Inhibitory Effect of Different Essential Oils on *Aspergillus Niger* of Postharvest Grape

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Abstract: *Aspergillus Niger is the main pathogen causing grape smut disease*. A vitro assay was conducted to screen out the essential oils with better inhibitory effect on grape *A. Niger*. The results showed that among 31 essential oils, nine essential oils at 1000 μL/L can kill *A. Niger*. Reduced the concentration of these nine essential oils to 500 μL/L, only basil and oregano essential oils had continuous antifungal effect on *A. Niger*. But if the concentration of basil and oregano essential oils reduced to 250 μL/L, the antifungal effect was weakened. Therefore, in order to maintain continuous antifungal effect, the concentration of essential oils should more than 250 μL/L.

1 INTRODUCTION

Grapes are berry fruits that mainly distributed in temperate and subtropical regions. Although grape industry is developing rapidly and grape production is increasing day by day, table grapes are still the most important part of Chinese grape industry (Meng, 2017). Grape fruits are easily infected by pathogenic fungus during postharvest storage due to their high-water content and thin peel, which strongly affects the edible taste and nutritional value of grapes (Guerra, 2016). If the fungus is not inhibited and preserved in time, it will not only cause significant economic losses, but also seriously restrict the development of the grape industry (Xu, 2007).

Aspergillus niger is a dominant pathogen at high temperature after harvesting fresh table grapes. They can cause smut disease, often infecting grapes through wounds. After grape fruit infected *A. niger*, it will lead to grape corruption, dehydration, and reduced nutritional value (Xu, 2007). For the consideration of environmental protection and human health, in recent years, natural preservatives extracted from animals and plants have emerged in endlessly, and plant extracts have been used in the preservation of fruits and vegetables (Tanapichatsakul, 2020). Singh found that most aromatic essential oil aqueous emulsions with a concentration of 0.1-10 μg/g can inhibit the growth of decay-causing fungus and food-borne pathogenic fungus to a certain extent on the immersion processing of fruit and vegetables. (Singh, 2002). However, effective concentration and side effect should be considered before large-scale application of plant essential oils.

In this study, the essential oils with inhibitory effect on *A. Niger* of postharvest grape were screened from 31 kinds of plant essential oils through in vitro tests, and their concentration was gradually reduced to determine the minimum concentration, which provided ideas for the postharvest preservation of grapes.

2 MATERIALS AND METHODS

2.1 Materials

Essential oils were provided by Ji'an Guoguang Spice Oil Co., Ltd. (Ji'an, Jiangxi, China). Potato dextrose agar (PDA) and potato dextrose broth (PDB) medium were provided by Shanghai Bio-way technology Co.,

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Ltd. (Shanghai, China). *A. Niger* was isolated from the mature grapes.

2.2 Methods

2.2.1 Propagation of *A. Niger*

The isolated and purified *A. niger* from tables grapes was inoculated on the PDA medium and cultured at 26°C for 48h. The mycelium was picked to a conical flask containing 50 mL PDB liquid medium with an inoculating loop, and cultured at 26°C for 48h.

2.2.2 Preparation of Essential Oil Medium and Determination of Colony Diameter

The stock solution of 31 kinds of plant essential oils was diluted with 5% Tween solution, mixed with the medium according to the required concentration, poured into a petri dish, and repeated three times for each essential oil. After the medium was solidified, a puncher was used to punch out 0.7 cm stipe from the prepared fungus-containing medium, and inoculated them on the medium containing different essential oils, and the medium without any essential oil but inoculated with *A. Niger* was used as control (CK). Then, all the inoculated culture dishes were placed in a constant temperature incubator at 26 °C, and the diameter of the colony was measured every 24h using a millimeter scale.

2.3 Statistical Analysis

Statistical analyses were conducted using SPSS 20.0 statistical software (IBM Corporation, Armonk, New York, USA). The data was analyzed using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test applied at 5% significance level.

3 TEST RESULTS AND DISCUSSIONS

3.1 Inhibitory Effect of Different Essential Oils on Grape *A. Niger*

Table 1 showed that after culturing for 72 hours, the colony diameter in the control is the largest, and except for the three essential oil treatments, the colony diameters in the other essential oil treatments were significantly lower than those in the control. Among the 31 essential oils, the colonies treated with 9 essential oils did not grow, and the diameter of the colonies was only the diameter of the stipe. It may be that 1000 μL/L of these 9 essential oils had killed *A. Niger*, which can be used for follow-up research.

