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Original article

Study of soil bacteria and fungi population in oil palm with big hole planting system

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Abstract

The big hole planting system for oil palm is one of the efforts to control the infection of *Ganoderma boninense.* The big hole method can also improve physical and chemical soil properties that affect the soil microbes population and in turn suppress the soil pathogens. However, the information about soil microbes population under the big hole planting method is still limited. This study aimed to compare the population of soil bacteria and fungi in soil samples from oil palm plantations that applied the big hole for 15 years and non big hole (standard) planting method. The population of bacteria and fungi was analyzed using the dilution plate method, while the calculation was based on the total plate count. Based on the results of statistical analysis (P-Value = 0.05), it is known that the mean population of bacteria is significantly different but the mean population of fungi is not significantly different between the big hole and non-big hole planting systems. The population of soil bacteria under the big hole and standard method was 1.6 x 106 cfu/g and 1.1 x 106 cfu/g, respectively. Meanwhile, the population of soil fungi under the big hole method was 9.7 x 104 cfu/g and 7.2 x 104 cfu/g under the standard planting method. Microorganisms in soil are considered important for maintaining soil health and quality, because various microorganisms are involved in important soil functions. In addition, high soil microbial populations will contribute to reducing soil base disease or suppression of soil pathogens.

Keywords: big hole, bacteria, fungi, oil palm, soil biology

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Introduction

Basal stem rot disease caused by the fungus *Ganoderma boninense* is known as a deadly and most destructive disease in oil palm plantations. This disease causes a significant decline in the oil palm population (Wiratmoko et al., 2018). *G. boninense* is a soil-borne pathogen, which attacks oil palm, starting from the root system, and quickly spreads to plant stem tissues and other plant organs (Assis et al., 2015). Several management practices have been made to prevent and control the *G. boninense* infection, such as physical, chemical, and biological control (Yanti et al., 2019; Siddiqui et al., 2021).

The combinations of physical and biological control are carried out by making larger planting holes and applying empty fruit bunches (EFB) and *Trichoderma* sp. at the beginning of oil palm planting. The big hole is one of the planting systems with a larger planting hole size than the standard, which is ± 3 m x 3 m x 0.8 m (Prasetyo et al., 2008). The purpose of making a larger planting hole is to sanitize the remaining stems and roots infected by *G. boninense.* Any contact between healthy roots and infected roots is one of the primary mechanisms for *G. boninense* spreading in the field (Naher et al., 2013). According to Priwiratama et al. (2014), the rate of *G. boninense* infection becomes faster in the endemic areas, especially in plants of the second generation (more than >50 years planting years) or with the planting pattern using standard size planting holes.

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In the previous study, the addition of EFB in the big hole system was reported to improve the soil physical and chemical properties. The big hole system increases the soil water content, improves soil porosity, soil permeability, and reduces soil density (Pradiko et al., 2016b). Furthermore, Surianto et al. (2015) also mention that the big hole causes the changes in soil organic carbon (SOC) content and soil pH. Changes in the physical and chemical properties of the soil are also influence the biological properties of the soil, such as the population and variety of soil microbes (Walsh & McDonnell, 2012; König et al., 2020).

In some cases, soil with a high population and microbial diversity has been reported to increase plant survival (Goh et al., 2020; Wang et al., 2017). It occurs due to the vital role of soil microbes as bioagents capable of suppressing pathogens in the soil (Schlatter et al., 2017). In soils with a fairly massive *G. boninense* invasion, Chung (2011) explained that the population and diversity of soil bacteria were lower than in healthy soils and had a minimal incidence of basal stem rot disease. However, the information about soil microbes population in the endemic soil of *G. boninense* that implementing the big hole planting system in a long time has not been widely reported. Hence, this study aimed to analyse the total population of soil microbes (bacteria and fungi) in oil palm plantations implementing the big hole planting system for 15 years compared to the standard planting system. In addition, we also observed information about incidence of oil palm tree mortality after 15 years implementation of different planting systems in the endemic soils that infected with *G. boninense*.

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Methods

Study area

The research carried out in April - June 2021 at Sei Pancur Plantation - Tanjung Morawa, North Sumatra, Indonesia. The research was conducted in plots with 15 years old oil palm trees which were planted with a big hole system and a standard hole (non-big hole) as a comparison. The big hole system is a planting hole with 2.8 m x 2.8 m on top and bottom side and 0.8 m depth with the addition of 400 kg of empty fruit bunches + *Trichoderma* sp at the beginning of planting, while the non-big hole planting system is 0.6 m x 0.6 m x 0.6 m without application of empty fruit bunches (EFB) and *Trichoderma* sp.

