J. Trop. Plant Pests Dis.
Vol. 24, No. 1, March 2024
Pages: 75–81

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.12475-81

RESEARCH PAPER

Antifungal evaluation of turmeric rhizome extract against *Colletotrichum capsici*, the causal agent of anthracnose on red-chili peppers (*Capsicum annuum L.*)

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Manuscript received: 1 March 2023. Revision accepted: 12 November 2023. Available online: 29 February 2024.

ABSTRACT

This study was conducted to evaluate the antifungal activity of the ethanolic extract of turmeric rhizome against *Colletotrichum capsici* Syd. and its efficacy in reducing anthracnose disease development on red chili peppers. The in vitro experiment aimed to determine the effect of the extract on inhibiting *C. capsici* growth in potato sucrose agar (PSA) medium incorporating the turmeric rhizome extract. The variables examined were mycelial growth, fungal sporulation, and spore germination. The in vivo experiment aimed to evaluate the effectiveness of the extract in reducing disease development on red chili peppers. The treatments were conducted using a completely randomized design with five concentration treatments and three replicates. The variables examined were radial mycelial growth zone diameter, fungal sporulation, spore germination, and disease intensity. Data were analyzed by analysis of variance, and the least significant difference was used for comparing means between treatments. The results of these experiments showed that ethanolic turmeric rhizome extract exhibited antifungal activity against *C. capsici* both in vitro and in vivo conditions. Turmeric rhizome extracts with concentrations of 1%, 2%, 3%, and 4% significantly inhibited mycelial growth, and even at a 4% concentration, *C. capsici* fungal colonies did not grow. Evaluation of the effect of the extract on fungal sporulation showed that inhibition occurred at a concentration of 3%, while inhibition of spore germination occurred at concentrations of 1%, 2%, and 3%. Application of turmeric extract at concentrations of 1%, 2%, 3%, and 4% effectively reduced the intensity of anthracnose on red chili.

Key words: Anthracnose, antifungal, *Capsicum annuum*, *Colletotrichum capsici*, turmeric extract

INTRODUCTION

Colletotrichum spp. is one of the most important plant pathogens, causing economically significant diseases in chili production in tropical and subtropical regions (Montri et al., 2009). Anthracnose of chili peppers, caused by *Colletotrichum* spp., is considered a major threat to chili production in Indonesia, leading to losses ranging from approximately 10% to 80% in the wet season and 2% to 35% in the dry season (Widodo, 2007). The pathogen spreads very rapidly during moist-humid conditions, leaving very little scope for farmers to protect the crop. Additionally, indiscriminate usage of pesticides has been increasing production costs for farmers and alarming environmental issues (Srideepthi et al., 2017).

In traditional agricultural systems, chemical pesticides are the primary means of controlling plant pests and diseases. Intensive application of chemical pesticides has been shown to have significant negative environmental impacts, potential human exposure to pesticides, and deposition of residues on agricultural products (Lee et al., 2007; Bakirci et al., 2014; Odukkathil & Vasudevan, 2016). To mitigate the adverse effects of synthetic pesticides, it is important to develop environmentally friendly control methods. One alternative to mitigate the adverse effects of synthetic fungicides used to control anthracnose disease in chili, such as benomyl, mancozeb and carbendazim, is to use botanical fungicides against anthracnose pathogens. The ultimate aim of recent research in this area has been the development of alternative control strategies to reduce dependency on synthetic fungicides. Plants have the ability to synthesize aromatic secondary metabolites, such as phenols, phenolic acids, quinones, flavones, flavonoids, tannins, and coumarins (Cowan, 1999).

Turmeric (*Curcuma longa* Linnaeus) is botanically related to the Zingiberaceae family. Turmeric is a spice obtained from the rhizome of *C. longa* that has multiple medicinal properties, including antibacterial and antifungal effects (Liu et al., 2018). Two compounds isolated from the ethanolic *C. longa* extract, ar-turmerone and 8-hydroxyl-ar-

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turmerone, have both been found to have insecticidal effects on *Culex pipiens* (Liu et al., 2018). Dried powder was extracted with ethanol and yielded an extract composed of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. These biochemical compounds have antifungal effects (Wuthi-udomlert et al., 2020). Kim et al. (2003) reported that curcumin showed in vivo antifungal activity against *Phytophthora infestans*, *Rhizoctonia solani*, and *Puccinia recondita*.

