

PAPER • OPEN ACCESS

The application of different *Bacillus subtilis* contained formula as bio fungicide tablet to control *Ganoderma boninense* in oil palm nurseries

To cite this article: F Puspita *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **250** 012052

View the [article online](#) for updates and enhancements.



IOP | ebooks™

Bringing you innovative digital publishing with leading voices to create your essential collection of books in STEM research.

Start exploring the collection - download the first chapter of every title for free.

The application of different *Bacillus subtilis* contained formula as bio fungicide tablet to control *Ganoderma boninense* in oil palm nurseries

F Puspita^{1,2}, Hadiwiyono^{1*}, S H Poromorto¹ and D I Roslim³

¹Department of Agriculture Science, Graduated School of Universitas Sebelas Maret, Surakarta, Indonesia.

²Department of Agrotechnology, Faculty of Agriculture, Universitas Riau, Pekanbaru, Indonesia.

³Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau, Pekanbaru, Indonesia.

Email: hadi_hpt@yahoo.com

Abstract. This study aimed to determine effect of different *Bacillus* sp. endophytic contained formula as bio fungicide tablet to control *Ganoderma boninense* in oil palm nursery. A Complete Randomized Design, 5 treatments and 4 replicates were used. The treatments were different formula namely: F₀ = *Bacillus* sp. endophytic, F₁ = *Bacillus* sp. endophytic + sago starch + peat + tapioca flour, F₂ = *Bacillus* sp. endophytic + corncobs flour + sago dregs + tapioca flour, F₃ = *Bacillus* sp. endophytic + solid + talk + tapioca flour, F₄ = *Bacillus* sp. endophytic + pineapple skin flour + zeolite + tapioca flour. Data were analyzed using ANOVA at $\alpha = 5\%$. The results showed that all formulas were able to slow down incubation period and reduce disease intensity. However, the increase of seedlings height, number of leaves, increase in tuber diameter, root volume, root canopy ratio and dry weight of roots is not affected. F₃ was found as best formula due to able control *G. boninense* by slowing down disease incubation period and reduce its intensity to 0% within 140 days. That formula also shows better in increasing seedling height, number of leaves, diameter of stem, root volume, and weight dried root.

1. Introduction

Basal Stem Rot disease, caused by *Ganoderma boninense*, is a major disease in oil palm plantations. *Ganoderma* is a pathogen attacking oil palm trees and causing major lost in older palm. But now on oil palm plantations that have entered the replanting stage. *Ganoderma* has attacked immature oil palms aged 1 to 2 years and plants produce 4 to 5 years of age and in nurseries. This disease has caused oil palm deaths in Indonesian plantations up to 80% or more than oil palm population, causing a decrease in palm oil production per unit area [1].

In Riau province oil palm plantations covering 533.8 ha have been attacked by *Ganoderma boninense* and the largest attack is in Kampar district which is 211 ha. The area of Old or Damaged Plants reached 36,551 ha, of which the total area of oil palm plantations was 2,399,172 ha. This condition can be a source of inoculum for oil palm plants that enter the replanting phase. Several controls have been suggested to control Basal Stem Rot disease include controlling technical, chemical



and biological cultures by using biological agents from the rhizosphere of plants, but all control techniques that have been implemented have not succeeded in controlling it maximally. Control by using biological agents to control *Ganoderma boninense* is an alternative approach to reduce the use of synthetic chemical fungicides that can lead to the development of pathogenic resistance, and disruption of ecolytic balance [2]. Biological control that has been done a lot is controlling using bio fungicides with active ingredients *Bacillus* sp. originating from the rhizosphere of oil palm plants. Use of *Bacillus* sp. both in the form of live bacterial cells and in the form of filtrate culture have not shown significant results. This is consistent with the results previous study [3][4] that *Bacillus* sp. at a concentration of 3.21×10^{10} cells / ml still showed symptoms with an average intensity of attack of *G. boninense* by 5% 90 days after inoculation.

