



RESEARCH ARTICLE

# Antifungal potential of biobased oils from *Citrus sinensis* peels and *Eucalyptus globulus* leaves *in vitro* against fungal isolates.

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## Abstract

Biobased oils found in the leaves of many plants used as coating materials for the preservation of fruits influence the handling of citrus fruits. The effectiveness of these oils on the target organisms is associated with the ability to develop resistant strains. In the present study the antifungal activity of the biobased oils obtained from orange peel and eucalyptus leaves against *Aspergillus niger*, *Aspergillus flavus*, *Rhizomucor pusillus* and *Penicillium citrinum* was tested at various concentrations *in vitro*. Sample fruits showing signs of decay during postharvest storage were selected for isolation and identification of the fungi. A serial dilution method was applied at 10 fold for fungi isolates and concentrations were plated onto Potato Dextrose Agar (PDA) media 15 mL. The plates were incubated at room temperature (28°C) and observed every 24 hrs for possible microbial growth. The pour plate method was used to investigate the antifungal activity of the oils on the test fungi *in vitro*. The results indicate a continuous decline in the inhibition of the biobased oil treatment during the period of inoculation. The interaction effect of the different biobased oil, their concentrations and the different isolates was significant ( $P \leq 0.05$ ) from day 1 to day 7 during the inoculation. The combined orange peels and eucalyptus leaves at 100% concentration recorded (69.35%) as the highest percent of inhibition which was significantly higher than all other interactions. The least percent inhibition was found with orange peels biobased oil at 1:1 v/v concentration against *Aspergillus flavus* (24.23%). Biobased oils at full strength from eucalyptus combined with oil from orange peels demonstrated significant potential against *Penicillium citrinum*. This research revealed the potential antifungal properties *in vitro* of the biobased oils against pathogenic fungi. These findings provide the basis for the application of combined biobased oil as an effective antifungal remedy against pathogenic fungi.

## Keywords

Fungal isolation, postharvest, inoculation, *Aspergillus flavus*, *Penicillium citrinum*, inhibition

## Introduction

Fungi are the main spoilage agents that affect the quality of food during handling and storage. Biodeterioration caused by fungi results in the reduction of nutritional and economic values of organic materials. Bioactive volatile compounds are usually applied to prevent pathogenic fungi and

reduce the number of conidia that are likely to grow on produce. Plant-based oil are volatile aromatic compounds that have effective biological activity in preventing spoilage of different produce during handling (1).

The oils can be found in different parts of fruits as well as in the leaves of many plants. The major bioactive compounds of biobased oils are the terpenoids, terpenes and perylpropanoids needed for the antifungal activity (2). Biobased oil containing flavonoids, cumarins and phenols provides chemical defense by providing fungitoxic action in controlling postharvest disease (3, 4). Extraction from different plant sources produces biological and fungicidal properties for *in vitro* and *in vivo* applications. Biobased oil helps to prevent oxidation and act as antioxidants (5). Biobased oils extracted from the leaves having an antioxidant and antifungal properties due to the presence of eucalyptol and limonene that influence biological activity of fungal isolates (6, 7).

The effectiveness of the biobased oils on the target organisms is associated with the cell structure of the organisms and the ability to develop resistant strains. The antifungal activities of biobased oil is achieved by a mixture of volatile organic compounds that serve as precursors to disintegrate the cell structures of pathogenic fungi (8-10).

*In vitro* antifungal assays of oils inhibited mycelia growth of plant pathogenic fungi (11, 12). Plant-based oils from citrus reticulate mandarin and bitter orange tested *in vitro* demonstrated antimicrobial activity against *Aspergillus niger*, *Penicillium verrucosum* and *Aspergillus flavus* (13). The antimicrobial activity of a combination of Dimethyl sulphoxide (DMSO) and plant-based oils has been reported for preservative and antimicrobial activities. Studies of the hydrophobic nature of some biobased oils and their antimicrobial activities have been reported (14, 15). Antimicrobial treatments with DMSO as organic solvent showed higher percentages of fungal mycelia growth inhibition *in vitro* against the growth of *Aspergillus niger* and *Aspergillus flavus* (15, 16). The concentration of the solvents and the methodology used in antimicrobial analysis can influence the results. Dimethyl sulphoxide as a solvent was used to ensure the oils were appropriately disseminated into agar wells to assess *in vitro* antimicrobial activities against fungal organisms (17). There have been some studies on the activities of plant-based oils against different fungal pathogens. Although food-grade waxes and coating materials are available for the preservation of fruits, these materials are either expensive to purchase for commercial application or require a lot of waiting period in the process. However, there is no study conducted on antifungal activity of the combined effect of eucalyptus leaves and orange peels. The aim of the research was to evaluate the antimicrobial potential of oils from eucalyptus leaves and orange peels on isolated pathogenic fungi *in vitro*. The results provide a theoretical and practical basis for the application of biobased oils of orange peels and eucalyptus leaves for antifungal properties against pathogenic fungi.

