RESEARCH ARTICLE

Basil oil incorporated beeswax coating to increase shelf life and reduce anthracnose development in mango cv. Willard

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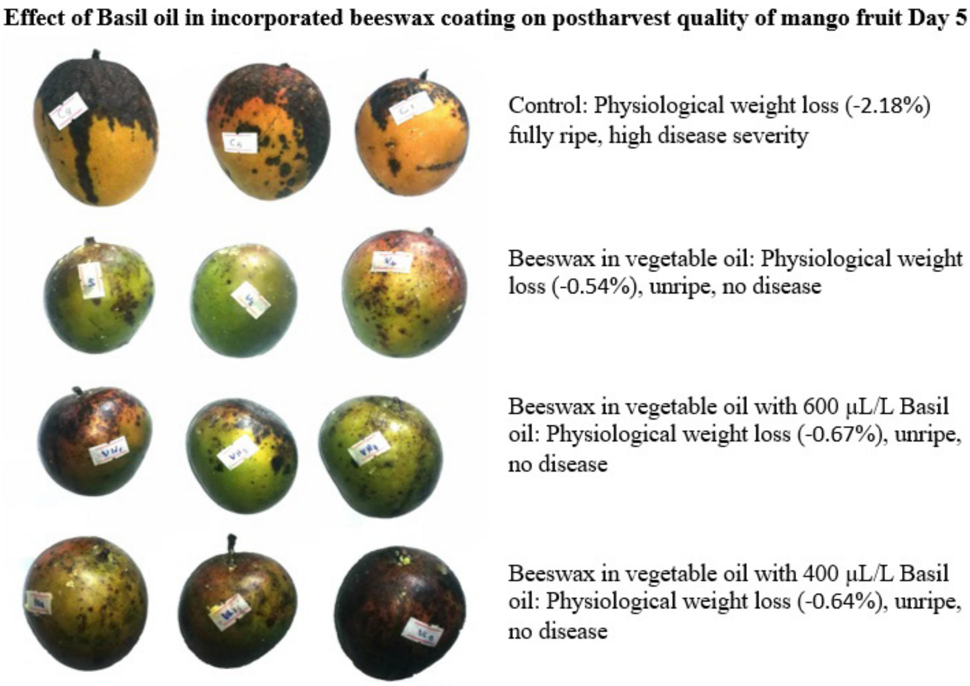

Highlights

- Melted beeswax in vegetable oil or pet ether can be applied to fruits as a coating to significantly reduce % weight loss and significantly increase the number of days taken to reach table ripe maturity in fruits of cv. 'Willard'
- Melted beeswax in vegetable oil (1:3 w/v) with 600 µL L^{-1} basil oil significantly reduces anthracnose development in mango fruits of cv. 'Willard'
- Melted beeswax in vegetable oil (1:3 w/v) with 600 µL L^{-1} basil oil is preferred by taste panelist over the pet ether solvent.

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Basil oil incorporated beeswax coating to increase shelf life and reduce anthracnose development in mango cv. Willard

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Abstract: Use of biologically safe, essential oil-incorporated fruit coatings to reduce postharvest losses has become an attractive alternative to synthetic fungicides in recent years. Increase in shelf life due to reduced water loss and reduced evaporation of essential oils (EO) within the coating are key advantages of incorporating EOs to fruit coatings. The effect of beeswax as a fruit coating with and without essential oil on the postharvest quality and natural disease development of mango (cv. Willard) was evaluated by conducting a laboratory experiment. Pure Beeswax, dissolved either in vegetable oil (1:3 w/v) or petroleum ether (1:4 w/v), served as the coating material. Basil oil at 400 or 600 µL L^{-1} was incorporated to the different coating material. Fruits with no coating and no essential oil served as controls. Eight replicate fruits were used per treatment and three trials were performed. Treated fruits were arranged in a Completely Randomized Design and kept under ambient conditions. Daily observations were made on natural disease development and weight loss. The Total Soluble Solid content and titrable acidity were measured at eating ripe stage and sensory evaluation by a taste panel was also obtained. Data were analyzed using ANOVA with SPSS software version 20.0 package. Results indicated that basil oil in beeswax significantly reduced physiological weight loss, delayed fruit ripening and also delayed and reduced postharvest disease development.

