In vitro antibacterial activity of 34 plant essential oils against *Alternaria alternata*

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Abstract. To determine the antibacterial effect of 34 plant essential oils on *Alternaria alternata*, 34 plant essential oils such as asarum essential oil, garlic essential oil, and mustard essential oil are used as inhibition agents to isolate *A. alternata* from citrus as indicator bacteria, through the bacteriostasis test and drug susceptibility test, the types of essential oils with the best inhibitory effect were screened and their concentration was determined. The results showed that the best inhibition effect was mustard essential oil with a minimum inhibitory concentration of 250 µl/L and a minimum bactericidal concentration of 250 µl/L. Followed by the Litsea cubeba essential oil and basil oil, the minimum inhibitory concentration is 500 µl/L.

1 Introduction

Citrus is a general term for orange, mandarin, orange, kumquat, pomelo, and medlar. Citrus is the world's largest fruit, rich in sugar, organic acids, mineral elements and other substances, because of its high nutritional value and special taste odor and is deeply loved by consumers [1-2]. At the same time, *A. alternata* can cause black rot and brown spot disease in lemon, golden pomelo and orange [3-5]. It can be seen that *A. alternata* is one of the main diseases of citrus after harvest. At present, chemical fungicides are widely used, and imazalil, carbendazim, etc. are used in the postharvest control of citrus [6]. Most fungicides are sprayed directly onto the skin of fruits and vegetables, which cause serious health effects after eating and have an impact on the environment [7]. What is even more worrying is that most fungicides have irritating odors, are easy to pollute the ecology, and have disadvantages such as being difficult to be broken down. Therefore, it is increasingly urgent to explore new environmentally friendly fungicides that are low in toxicity and effective.

In recent years, plant essential oils have been widely studied as an emerging natural environmentally-friendly and antibacterial substance. Plant essential oil is a unique aromatic substance extracted from plants, containing alcohols, aldehydes and other substances [8]. Plant essential oils have broad-spectrum antibacterial properties, and have good effects in the preservation of fruits and vegetables and the prevention and treatment of pathogenic bacteria [9]. The use of new pesticides in plant extracts during the cultivation and storage periods can reduce environmental pollution and improve food

quality and safety. *A. alternata* is the main fungus causing post-harvest disease of Pitaya, and the antibacterial effect of using cinnamon oil and clove oil is good [10]. Clove oil can effectively inhibit the growth of *A. alternata* on citrus [11]. The combination of cinnamaldehyde and chitosan, the main component of cinnamon essential oil, can effectively reduce the senescence and decay of fruits by improving the disease resistance of the fruit [12].

The essential oils of plants have a certain inhibitory effect on *A. alternata*, but the types of essential oils used by the predecessors are few, which are not continuously observed. The sustained antibacterial effect of various essential oils cannot be determined. Therefore, this experiment selected 1000 µl/L of 30 plant essential oil antibacterial data within 72 hours to screen effective plant essential oils. Observing the effective and effective antibacterial activity of effective essential oils against *A. alternata* at 500 µl/L, in order to provide a reference for the method and ideas of citrus preservation.

2 Materials and Method

2.1 Experimental materials

2.1.1 Experimental essential oil. Asarum essential oil, Garlic essential oil, Litsea cubeba essential oil, Mustard essential oil, Basil essential oil, Angelica pubescens essential oil, Thyme essential oil, Patchouli essential oil, Citronella essential oil, Clove oil, Peppermint essential oil, Lemon oil, Lavender oil, Pepper oil, Tea tree oil,

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Cinnamon oil, Fennel oil, Agarwood oil, Water squid oil, Rosemary essential oil, Asarum essential oil, Chamomile oil, Eucalyptus essential oil, Perilla leaf essential oil, Coptis essential oil, Nutmeg essential oil, Ginger essential oil, Blumea essential oil, Forsythia essential oil, Atractylodes essential oil, Rhubarb essential oil, Capsicum essential oil, Tangerine essential oil, Clausena lansium essential oil, purchased from Jiangxi Ji'an Grand Spice Oil Company.

2.1.2 Experimental medium and strain source. (1) potato dextrose agar medium (PDA): potato dextrose agar medium was weighed 46.0 g, added to 1000 ml of distilled water, autoclaved at 115 °C for 20 minutes, cooled and placed in a refrigerator at 4 °C for use. (2) *Alternaria alternata*, a pathogenic fungus isolated from the citrus fruits stored after harvest, was deposited in the laboratory of the Institute of Food Science and Technology, Chengdu Academy of Agriculture and Forestry Sciences.

