Aktivitas antijamur in vitro ekstrak kulit jeruk nipis (*Citrus aurantifolia* (Cristm.) Swingle.) Terhadap *Fusarium oxysporum* Schlecht. em. Snyd. & Hans.

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Abstrak

Fusarium layu pada tanaman disebabkan oleh *Fusarium oxysporum*. Pencegahan penyakit layu Fusarium dengan menggunakan fungisida sintetik menyebabkan berbagai masalah di lingkungan dan kesehatan manusia, maka perlu diubah menjadi fungisida alami seperti kulit jeruk nipis. Penelitian ini dilakukan untuk mengetahui persentase aktivitas antijamur pada konsentrasi optimum ekstrak etanol kulit jeruk nipis (*Citrus aurantifolia*) untuk menghambat pertumbuhan *Fusarium oxysporum*. Ekstrak kulit jeruk nipis diperoleh dari proses maserasi menggunakan pelarut etanol 96%. Uji aktivitas antijamur menggunakan metode keracunan makanan dengan parameter diameter miselium yang tumbuh di media. Konsentrasi ekstrak etanol kulit jeruk nipis yang diuji adalah 0%, 15%, 30%, 45%, dan 60%, dan kontrol positif yang digunakan adalah fungisida antracol sintetik 0,3%. Hasil penelitian menunjukkan konsentrasi optimum ekstrak etanol kulit jeruk nipis untuk menghambat pertumbuhan *Fusarium oxysporum* adalah konsentrasi 15% dengan persentase aktivitas antijamur adalah 56,34%. Ini karena konsentrasi adalah konsentrasi terendah yang dapat menghambat pertumbuhan *Fusarium oxysporum* dengan tingkat aktivitas yang kuat.

Kata kunci: *Citrus aurantifolia, Fusarium oxysporum, aktivitas antijamur*

Abstract

Fusarium wilt in plants is caused by *Fusarium oxysporum*. Fusarium wilt disease prevention using synthetic fungicides cause various problems in the environment and human health, it is necessary to change to natural fungicides like lime peels. This study was conducted to determine the percentage of antifungal activity at the optimum concentration of ethanol extract of lime peel (*Citrus aurantifolia*) to inhibit the growth of *Fusarium oxysporum*. Lime peel extract was obtained from the maceration process using 96% ethanol solvent. Antifungal activity test uses a food poisoning method with mycelium diameter parameters that grow on the media. The concentration of ethanol extract of lime peels tested was 0%, 15%, 30%, 45%, and 60%, and the positive control used was synthetic antracol fungicide 0.3%. The results showed the optimum concentration of ethanol extract of lime peel to inhibit the growth of *Fusarium oxysporum* was a concentration of 15% with a percentage of antifungal activity is 56.34%. This is because the concentration is the lowest concentration that can inhibit the growth of *Fusarium oxysporum* with a strong level of activity.

Keywords: *Citrus aurantifolia, Fusarium oxysporum, antifungal activity*

INTRODUCTION

Most of the genus Fusarium is a saprophytic fungus, but some are parasitic, especially in plants. *Fusarium oxysporum* is a species of Fusarium fungus that is commonly found as a cause of vascular wilt disease or often known as fusarium wilt disease (Kidd *et al*, 2016). Fusarium wilt has become a major limiting factor in the production of many agricultural and horticultural crops including bananas, cabbage, and beans (Kadja, 2013). The fungus infects plants through the roots mainly through wounds, settles and develops in the bundle of vessels until the network of dead vessels. Fusarium fungi from purplish-white spores on the infected roots in moist air. Spore spread can occur through the wind, water, irrigation and agricultural equipment (Semangun, 2001). A common treatment of Fusarium wilt disease used a synthetic fungicide. However, the use of synthetic fungicides causes many problems that are detrimental to humans or the environment because they cause residues attached to plants that interfere with human health. Therefore, prevention of plant diseases caused by fungi, that are environmentally friendly needs to be done, one of them with natural fungicides from the peels of lime fruit. Natural fungicides have the advantage of being biodegradable, easy to apply, easy to obtain and safe for the environment and human health. Lime peels have the main content of essential oils, which have the antifungal activity to control the growth of pathogenic fungi. In addition to essential oils, there are also other compounds in the peels of lime fruit, namely flavonoids, tannins, alkaloids, and saponins (Okwu *et al*, 2007). This study was conducted to determine the percentage of antifungal activity at the optimum concentration of ethanol extract of lime peel (*Citrus aurantifolia*) to inhibit the growth of *Fusarium oxysporum*.

