INTERNATIONAL JOURNAL of OIL PALM

ISSN: 2599-3496 print
ISSN: 2614-2376 online

Volume 5, Number 1, 2022
Page 16-25
DOI: https://doi.org/10.35876/ijop.v5i1.70

Efficacy of Microbial Concortia with Liquid Organic Fertilizer for Leaf Spot Disease Control on Oil Palm Nursery

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ABSTRACT

Leaf spot is the primary disease on oil palm nurseries. The symptom of the disease is leaf spots are round to oval in shape and vary in color from yellow to brown to black. The initial size may be as small as a pin point. Some leaf spots initially appear as lesions. During disease development, leaf spots will have a contrasting colored edge or halo - e.g., brown spot with a yellow halo, tan center with brown edge or gray center with black edge and a yellow halo. As the leaf spots expand in size, the shape and coloration may change. As the disease progresses, leaf spots often coalesce (merge together) to form large areas of blighted tissue. If the disease continues to develop, leaflets or the entire leaf may die prematurely. The disease also causes stunted and even plant death. Disease control methods that rely primarily on biological agents and do not include fertilizers are less effective in the field. Therefore, the development of organic fertilizer formulations and the use of biocontrol agents are expected to assist oil palm nurseries in suppressing leaf spot disease. The purpose of this study is to investigate the effect of organic fertilizer and biocontrol agents on leaf spot disease. The results showed that the treatment of Organic Fertilizer and biocontrol agents was able to reduce 47.19% leaf spot disease after five weeks of application.

Key words: disease intensity, organic waste, *Pseudomonas fluorescens, Trichoderma atroviride*

INTRODUCTION

Leaf spot disease was attributed to a fungal complex involving *P. glandicula, P. palmarum, Colletotrichum, Curvularia, Gloesporium* and *Helminthosporium* and the inductive insect in the zone was *Leptopharsa gibbicarina* (Escalante *et al.* 2010). Further reported by Nasehi *et al*. (2019) that *Phyllosticta capitalensis* as one of the pathogen in Malaysia.

Fertilizers and biocontrol agents are two important components in the control of plant diseases that can not be separated. Proper fertilization can help plants become more resistant to pests and diseases, as well as enhance disease recovery. Biocontrol agents, on the other hand can

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inhibit pathogens and reducing the severity of disease. Rosa and Blanco (2015) reported that soil amendments, particularly organic amandements have the capability to modify soil characteristics and eventually affect microbial communities and their activity. Further reported that combination organic amandements and biocontrol agents can provide an adequate nutrient reservoir to the biocontrol agents thereby enhancing its survival in a hostile environment. In practical, these two components are frequently used in separation, creating new problems for farmers. In this case, the control results are less effective, the cost is quite high, there is a need for more manpower, and the time required is also relatively long.

Pseudomonas fluorescens and *Trichoderma* sp. were extensively studied for their ability to suppress plant pathogens, with positive results for both soil-borne and air-borne pathogens. Shalini and Srivastava (2007) also reported that *P. fluorescens* inhibited *Curvularia lunata* growth by 96.07% in vitro. In the meantime, Iftikhar *et al.* (2017) found that *Trichoderma* was able to inhibit *C. lunata* growth by 92.2% in vitro. Combine of organic fertilizer and biocontrol agents (biofertilizer) is an alternative disease control that is expected to be able to effectively and efficiently control leaf spot disease.

MATERIALS AND METHODS

Microbial Strains and Plant Seeds

Pseudomonas fluorescens and *Trichoderma atroviride* were the microbial strains in this study. In Sanggau Regency, West Kalimantan, *P. fluorescens* was isolated in the rhizosphere of *Mimmosa pudica*. Meanwhile, *T. atroviride* was isolated from rotten bamboo plant tissue in Singkawang City, West Kalimantan. In previous studies, both strains were identified morphologically, biochemically, and molecularly. For propagation, *P. fluorescens* inoculum was cultured on nutrient broth (NB) for 48 hours by shaking at 150 rpm, while *T. atroviride inoculum* was grown on slanted potato dextrose agar for 5-7 days. Moreover, three-month-old oil palm seeds (Sriwijaya variety) were used in this study, which were obtained from nursery breeders in the Bengkayang Regency, West Kalimantan.