Observed parameters

Incidance of tree mortality

The incidence of oil palm mortality is calculated from the observation of the number of dead trees on plots with big hole and non-big hole planting systems. The incidence of tree mortality was calculated using a formula that refers to Wiguna et al. (2015):

$$\text{Incidence of tree mortality} = \frac{\text{Number of dead trees}}{\text{Total number of trees}} \times 100\% \qquad (1)$$

Soil Sampling

Soil samples were collected from the weeding circle (± 50 cm from the oil palm trunks) at 3 sampling points per treatment. At each sampling point, soil sample were taken at 0-20 cm, 20-40 cm and 40-60 cm depth (Figure 1). Soil samples were taken by disturbed method for soil physical analysis and undisturbed method for soil chemical and biological analysis. The undisturbed method was carried out using a ring sample with a diameter and height of 5 cm (Kurnia et al., 2017). Meanwhile, the disturbed method was carried out using a soil auger. Furthermore, the soil samples from 3 observation points were composited for chemical analysis, while soil samples for physical and biological analysis were not composited.

Soil physical, chemical, and microbial analysis

The soil physical analysis consists of texture, porosity, and soil moisture content analysis. Soil texture analysis was carried out using the hydrometer method, soil moisture content was measured using the gravimetric method, and porosity was measured using an air pycnometer (Kurnia et al., 2006). Meanwhile, soil chemical analysis consisted of soil organic carbon measured using a spectrophotometric method and soil pH measured using a pH meter (Kurnia et al., 2016; Purbaningtias, 2018). Soil microbial analysis consisted of bacterial and fungal population analysis using the dilution plate method (Kanna et al., 2018; Aziz & Zainol, 2018). Soil samples were inoculated into the medium at dilution series 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}. The growth medium used in this study was NA (Nutrient Agar) for bacteria and PDA (Potatoes Dextrose Agar) for fungi (Safriani et al., 2020). The total population of soil fungi and bacteria was calculated using the total plate count method, with the following formula (Saraswati et al., 2007):

Total population (CFU/ g^{-1} dry soil) =

$$\frac{\text{(number of colonies) x (fp)}}{\text{bk}} \qquad (2)$$

Information:
fp : dilution factor in the petri dish whose colonies are counted; bk : dry weight of soil sample (g) = wet weight x (1-moisture content)

Data analysis

The total population of bacteria and fungi in the big hole and non-big hole system is presented with a bar chart using Microsoft Excel software. The effect of different planting holes on the total population of bacteria and fungi was determined using t-test statistical analysis with a significance level of = 5% using Software R (R Core Team 2020; R Studio 2020).

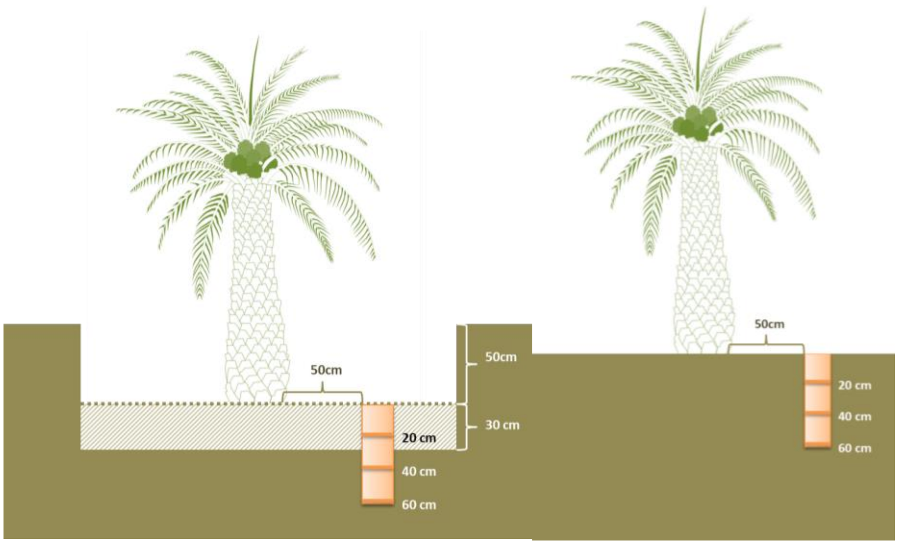

Figure 1. Soil sampling scheme (a) big hole and (b) non-big hole, at depth intervals of 0 20 cm, 20-40 cm and 40-60 cm. The shaded area represents the increase in soil height over 15 years. (Source of oil palm illustration: PPKS documentation)

