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Test of the Effectiveness of Senduduk Leaf Extract Concentration (*Melastoma Malabrhaticum* L.) Against Anthracnose Disease in Red Chili Commodities (*Capsicum annuum* L.)

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ABSTRACT

The disease that causes low chili productivity in Indonesia is anthracnose, anthracnose disease caused by the fungus *Colletotrichum* spp. Efforts to control anthracnose disease are currently still being made using many synthetic pesticides. The use of synthetic pesticides can have a negative impact on the environment. Vegetable functions can be used as another effort to control anthracnose. Plant extracts can be used in anthracnose disease control are Senduduk leaf extracts. This study aims to obtain the concentration of Senduduk plant leaf extract (*Melastoma malabathricum* L) in inhibiting the growth of *Colletotrichum* spp. Research is carried out in the field and laboratory. The design used in this research was a complete randomized design (RAL) with 5 treatments of Senduduk leaf extract, namely K0 = 0%, K1 = 3%, K2 = 6%, K3 = 9%, and K4 = 12%. With 3 repetitions. The results showed that Senduduk leaf extract treatment with a concentration of 12% effective could inhibit the growth of *Colletotrichum capsici* fungus.

Keywords: anthracnose, *Melastoma, Malabrhaticum* L, *Capsicum annuum*, *Colletotrichum capsici.*

1. INTRODUCTION

Red chilies (*Capsicum annuum* L.) hold significant importance in Indonesia, ranking second to legumes. According to BPS data from January to June 2021, Indonesia imported 27,851.98 tons of chilies due to the high demand and insufficient local production. Various factors, such as pest infestations, diseases, and weed growth hinder efforts to boost red chile productivity.

Diseases caused by fungi have a high attack level in the field, during transit, and storage. One of the important diseases that attack and are feared in chili plantations is anthracnose. This disease is caused by Colletotrichum spp., which can significantly reduce yields (Gusmarini et al. 2014).

Anthracnose disease caused by the fungus Colletotrichum spp. is the main disease in chili plants. Attack of the fungus Colletotrichum spp. in chili plants causes damage to the shoots, stems, leaves, and chili fruit both in the field and after harvest and storage. In Indonesia, anthracnose can cause a reduction in yield of up to 90%, especially in the rainy season (Wakhidah et al., 2021). Apart from reducing the quantity of chili fruit, anthracnose disease also reduces the quality of chilies because it causes a decrease in phenol levels by 16-69%, capsaicin levels by 20-60%, and oleoresin levels by 17-55%. (Kirana *et al*. 2014).

Efforts to manage and prevent anthracnose currently rely on the use of synthetic pesticides. The utilization of synthetic fungicides may have adverse impacts on the environment. Hence, it is imperative to exercise caution when applying synthetic pesticides to mitigate environmental contamination. In order to address this issue, it is essential to explore alternative methods that are both biologically and environmentally sustainable (Imansyah et al., 2013).

The leaf extract derived from the Senduduk plant has been identified as a viable botanical pesticide, as evidenced by the research conducted by Chatri et al. (2022). The application of Senduduk leaf extract at varying concentrations has demonstrated significant anti-fungal properties capable of inhibiting the growth of the Fusarium oxysporum fungus. Furthermore, the findings from the study conducted by Laeshita et al. (2022) suggest that concentrated extracts of betel leaves and noni leaves can effectively suppress Colletotrichum spp.'s growth, the anthracnose disease causative agent. According to Hayati (2018), the leaves of the Senduduk plant contain a variety of chemical compounds, such as saponins, flavonoids, tannins, steroids, alkaloids, phenolics, and glycosides, which play a crucial role in eradicating or impeding the growth of microorganisms.

2. MATERIALS AND METHOD

Place and time of research

Isolation and identification of the fungus Colletotrichum spp. Macroscopic and microscopic examinations were carried out at the Integrated Agrotechnology Laboratory, Faculty of Agriculture, Pasir Pengaraian University. The research period was carried out from October to December 2023.