METHOD

Materials used in this research are *Fusarium oxysporum*, lime peels, PDA (*Potato Dextrose Agar*) media, 70% alcohol, 96% ethanol, distilled water, and synthetic *antracol* fungicide.

Lime peels extraction

Extraction of lime peels using maceration method with 96% ethanol solvent. First, the lime peels are dried in an oven at 60ºC for 15 hours, then mashed to powder. A total of 200gr of lime peels powder was macerated with 1000ml of 96% ethanol for 24 hours. The maceration filtrate was evaporated using a rotary evaporator at a temperature of 60ºC with a

rotation speed of 100 rpm, then evaporated again using a water bath to obtain a thick extract of lime fruit peels.

Antifungal activity test

The design used was a completely randomized design with 5 variations of the ethanol extract concentration of lime peels (0%, 15%, 30%, 45%, 60%), and 4 replications. The activity of ethanol extract of lime peels on *Fusarium oxysporum* was carried out by food poisoning technique. As much as 5 ml of PDA medium in the test tube was thawed, then mixed with 1 ml of ethanol extract of lime peels at various concentrations. A mixture of PDA and ethanol extract of lime peels was poured into a sterile petri dish and allowed to condense. Pure *Fusarium oxysporum* culture is printed in a circle. Culture is placed in the middle of a 9 cm diameter petri dish which already contains the medium and extract. Incubation is carried out for 12 days. Observations were made on the diameter of the fungus mycelium. The data then analyzed using Analysis of Variance (ANOVA) using SPSS 22. The results that showed real differences were continued with the Duncan test at 95% confidence level.

RESULTS AND DISCUSSION

The diameter of *Fusarium oxysporum* mycelium was measured until the twelfth day because the fungus that grew at 0% concentration (negative control) had almost filled the petri dish. The mean diameter of *Fusarium oxysporum* mycelium was calculated every day for each treatment. The diameter of *Fusarium oxysporum* mycelium is presented in Figure 1 as follows.

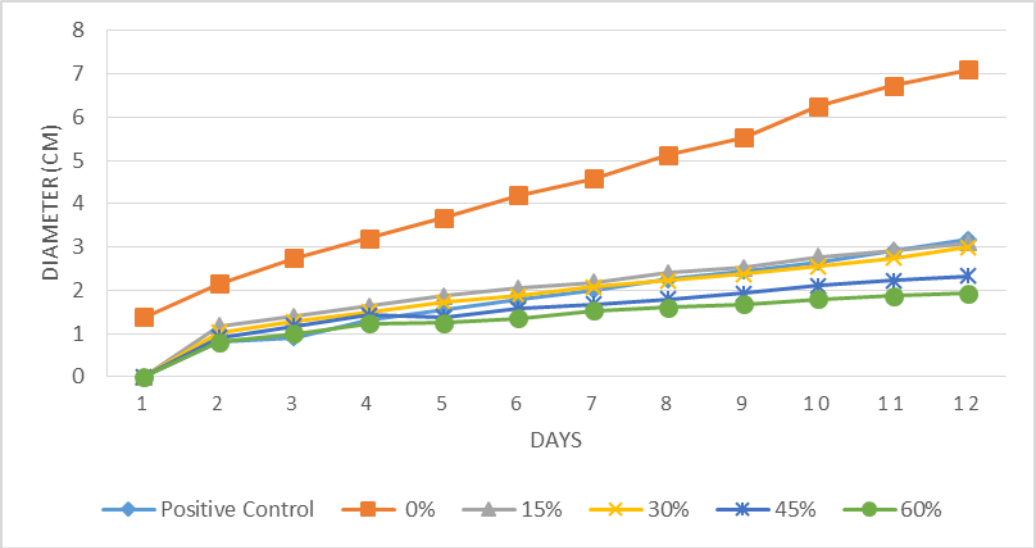

Figure 1. Mean diameter of *Fusarium oxysporum* mycelium

Fusarium oxysporum at 0% concentration treatment has a different color of mycelium with fungi in the treatment which is given extracts with various concentrations. *Fusarium oxysporum* mycelium at 0% concentration treatment is purple, thin and does not expand upwards. Mycelium on positive control is thin and slightly purple. Whereas in the extract treatment, the mycelium grows in white, thick and expands upward (Figure 2).

