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Research article

Potential application of dry medicinal plant powders in controlling black spot on Chinese kale and chili anthracnose diseases

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Abstract

Importance of the work: Chinese kale and chili are important vegetables in Thailand; however, their production is affected by diseases.

Objective: To evaluate the efficacy of plant powders against black spot disease on Chinese kale and chili anthracnose disease through greenhouse tests and field trials.

Materials and Methods: The efficacy was evaluated of 20 plant powders in inhibiting *Alternaria brassicicola* (cause of black spot disease) and *Colletotrichum truncatum* (cause of anthracnose disease) at 50 g/L and 25 g/L *in vitro* along with testing the protective and curative activities of the selected plant powders against black spot disease on Chinese kale and chili anthracnose disease under greenhouse and field conditions.

Results: The results from the greenhouse conditions showed that the *Syzygium aromaticum* (clove) and *Zingiber cassumunar* (phlai) powders had significant fungicidal activity in controlling black spot disease on Chinese kale with disease reduction ranges of 38.64–41.95% and 29.87–33.95% in the protective and curative tests, respectively. In addition, both plant powders demonstrated high levels of activity in controlling black spot on Chinese kale (36.3% and 32.76%, respectively), whereas the *Coscinium fenestratum* and *Curcuma longa* powders reduced the incidence of this disease by 29.43% and 26.83%, respectively, compared to the negative results from the controls (with water) in the field trials. The *S. aromaticum* and *Z. cassumunar* plant powders both showed strong activity in controlling anthracnose disease in leaves and detached fruit.

Main finding: *Syzygium aromaticum* and *Zingiber cassumunar* powders showed potent fungicidal activity in controlling Chinese kale and chili diseases.

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Introduction

Thailand is an agricultural country, with Thai agricultural policies seeking to promote Thai agricultural products through the concept of a world kitchen, serving field crops, fruit and vegetables and other food to people worldwide through sustainable farming (Yaklai et al., 2018). However, intensive and continual cultivation practices have resulted in the increasing severity of plant disease and infection outbreaks in crop production (Rodríguez et al., 2008; He et al., 2016; Jayaraman et al., 2021). This is especially the case in the production of fast-growing vegetables or raw vegetables, where the application of fungicides may lead to chemical residues which can directly affect consumers (Keinath, 2019; Bai et al., 2021). Chinese kale and chili are important vegetables in Thailand, with production of 167,435 t and 273,515 t, respectively, in 2020 (https://www.doa.go.th/hort/wp-content/uploads/2020. These vegetables, with their medicinal and health-promoting properties, are mainly used as food ingredients.

Black spot disease, caused by *Alternaria brassicicola*, is the main destructive disease in *Brassica* spp., namely, Chinese kale, cabbage, cauliflower and broccoli (Nowick et al., 2012; Dethoup et al., 2018). The first typical symptom observed comprises small black specks on the leaf surface or stem. These become spots that spread concentrically, developing yellow halos and becoming necrotic lesions on older leaves. Severe infections result in wilted black leaves which fall from the plant. Under wet and humid conditions, black spores are produced on the entire diseased leaf surface (Agrios, 2005; Nowicki et al., 2012).

Anthracnose disease is a major constraint in chili production in tropical and subtropical countries, causing an enormous amount of damage and reduced yields in the hot and humid environmental conditions that support infection, disease severity and dissemination (Ranathunge et al., 2012; De Silva et al., 2021). *Colletotrichum* spp. Have been reported to be causal agents of anthracnose disease in chili fruit. For example, in Thailand, four species of anthracnose pathogens have been recorded, namely, *C. capsici, C. acutatum, C. gloeosporioides*, and *C. siamense* (Suwannarat et al., 2017). These pathogens infect the stems, leaves and fruits. Typical symptoms appear on ripe chili fruit, starting with small spots, which develop into sunken circular or angular lesions, each of which extends in concentric rings at maturity and then coalescing to form severe fruit rot (Saxena et al., 2016; Mongkolporn and Taylor, 2018).

Thai medicinal plants have proven to be a source of antimicrobial agents for the control of many plant diseases (Dethoup et al., 2018, 2020; Kokkrua et al., 2020). Other studies showed that the ethanol extracts from the leaves of *Piper betle* Linn. (betel vine) and the stems of *Coscinium fenestratum* (Goetgh.) Colebr. (tree turmeric) exhibited potent and promising eco-friendly properties, which made them candidates for controlling black spot disease in Chinese kale, caused by *A. brassicicola* (Dethoup et al., 2018; Ounchokdee and Dethoup, 2020), as well as rice diseases caused by *Bipolaris oryzae*, *Curvularia lunata*, *Pyricularia oryzae*, and *Rhizoctonia solani* (Dethoup et al., 2019; Kokkrua et al., 2020).

Although plant extracts demonstrate potent activity against plant diseases, their extraction from plant material requires organic solvents, as well as a rotary evaporator to remove solvent from the extracts (Houlihan et al., 2019). These factors are obstacles for farmers who cannot afford either the expensive equipment or the solvents, resulting in limited use of plant extracts for disease management in farmers' fields as a replacement for synthetic fungicides. Therefore, new, easy-to-use and cost-effective methods for applying medicinal plants to control plant diseases should be developed and studied. Thus, the objectives of the current study were: 1) to evaluate the efficacy of 20 plant powders in inhibiting *Alternaria* and *Colletotrichum in vitro*; 2) to determine the antifungal activity of 10 selected plant powders against black spot disease on Chinese kale and chili anthracnose disease; and 3) to test the fungicidal activity of the selected plant powders against black spot disease on Chinese kale and chili anthracnose disease under greenhouse and field conditions.

