Compost Oil Palm and Indigenous Endophytic Fungi Effect on Basal Stem Rot in Oil Palm Seedling

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Abstract
Basal stem rot (BSR) caused by Ganoderma is an important disease of oil palm in North Sumatra, Indonesia. Control of BSR is complex, because the disease occurs in the dynamic environment at the interface of the roots with soil. Compost with endophytic fungi as a biological fertilizer offer possible advantages and eventually suppressed ganoderma growth. A study using two compost oil palm (empty fruit bunch and palm oil midrib-leaf compost) containing indigenous endophytic fungi (*Trichoderma koningii, T. viride*, and *Aspergillus* sp.) singly and in a mixture was conducted to determine potential of them to control *Ganoderma*. The result showed that 16 weeks after artificial inoculation, all treatments aren't significant difference reduced BSR incidence but compost containing endophytic fungi have potential to inhibit the pathogen showed BSR incidence in a mixture treated lower than singly treated seedlings.
Key words: *Aspergillus* sp.**,** compost, *Ganoderma*, oil palm, *Trichoderma koningii, T. viride*

Introduction

Oil palm (*Elaeis guineensis*) is one of the largest plantation commodity industries in Indonesia. Currently, Indonesia is one of the world's largest producer and exporter of oil palm. Areas of oil palm have increased from 135.000 hectares in 1970 to 8.91 million in 2011 (Pusat Data dan Sistem Informasi Pertanian, 2013), which producing an average palm oil yield approximately 31 tonnes in 2014 (http://www.indonesia-investments.com, 2015). One of province with the second largest oil palm planted area in Indonesia is North Sumatra with oil palm planted area is 1.2 million hectares (Direktorat Jenderal Perkebunan, 2012).

One major problem in oil palm is basal stem rot (BSR) disease, caused by *Ganoderma* spp., which is a serious threat to the oil palm industry in South East Asian countries, especially Malaysia and Indonesia. The disease is lethal and in the past few decades has been spreading rapidly. The disease spreads through root to root contact. During planting any little piece of rotting trunk is removed as this could be a potential source of *Ganoderma* in the current stand (Hasan and Turner, 1998). The disease progresses slowly, but eventually all infected plants become dead. This is because the disease cannot be detected at the early stages.

Field control of BSR by contact chemicals have not been very successful (Soepena *et al.*, 2000) even with those *in vitro* screened effective against the fungus (Khairudin, 1990; Teh, 1996). The current interest in reducing the application of chemical fertilizer and increasing demand for combined effects of beneficial fungi and organic compost can have great impact on crop production and sustainable agriculture. Compost with endophytic fungi as a biological fertilizer offer possible advantages and eventually suppressed ganoderma growth. A study using two compost oil palm (empty fruit bunch and palm oil midrib-leaf compost) containing indigenous endophytic fungi (*Trichoderma koningii, T. viride*, and *Aspergillus* sp.) singly and in a mixture was conducted to determine potential of them to control *Ganoderma*.

Materials and Methods

Preparation of *Ganoderma* inoculum on rubber wood blocks

Fresh rubber wood blocks (12 cm x 6 cm x 6 cm, weighing approximately 450 g) were washed with water and autoclaved for 20 min at 121 ^{o}C. One block each was put in heat-resistant polypropylene bags (15 cm x 33 cm x 0.05 mm thick material) and 100 mL of potato dextrose agar (PDA) added as supplementary nutrient for *Ganoderma.* Since inoculation of the blocks with *Ganoderma* plugs can introduce contamination, the bags were closed by drawing its open end through a 4 cm diameter polyvinyl chloride (PVC) tubing 2 cm long and the remaining hole plugged with cotton. The bags with rubber wood blocks and PDA were autoclaved at 121 ^{o}C for 30 min. After sterilization and cooling, the rubber wood block in the bag was rotated to ensure that it was well covered with the agar before the latter solidified. When the agar had solidified, five pieces five day-old *Ganoderma* culture taken with cork borer and inoculated on each rubber wood block. The inoculated blocks in the bags were then incubated in a dark cabinet at 28 ± 2 ^{o}C for three weeks until fully colonized by *Ganoderma* mycelium

Preparation of endophytic fungi
Trichoderma koningii, Trichoderma viride, and *Aspergillus* sp. were isolated from the oil palm roots in Gunung Melayu, Asahan, North Sumatra and cultured on PDA medium. All of endophyt fungi were prepared using rice medium, then formulated with zeolite powder (1:9 /v:v).

