

Evaluation Growth of Tea Seedling and Population of *Azotobacter* sp. from Application Compost of Green Tea Factory Waste (*Tea Fluff*) and *Azotobacter* sp. on Andisols

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Abstract: Tea plants are generally propagated through vegetative propagation leaf cutting and planting on mixed of topsoil and subsoil. To obtain the soil media from top soil began to feel difficult. In order to overcome these problems and preserve the environment, it is necessary to find alternative planting medium of tea seedling has to be explored to reduce the use of soil, one of which is the use of green tea factory waste compost *Tea Fluff* (TF). In addition, the fulfillment of nitrogen with use *Azotobacter* sp. This study aims to determine the effect of giving *Tea Fluff* compost and *Azotobacter* sp. on the growth of tea seedling and soil nutrients. This research was conducted from April 2020 to January 2021. The experiment will be carried out at Nursery in Research Institute for Tea and Chincona (RITC) Gambung, West Java. The planting material used was superior GMB 7 clones. The study used a factorial randomized block design (6 x 2) with 3 replications. Factor I is the combination of subsoil, (F1) 100% Topsoil + 0% TF; (70% Topsoil + 30% TF; 60% Topsoil + 40% TF; 50% Topsoil + 50% TF; 40% Topsoil + 60% TF; and 30% Topsoil + 70% TF, as a factor II is the *Azotobacter* sp dose, consisting of without *Azotobacter* sp. and 3 mL *Azotobacter* sp. The variables observed included the growth of tea seeds (18 WAP), analysis of soil chemistry and the microbial population of *Azotobacter* sp. The results showed that the combination of *Tea Fluff* compost with soil media had an effect on increasing the number of leaves, stem diameter, root length, root volume, percentage of live seedling, C-organic, N total, dan P available. *Azotobacter* sp. affect the number of microbial populations. There is an interaction between the combination of *Tea Fluff* compost with *Azotobacter* sp. on population of *Azotobacter* sp. The highest percentage of live seeds in control treatment was 14.83% followed by treatment 70% Topsoil + 30%TF is 14.17%. The composition of the planting medium with *Tea Fluff* compost planting media affects microbial populations differently and the growth of clones GMB 7 on Andisols.

Keywords: *Azotobacter* sp., Tea Seedling, *Tea fluff* Compost, Andisols

1. Introduction

Soil is one of the factors that is quite decisive for the growth of tea plants, so it is necessary to choose a suitable soil to meet the needs of tea plant growth. Soil that is suitable or meets the requirements for tea plants is fertile soil, contains lots of organic matter and has a soil pH between 4.5-5.6. Generally good soil for growing tea is located on the slopes of the volcano which is commonly called Andisol soil

(young volcanic).

Tea plants absorb nutrients from the soil continuously, so that the availability of nutrients in the soil decreases over time. Nutrients in the soil can also be reduced due to the leaching process by rainwater, evaporation, and erosion. Therefore, if the soil is left untreated, it will become damaged or become critical soil. Damaged soil results in disrupted plant growth and production will decrease, and plants will die, it is necessary to carry out soil management

as well as possible [16].

Tea nursery is the first step to get a good quality and sustainable tea plant. The success of tea seed growth in the nursery is expected to reach more than 80%. Problems that usually occur in tea nurseries are the percentage of seed growing which only reaches 40-50% and the low percentage of seeds ready for distribution. This problem is caused by the quality of the planting material is not good, the planting media is not suitable, and the lack of soil nutrients.

Green tea factory waste (*Tea Fluff*) is the rest of the tea factory processing which amounts to 1-3% of the total tea production in green tea factories. In addition, *Tea Fluff* is the result of sorting from the manufacture of green tea which consists of solids. According to [2] one way to reduce production costs in tea cultivation is the use of organic matter as fertilizer for sustainable tea.

