The potency of antagonistic microbes as plant growth-promoting on oil palm seedling infected with basal stem rot disease (*Ganoderma boninense*).

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Abstract. Basal stem rot disease (BSR) is one of the significant oil palm plant diseases triggered by the presence of *Ganoderma boninense*. This poses a major risk in terms of production oil palm sustainability in Southeast Asia, particularly Indonesia, and is implicated in great economic loss. Therefore, the aim of this study was to determine the antagonistic potential of plant growth-promoting microbes on oil palm seedlings infected by BSR disease which involved the use of four antagonistic microbial agents, including *Trichoderma harzianum* (TF), *Trichoderma viride* (TC), *Stenotrophomonas* sp1. (S51-3), and *Stenotrophomonas* sp2. (S81-1). The results showed that in dual culture antagonistic test, it was found that all antagonistic microbes used had the ability to inhibit the growth of *G. boninense* in vitro with the percentage of inhibition from 38-60%. *T. viride* and *T. harzianum* combined (A6), as well as a mixture of *Trichoderma* and *Stenotrophomonas* (A12, A13, and A16), can significantly increase the height of oil palm seedlings infected with *G. boninense*. The use of *T. viride* and *T. harzianum* were singly (A2 and A3) and combined with *Stenotrophomonas* sp1. and *Stenotrophomonas* sp2. (A12, A13, and A16) had increased number of leaves and leaf colours.

1. Introduction

The oil palm (*Elaeis guineensis* Jacq.) commodity is a highly significant plantation crops, estimated to play a substantial role in the economic activities of Indonesia. The Indonesian Plantation Statistics or Statistik Perkebunan Indonesia data, showed a production of 42.87 million tons in 2019, observed within an expanse of about 14.68 million ha [1]. Therefore, the country is considered the greatest producer and exporter worldwide (49%), and is subsequently followed by Malaysia (33%) [2]. However, the productivity value is relatively lower, with respect to the correlations with the areas cultivated.

This challenge is possibly attributed to pathogenic microorganism, including *Ganoderma boninense* implicated in basal stem rot (BSR) disease [3]. In addition, this manifestation has been reported in both young and older plants. Based on a survey result, the incidence rate in specimens aged 17 years was up to 50%, and the characteristic symptoms include leaf chlorosis, fallen plants, fruiting
body formation, and the presence of holes in the rootstock [4]. In addition, BSR disease has been implicated in reduced fruit output, and sometimes none. This phenomenon potentially instigates a decline in oil palm production [5].

There have been several efforts aimed at controlling BSR and reducing the impact, including through the use of resistant crops, technical culture, and also by applying chemical-based fungicides. However, a satisfactory and effective outcome has not been observed after the use of these techniques. Particularly, chemical fungicides tend to be less successful by virtue of the action mechanism, which involves slowing down the *G. boninense* infection. This agent is known to experience active ingredient degradation in soil before contacting the specific target [5]. In addition, it is essential to publicize the numerous negative impacts on unintended organisms as well as environmental concerns, encompassing health issues and possible groundwater contamination [6]. Therefore, adopting a biological control measure as a cheap, ecologically safe and easily accessible option is promising [7].

There is wide variance between the microorganisms present in the rhizosphere, with respect to plant type, growth stage, and also with the environmental factor. In addition, some fungi and bacteria species are known to colonize the roots of plants, and are therefore categorized as plant growth-promoting Fungi (PGPF) and *Rhizobacteria* (PGPR). These include *Stenotrophomonas* sp., which is often applied as a bio-agent to promote growth and control other organisms, as reported by [8] and [9]. [10] reported on the possible application in the invitro assay of potatoes, for brown rot disease control.

[11] reported on the ability for *Stenotrophomonas* spp. to produce extracellular proteases and chitinase, and is possibly use in for controlling *Pythium ultimum* in sugarcane. In addition, [12] showed the propensity to improve plant tolerance and antioxidant production under conditions with low N2. This microorganism is potentially used as bioinoculant for stimulating growth in plant, and also as biocontrol against pathogenic fungi. Moreover, the plant rhizosphere is known to encompass other microorganisms, including Trichoderma spp. which demonstrates the capacity to act as PGPF [7]. This study is, therefore, aimed at determining the potentials of *Stenotrophomonas* spp. and *Trichoderma* spp. as growth stimulation agents in oil palm seedlings infected with stem rot pathogens, after single and mixed applications.

