

Using plant volatiles in biodegradable sachets and exposure of volatiles under pallet covers to control postharvest decay in avocados

M Bill^{1,2}, PC Obianom¹, L Korsten², Z Mavuso³,
Z van Rooyen³ and D Sivakumar¹

¹Postharvest Technology Group, Department of Crop Sciences
Tshwane University of Technology, Private Bag X680, Pretoria 0001, SOUTH AFRICA

²Plant Sciences, University of Pretoria
Private Bag X20, Hatfield 0028, SOUTH AFRICA

³Westfalia Research Technologies
Westfalia, Tzaneen, SOUTH AFRICA
E-mail: sivakumarD@tut.ac.za

ABSTRACT

Anthrachnose caused by *Colletotrichum gloeosporioides* Penz. is the predominant postharvest pathogen resulting in severe postharvest losses during the supply chain. A synthetic non-systemic fungicide, prochloraz, is used for postharvest application to control anthracnose. An increase in consumer concern regarding food safety has necessitated the development of safer methods to control postharvest decay. In our previous investigations, the volatile phase of thyme oil was selected as a suitable plant volatile to control anthracnose in avocados during postharvest storage. Therefore, thyme oil was administered during commercial application in the currently used tray-packs in the volatile phase by using thyme oil impregnated polylactic acid sachets at 10% and 5% concentrations. Thyme oil (10%) polylactic acid sachets significantly reduced the anthracnose incidence and severity in artificially inoculated fruit (cv. Hass) after 12 days at 10°C and thereafter 24 h at 20°C. However, in naturally infected fruit both anthracnose and stem-end rot diseases were controlled completely with thyme oil (10%) polylactic acid sachets and prochloraz (half strength) after 5 days at 10°C and thereafter 5 days at 20°C (ready to eat programme). Thyme oil (10%) polylactic acid sachets and prochloraz (half strength) treatment retained the appropriate firmness for ready to eat fruits.

With a view to controlling anthracnose during large volume shipment, 20 cartons of naturally infected cv. Ryan stacked in a pallet (L = 1.3 m, W = 0.85 m, H = 0.95 m) were exposed to thyme oil vapours (78.9 mL) at 7.5°C (pack house holding temperature) and also at 25°C (room temperature) for 24 h under a pallet cover to determine the effectiveness of the treatment during the laboratory trial. Thereafter, fruits were removed from the pallet cover and stored at 7.5°C and 85% RH for 21 days and at 15°C for 5 days to simulate the market shelf conditions. Fruits exposed to thyme oil vapours at 7.5°C for 24 h showed significant control of anthracnose. The sensory properties of the fruits were not altered due to the exposure to thyme oil vapours. Effective control of anthracnose at postharvest stage can be only achieved, however, if good orchard sanitation practices are adopted.

In addition to the above, different defence inducers and combinations of plant volatiles in the liquid phase were tested on infected fruits to control anthracnose and stem-end rot during postharvest storage. This application can be used in dipping tanks at the pack house. The preliminary investigations suggest that the following applications, thyme oil (0.1%) and half strength prochloraz, Y-extract and half strength prochloraz, reduced the anthracnose and stem-end incidence to 50% in infected fruits. Alternatively, citral and half strength prochloraz could be considered as a favourable treatment to control anthracnose and stem-end decay.



INTRODUCTION

Avocado is a nutrient rich fruit, having high contents of lipophilic, bioactive phytochemicals including vitamin E, carotenoids and sterols that display antioxidant and radical scavenging activities (Torres *et al.*, 1987). Although the South African avocado industry is export-oriented, the local market still plays an important role in the industry. Recently there has been a strong growth in sales of avocados that are sold ripe and ready to eat in the local market. Anthracnose caused by *Colletotrichum gloeosporioides* Penz. and stem-end rot caused by *Lasiodiplodia theobromae* are the predominant postharvest pathogens resulting in severe postharvest losses during the supply chain.

