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# Application of cost-effective coating materials supplemented with different types of local essential oil to control *Fusarium verticillioides* (Sacc.) Nerenberg from post-harvest avocado fruits

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**Abstract** Post-harvest disease of avocado fruits was isolated for the causal agent, *Fusarium verticillioides* (Sacc.) Nirenberg which was molecularly identified using internal transcribed spacer (ITS). Among 4 types of local essential oils (0, 0.1, 0.25, 0.5, 1 and 2%) from *Citrus* sp., *Cocos nucifera*, *Cymbopogon flexuosus* and *Syzygium aromaticum* and 4 coating materials, beeswax (0, 5, 10 and 20%), chitosan (0, 0.5, 1 and 2%), gelatin (0, 2, 5 and 10%) and paraffin wax (0, 5, 10 and 20%), oil from *S. aromaticum*, beeswax and chitonsan exhibited antifubgal activities against *F. verticillioides* (postharvest disease of avocado fruits). The result revealed that the higher concentrations of coating materials (5, 10 and 20% beeswax and 0.5, 1 and 2% chitosan) and *S. aromaticum* oil (0.5 and 1%) showed the greater fungal inhibition. In conclusion, the local essential oil from *S. aromaticum* and coating materials, beeswax and chitosan gave the potential prevention against the fruit rot disease on avocadoes.

**Keywords:** Avocado fruits, Essential oil, Plant fungi, Post-harvest

## Introduction

Avocado has proved to be a valuable economic commodity in Thailand. It has become favorable to consumers because of its high nutritional value (Sukmak, 2016). One important area for growing avocados is in the northern part of Thailand, which is located in the tropics. The area is filled with many major fungal diseases such as scab causd by *Sphaceloma perseae*, black spot disease by *Cercospora purpurea*, anthracnose *Colletotrichum gleosporioides* as well as fungal rot diseases caused by various fungi such as *Botryodiplodia*

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*theobromae, Fusarium culmorum, Thyronectria pseudotrichia, Phomopsis perseae, Dothiorella aromatic* and *Lasiodiplodia theobromae* (Korsten and Kotzé, 1992). The causal agents can come from the cultivation sites, harvesting, transportation, and point of sale. Therefore, the occurrence of these diseases must be controlled from the period of harvesting and extended to the storage period before consumption.

There are many ways to control the diseases in post-harvest fruits, such as coating. The simple and common approach is to coat the fruits with fungi-resistant materials such as chitosan, chitosan wax, various polysaccharides e.g. chitosan and chitosan - common polymers in shrimps and crabs (Dhall, 2013). The chitosan is very flexible and well-ventilated. Therefore, it is used to coat fruits such as strawberries, cucumbers, apples and pears (Davies *et al*., 1989, El-Ghaouth *et al*., 1991, Dhall, 2013). Gelatin is a substance in the connective tissue, bones and skin able to be converted into a film by adding glycerin and sorbitol. It is used for coating products with low humidity and moisture (Dhall, 2013). Another coating material commonly applied to both fruits and sweets is made from waxes, lanolin, paraffin and other lipids. This material type can prevent moisture from outside and also keep moisture inside the products (Dhall, 2013; Hernandez, 1991).

Reportedly, essential oils from plants can also inhibit the growth of many kinds of plant pathogenic fungi (Combrinck *et al*., 2011). Controls of fungal pathogens can be achieved differently. The use of essential oil extracted from medicinal plants in both *in vitro* experiments and actual samples were reported to control the fungal pathogens on fruits (Combrinck *et al*., 2011). For example, essential oils can inhibit the fungal growth on mangoes and oranges (DuPlooy, 2007; Regnier *et al*., 2008). Essential oils from the lemongrass (*Cymbopogon* spp.), coriander (*Coriandrum sativum*), and *Lippia alba* are able to control anthracnose in passion fruits (Amini *et al*., 2016, Anaruma *et al*., 2010). Jatropha and Jojoba oils reported to be antifungal properties (Dayan *et al*., 2009). Another study found that the essential oil containing carvone which comes from mint could inhibit the growth of many fungi including *Colletotrichum gloeosporioides, Lasodiplodia theobromae, Alternaria alternata* and *Penicillium digitatum* in mango, avocado, and pear (Combrinck *et al*., 2011). In addition, coconut oil could inhibit the growth of *Polyporus sanguineu* (Shiny *et al*., 2014). The use of citrus oil can reduce the growth of *Colletotrichum musae* in bananas and *Botrytis cinerea, Penicillium italicum* and *Penicillium digitatum*, and clove oil can retard *Penicillium* sp., *Aspergillus niger* and *A. versicolor*, which cause diseases in post-harvest apples (Amiri *et al*., 2008; Vitoratos *et al*., 2013; Ma-in *et al*., 2014). It can be seen that many plant essential oils have potential to inhibit the fungi on agricultural products.

