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Potency of indigenous plant extracts as botanical pesticide to control anthracnose of cayenne pepper by *Colletotrichum* sp.

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Abstract

Cayenne Pepper (*Capsicum frutescens* L.) is one of the most economically important vegetable crops in Indonesia. Among different biotic constraints, anthracnose disease is the major limiting factor affecting yield and production of chilli crop. This fruit rot disease might cause yield loss around 50% - 100%. Botanical pesticide is an economically and environmentally viable alternative for the disease control, and also reduces negative effects towards farmers and consumers. Extracts of Neem (*Azadirachta indica*), Gulinggang (*Senna Alata*), and mixture of turmeric rhizome (*Curcuma domestica*), Ginger (*Zingiber officinale*), Galangal (*Alpinia galanga*) were assessed in vitro and in vivo against *Colletotrichum sp.*in local cayenne pepper. *In vitro* test is conduct to observe the inhibition of test plant extracts against *Colletotrichum* sp. The fungi has been isolated from local cayenne pepper of the Hiyung variety with anthracnose. The inhibitory effect was assessed by mycelium growth assay or Poisoned food method. The effect of test plant extracts on disease intensity was observed *in vivo* using the capsicum fruit maceration method by observing the incidence of anthracnose in each treatment. Rhizome mixture (turmeric, ginger and galangal) which has the potential as a botanical pesticide. This is due to the in vitro test of the plant extract has ability to inhibit the growth of *Colletotrichum* sp by 51,38%. In the in vivo test, the mixture of rhizome extract was also effective in suppressing anthracnose disease in local cayenne pepper by 65.82% while the application of neem and gulinggang leaf extracts was less effective, with effectiveness values of only 59.38% and 43.04%, respectively.

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Introduction

Chili anthracnose is one of the most devastating fungal diseases affecting the quality and yield production of chili. This disease is caused by the fungus *Colletotrichum* sp which can attack cayenne pepper (*Capsicum frutescens*), large chili (*Capsicum annum*) and curly chili (*Capsicum annum var. longum*) which are widely grown in Indonesia. The fruit that is attacked becomes rotten so that there can be direct losses to the resulting product. Losses can reach 50% - 100% especially during the rainy season. (Hakim *et al.*, 2014). Extensive anthracnose causes pre- and post-harvest damage to chilies. Even small anthracnose lesions on chili fruits can reduce their marke**table** value.. Pathogens are becoming increasingly important because they can infect seeds that will be used as seedlings. (Soesanto, 2019; Silva *et al.*, 2019; Saxena *et al.*, 2016).

This anthracnose disease also attacks the hiyung variety in South Kalimantan (Budi and Mariana, 2016), and farmers generally control the disease with synthetic pesticides. The unwise use of synthetic pesticides can have a negative impact on both humans and the environment. The use of synthetic pesticides for disease control has become a necessity for chili cultivation in Indonesia, both in Sumatra (Yasid, 2010) Java (Andriani, 2017) and South Kalimantan (Mariana *et al.*, 2021). This situation encourages many researchers to use natural ingredients to control pests and plant diseases. Natural ingredients are not only environmentally friendly but also safe for humans as farmers and consumers. (Mandava 2018).

Thus far, the control of anthracnose in chili farmers has not been controlled well. At chili cultivation center in South Kalimantan, the incidence of disease is quite high, 45.59% in Hiyung Village and 57.54% in Marabahan District (Mariana *et al.*, 2021). Meanwhile, the use of chemical pesticides was still carried out. Farmers only rely on chemical pesticides to control anthracnose. The use of botanical pesticides is economically and environmentally friendly solution that are easily available to farmers. Organic farming is losing its relevance to make space for intensive agriculture. However, repeated use of certain chemical fungicides has led to the emergence of pesticide-resistant populations of plant pathogens (Kumar and Singh, 2012). The bioactive content is used as a botanical pesticide to control plant diseases. Propyl disulfide from neem extract (*Azadirachta indica*) was effective inhibiting the mycelial growth *C. gloeosporioides* and *C. acutatum* in mango (Khan *et al*, 2021). While Yendi *et al*. (2015) proved that in banana anthracnose, the application of extracts of ginger, turmeric and galangal could inhibit the growth and development of the fungus C. musae in vitro. Asikin (2013) stated that gulinggang leaves are able to suppress chili anthracnose attacks by about 5%-10% while at the same time the controlled samples are attacked by about 80%-100%.

These local plants are easy to obtain and have the prospect of being used as botanical pesticides, usually the chilies are consumed fresh without being processed. This study aimed to examine the potential of extracts of neem, gulinggang, and a rhizomes mixture of turmeric, ginger, and galangal in suppressing anthracnose (*Colletotrichum* sp.) in cayenne pepper.

