

Research Article

The Potency Purple Sweet Potato (*Ipomoea batatas*) Leaf Extract as Biofungicide for Controlling *Fusarium* Rot on Chili

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ABSTRACT

Fusarium sp. has been recognized as a pathogenic agent causing fruit rot in chili. The disease significantly decreases the annual chili production rate. One of the plants potentially utilized as biofungicide to control *Fusarium* fruit rot disease in chili is purple sweet potato (*Ipomoea batatas* L.). Purple sweet potato contains secondary metabolites, mainly the active compounds in the form of flavonoids, which has been previously revealed to perform antifungal activity. This study aimed to examine the potential of purple sweet potato (*Ipomoea batatas* L.) leaf extract as biofungicide to inhibit the growth of chili rot disease caused by *Fusarium* sp. within vitro and in vivo assay. This work was conducted by testing the ethanol-extracted purple sweet potato leaves extracts against *Fusarium* sp. with various concentrations, i.e., 5%, 10%, 20%, and 40% (w/v) for in vitro assay. Then antifungal test against *Fusarium* sp. was conducted using preventive and curative test for in vivo assay. Observations were performed for 7 days. The result of in vitro assay showed inhibitory ability of the leaves extract against the growth of *Fusarium* sp. to range between 35.2-76.6% with potential concentration 40% (w/v). Based on the results of in vivo assay, the preventive test using 40% concentration of purple sweet potato leaves extract showed significant different against negative control through Duncan test ($\alpha = 0.05$). Purple sweet potato leaves extract with 40% concentration can reduce the intensity of *Fusarium* fruit rot disease and potentially utilized as biofungicide.

Keywords: biofungicide, chili, *Fusarium*, *Ipomoea batatas*

Introduction

Fusarium has been recognized as a pathogenic fungus causing wilt and fruit rot. In Bogor, Indonesia, *Fusarium* sp. had been isolated as the cause of wilting of leaves on infected chili plants in various villages (Utami, 2018). The disease resulted in reduced chili production, reaching 30-40% below normal production levels (Pandey & Gupta, 2013). Hence, efforts to increase chili production yields could begin with the use of fungicides to inhibit the growth of *Fusarium* sp. at chili plants. However, continuous uses of synthetic fungicides in a long period had expectedly been causing a disturbed ecological balance (Tando, 2018). Biofungicide, therefore, has been as an alternative to control

the disease by offering various advantages, including environmentally friendly characteristics, high degradability, abundant in nature, and line with the concept of sustainable agriculture (Dalimunthe & Rachmawan, 2017).

Among others, purple sweet potato (*Ipomoea batatas* L.) has been considered as a potential biofungicide. Purple sweet potato contains the highest anthocyanin content (± 110.51 mg/100 g) higher compared to other types of purple sweet potatoes. Anthocyanin is a secondary metabolite of flavonoids and polyphenols, which have been recognized to act as an antioxidant (Ginting et al., 2011). Extracting of purple sweet potato leaves by applying ethanol solvent has discovered secondary metabolites

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in the forms of alkaloids, steroids-triterpenoids, saponins, tannins, and flavonoids (Saputri & Kurniasih, 2018). Another research on an ethanol extract of purple sweet potato leaves has further revealed its potential as an inhibiting agent to the growth of *Fusarium* sp. (Saputri, 2018). In chili plants, the applications of bio fungicides are currently limited in laboratory uses only and have not reached public releases in the form of commercial products. Therefore, strategic steps in bio fungicide researches are required to explore the practical uses of purple sweet potato leaves, which have been experimentally proven to inhibit the growth of *Fusarium* sp. in general, on chili plants. Purple sweet potato leaf is particularly chosen due to its abundant presence in Indonesia, especially in the Bogor area. Its current utilization, however, has not been taken to its optimum potentials, in which the leaves are currently been used only as animal feeds. It is a contrast to the discovery of metabolites of sweet potato extract that perform bioactivity as antifungal agents. This study was aimed to determine the potency of purple sweet potato (*Ipomoea batatas* L.) leaves as a biofungicide for inhibiting the growth of *Fusarium* sp. causal agent of chili fruit rot.

Material and Methods

Purple Sweet Potato Leaves Extraction

Samples of purple sweet potato leaves used in this study were obtained from a farm in Bogor, West Java, Indonesia. In this step, ethanol 70% was taken as a solvent. Samples were macerated with the solvent in a 1:10 ratio at room temperature for 5 days. Next, the macerated samples were filtered. The filtrate was later evaporated in a rotary evaporator IKA RV6 at 60 °C to evaporate, resulting in a concentrated extract. Then, the concentrated extract was weighed to measure the extract yield of maceration.