Therefore, this research sought to choose essential oils that could be obtained and locally mixed with the coating materials in order to increase the ability to inhibit *F. verticillioides* causing postharvest disease of avocado fruits.

## Materials and methods

### *Fungal isolation and identification*

Avocado fruits were randomly collected in the market regardless of cultivars and sources, then incubated at room temperature and observed for disease. Both external and internal layers of the fruits were observed for signs and symptoms. Then, the diseased parts were isolated for the causal agent using tissue transplanting technique. Diseased tissues ($1 \times 1$ $mm^2$) on the fruits were dissected, transferred onto water agar plates and incubated for 3-7 days. The germinating mycelia were then taken to new petri dishes containing potato dextrose agar (PDA) to obtain pure fungal isolates. Then, the fungal mycelia were inoculated on the disease-free avocados and incubated in moist chambers to observe and confirm the pathogenicity of the isolated fungi. The fungal mycelia were also kept for identification and further experimentations.

### *Isolation of fungi and DNA extraction*

The fungal isolates from the previous experiment were cultured in potato dextrose broth for 7days and mashed in liquid nitrogen. Then, genomic DNA was extracted by using the standard method (White *et al.*, 1990) as follows: the grounded sample was incubated in the lysis buffer ( 200mM, Tris-HCl, pH 8.0; 250mM NaCl, 25mM EDTA, pH 8.0, 1%sodium dodecyl sulfate) with ß-mercaptoethanol at 60 °C for 60 min, then chloroform: isoamyl alcohol (24:1) was added and centrifuged at 12,000 rpm for 5 min. The clear supernatant was followed by adding isopropanol and stored at - 20 ° C to precipitate the DNA. After that the centrifugation at 12, 000 rpm for 5 min was performed to obtain the DNA pellets. The pellets were washed with 70% ethanol then air dried before being dissolved in TE buffer ( 10mM Tris-HCl, 1 mM EDTA). RNase A, 1 µL ( 10ng / µL) was added and incubated at 37 °C 30 min followed by Proteinase K, 1µL ( 10ng / µL) with incubation at 37 °C for 30 min. The DNA was washed again by adding chloroform: isoamyl alcohol (24:1) and centrifuged at 12,000 rpm for 5min. The clear supernatant was kept and added with 3M sodium acetate and absolute ethanol before being stored in - 20 °C to precipitate the DNA, The centrifugation at 12,000 rpm for 10min was

done to obtain the genomic DNA, and air-dried before adding TE buffer to dissolve the pellets and at - 20 °C for the next use.

***Polymerase chain reaction (PCR)***

The identification of the fungus was required to DNA sequence of the internal transcribed spacer (ITS) using ITS-4TCCTCCGCTTATTGATATGC and ITS -5GGAAGTAAAAGTCGTAACAAGG primers (White *et al*., 1990). Both primers amplified the identification region with PCR conditions as follows: 95$^{o}$C for 3 minutes followed by 35cycles of 95$^{o}$C for 1 min, 55$^{o}$C 1 min, 72$^{o}$C for 2min and 72$^{o}$C for 10 minutes for final extension (White *et al*, 1990). In 1 reaction of PCR ( 50µl), it contained the master mix with standard substances according to White *et al.* (1990), then PCR products was detected with electrophoresis in the TBE buffer ( 1M Tris, 9 .0M boric acid, and 0.01M EDTA, pH 8.3) before sequencing.

***Phylogenetic trees analysis***

The process confirmed the type of the fungus from the ITS sequences derived from successful PCR products. Once the DNA sequence obtained, it was submitted to the Sequence Scanner Software v2.0 to detect an unclear chromatogram which must be removed before analysis. DNA sequences of referencing fungus were taken from GenBank. (www.ncbi.nlm.nih.gov) to align with the DNA sequences of this study by using MEGA 6 (Table 1). The Neighbor-joining analysis was achieved for fungal identification. With 1000 replicates of bootstrap test, the evolutionary distances were computed using the p-distance method in the units of the number of base differences per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA 6 (Tamura *et al*., 2013).

