New methods of post-harvest disease control: Using thyme oil fumigation

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ABSTRACT

Avocado fruit has high economic value; however, major postharvest losses are encountered throughout the supply chain, mostly due to anthracnose (*Colletotrichum gloeosporioides*) and stem-end rot (*Lasiodiplodia theobromae*).

Antifungal activity of 20 essential oils was tested under this investigation during 2014. An *in vitro* study revealed that in the vapour phase, thyme oil at 5 µL plate^{-1} inhibited the radial mycelia growth of *C. gloeosporioides* and *L. theobromae* and revealed fungicidal activity. Thyme oil vapour at 66.7 µL L^{-1} significantly reduced the anthracnose and stem-end rot in artificially wounded and infected fruit (curative treatment).

During preventative treatment, freshly harvested avocados 'Carmen®-Hass' (15-29 April 2014 from Wes36B with 75.7-74.4% moisture content [Mc]), 'Hass' (15-28 April from Wes36B with 75.6-72.2% Mc; 4 Sept from Evenrond 64.1% Mc) and 'Ryan' (15 July from Westfalia [Fowey] with 67.4% Mc; 4 Sept from Groenfontein with 67.7% Mc) were fumigated with thyme oil (96 µL L^{-1}) for 24 hours at 20°C (ambient temperature) and thereafter, inoculated with 10^6 spores' mL-1 and incubated at 20°C for 5 to 6 days.

The untreated fruit and the commercial prochloraz treatments were included for comparison. The results indicated that the thyme oil treatment (fumigation) significantly reduced the anthracnose incidence ~ 65% compared with the untreated control fruit and by 23% in comparison to the prochloraz treatment in all three cultivars. Furthermore, the disease severity (lesion diameter) was significantly reduced in all three cultivars subjected to thyme oil treatment. In addition, the fruits subjected to thyme oil treatment were firmer than the prochloraz treated fruits. The gas chromatograph/mass spectrometry analysis showed the presence of thymol (58.77% RA).

Therefore, these results suggest that thyme oil vapour could be used as an alternate natural fungicide to control the anthracnose disease in avocado. However, further investigations need to be conducted with cv. Fuerte, as well as the effect on the stem-end rot pathogen (*L. theobromae*). The effect of thyme oil on overall quality, especially sensory quality and ripening patterns, also needs to be investigated.

INTRODUCTION

The avocado fruit is highly nutritious being rich in oleic, palmitic, linoleic and palmitoleic acids, vitamins A, B, C, E, K, minerals, potassium, phosphorus, magnesium and iron (Lu *et al*., 2009).

In South Africa, the avocado production is mainly aimed at exporting the fruit to the overseas markets such as the European Union and the United States (Bill *et al*., 2014). The largest market for South Africa is the European Union which requires high fruit quality standards. Major postharvest losses are encountered throughout the supply chain, mostly due to anthracnose (*Colletotrichum gloeosporioides Penz.*) and stem-end rot (*Lasiodiplodia theobromae*). Both field spraying and postharvest treatments are necessary to achieve quality fruit. Copper sprays are commonly used in the orchard to control postharvest diseases of avocado. A synthetic non-systemic fungicide, prochloraz, is used as a first defence mechanism in the packing line to control anthracnose, a common commercial pack house treatment adopted in South Africa. The development of acidified prochloraz treatments has been shown to reduce the concentration of active prochloraz (Mavuso & Van Niekerk, 2013),

but the disposal of the low pH solution remains a problem. Apart from the above mentioned challenges, a commercial wax coating, Avoshine® Carnauba Wax, is used for green-skinned avocados. If the proper wax formulation and application methods are not employed, green-skinned cultivars may develop surface discolouration; it is essential that the applied wax coating must not leave any deleterious residues or affect the natural glossiness of the fruit (Kruger, 2013) or the eating quality, or alter the characteristic fruit flavour.