Materials and Methods

Plant samples

For this study, 20 medicinal plants were purchased from Saphanmai Market, a famous medicinal plant market in Bangkok, Thailand (Table 1). The plant samples were washed

Table 1 Plants and efficacy of plant powder at 50 g/L against *Alternaria brassicicola* and *Colletotrichum truncatum*

Plant used	Family	Plant part used	% Mycelial growth inhibition	
			Alternaria brassicicola	*Colletotrichum truncatum*
Acanthus ebracteatus Vahl.	Acanthaceae	Leaves	25.071.56^{k}	30.25±1.34^{f}
Allium sativum L.	Amaryllidaceae	Bulbs	70.61±5.78^{e}	69.63±3.24^{c}
Alpinia galanga (L.)Willd.	Zingiberaceae	Rhizomes	32.22±2.05^{j}	11.85±1.06^{i}
Areca catechu L.	Arecaceae	Seeds	0^{m}	0^{j}
Cissus quadrangularis L.	Vitaceae	Stems	0^{m}	12.5±0.89^{h}
Citrus hystrix DC.	Rutaceae	Leaves	0^{m}	0^{j}
Coscinium fenestratum (Goetgh.) Colebr.	Menispermaceae	Stems	95.67±8.16^{b}	100±0^{a}
Curcuma longa L.	Zingiberaceae	Rhizomes	100±0^{a}	100±0^{a}
Derris scandens (Roxb.) Benth.	Fabaceae	Stems	87.04±4.32^{c}	27.04±1.08^{g}
Kaempferia parviflora Wallich. ex Baker.	Zingiberaceae	Rhizomes	68.52±4.55^{f}	67.41±5.26^{d}
Ocimum tenuiflorum L.	Lamiaceae	Leaves	0^{m}	0^{j}
Piper betle Linn.	Piperaceae	Leaves	82.56±5.63^{d}	95.67±4.30^{b}
Piper nigrum L.	Piperaceae	Leaves	54.44±2.81^{h}	52.96±2.50^{e}
Piper retrofractum Vahl.	Piperaceae	Fruits	61.11±5.11^{g}	53.70±2.34^{e}
Piper sarmentosum Roxb.	Piperaceae	Flower	5.67±0.44^{l}	11.11±0.57^{i}
Phyllanthus amarus Schum & Thonn.	Euphorbiaceae	All Plant Parts	0^{m}	0^{j}
Syzygium aromaticum (L.)	Myrtaceae	Flowers	100±0^{a}	100±0^{a}
Thunbergia laurifolia Lindl.	Acanthaceae	Leaves	5.56±0.15^{l}	0±0^{j}
Zingiber cassumunar Roxb.	Zingiberaceae	Rhizomes	100±0^{a}	100±0^{a}
Zingiber officinale Roscoe.	Zingiberaceae	Rhizomes	34.07±1.33^{i}	11.15±0.85^{i}
CV (%)			4.54	2.77
LSD (p = 0.05)			7.4	5.3

CV = coefficient of variation; LSD = least significant difference.

with running water for 10 min and then allowed to dry in the shade for a few hours, before being cut into small, thin pieces and allowed to dry for 5 d in the shade. Then, using a high-power grinder, they were made into a fine powder and passed through a sieve of #35 mesh size with a hole diameter of 0.5 mm.

Pathogen isolation and pathogenicity test

The pathogen strains of 26 *A. brassicicola* (Schweinitz, Wiltshire) were isolated from 52 diseased Chinese kale leaves collected from Tha Muang district, Kanchanaburi province (13°59'07.5"N 99°35'56.5"E), Thailand. Furthermore, the 52 *C. truncatum* (Schwein., A. & Moore, W. D.) strains (syn. *C. capsici* (Syd. & P. Syd.; E. J. Butler & Bisby 1931) in this study were isolated from the 121 diseased chili var. Jinda collected from a chili field at Nong Bua Yai, Chatturat district, Chaiyaphom province (15°36'24.8"N 101°51'37.4"E), Thailand, with these being primarily identified based on morphological analysis. Their pathogenicity test was conducted following Agrios (2005), on the Chinese kale and chili plants. Spore suspension of each pathogen at 1 × 10^{6} spores/mL was applied at 4 wk to the host Chinese kale and chili plants at 10 mL/plant with 10 plant replications per treatment. One strain of black spot disease on Chinese kale and one strain of chili anthracnose disease, each showing the most virulent symptoms on these host plants, were collected and further analyzed using a molecular technique based on the universal primers ITS1 and ITS4 (White et al., 1990). DNA was extracted from young mycelia following a modified method of Murray and Thompson (1980). Polymerase chain reactions (PCRs) were conducted on a thermal cycler, with the amplification process consisting of initial denaturation at 95 °C for 5 min.; 34 cycles at 95 °C for 1 min. (denaturation), at 55 °C for 1 min. (annealing) and at 72 °C for 1.5 min. (extension), followed by a final extension at 72 °C for 10 min. The PCR products were examined using agarose gel electrophoresis (1% agarose with 1 × tris-b ethylenediaminetetraacetic acid orate-EDTA [TBE] buffer) and visualized under ultraviolet light after staining with ethidium bromide. Analyses using DNA sequencing utilized the dideoxyribonucleotide chain termination method for sequencing by Macrogen Inc. (Seoul, South Korea). The DNA sequences were edited