Nitrogen (N) fixing soil microbes have an important role in helping the availability of N nutrients that are useful for plants. One of the non-symbiotic N-fixing microbes is *Azotobacter* sp. Some isolates of *Azotobacter* sp. and endophytic bacteria have been isolated from the tea rhizosphere using an acid pH medium (pH<6.5) (Setiawati *et al.*, 2013). Utilization of *Azotobacter* sp. and also as a biofertilizer that has been identified as an alternative to chemical fertilizers for crop production and to increase soil fertility [5]. Indigenous N-fixing bacteria were obtained from the isolation of the tea plant rhizosphere, namely *Azotobacter* sp. with code/strain II-1 (RTF-1) [15]. The role of *Tea Fluff* compost as a combination of soil media and the application of *Azotobacter* sp. in tea nurseries has not been studied, therefore as a source of organic matter and biological fertilization in tea nurseries on GMB 7 clones need to be done. The potency of compost and indigenous microbes originating from the tea plantation area is expected to increase the initial growth of tea seeds. This study aims to obtain the formulation of *Tea Fluff* on composting and determine the effect of giving the right compost dose of *Tea Fluff* and *Azotobacter* sp. on the early growth of tea seeds.

2. Materials and Methods

The research was conducted at the Tea Nursery, Research Institute for Tea and Chincona, Gambung, West Java. The experimental location is located at an altitude of 1,350 masl with Andisols. The research method used was a factorial randomized block design and three replications. All treatments used 30% of the top layer (subsoil) and 70% (bottom layer). Several combinations of undercoat treatment with *Tea Fluff* (TF) compost and *Azotobacter* sp. is as follows.

Research treatment:

Factor 1 (Bottom layer combination):

f1 = 100% Topsoil + 0% TF

f2 = 70% Topsoil + 30% TF

f3 = 60% Topsoil + 40% TF

f4 = 50% Topsoil + 50% TF

f4 = 40% Topsoil + 60% TF

f5 = 30% Topsoil + 70% TF.

Factor 2 (*Azotobacter* sp. application):

a1 = without *Azotobacter* sp.

a2 = inoculated with *Azotobacter* sp.

Azotobacter sp. was applied as much as 3 mL through the soil at the beginning of planting in each treatment. *Azotobacter* sp. Indigen N fixation was obtained from the results of preliminary studies isolated from the rhizosphere of tea plants, is *Azotobacter* sp. with code/strain II-1 (RTG-1) with a population of 1.21×10^9 cfu mL⁻¹. Total N content of *Azotobacter* sp. Indigen code II-1 at pH 5.0 medium was 1.46% from 0.12×10^8 CFU mL⁻¹ and the IAA content was 15 ppm (Pranoto, 2015). The results of the *Tea Fluff* compost analysis used in this study are shown in Table 1 and according to Compost Indonesian National Standard [6].

Table 1. *Tea Fluff* Compost Analysis.

Parameter	Results
pH H ₂ O	7,08
C-Organic (%)	20,61
Total N (%)	1,835
C/N	11,2
<i>Azotobacter</i> sp. (CFU mL ⁻¹)	$1,4 \times 10^8$

*) The results of the laboratory analysis of the Institute of Vegetable Crops Research, 2020.

The number of treatments was 12 repeated three times so that the total treatment was 36 experimental units. The time of the study was carried out for 4 months, starting from planting tea seeds to opening the lid. Each treatment as many as 15 tea cuttings seeds so that the total need for tea cuttings seeds is 450 seeds. The number of sample plants was 5 plants. Each plant sample was given *Azotobacter* sp. according to each treatment at the beginning of tea seed planting. Observation parameters were observed as follows:

1. Plant Height (plant height measured with a ruler from the ground to the highest leaf)
2. Number of Leaves (counting all the leaves that have opened completely).
3. Rooting (root length and root weight). At the age of 4 months (open the lid) was carried out destructively on 3 sample plants for each treatment. Root length (root length was measured using a measuring instrument/ruler, measured from the base to the tip of the root). Root weight (wet weight of roots dried in an oven at 80-90°C, then recorded is root dry weight).
4. Percentage of live seeds (number of viable seeds of the initial population). Observation of the number of live seeds was carried out on all experimental materials.
5. The *Azotobacter* sp. population was calculated at the beginning and end of the observation using the TPC (Total Plate Count) method.

