**Ganoderma boninense** control in palm oil plantations using **Trichoderma harzianum** in various Media

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**Abstract.** *Ganoderma boninense* is a pathogen that can attack palm oil plants. This fungus can cause death, causing a decrease in the number of stands and palm oil production. One alternative to control *Ganoderma boninense* in palm oil is using the biological agent; 'Trichoderma harzianum'. This research aimed to examine the inhibition of *T. harzianum* in various media on different spear leaves accumulations on *G. boninense* in palm oil plants. The research was arranged in a factorial randomized block design. The first factor was *T. harzianum* application with different media, the second factor was the accumulation of spear leaves on palm oil trees. The data from the observations were analyzed using the analysis of variance, followed by the DMRT at 5% level. The results of statistical tests showed that the application of *T. harzianum* in various media had no significant effect on the number of spear leaves at 1.5 months after application (MAA) and *G. boninense* mycelium at 3 MAA, but had a significant effect on the number of spear leaves at 3 MAA, *G. boninense* mycelium at 1.5 MAA, *T. harzianum* mycelium at 1.5 and 3 MAA and also significantly affected the root length at 1.5 and 3 MAA. The accumulation of spear leaves significantly affected at 1.5 and 3 MAA but had no significant effect on the observation of *G. boninense* mycelium, *T. harzianum* mycelium and the root lengths at 1.5 and 3 MAA. The interaction between *T. harzianum* in various media and the accumulation of spear leaves had a significant effect on decreasing the number of spear leaves at 1.5 MAA but had no significant effect on *G. boninense* mycelium, *T. harzianum* mycelium and the root lengths at 1.5 and 3 MAA. The application of *T. harzianum*, which was cultured in various media, was able to reduce the accumulation of spear leaves after 3 months of application. The lowest number of spear leaves was found in *T. harzianum* application with 25% OPEFB + 75% cow dung compost. The best of *T. harzianum* media in stimulating palm oil's root growth was 50% OPEFB + 50% cow dung compost.

1. **Introduction**

Palm oil (*Elaeis guineensis* Jacq) is one of the important plantation crops in Indonesia. This plant produces vegetable oil for the food industry as well as for fuel (biodiesel) [1]. Based on data from the Directorate General of Plantations at the Ministry of Agriculture, Indonesia's palm oil area in 2018 reached 14.23 million hectares with a production of 48.68 million tons of oil palm.

In the effort to achieve production, palm oil planters face various challenges, including handling the attack of the pathogenic fungus 'Ganoderma boninense' as a cause of stem rot disease [2]. This disease has caused the death of up to 50% of the plant population in several palm oil plantations in Indonesia, resulting in a decrease in oil palm production per unit area [3].
It has been reported that soils with low fertility are prone to a relatively high incidence of stem rot disease. The results of the Ganoderma infection rate study in four soil texture classes [4] showed that the infection rate in the sand planting medium was 1.77-1.83 plants per month per 100 plants. In other studies, it was stated that K content in the soil had a positive correlation with the intensity of stem rot disease [5]. Based on plant age, the rate of Ganoderma attack is higher in old plants than in young plants, the spread of stem rot disease occurs due to contact between healthy plants and diseased plants, the roots of diseased plants will be the source of inoculum in the soil [6].

Research on the control of G. boninense attack that causes stem rot disease based on technical culture was carried out by several methods, namely sanitation of the inoculum source, hole in hole planting system, surgery and burial and the making of isolation trenches [7]. The application of these four control methods can reduce the rate of G. boninense infection, sequentially; 2 years after treatment (YAT), 7 YAT, 3 YAT and 2 YAT [7]. Another study that is the control of Ganoderma using an organic fungicide which was applied to the base of the stem which had been cleaned from the fruiting bodies of Ganoderma sp. and Ganoderma sp colonized stem tissue, showed the opening of spear leaves and increased palm oil trees in forming female flowers, increasing the N, P and K nutrients content by applying organic fungicides every two weeks [8].

