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The Effectiveness of *Kenikir* and Betel Leaves Extract as Bio Fungicide to the Causes of Anthracnose Disease (*Colletotrichum Capsici*) on Chili Plants (*Capsicum annum* L.) with In vitro

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Abstract *: Effectiveness Kenikir and Betle Leaft Extraction as Biofungicide to Cause Disease Antraknosa (Colletotricum capsici) On Chili (Capsicum annuum) in In vitro. The research was done in the Laboratory Protection Plant Agriculture Faculty University of Medan Area, was held since Mei to July 2018. The research use Design Random Complete (RAL) Non Factorial with treatment F0 = negative control (PDA Media 100 %) F1 = Positive control (Synthetic fungicide 0.2%), F2 = 20% kenikir+ 10%betel, F3 = 30 % kenikir+ 10%betel,, F4 = 40% kenikir+ 10%betel, F5 = 20% kenikir+ 20%betel, F6 = 30 % kenikir+ 20%betel, F7 = 40% kenikir+ 20%betel, F8 = 20% kenikir+ 30%betel, F9 = 30% kenikir+ 30%betel, F10 = 40 % kenikir+ 30%betel. The results of the study: the tested extract of kenikir and betel leaf leaves showed the same results for inhibiting the growth of colony diameter and percentage of fungi growth.*
Keywords *: effectiveness; Kenikir and betel leaves extract; anthracnose disease*

I. Introduction

Red chili is one of the horticultural commodities that has an important meaning because it has high economic in Indonesia, both as a commodity consumed domestically and as an export commodity. Red chili has a fairly high nutritional value generally used as a spice in cooking, medicines, cosmetics, coloring agents and industrial materials (Harpenas and Dermawan, 2011).

North Sumatra Province is one of the centers of red chili production in Indonesia. Based on data from the National Statistics Center of North Sumatra Province, it produced red chili in 2012 amounted to 197,411 tons, 2013: 161,933 tons, 2014: 147,812 tons (National Statistics Agency, 2016). When viewed from these data, red chili production experienced fluctuations in production, in 2014 the production of red chili in North Sumatra Province decreased by 14,123 tons or 8.72% compared to 2013. One of the causes of the decline in red chili production was due to anthrax. caused by *Colletotrichum* fungus and can cause crop losses of up to 65% (Hersanti et al., 2001). *Colletotrichum* mushrooms can infect the organs of red chili plants, especially the fruit. This fungal infection in red chili is characterized by initial symptoms of small spots that are blackish and slightly curved. Further attacks cause the fruit to shrink, dry and rot (Syamsudin, 2007).

Until now, most farmers still use fungicides to control the fungal pathogens. Continuous and excessive use of fungicides will result in disruption of the environmental balance and directly also very harmful to the health of consumers. One alternative in controlling anthrax disease is by using natural ingredients as bio fungicides, namely *kenikir* leaves and betel leaves.

Preliminary studies on phytochemicals of *kenikir* leaves showed the presence of active compounds of *flavonoid, saponin, terpenoid, alkaloid, tanin* and essential oils that have the potential as antimicrobials (Rasdi et al., 2010). Flavonoid compounds are polyphenol group

compounds that have the potential as antioxidants and have other benefits, namely as an antifungal agent (Harborne & Williams, 2000).

Nurhayati's research (2006) states that treatment with betel leaf extract gives the best results in terms of suppressing the growth of colony diameter and the number of conidia of C. *Capsici*, because the administration of betel leaf extract can kill the pathogenic fungi. Triono (2017) stated that betel leaf extract is able suppress the intensity of anthracnose. Betel without fractionation and betel fractionation with water solvents is comparable to the ability of *probineb* fungicides to suppress anthracnose disease intensity.

This is in line with Lestari's research (2014) which states that betel leaf extract can inhibit the growth of *Colletotrichum capsicidan* fungi compared to synthetic fungicides. The results of the LSD test showed that the treatment of 20% concentration of betel leaf extract was proportional to the effective concentration of 1% synthetic fungicide.

The Astutiningrum (2016) study states that *kenikir* leaf extract has anti-bacterial power. *Kenikir* leaf mash extract has the smallest bacterial inhibition zone at 30% concentration of 6.76 mm and the largest inhibition zone is 7.58 mm at a concentration of 60%. Ethanol extract of the leaves of *kenikir* 30% concentration has a inhibition zone of 7.25 mm and at a concentration of 60% at 8.59 mm. In accordance with the preliminary research conducted by the author, the use of *kenikir* extract as a bio fungicide on the *Colletotrichum capsici* fungus showed that the extract of effective *kenikir* leaves began at a concentration of 40%.

Based on the description above, the authors conducted a study on the effectiveness of *kenikir* and betel leaf extract as a bio fungicide against the causes of anthracnose (*Colletottrichum capsici*) in red chili (*Capsicum annuum L*).