Materials and methods

This research was an in vitro and in vivo study. It has been carried out from April 2020 to December 2020, in the phytopathology laboratory of the agricultural faculty of Lambung Mangkurat University. The research design used was a completely randomized design with five treatments and three replications. Five treatments were tested, namely neem leaf extract, Gulinggang leaf, rhizome mixture (turmeric, ginger, and galangal), benomyl (synthetic pesticide) (positive control) and sterile distilled water (negative control). The data were analyzed by analysis of variance and then followed by Fisher's LSD (Least Significant Diffirence) test using Minitab 19 software.

Preparation of plant extracts.

Fresh plants leaves, Neem, Gulinggang, rhizome mixture (turmeric + ginger + galangal) was collected from around the chili plantation and washed thoroughly in tap water. The test material were surface sterilized by washing with 70% ethanol for 1 min followed by three washings with sterile distilled water. The test material was ground with sterile water at the rate of 1 g plant material in 1 ml of water using a pestle and mortar and the macerate for 72 hrs was filtered through double muslin cloth to get the crude extract (Rajeswari *et al.*, 2019).

In-vitro Test of Fungal Growth Inhibition

Colletotricum sp. were isolated from anthracnose lesion on cayanne pepper planted in Tungkaran village, south Borneo, Indonesia. Pieces of lesion tissue were surface-disinfected with 70% ethanol for 1 minute, rinsed with sterilized distilled water, and then air-dried on a clean bench. Dried samples were placed on potato dextrose agar (PDA) amended with streptomycin 100 µg/ml and lactic acid 25 µg/ml and incubated at 250 C. After 5 days of incubation, growing mycelial tips were transcultured on new PDA medium (Lim *et al.*, 2002).

The method used to test the inhibition of fungal growth by the plant extracts tested was the Mycelial Growth Assay or Poison Food Technique (Sattar *et al.*, 2018; Su *et al.*, 2019). Procedur for the technique according to Pandey *et al.*, (2021) 10 ml of PDA media at a temperature of ± 40^{o}C was put into a Petri dish. Then mixed with 0.5 ml of extract for each treatment. Mixing is done with the Pour plate technique the molten media were poured into three sterilized glass petri plates (90 mm) considering each as a replication. After solidification of the agar plate's mycelial plugs (5 mm) from the edge of the 7-day-old colony on PDA were transferred to the medium containing plant extract. The media without the botanical extract served as check. The plates were incubated at 29±1°C till the complete growth was observed in control plates. According to Krutmuang *et al.* (2022) effect of botanical extract measuring with colony diameter. Measuring the colony diameter of the fungal on both the X and Y axis was done and then the average percent inhibition of radical growth (PIRG) calculated as follows (Fig. 1). Formula for calculating the percentage of inhibition of mycelial growth (PIRG) according to Krutmuang *et al.*(2022) : PIRG = (R1 – R2)x100 / R1 Where R1: Radius length of the fungal colony in control. R2: Radius length of the fungal colony in the experiment. Estimated inhibition values were as follows. > 75% Very high inhibitory effectiveness. 61 – 75%. High inhibitory efficiency. 51 – 60% Moderate inhibition. ≤ 50% Low inhibitory efficiency.

In vivo evaluation

Colletotrichum cultures and inoculum preparation. A conidial suspension of each *C. capsici* isolate was prepared from conidia that were harvested from 7-day-old cultures grown on potato dextrose agar under 12 h of darkness and 12 h of light at 28 to 30°C. The C. capsici culture plates were flooded with sterilized water and the conidia gently scraped from the culture plate using a sterilized loop. The conidial suspension was adjusted to a concentration of approximately 10^6 conidia/ml (Montri *et al.*, 2009).

Fruits at redripe stage (40 to 50 days after flowering) harvested from chili plantation were inoculated with *Colletotricum* sp isolate using a dipping method. After the calyxes were removed, the fruits were injured by stabbing it with a sterile syringe, and the fruit were surface sterilized with 1% (wt/vol) sodium hypochlorite for 5 min and then rinsed twice with distilled water.

The chilli fruits were first dipped for 2-3 min in spore suspension of *Colletotrichum* sp. and kept at room temperature for 1 hour for complete drying. Pathogen inoculated air-dried fruits were then dipped for 2-3 min in 0.2 per cent w/v of the extracts for 2 minutes each. Control fruits were dipped in sterile distilled water.