## Materials and Methods

### Biobased oil extraction

Orange peels and eucalyptus leaves were obtained from Makurdi Metropolis and dried at room temperature 32°C. The samples were crushed before the commencement of extraction. The soxhlets extraction method was used with 15 kg and 20 kg of peels or leaves respectively by sample to solvent ratio of (1:10). The solvent used for the soxhlets extraction was absolute ethanol (Camlab UK). The soxhlets apparatus was set up as described earlier (18).

### Isolation of fungal organisms

Sample fruits showing signs of decay were selected and cut into small segments (3 cm in diameter) with a sterilized blade, surface sterilized in 1% hypochlorite for 2 min, crushed and ground. Each of the samples was dissolved in distilled water and added to a peptone solution. A serial dilution method as described earlier (19) was applied at 10 folds. The various concentrations were plated onto Potato Dextrose Agar (PDA) media 15 mL and incubated at room temperature (28°C) and observed every 24 hrs. for possible microbial growth. A pure culture was obtained and maintained by sub-culturing each of the different colonies that emerged onto the PDA plates and incubating at 28°C for 4 days. A portion of the pure culture was streaked while the media surface was streaked with a sterile loop with the pure culture incubated at a temperature (of 37°C). The methods used for the isolation were earlier suggested (20). A procedure described (21) was used for the identification of the isolates both macroscopically and microscopically with emphasis on cultural and morphological features such as colony growth pattern, conidial morphology and pigmentation.

### Antifungal Activity of biobased oils on Fungal Isolates *in vitro*

Pour plate method reported (22) was used to investigate the antifungal activity of the oils on the test fungi *in vitro*. Briefly, four milliliters each of 100%, 1:1 v/v, 2:1 v/v 3:1 v/v of the oils were dispensed in sterile petri dishes after which 15 mLs of molten PDA was added. The mixture was swirled gently on the workbench and allowed to be set. The medium was inoculated centrally with 3 mm discs obtained from 5 days old cultures of the test fungi (Fig. 1, 2). Each of the experiments involves 3 replications and controls were petri plates containing PDA without biobased essential oils inoculated with the test fungi. The plates were arranged in completely randomized design at room temperature for 7 days in BSU Laboratory. Measurement of growth of the fungi colony was carried out using a meter rule every 24 hours. Growth inhibition of the fungi was calculated using the formula described (23).

$$\text{Percent of growth inhibition (I)} = \frac{R_1 - R_2}{R_1} \times 100$$

Where I = Percent inhibition,  $R_1$  = Radial growth of the isolated fungi in control (mm),  $R_2$  = Radial growth of isolated fungi in treatment (mm)