The objective of this study was to explore the in vitro antifungal potential of ethanol turmeric rhizome extracts against *Colletotrichum capsici* Syd. and the in vivo antifungal efficacy of the extract in the development of red pepper anthracnose disease caused by *C. capsici*.

MATERIALS AND METHODS

Research Site. The experiments were conducted at the Agricultural Biotechnology Laboratory, Faculty of Agriculture, Universitas Lampung. The research started from February to Mei 2022.

Fungal isolation and culture. The *C. capsici* D623 (hereafter referred to as *C. capsici*) was isolated from field samples of red chili peppers with anthracnose symptoms, and pure cultures were used in these studies. The isolates were grown on solid potato sucrose agar (PSA) medium (200 g of potato, 20 g agar, 20 g sucrose, 1000 mL distilled water). All cultures were incubated at room temperature on PSA during activity assays.

Preparation of ethanol turmeric rhizome extract. Turmeric rhizomes were collected from the field, washed, dried, and cut into small pieces (1–2 cm). The small pieces of turmeric rhizome were dried using an electrical oven (Innotech, USA) at 50 ºC for five days. The rhizomes were mechanically powdered and macerated in ethanol (90%) at a ratio of 1:5 (v/v), then incubated at room temperature for 48 hours. The suspension was filtered through a membrane filter (Qualitative Filter Paper, China), and the filtrate was evaporated using a rotary evaporator (IKA RV 10 digital, Germany) at 100 rpm and 40 ºC to obtain pure extracts. The extract was stored at 5 ºC before being used in this experiment (Karmila et al., 2017).

Effect of the turmeric extract on fungal mycelial growth inhibition. The inhibitory effect of the turmeric extract on its antifungal potentials against *C. capsici* was assessed using the poisoned food technique (Nene & Thapliyal, 1993) with five treatments and three replicates. PSA was utilized as a basal medium for the growth of fungi. The PSA media incorporating the turmeric extract at concentrations of 0%, 1%, 2%, 3%, and 4% were inoculated at the center with *C. capsici* propagules (mycelial plug with a diameter of 0.5 mm). Three replicates of inoculated media for each concentration were incubated at room temperature for the entire duration of the test. The variables examined were the radial mycelial growth zones for each treatment concentration measured at 6, 8, 10, 12, and 14 days after the incubation period. Radial mycelial growth was recorded as the diameter of the colony using the following formula:

$$CD = \frac{d_1 + d_2}{2}$$

CD = Colony diameter (cm);
d_1 = Horizontal diameter of colony (cm);
d_2 = Vertical diameter of colony (cm).

Effect of turmeric rhizome extract on the fungal sporulation. The effect of ethanol turmeric extract against *C. capsici* sporulation was determined by growing the fungus on PSA media in the absence (control) and presence (treatments) of the turmeric extract. The media were inoculated with a single culture at the center of the plates. For this purpose, the fungi had been previously cultured in PDA (200 g potato, 20 g agar, 20 g dextrose, 1000 mL distillate water) in Petri dishes and subsequently incubated at room temperature for 14 days. Each treatment was replicated on three plates and were used for sporulation measurements. Three plate agar discs were aseptically removed from the central, intermediate, and peripheral zones of each plate using a cork borer and transferred to tube contain 90 mL sterile distillate water. The spores were estimated by counting in a Neubauer's hemocytometer in 1 mL sample using binocular microscope (Leica, Switzerland) with 400× of magnification. Total spore production was analyzed using formula Gabriel & Riyatno (1989):

$$C = \frac{t}{n \times 0.25} \times 10^6$$

C = Spore density (spores/mL);
t = Number of total spores in each sample observed;
n = Number of samples observed (5 big square × 16 small square;
0.25 = The correction factor for using small-scale square on a haemocytometer;
10^6 = Constanta.