Currently, the use of biocontrol agents such as endophytic fungi and endophytic bacteria for suppressing plant disease has gained much attention in pathological research. *Bacillus* sp. colonize plant roots thereby inhibiting the growth of *Ganoderma boninense* through the production of antimicrobial substances, competition space and nutrients and can cause a host plant becomes resistant to attack by pathogens [5]. Use of *Bacillus* sp. as a biological agent, it is generally carried out in the form of live bacterial cells and is considered less practical and less efficient for applications in the field, especially for large-scale application purposes. Therefore, it is necessary to develop a technique for packaging biological agents in the form of formulation. Earlier report said that in a formulation there must be active ingredients, nutrients, carrier material and mixing material [6]. This study was conducted to determine the effect of application formula contained *Bacillus* sp. endophytic as bio fungicide to control *Ganoderma boninense* in oil palm nurseries

2. Materials and methods

The research was conducted in the laboratory of plant diseases and Experimental Station, Faculty of Agriculture, Riau University from June 2017 to December 2017. The material used in this study was 3 months old Topaz 3 variety of palm oil seedlings (the result of a crossing between Dura Deli and Pisifera Ekona) from PT. Tunggal Yunus Estate Pekanbaru-Riau. The isolate of *Bacillus* sp. endophytic from oil palm plants originated from Teluk Dalam District, Asahan Regency, North Sumatra. The isolate of *G. boninense* is the collection from Biofertilizer Industry Business Unit and Biopesticide, Faculty of Agriculture, University of Riau.

This research was conducted experimentally using a Completely Randomized Design (CRD) consisting of 5 treatments and 4 replications to obtain 20 experiment units. Each experiment unit consists of 2 seedlings. The treatments were different formula containing *Bacillus* sp. (F) namely: F0 = *Bacillus* sp. endophytic without formulation, F1 = 15 ml of inoculant *Bacillus* sp. endophytic + 100 g of sago starch + 25 g of peat + 25 g of tapioca flour, F2 = 15 ml of inoculant *Bacillus* sp. endophytic + 100 g of corn cobs flour + 25 g sago + 25 g of tapioca flour, F3 = 15 ml of inoculant *Bacillus* sp. endophytic + 100 g solid + 25 g talk + 25 g tapioca flour, F4 = 15 ml inoculant *Bacillus* sp. endophytic + 100 g pineapple skin flour + 25 g zeolite + 25 g tapioca flour. Data were analyzed using analysis of variance, then followed by an Honestly Significant Difference Test (HSD) at the 5% level

2.1. Rejuvenation of *Bacillus subtilis*

B. subtilis endophytic were isolated on Nutrient Agar (NA) medium using a scratch method, followed with incubation at 27°C for 48 hours.

2.2. Rejuvenation of *G. boninense*

The *G. boninense* was isolated from the collection of the Biofertilizer Industry Business Unit and Biopesticide from the Faculty of Agriculture, University of Riau. Isolates were isolated by removing hyphae grown on PDA medium on petri dishes then stored at room temperature.

2.3. Substrate preparation of inoculum *Ganoderma boninense*

Fungus *G. boninense* of oil palm plantations have been isolated from people in the region Kampar Riau Province. The samples derived from healthy plant parts which are located around diseased plants. Then isolated with PDA media after 1 week purified and then identified under a microscope. Breeding *G. boninense* substrate inoculum was done with the palm midrib pieces at size 2 cm x 2 cm x 2 cm, soaked overnight using sterile distilled water then put in a heat-resistant polypropylene plastic that is topped onion 1-inch diameter PVC pipe and closed cotton, then sterilized by autoclaving at 121°C for 60 minutes. Cultures of *G. boninense* aged 7 days on PDA infested on a substrate that has been sterilized and incubated at room temperature for 4 weeks, to colonize all parts of the substrate, and then can be directly used as a source of inoculum in a polybag.

2.4. Spore production of *Bacillus subtilis* endophytic

The spore production of *B. subtilis* endophytic are used by Luria Bertani (LB) medium. *Bacillus subtilis* endophytic which have been grown on NA media for 48 hours are taken as much as one loz and inoculated on LB media. incubated using a shaker for 3 days at a speed of 150 rpm.

