

## FOLIAR APPLICATION OF BIONUTRIENT-C213 ON COFFEE PLANT

HENDRAWAN\*, YAYA SONJAYA, FITRI KHOERUNNISA,  
CAHYANING RISKI WIJAYANTI, DINAR ANUGRAH,  
MUHAMAD NURUL HANA, IQBAL MUSTHAPA

Department of Chemistry, Universitas Pendidikan Indonesia,  
Jl. Dr. Setiabudi No. 229 Bandung – 40154

\*Corresponding Author: hendrawan@upi.edu

### Abstract

This work aims to study the performance of the foliar technique of Bionutrient-C213 on the coffee plant. Bionutrient-C213 is a liquid prepared from a variety of biodiversities, which is treated chemically in various acidity conditions, mixed with a variety of inorganic materials in a certain proportion. This research was started by optimizing of the dose, consisted of doses 3.0 mL/L (means 3 mL of liquid was diluted until 1 liter in water); 3.5 mL/L; 4.0 mL/L; 4.5 mL/L, 5.0 mL/L; 5.5 mL/L; 6.0 mL/L; 6.5 mL/L; and 7.0 mL/L. In this stage, the parameters in concern are the number of productive branches and the number of fruit group in the productive branch. Each group of treatment and control consisted of ten trees which were separated by a series of barrier plants. They were sprayed by 20 liters of each dose of Bionutrient-C213. The control group was left as it usually treated by the farmer. The next step was applying the optimum dose, carried out in the following year, in a different coffee plantation. In this stage, the parameters in concern are the level of nitrogen and the level of chlorophyll in leaves, the average mass of the fruit, the average mass of dried beans, glucose level in fresh fruit flesh, caffeine level in dried beans. We found here that the optimum dose is 5 mL/L, which gives the highest number of productive branches and the number of the fruit group. Also, it is evidence that Bionutrient-C213 application on the Arabica coffee plants are able to improve the content of chlorophyll and nitrogen level in the coffee plant, increasing the average fruit mass of coffee per 100 pieces, increasing the average mass of dried seed per 100 coffees, accelerating of fruit ripening, increase the level of glucose, and increasing the level of caffeine.

Keywords: Biodiversity, Bionutrient-C213, Coffee.

## 1. Introduction

It is a common acquaintance that organic agriculture, which depends strongly on the fertilizer provided by composting, is considered as an accurate response to realizing high-level productivity while conserving the environmental-friendly agricultural practices. Nevertheless, we cannot resist that compost-based fertilizer has a variety of weaknesses, including invariability in chemical content, voluminous in quantity, time consume in process, possible releasing of greenhouse gases and potential risk of uncontrollable of growth of microbes. Concerning the potential release of methane gas into the air, people in some areas in this hemisphere has taken into account the issues in dealing with environmental policy [1-3].

The importance of such issues has led many researchers and practitioners to explore and develop methods or ways of composting and producing organic fertilizer while minimizing the risk of greenhouse gas emissions [4-6]. Excessive fertilization has an effect on soil salinity, eutrophication, nitrate accumulation, and also has a risk on sulfur and nitrogen liberation that eventually causes environmental problems [7]. Therefore, innovation in providing biodiversity-based farming materials which minimize the environmental risk needs to get further serious attention.

In terms of the method of fertilizer application, the foliar technique has recently become a more frequent use, therefore, justified as an alternative way to overcome much weakness in fertilizer application through the rooting. There are several potential advantages of providing nutrients through foliar applications, in particular for nitrogen, such as reducing nitrogen loss due to denitrification and leaching compared with nitrogen fertilizers applied through the land, and to enhance the ability of providing nutrient when the rooting activity is interrupted, i.e., on saline condition and drought, and when the nutrient up taking are retarded for certain season [8].

Some scientific shreds of evidence of the effect of foliar application of various nutrients in terms of crop production have been published by some researchers. Foliar application of fermented calcium mono-di-hydrogen phosphate (FCMP) came to the conclusion that the use of FCMP with foliar technique not only increases the quantity of the harvest of sweet persimmon but also improves its quality [9]. Other researchers found that the results and quality of wheat are levered while the foliar application of fungicide triazole containing macro-and micro-nutrients, without need to worry against deoxynivalenol contamination [10]. Hamed et al. [11] reported that the foliar application of micro-elements and bio-fertilizers gives a positive effect on crop yields of nuts. Similar results were obtained by Afshari et al. [12] when organic fertilizers that have enriched and sprayed against the tomato plants in a greenhouse. The effectiveness of the application of foliar nutrients depends on ingredients that rely upon being used and the type of plants that become the target of application.

Coffee is one of the important products among natural commodities. The demand for the commodity is getting an increase from time to time. The price also tends to get better as the public and the market demand increase. In this realm, the development of a new kind of nutrition for coffee plantation is somewhat challenging.

## 2. Method

### 2.1. Material

Materials used consisted of Bionutrien-C213, hydrophilic gel, Aquadest, citric acid, ethanol, methanol, n-hexane, acetic acid, hydrochloric acid, sulfuricacide, potassium chloride, potassium hydroxide, zinc chloride, natrium chloride, cupric sulfate, sodium carbonate, sodium sulfite, potassium iodide, potassium iodate, starch, universal indicator, phenolphthalein, DNS, Na-metabisulfite, and NaK-tartrate.

