et al., 2003; Serrano et al., 2005; Tzortzakis, 2007a, 2010; Stavropoulou et al., 2014; Aguilar-González et al., 2015). Such new applications could have a wide approval because of consumer's preference for natural plant products, *e.g.* EOs compared with chemicals fungicides (Anthony et al., 2003).

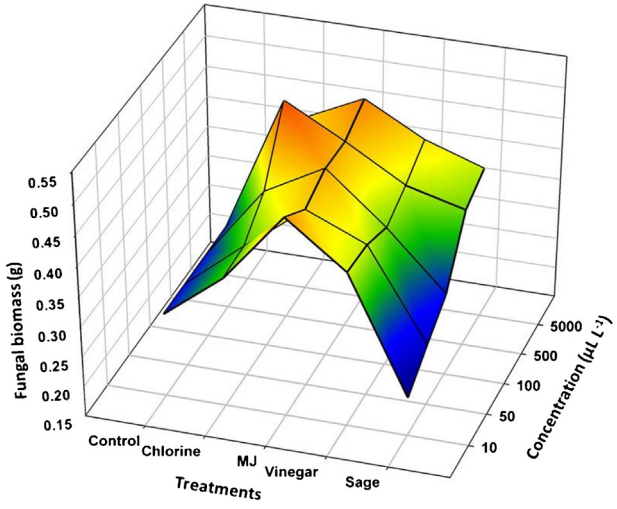

Fig. 1. Effect of chlorine, methyl jasmonate, vinegar, or sage essential oil on fungal biomass (g) of *Botrytis cinerea* grown in PDB. Tubes were incubated in controlled environment chambers maintained at 11 °C and 95% RH. PDB inoculated with *B. cinerea* and then exposed to vapours. Values represent means of measurements made on six independent tubes per treatment.

Pepper (*Capsicum annuum*) is one of the most grown vegetable and is consumed as a vegetable or fresh-cut produce (Pickersgill, 1997). It has also been associated with reducing the risk of cancer and/or other diseases due to attributes such as capsaicin, phenolic compounds and vitamin A and C.

The most significant reasons for postharvest loss of peppers are due to their susceptibility to chilling injury (when stored below 7 °C), and microbial infection before or during storage that may cause tissue damage or changes in secondary metabolism due to physiological changes that facilitate pathogen development (Eckert and Ogawa, 1988). Grey mould (*B. cinerea* Pers.: Fr (teleomorph: *Botryotinia fuckeliana*), one of the most important diseases of fruit worldwide) is a common and wide-spread rot occurring in a range of vegetables (Sanogo et al., 2003).

Therefore, the main objective of the present study was to examine if natural products (MJ, VIN or sage oil-SAG), as well as our sanitation technique would suppress *B. cinerea* spoilage in pepper fruit, in comparison with chlorine applications, a common, well-known sanitising agent.

2. Materials and methods

2.1. Plant material and inocula

Pepper (*C. annuum* cv. Sammy) fruit were obtained directly by a commercial field crop in Heraklion, Greece. Harvested fruit were randomised in uniformity of size, colour, ripeness (green stage) and checked for no defects or injuries. Natural sanitisers employed in this study consisted of MJ (Sigma-Aldrich, Larnaca, Cyprus), VIN (commercial product ~5% acetic acid) and SAG (*Salvia trilova*) essential oil, while chlorine (CHL; 5% NaOCl) was used as commercial control.

Essential oils derived from naturally growing sage, harvested in a hilly area of Crete, were extracted by hydrodistillation (clevenger apparatus for 3 h). The composition of the EOs was analysed by Gas Chromatography-Mass Spectroscopy (GC–MS). Analytical gas chromatography was carried out on a Shimadzu GC2010 gas chromatograph interfaced Shimadzu GC/MS QP2010 plus mass spectrometer. A sample volume of 2 μL was injected in a split mode (split ratio 20:1) into the gas chromatograph fitted with a ZB-5 column (Zebron, Phenomenex, USA) coated with 5% pheny-95% dimethylpolysiloxane with film thickness of 0.25 μm, length of 30.0 m and a diameter of 0.25 mm. The flow of the carrier gas (helium) was 1.03 mL min^{-1}. The injector temperature was set at 230 °C. Electron impact mass spectra with ionisation energy of 70 eV was recorded at the 35–400 *m/z*. The column temperature was programmed to rise from 60 °C to 240 °C at a rate of 5 °C min^{-1}, with a 5 min hold at 240 °C. The identity of the oil components was assigned by comparison of their retention indices relative to (C_8–C_{20}) *n*-alkanes with those of literature or with those of authentic compounds available in our laboratory. Further identification was made by matching their recorded mass spectra with those stored in the NIST08 mass spectral library of the GC–MS data system and other published mass spectra (Adams, 2012). The percentage determination was based on peak area normalisation without using correction factors. The composition (see Table 1S-Supplementary file in the online version at DOI: 10.1016/j.postharvbio.2016.04.004) of the main (>2.0%) essential oil constitutes was: α-Pinene (3.1%), Camphene (2.3%), β-Pinene (4.1%), Eucalyptol (53.5%), *cis*-Thujone (6.7%), *trans*-Thujone (3.3%) and Camphor (7.9%).

