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Rat Taro Extract (*Typhonium flagelliforme* (Lodd.)) as A Biofungicide Against the Cause of Leaf Fall (*Pestalotiopsis sp.*) on Rubber Plants (*Hevea brasiliensis*)

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Abstract: *Typhonium flagelliforme* or known as rat taro is a herbaceous plant that belongs to the taro tribe. The results of the phytochemical analysis of the leaves and tuber extracts of rat taro are known to contain secondary metabolite compounds belonging to the class of flavonoids, alkaloids, triterpenoids, steroids, saponins, and tannins which act as antimicrobials. This study aims to determine the effectiveness of leaf, stem and tuber extracts of rat taro (*Typhonium flagelliforme* (Lodd)) as a biofungicide against the cause of leaf fall (*Pestalotiopsis sp.*) on rubber plants (*Hevea brasiliensis*). Leaf samples came from rubber plants at PTPN III Karet Sei Putih, Galan District, Deli Serdang Regency, North Sumatra. *Pestalotiopsis sp.* was isolated from plant parts showing leaf fall symptoms. Pieces of rubber leaves infected with *Pestalotiopsis sp.* was grown on PDA media in a petri dish and incubated for ±7 days at room temperature 26–28^{o}C. After incubation, microscopic identification of the conidia of the fungus was carried out. In vitro test of the inhibition power of extracts of leaves, stems and tubers of rat taro against the fungus *Pestalotiopsis sp.* was carried out in a petri dish using the fungus culture method. The results of the phytochemical screening showed that the leaf, stem and tuber extracts of rat taro contained chemical compounds of flavonoids, tannins, saponins, alkoloids, and steroids/triterpenoids. The results showed that the leaves, stems and tuber extracts of rat taro (*T. flagelliforme L.*) have potential as biofungicides, K13 treatment of tuber extract of rat taro (*Typhonium flagelliforme L.*) 9% was the best treatment which could inhibit the growth of *Pestalotiopsis sp.*

Keywords: Bioactive; Biofungicide; Extract; Pestalotiopsis

Introduction

Diseases in rubber plants are generally caused by fungi. One very important disease that attacks them is leaf fall disease. This disease is caused by the fungus *Pestalotiopsis sp.* and can cause rubber production losses reaching more than 25% (Febbiyanti & Fairuza, 2019). The fungus *Pestalotiopsis sp.* is known that its presence has increased in Indonesian rubber plantations in 2016, to be precise, it was first detected in North Sumatra and spread to other provinces (Direktorat Jenderal Perkebunan Kementerian Pertanian, 2019; Badan Pusat Statistik Nasional, 2019). According to Febbiyanti et al. (2018) reported that this leaf fall disease has spread to several regions in Indonesia with different levels of attack, namely North Sumatra 50%, South Sumatra 25-75%, East Java 25%, Lampung 50% and others.

Generally, farmers still use fungicides to control these pathogenic fungi, because farmers consider this method to be the most effective (Direktorat Jenderal Perkebunan Kementerian Pertanian, 2019). The results of research on the continuous and excessive use of fungicides will have a negative impact on the environment and humans. Thus causing environmental pollution and including pests to become resistant and a threat to predators, parasites, fish, birds, and wild

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animals that are polluted by pesticides (Siswandi, 2019). Therefore it is necessary to find other alternatives that are considered environmentally friendly, cheap and easy.

Rat taro (*Typhonium flagelliforme* (Lodd) is a herbaceous plant belonging to the taro tribe (Araceae). In the Araceae family, all parts of the plant, including leaves, stalks/stems and tubers, are thought to contain different amounts of secondary metabolites. Farida et al. (2010) and Mankaran et al. (2013) stated that the results of the phytochemical analysis of taro leaf and tuber extracts contained secondary metabolites of flavonoids, alkaloids, triterpenoids, steroids, saponins, and tannins which act as antimicrobials. Previous research on mouse taro extract functions as an anti-bacterial, namely the bacteria *Pseudomonas aeruginosa* and *Salmonella choleraesuis* (Mohan et al., 2008). This study aims to test the effectiveness of extracts of leaves, stems and tubers of taro mice (*Typhonium flagelliforme* (Lodd)) as biofungicides against cause of leaf fall (*Pestalotiopsis sp.*) on rubber plants (*Hevea brasiliensis*).

