

## Antimicrobial Activities of Chili and Black Pepper Extracts on Pathogens of Chinese Kale

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### ABSTRACT

*Antimicrobial activities of chili and black pepper extracts were evaluated on *Xanthomonas campestris*, a pathogenic bacteria causing black rot, and *Alternaria brassicicola*, a pathogenic fungi causing leaf spot disease, both of which can cause severe damage to Chinese Kale crop production. The extracts were diluted with sterile distilled water in concentrations of 3.0%, 1.5% and 0.75% (w/v). After soaking in a 1:1 mixture (v/v) of bacterial suspension ( $1 \times 10^4$  cfu/ml) and diluted extracts, all tested concentrations of black pepper extract completely inhibited the bacteria. In contrast, the chili extract concentrations varied in their inhibition ability, from highest (3.0%) to moderate (1.5%) to none (0.75%). These results were also observed in the trials of fungal inhibitory effects. Radial growth of *A. brassicicola* were completely inhibited after the cultured disc was incubated on PDA agar supplemented with all tested concentrations of black pepper extract for 15 days, while the chili extracts caused less inhibition effect than the pepper extracts. Only the 3.0% chili extract, after 3 days incubation, demonstrated a 100% inhibition effect. Significant reductions of the inhibition percentages for the 1.5% and 0.75% trials were observed, 61.8% and 55.5%, respectively, after incubation for three days. In addition, microscopic observations of the fungal growth clearly showed an effect on the disorders causing swollen and frequently septa mycelia. Increasing extract concentrations resulted in fewer conidia and malformed germ tube developments. Spraying 3.0% diluted black pepper extract was the most effective for reducing the number, size and severity of leaf spot lesions with non-phytotoxicity*

**Keywords:** Chili extract, Black pepper extract, *Alternaria brassicicola*, *Xanthomonas campestris*

### INTRODUCTION

Chinese Kale, in the *Alboglabra* group of *Brassica oleracea* is a popular leaf consuming vegetable crop grown throughout Asia. *Brassica* crop yields have been reduced worldwide by pathogenic *Xanthomonas campestris* and *Alternaria brassicicola*, a bacterial black rot and fungal leaf spot pathogen, respectively (Westman et al., 1999). Controlling the diseases chemically by application of

several fungicides and bactericides has long been recommended. However, the chemicals used are indiscriminately toxic to beneficial or non-target organisms, and over the long term can pollute ecosystems.

Natural plant products, including medicinal plant extracts, are increasingly being used as agrochemicals for controlling diseases due to their non-phytotoxicity and easy bio-degradation (San Aye and Matsumoto, 2011; Mudalige et al., 2011). Chili and black pepper are well-known cooking herbs and the extracts are used as medicinal products. In addition, successful use of some extracts for inhibition of some food bacteria and fungi have been reported (Dorantes et al., 2000; Moreira et al., 2004). The objective of this study is to evaluate the antimicrobial activities of these extracts against pathogenic fungi and bacteria, and to study their effects on some morphological changes of fungi, which have not been previously studied under microscope.

## MATERIALS AND METHODS

### Plant extract and pathogen preparations

The chili and black pepper extracts were obtained in paste form from the Research and Development Institute, Government Pharmaceutical Organization (GPO), Thailand. The plant extracts were diluted (w/v) with sterile distilled water to three concentrations, 0.75%, 1.5% and 3.0%, for the pathogen and disease control trials.

Bacterial black rot pathogen, *X. campestris*, was isolated from infected plant samples collected from Chinese Kale grown in Nong Hoi, Chiang Mai Province, Thailand. The infected leaves were surface sterilized with 1% sodium hypochlorite for 3 minutes and washed two times with sterile distilled water. A piece of early infection lesion was put into a drop of sterile distilled water, then crushed. The obtained plant sap was streaked on nutrient agar (NA) for pathogenic bacteria isolation and incubated at room temperature for 24-36 hours. The single colony of pure culture was transferred to sterile distilled water and kept at 4°C until use. Fungal leaf spot pathogen, *A. brassicicola*, was isolated from lesions on infected leaves by tissue transplanting technique (Keitt, 1915) and incubated at 28°C. A pure culture of the fungus was obtained using hyphal tip isolation on potato dextrose agar (PDA). The pure culture was maintained in PDA agar slants and kept at 4°C. Mycelia and spores of the fungus were transferred to PDA agar two weeks before use.

### Growth inhibition of pathogens

*Xanthomonas campestris*. A single colony of bacteria, *X. campestris*, was suspended in 10 ml sterile distilled water, then mixed well, before 0.1 ml of the bacterial suspension was added to 1 ml of the diluted extract in a test tube at each concentration and mixed thoroughly for 5 minutes before spreading on NA. Each treatment was replicated two times with four plates per replication. The evaluation was carried out based on the colony counting method, 24 and 48 hours after incubation at room temperature.