2.2 Experimental method

2.2.1. Preparation of bacteria-containing plates. The *A. alternata* mycelium was picked and placed in an Erlenmeyer flask containing 50 ml of PDB, and cultured at 26 °C for about 48 hours. 1 ml of the bacterial suspension was pipetted onto a PDA plate and cultured at 26 °C for 48 h.

2.2.2. Preparation of essential oil plate and determination of inhibition rate. The 34 essential oil crude oil was dissolved in 5% Tween-80 and fully emulsified to a final concentration of 20000 µl/L. Under sterile conditions, absorb 1.5 ml of 20,000 µl/L essential oil in a centrifuge tube, add 28.5 ml of fungal medium, shake well, pour into a Petri dish, and prepare a 1000 µl/L essential oil plate. A blank PDA was used as a control, and each treatment was repeated 3 times. The bacterium having a diameter of 7.5 mm was placed on the bacterium-containing plate by a sterile puncher and placed in the center of the essential oil plate, and cultured at 26 °C for 24 hours, and the plaque diameter was measured.

$$\text{Inhibition rate} = \frac{C - T}{C} \times 100\%$$

C: Control plaque diameter (mm); T: different plant essential oil treatment plaque diameter (mm).

2.2.3. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in vivo. The essential oil with inhibition effect was screened by 2.2.2. Under aseptic conditions, separately absorb 20,000 µl/L of essential oil 0.75 ml or 0.375 ml in a centrifuge tube, add PDA medium 29.25 ml or 29.625 ml respectively, mix and pour into the culture dish, respectively, into 500 µl/L or 250 µl/L essential oil plate. A blank PDA was used as a control, and each treatment was repeated 3 times. Using

a sterile puncher, a 7.5 mm diameter bacterium was placed on the bacterium-containing plate and placed in the center of the essential oil plate, and cultured at 26 °C for 5 days, with the lowest concentration of no bacteria at all as the MIC of the essential oil [13]. The bacteria cake on the whole long-lasting essential oil plate was transferred to a blank PDA plate, and if it was still not grown for 4 days, it was MBC [14].

2.3 Statistical analysis

The data was analyzed using SPSS software.

3 Results and Discussion

3.1 Inhibition effect of 34 essential oils on citrus *A. alternata*

In Table 1, compared with the control, Essential oils of Asarum, Garlic, Litsea cubeba and Mustard had the best antibacterial effect, and the inhibition rate was 100% ($P < 0.01$). The inhibition rate of the positive critical value of Atractylodes essential oil was 1.77%, and the negative threshold value of Rhubarb essential oil was -2.40%. The inhibitory rates of Basil essential oil, Alive essential oil and Thyme essential oil were 99.27%, 97.90% and 90.90%, respectively, and the inhibitory effect on *A. alternata* was better. Essential oils of Patchouli, Citronella, Clove, Peppermint, Lemon, Lavender, Pepper, Tea tree, Cinnamon, Fennel, Agarwood, Water squid, Rosemary, Asarum, Chamomile, Eucalyptus, Perilla leaf and Coptis general antibacterial effect, and significantly different from thyme essential oil ($P < 0.01$). Essential oils of Nutmeg, Ginger, Blumea and Forsythia may inhibit the growth of *A. alternata*, and there was no significant difference compared with atractylodes essential oil ($P < 0.01$). Essential oils of Capsicum, Tangerine peel, Clausena lansium and Rhubarb may promote the growth of pathogenic bacteria, and there is no significant difference with rhubarb essential oil ($P < 0.01$).

3.2 500 µl/L 6 essential oils for continuous inhibition of Citrus *A. alternata*

In Table 2, the inhibition rate of Mustard essential oil to *A. alternata* for eight days was 100%, and the sustained bacteriostatic effect was the best. The inhibition rate of the first five days of Litsea cubeba oil was 100%, and the inhibition rate of the eighth day was 83.13%, which was significantly different from that of mustard essential oil ($P < 0.05$). The inhibition rate of Basil essential oil on the second day was 100%, and the inhibition rates on the fifth and eighth days were 78.63% and 81.49%, respectively, and the difference in inhibition rate between the two days was not significant ($P < 0.05$). The inhibition rate of Asarum essential oil and garlic essential oil in the first five days was 100%, and the inhibition rate on the eighth day was 76.89% and 69.45% ($P < 0.05$), respectively.