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 – a common commercial pack house treatment adopted in South Africa – is used as the first defence mechanism in the packing line to control anthracnose. To date, several studies have been done to either reduce the amount of the active prochloraz by using acidified solutions (Mavuso & Van Niekerk, 2013) or find a total replacement by using a commercial Avoshine® canuba wax coating for green-skinned avocados (Kruger, 2013). However, each method was accompanied by certain disadvantages that restrict its adoption as a commercial alternative.

An increase in consumer concern regarding food safety and demand for organically produced fruit have created 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, the 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.

Essential oils and natural plant volatiles are considered to be a promising alternative with many having antifungal properties (Feng & Zheng, 2007). Essential oils, as well as plant volatiles and their components, are gaining interest because of their relatively safe status, their wide acceptance by consumers and their exploitation for potential multi-purpose functional use (Sawamura, 2000). A major advantage of these compounds is their bioactivity during the vapour phase, a characteristic which renders them attractive as possible fumigants for the protection of stored products (Dixit *et al.*, 1995). On the other hand, essential oils such as eugenol, menthol or thymol in combination with modified atmosphere packaging (MAP) were reported to maintain quality and safety by reducing microbial spoilage in sweet cherry (Serrano *et al.*, 2005) and 'Crimson' table grapes (Valverde *et al.*, 2005). Sellamuthu *et al.* (2013) also indicated that the addition of thyme oil impregnated

pads to MAP had a further positive impact on the reduction of anthracnose in cold stored mature and unripe avocados after ripening. Since plant volatiles can be effective when applied in small amounts, they could possibly be used in cocktail solutions to control decay incidence in avocado fruit. 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. Alternatively, a combination of reduced concentrations of the active prochloraz and essential oil/plant volatile solutions might help control decay in avocados. This study was therefore carried out to investigate 1) the effect of thyme oil impregnated polylactic acid sachets on decay inhibition in artificially inoculated (*C. gloeosporioides*) fruit, as well as naturally infected fruit cv. Hass; 2) the effect of thyme oil vapour on decay inhibition in naturally infected avocado fruits cv. Ryan in a large volume (simulated shipment) setup; and 3) the effects of selected defence inducers on disease development (anthracnose and stem-end rot) in artificially infected avocado fruits cv. Hass.

MATERIALS AND METHODS

Trial 1: Artificially inoculated fruits

Freshly harvested, unblemished avocado fruit of cv. Hass were obtained from Bassan packers (Limpopo Province, South Africa). Fruits 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). The fruits were trigger ripened at room temperature until they had reached a finger feel firmness of approximately 2 and thereafter, surface sterilised by dipping in sodium hypochlorite (0.01%), for 5 min and air-dried at room temperature (~20°C). The fruits were then placed on a sterile paper towel on the bench tops for inoculation. Fruit inoculation was performed according to Sellamuthu *et al.* (2013), by uniformly wounding with a sterilised needle (1 mm x 1 mm) and inoculating with 20 µL of a spore suspension of *C. gloeosporioides* (10^5 spores mL⁻¹) at the equatorial region and left for 3 hours to initial infection. Subsequently, the fruits were placed carefully in the punnets, 4 fruits in each punnet. For the TO sachet treatments, each sachet was positioned at the centre of the punnet to avoid contact between the fruit and the sachet (Fig. 1). Thereafter, the punnets were sealed with micro perforated cling film to capture the released volatiles in the headspace of the punnet. Treatments included: 1) the commercial treatment (prochloraz 0.05% for 5 min dip) packed in commercial tray-packs (P); 2) sterile distilled water dipped fruit packed in commercial tray-packs (untreated control) (C); 3) 5% TO polylactic acid sachet and packed in commercial tray-packs (5TO_Pol); 4) 10% TO polylactic acid sachet and packed in commercial tray-packs (10TO_Pol); and neat polylactic acid sachet and packed in commercial tray-packs (Pol). Each treatment had ten replicate



punnets, each containing four fruits. The experiment was repeated twice. The fruits were cold stored at 10°C for 10 days. Soon after storage the punnets were opened and stored at room temperature (~ 20°C) for 24 hours. Observations on disease incidence and severity (lesion diameter in mm) were recorded. The disease incidence was determined according to Sellamuthu *et al.* (2013).