The main apparatus used in this research include a set of the water jet, generator set, water pump, laboratory equipment such as analytical balance, electric heating, crusher, HPLC D-7000 Hitachi, etc.

### 2.2. Procedure

#### 2.2.1. Optimization of the dose of bionutrient-C213

The application of Bionutrient-C213 was done on Arabica coffee plant (age around 3 years old), situated in Northern Bandung. The observed plants were divided into 10 groups, each group consisting of 10 trees. Nine groups of trees were treated by Bionutrient-C213 in different dose, ranging from 3 mL/L (mL Bionutrient-C213 per L of water), 3.5 mL/L, 4 mL/L, 4.5 mL/L, 5 mL/L, 5.5 mL/L, 6 L, mL/6.5 mL/L and 7 mL/L, while the control group was left as it usual treatment. To avoid any possible bias, the groups were separated by a single line border tree, named tree barrier. Each group was sprayed with 20 liters of Bionutrient-C213. Spraying was done once every two weeks, in the morning. In this stage, the parameters in concern are the number of productive branches and the number of the bunch in every productive branch.

#### 2.2.2. Application of bionutrient-C213

Further applications were carried out on coffee Arabica plants, in another coffee plantation, situated in southern Bandung. The plants were divided into two groups, namely the treated by Bionutrient-C213 in its optimum dose, and the control group. Observations were carried out on the two groups, where the parameters in concern include the level of nitrogen and chlorophyll in leaves, fresh fruit mass, dried seeds mass, glucose level, and caffeine level.

#### 2.2.3. Laboratory test

Laboratory tests include 1) examination of chlorophyll level in the leaves; 2) examination of total N content in the leaves; 3) examination of glucose level in the flesh of the fruit, and 4) examination of caffeine level in the dried beans.

**Examination of chlorophyll level.** A number of leaf samples were taken randomly, where the leaves are pair on the third and fourth range from the base of the branch. Each sample consisted of 4 leaves taken from 4 different branches. The fresh leaves were mixed and ground in a mortar, then extracted with methanol 96%. The chlorophyll level in the extract was determined using a UV-Vis spectrometer at wavelength 649 and 665 nm.

**Examination of total nitrogen level.** A number of leaf samples were taken in the same way as on the determination of the chlorophyll. Furthermore, the total nitrogen level was determined by the Kjeldahl method.

**Examination of glucose level.** The flesh of fruit on the coffee plant was separated from the seeds, then dried by means of exposing to sunlight. The dried flesh was ground using a mortar, then extracted in Aquadest. The glucose level was determined using the DNS method, colorimetrically, using spectrophotometer UV-Vis, at wavelength 540 nm.

**Examination of caffeine level.** As much as 25-gram caffeine in powder form was dissolved in a solvent, mixed between Aquadest and methanol 96% in ratio 9:1 (v/v), in a flask, so that the final volume was exactly 50 mL. The mixture was homogenized for about 3 minutes using an ultrasonic vibrator. From the solution, a series of the standard solution was prepared with concentration ranging from 10 ppm, 15 ppm, 20 ppm, 24 ppm, 30 ppm, and 34 ppm. Samples consisted of roasted coffee beans from the experiment group, roasted coffee beans from the control group, the green beans from the experiment group, and green beans from the control group. For each sample, as much as 90 grams of their powder form was shocked with 250 mL of methanol 96% for about 6 hours, with 5 times repetition. The resulted liquid was then directly evaporated, and weighed, we called it as stock samples. Two drops of liquid taken from the stock samples were weighed, then diluted in a water-methanol mixture (9:1 v/v) up to 10 mL in a flask. Before being examined, the samples were priorly filtered using the PTFE membrane. Finally, the samples were examined using the HPLC instrument.

### 3. Results and Discussion

#### 3.1. Optimization of the dose of bionutrient-C213

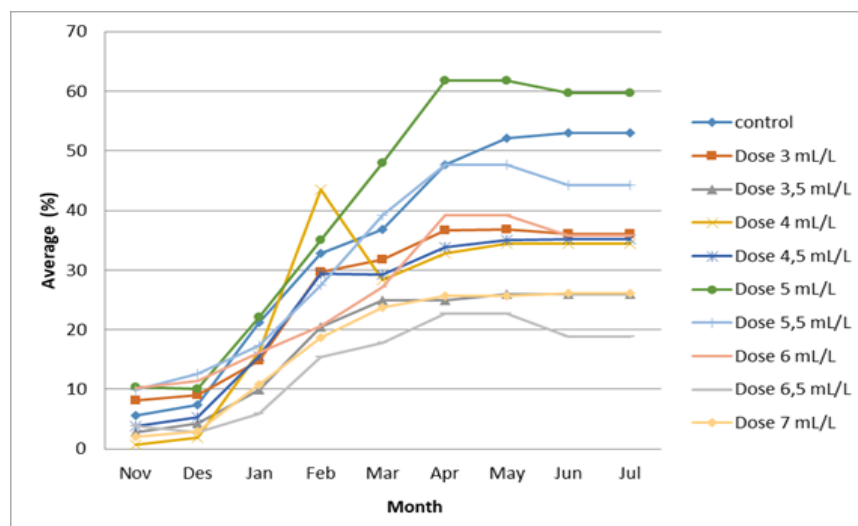
##### 3.1.1. Growth of productive branch