3.3 MIC and MBC of different essential oils against the citrus *A. alternate*

In Table 3, the MIC of Mustard essential oil on citrus *A. alternate* is 250 μL . The MIC of Litsea cubeba essential oil and Basil essential oil on citrus *A. alternate* is 500 μL . The MIC of Asarum essential oil, garlic essential oil and Alive essential oil on citrus *A. alternate* is 1000 μL . The MBC of Mustard essential oil against *A. alternate* is 250 μL . The MBC of Garlic essential oil and Litsea cubeba essential oil on *A. alternate* is 1000 μL . The MBC of Basil essential oil against *A. alternate* is 2000 μL . The MBC of Asarum essential oil and Angelica pubescens essential oil on *A. alternate* exceeds 2000 μL , and the value is too large to be practical.

4 Conclusions

In this experiment, the Mustard essential oil of 34 essential oils had the best inhibition effect against *A. alternate* on citrus, and the inhibition effect was 100% within 8 days, and the continuous inhibition effect of Litsea cubeba oil and basil essential oil was better. Therefore, in practical applications, Mustard essential oil, Litsea cubeba oil and Basil oil can effectively inhibit the growth of *A. alternate* on citrus, providing a methodological and technical reference for citrus storage and pathogen inhibition.

Table 1. 1000 μL 34 essential oils on the inhibition of *A. alternate*.

Essential oil type	Inhibition rate	Essential oil type	Inhibition rate
Asarum essential oil	100.00 $\pm$ 0.00 ^A	Agarwood essential oil	54.57 $\pm$ 9.26 ^{GHI}
Garlic essential oil	100.00 $\pm$ 0.00 ^A	Water squid oil	45.60 $\pm$ 5.06 ^{HIJ}
Litsea cubeba essential oil	100.00 $\pm$ 0.00 ^A	Rosemary essential oil	40.37 $\pm$ 8.64 ^{JK}
Mustard essential oil	100.00 $\pm$ 0.00 ^A	Asarum essential oil	36.37 $\pm$ 2.72 ^{JKL}
Basil essential oil	99.27 $\pm$ 0.12 ^A	Chamomile essential oil	33.07 $\pm$ 4.62 ^{JKL}
Angelica pubescens essential oil	97.90 $\pm$ 0.20 ^{AB}	Eucalyptus essential oil	31.23 $\pm$ 8.81 ^{JKL}
Thyme essential oil	90.90 $\pm$ 8.77 ^{ABC}	Perilla leaf essential oil	28.70 $\pm$ 1.97 ^{KLM}
Patchouli essential oil	83.13 $\pm$ 8.30 ^{BCD}	Coptis essential oil	23.87 $\pm$ 5.75 ^{LMN}
Citronella essential oil	82.60 $\pm$ 19.3 ^{BCD}	Nutmeg essential oil	14.57 $\pm$ 3.76 ^{MNO}
Clove essential oil	80.00 $\pm$ 8.60 ^{CD}	Ginger essential oil	13.30 $\pm$ 2.41 ^{MNOP}
Peppermint essential oil	78.43 $\pm$ 6.80 ^{CDE}	Blumea essential oil	11.23 $\pm$ 6.48 ^{NO PQ}
Lemon essential oil	77.17 $\pm$ 11.43 ^{CDEF}	Forsythia essential oil	2.33 $\pm$ 1.36 ^{OPQR}
Lavender essential oil	76.37 $\pm$ 6.72 ^{CDEF}	Atractylodes essential oil	1.77 $\pm$ 1.46 ^{OPQR}
Pepper essential oil	66.93 $\pm$ 9.13 ^{DEFG}	Rhubarb essential oil	-2.40 $\pm$ 4.02 ^{PQR}
Tea tree oil	63.90 $\pm$ 1.57 ^{EFG}	Capsicum essential oil	-3.07 $\pm$ 3.45 ^{QR}
Cinnamon essential oil	61.78 $\pm$ 5.78 ^{FG}	Tangerine essential oil	-5.53 $\pm$ 2.89 ^R
Fennel essential oil	56.43 $\pm$ 13.61 ^{GH}	Clausena lansium essential oil	-5.70 $\pm$ 2.81 ^R

Note: Different lowercase letters indicate significant differences at the $P < 0.05$ level, different uppercase letters indicate significant differences at the $P < 0.01$ level, the table below is the same.

Table 2. 500 μL 6 essential oils for continuous inhibition of *A. alternate*.

