Biodiesel Industrial Waste based on *Jatropha curcas* as a Fungicide to Control *Fusarium oxysporum* and *Alternaria solani*

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Abstract. *Fusarium oxysporum* (Schlecht. emend. Snyder & Hansen.) is the most devastating pathogens causing wilt disease on the tomatoes (*Solanum lycopersicum* L.) plant, whereas *Alternaria solani* (Sorauer), is a pathogen that caused early blight on potatoes (*Solanum tuberosum* L.) *Jatropha curcas* L. is a biodiesel material known as a potential fungicide. The industrial biodiesel waste based on *J. curcas* was not yet observed, particularly on the utilization of waste mainly for green manure and biogas. This research aimed to evaluate *the* extract of industrial biodiesel waste based on *J. curcas* against *F. oxysporum* and *A. solani*. There were 2×10^3 mg L^{-1}, 4×10^3 mg L^{-1}, 6×10^3 mg L^{-1}, 8×10^3 mg L^{-1}, and 10×10^3 mg L^{-1} of crude extract that were tested on both pathogens *in vitro* and *in vivo*. The concentration of the extract was in an effective range of 10×10^3 mg L^{-1} and was able to inhibit the growth of all isolates of *F. oxysporum* from both locations by more than 80 %, but the inhibition of the pathogen *A. solani* was less than 76 %.

Keywords: Environmentally friendly, pathogen control, waste to fungicide, waste utilization

1. Introduction

Jatropha curcas L. is a bioenergy resource in the family Euphorbiaceae. It is a perennial bush that is easy to grow in tropical and subtropical regions and resistant to drought. This plant is native to Mexico and Central America but can be cultivated in South East Asia [1, 2]. Indonesia has started to develop *J. curcas-based* energy to reduce dependence on fossil energy. It is possible because *J. curcas* can be grown on marginal lands that do not compete with food crops [3–6].

Tomato (*Solanum lycopersicum* L.) plants often experience decreased production due mainly to *Fusarium* wilt disease. *Fusarium* attacks tomatoes with a yield reduction of up to 85 % [3]. Until now, this disease is very difficult to control even with synthetic pesticides. Various ways have been done including using antagonistic microorganisms, but the results have not been able to effectively control this disease. The other disease is early blight. Early blight is one of the important diseases in potato plants after *Phytophthora.* The attack rate of this disease reaches 65 % [4].

In the processing of *J. curcas* as a biofuel, the process generates waste, such as seed coat waste, liquid waste, and husk. *J. curcas* seed cake is generated in considerable quantities as a by-product of *J. curcas* seed oil extraction [7, 8]. This biodiesel waste contains toxic ingredients so it cannot be used immediately. Generally, the collection and disposal

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of residues is a separate issue because the longer it is stacked, it would be problematic for the environment [9]. Burning the wastes would add more to the global CO_2 level resulting in the enhanced greenhouse effect and climate change [9]. On the other hand, *Jatropha* plant potentially as a fungicide, the extract of ethanol of the Jatropha seed showed a maximum zone of inhibitory potentials at 100 % [10], *J. curcas* seed extract showed significant antifungal activities with a growth inhibition zone of 5.6 mm or equivalent to 78.87 % inhibition followed by pulp with the zone of 7.4 mm or equivalent to 72.07 %, and whole fruits with the zone of 14.2 mm or equivalent to 46.42 % as compared to the control with the zone of 26.5 mm or equivalent to 100 % [11], as antimicrobial and insecticidal [12]. Study in utilizing biodiesel processing waste from *Jatropha* plants still have a wide opportunity, the recent study by [13–15], used two wastes of *Jatropha* that are relevant in both crop and animal production when used as organic manure and animal feeds respectively, none of the previous studies that use waste as a fungicide.

2 Material and methods

2.1 Pathogen

Pathogen *Fusarium oxysporum* (Schlecht. emend. Snyder & Hansen) causes wilt disease in tomato plants obtained from tomato production centers in Malang, Batu, and Blitar, East Java, Indonesia. Meanwhile, *Alternaria solani* (Sorauer) , was isolated from the potato (*Solanum tuberosum* L.) production center of Tosari, Pasuruan, and Cangar, Batu, East Java, Indonesia. The parts of the plant which isolated were stems, leaves, roots, and soil. All isolates were pathogenic isolates. Pathogenic *F.oxysporum* and *A. solani* isolates were grown on PDA Merck for 7 d then used in a laboratory and greenhouse test.

