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Growth response of not-ready-to-distribute tea (*Camellia sinensis* (L) O. Kuntze) seedlings due to application of biofertilizer at various concentrations and intervals

Abstract. The plantation rejuvenation program makes the need for ready-to-distribute tea seedlings getting higher. Meanwhile, the nursery's seedlings are mostly not ready to distribute. This study aimed to determine the response of not-ready-distribute tea seedlings due to the application of biofertilizers at various concentrations and intervals. This experiment was carried out at Gambung Tea and Quinine Research Center Nursery, conducted from December 2021 to February 2022 at an altitude of 1,350 meters above sea level (asl). The experimental method used was a randomized block design with eight treatment combinations, namely: control (no fertilizer); urea fertilizer every two weeks; Biofertilizer 5 mL L^{-1} + interval once a week; Biofertilizer 5 mL L^{-1} + interval every two weeks; Biofertilizer 10 mL L^{-1} + break once a week; Biofertilizer 10 mL L^{-1} + interval every two weeks; Biofertilizer 15 mL L^{-1} + interval once a week; Biofertilizer 15 mL L^{-1} + interval every two weeks, all repeated four times. The experimental results showed that treatment of biological fertilizers influenced the parameter number of leaves and chlorophyll index. The application of biofertilizer with a concentration of 15 mL L^{-1} + interval of 2 weeks greatly influenced the parameters of leaf number and chlorophyll content index.

Keywords: Biofertilizer · Concentration · Interval · Not ready to distribute tea seedlings

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Introduction

The problem tea commodities face today in Indonesia is the yearly decline in tea production. Contrary, the high demand for tea products must be fulfilled. Statistics Indonesia noted that the volume of tea production in 2018 decreased by 4.11% from 2017, followed by a decline in export volume of 9.52% (Statistics Indonesia, 2020). Some factors have contributed to this, namely a decrease in the area of the plantation, the unproductive age of plants, and the plant population of fewer than 10,000 trees/ha (Fitria, 2016). One solution to these problems is by rejuvenating old tea plants through replanting. The rejuvenation of old tea plants and the increasing population requires the availability of quality seeds and adequate quantities (Suherman et al., 2015).

A suitable tea nursery is the first step to getting high-quality and sustainable tea plants. Salim et al. (1996) stated that the balanced development of roots, stems, and leaves characterized seedlings growth. With a survival percentage reached > 95% when transferred to the field. Tea nursery is expected to produce a success rate of 80% of tea seedling growth; the percentage of tea seedlings growth only reaches 40-50%, which causes a low percentage of seedlings ready for distribution (Wulansari *et al*., 2016). According to Suherman et al. (2015), the percentage of tea seedlings that are not ready for distribution is 40 - 50%. Characteristics of tea seedlings ready to distributed have a minimum height of 25 cm, a minimum number of leaves of 5-6 leaves, and 1 year old, while the seedlings that do not meet these criteria are included in the seedlings not ready to distributed (Suherman et al., 2015). The slow-growing rate of seedlings at the nursery causes the seedlings not to be ready for distribution. Several factors drive the high number of not-ready-to-distribute seedlings: inappropriate planting media, poor plant material, and unfulfilled nutrients (Wulansari *et al*., 2016). If one of the needed nutrients is not fulfilled, the plant metabolic processes will be inhibited, affecting plant growth and development (Salisbury & Ross, 1992). One of the efforts that can be made to improve the quality of tea seedlings that are not ready for distribution is fertilizing.

Generally, the not-ready-to-distribute tea seedlings are treated with urea fertilizer with a concentration of 2% until 1-year-old seedlings are ready for distribution (Naomi & Rahadi, 2019). Urea is easily dissolved, but the application of it on the surface can cause N loss to the air up to 40% of the applied N (Ramadhani et al., 2016). Using inorganic fertilizers should be balanced with organic fertilizers, one of which is using biological fertilizers. Biofertilizers are inoculants that contain active living organisms in solid or liquid form. When applied to seeds, plant surfaces, or soil can stimulate the growth of these plants (Vessey, 2003). Biofertilizers can accelerate plant growth because they contain nitrogen-fixing microorganisms (Prasetyo, 2018). Biological fertilizers can reduce the use of inorganic fertilizers to protect the environment from the impact of excessive use of inorganic fertilizers (El Salam, 2007). According to Enice et al. (2019), the right time for fertilizer application will increase plant growth and production. If the interval is too frequent, it can lead to too luxurious consumption, causing fertilizer wastage. On the other hand, the infrequent interval can lead to insufficient plant nutrient requirements (Rajak et al., 2016). This research was carried out to determine the effect of concentration and intervals of biological fertilizers to accelerate the growth of not ready-to-distribute tea seedlings.

