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To cite this article: D D Saputri and A W A Utami 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **468** 012031

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# In vitro assays to investigate ethanol extract of *Ipomoea batatas* leaves as potential biofungicide for controlling *Fusarium*

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**Abstract.** *Fusarium sp.* has been known as a pathogenic agent causing fruit rots in chili and potentially decrease annual chili production rates. One plant potentially utilized for controlling *Fusarium* is purple sweet potato (*Ipomoea batatas* L.). Purple sweet potato contain active compounds in the form of flavonoids, which has been previously revealed to perform antifungal activity. This study aimed to determine antifungal activity of purple sweet potato (*Ipomoea batatas* L.) leaves as a bio fungicide for inhibiting the growth of *Fusarium sp.*-caused chili fruit rots. This work was conducted by testing the ethanol-extracted purple sweet potato leaves extracts against *Fusarium sp.* with *in vitro* assays. It was conducted by applying disc-diffusion method to observe the antifungal activity against *Fusarium sp.* This study showed inhibitory ability of the leaves extract against the growth of *Fusarium sp.* to range between 46.9 to 89.2%. Besides, *in vitro* treatment of the leaves extract at 40% concentration was discovered to significantly inhibit the incubation time of *Fusarium sp.* fruit rot compared to other treatment groups. These findings suggested the leaves extract at 40% concentration to be a potential bio fungicide for controlling *Fusarium* fruit rots on chili.

## 1. Introduction

*Fusarium* has been recognized as a pathogenic fungus causing wilting leaves and fruit rot. In Bogor, Indonesia, *Fusarium sp.* had been isolated as the cause of wilting leaves on infected chili plants in various villages [1]. The disease resulted in a reduced chili production, reaching 30-40% below normal production levels [2]. Hence, efforts to increase chili production yields could begin with the uses of fungicides to inhibit the growth of *Fusarium sp.* at chili plants. However, continuous uses of synthetic fungicides in a long period of time had expectedly been causing a disturbed ecological balance [3]. Bio fungicide, therefore, had risen as an alternative in controlling the disease by offering various advantages, including environmentally friendly characteristics, high degradability, abundant in nature, and in line with the concept of sustainable agriculture [4].

Among others, purple sweet potato (*Ipomoea batatas* L.) has been considered as a potential bio fungicide. Purple sweet potato contains highest anthocyanin content ( $\pm 110.51$  mg/100 g) compared to other types of purple sweet potatoes. Anthocyanin is a secondary metabolite of flavonoids and polyphenols, which have been recognized to act as antioxidants [5]. Extracting purple sweet potato leaves by applying ethanol solvent has discovered secondary metabolites in the forms of alkaloids, steroids-triterpenoids, saponins, tannins and flavonoids [6]. Another research on ethanol extract of purple sweet potato leaves has further revealed its potential as an inhibiting agent to the growth of *Fusarium sp.* [7]. 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 contrast to the discovery of metabolites of sweet potato extract that perform bioactivity as antifungal agents. This study, therefore, was aimed at determining potential antifungal activity of purple sweet potato (*Ipomoea batatas* L.) leaves as a bio fungicide for inhibiting the growth of *Fusarium sp.*-caused chili fruit rot caused by applying *in vitro* assays of the leaves.

## 2. Methods

### 2.1. 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 solvent. Samples were macerated with ethanol 70% in 1:10 (b/v) ratio at room temperature for 5 d [8]. Next, the macerated samples were filtered. The filtrate was later evaporated in a rotary evaporator IKA RV 8 at 60 °C, resulting in a concentrated extract. Then, the concentrated extract was weighed to measure the yield. Extracts 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% (weigh/vol) [9]. Besides, 2 control treatments included a positive control with propineb 70% at 0.2% concentration and a negative control with no added extract. In total, there were 6 treatment groups tested on *Fusarium sp.* Each of the 6 treatment groups were repeated 5 times by following the Federer's formula [10], hence totaling in 30 treatment samples.

### 2.2. *Fusarium sp.* regeneration

*Fusarium sp.* collected from Microbiology Laboratory of State University of Jakarta (UNJ) was rejuvenated by etching it using an inoculating loop (ose needle) on a sloping medium [11]. Then, it was incubated at room temperature for 7 d [12].

### 2.3. *In vitro* assay

The assays of ethanol-extracted purple sweet potato leaves on the growth of *Fusarium sp.* were performed on PDA medium. 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.* with 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.

## 3. Results and discussion

Extraction of purple sweet potato leaves was carried out using ethanol 70%. *In vitro* test showed that purple sweet potato leaves extract using ethanol 70% solvent inhibited the growth of *Fusarium sp.* Table 1 exhibits the measured diameters of *Fusarium sp.* after a 7-d treatment period.

**Table 1.** Average diameters of *Fusarium sp.* after a 7-d treatment period.

Treatments	Average	Inhibition ability
Control (-)	3.62 ± 0,192	Weak
Control (+)	2.14 ± 0,378	Medium
5%	1.24 ± 0,114	Strong
10%	1.18 ± 0,083	Strong
20%	1.04 ± 0,114	Strong
40%	0.62 ± 0,044	Very strong

Looking at Table 1, 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 treatment of administering purple sweet potato leaves extract at 40% concentration was observed to perform a very strong inhibitory ability. 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) [13].

After observing the averaged diameters of *Fusarium sp.*, these data were processed in ANSIRA calculation (Analysis of Variance) of 1 factor (Table 2).

**Table 2.** ANSIRA results on the effect of purple sweet potato leaves extract on the growth of *Fusarium sp.*<sup>a</sup>.