Application of compost oil palm containing indigenous endophytic fungi
Experiments carried out in the glasshouse at Faculty of Agriculture, University of Sumatra Utara, Medan, Indonesia, using a factorial randomized block design. A total of 72 healthy oil palm seedlings three months old were used in this experiment. 300 g of compost single or combination with 30 g of endophytic fungi inoculated before or after artificial inoculation of *Ganoderma* on oil palm seedlings.

Effect of compost oil palm and indigenous endophytic fungi on BSR incidence
At the end of the experiment (4 months/16 weeks), the oil palm seedlings were split longitudinally to observe for root and stem decay and to visually assess the severity of the internal symptoms based on the proportion of root and bole tissues damaged by *Ganoderma*. The estimation was based on the following modified scale (Abdullah *et al.,* 2003; Ilias 2000). 0 = Healthy plants, 1 = Appearance of white fungal mass on any part of plants, with or without chlorotic leaves, 2 = Appearance of basidioma on any part of plants with chlorotic leaves (1–3 leaves), 3 = Formation of basidioma on any part of plants with chlorotic leaves (> 3 leaves), 4 = Formation of well-developed basidioma and the plants dried. Disease severity (DS) for the internal symptoms was calculated by the formula of Liu *et al.* (1995) as under. $DS_{(internal)}$ = (number of seedlings in the rating x rating number)/(total number of seedlings assessed x highest rating)x 100.The disease incidence and disease severity percentages were transformed by arcsine and analyzed using ANOVA with the means compared by the LSD ($P \leq 0.05$).

Results and Discussion
Artificial infection of plants by contact with the inoculum block carrying *Ganoderma* is an effective strategy for inducing the infection. The assessment of disease severity was based on the sequential progression of disease using index values 0-4. Lower disease severity indicates the effectiveness of the treatments in suppressing *Ganoderma* BSR in oil palm through *in vivo* assessment. In general, 16 weeks after artificial inoculation, all treatments aren't significant difference reduced BSR incidence but compost containing endophytic fungi have potential to inhibit the pathogen showed BSR incidence in a mixture treated lower than singly treated seedlings (Table 1). This study showed that compost containing endophytic fungus (*T. koningii, T. viride,* and *Aspergillus* sp.) have a good potential as biological agents to inhibit *Ganoderma.*

Table.1. Disease severity of oil palm plants at BSR 16 weeks after artificial inoculation

Treatments	Disease severity (%)
K0W1E0	33.3
K0W1E1	33.3
K0W1E2	25.0
K0W1E3	25.0
K0W2E0	25.0
K0W2E1	25.0
K0W2E2	25.0
K0W2E3	25.0
K1W1E0	25.0
K1W1E1	8.3
K1W1E2	16.7
K1W1E3	16.7
K1W2E0	25.0
K1W2E1	16.7
K1W2E2	16.7

K1W2E3	8.3
K2W1E0	25.0
K2W1E1	16.7
K2W1E2	16.7
K2W1E3	8.3
K2W2E0	25.0
K2W2E1	16.7
K2W2E2	8.3
K2W2E3	16.7

Note : K0 : without compost,
K1 : empty fruit bunch compost,
K2 : palm oil midrib-leaf compost,
W1 : inoculation *Ganoderma* before endophytic fungi
W2 : inoculation *Ganoderma* after endophytic fungi
E0 : withoout endpohytic fungi
E1 : *T. viride*
E2 : *T. koningii*
E3 : *Aspergillus* sp.