2.2 Isolation of active ingredients of industrial biodiesel waste based on *Jatropha* seed

The waste of biodiesel based on *Jatropha* was obtained from the biodiesel factory. The waste taken was still fresh out of the factory. Waste extraction to obtain the active ingredients is performed by the method described by [16]. Waste of industrial biofuels from *Jatropha* seed was crushed, dried, weighed 300 g, and deep in 96 % methanol (Merck) for 3 d, then filtered by using Whatman paper No. 1. The filtrate was evaporated at 40 °C with a rotary evaporator RE-1020 at 200 mbar (1 mbar = 100 Pa). Furthermore, the dried crude extract was separated between hydrophobic and hydrophilic with dichloromethane (Merck), then the part that dissolved in dichloromethane (Merck) was evaporated and used for the antifungal test.

2.3 Bioassay test (*In-vitro*)

This bioassay was done to determine the inhibition ability of the crude extract to pathogens *in vitro*. Screening the best crude extract concentration were started at 0 mg L^{-1}, 1 × 10^3 mg L^{-1}, 2 × 10^3 mg L^{-1}, 3 × 10^3 mg L^{-1}, 4 × 10^3 mg L^{-1}, 5 × 10^3 mg L^{-1}, 6 × 10^3 mg L^{-1}, 7 × 10^3 mg L^{-1}, 8 × 10^3 mg L^{-1}, 9 × 10^3 mg L^{-1}, 10 × 10^3 mg L^{-1}. The poisoning food method was used for screening by adding the crude extract on each concentration into a PDA medium at a Petri dish of 9 mm diameter, and then a diameter of 5 mm pathogen was placed in the center. The control treatment was by growing the pathogen on a PDA medium. All treatment was repeated 10 times. All treatments were incubated for 14 d at 27 °C, then observed for the percentage of inhibition. The formula to calculate inhibition of growth (Equation 1):

$$Growth\ (\%)\frac{(Colony\ diameter\ (control-treatment)}{Diameter\ colony\ on\ the\ control}\times 100\ \% \quad (1)$$

The result of the screening was used for the greenhouse test.

2.4 The greenhouse test

A completely randomized design was used in this test. All treatments were repeated three times. *F. oxysporum* isolates were tested on tomato plants aged 1 mo after planting, whereas isolates of *A. solani* were tested on potato plants aged 2 wk. The 100 mL isolates with a population of 10^8 were invested in the soil. A day after pathogen application, 100 mL of crude extract was poured into the planting medium of tomatoes or potatoes. The concentration of crude extract used in this research was the most effective concentration for inhibiting the growth of both types of pathogen in the in vitro test. As a positive control was a chemical pesticide, a negative control was not added to the plants. Observation variables were the incubation period and the intense attack of the disease. The incubation period was calculated from the time of inoculation until the appearance of the first symptoms of the disease. The intensity attack was calculated using the Equation 2:

$$Intensity\ attack\ (\%)\frac{(Total\ plant\ diseased)}{(Total\ plant\ observerd)} \times 100\ \% \quad (2)$$

Statistical analysis

The data collected were analyzed using SPSS statistical package, while means were separated using the least significant difference (LSD) test at 5 % level of significance [17, 18]

3 Result and discussion

3.1 *In vitro* test

The crude extracts 10×10^3 mg L^{-1} of concentration were able to suppress the growth of isolates from all three origin locations of *F.oxysporum* isolates, namely the isolates from Batu (leaves) of 80.36 %, Malang (stems) of 78.46 %, Malang (roots) of 80.84 %, Blitar (stems) of 80.81 %, and Blitar leaves of 81.58 %. While the percentage of inhibition to isolate *A. solani* from Cangar (stems) of 62.2 %, Cangar (leaves) of 57.6 %, Tosari (stems and leaves) is 69.84 % and 76 % respectively (Figure 1 and Figure 2).