Ingredients of Biofertilizer and Their Analysis

Biofertilizer made by combining liquid organic fertilizer (LOF) and biocontrol agents. The liquid organic fertilizer made through aerobic decomposition based on organic waste and other materials which consist of water, rice flour, bagasse, duck eggs, granulated sugar, banana stem ares, coconut fiber, *Arachis pintoi*, banana peel, tofu dregs, green vegetable waste, egg shells, bran, and coconut water (Table 1). Organic waste of sugarcane bagasse, banana stem ares, coconut fiber, *A. pintoi*, banana peel, green vegetable waste, and egg shells are chopped first and then mixed with other components in a composter. The suspension of *P. fluorescens* was poured over all materials in the composter as a decomposer, then mixed until evenly distributed and incubated for 6 weeks. The composter's contents are mixed once a week to ensure complete decomposition. whereas, *T. atroviride* suspension was added to the fertilizer after harvesting. The population number of *P. fluorescens* and *T. atroviride* in this product had been tested at the Soil Research Institute, Bogor (Table 1) as well as the composition of

Table 1 Organic material composition

Sources	Ingredients	Amount
Main ingredient		
Decomposer and biocontrol agent (mL)	*Pseudomonas fluorescens*	250 (108 cfu/mL)
Biocontrol agent (mL)	*Trichoderma atrov*iride	250 (108 conidia/mL)
Carbohydrate (g)	Rice flour+ water	240
Glucose (g)	Baggase/cane sugar	200
Albumine (egg)	Duck eggs	1
Diluent (L)	Water	1
Macro-element		
N (g)	*Arachis pintoi*	150
P (g)	Banana tree base	150
K (g)	Coconut fiber	150
Micro-element		
Mg, Na, S (g)	Banana peel	30
Fe, Ca, Ca, C (g)	Tofu dregs	30
Mn, Cu, Zn (g)	Green vegetable waste	30
Ca (g)	Egg shells	30
Vitamin A & E, protein, lipid carbohydrate (g)	Bran	60

nutritional levels, had to meet certain quality requirements in according to the Minister of Agriculture's Regulation No. 261 of 2019 and also the contamination level of *Escerichia coli* and *Salmonella* sp. for biosafety requirements.

Inoculum of Leaf Spot Disease

The inoculum was obtained using symptomatic oil palm leaves that had been chopped into pieces according to the symptoms. The samples were crushed with a sterile mortar and then being mixed with sterile distilled water and sprayed into the healthy leaf (Susanto and Prasetyo 2013) until the seedlings have the moderately symptoms category (26-50 %).

Research Design and Application

Randomized Block Design was used to design the experimental units in a Greenhouse. There were six levels of treatment and each treatment has been repeated four times. The treatmens that are A: (LOF), B: (LOF+*P. fluorescens*), C: (LOF+*T. atroviride*), D: (LOF+*P. fluorescens*+*T. atroviride*), E: (commercial LOF product as comparison), and Control (distilled water). Each treatment (except control) diluted with distilled water (2 mL per litre) and the solution applied 250 mL per plant with a hand sprayer through rizosphere and the underside of the leaves after the disease symptoms are visible. During the experiment, each treatment was applicated for six times with interval every two weeks.

Observation

Data observations were taken three times, starting from one week before application and with an interval every two weeks. Disease level, plant height, and the number of leaves are some of the observed variables. The percentage of spotting was used to calculate disease intensity (Table 2), based on Susanto and Prasetyo (2013).

Tabel 2 Leaf spot percentage score

Score category	Leaf spot (%)
0	0
1	1 – 25
2	26 – 50
3	51 – 75
4	76 - 100

Furthermore, the disease's intensity is calculated below:

$$DI = \frac{\Sigma(n \times v) \times 100\%}{N \times V}$$

Where:
DI, disease intensity; n, number of leaf based on score category; v, score category; N, number of leaf; V, highest score category.