Manufacture of bio fungicide formulations with active ingredients *Bacillus subtilis*. Endophytic. *Bacillus subtilis* endophytic spores that are 3 days old are harvested by centrifuging at 7,500 rpm at room temperature for 6 minutes. The bacterial spores were then washed with Sodium Broth (NB) media, then washed again with a solution of 0.05 molar phosphate buffer saline (PBS) pH 7.0. Bacterial spores that have been washed with PBS are resuspended in PBS solution.

Each organic material is weighed starting from sago starch flour, corn cobs flour, solid waste, and pineapple skin waste each weighing as much as 800 g, each unit. Carrier materials such as peat, zeolite, sago pulp and talc. Adhesive materials such as tapioca flour was 200 g for each treatment unit. The weighed ingredients are put into polypropylene plastic and wrapped in aluminum foil and then sterilized by using an autoclave at a pressure of 1.5 psi, temperature of 121°C for 15 minutes. Refrigerated flour for use in making tablet bio fungicides.

2.5. Manufacture of bio fungicide tablet formula contained active ingredients of *Bacillus subtilis*. Endophytic

Spore *Bacillus subtilis* was manufactured of bio fungicide formulations with active ingredients *Bacillus subtilis*. Endophytic. *Bacillus subtilis* spores. endophytic that are 3 days old are harvested by centrifuging at 7,500 rpm at room temperature for 6 minutes. The bacterial spores were then washed with Sodium Broth (NB) media, then washed again with a solution of 0.05 molar phosphate buffer saline (PBS) pH 7.0. Bacterial spores that have been washed with PBS are resuspended in PBS solution. Each organic material is weighed starting from sago starch flour, corn cobs flour, solid waste, and pineapple skin waste each weighing as much as 800 g, each unit. Carrier materials such as peat, zeolite, sago pulp and talc. Each of them is used as much as 200 g. Adhesive materials such as tapioca flour are needed as much as 200 g, for each treatment unit. The weighed ingredients are put into polypropylene plastic and wrapped in aluminum foil and then sterilized by using an autoclave at a pressure of 1.5 psi, temperature of 121 ° C for 15 minutes. Endophytic hat are 3 days old are harvested by centrifuging at 7,500 rpm at room temperature for 6 minutes. The bacterial spores were then washed with Sodium Broth (NB) media, then washed again with a solution of 0.05 molar phosphate buffer saline (PBS) pH 7.0. Bacterial spores that have been washed with PBS are resuspended in PBS solution.

Each organic material (sago starch flour, solid waste, pineapple skin waste, corn cobs flour) was weighed as much as 800 g, mixed with 200 g of carrier material and 200 g of tapioca flour. Formulation is carried out by spreading the spore suspension evenly in a ratio of 15 ml of spores for every 150 g of carrier material. The formulation is stirred to be homogeneous and the bacterium *Bacillus* sp. spread evenly in the media, after which the formulation was incubated for 3 weeks. Then the formulation was weighed using an analytical scale of 5 g then put into a tablet printing machine that had been sterilized by spraying 10% chlorox. So that a tablet with a weight of 5 g is obtained and

a diameter of 1 cm. The tablet is then dried at a temperature of 27°C for 2 hours. The tablet is placed in polyethylene plastic and removed at room temperature. Tablets are ready to be used to test directly in the field in oil palm nurseries.

Research in the field included seedling planting media preparation, research site preparation, fertilization, planting, oil palm seedling inoculation with *G. boninense*, giving tablet bio fungicide with active ingredients *Bacillus subtilis* and maintenance. Variables observed were incubation period, attack intensity, seedling height, number of midribs, increase in tuber diameter, root volume, root canopy ratio and root dry weight.

3. Results and discussion

3.1. Incubation period and disease intensity

The results of variance showed that the application of several formula contained *Bacillus sp.* significantly affect to the incubation period and the disease intensity in oil palm seedlings. The mean intensity of the disease after shown in Table 1.