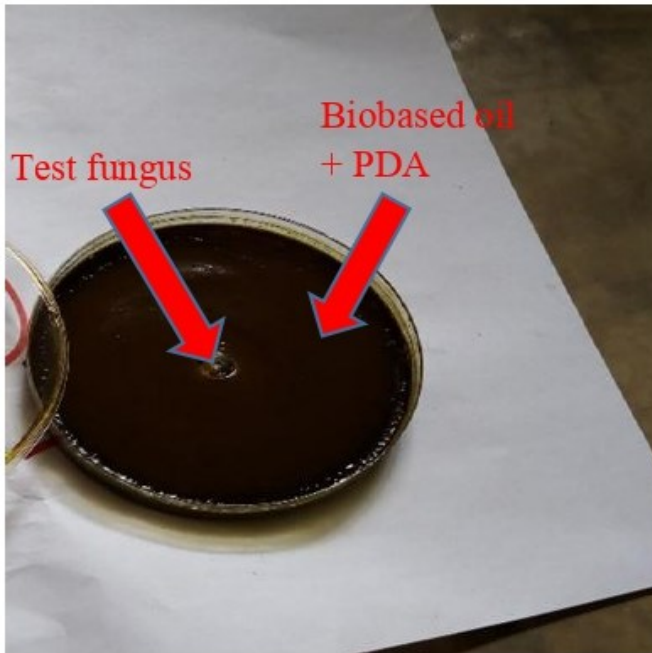


Fig. 1. Medium inoculated centrally with test fungus

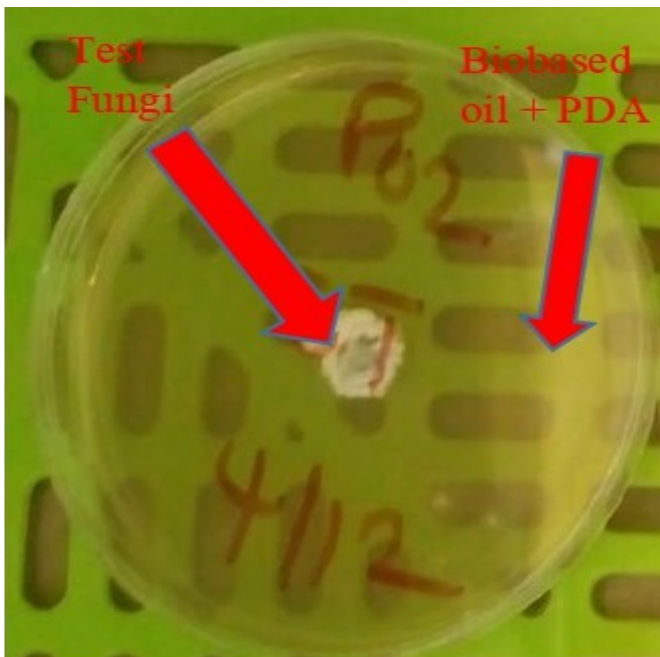


Fig. 2. Test fungus on PDA.

## Experimental Design

Factors in the experiment

3 oils

5 concentrations

4 fungi

Experimental design: 3 x 5 x 4 factorial in completely randomised design

Treatment combinations = 3 x 5 x 4 = 60

Replications 3

Total units 3 x 60 = 180 units

## Data Analysis

The data obtained from the study were subjected to an Analysis of Variance (ANOVA) to test whether there exist any significant differences at  $p \leq 0.05$  alpha level among the treatment means. The analyses were performed using the Gentsat Discovery Edition 7 for 2014 VSN International Limited. Means separation for microbial data analysis was done using the Duncan multiple range test.

## Results

### Effect of biobased oil type on % inhibition

The results indicate a continuous decline in the inhibition of the biobased oil treatment during the period of inoculation. The main effect of biobased oil treatment was significant ( $P \leq 0.05$ ) from day 1 to day 7 during the inoculation (Table 1). Effect of biobased oil on day 1 indicates that the highest percent inhibition was produced by eucalyptus leaves and orange peel combined (61.56%) which was significantly higher in comparison with eucalyptus (60.93%) and orange peels only (59.24%). On day 3, the percent inhibition for eucalyptus (58.68%) was significantly higher ( $P \leq 0.05$ ) than for eucalyptus leaves and orange peel combined (56.37 %) and orange peel (52.98%). On day 5, the % inhibition for eucalyptus (49.73%) was significantly higher ( $P \leq 0.05$ ) than for eucalyptus leaves and orange peel combined (46.97 %) and orange peel (46.23%). On day 7 after inoculation, the % inhibition produced by eucalyptus leaves and

Table 1. Main effect and interaction of different bio-based oils, concentration and fungal isolates on % inhibition of the growth of test fungi