B. cinerea isolated from Solanaceae species was supplied by CABI (CABI Bioscience UK Centre, Bakeham Lane, Egham, UK) and aseptically sub-cultured in Potato Dextrose Agar (PDA, Oxoid Ltd, Hampshire, UK), purified and cultures stored at 4 °C for long-term use, as described previously (Stavropoulou et al., 2014).

2.2. Treatments with natural compounds

Examining the impacts of natural compounds (MJ, VIN, SAG) and CHL on fungal development (on a biomass base) and sporulation, a range of concentrations (10-50-100-500-5000 μL L^{-1}) were studied in Potato Dextrose Broth (PDB, Oxoid Ltd, Hampshire, UK). For the *in vitro* studies, two mycelia plugs inoculated in 30 mL PDB medium in a 200 mL glass container, consisting of different natural compound concentrations. Following inoculation, the containers were closed and sealed with parafilm.

Considering fungal development (on an area base), concentrations used were based on previous experiments and/or published data (Tzortzakis, 2007b; 2010) for PDA media. Thus, natural compounds used in this experimental set with PDA include MJ (44.8 μL L^{-1}), VIN (16 mL L^{-1}), or SAG (0.4 mL L^{-1}) diluted in distilled H_2O [with 5% (v/v) Tween-20 for SAG oil; vortexed in order to ensure thorough mixing] compared with CHL (48 mL L^{-1}) as chemical control and a negative control treatment. For the *in vitro* studies, the disc diffusion method used for colony development on PDA medium as described in previous studies (Stavropoulou et al., 2014). In brief, PDA medium inoculated with a mycelial disc from 4 to 5 d-old culture of *B. cinerea*, aliquots (0.1 mL) of each volatile solution (MJ, VIN and SAG) were soaked into individual filter paper (3.5 × 3.5 cm). The paper discs were placed on the inverted lid of each Petri dish, the lids were closed and sealed with parafilm. For spore production and spore germination estimation, aliquots (30 mL) of each volatile solution, in individual small beakers, were placed inside the plastic containers just before the lids were closed. Wet filter paper was placed in each container to maintain high relative humidity (RH∼ 95%) during the storage period.

In order to examine the appropriate type of natural compound application *in vivo* (either volatiles or dipping), two sub-experiments were conducted for pepper fruit as described in Sections 2.4–2.5. In sub-experiment I, six individual fruit exposed to each volatile compound treatment [untreated control; CHL-treated chemical control (48 mL L^{-1}); MJ-treated (44.8 μL L^{-1}); VIN-treated (16 mL L^{-1}) and SAG-treated fruit (0.4 mL L^{-1})]. Fruit were placed into 2.9 L polystyrene containers (2 fruit/container) with snap-on lids for each individual experiment. Aliquots (5 mL) for the desirable concentration of each volatile solution were soaked into individual Whatman No.1 filter paper (3.5 × 3.5 cm), placed into individual small beakers and subsequently placed inside the plastic containers. Wet filter paper was used for 95% RH maintenance. The volatiles were allowed to vaporise inside the containers spontaneously at 20 °C for 2 h. Control samples were handled similarly with the exception of the volatile treatment [controls consisted of distilled H_2O with 5% (v/v) Tween-20]. *In vitro* and *in vivo* experiments were repeated twice.

In sub-experiment II, six individual fruit per treatment were dipped into the natural compound solution (same concentration as vapour treatments) for 30 min, dried for 1 h at room temperature (RT) and transferred to storage at ambient air (AA) conditions for up to 12 d.