II. Research Methods

The research was conducted at the Plant Protection Laboratory of the Faculty of Agriculture, Medan Area University and University of North Sumatera (USU) Laboratory from May to July 2018.

The research was carried out by the experimental method of conducting direct experiments and conducted in vitro. This study used a completely non-factorial randomized design (Non Factorial CRD) as for the levels of concentration factors of *kenikir* and betel extract as follows:

F0 = negative control (100% PDA media) + *Colletottrichum capsici*
F1 = Positive control (*Benlox* 50 WP 0.2% synthetic fungicide) + *Colletottrichum capsici*
F2 = PDA media with 20% *kenikir* leaf extract + 10% betel leaf extract + *Colletottrichum capsici*
F3 = PDA Media with treatment of 30% *kenikir* leaf extract + 10% betel leaf extract + *Colletotrichum capsici*
F4 = PDA media with 40% *kenikir* leaf extract + 10% betel leaf extract + *Colletotrichum capsici*
F5 = PDA media with 20% *kenikir* leaf extract + 20% betel leaf extract + *Colletotrichum capsici*
F6 = PDA Media with treatment of 30% *kenikir* leaf extract + 20% betel leaf extract + *Colletotrichum capsici*
F7 = PDA media with 40% *kenikir* leaf extract + 20% betel leaf extract + *Colletotrichum capsici*

F8 = PDA media with 20% *kenikir* leaf extract + 30% betel leaf extract + *Colletotrichum capsici*
F9 = PDA Media with 30% *kenikir* leaf extract + 30% betel leaf extract + *Colletotrichum capsici*
F10 = PDA media with 40% *kenikir* leaf extract + 30% betel leaf extract + *Colletotrichum capsici*

The work procedure in this study begins with the provision of 560 grams of *kenikir* leaf extract and 360 grams of betel leaf extract which has been dried and mashed, then immersed with methanol 12 L solvent for *kenikir* and 10 L for betel for 3 x 24 hours. The solution was filtered using filter paper, then evaporated using a vacuum rotary evaporator (Buchii / R205). The filtered liquid is put together and inserted into a weighed evaporating flask, then methanol is evaporated using a rotary evaporator at temperatures (45-50) ^{0}C, rotation speed (50 - 60) rpm, and low pressure (150-200) mm Hg. After evaporation is complete, the pumpkin containing the extract is weighed and the difference between the results of the two weighing is the weight of the extract to get the concentrated solution of the extract and add *aquades* with a ratio of 1: 1 (b / v) after being stored in a refrigerator (± 40C) for biological testing. The extract was then made extract dilution on the concentration treatment namely *Kenikir* 20% + 10% betel nut, 30% *kenikir* + 10% betel nut, *Kenikir* 40% + 10% betel nut, 20% *kenikir* + 20% betel nut, 20% betel nut + betel 20%,% , 40% tasting + betel 20%,%, 20% betel + 30% betel,% 30% + 30% betel nut, 40% + 30% betel nut in making agar media as much as 100 ml from each treatment. Next was isolation of C*olletotrichum capsici*, which was obtained from parts of the plant which showed symptoms of anthracnose attack. Then cut to size ± 0.5 x 0.5 cm² and soaked in 70% alkaline for 2.5 minutes to reduce contaminants of other organisms and then rinsed with distilled water and dried using tissue to grow in PDA media in petri dishes and incubated for ± 7 days in a 26-28oC temperature room.

After incubation then microscopic identification of conidial forms of fungi after culture was then obtained and then performed in vitro testing to determine the inhibitory test of leaf extracts of *kenikir* and betel leaves on *Colletotrichum capsici* carried out in petri dishes using the culture method of fungi, then prepared media culture according to the subsequent treatment incubation for 2 x24 hours a day then observing the parameters and the observational parameters consisted of phytochemical screening tests to analyze the bioactive content that is useful for testing anti-fungal pathogens. The phytochemical screening test of leaf powder *kenikir* and betel leaf, namely examination of *flavonoids, tannins, saponins, alkaloids, steroids / triterpenoids* and *glycosides*, while for culture, observation of colony diameter, and percentage of inhibition.

III. Result and Discussion

Phytochemical testing (screening) is one of the important steps in an effort to uncover the potential of plant resources. The results of the chemical content of phytochemical screening on the leaves of *kenikir* and betel leaf are in Table 1.

