Potential of *Trichoderma* and mycorrhizae as biological agents for controlling *Ganoderma boninense* in oil palm

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**Abstract.** One of the problems in oil palm cultivation is *Ganoderma boninense* attack, the causal agent of basal stem rot (BSR) disease. Basal stem rot (BSR) is the most feared disease because the plant death rate can reach 80% in one area. The spread of this pathogen is relatively fast and not easy to control because it occurs through root contact between oil palm plants. Alternatives for controlling this disease are by using biological control agents. The purpose of this paper is to analyze the potential of *Trichoderma* and Arbuscular Mycorrhiza Fungi (AMF) as biological agents and find out the recommendations for the formulation that is effective to control BSR of oil palm. *Trichoderma* and AMF can inhibit *Ganoderma boninense*. Recommendations for the best carrier material for the growth of *Trichoderma* in the field are bran and empty fruit bunch (EFB), while the carrier material for AMF growth is zeolite so that it can extend the latent period to 71 days, disease severity to 38.3%, disease incidence to 46.7%, infection rate to 0.23. The combined application of the AMF formulation and the *Trichoderma* spp formulation can be recommended as a preventive measure for basal stem rot in oil palm plantations.

**Keywords:** Basal Stem Rot, Biological Control, Fungi

1. Introduction

Basal Stem Rot (BSR) of oil palm caused by *Ganoderma boninense* is the most destructive disease in oil palm plantations in Indonesia and Malaysia. This pathogen attacks not only old plants but also young ones. Currently, the rate of BSR disease infection is running faster, especially on soils with sandy textures [1]. Several decades ago, height incidence was only found in gardens with more than twice replanting, but now the disease incidence is relatively high. Thus, many areas are found oil palm plantations with criteria endemic to *G. boninense* and suffer losses the big one. BSR may affect a plantation by reducing the number of standing trees and the weight of fresh fruit bunches. According to Paterson [2], fresh fruit bunches (FFB) yield decreases by an average of 0.16 tons per hectare for every dead palm or equivalent to 35% when half of the planted trees have died. Based on the BSR incident rate, the total area affected in 2020 was estimated to be 443,440 hectares, equivalent to 65.6 million oil palms, which is worrying if preventive measures are not implemented [3]. BSR may affect up to 80% of the trees to die [4]. However, the trees with less than 20% infection can still be treated [5]. Various agronomic efforts to control BSR disease have among other things, land sanitation, namely cleaning the source of infection before planting (replanting). Preventing disease transmission in the garden is conducted by making isolation trenches and a hole-in-hole planting system. Even removing the base of the infected stem (surgery mounding) requires huge costs [6, 7]. Although *Ganoderma* has been studied...
and remains a problem, none of the applications is effective in controlling the epidemic of BPB disease, and remains a problem to this day because of *G. boninense*, which has been that infects oil palm stump can live and thrive in the tissue of live and dead plants hence it needs to be done development of early prevention techniques by utilizing soil microbes that have potential as a biological agent and can reduce the attack of *G. boninense* in oil palm [8]. Agency biological agents or biocontrol agents are the organisms that interact with components of the disease triangle for managing disease [9].

Biological control aims to suppress disease by reducing pathogen inoculum, reducing infection of host plants by pathogens, and reducing the severity of pathogen attack by living microorganisms. Biological control developed as an alternative for basal stem rot control in oil palms, such as the application of *Trichoderma harzianum*, Arbuscular mycorrhizal fungi (AMF), and endosymbiotic bacteria isolated from AMF spores in coconut rhizosphere palm oil can inhibit the growth of the pathogen *G. boninense* in vitro [10].

Biological control mechanisms can occur through several modes of action. The antagonism is one of the modes of action that includes: (a) competition for nutrients or something else in limited quantities but required by the pest, (b) antibiosis as a result of the release of antibiotics or other chemical compounds by microorganisms and suppresses the pest, (c) predation, hyperparasitism, microparasites or other forms others from direct exploitation of pests by other microorganisms and resistance induction in plants [11]. Understanding how biocontrol agents work can make it easier to optimize control while screening more effective strain agents. Understanding the biological control mechanism of plant diseases through interactions between biocontrol agents and pathogens allows us to manipulate the soil environment to create conditions conducive to agents biocontrol or improve biological control strategies.

The purpose of this paper is to analyze the potential of *Trichoderma* and arbuscular mycorrhizal fungi as biological agents and find out the recommendations for the formulation of *Trichoderma* spp. and AMF, which is effective in controlling basal stem rot of oil palm by *G. boninense*.