Effect of turmeric rhizome extract on spore viability. The spore germination assay was used to evaluate the effect of different extract concentrations on spore germination of *C. capsici*. Spore viability was determined by measuring the rate of spore germination. Extracts with different concentrations (1%, 2%, 3%, and 4% v/v) were prepared in sterilized distilled water. One drop of *C. capsici* spore suspension (25 µL) was then transferred to PSA media and incubated at room temperature for 14 hours. A spore was considered germinated if the length of the germ tube was at least twice the diameter of the spore. Germinated spores were counted using a binocular microscope (Leica, Switzerland) at 400× magnification. The data were expressed as percent spore germination inhibition, calculated according to the following formula:

$$Sg\ (\%) = \frac{Gs}{So} \times 100\%$$

Sg = Spore germination;
Gs = Germination spore;
So = Total spores observed.

Effect of turmeric rhizome extract on anthracnose intensity. The red chili peppers, Lembang 1 variety, were sterilized with ethanol 70% and air dried in room temperature. The turmeric rhizome extract at five concentrations (0, 1, 2, 3, and 4% v/v) was sprayed onto the red chili peppers. Inoculum of *C. capsici* was collected as spores from fungal cultures and suspended in sterilized water. Red chillies were then inoculated by spraying with a solution containing *C. capsici* conidia at a density of 1.25×10^6 conidia/mL and 0.02% Tween 80% (v/v). The inoculated red chili peppers were incubated at room temperature for six days, and the variable observed was the intensity of disease symptoms on the red chili peppers. The score levels of symptoms for assessing the disease intensity caused by *C. capsici* can be seen in Table 1. The disease intensity was calculated using the formula:

$$DI = \frac{\sum (n \times v)}{N \times V} \times 100\%$$

DI = Disease intensity (%);
Σ = Summation;
n = Number of samples in each score level of disease symptoms;
v = Score levels of disease symptoms;
N = Total number of samples examined;
V = The highest score levels of disease symptoms.

Data analysis. The collected data of mycelial growth, fungal sporulation, spore viability, and disease intensity were analysed by analysis of variance (ANOVA). The calculated value of F was compared with the tabulated values at 5% level of significance for an appropriate degree of freedom. The comparisons of means between treatments were determined according to the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

In vitro antifungal activity. The antifungal activities of turmeric rhizome extracted by ethanol were determined against *C. capsici* in laboratory experiments. The results showed that ethanol turmeric rhizome extract at concentrations of 1%, 2%, 3%, and 4% in PSA medium significantly inhibited the mycelial growth of *C. capsici*. The strongest inhibitory activity of the extract on fungal growth was observed at a concentration of 4%, with no visible fungal mycelial growth observed at this concentration (Table 2; Figure 1).

Table 1. The score level of disease symptoms

Score levels	Symptom description of *Colletotrichum capsici* infection on red chili peppers
0	No symptom;
1	1–10 % of the fruit area shows necrotic lesion or a larger water-soaked lesion surrounding the infection site;
2	10–25 % of the fruit area shows necrotic lesion, acervuli may be present, or water-soaked lesion up to 5 % of the fruit surface of fruit surface;
3	25–50% of the fruit area shows necrotic lesion, acervuli present, or water-soaked lesion up to 25 % of the fruit surface of fruit surface;
4	More than 50 % of the fruit area shows necrosis, lesion often encircling the fruit; abundant acervuli.

Means with the same letter in a colum are not significantly different (p= 0.05) using LSD (least significant difference); dai: days after inocubation.

The results of the experiment showed that the turmeric rhizome extract was most effective in restricting the sporulation and spore germination of *C. capsici*. The experiments demonstrated that the turmeric extract significantly inhibited *C. capsici* sporulation at a 3% concentration and also significantly inhibited spore germination at concentrations of 1%, 2%, and 3% (Table 3; Figure 1).