**Table 1.** ITS sequences of *Fusarium* species retrieved from the database

| **Species** | **Accession no.** |
|---|---|
| *Fusarium oxysporum* | JX406553, JX406508, JX406507 |
| *Fusarium solani* | MF688989, MF688988, KC013591, JX406551 |
| *Fusarium proliferatum* | EU151488, EU151487, EU151486, EU151489 |
| *Fusarium verticillioides* | KR052812, KR020684, LC363503 |
| *Fusarium fujikuroi* | MG654673, KX878924, KU991656, KU604035 |
| *Gibberella avenacea* | AB272122, AB272121, AB272120, JX406576 |
| *Fusarium culmorum* | AB272116, AB272115 |
| Isolates for this study | H4, H16, P10 |

***Preparation of coating materials***

The local coating materials were selected from 3 different groups, 1) protein (gelatin), 2) polysaccharide (chitosan), and 3) fat and lipid (beeswax and paraffin). Gelatin solutions of 2%, 5% and 10% were prepared in heated sterize distilled water at 70 $^{o}$C until completely dissolved. Then, glycerol 10 g per 100 g of gelatin solution was added (Fakhouri *et al*., 2014). The chitosan solutions of 0.5%, 1% and 2% were prepared as follows: chitosan powder was dissolved in 0.6% acetic acid and heated until 90 $^{o}$C using triethanolamine (0.15%) as an emulsifier and continuously stirred until well mixed, before adding 2% glycerol (Tzoumaki *et al*., 2009; Cerqueira *et al*., 2009; Vasconez *et al*., 2009; Suseno *et al*., 2014). The beeswax and paraffin emulsions were prepared separately into 3 concentrations (5%, 10% and 20%) by melting bees wax and paraffin until they became completely liquid at 90 ℃. Then, 20 ml oleic acid and 60 ml triethanolamine (TE) were added and adding water to comprise a final volume of 1000 ml (Hagenmaier and Baker, 1996; Hassan *et al*., 2014).

***Inhibitory activities of different plant essential oils in vitro***

Four essential oils from plants, *Citrus* sp. (citrus plants), *Cocos nucifera* L. (coconut), *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson (lemon grass) and *Syzygium aromaticum* (L.) Merrill & Perry (clove) were tested the fungal inhibition in different concentrations (0.1, 0.25, 0.5, 1 and 2%). They were mixed into PDA agar containing 0.1% tween80 before the test. The fungal plugs from the pure culture were placed onto the PDA plates containing different essential oil concentrations and incubated at room temperature for 120 hr. The radial measurement of fungal growth was conducted every 24 hr in comparison with the control (0% essential oil). This experiment was done in 5 replications. The average lengths of the radial fungal growth were compared using ANOVA at 95% confidence.

***Inhibitory activities of different coating materials in vitro***

Each coating material previously prepared with an equal volume (200 µl) was added to PDA plate and spreaded using a sterilized triangular glass rod. After the plates were prepared with the dried coating materials, the fungal plugs were placed onto the plate and incubated at room temperature ranging from 28

-35 $^{o}$C for 120 hr. A radial measurement of fungal growth was conducted every 24 hr in comparison with the control (0% coating material). The experiment was done in 3 replications. The average lengths of the radial fungal growth were compared using ANOVA at 95% confidence.

### *Inhibitory activities of coating materials combined with essential oil in vitro*

The best essential oil (clove) and coating materials (chitosan and beeswax) performing the most inhibitory activities were selected for this experiment. Beeswax (5, 10 and 20%) and chitosan solution (0.5, 1 and 2%) were prepared as previously described and homogenously incorporated with clove oil (0.5 and 1%). An equal volume (200 µl) of the coating materials containing clove oil was poured to PDA and spreaded on the surface before placing the fungal plugs. The plates were incubated at room temperature (28 - 35 $^{o}$C) for 120 hr. A radial measurement of fungal growth was conducted every 24 hr in comparison with the control (0% coating materials and essential oil). The experiment was done in 3 replications. The average radiuses of the fungal growth were compared using ANOVA at 95% confidence.

## Results

### *Fungal isolation and identification*

The fungus isolated from the diseased avocado fruit (Figure 1. A-E) was inoculated on healthy avocados and observed for fruit rot symptoms using the mycelail plugs. After 6 days of inoculation, the disease severity was visible compared to the naturally diseased fruits (Figure 1. F and G) with scabbing, fractured peeling, and damaged flesh underneath. The causal agent of avocado fruit rot was confirmed by pathogenecity test.

