# Antifungal Activity of Leaf Extract of Ficus Septica Against Colletotrichum Acutatum the Cause of Anthracnose Disease on Chili Pepper

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## Abstract

Antifungal activity of the leaf extract of *Ficus septica* Burm.F. was done against *Colletotrichum acutatum*. the cause of anthracnose disease on chili pepper. The *in vitro* experiment was conducted on potato dextrose medium (PDA) and potato dextrose broth (PDB) medium. Fifteen levels of extract concentrations, ranged from 0.1 to 5% (w/v) were tested for determining the minimum inhibitory concentration (MIC) on PDA. Six levels of extract concentration (w/v) viz. 0%, 1%, 2%, 3%, 4% and 5% were tested using completely randomized design with four replications. The antifungal activity was determined based on the effects of plant extract to the fungal growth, spore's formation, spore's germination and total biomass. Results of this study showed that the crude extract of *F. septica* obviously inhibited the growth of *C. acutatum* on PDA with MIC by 0.9% (w/v). Treatment with leaf extract of *F. septica* significantly (P<0.05) inhibited the fungal growth, spore's formation, spore's germination, and fungal biomass were respectively ranged from 29.72 to 81.39%, 63.27 to 99.11%, 61.70 to 100% and 38.52 to 99.91%. This result suggested that the leaf extract of *F. séptica* contains the antifungal subtances that responsable for the antifungal activity, and this extract can be considered as one of alternative measures to control the anthracnose disease on chili pepper.

Keywords : anthracnose disease, antifungal activity, Colletotrchum spp., Ficus septica

## 1. Introduction

Anthracnose disease is one of important devastating diseases on chilli pepper and may cause remarkable yield losses. The disease is caused by *Colletotrichum* spp. The most common cause of anthracnose disease in Indonesia is *Colletotrichum capsici* and *Colletotrichum gloeosporioides* (Suryaningsih *et al.*, 1996), while the anthracnose disease in Bali Island is mostly caused by *Colletotrichum acutatum* (Sudiarta, 2012). The disease is started from the inoculation of fungal propagules to the chili pepper, and when the environmental condition is suitable for further development of the disease, then would be followed by penetration, infection, colonization, incubation and dissemination (Sinaga, 2006). The disease causes yield losses through the decrease of the number of marketable chili pepper fruits. In general, the yield losses resulted from anthracnose disease may reach 50% or even more (Semangun, 2004).

To control the anthracnose disease, the farmers are mainly rely on the use of synthetic chemical fungicides, however improper use of synthetic chemical fungicides over long period may cause resistance of fungus to synthetic fungicide, environmental hazard as well as consumer's hazard (Soesanto, 2013). The fungicide residue may flows into water bodies and accumulated on agricultural produces that in turn may cause the hazard against human and other organisms (Sa'id, 1994). Based on this consideration, it is necessary to find an alternative measure to control anthracnose disease on chili pepper which is environmentally friendly by using plant extracts from the higher plants. There are many higher plants have been proven to contain chemical compounds to protect the plants from animal attack and microorganisms infection. These compounds can be used as sources of botanical pesticides to control plant pests and diseases (Nurmansyah, 1997; Suprapta and Khalimi, 2012; Dixit et al., 1995, Suprapta et al., 2005; Nwachukwu and Osuji, 2008). Leaf extract of Ageratum conyzoides inhibited the mycelial growth of Penicillium italicum the cause of blue mold rot on citrus cultivar Mandarin (Dixit et al., 1995). Leaf extract of Cassia alata and Dennetia tripetala showed antifungal activity against Sclerotium rolfsii the cause of the rot disease on Cocoyam during storage (Nwachukwu and Osuji, 2008). Leaf extracts of Albizia saman, Syzygium aromaticum and Piper betle effectively suppressed the growth of Fusarium oxysporum f.sp.capsici the cause of Fusarium wilt on chili pepper (Suprapta dan Khalimi, 2012). Suprapta et al. (2005) proved that combination of leaf extract of Piper betle and rhizome of Alpinia galanga effectively suppressed the development of banana wilt disease on banana seedlings caused by Fusarium oxysporum and Ralstonia solanacearum.