Method

Sampling

Leaf samples were taken from rubber plants at PTPN III Karet Sei Putih, Galan District, Deli Serdang Regency, North Sumatra. Samples are put in plastic and labeled, and taken to the laboratory for further research.

Isolation of Pestalotiopsis sp.

Part of the plant showing leaf fall of *Pestalotiopsis sp.* leaves were taken, and cut into lengths of ± 0.5 x 0.5 cm and soaked in 70% alcohol for 1.5-2 minutes to reduce contaminants from other organisms. The pieces of rubber leaves are rinsed with distilled water and air-dried using a tissue. The rubber leaf pieces were then grown on PDA media in a petri dish and incubated for ±7 days at room temperature 26–28^{o}C. After incubation, microscopic identification of the conidia of the fungus was carried out (Barnet & Hunter 1972).

Antagonist Test of Mouse Tuber Extract Against Pestalotiopsis sp In Vitro

Test of the inhibition of rat taro leaf and tuber extracts against the fungus *Pestalotiopsis sp.* in vitro was carried out in a petri dish using the fungus culture method. Each extract of rat taro leaves, stems and tubers according to different treatment concentrations was mixed into PDA media. Treatment K0 (control -), K1 (Control +, Benlox 50 WP 0.2%), K2 (3% leaf), K3 (5% leaf), K4 (7% leaf), K5 (9% leaf), K6 (3% stems), K7 (5% stems), K8 (7% stems), K9 (9% stems) K10 (3% tubers), K11(5% tubers), K12 (7% tubers) and K13 (Tubers 9%). After that, in the center of the frozen PDA media, a piece of the *Pestalotiopsis sp.* with a 1 mm diameter cork borer. After incubation for 2 x 24 hours/day, the diameter growth of *Pestalotiopsis sp.* was observed. Drag power can be calculated by the formula:

$$T = \frac{D0 - Dn}{D0} \times 100\% \quad (1)$$

Description: T= inhibition rate (%), D0= diameter of fungal growth in control petri dishes, Dn= diameter of fungal growth in treated petri dishes.

Phytochemical Screening Test

Phytochemical screening was carried out to analyze the bioactive content which is useful for testing against pathogenic fungi. As for the phytochemical screening test of leaf powder, stems and tubers of rat taro, namely: flavonoids, tannins, safonins, alkaloids and steroids/triterpenoids.

Data Analysis

The antagonism test data obtained were analyzed using ANOVA and Duncan's test at 5% level to determine differences in test treatment using the SPSS Ver. program. 23.

Results and Discussion

Phytochemical Screening of Mouse Tuber Extract

Based on the results of the phytochemical screening test for leaves, stems and tubers of rat taro (*T. flagelliforme* L.) in Table 1 it can be seen that the leaves, stems and tubers of rat taro contain chemical compounds of flavonoids, tannins, saponins, alkoloids and steroids/triterpenoids. Results of Phytochemical Screening of methanol extract of leaves, stems and tubers of rat taro (*Typhonium flagelliforme* Lodd.) can be seen in Table 1.

Table 1. Results of Phytochemical Screening of Methanol Extract of Leaves, Stems and Tubers of Rat Taro (*Typhonium flagelliforme* Lodd.)

Secondary Metabolites	Result
Flavonoids	+
Alkaloids	+
Terpenoid/Streroid	+
Tanin	+
Saponin	+

Observation of Fungal Colony Diameter

The average growth diameter of the *Pestalotiopsis sp.* mushroom colony was treated with extracts of leaves, stems and tubers of rat taro (*T. flagelliforme* L.) on mushroom growth media 2-8 days after inoculation (HSI) during observations and the notation can be seen in Table 2 as follows.

