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

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ABSTRACT

Anthracnose 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. Consumer concerns regarding food safety and the proposed phasing out of Prochloraz® by 2020 have made the development of alternative novel fungicides a priority in the avocado industry. In our previous investigations, the volatile phase of thyme oil was selected as a suitable plant volatile to control anthracnose in avocado during postharvest storage. *In vivo* studies indicated that a natural fruit volatile 10% citral was also effective in controlling the postharvest diseases. Therefore, the effectiveness of 5% or 10% citral (C-LDPE-P) or 5% or 10% thyme oil incorporated into low-density polyethylene impregnated pellets (TO-LDPE-P) in polylactic acid (PLA) sachets were compared separately within cling film wrapped tray packs for trigger-ripened healthy fruit displayed at the simulated retail shelf conditions. However, the effectiveness of 10% C-LDPE-P/ PLA used for postharvest decay control was lower than the 10% TO-LDPE-P)/PLA sachets.

The trials with the defence inducers (Chitoplant® or Yucca extract) were also repeated during the 2016 growing season. The stand-alone or combination treatments of Chitoplant® or Yucca extract were not as effective as the prochloraz treatment. The effectiveness of thyme oil vapours for the control of anthracnose decay under the pallet covers was higher, compared to the stem-end rot control. However, the stem-end rot control was similar to that of the prochloraz treatment. It was also observed that the incorporation of half strength prochloraz with thyme oil vapour under the pallet cover remarkably reduced the incidence of stem-end rot. Furthermore, Chitoplant® or Yucca extract as a dip treatment was only effective in combination with 50% prochloraz. Finally, naturally infected 'Hass' avocados exposed to thyme oil vapour under the pallet cover as a large volume treatment (bulk shipment) or in tray packs (TO-LDPE-P)/PLA, significantly reduced the incidence of postharvest decay during small scale semi-commercial trials. The taste and flavour of the fruit were not affected by the thyme oil vapour. Thyme oil vapour effectively induced the defence mechanism and provided residual protection to the fruit. Therefore, thyme oil vapour can be recommended as a positive postharvest treatment to replace the currently used prochloraz treatment. Thymol residues in fruit fall below the recommended limit and due to the high volatility of the compound, the food safety risk for the consumer could be lower than that of the prochloraz fungicide application.

INTRODUCTION

The trials were repeated during the 2016 growing season in order to confirm the observations recorded during the year 2015. This study was carried out to investigate: 1) the effect of thyme oil impregnated low-density polyethylene pellets in polylactic acid sachets (TO-LDPE-P)/PLA) or 5% citral impregnated low-density polyethylene pellets in polylactic acid sachets (5%C-LDPE-P/ PLA) or 10%C-LDPE-P/ PLA on naturally infected fruit cv. Hass; 2) the effect of thyme oil vapour on decay inhibition in naturally infected avocado fruits cv. Hass in a large volume (simulated shipment) setup; and 3) the effects of selected defence inducers (Chitoplant® or Yucca extract) as stand-alone or in combination with half strength prochloraz on anthracnose and stem-end rot disease development in naturally infected avocado fruits cv. Hass.

MATERIALS AND METHODS

Trial 1: Evaluating the effectiveness of thyme oil or citral impregnated low-density polyethylene pellets in polylactic acid sachets on the control of postharvest disease anthracnose

Naturally infected fruits

Naturally infected fruits obtained from Westfalia, at commercial maturity, were exposed to 'triggered ripening' at room temperature for three days. For all the prochloraz dipped fruit, either as a stand-alone (full strength) treatment or a combination treatment (half strength) with either of the (TO-LDPE-P)/PLA sachet treatments, the prochloraz was applied soon after harvesting as a commercial standard procedure. Immediately the triggered ripening occurred, the fruit were placed carefully in the punnets, four fruit in each punnet. For the (TO-LDPE-P)/PLA sachet treatments or the combination treatments, each sachet was positioned at the centre 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 include: 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-LDPE-P)/PLA sachet and packed in commercial tray-packs; 4) 10% (TO-LDPE-P)/PLA sachet and packed in commercial tray-packs; 5) control (neat sachets) packed in commercial tray-packs; and 6) 5%C-LDPE-P/ PLA sachet; 10%C-LDPE-P/ PLA sachet. Each treatment had ten replicate punnets, each containing four fruit. The experiment was repeated twice. The fruit were cold stored at 12°C for 10 days. Soon after storage the punnets were opened and stored at 20°C for 5 days to allow further ripening (ready to eat programme). Observations on disease incidence, severity, fruit firmness and sensory properties (smell, appearance, flesh colour, taste and mouth feel) were recorded according to 1-5 scale (1-Hate, 2-Do not like, 3-Do not mind, 4-Like, 5-Love).