3. Result and Discussion

3.1. Plant Height

The composition of the growing media and *Azotobacter*

sp. showed no significant effect on tea seed plant height at 18 WAP (Table 3). The average plant height at 18 WAP can be seen in the combination treatment of 60% Topsoil + 40% Compost *Tea Fluff* of 8 cm. At the age of tea seeds after opening the lid (18 WAP), plant height in soil media with and without *Azotobacter* sp did not differ, which explains that in soil media, *Azotobacter* sp. doesn't do much. This is in line with the results of research [17] which stated that the application of *Azotobacter* sp. had no significant effect on the growth of seedling height, both oil palm and tea. Under conditions of sufficient N from other sources, there is no stimulus to *Azotobacter* sp. activity.

Table 2. Effect of Various Growing Media and *Azotobacter* sp. on tea seed plant height.

Treatment	Plant Height (cm)
Bottom Layer Combination	
f0 = 100% Topsoil + 0% <i>Tea Fluff</i> compost	6.50 a
f1 = 70% Topsoil + 30% <i>Tea Fluff</i> compost	6.83 a
f2 = 60% Topsoil + 40% <i>Tea Fluff</i> compost	8.00 a
f3 = 50% Topsoil + 50% <i>Tea Fluff</i> compost	6.50 a
f4 = 40% Topsoil + 60% <i>Tea Fluff</i> compost	6.50 a
f5 = 30% Topsoil + 70% <i>Tea Fluff</i> compost	7.00 a
<i>Azotobactersp</i>	
a0 = without <i>Azotobacter</i> sp.	7.22 a
a1 = inoculated <i>Azotobacter</i> sp.	6.56 a

Note: Numbers followed by the same letter in the same column indicate that the treatment is not significantly different based on Duncan's multiple-distance test with a significance level of 0.05%.

The increase in plant height is in accordance with the development of tea seed rooting. According to [13] dry matter resulting from photosynthesis is a source of energy for cell division and enlargement which results in an increase in plant height. Roots grew better on tea seeds grown in soil without or with *Azotobacter* sp. Plants will be better able to absorb nutrients from the soil and contribute to plant height. These results are in accordance with the research of [20] that the application of *Tea Fluff* compost derived from black tea as organic material up to 40% mixed with topsoil can provide the highest tea seed plant height in the nursery. Effect of *Tea Fluff* compost and *Azotobacter* sp. to plant height at 18 WAP is presented in Figure 1.

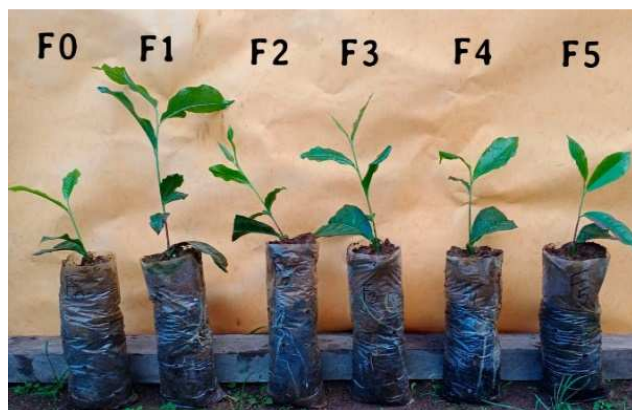


Figure 1. Tea seedling at 18 weeks after planting (WAP).

The tea plant is able to grow and form new shoots even though the seed roots have not yet grown, this is due to the presence of food reserves in the leaves and stems, and also plants photosynthesize through one leaf in the tea seed. Planting media with the addition of fluff compost decreased plant height in callus plants (roots did not grow) so that although the N content in fluff compost was very high, without roots, there were obstacles in the absorption of N by plants and further stunted plant growth. According to [17] stated that the effect of *Azotobacter* sp. may not have experienced real development and activity in N fixation and synthesis of organic compounds that are beneficial for plant growth.