Ficus septica Burm.f. is a plant belong to the family Moraceae which is commonly grown wildly on the

bare land and in the forest in Bali Island. The leaf of this plant is often used as traditional medicine such as to cure foxes, mechanical injuries, and to neutralize the poison resulted from poisonous animals. In addition, this plant is also a potent anti-cancer and anti-imflamation (Lansky *et al.*, 2008). The leaf, fruit and root of *F. septica* contains alkaloid, saponin, flavonoid, tannin dan polyphenol (de Padua *et al.*, 1999). These compounds probably related to the bioactivity of the leaf of *F. séptica*.

This study was done in order to test the antifungal activity of the leaf extract of *F. séptica* against *Colletotrichum* acutatum the cause of anthracnose disease on chili pepper (*Capsicum annuum* L.).

## 2. Materials and Methods

#### 2.1. Plant extract preparation

The leaves (number four or five from the tip) of *Ficus séptica* was collected from the field in Ungasan Village, Badung Regency, Bali Indonesia. The leaves were washed in tap water to remove the surface contamination and then chopped off into small pieces using Sharp knife. This material was air dried for three days under room temperature and then extracted with pro analysis grade metanol. One hundred gram of dried leaf was soaked in 1.000 ml metahol and put in the dark under room temperature for 72 h. The filtration was done using Whatman No.1 filter paper to get the filtrate. This procedure was repeated three times, and the filtrates were combined and evaporated in a vaccum rotary evaporator (Iwaki, Tokyo Japan) on water bath at water temperature 40°C to get the crude extract. This crude extract was used for further test.

#### 2.2. Determination of minimum inhibitory concentration (MIC)

The fungus of *Colletotrichum acutatum* was isolated from chili paper fruit with anthracnose disease symptom and maintained in the Laboratory of Biopesticide Faculty of Agriculture Udayana University. The fungus was recultured on PDA médium to allow it to produce mycelia and spores. The propagules (mycelia and spores) were harvested in sterile distilled water and then this suspension (200 l) were spread on melted PDA médium in a laminar flow cabinet. Two diffusion wells were made on PDA using cork borer (5 mm diam.) in which 20 l of crude extract of *F. séptica* was put using micro pipette at concentrations of 0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5% (w/v). Five Petri dishes were prepared for each concentration. The cultures were incubated for 48 h in the dark under room temperature ( $28 \pm 2^{\circ}$ C). The formation of inhibition zone around the diffusion well was observed and was used to determine the antifungal activity. The lowest concentration in which the extract of *F. septica* produced inhibition zone is considered as minimum inhibitory concentration (MIC).

#### 2.3. Determination of antifungal activity of extract against radial growth

The method developed by Astiti and Suprapta (2012) was used to measure the effect of extract of *F. septica* to the radial growth of *C. acutatum*. Various concentrations of leaf extract of *F. septica viz.* 0%, 0%, 1%; 2%; 3%; 4% and 5% (w/v) were put in Petri dishes and added with 10 ml melted PDA medium. The Petri dishes were shaken gently to allow the extract to distribute evenly in the medium. A mycelial plug (5 mm diam.) of *C. acutatum* taken from the edge of a 5-day old culture was put in the center of PDA. Five Petri dishes were prepared for each concentration. The culture were incubated for seven days in the dark under room temperature. The diameter of fungal colony was measured daily. The inhibitory activity to the radial growth (IR) was determined according to the following formula (Pinto *et al.*, 1998).

$$IR (\%) = \frac{dc - dt}{dc} \times 100$$

where

IR = inhibitory activity to the radial growth dc = average increase in mycelia growth in control plates dt = average increase in mycelia growth in treated plates.