Fusarium sp. Regeneration

Fusarium sp. was rejuvenated by etching it using an inoculating loop (one needle) on a sloping medium (Berlian et al., 2016). Then, it was incubated at room temperature for 7 days (Setyaningsih et al., 2012).

Preparation of Extracts Concentration

Extracts concentration of purple sweet potato were made by dissolving previously concentrated extract with DMSO 10% solvent at various concentrations, i.e., 5%, 10%, 20%, and 40% (w/v) (Hidayati & Harjono, 2017).

In Vitro Assay of Purple Sweet Potato Leaf Extract Against Fusarium sp.

Furthermore, in vitro assays of ethanol-extracted purple sweet potato leaves on the growth of *Fusarium* sp. were performed on PDA mediums aseptically. Each extract was mixed with liquid PDA to form the medium with a specific sweet potato extract concentration of 5%, 10%, 20%, or 40%. Furthermore, isolates of *Fusarium* sp. in 5 mm diameter were grown on all test media. As a negative control, *Fusarium* sp. isolates were grown on a non-extract-added PDA medium, which was without the addition of purple sweet potato leaves extract. For positive control, isolates of *Fusarium* sp. were also grown on a non-extract-added PDA medium mixed with a Propineb 70% fungicide at 0.2% concentration (w/v). Each *in vitro* test treatment was repeated 5 times.

In Vivo Inhibition Assay

In vivo inhibition assays of purple sweet potato leaf extract with 40% concentration were conducted in curative and preventive tests. A curative test was performed to determine the ability of purple sweet potato leaf extract to suppress the incidence of chili rot disease after *Fusarium* sp. attack. Curative treatment was performed after the first symptoms arose and seven days after the fruit rot. Rot chili fruits were soaked in purple sweet potato leaf extract suspension for 10 minutes, dried, placed on the tray, and subsequently incubated for 7 days. As a negative control, chilis were incubated without soaking in the purple sweet potato leaf extract. A preventive test was performed to determine the potency of purple sweet potato leaf extract to prevent *Fusarium* infection in chili. The purple sweet potato leaf extract was applied before rot chili disease symptoms appeared.

Results and Discussion

In vitro assays of purple sweet potato leaves ethanol extract on the growth of *Fusarium* sp. The negative control group was observed to show a weak inhibitory ability. Meanwhile, treatment groups with 5%, 10%, and 20% added purple sweet potato leaves extract were observed to deliver a strong inhibitory ability. Then, the application of purple sweet potato leaves extracts at 40% concentration was observed to perform a very strong inhibitory ability (Table 1). It followed an existing classification of inhibitory ability that suggested the ability of fungal response to growth as weak (> 3 cm diameter), medium (2-3 cm),

strong (1-2 cm), or very strong (< 1 cm) (Alfian et al., 2015).

Based on Table 2, the Duncan-based statistical test with a 5% significance level discovered the treatment of purple sweet potato leaves extracts at 40% concentration to be significantly different compared to other treatments. It revealed treatments at all extract concentrations (5%, 10%, 20%, 40%) to deliver considerably good inhibitory influences on the growth of *Fusarium* sp. Table 1 presents the results of the influence of purple sweet potato leaf extract on the growth of *Fusarium* sp. After that, the percentage of inhibitory ability for each treatment group was calculated (Table 2).

Table 1. Diameter (cm) of *Fusarium* sp. under various treatments of purple sweet potato leaf extract concentration

Treatment	Average	Duncan Test _{0.05}	
			Inhibition Ability
40%	0.62	a	Very Strong
20%	1.04	b	Strong
10%	1.18	b	Strong
5%	1.24	b	Strong
Control (+)	2.14	c	Medium
Control (-)	3.62	d	Weak

Note numbers followed by the same alphabet refer to insignificances according to the Duncan test at the 5% significant level. Propineb 70% as control positive and no treatment as control negative

Table 1 shows that purple sweet potato leaves extract at 40% concentration best decreased significantly the growth of *Fusarium* sp. compared to other treatments, including positive control. It revealed that treatments at

all extract concentrations (5%, 10%, 20%, 40%) to deliver considerably good inhibitory influences on the growth of *Fusarium* sp. Table 1 presents the results of the calculation. After that, the percentage of inhibitory ability for each treatment group was calculated in Table 2.