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Table 1. Results of phytochemical screening on methanol extract of *kenikir* leaves *(Cosmos caudatus)* and betel leaf *(Piper betle)*

No	Phytochemical Test	Result	Conclusion	
			Kenikir	betel
1	Flavonoid	Red, yellow, orange occurs in the amyl alcohol layer	+	+
2	Tanin	Blue or blackish green occurs	+	+
3	Saponin	Froth does not disappear as high as 1-10 cm	+	+
4	Alkaloida	2 of 3 reagents produce the same deposits	+	+
5	Steroid/triterpenoid	Purple or red occurs and then turns blue green	+	+
6	Glikosida	A purple ring is formed	+	+

Description: + (positive) = exists; - (negative) = none

Based on the results of the phytochemical screening test in Table 1 it can be seen that the leaves of *kenikir* and betel leaf contain chemical compounds of *flavonoids*, *tannins*, *saponins*, alkaloids, steroids / *triterpenoids* and *glycosides*. The results of this phytochemical screening test showed the same results with the phytochemical screening test conducted by Rasdi, et al. (2015) and Safita et al. (2017) that the phytochemical test results on *kenikir* leaf extract showed the presence of chemical compounds of *flavonoids, alkaloids, steroids / terpenoids, tannins / polyphenols, and saponins*.

Chemical compounds in these plants are known to have physiological activity as an antifungal. Anti-microbial activity of *terpenoids* by disrupting the growth and development of fungal spores due to the toxic properties of *triterpenoid* compounds (Ismaini 2011).

The extract of *kenikir* and betel leaf leaves has the potential to be anti-fungal, because it contains the active compounds of secondary *flavonoids*, *saponins*, *triterpeoid alkaloids* and *steroids* from the results of phytochemical screening tests that will be carried out for anti-pathogenic fungi causing anthracnose disease *(Colletotrichum capsici*) in red vitro (Capsicum annuum L.) in vitro.

Colony Diameter

Table 2. Diameter of Mushroom Colony Colletotrichum capsici in 2 to 8 days after inoculation (HSI) Treatment of Giving *Kenikir* Leaf and Betel Leaves

Treatment	Mushroom Colony Diameter *Colletotrichum capsici*(cm)													
	2 Hsi		3 Hsi		4 Hsi		5 Hsi		6 Hsi		7 Hsi		8 Hsi	
F0	1.23	B	1.97	B	2.72	B	3.18	B	4.00	B	4.67	B	5.2	B
F1	1	A	1	A	1	A	1	A	1	A	1	A	1	A
F2	1	A	1	A	1	A	1	A	1	A	1	A	1	A
F3	1	A	1	A	1	A	1	A	1	A	1	A	1	A
F4	1	A	1	A	1	A	1	A	1	A	1	A	1	A
F5	1	A	1	A	1	A	1	A	1	A	1	A	1	A
F6	1	A	1	A	1	A	1	A	1	A	1	A	1	A

F7	1	A	1	A	1	A	1	A	1	A	1	A	1	A
F8	1	A	1	A	1	A	1	A	1	A	1	A	1	A
F9	1	A	1	A	1	A	1	A	1	A	1	A	1	A
F10	1	A	1	A	1	A	1	A	1	A	1	A	1	A

Description: Numbers followed by the same letters in the same column are not significantly different at the level of α = 0.01

From Table 2 the data on the diameter of the fungus colonies treated with the extract of *kenikir* leaves and betel leaves gave a very significant influence on the diameter of the fungi colony *Colletotrichum capsici*. It can be seen from the results of observations of 2-8 hsi, namely the treatment of F2 extract of *kenikir* leaves and betel leaves with concentrations (20% + betel 10%) differed very significantly from treatment F0 which was without vegetable pesticide treatment. The F2 treatment was not significantly different from the F1 treatment of *benlox* pesticide 50 WP 0.2% and treatment F3 to treatment F10. These results indicate that all test treatments have the same ability as *Benlox* 50 WP 0.2% (F1) synthetic pesticides in suppressing the growth of the *Colletotrichum capsici* mushroom colony diameter.

Based on the observations in Table 2, it can be concluded that the administration of the leaves of *kenikir* and betel leaves can inhibit the growth of the diameter of the fungus colonies. This is presumably because the leaves of *kenikir* and betel leaves contain substances that can inhibit the growth of *Colletotrichum capsici* fungi such as *flavonoids, tannins, saponins, alkaloids, steroids / triterpenoids,* and *glycosides* as evidenced by the results of phytochemical analysis test leaves of *kenikir* and betel leaves. This also affects the percentage growth inhibition of the *Colletotrichum capsic*i fungus colonies.

