The Effect of Mulch, *Trichoderma,* and Arbuscular Mycorrhizal Fungi (AMF) Biofertilizer on the Growth of Oil Palm Seedlings Inoculated with *Ganoderma*

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Abstract

A Greenhouse experiment was carried out to determine the effect of mulching, *Trichoderma* sp., *Metharizium anisopilae*,. arbuscular mycorrhizal fungi (AMF) biofertilizer on the growth of oil palm seedlings. The experiment was conducted in 70x80 cm sized of polybags filled with 50 kg of Latosol Ciomas soil. Mycorrhizal inoculum is in the form of spores, hyphae and infected roots. Germinated oil palm was planted in polybag, then covered with empty fruit bunches of oil palm (EFBOP) as mulch. The dose of inorganic fertilizer was 50% for the application of AMF biofertilizer while on the other treatment the dose of fertilizer was 100%. The experimental design was complete randomized design to test nine treatments: 1) without any fertilizer, 2) 100% inorganic fertilizer, 3) EFBOP, 4) EFBOP+*Trichoderma pseudokoningii*+*Trichoderma* DT38, 5) EFBOP+*T. pseudokoningii*+ *Trichoderma* DT41, 6) EFBOP+*T. pseudokoningii* + *Trichoderma* DT38 + *Metharizium anisopilae*, 7) EFBOP+*T. pseudokoningii*+*Trichoderma* DT41+*Metharizium anisopilae,* 8) EFBOP+*T. pseudokoningii*+*Trichoderma* DT38+ *Metharizium anisopilae+AMF biofertilizer*, 9) EFBOP+*T. pseudokoningii*+*Trichoderma* DT41+*M. anisopilae+AMF biofertilizer*. The results showed that application of EFBOP as mulch enhanced the gowth of oil palm seedling and combination with DT 41 and both *T. pseudokoningii* and *Metharizium* produce the similar growth with those 100% inorganic fertilizer treatment. While the addition of EFBOP+*Trichoderma* DT41+*T. pesudokoningii* produced the highest seedling dry weight. The application of AMF biofertilizaer reduced the dose of inorganic fertilizer yielded a similar seedling dry weight with those fertlized with 100% of inorganic fertilizer.

Key words: oil palm, mulch, empty fruit bunches oil palm, arbuscular mycorrhizal fungi, acid soil

Introduction

One method of organic matter application is to use it as mulch. Mulch is organic or inorganic materials either natural or artificial which is placed above the soil and have a role to protect and cover it. Some of the benefits of mulching is to minimize the occurrence of surface runoff, increase water infiltration, protect the soil against rain, modify soil temperature, suppress weeds, reduce the rate of dry surface soil, increase soil biological activity and modifies the level of nutrient availability as well as to maintain or increase the availability of organic material soil (Baon, 1998). Basically the main function of mulching is to maintain the soil moisture. Water is a very important factor in the cultivation of plants including crops. The application of mulch is not accompanied with tillage practice. Muruganandam *et al* (2009) showed that the activity of enzymes that play a role in N mineralization in no-tillage soil is significantly higher compared to conventionally cultivated soil. While White & Rice (2009) suggested that the biological activity in no-tillage soil was detected higher compared to conventional cultivated soil.

In the microbiological aspects, organic materials addition means the addition of carbon and energy sources to improve microbe activities. The same thing seems to occur in peat soil. Komariah *et al* (1993), Nurani *et al.* (2007) reported that the use of microbial consortia and EFBOP increase soil pH and base saturation and lower CEC of peat soil as well as the C/N ratio. Microbes, in addition to functioning release nutrients or nutrient mineralization in the process of decomposition is also able to degrade toxic compounds (Sparling, 1998). However, microbe use root exudate as source of carbon, energy and other nutrients to support their live. Brockling *et al*. (2008) suggests that plants can regulate microbial communities through root exudates.

