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Effectiveness Concentration of Betel Leaf Extract (Peperbetle L.) on the Growth of Fusarium sp. in Vitro

Jasmi, Lola Adres Yanti, Chairuddin, Eka Suri Marlinda and Yuliatul Muslimah

Abstract--- *Fusariumsp is a fungus that has an important influence on human life, because it acts as a pathogen in plants and humans, and produces toxins. Fusarium sp. causes the permeability of paremkin cells to increase, thereby losing osmotic efficiency resulting in wilting. Betel leaf extract contains phenyl propanoid compounds and tannins. This compound is antimicrobial and antifungal which is strong and can inhibit the growth of several types of fungi such as Escherichia coli, Salmonella and Fusarium sp. The study was conducted in December 2018 in the Plant Physiology Lab, Faculty of Agriculture, UTU Aceh Barat. Material used; distilled water, 96% alcohol, PDA media, betel plant and Fusariumsp, tools used; petri dishes, bunsen lamps, piset, measuring cups, spatulas, ovens, microscopes, autoclaves, blenders and aluminum foil. This research uses a non factorial Completely Randomized Design (RAL) which is the concentration factor of betel leaf extract (K) consisting of three levels, namely: K0: 0%, K1: 10%, K2: 20%, K3: 30%. The results showed the percentage of Fusarium sp. the largest was found in the concentration of betel leaf extract 30%.*

Keywords--- *Betel Leaf Extract, Concentration, Fusarium, sp.*

I. INTRODUCTION

1.1 Background

Fusarium mushroom spp. is one type of deadly soil-borne pathogen, because this pathogen has a strain that can be dormant (rested) for 30 years before continuing virulence and infecting plants. Fusarium wilt caused by *Fusarium* spp. Cases of this disease often occur in the lowlands. Generally, these plants will wither and die within 14-90 days. Water infiltration in badland or land with lots of water will increase the risk of this disease(Mukarlina, 2010).

Fusarium sp. causes the permeability of parents cells to increase, thereby losing osmotic efficiency resulting in wilting. Also, causing vascular tissue cells can not compensate for transpiration and can no longer maintain tissue turgidity ((Wibowo, 2005). The vessels of the vessels will turn brown due to phenols released into the vessel bundles. These phenols by the enzyme phenoloxidase produced by host plants will undergo polymerization into melanin which is brown(Agriculture, 2019).

The attack of *Fusarium* sp. In the field can reduce the quality and quantity of banana or chili production decreased Semangun (2001).

Jasmi, Agrotechnology Study Program, Faculty of Agriculture, Teuku Umar University. E-mail: jasmi@utu.ac.id
Lola Adres Yanti, Agrotechnology Study Program, Faculty of Agriculture, Teuku Umar University.
Chairuddin, Agrotechnology Study Program, Faculty of Agriculture, Teuku Umar University.
Eka Suri Marlinda, Agrotechnology Study Program, Faculty of Agriculture, Teuku Umar University.
Yuliatul Muslimah, Agrotechnology Study Program, Faculty of Agriculture, Teuku Umar University.

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Chemical control is usually the first choice for farmers in controlling fungi, because chemical fungicides are easier to obtain and practical in their application. However chemical fungicides have negative impacts on the environment and public health and even impacts on non-target microorganisms (Nasution, 2013).

Therefore, it is necessary to look for environmentally friendly controls, namely biological control using plant-based pesticides such as betel leaf extract.

Betel (Piper betle L.) has the potential as a vegetable fungicide that is safe for the environment and humans. Betel leaf extract, it contains essential oils up to 4.2%, phenylpropanoid compounds, and tannins. This compound is antimicrobial and antifungal which is strong and can inhibit the growth of several types of fungi, including Escherichia coli, Salmonella and *Fusarium*(Kursia, Sukriani Lebang, Julianri S Taebe, Burhanuddin Burhan, Asril Wa, O R Rahim, 2016). Effendi research results (2013) states that betel leaf extract 10% can suppress the growth of fungi colony diameter *Fusarium* sp. equal to 1.12 at 5 (HSI).

Based on the description above, it is necessary to research to find out the betel leaf extract, in inhibiting the growth of *Fusarium* sp.

