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In Vitro effects of clove and turmeric extracts controlling crucifer pathogens

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Clove (*Syzygium aromaticum*) and turmeric (*Curcuma longa*) extracts were tested against crucifer pathogens using soaking method. Both extracts showed inhibitory effects on the pathogens. The results of antifungal activities revealed that clove extract was indicated the minimum inhibitory concentration (MIC) of *Alternaria brassicicola* and *Fusarium oxyporum* at 1900 ppm and 2300 ppm respectively. While turmeric extract was indicated at 7500 ppm and 13200 ppm respectively. Whereas, the lower MIC at 470 ppm and 230 ppm of clove and turmeric extracts respectively were observed with *Xanthomonas campestris* inhibitions. Microscopic monitoring of fungal growths on PDA supplemented with the extracts showed abnormal conidia and malformations as swollen, often septated and pale color hypha.

Key words: *Alternaria brassicicola, Fusarium oxysporum, Xanthomonas campestris*, clove, turmeric

Introduction

Crucifers are important vegetable crops and increasingly important crop group especially the genus *Brassica*. There are subject to attack by pathogens. Diseases are important factors limiting the production of leafy greens and mainly cause damage by reducing crop quality. Anne (1998) reported *Brassica* crop yields are reduced worldwide by leaf spot (*Alternaria brassicicola*) and black rot (*Xanthomonas campestris* pv. *campestris*). Synthetic chemicals are often used as antimicrobials but many of which have built resistance to the antibiotics. For this reason, consumers tend to be suspicious of chemical additives and thus the demand for natural and socially more acceptable preservative has been intensified because of their relatively safe status, their wide acceptance by consumers (Skandamis *et al*., 2001; Ormancey *et al*., 2001; Sawanura, 2000). Many spices and herbs exert antimicrobial activity due to their essential oil fractions.

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Vuida (2007) reported antifungal potential of essential oils of oregano, thyme and clove presented inhibitory effects on food spoilage, *Aspergillus niger* and *Aspergillus flavus*. Oregano essential oil showed the highest inhibition of mold growth. Clove essential oil was a stronger inhibitor against *A. niger* than against *A. flavus*. In this study, clove and turmeric extracts were examined for their efficiency to control crucifer pathogens using minimum inhibitory concentration (MIC), microscopic observations and seed treatments to evaluate their possibility for disease control.

Materials and methods

Isolation of fungal and bacterial

The pathogens used in this study were isolated from infected crucifer. Leaf spot, wilt and black rot diseases were diagnosed as *Alternaria brassicicola*, *Fusarium oxysporum* and *Xanthomonas campestris* respectively.

Antimicrobial activity assay

Antimicrobial activity was determined by soaking method using 8 concentrations of clove and turmeric extracts at two-fold dilutions of 3% of each extract. Fungal disc culture and single colony of bacterial were soaked in the extracts for 10 minutes. Inhibition percentages of colony diameters and colony numbers were measure after 7 days cultured of fungi and 48 hours for bacteria respectively.

Minimum inhibitory concentration (MIC) was determined using 3 concentrations of four-fold dilution of the inhibited concentration of each extract. The minimum concentration that 90% reduced growth of microbial colonies was defined as MIC (Moreira *et al.*, 2004).

Microscopic observation of fungal growth

In order to investigate the effect of extracts on fungal growth, *Alternaria brassicicola* and *Fusarium oxysporum* were cultured on PDA supplemented with the extracts using slide culture technique and incubated at room temperature for 5 days. Fungal morphology observation was done under compound microscope.

Percentage seed germination and disease of seedling

Seeds were soaked in 1% sodium hypochlorite for 2 minutes and washed two times in distilled water. Sterilized seeds were soaked in the mixture

of extracts at MIC value and each pathogen for 2 hours. Number of germinated seeds was counted by blotter method and the occurrence of seedling diseases were determined after grown in sterile soil for 7-14 days.