The control of G. boninense still needs to be investigated in order to overcome the high rate of plant destruction due to stem rot disease in palm oil plantations. This research aimed to determine the effect of Trichoderma harzianum on various media on the inhibition of G. boninense development which was observed through the number of spear leaves, G. boninense and T. harzianum mycelium development, and also palm oil root growth. This research is expected to find the best medium for T. harzianum in suppressing the growth of G. boninense which is visualized from a decrease in the number of spear leaves hence the stem rot disease of palm oil can be controlled.

2. Research Methods

This research is an experimental research design, arranged in a factorial randomized block design. Factor I was the application of T. harzianum with different media, consisting of seven levels, namely M0 (as control), M1 (Trichoderma sp. + Water), M2 (empty bunches + Trichoderma sp. + Liquid Organic Fertilizer/LOF), M3 (cow dung compost + Trichoderma sp. + LOF), M4 (cow dung compost + chopped empty bunches + Trichoderma sp. + LOF), M5 (75% chopped empty bunches + 25% cow dung compost + Trichoderma sp. + LOF), M6 (75% cow dung compost + 25% chopped empty bunches + Trichoderma sp. + LOF). Factor II was the accumulation of spear leaves on palm oil trees, consisting of 3 levels, namely T2 (accumulation of spear leaves 2), T3 (accumulation of spear leaves 3) and T4 (accumulation of spear leaves 4). The treatment combination was 21, replicates 5, the sample plants in one plot 2, thus there were 210 sample plants.

2.1 Materials and Tools

The materials used in this research were plants attacked by G. boninense, Trichoderma harzianum inoculum with $10^{12}$ population, cow dung compost, palm oil empty fruit bunches, Liquid Organic Fertilizer (LOF), bran, water and chemicals for total microbial observation in the laboratory. The tools used were gunny, hoe, spade, rickshaw, broomstick, scale, measuring cup and colony counter meter.

2.2 Research Implementation

The research was conducted at Afdeling IV Kebun Gunung Pamela PT. Perkebunan Nusantara III Sumatera Utara Province from January to August 2019. The research was started by conducting an inventory of blocks affected by stem rot disease with the criteria of having a population of 90-110 trees ha-1. Furthermore, identifying the symptoms of G. boninense with the characteristics of the accumulation of spear leaves, broken fronds and mycelium/fruiting bodies. The treatments were selected with 3 criteria, that is plants with the accumulation of spear leaves 2, 3 and 4 (as T2, T3 and T4). Then, the media preparation was carried out, that was palm oil empty fruit bunches (OPEFB) that have been chopped, cow dung compost, and liquid organic fertilizer. The media that had been enriched with T. harzianum were weighed, that were 25 kg of OPEFB (M2), 25 kg of cow dung compost (M3), 25 kg mixed media of 50% of OPEFB and 50% of cow dung compost (M4), 25 kg of 75% of OPEFB and 25% of cow dung compost (M5), and 25 kg of 75% of compost and 25% of OPEFB (M6). The M0 treatment; without the application of T. harzianum (control) and the M1
treatment; *T. harzianum* + water, was applied by sprinkling it on the base of the palm oil plant, with a dose of 1 liter tree-1.

2.3 Observation parameters

*Spear leaf observation*

Spear leaves are leaves that do not open. Observations were made by counting the number of spear leaves contained in each sample plant at 1.5 months and three months after the treatment application.

*Ganoderma boninense mycelium observations*

Visual observation of *G. boninense* mycelium (fruiting body) was using qualitative methods, namely 0 = none, 1 = few, 2 = moderate, 3 = a lot, 4 = fruiting body, carried out 1.5 months and 3 months after treatment application.

*Trichoderma harzianum mycelium observations*

Visual observation of *T. harzianum* mycelium was using qualitative methods, namely 0 = none, 1 = few, 2 = moderate, 3 = a lot, carried out 1.5 months and 3 months after treatment application.