Keywords: Basil oil, anthracnose, mango, fruit coating, beeswax.

INTRODUCTION

Due to increasing awareness of the benefits of a balanced diet and healthy eating habits, there is a high consumer demand for fresh fruits and vegetables. Although the production of fresh commodities is high, a large percentage of fresh produce is lost postharvest, due to diseases caused by microorganisms. Except for the 'organic' food products, the remainder are highly polluted with chemical fungicides. Application of synthetic chemicals for prevention of postharvest diseases is at present being misused. The use of essential oils (EO) for the control of disease has become an attractive alternative to synthetic chemicals due to its therapeutic activity and toxicity to fungi, bacteria and insects (Delespaul *et al*., 2000). The oils of herbs such as thyme (*Thymus*), marjoram (*Origanum*) and basil (*Ocimum*) are generally regarded as safe (GRAS) and contain compounds such us cinnamaldehyde, acetaldehyde and eugenol. Use of essential oils for the control of postharvest diseases has been tried out with promising results on fruits such as mandarin, kiwi and rambutan (Arras, 1988; Thanassoulopoulos and Yanna, 1997; Sivakumar *et al*., 2002).

Anthracnose of mango has been effectively controlled by the use of essential oils, ginger and cinnamon (Sefu *et al*., 2015), lemongrass (Duamkhanmane, 2008), basil oil (*Ocimum basillicum*), orange oil (*Citrus sinensis*), lemon oil (*Citrus medica*) and mustard oil (*Brassica juncea*) (Abd-alla and Haggag, 2013). In Sri Lanka, extensive work has been carried out by Abeywickrama and co-workers (Abeywickrama *et al*., 2003; Anthony *et al*., 2004; Anthony *et al*., 2003) on the use of essential oils for the control of postharvest diseases of banana fruit. Treatment with oil of *Ocimum basilicum* controlled crown rot and anthracnose of banana with no detrimental effect on organoleptic properties (Anthony *et al*., 2004) while *Cymbopogon nardus* and *O. basilicum* oils were directly fungitoxic to common postharvest pathogens in banana, *Colletotrichum musae*, *Lasiodiplodia theobromae* and *Fusarium proliferatum* (Anthony *et al*., 2004). Cardamom oil in warm water (45 ˚C), used as a dip treatment *in vivo,* significantly reduced Stem-end rot development in mango cv 'Karuthacolomban' (Kulasinghe *et al*., 2019) and spray and fumigation treatments with basil, clove, cinnamon leaf and cinnamon bark oils effectively controlled SER in mango cv 'Karthacolomban' stored at 12 – 14 ºC (Kodituwakku *et al*., 2020).

However, due to their high volatile nature, the essential oils are more effective when they are incorporated into a coating material and then applied to a fruit. Edible coatings, which slowdown the ripening process, protect the fruit from water loss and spoilage, may be a good way to increase the shelf life of fresh produce (Antunesa *et al*., 2012). More recently, the inclusion of additives such as essential oils and other constituents with antimicrobial or antioxidant properties, into edible coatings to enhance their effectiveness, has been reported and patented (Antunesa *et al*., 2012). Cinnamon oil incorporated to beeswax (BW) has been found to reduce microbial contaminations and increase shelf life of sweet peppers (Yimtoe *et al*., 2014). BW and chitosan as fruit coatings, at different concentrations (0.5%, 1.5% and 2%), were applied on

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two mango varieties 'Apple' and 'Tommy Atkins' (Eshetu *et al*., 2018). Application of beeswax and chitosan at 2%, significantly reduced physiological weight loss, disease incidence, maintained firmness and prolonged shelf life of fruits compared with untreated control (Eshetu *et al*., 2018).

The present research was conducted to investigate the effect of basil oil-enriched beeswax coating material in controlling anthracnose disease caused by *Colletotrichum* spp. and also evaluate its effect on ripening and physicochemical, organoleptic parameters of the fruit. Anthracnose is one of the most important and widely distributed diseases of mango cv. Willard (Karunanayake *et al*., 2013).

MATERIALS AND METHODS

Essential oil

Pure-grade essential oil of basil (*Ocimum basilicum*) was obtained from Herbal Exotics, Pugoda, Sri Lanka.