2.3. Impact of natural compounds-enrichment on pathogen development in vitro

Two mycelial plugs (1 cm^2), obtained from the periphery of 4–5 d old culture of *B. cinerea* at 25 °C, were placed in the centre of containers with 30 mL PDB. Following inoculation, containers were incubated for 8 d in the dark at 11 °C, 95% RH. Fungal biomass was dried, weighed and data expressed as dry weight (g), while inhibition of sporulation was calculated [considering the area measured using digital imaging tools (Adobe Photoshop CS6 analysis tools] in comparison to sporulation of the control (without natural compounds).

A mycelial plug (2.5 mm diameter), as described above, was placed in the centre of PDA plates. Following inoculation, plates were incubated for 6 d in the containers exposed to AA or volatiles in the dark at 11 °C, 95% RH (curative effect). In a second experiment, PDA plates were exposed to AA or volatiles for 6 d. Following exposure, culture medium was inoculated (as described above), lids were replaced on the plates and plates were transferred to AA for an additional 6 d period (residual effect). Colony diameter was measured and results were monitored as colony area (cm^2) development.

2.4. Impact of volatile enrichment on grey mould development in wound-inoculated fruit

Two wounds (3 mm diameter and 1–2 mm deep) were made on opposite sides, and also at the top and bottom, of commercial immature green pepper, using a sterilised spike. Inoculation with *B. cinerea* took place as described previously (Tzortzakis, 2010). Briefly, a mycelial plug (2.5 mm diameter) was taken from the

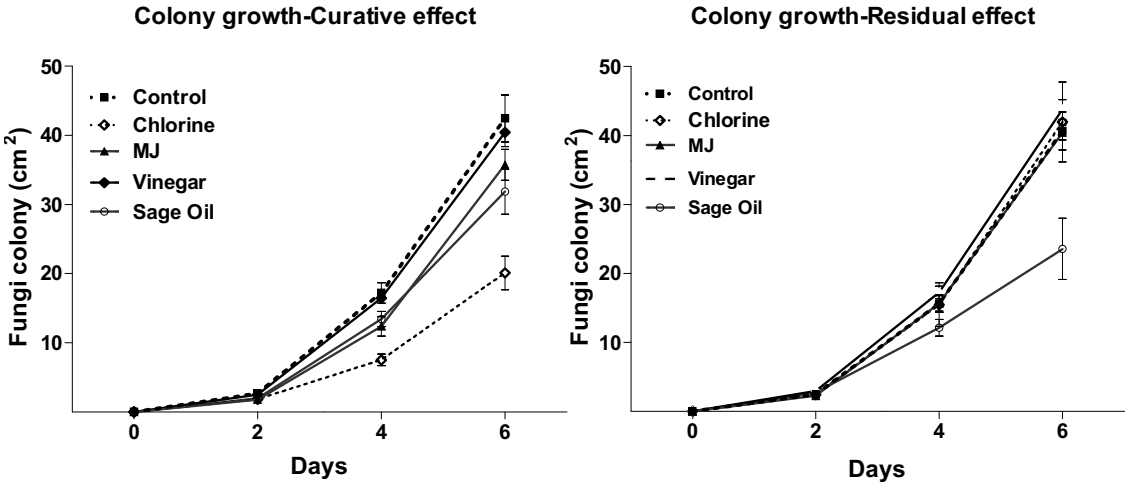

Fig. 2. Effect of curative or residual vapour enrichment of chlorine, methyl jasmonate, vinegar, or sage essential oil on colony growth (cm^2) of *Botrytis cinerea* grown on PDA. Values represent means (±SE) of measurements made on six independent plates per treatment.

actively growing edge of a 4–5 d old culture of *B. cinerea* and placed in each of the superficial wounds of the fruit. The following sub-experiments were conducted:

a) Pepper fruit inoculated with *B. cinerea* were placed in containers and exposed continually to AA or volatiles for 6 d or 12 d (examine curative effect).
b) Pepper fruit inoculated with *B. cinerea* were placed in containers and exposed to AA or volatiles for 6 d and then transferred to AA for an additional 6 d (examine residual effect due to partial exposure to volatiles).
c) Pepper fruit first exposed to AA or volatiles for 6 d, then removed from volatiles and inoculated with *B. cinerea* and transferred to AA for an additional 6 d (examine residual effect on fruit; no fungal exposure to volatiles).