TREATMENT	DAYS						
OIL TYPE (T)	1	2	3	4	5	6	7
orange peel	59.2 <sup>ab</sup>	55.89 <sup>c</sup>	52.98 <sup>c</sup>	49.27 <sup>c</sup>	46.23 <sup>b</sup>	42.08 <sup>b</sup>	39.01 <sup>b</sup>
orange peel + eucalyptus	61.56 <sup>a</sup>	59.86 <sup>b</sup>	56.37 <sup>b</sup>	52.50 <sup>b</sup>	46.97 <sup>b</sup>	44.20 <sup>a</sup>	41.75 <sup>a</sup>
eucalyptus leaves	60.93 <sup>a</sup>	61.00 <sup>a</sup>	58.68 <sup>a</sup>	54.05 <sup>a</sup>	49.73 <sup>a</sup>	44.74 <sup>a</sup>	40.78 <sup>a</sup>
SE±	0.267	0.352	0.434	0.532	0.594	0.601	0.539
OIL CONCENTRATION (C)							
control	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>
100%	80.19 <sup>a</sup>	79.60 <sup>a</sup>	76.33 <sup>a</sup>	73.29 <sup>a</sup>	70.20 <sup>a</sup>	67.37 <sup>a</sup>	66.20 <sup>a</sup>
3:1 v/v	75.92 <sup>c</sup>	74.46 <sup>b</sup>	72.01 <sup>b</sup>	67.94 <sup>b</sup>	63.00 <sup>b</sup>	58.04 <sup>b</sup>	51.88 <sup>b</sup>
2:1 v/v	77.86 <sup>b</sup>	74.98 <sup>b</sup>	69.30 <sup>c</sup>	64.28 <sup>c</sup>	57.92 <sup>c</sup>	54.51 <sup>c</sup>	49.96 <sup>b</sup>
1:1 v/v	68.91 <sup>d</sup>	65.54 <sup>c</sup>	62.30 <sup>d</sup>	54.17 <sup>d</sup>	47.08 <sup>d</sup>	38.44 <sup>d</sup>	34.59 <sup>c</sup>
SE±	0.345	0.455	1.372	0.687	0.246	0.776	0.696
ISOLATES (I)							
<i>Penicillium citrinium</i>	61.00	58.77 <sup>a</sup>	56.94 <sup>a</sup>	51.46 <sup>ab</sup>	49.22 <sup>a</sup>	45.72 <sup>a</sup>	42.66 <sup>a</sup>
<i>Rhizomucor pusillus</i> .	60.00	59.90 <sup>a</sup>	57.17 <sup>a</sup>	52.40 <sup>ab</sup>	47.36 <sup>ab</sup>	45.60 <sup>a</sup>	40.80 <sup>b</sup>

<i>Aspergillus niger</i>	60.65	57.54 <sup>b</sup>	55.23 <sup>b</sup>	50.98 <sup>b</sup>	48.50 <sup>a</sup>	44.22 <sup>ab</sup>	40.36 <sup>b</sup>
<i>Aspergillus Flavus</i>	60.25	59.46 <sup>a</sup>	54.63 <sup>b</sup>	52.91 <sup>a</sup>	45.50 <sup>b</sup>	42.15 <sup>c</sup>	38.24 <sup>c</sup>
SE±	0.308	0.407	0.501	0.614	0.686	0.694	0.622
<b>INTERACTION</b>							
T × C	**	**	**	**	**	**	**
T × I	NS	NS	NS	NS	NS	NS	NS
C × I	**	**	**	**	**	**	**
T × C × I	NS	NS	NS	NS	NS	NS	NS

Means in each column followed by the same letter are not significantly different ( $p \leq 0.05$ ) according to the ANOVA and DMRT tests. Means are average values of triplicates. NS = Not significant, \*\* = highly significant.

orange peel combined (41.75%) was higher than eucalyptus (40.78%) which differed significantly from orange peels only (39.01%). Also, the results demonstrated there was a significant interaction effect between oil type and concentration at ( $P \leq 0.05$ ). The combined oils from eucalyptus and orange peels at full strength concentration (69.35) was significantly higher than all other interactions and 1:1v/v of orange peel (25.75) recorded the least (Table 2).

#### Effect of biobased oil concentration on % inhibition

The results show a gradual decrease in % inhibition of the

biobased oil treatment at different concentrations throughout inoculation. The main effect of different concentrations of the biobased oil treatment was significant ( $P \leq 0.05$ ) from day 1 to day 7 during the inoculation. Effect of different concentrations of biobased oil on day 1 shows that the highest percent inhibition was produced by 100% (80.19%) which was significantly higher in comparison with 2:1v/v (77.86%), 3:1 v/v (68.91%) and 1:1v/v (%) and the control gave no inhibition (Table 1). On day 3 after inoculation, the effect of different concentrations of