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Table 1: Effects of different essential oils at 1000 μL/L on colony growth of *A. Niger* at 72h.

Essential oil	Colony Diameter (cm)	Essential oil	Colony Diameter (cm)
CK	5.68±0.34a	Tea tree	5.21±0.45ab
Lemon	5.16±0.27ab	Ginger	5.05±0.46ab
Zanthoxylum	5.09±0.19b	Folium eucalypti	4.82±0.29b
Rhizoma calami	4.84±0.37bc	Perilla leaf	4.75±0.29bc
Artemisiae argyi	4.46±0.45c	Rhubarb	4.45±0.32c
Myristica fragrans	4.32±0.31c	Fructus forsythiae	4.15±0.32c
Rhizoma atractylodis	4.06±0.22cd	Chamomile	4.07±0.31cd
Lophatherum gracile	3.96±0.18d	Clausena lansium	3.82±0.27d
Rhizoma Coptidis	3.48±0.26de	Tangerine Peel	3.52±0.26de
Rosemary	3.19±0.27ef	Mentha	3.25±0.18ef
Lavender	2.82±0.16f	Pogostemon cablin	2.83±0.16f
Agilawood	1.73±0.06g	Thyme	0.70±0.00h
Citronella	0.70±0.00h	Clove	0.70±0.00h
Oregano	0.70±0.00h	Cinnamon	0.70±0.00h
Asarum	0.70±0.00h	Basil	0.70±0.00h
Garlic	0.70±0.00h	litsea cubeba	0.70±0.00h

Different lowercase letters within a column indicate significant differences based on one-way analysis of variance and the least significant difference test (95% confidence level). The same as below.

3.2 500 μL/L of 9 Essential Oils for Continuous Inhibition on Grape *A. Niger*

Table 2 showed that when the concentration of essential oil was reduced to 500 μL/L, Cinnamon essential oil lost its antifungal effect. Although the five essential oils, citronella, clove, garlic, asarum and basil had a certain antifungal effect, their colonies had begun to grow. However, two essential oils, litsea cubeba and oregano, still had good antifungal effects, and the colony diameter had always been only the diameter of the stipe, which were available for further research.

Table 2: Effects of 9 essential oils at 500 μL/L on continuous colony growth of *A. Niger.*

Essential oil	Colony Diameter (cm)		
	24h	48h	72h
CK	1.51±0.14a	3.16±0.18a	4.69±0.35a
Cinnamon	1.14±0.08b	2.16±0.15b	4.08±0.29a
Citronella	0.70±0.00d	1.27±0.03e	2.16±0.11c
Clove	0.94±0.01c	1.38±0.06d	1.64±0.08d
Garlic	0.95±0.02c	1.66±0.09c	2.08±0.12c
Asarum	0.92±0.03c	1.67±0.06c	2.68±0.11b
Basil	0.70±0.00d	0.82±0.01f	0.91±0.02e
Litsea cubeba	0.70±0.00d	0.70±0.00g	0.70±0.00f
Oregano	0.70±0.00d	0.70±0.00g	0.70±0.00f

3.3 250μL/L of Basil and Oregano Essential Oil for Continuous Inhibition on *A. Niger*

It can be seen from table 3 that although the colony diameters of basil and oregano essential oils were significantly lower than those of the control in each period, *A. Niger* had begun to grow, indicating that 250 μL/L of these two essential oils had lost their continuous antifungal effect.

Table 3: Effects of 250 μL/L basil and oregano essential oil on colony growth of grape *A. Niger.*

Essential oil	Colony Diameter (cm)		
	24h	48h	72h
CK	1.78±0.10a	2.96±0.13a	5.46±0.35a
Basil	1.36±0.04b	2.36±0.08b	4.67±0.26b
Oregano	1.02±0.01c	1.89±0.05c	3.35±0.18c

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4 CONCLUSION

Based on the results presented above, the conclusions are obtained as below:

(1) Most essential oils at high concentration have antifungal effect on table grape *A. Niger*, but its antifungal effect decreased with the decrease of essential oil concentration.

(2) In vitro assay, basil and oregano essential oil has good continuous antifungal effect on grape *A. Niger*, but its concentration should be higher than 250 μL/L.

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