Evaluation of Lippia scaberrima essential oil and some pure terpenoid constituents as postharvest mycobiocides for avocado fruit

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a b s t r a c t

Mycobiocides are attracting research interest worldwide as possible postharvest pathogen control measures to replace synthetic fungicides. In this study, the application of two essential oils as fungicides was evaluated. Initially, the in vitro antifungal effects of Lippia scaberrima essential oil and three of the major oil components, (d)-limonene, R-(−)-carvone, and 1,8-cineole, as well as that of S-(+)-carvone, were investigated against Colletotrichum gloeosporioides, Lasiodiplodia theobromae, and an Alternaria isolate. The oil and terpenoids caused significant inhibition of the mycelial growth of all the pathogens when applied at a concentration of 2000 μL L^{-1}. The most potent volatile component of L. scaberrima essential oil, able to inhibit all the pathogens tested, proved to be R-(−)-carvone. The efficacy of the essential oil (1000 and 2000 μL L^{-1}) incorporated into the commercial coating was confirmed on fruit inoculated with two of the pathogens. A simulated export trial was done using Lippia essential oil, in addition to Mentha spicata (spearmint) essential oil, as supplements for fruit coatings. Results indicate that essential oils rich in R-(−)-carvone could be valuable alternatives to synthetic fungicides for the postharvest management of avocado fruit. The combination of essential oils with a commercial coating, acceptable to the organic market, offers additional protection to this vulnerable commodity.

1. Introduction

Pre- and postharvest spoilage of avocado (Persea americana Mill.) is caused by a number of well-known pathogens. Most losses are ascribed to Colletotrichum gloeosporioides (Penz.) (Jeffries et al., 1990; Sanders and Korsten, 2003), L. theobromae (Pat.) Griffon and Maubl. (Maftoonazad et al., 2007), and to a lesser extent to Alternaria sp. (Darvas et al., 1990). Despite not yet being classified as major phytosanitary risks in South Africa, the potential economic impact of the diseases caused by these pathogens is significant (Sanders et al., 2000). The use of environmentally toxic fungicides to control fruit pathogens is currently under review (Plaza et al., 2004). Accumulation of harmful residues, such as copper (II) originating from copper oxychloride, as a result of repeated orchard applications throughout the season, could lead to a reduction in the phylloplane population diversity present on the fruit. According to Stirling et al. (1999), the non-specificity of the fungicide eliminates beneficial microorganisms, thereby allowing pathogenic species to flourish during postharvest storage. The presence of copper ions on edible fruit could also pose a threat to human health as long-term exposure can compromise immunity by effecting copper absorption and retention, and antioxidant status of individuals (Turnlund et al., 2004).

Plants control phylloplane populations by producing secondary metabolites such as terpenoids (Shepherd and Wagner, 2007). These compounds form the basis of essential oils that are obtained from aromatic plants through steam- or hydrodistillation. The ability of the plant for self-protection against invasive fungi has prompted researchers to consider these oils for the development of safer antifungal agents (Feng and Zheng, 2007) in postharvest applications (Yigit et al., 2000; Klieber et al., 2002; Chebli et al., 2004). Effective postharvest mycobiocides can reduce the necessity for repeated application of fungicides in the orchard, thereby reducing harmful residues in the fruit. Lippia scaberrima Sond., a medicinal aromatic plant indigenous to South Africa, yields an essential oil rich in R-(−)-carvone, (d)-limonene and 1,8-cineole (Combrinck et al., 2006). This oil has been reported to inhibit the in vitro and in vivo growth of two mango postharvest spoilage pathogens (Regnier et al., 2008; Du Plooy et al., 2009).

This study had a threefold objective. Firstly, the application of L. scaberrima essential oil as an alternative means of controlling three major avocado postharvest pathogens was investigated. Secondly, the antifungal activity of the oil was compared to that of R-(−)-carvone, (d)-limonene and 1,8-cineole (some of the pure major components of the oil) as well as S-(+)-carvone. Lastly, the effectiveness of the essential oil of L. scaberrima incorporated into

commercial coatings, to improve the postharvest quality of avocado fruit, was compared to that of spearmint oil.

