



Study on the potential of antifungal activity of essential oils against fungal pathogens of fruits and vegetables

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ABSTRACT

This research was undertaken to assess the *in vitro* antifungal potential of the essential oils of clove, cinnamon, aniseed, green pricklyash, Pricklyash peel, cumin and fennel against the five major fungal pathogens of fruits and vegetables using the method of inhibitory zone, the inhibition to the mycelial growth and the minimal inhibitory concentration. i.e, *Penicillium italicum*, *penicillium expansum*, *Monilinia fructigena*, *Penicillium sp.* and *Fusarium spp.* The results indicated that clove had significant antifungal activity to all strains, especially against *Penicillium italicum*. Cinnamon and aniseed had strong antifungal activity to all strains tested except *Fusarium spp.*, especially against *Monilinia fructigena*. Moreover, Clove could inhibit mycelial growth of all pathogens completely and the minimal inhibitory concentration of clove was less than or equal to 0.5% (v/v). The present results demonstrated that clove oil could be potential sources of natural fungicides to control certain important fungal pathogens of fruits and vegetables.

Key words: antifungal activity, essential oil, fungal pathogens.

INTRODUCTION

In Postharvest, fruits and vegetables were infected by fungal pathogens easily and often seriously, causing hundred millions of dollars worth of economic damage each year[1].and the focus of attention is to control these fungal pathogens and prolong the shelf-life of fruits and vegetables. Application of chemical germicides is the effective measure to control the fungal infection and plays an important role in preventive defense[2].However, the leftover of germicides imperils human health and results in the unbalance of ecological environment directly, and many of fungal pathogens have the resistance to germicides, which have caused the withdrawal of some of them[3]. So, new and safe alternative control measures are much sought after. And the chemicals from natural sources (particularly of plant resources) is gaining attention as alternative to synthetic germicides ,which is perceived as safe to the environment and human health and can avoid the appearance of drug-resistance.

Antifungal features of essential oils isolated are well documented from the different plant species [4]. Since it is important to test the effects of essential oils of many plants on different microorganisms, *in vitro* laboratory research is being continuously done. Clove, cinnamon, aniseed, green pricklyash, Pricklyash peel, cumin and fennel are native spices, having high amounts of essential oil, naturally grown in China. The essential oils of these seven spices are well known for their antimicrobial activities. However, their antifungal activities against the major fungal pathogens of the staple fruits in China are not well documented.

In this research, the antifungal effects of clove, cinnamon, aniseed, green pricklyash, Pricklyash peel, cumin and fennel were studied for the pathogens of the major disease of citrus, pear, longan and tomato in China. And the inhibitory zone diameters, growth rate reduction assay and minimal inhibitory concentration (MIC) were adopted.

EXPERIMENTAL SECTION

Source of Pathogen Isolates

The fungal pathogens were *Penicillium italicum*, *penicillium expansum*, *Monilinia fructigena*, *Penicillium sp.* and *Fusarium spp.*, which isolated from rot citrus, pear, pear, longan and tomato respectively. Those fungal pathogens were determined as leading to citrus blue mold, pear blue mold, brown rot of pear, longan blue mold and fusarium fruit rot of tomato respectively. All isolates were maintained on potato dextrose agar (PDA) in Petri dishes (100×15 mm) for routine use and on slants of PDA at 4°C for long-term storage.

Preparation of the spore suspension

The fungal pathogens were grown on PDA plates at 28±2°C for 7–9 days, after which time, spores were harvested from sporulating colonies and suspended in sterile distilled water containing 0.1% (v/v) Tween 20. The concentration of spores in suspension was determined using a hemacytometer and adjusted to 1.0×10⁸ spores/ml for each fungal pathogen.

Inhibitory zone diameters

The inhibitory zone diameters of spice essential oils against five strains were assessed by disc diffusion method using PDA in 9cm petri dishes[5]. Sterile PDA was poured into 9cm diameter sterile Petri dishes. After solidifying, the spore suspension was coated on the PDA plates. A sterilized filter paper disc of 6 mm diameter with essential oil (1,000 µl/disc) was placed in the centre of the lid. Control plates contained equivalent amounts of distilled water. Plates were tightly sealed with parafilm and incubated at 28±2°C for 3 days. The average diameters of the inhibition zone surrounding the discs were measured visually. The assays were carried out in triplicate.