The application of inorganic fertilizer as well as pesticide in higher dose will disturb the activities of soil microbes caused declining biological soil properties. These imbalances soil properties affect the soil microbial communities to control soil borne pathogens and microbes that play a role in the health of the land and crops. This is supported by research conducted by Vallad *et. al.* (2003) which proves that the application of organic fertilizer (compost) reduce plant disease attack. Rhizosfer manipulation seems necessary to maintain land productivity. Rousk *et al* (2009) suggested that the decrease in pH will increase five times the population of fungi, and *vice versa* for the bacteria. The aim of the research was to determine the effect of mulching*, Trichoderma* sp. , *M. anisopilae*, and AMF biofertilizers on the growth of oil palm seedlings.

Materials and Methods

The experiment was conducted in a greenhouse using a 70x80 cm sized polybags filled with 50 kg of Ciomas soil. Germinated oil palm was used as plant material and planted in polybag and add with empty fruit bunches oil palm (EFBOP) as mulch. DT38 and DT41 isolates were cultured in the media then transferred to PDA medium and inoculated into polybags. Mycorrhizal inoculum is in the form of spores, hyphae and infected roots. Germinated oil palm was planted on the polybag and the EFBOP was added as mulch. In the treatment of mycorrhizal fertilizer, the dosage of inorganic fertilizer was 50% of the recommendation dose while others were 100% of inorganic fertilizer. The experiment was arranged in completely randomized design to test nine treatments i.e. 1) without fertilizer, 2) 100% dose of inorganic fertilizer, 3) EFBOP, 4) EFBOP+*T. pseudokoningii*+DT38, 5) EFBOP+*T. pseudokoningii*+DT41, 6) EFBOP+*T. pseudokoningii*+DT38+ *Metharizium anisopilae*, 7) EFBOP+*T. pseudokoningii*+DT41+*Metharizium anisopilae*, 8) EFBOP+ *T. pseudokoningii*+DT38+*Metharizium anisopilae*+AM fungi, 9) EFBOP+*T. pseudokoningii* DT41+ *M. anisopilae*+AM fungi. Each treatment was repeated 3 times. Observations made on the growth of oil palm.

Results and Discussion

Observations of plant height showed that fertilization increased height of plants while EFBOP solely suppressed the height growth of plant (Table 1). The application of *Trichoderma* sp. and *M. anisopilae* and arbuscullar mycorrhizal fungi (AMF) enhanced the height of palm seedling.

Table 1. The height and leaf number of oil palm seedlings (9 months) in each treatment tested

Treatment	Height (cm)	Leave number
Withou fertilizer	106 ab	19 a
100% inorganic fertilizer	119 ab	19 a
EFBOP	81 a	20 a
EFBOP+Trichoderma DT38+T pseudokoningii	130 b	20 a
EFBOP+ Trichoderma DT41+T pseudokoningii	130 b	20 a
EFBOP+ Trichoderma DT38+T pseudokoningii +Meth	129 b	19 a
EFBOP+ Trichoderma DT41+T p+Meth	133 b	20 a
EFBOP+ Trichoderma DT38+T p+Meth+AMF	126 b	20 a
EFBOP+ Trichoderma DT41+T p+Meth+AMF	123 ab	18 a

Note: Figure (s) in each column followed by the same letter is not different according to Duncan ($P < 0.05$).