Materials and Tools

The research utilized Senduduk leaves, Colletotrichum capsici isolate, Potato Dextrose Agar (PDA), ethyl acetate, distilled water, dextrose, tissue, NaOCl, methylene blue, magnesium, amyl alcohol, alcohol, FeCl, hot water, 2 N HCl, acetic acid, sulfuric acid, mayer, aluminum foil, plastic, and filter paper as the materials.

Additionally, the tools employed in the study included an autoclave, petri dish, test tube, element tube, microscope, tube needle, cork borer, oven, blender, vacuum rotary evaporator, laminar airflow, micro pipette, busen lamp, dropper pipette, knife, opaque bottle, digital scales, cameras, and stationery.

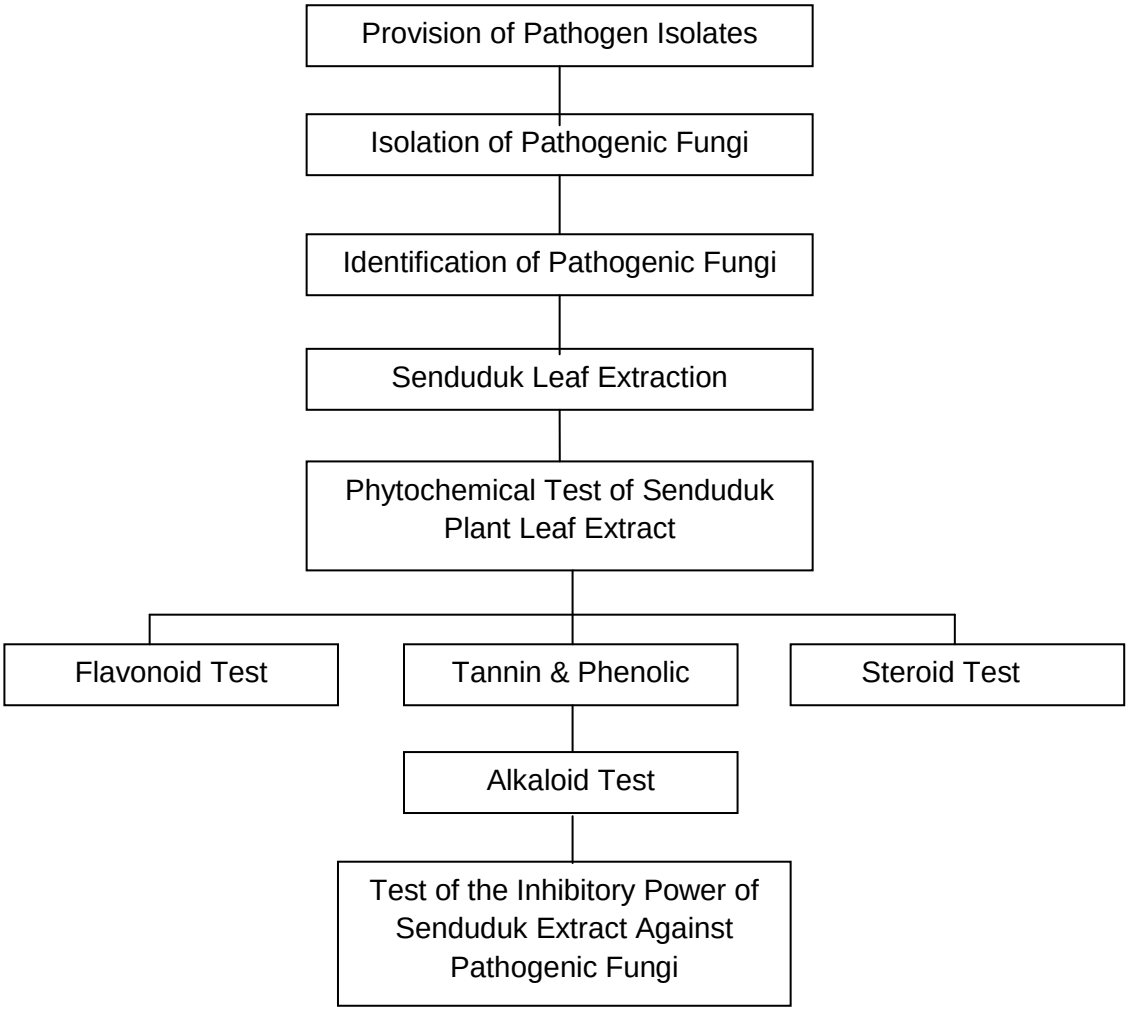

Figure 1. Flow diagram of research implementation

Provision of Pathogen Isolates

Anthracnose-symptomatic red chili fruits were obtained from chili plantations in Rambah Baru Village, Rambah Samo Subdistrict, Rokan Hulu Regency. The anthracnose-infected chili fruit was collected using the roaming method and the direct sampling technique.

Isolation of Pathogenic Fungi

Chili fruits are washed with running water. The infected or symptomatic parts were then cut into 1x1 cm size. The part taken is between the symptomatic part of the fruit and the healthy part of the fruit. Then, surface sterilization was carried out with 1% NaOCl for approximately 15 seconds, and the mixture was rinsed 3 times with distilled water. In the next stage, the chili pieces were planted on PDA media and incubated for 7 days. After the mycelium grows, it is incubated again for pure culture (Laeshita *et al*., 2022).

Identification of Pathogenic Fungi

Fungal identification includes macroscopic and microscopic characters (Watanabe, 2002). Macroscopic characteristics were obtained by making pure cultures in PDA media. Microscopic characteristics are obtained by making preparations. Next, covered with a covered glass, the colonies were observed under a microscope with a magnification of 400 times.

Senduduk Leaf Extraction