Fumigation was performed in the dark at 11 °C, 95% RH. Lesion development (expressed in cm^2) was measured at the end of the experiments.

2.5. Impact of sanitary dips on grey mould development in wound-inoculated fruit

In sub-experiment II, fruit were dipped into natural compounds. In the first treatment, the fruit were inoculated with *B. cinerea*, incubated for 24 h at RT and then dipped into either the appropriate natural compounds or chlorine solution for 30 min, dried for 1 h at RT and transferred to AA for 12 d (curative effect). In the second treatment, fruit were dipped in the volatile solutions for 30 min, dried for 1 h at RT, inoculated with *B. cinerea*, incubated for 24 h at RT and transferred to storage conditions AA for 12 d (preventative effect).

Incubation was performed in the dark at 11 °C, 95% RH and lesion development (cm^2) was measured every 2 d, throughout the storage period. Both experiments were repeated twice.

2.6. Effect of volatile enrichment on spore production

The *B. cinerea* spore suspension was collected (as described in Tzortzakis, 2010) by harvesting the spores from inoculated PDA medium with an L-shaped spreader, concentrated and counted using a haemocytometer slide. Plates (PDA medium) were inoculated in the centre with 20 μL spore solution (2×10^4 spores mL^{-1}), transferred to containers and exposed to AA or volatiles (CHL, MJ, VIN or SAG) for 10 d (curative effect). In the second experiment, plates were first placed in containers, exposed to AA or volatiles for 6 d, and then inoculated with freshly prepared fungal suspension (preventative effect). In both experiments, the lids were removed from the plates during incubation. After exposure, the plates were closed and transferred to AA for 10 d (residual effect). Following exposure to volatiles or AA, spores were collected with an L-shaped spreader with 20 mL dH_2O (with Tween 80; 0.1% v/v) for 5 min and concentrated to a final 1 mL volume, as described by Tzortzakis (2010).

Wounded pepper fruit were inoculated with 15 μL spore suspension (2×10^4 spores mL^{-1}) of *B. cinerea* in each wound, placed in containers and exposed to AA or volatiles for 10 d until spores formed. In the second experiment, healthy fruit were exposed to AA or volatiles for 6 d, inoculated with *B. cinerea* (as above) and transferred to AA for 10 d until spores formed. Fumigation was performed in the dark at 11 °C, 95% RH. Following exposure to volatiles or AA, fruit were shaken for 20 min in 70 mL dH_2O [with Tween 80 (0.1% v/v) for better spore separation] to remove the spores. Spore suspensions were concentrated to a final volume of 1 mL. A haemocytometer slide was used for microscope quantification counts.

2.7. Effect of volatile enrichment on spore germination

Spore germination studies were carried out in several treatments:

a) Spores from 9 d old *B. cinerea* colonies were collected with an L-shaped spreader and inoculated on PDA medium (2–3 mm thick). Plates were placed in containers, the lids removed, and exposed to AA or volatiles (CHL, MJ, VIN, or SAG) for 24 h (curative effect).
b) Plates were placed in containers, the lids removed, and were exposed to AA or volatiles for 24 h and then inoculated with the fungal suspension. Following exposure, lids were replaced and plates were transferred to AA for 24 h (residual effect).
c) Spore suspensions were made from inoculated and volatile-exposed tomato fruits, inoculated on PDA medium and incubated in AA for 24 h in the dark at 11 °C, 95% RH. Measurements were recorded after 24 h incubation. Within each of the 6 replicates (4 sets of 16 squares; 44% of the haemocytometer slide was measured, in 2 different areas for the PDA medium or the fruit per replicate), 100 spores were examined and the percentage of germinated spores was calculated.

2.8. Statistical analysis

Values were presented as means ± standard errors (SE) from six independent values per treatment. These data were subjected to analysis of variance, and Duncan's Multiple Range test ($P < 0.05$) using IBM SPSS v.22 for Windows.

Table 1
Effect of curative vs. residual vapour enrichment of chlorine, methyl jasmonate, vinegar and sage essential oil on grey mould (*Botrytis cinerea*) spore germination and spore production *in vitro* on PDA medium. In each row, mean (±SE) values (n = 6) of plates for the individual vapour enrichment, followed by the same letter do not differ significantly at P = 0.05.