In vivo antifungal activity. The ethanolic extract of turmeric rhizome, which has inhibitory effects on *C. capsici*, was subjected to in vivo testing to evaluate its efficacy in inhibiting disease development on red chili peppers. The results indicated that the antifungal activity of the turmeric rhizome extract significantly reduced disease intensity caused by *C. capsici* on red chili peppers. The turmeric rhizome extract showed a significantly reduced disease intensity compared to control treatments. Depending on the extract concentrations, the reduction in disease intensity caused by *C. capsici* infection was 8.67% at a 4% concentration compared to the treatment control of 95.67% (Table 4, Figure 2).

These experiments found that the ethanol turmeric rhizome extract has antifungal activities in both in vitro and in vivo experiments. The extract significantly inhibited the mycelial growth, fungal sporulation, and spore germination of *C. capsici* under in vitro conditions. Additionally, the extract significantly reduced disease intensity on red chili peppers under in vivo conditions. A similar study performed by Cho et al. (2006) also indicated that curcuminoids of turmeric rhizome extract effectively inhibited spore germination and mycelial growth of three red pepper anthracnose pathogens, *C. coccodes*, *C. gloeosporioides*, and *C. acutatum.*

Plant extracts from organic solvents have been reported to provide more consistent antimicrobial activity compared to water extracts. Gurjar et al. (2012) reported that the ethanolic extract of turmeric contains curcumin compounds belonging to the terpenoid class, which exhibit antifungal activity against fungi, bacteria, and protozoa. Water extracts contain soluble flavonoids (mostly anthocyanins) that have no antimicrobial significance, and water-soluble phenolics are only important as antioxidant compounds (Parekh et al., 2005). It has been reported that the *C. longa* extract has inhibitory effects on over twenty pathogenic fungi, including *Aspergillus flavus* (Hu et al., 2017), *Curvularia pallescens, Colletotrichum falcatum, Aspergillus niger, Aspergillus terreus, Fusarium oxysporum,* and *Fusarium moniliforme*

Table 2. Inhibition of the mycelial growth of *Colletotrichum capsici* on potato sucrose agar media containing ethanolic extract of turmeric rhizome

Extract concentration (%)	Radial mycelial growth diameter (cm)				
	6 dai	8 dai	10 dai	12 dai	14 dai
0	4.43 a	6.20 a	6.90 a	7.30 a	7.60 a
1	2.20 b	3.50 b	4.57 b	5.70 b	6.33 b
2	1.62 c	2.53 c	3.25 c	3.73 c	4.63 c
3	1.50 c	2.45 c	3.40 c	4.17 c	5.20 bc
4	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d
LSD	0.41	0.56	0.70	1.16	1.19

Means with the same letter in a colum are not significantly different (p= 0.05) based on the LSD (least significant difference) test; dai: days after inoculation.

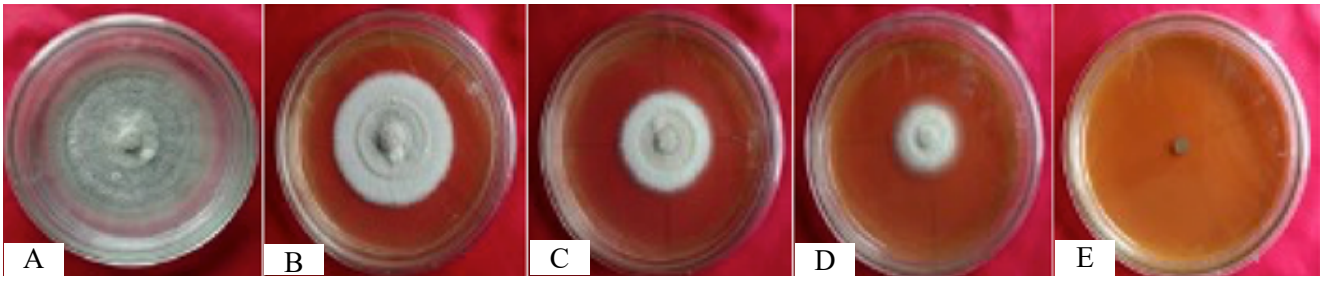

Figure 1. A representative mycelial growth zone on PSA medium containing turmeric rhizome extract at six days after incubation. The growth of mycelium became increasingly inhibited with higher concentrations of the extract. A. 0%; B. 1%; C. 2%; D. 3%; E. 4%.