Results

Antimicrobial activity of extracts against pathogens

Inhibitory concentration ranges in Table 1 revealed that antimicrobial activity of clove and turmeric extracts, fungal pathogens were inhibited in the rages of 950-3800 ppm of clove and 3800-15000 ppm of turmeric extracts. While the revising of their antimicrobial on bacteria pathogen was 230-470 ppm and 470-950 ppm of turmeric and clove extracts respectively. Minimum inhibitory concentrations (MICs) tested for each couple of pathogen and extract were done in the ranges of inhibitory concentration and the results shown that MICs of clove extract were 1900, 2300 and 470 ppm inhibited *A. brassicicola*, *F. oxysporum* and *X. campestris* respectively while clearly observed MICs of turmeric extract were 7500, 13200 and 230 ppm respectively Fig.1.

Table 1. Inhibitory concentrations range and minimum inhibitory concentrations (MIC) of clove and turmeric extracts on crucifer pathogens.

Pathogens	Inhibitory concentration ranges/MIC (ppm)	
	Clove/MIC	Turmeric/ MIC
Alternaria brassicicola	950-1900/1900	3800-7500/7500
Fusarium oxysporum	1900-3800/2300	7500-15000/13200
Xanthomonas campestris	470-950/470	230-470/230

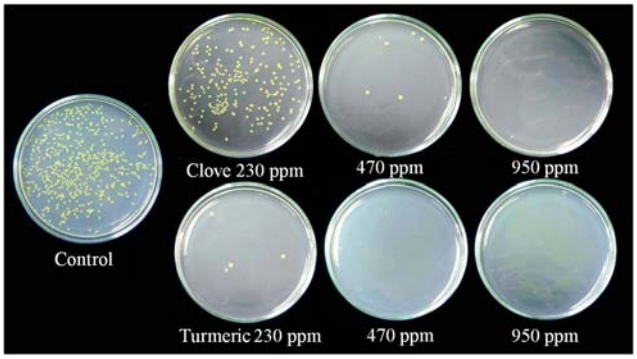

Fig. 1. Number of colony-forming of *Xanthomonas campestris* treated with clove and turmeric extracts by soaking method at 48 hours.

Effects on fungal growth

Abnormal growths of mycelia, swollen hypha, often septum, pale color, conidial malformation and decreasing of conidial number of both fungal pathogens were determined as the effects of clove and turmeric extracts under microscopic observation. Particularly, macro-conidia of *F. oxysporum* treated with clove extract at 1900 ppm generated large numbers of swollen and non-septum conidia (Fig. 2) and these would be severely occurred when treated with 7500 ppm of turmeric extract.

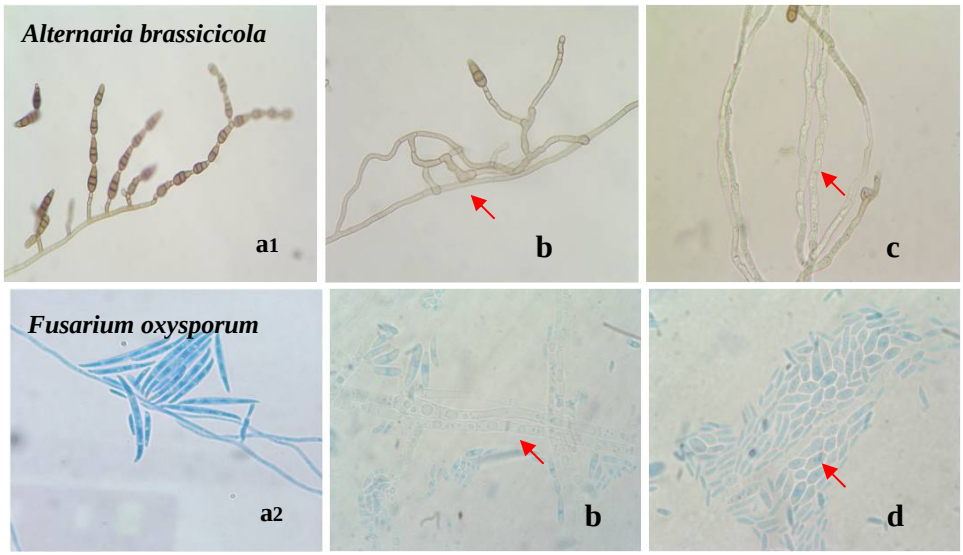

Fig. 2. Abnormal morphology of fungi observed under compound microscope, swollen hypha treated with clove (b) and turmeric (c), swollen and non-septum conidia treated with turmeric (d), compared to untreated fungi (a1, a2).