3.2. Number of Leaves and Stem Diameter

Based on the results of statistical analysis, the combination of *Tea Fluff* compost with soil media had a significant effect ($P < 0.05$) on the number of leaves and stem diameter. The number of leaves is an important parameter as a condition for tea plant seeds to be transferred to the field. The average number of leaves at the age of 18 WAP was 2-4 leaves, this did not meet the requirements for transplanting because it had just been opened in the tea nursery. The length of the tea seedling until it is ready for planting is 8-12 months. At the age of 18 WAP, the number of leaves showed normal growth.

Table 3. Independent Influence of Various Growing Media and *Azotobacter* sp on the number of leaves and stem diameter of tea seeds.

Treatment	Number of leaves (leaves)	Stem Diameter (cm)
Bottom Layer Combination		
f0 = 100% Topsoil + 0% <i>Tea Fluff</i> compost	2.57 ab	1.83 a
f1 = 70% Topsoil + 30% <i>Tea Fluff</i> compost	3.10 b	1.91 ab
f2 = 60% Topsoil + 40% <i>Tea Fluff</i> compost	3.03 b	1.99 ab
f3 = 50% Topsoil + 50% <i>Tea Fluff</i> compost	2.27 ab	1.93 ab
f4 = 40% Topsoil + 60% <i>Tea Fluff</i> compost	2.13 a	1.92 ab
f5 = 30% Topsoil + 70% <i>Tea Fluff</i> compost	1.70 a	2.11 b
<i>Azotobactersp</i>		
a0 = without <i>Azotobacter</i> sp.	2.47 a	2.02 a
a1 = inoculated <i>Azotobacter</i> sp.	2.47 a	1.88 a

Note: Numbers followed by the same letter in the same column indicate that the treatment is not significantly different based on Duncan's multiple-distance test with a significance level of 0.05%.

The growth of tea cuttings begins with shoot growth and leaf growth. Parameter number of leaves is closely related to the process of photosynthesis. The more the number of leaves, the more photosynthate produced to support the growth and development of tea cuttings into ready-to-plant tea seeds [8]. At the initial growth of the seed age 3 months after planting (120 days after planting), leaf formation is not optimal and the energy used for the growth of shoot cuttings and leaves comes from food reserves contained in the stems of tea cuttings [19]. After the leaves are formed, the plant can carry out the process of photosynthesis and the growth of cuttings leads to root growth [20].

The diameter of the stem needs to be observed to see the health of the plant. The effect of the organic growing media tested was not significantly different in stem diameter. The combination of media 30% Topsoil + 70% *Tea Fluff* compost gave more influence on the development of stem diameter of tea plants in the nursery (see Table 4). This is due to the combination treatment of 30% Topsoil + 70% *Tea Fluff* compost has the highest organic matter composition compared to other treatments. The physical properties of organic media further strengthen the growth of plant seeds, the structure, and texture of organic media is also more able to maintain aeration balance, so that the growth of stem diameter is greater than the treatment with no or little organic matter (*Tea Fluff* compost) [8].

So far, the planting media in tea nurseries only uses two types of soil layers, namely topsoil and subsoil. Apparently, the use of waste in the form of organic materials such as green tea factory waste (*Tea Fluff*) can be considered as a

mixture of planting media in tea plant nurseries. Organic materials, especially those that are waste, which are abundantly available and inexpensive, can be used as an alternative to soil media that is difficult/slow to replace.