In vitro		Curative vapour enrichment*			
	Control	Chlorine	Methyl jasmonate	Vinegar	Sage oil
Spore production × 10^5 (mL^{-1})	93.9 ± 8.33^{ab}	105.1 ± 11.29^{a}	109.3 ± 17.19^{a}	64.5 ± 11.69^{b}	33.4 ± 4.18^{c}
Spore germination (%)	66.3 ± 5.09^{a}	59.4 ± 7.80^{a}	63.7 ± 3.13^{a}	64.7 ± 3.48^{a}	72.1 ± 5.34^{a}
		Vapour-induced residual effect**			
Spore production × 10^5 (mL^{-1})	142.8 ± 16.12^{a}	61.8 ± 8.43^{b}	73.7 ± 13.46^{b}	74.5 ± 19.36^{b}	82.6 ± 4.58^{b}
Spore germination (%)	87.1 ± 2.92^{a}	72.2 ± 8.23^{a}	75.7 ± 2.91^{a}	76.4 ± 6.77^{a}	86.6 ± 5.21^{a}

Treatments were maintained throughout at 11 °C and 95% RH.
* Curative vapour enrichment: plates were first inoculated with *B. cinerea* and then exposed to vapours.
** Vapour-induced residual effect: plates pre-exposed to vapours, inoculated with fungi and transferred to ambient air (control).

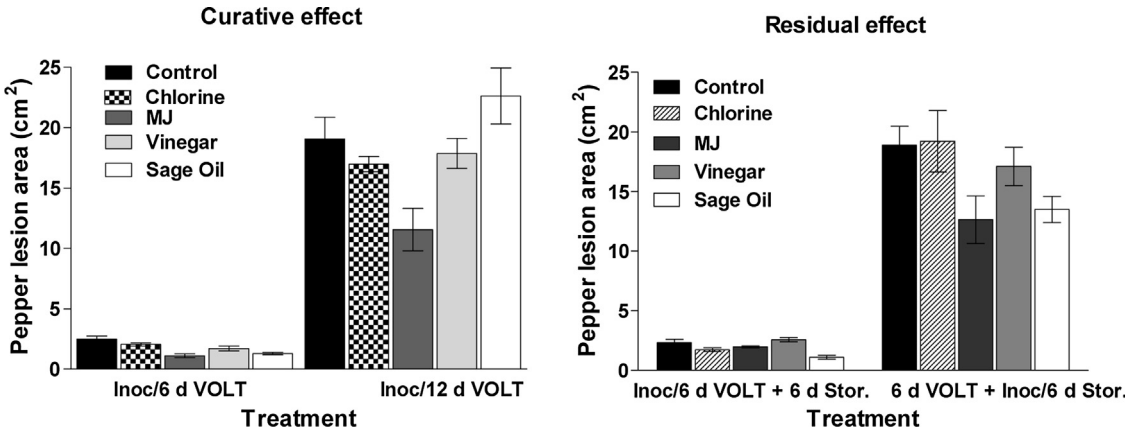

Fig. 3. Effect of curative or residual vapour (VOLT) enrichment of chlorine, methyl jasmonate, vinegar, or sage essential oil on colony growth (cm^2) of *Botrytis cinerea* grown on PDA. Values represent means (±SE) of measurements made on six independent plates per treatment.

3. Results

3.1. Impact of natural compounds on fungal biomass in vitro

B. cinerea biomass decreased (up to 26%) following SAG treatments of 10 μL L^{-1} and 50 μL L^{-1}, while a higher SAG concentration accelerated fungal growth (up to 39%, Fig. 1). The application of CHL, MJ and VIN increased fungal biomass for all the concentrations tested. However, spore production reduced as VIN concentration increased, while spores were not produced in MJ > 50 μL L^{-1} and SAG > 100 μL L^{-1} (data not presented; see Fig. 1S in the online version at DOI: 10.1016/j.postharvbio.2016.04.004).

3.2. Impact of volatile vapour enrichment on grey mould development in vitro

SAG and MJ vapour enrichment suppressed colony growth (vegetative phase) of *B. cinerea in vitro* (*i.e.* fungi were cultured before exposure to volatiles on PDA) following 4 and 6 d of incubation (Fig. 2). Chlorine treatment supressed colony growth up to 52% compared to the control. No differences were observed when VIN was used against *B. cinerea*. Similarly, in pre-exposed PDA with volatiles, SAG vapour enrichment suppressed fungal colony growth, while neither CHL, MJ nor VIG suppressed fungal colony growth (Fig. 2).