Growth rate reduction assay

Growth inhibitions of essential oils against five strains were started by measuring the diameter of fungal colonies after 3 days of incubation and by comparing them to the control plates. Sterile PDA with essential oil (1%) was poured into 9cm diameter sterile Petri dishes. Control plates without essential oil. After solidifying, an agar plug of fungal inoculum (6 mm in diameter) was removed from a 10-day-old previous culture of all the fungal pathogens tested and placed upside down in the center of the PDA plate. Plates were tightly sealed with parafilm and incubated at 28±2°C. The average growth rates of mycelium after 3 days of incubation were measured. The inhibitory activeness was calculated to assess the effect of the essential oils on the mycelium growth of fungal pathogens using the following formula [6].

$$\text{Inhibition (\%)} = [(C-T)/C] \times 100$$

C was the diameter of the control colony (mm) and T was the diameter of the essential oil treated colony (mm).

Evaluation of MIC (minimal inhibitory concentration)

The MIC was defined as the lowest concentration of the test essential oil sufficient to prevent fungi growth in vitro. This test was determined by double broth dilution method [7]. Essential oils were diluted twofold serially with sterile PDA so that the concentration of essential oils were 2%, 1%, 0.5%, 0.25%, 0.125% and 0.0625%, respectively. Next, the sterile PDA with different concentrate essential oil was poured into 9cm diameter sterile Petri dishes. After solidifying, 1 ml of the suspension was inoculated on the plate. Plates were tightly sealed with parafilm and incubated at 28±2°C for 3 days. The experiments were carried out in triplicate.

Statistical analysis

The data obtained in this research were evaluated using the one-way ANOVA of variance test. Differences between means were tested through Bonferroni and values of P<0.05 were considered significantly different.

RESULTS AND DISCUSSION

Determination of inhibition diameters

Inhibition zone diameters of clove, cinnamon, aniseed, green prickleyash, Pricklyash peel, cumin and fennel oil against five fungal pathogens are presented in Table 1. Clove, cinnamon and aniseed oils showed potent inhibitory effect on *Penicillium italicum*, among which clove oil had the strongest fungistatical effect. Conversely, aniseed oil had the lowest inhibition activity. Furthermore, the mean difference between clove and cinnamon oils was significant. The mean difference was also applicable to cinnamon and aniseed oils. The tested essential oils presented good antifungal activity against *penicillium expansum* except fennel oil. And the mean difference was not significant. The essential oils tested presented good antifungal activity against *Monilinia fructigena* except green prickleyash and cumin oils, among which, cinnamon oil had the strongest fungistatical effect and the mean

difference was significant between cinnamon and other essential oils tested. The tested essential oils presented excellent antifungal activity against *Penicillium sp.* except cumin and fennel oils. And clove oil was most remarkable. When it came to *Fusarium spp.*, clove oil was the only essential oil for the tested oils which had the inhibition activity.

Table-1 Inhibitory zone diameters of seven spice essential oils toward some pathogen fungi

	pathogen fungi				
	<i>Penicillium italicum</i>	<i>Penicillium expansum</i>	<i>Monilinia fructigena</i>	<i>Penicillium sp.</i>	<i>Fusarium spp.</i>
Control	-	-	-	-	-
clove	42.8±8.9Cb	16.7±3.5 Aa	17.0±4.2A Ba	33.3±6.7 Bab	32.5±5.9 ab
cinnamon	27.9±7.9Ba	22.7±6.8 Aa	33.0±0.0 Ca	18.3±2.9 Aa	-
aniseed	12.3±2.1Aa	11.3±2.8 Aa	22.0±0.0 Bb	16.0±3.6 Aab	-
Green pricklyash	-	17.2±6.8 Aa	-	15.7±2.6 Aa	-
Pricklyash peel	-	16.0±1.0 Ab	10.5±0.7 Aa	19.8±1.6 Ab	-
cumin	-	15.0±5.0 A	-	-	-
fennel	-	-	11.0±0.0 A	-	-

Notes: A, B, C means the significance of difference between essential oils towards the same strain;
a, b means the significance of difference between strains for the same essential oil;
“-” means no Inhibitory zone

Clove oil exhibited excellent antifungal activity against the five fungal pathogens. But, it exhibited the best antifungal activity against *Penicillium italicum* than to other fungal pathogens tested. Cinnamon and aniseed oils showed fine antifungal activity against fungal pathogens tested except *Fusarium spp.*, which both exhibited the best antifungal activity against *Monilinia fructigena* than to other fungal pathogens tested. Green pricklyash, Pricklyash peel, cumin and fennel oils exhibited antifungal activity against some of the fungal pathogens tested only.