Table 1. The incubation period and the intensity of the disease (%) *G. boninense* in main nursery with different formulation of bio fungicides tablet contained *Bacillus subtilis*

Formulation of bio fungicides tablet	Incubation period	Intensity of the disease
F0	101.75 b	26.89 b
F1	120.13 ab	11.86 ab
F2	123.63 ab	13.71 ab
F3	140.00 a	0.00 a
F4	132.63 a	17.05ab

The numbers followed by lower case letters are not the same is significantly different according to the results of a further test honestly significant difference at 5% level after transformed into $\sqrt{Y + 0.5}$

Table 1 showed that the best formula was F3. This is presumably because complex nutrient content in solid compared with other organic materials in the formulation, so that the effectiveness and viability of *Bacillus subtilis* endophyticin suppressing the development of *G. boninense* maintained. Utomo and Widjaja, suggested that solid contains 12.63% protein, 9.98% crude fiber, 7.12% crude fat, 0.03% calcium, and 0.003% phosphorus [7].

The addition of carrier material in the form of talk F3 formulation could be expected to further enrich the organic material (solid), and to improve and stabilize the pH, as well stabilizing water content in the formulation for maintaining the effectiveness and viability of *Bacillus sp.* endophytic. The formulation of flour with talc carrier material at 20% moisture content can still maintain a population of *Bacillus polymixa* BG 25 and *Pseudomonas fluorescens* PG 01 to 8-month shelf life [8]. A formulation able to maintain an average population rhizobacteria range 10^7 - 10^8 cells / g of carrier material for each type of bacteria during 6 months of storage.

The lowest average disease intensity was found F3 with a mean intensity of 0% at 140 days of observation. This is presumably because the nutrient content in F3 was more complex that serves as a source of nutrients *Bacillus sp.* endophytes in carrying out their life activities so they can maintain their population. This can be seen from the results of the calculation of the colony after 3 weeks fermentation showed that F3 application was more (38.5×10^8 cfu/g) compared to other formulations (Table 1). The solids having a dry matter content 81.56%, which included a 12.63% protein, 9.98% crude fiber, crude lipid 7.12%, 0.03% calcium, and 0.003% phosphorus [7]. Weller and Cook stated that the competition space between *Bacillus spp.* occur because of their ability to colonize along the root system resulting in inhibition of proliferation and spread *G. boninense*. The competition of nutrients also occurred as a result of the increase in population of *Bacillus sp.* which can lead to the source of nutrients available for the pathogen needs to be limited [10].

3.2. Height increment of nursery

The data analysis showed that the using of several formulations of *Bacillus subtilis* endophytic affect to non-significant the height increment of oil palm nursery. Average height increment of nursery after being tested further by using HSD at 5% level can be seen in Table 2.

Table 2. The increase of seed height and the number of palm oil leaves by giving some formulations of *Bacillus* sp. endophytic

Formulation of bio fungicides tablet	Height increment of nursery	Number of palm oil leaves
F0	35.125 a	6.50 a
F1	33.875 a	6.63 a
F2	33.375 a	6.88 a
F3	36.125 a	7.13 a
F4	35.250 a	6.75 a

The numbers followed by unequal lowercase letters are significantly different according to the results of HSD test at the 5% level

Data from Table 2 showed that all bio fungicide tablet formulations contained *Bacillus subtilis* endophytic and without formulation were not significantly different in seed height and the number of oil palm leaves. This is presumably because oil palm is an annual plant that has a relatively slow increase in height so that the increase in oil palm seedlings will be more influenced by genetic characteristics. This can be seen from the description of oil palm plants that has a high increase of 58 cm / year or in other words has a high increase of 4.83 cm/month. High accretion of oil palm nursery are relatively similar in all treatments that increase the number of leaves that form will be the same anyway because of the high gain influence in the formation of leaf plant oil palm nursery. *Bacillus* sp. in increasing the number of leaves of oil palm seedlings associated with its ability to increase the height of oil palm nursery [11].

The treatment of F3 formulation has a mean increase in seed height and the number of leaves tends to be more than other formulations and without formulation. This is presumably because in the formulation of F3, oil palm seedlings have higher seedlings which tend to be higher so that the leaves formed will be more than other treatments. The number of leaves is related to the height of the plant where the higher the plant, the more leaves are formed [12].