2. Materials and methods
This research is a literature study obtained from several sources, namely the Research, Development Agency Library, journals in the fields of plantations, Plant Pathology, biology, and sources from e-journals and scientific magazines. This literature study is carried out in 2021.

Bibliography search using the google search engine. The keywords used in Indonesian and English include Basal stem rot, *G. boninense*, oil palm, fungi, *Trichoderma* sp, AMF, biological control agents, dual culture, in planta, in vitro, volatile compound, rhizosphere fungi, mechanism, formulation, and antifungal. The search results are then selected based on the category of the posts found. Articles from blogs and news are not used in the analysis/review process. Scientific writings/papers are then analyzed by tabulation, especially to analyze the potential of *Trichoderma* and AMF as biological agents and find out the recommendations for the formulation of *Trichoderma* spp. and AMF is effective in controlling basal stem rot of oil palm by *G. boninense*.

3. Results and discussion
3.1 *Ganoderma boninense*, the causal agent Basal Stem Rot in oil palm
Both asymptomatic and symptomatic plants of various levels severity of BSR were found in the field. In asymptomatic plants, the center of the spear leaves is perfectly open (Fig 1A), while in the symptomatic plants, spear leaves at the center were unopened (Figure 1B). Then the leaves skirt–like appearance and the symptomatic palms had a pale appearance (Figure 1C); other symptoms observed were the production of fruiting bodies (Figure 1D), bole creation on the base of the trunk (Fig 1E), and finally death of the palms (Figure 1F). The symptoms were typical of those described for the disease. These are different from symptoms of vascular wilt of oil palm in that there is dryness of the lower leaves, the breaking of the rachis at about one third the length from the trunk, the hanging of the dry leaves along the trunk for the typical or acute form, the narrowing of the trunk at the top taking a "pencil – point" plants appearance, and the cracking of the trunk resulting from deterioration of the vessels for the chronic form [12].
Symptoms of basal stem rot disease on oil palm roots can be seen more evident than the symptoms on the leaves, observations on the roots should be done by dismantling the plant from the soil medium. Root necrotic symptoms often encountered are the roots turn blackish brown (Figure 2). Necrotic roots significantly affect the seeds in absorbing nutrients and water that are needed then it will have an impact on the disruption of crown growth [13].

**Figure 2.** Symptoms on the roots of seedlings aged six months (3 mai): (A) plant roots healthy, (B) root necrotic symptoms [13].
*mai: month after inoculation

### 3.2 The role of Trichoderma and AMF as biocontrol agents

The genus *Trichoderma* consists of a large number of fungal strains that act as a biocontrol agent. *Trichoderma* spp. is a biocontrol agent against fungal and bacterial pathogens either indirectly by competing for nutrients and space, modifying environmental conditions, or directly by mechanisms such as mycoparasitism. The importance of *Trichoderma* spp. in the biocontrol process depends on *Trichoderma* strains, antagonist fungi, plants, and environment. Activation of each mechanism implies the production of environmental conditions, including nutrient availability, pH, temperature, and iron concentration. Specific compounds and metabolites, such as plant growth factors, hydrolytic enzymes, siderophores, antibiotics, and carbon and nitrogen. These metabolites can be in the form of overproduction from plants or in combination with appropriate biocontrol strains to obtain new formulations for use in the more efficient control of disease crops and postharvest applications [14].

*T. harzianum* has been reported effectively control various types of fungal pathogens because it
secretes an antibiotic or antifungal compound called trichodermin. *T. harzianum* can also trigger the biosynthesis of the chitinase enzyme, can be highly competitive, and is hyperparasite. In addition, *T. harzianum* has the potential to induce plant resistance to pathogenic infection. Some of the soil-borne fungi that *T. harzianum* can control are *G. boninense* in oil palm, *Sclerotium* spp. on peanuts, and *Colletotrichum truncatum* on soybeans [15].