using the FinchTV software (Dethoup et al., 2018) and submitted to the Basic Local Alignment Search Tool (BLAST) program for alignment, as well as comparison being undertaken with fungal species in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/). These pathogens were identified as *A brassicicola* and *C. truncatum* (syn. *C. capsici*), with accession numbers ON222525 and ON222524, respectively.

Effect of dry plant powders on mycelial growth of pathogens

The 20 prepared Thai medicinal plant powders were sterilized using autoclaving at 121 °C for 15 min. Their fungicidal activity on *A. brassicicola* and *C. truncatum* was evaluated using the poison food method and a completely randomized design (CRD), as described by Dethoup et al. (2018). Other studies have reported that a concentration of 50 g/L was the maximum concentration that did not cause phytotoxicity in the tested plants (Dethoup et al., 2018; Kokkrua et al., 2020). Thus, each sterile plant powder was mixed with warmed potato dextrose agar (PDA), vortex mixed well and poured into a sterile Petri dish to obtain a final concentration of 50 g/L of the plant powder on the PDA plates. PDA plates void of plant powder served as negative controls. Then, a mycelial disc (0.5 cm in diameter) of each pathogen was placed in the center of each prepared PDA plate and incubated for 10 d at 28 ± 2°C. The percentage of radial growth inhibition was computed as % inhibition = (R1 - R2) / R1 * 100, where R1 is the radial growth of the pathogen in the control treatment and R2 is the radial growth of the pathogen in the dual culture test. In all, 10 of the 20 plant powders that displayed effective mycelial growth on the pathogens were selected and further tested using the poison food method at 10 g/L and 25 g/L. Each assay was performed on five plates (replications) and repeated three times.

Preparation of spore suspension

The pathogens, *A. brassicicola* and *C. truncatum*, were cultured on PDA for 10 d, with 15 mL of sterile water then poured into each cultured plate. The spores were collected by gentle scraping of the colonies, filtered through sterile muslin cheesecloth and adjusted with sterile water to 1.13×10^6 spores/mL of each pathogen using a hemocytometer.

Protective and curative activities of dry plant powders against black spot disease under greenhouse conditions

Chinese kale (*Brassica oleracea* L. cv. *alboglabra* group) plants were planted in plastic pots 15 cm in diameter, each containing 1 kg of potting soil. Four plants per pot were planted and placed in a greenhouse at an average temperature of 30–32 °C for 7 wk. Each pot was applied with 5 g of 15-15-15 N-P-K (nitrogen-phosphorus-potassium) every week and watered every day until the water ran off. A concentration of plant powder at 25 g/L was used in all experiments as applying plant powders at more than this amount may have caused yellow or green stains on leaf surfaces (Dethoup et al., 2018). For the protective activity tests, both sides of the leaves of the prepared Chinese kale plants were sprayed with 10 mL of each plant powder at 25 g/L mixed with 2% Tween 80 per pot. After 24 hr, the treated plants were inoculated with 10 mL of *A. brassicicola* spore suspension at 1×10^6 spores/mL per pot. Negative and positive controls comprised water mixed with 2% Tween 80 and a fungicide (iprodione 50%WP) at 1.5 g/L, respectively.

For the curative activity tests, Chinese kale plants were foliar-spayed with 10 mL of pathogen spore suspension at 1×10^6 spores/mL per pot and incubated for 24 hr under moist conditions. Then, separate treated plants were sprayed with the respective plant powders at 25 g/L mixed with 2% Tween 80. Chinese kale plants sprayed with a fungicide (iprodione 50%WP, at 1.5 g/L) and plants treated only with the pathogen served as the controls. Five pots (replications) were set up for each treatment and were placed in a CRD in a greenhouse. The disease incidence was measured as a percentage of the lesion area over the total leaf surface 7 d after pathogen inoculation, with six levels of classification of the disease symptom on the leaf area: 0 = no disease; 1 = 1–20%; 2 = 21–40%; 3 =41–60%; 4 = 61–80%; and 5 = 81–100% (Panwar et al., 2013). The percentage of disease severity was calculated using Equation 1:

$$[\Sigma(r \times nr) / 5 \times Nr] \times 100 \quad (1)$$

where r is the rating value (0–5); nr is the number of infected leaves with a rating of r; and Nr is the total number of leaves tested in each replication. The percentage of disease reduction was calculated by comparison with the control based on:

% Disease reduction = [(S1 × 100) / S2] - 100

where S1 is the percentage of disease severity in the treatment and S2 is the percentage of disease severity in the negative control. The entire experiment was carried out twice using a RCB design during June–July, 2019 and August–September 2019.

