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Effects of plant extracts and chitosan against Alternaria leaf spot of Chinese kale

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ABSTRACT

Ethanol extracts of *Alpinia galanga* (L.)Willd. (rhizome), *Coscinium fenestratum* (Goetgh.) Colebr. (stem), *Piper betle* Linn. (leave), *Piper longum* L. (fruit), *Zingiber cassumunar* Roxb.(rhizome) and chitosan were evaluated for their fungicidal activities in controlling Alternaria leaf spot of Chinese kale, caused by *Alternaria brassicicola*, under field conditions. The results showed that the application of these plant extracts resulted in reduction of disease severity in a concentration-dependent manner. Among the five plant extracts tested, the extracts of *P. betle* and *C. fenestratum* at a concentration of 10,000 ppm displayed potent fungicidal activity in the suppression of disease severity, causing 43% and 41% disease reduction, respectively. The extracts of *Z. cassumunar* and *A. galanga* at 10,000 ppm showed moderate effectiveness in controlling the disease, causing 28% and 25% disease reduction, respectively, whereas the extract of *P. longum* at the same concentration possessed the lowest antifungal activity, reducing the disease severity by less than 20% at the highest dosed tested. The application of chitosan at 2% had a moderate effect; it reduced the disease severity by 30%. However, the application of the systemic fungicide, iprodione, displayed the greatest fungicidal activity in suppression disease severity**,** causing 65% disease reduction. The results in this study indicated that the *P. betle* and *C. fenestratum* may be promising eco-friendly candidates for controlling Alternaria leaf spot disease in Chinese kale production.

Keywords: Medicinal plant, Phytopathogen control

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INTRODUCTION

Alternaria brassicicola is one of the most devastating plant pathogenic fungi. Infection with this fungus results in losses in the yields of host plants. It causes leaf spot disease in wide range of hosts, especially in Brassicaceae including Alternaria blight of rapeseed and mustard (Singh *et al*., 2017), black leaf spot of cabbage and Chinese kale (Lin *et al*., 2011; Dethoup *et al.*, 2018), and leaf spot of sugar beet (Rosenzweig *et al*., 2019). Recently, it was reported as a causal agent of leaf blight on *Orychoophragmus violaceus* in China (Guo *et al*., 2019). Under favorable conditions, this plant pathogenic fungus can produce great numbers of spores which infect plant parts and can spread by wind, rain or insects and can infect a crop multiple times (Lin *et al*., 2011; Siciliano *et al*., 2017). Moreover, the fungus can infest host plant seeds and can disseminate as a seed-borne fungus to new crops. The typical symptoms of brown concentric ring on infected leaves are observed within seven days after infection (Macioszek*et al*., 2018). Moreove, this fungus can produce several mycotoxins such as tenuazonic acid, alternariol, alternariol monomethyl ether, altenuene and tentoxin (Pedras and Park, 2015; Siciliano *et al*., 2017).

Researchers have been seeking eco-friendly strategies for controlling this pathogen by sprayingcrops with botanical fungicides, plant extracts and biological control agents or treating seed with biological agents, heat or chemicals (Hassan *et al*., 2017; Dethoup *et al*., 2018; Rosenzweig *et al*., 2019; Kokkrua *et al*., 2020). Plant extracts have been reported to have potent antifungal activity against this pathogen because plants are rich in bioactive compounds (Ounchokdee *et al*., 2016).The

antifungal activity against this pathogen of extracts of many plant species have been evaluated (Singburaudom, 2015, Rueangrit *et al*., 2019). Notably, Lin *et al*. (2011) reported the efficacy of *Solanum nigrum* ethanol extract exhibited completely inhibited spore germination of *A. brassicicola* at 500 mg/L whereas the n-butanol fraction of the ethanol extract exhibited 100% spore germination inhibition of *A. brassicicola* at a low concentration of 25 mg/L.

Likewise, chitosan has been reported as a promising biological control agent against many plant diseases (Hadrami *et al*., 2010). Chitosan is a linear unbranched biopolymer of β-1,4-D- glucosamine derived from a co-polymer of D-glucosamine and N-acetyl-D-glucosamine linked with 1,4-glycosidic bonds (Malerba and Cerana, 2018). There are many reports of the potential of chitosan and its mechanisms in controlling plant diseases in many crops (Sharif *et al*., 2018; Malerba and Cerana, 2018). Multifunctional actions of chitosan in plants have been reported including antifungal and antibacterial activities (Sharif *et al*., 2018), induced resistance in host plants (Parada *et al*., 2018), promotion of plant growth (Egusa *et al*., 2019) and increase in seed germination (Zhou *et al*., 2017).