2. Materials and methods

2.1. Essential oil

Essential oil of L. scaberrima was obtained by steam-distillation of the aerial parts of plants collected in the Wolmaransstad region (North West Province, South Africa) as described by Combrinck et al. (2006). Mentha spicata L. (spearmint) essential oil was obtained from Holistic Emporium (Johannesburg, South Africa) and standards (R-(−)-carvone, S-(+)-carvone, (d)-limonene and 1,8-cineole), with purities exceeding 98%, were purchased from Sigma–Aldrich (Pty) Ltd. (Johannesburg, South Africa).

2.2. Pathogens

The avocado fruit pathogens (C. gloeosporioides, L. theobromae and an Alternaria strain), isolated from infected fruit, were kindly provided by Westfalia Laboratories (Tzaneen, South Africa). All isolates were cultured and maintained on malt extract agar (MEA) (Biolab, Johannesburg) in a temperature controlled environment at 23–25 °C. Spore suspensions (10^7 spores mL^{-1}) of each isolate were prepared as described by Regnier et al. (2008).

2.3. In vitro antifungal assays

The in vitro inhibitory effects of the essential oils and individual terpenoids were evaluated using both toxic medium and vapour exposure. For the toxic medium, MEA was sterilized and supplemented with 400 μL L^{-1} of the emulsifier Triton X-100 (Ajax Laboratory Chemicals, Philadelphia, USA) mixed with the essential oil or individual terpenoid components. Controls were prepared using emulsifier only. The essential oil and terpenoids were each evaluated at concentrations of 500, 1000, 2000, 2500 and 3000 μL L^{-1}. The agar was poured into Petri dishes (90 mm) and after setting, a 5 mm agar plug of fungal mycelia from a 7 d old culture were placed in the centre of each MEA plate. This was done for each of C. gloeosporioides, L. theobromae, and the Alternaria sp. After six days of incubation, mycelial growth was measured (in mm) with digital callipers (Absolute Digimatic-Mitutoyo Corp. Japan). For each isolate, 10 replicates of each concentration tested were prepared. Percentage inhibition of mycelial growth was determined as described by Plaza et al. (2004).

For the vapour exposure treatments, the three test organisms were plug inoculated as before. This was done 4 h prior to exposure of the pathogens to the essential oils and terpenoids, to allow for regeneration of damaged hyphae. Droplets of undiluted test substances (10, 20 or 40 μL) were placed onto glass cover slips fixed to Petri dish lids with glycerol. Five replicates per treatment were done and sunflower oil served as a control. The Petri dishes were inverted and sealed with parafilm (American Natural, Chicago, USA) to prevent vapour loss. Incubation of the treated cultures was done at 23–25 °C for two and four days respectively, and the fungal growth recorded. Growth inhibition was calculated as the percentage of radial mycelial growth of the cultures exposed to the treatments, relative to the growth of those on the control plates (Plaza et al., 2004). To distinguish between fungicidal and fungistatic activity of the vapour against the target organism, mycelial plugs that did not grow were transferred to fresh MEA, after two and four days exposure. Any observed radial fungal growth was measured after a total of six days incubation at 23–25 °C.

2.4. Preliminary in vivo investigations using inoculated fruit

Freshly harvested, unblemished 'Fuerte' avocado fruit were selected from Bassan Fruit Packers (Tzaneen, Limpopo Province, South Africa). Fruit were surface sterilized by dipping in 70% ethanol for 1 min, and air-dried. Thereafter, the prepared fruit were inoculated with C. gloeosporioides (equatorial region) and L. theobromae (stem-end) for evaluation of the pathogen virulence, using a 20 μL droplet of a conidial suspension (5×10^4 spores mL^{-1}) (Yakoby et al., 2001). After 12 h at ambient temperature, fruit were dipped into one of the following: distilled water, coating (Avoshine®, Citrushine (Pty) Ltd.), coating supplemented with 1000 μL L^{-1} or with 2000 μL L^{-1} L. scaberrima oil. Each dip treatment was conducted on 20 fruit, while 20 untreated un-inoculated fruit served as the negative control. An additional 20 un-inoculated fruit were dipped in unsupplemented Avoshine®. Fruit of individual treatments were placed in bags and stored for six days at 23 °C and high humidity. The decay was observed after halving each fruit transversally through the equatorial inoculation site. For each pathogen, infection was classified according to the percentage internal surface area of the halved fruit covered by the blemish: clean fruit (rating 0), 1–10% surface area (rating 1), 11–20% (rating 2), 21–40% (rating 3), 41–70% (rating 4) and 71–100% surface area (rating 5).