In addition, an increase in consumer concern regarding food safety and demand for organically produced fruit have brought a need for safer methods to control postharvest decay development. Furthermore, the importing countries have enforced stringent regulations regarding the maximum residue limits (MRL). In addition to this, development of fungicide resistant strains and growing global pressure on the fruit industry to reduce the associated environmental pollution footprint have necessitated the search for natural novel products to replace the prochloraz fungicide application at postharvest stage.

Meanwhile, essential oils are considered to be a promising alternative with many having antifungal properties (Feng & Zheng, 2007). Essential oils and its components are gaining interest because of their relatively safe status, its wide acceptance by consumers and its exploitation for potential multipurpose functional use (Sawamura, 2000). A major advantage of essential oils is its bioactivity during the vapour phase, a characteristic which renders it attractive as a possible fumigant for the protection of stored products (Dixit *et al*., 1995). This study was therefore carried out to investigate 1) the antifungal effects of different essential oils on *C. gloeosporioides* and *L. theobromae* (*in vitro*), as well as 2) the effect of fumigation on decay inhibition in artificially *C. gloeosporioides* inoculated avocado fruits (*in vivo*) cv. Carmen®-Hass, Hass and Ryan.

MATERIALS AND METHODS

Pathogen

C. gloeosporioides was obtained from the Fruit and Vegetable Technology Laboratories of Tshwane University of Technology, South Africa, and *L. theobromae* was obtained from Westfalia Fruit (Limpopo Province, South Africa). The *C. gloeosporioides* and *L. theobromae* isolates were cultured and maintained on potato dextrose agar (PDA) (Merck, Johannesburg, South Africa) and incubated at 25°C for 12 to 13 days. A spore suspension was prepared according to standard procedures and the mycelia fragments were removed from the suspension by filtering through three layers of muslin cloth. The spores were counted using a haemocytometer and adjusted to 1 x 10^5 spore mL^{-1}.

In vitro screening of essential oils

The essential oils (20) including thyme, clove bud, cinnamon, basil, camphor and ginger were tested for antifungal activity *in vitro* by adopting a disc volatilisation method (Dafarera *et al*., 2000). The experiments were conducted in Petri plates (80 mm in diameter) of PDA (10 mL $plate^{-1}$), inoculated with 6 mm plugs from cultures (7 days old). The plates were kept in an inverted position. A sterilised filter paper disc (diameter 80 mm) was placed in the lid and different volumes (5-10 µL $plate^{-1}$) of essential oil were added to the paper. A blank plate served as the control sample. Five replicates were used per treatment. The plates were tightly sealed with parafilm and incubated for 7 days at 25°C. Radial mycelial growth was measured by measuring the colony diameter along the two axes at right angles to each other using a Vernier calliper (Digimatic; Itutoyo Co., Japan) in mm on a daily basis until the control Petri dishes were fully covered (7 days) with mycelia. The fungi toxicity was expressed as a percentage inhibition of radial mycelial growth (IMG %) using the formula according to IMG (%) = [(dc - dt)/dc] x 100, where dc and dt are the radial mycelial growth measurements in the control sample.

In order to distinguish between fungicidal and fungistatic activity of the selected essential oil treatment against the *C. gloeosporioides* and *L. theobromae*, the mycelia discs that did not show any growth were transferred to a freshly poured PDA plate and incubated for 7 days at 25°C to observe the recovery of the growth. This evaluation was carried out in terms of mycelial growth in millimetres (mm) (Table 1). The fungicidal effect was classified as an absence of growth, whereas any observed growth was classified as fungistatic. The essential oil treatment that showed fungicidal effects on *C. gloeosporioides* and *L. theobromae* were further investigated regarding the control of postharvest disease in artificially infected fruit.