	DF	Sum Sq	Mean Sq	F value	F <sub>0.05</sub>	F <sub>0.01</sub>
Total	29	43.15	1.48792	6.45643*	2.6206541	3.89507
Treatment	5	24.75	4.949933			
Error	24	18.40	0.766667			

<sup>a</sup> Notes:  $F_{\text{value}} > F_{\text{table}}$  with a level of 0.05, \* significantly different.

Based on these calculations, the purple sweet potato leaves extract could be stated as having a significant influence on the growth of *Fusarium sp.* Further calculations were performed by applying the Duncan test formula (Table 3). Duncan-based statistical test with 5% significance level discovered the treatment of purple sweet potato leaves extract at 40% concentration to be significantly different compared to other treatments. In fact, it revealed that treatments at all extract concentrations (5%, 10%, 20%, 40%) to deliver considerably good inhibitory influences on the growth of *Fusarium sp.* After that, the percentage of inhibitory ability for each treatment group was calculated (Table 4).

**Table 3.** Duncan test influence purple sweet potato leaf extract on the growth of *Fusarium sp.*

Treatment	Average	Real difference at distance				BJND <sub>0.05</sub> <sup>a</sup>
		P = 1	P = 2	P = 3	P = 4	
40%	0.62	0.62				a
20%	1.04		1.04			b
10%	1.18		1.18			b
5%	1.24		1.24			b
Control (+)	2.14			2.14		c
Control (-)	3.62				3.62	d

<sup>a</sup> Note: numbers followed by the same alphabet refer to insignificances at the 5% test level.

Looking at Table 4, 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 46.9 to 89.2%. 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 on growing media indicated the addition of leaves extract 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 optimum diffusion of leaves extract into the growing medium containing inoculated *Fusarium sp.* It was observed to produce an increasingly inhibited growth of

*Fusarium sp.*, which would be highly desired for horticulture plants infected by the disease [14]. *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 [1]. It had particularly been observed to cause infection if a chili storage system was not good enough to prevent the growth and spread of *Fusarium* [14]. 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% [2].

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 [15]. 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 in line with the concept of sustainable agriculture [4]. One of the potential plants to be used as material for controlling *Fusarium* fruit rot disease is purple sweet potato plant.

**Table 4.** The percentage of inhibitory ability of purple sweet potato leaves extract against *Fusarium sp.*

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

Purple sweet potato leaves can be used as an antifungal because they contain anthocyanin compounds, which are secondary metabolites of flavonoid and polyphenol groups [8]. Basically, plants have a natural defense mechanism against pathogen infections by producing compounds such as peptides, protein, flavonoids, and other organic compounds [16]. 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 [17].

#### 4. Conclusion

This research had discovered the extracts of purple sweet potato leaves to have an ability in inhibiting the growth of *Fusarium sp.* The peak inhibition rate was observed to reach 89.2%. 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 the *Fusarium* fruit rot disease on chili plants.

#### Acknowledgments

The authors would like to thank to the Faculty of Teacher Training and Education, the University of Pakuan, Indonesia, for providing facilities required in conducting this research. This work was

financially supported by the Ministry of Research, Technology and Higher Education (*Kementrian Riset, Teknologi & Pendidikan Tinggi – Ristekdikti*), the Republic of Indonesia, through the DIKTI Competitive Research funding program in 2019 fiscal year with grant no. 7/E/KPT/2019.

## References

- [1] Utami A W A 2018 *Journal of Pedagogia* **10**(1) 34-43
- [2] Pandey K K and Gupta R C 2013 *J. Mycol. Plant. Pathol.* **43** 409-413
- [3] Tando E 2018 *Biotropika Journal* **6**(1) 21-27
- [4] Dalimunthe C I and Rachmawan A 2017 *Warta Perkaratan* **36**(1) 15-28
- [5] Ginting E, Utomo J S, Yulifianti R and Jusuf M 2011 *Food Crop Science and Technology* **6**(1) 116-138
- [6] Saputri D D and Kurniasih S 2018 *Phytochemical Screening and Analysis of Gass Cromatography – Mass Spectrometer (GC-MS) Ethanol Extract of Purple Sweet Potato (Ipomoea batatas L.) Leaves* (Bogor: University of Pakuan)
- [7] Saputri D D 2018 *Pedagogia Journal* **10**(1) 84-91
- [8] Azwanida N N 2015 *Med. Aromat/. Plants.* **4**(3) 1-6
- [9] Hidayati A S and Harjono 2017 *MIPA Journal* 4(1)
- [10] Harjosuwono B A, Arnata I W and Puspawati G A K D 2011 *Design of Theory Experiment, SPSS and Excel Applications* (Malang: Lintas Kata Publishing)
- [11] Berlian Z, Aimi F and Lestari W 2016 *Biota Jurnal* 2(1)
- [12] Setyaningsih I, Desniar and Purnamasari E 2012 *Akuatika Journal* **3**(2) 183
- [13] Alfiah R R, Khotima S and Turnip M 2015 *Protobiont. Journ.* **4**(1) 53
- [14] Dani J and Rosmaiti 2015 *Agrosamudra Journal* **2**(2) 10-18
- [15] Riansyah Y, Mulqie L and Choesrina L 2015 *Antiinflammation Activity Examination of Sweet Potato Leaf Ethanol Extract on Wistar Rat Male* (Bandung: University of Islam)
- [16] Soekarno, Bonny P W, Suroño and Marhaenis E 2012 *Fitopatologi Journal* **8**(5) 121-127
- [17] Muthukumarán P, Padmapriya P, Salomi S, Umamaheswari R, Kalaiarasan P and Malarvizhi C 2011 *Asian J. Pharm. Res.* **1**(4) 108-110