Senduduk leaves were cut, washed, and dried in the oven at 80°C. The dried sample is crushed using a blender until it is crushed and becomes powder and then ready for extraction. The simplicia powder is put into a container and soaked (macerated) at room temperature with ethyl acetate solvent. After 5 days of maceration, it is filtered, the filtrate is separated, and the dregs are soaked again in a new solution. Maceration was carried out 2 times. The filtrate obtained was then separated from the solvent and extract using a vacuum rotary evaporator at a temperature of 40˚C and evaporated so that the solvent was separated from the Senduduk leaf extract (Muzafri *et al*. 2022).

Phytochemical Test of Senduduk Plant Leaf Extract

Flavonoid Test

10 mg of self-extract, 0.1 mg of magnesium powder, and a 1:1 amyl alcohol solution were put into a test tube. Next, add 4 ml of 96% alcohol. The test is said to be positive if the solution changes color to yellow, orange, or red (Elisa *et al*., 2018).

Tannin & Phenolic Test

The tannin test was carried out by adding 10 mg of Senduduk extract into a test tube and 3 drops of 5% FeCl3. The test is said to be positive if the solution changes color to blackish green. In the phenolic test, 10 mg of extract was put into a test tube, then 3 drops of 1% FeCl3 were added. The test is said to be positive if the solution changes color from green to red (Elisa *et al*., 2018).

Saponin Test

A total of 10 ml of hot water and 0.5 g of Senduduk extract were put into an elemeyer tube, then cooled and homogenized for 10 seconds. If foam forms for no less than 10 minutes, 1 cm to 10 cm high, and if 1 drop of 2 N HCl is added. The foam does not disappear, and the extract contains saponin (Depkes RI, 1995).

Steroid Test

Ekstrak senduduk sebanyak 50-100 mg diletakkan pada plat tetes dan ditambahkan asam asetat sampai semua sampel terendam, dibiarkan 15 menit kemudian 6 tetes larutan dipindahkan ke dalam tabung reaksi dan ditambahkan 2-3 tetes asam sulfat pekat. Perubahan warna yang terjadi diamati dan digunakan sebagai ukuran relatif kandungan steroid dalam sampel. Adanya warna biru. (Sangi *et al*. 2012).