Fruits

Mature, unblemish-free mango fruits of cv. 'Willard' at harvesting maturity were obtained from Anuradhapura (8.3114˚ N, 80.4037˚ E) (North Central Province) within 24 h of harvest. The fruits were transported to the Department of Botany Research Laboratory in The Open University of Sri Lanka, washed in tap water and allowed to dry under ambient conditions. Healthy, unripe fruits of uniform maturity were used in experiments.

Preparation of bio-safe coatings

Beeswax (Beeswax pure for histology, 24031-0247368-8012-89-3, SRL) was melted at 65 ^{o}C in a water bath as double boiled. Beeswax (BW) in vegetable oil (1:3 w/v) and BW in pet ether (1:4 w/v) were prepared with slight modifications to the method described by Mladenoska (2012).

Preparation of essential oil mix with coating

Basil oil and the suitable concentration were selected based on preliminary trials by Kulasinghe (2016). Although a higher concentration of the EO was necessary to retard the growth of *C. gloeosporioides in vitro*, a lower concentration (600 $\mu L\ L^{-1}$) of EO was sufficient to retard disease development when incorporated as an emulsion with a coating material as identified in preliminary trials. One litre of each coating material was prepared by mixing coating material and essential oil as follows: BW with pet ether 1:4 without basil oil, BW with pet ether 1:4 + 600 $\mu L\ L^{-1}$ basil oil, BW with pet ether 1:4 + 400 $\mu L\ L^{-1}$ basil oil, BW with vegetable oil 1:3 without basil oil, BW with vegetable oil 1:3+ 600 $\mu L\ L^{-1}$ basil oil, BW with vegetable oil 1:3 +400 $\mu L\ L^{-1}$ basil oil and untreated control dipped in distilled water alone.

Effect of the *in vivo* essential oil treatments on physiological weight loss (%)

Weight loss was determined by weighing of fruits daily for 5 days using a digital balance (Radwag, PS6000.R2). The average weight of all fruits in each treatment was taken and at the end of the experiment % weight loss was calculated by the formula of Gerefa *et al*. (2015),

$$\text{Physiological weight loss} = \frac{\text{Initial weight of fruits- Final weight of fruits}}{\text{Initial weight of fruits}} \times 100$$

Effect of *in vivo* treatment on disease incidence and severity

The number of fruits showing any visual sign of disease out of the eight replicates was recorded on a daily basis. The number of fruits with symptoms of disease served as the disease incidence and this was presented as a percentage. Anthracnose severity was determined visually for each fruit subjected to different treatments based on the digital image scale (Corkidi *et al*., 2006). The disease severity was recorded daily as a percentage and the average value was determined per day per treatment.

Effect of treatments on physicochemical properties

Pulp pH

Four (4) fruits of mango, at table ripe stage, were selected from each treatment and fruit pulp was made into juice without adding water. Pulp pH was measured using a calibrated digital pH meter (IQ150 Spectrum Technologies Inc.).

Flesh firmness

Flesh firmness was determined in mango fruit (4 replicate fruits) at table ripe stage using a hand-held penetrometer (Model FT 40 Wagner instruments Greenwich CT).

Total Soluble Solids (°Brix)

To determine the total soluble solids content (TSS), four mango fruits at table ripe stage from each treatment were selected and fruit pulp was made into juice without adding water. The Brix value of the pulp of each treatment was recorded using a portable refractometer (WZ113- Zhejiang top instruments Co Ltd, Brix/ATC 0 ~ 32%) of 0-32 Brix range at RT (25 ± 2 °C) and expressed as °Brix (Samane *et al*., 2012).

Effect of treatments on fruit ripening

Mango fruits cv. 'Willard', which changed their peel colour from green-red to yellow-red, were considered to have reached table ripe stage of maturity. The number of fruits that have undergone the desired colour change were noted daily from day 1 to 5 after treatment. The average number of ripe fruits per day for each treatment was recorded.

Effect of treatments on sensory properties – Taste panel

A panel of untrained 27 adults (aged above 21 years), comprising both male and female, was randomly selected for sensory evaluation. A five-point hedonic scale, described by Larmond (1977) was used where 5 = very high, 4 = high, 3 = moderate, 2 = low and 1 = very low. The samples were served in identical plates. The organoleptic properties of interest were: color (both peel and flesh color), appearance, texture, odor and taste (Umuhozariho *et al*., 2013).