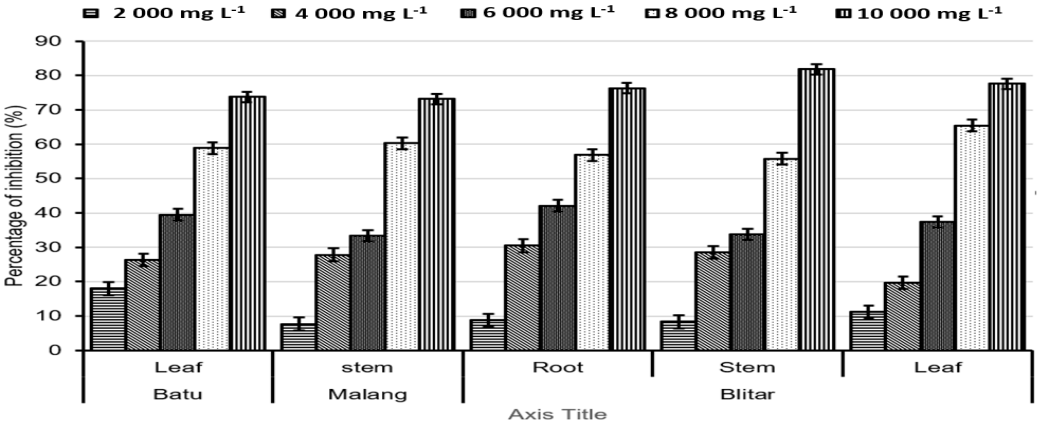

Fig. 1. The percentage of inhibition of crude extracts of biodiesel waste to the growth of various *F.oxysporum* isolates (Batu: leaf, Malang: stem, and Blitar: stem and leaf) through *in vitro* at various concentrations 2×10^3 mg L^{-1}, 4×10^3 mg L^{-1}, 6×10^3 mg L^{-1}, 8×10^3 mg L^{-1}, and 10×10^3 mg L^{-}

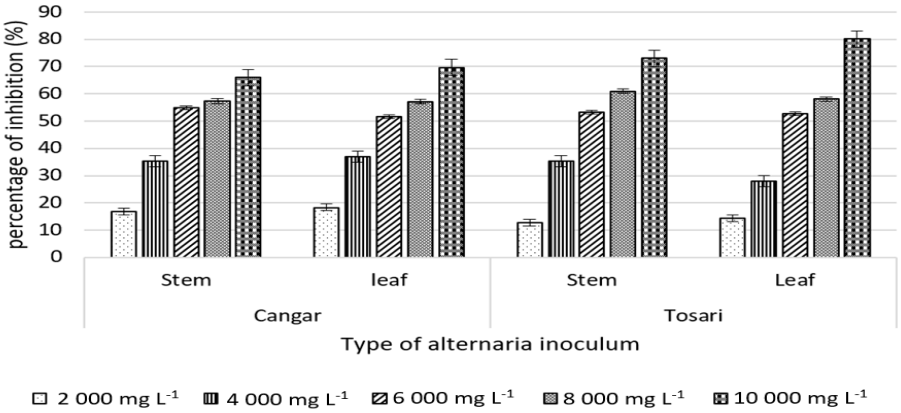

Fig. 2 The percentage of inhibition of crude extracts of biodiesel waste to the growth of various *A. solani* isolates (stem and leaf of potatoes from Cangar and Tosari areas) through *in vitro* at various concentrations 2×10^3 mg L^{-1}, 4×10^3 mg L^{-1}, 6×10^3 mg L^{-1}, 8×10^3 mg L^{-1}, and 10×10^3 mg L^{-1}

A concentration of 10×10^3 mg L^{-1} can inhibit the growth of both types of pathogens by more than 80 %. These results indicate that the biodiesel waste made from Jatropha still has the potential as a fungicide as it was before the oil

was taken. In line with [19], used four extracts were acetone, methanol, ethyl acetate, aqueous, one negative control (0 µL mL^{-1}), and one positive control used fungicide enriched medium (Monchamp) (3.33 g L^{-1}). The concentrations of various extract were 7.5 µL mL^{-1}, 15 µL mL^{-1}, 30 µL mL^{-1}, 60 µL $mL^{-1}$and 120 µL mL^{-1}. The radial growth inhibition test was carried out. The concentration of 120 µL mL^{-1} had an inhibitory effect on the radial growth of the various organic extracts. Along with this result, extracts of Organic and aqueous *J. curcas* seeds could as present a fungicidal potential. Moreover, *J. curcas* leaf extracts highly inhibited mycelial growth by (85.78 %) similar to standard fungicide (Chlorothalonil) (88.37 %) in this experiment against *Phaeosariopsis personata* (Berk. & M.A. Curtis) Arx) pathogen [20].

Jatropha contains active ingredients that have the potential as antimicrobials. The content in castor bean extract is phenol and flavonoids which function as antimicrobial. This is consistent with the results of research from [11], in which seed extract has the potential as a natural fungicide that can replace synthetic fungicides. According to Cushnie and Lamb [21], gallic acid and pyrogallol compounds are phenolic groups that can inhibit microbial activity by acting as a chemical inhibiting the growth of microorganisms. Daidzein as isoflavonoids and routine and myricetin as flavonoids can inhibit bacterial growth by inhibiting DNA gyrase, metabolism energy, and cytoplasmic membrane. But, [20], found that among the major compounds identified with antifungal activity were hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, hexadecane, n-hexadecanoic acid, octadecanoic acid ethyl ester, phytol, and 9, 12-octadecadienoic acid (Z,Z)-methyl ester.