Alkanoid Test

10 mg of self-extract was put into a test tube, and 1 mL of Mayer's reagent was added. After that, wait a few moments. The test is positive if an orange and yellow precipitate forms. (Elisa *et al*., 2018).

Test of the Inhibitory Power of Senduduk Extract Against Pathogenic Fungi

The test used a Completely Randomized Design (CRD) with 5 treatments and 3 repetitions so that 15 experimental units were obtained. The experimental design consisted of 5 concentrations of Senduduk leaf extract, i.e., K0 = 0 %, K1 = 3 %, K2= 6 %, K3: 9 %, K4= 12 %.

RESULT AND DISCUSSION

Symptoms of Anthracnose Disease in Red Chilies

The results of observations carried out on chili plantations showed that there was an anthracnose attack. Symptoms of anthracnose on red chilies can be seen in Figure 1.

Figure 1. Symptoms of Anthracnose Disease on Chili Fruit

Chili plants indicated to be infected with anthracnose disease have several characteristics, namely, oval-shaped and black spots on the skin, which gradually enlarge, become slightly watery, then form concave lesions. On the surface of the fruit, it looks like necrosis, as seen in Figure 1. This follows the statement of Harahap et al. (2013) that parts of the fruit or pods show symptoms of anthracnose, namely the presence of black spots on the skin, which will enlarge, merge, and become concave over time, causing the fruit to rot.

Identification of Anthracnose Disease on Red Chili Fruit

The results of identifying anthracnose obtained from red chilies showed that the pathogen was Colletotrichum capsici. Macroscopic and microscopic characterization of the pathogenic fungal isolate C. capsici can be seen in Figure 2.

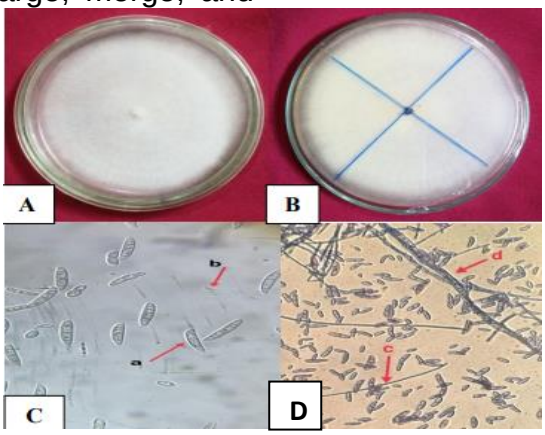

Figure 2. Macroscopic and Microscopic of Colletotrichum and capsici fungi (A) Macroscopic view of the fungus Colletotrichum capsici top view, (B) Macroscopic view of the fungus Colletotrichum capsici bottom view (C), Microscopic, (a) Conidia, (b) Microconidia, (c) Conidiophores, (d) Insulated hyphae

Based on the macroscopic characterization of the fungus, Colletotrichum capsici has the characteristics of white colonies, a cotton-like texture, a lateral growth direction, and slow growth. Meanwhile, microscopically, the shape of the conidia is like a crescent moon; it does not have a septa and has a hyphae.

The book Barnet and Hunter (1972) says that the fungus Colletotrichum capsici has macroconidia shaped like a crescent moon. This is also supported by the statement of Sudirga et al. (2016) that the fungus Colletotrichum capsici has white, pink, orange, and gray colonies. Meanwhile, microscopically, the fungus Colletotrichum capsici has a septate and

branched fungal mycelium. The shape of the spores resembles a sickle, and the spores are clear in color and have no septa.

Phytochemical Content of Senduduk Plant Leaf Extract

The results of the phytochemical testing of the Senduduk leaf extract can be seen in Table 1. The phytochemical screening test on the Senduduk leaf extract aims to determine the class of secondary metabolite compounds contained in the extract (Muzafri, 2019).

