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The Influence of pH and Temperature on Development of Pathogens of Dieback Disease on The Nutmeg Tree In Aceh Selatan In Vitro

Susanna[1], Meity S. Sinaga[2], Suryo Wiyono[3] and Hermanu Triwidodo[4]

Research Scholar[1] and Professor[2-4]

Department of Plant Protection

Agriculture Faculty of Syiah Kuala University

Banda Aceh, Aceh

Indonesia

ABSTRACT

The ability of microorganisms to grow and stay alive is an important thing to know. Factors that play a role in the development of life of the pathogen can be used as a bases to inhibit the development or decrease inoculums population in the field. The objectives of this study were to determine the effect of pH and temperature in vitro. Four of pathogenic fungi (Lasiodiplodia theobromae, Graphium euwalaceae, Fusarium solani, dan Rigidoporus microporus) were tested for growth at pH media include 3, 4, 5, and 6 while the effect of temperature include 16, 22, 28, and 34 ^{o}C. The results showed that L. theobromae had a wide range of pH and temperature compared to three other fungi. Optimum pH medium for mycelia growth is 4 to 6 and the optimum temperature is 28 ^{o}C. The lower the pH causes the mycelial growth to be inhibited, as well as the temperature. When the temperature is lowered or increased, the mycelial growth is disturbed.

Key Words: *Fusarium solani, Graphium euwallaceae, Lasiodiplodia theobromae, pH, Rigidoporus microporus, temperature.*

1. INTRODUCTION

Nutmeg developed by the people of Aceh Selatan is faced with new diseases that are expanding rapidly. The disease initially attacks twigs, branches and stems. Almost all farmers do not know the pathogens that attack and how to control them so that the disease continues to develop. As time goes by, the incidence of this disease continues to spread in Aceh Selatan District with the incidence of the disease already in the range of 5 - 100% [9]. Therefore, it is feared that the nutmeg epidemic continues to grow and causes nutmeg plants to become extinct in Aceh Selatan. There is currently no control strategy that effectively controls the dieback disease of the nutmeg tree in Aceh Selatan. Losses due to the disease are significant enough for the welfare of the farming community. In 2014 the number of people involved in nutmeg plantation reached 18 165 households and the level of nutmeg production reached 7 565 tons of yielding crops, namely 9 226 [2]. The impact of a pathogen attack is not only on farmers but also on the state. Most of the nutmeg seeds produced by farmers (70-75%) are designated as export commodities. Seeing this fact, the country's foreign exchange is potentially large enough to be lost. According to Harni *et.al*., yield loss due to dieback disease can reach 70%. This large level of loss is partly due to direct and indirect effects as a result of plant growth disturbances [9]. Based on Koch's postulate test, morphological and molecular identification, dieback disease on the nutmeg tree can be caused by *Lasiodiplodia theobromae*, *Fusarium solani*, *Graphium euwallaceae*, and *Rigidoporus microporus* [10].

The development of disease in the plant population is closely related to physical environmental factors namely temperature and pH. Kausar *et al*. stated that differences in temperature and media could influence the growth of mycelia of *L. theobromae* and *F. solani* cultured on PDA (potato dextrose agar) and WA (water agar) media. Responding to physical environmental factors is very important in order to control pathogen attacks in plants [8]. Information about dieback pathogens that attack the nutmeg tree in Aceh Selatan has never been present and published, so it becomes important to conduct research on physical environmental factors that influence the development of dieback causes in the nutmeg tree, and this can be used as a basis for developing control strategies. Based on the description above, it is necessary to conduct a study that aims to determine the effect of pH and temperature on in vitro growth of dieback pathogens in the nutmeg tree in Aceh Selatan.

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2. EXPERIMENTAL/RESEARCH WORK

The study consisted of in vitro testing of pH and temperature treatment for the growth of pathogenic mycelia.

Effect of pH of Media and Temperature ob Growth of Pathogen in Vitro

Test of pathogenic isolates (*L. theobromae*, *G. euwallaceae*, *F. solani*, and *R. microporus*) were cultured on PDA for 4 days. Especially for pH treatment, the composition of the PDA became increased so that the test media remains solid at low or high pH. Addition so that the media more and more if the pH is lower. Setting the pH of PDA of media according to the treatment, carried out by adding 2% HCl solution. Addition of HCl is done before media sterilization. The pH treatment consisted of pH 3, 4, 5, and 6. Each treatment was repeated 3 times. The pH value of the media is determined using a pH meter. Temperature treatment using PDA medium with pH 6.8. On each medium in the petri dish, a disc-shaped diameter of mycelia culture is ± 0.5 cm in diameter in the middle. The temperature treatment is carried out by placing a petri dish that has been inoculated with a culture of pathogenic mycelia in an incubator. The treatment temperature level consisted of 16 ^{o}C, 22 ^{o}C, 28 ^{o}C, and 34 ^{o}C.

Observations made in the form of measurements of the diameter of the growing colonies. Measurements are carried out starting one day after inoculation (dai) until the colony one of the treatment levels reaches the edge of the petri dish. Colony diameter measurements were carried out on each treatment (influence of temperature and pH).