The phylogenetic analysis of the fungus was conducted using ITS regions using the Neighbor-Joining method. The optimal tree with the sum of branch length (0.26654412) is shown. The analysis involved 27 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 391 positions in the final dataset. As the results, it was found that the fungal isolate from the diseased avocado was *Fusarium verticillioides* with bootstrap score at 83 (Figure 2).

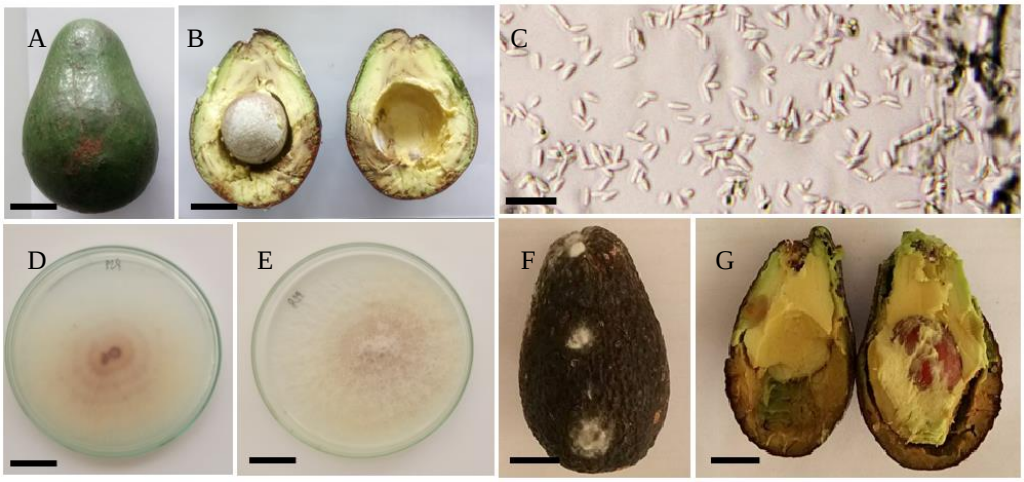


**Figure 1.** Fruit rot disease of avocado(A & B), Spores and pure culture of *Fusarium verticillioides* isolated from the diseased fruit (C & D), the inoculated Avocado fruits by *F. verticillioides* (F & G), Scale bars A, B, F & G = 3 cm, C = 5 µm, D & E = 2 cm

***Inhibitory activities of different plant essential oils in vitro***

Four types of essential oil (citrus, clove, coconut and lemon grass) were tested for their inhibition activities against *F. verticillioides* at 24, 48, 72, 96 and 120 hours. It was found that the clove oil showed the greatest inhibitory effect at all interval time which the tested pathogen did not grow (0.00 cm) in any concentration, and followed by lemongrass, citrus and coconut oil (Table 2. A-E).

The clove oil performed the highest ability to inhibit the growth of *F. verticillioides* at the lowest concentration of 0.1% because the tested pathogen did not grow followed by the lemon grass essential oil at the lowest concentration (0.25%). It completely inhibited *F. verticillioides* in 5 days. Thus, the best result to inhibit *F. verticillioides* was chosen to test for combination with coating materials in the next experiment.

***Inhibitory activities of different coating materials in vitro***

The four coating materials were beeswax, chitosan, gelatin and paraffin wax, were tested for their inhibition activities against *F. verticillioides* at 5 intervals (24, 48, 72, 96 and 120 hours). It was found that the beeswax showed the greatest inhibitory effect at all 5 intervals, and followed by chitin, paraffin wax, and gelatin (Table 3. A-E).

Beeswax is an edible coating material showed the highest fungal growth inhibition compared to other materials. After coating on the media surface, *F. verticillioides* was slow growing as the radial growth was shorter than the others at all 5 intervals, and followed by either chitosan or paraffin wax which was found that chitosan had the better inhibition than paraffin. Accordingly, the beeswax was selected as a representative of a fat and lipid, and chitosan was selected as another candidate from the polysaccharide group. Therefore, in the next experiment, beeswax and chitosan were used to combine with the clove oil.