3.3. Increase in diameter of hump

The results of analysis variance showed that the formula contained *Bacillus subtilis*. endophytic have no significant effect on the increase in diameter of bulbs of oil palm seedlings. The mean height increase of seeds can be seen in Table 3

Table 3. Increasing the diameter of the bulb of oil palm seedlings by giving some formulations of *Bacillus subtilis* endophytic

Formulation of bio fungicides tablet	Diameter of bulb
F0	1.686 a
F1	1.750 a
F2	1.925 a
F3	2.313 a
F4	2.150 a

The numbers followed by unequal lowercase letters are significantly different according to the results of HSD test at the 5% level

Table 3 showed that all formulations of *Bacillus subtilis* endophytic and without the formulation given to oil palm nursery are not significantly different from the increase in diameter of the seedlings of oil palm nursery. This is presumably because oil palm is an annual plant with a relatively slow growth rate so that the increase in diameter of the stump of oil palm nursery will be relatively the same. Oil palm plants are annual crops with relatively slow growth rates [13]. In addition, *Bacillus subtilis* endophytic which are contained in all treatments besides being instrumental in protecting the roots of oil palm seedlings from *G. boninense* infection through their ability to colonize the roots of oil palm nursery, *Bacillus subtilis* endophytic can also stimulate lateral root growth of oil palm nursery by producing hormones that promote plant growth so that absorption of nutrients is more optimal. The optimal absorption of nutrients will affect the ability of plants to carry out physiological processes such as cell division, cell extension, cell differentiation, and cell enlargement so that the increase in diameter of the hump in each treatment is relatively the same. The roots of plants develop well, the growth of the canopy parts of plants (stems and leaves) is well developed because the roots are able to absorb nutrients needed by plants [11].

3.4. Root volume (ml), root canopy ratio and dry weight of oil palm nursery

The results of variance showed that the using of formula contained *Bacillus subtilis*. Endophytic have no significant effect on root volume, root canopy ratio and dry weight of oil palm seedlings. The average root volume, root canopy ratio and dry weight of oil palm nursery can be seen in Table 4

Table 4. Volume roots, roots and canopy ratio root dry weight in palm oil under the using of several formulations of tablets bio fungicide active ingredient *Bacillus subtilis* endophytic

Formulation of bio fungicides tablet	Root volume	Root canopy ratio	Dry weight of oil palm nursery
F0	40.13 a	2.290 a	14.881 a
F1	44.25 a	2.537 a	14.728 a
F2	45.29 a	2.165 a	15.178 a
F3	47.75 a	2.201 a	15.811 a
F4	44.75 a	2.102 a	15.198 a

The numbers followed by unequal lowercase letters are significantly different according to the results of HSD test at the 5% level

As shown in Table 4, all formulas tested were not significantly affect to the volume of oil palm roots, root canopy ratio and dry weight in oil palm seedlings. This is thought to be *Bacillus subtilis* endophytic can also promote the growth of lateral roots of oil palm seeds by producing hormones that promote plant growth then they have an impact on root volume. Using of *Bacillus* sp. the origin of Rhizosphere of palm oil can improve root development which has an impact on root volume [14]. The addition of *Bacillus* sp. as active ingredients contained inside can increasing the root volume [11].

F3 formula has mean root volume which tends to be greater than other formulations and without formulations. This is presumably because F3 treatment has a lower disease intensity so that oil palm seedling can grow better than the other treatments. Addition of organic material (solid) and carrier material (talk) in F3 formula in addition to functioning as an energy source for *Bacillus subtilis*. Endophytic can also function as a nutrient provider for plants. The results of the F3 formula analysis have a nutrient content of N (1.55%) which tends to be higher than other formulas. Root volume is very closely related to the availability of N nutrients. N elements play a role in synthesizing carbohydrates into proteins and protoplasm (through the mechanism of respiration) which play a role in the formation of vegetative tissue plants. The N elements absorbed by plants play a role in supporting vegetative growth of plants such as roots [11].