*Increased root length observations*

Root observations were carried out visually. Root growth was measured with a ruler (cm). The measurements were carried out at 1.5 months and 3 months after treatment application.

2.4 Data analysis

The results of the observations were analyzed with Analysis of Variance if F count > F table then it is followed by the Duncan Multiple Range Test (DMRT) with a level of 5% [9].

3. Results and Discussion

The results of statistical tests showed that the application of *T. harzianum* in various media had no significant effect on the number of spear leaves at 1.5 MAA and *G. boninense* mycelium at 3 MAA, but had a significant effect on the number of spear leaves at 3 MAA, *G. boninense* mycelium at 1.5 MAA, *T. harzianum* mycelium at 1.5 and 3 MAA and also significantly affected root length at 1.5 and 3 MAA. The accumulation of spear leaves significantly affected at 1.5 and 3 MAA but did not significantly affect the observation of *G. boninense* mycelium, *T. harzianum* mycelium and root lengths at 1.5 and 3 MAA. The interaction between *T. harzianum* in various media and the accumulation of spear leaves had a significant effect on decreasing the number of spear leaves at 1.5 MAA but had no significant effect on *G. boninense* mycelium, *T. harzianum* mycelium and root lengths at 1.5 and 3 MAA.
Table 1. The Mean of Spear leaves, *G. boninense* mycelium, *T. harzianum* mycelium, and palm oil root length