Table 2. Data on Diameter (cm) of *Pestalotiopsis sp.* Mushroom Colonies at 2-8 Days After Inoculation (HSI) by Administration of Extracts of Leaves, Stems and Tubers of Mouse Taro (*Typhonium flagelliforme* L.)

Treatment	Data Diameter of Fungal colonies *Pestalotiopsis sp.* (cm)													
	2 HSI		3 HIS		4 HSI		5 HSI		6 HSI		7 HSI		8 HSI	
K0	2.53	ab	4.07	cde	4.72	cde	6.10	cde	7.25	cde	8.27	cde	8.50	cde
K1	0.50	mn	0.50	mn	0.50	n	0.50	n	0.50	n	0.50	n	0.50	n
K2	1.97	fg	3.27	fg	3.98	fg	5.05	fg	6.03	fg	7.03	fg	7.68	fg
K3	1.65	fghi	2.50	ghi	2.88	ij	3.60	jk	4.37	jk	5.17	jk	5.53	jk
K4	0.97	l	1.55	l	1.83	l	2.37	l	2.80	l	3.38	l	3.75	l
K5	0.80	lm	1.03	lm	1.70	lm	2.02	lm	2.27	lm	2.57	m	2.83	m
K6	2.78	a	4.48	a	5.50	a	6.73	a	8.33	a	8.50	a	8.50	a
K7	2.52	abc	4.22	ab	2.20	ab	6.72	ab	8.17	ab	8.50	ab	8.50	ab
K8	2.49	bcd	4.15	bcd	5.17	bcd	6.70	abc	8.07	abc	8.50	abc	8.50	abc
K9	2.48	cde	4.17	bcd	5.25	bcd	6.55	bcd	8.05	bcd	8.50	bcd	8.50	bcd
K10	2.02	f	3.55	f	4.00	f	5.42	f	6.17	f	7.23	f	7.88	f
K11	1.88	fgh	2.82	fgh	3.57	fgh	4.43	gh	5.30	gh	6.00	h	6.95	h
K12	1.60	ghij	2.43	ghij	3.28	hi	4.10	hi	4.93	hi	6.00	hi	6.83	hi
K13	1.43	ijk	2.33	ijk	2.87	ijk	3.67	hij	4.48	ij	5.28	j	5.92	J

Note: Numbers followed by the same letter in the same column are not significantly different at the level α = 0.05.

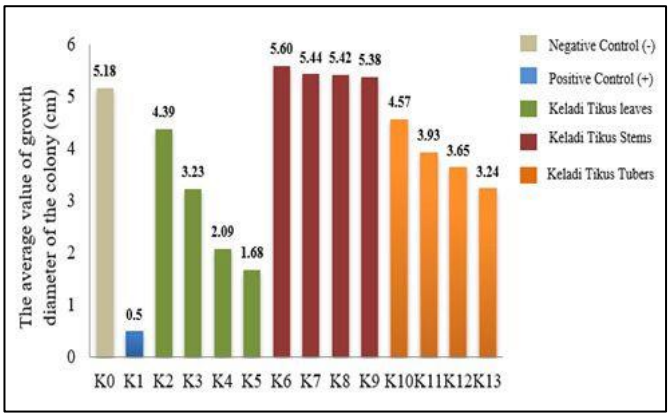

Figure 1. Stem diagram of the growth of the diameter of the *Pestalotiopsis sp.* mushroom colony against extracts of leaves, stems and tubers of rat taro (*Typhonium flagelliforme L.*)

Morphology Characteristics of Pestalotiopsis sp. Fungus Colonies

Based on the results of macroscopic identification of the morphological characteristics of the *Pestalotiopsis sp.* mushroom on the 8th day after incubation on PDA media, they are as follows.