Effects on seed germination and disease control

The germination percentages of Chinese kale seed treated with mixture of clove extract and each pathogen; *X. campestris*, *A. brassicicola* and *F. oxysporum* were lower than the treated with turmeric extract at 82%, 58%, 64% and 92%, 80%, 78% respectively.

After treated seeds were sown in sterile soil for 7-14 days, turmeric extract was shown effectiveness to decrease the percentage of infected seedling with *X. campestris* more than clove extract at 4% and 15% respectively. Whereas, the occurrence of infected seedling treated with clove extract was lower percentage in *A. brassicicola* and *F. oxysporum* than that treated of turmeric extract at rate of 10%, 6% and 75%, 13% respectively compared to

33% infected seedling was found with small lesion of black rot disease at margins of Chinese kale leaves (Tables 2, 3 and 4) and clearly dwarf seedling were shown in pathogen treated on control treatment (Fig. 3).

Table 2. Percentage of seed germination and infected seedling treated with the mixture of *Xanthomonas campestris* and extract at minimum inhibitory concentration for 2 hours.

Treatments	Seed germination Percentage (%)	Seedling disease Percentage (%)
Control $^{+}$ (untreated)	96	0
Control $^{-}$ (only pathogen treated)	92	33
Clove 470 ppm	82	15
Turmeric 230 ppm	92	4

Table 3. Percentage of seed germination and infected seedling treated with the mixture of *Alternaria brassicicola* and extract at minimum inhibitory concentration for 2 hours.

Treatments	Seed germination Percentage (%)	Seedling disease Percentage (%)
Control $^{+}$ (untreated)	84	0
Control $^{-}$ (only pathogen treated)	70	100
Clove 1900 ppm	58	10
Turmeric 7500 ppm	80	75

Table 4. Percentage of seed germination and infected seedling treated with the mixture of *Fusarium oxysporum* and extract at minimum inhibitory concentration for 2 hours.

Treatments	Seed germination Percentage (%)	Seedling disease Percentage (%)
Control $^{+}$ (untreated)	94	0
Control^{-} (only pathogen treated)	84	36
Clove 2300 ppm	64	6
Turmeric 13200 ppm	78	13

Fig. 3. Chinese kale seedlings treated with the mixture of clove or turmeric extract at minimum inhibitory concentration and *Alternaria brassicicola* after sown in sterile soil for 7 days compared to healthy seedlings in untreated seed (control^{+}) and severely diseased seedlings in only pathogen treated seed (control^{-}).

Discussion

Antimicrobial activities of clove and turmeric extracts were proved against pathogens of Chinese kale. Comparing between fungi and bacteria pathogens, result of MIC tests was shown that *X. campestris* was more sensitive inhibited with very low concentrations (less than 500 ppm) of clove and turmeric extracts than fungi. And between two fungal pathogens, *A. brassicicola* was more sensitive inhibited by the extracts than *F. oxysporum* that have also been reported by Pawar and Thaker (2006) in *A. porri* and *F. oxysporum* f. sp. *cicer*. Chami *et al*. (2005) has attributed this inhibitory capacity to eugenol, the major component of clove oil. The difference of diseases controlling, clove extract slightly effect on seed germination but has more effective to control fungi than bacteria pathogens. In case of *A. brassicicola* and *F. oxysporum*, clove extract could decrease numbers of seedling leaf spot disease and wilt disease reach to ten times and six times respectively compared to only pathogens treated controls that also revealed seedling dwarfing. Possible modes of action of the extract constituents have been reported by Nychas (1995) indicated that phenolic compounds (eugenol, thymol, etc.) could denature the enzymes responsible for spore germination or interfere with the amino acids involved in germination. That would be impacted to their pathogenicity. In opposite, turmeric extract was particularly more efficiency to control bacteria than fungi. Additional, phytotoxic was not determined on seedling germinated from seeds treated with both extracts. However, sufficient testing of these two extracts will be indicated the possibility for further field using.

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