1.2 Purpose

This research was conducted to determine the effectiveness of betel leaf extract on the growth of Fusariumsp

II. LITERATURE REVIEW

2.1 Fusarium sp.

Fusarium sp. is one of the filamentous fungus genums commonly found in plants and soils (Summerell, 2011). *Fusarium* spp. This fungus has a very small body size and its life is parasitoid which is an organism that depends on other organisms and is supported by warm soil temperatures and very low soil moisture. The population will increase if in the same place plant plants which are its host and this fungus infects plants through meristem tissue at the root tip.

This fungus is insulated mainly between cells, especially in wooden vessels. This fungus also forms mycelium which is found between cells, namely in the skin and in the parenchymal tissue near the site of infection.

In the medium potato dextrose agar (PDA) in a petri dish Fusarium sp. this forms conidium in a fruit body formed on the surface of the stalk or media. Macroscopic appearance by looking at the color of the colony, the shape of the colony, the surface of the colony, the growth pattern of the colony and the diameter of the colony that shows the same morphological characteristics of the colony was identified that Fusarium sp. has a white colony color, jagged colony shape, flat surface, colony and round growth patterns have a diameter of 0.5 mm. Yellow growth spreads and wavy surfaces form jagged colonies, colonized growth patterns and colony diameters of 0.4 mm (Inaly, 2003).

Fusarium mushroom spp. has a structure consisting of micronidia and macronidia. The surface of the colony is purple and the edges are jagged and have rough, stringy and bumpy surfaces. In nature, this fungus forms conidium. Branched conidiophores and macroconidium are crescent shaped, small-stemmed and often paired.

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This fungi plants well on various types of plants, namely with a pH range of 4.5-6.0 and on pure cultures with a pH range of 3.6-8.4. The life cycle of Fusarium sp. Experiences the pathogenesis and sporogenesis phases. In phasepathogenesis, fungi live as parasites on host plants. Fungi Fusarium sp. It can live at soil temperatures between 28 ° C, although this depends also on the isolates of mycelium fungi which are insulated mainly in cells, especially in banana, tomato, and chili plants. Also, these fungi form mycelium which is found between cells, namely in the skin and in the parenkim tissue near the site of infection in plants.

This fungus is a weak parasite which means it can only attack plants that are in a weak condition (sensitive) due to drought, nutrient deficiency, too much fruit.

This fungus is very suitable in acid soils which have a pH range of 4.5 to 6.0, this fungal attack is more determined by temperatures that are less favorable to the host plant. This fungus infects plants through the skin, lenticels, cuticles, wounds in plants and attacks through the plant tissue (Burlakoti R.R., S. Ali, G. A. Secor, S. M. Neate, 2008).

Fusarium sp. Is also influenced by pH, which is from the range of acidity of the soil that allows fusarium sp. grow and do its activities. Meanwhile, the temperature in the soil is closely related to the temperature of the air above the ground surface. Low air temperature will cause low soil temperature, Fungi Fusarium sp. able to live at soil temperatures between 28 ° C, although this also depends on the isolate function (R, 2002).

Table 1: Macroscopic characteristics of Fusarium sp.

isolate code	Colony Characteristic				
	Color	shape	Surface	Growth Pattern	Diameter (cm)
1	White	Jagged	Align	Colonize, round	0,5
2	White	Jagged	Align	Colonize, round	0,3
3	White	Jagged	Align	Colony, spread	0,2
4	Yellow	Wavy	Jagged	Colonize	0,7
5	Yellow	Wavy	Jagged	Colonize	0,4
6	Yellow	Wavy	Jagged	Colonize	0,3

As a primary pathogen, fungi can infect host tissues before there is another pathogenic fungal attack and can cause symptoms. As a secondary pathogen when fungi infect host plants after an attack of other pathogenic fungi, so the level of attack becomes so severe. Fungi can spread through the transportation of seeds and soil carried by wind or water and agricultural equipment. Pathogenic populations can survive naturally in the soil and the roots of diseased plants. If there are sensitive plants, if there is a wound at the root, the roots immediately infect it (Isnaini, M. Rohyadi, n.d.).

2.2 Betel Plant (Piper betle L)

Betel is an herbal plant, which extends to the height of the plant can reach 2-4 m. The stem of the plant is round and soft, broad, grooved and gray-green.

Betel has a single leaf and is located alternately with varying shapes ranging from round to oval, pointed leaf tips, leaf base heart-shaped or somewhat round asymmetric(Harman, 2016).