Based on the research results of the rat taro phytochemical screening test showed the same results as the phytochemical screening test conducted by Nobakht et al. (2010) stated that the results of rat taro plant extract contained the most flavonoid and alkaloid compounds in the leaves and tubers. The same thing was done by Widowati & Mudahar (2009), Farida et al. (2010), Sukardi (2011), Sukiman & Nuriyanah (2016), and Hesananda et al. (2017) who showed that the chemical content of rat taro leaf extract, both powder and methanol extract, contained a class of flavonoids, saponins and steroid/triterpenoid compounds, and also Iswantini (2009) and Syahid et al. (2007) stated that the phytochemical tests on fresh and dry showed the presence of alkaloids, triterpenoids, saponins, and tannins. Flavonoid compounds are the largest group of polyphenol compounds present in rat taro leaves (Nobakht, et al., 2010). So that the class of flavonoids contained in the extract can damage the cell walls causing lysis of the fungal cell walls (Firdaus, 2015).

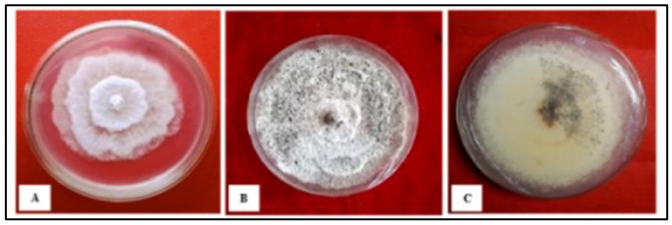

Figure 2. Macroscopic characteristics of Pestalotiopsis sp: a. mushroom colony at 4 HSI, b. top of mushroom colony at 25 HSI, c. bottom of mushroom colony at 25 HSI.

Tannins have a mechanism as an antifungal which damages cell membranes. This results in the cell not being able to carry out living activities so that fungal growth is inhibited and also hoff Saponin has activity as an antifungal by destroying proteins and enzymes from inside the cell so that the cell membrane is unstable. This can happen because the active substances found on the surface of saponins are similar to detergents, as a result saponins will reduce the surface tension of the fungal cell wall and damage the permeability of the fungal plasma membrane. This can result in fungal cell damage and also fungal cell death (Fosgerau & Hoffmann, 2015). According to Gholib (2009) alkaloids are also one of the compounds that have antimicrobial activity. Therefore, the alkaloids contained in plants (Osbourn, 1996). Steroids/terpenoids work by interfering with the growth and development of fungal spores due to the toxicity of the triperpenoid compounds (Ismaini, 2011).

Extracts of leaves, stems and tubers of taro mice (*T. flagelliforme* L.) have the potential as an antifungal, because the results of the phytochemical screening tests that have been carried out extracts of leaves, stems and tubers of taro mice (*T. flagelliforme* L.) contain active compounds secondary metabolites flavonoids, tannins, saponins, alkaloids and steroids/triterpenoids. Therefore, leaf, stem and tuber extracts of mouse taro (*T. flagelliforme* L.) can be used as a biofungicide against the cause of leaf fall (*Pestalotiopsis sp.*) on rubber plants (*Hevea brasiliensis*).

Table 2 shows the results of observations showing that the K5 treatment = 9% taro leaf extract had yielded results that inhibited the *Pestalotiopsis sp.* mushroom colonies in petri dishes, the 9% rat taro leaf extract treatment showed a very significant difference with K0 = negative control (-) without treatment and significantly different from several treatments K2 to K4, but the K5 treatment was not significantly different from the K1 treatment positive control (+) synthetic fungicide Benlox 50 WP 0.2%. K5 treatment of rat taro leaf extract (*T. flagelliforme* L.) 9% was the best treatment that could inhibit colony growth until day 8 (HSI). However, in the K2 treatment, 3% taro leaf extract (*T. flagelliforme* L.) was able to inhibit the growth of fungal colonies, but the K5 treatment had a better inhibition level. This is because the K5 treatment of rat taro leaf extract (*T. flagelliforme* L.) 9% is equivalent to the K1 treatment of positive control (+) synthetic fungicide Benlox 50 WP 0.2%.