2.5. Quality retention using coatings amended with essential oils

Fruit were randomly selected from the same freshly harvested consignment and sorted by size. Fifteen cartons (12 fruit per carton) were used for each of the six treatments and the untreated control. Two commercial coatings, Carnauba Tropical® and Midseason 865® (MS865) supplied by John Bean Technologies (Pty) Ltd., were applied by spray application on the packline. Treatments consisted of coating amended with L. scaberrima or spearmint essential oil at a final concentration of 2500 μL L^{-1}, without the use of any synthetic fungicide in the dip tank. These treatments were compared to the standard commercial process using a prochloraz dip (final concentration 1.8%, v/v), followed by the application of the unsupplemented coating. The boxes were placed in cold storage (10 °C and R.H. 65–75%) for four weeks to simulate typical export conditions from South Africa to Europe. Thereafter, fruit were ripened at ambient temperature (22 °C) for seven days and fruit quality parameters were subsequently evaluated.

Cartons containing fruit were weighed immediately following application of the appropriate treatment. These cartons were reweighed upon evaluation to determine the average percentage moisture loss of the fruit for each treatment. Fruit displaying any visible sign of infection were considered to be diseased, regardless of the size of the lesion, while fruit without lesions were considered "clean". Disease control was reflected by calculating the average percentage of clean fruit per treatment. Approximately two 5 cm^3 cubes of pulp were removed from each fruit for tasting.

2.6. Statistical analysis

The statistical package, SPSS Version 16, was used for all data analyses. The normality and homogeneity of each set of data were tested using the Kolmogorov–Smirnov and Levine tests, respectively (Carver and Nash, 2009). Data were regarded as normally distributed if the P-values obtained were greater than $\alpha = 0.05$. One-way ANOVA (single factor) was used to determine significant differences for normally distributed data (in vitro data). Analysis results obtained with $P \leq 0.05$ were considered as significant. For non-parametric data (in vivo data), comparisons were made using the Wilcoxon Signed Ranks Test (two-tailed). If the P-value obtained was smaller than 0.05, differences were regarded as significant.

3. Results

3.1. In vitro antifungal assays

The in vitro antifungal activities of the toxic media are presented in Table 1. Lippia oil and the individual terpenoids, with the exception of (d)-limonene, were effective against all three pathogens tested as reflected by their complete inhibition at a concentration of 2500 j.LL L^{-1}. Both enantiomers of carvone caused total growth inhibition of all the fungi at a concentration of 1000 j.LL L^{-1}. (d)-Limonene was unable to totally inhibit the growth of L. theobromae, even at 3000 j.LL L^{-1}.

The nature of the vapour toxicity (fungicidal or fungitoxic) was distinguished through two and four days exposure of the pathogens to the vapour phase of the various inhibitors (Table 2). L. scaberrima oil volatiles proved fungistatic to C. gloeosporioides after exposure to 40 j.LL for two days. This was demonstrated by the observed reduction in pathogen growth after transfer to new medium. The vapour of R-(−)-carvone proved to have the highest fungicidal activity against this pathogen at all amounts applied after two and four days exposure. S-(+)-carvone was far less active than its enantiomer, while limonene exhibited limited inhibition ability at all volumes tested. Furthermore, the vapour of 1,8-cineole was slightly more active against C. gloeosporioides than that of L. scaberrima essential oil.