2. Materials and methods

2.1. Stock culture
The microorganisms, including *Trichoderma viride* (TC), *T. harzianum* (TF) *Stenotrophomonas* sp1. (S51-3), and *Stenotrophomonas* sp2. (S81-1) were isolated from the oil palm rhizosphere in Kebun Bahilang and Bukit Kijang, North Sumatra. The causative organism, termed *Ganoderma boninense* was obtained at the Laboratory of Plant Diseases, Faculty of Agriculture, Universitas Sumatera Utara. Therefore, all bacterial and fungal cultures were grown in Nutrient Broth (NB) (Difco ™) and Potato Dextrose Agar (PDA) (Difco ™), respectively, prior to subsequent testing.

2.2. Antagonistic activity against test-cultures of *Ganoderma boninense* in vitro
A dual culture method with modifications was adopted during the vitro testing, as described by [13]. This involved using a petri plate with 9 cm diameter and comprising PDA media to develop *G. boninense* measuring 0.5 m in diameter. Subsequently, *T. viride* and *T. harzianum*, 0.5 cm in diameter were added at a 6 cm distance from one another as antagonistic fungi after 5 days. The antagonistic bacteria test-cultures (*Stenotrophomonas* sp1. and *Stenotrophomonas* sp2.) were evaluated with an 0.5 cm diameter sterile filter paper saturated with bacterial suspension (10⁶ CFU). This was then placed in the same 9 cm petri dish containing PDA media and the *G. boninense* culture, at a 6 cm distance from one another. Furthermore, all cultures were then placed in an incubator set at 27 ± 2 °C for 21 days. The control treatment involved growing *G. boninense* on petri plate measuring 9 cm in diameter, and characterized by PDA media, and all treatment were performed with four replications. The radial growth inhibition (PIRG) percentage was evaluated every 2 days, using the formula as shown in [14]:

\[
\text{PIRG} = \frac{\text{Radial growth of control}}{\text{Radial growth of test culture}} \times 100
\]
where: R1 denotes the radial growth of *G. boninense* in the control experiment, and R2 represents the colony growth radius in relation to the antagonist.

### 2.3. *Ganoderma boninense* inoculum

The Rubber Wood Block (RWB) was used to propagate the *G. boninesne* inoculum [15]. This involved cutting the Fresh RWB to the specified measurement (6cm x 6cm x 6cm), followed by washing and reserving in polypropylene plastic bags comprising 2% extra malt agar (MEA). Therefore, the entire set up was treated with 12 hours of incubation, before autoclaving at 121°C for one hour. The cooling process involved rotating RWB to ascertain total surface coverage with MEA. Subsequently, *G. boninense* was inoculated in the sterile RWB preserved in polypropylene plastic bags, before incubating for 60 days in a dark room set at 27 ± 2°C. This was to ensure a total surface coverage by mycelium growth from the inoculum.

### 2.4. Greenhouse test

The impact of antagonistic microbes on the oil palm seedling vegetative growth was evaluated using a greenhouse test. The seedlings used were 3 months old, and obtained from PT. Socfindo Indonesia. These were then implanted on sterile soil present in polybags measuring 15 cm x 23 cm.

| Code | Treatment |
|------|-----------|
| A0   | Negative control (not inoculated by *G. boninense*) |
| A1   | Positive control (inoculated by *G. boninense*) |
| A2   | *T. harzianum* |
| A3   | *T. viride* |
| A4   | *Stenotrophomonas* sp.1 |
| A5   | *Stenotrophomonas* sp.2 |
| A6   | *T. harzianum* + *T. viride* |
| A7   | *T. harzianum* + *Stenotrophomonas* sp.1 |
| A8   | *T. harzianum* + *Stenotrophomonas* sp.2 |
| A9   | *T. viride* + *Stenotrophomonas* sp.1 |
| A10  | *T. viride* + *Stenotrophomonas* sp.2 |
| A11  | *Stenotrophomonas* sp.1 + *Stenotrophomonas* sp.2 |
| A12  | *T. harzianum* + *T. viride* + *Stenotrophomonas* sp.1 |
| A13  | *T. harzianum* + *T. viride* + *Stenotrophomonas* sp.2 |
| A14  | *T. harzianum* + *Stenotrophomonas* sp.1 + *Stenotrophomonas* sp.2 |
| A15  | *T. viride* + *Stenotrophomonas* sp.1 + *Stenotrophomonas* sp.2 |
| A16  | *T. viride* + *T. harzianum* + *Stenotrophomonas* sp.1 + *Stenotrophomonas* sp.2 |

The vegetative growth was then assessed to determine some parameters after four months of first applying the antagonistic microbes. The evaluated characters include: (i) plant height obtained by attaching a measuring tape at the base of the plant for the tip of the first midrib. (ii) number of leaves, assessed by counting only the perfectly open leaves. (iii) Leaf colour, was appraised based on a scoring method. This involved matching the colours of perfectly opened leaf samples with the shades on a Leaf Colour Chart tool.

