Polyphenols Content of Indonesian Tea Clones on Optimum Manuring Condition

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Abstract. Tea (\textit{Camellia sinensis} (L.) Kuntze) has been known as a functional drink containing bioactive polyphenols with health benefits effect. Polyphenols in tea are not only influenced by climate but also by clone types and cultivation techniques. Recently, technological developments in tea commodities are not only focused on the quantity of production but also the content of polyphenols. Previous studies reported that the application of Nitrogen (N), Phosphorus (P), and Potassium (K) fertilizers played a role in increasing the polyphenol content in tea. Research Institute for Tea and Cinchona (RITC) has released GMB Series clones that have high productivity compared to other clones. The objectives of this study were to determine the content of tea polyphenols of various GMB series clones on optimum manuring conditions. This research was carried out in 2018 in the high elevation areas (1,350 m masl) and medium elevation (800 m masl). At the high elevation area at RITC Gambung Experimental Plantation, showed that on optimum manuring condition had an effect on the increase in polyphenol content in most GMB clones (14.83 - 15.43\% dw) compared to control (10.56\% dw) and GMB 1, GMB 4, GMB 7 and 9 clones showed significantly different effect on the content of total polyphenols. Tests which conducted on the medium elevation area at Cianjur, West Java, showed that under optimum manuring condition, the range of total above 15\% with the highest polyphenols were obtained in GMB 1 clone at the dosage application of NPKMg (300:30:249:50) kg ha\textsuperscript{-1} year\textsuperscript{-1}

1. Introduction
Tea is one of the foreign exchange producing commodities for Indonesia and absorbs a lot of labor because it is labor-intensive. Recently, Indonesia is the seventh tea producer in the world with production in 2017 of 139,362,000 kg which contributes around 2.3\% of total tea production worldwide [1]. Tea plants have been recognized by the community as a healthy drink for the human body.

The chemical content of tea plants that are considered healthy for humans are polyphenol compounds [2]. This is certainly a challenge for the world of tea research to conduct various studies so that polyphenols in tea plants to be higher, one of which is to modify the tea cultivation system to produce high polyphenols. Polyphenol compounds are useful as antioxidants to prevent free radicals in the body, reduce cholesterol content in the body, prevent cancer and heart attacks and are good for people with...
high blood pressure [3] and anti-obesity. In tea plants, the polyphenol content is influenced by several factors namely the type of tea clones, seasons [4] and cultivation techniques [5].

The GMB series clones produced by the Research Institute of Tea and Cinchona. It has been recognized by the Government of the Republic of Indonesia as a superior clone with various characteristics, high productivity, and good quality. GMB 1 clones up to GMB 5 were released by the Minister of Agriculture in 1988 on April 21, 1988 with numbers SK 260, 267, 266, 265 and 264 as superior clones with high yield potential (4,000 kg ha\(^{-1}\) year\(^{-1}\)), blister blight resistant, and can grow both in the medium and high land [6]. GMB 6 clones until GMB 11 were released on October 9, 1998 with numbers SK 684, 684.a, 684.b, 684.c, 684.d, and 684.e as superior potentially high-yielding clones (5,000 kg ha\(^{-1}\) year\(^{-1}\)), blister blight resistant, and initial growth both in the medium and high land [7]. Apart from depending on the environment, tea shoots are also influenced by the cultivated clones, Camellia sinensis var. Assamica tends to be able to give higher shoots [8].

Until now there has been no research on cultivation systems specifically to increase the content of tea plant polyphenols on a large scale. Research on a scale experiment to increase the content of polyphenols in the field, it has been known that fertilizing N and K with a ratio of 1: 0.83 with N nutrient doses of 300 Kg/ha/year and nutrient doses of 250 Kg/ha /year can increase the content of polyphenols on tea plants [8].

Indonesia is one of tea producing countries in the world, but until now it does not have special crop farming technology in GMB series clones that have high polyphenol content. This study aims to develop a technology package to increase the content of polyphenols in tea plants. Research on cultivation technology to increase the content of polyphenols in plants will be the latest innovation and technological breakthrough that will help planters to increase economic added value that can improve welfare, especially for community tea growers in Indonesia. This study aims to produce a technology package for tea cultivation, especially in several GMB series clones to increase the polyphenol content of tea plants.