Table 2. The percentage of the inhibitory ability of purple sweet potato leaves extract against *Fusarium* sp.

Treatment	Inhibition ability after the 7-day treatment period							Average (%)
	1	2	3	4	5	6	7	
Control (-)	0	0	0	0	0	0	0	0
Control (+)	11.5	21.8	30.8	38.8	48.3	48.3	46.9	35.2
5%	1.9	47.0	58.8	65.6	73.5	73.5	76.5	56.7
10%	21.2	39.6	55.4	67.8	76.0	75.6	77.9	59.1
20%	39.4	42.6	58.1	69.6	78.6	78.6	80.9	64.0
40%	44.2	66.8	78.5	82.9	87.4	87.2	89.2	76.6

Based on the result which has shown in Table 2, the average inhibitory abilities of purple sweet potato leaves extract on the growth of *Fusarium* sp. by *in vitro* tests were discovered to range between 56.7-76.6%. Technically, the potentials of purple sweet potato leaves extract as a bio fungicide were measured from the inhibited growth of *Fusarium* sp. due to the addition of purple sweet potato leaves extract. Observations of *in vitro* test results on growing media indicated the addition of leaves extracts at 40% concentration to be more effective in inhibiting the growth of *Fusarium* sp., resulting in the highest inhibition percentage at 76.6% compared to other treatment groups.

At 40% concentration of the leaves extract, the inhibitory ability was observed as being able to diffuse very well, resulting in the opti-

um diffusion of leaves extract into the growing medium containing *Fusarium* sp (Figure 1). It was observed to produce an increasingly inhibited growth of *Fusarium* sp., which would be highly desired for horticulture plants infected by the disease (Dani & Rosmaiti, 2015). *Fusarium* caused fruit rot had been consistently taking attention as the most common disease found in chili during peak harvest season in various areas in Bogor, Indonesia (Utami, 2018). It had particularly been observed to attack if a chili storage system was not good enough to prevent the growth and spread of *Fusarium* (Riansyah et al., 2015). It could then cause significant economic losses due to unusable harvested chili. In general, *Fusarium* fruit rot disease in chili plants could reduce chili production by 30-40% (Pandey & Gupta, 2013).

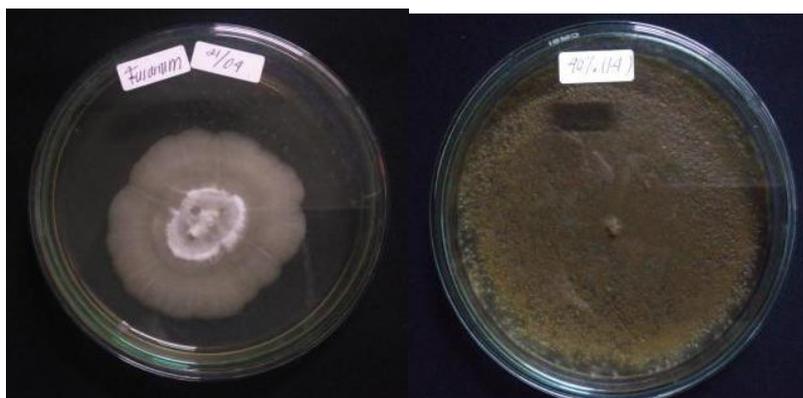


Figure 1. Antifungal activity under optimum conditions (a) 0% (Negative control), (b) 40% *Ipomoea batatas* L. leaves extract to inhibit *Fusarium* sp. within *in vivo* assay

In general, the uses of chemical fungicides had been stated to affect the conditions of soil, water, treated agriculture products, and human users of these fungicides, hence causing further problems such as unbalanced ecosystems (Riansyah et al., 2015). Therefore, the uses of biofungicide had been stated to act as an alternative treatment in controlling diseases in plants. Biofungicide offered several advantages, *i.e.*, being environmentally friendly, easily degraded, abundant in nature, and line with the concept of sustainable agriculture (Dalimunthe & Rachmawan, 2017). One of the potential plants to be used as material for controlling *Fusarium* fruit rot disease is a purple sweet potato plant.