Because Chinese kale is an important vegetable in Thailand and because continuous cropping has increased the severity of Alternaria leaf spot disease of Chinese kale under field conditions, ecofriendly approaches to control of this disease have been intensively investigated. In our previous study, we evaluated the antifungal activity of 10 plant extracts against this disease and found that crude ethanol extracts of *C. fenestratum* and *P. betle* at 10,000 ppm exhibited significant ($P < 0.05$) reduction of the disease incidence of up to 67% and showed promising preventive and curative activities against *A. brassicicola* under greenhouse conditions (Dethoup *et al*., 2018).However, further studies need to evaluate the antifungal activities of these extracts against this disease under field conditions. In addition, chitosan under various brands has been promoted for controlling this disease in Thailand. Therefore, the objective of this study was to determine the effects of effective five plant extracts and chitosan at various concentrations in controlling Alternaria leaf spot of Chinese kale under field conditions.

MATERIALS AND METHODS

Plant Extractions and Chitosan

The five medicinal plant extracts were prepared as described previously by Dethoup *et al*. (2018). Briefly, the plants were purchased from a medicinal plant market, in Bangkok, Thailand. Each plant sample was cleaned by washing with tap water thrice, dried in the shade, cut into small pieces, and ground into fine powder with a high-powder grinder. Then, each dried plant preparation was extracted thrice with 70% ethanol at room temperature. The ethanol extract of each plant was filtered through three layers of cheesecloth and then concentrated using a rotary evaporator to obtain a crude ethanol extract. The chitosan 2% used in this study was purchased from GEN Technology Co., Ltd., Thailand.

Fungal Pathogen

The strain of *A. brassicicola* KUFA 1079 was isolated from an infected Chinese kale plant, and its pathogenicity was confirmed according to Koch's postulates. The fungus was cultured on potato dextrose agar for 14 days at room temperature. Fifteen mL of sterile water was poured into a petri dish and gently scraped with a sterile glass rod to obtain a spore suspension. The spore suspension was filtered through three layers of sterile cheesecloth and adjusted to 10^6 spores/ mL using a hemocytometer.

Black spot under field conditions

Field trials were conducted at Amphur Tha Maka, Kanchanaburi province, in the western part of Thailand. Chinese kale seeds were surface disinfected with 0.1% (v/v) sodium hypochlorite for 2 min., washed three times with sterile water, and planted in tray nurseries. The plot size was 1.5 x 1.5 m with 0.1 m within the row and between rows, for a total of 14 rows per plot arranged as a completely randomized design with the distance between plots was 0.5 m.Seven-day-

old seedlings of the same height and vigor were transplanted to plots. Thirty days after planting, the Chinese kale plants were sprayed with one of the following: 1. plant extract at 5,000 ppm; 2. plant extract at 10,000 ppm, 3. chitosan 2%, 4. iprodione 50%WP (1.5 g/L positive control) and 5. water + Tween-20 (negative control). The plants were sprayed with 1 L of each treatment per plot with separate sprayers, and each treatment consisted of three plots (replicates). After 1 hour, the treated plants were inoculated with 1 L of the spore suspension (10^6 spores/ mL) of *A. brassicicola* per plot.

Seven days after applications, thirty leaves were randomly collected from plants in the middle of each test plot. The disease severity was recorded as a percentage of lesion area over the total leaf surface. The disease severity was classified into six levels: 0 = no lesion area; 1 = lesion area 1–20 %; 2 = lesion area 21–40 %; 3 = lesion area 41–60 %; 4 = lesion area 61–80 %; 5 = lesion area >80 % (Panwar et al. 2013). The experiments were performed twice during July-August, 2017 and repeated once during August-September, 2018.

Statistical analysis

The experiments in this study were conducted twice. Due to there being no significant difference between the repetitions of each experiment, the separate data of each experiment were pooled and then submitted to analysis of variance, and means were compared by Duncan's multiple range test ($P < 0.05$), using the statistical program SPSS version 19 (IBM Corporation, Somers, NY).