Headspace analysis of thymol concentration within the punnets

The change in the major volatiles released in the headspace of the tray-packs was measured over 10 days at 12°C at a 2 day interval using a GC/MS (Agilent 7890A gas chromatograph equipped in combination with an Agilent 5973N MSD). The experimental conditions for GC/MS analysis of thyme oil volatiles were mentioned by Sellamuthu *et al*. (2013). Volatiles were extracted using a solid phase micro-extractor (SPME) fibre holder containing a PDMS/CAR fibre. The SPME fibre was exposed to volatiles within the headspace of the tray-packs for 1 h at 12°C before each storage sampling interval. Thereafter, the fibre was placed in the injector port for 25 min for completion of thermal desorption of its compounds as stated by Sellamuthu *et al*. (2013). Compound identification was confirmed by comparison of the mass spectra with NIST08 (National Institute of Standards and Technology 08) and also those published in the literature.

Trial 2: Evaluating the effectiveness of thyme oil vapour on anthracnose or stem-end rot inhibition in naturally infected avocado fruit

Freshly harvested, unblemished avocado fruit of cv. Hass (late season) were obtained from Westfalia (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). Twenty cartons of naturally infected cv. Hass fruit (count 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 (packhouse holding temperature- CS) and also at 25°C (room temperature- RT) for 24 h under a pallet cover to determine the effectiveness of the treatment during the laboratory trial. Thereafter, fruit were removed from the pallet cover and stored at 5.5°C and 85% RH for 28 days and at 20°C for 5 days to simulate the market shelf conditions.

Trial 3: Evaluation the effects of selected defence inducers on the control of anthracnose and stem-end rot

Fruit were selected for this trial according to the methodology adopted in trial 2 and later subjected to the following treatments: (Repeat trial 2016)

I. Untreated control
II. Full strength prochloraz solution (commercial treatment)
III. 1% v/v citral solution
IV. 0.1% v/v thyme oil solution
V. Yucca extract solution
VI. 0.1% v/v citral + half strength prochloraz solution
VII. 0.1% v/v thyme oil + half strength prochloraz solution
VIII. Yucca extract + half strength prochloraz solution
IX. Yucca extract + 0.1% v/v thyme oil
X. Yucca extract + 0.1% v/v citral solution
XI. Chitoplant + 0.1% v/v thyme oil
XII. Chitoplant + 0.1% v/v citral solution
XIII. Chitoplant + half strength prochloraz solution.

Naturally infected fruit were dipped in the above solutions for 1 min. After completion of the dipping treatments, fruit were air dried and stored held at 5.5°C for 28 days. Prochloraz was used as the standard commercial treatment and the fruit 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 ripening (28 days + 5 days), and fruit firmness was determined on the 5th day, i.e. 28 days + 5 days. Fruit firmness in all three trials was determined by two points at the equatorial region 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). 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

Naturally infected fruit

Anthracnose and stem-end rot incidence was 15% and 25% respectively in naturally infected cv. Hass fruit exposed to 10% (TO-LDPE-P)/PLA sachets (Fig. 1). The observation was more or less similar to the 2016 trials.

However, when compared to the 5% or 10%C-LDPE-P/ PLA sachet, the 10% (TO-LDPE-P)/PLA sachets effectively controlled the anthracnose during storage (Fig. 2). It is interesting to note that the stem-end rot was reduced to 25% when the fruit were exposed to 10% (TO-LDPE-P)/ PLA sachets during storage. The effective control of 10%C-LDPE-P/ PLA sachet on stem-end rot was more or less similar to the 10% (TO-LDPE-P)/PLA sachets. However, application of (10%TO-LDPE-P)/PLA sachets is recommended compared to 10% C-LDPE-P/ PLA due to its cost effectiveness during the application.

On the other hand, the prochloraz dipped fruit packed in commercial packing trays showed 20% of stem-end rot and 35.7% anthracnose incidence by the end of the experiment. Fruit exposed to 10% (TO-LDPE-P)/PLA (0.98 kg) sachets during storage and thereafter at the retailer's shelf showed higher firmness than the 10%C-LDPE-P/ PLA sachet (Fig. 3).