RESULT AND DISCUSSION

Biological Analysis and Nutrient Levels of Biofertilizer

Analysis result showed the amount of *P. fluorescens* after the composting process was still above the required standard which is 1.81 x108 cfu/mL. Organic matter decomposition by *P. fluorescens* resulted in high levels of macro and micronutrients, especially Fe^{3+} and phosphorus. According to Trapet *et al.* (2016), *P. fluorescens* was able to produce pyovirdin, which is a siderophore synthesized under iron (Fe^{3+}) poor conditions. Bacteria excrete these high-affinity iron-adhesive compounds produced from degraded organic matter in fertilizers. Meanwhile, *P. fluorescens* also aids in the dissolution of phosphate compounds by producing organic acids (gluconic acid) that dissolve phosphate complexes into usable ortho-phosphates for plants (Oteino *et al.* 2015). Furthermore, Kalayu (2019) stated that the dissolving phosphorus process occurs when organic acids or protons produced by *P. fluorescens* create a reduction in pH in the surroundings. Heavy metal levels in the fertilizer produced were also low, namely 7 ppm (available Pb), 1 ppm (available Cd), and 1.4 ppm (available Co). Meanwhile, the total As and total Hg elements were not detected. This is most likely due to the organic components utilized, which are low in nature in heavy metals. Furthermore, it was suspected due to *P. fluorescens*' role as a bioremediator. This study was supported by Khan and Ahmad (2006), *P. fluorescens* was able to reduce cadmium, hexavalent chromium, and lead within 24 hours, and according to the fertilizer manufacturer, it is free of chemicals, and livestock dung also contributes to the low *E. coli* and *Salmonella* sp. concentration (<30 MPN/mL) in the final composting yield. The results of our study's analysis of nutrient and biological levels of liquid organic fertilizer are shown in Table 3.

Decreased Intensity of Leaf Spot Disease

In this study, the application of D treatment (LOF+*P. fluorescens*+*T. atroviride*) was able to reduce the intensity of leaf spot disease by 47.19% after five week application and significantly different when compared to the control and commercial liquid organic fertilizer (E) according to the Table 4.

Table 3 The analysis of nutrient and biological levels of biofertilizer

Composition	Rate		Method
	Result	Standard**	
Organic carbon (%)	1.22	> 4.5	Walkley & Black Spectrophotometry
Cation exchange capacity	-		NH_4 OAC pH 7/ Spectrophotometry
Macro-elements			
N-total (%)	0.42	> 0.5	Kjeldah/Destilation
P2O5-total (%)	0.03	< 5	HNO_3/Spectrophotometry
P2O5 - available (%)	0.01	-	Citrate 2%/Spectrophotometry
K2O-total (%)	0.07	<5	HNO_3/F-AAS
K2O-available (%)	0.06	-	Citrate 2%/F-AAS
Secondary-elements			
Ca-available (%)	0.26	-	Citrate 2%/F-AAS
Mg-available (%)	0.03	-	Citrate 2%/F-AAS
S-available (ppm)	41	-	Citrate 2%/Spectrophotometry
Micro-elements			
Fe-available (ppm)	8	5-50	Citrate 2%/F-AAS
Mn-available (ppm)	4	0.25	Citrate 2%/F-AAS
Zn-available (ppm)	2	0.25	Citrate 2%/F-AAS
B-available (ppm)	5	125-2500	Citrate 2%/Spektrofotometri
Mo-total (ppm)	25	< 10-3	HNO_3/F-AAS
Cu-available (ppm)	*	0.25	Citrate 2%/F-AAS
Heavy Metals			
Pb-available (ppm)	7	< 12.5	Citrate 2%/F-AAS
Cd-available (ppm)	1	< 1	Citrate 2%/F-AAS
Co-tersedia (ppm)	1.4	< 700	Citrate 2%/F-AAS
As-total (ppm)	*	< 10	HNO_3/F-AAS
Hg-total (ppm)	*	< 1	HNO_3/F-AAS
Biological Analysis			
Escerichia coli (MPN/mL)	<30	<102	MPN
Salmonella sp. (MPN/mL)	<30	<102	MPN
Pseudomonas sp. (cfu/mL)	1.81x108	> 105	MPN
Trichoderma atroviride (cfu/mL)	*	> 105	MPN