Inoculation of AMF on oil palm seedlings followed by inoculation of the *Ganoderma* was able to prolong the incubation period of pathogenic fungi to cause infection or cause the death of seedlings. After nine months, all coconut seeds palm oil that was not inoculated with mycorrhizae showed symptoms of the disease by *Ganoderma* fungus. Meanwhile, only 20% of seedlings were inoculated with mycorrhizae show symptoms of the disease, and only 10% cause mortality in oil palm seedlings [16]. The role of AMF in pathogenic biotic stress of *G. boninense* is also reported [17], where AMF inoculation on oil palm seeds can prevent *G. boninense* infection 100%. AMF Inoculation on 20-year-old producing (TM) plants heavily attacked by *G. boninense* in the field, the FMA application cannot turn off *G. boninense* but can extend the production life of the coconut plant palm. Arbuscular mycorrhizal fungi form a symbiotic mutualism or mutual benefit with the roots plants, where AMF helps plants in the absorption of nutrients from in the soil, especially P, increases plant resistance to stress abiotic and biotic and vice versa plants provide root exudates that can be used by AMF for its metabolism. Mycorrhizal endosymbiotic bacteria *Bacillus subtilis* ZJ06 isolated from AMF spores in oil palm rhizosphere able to inhibit the growth of the pathogen *G. boninense* in vitro tests even the inhibitory power of these bacteria is much higher than that of fungicides commonly used nystatin [10]. Microrhiza endosymbiotic bacteria *Bacillus subtilis* B10 compounds are active compounds in vitro which have high inhibitory power very high on the growth of *G. boninense* in vitro tests.

### 3.3 Mode of action of Trichoderma and AMF invitro

Visual observation of inhibition test *Trichoderma* spp. against *Ganoderma* sp. colony control treatment is seen *Ganoderma* sp. mycelium growth faster than growth mycelium *Ganoderma* sp. on treatment *Trichoderma* spp (Figure 3) [13]. The five *Trichoderma* isolates can cover the surface of the petri dish 9 cm in diameter within four days after inoculation. This is because *Trichoderma* spp. able to inhibit growth colony of *Ganoderma* sp. with mechanism competition for nutrition based on in vitro tests on PDA. Competition mechanism is competition growth between antagonists and test pathogens for getting the nutrients and space limited availability [18].

**Figure 3.** The inhibitory ability of *Trichoderma* in vitro: (A) *G. boninense* with *T. asperellum*, (B) *G. boninense* with *T. atroviridae* (C) *G. boninense* with *T. asperellum*, (D) *G. boninense* with *T. virens*. (E) *G. boninense* with *T. hazianum*, (F) Control of *G. boninense*. i: *Trichoderma* spp. ii: *G. boninense* [13]

The results of the multiple culture test obtained two *Trichoderma* spp., which can inhibit *G. boninense* better than other isolates. Hyperparasites test of *T. asperellum* and *T. harzianum* showed a confluence between *Trichoderma* hyphae and *G. boninense* which causes lysis hyphae of *G. boninense* (Figure 4).
Figure 4. Hyperparasitism activity: (A) *T. asperellum*, (B) *T. harzianum* with *G. boninense* on water agar medium [13]

Colony radial growth *Ganoderma* sp. slow to treatment presents *Trichoderma* spp. because *Trichoderma* sp. has the ability to degradation of pathogenic cell walls *Ganoderma* sp. through the enzyme chitinase, glucanase, and protease [19]. Hyphae of *Trichoderma* sp. are attached to and wrapped around the hyphae of *Ganoderma* sp. to make lysis and then destroy pathogenic cell walls *Ganoderma* sp. consisting of chitin and glucan. The molecular mechanism of lytic enzymes involved in the activity of biological agents *T. harzianum* and stated that fungal cell wall degradation, especially chitinases, glucanases, and proteases [20]. If the hyphae of *Trichoderma* spp. stick and wrapped around the hyphae of the host fungus, then the hyphae of the host undergo vacuolation, lysis, and finally destruction. *Trichoderma* sp. to do penetration into the host cell wall with the help of wall-degrading enzymes cells such as chitinase, glucanase, and proteases and use its contents as a source food.

AMF is known to inhibit the growth of *G. boninense* known because of the role of AMF symbiont bacteria because AMF itself could not directly inhibit the growth of *G. boninense*. Antibiosis is indicated by the occurrence of a zone of inhibition of *G. boninense* against AMF symbiont bacteria, and it is suspected that the activity of volatile compounds indicated by inhibition of growth of *G. boninense*, both close to and away from bacteria (BS1), almost all sides of its growth is inhibited (Figure 5). Several previous studies and identified various antibiotics, including compounds such as kanosamine, and zwittermicin A produced by *Bacillus* spp., which can inhibit the growth of pathogens [21].