3.2 Greenhouse test

For all types of isolates, the concentration of 10×10^3 mg L^{-1} may postpone the incubation period of *F.oxysporum* wilt disease more than 30 d, and it was longer than chemical pesticides by 20 d. The crude extract could delay symptom appearance of *A. solani* in more than 30 d, and it was longer than chemical pesticides (18 d). More results were presented in Figure 3 and Figure 4.

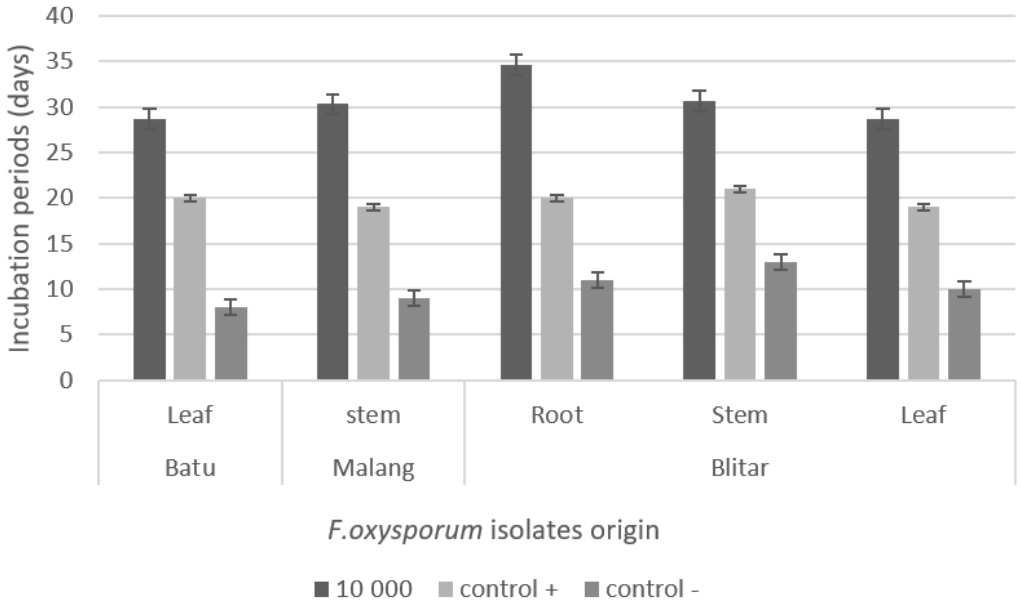

Fig. 3. The incubation period of *F.oxysporum* from different regions and several parts of the plant at the concentrations 10×10^3 mg L^{-1}

Percentage of xylem discoloration due to the attack of *F.oxysporum* origin from Batu (leaves), Blitar (Root), Blitar (leaves and stem) at a concentration of crude extract 10×10^3 mg L^{-1} were respectively 4.3 cm, 4.2 cm, 4.3 cm, 4.2 cm, and 4.2 cm. It was almost the same as a positive control with chemical pesticides were an average of 4.2 cm. whereas the negative control without any application of its percentage discoloration by 9 cm (Figure 5).

Extract of biodiesel waste based on *Jatropha* was able to suppress the growth of pathogenic fungi because the extract may contain active ingredients. The presence of a phytochemical of the extract of biodiesel based on *Jatropha* can function as an antifungal [22]. On further research fractionation of industrial biodiesel extract with 80 % Me-OH using an open column chromatography could inhibit *S. rolfsii*. From this, it can be concluded that there was a secondary metabolite issued by Jatropha seeds that were able to inhibit the growth of *S. rolfsii* [23]. Francis [20], found that *in vitro* trial showed the antifungal activity of *J. curcas* extracts varied with the type of solvent used for extractions.

Besides, the study showed that the mycelial growth of *P. personata* was highly inhibited at the highest concentration of *J. curcas* extracts than at the lowest. This study correlated with the investigation by [24], the highest concentration (80 mg mL^{-1}) of *J. curcas* extract inhibited the growth of *F. oxysporum* by 54 % as compared with 10 % inhibition at the lowest concentration of 20 mg mL^{-1}. Likewise, according to [25]; disease severity was lowered as the concentration of plant extracts increased [26].