**Table 2.** Interaction effect of oil type and concentration on % inhibition on the growth of test fungi

Treatments	CONCENTRATION (C)				
	3:1 v/v	2:1 v/v	1:1 v/v	100%	Control
<b>Day 1</b>					
<b>OIL TYPE (T)</b>					
eucalyptus	73.75 <sup>e</sup>	76.25 <sup>d</sup>	65.63 <sup>f</sup>	80.56 <sup>b</sup>	0.00 <sup>h</sup>
orange peel +eucalyptus	79.63 <sup>bc</sup>	82.50 <sup>a</sup>	62.50 <sup>g</sup>	80.00 <sup>bc</sup>	0.00 <sup>h</sup>
orange peel	74.38 <sup>e</sup>	74.83 <sup>de</sup>	78.58 <sup>c</sup>	80.00 <sup>bc</sup>	0.00 <sup>h</sup>
SE±			0.5970		
<b>Day 2</b>					
eucalyptus	70.74 <sup>e</sup>	73.97 <sup>cd</sup>	56.43 <sup>g</sup>	78.30 <sup>b</sup>	0.00 <sup>h</sup>
orange peel +eucalyptus	80.35 <sup>ab</sup>	79.10 <sup>ab</sup>	64.56 <sup>f</sup>	81.00 <sup>a</sup>	0.00 <sup>h</sup>
orange peel	72.30 <sup>de</sup>	71.88 <sup>e</sup>	75.63 <sup>c</sup>	79.50 <sup>ab</sup>	0.00 <sup>h</sup>
SE±			0.7872		
<b>Day 3</b>					
eucalyptus	69.03 <sup>e</sup>	67.42 <sup>e</sup>	53.88 <sup>h</sup>	74.58 <sup>cd</sup>	0.00 <sup>i</sup>
orange peel +eucalyptus	77.39 <sup>ab</sup>	76.21 <sup>bc</sup>	60.81 <sup>g</sup>	79.00 <sup>a</sup>	0.00 <sup>i</sup>
orange peel	69.62 <sup>e</sup>	64.29 <sup>f</sup>	72.22 <sup>d</sup>	75.41 <sup>bc</sup>	0.00 <sup>i</sup>
SE±			0.9699		
<b>Day 4</b>					
eucalyptus	65.24 <sup>c</sup>	61.66 <sup>d</sup>	48.70 <sup>e</sup>	70.74 <sup>b</sup>	0.00 <sup>f</sup>
orange peel +eucalyptus	73.27 <sup>ab</sup>	71.17 <sup>b</sup>	51.04 <sup>e</sup>	74.74 <sup>a</sup>	0.00 <sup>f</sup>
orange peel	65.35 <sup>c</sup>	60.00 <sup>d</sup>	62.76 <sup>cd</sup>	74.39 <sup>a</sup>	0.00 <sup>f</sup>
SE±			1.1890		
<b>Day 5</b>					
eucalyptus	61.10 <sup>c</sup>	57.04 <sup>d</sup>	45.09 <sup>f</sup>	67.94 <sup>b</sup>	0.00 <sup>g</sup>
orange peel +eucalyptus	67.38 <sup>b</sup>	62.82 <sup>c</sup>	46.45 <sup>ef</sup>	71.98 <sup>a</sup>	0.00 <sup>g</sup>
orange peel	60.53 <sup>c</sup>	53.91 <sup>d</sup>	49.71 <sup>e</sup>	70.70 <sup>ab</sup>	0.00 <sup>g</sup>
SE±			1.3282		
<b>Day 6</b>					
eucalyptus	54.93 <sup>cd</sup>	55.90 <sup>c</sup>	36.15 <sup>f</sup>	63.39 <sup>b</sup>	0.00 <sup>g</sup>

orange peel +eucalyptus	62.43 <sup>b</sup>	55.90 <sup>c</sup>	36.11 <sup>f</sup>	69.49 <sup>a</sup>	0.00 <sup>g</sup>
orange peel	56.74 <sup>c</sup>	51.74 <sup>d</sup>	43.04 <sup>e</sup>	69.23 <sup>a</sup>	0.00 <sup>g</sup>
SE±			1.3439		
<b>Day 7</b>					
eucalyptus	44.70 <sup>f</sup>	52.82 <sup>d</sup>	36.44 <sup>h</sup>	61.10 <sup>b</sup>	0.00 <sup>j</sup>
orange peel +eucalyptus	56.27 <sup>c</sup>	53.75 <sup>cd</sup>	25.75 <sup>i</sup>	69.35 <sup>a</sup>	0.00 <sup>j</sup>
orange peel	48.75 <sup>e</sup>	49.06 <sup>e</sup>	41.59 <sup>g</sup>	68.14 <sup>a</sup>	0.00 <sup>j</sup>
SE±			1.2045		

Means in each column followed by the same letter are not significantly different ( $p \leq 0.05$ ) according to the ANOVA and DMRT tests.

biobased oil shows that the highest % inhibition was produced by 100% concentration (76.33 %) which was significantly higher in comparison with 3:1 v/v (72.01%), 2:1v/v (69.30%), 1:1v/v(62.30%) and the control gave no inhibition.