Observation days	Essential oil type	Inhibition rate
Second day	Asarum essential oil	100 ^a
	Garlic essential oil	100 ^a
	Litsea cubeba essential oil	100 ^a
	Mustard essential oil	100 ^a
	Angelica pubescens essential oil	100 ^a
	Basil essential oil	100 ^a
Fifth day	Asarum essential oil	100 ^a
	Garlic essential oil	100 ^a
	Litsea cubeba essential oil	100 ^a
	Mustard essential oil	100 ^a
	Angelica pubescens essential oil	43.17 ^g
	Basil essential oil	78.63 ^{cd}
Eighth day	Asarum essential oil	76.9 ^d
	Garlic essential oil	69.45 ^e
	Litsea cubeba essential oil	83.13 ^c
	Mustard essential oil	100 ^a
	Angelica pubescens essential oil	49.17 ^f
	Basil essential oil	81.49 ^{cd}

Table 3.MIC and MBC of essential oils against *A. alternata*.

Essential oil type	Asarum essential oil	Garlic essential oil	Litsea cubeba essential oil	Mustard essential oil	Angelica pubescens essential oil	Basil essential oil
MIC (μl/L)	1000	1000	500	250	1000	500
MBC (μl/L)	>2000	1000	1000	250	>2000	2000

References

- Ding, X.B., Zhang, H., Liu, S.Y., Liao, Y.J., Zhou, Z.Q. (2012) Current status of the study in citrus nutriology. *Acta Horticulturae Sinica.*, 39: 1687-1702.
- Li, H.Y., Mei, X.F., Fu, Y.S., Huang, F., Chen, Z.L., Yang, X.P., Wang, Y.G., Huang, H.H. (2015) *Alternaria* brown spot of citrus: the risk and management strategy. *Journal of Fruit Science.*, 32: 969-976.
- Yang, X.Y., Yang, C., Wang, L., Yang, X.J., Tang, J., Tang, J., He, S.G. (2016) Main diseases and control measures of An Yue lemon during storage. *Science and technology of Sichuan agriculture.*, 03: 32-33.
- Chen, Y.B., OuYang, Y.S., Xie, X.B., Huang, X.R., Chen, J.D. (2001) Study on Storage Disease and Antisepsis and Fresh-keeping Technology of Golden Pomelo. *Plant Protection.*, 06: 19-21.
- Santos Dória, M., Silva Guedes, M., de Andrade Silva, E.M., Magalhães De Oliveira, T., Pirovani, C.P., Kupper, K.C., Bastianel, M., Micheli, F. (2019) Comparative proteomics of two citrus varieties in response to infection by the fungus *Alternaria alternata*. *International Journal of Biological Macromolecules.*, 136: 410-423.
- Tuffnail, W., Mills, G.A., Cary, P., Greenwood, R. (2009) An environmental H-1 NMR metabolomic study of the exposure of the marine mussel *Mytilus edulis* to atrazine, lindane, hypoxia and starvation. *METABOLOMICS.*, 5: 33-43.
- Jiang, G.S. (2017) Study on the inhibition of citrus acid rot and its metabolites by peppermint essential oil. Shaanxi Normal University, Xi'an, China.
- Liu, M., Li, Z.Y. (2011) Research progress on plant essential oils. *Chinese animal husbandry veterinarian.*, 38: 252-254.
- Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. (2008) Biological effects of essential oils - A review. *Food and Chemical Toxicology.*, 46: 446-475.
- Castro, J.C., Endo, E.H., de Souza, M.R., Zanqueta, E.B., Polonio, J.C., Pamphile, J.A., Ueda-Nakamura, T., Nakamura, C.V., Dias Filho, B.P., Abreu Filho, B.A.D. (2017) Bioactivity of essential oils in the control of *Alternaria alternata* in dragon fruit (*Hylocereus undatus* Haw.). *Industrial Crops and Products.*, 97: 101-109.
- Xei, S.H., Shao, X.F., Wang, K., Zhang, X.L., Wang, H.F. (2013) Isolation and identification of dominant pathogen on citrus fruit and the antifungal effect of clove oil on these fungi. *Journal of Fruit Science.*, 30: 134-139.
- Gao, Y., Kan, C.N., Wan, C.P., Chen, C.Y., Chen, M., Chen, J.Y. (2018) Quality and biochemical changes of navel orange fruits during storage as affected by cinnamaldehyde-chitosan coating. *Scientia Horticulturae*, 239: 80-86.
- Kong, J.S., Chen, J.W., Wang, B.Z., Yao, L. (2011) Antibacterial comparison and GC /MS analysis of three aromatic plants. *Food industry technology.*, 32: 151-155.
- Duan, X.A., Wu, K.G., Chai, X.H. (2012) Bactericidal activities of spicy essential oils on microorganisms in crude food. *Chemistry and Industry of Forest Products.*, 32: 87-91.