Figure 5. Inhibitory activity of AMF symbiont bacteria against *G. boninense*: *Bordetella avium* (BS1), *Bacillus subtilis* (BS2), and *Bacillus* sp. (BS3), *G. boninense* (control), i, ii: AMF symbiont bacteria *G. boninense* [13]

AMF symbiont bacteria obtained from *Glomus* sp. and *Gigaspora* sp. are known to inhibit the pathogen *G. boninense*. So AMF needs to be in symbiosis with AMF symbiont. There are 11 genera *Bacillus* of 20 AMF symbiont bacteria [10]. Isolates B5, B14 and B16 are types of the same, namely *Bacillus thuringiensis* CCM11B with 100% homology, and isolates B4 and B17 also showed the same type, namely *Bacillus pumilus* CrK08 with 100% homology, but morphologically has the shape and colony colors are also different.

The interaction between bacteria and mycorrhizal fungi is called "mycorrhiza helper bacteria". These bacteria can help germination of AMF and have antagonistic activity against fungi pathogenic [22]. The mechanisms of bacteria in inhibiting fungi that cause disease are very diverse, including degradation of
virulence, fungi, or the production of various antifungal compounds. Symbiont bacteria such as Burkholderia spp. and Bacillus spp. are capable of producing various volatile compounds of the aldehyde group, alcohol, dimethyl trisulfide, 2-nonanone, 2-undecanone, ketones, and sulfides. Test results prove that bacterial symbionts can inhibit the growth of pathogens. Symbiont bacteria mycorrhizae always be present in AMF spores at the time of isolation, propagation, formulation, and application. Mycorrhizal endosymbiotic bacteria Bacillus subtilis isolated from AMF spores in oil palm rhizosphere is able to inhibit the growth of the pathogen G. boninense in vitro, and even the inhibition of these bacteria is much higher than that of the nystatin fungicide.

3.4 Formulation Trichoderma and AMF in the field
The biocontrol agents such as Trichoderma spp. and arbuscular mycorrhizal fungi are known to inhibit the development of G. boninense. The results of biological control are still not consistent in the field. Biological control with biocontrol agents such as Trichoderma sp. and AMF and other beneficial organisms are very effective in the laboratory but failed at some stage in the field, even after product development. One of the biggest obstacles to biological control is the mass production of biocontrol agents. A significant obstacle to the commercialization of biocontrol agents is the loss of propagule viability over time. Product shelf life biocontrol is highly dependent on the storage temperature and the carrier used to formulate biocontrol products [23].

Characteristics or requirements of an ideal formulation are not allowed phytotoxic to plants and the environment, can increase shelf life, can tolerate adverse environmental conditions, cost-effective, effective for plant disease control, well soluble in water, must be cheap and readily available for formulation development, and product compatible other agrochemicals [24]. The formulation is not only determined by scientific requirements but also by commercial requirements, e.g., easy to store and use and compatible with the equipment used. Necessary formulation requirements are determined by the characteristics of the organism itself and its environment.

Several organic and inorganic materials are known to be propagators and carriers in the formulation of biological agents. Rice bran, crushed corn, and bran can be used for mass propagation of Trichoderma spp. while husks, sawdust, or coconut coir powder can be used as a carrier. Zeolite, sand, and soil as AMF carrier material. The conditions of good characterization for inoculants include: non-toxic to microbial inoculants, absorption capacity and moisture relatively good, easy to accept and not lumpy, easy to sterilize with using either autoclave or Gamma-ray irradiation, available in resources sufficient (unlimited), inexpensive, neutral pH range, non-toxic to plants.

The latent period of G. boninense in infecting oil palm seedlings of susceptible progeny is 40 days after infection. The most extended incubation period is a combination of AMF and Trichoderma spp. with the formulation on EFB compost, which is 71 days or more than two months and significantly different from the control infected with G. boninense (G1) or without bio fungicide application (Table 1). Extending the latency period is one of the control tactics so that energy released by pathogens is not balanced (greater) with energy obtained from plants during infection.
The Role of *Trichoderma* spp. in controlling *Ganoderma* by producing the enzyme -1,3- glucoanase and

| Treatment + *G. boninense* | Latent period (Days) | Disease severity (%) | Disease Incidence (%) | Infection rate (%) |
|----------------------------|----------------------|----------------------|-----------------------|-------------------|
| Control (G1)               | 40.2 c               | 78.3 a               | 100.0 a               | 0.77 a            |
| T1                         | 45.7 abc             | 45.0 bc              | 73.3 abc              | 0.28 c            |
| AMF 1                      | 54.1 bc              | 50.0 bc              | 73.3 abc              | 0.33 bc           |
| AMF 1+T1                   | 59.2 bc              | 61.7 ab              | 60.0 bc               | 0.46 b            |
| AMF 1+T3                   | 54.1 ab              | 66.7 ab              | 86.7 ab               | 0.63 a            |
| AMF 2                      | 42.3 abc             | 61.7 ab              | 86.7 ab               | 0.35 bc           |
| AMF 2+T1                   | 46.7 abc             | 63.3 ab              | 73.3 abc              | 0.51 b            |
| AMF 2+T3                   | 71.5 a               | 38.3 c               | 46.7 c                | 0.23 c            |