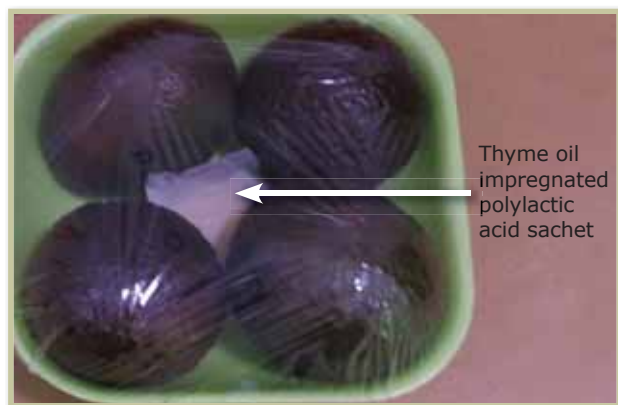


Figure 1. Avocado packaging with thyme oil impregnated poly(lactic acid) sachets.

Trial 1: Naturally infected fruits

Naturally infected fruits obtained from Westfalia, at commercial maturity, were exposed to 'triggered ripening' at room temperature for three days as above. For all the prochloraz dipped fruit, either as a standalone (full strength) treatment or a combination treatment (half strength) with either of the TO poly(lactic acid) sachet treatments, the prochloraz was applied soon after harvesting as a commercial standard procedure. Immediately after triggered ripening, the fruits were placed carefully in the punnets, four fruits in each punnet. For the TO poly(lactic acid) sachet treatments or the combination treatments, each sachet was positioned at the center of the punnet to avoid contact between the fruit and the sachet. Thereafter, the punnets were sealed with micro perforated cling film to capture the released volatiles in the headspace of the punnet. Treatments included: 1) the commercial treatment (prochloraz 0.05% for 5 min dip) packed in commercial tray-packs (P); 2) sterile distilled water dipped fruit packed in commercial tray-packs (untreated control) (C); 3) 5% TO poly(lactic acid) sachet and packed in commercial tray-packs (5TO_Pol); 4) 10% TO poly(lactic acid) sachet and packed in commercial tray-packs (10TO_Pol); prochloraz (50%) dip and 5% TO poly(lactic acid) sachet and packed in commercial tray-packs (50%P + 5TO_Pol); and prochloraz (50%) dip and 10% TO poly(lactic acid) sachet and packed in commercial tray-packs (50%P + 10TO_Pol). Each treatment had ten replicate punnets, each containing four fruits. The experiment was repeated twice. The fruits were cold stored at 10°C for 5 days. Soon after storage the punnets were opened and stored at RT for 5 days to allow further ripening (ready to eat programme). Observations on disease incidence,

severity, fruit firmness and sensory properties were recorded.

Trial 2:

Freshly harvested, unblemished avocado fruits of cv. Ryan were obtained from Bassan packers (Limpopo Province, South Africa). Fruits 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). Twenty cartons of naturally infected cv. Ryan fruit (count 14-18) stacked in a pallet (L = 1.3 m, W = 0.85 m, H = 0.95 m) were exposed to thyme oil vapours (78.9 mL) at 7.5°C (pack house holding temperature - CS) and also at 25°C (room temperature - RT) for 24 hours under a pallet cover to determine the effectiveness of the treatment during the laboratory trial. Thereafter, fruits were removed from the pallet cover and stored at 7.5°C and 85% RH for 21 days and at 15°C for 5 days to simulate the market shelf conditions.