Five days after inoculation, the effect of different concentrations of biobased oil shows that the highest percent inhibition was produced by 100% concentration (70.20%) which was significantly higher in comparison with 3:1 v/v (63.00%), 2:1v/v (57.92%), 1:1v/v(47.08%) and the control gave no inhibition. Seven days after inoculation, the effect of different concentrations of biobased oil indicates that the highest % inhibition was produced by 100% concentration (66.20%) which was significantly higher in comparison with 3:1 v/v (51.88%), 2:1v/v (49.96%), 1:1v/v(34.59%) and the control gave no inhibition.

#### Effect of different inoculated fungi on % inhibition

The results show a general decrease in percent inhibition of the different isolated fungi throughout inoculation. Isolated fungi's main effect was significant ( $P \leq 0.05$ ) from day 1 to day 7 during the inoculation. Effect of different isolated fungi on day 1 shows that the highest % inhibition was produced by *Penicillium citrinium* (61.00%) which was sig-

nificantly higher in comparison with, *Aspergillus niger* (60.65%), *Aspergillus flavus* (60.25%), *Rhizomucor pusillus* (60.00%) (Table 1). Three days after inoculation, the main effects of the different isolates show that the highest % inhibition was produced by *R. pusillus* (57.17%) which was significantly higher in comparison with *P. citrinium* (56.94%), *A. niger* (55.23%) and *A. flavus* (54.63%). On day 5 of inoculation, the main effect of the different isolates shows that *P. citrinium* (49.22%) was significantly higher in comparison with *R. pusillus* (47.36%), *A. niger* (48.50%), *A. flavus* (45.50%). On day 7 of inoculation, the main effect of the different isolates shows that *P. citrinium* (42.66%) was significantly higher in comparison with *R. pusillus* (40.80%), *A. niger* (40.32%), *A. flavus* (38.24%) (Table 1). The oils at full strength concentration effectively inhibited *P. citrinium* (69.35) which was significantly higher than all other interactions and 1:1v/v of orange peel (24.23) recorded the least (Table 3).

#### Discussion

The results demonstrated that the combined effect of orange peels and eucalyptus leaves effectively inhibited the fungal growth better than eucalyptus leaves only and orange peels singularly. This could probably be attributed

**Table 3.** Interaction effects of isolates and oil concentration on % inhibition of the growth of test fungi

Treatments	CONCENTRATION (C)				
	3:1 v/v	2:1 v/v	1:1 v/v	100%	Control
<b>Day 1</b>					
<b>ISOLATES (I)</b>					
<i>Penicillium citrinium</i>	73.33 <sup>g</sup>	74.78 <sup>f</sup>	73.11 <sup>g</sup>	80.00 <sup>bc</sup>	0.00 <sup>j</sup>
<i>Aspergillus .niger</i>	76.67 <sup>e</sup>	78.33 <sup>d</sup>	67.50 <sup>h</sup>	80.75 <sup>ab</sup>	0.00 <sup>j</sup>
<i>Aspergillus flavus</i>	75.00 <sup>f</sup>	76.67 <sup>e</sup>	73.33 <sup>g</sup>	80.00 <sup>bc</sup>	0.00 <sup>j</sup>
<i>Rhizomucor pusillus.</i>	78.67 <sup>cd</sup>	81.67 <sup>a</sup>	61.67 <sup>i</sup>	80.00 <sup>bc</sup>	0.00 <sup>j</sup>
SE±			0.6894		
<b>Day 2</b>					
<i>Penicillium citrinium</i>	74.38 <sup>fg</sup>	70.83 <sup>h</sup>	71.43 <sup>h</sup>	80.67 <sup>a</sup>	0.00 <sup>k</sup>
<i>Aspergillus .niger</i>	73.36 <sup>g</sup>	76.40 <sup>de</sup>	56.20 <sup>j</sup>	81.73 <sup>a</sup>	0.00 <sup>k</sup>
<i>Aspergillus flavus</i>	71.54 <sup>h</sup>	75.01 <sup>e-g</sup>	71.31 <sup>h</sup>	76.00 <sup>d-f</sup>	0.00 <sup>k</sup>
<i>Rhizomucor pusillus.</i>	78.57 <sup>bc</sup>	77.70 <sup>cd</sup>	63.22 <sup>i</sup>	80.00 <sup>ab</sup>	0.00 <sup>k</sup>
SE±			0.9090		
<b>Day 3</b>					
<i>Penicillium citrinium</i>	73.38 <sup>d</sup>	66.77 <sup>g</sup>	67.52 <sup>g</sup>	77.01 <sup>ab</sup>	0.00 <sup>j</sup>