The major component of TO is thymol and its concentration within the headspace of the tray-pack was higher on day 0 (Fig. 4). The 10% (TO-LDPE-P)/PLA showed a higher release of thymol concentration than the 5% TO-LDPE-P. On day 2 the headspace thymol concentration drastically dropped to 22.88% and 29.75% in tray-packs containing 5% and 10% (TO-LDPE-P)/PLA respectively. After day 2 the release of thymol gradually stabilised and finally on day 10, 21.02% (5% TO-LDPE-P)/PLA) and 27.8% (10% TO-LDPE-P)/PLA) in the tray-pack headspace (Fig. 4). Also, with the TO (TO-LDPE-P)/ PLA (0.20 mg/kg), thymol was detected after

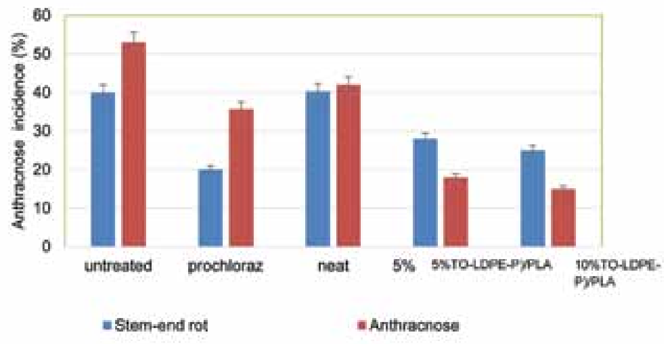

Figure 1. Effect of TO-LDPE-P)/PLA sachets on the control of anthracnose and stem-end rot in 2016 trails.

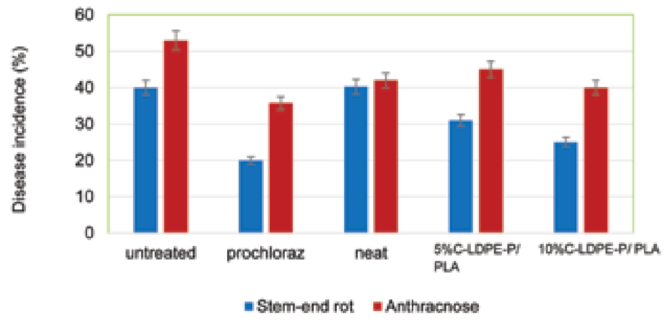

Figure 2. Effect of C-LDPE-P/PLA on the control of anthracnose and stem-end rot in 2016 trails.

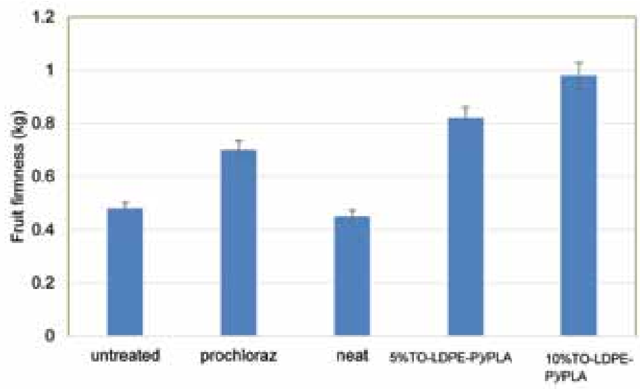

Figure 3. Effect of (TO-LDPE-P)/PLA sachets on the maintenance of fruit firmness.

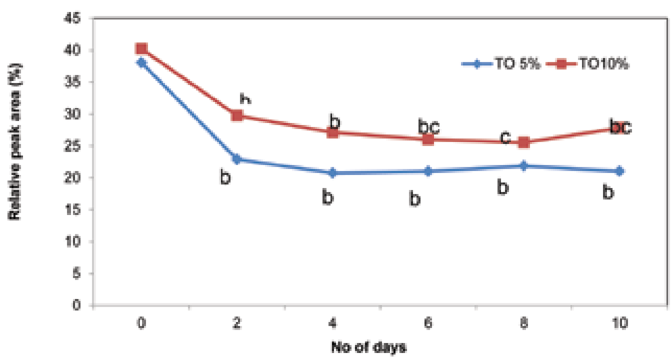

Figure 4. Head space analysis of thymol (active ingredient) in thyme oil vapour released from the 10% (TO-LDPE-P)/PLA or 5% (TO-LDPE-P)/PLA during storage.

the 12 days storage and 3rd day at the retail shelf condition.