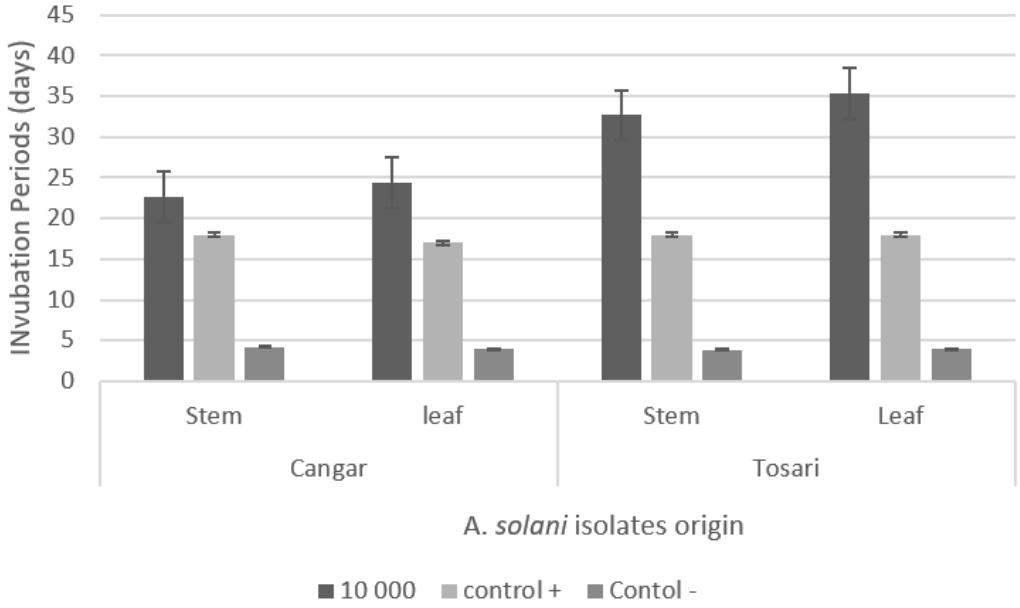

Fig. 4. The incubation period of *A. solani* from different regions and from several parts of the plant at at the concentrations 10×10^3 mg L^{-1}

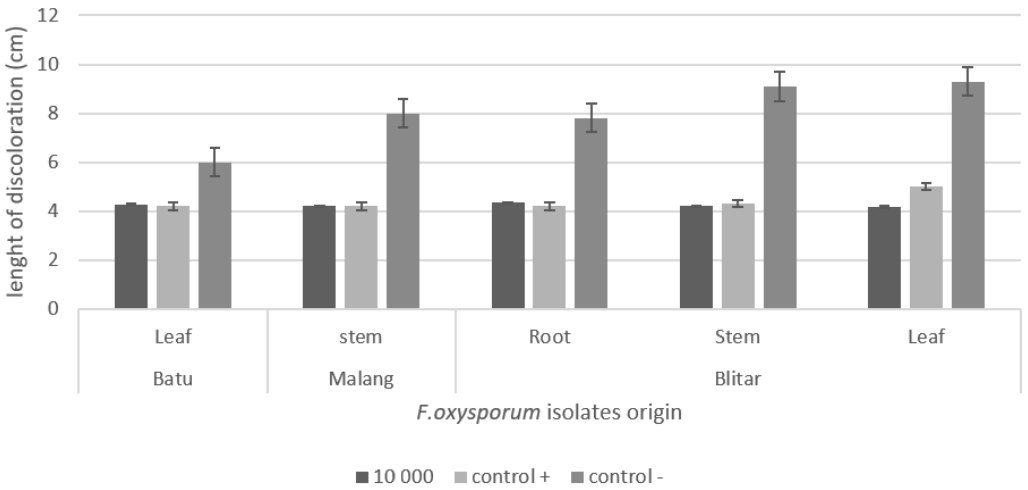

Fig. 5. Percentage of discoloration of tomato xylem due to Fusarium wilt disease from different regions and several parts at the concentrations 10×10^3 mg L^{-1}

Industrial waste extract applications at a concentration of 10×10^3 mg L^{-1} could only delay However, if the extract was periodically applied, as well as chemical pesticides, it was likely to be able to prevent the occurrence of these two diseases. However, when compared with the application of chemical pesticides, the extract can be an advanced alternative for environmentally-friendly disease control. Therefore, fertilizing with *jatropha*-based biodiesel production is very promising.

4 Conclusion

The positive results in suppressing two pathogens by crude extract of industrial biofuel waste can be an alternative for reducing chemical fungicide usage, but it is needed to be further developed and extended to make commercial fungicides.

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