<i>Aspergillus niger</i>	71.41 <sup>ef</sup>	70.86 <sup>f</sup>	55.54 <sup>i</sup>	78.34 <sup>a</sup>	0.00 <sup>i</sup>
<i>Aspergillus flavus</i>	67.44 <sup>e</sup>	65.47 <sup>e</sup>	66.67 <sup>e</sup>	73.55 <sup>de</sup>	0.00 <sup>i</sup>
<i>Rhizomucor pusillus</i> .	75.81 <sup>bc</sup>	74.13 <sup>cd</sup>	59.48 <sup>h</sup>	76.44 <sup>ab</sup>	0.00 <sup>i</sup>
SE±			1.1199		
<b>Day 4</b>					
<i>Penicillium citrinium</i>	70.57 <sup>cd</sup>	62.77 <sup>fe</sup>	56.46 <sup>j</sup>	74.74 <sup>a</sup>	0.00 <sup>k</sup>
<i>Aspergillus niger</i>	67.66 <sup>e</sup>	64.44 <sup>f</sup>	51.04 <sup>j</sup>	74.16 <sup>ab</sup>	0.00 <sup>k</sup>
<i>Aspergillus flavus</i>	61.67 <sup>gh</sup>	61.10 <sup>gh</sup>	59.17 <sup>h</sup>	72.98 <sup>a-c</sup>	0.00 <sup>k</sup>
<i>Rhizomucor pusillus</i> .	71.93 <sup>bc</sup>	68.79 <sup>de</sup>	50.00 <sup>i</sup>	71.28 <sup>cd</sup>	0.00 <sup>k</sup>
SE±			1.3729		
<b>Day 5</b>					
<i>Penicillium citrinium</i>	66.87 <sup>cd</sup>	57.92 <sup>ef</sup>	48.81 <sup>e</sup>	72.47 <sup>a</sup>	0.00 <sup>i</sup>
<i>Aspergillus niger</i>	64.43 <sup>d</sup>	60.42 <sup>e</sup>	46.70 <sup>gh</sup>	70.92 <sup>ab</sup>	0.00 <sup>i</sup>
<i>Aspergillus flavus</i>	55.31 <sup>f</sup>	55.22 <sup>f</sup>	48.18 <sup>e</sup>	68.82 <sup>bc</sup>	0.00 <sup>i</sup>
<i>Rhizomucor pusillus</i> .	65.40 <sup>d</sup>	58.13 <sup>ef</sup>	44.66 <sup>h</sup>	68.60 <sup>bc</sup>	0.00 <sup>i</sup>
SE±			1.5337		
<b>Day 6</b>					
<i>Penicillium citrinium</i>	62.66 <sup>c</sup>	55.55 <sup>ef</sup>	39.41 <sup>j</sup>	70.36 <sup>a</sup>	0.00 <sup>i</sup>
<i>Aspergillus niger</i>	58.35 <sup>de</sup>	59.73 <sup>d</sup>	36.90 <sup>jk</sup>	66.10 <sup>b</sup>	0.00 <sup>i</sup>
<i>Aspergillus flavus</i>	51.55 <sup>gh</sup>	53.23 <sup>fe</sup>	42.38 <sup>i</sup>	66.44 <sup>b</sup>	0.00 <sup>i</sup>
<i>Rhizomucor pusillus</i> .	59.59 <sup>d</sup>	49.53 <sup>h</sup>	35.05 <sup>k</sup>	66.58 <sup>b</sup>	0.00 <sup>i</sup>
SE±			1.5518		
<b>Day 7</b>					
<i>Penicillium citrinium</i>	56.75 <sup>d</sup>	51.67 <sup>ef</sup>	43.13 <sup>h</sup>	69.35 <sup>a</sup>	0.00 <sup>k</sup>
<i>Aspergillus niger</i>	45.61 <sup>h</sup>	57.09 <sup>d</sup>	35.46 <sup>j</sup>	66.66 <sup>b</sup>	0.00 <sup>k</sup>
<i>Aspergillus flavus</i>	43.78 <sup>h</sup>	48.33 <sup>e</sup>	24.23 <sup>j</sup>	63.64 <sup>c</sup>	0.00 <sup>k</sup>
<i>Rhizomucor pusillus</i> .	53.49 <sup>e</sup>	50.42 <sup>fe</sup>	35.55 <sup>j</sup>	65.13 <sup>b</sup>	0.00 <sup>k</sup>
SE±			1.3908		