| Treatment | Spear Leaf | *G. boninense* mycelium | *T. harzianum* mycelium | Root Length |
|-----------|------------|-------------------------|-------------------------|-------------|
|           | 1.5 MAA    | 3 MAA                   | 1.5 MAA                 | 3 MAA       | 1.5 MAA | 3 MAA | 1.5 MAA | 3 MAA | 1.5 MAA | 3 MAA | 1.5 MAA | 3 MAA |
| M0        | 2.55       | 2.60a                   | 0.25cd                  | 0.30        | 0d      | 0e    | 0e      | 0.09d | 0.02d |
| M1        | 2.03       | 1.63cd                  | 0.77a                   | 0.23        | 0.07d   | 0.3e  | 0.41de  | 0.57ed | 0.67ed |
| M2        | 2.03       | 2.00bc                  | 0.51abc                 | 0.53        | 1.33a   | 1.33a | 2.91a   | 4.62a  |         |
| M3        | 2.27       | 2.33ab                  | 0.23cd                  | 0.33        | 0.63bc  | 0.57cd| 2.73ab  | 4.83a  |         |
| M4        | 2.57       | 2.07abc                 | 0.03d                   | 0.40        | 0.58bc  | 0.57cd| 3.56a   | 5.50a  |         |
| M5        | 2.17       | 1.50d                   | 0.33bcd                 | 0.13        | 0.93b   | 1ab   | 1.82bc  | 2.76b  |         |
| M6        | 2.13       | 1.47d                   | 0.7ab                   | 0.47        | 0.5c    | 0.77bc| 1.18ed  | 1.72bc |         |
| T2        | 1.93b      | 1.6b                    | 0.45                    | 0.25        | 0.60    | 0.65 | 1.96    | 3.14   |         |
| T3        | 2.18b      | 1.78b                   | 0.54                    | 0.37        | 0.77    | 0.85 | 2.19    | 3.73   |         |
| T4        | 2.48a      | 2.12a                   | 0.30                    | 0.43        | 0.65    | 0.77 | 2.16    | 3.13   |         |
| M0T2      | 2.2b-f     | 2.0                     | 0.3                     | 0.3         | 0.0     | 0.2  | 0.0     | 0.1    |         |
| M0T3      | 2.2b-f     | 2.5                     | 0.2                     | 0.4         | 0.0     | 0.0  | 0.0     | 0.0    |         |
| M0T4      | 2.9ab      | 2.7                     | 0.3                     | 0.2         | 0.0     | 0.0  | 0.0     | 0.0    |         |
| M1T2      | 2.3b-f     | 1.5                     | 0.6                     | 0.2         | 0.0     | 0.2  | 0.6     | 0.74   |         |
| M1T3      | 2.3b-f     | 1.6                     | 0.8                     | 0.2         | 0.2     | 0.3  | 0.0     | 0.0    |         |
| M1T4      | 1.5f       | 1.8                     | 0.9                     | 0.3         | 0.0     | 0.4  | 0.62    | 0.97   |         |
| M2T2      | 1.6ef      | 1.5                     | 0.7                     | 0.6         | 1.2     | 1.2  | 2.74    | 4.94   |         |
| M2T3      | 2c-f       | 2.3                     | 0.8                     | 0.7         | 1.4     | 1.4  | 2.76    | 4.58   |         |
| M2T4      | 2.5b-e     | 2.2                     | 0.0                     | 0.3         | 1.4     | 1.4  | 3.24    | 4.34   |         |
| M3T2      | 1.9c-f     | 2.3                     | 0.5                     | 0.5         | 0.4     | 0.2  | 2.04    | 3.68   |         |
| M3T3      | 2.3b-f     | 2.0                     | 0.2                     | 0.2         | 0.8     | 0.8  | 2.84    | 6.04   |         |
| M3T4      | 2.6abc     | 2.7                     | 0.0                     | 0.3         | 0.7     | 0.7  | 3.32    | 4.76   |         |
| M4T2      | 2.1c-f     | 1.8                     | 0.1                     | 0.2         | 0.6     | 0.6  | 3.52    | 5.08   |         |
| M4T3      | 2.3b-f     | 2.0                     | 0.0                     | 0.3         | 0.5     | 0.5  | 4.14    | 6.78   |         |
| M4T4      | 3.3a       | 2.4                     | 0.0                     | 0.7         | 0.6     | 0.6  | 3.02    | 4.64   |         |
| M5T2      | 1.7def     | 1.4                     | 0.3                     | 0.0         | 0.9     | .01  | 1.49    | 2.49   |         |
| M5T3      | 2.4b-e     | 1.4                     | 0.5                     | 0.2         | 1.2     | 1.4  | 1.82    | 2.66   |         |
| M5T4      | 2.4b-e     | 1.7                     | 0.2                     | 0.2         | 0.7     | 0.6  | 2.16    | 3.14   |         |
| M6T2      | 2c-f       | 1.1                     | 0.5                     | 0.0         | 0.5     | 0.7  | 1.37    | 1.93   |         |
| M6T3      | 1.8c-f     | 1.4                     | 0.9                     | 0.6         | 0.5     | 0.7  | 1.58    | 2.33   |         |
| M6T4      | 2.6a-d     | 1.9                     | 0.7                     | 0.8         | 0.5     | 0.9  | 0.58    | 0.91   |         |

Information: numbers followed by different letters in the same column are significantly different according to the DMRT at the 5% level

MAA : Month(s) After Application  
M1 : *T. harzianum* without mixed media  
M2 : *T. harzianum* with the oil palm empty fruit bunches media  
M3 : *T. harzianum* with cow dung compost media  
M4 : *T. harzianum* with 50% of OPEFB + 50% of cow dung compost media  
M5 : *T. harzianum* with 75% of OPEFB + 25% of cow dung compost media  
M6 : *T. harzianum* with 25% of OPEFB + 75% of cow dung compost media  
T1 : Accumulation of spear leaves = 2  
T2 : Accumulation of spear leaves = 3  
T3 : Accumulation of spear leaves = 4
3.1 Spear leaves

The results showed that the application of *T. harzianum* in various media was able to suppress the development of *G. boninense*. This is indicated by the reduced accumulation of spear leaves of palm oil plants at 3 months after the treatment application. The decrease in the number of spear leaves was influenced by *T. harzianum* cultured in various media. The optimal reduction in the number of spear leaves requires *T. harzianum* to inhibit the development of *G. boninense* pathogen hence plant growth is not inhibited by stem rot. Arya and Perello stated that Trichoderma sp was able to release antibiotic compounds such as gliotoxin and glioviridin. These antibiotic compounds affect and inhibit many functional systems and make pathogens susceptible [10].