Trial 3:

Previously, citral, thyme oil and Y-extract in combination with half strength prochloraz were selected based on their effectiveness in inhibiting the radial mycelial growth of *C. gloeosporioides* and *L. theobromae* using food poison technique (*in vitro*). Freshly harvested avocado fruits of cv. Hass were obtained from Bassan Fruit Packers (Tzaneen, Limpopo Province, South Africa). The fruits were selected at maturity stage by finger feel method (1 = hard, 2 = slightly soft, just starting to ripen, 3 = very soft), surface sterilised by dipping in 0.01% sodium hypochlorite for 5 min and air-dried before being subjected to preventive and curative treatment (Bill *et al.*, 2014; Fan *et al.*, 2014). The fruits were subjected to the following treatments:

- i. Untreated control,
- ii. Full strength prochloraz solution,
- iii. 0.1% v/v citral solution,
- iv. 0.1% v/v thyme oil solution,
- v. Y-extract solution,
- vi. 0.1% v/v citral + half strength prochloraz solution,
- vii. 0.1% v/v thyme oil + half strength prochloraz solution,
- viii. Y-extract + half strength prochloraz solution,
- ix. 0.1% v/v citral + 0.1% v/v thyme oil solution.

The fruits dipped in the above solutions were uniformly wounded with a sterile needle (2 x 2 mm), inoculated with 20 µL of *C. gloeosporioides* and *L. theobromae* spore suspension (10^5 spores mL⁻¹) at the equatorial and stem-end regions of the avocado fruits and thereafter, incubated for 12 hours at 20°C. In the curative method, the sterilised fruits were aseptically wounded and infected with 20 µL of *C. gloeosporioides* and *L. theobromae* spore suspension (10^5 spore mL⁻¹). Thereafter, infected fruits were subjected to (dipped in) abovementioned stand-alone or combination treatments. After completion of the dipping



treatments, fruits were air dried and held at 20°C for 8 days. Prochloraz was used as the standard commercial treatment and the fruits dipped in sterile distilled water alone (untreated control) were included for comparison. Observations were made on disease anthracnose and stem-end rot incidence; severity was recorded after 2, 4, 6 and 8 days, and fruit firmness was determined on the 8th day.

Fruit firmness in all three trials was determined on two points at the equatorial point of the fruit using a Chitillon 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 5 days. The results were reported in terms of kilograms (kg).

Statistical analysis

A complete randomised design was adopted in this study. Data of the experiment were analysed with the General Linear Models (GLM) procedure in the SAS (Statistical Analysis System) programme (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

Trial 1

Artificially inoculated fruits

In the first trial, significant ($P < 0.05$) differences were found on anthracnose incidence and severity between different treatments in artificially inoculated fruit. The 10TO_Pol sachet and 5TO_Pol sachet combination treatments were the most effective treatments, reducing the % disease incidence by 50% and 25% respectively in artificially inoculated fruit cv. Hass (Table 1). On the other hand, half of the prochloraz dipped fruits packed in commercial packing trays were infected by the end of the experiment. The results of this study showed that the incorporation of thyme oil in polylactic acid sachets has a positive effect on reducing the incidence and severity of

Table 1. Effect of thyme oil-polylactic acid sachets on the incidence and severity of anthracnose in artificially inoculated avocado fruit cv. Hass.

Treatmentx	Incidence of anthracnose (%)	Severity of anthracnose (mm)
P	60 a	10.0 a
Prochloraz 0.05% for 5 min dip (P)	50 b	5.9 abc
Neat polylactic acid sachet	55 c	8.1 ab
5% TO polylactic acid sachet	35 d	4.3 bc
10% TO polylactic acid sachet	10 e	1.5 c

Means in the same column with different letters are significantly different ($P < 0.05$).

anthracnose compared to the commercially treated fruit, with the highest concentration of 10% TO giving the best results. Significantly ($P < 0.05$) higher fruit firmness (1.18 kg) was retained in fruits kept in commercial packing trays with 10TO_Pol sachets followed by the 5TO_Pol sachets (1.13 kg) and prochloraz fungicide (0.81 kg) treatments (Fig. 2). No significant differences ($P < 0.05$) in firmness were noticed between neat_Pol (control) and untreated fruit which both showed the lowest firmness values of 0.74 kg as a result of excessive softness due to faster ripening (Bill *et al.*, 2014). Anthracnose incidences were absent in naturally infected cv. Hass fruit in all the treatments after shelf life and therefore the data is not shown.