Statistical analysis

Eight replicate fruits were used per treatment and all experiments were performed thrice. Both treated and control fruits were arranged in a completely randomized design (CRD) and kept on laboratory tables in clean plastic trays under ambient conditions for 5 days after treatment. Data were analyzed as a factorial ANOVA using the statistical package, IBM SPSS version 20.0. After applying the Least Significant Difference (LSD) test, differences of $P \leq 0.05$ were considered as significant. For sensory evaluation data, Principal Component Analysis (PCA) and cluster analysis were used to identify the preferences of the panelists for the samples.

RESULTS AND DISCUSSION

Physiological weight loss

Physiological weight loss was determined in treated and control fruits from day 1 to day 5 after treatment. The physiological weight loss was significantly higher in the control fruits which had no coating material or EO treatment on all days considered as opposed to the fruits receiving treatments (Figure 1). Physiological weight loss among treatments (except the control) were not significantly different (Figure 1). Either BW coating in pet ether or vegetable oil or the same coatings incorporated with EO had significantly lesser weight loss when compared with the control. Mladenoska (2012), Foo (2019) and Yimtoe *et al*. (2014) also reported that beeswax when applied as a fruit coating significantly reduced the weight loss of fruits.

Disease incidence and severity

The disease incidence and severity were determined in controls and fruits treated with essential oil incorporated coating material from day 1 to 5 after treatment. Anthracnose symptoms were visible in fruits following 3 days of incubation (Table 1a).

The highest disease incidence and severity were seen in control fruits, on days 4 and 5, this was followed by fruits which received only the coating treatment, either beeswax in vegetable oil or beeswax in pet ether; the fruits which received basil oil incorporated coating had the lowest disease incidence and severity (Table 1a, b). Of the two basil oil treatments, the lowest disease incidence and severity were seen in the higher (600 µL L^{-1}) basil oil treatment. Control fruits had significantly higher disease severity compared to all other treated fruits. By the day 5, an average of 50% disease severity was observed in the control fruits whereas all treated fruits had less than 10% disease severity (Table 1b). Similarly, Yimtoe *et al*. (2014) reported that application of cinnamon oil incorporated beeswax on sweet peppers reduced microbial development and also reduced weight loss when compared with uncoated controls.

The lowered disease incidence and severity in fruits that received basil oil incorporated coating, could be due to the antimicrobial activity found in several components of basil oil. The antimicrobial activity of basil against various microbes has been reported in previous studies (Bozin *et al*., 2006; Sokovic and Griensven, 2006) and many scientists have linked the antimicrobial effects of basil to the higher level of linalool which is the main component of the oil (Juliani and Simon, 2002).

Physicochemical properties

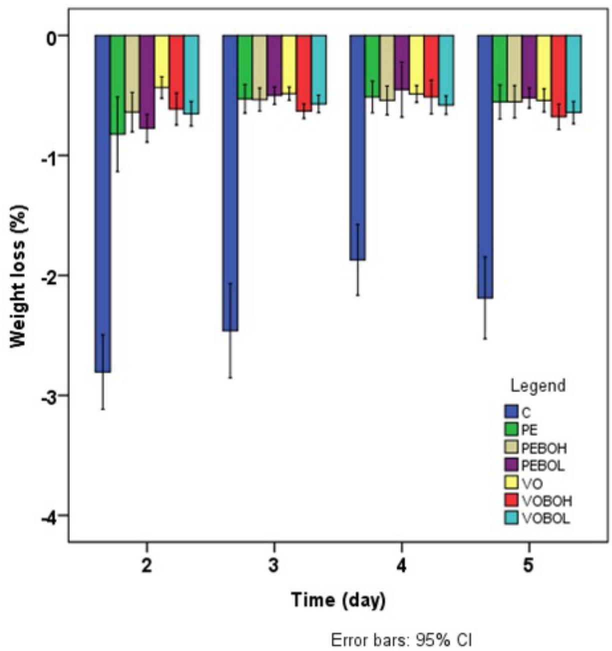


Figure 1: Physiological weight loss (%) in control fruits and fruits coated with varying beeswax coatings from day 1 to 5 after treatment, C- control, PE- beeswax dissolved in Pet ether, PEBOH- beeswax in pet ether with 600 µL L^{-1} basil oil, PEBOL- beeswax in pet ether with 400 µL L^{-1} basil oil, VO- beeswax dissolved in vegetable oil, VOBOH- beeswax in vegetable oil with 600 µL L^{-1} basil oil, VOBOL- beeswax in vegetable oil with 400 µL L^{-1} basil oil. Error bars at 95% confidence interval.