## 2.4. Determination of antifungal activity against spore's formation

Spores of *C. acutatum* were harvested in sterile distilled water from a culture maintained in a slant PDA. To obtain the spores, the suspension was sieved using a filter paper (Whatman No.2). A 100 l spore suspension  $(10^5 \text{ spores/ml})$  was added into 10 ml potato dextrose broth in test tubes containing various concentrations of extract of *F. septica viz.* 0%, 1%, 2%, 3%, 4% and 5% (w/v). The cultures were maintained in the dark under room temperature (28±2°C) for five days. The number of spore was counted using hemocytometer under light microscope. The inhibitory activity on spore's formation was calculated according to the following formula (Astiti and Suprapta, 2012) :

$$IS (\%) = \frac{dc - dt}{dc} x \ 100$$

where

IS = inhibitory activity against spore's formation dc = spore's density on control (without extract treatment) dt = spore's density with extract treatment.

#### 2.5. Determination of antifungal activity against spore's germination

Spores of *C. acutatum* were harvested from cultures maintained on a slant PDA using sterile distilled water. The suspension was sieved through Whatman No. 2 filter paper to separate the spores and mycelia or hypae. A 100 1 spore suspension (10<sup>5</sup> spores/ml) was inoculated into 10 ml PDB medium in test tubes with various concentrations of extract *viz*. 0%, 1%, 2%, 3%, 4% and 5% (w/v). Five tubes were prepared for each concentration. The cultures were incubated in the dark under room temperature (28±2°C) for 12 h. The number of germinated spores were observed and calculated under light microscope using hemocytometer. Inhibitory activity to the spore germination was determined according to the following formula :

$$IG (\%) = \frac{GC - GT}{GC} \times 100$$

where

IG = inhibitory activity to the spore germination

GC = germinated spores on control

WT = germinated spores on treatment with extract.

## 2.6. Determination of antifungal activity against fungal biomass

A 100 ml potato dextrose broth (PDB) medium was placed in 200-ml Erlenmeyer flasks and various concentrations of extract of *F. septica viz.* 0%, 1%, 2%, 3%, 4% and 5% (w/v) were added into the flask. The medium was inoculated with 1 ml of spore suspension ( $10^5$  spores/ml). The final volume of the culture was 100 ml. Five flasks were prepared for each concentration. The culture were incubated in the dark under room temperature ( $28\pm2^{\circ}$ C) for 8 days. The biomass was harvested through centrifugation at 5,000 rpm for 5 minutes. The biomass was taken and placed on glass filter paper and dried in an oven at 60°C until constant weight. The inhibitory activity to the fungal biomass was calculated according the following formula (Astiti and Suprapta, 2012) :

$$IB (\%) = \frac{WC - WT}{WC} \times 100$$

where

IB = inhibitory activity to the fungal biomassWC = dry weight of biomass on controlWT = dry weight of biomass with extract treatment.

## 3. Results and Discussion

Results of this study showed that the crude extract of *P. septica* effectively inhibited the growth of *Colletotrichum acutatum*. on PDA médium. The diameter of inhibition zone around diffusion well was 30 mm as presented in Fig. 1. According to Ardiansyah (2005) when the diameter of inhibition zone around diffusion well  $\geq 20$  mm indicated that the inhibitory activity is very strong, between 10-20 mm is strong, between 5-10 mm is intermediate, and  $\leq 5$  mm indicated the inhibitory activity is weak. Base don this standard, the inhibitory activity of leaf extract of F. séptica is strong with the mínimum inhibitory concentration (MIC) by 0.9% (w/v).