*: unidentified (so, added become 108 cfu/mL after decomposition process); **: according to: a. The Minister of Agriculture's Regulation No. 261/Kpts/Sr.310/M/4/2019 about organic fertilizer, biofertilizer, and soil repairer requirements, b. Indonesian Standard (SNI) 19-7030-2004; Specifications of compost from domestic organic waste.

Table 4 The effect of treatments on disease intensity and plant vegetative

Treatments	Decreased of disease intensity (%)*	Increase of plant height (cm)	Increase of leaf number (%)*
Control	33.97 c	1.93 b	18.31 d
LOF	3283 d	1.07 c	20.70 c
LOF+P. fluorescens	46.89 a	0.34 d	14.38 f
LOF+T. atroviride	43.07 b	1.30 c	20.95 a
LOF+P. fluorescens+T. atroviride	47.19 a	2.77 a	20.82 b
Commercial LOF product	33.97 c	2.19 b	17.20 e

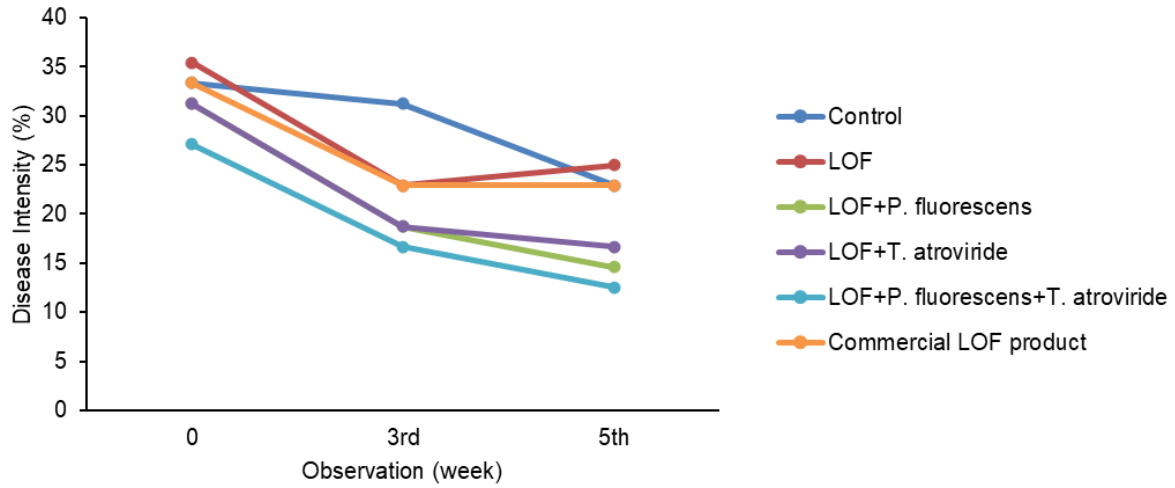

Figure 1 Leaf spot disease intensity development during experiment.

Among all of the treatments, LOF+*P. fluorescens* and LOF+*P. fluorescens*+*T. atroviride* gave reduction significantly in the disease than other treatments (Figures 1). This is thought to be due to the synergistic role of LOF, *P. fluorescens* and *T. Atroviride* and LOF+*P. fluorescens* in plant physiological systems. Both were able to suppress leaf spot disease through secondary metabolites produced by *P. fluorescens* and *T. atroviride*. According to Simamora *et al*. (2021), secondary metabolites of biocontrol agents can increase the phenol content in plants. These phenolic compounds are translocated systemically to all parts of the plant and have an effect on increasing plant resistance.