***Alternaria brassicicola*.** The effects of plant extracts on *A. brassicicola* were determined by the poisonous agar method (Mishra and Tiwari, 1992; Nene and Thapilyal, 2002) using PDA supplemented with each plant extract at concentrations of 0.75%, 1.5% and 3.0%. One cultured disc of fungus grown on PDA was transferred to the center of each poisonous agar plate and incubated at room temperature. The diameter of the fungal colony in each plate, after incubation for 3, 5, 7, 10 and 15 days, was measured. Percent inhibition of radial growths in each treatment compared with the untreated control was calculated using the following formula:

$$\text{Percent inhibition of radial growth (\%PIRG)} = \frac{R1 - R2}{R1} \times 100$$

R1 = radial growth of control

R2 = radial growth of treatments

### Observation on affected fungi

The slide culture of *A. brassicicola* was prepared using water agar supplemented with each concentration of the extracts. A piece of 1x1 cm<sup>2</sup> water agar was placed on microscope slides then mycelia of the fungus were inoculated before being covered with cover glass and incubated at 28°C. A compound and scanning electron microscopes were used to determine the growth and morphological changes of *A. brassicicola* after incubation for 7 days.

### Effects on fungal disease control

One-month-old Chinese Kale seedlings grown in plastic pots were used for testing the effects of plant extracts on controlling Alternaria leaf spot. The concentrations of chili and black pepper extract which showed the highest inhibitory effects for controlling mycelia radial growth of *A. brassicicola* were used for disease control trials. Fungal inoculums were prepared using 1x10<sup>5</sup> spore/ml spore suspension. Forty seedlings were sprayed until the leaves were thoroughly imbued with the inoculums. After inoculation, 10 seedlings were separately sprayed with the diluted extract in the first dose at 3 days and repeated doses at 7 and 10 days. Then, sizes and numbers of the disease lesions were observed for evaluating the disease control potential of the extracts compared with the non-sprayed control trial.

## RESULTS

### Growth inhibition of *A. brassicicola* and *X. campestris*

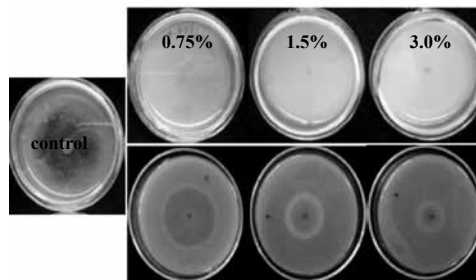
Percent inhibition of fungal growth increased significantly after the fungus was grown on the cultured medium supplemented with the higher concentrations of chili or black pepper extract. In the case of black pepper extract, 100% inhibition of fungal growth was observed in all tested concentrations, whereas, chili extract was less effective. The chili extract trial at a concentration of 0.75% failed to inhibit radial growth of *A. brassicicola* after incubation for 15 days (Table 1, Figure 1), while trials of black pepper extract significantly inhibited *X.*

*campestris* for all tested concentrations (Figure 2, Table 2). Chili extract was less effective than black pepper extract for controlling the bacteria *X. campestris* since chili extract at 0.75% could only delay, but not significantly inhibit the bacterial colonies after 12 hours of incubation.

**Table 1.** Percent inhibitions of radial growth of *Alternaria brassicicola* colonies after cultured on PDA supplemented with chili extract at various incubation periods.

Extract concentrations	Percent inhibition of radial growth (%) <sup>1</sup>				
	3 days	5 days	7 days	10 days	15 days
0.75%	55.54 <sup>c</sup>	52.14 <sup>c</sup>	35.50 <sup>c</sup>	10.75 <sup>c</sup>	0.00 <sup>c</sup>
1.5%	61.75 <sup>b</sup>	61.03 <sup>b</sup>	48.00 <sup>b</sup>	29.00 <sup>b</sup>	14.50 <sup>b</sup>
3.0%	100.00 <sup>a</sup>	74.84 <sup>a</sup>	68.00 <sup>a</sup>	51.25 <sup>a</sup>	39.25 <sup>a</sup>
CV%	0.15	1.29	2.88	8.48	15.98

Note: <sup>1</sup>Means of percent inhibition of radial growth (%). Different superscripts within the same column are significantly different ( $P<0.05$ ).

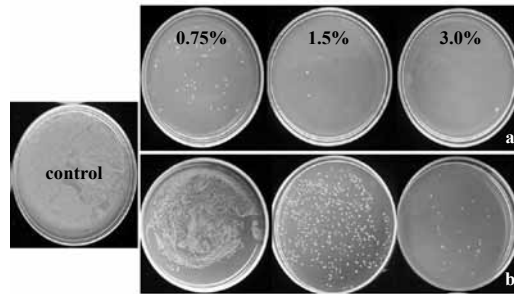


**Figure 1.** Inhibition effects of various concentrations of black pepper (a) and chili (b) extract supplemented in PDA on colony diameters of *Alternaria brassicicola* after incubation for 15 days.

**Table 2.** Colony numbers of *Xanthomonas campestris* after soaking in black pepper and chili extract for 5 minutes then grown on nutrient agar for 48 hours.