Efficacy of dry plant powder for controlling black spot disease in field trial

The field trial was conducted in Tha Muang district, Kanchanaburi province (13°59'07.5"N 99°35'56.5"E), in the main area for Chinese kale cultivation in Thailand. A severe natural infection of black spot disease was present, due to repeated and continual crop production in the fields (Yuttitham, 2019; Ounchokdee and Dethoup, 2020). The field trial adopted a randomized complete block (RCD) design. The plot size was set as 1 m × 2 m with 1 m between each of the three plots (replications) that were arranged with a CRD. Chinese kale was planted in nursery trays for 14 d and then transplanted into holes in the plots, with two plants per hole and 20 cm spacing between plants and between rows. When typical symptoms of the disease were observed at a level of 10%, about 40 d after planting, the Chinese kale plants were sprayed with each of the plant powders at 25 g/L at 2 L per plot. Applications of water and the fungicide, iprodione (50%WP), at 1.5 g/L were set as negative and positive controls, respectively. The third leaves were randomly collected 10 d after spraying; in total, 20 leaves per plot (60 leaves per treatment) were collected. The experiment recorded the percentages of disease severity and disease reduction, as reported in the greenhouse test. The whole trial was carried out twice during July–September 2020 and August–October 2021.

Effect of dry plant powder against anthracnose disease on chili fruit

Ripe green chili fruits cv. Jinda were purchased from an organic farm in Chaiyaphom province (15°36'24.8"N, 101°51'37.4"E). The chili fruit were cleaned with tap water and allowed to dry for 2 hr, with surface disinfection carried out by soaking in 70% ethyl alcohol for 3 min. Then, they were washed with sterile water for 5 min, soaked in 10% sodium hypochlorite (NaOCl) for 3 min and finally washed with sterile water for 5 min, before being allowed to dry under a laminar flow. The chili fruit were soaked in solutions of dry plant powder at 2.5 g per 100 mL water and placed in a plastic box (10 cm wide × 1 cm long × 20 cm high) with 10 chili fruit per box. Chili fruit treated with water served as controls. The treated chili fruit were wounded by puncturing 10 holes in an area 1 cm in diameter using a sterile needle at the center of the fruit. Then, a mycelial plug of *C. truncatum* (0.5 cm) was inoculated into the central part of each chili fruit. The box was kept moist and incubated at room temperature. After 4 d of incubation, the size of the disease symptom on each chili fruit was recorded. The percentage of disease reduction was calculated using Equation 2:

% Disease reduction = [(R1 × 100) / R2] - 100 (2)

where R1 is the average size of disease symptom in the treatment and S2 is the average size of the disease symptom in negative control. Each treatment used 30 chili fruit, with the experiment repeated three times as an RCB design.

Effect of dry medicinal plant powder in controlling anthracnose disease on chili plants

The chili var. Jinda seeds were planted in trays in a nursery for 14 d and then transplanted into 25 cm diameter pots. One plant was planted per pot, each containing 1 kg of potting soil; all pots were placed in a greenhouse at an average temperature of about 30–32°C for 30 d. The plants were watered till runoff every day and 5 g of 15-15-15 (N-P-K) was applied per pot every week. The chili plants were sprayed with: 1) 2.5 g per 100 mL mixed in 1% Tween 80; 2) 1% Tween 80 water; or; 3) phochloraz 45%EC 1.5 mL in 1 L water at 20 mL per plant, then allowed to dry for 2 hr. The treated chili plants were sprayed with a *C. truncatum* spore suspension at 1×10^6 spores/mL in the same volume. The disease severity was determined 14 d after application by collecting five leaves at the central part of each plant. The numbers of diseased spots per leaf were counted and compared with the negative controls. The percentage of disease reduction was calculated using Equation 3:

% Disease reduction = [(R1 × 100) / R2] - 100 (3)

where R1 is the average of number of diseased spots per leaf in the treatment and S2 is the average of number of diseased spots per leaf in the negative control. All experiments consisted of five pots and were conducted twice independently during June–August 2019 and August–October 2019 based on an RCB design.

All experiments were conducted at least twice.

Data analyses

Data were subjected to analysis of variance and subsequently means were compared utilizing Duncan's multiple range test ($p < 0.05$), in the SPSS version 19 statistical program (IBM Corporation; Somers, NY, USA).

Results

Effects of plant powders on plant pathogens

The effects of the 20 plant powders mixed in PDA at 50 g/L in inhibiting the mycelial growth of *A. brassicicola* and *C. truncatum* are shown in Table 1. In all, 10 of the 20 plant powders displayed potent activity with more than 50% inhibition of one pathogen and thus they were selected (Table 2). Their activity was further evaluated against these pathogens in PDA at 25 g/L and 10 g/L. The *S. aromaticum* powder showed complete inhibition of both pathogens even at the low concentration of 10 g/L. The other plant powders showed varied levels of inhibition of both pathogens. The *D. scandens*, *P. betle* and *Z. cassumunar* powders exhibited low activity against both pathogens, whereas the remaining plant powders inhibited the mycelial growth of *A. brassicicola* by more than 50% at 10 g/L. However, the *C. fenestratum*, *C. longa*, *D. scandens*, *K. parviflora* and *Z. cassumunar* powders displayed mycelial growth inhibition of *C. truncatum* by more than 50% at 25 g/L.