The results of growth rate reduction

On the basis of inhibition zone diameters results, clove, cinnamon and aniseed oils were choose to test the evaluation of growth rate reduction on the five fungal pathogens. Clove, cinnamon and aniseed oils all could reduce the mycelium growth of the five fungal pathogens tested to some extent. As shown in Table 2, Clove oil showed excellent antifungal activity against all the tested fungal pathogens. This oil showed 100.0% of fungistatic effect as fungal mycelial growth inhibition percentage against all the fungal pathogens tested. Besides, cinnamon oil also exhibited remarkable fungistatic effect (33.3% to 100%) against all the fungal pathogens tested. Aniseed oil also showed fungistatic effect against all the fungal mycelial growth. When it came to fungal pathogen, *Penicillium italicum* and *Penicillium sp.* displayed less susceptibility to aniseed oil. There existed significant statistical differences between clove and cinnamon oils, cinnamon and aniseed oils. *Penicillium expansum* and *Monilinia fructigena* were found to be the most inhibited fungal pathogens by clove and cinnamon oils. And the mean difference was significant. *Fusarium spp.* was sensitive to the essential oils tested. But clove oil was the most sensitive essential oil tested. And there existed significant statistical differences between clove and cinnamon oils, cinnamon and aniseed oils.

Table-2 Effects of clove, cinnamon and aniseed oils on mycelial growth of pathogenic strains

	Radial growth (mm)				Radial growth Inhibition (%)		
	control	clove	cinnamon	aniseed	clove	Cinnamon	aniseed
<i>Penicillium italicum</i>	13.5±0.7	0.0±0	9.0±0	12.0±1.4	100.0 a	33.3 b	11.1 c
<i>Penicillium expansum</i>	2.0±0	0±0	0.0±0	0.75±0.35	100.0 a	100.0 a	62.5 b
<i>Monilinia fructigena</i>	3.0±0	0.0±0	0.0±0b	1.5±0.7	100.0 a	100.0 a	50.0 b
<i>Penicillium sp.</i>	3.5±0.3	0.0±0	2.0±0.3	3.0±0.4	100.0 a	42.9 b	14.3 b
<i>Fusarium spp.</i>	74.5±0.7	0.0±0	16.0±1.4	22.0±0.7	100.0 a	78.5 b	70.5 c

Notes: a, b, c means the significance of difference between essential oils towards the same strain.

Determination of MIC

On the basis of inhibition zone diameters results and the results of growth rate reduction, the MICs of clove and cinnamon oils were tested. As shown in Table 3, the MICs were found to be 0.25–2.00% (v/v). *Penicillium italicum* and *Penicillium sp.* were found to be the susceptible fungal pathogens to clove oil with their MIC value as 0.25% (v/v). Cinnamon oil also displayed potential effect of antifungal activity against the tested fungal pathogens. And the most susceptible fungal pathogens were *Penicillium expansum* and *Monilinia fructigena*. And the MIC value was 1.0% (v/v). However, the antifungal activity of cinnamon oil was weaker than that of clove oil against the tested fungal pathogens as a MIC.

The results indicate that different essential oils have different efficacy. Different essential oil owns various components which may be active against different fungal pathogens. It has been reported that the active ingredient

of clove oil is eugenol, and cinnamaldehyde is the major antifungal component in cinnamon oil. It seems that there is a correlation of the chemical structure of the essential oil constituents with antifungal activity[8]. Although some scientific researches have reported that clove oil displays antimicrobial and antifungal activities [9], Our experiments are shown that the essential oil from clove have the best in vitro effect of the series of oils, we detected against the five major diseases of fruits and vegetables in all methods used.

Table 3 MIC of clove and cinnamon towards pathogenic strains

	MIC (%v/v)	
	clove	cinnamon
<i>Penicillium italicum</i>	0.25	2.0
<i>Penicillium expansum</i>	0.5	1.0
<i>Monilinia fructigena</i>	0.5	1.0
<i>Penicillium sp.</i>	0.25	2.0
<i>Fusarium spp.</i>	0.5	2.0

CONCLUSION

The concentration at which clove oil was active in our research was very low, ranging from 0.25–0.5%(v/v). Oils that are rich in phenolic components were very active against microorganisms[10].

The oral LD50 value in rats for eugenol is 1930 mg kg⁻¹ body weight. The acute toxicity experiment suggest that clove oil can be classified as a low toxic substance for *P. semisulcatus* weighing 1.8-2.1g[11]. This shows that clove oil is considered less harmful than synthetic chemicals, which has been restricted in many countries for side effects, such as residual toxicity, carcinogenicity and teratogenicity[12]. Furthermore, the clove plant is the native plant in China. So, clove oil may be used as an alternative for the synthetic chemicals that are applied at present in post harvest of fruits and vegetables to prevent the most important diseases.

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