All the treatments of bio fungicide table formula contained *Bacillus subtilis* endophytes and without different formula were not significant on root canopy ratio. This is presumably due to the increase in seedling height, increase in leaf number and root volume of all treatments relatively the

same so that the root canopy ratio in all treatments is relatively the same. The root canopy ratio is the ratio between canopy dry weight (stems and leaves) with root dry weight so that it is related to seed height, leaf number and root weight. Canopy weight increases linearly following the increase in root weight [15]. The formula of *Bacillus subtilis* endophytic and without bio fungicide formulations showed non significant differences in dry weight of the roots of oil palm seedling. This is presumably because the root volume in all treatments is relatively the same.

4. Conclusions

Formulation of *Bacillus subtilis* endophytic affect the incubation period and reduce the intensity of the disease but have no effect on height, number of leaves, increase in diameter, root volume, root canopy ratio and dry weight of roots in oil palm seedlings. Formulation of *Bacillus subtilis* endophytic with the addition of solid + talk + tapioca flour (F3) is the best formulation because it can slow the incubation period to 140 hsi and reduce the intensity of the disease 0% after 140 days of observation on oil palm seeds.

References

- [1] Susanto A 2002 *Study of Biological Control of Ganoderma boninense Pat. Causes of Palm Oil Stumps* (Bogor: Dissertation IPB)
- [2] Harjono and Widyastuti S M 2001 *J. Indonesian Crop Protection* **7** 55–58
- [3] Puspita F, Zul D and Khoiri M A 2011 *Proceedings of the National Seminar on the Role of Technology and Agricultural Institutions in Realizing Tough and Sustainable Agricultural Development 2013*
- [4] Lusiyantri 2011 *Resistance Test for Resilience with Bacillus sp. and Fitrat Culture to the Attack of Ganoderma boninense Mushrooms and Growth of Palm Oil Seedlings in Early Nursery* (Pekanbaru: Essay Faculty of Agriculture University of Riau) Not Published
- [5] Bacon C W and Hinton D M 2006 *Bacterial Endophytes: The Endophytic Niche, Its Occupants, And Its Utility* ed Gnanamanickam S S (Dordrecht: Springer)
- [6] Purwantisari S, Ferniah R S and Raharjo B 2008 *Bioma* **10** 13–19
- [7] Widjaja E and Utomo B N 2005 *Pemanfaatan limbah pengolahan minyak kelapa sawit yang berupa solid untuk pakan ternak (sapi, domba dan ayam potong). Success Story Pengembangan Teknologi Inovatif Spesifik Lokasi* (Jakarta: Badan Litbang Pertanian) pp 173–185
- [8] Widodo SW 2012 *Jurnal Ilmu Pertanian Indonesia* **17** 180–185
- [9] Siregar B A 2011 *Technology of Formulation Rhizobacteria Biofertilizer and its Application as Plant Growth Promoters on Soybean and Biofertilizer in Acid Soils* (Bogor: Thesis Agriculture Faculty IPB)
- [10] Puspita F, Zul D and Restuhadi F 2013 *National Seminar on Indonesian Agricultural University Cooperation Agency* (Pontianak) March 19-20 2013
- [11] Hutabarat R 2014 *Online Journal of Students of the Faculty of Agriculture, University of Riau* **1**
- [12] Rumapea D S 2015 *Formulation Test for Flour Biofertilizer Made Active from Bacillus sp. In Rubber Nursery (Hevea brailliensis)* (Pekanbaru : Essay Riau University) Not Published
- [13] Gusmawartati, Hapsoh and Rambe W P D 2013 *Journal of Agrotechnology* **3**
- [14] Hadda I A 2010 *Antagonistic Indications Test of Bacillus Sp. Local Riau Against Mushrooms Ganoderma boninense Causes of Stinking Oil Palm Stems in Early Nursery* (Pekanbaru : Essay Faculty of Agriculture University of Riau) Not Published
- [15] Gunawan A 2013 *Effectiveness of Human Urine Fermentation as a Substitute for Nitrogen Fertilizer in Cocoa Plant Nursery (Theobroma cacao, L)* (Pekanbaru : Essay Faculty of Agriculture University of Riau) Not Published