The selected plant powders were further evaluated on their activity against these pathogens at 25 g/L and 10 g/L in PDA. Powder of *S. aromaticum* showed complete inhibition of both pathogens, even at the low concentration of 10 g/L, while the remaining plant powders showed varied inhibition activity against both pathogens. The powders of *D. scandens*, *P. betle* and *Z. cassumunar* exhibited low activity against both pathogens, whereas the remaining plant powders inhibited the mycelial growth of *A. brassicicola* by more than 50% at 10 g/L. However, the *C. fenestratum*, *C. longa*, *D. scandens*, *K. parviflora* and *Z. cassumunar* powders displayed mycelial growth inhibition of *C. truncatum* by more than 50% at 25 g/L.

Efficacy of plant powders in controlling black spot disease on Chinese kale

The plant powders showed better efficacy in controlling black spot disease on Chinese kale through protective activity or when applied before pathogen inoculation, compared to through curative activity. Among the plant powders tested, *S. aromaticum* and *Z. cassumunar* powders demonstrated the best level of activity in controlling black spot disease on Chinese kale. They reduced disease by 38.64–41.95% and

Table 2 Plants and efficacy of plant powder at 25 g/L and 10 g/L against *A. brassicicola* and *C. truncatum*

Plant	% Mycelial growth inhibition			
	Alternaria brassicicola		*Colletotrichum truncatum*	
	25 g/L	10 g/L	25 g/L	10 g/L
Allium sativum L.	76.11±3.67^{c}	59.11±3.21^{d}	0^{h}	0^{g}
Coscinium fenestratum (Goetgh.) Colebr.	96.33±6.05^{b}	74.67±4.60^{b}	65.11±4.25^{c}	35.19±2.16^{e}
Curcuma longa L.	62.78±4.11^{e}	56.89±3.12^{e}	100±0^{a}	81.85±6.15^{b}
Derris scandens (Roxb.) Benth.	31.112.03^{h}	29.44±2.08^{h}	50.26±2.05^{e}	22.22±1.07^{g}
Kaempferia parviflora Wallich. ex Baker.	69.67±5.48^{d}	65.89±4.33^{c}	60.37±4.28^{d}	45.56±3.12^{d}
Piper betle Linn.	0^{i}	0^{i}	42.96±2.36^{g}	29.63±1.90^{f}
Piper nigrum L.	57.44±2.41^{f}	50.78±3.56^{f}	44.44±2.11^{f}	20.11±1.02^{h}
Piper retrofractum Vahl.	58.33±2.33^{f}	51.25±3.24^{f}	44.44±2.06^{f}	29.63±2.08^{f}
Syzygium aromaticum L.	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
Zingiber cassumunar Roxb.	55±2.46^{g}	34.11±2.07^{g}	70.37±5.26^{b}	66.67±4.13^{c}
CV (%)	4.07	4.11	3.87	4.01
LSD ($p = 5$)	5.9	6.2	5.3	6.5

CV = coefficient of variation; LSD = least significant difference.

Mean ± SD in each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

29.87–33.95% in the protective and curative tests, respectively. The *C. fenestratum* and *C. longa* powders showed significant fungicidal activity against the disease by reducing disease incidence levels by 31.67–34.99% and 23.17–26.18% in the protective and curative tests, respectively, when compared with the negative (water) controls. The remaining plant powders reduced disease incidence by 22.15–26.75% and 13.02–18.853% in the protective and curative tests, respectively. However, the application of the fungicide, iprodione (50%WP), resulted in the highest level of disease reduction in both tests under greenhouse conditions (Fig. 1).

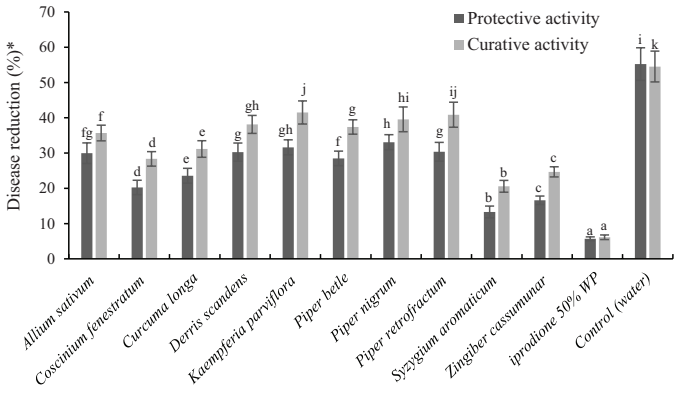

Fig. 1 Effects of plant powders in controlling black spot of Chinese kale under greenhouse conditions; bars represent mean; error bars represent SD; different lowercase letters above bars indicate significant ($p < 0.05$) differences among means of each parameter.
* % disease reduction = [(S1 × 100) / S2] - 100 where S1 is the percentage of disease severity in the treatment and S2 is the percentage of disease severity in the negative control.

Efficacy of plant powders in controlling black spot disease on Chinese kale in field trials

Fig. 4 shows the effects of plant powders in controlling black spot disease on Chinese kale under field conditions that were similar to those under greenhouse conditions. The *S. aromaticum* and *Z. cassumunar* powders demonstrated a similar level of fungicidal activity in controlling black spot disease on Chinese kale in the greenhouse and field conditions by 36.3% and 32.76%, respectively, whereas the *C. fenestratum* and *C. longa* powders reduced disease incidence by 29.43% and 26.83%, respectively, compared to the control. The remaining plant powders produced 12.99–17.78% disease reductions with the application of the fungicide, iprodione (50%WP), having the highest level of disease reduction at up to 50% (Figs. 2–3).