Table 1 shows the study method, performed using 17 treatments, based on a non-factorial randomized block design (RBD), with single and mixed antagonistic microbes. Therefore, each treatment respectively comprised of 3 sample units and 3 replications. The seedlings were then
inoculated using a modified [16] method. This involved the placement at a 2.5 cm distance from the RWB already covered by the *G. boninense* mycelium, before this set up was inserted in a polybag. Furthermore, the seeds cultured in the absence of any inoculum served as the control experiment.

Some of the antagonistic microorganisms selected for this research, including *T. harzianum* and *T. viride* were applied 3 times, particularly at 1, 2, and 3 months after inoculating the media with *G. boninense*. Moreover, milled corn medium was used to grow about 30 g of each (106 CFU / mL), which were then scattered at the root neck in a 2.5 cm depth distance from the RWB, before covering with soil. A similar experiment was performed for the evaluation of *Stenotrophomonas* sp1 and sp2. as antagonistic bacteria. This involved splashing about 30 ml of the suspension (108 CFU / mL) developed at the root neck. These treatments were introduced at monthly intervals, and a total of three applications were performed during this experiment.

### 3. Results and discussion

#### 3.1. Antagonistic activity against test-cultures of *Ganoderma boninense* in vitro

The in vitro assessment of microbial antagonistic effects against *G. boninense* indicate different growth inhibition rates. This indicates a positive activity in vitro, and the final observation after a 21 day incubation period, showed 28% to 60% inhibition. Moreover, the patterns of inhibition recorded in all treatments from day 3 to 21 post-incubation was similar especially for *T. harzianum* (60%) and *T. viride* (55%). This value was relatively higher than the outcome with *Stenotrophomonas* sp1. and *Stenotrophomonas* sp2., as observed in figures 1 and 2.

![Figure 1](image-url)

*Figure 1.* The growth of *G. boninense* and antagonistic microbes to test-cultures on PDA media during 21 days after incubation, (a) *G. boninense* colony in control; (b) *G. boninense* and *Stenotrophomonas* sp1.; (c) *G. boninense* and *Stenotrophomonas* sp2.; (d) *G. boninense* and *T. viride*; and (e) *G. boninense* and *T. harzianum*, where R1 is the radial growth of *G. boninense* in the control petri dish, and R2 is the radius of the *G. boninense* colony toward the antagonist.

The results of related studies indicated the antagonistic characteristics of *Trichoderma* sp. towards *G. boninense* [17,18], while others acknowledged the effectiveness of *Stenotrophomonas maltophilia* in biocontrol against *Ralstonia solanacearum* race 3 biovar 2. This outcome was ascertained using by the in vivo and in vitro assay of brown rot disease in potatoes [10]. In addition, the results obtained were highly similar, while dual culture-test in vitro demonstrated the ability for the respective organisms to hinder the development of *G. boninense* at the following inhibition percentage: *Stenotrophomonas* sp1. (28%), *Stenotrophomonas* sp2. (38%), *T. viride* (57%), and *T. harzianum* (60%).

Based on the inhibition percentage result, *T. harzianum* and *T. viride* were determined to have greater effectiveness against *G. boninense* compared to *Stenotrophomonas* sp1. and sp2. [19]. This evaluation required the use of growth inhibition category scale (GIC) in a range of 0 to 4, where 0 = no inhibition of growth, 1 = inhibition of growth is 1-25%, 2 = inhibition of growth is 26-50%, 3 = inhibition of growth is 51-75%, and 4 = inhibition of growth is 76-100%. Scale 0 shows no inhibition, while scale 4 shows the ability to inhibit maximum growth.
Inhibition percentage of antagonistic microbe against G. boninense on dual-culture test

Table 2. Effect of antagonistic microbe addition to oil palm seedlings infected by G. boninense.