Purple sweet potato leaves can be used as an antifungal because they contain anthocyanin compounds, which are secondary metabolites of flavonoid and polyphenol groups (Berlian et al., 2016). Plants have a natural defense mechanism against pathogen infections by producing compounds such as peptides, protein, flavonoids, and other organic compounds (Soekarno et al., 2012). As an antifungal, flavonoid and phenol can damage pathogenic cell membranes, resulting in changes in cell growth or deaths of fungal cells. These compounds can also desaturate pathogenic cell proteins and shrink cell walls to then lyse fungal cell walls. Besides, flavonoid compounds and phenols can diffuse into fungal cell membranes and disrupt

metabolic pathways in fungi, including the synthesis of ergosterol, glucans, chitin, proteins, and glucosamine. The compounds will bind with ergosterol, which is a constituent of fungal cell membranes, causing the formation of a pore in the cell membranes. Then, the formed pores cause fungal cell components, *i.e.*, amino acids, carboxylic acids, inorganic phosphates, and phosphates esters, out of the cells to eventually cause fungal cell deaths (Muthukumaran Et al., 2011).

***Ipomoea batatas* L. Leaves Extract to Inhibit *Fusarium* sp. within vivo assay**

Ipomoea batatas L. leaves extract to inhibit *Fusarium* sp. within vivo assay incubation period is the time required for *Fusarium* sp. to cause the first symptoms on chili after inoculation. Resistant induction influence test on 40%

purple sweet potato leaf extract was capable of expanding the rot disease caused by *Fusarium* compared to other treatments (Figure 2).

Resistant induction influence preventive test could expand the incubation period of *Fusarium* rot disease better than positive control as there were fruit rot symptoms that emerged on the 5th day of incubation. Residual influence preventive test could expand the incubation period *Fusarium* rot disease compared with the positive control using a *propineb* fungicide. The curative test could suppress *Fusarium* growth better than the negative control, proven that a 40% concentration of purple sweet potato leaf extract is potentially utilized as biofungicide (Figure 2).

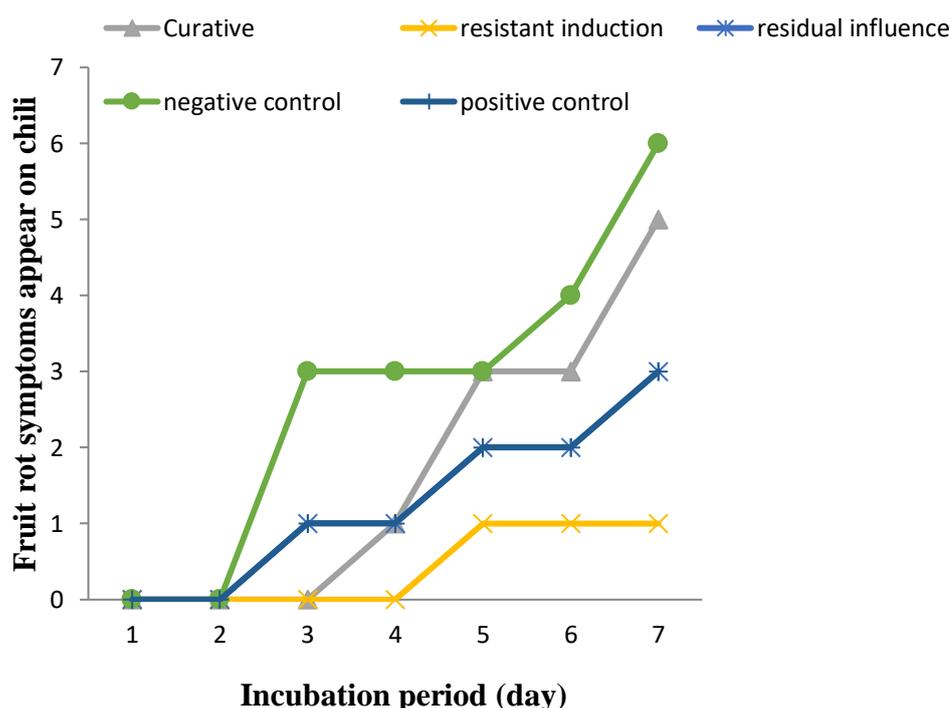


Figure 2. The incubation period for *Fusarium* sp. to cause the first symptoms on chili

Purple Sweet Potato Leaf Extract Influence on Disease Incidence and Intensity

Based on this study, the most severe disease occurred on the negative control treatment,

while the least occurred on resistant induction influence treatment. Duncan's statistical test with 5% significant degree indicated that resistant induction influence treatment did not

differ significantly against the residual influence and positive control. The curative test showed no significant difference in the negative control. Disease incidence on preventive treatment of resistant induction and residual influence differed significantly with negative control and did not differ significantly with

positive control using b (Table 3). This indicates that rot disease *Fusarium* growth in chili can be suppressed due to resistant induction and residual influence. Preventive treatment with resistant induction and residual influence decrease disease intensity compared to negative control (Table 4).