However, the application of EFBOP as mulch in term of plant height was not significantly different compared with those were not treated (without fertilizer) and 100% inorganic fertilizer. These results indicated that the plant height variable is not affected by EFBOP and inorganic fertilizer application. The absence of the influence of fertilization and the addition of EFBOP as mulch plants may caused the soil already contains enough nutrients. Inoculation treatments DT 41 + *T. pseudokoningii* or DT 38 + *T. pseudokoningii* without or in combination with *M. anisopilae*, and EFBOP as mulch significantly resulted in higher plant height compared to the application of EFBOP. These results indicated that the application of *Trichoderma* sp. DT 38, DT 41, *M. anisopilae*, *T. pseudokoningii* in combination with EFBOP yield positive effect on plant height. Positive influence is probably due to the decomposition process carried out by DT 38 and DT 41, while *M. anisopilae* seems to have an effect on the prevention of *Oryctes*.as a plant pest. The same was observed in the treatment of DT 38 + *T. pseudokoningii* + plus *M. anisopilae* and AM fungi. AM fungi can be symbiotic with the roots of oil palm and the symbiosis can enhance nutrient uptakes, especially P which contributes to increase plant height. In addition, the treatment was shown that application of AMF (fertilizer 50%) showed a similar plant height with plants that 100% fertilized .These results demonstrated that the application of AMF biofertilizer increased the efficiency of inorganic fertilizer by 50%. The increasing inorganic fertilizer efficiency by using AMF symbiosis has also been reported by other researchers. Some mechanisms that occur are by increasing the reach of roots, and mineralization of organic P through the extraction phosphatase. The application of DT 41 + *T. pseudokoningii* + *M. anisopilae* + and AMF biofertilizer was not significantly different with those of DT 38 + *T. pseudokoningii* + *M. anisopilae* + and AM fungi in term of plant height.

Treatment of EFBOP increased the number of leaves compared to 100% inorganic fertilizer and the unfertilizer treatment (Table 1). The application of EFBOP provides the same number of leaves with the inoculation of DT41 or DT38 and the *T pseudokoningii* accompanied by the application of *M. anisopilae* and AMF biofertilizer. However there is no significantly differences between the number of leaves of all treatments tested in the blank, 100% inorganic fertilizer, or mulching EFBOP. Interaction between AM fungi with DT 38 is better compared to the interaction of those fungi with DT41 especially demonstrated by the number of leaves.

The fresh weight of leaves increased significantly with the addition of inorganic fertilizers as well as giving EFBOP as mulch. Similar results were also observed in the treatment of EFBOP addition which is accompanied by DT 38, DT 41, *T. pseudokoningii, M. anisopilae* and AMF biofertilizer. It was shown that the efficiency of inorganic fertilizer can be achieved by the application of AMF biofertilizer combined with either DT 41 or DT 38. Similar results are also shown on the variables fresh weight of stem. Fresh weights of oil palm seedlings stem with inorganic fertilizer is higher than thethe blank (Table 2). Nevertheless, the fresh weight of stem between 100% inorganic fertilizer and application of EFBOP as mulch and blank werenot significantly different. The addition of DT 41 and DT 38, which is accompanied by *T. pseudokoningii* and *M. anisopilae* results a fresh weight of stem that were not significantly different compared to those with the addition of EFBOP as mulch or 100% inorganic fertilizer. The application of EFBOP as mulch, AMF biofertilizer and 50% inorganic fertilizer yield fresh weight of stem that is not significantly different to 100% inorganic fertilizer treatment. These results indicated that the application of AMF biofertilizer improves inorganic fertilizer efficiency as much as 50%.

Compared to the fresh weight of the stem appears that the effect of the treatments being tested was more visible on the fresh weight of leaf variables. Application of EFBOP accompanied by DT 41 and *T. pseudokoningii* addition produced the highest fresh weight of leaves. Similar results were also observed in the treatment of EFBOP as mulch with DT 38, DT 41 or accompanied by *M. anisopilae*. As for DT 38 leaf fresh weight significantly higher compared with the blank when combined with *M. anisopilae* and AMF biofertilizer.

Table 2. The fresh weight of oil palm seedlings (9 months) in each treatment tested

Treatment	Fresh weight (g)				
	Leaf	Stem	Shoot	Root	Total
Without fertilizer	450 a	833,33 a	1283,33 a	783 a	2066 a
100% inorganic fertilizer	850 b	1433,33 ab	2250 ab	1017 ab	3267 b
EFBOP	863,33 b	1583,33 ab	2466,67 ab	967 ab	3433 b
EFBOP+DT38+T p.	733,33 ab	1683,33 ab	2416,67 ab	933 ab	3349 b
EFBOP+DT41+T p.	1066,67 b	2000 b	2733,33 b	1183 b	3916 b
EFBOP+DT38+T p+Meth	833 b	1566,67 ab	2400 ab	933 ab	3333 b
EFBOP+DT41+T p+Meth	883,33 b	1750 ab	2633,33 ab	1000 ab	3633 b
EFBOP+DT38+T p+Meth+AMF	900 b	1533,33 ab	3433,33 ab	783 a	4216 b
EFBOP+DT41+T p+Meth+AMF	716,67 ab	1416,67 ab	2133,33 ab	1017 ab	3150 b

Note: Figure (s) in each column followed by the same letter is not different according to Duncan ($P < 0.05$).