Spore production decreased in SAG vapour enrichments in comparison to the control (Table 1), while both SAG and VIN proved to be more effective sporulation inhibitors than CHL. When PDA was pre-exposed to vapours, spore production was suppressed (up to 48%) compared with the control. However, curative vapour treated spores (vapour pre-exposed PDA medium) did not affect spore germination (reproductive phase).

3.3. Effects of volatile vapour enrichment on anthracnose development in vivo

Fungal mycelial growth, measured as lesion area on pepper fruit, was affected during or following exposure to volatile vapour treatment (see Fig. 2S in the online version at DOI: 10.1016/j.postharvbio.2016.04.004). Moreover, in MJ- and SAG-treated fruit, lesion development decreased by 56% and 48% respectively compared with control fruit (maintained in AA throughout) at 6 d exposure, which was evident only for MJ-treated fruit (with a 39% reduction) after 12 d exposure (Fig. 3). In case of partial colony exposure to volatiles and subsequent transfer to AA, a lesion area reduction was observed only in wound-inoculated SAG-treated fruit for 6 d with an additional 6 d in AA. The lesion area was reduced (up to 29%) in pre-exposed fruits to SAG vapours for 6 d followed by inoculation with *B. cinerea* and then stored for 6 d in AA. However, no differences were observed in fruit pre-exposed to CHL-, VIN- and MJ-vapours for mycelial growth compared with the control group.

The effect of natural volatiles on the proliferation of fungal flora on pepper fruit are presented in Table 2. Interestingly, spore production significantly ($P < 0.05$) reduced during exposure of wound-inoculated fruit to MJ, VIN and SAG, being as effective as CHL when compared to the control (peppers exposed to AA throughout). In peppers pre-exposed to volatiles (residual effect), MJ, VIN and SAG treatments suppressed (up to 74%) spore production compared to the untreated peppers. Spore germination indicating spore viability was not influenced by vapour-enrichment during exposure, while a significant ($P < 0.05$) decrease was observed in spore viability on pre-exposed peppers to VIN- and SAG-vapours (Table 2).

3.4. Sanitary dips on grey mould development in vivo

Sanitary dips of fruit in MJ after wound-inoculation with the fungus increased lesion development, while no differences were found with CHL, VIN and SAG treatments. However, sanitary dips in MJ solution before wound-inoculation of pepper fruit with *B. cinerea* (residual effect) suppressed lesions by approximately 31%, whereas no differences were found for sanitary dips in CHL, VIN and SAG after 12 d at 11 °C (Fig. 4). In all sanitary dip treatments, fruit retained a negligible odour of the sanitary solution, but no fruit discolouration was taking place (data no presented).

4. Discussion

This work highlights the potential for using natural products for postharvest disease control of fresh fruit and vegetables. Natural volatiles reduced spoilage by grey mould during the vegetative (mycelial growth) and reproductive (spore production) phase of *B. cinerea* in pepper fruit, which is considered of great importance for disease control, by interfering with the fungal disease cycle. This was evident mainly for pepper fruit treated with SAG vapours, followed by MJ. The EO of *Salvia officinalis* has also shown practical potency in enhancing the storage life of some vegetables and fruits posing antifungal and antibacterial activities (Tornuk et al., 2011; Lopez-Reyes et al., 2013). It has been reported that MJ vapour or emulsion inhibited microbial contamination of fresh-cut celery and peppers (Buta and Moline, 1998), grey mould in strawberries (Moline et al., 1997), green mould in grapefruit (Droby et al., 1999), anthracnose rot in tomatoes (Tzortzakis, 2007b) and Botrytis rot in

Table 2
Effect of curative vs. residual vapour enrichment of chlorine, methyl jasmonate, vinegar and sage essential oil on grey mould (*Botrytis cinerea*) spore germination and spore production *in vivo* (pepper fruit). In each row, mean (± SE) values (n = 6) of fruits (two inoculations per fruit) for the individual vapour enrichment, followed by the same letter do not differ significantly at P = 0.05.