Table 3. Purple sweet potato leaf extract with 40% on disease incidence of rot disease *Fusarium* using preventive and curative test

Treatment	Disease Incidence (%)*
Resistant induction	2.23a
Residual influence	6.67ab
Positive control	6.67ab
Curative test	11.09bc
Negative control	13.30c

*Numbers followed with different letters indicate significant difference (Duncan test $\alpha = 0.05$).

Table 4. Purple sweet potato leaf extract with 40% on disease severity of *Fusarium* rot disease using preventive and curative test

Treatment	Disease Severity (%)*
Resistant induction	0.43a
Residual influence	1.33a
Positive control	1.33a
Curative test	3.99b
Negative control	5.33b

* Numbers followed with different letters indicate significant difference (Duncan test $\alpha = 0.05$).

Fusarium rot disease attack chili during the abundant harvest season. This disease affects whether the unwell storage system performs, encouraging the growth and dissemination of *Fusarium* (Soekarno et al., 2012). *Fusarium* fungus enters the vessel tissue through root tissues and subsequently uses xylem vessel as a way to rapidly form colonies in the plant, causing typical rot symptom (Wongpie & Lomthaisong, 2010).

Rot disease caused by *F. oxysporum* often encounters in chili. This disease plays an important role to reduce chili production (Nurzannah et al., 2014). Efforts to increase chili production can be performed using *Fusarium* sp. fungicide in chili. However continuous use of synthetic fungicides over long period will cause disrupted ecological balance. Biofungicide utilization becomes an alternative disease control in plant. Biofungicide is environmentally friendly, easily degraded, abundant local

resources, and following sustainable agriculture concept (Dalimunthe & Rachmawan, 2017).

One plant that is abundant and potentially developed as biofungicide is purple sweet potato (*I. batatas* L.). Purple sweet potato has the highest content of anthocyanins among other types of purple sweet potato, i.e 110.51 mg/100 g. Anthocyanins are secondary metabolite of flavonoids and polyphenols act as antioxidants (Ginting et al., 2011). Purple sweet potato leaf ethanolic extract has been proven to be capable of inhibiting *Fusarium* sp. growth (Saputri, 2018). Purple sweet potato leaf ethanolic extract contains secondary metabolites, namely flavonoids and tannins, with relatively higher antioxidant activity than alpha tocopherols which are popular as antioxidant compounds (Sulastri et al., 2013). The highest compounds detected in purple sweet potato leaf

ethanolic extract through Gas Chromatography – Mass Spectrometer (GC-MS) analysis are 1,4-benzenediol (CAS) hydroquinone with 19.70% concentration (Saputri & Kurniasih, 2018). 1,4-Benzenediol (CAS) hydroquinone is phenolic compound group with C₆H₆O₂ chemical formula (National Chemical Inventories (NCI), 2007). Phenol is bioactive compound as an antibacterial in medicinal herbs (Proestos et al., 2005).

Plants have defense mechanism against pathogenic infection attack by producing compounds, such as peptides, proteins, flavanoids, and other organic compounds (Muthukumaran et al., 2011). Secondary metabolite compounds defend the plants from less favorable environmental conditions, e.g. capable of overcoming pest and disease attack, attracting pollinators, and producing molecular signal. Secondary metabolites are used in organisms to interact with the environment (Putri et al., 2014). Purple sweet potato have anthocyanins as antimicrobial agents in foods. The anthocyanins from purple sweet potato (APSP) against the fungal growth of *Geotricum candidum* and *Candida albicans* (Wen et al., 2016).

The results, including preventive test both on resistant induction and residual influence and curative test indicates the capability of suppressing *Fusarium* rot disease intensity better than the negative control. This was shown as lower disease incidence rate than negative control. Based on the study results, purple sweet potato leaf extract is capable of suppressing growth and expansion of *Fusarium* sp. in chili.

Conclusion

Extracts of purple sweet potato leaves inhibited the growth of *Fusarium* sp. The peak inhibition rate was observed to reach 76.6%. In particular, the addition of purple sweet potato leaves extract at 40% concentration was observed to potentially act as a high performance and environmentally friendly bio fungicide to control *Fusarium* fruit rot disease on chili plants.

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