2. Materials and Methods
Research was conducted in Gambung Tea Plantation, Bandung District, West java with 1.350 m mean above sea level (masl) altitude, Andisol soil and type B rainfall according to Schmidt and Ferguson classification. The experiments were conducted from June to October 2018. The design of experiments in this research is was split plot using superior Indonesian Assamica tea clones series (GMB 1, GMB 3, GMB 4, GMB 7, dan GMB 9) as main plot and four manuring packages as subplot consists of Package 0 (P0) without manure as control, Package 1 (P1) [9], Package 2 (P2) [9] and Package 3 (P3) [8]. All plots replicated by 3 replications. Plucking is done by scissors plucking with the plucking period of 28-30 days.

| Fertilizer Packages | Dosages (kg\(^{-1}\)ha\(^{-1}\)year\(^{-1}\)) |
|---------------------|---------------------------------|
|                     | N     | P     | K     | Mg    |
| P1                  | 300   | 25    | 120   | 50    |
| P2                  | 300   | 90    | 120   | 50    |
| P3                  | 300   | 30    | 249   | 50    |

The parameter observed was the polyphenol content. The polyphenol content was analyzed using the colorimeter method using Folin-Ciocalteu reagents [10] with the procedure consisting of two stages namely standard series making and determination of fresh tea leaf polyphenols. Level the polyphenols in the example using the following formula:
Polyphenol content (% dry weight (dw)) = \frac{(Abs−a)/b \times 100/5 \times 50/1000}{W} \times 100\%

3. Result and Discussion
In observations of the tea shoots plucked at an altitude of 1,350 m masl, based on statistical analysis (Table 2), the treatment of all manuring packages have significant differences in the content of the polyphenols, as well as the treatment of GMB clones, were GMB 1, GMB 4, GMB 7 and GMB 9 showing significant differences in polyphenol content.

Table 2. The effect of manuring treatment and clones on total polyphenols (% dw) at high elevation (1,350 m masl)

| Clones | Manuring Dosage Packages | Mean |
|--------|-------------------------|------|
|        | Control* | P1** | P2*** | P3**** |
| GMB 1  | 9.89     | 16.44 | 16.77 | 16.34 | 14.86b |
| GMB 3  | 6.83     | 13.17 | 14.01 | 14.08 | 12.02a |
| GMB 4  | 10.63    | 14.05 | 15.93 | 15.88 | 14.12b |
| GMB 7  | 13.63    | 16.11 | 15.38 | 15.99 | 15.28b |
| GMB 9  | 11.80    | 14.40 | 14.74 | 14.87 | 13.95b |
| Mean   | 10.56a   | 14.83b| 15.36b| 15.43b|

Note: Number followed by the same letter in the same column shows no significant difference based on Duncan’s Multiple Range Test (DMRT) test at the 95% confidence level

*Control (without manuring); **P1 (N300, P25, K120, Mg50) kg ha⁻¹ year⁻¹; ***P2 (N300, P90, K120, Mg50) kg ha⁻¹ year⁻¹; ****P3 (N300, P30, K249, Mg50) kg ha⁻¹ year⁻¹

At the high elevation area (1,350 m masl) There is no interaction between clone types and manuring packages. Manuring packages of all type can increase the total polyphenols in the fresh tea leaves in clones GMB1, GMB3, GMB4, GMB7 and GMB 9 by 4.28 to 4.88% dw compared to without fertilization. GMB 3 tea clones fresh leaves have the lowest polyphenol content of 12.02% dw and GMB 7 clone have the highest total fresh tea leaves polyphenol content of 15.28% dw compared to GMB 1 clones (14.86% dw), GMB 4 (14.12% dw), and GMB 9 (13.95% dw) in all manuring packages (Figure 1).
Figure 1. Effect of tea clones on total polyphenols at high elevation (1,350 m asl)

Figure 2. Effect of manuring package on total polyphenols at high elevation (1,350 m asl)

Observations made in areas with medium elevation (800 m asl) in GMB1 and GMB7 clones, where these clones have the highest polyphenol content in the highlands (1,350 m asl) show interactions between clones and fertilizer packages (Table 3).

Table 3. The effect of manuring treatment and clones on total polyphenols (%) at middle elevation (800 m asl)

| Clones  | Manuring Dosage Packages | Control* | P1** | P2*** | P3**** |
|---------|--------------------------|---------|------|-------|--------|
| GMB 1   |                          | 16.45b  | 18.53c| 17.83cd| 18.71d |
| GMB 7   |                          | 15.65ab | 16.15ab| 15.37a| 15.71ab |
Note: Number followed by the same letter in the same column shows no significant difference based on Duncan’s Multiple Range Test (DMRT) test at the 95% confidence level

*Control (without manuring); **P1 (N300, P25, K120, Mg50) kg ha⁻¹ year⁻¹; ***P2 (N300, P90, K120, Mg50) kg ha⁻¹ year⁻¹; ****P3 (N300, P30, K249, Mg50) kg ha⁻¹ year⁻¹

Figure 3. Effect of clones and manuring package on total polyphenols at middle elevation (800 m masl)

At the medium elevation area (800 m masl) GMB 7 clones in all fertilizer packages have lower polyphenol content compared to GMB 1 clones. The lowest total polyphenols obtained in GMB 7 clones with P2 (N300, P25, K120, Mg50 kg ha⁻¹ year⁻¹) fertilizer package, meanwhile, the highest polyphenols were obtained in GMB 1 clone which fertilized using P1 (N300, P30, K249, Mg50 kg ha⁻¹ year⁻¹) manuring package.