The sensory evaluation by 80-90 of the panellist showed higher preference in terms of taste, appearance and aroma for the fruit treated with thyme oil vapour released from 5% (TO-LDPE-P)/PLA sachets after low-temperature storage and ripening. Most of the participants preferred the internal fruit colour in fruit exposed to thyme oil vapour released from 10% (TO-LDPE-P)/PLA sachets due to its comparability to prochloraz treated fruit. The panellist also mentioned that the thyme oil aroma was not present in the fruit exposed to (TO-LDPE-P)/PLA sachets. Also, there was absence of deleterious effects on the mouth feel and overall quality, hence the panellist preference for the fruit exposed to 5% (TO-LDPE-P)/PLA sachets.

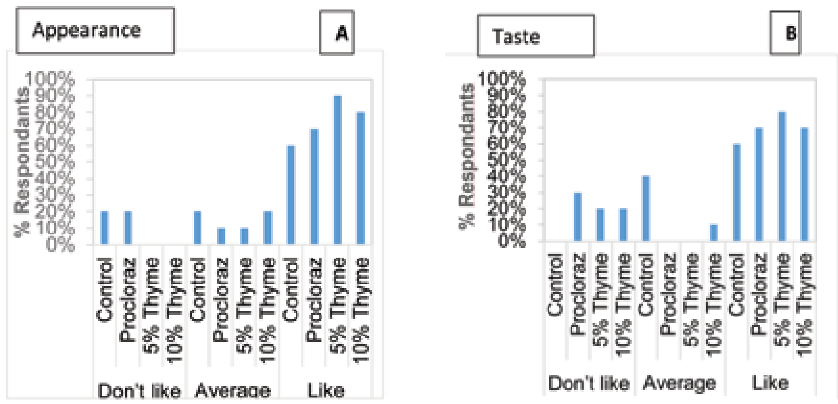

Figure 5. Effect of 5% or 10% 5TO-LDPE-P)/PLA sachets on (A) appearance and (B) taste of 'Hass' avocados after 10 days at 12°C and thereafter on the 5th day at 20°C (at the retail shelf).

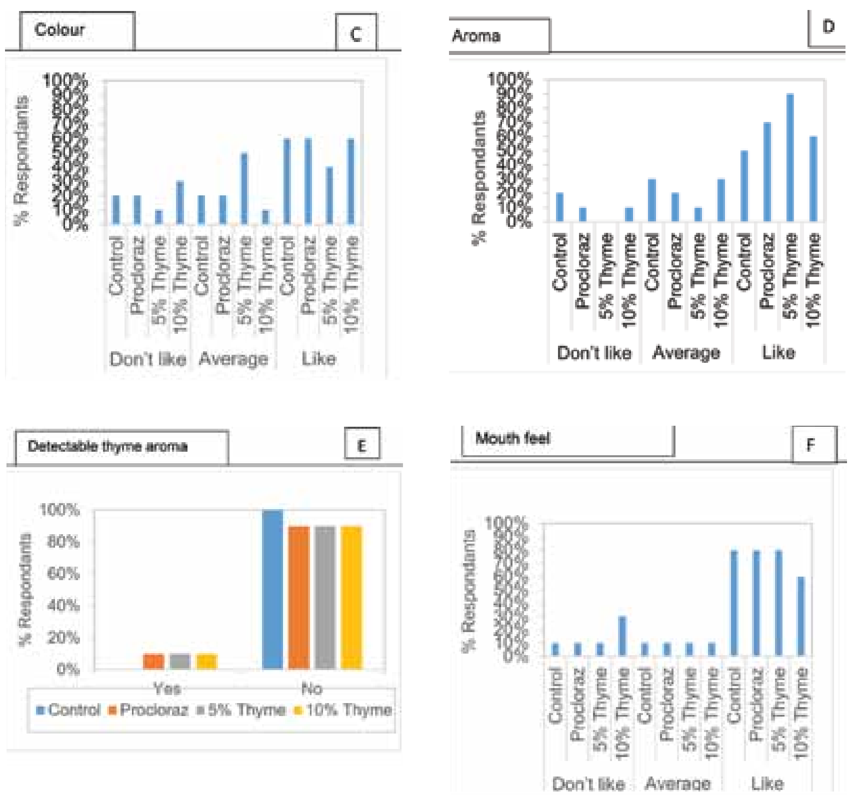

Figure 6. Effect of 5% or 10% 5TO-LDPE-P)/PLA sachets on (C) colour, (D) aroma (E) detectable thyme aroma and (F) mouth feel of 'Hass' avocados after 10 days at 12°C and thereafter on the 5th day at 20°C (at the retail shelf).