to the presence of phenolics and terpenoids which cause structural and functional damage to the cell. This result confirms previous reports on the effectiveness of antimicrobial activity of plant-based oils with higher inhibition percentages (24–26). The results for only orange peels biobased oils in this current study were probably due to the presence of limonene and that collaborates with related research which reported the presence of limonene with antioxidant properties from citrus oils. The % inhibition exhibited by eucalyptus oil only was attributable to the phytochemical factors with an abundance of eucalyptol,  $\gamma$ -terpinene and  $\alpha$ -pinene (27, 28). In the current study, it was found that *P. citrinium* growth was highly inhibited by the biobased oils and *R. pusillus*, *A. niger* and *A. flavus* followed respectively.

The results are in agreement with related studies which reported that *Penicillium* spp. growth was synergistically suppressed that produced the highest inhibitory % (29). The % inhibition of biobased oils against *R. pusillus* gave a lower inhibitory % relative to *Penicillium* spp. but was higher than the other isolates resulting from the active provision of natural antifungal constituents. The results of

the current study are in agreement with the earlier report (24). Although the inhibitory effects of the biobased oil on *A. niger* were not the highest in the current study, the results confirm that the morphology, colony diameter and sporulation of the cell could be altered by the oils to affect the percent inhibition. (30–32).

The least inhibitory percent was found with *A. flavus* which collaborates with the findings of inhibition of *Aspergillus flavus* following the application of citrus essential oil (33, 34). In general, the main and interaction effects of the biobased oils, type of isolates, and the concentrations on the percent inhibition suggest that higher concentrations gave better inhibitions against all isolates examined. The effect of combined orange peels and eucalyptus in undiluted oil gave the highest inhibition against *P. citrinium* when the experiment was terminated. The performance of the combinations of biobased oils at higher concentrations was possibly due to mycelial destruction and control of lesion diameter growth resulting in effective inhibition of the fungal growth.

Similarly, except that percent inhibition values were less than that of the current study, it has previously been

suggested that higher quantities of plant-based oils are required for effective inhibition against *Penicillium* spp. (35, 36). Except for *A. flavus*, all other singularly applied or combinations at different concentrations of biobased oils with DMSO against the fungal isolates inhibited mycelia growth by more than 40%. The oils with DMSO 1:1v/v against *A. flavus* was 24.23% inhibition. The fungicidal activity of the DMSO diluted oils was probably less suppressive to spore elongation of *A. flavus*. The results concur with those reported on the application of plant-based oils by direct contact with *A. flavus* with less inhibitory effect (37).

## Conclusion

Antimicrobial activity of this study demonstrated that the biobased oils from eucalyptus leaves combined with orange peels gave a significant fungal activity against the mycelial growth and spores' germination of *Aspergillus niger*, *A. flavus*, *Penicillium citrinum* and *Rhizomucor pusillus*. It was established that *P. citrinum*, was the most inhibited isolate at full strength of the combined oils. Based on findings of the current study, biobased oil proved to be effective in antifungal activity for all tested pathogenic fungi. The potential application was based on the selected concentrations and duration of inoculation. The use of biobased oil treatments singularly and in combination for controlling pathogenic fungi is promising for small scale commercial application in handling fruits.

## Data availability statement

Data supporting the findings of the study are part of the analysis in this article.

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## Authors contributions

Adams AR designed the experiment, data collection, analysis and drafted the manuscript, Umar Makinta helped in the experiment and data collection, Kortse PA and Simon VI supervised and helped in the design and contributed to compilation and revision of the manuscript. All authors read and approved the final version of the manuscript.

## Compliance with ethical standards

**Conflict of interest:** No potential conflict of interest was reported by the authors.

**Ethical issues:** Not applicable.

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