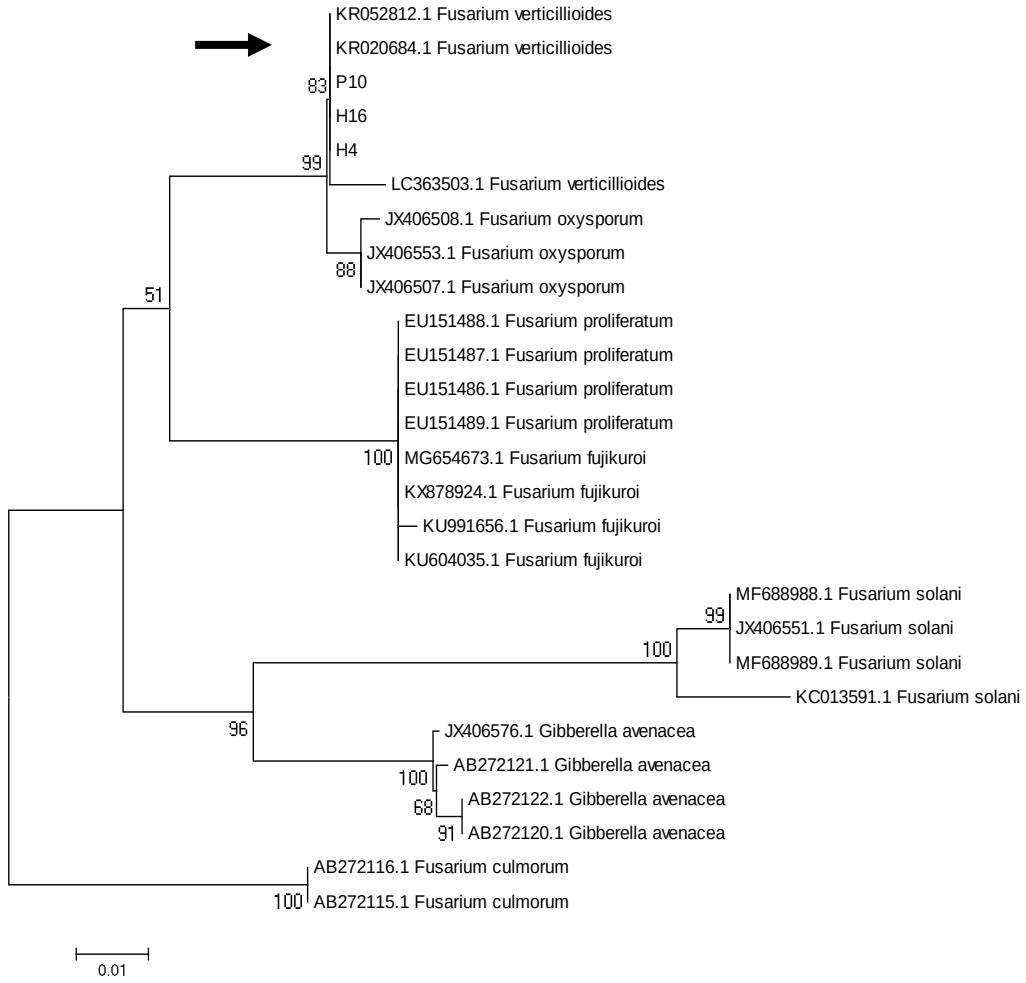


**Figure 2.** Phylogenetic tree of *Fusarium verticillioides* using ITS DNA sequence with bootstrap score at 83 (arrowhead)

**Table 2.** Average radial growth (cm) of *F. verticillioides* on the medium containing different concentrations and types of essential oils at 24 (A), 48 (B), 72 (C), 96 (D) and 120 hours (E)