In infected plants, *G. boninense* fruit bodies are not necessarily found at the base of the stem, but it can be identified by the number of spear leaves that do not open as many as ± 3 leaves. Basidiocarps that are formed initially are small, round, and white in color, with rapid growth, hence form adult basidiocarps that vary in shape, size and color. Generally, the basidiocarp develops slightly above and around the base of the affected stem. The larger basidiocarp size indicates further disease progression and eventually causes death in the plant [11].

3.2 *G. boninense* mycelium

Application of *T. harzianum* in various media was able to suppress the growth of *G. boninense* mycelium at 1.5 MAA. This is in line with Purwanto et al’s statement that the application of the fungus Trichoderma spp which is applied together with the pathogenic fungus *G. boninense* can reduce the distribution area of *G. boninense* [12]. *Trichoderma spp* produces β-1.3-glucanase and chitinase enzymes which are able to hydrolyze chitin from the hyphal wall of pathogenic fungi, causing lysis [13]. Inhibition mechanism of Trichoderma spp. can occur through hyperparasites (parasitizing the mycelium of other fungi by penetrating the cell wall and entering the cell to take nutrients from the cell hence the fungus will die). Trichoderma spp produces antibiotics such as alamethicin, paracelsian, trichotoxin which can destroy fungal cells through damage to cell membrane permeability [14]. In unfavorable environmental conditions, poor nutrients or drought, Trichoderma spp. will form chlamydospores as propagules to survive and develop again if environmental conditions are favorable [15].

3.3 *T. harzianum* mycelium

Application of *T. harzianum* in various media significantly increased the development of *T. harzianum* mycelium on observations of 1.5 and 3 MAA. The best development of mycelium was found in M2 (*T. harzianum* with oil palm empty fruit bunches) treatment. Environmental conditions greatly affect the development of *T. harzianum*. Soil pH, aeration, and nutrient sources are factors that influence the development of Trichoderma spp. in the field. At low pH and humid conditions, Trichoderma spp. will develop well. The advantages of using Trichoderma spp. which has the potential as a biological agent is a fast growth, easy to culture in natural and cultured conditions. In addition, several types of Trichoderma spp. can survive by forming chlamydospores under unfavourable conditions and sufficiently resistant to fungicides and herbicides [15].

3.4 Increased length of palm oil roots

The increase in palm oil root length after application of *T. harzianum* cultured in various media at 1.5 and 3 MAA showed differences. The highest root length gain was found in the treatment of M4 (*T. harzianum* with 50% of OPEFB + 50% of cow dung compost). While the root length increase in the treatment of spear leaf accumulation showed no differences in T1, T2 and T3. Plant roots colonized by Trichoderma sp. and Glioolidium sp. can protect plants from pathogens, especially soil-borne pathogens [16]. Based on the results of research by Elfina et al, it was found that *T. harzianum* application to early palm oil nurseries increased root volume [17]. This was confirmed by the results of the research by Naher et al., stated that the chitinase gene produced by *T. harzianum* was able to suppress *G. boninense*. The chitinase gene is a gene expressed by *T. harzianum* against *G. boninense* [18]. *T. harzianum* culture media such as OPEFB and cow dung compost used in this research are organic matters which also play a role in helping plant root development. Organic matter contains nutrients needed by plants and also organic acids that play a role in dissolving nutrients. In addition,
organic matter can increase the availability of water in the soil, improve soil structure, hence plant roots can grow and develop [19].

4. Conclusion

The application of T. harzianum which was cultured in various media was able to reduce the accumulation of spear leaves after 3 months of application on palm oil attacked by G. boninense. T. harzianum media which is best in stimulating palm oil root growth is 50% of OPEFB + 50% of cow dung compost. OPEFB and cow dung act as T. harzianum media as well as a source of soil organic matter that can stimulate the growth of palm oil plant roots.

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