Processing colony diameter data from each treatment using SAS program Anova 9.1. Duncan's multiple range test = 0.05 was done to see the possibility of differences between isolates at the level of each treatment.

3. RESULTS AND DISCUSSION

3.1 Growth of pathogenic fungi at various pH medium

The pH level of the media has a very real effect on the mycelia of the pathogen. The lower the pH of the media, the growth of fungal mycelia is inhibited. Each isolate tested showed differences in the development of colony diameter with different pH of the media. The growth of L. theobromae colony diameter can grow well from pH 4 to 6, and can fill up the petri dish on the third day when compared to the three other pathogenic fungi (Figure 1). The figure shows that *L. theobromae* can grow at a wide pH range. However, when the pH drops to 3, the development of the colonies is hampered. This fact is in accordance with the results of research conducted by Saha *et al.* who reported that *L. theobromae* can grow in the range of pH 3-8, optimum growth at pH 6 [4]. While Febbiyanti reported that the optimum growth of *L. theobromae* from rubber plants occurred at pH 5.56 [7]. Each fungus has the power to grow, and has a certain pH range. Media acidity will affect the availability of minerals and nutrients that can be absorbed by the fungus, and also the work of enzymes produced by fungi in helping to destroy the substrate that can be used for its growth. The media environment for growing fungi which is classified as acidic (pH ≤ 4) causes some nutrients such as K, S, Mo, N, Ca, Mg, and P to be less able to be used by fungi. The indirect effect of pH is on the surface performance of fungus cells. Fungus to grow well requires large amounts of nutrients such as C, H, O, N, S, P, Mg, K as well as micro elements which are useful as enzyme cofactors and functional proteins [6]. Usually the solubility/availability of nutrients will be high if the pH of the media is in neutral conditions, ranging from 5-6.8.

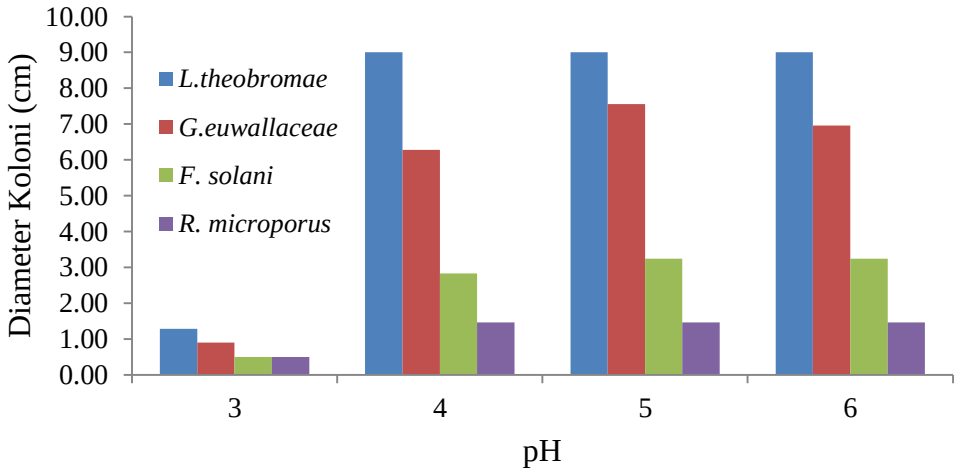

Figure 1 Effect of media pH on the growth of dead pathogenic fungi with nutmeg in Aceh Selatan on the third day

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The effect of pH on the media also occurs on solubility of anions and cations, and this will certainly determine the ability of fungi to absorb. At neutral pH the solubility of some cations such as Fe, Zn, and Ca increases so that is easily absorbed by fungi. If the pH is acidic the absorption of cations by hyphae is disrupted, because on the surface of the hyphae it will be occupied by H ions [12]. In *Trichoderma reesei* pH plays a role in its ability to produce cellulase enzyme components (endoglucanase, exoglucanase and β glucoside). This enzyme is used to destroy substrate containing cellulose [1].

3.2 Growth of pathogenic fungi at various temperatures

The growth of mycelia is different from isolates that differ from temperature differences, with isolates that can grow well are *Lasiodiplodia theobromae*. The optimum growth of *L. theobromae* occurs at a temperature of 28 ^{o}C, if lower or higher growth is disturbed. Treatment of temperatures above 34 ^{o}C can inhibit growth more than if it is at a low temperature. Almost the same as in the pH treatment of the media, between new isolates show a difference if the ambient temperature is not good for growth. At a temperature of 16 ^{o}C or 34 ^{o}C there is a difference between the isolates used in the test. The results of the diversity analysis also show that the effect of temperature causes a difference between the temperature of 28 ^{o}C and 34 ^{o}C. At low temperatures (16 ^{o}C) there was no difference between isolate colonies (Figure 2). Temperature will have a major effect on the growth of fungus colonies.