| **(A)** | **Oil concentrations** | | | | | |
|---|---|---|---|---|---|---|
| **Oil types** | **0%** | **0.1%** | **0.25%** | **0.5%** | **1%** | **2%** |
| Citrus | 0.3200 a | 0.2050 b | 0.0000 b | 0.0000 b | 0.0000 b | 0.0000 b |
| Clove | 0.2550 bc | 0.0000 c | 0.0000 b | 0.0000 b | 0.0000 b | 0.0000 b |
| Coconut | 0.2850 ab | 0.3300 a | 0.3050 a | 0.3350 a | 0.3050 a | 0.3250 a |
| Lemon grass | 0.2400 c | 0.0000 c | 0.0000 b | 0.0000 b | 0.0000 b | 0.0000 b |
| **(B)** | | | | | | |
| **Oil types** | **0%** | **0.1%** | **0.25%** | **0.5%** | **1%** | **2%** |
| Citrus | 0.9350 ab | 0.5950 b | 0.0800 b | 0.0000 b | 0.0000 b | 0.0000 b |
| Clove | 0.8650 c | 0.0000 c | 0.0000 c | 0.0000 b | 0.0000 b | 0.0000 b |
| Coconut | 0.9450 a | 0.8900 a | 0.9450 a | 0.9350 a | 0.9000 a | 0.9700 a |
| Lemon grass | 0.8950 bc | 0.0000 c | 0.0000 c | 0.0000 b | 0.0000 b | 0.0000 b |
| **(C)** | | | | | | |
| **Oil types** | **0%** | **0.1%** | **0.25%** | **0.5%** | **1%** | **2%** |
| Citrus | 1.6200 a | 1.0250 b | 0.2950 b | 0.0500 b | 0.0000 b | 0.0000 b |
| Clove | 1.6350 a | 0.0000 d | 0.0000 c | 0.0000 c | 0.0000 b | 0.0000 b |
| Coconut | 1.6250 a | 1.6200 a | 1.6000 a | 1.6100 a | 1.5850 a | 1.6300 a |
| Lemon grass | 1.5400 b | 0.0500 c | 0.0000 c | 0.0000 c | 0.0000 b | 0.0000 b |
| **(D)** | | | | | | |
| **Oil types** | **0%** | **0.1%** | **0.25%** | **0.5%** | **1%** | **2%** |
| Citrus | 2.2550 b | 1.5000 b | 0.5600 b | 0.0650 b | 0.0000 b | 0.0000 b |
| Clove | 2.4150 a | 0.0000 d | 0.0000 c | 0.0000 b | 0.0000 b | 0.0000 b |
| Coconut | 2.1050 c | 2.2900 a | 2.2350 a | 2.3100 a | 2.3500 a | 2.2500 a |
| Lemon grass | 2.2300 b | 0.1000 c | 0.0000 c | 0.0000 b | 0.0000 b | 0.0000 b |
| **(E)** | | | | | | |
| **Oil types** | **0%** | **0.1%** | **0.25%** | **0.5%** | **1%** | **2%** |
| Citrus | 2.7700 b | 1.9600 b | 0.8250 b | 0.2100 b | 0.0000 b | 0.0000 b |
| Clove | 3.0450 a | 0.0000 d | 0.0000 c | 0.0000 c | 0.0000 b | 0.0000 b |
| Coconut | 2.7000 b | 2.8350 a | 2.8150 a | 2.7300 a | 2.8250 a | 2.7250 a |
| Lemon grass | 2.7700 b | 0.2350 c | 0.0000 c | 0.0000 c | 0.0000 b | 0.0000 b |

Means with the same letter are not significantly different.

**Table 3.** Average radial growth (cm) of *F. verticillioides* on the medium containing different coating materials at 24 (A), 48 (B), 72 (C), 96 (D) and 120 hours (E)

| **(A)** **Coating types** | **Concentration 1** | **Concentration 2** | **Concentration 3** | **Concentration 4** |
|---|---|---|---|---|
| Beeswax | 0.3667 b | 0.3917 b | 0.3417 b | 0.2083 b |
| Chitosan | 0.3333 b | 0.4500 b | 0.3167 b | 0.2167 b |
| Gelatin | 0.4000 a | 0.4500 a | 0.4167 a | 0.3750 a |
| Paraffin wax | 0.3667 b | 0.4250 b | 0.3167 b | 0.2833 b |
| **(B)** **Coating types** | | | | |
| Beeswax | 0.9167 a | 0.9500 a | 0.7917 a | 0.3917 b |
| Chitosan | 0.8333 a | 0.9833 a | 0.8833 a | 0.4500 b |
| Gelatin | 0.8417 a | 0.9667 a | 0.9000 a | 0.8750 a |
| Paraffin wax | 0.9000 a | 0.9083 a | 0.7500 a | 0.7167 a |
| **(C)** **Coating types** | | | | |
| Beeswax | 1.6083 a | 1.5417 a | 1.4667 b | 0.8000 d |
| Chitosan | 1.4583 b | 1.6167 a | 1.5083 ab | 1.2000 c |
| Gelatin | 1.5833 ab | 1.6167 a | 1.6167 a | 1.6083 a |
| Paraffin wax | 1.7167 a | 1.6667 a | 1.5250 ab | 1.3917 b |
| **(D)** **Coating types** | | | | |
| Beeswax | 2.0583 b | 2.1667 b | 2.0917 a | 1.2667 d |
| Chitosan | 2.0000 b | 2.1667 b | 2.1000 a | 1.6750 c |
| Gelatin | 2.2167 a | 2.2083 b | 2.1000 a | 2.1500 a |
| Paraffin wax | 2.3333 a | 2.3583 a | 2.0417 a | 1.8167 b |
| **(E)** **Coating types** | | | | |
| Beeswax | 2.6333 bc | 2.4917 b | 2.6167 a | 1.7500 c |
| Chitosan | 2.4917 c | 2.6500 a | 2.5417 ab | 2.2667 b |
| Gelatin | 2.6417 b | 2.7000 a | 2.6583 a | 2.6500 a |
| Paraffin wax | 2.8667 a | 2.7250 a | 2.4667 b | 2.2500 b |