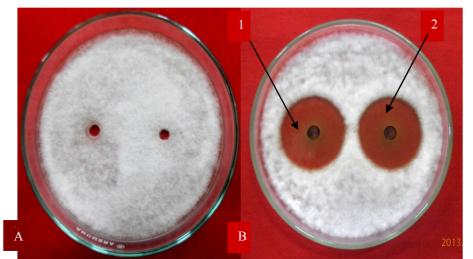


Figure 1. Inhibitory activity of the leaf extract of *F. séptica* against *Colletotricum* spp. on PDA medium

A = Control, B = Treatment with extract, 1 = Diffusion well,

2 = Inhibition zone

This extract significantly (P<0.05) inhibited the growth of colony on PDA, spore's density, germinated spores and biomass of *Colletotrichum* spp. as shown in Table 1. In general, the higher the concentration of extract between 1 to 5% (w/v) the higher the inhibitory activity against all parameters. The inhibitory activity of the extract to the fungal radial growth indicated by the diameter of colony was in the range between 29.72% to 81.39% when compared to control. Treatment with leaf extract of *F. séptica* significantly (P <0.05) inhibited the spore's formation indicated by the spore's density on cultural medium. The inhibitory activity ranged from 63.21% for extract treatment at concentration of 1% (w/v) to 99.11% for extract treatment at concentration of 5% (w/v). Spore's germination was also significantly (P<0.05) suppressed by the leaf extract treatment with the inhibitory activity ranged from 61.70% to 100%. The fungal biomass was also significantly (P<0.05) reduced by the leaf extract treatment with the inhibitory activity ranged from 39.53% to 99.91%.

Several researchers successfully proven the inhibitory activities of crude extracts of higher plants against fungal pathogens of several crops (Doughari and Obidah, 2008; Astiti and Suprapta, 2012; Bajwa *et al.*, 2004; Okigbo dan Ogbonnaya, 2006; Damu *et al.*, 2005). Doughari and Obidah (2008) reported that, the metanol extract of stem of *Leptadenia lancifolia* showed antifungal activity against two fungi *viz. Crytococcus neoformans* and *Candida albicans* with the diameters of inhibition zones by 30 mm and 28 mm respectively. According to Astiti and Suprapta (2012), Leaf extract of teak (*Tectona grandis*) at concentration of 1% (w/v) inhibited the gorwth of several fungi the cause of Wood decay such as *Nigrospora* sp., *P. ctrinum, A. flavus, A. phaeospermum* and *A. butyri* with the inhibitory activity respectively by 95.76%., 63.12%, 90.59%., 92.0% and 77.43% (Astiti, 2012).

Extract concentration (%,w/v)	Diameter of colony (mm)	Spore's density (spores/ml x 10 <sup>5</sup> )	Germinated spores (spores/ml x 10 <sup>4</sup> )	Biomass (g/100 ml)
0	90,00a*	11,38a	6,76a	0,86a
1	63.25b (29.72)	4.19b (63.21)	2.59b (61.70)	0.52b (39.53)
2	55.00c (38.89)	2.77c (75.61)	1.66c (75.46)	0.41c (52.33)
3	47.75d (46.95)	0.91d (91.96)	0.56d (91.74)	0.33d (61.63)
4	38.50e (57.23)	0.33e (97.14)	0.09e (99.08)	0.12e (86.05)
5	17.00f (81.39)	0.10f (99.11)	0.00e (100)	0.07f (99.91)

 Table 1. Inhibitory activity of the leaf extract of F. séptica against diameter of colony on PDA, spore's density, germinated spores and biomass of Collectorichum acutatum

\*Means followed by the same letters in the same column are not significantly different according to the Duncan's Multiple Range Test at 5% level.

\*\*Values in the parenthesis indicate the percentage of inhibitiory activity when compared to control Silva *et al.* (2008) reported that, the leaf extract of *Origanum majorana* L. could inhibit dpore's germination of *Colletotrichum gloeosporioides* Penz with the inhibitory activity by 96%. According to Bajwa *et al.* (2004) allelopathy counpound in the extract of *Parthenium hysterophorus* could reduce the biomass of *Drechslera hawaiiensis, Altenaria alternata* and *Fusarium monilifrome* at concentration of 10, 20, 30 and 50% respectively. Okigbo dan Ogbonnaya (2006) reported that the etanol extracts of the leaves of *Ocimum gratissimum* and *Aframomum melegueta* were more effective to inhibit the spore's germination and micelial formation of *Aspergillus niger, A. flavus, Fusarium oxsporium, Rhizopus stolonifer, Botryodiplodia theobromae* and jamur *Penicillium chrysogenum* when compared to the hot water extracts or cold water extract.