Fertilization treatments increased fresh weight of shoots as well as the addition of EFBOP as mulch. The application of DT 41+*T. pseudokoningii* and DT 38+*T. pseudokoningii* either accompanied by *M. anisopilae* or not resulted in fresh weight of shoots that are not significantly different compared to those with 100% inorganic fertilizer and EFBOP as mulch. This result shows that the effect of DT 41, DT 38 and *M. anisopilae* is not significantly different on the fresh weight of shoots. However the application of DT 41+*T. pseudokoningii* and DT 38+*T. pseudokoningii* accompanied by *M. anisopilae* and AMF biofertilizer yield shoots fresh weight equal to 100% fertilization. These results indicated that AMF biofertilizer application increased the efficiency of inorganic fertilizer, although this treatment may not significantly increased the fresh weight of shoots of oil palm seedlings.

Root fresh weights were not significantly different in the treatment of 100% inorganic fertilizer, EFBOP as mulch, and EFBOP+DT 38 + *T. pseudokoningii* and DT 41+*T. pseudokoningii* accompanied by *M. anisopilae* or without *M. anisopilae*. The same result observed in the treatment of DT 41 or DT 38+*T. pseudokoningii*+*M. anisopilae* accompanied wth AMF biofertilizer. Other study demonstrated that the application of DT 41 and DT 38 or AMF biofertilizer increase the root fresh weight. The same result was observed in the total oil palm seedlings fresh weight.

Table 3. Dry weight of oil palm seedlings (9 months) in each treatment tested

Treatment	Dry weight (g)				
	Leaf	Stem	Shoot	Root	Total
Without fertilizer	123,33 a	231,67 a	355 a	138 a	493 a
100% inorganic fertilizer	220 a	361,67 a	582 ab	215 ab	797 b
EFBOP	205 a	453,33 a	658 ab	182 ab	840 b
EFBOP+DT38+T p.	201,67 a	386,67 a	588 ab	278 b	866 b
EFBOP+DT41+T p.	225 a	463,33 a	688 b	212 ab	900 b
EFBOP+DT38+T p+Meth	196,67 a	460 a	657 b	140 ab	797 b
EFBOP+DT41+T p+Meth	188,33 a	438,33 a	627 ab	165 ab	792 b
EFBOP+DT38+T p+Meth+AMF	168,33 a	410 a	578 ab	128 a	726 b
EFBOP+DT41+T p+Meth+AMF	126,67 a	425 a	551 ab	145 ab	698 b

Note: Figure (s) in each column followed by the same letter is not different according to Duncan ($P < 0.05$).

The addition of 100% inorganic fertilizer increased dry weight of oil palm stems as well as the treatment of EFBOP as mulch (Table 3). Similar result was observed in mulching treatments EFBOP with by DT 41, DT 38 which with *T. pseudokoningii* and *M. anisopilae* and AMF biofertilizer. Nevertheless, the results of statistical tests indicated that there is no significanly differences between dry weight of plants fertilized with 100% treatment and other treatments. Similar results were also found in variable weight of dry leaves, shoot, roots, and oil palm seedling. It seems that the application of EFBOP as mulch can be combined with the inoculation of *Trichoderma* sp, *M. anisopilae* and AMF biofertilizer. Hence the application of AMF biofertilizer reduced the doseage of inorganic fertilizer as much as 50%. The effect of treatment to *Ganoderma* infection is likely could be suppressed in this experiment.

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