| No | Treatment | Plant Height (cm) | Number of Leaves | Leaf Colour |
|----|-----------|-------------------|------------------|-------------|
| 1  | A0        | 73.9 efg          | 10.4 defg        | 4.0 a       |
| 2  | A1        | 64.2 h            | 9.7 g            | 2.9 defg    |
| 3  | A2        | 78.3 cdef         | 11.3 abcde       | 3.4 abede   |
| 4  | A3        | 78.6 cdef         | 11.2 abcde       | 3.4 abed    |
| 5  | A4        | 70.8 fgh          | 10.0 fg          | 2.7 fgh     |
| 6  | A5        | 64.8 h            | 10.3 efg         | 2.3 h       |
| 7  | A6        | 90.0 ab           | 11.9 ab          | 3.6 abc     |
| 8  | A7        | 79.8 cde          | 10.8 bcdef       | 3.1 cdefg   |
| 9  | A8        | 83.6 bc           | 11.0 abcdef      | 2.9 cefgh   |
| 10 | A9        | 79.3 cde          | 10.7 cdefg       | 3.1 cdefg   |
| 11 | A10       | 77.6 def          | 11.0 abcdef      | 3.1 cdefg   |
| 12 | A11       | 67.1 gh           | 10.0 fg          | 2.6 gh      |
| 13 | A12       | 92.0 ab           | 11.7 abc         | 3.4 abede   |
| 14 | A13       | 91.2 a            | 12.1 a           | 4.0 a       |
| 15 | A14       | 86.0 abc          | 11.6 abcd        | 3.2 bcdef   |
| 16 | A15       | 81.1 cde          | 11.1 abcdef      | 3.0 cdefg   |
| 17 | A16       | 91.8 a            | 11.9 ab          | 3.8 ab      |

where: numbers without the same letters in the same columns shows significant difference through Duncan test in level of significance (α) 5%.

3.2. Vegetative growth and development

Table 1 shows the observations of parameters evaluated to estimate the vegetative growth and development. These include significant increase in the average plant height after treatment with the combination of T. viride and T. harzianum (A6), as well as the mixture of Trichoderma sp. and Stenotrophomonas sp. (A12, A13, and A16), in contrast with single applications. Furthermore, the lowest plant height values were observed in seedlings infected with G. boninense (A1), while similar outcome was recorded in treatments with the addition of T. viride and T. harzianum (A2 and A3). This phenomenon, especially in terms of number and colour of leaves was attributed to the absence of any combinations. However, treatments with interactions Stenotrophomonas sp1. and Stenotrophomonas sp2. (A12, A13, and A16) yielded good and not significantly different results. Therefore T. harzianum, T. viride, Stenotrophomonas sp1. and sp2. possess the capacity to positively influence spurring in seedlings. These four microorganisms facilitate plant growth after a combined application. Also,
Numerous studies have indicated the potential use of *Trichoderma* sp. as a growth booster or enhancer in palm plants. There have also been reports on the intrinsic capacity to expand the biomass of shoots, roots and the plant’s basal stem [20,21]. In addition, *Trichoderma* sp. also possibly boosts growth stimulates seedling development by influencing the balance in hormone, as observed with IAA, ethylene, and gibberellic acid. The other related studies identified *T. harzianum* as one of the most effective biocontrol agents, with potential for commercial exploitation, particularly to confer protection against pathogenic fungi in soil [22]. This microorganism also has the capacity to improve production quantity and quality by enhancing the length of shoot and root, dissolution of phosphates and micronutrients, elevated nitrogen fixation, and through the promotion of healthy growth in early plant development stages.

In addition, similar outcome was observed after treatment with *Stenotrophomonas*. This is a known gram-negative bacteria, belonging to the family *Xanthomonadaceae*. Prior reports have highlighted the possible uses of *S. maltophilia* to promote plant growth, particularly with wheat, and also the potential resistance to both abiotic and biotic stress were evaluated [23]. Moreover, other studies have shown the bioremediation and biological control applications against diseases instigated by the presence of fungi [8]. Also, one of the action mechanisms of *S. maltophilia* BJ01 include free radical scavenging or antioxidant capacity (DPPH, hydroxyl, and H$_2$O$_2$), as observed in *Arachis hypogea* [12]. Furthermore, the *Stenotrophophome* species enhance the osmo-protectants (proline, total amino acids and total dissolved sugar), and are able to elevate auxins as growth hormone, alongside the total flavonoids and total phenolic compounds during vegetative growth and development of plants.

### 4. Conclusions

The results obtained confirmed the ability for all antagonistic microbes evaluated to hinder *G. boninense* growth in vitro, using dual test-cultures. This was observed at the inhibition percentage of 57% for *T. viride*, 60% for *T. harzianum*, while *Stenotrophomonas* sp1., and sp2. were 28% and 38%, correspondingly. In addition, these microbial agents collectively and respectively produced improved outcomes in oil palm seedlings affected by *G. boninense*, particularly in the aspect of growth stimulation.

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