S-(+)-carvone proved to be fungicidal to L. theobromae after four days of exposure to 40 j.LL of the terpenoid (Table 2). The efficacy of the Lippia oil and the other individual terpenoids against the pathogen was found to be fungistatic only, since the pathogen recovered fully following transfer to fresh medium. In fact, limonene appeared to promote the growth of the fungus. R-(−)-carvone was less active against this pathogen than its enantiomer.

Complete inhibition of Alternaria sp. was observed after two days exposure to the vapour of 20 and 40 j.LL applications of L. scaberrima oil (Table 2). However, no fungicidal activity was observed after transfer to a fresh medium. R-(−)-carvone was the only pure terpenoid component that was able to demonstrate fungicidal activity (using 40 j.LL) after two and four days of contact with the pathogen. Although S-(+)-carvone and 1,8-cineole were able to suppress the mycelial growth of the pathogen at 20 and 40 j.LL, none of them were fungicidal. A stimulation of the mycelial growth was again observed when limonene was used for four days at volumes of 10 and 20 j.LL.

3.2. Preliminary in vivo investigations using inoculated fruit

Results presented in Fig. 1 indicate the ability of coatings amended with L. scaberrima essential oil to inhibit disease development of avocado fruit inoculated with C. gloeosporioides and L. theobromae. The virulence of both pathogens was confirmed by the presence of typical lesions on the positive (inoculation + water; Treatment 1) control fruit. Disease development in inoculated fruit was markedly reduced by the application of coating supplemented with L. scaberrima essential oil (Treatments 4 and 5) when compared to inoculated fruit dipped in unamended coating (Treatment 2). Although curative controls against the two pathogens were not similar, the efficacy of Treatments 4 and 5 was comparable to that obtained for un-inoculated coated fruit (Treatment 6).

3.3. Quality retention using coatings amended with essential oils

In the simulated export trial, all treated fruit displayed significantly better quality retention when compared to untreated fruit (Fig. 2). Standard commercial practise using prochloraz dip for fungal control in combination with Carnauba Tropical® coating

Table 1
Percentage inhibition of toxic media (MEA, incorporating different concentrations of inhibitors) on mycelial growth of C. gloeosporioides, L. theobromae and Alternaria sp. after six days at 23 °C. Averages (n = 10) of percentage inhibition are followed by standard errors in brackets.

Inhibitor	Inhibition (%) at concentration (j.LL L^{-1}) of														
	C. gloeosporioide					L. theobromae					Alternaria sp				
	500	1000	2000	2500	3000	500	1000	2000	2500	3000	500	1000	2000	2500	3000
L. scaberrima	64 (0.21)	100 (0.00)	100 (0.00)	100 (0.00)	100 (0.00)	83 (0.19)	87 (0.26)	90 (0.32)	100 (0.00)	100 (0.00)	75 (0.18)	82 (0.13)	100 (0.00)	100 (0.00)	100 (0.00)
R-(−)-carvone	83 (0.32)	100 (0.00)	100 (0.00)	100 (0.00)	100 (0.00)	95 (0.11)	100 (0.00)	100 (0.00)	100 (0.00)	100 (0.00)	81 (0.23)	100 (0.00)	100 (0.00)	100 (0.00)	100 (0.00)
S-(+)-carvone	80 (0.37)	100 (0.00)	100 (0.00)	100 (0.00)	100 (0.00)	93 (0.11)	100 (0.00)	100 (0.00)	100 (0.00)	100 (0.00)	82 (0.44)	100 (0.00)	100 (0.00)	100 (0.00)	100 (0.00)
(d)-Limonene	65 (0.34)	78 (0.22)	82 (0.13)	87 (0.19)	100 (0.00)	70 (0.15)	73 (0.18)	78 (0.05)	88 (0.21)	89 (0.01)	71 (0.11)	75 (0.15)	80 (0.05)	85 (0.29)	100 (0.00)
1,8-Cineole	74 (0.31)	92 (0.21)	100 (0.00)	100 (0.00)	100 (0.00)	82 (0.25)	86 (0.29)	100 (0.00)	100 (0.00)	100 (0.00)	82 (0.21)	100 (0.00)	100 (0.00)	100 (0.00)	100 (0.00)

Table 2
Inhibitory effects of vapourised L. scaberrima oil and its main constituents R-(−)-carvone, S-(+)-carvone, (d)-limonene and 1,8-cineole on mycelial growth inhibition of C. gloeosporioides, L. theobromae and Alternaria sp. Pathogens were exposed to oil and terpenoid vapours (n = 5) at 10, 20 and 40 μL for two and four days and subsequently transferred to fresh medium.