Materials and Methods

This research was conducted at the Experimental Field of Tea and Quinine Research Center (PPTK) Gambung, at an altitude of 1,350 meters above sea level, Andisol soil order and climate type B according to the Schmidt-Ferguson classification, from December 2021 to February 2022. The materials used were GMB 7 clone tea seedlings aged eight months for the class C seed category or not ready to distribute, urea fertilizer, and biological fertilizers with the trademark Bion-Up that contains *Azotobacter* sp *Azospirillum* sp, *Pseudomonas* sp, *Acinetobacter* sp, *Penicillium* sp, Gibberellin Hormones, Auxin Hormones, Cytokinin Hormones based on analysis of UNPAD Soil Chemistry and Plant Nutrition Laboratory. The tools used were writing instruments, rulers, scales, a caliper, a hand sprayer, a chlorophyll meter with SPAD 502 type, and an oven.

The experimental method used was a randomized block design (RBD) with eight treatment combinations. Each treatment was repeated 4 times so that there were 32 experimental units. Each experimental unit contained 5 plants, so the total number of plants was 160 plants. The treatments are arranged as follows: A = control (no fertilizer); B = urea fertilizer every two weeks; C = biofertilizer 5 mL L^{-1} + interval once a week; D = biofertilizer 5 mL L^{-1} + interval every two weeks; E = biofertilizer 10 mL L^{-1} + interval once a week; F = biofertilizer 10 mL L^{-1} + interval every two weeks; G = biofertilizer 15 mL L+ interval once a week; H = biofertilizer 15 mL L^{-1} + interval every two weeks Data were analyzed using Analysis of Variance (ANOVA) for the F test at a 95% confidence level. Duncan's further test was carried out at a 95% confidence level if there were differences between treatments.

The first step was land preparation making a shade made of bamboo and a roof using paranet with a 50-60% density. Preparation of Class C or not ready to distribute tea seedlings were picked eight months old, 12-13 cm tall, and with less than five leaves. Seedlings were in polybags with the same topsoil and subsoil planting media 14 cm x 20 cm as the nursery. Then the treatment application, urea fertilizer, was given by dissolving it in 1 liter of water and watering the soil. The application of biological fertilizers was carried out for ten weeks, with ten applications for the once-a-week treatment and five times every two weeks.

The biofertilizer was dissolved with 1 liter of water, then watered into the soil. The maintenance included watering, weeding, and controlling pests and diseases. Watering was done every six days. Weeding was done mechanically when weeds were growing around the polybags. Pest control was carried out by spraying pesticides when the tea seedlings had been damaged by >50%. The parameters observed consisted of supporting and main observations. The main observations were made on tea seedlings' morphological and physiological conditions, including plant height was calculated using ruler, leaf area was calculated using Image J software, number of leaves, leaf chlorophyll index was calculated using Digital Chlorophyll Meter clamped on the third leaf of the sample plant until a number appears on the monitor expressed in Chlorophyll Content Index (CCI) units, and root length observation of the length of the root is done in a way dismantling sample plants. Roots were taken from the media then the measurement was made every start from the base of the stem to the longest root end. The measurement of parameters is carried out every two weeks on three plants per treatment, but the root length measurement was carried out at the end of the study. Supporting observations included soil analysis, rainfall, air temperature, humidity, and plant pests and diseases.