Concentrations 1, 2, 3 and 4 for beeswax are 0, 5, 10 and 20% respectively.
Concentrations 1, 2, 3 and 4 for chitosan are 0, 0.5, 1 and 2% respectively.
Concentrations 1, 2, 3 and 4 for gelatin are 0, 2, 5 and 10% respectively.
Concentrations 1, 2, 3 and 4 for paraffin wax are 0, 5, 10 and 20% respectively.

Means with the same letter are not significantly different.

### *Fungal inhibitory activities of essential oil and coating materials in vitro*

Three concentrations of coating materials, beeswax, and chitosan were combined with clove oil and tested for their inhibition activities against *F. verticillioides* at 5 intervals (24, 48, 72, 96 and 120 hours), as shown in Table 4A-E. It was found that the inhibitory activity was related to the concentrations

of the coating material and clove oil i.e. the highest inhibitory activity was observed in the treatment containing 1% clove oil and both coating materials.

**Table 4.** Average radial growth (cm) of *F. verticillioides* on the medium containing clove oil (0.5 and 1%) and coating materials, beeswax and chitosan in different concentrations at 24 (A), 48 (B), 72 (C), 96 (D) and 120 hours (E)

| **(A) Essential oil + coating materials** | **Coating material concentration 1** | **Coating material concentration 2** | **Coating material concentration 3** |
|---|---|---|---|
| Clove (0.5%) + Beeswax | 0.2500 a | 0.2000 a | 0.1167 a |
| Clove (0.5%) + Chitosan | 0.1667 a | 0.1750 a | 0.1417 a |
| Clove (1%) + Beeswax | 0.3500 a | 0.1333 b | 0.0917 b |
| Clove (1%) + Chitosan | 0.2333 a | 0.1083 b | 0.1417 a |
| **(B) Essential oil + coating materials** | | | |
| Clove (0.5%) + Beeswax | 0.6583 a | 0.7333 a | 0.6167 a |
| Clove (0.5%) + Chitosan | 0.7000 a | 0.7250 a | 0.7250 a |
| Clove (1%) + Beeswax | 0.6250 a | 0.3583 b | 0.4750 b |
| Clove (1%) + Chitosan | 0.6083 a | 0.4083 b | 0.4000 b |
| **(C) Essential oil + coating materials** | | | |
| Clove (0.5%) + Beeswax | 1.0250 ab | 1.1250 a | 0.9833 a |
| Clove (0.5%) + Chitosan | 1.0833 a | 1.1750 a | 1.1500 a |
| Clove (1%) + Beeswax | 0.9333 ab | 0.7000 b | 0.7333 b |
| Clove (1%) + Chitosan | 0.8917 b | 0.6667 b | 0.5667 b |
| **(D) Essential oil + coating materials** | | | |
| Clove (0.5%) + Beeswax | 1.4750 a | 1.6417 a | 1.5000 a |
| Clove (0.5%) + Chitosan | 1.6083 a | 1.6583 a | 1.6167 a |
| Clove (1%) + Beeswax | 1.4417 a | 1.1333 b | 1.1833 b |
| Clove (1%) + Chitosan | 1.2667 a | 1.1333 b | 1.1000 b |
| **(E) Essential oil + coating materials** | | | |
| Clove (0.5%) + Beeswax | 2.0000 a | 2.1583 a | 1.9667 a |
| Clove (0.5%) + Chitosan | 2.0000 a | 2.0167 a | 2.0667 a |
| Clove (1%) + Beeswax | 1.9500 a | 1.4583 b | 1.5583 b |
| Clove (1%) + Chitosan | 1.7417 b | 1.5583 b | 1.4833 b |

Coating material concentrations 1 for beeswax and chitosan are 5 and 0.5% respectively.
Coating material concentrations 2 for beeswax and chitosan are 10 and 1% respectively.
Coating material concentrations 3 for beeswax and chitosan are 20 and 2% respectively.

Means with the same letter are not significantly different.

According to the findings, the treatments containing 1% clove oil and both coating materials were not statically different at all time intervals, except at 24 hours. It implies that the inhibitory effect against *F. verticillioides* was enhanced by the essential oil.