In vivo		Curative vapour enrichment*			
	Control	Chlorine	Methyl jasmonate	Vinegar	Sage oil
Spore production × 10^5 (mL^{-1})	24.4 ± 7.80^a	11.5 ± 3.73^b	9.1 ± 3.38^b	9.8 ± 2.76^b	12.1 ± 3.12^b
Spore germination (%)	99.5 ± 0.28^a	97.2 ± 1.31^a	98.0 ± 1.68^a	95.2 ± 1.57^a	96.9 ± 2.06^a
		Vapour-induced residual effect**			
Spore production × 10^5 (mL^{-1})	3.1 ± 0.96^a	2.4 ± 0.92^{ab}	1.1 ± 0.41^b	0.8 ± 0.19^b	1.1 ± 0.12^b
Spore germination (%)	99.7 ± 1.93^a	99.2 ± 0.25^a	98.5 ± 0.86^a	95.0 ± 2.04^b	95.7 ± 0.94^b

Treatments were maintained throughout at 11 °C and 95% RH.
* Curative vapour enrichment: fruits inoculated with *B. cinerea* and then exposed to vapours.
** Vapour-induced residual effect: fruits pre-exposed to vapours, inoculated with fungi, transferred and stored in ambient air.

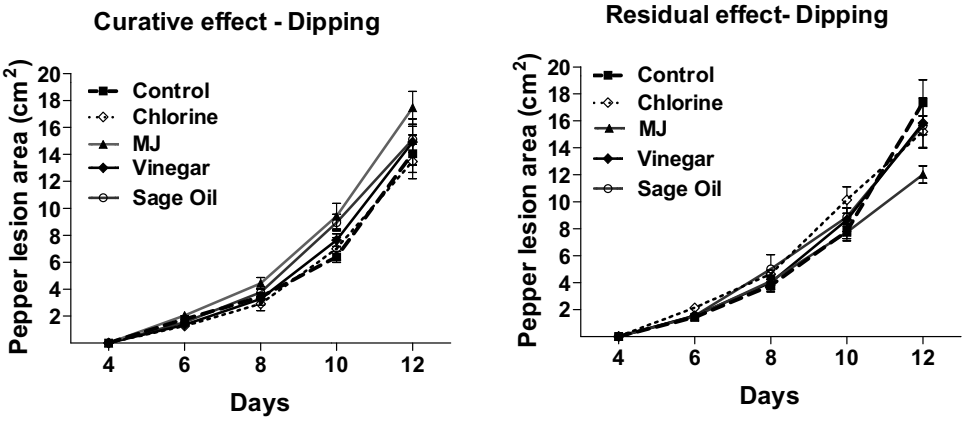

Fig. 4. Effect of curative or residual dipping-enrichment of chlorine, methyl jasmonate, vinegar, or sage essential oil on lesion growth (cm^2) of *Botrytis cinerea* grown on pepper fruits at 11 °C and 95% RH. Values represent means (±SE) of measurements made on six independent fruits per treatment.

cut rose flowers (Meir et al., 1998). Fumigation of sweet cherries with thymol was effective in controlling rots caused by *B. cinerea* (Chu et al., 1999) and *M. fructicola* (Chu et al., 2001) during storage. Application of MJ as a co-fumigant with thymol or carvacrol decreased the brown rot decay in sweet cherries (Tsao and Zhou, 2000) and inhibited aflatoxin production by *Aspergillus flavus* (Goodrich-Tanrikulu et al., 1995). Moreover, MJ application could reduce chilling injury and extend storage period of fresh products by inducing the accumulation of produce heat shock proteins, as correlated with the protection against chilling injury (Ding et al., 2001).