Trial 2

The results of Trial 2 indicated that fumigating at 5.5°C (packhouse holding temperature) under a pallet cover, significantly reduced the incidence of anthracnose and stem-end rot in naturally infected cv. Hass avocado fruit (Figs. 5 and 6). Thyme oil vapour reduced the anthracnose to ~15% on day 7 at the simulated retail shelf condition, whereas the prochloraz treatment showed that 25% of the fruit were infected with anthracnose. However, the influence of thyme oil vapour on the control of stem-end rot was lower compared to the control of anthracnose, while thyme oil vapour showed that approximately 22.9% of the fruit were infected with stem-end rot (Fig. 6). Fruit exposed to thyme oil vapour were slightly firmer than the fruit exposed to other treatments (data not shown).

Trial 3

It was concluded from the 2016 trials that the thyme oil treatment is effective in vapour phase than in the aqueous phase. The chitoplant and yucca extracts failed to show effective control on the anthracnose and stem-end rot as stand-alone treatments. The observation was similar to the 2016 trials and inclusion of half strength prochloraz in 1% chitoplant and yucca extracts respectively significantly reduced stem-end rot development. Therefore detailed results are not reported here.

CONCLUSION

The second year data clearly stated the inclusion of 10% LDPE-P in polylactic acid sachets in commercial tray-packs containing trigger-ripened avocado fruit as a suitable postharvest alternative to prochloraz treatment. Also, the thyme oil vapour treatment under the pallet cover also showed a potential treatment to replace the currently adopted prochloraz treatment. However, trials need to be conducted in different packhouses under different conditions in order to confirm the effectiveness of the treatment under different conditions, such as fruit

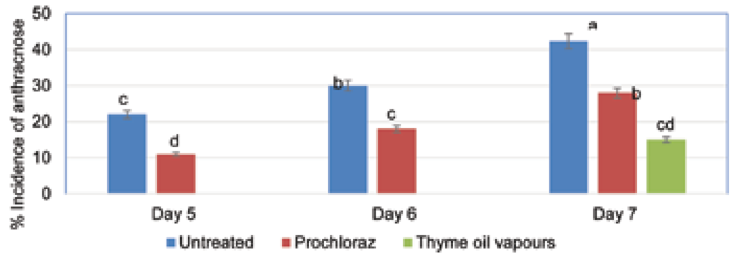

Figure 7. Effect of thyme oil vapours applied under the pallet wraps on the incidence of anthracnose after 28 days cold storage and thereafter on the 5th day at the market shelf.

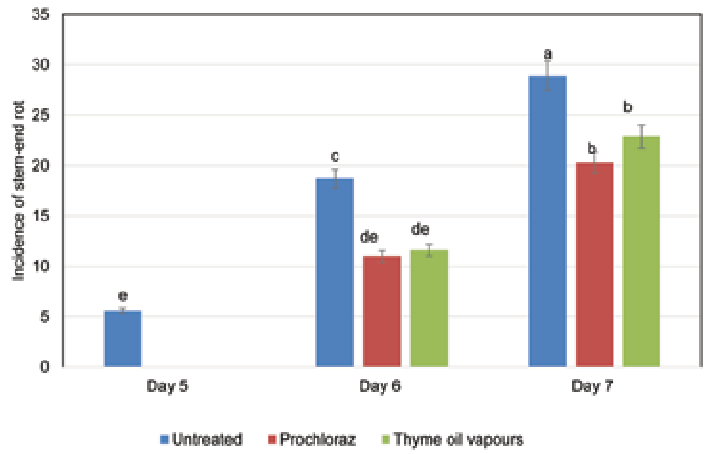

Figure 8. Effect of thyme oil vapours applied under the pallet wraps on the incidence of stem-end rot after 28 days cold storage and thereafter on the 5th day at the market shelf.

from orchards in different sanitation conditions, fruit counts, etc. prior to commercialisation.

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