Results

Incidence of tree mortality

Based on Figure 2, it can be seen that the number of oil palm planted with the big hole system is more than the number of planted with the non-big hole system, which indicates that the mortality rate of oil palm after 15 years planting with a big hole system was lower than on field with non-big hole system. The mortality rate of oil palm tree in plants with a big hole system is 24% dan the mortality rate of oil palm trees with a non-big hole system resulted in 44%.

Soil physical and chemical properties

The results of soil texture analysis showed that the soil texture on plot with big hole and non-big hole systems was sandy clay loam at a depth of 0-20 cm and 20-40 cm. Meanwhile, in the soil depth of 40-60 cm was sandy clay. The results of soil physics and chemical analysis showed that the soil water content and porosity of the field with the big hole and non-big hole planting system were relatively similar (Table 1 and Table 2)

Table 1. Soil texture on land with big hole and non-big hole systems

Planting hole	Depth (cm)	Sand (%)	Dust (%)	Clay (%)	Soil Terxture
Big hole	0-20	63.84	13.84	22.32	Sandy clay loam
	20-40	59.84	7.84	32.32	Sandy clay loam
	40-60	55.84	5.84	38.32	Sandy clay
Non-big hole	0-20	61.84	13.84	24.32	Sandy clay loam
	20-40	59.84	9.84	30.32	Sandy clay loam
	40-60	47.84	9.84	42.32	Sandy clay

Table 2. Soil moisture, porosity, pH , and SOC on land with big hole and non-big hole systems

Planting hole	Depth (cm)	Soil Moisture (%)	Porosity	pH	SOC (%)
Big hole	0-20	31.78	59.04	6	1.50
	20-40	42.68	60.04	6.2	0.92
	40-60	46.89	65.03	5.4	0.57
Non-big hole	0-20	30.80	65.03	4.8	1.10
	20-40	38.72	63.03	4.7	0.85
	40-60	47.29	66.03	4.7	0.49

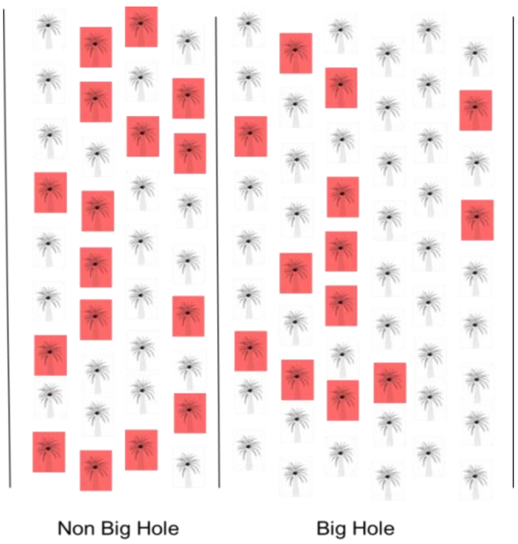

Figure 2. Population of oil palm trees in big hole and non-big hole planting systems. The red color indicates a dead tree

Population of soil fungal and bacteria

The results of soil bacterial and fungal populations on the big hole and non-big hole systems are shown in Figures 3 and 4. Based on the results of statistical analysis (P-Value = 0.05), after 15 years of implementation, it is known that the mean population of bacteria is significantly different but the mean population of fungi is not significantly different between the big hole and non-big hole planting systems. However, the highest populations of bacteria and fungi were found in field with a big hole planting system in soil depth of 0-20 cm, 1.6 x 106 cfu/g, and 9.7 x 104 cfu/g, respectively. On average, from the three soil depths (0-60 cm), the population of bacteria and fungi in standard holes differed 19.28% and 15%, respectively, compared to the population in the big hole system. Furthermore, based on the depth of the soil, the abundance of bacteria and fungi populations showed almost the same trend (in big holes and non-big holes) where the populations of bacteria and fungi were most abundant at a depth of 0-20 cm and decreased at a depth of 20-40 cm and 40 cm. -60 cm.