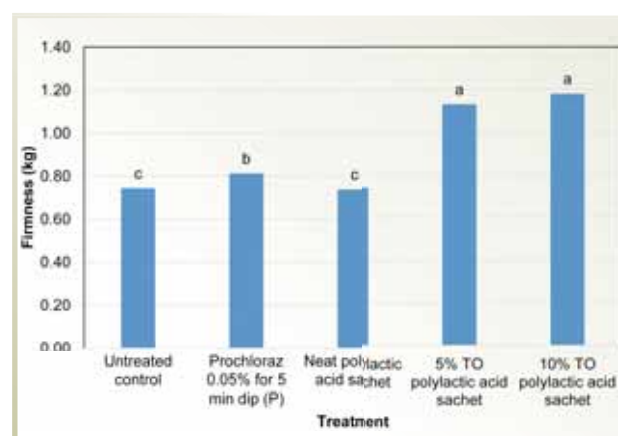


Figure 2. Effect of thyme oil-polylactic acid sachets on fruit firmness in artificially inoculated avocado fruit cv. Hass.

Means in the same bar with different letters are significantly different ($P < 0.05$).

Naturally infected fruits

Anthracnose incidences were absent in naturally infected cv. Hass fruit in all the treatments after shelf life and therefore the data is not shown. However, in naturally infected fruit, the stem-end rot disease was controlled completely with 10TO_Pol + prochloraz (half strength) after 5 days at 10°C and thereafter 5 days at 20°C (ready to eat programme) (Fig. 3). It is important, therefore, to point out that dipping the fruits in half strength prochloraz before keeping them in the packing trays with TO polylactic acid sachets augments the effectiveness of the treatment in controlling stem-end rot. Almost half of the untreated fruits kept in packing trays were infected by stem-end rot. Thyme oil (10%) polylactic acid sachets and prochloraz (half strength) treatment retained the appropriate firmness (0.85 kg) for ready to eat fruits (Fig. 4).

Trial 2

The results of Trial 2 indicated that fumigating at 7.5°C (pack house holding temperature) and also at 25°C (room temperature) for 24 hours under a pallet cover both completely reduced the incidence and severity of anthracnose and stem-end rot