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The content of chemical compounds that are easily found in green betel leaves is essential oil compounds. Essential oils consist of hydroxycavikol, cavibetol, estragole, eugenol, metileugenol. Kavikol is the most abundant component in essential oils which gives the betel a distinctive odor. Kavikol is easily oxidized and can cause discoloration (Moeljanto, R. D., 2003).

In addition to essential oils, green betel leaves also contain phenolic compounds. Phenolic compounds are antioxidants that are commonly found in plants. Phenolic compounds are flavonoids, cinnamic acid derivatives, coumarin, tocopherols, and polyfunctional acids.

III. MATERIALS AND METHODS

3.1 Research Time and Place

This research was conducted in December 2018 at the Laboratory, Faculty of Agriculture, Teuku Umar Meulaboh University, West Aceh.

3.2 Materials and Tools

The materials used are aquades, cotton, 96% alcohol, PDA media, betel plants and Fusariumsp tools used; petridishes, bunsen lamps, tweezers, scales, measuring cups, spatulas, ovens, microscopes, autoclaves, Erlenmeyers, blenders, plastic wrap, and aluminum foil.

3.3 Research Design

The design used is a non-factorial Completely Randomized Design (RAL) consisting of 4 levels in 3 replications. Observed factors.

The concentration factor of betel leaf extract (K), consists of three levels, namely:

K0: 0%

K1: 10%

K2: 20%

K3: 30%

If the F test shows a real effect, then it is continued with a follow-up test, which is the real honest difference test (BNJ) at the 5% level.

3.4 Implementation of research

3.4.1 Equipment sterilization

Sterilized instruments are petridishes, tweezers, elemeyers and spatulas. Sterilization is done by soaking in the bayclin, after which it is rinsed with sterile water. Then each appliance, boiled with a stove until boiling water. Drain on a tissue by spraying alcohol and wrapped it in paper and then dried.

3.4.2 Fusarium Isolates sp.

Fungi Fusarium sp. obtained from the Laboratory of the Faculty of Agriculture and conducted Fusarium sp. using aquades to be easily observed under a microscope.

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Fusarium sp. Identification at the Teuku Umar University MIPA Laboratory using the Berner and Hunter identification book (1998).

3.4.3 Media Making

Prepare ingredients consisting of 200 grams of potatoes, 20 grams of sugar, 15 grams of agar, 2 grams of vancomyci and 1000 ml of distilled water.

Cook the potatoes until soft, then take the water from the boiled potatoes again by adding sugar, agar, and vancomyci, stirring until they boil. The sea of PDA was poured into a 1000 ml erlemeyer covered with cotton. Let stand until a little cold. then put it in the autoclave for 15 minutes at 120 ° C.

3.4.4 Making betel leaf extract

The extract used in this study came from betel plants. Betel leaves used fresh leaves and have been cleaned as much as 100 g mixed with distilled water as much as 100 ml and then blended (Hasanah 2017).

The solution of the betel leaf is then extracted using a filter tool, namely gauze. Betel plant extract obtained at a concentration of 100% and then diluted to get a concentration of 10% (10 ml extract + 90 ml media), 20% (20 ml extract + 80 ml media), and 30% (extra 30 ml + 70 ml media) .

3.5 Observations

1. Diameter of the colony

The isolate colony diameter was obtained by observing and measuring the growth diameter of Fusarium sp. which formed every day until 7 days after inoculation, the area of diameter of the growth of fungi isolate colonies was carried out by drawing a cross-line to measure the growth area of Fusarium sp. Use a transparent ruler and use the following formula:

The Diameter of Fusarium Sp. $Nr = \frac{N1 - N2X}{N1}$ 100%

Information: Nr = Percentage of barrier area (%) N1 = Growth of fungal colonies on media not given betel leaf extract N2 = Growth of fungal colonies in media which were given betel leaf extract

2. Percentage of growth inhibition of Fusarium sp. calculated according to the formula: Percentage of fungi Fusarium sp. = (Control - treatment) x 100% Control

3. Shape Fusarium sp.

This test was carried out to see the interaction of Fusarium sp. in observing this form of interaction is done after the meeting of both ends of Fusarium sp.

when growth encircles all media in the petri dish and observed under a microscope (40x10) the form of the interaction growth of fungi Fusarium sp. in the antagonist of its growth acceleration. Observations were made after Fusarium sp. applied to PDA media one day after planting Fusarium sp. The treatment was observed with the following diameter.