Table 1. Results of Phytochemical Content Analysis of Senduduk Plant Leaf Extract

Compound Type	Result
Flavonoids	+
Tannin	+
Phenolic	+
Saponins	+
Steroids	+
Alkaloids	-

Note: (+) = Contain compound type
(-) = Not contain a compound type

The Senduduk leaf extract underwent an analysis to determine its flavonoid content, and the findings revealed a noticeable shift in color towards orange. Elisa et al. (2018) corroborated this observation by stating that a positive flavonoid test involves a color change to yellow, orange, or red. Furthermore, Komala et al. (2019) explained that flavonoid compounds exhibit anti-fungal properties by impeding mitochondrial electron transport, leading to a decline in mitochondrial membrane potential. This inhibition can be attributed to the hindrance of protons in the respiratory chain, subsequently causing a reduction in ATP production and ultimately resulting in the demise of fungal cells.

Tannin is said to be positive if the solution changes color to blackish green (Elisa et al. 2018). The tannin compound functions as an inhibitor of chitin synthesis, which formsm cell walls in fungi and damages cell membranes so that fungal growth is hampered. Apart from that, tannins are also lipophilic, so they easily bind to cell walls and cause damage to fungal cell walls (Watson and Preedy, 2007).

Phenolics exhibit a positive reaction when the color of the solution transitions from green to red (Elisa et al., 2018). These phenolic compounds act as anti-fungal by interfering with the cytoplasmic membrane, leading to leaks in the cell wall (Ansari et al. 2013).

According to the Indonesian Ministry of Health (1995), saponin compounds are deemed positive if foam persists for at least 10 minutes. Saponin itself functions as an anti-fungal agent by disrupting the lipid layer of the cell membrane, ultimately causing a breakdown in the permeability of the cell membrane. This disruption hinders the diffusion process of necessary materials or substances for the fungus, resulting in cell swelling and eventual bursting. (Sugianitri, 2011). Steroid showing positive result. The presence of steroid compounds can be determined by observing a color change, specifically a green color, in the Senduduk leaf extract. This observation aligns with the findings of Koleangan et al. (2014), who stated that a change in red or green indicates steroid compounds' presence. Mandduluri (2013) further explains that steroid compounds exhibit anti-fungal properties due to their lipophilic nature, which allows them to disrupt lipid cell membranes, leading to cell lysis and wilting.

On the other hand, the alkaloid test yielded negative results, as evidenced by the absence of orange and yellow deposits. This suggests that the formation of the potassium alkaloid complex may

not have reached its saturation limit, preventing the formation of a precipitate. Salim et al. (2016) highlight that both internal and external factors influence the phytochemical content of plants. Internal factors include genes, while external factors encompass light, temperature, pH, humidity, nutrients, and altitude.

Growth Rate of Colletotrichum Capsici

Observation of the growth rate of the fungus Colletotrichum capsici began 1 day after incubation (HSI) until the fungus filled the petri dish. The results of observations of the growth rate of C. capsici until it filled the petri dish, occurred over 10 days. The growth rate of C. capsici can be seen in Figure 2.