The composition of the microorganisms in composts is affected by the chemistry of the materials from which the compost is prepared (Castaño *et al.*, 2011). Composts with high lignocellulosic substances (tree barks) are mostly colonized by *Trichoderma* spp. In contrast, grape pomace, with low cellulosic substances and high sugars, becomes colonized by *Penicillium* spp. and *Aspergillus* spp. (Kutter *et al.* 1983; Gorodecki and Hadar 1990). In composts, in addition to the well-documented effects of microbial populations against several soil-borne diseases, induction of resistance has also been reported as an additional biocontrol mechanism against both foliar and root diseases (Zhang *et al.* 1996, 1998; Kavroulakis *et al.* 2005; Ntougias *et al.*, 2008). Reduction in disease severity was demonstrated against *Septoria lycopersici* in tomato (Kavroulakis *et al.*, 2005), bacterial leaf spot in radish caused by *Xanthomonas camperstris* pv. *Armoraciae* (Krause *et al.*, 2003), anthracnose in cucumber caused by *Colletotrichum orbiculare* (Zhang *et al.*, 1996) and *Botrytis cinerea* in cucumber and melon (Segarra *et al.* 2007; Yogev *et al.*, 2010). Moreover compost water extract root treatment induce systemic resistance to anthracnose caused by *C. orbiculare* in cucumber and *C. coccodes* in pepper (Sang and Kim 2011).

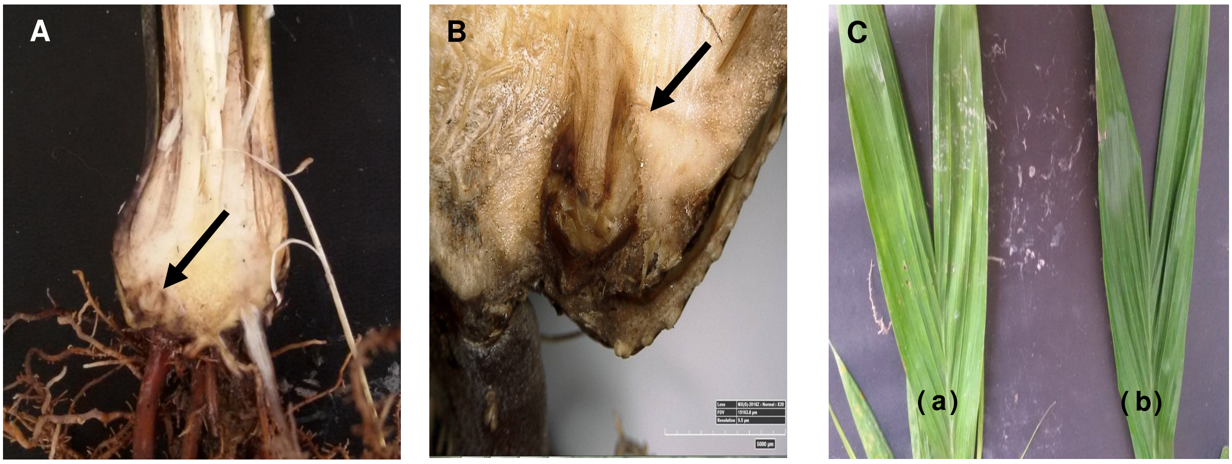

Figure 1. Signs and symptoms of BSR, caused by *Ganoderma*; A, B. necrotic lesion on infected plant (arrow), C. The leaves of infected plants were chlorotic (a), and healthy leaves (b)

The ideal of biocontrol strategy attempts to introduce or promote the activity of biocontrol agents only when and where they are needed or are most effective, and minimises wasteful application of inoculum on non-target habitats. Different mechanisms have been suggested as being responsible for the effects of biocontrol agents; they include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of an inhibitory compound (Haram *et al.,* 1996; Zimand *et al.,* 1996). Thus, the timing of when to apply the treatments is important due to the probability of changes brought about by the biocontrol agent, e.g. by the latter's dominating requirements for space and nutrients.

Artificial inoculation and following very close contact with the root surface, penetration of the root epidermis and exodermis occurred. Heavily infected tissue within the root was evident on sectioning by brown discoloration primarily in the cortex, but in some instances *Ganoderma* mycelium re-emerged through ruptures in the epidermis of infected roots. The cross-section of an infected plant stem showed a necrotic lesion at the vascular region (Figure 1A and B). Immediately in advance of infection was often a small area of yellow tissue which has been observed by other authors including Darmono (1998). The leaves of infected plants were chlorotic and had a white fungal mass on any part of the plants, then formed basidioma before the plants dried (Figure 1C).

Conclusion

Amount of 16 weeks after artificial inoculation, all treatments aren't significant difference reduced BSR incidence but compost (empty fruit bunch and palm oil midrib-leaf compost) containing indigenous endophytic fungi (*Trichoderma koningii, T. viride*, and *Aspergillus* sp.) have potential to inhibit the pathogen showed BSR incidence in a mixture treated lower than singly treated seedlings.

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