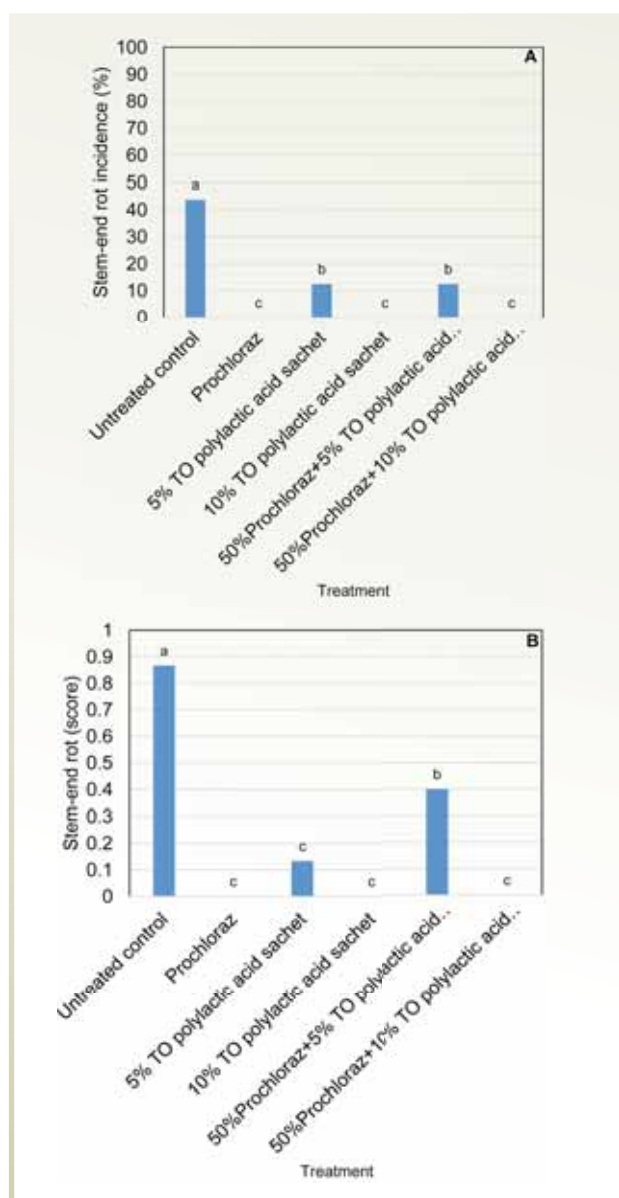


Figure 3. Effect of thyme oil-poly-lactic acid sachets on the incidence (A) and severity (B) of stem-end rot in naturally infected avocado fruit cv. Hass.

Means in the same bar with different letters are significantly different ($P < 0.05$).

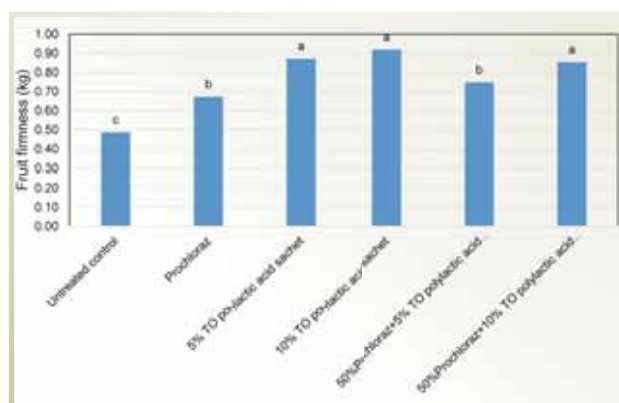


Figure 4. Effect of thyme oil-poly-lactic acid sachets on fruit firmness in naturally infected avocado fruit cv. Hass.

Means in the same bar with different letters are significantly different ($P < 0.05$).

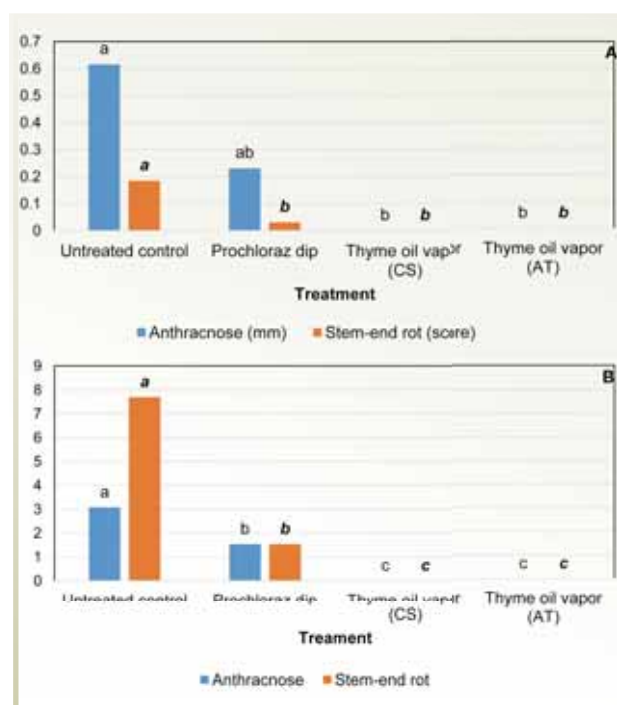


Figure 5. Effect of thyme oil vapour on the incidence (A) and severity (B) of anthracnose and stem-end rot in naturally infected avocado fruit cv. Ryan.