The viability of spores from wound-inoculated pepper fruit was affected to a lesser extent compared with spore production. It was notable that the benefits related with volatile enrichment was maintained in fruit pre-exposed to SAG oil vapours, resulting in lesion growth suppression with direct effects on the fungal reproductive stage (spore germination/production). These responses indicated that vapour treatments may exert a residual effect, potentially through fruit priming in response to subsequent challenge (Conrath et al., 2002) and possibly developing defence-related mechanisms in fruit (Farmer, 1994). These findings are in agreement with previous reports on apple (*Malus pumilo*) and on tomato, highlighting the beneficial effects of lemongrass oil (*Cymbopogon flexuosus*) or oregano oil (*Origanum vulgare*) and MJ, respectively, against fruit rotting (Shahi et al., 2003; Tzortzakis, 2007b, 2010; Wang et al., 2015). Molecular base studies would be valuable to understand the secondary metabolisms and putative mechanisms involved. It has been suggested that jasmonate and EO treatments probably reduced microbial decay in fruit indirectly by enhancing the natural resistance of the fruit to the pathogen (Droby et al., 1999; Moline et al., 1997; Tzortzakis, 2007b; Wang et al., 2015) and triggering increased accumulation and expression of pathogenesis-related (PR) proteins (chitinases and glucanases), heat shock proteins (Ding et al., 2001; Wang et al., 2015) and antioxidative status, ascorbate and phenolic concentrations shown by fruit (Tzortzakis and Economakis, 2007).

Considering the effectiveness of natural product applications in this study, fungal growth accelerated during fruit dipping application in wound-inoculated pepper fruit, while sanitary dips in MJ solution before wound-inoculation suppressed *B. cinerea* lesion development. Indeed, sanitary dips were less effective in fruit sanitation compared to vapour application, whereas fruit dipped in natural products retained a slight odour of the sanitary solution. The MJ application (dipping or vapour fumigation) effectively inhibited re-growth of tops and roots of harvested radishes (*Raphanus sativus*), improving quality maintenance (Wang, 1998).

Jasmonic acid and derivatives such as MJ have been described as signaling compounds that stimulated the expression of wound-inducible and defense-related genes, as well as involvement in many developmental processes in plants (Farmer, 1994). Several reports underline the beneficial effects of MJ in fruit quality (Gonzalez-Aguilar et al., 2003; Wang, 2003; Tzortzakis, 2007b). In another study by Thanassopoulos and Yanna (1997) on sanitary dip treatment of kiwi (*Actinidia chinensis*) fruit against *Botrytis*, 500 $\mu L L^{-1}$ aqueous solution of oregano oil had some effect on fungus growth in the fruit flesh, but reduced fruit quality severely.

Low SAG concentrations (up to 50 $\mu L L^{-1}$) decreased fungal biomass, while a concentration increase was less effective, possibly due to alteration of the culture medium, which was confirmed by

the pre-exposed PDA medium to vapours. Further examination might be useful for the examined concentrations/duration.

The mode of action of volatiles on fungal spores is poorly understood. The fungitoxic potential of EOs is possibly correlated with the joint action of several constituents present in the oils (Scardavi, 1966), hence the enhanced effectivity of EOs as such, compared with that of its main constitute only. This complexity of EO constituents could prevent pathogens from building up resistance, a valuable postharvest storage benefit. More research is required on the synergistic action of plant products, both *in vitro* and *in vivo*. Limited information was found on the mode of action of EOs when used as postharvest fungitoxicants (Tripathi and Dubey, 2004).

Decreased spore production mainly, but also spore germination to a lesser extent, as featured in the present study, would suppress fungal proliferation, making natural products a valuable postharvest sanitation tool and alternative to commercial chlorine. Essential oils, which have been registered as food additives, are much easier to register for postharvest use than new synthetic pesticides. The US Food and Drug Administration lists thymol, thymol EO and thyme (spice) as food, as well as food additives. Thymol was initially registered as a pesticide in the US in 1964 (Tripathi and Dubey, 2004). During EO application, an optimised concentration, duration and method of exposure are important, in order to prevent tainting of the product. Fresh produce thickness is correlated to tainting, thicker fruit skins being less prone to tainting.

Natural volatiles maybe considered for postharvest disease control during storage and/or transit. These compounds are not as effective on a broad spectrum as synthetic pesticides, but their effectivity can be improved by using them in combination with specifically designed packaging, as no detectable residues (a growing concern as current sanitation techniques are mainly chlorine-based) are left on the surface, with the potential to improve fruit quality and storage of fresh produce. Application of natural products *via* the vapour phase should also be more cost-effective than dipping.

5. Conclusion

The current study highlights the promising utilization of natural volatiles for postharvest disease control, both in vegetative and reproductive phase. SAG-, followed by MJ volatiles may be considered as alternatives to the traditional fresh produce preservation techniques, with curative and residual effect. Vapour application proved to be more effective in fruit sanitation compared to sanitary dips. Each commodity needs to be individually assessed, and the volatile concentration and sanitising technique optimised, before commercial use.

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