Row numbers followed by different letters indicate a significant difference in the distance test Duncan multiples (DMRT) = 5%. *T*: *T. asperellum* & *T. harzianum* in millet + bran, T3: *T. asperellum* & *T. harzianum* in millet + bran + EFB compost. AMF 1: sorghum and zeolite, AMF 2: sorghum, zeolite, EFB compost. G0: without inoculation *G. boninense*, G1: by inoculation of *G. boninense*.

Based on Table 1, the combination of Arbuscular Mycorrhiza Fungi (AMF) formulations in zeolite and EFB compost carrier (AMF2) with *Trichoderma* spp. the formulation in rice bran and EFB compost carrier (T3) reduced disease severity up to compared to with the control treatment, in which the disease severity rate of FMA2 combined with T3 was 38.3% while control was 78.3%. This combination also suppressed the disease incidence by 46.7% compared with control (100%) and also prolonged the latent period of BPB disease until 71 days. Applications of AMF, either alone or in combination with formulations Trichoderma are known to worsen the severity of the disease. This is related to the ability of AMF, which can improve plant resistance to pathogens, by increasing the plant's ability to absorb nutrients. Increased tissue lignification, pathogen competition with AMF against plant photosynthesis, antagonist microbial population and activity, and production of several chemical compounds such as phenols, chitinase enzymes, and -1,3 glucanase enzymes thought to be a factor in controlling pathogens [25].

Empty Fruit Bunch (EFB) as solid waste can be burned and produce bunch ash containing 30%-40% K2O, 7% P2O5, 9% CaO, and 3% MgO. Also contains micronutrients Fe 1,200 ppm, 1,000 ppm Mn, 400 ppm Zn, and 100 ppm Cu. These materials contain ingredients organic as food for agents. Dry organic matter can keep moisture content and viability of dormant propagules, as well as optimizing agent application biological activity to reduce the intensity of *G. boninense* disease in oil palm.

Addition of bran and EFB compost to the formulation with the aim of addition of natural organic ingredients that can increase effectiveness *Trichoderma* sp. because in some plantations it is known that bran is difficult to obtain and EFB is abundantly available in oil palm plantations. EFB is known to have the content of nutrients needed by plants, so that they can be utilized as a carrier for biocontrol agents. EFB has a high C/N content, which is 38. C/N content of ingredients organic matter as a carrier for biocontrol agents and will be applied to seeds must be removed so as not to become toxic to plants and biocontrol agents. EFB that has become compost, the C/N content drops to 7.53, so that it can be directly used by plants. Nitrogen Content EFB compost increased up to 4% from 0.9% and can be utilized by biocontrol agents. Water content of EFB compost increased to 2.09% from before the occurrence of the decomposition process, to reduce the water content in the EFB compost need oven drying.

4. Conclusion

The Role of *Trichoderma* spp. in controlling *Ganoderma* by producing the enzyme -1,3- gluconase and
chitinase capable of hydrolyzing chitin from hyphae walls pathogenic fungi that cause lysis while the role of AMF in controlling Ganoderma is by increasing tissue lignification, competition of pathogens with AMF against plant photosynthesis, antagonist microbial population and activity, and production of several chemical compounds such as phenols, chitinase enzymes, and -1,3 glucanase enzymes suspected as a factor in controlling pathogens Trichoderma spp and AMF both applied singly or in combination can inhibit the development of Ganoderma. Recommendations for the best carrier material for the growth of Trichoderma spp in the field are bran and EFB while the carrier material for AMF growth is zeolite so that it can extend the latent period to 71 days, disease severity to 38.3%, disease incidence to 46.7%, infection rate to 0.23. The combined application of the AMF formulation and the Trichoderma spp formulation can be recommended as a preventive measure for basal stem rot with AMF application on later seed planting Trichoderma spp when transplanting seedlings from pre-nursery to main nursery.

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