Treatments	L. scaberrima			R-(−)-carvone			S-(+)-carvone			(d)-Limonene			1,8-Cineole		
	10	20	40	10	20	40	10	20	40	10	20	40	10	20	40
Inhibition (%) for volume specified (μL) for each inhibitor															
C. gloeosporioides															
Two days	99.04 A	100 A	100 A	100 A	100 A	100 A	100 A	100 A	100 A	−22.32 A	−23.34 C	1.02 B	35.71 A	100 A	100 A
	(1.04)		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(2.56)	(2.12)	(1.49)	(2.26)	(0.00)	(0.00)
Two days + transfer	0 D	3.81 D	100 A	100 A	100 A	100 A	6.66 B	9.80 C	27.84 B	0 B	0 B	0 C	0 C	6.93 B	100 A
	(0.00)	(1.31)	(0.00)	(0.00)	(0.00)	(0.00)	(1.61)	(0.80)	(3.08)	(0.00)	(0.00)	(0.00)	(0.00)	(2.69)	(0.00)
Four days	68.27 B	78.76 B	99.73 A	100 A	100 A	100 A	100 A	100 A	100 A	−19.66 A	−2.07 A	22.53 A	31.55 B	100 A	100 A
	(1.08)	(5.22)	(0.58)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(3.38)	(10.13)	(6.36)	(0.37)	(0.00)	(0.00)
Four days + transfer	3.33 C	11.09 C	17.18 B	100 A	100 A	100 A	0 C	52.66 B	100 A	0 B	0 B	0 C	0 C	0 C	100 A
	(3.07)	(2.26)	(2.57)	(0.00)	(0.00)	(0.00)	(0.00)	(4.78)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
L. theobromae															
Two days	97.39 A	100 A	100 A	100 A	100 A	100 A	100 A	100 A	100 A	−44.47 C	18.45 A	32.64 A	24.54 B	100 A	100 A
	(4.16)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(2.25)	(6.15)d	(9.16)	(8.98)	(0.00)	(0.00)
Two days + transfer	0 B	0 B	0 B	0 B	0 B	0 C	0 B	0 B	0 B	0 A	0 B	0 B	0 C	0 C	0 C
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Four days	98.80 A	99.07 A	100 A	100 A	100 A	100 A	100 A	100 A	100 A	−15.66 B	−6.23 C	−0.08 B	82.46 A	85.3 B	90.82 B
	(1.18)	(1.46)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(1.61)	(0.81)	(2.90)	(2.14)	(1.09)	(1.33)
Four days + transfer	0 B	0 B	0 B	0 B	0 B	53.83 B	0 B	0 B	100 A	0 A	0 B	0 B	0 C	0 C	0 C
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(1.37)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Alternaria sp.															
Two days	88.81 A	100 A	100 A	100 A	100 A	100 A	100 A	100 A	100 A	0.11 A	−1.34 A	7.63 A	77.99 A	100 A	100 A
	(3.25)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(1.54)	(2.28)	(0.31)	(1.56)	(0.00)	(0.00)
Two days + transfer	0 C	2.79 C	10.79 C	6.69 C	14.72 C	100 A	8.44 B	7.94 C	10.80 C	0 A	0 A	0 C	5.55 C	15.54 B	54.41 B
	(0.00)	(1.75)	(0.83)	(4.06)	(1.56)	(0.00)	(2.15)	(2.99)	(2.09)	(0.00)	(0.00)	(0.00)	(1.14)	(2.75)	(2.40)
Four days	50.13 B	71.31 B	100 A	100 A	100 A	100 A	100 A	100 A	100 A	−20.68 B	−15.56 B	3.17 B	65.16 B	100 A	100 A
	(2.19)	(1.14)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(6.70)	(5.38)	(1.51)	(2.75)	(0.00)	(0.00)
Four days + transfer	0 C	0 D	41.62 B	35.48 B	53.03 B	100 A	10.44 B	22.35 B	25.06 B	0 A	0 A	0 C	2.00 C	2.37 C	53.83 B
	(0.00)	(0.00)	(2.57)	(2.71)	(3.32)	(0.00)	(2.07)	(2.27)	(1.39)	(0.00)	(0.00)	(0.00)	(1.47)	(1.77)	(1.47)