Mishra and Arora (2017) were reported that *P. fluorescens* is capable of producing important secondary metabolites in suppressing pathogens, including hydrogen cyanide (HCN), penazines, fluoroglukinol, pyoluteorin, and Pyrrolnitrin. HCN produced by *P. fluorescens* is toxic to pathogens by inhibiting cytochrome C (Arora 2017). However, *P. fluorescens* is resistant to cyanide due to the presence of RhdA, a thiosulfate that converts cyanide to thiocyanate making it less toxic to it. Apart from inhibiting most fungal pathogens, penazines also important in iron acquisition, cell signaling, and regulating gene expression.

On the other hand, Matloob and Baldawy (2020) were reported that the addition of organic fertilizers can change the efficacy environment so that it is not suitable for the growth of surrounding pathogens, increase the effectiveness of biocontrol agents because it provides a nutritional basis for growth, increases plant resistance to disease, and increases plant growth and reproduction.

Pseudomonas fluorescens was also known produces the mycolytic enzymes 1,3 glucanase and 1,4 glucanase (Karnwal 2010). Both enzymes are involved in the degradation of -glucan, which is one of the most important components of fungal cell walls, resulting in cell disintegration and pathogenic cytoplasm leakage (Chen *et al.* 2015). Furthermore, El-Benawy *et al.* (2020) reported that *T. atroviride* secretes chitinase and protease enzymes, which are implicated in pathogenic mycolytic events. Moreover, an important compound that is also produced by *T. atroviride* is 6-n-pentyl-2 H-pyran-2-1. These compounds have the ability to minimize toxin production and prevent pathogenic mycelium growth, as well as in plant induced resistance. Meanwhile, the nutrients contained in LOF are able to repair damaged cells found in the former symptoms. The nutrients in LOF can also supply plants with enough nourishment, making it immune to external diseases like pathogenic infections. *Pseudomonas fluorescens* has the ability to fix nitrogen and dissolve phosphate due to its natural characteristics.

A different case occurred in B treatment, although it involved LOF+*P. fluorescens* but did not gave significant results on plant height. This is presumably because the available nitrogen and phosphorus compounds dissolved by *P. fluorescens* was translocated for the recovery of symptomatic plant parts (disease intensity) so that the increase in plant height and number of leaves was not significant (Table 4). Miljakovic *et al.* (2020) reported that beside of nitrogen, plant growth directly depends on phosphorus (P). Meanwhile, synergistic LOF+*P. fluorescens*+*T. atroviride* (D treatment) was able to increase plant height significantly (Figure 2) among other treatments.

Pseudomonas fluorescens as plant growth promoting rhizobacteria can help the formation of growth hormones including indole acetic acid, auxins, gibberellins, and cytokinins which are involved in cell division, shoots growth, and other vegetative organs (Karnwal and Kaushik 2007).

As a Plant Growth Promoting Fungi (PGPF), *T. atroviride* also contributes to seedling growth (El-Benawy *et al.* 2020).