The purple color of the *Fusarium oxysporum* mycelium is caused by the anthraquinone produced by them. Anthraquinone in culture shows a pale yellowish-brown, orange, red or purple color. Generally, anthraquinone is the color at the sexual stage or resistant form (ascomata, spores, conidia), but sometimes gives color to the fungus mycelium. Changes in the color of the fungus *Fusarium oxysporum* on the treatment of ethanolic extract of lime peels in various concentrations can be caused by the disruption of the process of nucleic acid synthesis and fungal protein by active compounds in lime peels extracts. The presence of ethanol extract of lime peel inhibits the formation of the polyketide synthase gene so that the fungus mycelium is not purple but white (Fauillaud et al, 2016).

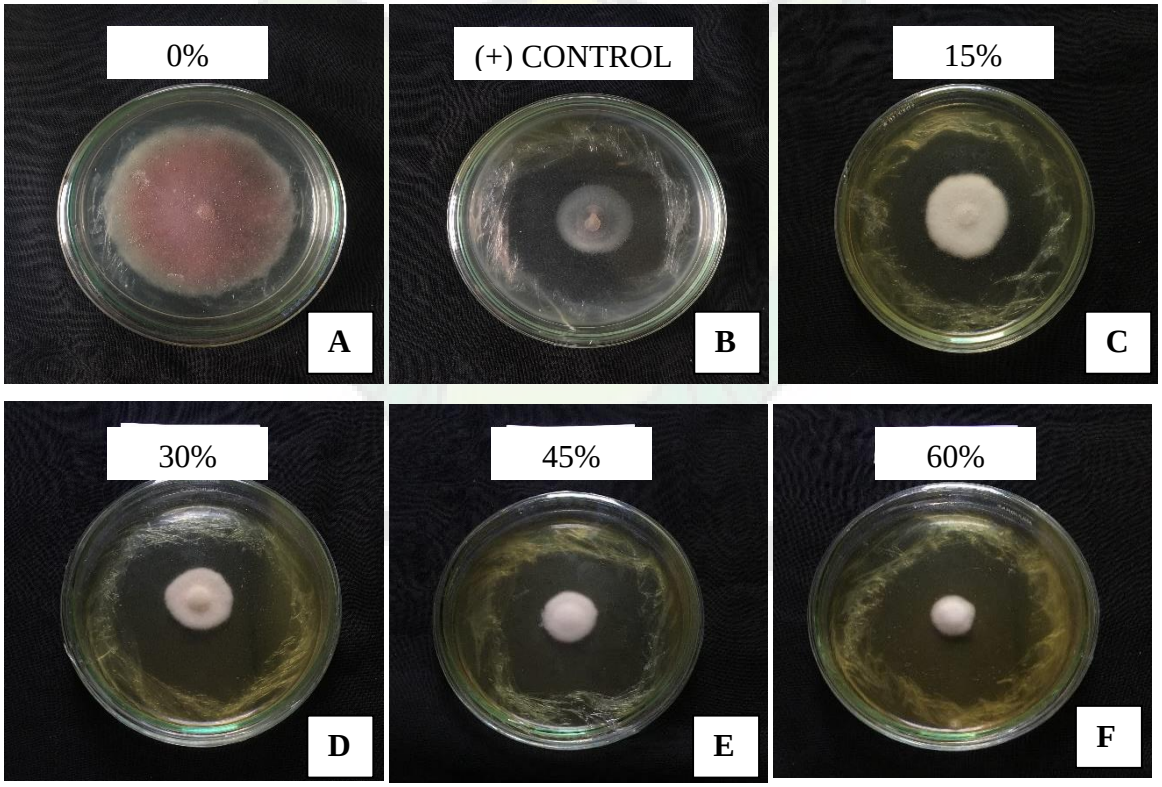

Table 1. Mean diameter of *Fusarium oxysporum* mycelium at twelfth day

Treatment	Mean Diameter of Mycelium (cm)
Positive Control	3,175^{c}
0%	7,100^{d}
15%	3,100^{c}
30%	3,000^{bc}
45%	2,325^{ab}
60%	1,925^{a}

In a column means followed by a common letter are not significantly different at the 5% level by Duncan Test.