(Singh et al., 2002). These reports indicate that the rhizome extract of *C. longa* exhibits a broad-spectrum antifungal effect. The ethanol extract of turmeric rhizome inhibited the growth of *C. gloeoporiodes* and anthracnose development on mango fruits (Alvindia et al., 2022).

Several studies have been conducted to determine secondary metabolites in turmeric rhizome extract with antimicrobial activities. Chen et al. (2018) reported on the mechanism by which turmeric extract disrupts the synthesis of critical proteins and enzymes, ultimately inhibiting fungal growth. The antifungal activity was found to be related to the disruption of fungal cell membrane systems, specifically the inhibition of ergosterol synthesis and the respiratory chain. Eight chemical constituents of *C. longa*, including curdione, isocurcumenol, curcumenol, curzerene, β-elemene, curcumin, germacrone, and curcumol, were all verified to exhibit inhibitory effects on the mycelial growth of *F. graminearum* (Chen et al., 2018). In vitro studies showed that alkaloids, flavonoids, terpenoids, and tannins contained in the *C. longa* has a strong fungicidal effect on *Sclerotium rolfsii*, the causal agent of damping off in soybeans (Fauzi & Sari, 2022). Hu et al. (2017) reported that the inhibitory effect of *C. longa* on *Aspergillus flavus* is involved in its ability to disrupt the integrity of the plasma membrane and induce mitochondrial dysfunction, leading to metabolic stagnation. Further studies need to be undertaken to isolate the active compounds from turmeric rhizome

Table 3. Inhibition of fungal sporulation and spore germination of *Colletotrichum capsici* on potato sucrose agar media containing ethanolic extract of turmeric rhizome

Extract concentration (%)	Sporulation (× 10^6 spores/mL)	Sporulation (%)
0	5.30 a	73.30 a
1	5.00 a	50.60 b
2	4.60 a	47.20 b
3	2.87 b	42.70 b
4	0.00 c	0.00 c
LSD	2.30	10.62

Means with the same letter in a colum are not significantly different (p= 0.05) based on the LSD (Least Significant Difference) test.

Table 4. The effect of turmeric rhizome extract on disease intensity was observed at six days after inoculation

Extract concentration (%)	Disease intensity (%)
0	95.67 a
1	25.67 b
2	20.00 b
3	12.00 b
4	8.67 b
LSD	45.24

Means with the same letter in a colum are not significantly different (p= 0.05) based on the LSD (Least Significant Difference) test.

Figure 2. Representative anthracnose symptoms on red chili peppers sprayed with turmeric extract, observed six days after application. Disease severity decreases with increasing concentration of the extract. The arrow indicates the symptom of the disease. A. 0%; B. 1%; C. 2%; D. 3%; E. 4%.

extracts with fungicidal potential especieally againts *C. capsici*.

CONCLUSION

The ethanolic extract of turmeric rhizome has potential antifungal activity against *C. capsici*, the causal agent of anthracnose in red chili peppers, under both in vitro and in vivo conditions. The extract effectively inhibited the mycelial growth, spore production, and spore germination of *C. capsici*. Additionally, the extract effectively reduced disease intensity on red chili peppers.

ACKNOWLEDGMENTS

Acknowledgments are extended to all those who assisted in this research. We extend our gratitude to the head and staff of the Agricultural Biotechnology Laboratory, Faculty of Agriculture, The University of Lampung, for providing the laboratory facilities used in this research.

FUNDING

Not applicable.

AUTHORS' CONTRIBUTIONS

HMA considered and planned the experiment. DA carried out the isolation and pathogenicity test. HMA, LW, JP, RS prepared the manuscript. The authors provided response and comments on the research flow, data analysis and interpretation as well as shape of the manuscript. All the authors have read and approved the final manuscript.

COMPETING INTEREST

The authors declare that they have no financial or non-financial competing interest in the publication of this article.

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