The inoculated fruit were placed in plastic boxes (20 by 30 by 10 cm) on a tray with 500 ml of water in the bottom and incubated at 28°C, 12 h of darkness and 12 h of light, and 100% relative humidity. After 3 days, lids were removed from the boxes to maintain 70 to 80% relative humidity. The effect of extract treatments on disease incidence and disease severity was evaluated daily for 5 days during storage at simulated marketing conditions (80-82 per cent R.H., 27,1ºC). (Bhutia *et al.*, 2016). Disease incidence data were expressed as the percentage of fruit showing anthracnose symptoms of the total number of fruit in each treatment. In this in vivo test, the intensity of the disease was observed which was calculated using formula for a Disease Severity Index (DSI) can be as follows: DSI (%) = ∑ (Class frequency × score of rating class)/ (Total number of observations) × (maximal disease index) × 100 % (Chiang *et al*, 2017), while disease severity was scored following a set of disease ratings of 0 to 9 was established based on the percent lesion size relative to the overall size of the fruit, appearance of infected tissue (necrotic or water soaked), and the presence of acervuli. A rating of 0 was no infection and 9 was infection greater than 25% (**Table** 1) (Montri *et al.*, 2009). The efficacy of fungicide was calculated according to Abbott`s formula: (degree of attack in untreated control - degree of attack in treated plot)/ degree of attack in untreated control) x 100%. (Jaloba *et al.*, 2019).

The treatments were arranged in a complete randomized block design with 30 fruits constituting a single replicate, and each treatment was replicated five times. The experiment was repeated twice.

Result

Inhibitory Effect of Plant Extract on fungal growth

The inhibition of the plant extracts tested against *Colletotrichum* sp was evaluated by the inhibitory effect. In this study, the highest inhibitory was the application of rhizome mixture (Ginger Turmeric and Galangal) (Figure 1) and **Table** 1.

Table 1. Anthracnose severity scores on chili fruits, and symptom description (Montri *et al.*, 2009).

score	Symptom details
0	No infection
1	1–2% of the fruit area shows necrotic lesion or a larger water-soaked lesion surrounding the infection site
3	>2–5% of the fruit area shows necrotic lesion, acervuli may be present, or water-soaked lesion up to 5% of the fruit Surface
5	>5–15% of the fruit area shows necrotic lesion, acervuli present, or water-soaked lesion up to 25% of the fruit surface
7	>15–25% of the fruit area shows necrotic lesion with acervuli
9	>25% of the fruit area shows necrosis, lesion often encircling the fruit; abundant acervuli

The ability of this rhizome is even higher, namely its inhibitory effect is 50.38% than the synthetic pesticide Benomil (30.04%). Meanwhile, Gulinggang and Neem have weaker inhibitory effect, which are 25.58% and 29.33%, respectively (**Table** 2).

Effect of Plant Extracts on the Intensity of Anthracnose Disease.

In vivo treatment of plant extracts on chillies showed that the mixture (Ginger Turmeric and Galangal) was able to suppress the intensity of anthracnose disease. This is significantly different from neem and gulinggang, even better than dyed with the synthetic pesticide/chemical Benomyl. At the time when the chili fruit that was not treated was attacked by disease up to an average of 87.78%, the fruit that was dyed with a mixture of rhizomes was only attacked by 30%. Thus, treatment with a mixture of rhizomes can suppress the intensity of anthracnose disease better than synthetic pesticides, both in vitro and in vivo.

The mixture of rhizomes has an effectiveness value of 65.82% so that it is included in the category of effectively resisting anthracnose attacks, while neem, benomyl and gulinggang are categorized as quite effective (**Table** 3.).

Tabel 2. In vitro efficacy of plant extracts in inhibition of Mycelial Growth of *Colletotrichum* sp.

No.	Plant extracts	Inhibition (%).	*Efficacy*
1	Neem	25.58 a	*Low inhibitory efficiency.*
2	Gulinggang	29.33b	*Low inhibitory efficiency.*
3	Benomyl	30.04 b	*Low inhibitory efficiency.*
4	Rhizome mixture (turmeric + ginger + galangal)	51.38c	*Moderate inhibition*

Remark : Values in the row with the same letter are not significantly different (P = 0.05) based on Least Significant Difference test level = 0.05.

The rhizome mixture able to inhibit the growth of *Colletotrichum* sp up to 50.38%. Which is significantly different from the synthetic pesticide benomyl which has an inhibitory effect of only 30.04%.

Discussion

In vitro, rhizome mixture has the highest inhibitory effect against mycelial growth of *Colletotrichum,* compared to benomyl (a chemical fungicide), gulinggang leaf, and neem leaf. This mixture has been used by farmers at several areas to control anthracnose in chili. The active ingredients of this rhizome have been shown to control plant pathogens.