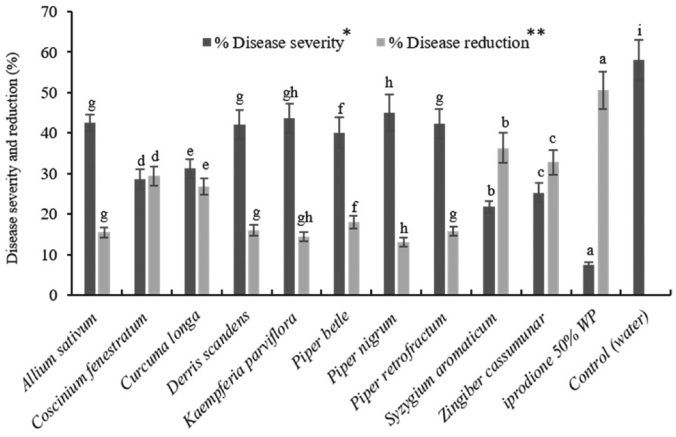

Fig. 2 Effects of plant powders in controlling against black spot of Chinese kale under field conditions; bars represent mean; error bars represent SD; different lowercase letters above bars indicate significant ($p < 0.05$) differences among means of each parameter.
* percentage of disease severity = [$\Sigma(r \times nr) / 5\ Nr$] × 100, where r is the rating value (0–5), nr is the number of infected leaves with a rating of r and Nr is the total number of leaves tested in each replication.
** a formula for % disease reduction is presented in Fig. 1.

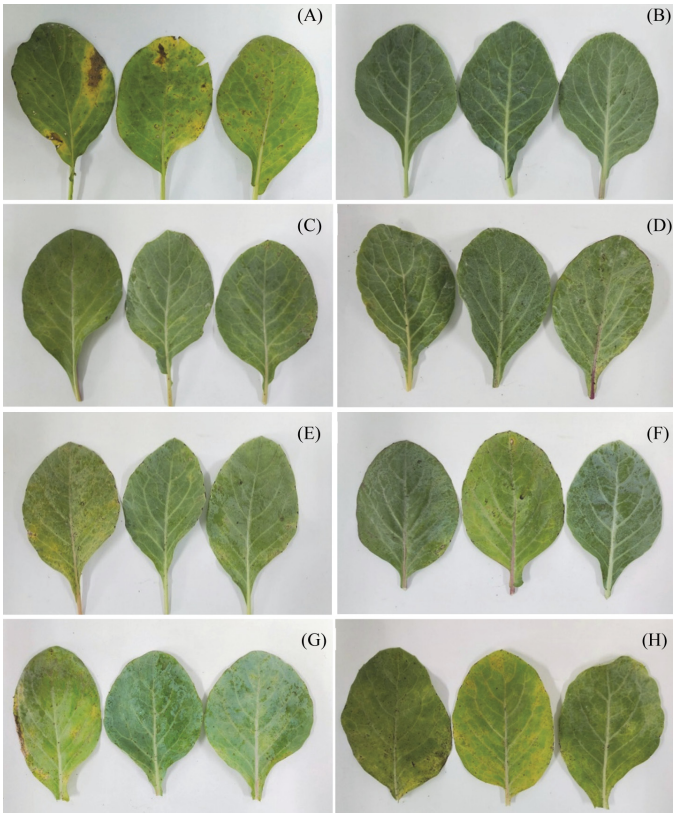

Fig. 3 Control efficacy of plant powders against black spot of Chinese kale under field trials: (A)control (water); (B) iprodione 50%WP; (C) *Syzygium aromaticum*; (D) *Zingiber cassumunar*; (E) *Coscinium fenestratum*; (F) *Curcuma longa*; (G) *Piper betle*; (H) *Piper nigrum*

Effect of plant powders in controlling anthracnose disease on chili fruit and leaves

Table 3 and Fig. 4 show the effects of plant powders in controlling anthracnose disease on chili fruit and leaves in a protective activity test. Among the plant powders tested, only the *S. aromaticum* and *C. longa* powders showed strong fungicidal activity in controlling anthracnose disease on chili fruit and leaves, while the remaining plant powders showed low-to-moderate activity against the disease. Significant control activity was shown with 52.85% and 44.79% disease reduction, respectively, on the leaves, and 69.68% and 65.61% disease reduction on detached fruit, respectively. However, the effects of *C. fenestratum* and *Z. cassumunar* displayed moderate control activity by 32.67% and 30.1% disease reduction, respectively, on leaves, and 29.86% and 27.15% disease reduction on detached fruit, respectively. The remaining plant powders showed control activity at less than 20% disease reduction. The treatment with the fungicide, iprodione (50%WP), displayed the best level of activity in controlling the disease. Although the effects of plant powders showed higher efficacy in controlling chili anthracnose disease than black spot disease on Chinese kale, their efficacy was not stable even in tests