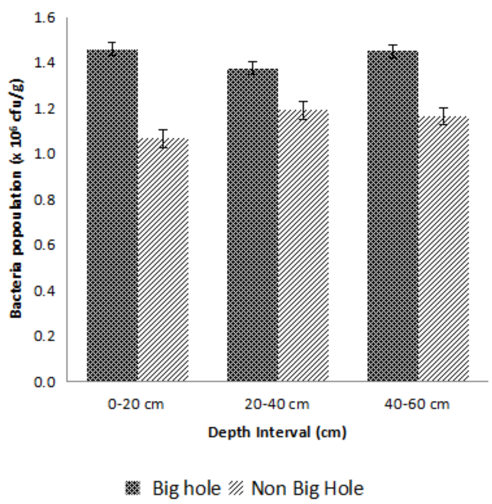

Figure 3. Soil bacteria population in the big hole and non-big hole system at depth intervals, 0-20 cm, 20-40 cm, and 40 60 cm

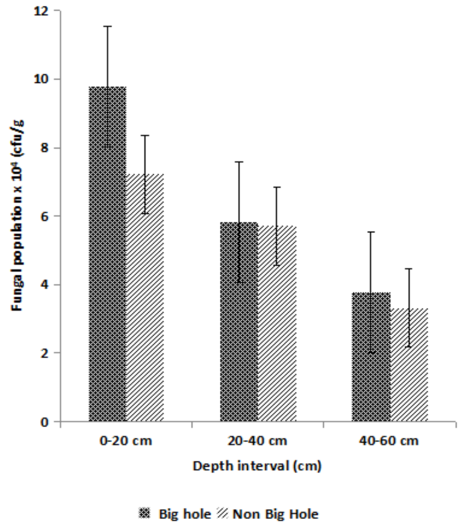

Figure 4. Soil fungal population in the big hole and non-big hole system at depth intervals, 0-20 cm, 20-40 cm, and 40-60 cm

Discussion

The mortality rate of oil palm after 15 years planting with big hole system was lower than on field with non-big hole system. Previously, at 10 years after planting, Pradiko et al. (2016a) have calculated the incidence of tree mortality in the same plot. In this study, the mortality rate of oil palm trees was 6.60% and 10.71% on field with big hole and non-big hole systems. The similar result was also informed in the study of Prasetyo et al. (2008) where the mortality rate of oil palm with a big hole system is lower than that of standard planting holes, with 0.87% and 2.31% respectively. This result showed that soil tillage with the big hole system has the potential to minimize the mortality of oil palm that infected with *G. boninense*. This could be due to the better physical, chemical and biological properties of the soil in oil palm plants with a big hole system, because healthy soil can increase soil disease supression (Janvier et al., 2007). Various potential fungal and bacterial biocontrol agents, namely Trichoderma harzianum, T. viride, Pseudomonas fluorescens, P. aeruginosa, Burkholderia cepacia, and Bacillus species, have been screened and studied for the control of Ganoderma disease (Norman et al., 2019; Susanto et al., 2005; Sapak et al., 2008).

The number of bacterial populations was significantly higher on land with large hole planting systems compared to non-big hole system, but the number of fungal populations was not significantly different. This is because bacteria are more sensitive to environmental changes than fungi, so that environmental changes do not cause significant differences in the total fungal population in fields with large hole systems (Clark, 1997). Even so, the number of fungal and bacterial populations on land with a large hole system still tends to be higher than that of a non big hole system. Chen et al. (2020) showed that conservation tillage greatly increased overall soil microbial biomass 37 %, including both fungal and bacterial biomass, which was caused by improvements in soil physical and chemical properties such as reducing erosion and increasing C-organic. Soil physical and chemical properties on the field with the big hole system is more preferable for growth and development of soil bacteria and fungi. The soil pH in big hole planting system was categorized to be more optimum for soil microbial growth. According to Msambira and Smith (2020) and Sullivan et al. (2016), the optimum pH for soil microbial growth is in the pH range of 5.5–7. At this soil pH value, the growth of the plants is improved which followed by the producing of more root exudates as a carbon source available for microbial survival and propagation (Msimbira & Smith, 2020).