Table 1: Average number of fruits showing disease symptoms expressed as a % (a), and the severity of the disease % (b), in the control fruits and fruits coated with beeswax either with or without EO.

(a) Disease incidence

Treatment code	Disease incidence (%)		
	Day 3	Day 4	Day 5
*Control	50	75	75
PE	50	50	50
PEBOH	25	37.5	37.5
PEBOL	37.5	37.5	50
VO	50	50	50
VOBOH	37.5	37.5	37.5
VOBOL	62.5	62.5	62.5

(b) Disease severity

Treatment code	Disease severity (%)			status of disease severity
	Day 3	Day 4	Day 5	
C	6-9	10-49	10-49	Severe
PE	1-5	6-9	6-9	Moderate
PEBOH	0-1	0-1	0-1	No disease
PEBOL	0-1	0-1	1-5	Slight
VO	0-1	1-5	1-5	Slight
VOBOH	0-1	0-1	0-1	No disease
VOBOL	0-1	0-1	0-1	No disease

*C - control, PE- beeswax dissolved in Pet ether, PEBOH- beeswax in pet ether with 600 µL L^{-1} basil oil, PEBOL- beeswax in pet ether with 400 µL L^{-1} basil oil, VO - beeswax dissolved in vegetable oil, VOBOH- beeswax in vegetable oil with 600 µL L^{-1} basil oil, VOBOL- beeswax in vegetable oil with 400 µL L^{-1} basil oil.

Pulp pH, flesh firmness and total soluble solids content

Ripe mango has a slightly acidic pH value range of 5.8-6.0. pH value in all treated and control fruits was within this range. Islam *et al*. (2013) also reported similar values for pulp pH in mango fruit. There was no significant difference in pulp pH between treated and control fruits (Table 2). Therefore, the coating material and the EO treatment did not affect the pH of fruits. Flesh firmness was not affected by treatments (Table 2). All treated and control fruits had flesh firmness ranging from 1.48 to 1.8 and there was no significant difference between treatments.

Total soluble solids in all treated and control fruits were in an acceptable range at table ripe stage. Control fruits had the highest TSS compared to the others (Table 2). The TSS, in fruits receiving different treatments, was not significantly different, however, the TSS in control fruits was significantly higher. Foo *et al.* (2019) also reported a lower breakdown in soluble solids in beeswax coated fruits. Since all treated fruits received a beeswax coating, this could be the reason for the lower TSS. Brix value for sugar content is given as 4% for poor, 6% for average, 10% for good and 14% for excellent in mango fruit (Harrill, 1998). The control fruits accordingly can receive excellent grading. However, all treated fruits are also in the 'good' category. Abeywickrama *et al*. (2009) also reported that no significant effects on physicochemical parameters, titrable acidity, pH, TSS and pulp firmness were visible in banana fruit treated with basil oil.

Effect of treatments on ripening

The number of days taken to reach table-ripe stage of maturity increased significantly in treated fruits receiving beeswax coating alone or with EO when compared with control fruits. Ripening commenced after 3 days in control fruits (37 %) while ripening was seen to commence only 4 days after treatment in the fruits which received coating treatments (Table 3). By the day 5, 100% of control fruits were ripe while only 25% of treated fruits were in ripe stage. Foo *et al*. (2019) also report that senescence, weight loss and breakdown of soluble solids in the fruit were slowed down by beeswax coatings. Yimtoe *et al*. (2014) reported that cinnamon oil incorporated beeswax coatings increased shelf life while maintaining quality of sweet pepper under ambient and cold storage conditions. Generally, edible coatings are known to create a modified atmosphere and reduce weight loss, metabolic activities and protect the commodity from microbial attack during transport and storage (Tripathi and Dubey, 2004).