In observing the growth diameter of the *Pestalotiopsis sp.* mushroom with the treatment of taro tuber extract (*T. flagelliforme* L.) in the mushroom growth medium, it had a very significant effect 2-8 days after inoculation (HSI). Based on the results of observations, the treatment of K10 = 3% taro tuber extract (*T. flagelliforme* L.) has produced results that can inhibit the colonies of *Pestalotiopsis sp.* fungi in petri dishes. Treatment of K10 = 3% taro tuber extract (*T. flagelliforme* L.) showed highly significant difference with K0 = negative control (-) without treatment, but not significantly different from treatment K11 = taro tuber extract (*T. flagelliforme* L.) 5% and 9% K13 treatment of rat tuber extract (*T. flagelliforme* L.) significantly different from treatment K0 = negative control (-) without treatment. K13 treatment of rat taro tuber extract (*T. flagelliforme* L.) 9% was the best treatment that could inhibit colony growth until day 8 (HSI). However, in the K10 treatment, 3% taro tuber extract (*T. flagelliforme* L.) was able to inhibit the growth of fungal colonies, but the K13 treatment had a better inhibition rate. This was because the K13 treatment of 9% taro tuber extract (*T. flagelliforme* L.) was more able to inhibit the growth of the diameter of the *Pestalotiopsis sp.* mushroom colonies compared to K0 = negative control (-) without treatment.

The results of observing the diameter of the growth of the fungus *Pestalotiopsis sp.* to the administration of the three treatments of extracts of leaves, stems and tubers of rat taro (*T. flagelliforme* L.) on PDA growth media showed that there was an effect on several different concentrations. This is presumably due to the ability of the fungus to grow and the effect of giving each of the different treatment concentrations causing the growth of a different diameter of the *Pestalotiopsis sp.* mushroom colony. This is shown in Figure 1, a graph of the growth of the *Pestalotiopsis sp*.

Based on the observations, Figure 1 shows the difference in diameter of the growth of the *Pestalotiopsis sp.* mushroom against the administration of the three treatments of taro leaf, stem and tuber extracts (*T. flagelliforme* L.) on PDA growth media, where the effect of giving several concentrations of taro leaf extract (*T. flagelliforme* L.) on PDA growth media was able to inhibit the growth of the *Pestalotiopsis sp.* fungus with 9% K5 treatment of rat taro leaf extract (*T. flagelliforme* L.) is the best treatment. This is because K5 with a concentration of 9% differed significantly from the K0 treatment = negative control (-) without treatment. Meanwhile, administration of several concentrations of rat taro stem extract (*T. flagelliforme* L.) on PDA growth media was not able to inhibit the growth of the fungus *Pestalotiopsis sp.* Based on observations from 2 HSI to 8 HSI, the average mushroom diameter did not show any difference in diameter between treatments. However, administration of several concentrations of taro tuber extract (*T. flagelliforme* L.) on PDA growth media was quite good at inhibiting the growth of the *Pestalotiopsis sp.* fungus with K13 treatment of taro tuber extract (*T. flagelliforme* L.) 9% was the best treatment.

The inhibition of the growth of the diameter of the *Pestalotiopsis sp.* mushroom colony was caused by the presence of secondary metabolite compounds which are antifungal in each part of the rodent tuber plant. The presence of antifungal compounds contained in a plant extract can potentially act as an inhibitor against fungal cells. Secondary metabolite compounds contained in the leaves, stems and tubers of rat taro (*T. flagelliforme* L.) according to the results of the phytochemical screening test showed the presence of chemical compounds in the form of flavonoids, tannins, saponins, alkoloids, and steroids/triterpenoids. The presence of secondary metabolite compounds contained in tuber plants (*T. flagelliforme* L.) is an antibacterial compound (Mankaran et al., 2013) and antifungal (Purnamasari, 2021; Gupita, 2021; Rusmin, 2019).