Concentration (%)	Colony numbers <sup>1</sup>	
	Black pepper	Chili
3.00	0.25 <sup>a</sup>	16.50 <sup>a</sup>
1.50	3.00 <sup>a</sup>	772.00 <sup>b</sup>
0.75	51.00 <sup>a</sup>	5,922.00 <sup>c</sup>
control	6,930.00 <sup>b</sup>	6,930.00 <sup>c</sup>

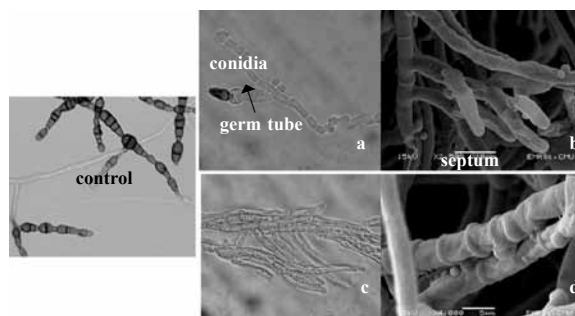
Note: <sup>1</sup>Means of colony numbers. Different superscripts within the same column are significantly different ( $P<0.05$ ).



**Figure 2.** Effects of various concentrations of black pepper (a) and chili (b) extract on colony formation of *Xanthomonas campestris* soaked in different concentrations for 5 minutes and then grown on nutrient agar for 48 hours.

### Malformations of *A. brassicicola* mycelia

Microscopic observations of slide cultures of *A. brassicicola* clearly indicated the effect of chili and black pepper extracts on morphological disorders of the mycelia. Swollen hypha and mycelium septa were frequently observed in all tested concentrations compared with no changes in the mycelia development in the control treatment (Figure 3). In addition, sporulation and conidial germ-tube development of *A. brassicicola* were affected. The conidial number decreased as the concentration increased. Malformed conidial germ-tube development was also observed.

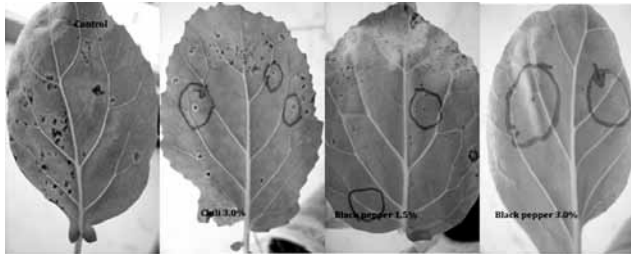


**Figure 3.** Malformation growths of *Alternaria brassicicola* caused by 0.75% black pepper extract supplemented in water agar performed on slide culture for 7 days. Swollen germ tube of conidia (a), abnormal and short branching of hypha (b, c) and frequent septa were clearly observed under a scanning electron microscope (d).

### Effects on fungal disease control

Depending on extracts and concentrations, disease controls by spraying the extracts on inoculated Chinese Kale seedlings were investigated in reducing the size and number of *A. brassicicola* leaf spot lesions. Concurring with the *in vitro* results, chili extract was less effective for controlling leaf spot than black

pepper extracts. The highest dose of chili extract, 3.0%, controlled the fungus *in vitro*, but was not high enough to control the disease *in vivo*. Black pepper extract in concentrations of 1.5% and 3.0% were more effective in reducing the yellow halo area around spot lesions of the disease. Spraying with 3.0% black pepper extract also decreased the number, size and severity of leaf spot lesions, while non-phytotoxicity was observed (Figure 4.)



**Figure 4.** Symptoms of *Alternaria brassicicola* leaf spot on Chinese Kale seedlings after spraying with chili and black pepper extracts two times at 3 and 7 days after inoculation.

## DISCUSSION

Many plant species have antimicrobial properties that can be utilized to manage plant diseases naturally. Chili and black pepper, common spices used to improve the odor and flavor of foods, contain metabolic substances that have been used pharmaceutically for a long time. This study has proven that extracts of these spices have inhibitory effects on both bacterial and fungal pathogens of Chinese Kale. These results are supported by the finding of Singh et al. (2004), who reported that the volatile oils, such as piperine, piperolein and piperamid major obtained from black pepper extract by acetone, were 100% effective in controlling the mycelial growth of many foodborne fungi. However, *X. campestris* and *A. brassicicola*, were more sensitive to black pepper than chili extract. The lowest effective concentration of the extracts examined in terms of pathogen propagule reduction was 1.5%, at which both bacterial colony numbers and the growth of fungal colonies and sporulation were inhibited. Mycelial disorders of the fungus were clearly observed with Khan and Zhihui (2010), who also found collapsed abnormal hypha (swelling, curling, short branching) of *Phytophthora capsici*, after treating with 0.08% w/v garlic root exudates. According to these results, a large reservoir of natural fungicides exists in plants such as garlic, alfalfa and cabbage, which have inhibitory effects on mycelial growth and disease severity caused by *P. capsici* (Demirci and Dollar, 2006) and are a safe and effective alternative to synthetic fungicides (El-Mougy et al., 2007). These extracts not only have the potential to control fungal and bacterial diseases in vegetable crops, but also are safe and organic. Biologically active compounds present in the extracts act as elicitors and induce resistance in the host plant, resulting in reduced disease development that can be exploited as an ideal treatment for future plant disease management.

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