Inoculation and measurement of disease progress

Freshly harvested, unblemished avocado fruits of cv. Carmen®-Hass (15-29 April 2014 from Wes36B with 75.7-74.4% moisture content [Mc]), Hass (15-28 April from Wes36B with 75.6-72.2% Mc; 4 Sept from Evenrond with 64.1% Mc) and Ryan (15 July from Westfalia [Fowey] with 67.4% Mc; 4 Sept from Groenfontein with 67.7% Mc) were obtained from Westfalia Fruit, Limpopo Province, South Africa. Fruit at the correct stage of maturity were selected according to a finger feel firmness score 2 (1 = hard, 2 = slightly soft, just starting to ripen, 3 = very soft) (Sellamuthu *et al.*, 2013). Thereafter, the surface was sterilised by dipping the fruit in sodium hypochlorite (0.01%) for 5 min and air-dried at room temperature. Subsequently, the avocado fruits were exposed to (i) the commercial treatment (prochloraz), (ii) thyme oil fumigation, and iii) sterile distilled water dip (untreated control). The thyme oil fumigation treatment was performed as follows: The thyme oil concentration was selected in the *in vitro* experiment (5 µL $plate^{-1}$) based on the minimal inhibitory concentration. The thyme oil (960 µL) calculated proportionally to the volume of the container was introduced into a 10 L translucent plastic container

(90% RH) by placing the thyme oil in a Petri plate (65 mm in diameter) lid inside the container of the fruits. Ten avocado fruit were carefully placed in each container so as to avoid contact between the fruit and the thyme oil. The container was immediately sealed with a slip on lid to start the fumigation process. The fruits were exposed to thyme oil vapour for 24 hours at 20°C and thereafter placed on sterile paper towel on bench tops for inoculation (after 0.5 h). The fruit inoculation was performed according to Sellamuthu *et al*. (2013) by uniformly wounding (2 mm deep x 6 mm wide) the fruit with a sterilised cork-borer and inoculating them with 20 µL of a spore suspension of *C. gloeosporioides* (10^5 spores' mL^{-1}) at the equatorial region. Inoculated and treated fruits were packed in standard corrugated cardboard cartons and held at ca. 20°C for five days. Five replicate boxes each containing 14 fruits was used for each treatment. The experiment was repeated twice. Observations of disease incidence and severity (lesion diameter in mm) were recorded at the end of the storage time (5 days). The disease incidence was determined according to Sellamuthu *et al*. (2013).

Flesh firmness was determined on two points at the equatorial point of the fruit using a Chatillon penetrometer, Model DFM50 (Ametek, Largo, Florida, USA), with an 8 mm diameter flat-head stainless steel cylindrical probe (puncture method) (Woolf *et al*., 2005) after five days and the results were reported in terms of kilograms (kg).

Statistical analysis

A complete randomised design was adopted in this study. Data of the experiment was analysed with the General Linear Models (GLM) procedure in the SAS (Statistical Analysis System) computer program (SAS Enterprise Guide 4.0; SAS Institute, 2006, Cary, NC). Means were separated by LSD (5%). All the experiments were repeated twice.

RESULTS AND DISCUSSION

Amongst the screened essential oils, thyme oil at 5 µL $plate^{-1}$ completely inhibited the radial mycelial growth of *C. gloeosporioides* and *L. theobromae* (Fig. 1). Arrebola *et al*. (2010) indicated that thyme oil directly inhibits pathogen growth and spore germination by affecting the active sites of enzymes and cellular metabolism. Furthermore, thyme oil also revealed fungicidal activity against the two pathogens (Table 1). During curative treatment, thyme oil fumigation significantly reduced the incidence of anthracnose and stem-end rot by approximately 74% and 66% in cv. Hass (Fig. 2). Thyme oil fumigation as a preventive treatment significantly reduced the percentage of anthracnose disease incidence in cv. Carmen®-Hass, Hass and Ryan avocado cultivars compared with the untreated control fruits. Also, the effectiveness of thyme oil treatment in anthracnose control was greater than the currently used prochloraz treatment during both trials as illustrated in Figure 3. The thyme oil fumigation treatment also significantly ($p < 0.05$) reduced the disease severity compared to both the prochloraz and untreated control treatments (Fig. 4). Thyme oil fumigation was also reported to significantly ($p < 0.05$) reduce the percentage of decayed fruit in cherry tomatoes compared to the control sample (Feng *et al*., 2011). Thyme oil has also been reported to possess the ability to act as a 'signalling compound' as methyl jasmonate vapours that triggers a signal similar to that of a mild stress condition in the fruit. In this investigation, it is therefore clearly evident that the thyme oil fumigation treatment consistently reduced the incidence as well as the severity of anthracnose in avocados compared to the currently commercially used prochloraz fungicide treatment.