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IV. RESULTS AND DISCUSSION

4.1. Isolate Colony Diameter

The average diameter of *Fusarium* sp. Isolates. at various extra concentrations of betel plants after being tested with BNT0.05 are presented in Table 2.

Table 2: Average diameter of Fusarium sp. Isolates. age 1, 2, 3, 4, 5, 6 and 7 days after isolation at various extra concentrations of betel plants

Isolation Day to	Extra Concentration of Betel Leaves				BNT 0,05
	0% (Control)	10 % (K_1)	20% (K_2)	30% (K_3)	
1	13,32 b	12,60 ab	11,51 a	11,24 a`	1,23
2	15,21 b	13,96 b	12,38 a	11,74 a	1,56
3	18,95 c	15,92 b	14,18 a	13,62 a	1,42
4	19,56 c	17,12 b	16,04 ab	15,29 a	1,55
5	20,85 c	18,75 b	17,32 a	16,53 a	1,03
6	21,88 c	19,65 b	18,41 a	17,65 a	1,19
7	22,88 c	20,46 b	19,55 b	18,41 a	0,91

Note: Numbers followed by the same letters on the same line that are not significantly different in the BNT0.05 test.

Table 3 shows that the diameter of *Fusarium* sp. The smallest on the day of 1-6 HSI observation was found in the treatment of betel leaf extract concentration of 30% (K3) which was not significantly different from the treatment of 20% (K2), but significantly different from 10% (K1) and 0% (Control). This is because the betel leaf extract contains phenols as anti-microbial substances contained in the betel leaf extract which can damage the fungal cell wall of *Fusarium* sp. causing slow mold growth. Furthermore, Ingram (1981) in (Arsih D W J Panggeso, 2015)explained that phenol compounds were able to break the peptidoglycan cross-linkage in an attempt to break through the fungal cell wall.

The results showed that the greater the betel leaf concentration, the *Fusarium* sp. getting smaller. This is consistent with the results of the study of(Sastrawati, 2011), that different betel leaf extract concentrations influence the growth of *Fusarium* sp. Mushroom diameter, which the greater the concentration of betel leaf extract, the growth of the diameter of Fusarium sp. the cause of Fusarium wilt in chili plants, is getting smaller. This means that increasing the concentration of betel leaf extract causes increased inhibition of betel leaf in suppressing the fungus *Fusarium* sp. In the treatment of betel leaf extract concentrations were compared without betel leaf extract growth of *Fusarium* sp. faster. This can be seen in the results of research in which the diameter of the fungus *Fusarium* sp. highest in the treatment without betel extract (K0). The greater the concentration of betel leaf extract given is suspected to be the higher phenol content and the resulting reaction will be stronger.

The results of Koesmiati research (1966) in (Arsih D W J Panggeso, 2015) showed that 82.8% of the constituent components of betel leaf essential oil consisted of phenol compounds, and only 18.2% were non-phenol compounds.

4.2. Percentage of Obstacles

The average percentage of inhibition fungi *Fusarium* sp. at various extra concentrations of betel plants after being tested with BNT0.05 are presented in Table 3.

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Table 3: The average percentage of inhibition of Fusarium f. age 1, 2, 3, 4, 5, 6 and 7 days after isolation at various extra concentrations of betel plants

Isolation Day to	**Extra Concentration of Betel Leaves**				BNT 0,05
	0% (Control)	10 % (K_1)	20% (K_2)	30% (K_3)	
1	0,57 a	18,54 b	29,61 c	31,97 c	10,68
2	0,57 a	22,56 b	34,85 c	38,78 c	9,22
3	0,57 a	32,00 b	40,79 c	43,40 c	6,69
4	0,57 a	28,19 b	33,64 bc	37,75 c	7,56
5	0,57 a	25,42 b	32,99 c	36,87 c	4,89
6	0,57 a	24,82 b	31,75 c	35,39 c	6,25
7	0,57 a	25,84 b	30,33 c	35,51 d	4,25

Note: Numbers followed by the same letters on the same line that are not significantly different in the BNT0.05 test

Percentage of inhibition of *Fusarium* sp. the largest on the day of observation 1-7 HSI was found in the treatment of betel leaf extract concentration of 30% (K3) which was not significantly different from the treatment of 20% (K2), but significantly different from 10% (K1) and 0% (Control), it can be seen in Table 4.

