In Vitro Screening of Antifungal Effects of Different Plant Essential Oils on White *Fusarium*

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Keywords: White *Fusarium*, Oregano Essential Oil, Inhibition Ratio, Grape.

Abstract: White *Fusarium* can easily lead to postharvest grape rot. By studying the inhibition rate of different essential oils on white *Fusarium*, the type and concentration of essential oils with better antifungal effect were screened out. The results showed that the concentration of essential oils was 1000 μL/L, and the inhibition ratio of 5 essential oils among the 32 essential oils was 100%. Further reducing the concentration of these five essential oils to 500 μL/L, cinnamon, basil, and oregano still had 100% continuous bacteriostatic ratio. But at the concentration of 250 μL/L, only oregano essential oil had a higher bacteriostatic rate, but it was still lower than 100%. It indicated that oregano essential oil higher than 250 μL/L had better antifungal effect on white *Fusarium*.

1 INTRODUCTION

Grape is a seasonal fruit which ripening period is mainly concentrated in the hot summer. The planting area of grapes in China is wide and the varieties are complete. They are mainly used for fresh food or made into raisins, grape juice, oil extraction, and winemaking. Grapes and their products are not only delicious, but also have high nutritional value and health care functions, so they are much loved by consumers and have great economic development value and broad market prospects (Rinaldi, 2017).

In the process of picking and harvesting, the surface of grapes will be damaged by mechanical friction; in addition, the fruit itself has the characteristics of soft pulp, and high-water content, making it vulnerable to pathogenic fungus infection (Sultan, 2010). Common pathogens that cause grape diseases include *Botrytis cinerea*, *Penicillium*, *Fusarium*, etc. These pathogens can not only cause grape diseases, but also secrete secondary metabolites that are unfavorable to the human body. Therefore, reasonable measures should be taken to control the postharvest disease of grapes (Leong, 2004).

Because natural fungicides are safe, effective, and environmentally friendly, they have attracted people's attention. With the deepening of relevant research, these natural substances, especially natural plant volatile substances, such as plant essential oils, are used in the prevention and control of postharvest diseases of fruits and vegetables (Rauha, 2000). Essential oils are secondary metabolites synthesised by plant themselves, and they play an important role in the protection of plants such as antifungal and insecticide (Bakkali, 2008). At present, there are some studies on the antibacterial properties of plant essential oils, but data on the effect of plant essential oils on postharvest table grape are scarce.

In this study, we studied the antibacterial effect of plant essential oils on white Fusarium, and screened out the types of plant essential oils with the best inhibitory effect on white Fusarium and their optimum concentrations, which provided a theoretical basis for the storage and preservation of grapes after harvest.

Xie, Y., Zhu, J., Liu, H., Lian, H. and Liu, J.
In Vitro Screening of Antifungal Effects of Different Plant Essential Oils on White Fusarium.
DOI: 10.5220/0012012000003633
In *Proceedings of the 4th International Conference on Biotechnology and Biomedicine (ICBB 2022)*, pages 17-20
ISBN: 978-989-758-637-8

2 MATERIALS AND METHODS

2.1 Materials

Potato dextrose agar (PDA) medium were provided by Shanghai Bio-way technology Co., Ltd. (Shanghai, China). Essential oils were provided by Ji'an Guoguang spice Co., Ltd. (Ji'an, Jiangxi, China).

2.2 Methods

White Fusarium was separated from the ripe grape fruit, and the purified mycelium was inoculated on the PDA medium, and cultivated at 26°C for 48h. The essential oils were diluted with 5% Tween solution, the diluted essential oils were mixed with unset PDA medium in a centrifuge tube according to requested concentration, poured into a petri dish, and each essential oil repeated for three times. Then 0.7 cm stipes were punched out by a puncher from the prepared fungus-containing medium, and inoculated them on the essential oils medium, the medium without any essential oil but inoculated with white *Fusarium* was control (CK). After inoculation, all petri dishes were placed in a constant temperature incubator with 26 °C, the inhibition ratio was measured daily.

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 interval. Inhibition ratio= $\frac{C-T}{C} \times 100\%$. C: colony diameter of control, T: colony diameter of treatments with essential oils.