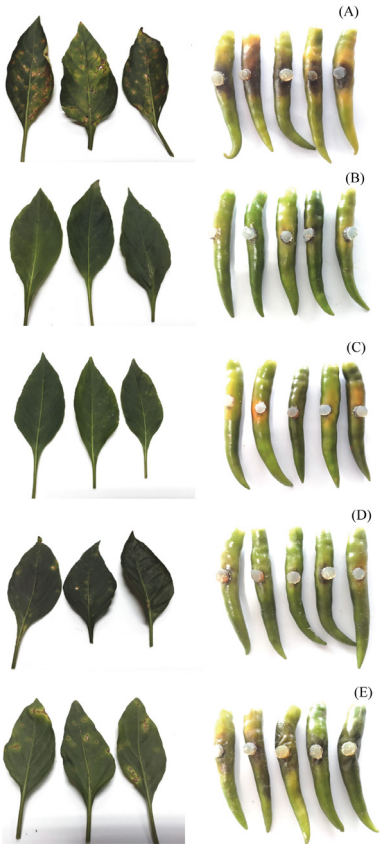

Fig. 4 Effect of plant powders against anthracnose on chili fruit and leaves: (A) control (water); (B) *Syzygium aromaticum*; (C) *Curcuma longa*; (D) *Coscinium fenestratum*; (E) *Zingiber cassumunar*

Table 3 Effects of plant powders in controlling anthracnose diseases on chili fruit and leaves

Treatment	Leaf		Fruit	
	Disease incidence (number of spots)	Disease reduction*	Disease incidence (cm)	Disease reduction**
Allium sativum L.	26.59±2.03^{de}	28.73±2.17^{e}	1.76±0.24^{bc}	20.36±2.04^{f}
Coscinium fenestratum (Goetgh.) Colebr.	25.12±1.56^{d}	32.67±2.50^{d}	1.55±0.56^{b}	29.86±2.64^{d}
Curcuma longa L.	20.6±2.01^{c}	44.79±3.44^{c}	0.76±0.25^{a}	65.61±5.21^{c}
Derris scandens (Roxb.) Benth.	29.07±2.24^{f}	22.09±2.03^{g}	1.86±0.16^{bcd}	15.84±1.30^{g}
Kaempferia parviflora Wallich. ex Baker.	34.09±3.50^{g}	8.68±0.76^{j}	2.24±0.22^{d}	***
Piper betle Linn.	32.58±2.69^{g}	12.68±1.12^{i}	2.07±0.21^{cd}	6.33±0.55^{h}
Piper nigrum L.	30.56±2.67^{f}	18.09±1.56^{h}	1.85±0.17^{bcd}	16.29±1.20^{g}
Piper retrofractum Vahl.	27.17±2.33^{e}	27.18±2.35^{f}	1.79±0.11^{bc}	19.00±1.35^{f}
Syzygium aromaticum L.	17.59±1.52^{b}	52.85±4.55^{b}	0.67±0.09^{a}	69.68±6.52^{b}
bZingiber cassumunar Roxb.	26.08±2.34^{de}	30.10±2.41^{e}	1.61±0.17^{b}	27.15±2.54^{e}
Prochloraz 45%EC	11.68±1.02^{a}	68.69±5.84^{a}	0.59±0.06^{a}	73.30±6.38^{a}
control	37.31±3.06^{h}		2.21±0.16^{d}	
CV (%)	8.47	8.52	12.65	12.70
LSD (p = 0.05)	3.1	3.7	2.1	4.6

CV = coefficient of variation; LSD = least significant difference.

Mean ± SD in each column superscripted with different lowercase letters indicate significant ($p < 0.05$) difference.

* % disease reduction = [(R1 × 100) / R2] - 100 where R1 and R2 are the average size of disease symptom in the treatment and the negative control, respectively.

** % disease reduction = [(R1 × 100) / R2] - 100 where R1 and R2 are the average number of disease spots per leaf in the treatment and the negative control, respectively.

*** the disease incidence had higher than that of the controls.

on detached fruit. Therefore, the antagonistic activity was studied only on black spot disease on Chinese kale in the field trial.

Discussion

Medicinal plant extracts have been reported to be potent botanical fungicides in controlling various plant diseases; however, the extraction process presents an obstacle that has resulted in limited use of medicinal plants in disease control (Dethoup et al., 2018). The current results showed the effects of the direct use of medicinal plant powders against diseases on Chinese kale and chili, with these plant powders being cost effective and easy to prepare and apply in the field. To the best of the authors' knowledge, the current study was the first to show the efficacy of the direct use of medicinal plant powders in controlling vegetable diseases. Here, the *S. aromaticum* (clove) powder had the best level of activity against the diseases, followed by the *C. longa*, *C. fenestratum* and *Z. cassumunar* powders, respectively, all of which had no phytotoxicity. Dried clove flower buds are a well-known spice and are also used in Asian medicine, with its extract reported to be a potent botanical fungicide against various diseases, such as late blight in potatoes, caused by *Phytophthora infestans* (Najdabbasi et al., 2020); sheath blight in rice, caused by *Rhizoctonia solani* (Persaud et al., 2019); and wilt disease in tomatoes, caused by *Fusarium oxysporum f.sp. lycopersici* (Sharma et al., 2018). Eugenol is one of the bioactive constituents contained in cloves at a concentration of at least 50%, comprising 10–40% eugenyl acetate, β-caryophyllene and α-humulene (Haro-González et al., 2021).