Among agronomic practices, application of N, P, and K fertilizers was reported to have a pronounced effect on leaf total polyphenol content [11]. N, P, and K application was also found to improve the accumulation of carbohydrates for plant growth [12] and to increase photosynthetic rates [13]. This resulted in the biosynthesis of carbon-based secondary metabolites, such as flavonoids, phenolic acids, and tannins, known as total polyphenols, which are antioxidant in nature [13].

At high elevation area, fertilization has an impact on increasing polyphenols compared to without fertilization (Table 2). Meanwhile GMB3 clones have the lowest polyphenol content in all fertilizer packages. The range of polyphenol content values in fertilized clones in this study was observed from 13.17% dw in the highlands (Table 2) to the highest 18.71% dw in the medium elevation (Table 3). This value is far from the polyphenol content of tea clones from India UPASI-9 with a range of 21.4% dw to 31.4% dw [8]. The minimum polyphenol content must be fulfilled for green tea products based on the Indonesian national standard (SNI) at least 15% [14]. The results of the study showed that there was an effect of fertilization on the increase in polyphenol content in all clones tested. Polyphenol contents of the crop shoots were also higher due to this ratio of NK manuring. A lower polyphenol content was recorded when the plots were supplied with less (or) no nitrogen and potash fertilisers [8].
Furthermore, in medium elevation area, the interactions that occur (Table 3) between clones and fertilization packages, indicate that fertilization packages containing 249 kg ha\(^{-1}\) year\(^{-1}\) potassium (K) dose have the highest polyphenol content with an increase of 3.78% compared dose K 120 kg ha\(^{-1}\) year\(^{-1}\) at the same nitrogen dose of 300 kg ha\(^{-1}\) year\(^{-1}\). Results of research conducted by Ruan et al. it was reported that the addition of K fertilizer in the form of KCl could increase 6.5% of total polyphenols from 228.7 ppm without K fertilizer to 244.6 ppm in the N: P: K fertilizer ratio 1: 0.22: 0.42 [15]. At the same level of nitrogen, when the potassium dose was increased there was a significant increase in polyphenols and free amino acid contents of flush shoots until a ratio is reached, beyond which there was a sharp decrease [8]. The quality of harvested shoots was improved by K-fertilizer application as revealed by increased concentrations of free amino acids, water-extractable dry matter, and total polyphenols [15].

From Tables 2 and 3 we can see the comparison of the total content of polyphenols in GMB 1 and GMB7 clones at two different altitudes. In the highlands (altitude of 1,350 m masl), GMB1 clones had a total polyphenol of 14.86% dw, but in the medium elevation (altitude of 800 m masl), the polyphenol content was 18.71% dw in the P3 treatment or an increase of 19.53%. This is in line with research conducted by Han et al. that the total polyphenol decreased with rise of altitude with a very strong positive correlation \(r = 0.941\) [16]. Research conducted at Lushan Mountain, eastern China shows the difference in the decrease in total polyphenol content by 16.59% on increasing altitude from 828 m asl to 1,020 m asl [16].

Different results are shown in GMB7 clones, the total content of polyphenols ranges from 15.38% dw (height elevation) to 16.15% dw (medium elevation), or the difference in increase of 4.77%. The difference in height between the plateau and the medium does not seem to provide a large enough difference in the GMB7 clone when compared to the GMB1 clone. The changes in yield and quality will change with altitude in an expected manner, though not necessarily at the same rates for different cultivars [17].

The sustainable development of the tea production and soil nutrient status is the decisive factor of whether tea grown vigorous and the tea quality [18]. Nitrogen (N) is the major nutrient affecting tea growth, yield, and quality. Application of nitrogenous fertilizer substantially increases the production of new shoots and the content of functional compounds, such as amino acids [19]. Phosphorus and potassium are also major nutrients for tea production. Tea soils are highly weathered and have kaolinite clay minerals, where there is hardly any binding site for K, which necessitated frequent application of K fertilizers. Therefore, balanced fertilizer application including K is important for getting high quality products [20], not only K but all nutrient which needed it for tea plant. The direct increase of polyphenols with theapplied potash fertilisers has already been reported by Ruan et al. [21]. Based on stated of Venkatesan and Ganapathy that application of large quantities of N fertiliser without balancing with K should be avoided [8].

### 4. Conclusion

Result of experiment at high elevation area (1.350 m masl) showed that on optimum manuring condition had an effect on the increase in polyphenol content in most GMB clones and four clones were GMB 1, GMB 4, GMB 7 and 9 showed significantly effect on the content of total polyphenols. Tests on the medium elevation area (800 m masl), showed on optimum manuring condition, the lowest total polyphenols obtained in GMB 7, the highest polyphenols were obtained in GMB 1 clone at the dosage application of N:P:K:Mg (300:30:249:50) kg ha\(^{-1}\) year\(^{-1}\).
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