Averages (n = 10) followed by the same upper-case letter did not differ significantly within a column at $P \leq 0.05$. The standard error for each set is given in brackets below the average.

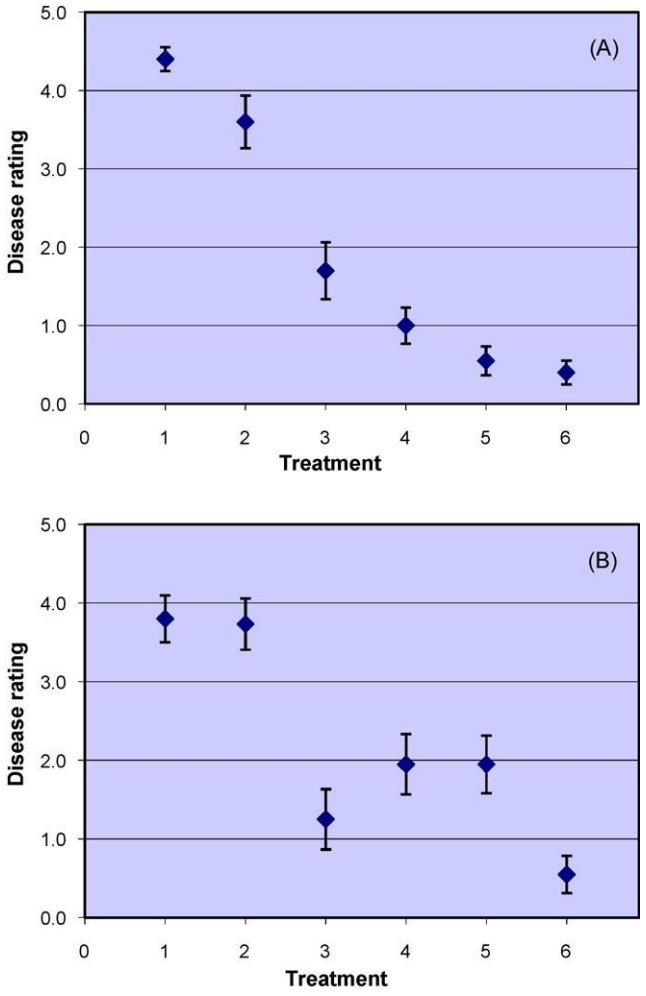

Fig. 1. Ability of coatings amended with L. scaberrima essential oil to inhibit disease development of avocado fruit (n = 20) inoculated with C. gloeosporioides (A) and L. theobromae (B) after six days storage at 23 °C. In Treatment 1 inoculated fruit were dipped in distilled water; Treatment 2 represents inoculated fruit dipped in unamended coating (Avoshine®); Treatment 3 is the negative control (un-inoculated and untreated). In Treatments 4 and 5, inoculated fruit were dipped in coating supplemented with 1000 and 2000 µL L^{-1} L. scaberrima essential oil respectively. Treatment 6 consisted of un-inoculated fruit dipped in unamended coating. The bars on the average rating values indicate standard error.