Table 2 shows that the higher the concentration of ethanol extract of lime peel, the lower the diameter of the growing fungus mycelium. A similar study using extract material with the same genus, siam oranges, was carried out by Khotimah *et al* (2017) showing similar results, that the higher the concentration of the extract produced the smaller diameter of the fungus growth. The decrease in mycelium diameter is due to the higher concentration of the extract, the more active substances that will inhibit fungal growth. Some active substances contained in the skin of lime fruit are secondary metabolites, namely terpenes in the form of essential oils such as limonene, eugenol, and citral. The results of research conducted by Okwu *et al* (2007) found that not only essential oils are contained in the skin of lime fruit, but there are also flavonoid compounds, tannins, alkaloids, and saponins. The content of secondary metabolites in the skin of lime fruit can be used as an antifungal ingredient.

Table 3. Antifungal activity percentage of ethanolic lime peels extract against *Fusarium oxysporum*

Treatment	Antifungal activity percentage (%)	Level of Activity
Positive Control	55,28^{c}	Strong
0%	0,00^{d}	Not active
15%	56,34 ^{c}	Strong
30%	57,75^{bc}	Strong
45%	67,25^{ab}	Strong
60%	72,89^{a}	Strong

In a column means followed by a common letter are not significantly different at the 5% level by Duncan Test.

The results of ethanol extracts of the lime peel on *Fusarium oxysporum* fungus showed that the higher the concentration of the extract provided the greater the percentage of antifungal activity. Inhibition was indicated by mycelium diameter which was getting smaller as the extract concentration increased (Figure 2). The results of the analysis by Duncan test

showed that the concentration of 15% and positive control had a mean diameter of mycelium that was not significantly different. The 15% concentration treatment is the best concentration in inhibiting the growth of *Fusarium oxysporum* fungi in this study with the percentage of antifungal activity 56.34%. That is because the 15% concentration is the lowest concentration that can inhibit the growth of *Fusarium oxysporum* fungi with a strong inhibitory rate and has the same inhibitory response with positive control of antracol 0.3%.

The antifungal mechanism of essential oils is to damage cell membranes and destroy proteins. Essential oils will disrupt the integrity and permeability of cell membranes that might cause permanent damage to the walls and cell membranes of fungi (Cai et al 2019). Flavonoid and saponin compounds in plant extracts can inhibit the growth of fungus cells by changing the composition of the fungus cell components. Ergosterol which is a constituent of the fungus cell membrane will bind to flavonoid compounds and saponins so that it results in the formation of a pore on the cell membrane, then through which the components of the fungus can get out (Wahyuningtyas, 2008; Suryana, 2004). Alkaloid compounds can inhibit fungal cell respiration (Aniszewki, 2007; Adegoke and Adebayo-tayo, 2009). The antifungal mechanism of tannin compounds is to damage the fungus cell membrane (Watson and Preedy, 2007).

Research conducted by Okwu et al (2007), showed that Citrus vitis and Citrus limonum orange peel extracts that have been tested to inhibit *Fusarium oxysporum* fungi using the same antifungal and solvent test methods with this study showed slightly different results. The concentration of 10% Citrus vitis and Citrus limonum orange peel extracts had a percentage of antifungal activity respectively 42.15% and 48.48% so that it was included in the moderate inhibition rate. Whereas in this study a slightly higher concentration of 15% produced a percentage of inhibition of 56.34% and included a strong inhibition rate. Thus it can be said that 96% ethanol extract of lime peel is better in inhibiting the growth of *Fusarium oxysporum* fungi.

CONCLUSIONS

Based on the results of research that has been done, it can be concluded the optimum concentration of ethanol extract of lime peel to inhibit *Fusarium oxysporum* fungi is a concentration of 15% because that concentration is the lowest concentration that can inhibit the growth of *Fusarium oxysporum* fungi and the optimum percentage of antifungal activity

from extract ethanol 96% of the skin of lime in inhibiting the growth of *Fusarium oxysporum* fungi was 56.34%.

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