Means in the same bar with different letters are significantly different ($P < 0.05$).

in naturally infected cv. Ryan avocado fruit (Fig. 5). Similar results were noticed in the previous study when thyme oil vapour was applied in small containers for 24 hours to artificially inoculated fruit (Bill *et al.*, 2015). This observation indicated that fumigation temperature did not seem to influence the effectiveness of thyme oil at vapour phase. Approximately 1.5% of the prochloraz dipped fruits were infected by both postharvest diseases, whereas 3% and 8% of the untreated fruits were infected by anthracnose and stem-end rot respectively. However, no significant ($P < 0.05$) differences were noticed between the treatments in fruit firmness at the end of market-shelf period. Sensory data after ripening revealed that the eating qualities were not affected by exposing the fruit to thyme oil vapours (Fig. 6).

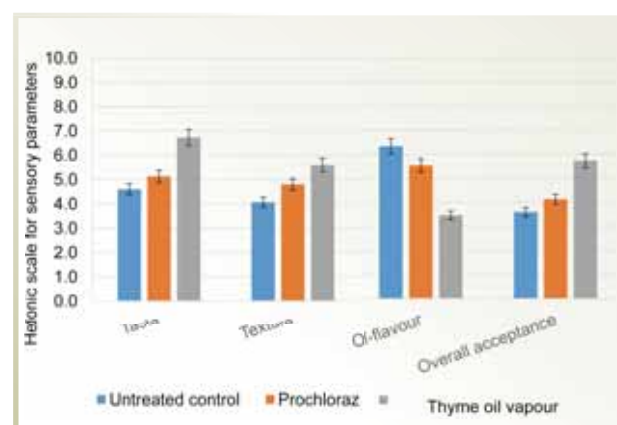


Figure 6. Effect of thyme oil vapour on eating quality in naturally infected avocado fruit cv. Ryan.



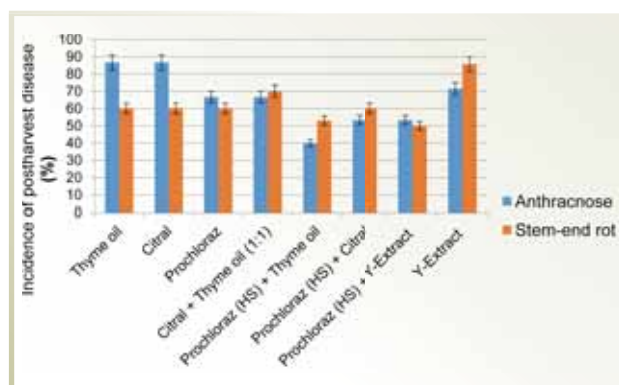


Figure 7. Effect of different defence inducers alone or in combination with half strength (HS) prochloraz on anthracnose and stem-end rot incidence in artificially inoculated avocado fruit cv. Hass.

Trial 3

The preliminary investigations done in Trial 3 indicated that the combination of half strength prochloraz with either TO or Y-extract or citral, significantly ($P < 0.05$) reduced the incidence of anthracnose to 40-53%, whereas stem-end rot was effectively reduced to 50% to 60% with half strength prochloraz with either TO or citral or Y-extract. However, the promising treatments need to be repeated during 2016 trials; trials will be conducted with cv. Fuerte and Hass on naturally infected fruits.

In conclusion, inserting thyme oil (10%) impregnated polylactic acid sachets in the currently used tray-packs after having dipped the fruit in half strength prochloraz during commercial application, could be a possible alternative for the commercial prochloraz for the ripe and ready to eat programme. However, further research is required to investigate the effectiveness of the treatment in decay control for the popular green skin cultivar Fuerte. The effect of this treatment on the sensory parameters of the fruit should also be further investigated. Though no differences were noticed when thyme oil vapour was applied either at pack house holding temperature or at room temperature while being effective in controlling decay incidence, further work has to be done with other susceptible cultivars, including cv. Hass and Fuerte, under the same setup and eventually under the current supply chain setup. The feasibility of this application should also be assessed. The use of defence inducers for postharvest decay control could be the ultimate promising alternative for the currently used prochloraz dip, considering the application method (dipping) and its effectiveness. Therefore, further research is required to determine the alternative that is most effective in controlling decay while retaining the desired overall fruit quality parameter (firmness, colour and eating quality) in all the current commercial cultivars, as well as in naturally infected fruit.

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