Result and Discussion

Soil Analysis. Based on initial soil analysis, the soil used had a pH value of 5.14. According to Thamrin *et al.* (2013), tea plants can grow optimally on land with a pH of 4.5 – 5.5. The C-Organic soil content of 8.87% is classified as very high because the Andisol order contains relatively high organic matter (Wulansari & Pranoto, 2018). The value of the cation exchange capacity (CEC) was 19 cmol $kg^{-1,}$ which was relatively high. A soil with a high CEC indicates that it is fertile because it has a high nutrient reserve (Susanto, 2005). The higher the organic matter content, the higher the CEC (Rosmarkam & Yuwono, 2002). C-Organic in the soil is useful as a source of energy for soil microorganisms, improving degraded land and increasing plant productivity (Nurida & Jubaedah, 2014).

N, P, and K Nutrients play an important role in plant growth and development. The total N content of the soil was 0.46% which was classified as moderate. P-total of 196.75 mg/100g, classified as very high, and available K-available of 18.28 mg/100g, classified as low. The available P of 0.03 ppm was very low. This is because the pH of the soil was acidic, and the soil type was Andisol. Firnia (2018) stated that P is well available in the soil at pH 6.0-7.5. Al and Fe content will be high when the pH is low and cause it to absorb very strongly but release P very slowly (Azurianti et al., 2022).

Plant height. Plant height observations were carried out 12 weeks after application (WAA). The results showed that the application of biological fertilizers did not significantly affect the height of tea seedlings at 12 WAA.

Biological fertilizers did not give a significantly different effect, probably because the biological fertilizers could not provide sufficient nutrients for the seedlings, especially P nutrients. In line with Antralina et al. (2015), microbes need a long process to adapt and grow, then develop and break down nutrients. Microbial life is influenced by soil organic matter, pH, temperature, aeration, and groundwater. The soil analysis results showed that the total N content of the soil was 0.46% which was classified as moderate. P-total of 196.75 mg/100g was classified as very high, and available K-available 18.28 mg/100g was classified as low. The available P of 0.03 ppm is very low.

Table 1. Effect of biofertilizer on plant height of tea seedlings 12 WAA

Treatments	Plant height (cm)
A: Control (without fertilizer)	13.09
B: Urea fertilizer every two weeks	13.70
C: Biofertilizer 5 mL L^{-1} + Interval once a week	13.53
D: Biofertilizer 5 mL L^{-1} + Interval every 2 week	14.49
E: Biofertilizer 10 mL L^{-1}+Interval once a week	13.80
F: Biofertilizer 10 mL L^{-1} + Interval every two week	14.09
G: Biofertilizer 15 mL L^{-1} + Interval once a week	14.42
H: Biofertilizer 15 mL L^{-1} +Interval every two week	14.13

Note: Numbers that are not followed by letters in the same column show no significant effect according to the F test at a 5% significance level

Furthermore, it is suspected there were indigenous microorganisms that made the plant height with and without the application of biological fertilizers not significantly different. Indigenous microorganisms are found naturally and benefit humans (Batubara et al., 2015) as decomposers of organic matter, stimulate plant growth, and control agents for plant diseases and pests (Hajama, 2014).

Leaf Area. Leaf area is one of the main observation parameters to determine the effect of biological fertilizers on leaves. The leaf area is related to plant growth because it is related to the ability of plants to photosynthesize. Based on the results, the application of biofertilizers did not significantly affect tea seedlings' leaf area (Table 2). The application of biological fertilizers was not able to increase leaf development. This was suspected due to indigenous microorganisms present in the soil. The microbial activity contained in biological, therefore fertilizers was not significant.

Table 2. Effect of biofertilizer on leaf area of tea seedlings

Treatments	Leaf area (cm^2)
A: Control (without fertilizer)	94.38
B: Urea fertilizer every two weeks	102.22
C: Biofertilizer 5 mL L^{-1} + Interval once a week	107.37
D: Biofertilizer 5 mL L^{-1} + Interval every 2 weeks	105.29
E: Biofertilizer 10 mL L^{-1}+Interval once a week	113.27
F: Biofertilizer 10 mL L^{-1} + Interval every 2 weeks	101.51
G: Biofertilizer 15 mL L^{-1} + Interval once a week	113.81
H: Biofertilizer 15 mL L^{-1} +Interval every 2 weeks	114.39

Note: Numbers not followed by letters in the same column show no significant effect according to the F test at a 5% significance level.