The mushroom culture of *Pestalotiopsis sp.* which has been successfully isolated on PDA media has a colony color that changes with increasing age of the culture, namely the color changes from white to creamy white. This can be seen in Figure 2A. The growth of the

fungal colonies was not perfect and the color of the colonies was white at 4 HSI, while in Figure 2B, the growth of the fungus *Pestalotiopsis sp.* perfect, this fungus produces lots of mycelium, creamy white colonies which over time black spots will appear and the base of the colonies is yellow-brown (Figure 2C).

Based on the results of research that has been done that the administration of extracts of leaves, stems and tubers of taro mice (*Typhonium flagelliforme* Lodd.) can be concluded that the active compounds contained in the extracts of leaves, stems and tubers of taro mice (*Typhonium flagelliforme* Lodd.) contain compounds the chemistry of flavonoids, tannins, saponins, alkoloids, and steroids/triterpenoids. Then the administration of extracts of leaves, stems and tubers of rat taro (*Typhonium flagelliforme* Lodd.) has a different ability to suppress the growth of the fungus *Pestalotiopsis sp.* in vitro. Administration of rat taro leaf extract (*Typhonium flagelliforme* Lodd.) affected the colony diameter, inhibition percentage and morphological characteristics of *Pestalotiopsis sp.* The results showed that K5 treatment of rat taro leaf extract (*Typhonium flagelliforme* L.) 9% was the best treatment that inhibited the diameter of colony growth until day 8 (HSI) with an inhibition percentage of 66.67% and the morphological characteristics of the fungus showed that the growth of the colony mycelium was visible loose and thin, rather smooth surface, has smooth margins, not even wavy, white colonies are thin and transparent, no discoloration on the surface of fungal colonies in old cultures. The administration of rat taro stem extract (*Typhonium flagelliforme* Lodd.) had a significant effect on the percentage of effectiveness of the inhibition of the growth of the *Pestalotiopsis sp.* mushroom, but had no significant effect on the colony diameter and morphological characteristics of the *Pestalotiopsis sp.* mushroom. Giving taro tuber extract (*Typhonium flagelliforme* Lodd.) had an effect on the diameter of the colony and the percentage of inhibition of the fungus *Pestalotiopsis sp*. The results showed that the K13 treatment of taro tuber extract (*Typhonium flagelliforme* L.) 9% was the best treatment that inhibited the diameter of colony growth until the 8th day (HSI) with an inhibition percentage of 33.39%.

Conclusion

Rat taro leaf extract (*Typhonium flagelliforme* Lodd.) has an effect on colony diameter, inhibition percentage and morphological characteristics of *Pestalotiopsis sp*. The results showed that the K5 treatment of rat taro leaf extract (*Typhonium flagelliforme* L.) 9% was the best treatment that could inhibit the diameter of colony growth until day 8 (HSI) with an inhibition percentage of 66.67% and the morphological characteristics of the fungus showed that the mycelium growth Colonies look tenuous and thin, the surface is rather smooth, has smooth margins, not even wavy, the colonies are thin white and transparent, there is no color change on the surface of the fungal colonies in old cultures. Stem extract of rat taro (*Typhonium flagelliforme* Lodd.) had a significant effect on the percentage of effectiveness of the inhibition of the growth of the *Pestalotiopsis sp.* fungus, but had no significant effect on the diameter of the colony and the morphological characteristics of the fungus *Pestalotiopsis sp*. Giving taro tuber extract (*Typhonium flagelliforme* Lodd.) has an effect on the diameter of the colony and the percentage of inhibition of the fungus *Pestalotiopsis sp*. The results showed that K13 treatment of taro tuber extract (*Typhonium flagelliforme* L.) 9% was the best treatment that could inhibit colony growth diameter until day 8 (HSI) with an inhibition percentage of 33.39%.

Author Contributions

Zulheri Noer conceptualized the research idea, design of methodology, management and coordination responsibility. Saipul sihotang conducted of research and investigated processes, literature review and analyzed data.

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Conflicts of Interest

The author declare no conflict of interest.

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