Loss of firmness affects the fruit quality during marketing. Significantly ($p < 0.05$) higher fruit firmness (1.28, 1.21 and 1.01 kg) was retained in fruits fumigated with thyme oil followed by the prochloraz fungicide (1.0, 0.96 and 0.76 kg) treatments in cv. Carmen®-Hass, Hass and Ryan

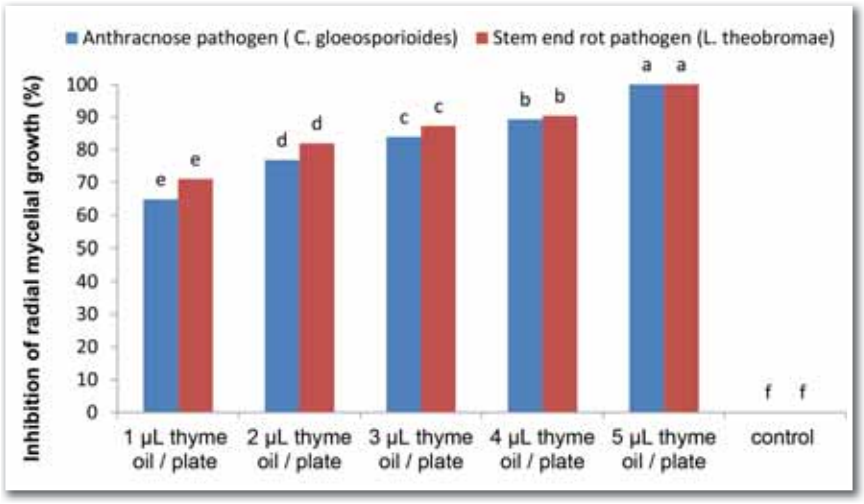

Figure 1. Effect of thyme oil fumigation treatment on radial mycelia growth of *C. gloeosporioides* and *L. theobromae* (*in vitro*).

Table 1. The fungicidal or fungistatic effect of thyme oil on *C. gloeosporioides* and *L. theobromae (in vitro)*.

	% Radial mycelial growth	
	Anthracnose pathogen *C. gloeosporioides*	Stem-end rot pathogen *L.theobromae*
Control	90±0.23a	80±0.3a
Exposed to 5 µL $plate^{-1}$ thyme oil for 7 days and transferred to freshly poured PDA	0.0±0.0b*	0.0±0.0b*

avocado fruit respectively (Fig. 5). The untreated control fruits showed the lowest firmness values of 0.81, 0.76 and 0.48 kg (cv. Carmen®-Hass, Hass and Ryan respectively) as a result of excessive softness induced by pathogen infection.

In conclusion, the thyme oil fumigation (24 h) at 25°C offers great practical potential in reducing the anthracnose incidence during the postharvest supply chain. In future, the application of thyme oil fumigation treatment should also be tested in naturally infected fruit in order to provide an effective decay control measure to the avocado fruit industry. Further work should be carried out to assess the effect of thyme oil fumigation on sensory and visual characteristics of the fruit, as well as cost benefit analysis. In addition, the residual effect of the thymol content (active ingredient of the thyme oil) on the fruit skin needs to be clarified.