The increase in leaf area is related to nutrients such as N, P, and Mg elements. This is the opinion of Lakitan (2001), who states that the element N greatly affects the growth and development of leaves.

The increase in plant leaf area can be determined by the number of carbohydrates allocated to the leaves, so the distribution of carbohydrates into leaves greatly determines plant development. The leaf area influences planting density and supply of nitrogen nutrients (Goldsworthy and Fisher, 1992).

Number of Leaves. Based on the statistical analysis test results, the application of biological fertilizers significantly affected the number of tea seedling leaves at 12 WAA (Table 3). This is presumably due to the role of cytokinin hormones contained in biofertilizers. The analysis of biological fertilizers showed a cytokinin content of 95.60 ppm. Cytokinin hormones have an important role in regulating cell division and stimulating the growth of the number of leaves (Widiastoety, 2014). Cytokinins are circulated to the leaves from the roots through the xylem vessels and balance the protein and chlorophyll content in the leaves (Loveless, 1991).

The application of urea fertilizer and biofertilizer had different effects on controlling or without fertilizer for the number of tea seedling leaves. Urea fertilizer which contains 46% of N nutrients, is used by seeds to stimulate the growth of the number of leaves. According to Widianto (2002), nitrogen is the nutrient that encourages leaf growth. Fauziah et al. (2015) stated that the growth of stems and leaves requires a lot of N. meanwhile, the provision of biofertilizer gave the same effect as urea giving; it showed that N content in biofertilizer gave the same effect as inorganic fertilizer, even more, significant for several treatments. This is probably supported by the function of biofertilizers that affected not only the soil's chemical properties but also improved the soil's physical character.

Table 3. Effect of biofertilizer on the number of tea seedling leaves

Treatments	Number of Leaves (strands)
A: Control (Without fertilizer)	6.00 a
B: Urea fertilizer every 2 weeks	7.00 b
C: Biofertilizer 5 mL L^{-1} + Interval once a week	7.17 b
D: Biofertilizer 5 mL L^{-1} + Interval every 2 weeks	6.67 b
E: Biofertilizer 10 mL L^{-1}+Interval once a week	7.92 b
F: Biofertilizer 10 mL L^{-1} + Interval every 2 weeks	6.83 b
G: Biofertilizer 15 mL L^{-1} + Interval once a week	7.25 b
H: Biofertilizer 15 mL L^{-1} +Interval every 2 weeks	8.08 b

Note: Numbers followed by the same letters in the same column show no significant effect according to the Duncan test at a 5% significance level.

The nutrients contained in the treatment can be absorbed by plants optimally. A large number of leaves made it possible to absorb more leaves so that the photosynthesis process could be faster, thus adding new leaves. On the other hand, the application without fertilizer showed a smaller number of leaves. This was because there was no addition of fertilizer for plant needs and only relied on the availability of nutrients in the soil. Hanafiah (2005) states that nitrogen fertilizer plays a prominent role in the vegetative parts of plants, namely leaves and shoots.

Chlorophyll content index. Based on the statistical analysis, the application of biological fertilizers significantly affected the chlorophyll index (Table 4).

Table 4. Effect of biofertilizer on chlorophyll content index

Treatments	Chlorophyll content index (cci)
A: Control (without fertilizer)	70.40 a
B: Urea fertilizer every 2 weeks	73.90 ab
C: Biofertilizer 5 mL L^{-1}+ Interval once a week	81.89 bc
D: Biofertilizer 5 mL L^{-1} + Interval every 2 weeks	80.20 abc
E: Biofertilizer 10 mL L^{-1}+Interval once a week	83.96 c
F: Biofertilizer 10 mL L^{-1} + Interval every 2 weeks	81.85 bc
G: Biofertilizer 15 mL L^{-1} + Interval once a week	82.94 bc
H: Biofertilizer 15 mL L^{-1} +Interval every 2 weeks	84.14 c

Note: Numbers followed by the same letters in the same column show no significant effect according to the Duncan test at a 5% significance level.