3.3. Root Length and Root Volume

Root parameters are a determinant of the success of plant propagation in nurseries. The plant root length measurements were carried out at four months after planting. The longer the roots, the more secondary roots are expected, so that the ability to take up nutrients and water by the roots is greater. Based on the results of statistical analysis, the combination of *Tea Fluff* compost with soil media had a significant effect ($P < 0.05$) on root length and root volume (Table 4). The use of 30-40% *Tea Fluff* compost from the planting medium was significantly different in growing tea seed roots. The application of *Tea Fluff* compost has a crumb structure, so that the roots of tea cuttings will easily penetrate the soil so that they can develop properly [20]. However, the addition of topsoil must still be done to get a dense planting medium and able to maintain soil moisture. Therefore, the combination of 100% topsoil and 70% topsoil with *Tea Fluff* compost gave better results in root length and root volume than other combination treatments. The plants roots are evenly distributed indicate that plants absorb nutrients well. In addition, PGPR such as *Azotobacter* sp can affect lateral root growth and root hair development. One other characteristic is the increased rate of root elongation, which results in more active roots [9].

Table 4. Effect of various growing media and *Azotobacter* sp. on root length and root volume of tea seeds.

Treatment	Root Length (cm)	Root Volume (cm)
Bottom Layer Combination		
f0 = 100% Topsoil + 0% <i>Tea Fluff</i> compost	10.18 c	0.95 abc
f1 = 70% Topsoil + 30% <i>Tea Fluff</i> compost	9.53 bc	1.33 c
f2 = 60% Topsoil + 40% <i>Tea Fluff</i> compost	9.03 bc	1.16 bc
f3 = 50% Topsoil + 50% <i>Tea Fluff</i> compost	3.24 a	0.44 a
f4 = 40% Topsoil + 60% <i>Tea Fluff</i> compost	5.25 ab	0.64 ab
f5 = 30% Topsoil + 70% <i>Tea Fluff</i> compost	3.20 a	0.43 a
<i>Azotobactersp</i>		
a0 = without <i>Azotobacter</i> sp.	7.11 a	0.85 a
a1 = inoculated <i>Azotobacter</i> sp.	6.36 a	0.80 a

Note: Numbers followed by the same letter in the same column indicate that the treatment is not significantly different based on Duncan's multiple-distance test with a significance level of 0.05%.

Root length growth is determined by the penetration of the roots to the soil which is influenced by the texture, structure and depth of groundwater [19]. Tea plants from cuttings have roots that are not as deep as the origin of seeds. Therefore, a longer root system helps the plant to absorb more nutrients and water. Root formation in the plant from the cuttings begins with callus formation, i.e. cell growth and multiplication will close the cutting wound. After the wound is closed, roots will form which is produced by the differentiation of the callus cells.

Organic matter plays a role in improving the physical

properties of the soil in terms of the ability to bind water by the soil, *Tea Fluff* compost has a high water binding ability so that the availability of water in the root zone is sufficient and can stimulate root growth properly. The composition of the growing media for tea cuttings consists of sand: soil: compost with a ratio of 1:3:1 and for rooting media it must have a sand:soil ratio of 1:1.

3.4. Root Dry Weight, Shoot Dry Weight and RS Ratio

Root observations were carried out starting from the age of 4-5 months and the destruction of 5 (five) tea cuttings seeds.

The results of the composition of the growing media and *Azotobacter* sp. showed no significant effect on root dry weight, shoot dry weight and RS ratio of tea seeds at 18 WAP (Table 5).

Table 5. Effect of various planting media and *Azotobacter* sp tea seeds on root dry weight, shoot dry weight and RS ratio.

Bottom Layer Combination	Root Dry Weight (g)	Shoot Dry Weight (g)	RS ratio
f0 = 100% Topsoil + 0% <i>Tea Fluff</i> compost	0.14 a	0.20 a	1.43 a
f1 = 70% Topsoil + 30% <i>Tea Fluff</i> compost	0.17 a	0.23 a	1.35 a
f2 = 60% Topsoil + 40% <i>Tea Fluff</i> compost	0.12 a	0.21 a	1.75 a
f3 = 50% Topsoil + 50% <i>Tea Fluff</i> compost	0.07 a	0.20 a	2.85 a
f4 = 40% Topsoil + 60% <i>Tea Fluff</i> compost	0.09 a	0.17 a	1.89 a
f5 = 30% Topsoil + 70% <i>Tea Fluff</i> compost	0.10 a	0.15 a	1.50 a
<i>Azotobacter</i> sp			
a0 = without <i>Azotobacter</i> sp.	0.12 a	0.19 a	1.58 a
a1 = inoculated <i>Azotobacter</i> sp.	0.11 a	0.19 a	1.72 a