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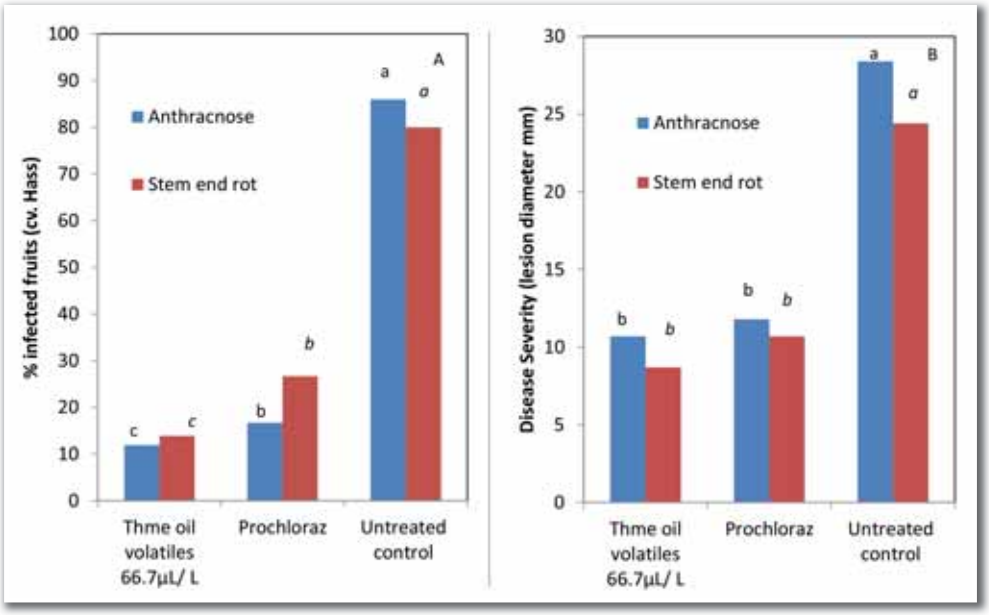

Figure 2. Effect of thyme oil fumigation treatment on the incidence (A) and severity (B) of anthracnose and stem-end rot in artificially inoculated avocado fruit cv. Hass.

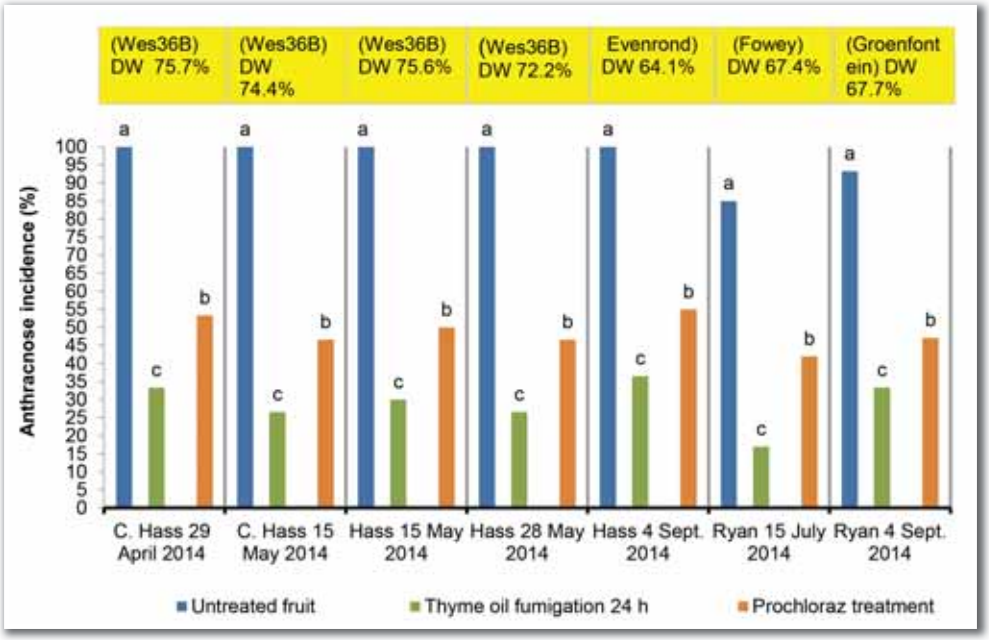

Figure 3. Effect of thyme oil fumigation treatment on the incidence of anthracnose in artificially inoculated avocado fruit cv. Carmen®-Hass, Hass and Ryan.