3 TEST RESULTS AND DISCUSSION

3.1 Effects of Different Essential Oils at 1000 μL/L on White *Fusarium*

It can be seen from table 1 that when the essential oil concentration was 1000 μL/L, almost all 32 essential oils hada certain antifungal effect, but the inhibition ratio of 19 essential oils was low (< 50%). There were 8 kinds of essential oils with high antifungal rate, and the antifungal ratio was 50%-90%. Only the essential oils of asarum, litsea cubeba, clove, basil, oregano, and cinnamon had 100% antifungal ratio, indicating that 1000 μL/L of these five essential oils can kill white *Fusarium* and can be used for further research.

Table 1: The antifungal effect of 32 essential oils at 1000 μL/L on white *Fusarium* of grape.

Essential oils	Inhibition ratio %	Essential oils	Inhibition ratio %
Tea tree	3.70±0.08l	Fructus forsythiae	11.11±0.26k
Folium eucalypti	11.11±0.85k	Rosemary	14.81±1.01j
Lemon	16.67±0.97ij	Rhubarb	18.52±0.94hi
Clausena lansium	18.52±0.84hi	Myristica fragrans	20.37±0.96h
Mentha	20.37±0.56h	Perilla leaf	20.37±1.09h
Pepper	25.93±1.06g	Lophatherum gracile	25.93±1.85g
Chamomile	27.78±0.73g	Rhizoma atractylodis	33.34±1.47f
Argy wormwood leaf	35.19±2.12f	Goldthread	35.19±2.04f
Tangerine peel	35.19±2.16f	Ginger	44.44±2.88e
Lavender	46.30±2.57e	Rhizoma calami	53.71±2.14d
Foeniculum vulgare	59.26±3.71c	Pogostemon cablin	61.11±3.48c
Tambac	64.81±3.08c	Citronella	74.07±5.84b
Thyme	75.93±4.52b	Garlic	77.78±5.09b
Asarum	83.33±5.46b	Basil	100.00±0.00a
Litsea cubeba	100.00±0.00a	Oregano	100.00±0.00a
Clove	100.00±0.00a	Cinnamon	100.00±0.00a

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 Effects of 5 Essential Oils at 500 µL/L on White *Fusarium* of Grape

The concentration of the above five essential oils was reduced to 500 µL/L. Table 2 showed that litsea cubeba had no continuous antifungal effect. Clove essential oil could completely inhibit the growth of white *Fusarium* in the early stage, but the inhibition ratio continues to decrease in the later stage. The continuous inhibition rate of cinnamon, oregano, and basil was consistently 100%, which implied that these three essential oils were still effective in killing white *Fusarium* at a concentration of 500 µL/L.

Table 2: The continuous antifungal effect of 5 essential oils at 500 µL/L on white *Fusarium.*

Essential oil	Inhibition ratio %			
	1d	2d	3d	4d
Litsea cubeba	0.00±0.02c	6.25±0.48e	3.25±0.19d	4.44±0.25e
Clove	100.00±0.00a	100.00±0.00a	68.75±3.89b	75.56±3.48b
Cinnamon	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a
Oregano	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a
Basil	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a

3.3 Effects of 3 Essential Oils at 250 µL/L on White *Fusarium* of Grape

In table 3, when the essential oil concentration was reduced to 250 µL/L, cinnamon essential oil almost lost its antifungal effect. Basil and oregano essential oils had better antifungal effect at the beginning, but the inhibition ratio decreased with time. The inhibition ratio of oregano was always significantly higher than those of basil essential oils.

Table 3: The continuous antifungal effect of 3 essential oils at 250 µL/L on white *Fusarium.*

Essential oil	Inhibition ratio %			
	1d	2d	3d	4d
Cinnamon	23.08±1.14b	12.56±0.67c	15.63±0.97c	2.22±0.02c
Basil	100.00±0.00a	25.09±1.29b	25.29±1.08b	17.78±0.86b
Oregano	100.00±0.00a	100.00±0.00a	93.75±5.94a	88.89±4.96a

4 CONCLUSION

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

(1) Five essential oils such as litsea cubeba, clove, basil, oregano, cinnamon at 1000 µL/L can effectively kill white *Fusarium* of table grapes.

(2) Among 32 essential oils, oregano essential oil has the best antifungal effect on white *Fusarium*, but its minimum concentration should not be lower than 250 µL/L.

ACKNOWLEDGMENTS

This work was supported by Local Financial Funds of National Agriculture Science and Technology Center, Chengdu (NASC2020AR05) and Sichuan Science and Technology Program (2021JDRC0134).

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