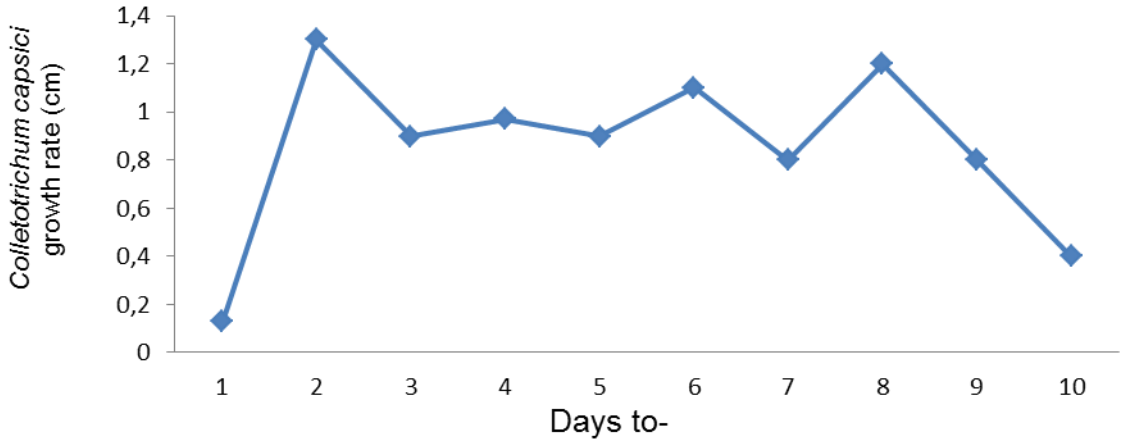

Figure 2. Growth of the Mushroom *Colletotrichum capsici*

The *C. capsici* fungus experienced its highest growth rate on day 2 of HSI, reaching 1.3 cm. Conversely, the lowest growth was observed on day 1 of HSI, attributed to the lag phase in the fungus. Riadi (2016) explains that the lag phase is when fungi acclimate to a new environment. The duration of the lag phase in fungi varies significantly, depending on factors such as media composition, pH, temperature, aeration, initial inoculum cell count, and the physiological characteristics of the microorganisms in the previous media. Subsequently, the log/exponential phase ensues, characterized by rapid growth. Genetic traits largely influence the extent of fungal growth during the exponential phase. This phase occurs from day 2 to day 5 of HSI. The stationary phase takes place from days 6 to 10, during which fungal growth stabilizes, balancing with the number of dead cells. Although carbon, an essential energy and nutrient source, may be depleted in the stationary phase, growth continues due to the lysis of dying cells, which serve as a nutritional source (Maier, 2000).

Inhibitory Power of Senduduk Plant Leaf Extract Against the Fungus Colletotrichum capsici

The results of observations of the inhibitory power of Senduduk leaf extract on the Colletotrichum capsici fungus with various concentrations had a real effect, as seen in Table 2.

Table 2. Inhibitory Power Rate of Senduduk Plant Leaf Extract Against Colletotrichum Capsici Fungus

P	Percentage of Inhibitory Power Per Day									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
K0	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
K1	20b	34b	48b	48b	43b	40b	38b	33b	24b	16.2b
K2	20b	74c	74c	61c	53c	45bc	39b	36c	35c	34c
K3	20b	74c	82d	86d	89d	82d	74c	64d	54d	45d
K4	66c	78d	85de	88de	89d	91e	92d	93e	94e	94.2e

Note: Numbers followed by the same letter in the same column are not significantly different based on the BNJ test at the 5% level. P: Treatment, K0 (0% concentration), K1 (3% concentration), K2 (6% concentration), K3 (9% concentration), K4 (12% concentration).

According to the results of the variance analysis, it is evident that each treatment exhibits distinct differences from the others. Notably, the K4 treatment displayed the highest inhibitory power, with a remarkable 94.222% inhibitory power on day 10. Consequently, the K4 treatment, which utilized a 12% concentration of Senduduk plant leaves, proved the most effective. The inhibitory power against fungi indicates an active anti-fungal compound in the extract of Senduduk plant leaves against the Colletotrichum sp. fungus. The concentration of the treatment plays a significant role in its effectiveness, as higher concentrations result in larger fungus-free areas. This aligns with the findings of Cahyani et al. (2015), who emphasized the close relationship between concentration and the amount of active ingredients in a formulation. The greater the concentration, the higher the number of active ingredients, leading to a more optimal ability to suppress pathogens. The number of compounds in each concentration directly influences the inhibitory power against fungal growth. Higher concentrations contain more active compounds, resulting in stronger inhibitory power. Conversely, lower concentrations have fewer active compounds, weakening inhibitory power (Durairaj, 2009).

4. CONCLUSION

1. The cause of anthracnose disease in chili plantations in Rambah Baru Village is Colletotrichum capsici.
2. Senduduk plant leaf extract contains active compounds, including flavonoids, tannins, phenolics, and steroids.
3. Senduduk plant leaf extract can inhibit the fungus Colletotrichum capsici's growth, which causes anthracnose disease.
4. Senduduk plant leaf extract with a concentration of 12% is effective in inhibiting the growth of the fungus Colletotrichum capsici

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