RERULTS

The effects of five plant crude ethanol extracts and chitosan at concentrations against Alternaria leaf spot of Chinese kale under field conditions are shown in Table 1 and Fig. 1. The application of the systemic fungicide iprodione showed the greatest fungicidal activity in the suppression of disease severity**,** causing 68% disease reduction. Among the five plant extracts tested, the extracts of *P. betle* and *C. fenestratum* exhibited significant ($P < 0.5$) reduction of the disease severity, causing 43% and 41% disease reduction, respectively, at a concentration of 10,000 ppm. Following this, the application of chitosan at 2% reduced the disease severity by 30%. The extracts of *Z. cassumunar* and *A. galanga* at 10,000 ppm showed moderate antifungal activity in controlling the disease, causing 28.54 and 25.42% disease reduction, respectively, whereas the extract of *P. longum* displayed the lowest activity, reducing the disease severity by less than 20% at 10,000 ppm. The extracts of *C. fenestratum* and *P. betle* at a concentration of 5,000 ppm showed moderate antifungal activity in controlling the disease, causing 26.42 and 29.16% disease reduction, respectively. When applied at 5,000 ppm, the extracts of *Z. cassumunar*, *A. galanga* and *P. longum* exhibited low activity in controlling this disease under field conditions.

Table 1 Effects of plant extracts and chitosan on the incidence of Alternaria leaf spot of Chinese kale under field conditions.

Treatment	Plant part	Concentration (ppm)	Disease Severity (%)
Alpinia galanga (L.)Willd.	Rhizome	10,000 5,000	48.32 ± 4.24^{f} 59.24 ± 5.50^{h}
Coscinium fenestratum (Goetgh.) Colebr.	Stem	10,000 5,000	32.57 ± 0.89^{c} 47.32 ± 3.09^{f}
Piper betle Linn.	Leaves	10,000 5,000	30.09 ± 2.93^{b} 44.58 ± 4.41^{de}
Piper longum L.	Fruit	10,000 5,000	54.27 ± 2.89^{g} 61.43 ± 3.62^{i}
Zingiber cassumunar Roxb.	Rhizome	10,000 5,000	45.20 ± 3.69^{e} 61.24 ± 6.14^{i}
Chitosan 2% Iprodione 50%WP Water	- - -	- -	43.28 ± 4.58^{d} 5.11 ± 1.04^{a} 73.74 ± 6.19^{j}

Means ± standard derivations followed by the same letter in each row do not significantly differ at $P < 0.05$, when analyzed using Duncan test of One-Way ANOVA.

Fig. 1 Effects of plant extracts and chitosan against Alternaria leaf spot disease of Chinese kale under field conditions**.** a. *Alpinia galanga* extract at 10,000 ppm, b. *Coscinium fenestratum* extract at 10,000 ppm, c. *Piper betle* extract at 10,000 ppm, d. *Piper longum* extract at 10,000 ppm, e. *Zingiber cassumunar* extract at 10,000 ppm f. chitosan at 20,000 ppm, g. iprodione 50%WP (1.5 g/ L) and h. water (negative control)

DISCUSSION

The results in this study showed the efficiency of *P. betle* and *C. fenestratum* extracts in controlling Alternaria leaf spot disease of Chinese kale caused by *A.brassicicola* under field conditions. However, the percentages of disease reduction under field conditions were lower than those of these extracts under greenhouse conditions, which we reported earlier (Dethoup *et al*., 2018). In our previous report, the applications of *C. fenestratum* and *P. betle* extracts were able to reduce the disease severity by up to 67% under greenhouse conditions; however, the extracts reduced the disease severityby43% and 41%, respectively when applied at 10,000 ppm under field conditions. This phenomenon may be the effect of light, water, wind, and other environmental factors on the performance of the plant extracts against the disease (Deberdt *et al*., 2008; Rojo Baio *et al*., 2019). Thus, it may be necessary to increase the concentration of the extracts when applied in field trials due such factors. In contrast, the fungicide, iprodione showed consistent fungicidal effects in controlling the disease under both greenhouse and field conditions (Dethoup *et al*., 2018).