In this study, neem and gulinggang leaves have not shown a high inhibitory effect on the *colletotrichum* isolates used, likewise benomyl pesticide, Inhibitory effect of benomyl was low, this may be due to the reduced sensitivity or increased resistance of *Colletotrichum* sp. In chili cultivation, the use of pesticides has been used intensively. Intensive use of pesticides results in the emergence of resistant strains of pathogens (Sumardiyono *et al.*, 1995). There may have been a change in the strain of the existing *colletotrichum.* It has long been reported that benomyl, which are a benzimidazole group, rank highest for the emergence of resistant strains (Dekker, 1977).

Tabel 3. Intensity of anthracnose on cayenne peppers fruits after dipping with plant extract.

No.	Perlakuan	% Disease intensity	Effectivity
1	Rhizome mixture (turmeric + ginger + galangal)	30.00^{a}	65.82 %
2	Neem	35.65^{b}	59.38 %
3	Benomyl	36.67^{b}	58.22 %
4	Gulinggang	50.00^{c}	43.04 %
5	Sterilized water (Control)	87.78^{d}	

Remark : Values in the row with the same letter are not significantly different (P = 0.05) based on Least Significant Difference test level = 0.05.

The intensive use of metalaxyl to control downy mildew in maize has resulted in resistant strains of *Peronosclerospora maydis.* Repeated use of metalaxyl fungicides on *P. palmivora* will result in resistant fungal strains (Sumardi yono *et al.*, 1995b).

In the chili cultivation, at the swamps land of South Kalimantan, local isolates of *Colletotrichum* were resistant to fungicides containing the active ingredient propineb 70%, while the fungicides containing the active ingredients azoxystrobin and diphenoconazole were still sensitive (Mariana *et al.*, 2021). The in vivo evidence that mixtures are more effective than Neem and Gulinggang. The ability of rhizome mixture (turmeric + ginger + galangal) is more effective in reducing disease intensity. In the interaction between plants and pathogens, several things that might happen are that the mixture of rhizomes contains active ingredients such as phenol, tannin and curcumin which can directly control the pathogen so that the intensity of anthracnose disease decreases. According to Otunala *et al.* (2010).

The tannin content in ginger is quite high, namely 3.54% while the alkaloids are 11.21%.. In white galangal reached 23.381%, in Turmeric (Malahayati *et al*., 2021) showed that the total phenol was (193.26 mg/kg) and curcuminoids (8.13 mg/L). Phenol Tannins and Curcumin have a role in the pathogenesis process. In Poplar (*Populus* sp) the phenol content in the form of salicylic acid, catechol and benzoic acid was significantly antifungal against *Botryosphaeria dothidea* which causes poplar cancer.

Fig. 1. In vitro evaluation of inhibitory effect of plant extracts on *Colletotrichum* sp.

These compounds can provide a signal for plant resistance so that plants become more resistant (Li *et al*., 2020). In resistant tissue, phenol and tannin were detected which can limit pathogen attack (Rioux *et al*., 2018). In resistant tissue, phenol and tannin were detected which can limit pathogen attack (Rioux *et al*., 2018). The results of Kim *et al* (2003) showed that hexane extract from turmeric at a concentration of 1000 mg/L was antifungal against *Rhizoctonia solani, Phytophthora infestans*, and *Erysiphe graminis*. In addition, ethyl acetate extract from turmeric also had an inhibitory effect on *R. solani*, *P. infestans*, *Puccinia recondita*, and *Botrytis cinerea* at a concentration of 1000 mg/L. The active substance curcumin in turmeric has antifungal activity at a concentration of 500 mg/L against *R. solani*, *Pu. recondita*, and *P. infestans*. Chowdhury *et al*, (2008) Curcumin and turmeric oil are antifungal against, *Fusarium solani* and *Helminthosporium oryzae*. Likewise, Turmeric Oil showed the most effective antifungal activity against *F. solani* and *H. oryzae* with IC50 of 19.73 and 12.7 g/mL, respectively.

Conclusions

Rhizome mixture (turmeric, ginger and galangal) which has the potential as a botanical pesticide. This is due to the in vitro test of the plant extract has ability to inhibit the growth of *Colletotrichum* sp by 51,38%. In the in vivo test, the mixture of rhizome extract was also effective in suppressing anthracnose disease in local cayenne pepper by 65.82% while the application of neem and gulinggang leaf extracts was less effective, with effectiveness values of only 59.38% and 43.04%, respectively.

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