Inhibition percentage

Table 3. Value of the percentage inhibition of fungi Colletotrichum capsici at 8 days after inoculation (HSI) Treatment of Kenikir Leaf Extract and Betel Leaves (%) (Transformation Results √x + 0.5)

Treatment	Average	Notation	
		0.5	0.1
F0	0.707	b	B
F1	9.013	a	A
F2	9.013	a	A
F3	9.013	a	A
F4	9.013	a	A
F5	9.013	a	A
F6	9.013	a	A
F7	9.013	a	A
F8	9.013	a	A
F9	9.013	a	A
F10	9.013	a	A

Based on Table 3 shows the percentage value of the treatment of *kenikir* leaves and betel leaf extract gave a very significant effect on the percentage of inhibition of the

Colletotrichum capsici fungus. It can be seen from the 8 values of inhibitory values, namely the treatment of F2 extract of *kenikir* leaves and betel leaves with concentrations (20% + betel 10%) different from the F0 treatment without vegetable pesticide treatment. The F2 treatment was not significantly different from the F1 treatment of *benlox* pesticide 50 WP 0.2% and treatment F3 to treatment F10. Fungal growth that does not occur due to physiological failure or tissue death

Antifungal compounds have various inhibitory mechanisms for fungal cells. Djunaedy (2008) states that antifungal compounds have a mechanism of action by neutralizing enzymes associated with invasion and colonization of fungi, damaging fungal cell membranes, inhibiting the fungal enzyme system so that it interferes with the formation of hyphae and influencing the synthesis of nucleic acids and proteins.

In line with the study of Astutiningrum (2016) states that *kenikir* leaf extract has anti-bacterial power at a concentration of 30% at 6.76 mm and the largest inhibition zone of 7.58 mm at a concentration of 60%. Whereas in previous studies conducted by single *kenikir* extract authors had a percentage of inhibitory power on the *Colletotrichum capsici* fungus at a concentration of 40%. This shows that *kenikir* extract has the potential as a bio fungicide. The results of the combination study of *kenikir* and betel extract as bio fungicides in F2 treatment (concentration of 20% 10% betel + betel leaf) shown in Tables 2 and 3 showed an increase in the effectiveness of inhibition of *kenikir* extract to a concentration of 20% and betel to 10%. The combination of *kenikir* extract and betel extract increases the active compound of secondary metabolites as bio fungicides including *flavonoids, saponins, tannins, alkaloids, triterpenoids* and *steroids*.

Flavonoid compounds are the largest group of polyphenol compounds. Flavonoids work by denaturing proteins to increase cell membrane permeability. Protein denaturation causes a disruption in cell formation, thus changing the composition of protein components (Wahyuningtyas, 2008). Cowan (1999) in Firdaus (2015), added that phenol compounds found in flavonoids can denaturate cell proteins and shrink cell walls causing lysis of fungal cell walls. In addition, phenolic compounds through hydroxy groups that will bind to the sulfihidril group of fungal proteins so as to be able to change the conformation of target cell membrane proteins resulting in impaired fungal cell growth can even experience death.

Saponins are water-soluble compounds and are like soap. Saponins are widespread in higher plants and have been detected in 70 plant families (Daniel 2006). Saponins are found as antimicrobials in nature. Saponin also has a function of biological activity as an antifungal (Kalaisezhiyen and Sasikumar 2012; Senthilkumar and Vijayakumari 2013). Wulansari (2009) also stated that saponin compounds have an antibacterial and anti-fungal effect. As an anti-fungal saponin, it results in microbial lysis cells which disrupt the stability of the cell membrane. The saponin mechanism as an anti fungus is the complex formation of saponins with sterols in the plasma membrane of fungi, then destroys semipermeable cells and causes death in fungal cells (Hoffmann 2003).

Tanin is an acidic polyphenol compound with a feeling of tightness. Tanin can be found in many plants and is spread in various plant organs such as stems, leaves and fruit. Tanin is anti-bacterial and anti-fungal. Tanin as an antifungal contributes a lot to plants to attack fungi and other microorganisms (Daniel 2006). The mechanism of tannin as an antifungal is inhibiting chitin synthesis which is used for the formation of cell walls in fungi and damaging cell membranes so that formation of fungi is inhibited (Watson and Preedy 2007).

While alkaloids are active substances from plants that function as drugs (Olivia, 2004). In general, plants that contain alkaloid compounds, physically can be identified with clear characteristics, such as gummy and bitter taste if tasted (Mustanir, 2013). According to Aniszewki (2007) in Gholib (2009), alkaloids are compounds that have anti-microbial activity, which inhibits esterase and also DNA and RNA polymerase, also inhibits cell respiration and plays a role in DNA intercalation.

Triterpenoid and steroid group compounds are known to have certain physiological activities as antifungal. Where the antifungal activity of terpenoids works by disrupting the growth and development of fungal spores due to the toxic properties possessed by triterpenoid compounds (Ismaini 2011).

IV. Conclusion

Effectiveness of extracts of kenikir leaves and betel leaves as biofungicides on the causes of anthracnose (Colletotrichum capsici) in vitro can reduce the growth of colony diameter and inhibit fungal growth. Inhibition of growth in colony diameter and percentage of fungal growth, all treatments tested for extracts of kenikir leaves and betel leaf showed the same results.

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