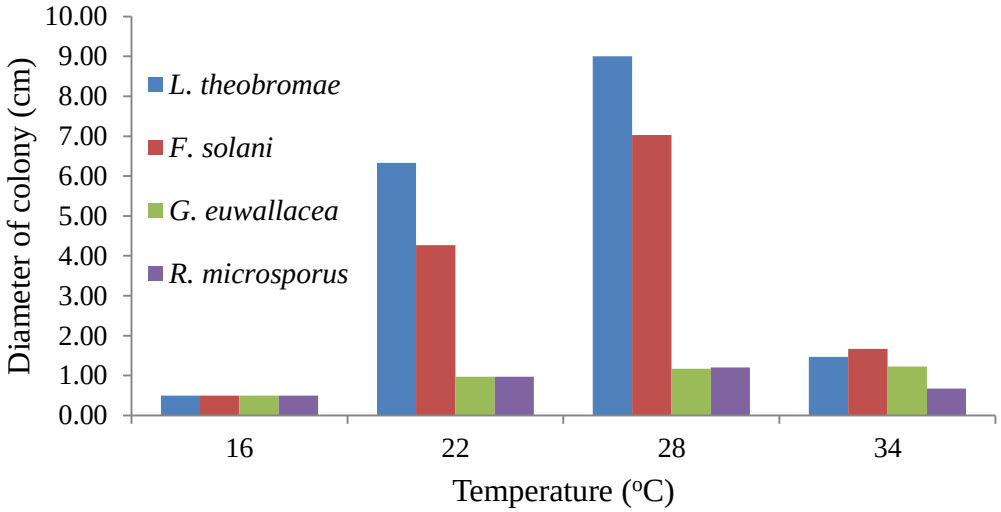

Figure 2 Effect of temperature on the growth of pathogenic fungus which causes dieback disease on the nutmeg tree in Aceh Selatan on the second day

The results of testing the effect of temperature showed that there was an increase in growth from a temperature of 22 ^{o}C to 28 ^{o}C but there was growth inhibition if the temperature rose to 34 ^{o}C. Temperature can increase the growth of pathogenic fungal colonies radial to a certain range, and after that can inhibit growth. The effect of temperature is not only on mycelia growth, but also on spore germination, reproduction and some metabolic processes. Among these metabolic activities are respiration, antibiotic production and vitamin synthesis [12]. Increased metabolism will have an impact on mycelial growth. Increased metabolism causes the absorption of nutrients also increases so that it will result in the growth of fungi.

The fungus responds to the influence of temperature can be different but has the same pattern, growth will stop if the temperature continues to be increased or vice versa. Research report Alam *et al*. stated that Botryodiplodia theobromae from bananas grew at an optimum temperature of 25-30 ^{o}C, which was tested in the temperature range of 10-40 ^{o}C [5]. According to Kausar *et al.*, *L. theobromae* and *Fusarium solani* are very good to grow at a temperature range of 25 - 30 ^{o}C and will decrease if the temperature is lower of higher [8]. While the research of Fovo *et al.* reported that *L. theobromae* can grow well at 21, 23 and 28 ^{o}C at PDA media with an optimum temperature of 23 ^{o}C and cannot grow at 33 ^{o}C. While the optimum temperature for the growth of *F. oxysporum* is 28 ^{o}C [3]. Jet and Ahir stated that, *F. solani* showed high radial mycelia growth at optimum temperatures, but if it was above or below the optimum temperature growth would be inhibited [7].

Observations of the time needed for fungus colonies to meet petri also varied at different temperatures for each type of fungus, which *L. theobromae* took 2.00 ± 0.00 days at 28 ^{o}C and increased with temperature decreasing or increasing (Table 1). This shows that *L. theobromae* takes a shorter time/grows and develops in the same temperature range when compared to the three causes of dieback disease on the nutmeg tree in Aceh selatan.

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Table 1 Effect of temperature on the growth of pathogenic fungal colonies (days)

Fungus	Time of colony growing (days)			
	16 °C	22 °C	28 °C	34 °C
L. theobromae	16.67 ± 0.58 e	3.67 ± 0.58 k	2.00 ± 0.00 l	5.67 ± 0.58 j
G. euwallaceae	18.67 ± 0.58 c	3.67 ± 0.58 k	4.00 ± 0.00 k	5.67 ± 0.58 j
F. solani	20.67 ± 0.58 b	9.67 ± 0.58 f	5.67 ± 0.58 j	7.67 ± 0.58 h
R. microporus	50.67 ± 0.58 a	8.67 ± 0.58 g	6.67 ± 0.58 i	17.67 ± 0.58 d

The numbers followed by the same letter are not significantly different after being tested by Duncan's multiple distance test (DMRT) α = 5%; n = 3; The average time needed for the fungus colony to fill up the petri dish (days).

4. CONCLUSION

Based on the testing of pH and temperature on the growth of colonies of four fungi that cause dieback disease on the nutmeg tree in Aceh Selatan, *L. theobromae* is more dominant in a wide range. The optimum growth of fungus *L. theobromae* in vitro was at pH 4-6 and temperature of 28 oC. The time needed by *L. theobromae* to fill up the media is 2.00 ± 0.001 days.

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