Note: Numbers followed by the same letter in the same column indicate that the treatment is not significantly different based on Duncan's multiple-distance test with a significance level of 0.05%.

The total dry weight reflects the accumulation of organic compounds that have been successfully synthesized by plants from inorganic compounds (nutrients, water and carbohydrates), the higher the total dry weight, the better the seed growth. Compost treatment is able to add nutrients to the growing media so that it can affect the formation of plant biomass.

The *Tea Fluff* compost added to the soil media is able to improve the soil structure, so that the soil becomes crumbly and loose and has the ability to store water (drainage) and air porosity (aeration). The physical condition of the media allows the plant roots to multiply well and has adequate water supply conditions. The growth of the root system will deviate from its ideal condition if the soil conditions as a place for growth are not optimal, but if the opposite occurs, it is certain that the plant root system is fully influenced by genetic factors.

Root crown ratio is a parameter that reflects the ability of plants to absorb nutrients and metabolism that supports plant growth. The plant root system is more influenced by the genetic nature of the plant and the soil conditions of the growing medium. The ratio of the average weight of shoots and roots (shoot/root) in the combination treatment of soil media with fluff compost was 1.80. Root development will

determine shoot development. The process of photosynthesis involves the role of roots, in good root conditions photosynthesis goes well and stops assimilation in the form of tissues or organs. The N_2 fixing microorganisms collectively referred to as "*diazotrophs*" are capable of biologically fixing N_2 in association with plant roots *Azotobacter* sp. have beneficial plant-promoting properties e.g., efficient use of nutrients, phytohormones [1]. Tea compost is very rich in phytohormones and growth regulators, stimulates microorganisms that have direct and indirect effects on plant rhizosphere, in addition to improving soil physical and chemical properties and suppressing several plant disease pathogens [11, 12].

3.5. The Population of *Azotobacter* sp

The interaction of the *Tea Fluff* compost combination with *Azotobacter* sp. significantly ($P < 0.05$) had an effect on the population of *Azotobacter* sp. The population of *Azotobacter* sp compost *Tea Fluff* used was 1.5×10^7 CFU g^{-1} . The bacterial population did not experience a large increase due to root growth that had not been intensive until 18 WAP. Root exudates of higher plants are needed by non-symbiotic heterotrophic N-fixing bacteria as an energy source [3, 4].

Table 6. Effect of Interaction of Various Growing Media and *Azotobacter* sp. tea seeds on the population of *Azotobacter* sp.

Bottom Layer Combination	Number of Azotobacter sp. (10 ⁶)		Average
	Azotobacter sp.		
	0 ml	3 ml	
f0 = 100% Topsoil + 0% <i>Tea Fluff</i> compost	1.40 a A	1.40 a A	1.40
f1 = 70% Topsoil + 30% <i>Tea Fluff</i> compost	1.47 a A	3.90 ab A	2.68
f2 = 60% Topsoil + 40% <i>Tea Fluff</i> compost	4.20 b A	1.83 ab A	3.02
f3 = 50% Topsoil + 50% <i>Tea Fluff</i> compost	1.73 a A	2.23 ab A	1.98
f4 = 40% Topsoil + 60% <i>Tea Fluff</i> compost	1.27 a A	4.43 ab A	2.85
f5 = 30% Topsoil + 70% <i>Tea Fluff</i> compost	1.37 a A	6.10 b A	3.73
Average	1.91	3.32	

Note: Numbers followed by the same letter were not significantly different according to Duncan's test at the 5% level. Capital letters are read horizontally (rows) and lowercase letters are read vertically (columns).