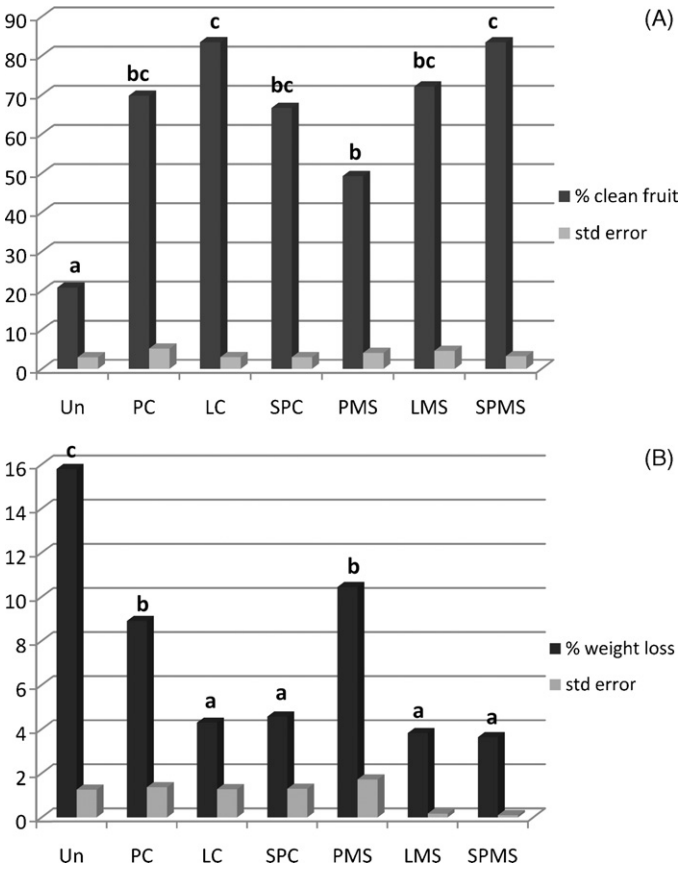

Fig. 2. Effect of essential oil amended coatings on disease control (A), and percentage moisture loss (B). Each of the following treatments were applied to fifteen cartons containing twelve fruit per carton: Un = untreated; PC = Prochloraz followed by coating with Carnauba Tropical®; LC = Lippia oil (2500 µL L^{-1}) in Carnauba Tropical®; SPC = spearmint oil (2500 µL L^{-1}) in Carnauba Tropical®; PMS = Prochloraz followed by coating with MS865®; LMS = Lippia oil (2500 µL L^{-1}) in MS865®; SPMS = spearmint oil (2500 µL L^{-1}) in MS865®. Bars with different lower case letters differed significantly according to the Wilcoxon Signed Ranks Test at 95% confidence level.

yielded almost 70% clean fruit (Fig. 2(A)). Although not significant, prochloraz in combination with MS865 coating resulted in diminished fungal control. Lippia essential oil provided more protection in combination with Carnauba Tropical®, while spearmint oil performed better when used as a supplement for MS865. In the commercial trial, the essential oil supplemented coatings significantly improved the water retention of the fruit (Fig. 2(B)), when compared to the conventional method of coating application.

4. Discussion

4.1. In vitro antifungal assays

The toxic medium trials clearly demonstrated the inhibitory role of carvone against all the pathogens investigated. R-(−)-carvone, present in L. scaberrima oil (Combrinck et al., 2006), was previously found to be effective against another strain of C. gloeosporioides, isolated from mango fruit (Regnier et al., 2008). Total inhibition of the pathogens was achieved at an essential oil concentration of 2500 µL L^{-1}; the latter then served as a guide to the concentrations used in the later in vivo trials. The effects of exposure of the pathogens to vapour were initiated to investigate the potential use of L. scaberrima essential oil as a fumigant during export of fruit. Both enantiomers of carvone were able to effectively control pathogen growth, in most cases, when large amounts were applied. L. theobromae was more resistant than the other pathogens against the inhibitors tested, although S-(+)-carvone was fungicidal towards the pathogen when larger volumes were applied. Lippia essential oil showed a trend of reduced efficacy as the period of exposure became longer. This observation indicates that the use of this oil as a fumigant may be limited. In addition, limonene, a major oil component, appeared to promote the growth of the fungi when applied as a vapour. This finding makes the use of limonene-rich essential oils, such as citrus oils (Shaw, 1979; Lan-Phi et al., 2009), a less attractive option for the control of postharvest pathogens.