## Discussion

This study found *Fusarium verticillioides* causing fruit rot on avocados during storage. Its inoculum might be airborne to infect the fruits which are already susceptible as they are post-harvested and aerially exposed. This fungus once successfully colonized, could lead to rotten fruits with obvious symptoms, changes in color, and fractures on the fruit skin with very soft and darkened flesh. *F. verticillioides* is one of common species causing rot diseases in plants. This fungus can cause rot diseases in fruits such as plantain fruit (*Musa paradisiaca*) (Aborisade and Akomolafe, 2011), banana (Hirata *et al*., 2001; Triest and Hendrickx, 2016), pineapple (Ibrahim *et al*., 2017). In avocado, different *Fusarium* species e.g. *F. solani, F. oxysporum* and *F. equiseti* (Corda) are the cause of avocado wilt (Ram ŕez-Gil, 2018). This *Fusarium* species is also able to produce a mycotoxin called fumonisin which is adverse to humans (Blacutt *et al*., 2018).

Clove oil showed the highest inhibitory ability against the growth of *F. verticillioides* based on the result of this study because it could completely inhibit the growth of the fungus at very low concentration (0.1%) compared to the other three, citrus, coconut and lemon grass oil. This clove oil with antifungal, antimicrobial and general stimulating, anesthetic, and carminative properties has been traditionally used as a food preservative. It chemically consists of eugenol (80-95%), acetyl eugenol (1-5%) and β- caryophyllene (4-12%). There were reported that the anti-fungal activity of clove oil against different *Fusarium* species e.g. *F. solani, F. redolens F. oxysproum, F. commune, F. verticillioides F. oxysporum* and *F. monliformi*. Similar to the study result, the inhibitory effect of the oil against plant pathogenic fungi including *Fusarium* could be seen at a very low concentration and progressively increased when higher oil concentrations were tested (Ćosić *et al*., 2010; Hamini-Kadar *et al*., 2010; Sharma *et al*., 2017). Apart from *Fusarium*, eugenol as the main compound in clove could inhibit *Aspergillus* group such as *A. acculeatus, A. versicolor, A. fumigates, A. niger* and *A. flavus* and other plant pathogentic fungi e.g. *Alternaria alternata, Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Phomopsis viticola* and *Rhizopus stolonifer* (Hitokoto *et al*., 1980; Martini *et al*., 1996; Hong *et al*., 2015).

Chitosan as a polysaccharide from chitin is largely known as having potential in anti-fungal activities. It is used to test against different fungi

including plant pathogenic fungi e.g. *A. alternata*, *Botrytis cinerea*, *R. stolonifer* and *C. gloeosporioides* (El-Ghaouth *et al*., 1992). It possesses negative effects to microbial permeable membrane, respiration system, and mRNA and protein synthesis (Peña *et al*., 2013). Because of this adeptness and its safety for consumption, chitosan is widely used to control plant pathogens. This study also found the similar results as a report by Zachetti *et al.* (2019). They found that chitosan was able to decrease the growth rate of *F. verticillioides* and its fumonisin production. Additionally, beeswax, another coating material, was also potential due to the result of this study. It is reported to have an anti-microbe property against fungi and bacteria in synergy with other natural products like honey and olive oil (Fratini *et al*., 2016).

The combination of clove oil with coating materials, chitosan, and beeswax could enhance the antifungal activity. This is similar to studies reporting that the synergic effect of chitosan and clove oil could perform inhibitory effects against *Penicillium digitatum* on citrus fruits *in vitro*, *F. verticillioides* and *A. parasiticus* (Shao *et al*., 2015; Villegas-Rascón *et al*., 2018). These results suggested that the essential oil alone or combination with chitosan was able to reduce the growth, conidial germination and fungal production. Apart from chitosan, beeswax incorporated with cinnamon oil to coat sweet peppers was proved to have ability to extend the quality and shelf life of the pepper because the cinnamon oil had a strong anti-fungal effect (Yimtoe *et al*., 2014).

This study is proved that the clove oil and coating materials, beeswax and chitosan have potential in the anti-fungal activity against *F. verticillioides*. However, this study was only conducted the inhibitory tests *in vitro*. In future studies, these ingredients (clove oil, chitosan and beeswax) shold therefore be formulated these results as the coating materials on actual avocado fruits in order to practically examine the ability of the formulated coatings to prevent the fungal infection on the fruits. This could bring the practical application of the local essesntial oil and materials which are cost-effective to extend the shelf life of the avocado fruits.

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