Plant extracts have been reported as promising agents for controlling plant diseases because the plants from which they are derived produce large groups of bioactive compounds

effective against plant pathogens (Jesonbabu *et al*., 2012; Dethoup *et al*., 2019; Kaewsalong *et al*., 2019). In this study, the extracts of *P. betle* and *C. fenestratum* showed potent fungicidal activity against Alternaria leaf spot disease under field conditions. *Piper betle*, belonging to the family Piperaceae, and its leaf extract have been reported to have potent antimicrobial activity against many plant pathogens and are also used in folk medicine in many countries (Valentão *et al*., 2010). Eugenol, β-caryophyllene and hydroxychavicol have been reported as the major bioactive compounds responsible for the antifungal activity of the extract of *P. betle* leaves (Ali *et al*., 2010; Valentão *et al*., 2010; Singburaudom, 2015). There are many reports of the effects of *P. betle* extract in inhibiting the growth of plant pathogens *in vitro* including *Phaeoisariopsis personata* and *Puccinia arachidis* (Kishore and Pande, 2005), *Colletotrichum capsici*, *C. gloeosporioides*, *Fusarium oxysporium* f.sp. *cubense* and *Pyricularia oryzae* (Singburaudom, 2015).

Meanwhile, *Coscinium fenestratum*, belonging to the family Menispermaceae, has been found to produce bioactive metabolites against plant diseases. Recently, Kaewsalong *et al*. (2019) reported the efficacy *C. fenestratum* extract against dirty panicle disease in rice. The authors found that the extract reduced the disease incidence by 32% when applied at 10,000 ppm under field conditions. Berberine has been reported to be a key bioactive compound in *C. fenestratum*extract and has also been reported as a main bioactive compound against *Alternaria brassicicola*, a causal agent of Alternaria leaf spot under greenhouse conditions (Dethoup *et al*., 2019), and *Monilinia fructicola*, a causal agent of peach brown rot under field conditions (Fu *et al*., 2017; Pei *et al*., 2019). The potency of berberine against plant pathogens results from its many modes of action such as affecting cell wall integrity and ergosterol biosynthesis (Dhamgaye *et al*., 2014) and binding target DNAs, RNAs and proteins in fungal cells (Fu *et al*., 2017).

Although the *A. galangal, P. longum* and *Z. Cassumunar* extracts showed only moderate antifungal activity against *A. brassicicola* in this study, there are many studies which reported theses extracts displayed potent antifungal activity against other plant pathogens. *P. longum* extract was reported as an effective agent in inhibiting the mycelial growth of *C. capsici*, *C. gloeosporioides* and *F. oxysporum* f.sp. *cubense in vitro* (Ounchokdee *et al*., 2016; Rueangrit *et al*., 2019). Mongkol *et al*. (2014) reported the dichloromethane extract of *A. galangal* effectively suppressed the growth of *Puccinia nicotianae* and *A. porri* with MIC31.5 μg/ mL and *C. gloeosporioides* and *F. oxysporum* with MIC250 and 500 μg/ mL, respectively. However, as yet there are no reports about the antifungal activity of these extracts in controlling any plant diseases under field conditions.

Moreover, in this study we also evaluated the efficacy of the commercial chitosan 2% against this disease. Although there are many reports of the potential of chitosan against many plant diseases, it showed moderate fungicide activity against this disease, causing 30% disease reduction. However, Sathiyabama *et al*. (2014) reported that the application of chitosan at 1 mg/mL could reduce the disease severity of early blight disease of tomato caused by *A. solani* by 75% while Abd-El-Kareem and Haggag (2014) reported that the application of chitosan at 2 g/L reduced the incidence of early blight of potato caused by *A. solani* by more than 76.6% and increased tuber yield by more than 80% over the untreated control.

The results in this study indicated that *P. betle* and *C. fenestratum* extracts are promising agents in controlling Alternaria leaf spot of Chinese kale caused by *A. brassicicola* under field conditions. Although the fungicide, iprodione gave the greatest activity in disease reduction, plant extracts are safer and lower in toxicity and negative effects on humans, animals and the environment than that of synthetic fungicides. These findings will support the potential of the extracts of *P. betle* and *C. fenestratum*as alternative approaches for controlling the Alternaria leaf spot disease

in Chinese kale production for sustainable agriculture.

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Conflict of Interest

The authors declare no conflict of interest.

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