4.2. Preliminary in vivo investigation using inoculated fruit

It has been demonstrated that essential oils incorporated into fruit coatings are effective in the packhouse environment to control postharvest pathogens of mango (Regnier et al., 2008) and citrus (Du Plooy et al., 2009). In our investigation, this application was extended to avocado fruit. For the initial in vivo trial, inoculated fruit were used to test the efficacy of the essential oil supplemented

coatings in the presence of pathogens. The Alternaria sp. was not included since the pathogen is currently not a major concern of South African producers. The supplementation of the wax coating provided enhanced control, when compared to the coating alone. Previous workers (Inouye et al., 2000) have suggested that the essential oil contributes to the creation of a lipophilic environment that may be detrimental to the development of the pathogen. The successful implementation of the oil into the commercial coating for pathogen control, further prompted us to conduct a simulated export trial using L. scaberrima essential oil. Since R-(−)-carvone appeared to be one of the active components responsible for the antifungal efficacy of the oil, an alternative commercial source of the terpenoid, spearmint oil, containing higher concentrations of carvone, but low levels of (d)-limonene (Kokkini et al., 1995), were added as an inhibitor. The use of an emulsifier was not necessary upon essential oil amendment of the coatings, since the coating formulations were compatible with the lipophilic nature of the oils.

4.3. Quality retention using coatings amended with essential oils

In the simulated export trial, the Avoshine® coating was replaced with the carnauba-based coatings, Carnauba Tropical® and MS865®, to align our trial with the commercial process. It was observed that severe infection developed in all treatments, including the conventional fungicide application. This indicated that the fruit was subjected to high pathogen pressure. In spite of this, the essential oil-enriched coatings performed equally well, or better, than the conventional disease control method. Contrary to expectations, spearmint oil containing higher levels of R-(−)-carvone, did not offer better pathogen control than Lippia oil (Fig. 2(A)). Irrespective of the essential oil and coating used, the integration of the two components promoted water retention in the fruit (Fig. 2(B)). This aspect is related to the compatibility of the essential oil with a specific commercial coating. This enhanced compatibility must be verified as part of product development of other coatings before these are applied on the packline.

Concerning the organoleptic aspects, the application of essential oils in the coating did not appear to affect the taste of the fruit in any way. The low essential oil concentrations applied probably explains the observed absence of undesirable flavours. At an application rate of one litre of coating per metric ton of fruit, containing 2500 $\mu L\,L^{-1}$ of essential oil, this result was expected.

5. Conclusion

Producers and distributors of fresh produce are increasingly faced with the dilemma of providing consumers with products that are attractive, free of disease and toxic residues, while having a long shelf life. In an environment where fungal pathogens are increasingly becoming resistant to fungicides, with ever stricter legislation regarding residue levels and waste disposal (Bankole, 1997), demands to lower the associated environmental footprint (Granatstein, 2007) are growing. A preference for organically produced commodities is continually expanding as consumers in first world countries become more health conscious. However, currently this mode of production is less cost effective, largely as a result of product losses due to spoilage by postharvest pathogens. Essential oils, generally recognized as safe, have the potential to address all of these problems. Simultaneously, essential oils are regarded as low risk targets for the development of microbial resistance (Wilson et al., 1997; Tatsadjieu et al., 2009) and can therefore contribute to a longer useful lifespan of currently used fungicides.

The major function of the currently used commercial coatings is to maintain fruit quality by decreasing the desiccation occurring during storage, while the sole purpose of the fungicide dip is to facilitate pathogen control. In this study an integrated alternative is proposed, to eliminate the use of synthetic crop protective products in avocado packhouses and reduce moisture loss during transport. Such an approach is imperative to alleviate problems such as waste disposal, toxic residues and a large environmental footprint.

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