Treatment H (application of 15 mL L^{-1} + interval of 2 weeks) and E (application of 10 mL L^{-1} + interval of 1 week) differed significantly from other treatments. This was because the biological fertilizer contains the microbe *Azotobacter* sp. This microbe is a non-symbiotic nitrogen-fixing bacterium that can increase and improve nitrogen content (Toago et al., 2017). The results of the biofertilizer analysis showed that several bacteria helped the N fixation process, including *Azotobacter* sp (2.6×10^8 CFU/mL) and *Azospirillum* sp (1.9×10^8 CFU/mL) (Laboratory of Soil Chemistry and Plant Nutrition UNPAD, 2022) The element nitrogen stimulates growth and functions for the synthesis of amino acids and proteins in plants (Subowo et al., 2010). Leaf chlorophyll content will increase if sufficient N element is available to plants to improve the photosynthesis process and produce more assimilation (Zahrah, 2011).

Treatment A had the lowest chlorophyll index. It was possibly due to green leaves, so the leaf chlorophyll was not as much as in other treatments. This is to the research of Setiawati et al. (2016), where the older the age of the plant leaves, the greener the leaf color and the higher the chlorophyll content. Leaf chlorophyll formation depends on various factors, such as temperature, light, elements of nitrogen (N), magnesium (Mg), iron (Fe), manganese (Mn),

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copper (Cu), zinc (Zn), Sulfur (S) and oxygen. (O2) (Curtis & Clark, 1950). Therefore, treatment A was insufficient in nutrition, especially N, for not being given fertilizer.

Root length. Roots are the entrance for nutrients and water from the soil, which are very important for the physiological process of plant growth. If root function is disturbed, it will cause growth disorders in the canopy. Based on the results, the application of biological fertilizers had a significantly different effect on root length (Table 5). The roots are deficient in nutrients at low nutrient concentrations and hinder nutrient distribution. P deficiency can affect root growth. This was in line with the results of the initial soil analysis that the available P content was relatively low at 0.03 ppm, even P-total was 196.75 mg/100g, which was very high, and the available K was 18.28 mg/100g which was low. According to Herrera et al. (2015), element P is a critical nutrient that affects initiation (low Pi supply). In addition, the formation is also influenced by the level also influenced by N and Fe.

Table 5. Effect of biofertilizer on the root length

Treatments	Root length (cm)
A: Control (without fertilizer)	22.03
B: Urea fertilizer every 2 weeks	26.30
C: Biofertilizer 5 mL L^{-1} + Interval once a week	25.16
D: Biofertilizer 5 mL L^{-1} + Interval every 2 weeks	23.21
E: Biofertilizer 10 mL L^{-1}+Interval once a week	22.70
F: Biofertilizer 10 mL L^{-1} + Interval every 2 weeks	23.14
G: Biofertilizer 15 mL L^{-1} + Interval once a week	26.17
H: Biofertilizer 15 mL L^{-1} +Interval every 2 weeks	27.48

Note: Numbers that are not followed by letters in the same column show no significant effect according to the F test at a 5% significance level

Another influential factor on root length is the dense soil structure, which will inhibit the rate of deeper root penetration. Because dense soil is difficult for roots to penetrate, the root elongation area is getting shorter. Soils that have a high-density level have a low total root length. Russel (1977) *in* Rusdiana et al. (2000) argue that if the soil density increases, the macro pore space decreases, and root penetration is inhibited. According to (Nugroho, 2004), the root system will grow optimally in suitable media conditions both physically and chemically.

The root length that matches the criteria for the ready-to-distribute seedling is maximum until the plant is 1 year old. The root system is positively correlated with the resulting growth. The longer the root of a plant, the higher the ability of the plant to absorb water and nutrients so that it will produce optimal growth, such as plant height, number of stalks, and number of leaflets.

Conclusion

Treatment of biofertilizers influenced the number of leaves and chlorophyll index. The application of biofertilizer with a concentration of 15 mL L^{-1} + interval of 2 weeks had a great value on the parameters of leaf number and chlorophyll content index. It is necessary to maintain optimal maintenance to support growth toward the tea seeds ready for distribution.

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