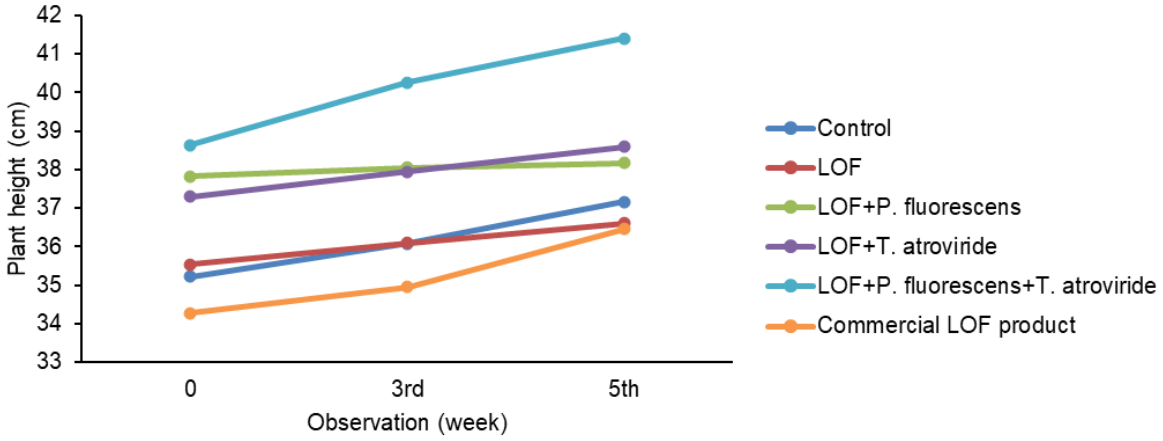

Figure 2 Average of plant height development during experiment.

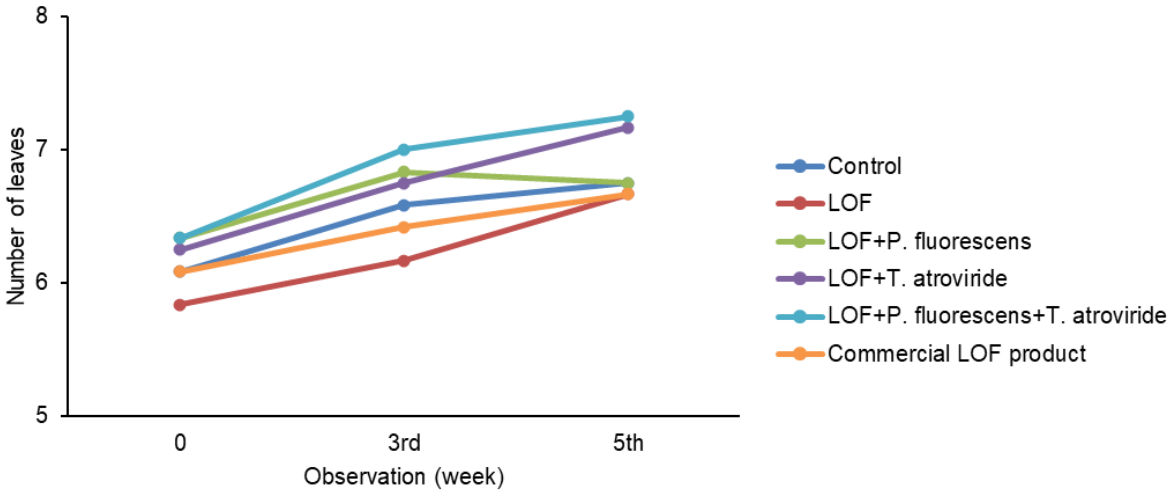

Figure 3 Average of leaves number during experiment.

The compound 6-n-pentyl-2 H-pyran-2-1, which is produced by *T. atroviride* and sprayed on the leaves can increase root growth, and allowing plants to produce more auxin hormones. According to Zin and Badaluddin (2020) *Trichoderma* sp. able to decompose organic matter in the medium so that it becomes a simpler structure, easily soluble, and can be used as a source of nutrients for plant growth. Furthermore, Halifu *et al.* (2019) reported that *Trichoderma* is able to change nitrogen compounds available to plants so that they can be used for the formation of plant cells, tissues, and organs.

Treatment C (LOF+*T. atroviride*) had the greatest percentage, increased in the number of leaves, and it was significantly different from other treatments. (Figure 3). The addition of *T. atroviride* to the LOF treatment stimulated leaf growth. Rahmad *et al*. (2018) states that the number of leaves produced by a plant has a synergistic relationship with nutrient abundance. The formation of cells, tissues, and plant organs is proceeding normally, as evidenced by the number of leaves produced. According to Azarmi *et al.* (2011), application of *Trichoderma sp.* can enhance the quantity and width of leaves as well as the amount of chlorophyll in the leaves.

CONCLUSION

Combination of organic fertilizer, *Trichoderma atroviride*, and *Pseudomonas fluorescens* were able to suppress leaf spot disease up to 47.19%, decrease in disease intensity, and had a positive effect on plant growth after five weeks application.

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