The efficacy has been reported of *C. longa* in pharmaceutical and agricultural uses (Ibáñez and Blázquez, 2021). Its extract showed potent biocidal activity against late blight in potatoes, caused by *P. infestans* (Najdabbasi et al., 2020); anthracnose in dragon fruit, caused by *C. gloeosporioides* (Bordoh et al., 2020); cucumber powdery mildew, caused by *Podosphaera xanthii* (Fu et al., 2018); fusarium root rot in sunflowers, caused by *F. solani* (Alsahli et al., 2018) and early blight disease in tomatoes, caused by *A. solani* (Ahmad et al., 2017). Furthermore, the active compound, curcumin, (+)-(S)-ar-turmerone, and the phenolics are the key compounds in the root of *C. fenestratum* (turmeric), according to Sabir et al. (2021).

Dethoup et al. (2018) showed that *C. fenestratum* and *P. betle* extracts displayed potent fungicidal activity against *A. brassicicola* in Chinese kale both *in vitro* and under greenhouse conditions, with these extracts suppressing disease incidence by up to 67%, demonstrating promising preventive and curative activities. The current results indicated that their powders had lower fungicidal activity than their crude extracts in controlling black spot disease, with disease reduction of 29% under field conditions. However, among the plant powders tested, the *C. fenestratum* (turmeric root) powder had strong fungicidal activity, followed by the *Z. cassumunar* (phlai) powder, with these plant powders possibly applicable for controlling black spot disease in sustainable crop production. Berberine was reported as a key biological compound in *C. fenestratum*, with its crude extract having potent activity on various plant diseases, such as dirty panicle in rice, caused by *Alternaria, Curvularia* and *Fusarium* (Kaewsalong et al., 2019; Kokkrua et al., 2020) and brown rot in peaches, caused by *Monilinia fructicola* (Fu et al., 2018). *C. fenestratum* and *P. betle* are economically and medicinally important plants in Southeast Asian countries, including Thailand (Rueangrit et al., 2019). Although the *P. betle* extract displayed good fungicidal activity against *A. brassicicola* in Chinese kale, its powder showed low efficacy in control against the disease in the current study. In addition, extracts from plants, such as *Piper* spp., had effective activity against many pathogens in other studies (Ounchokdee et al., 2016; Kokkrua et al., 2020); however, when applied in powder form, they showed low or moderate activity against black spot disease. This result may have been due to environmental effects, as the plant powder may suffer reduced concentration or activity due to the effects of rain, wind and sunshine (Dethoup et al., 2018; Kokkrua et al., 2020). The effects of plant powders showed greater fungicidal activity in controlling diseases as a protective activity rather than as a curative activity which was consistent with other studies (Dethoup et al., 2018; Rueangrit et al., 2019).

Plant powders had better fungicidal activity in controlling chili anthracnose disease than black spot disease on Chinese kale. However, their effects were not stable, even under laboratory conditions; thus, the effects of plant powders should be further evaluated to confirm their levels of activity.

In addition, the tested plant powders showed a lower level of activity against chili anthracnose disease than was the case with plant extracts, as found in another study on controlling plant diseases caused by the *Colletotrichum* species (Bhutia et al., 2016; Mokhtar et al., 2018). Recently, Janthong et al. (2021) revealed that the effects of *P. betle*, *P. retrofractum* and *P. griffithii* extracts had the highest reduction of disease severity of *C. capsici* and *C. gloeosporioides* on chili fruit and completely inhibited disease development on the leaves by inhibiting germination and mycelial growth. Mokhtar et al. (2018) reported the methanolic crude extracts of *Garcinia atroviridis* fruits effectively suppressed *C. capsici* growth, causing collapse and shrinkage of the spores and mycelia. Bhutia et al. (2016) reported that methanol extracted from *Zingiber officinale* effectively inhibited the mycelial growth of *C. musae*, the causal agent of banana anthracnose disease, by up to 88.89%, and reduced the disease's incidence on banana fruits. Bordoh et al. (2020) found that crude extracts of ginger, turmeric rhizome and "dukung anak" (a medicinal herb) had significant antifungal activity against *C. gloeosporioides* by inhibiting mycelial growth and conidial germination. The current authors found that all plant crude extracts at 15 g/L significantly controlled anthracnose disease in dragon fruit (Bordoh et al., 2020).

The current results indicated that the clove and turmeric powders, respectively, had potent fungicidal activity in controlling black spot in Chinese kale, caused by *A. brassicicola*, and anthracnose disease in chili fruit, caused by *C. truncatum*. Their application before pathogen infection is recommended because they showed more effective fungicidal activity in controlling these diseases through protective activity rather than curative activity. Both these medicinal plants have been used in folk medicine, indicating that their application may have low toxicity to humans. Overall, the current results supported the potential use of clove and turmeric powders in the development of plant-derived fungicides, with these powders being promising alternatives to the use of synthetic fungicides for the management of black spot on Chinese kale and anthracnose disease on chili fruit. It should be noted that these medicinal plant powders may stain plant leaves yellow when applied at rates of more than 25 g/L.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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