Effect of the treatments on sensory properties - Taste Panel

According to the results of the taste panel, the control (No wax, no oil) fruits were the most preferred (Figure 2) and the second most acceptable were the fruits coated with beeswax in vegetable oil with 600 µL L^{-1} basil oil (Figure 2). All the other treatments were not rated favourably by the panelists. Abeywickrama *et al*. (2009) also report that in banana treated with basil oil the most preferred by the panelists was the control.

CONCLUSIONS

Beeswax treatment significantly reduces weight loss, disease incidence, disease severity and significantly increases the number of days taken to reach table ripe stage of maturity in mango fruits of cv. 'Willard'. Incorporating basil oil at 600µL L^{-1} to beeswax coating was the most effective to control anthracnose and 600 µL L^{-1} beeswax in vegetable oil was also the most preferred by the taste panelists second only to the control. However, the method used to dissolve the beeswax needs to be considered. The pet ether solvent was not favourable to taste panelists while the vegetable oil solvent was preferred. The physicochemical properties except TSS were not affected by the treatment, however, there appears to be a slight effect on the organoleptic properties of the fruit as the control was the most preferred by the taste panelists.

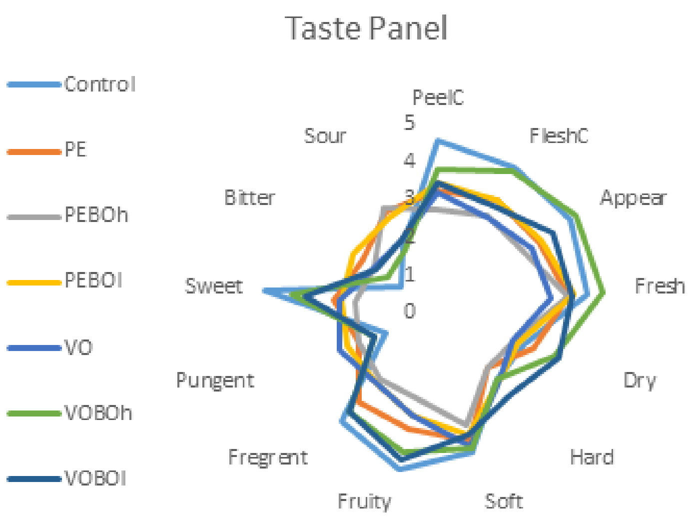
Table 2: Physicochemical parameters in the control fruits and fruits coated with beeswax, with or without EO treatment at table ripe stage of maturity. Table gives, mean value +or- standard deviation

Physicochemical parameter			
Treatment Code	pH	Firmness	TSS
Control	5.04±0.08	1.39±0.11	12.86±0.96
Beeswax in pet ether	4.80±0.20	1.85±0.25	11.00±1.00
Beeswax in pet ether 600µL L^{-1} basil oil	4.75±0.05	1.60±0.20	11.00±3.00
Beeswax in pet ether 400µL L^{-1} basil oil	4.85±0.05	1.45±0.85	11.00±1.00
Beeswax in veg oil	4.90±0.00	1.90±0.00	8.00±0.00
Beeswax in veg oil 600µL L^{-1} basil oil	4.70±0.00	1.90±0.00	11.00±0.00
Beeswax in veg oil 400µL L^{-1} basil oil	4.60±0.00	1.80±0.00	10.00±0.00

Table 3: The percentage (%) of fruits to reach table ripe stage of maturity from days 3 to 5 after treatment

Treatment Code	**D ay 3 %**	**Day 4 %**	**Day 5 %**
Control	37.50	62.50	100
Beeswax in pet ether	0	25	25
Beeswax in pet ether 600 µL L^{-1} basil oil	0	25	25
Beeswax in pet ether 400 µL L^{-1} basil oil	0	25	25
Beeswax in veg oil	0	12.50	12.50
Beeswax in veg oil 600µL/L basil oil	0	25	25
Beeswax in veg oil 400µL/L basil oil	0	25	25

Figure 2: Radar plot representation of the taste panel results.: Control- no coating or EO, PE – Bees wax in Pet ether, PEBOh– Bees wax in Pet ether: 600 µL L^{-1} Basil oil, PEBOl – Bees wax in Pet ether: 400 µL L^{-1} Basil oil, VO – Bees wax in Vegetable oil, VOBOh – Bees wax in Vegetable oil: 600 µL L^{-1} Basil oil, VOBOl– Bees wax in Vegetable oil: 400 µL L^{-1} Basil oil.



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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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