Increasing the percentage of fungi inhibition *Fusarium* sp. in the treatment of betel leaf extract, it is suspected that betel leaf extract contains antimicrobial substances so that it can suppress the area of *Fusarium* sp. This is consistent with the results of research by(Hasanah, 2017)also reported that betel leaf extract was able to suppress the fungus *Fusarium* sp. causes of *Fusarium* wilt in chili plants. According to (Windriyati YK Wahyuningrum, 2007), the ability of betel leaf extract as an antimicrobial agent is related to the content of betel leaf extracts such as Flavonoids, Polyphenols, Tannins and essential oils.

From the results of research that has been done, it can be seen that the area of *fusarium* f. where the treatment without betel extract (K0) can cover the entire surface of the cup this can be seen in Figure 1.

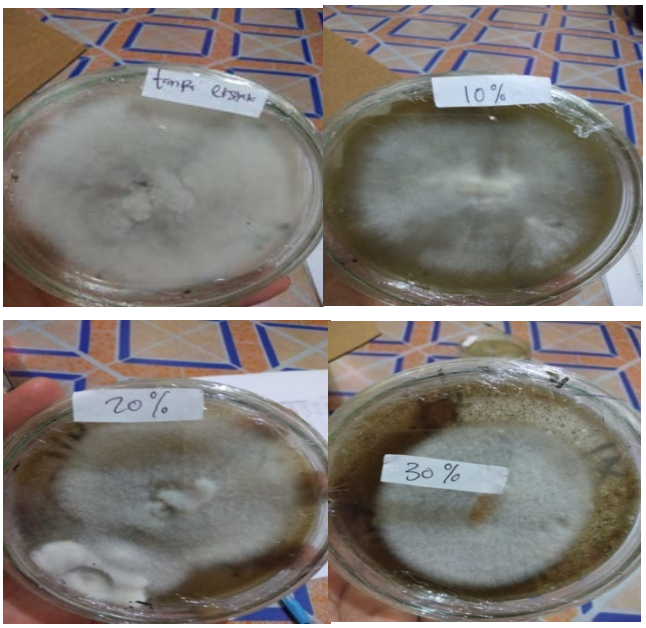

Figure 1: Fusarium fungi resistance area sp. on the 7th day

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From the picture above it can be seen that the area of inhibition on day 7 of *Fusarium* sp. in the treatment with betel leaf extract with various concentrations can inhibit the development of the fungus *Fusarium* sp. Whereas in the treatment without betel extract the development of *Fusarium* sp. cover the entire surface of the cup. According to (Arsih D W J Panggeso, 2015), that the betel leaf extract contained phenol as an anti-microbial agent which could inhibit the growth of the fungus *Fusarium* sp. by damaging the *Fusarium* sp. cell wall, causing fungal growth to slow.

4.3. Form of Fusarium sp.

The observations obtained by the general results of the growth of *Fusarium* sp. at various extra concentrations of betel plants can be seen in Figure 2.

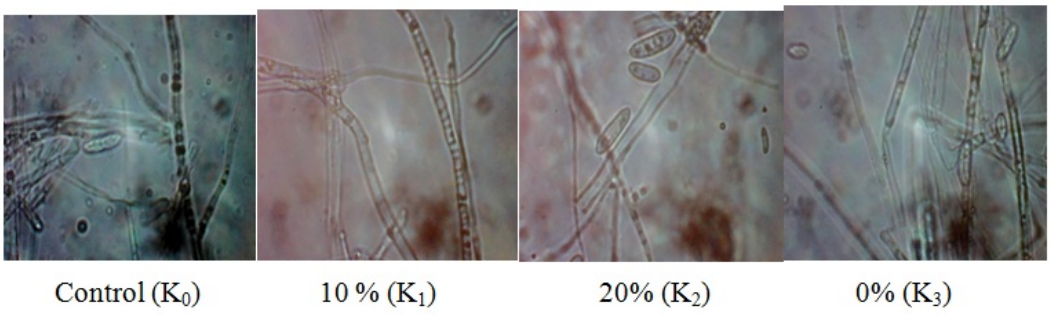

Figure 2: Macroscopic observation

The results of macroscopic observations showed that the shape of the fungus Fusarium sp. many branches are flat, insulated. The fungus Fusariumsp is white in color and has a rounded texture, forming a mass. fungus sporulation Fusarium sp. the number of branching control treatments more than the betel leaf extract treatment.

V. CONCLUSIONS

Based on the results of the study that the best betel leaf extract concentration was found in 30% concentration with the highest level of inhibition.

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