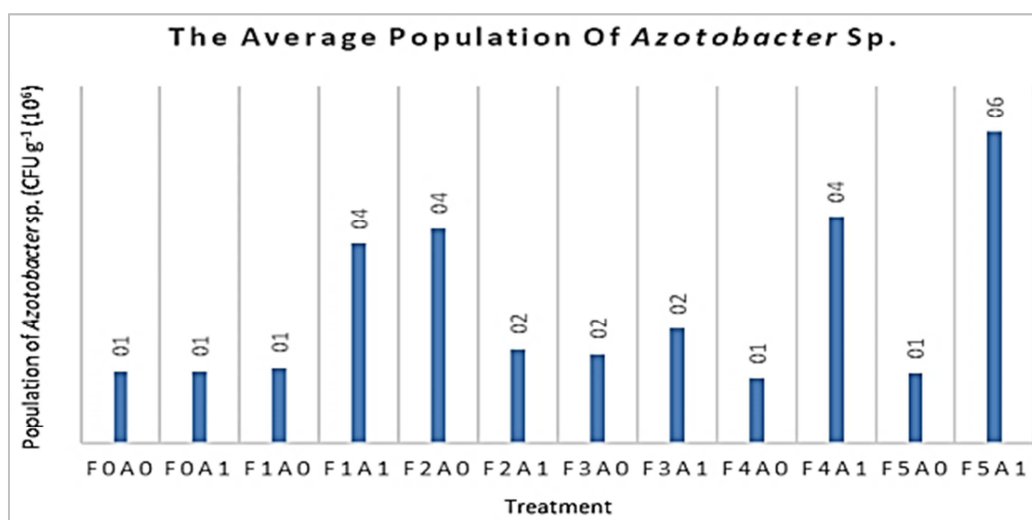


Figure 2. Average Population of Azotobacter sp.

The population of *Azotobacter* sp. in the combination treatment of soil media with *Azotobacter* sp. in the treatment of 30% Topsoil + 70% Compost *Tea Fluff* of 3.73×10^6 CFU g⁻¹ and the control treatment of 1.40×10^6 CFU g⁻¹ (Figure 2). Treatment of 70% Topsoil + 30% Compost *Tea Fluff* before the experiment was 9.40×10^7 CFU g⁻¹, while the population of *Azotobacter* sp. after the experiment was 2.68×10^6 CFU g⁻¹. The results showed that there was no high population increase between before and after the experiment. The carbon source in the soil in the form of organic carbon is in a form that is relatively more available to microbes [10]. *Azotobacter* sp. is found in soil with a pH of 6.0 or more, although at a pH of less than 6.0 can also live but not active. Soil reaction is a limiting factor for the development and spread of these bacteria [14], soil pH for tea plants is around 4.5-5.5.

The use of compost as organic fertilizer allows increased fertility, besides being an excellent soil conditioner, improving physical, chemical and biological properties, such as water retention, aggregation, porosity, cation exchange capacity, fertility and soil microbial life [7]. According to green waste is a safe substrate for the environment for composting because it does not contain pollutants and is beneficial for plants [18].

4. Conclusion

Combination of *Tea Fluff* compost and soil media with *Azotobacter* sp. The best treatment was in the treatment of 70% Topsoil + 30% TF with a population of *Azotobactersp.* of 2.68×10^6 CFU g⁻¹. The interaction of the combination of *Tea Fluff* compost with *Azotobactersp* affects the population of *Azotobacter* sp. The combination of *Tea Fluff* compost with soil media had an effect on increasing the number of leaves, stem diameter, root length, root volume and percentage of live seeds. The highest percentage of live seeds treated with 70% Topsoil + 30% TF and 70% Topsoil + 40% TF did not differ from the control soil growing media. The composition of the growing media with *Tea Fluff* compost affected the *Azotobacter* sp population and the growth of

GMB 7 clone tea seeds in Andisols.

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