In the present study we proved that the leaf extract of *F. sptica* significantly could inhibit the growth of *Colletotrichum* spp. This is probably due to the presence of the antifungal substances in the extract. According to Damu *et al.* (2005), the stem extract of *F. séptica* contained the compounds of alkaloid group phenanthroindolizidine that consisting of ficuseptines B-D (1-3), 10R,13aR-tylophorine *N*-oxide (4), 10R,13aR-ylocrebrine *N*-oxide (5), 10S,13aR-tylocrebrine *N*-oxide (6), 10S,13aR-isotylocrebrine *N*-oxide (7), and 10S,13aSisotylocrebrine *N*-oxide (8). Those alkaloid compounds showed citotoxic activity. According to Baumgartner *et al.* (1990) a fraction of metanol leaf extract of *F. sptica* contains 2 indolizidine alkaloid *viz.* ficuseptine, 4,6-bis-(4-methoxyphenyl)-1,2,3-trihydroindolizidinium chloride and antofine. Both of these compounds showed antifungal and antibacterial activity.

Castillo *et al.* (2012) reported that *F. séptica* contaning activive compounds, antofine and ficuseptine. Antofine potentially act as anti-cancer compound, while ficuseptine act as antifungal and antibacterial agent. Leaf, fruit and root of *F. sptica* contain alkaloid, saponin and flavonoid that act as antimicrobial compound (Nugroho *et al.*, 2011). Nogodula *et al.*(2012) reported that the crude extract of *F. séptica* could inhibit the growth of *Canida albican* with diameter of inhibition zone of  $16.67 \pm 5.38$  mm. The crude extract of *F. séptica* could inhibit the growth of *Staphylococcus aureus*, *Canida albican* and *Escheria coli* with the inhibition zone respectively  $13,83 \pm 4,01$  mm,  $17.67 \pm 1.53$  mm and  $13.00 \pm 1.00$  mm (Vital *et al.*, 2010). Sukadana (2005) reported that the etanol extract of the root of *F. séptica* could inhibit the growth of *Eschericea coli*.

Several plants belong to the family Moraceae produce antimicrobial compounds such as *F. septica*, *F. syocomorus*, *F. benjamina*, *F. religiosa*, *F. racemosa*, *F. pumila*, *F. vasta*, *F. thonningii*, *F. capensis* and *F. bengalensis*. Ethyl acetate leaf extract of *F. bengalensis* at concentration of 100  $\mu$ g/disc could inhibit *Bacillus subtilis* and *Salmonella typhi* with dimater of inhibition zone by 12 mm. In addition, this extract could inhibit the growth of *Colletotrichum* spp. and *Fusarium* spp. with diameter of inhibition zone by 8 mm and 14 mm respectively (Hossain and Shahadat, 2014).

#### 4. Conclusion

The crude extract of *F. séptica* showed a strong inhibitory activity against C. acutatum with MIC by 0.9% (w/v). Treatment with the leaf extract of *F. séptica* at concentrations of 1 to 5% (w/v) under *in vitro* test significantly suppressed the growth of *Colletotrichum* spp, the cause of anthracnose disease on chili pepper. This treatment effectively reduced the fungal radial growth on PDA, spore's formation and biomass formation on PD broth médium with the inhibitory activity ranged from 29.72% to 100%. This result suggested that the leaf extract of *F. séptica* contains antifungal substances responsable for the antifungal acivity against *C. acutatum* and can be considered as one of alternative tools to control anthracnose disease on chili pepper.

## Acknowledgement

We would like to express our appreciation to the Laboratory of Biopesticide, Faculty of Agriculture Udayana University for providing laboratory's facilities that made this study happened.

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