Another factor that affects the high population of fungi and bacteria on the field with the big hole system is the higher soil organic carbon content (SOC). Though this research took place after 15 years of planting, it is expected that the additional EFB in the big hole planting system was also acknowledged as the source of additional SOC. Feng et al. (2019) explain that the availability of SOC will result in changes to the diversity and total population of soil microbes. In this case, the total number of soil microbes will increase with SOC content (Prommer et al., 2020). This is also supported by the fact that SOC is a required substrate to support microbes' metabolism (Gougoulias et al., 2014).

Furthermore, the high population of fungi and bacteria on the field with a big hole system can be occurred by a higher soil water content. Aung et al. (2018) explained that water plays a vital role as a solvent and temperature buffer in microbial cells. Thus, lack of water content can decrease microbial activity and growth (Sorensen et al., 2013). According to Borowik and Wyszkowska (2016), the optimum moisture content for soil bacterial growth is 40%, while fungi grows best at 60% soil moisture content.

The soil texture is the next factor influencing the population of soil bacteria and fungi on the field with the big and non-big hole systems. The research data showed that the total population of soil bacteria and fungi also decreased with increasing depth and decreasing soil sand content. This is supported by Chau et al. (2011) which states that the total microbial population will decrease along with the decreasing soil sand content. High soil sand content is related to soil porosity (Stolf et al., 2011). The higher total population of soil bacteria and fungi in the big hole system can also be caused by higher soil porosity. High porosity is in line with higher water content and greater nutrients in the soil (König et al., 2020). Fichtner et al. (2019) study explained that the number of microbes would increase along with a greater soil porosity. However, since the soil texture in this study was slightly varied, it resulted in the small gap of bacteria and fungi in both observed planting systems.

The higher mean value of pH, soil organic carbon (SOC), water content, and porosity with a big hole system can be caused by the tillage and the addition of soil organic matter in the form of empty fruit bunch (EFB) at the beginning of the planting period. The addition of organic matter can prevent a decrease of soil pH due to the use of chemical fertilizers on the soil (Tao et al., 2018; Murphy, 2014). According to Rosenani et al. (2016) application of EFB can also improve the soil organic carbon as the decomposition process occurred. Additionally, Sakiah (2019) explained that applying oil palm empty fruit bunches on the land could increase the total number of microbes in the soil. Obour et al. (2018), Murphy (2015), and Waldron (2009) reported that decomposed organic matter would produce humus which accrues the water-holding capacity of the soil. The soil porosity is also influenced by the addition of organic matter and implemented tillage in the field (Surya et al., 2017; Robin et al., 2018). The indication of decomposed materials of EFB after 15 years was improving both physical, chemical, and biological soil properties under the big hole planting system. The decomposition process of organic matter consists of 3 times, namely: fast, medium, and slow, where slow decomposition of organic matter can reach >1000 years (Murphy, 2014; Clara et al., 2017).

Furthermore, the total number of fungi and bacteria in each soil depth showed almost the same trend in big hole and non big hole planting systems. The number of bacterial and fungal populations decreases in deeper soil layers. This is in accordance with the research of Feng et al. (2019) and Yao et al. (2018) which shows that the diversity of microbial communities decreased with depth of the plantation soil. Environmental conditions, such as oxygen levels and changes in soil physicochemical characteristics such as pH and SOC that change at each depth have a positive correlation with the diversity of microbial communities (Feng et al., 2019). In several studies, soil pH has been proposed as the main factor determining the diversity of soil microbial communities (Lauber et al., 2009). Soil pH tends to decrease at each depth in both big hole and non big hole planting systems as well as the population of soil bacteria and fungi. This is in accordance with the theory presented by König et al., (2020) that a lower pH will reduce the growth and activity of bacteria. Furthermore, the difference in SOC at each depth also causes a shift in the microbial community between surface and subsurface soils. Feng et al. (2019) stated that the amount of SOC greatly affects the structure of the soil microbial community.

Microorganisms in soil is seen to be critical to the maintenance of soil health and quality, as a wide range of microorganisms is involved in important soil functions. Research by Goh et al., (2020) explains the importance of high microbial abudance and diversity in reducing soil-borne diseases or contributing to soil pathogen suppression. A recent study in Sabah, Malaysia, showed that higher bacterial diversity was observed in soils. disease free compared to soils with a high incidence of root rot disease (Chung, 2011). In addition, soil microorganisms also play essential roles in the nutrient cycles that are fundamentally important to life on plant. Agricultural practices that have failed to promote healthy populations of microorganisms, limiting production yields and threatening sustainability.

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