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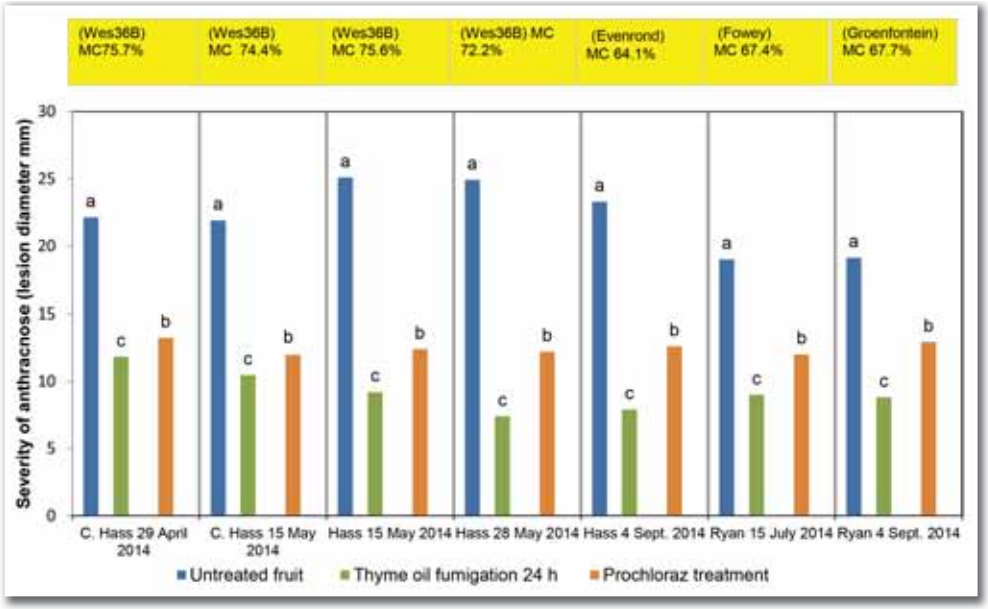

Figure 4. Effect of thyme oil fumigation treatment on the severity of anthracnose in artificially inoculated avocado fruit cv. Carmen®-Hass, Hass and Ryan.

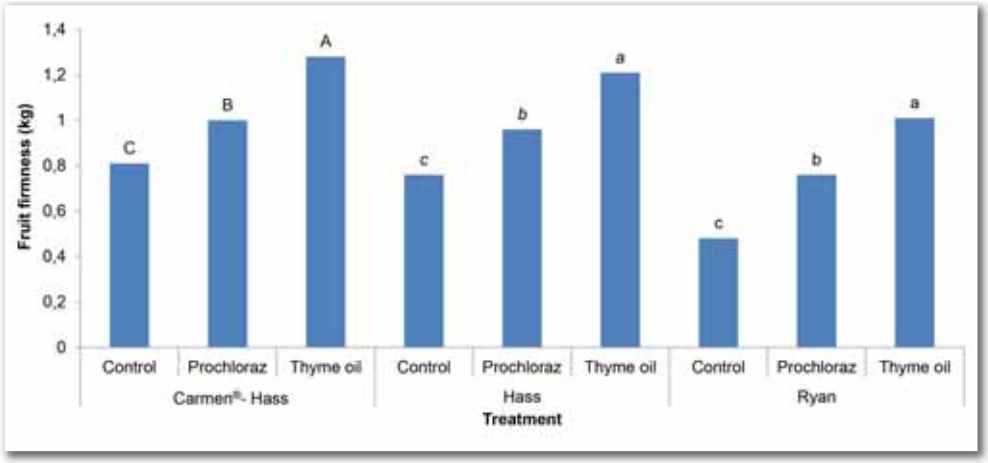

Figure 5. Effect of thyme oil fumigation treatment on the fruit firmness in artificially inoculated avocado fruit cv. Carmen®-Hass, Hass and Ryan.