The quality of growth and distribution of nutrition in a coffee plant are indicated by the abundance of fruit in a productive branch. Observations were conducted each week, calculating the productive branch, displayed in the form of a percent. Observations were taken as many as 33 times starting from November until July of the following year, which was subsequently stated percentage of the appearance of the productive branch in all groups for every month. Figure 1 represents the graphs of average of the number of productive branches taken from all ten groups including control group, i.e., the group starting from dose 3 mL/L, 3.5 mL/L, 4 mL/L, 4.5 mL/L, 5 mL/L, 5.5 mL/L, 6 L, mL/6.5 mL/L and 7 mL/L, and control in the period of observation.

As can be seen in Fig. 1, the average of productive branch growth gradually increased with time of application. In November, the percentage of the branch to be around 0.7 to 10%. Then in the following month, the percent of branches slightly increased. Percent of the branch continues to increase in January until March or April, then cease, and arrived in constant condition.

In the first month of observation, the highest growth average was demonstrated by the group treated with Bionutrient-C213 in dose 5 mL/L, i.e., 10.32%, while the lowest was achieved by the group of the dose of 4 mL/L and control, i.e., 0.77%. At the end of the observation period, i.e., in July, the highest productive branch

indicated by dose 5 mL/L i.e., 59.71%, while the lowest was shown by the dose of 6.5 mL/L, i.e., 18.86%. Starting in January, all groups of treatment for all doses of application showed a significant increment of the number average of the productive branch until April. In all periods of observation, the dose of 5 mL/L shows its superiority in productivity among others. The optimum dose for the growth of the productive branch indicated by dose 5 mL/L with the highest number 61.73%, while the control group showed the highest number 53.04%. This data shows that the group treated using Bionutrient-C213 has a higher percentage of branch compared to the control one.

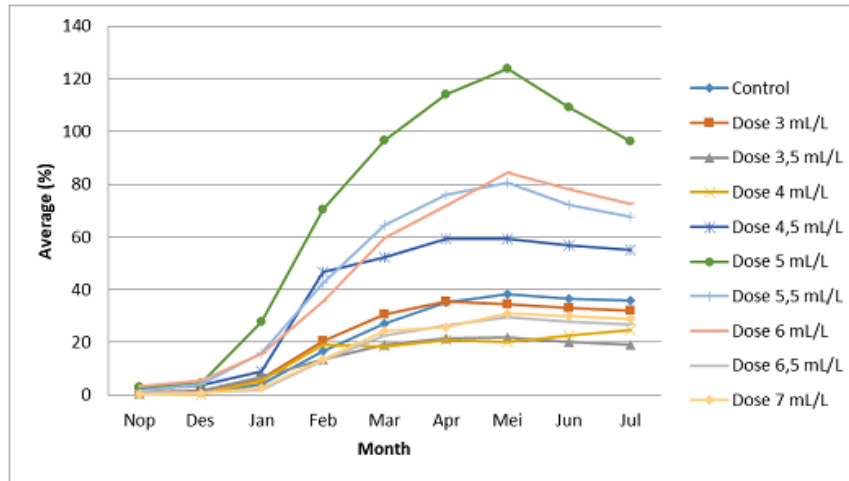


**Fig. 1. Number average of the productive branch during the period of observation.**

### 3.1.2. Bunch in productive branch

The number of the bunch is the number of fruit groups in a productive branch, represented in the number of a bunch that grows on each branch. Observations were conducted each week, calculating the number of the bunch in all branches of the tree in concern. Observations were taken as many as 33 times, starting from November until July in the following year. It was taken the average of the number of the bunch to represent its productivity during the period of observation. Then, it was converted to a percentage, as compared to the whole average number taken for all doses of application and control group, are graphically represented in Fig. 2.

The graph in Fig. 2 shows that the average of the bunch on each productive branch for each tree in their corresponding dose. There is a tendency that in both experimental and control group there is an increase in the average number of bunches. In November, the starting point of growth, the average number of the bunch is very low. In the following month, it is looked likely that the number of the bunch is getting higher. For several doses, the growth is continuously increased until May and reached a constant condition in the following month.



**Fig. 2. The average number of the bunch in each dose within a period of observation.**

We noticed here that the optimum doses for the parameter of the bunch have indicated by dose 5 mL/L, with the highest number is 17.69%, while the control group showed the highest number at 6.85%. This data shows that within the applied doses, the experiment group is more productive than the control one. This result is comparable to the research finding carried out by Araujo et al. [13] about the influence of organic compost and *Crotalaria Juncea* L compost on coffee plant growth. The research results show that the growth of the bunch per branch on a coffee plant rises up to 11.68%, while the control one only reaches to 11.44%.

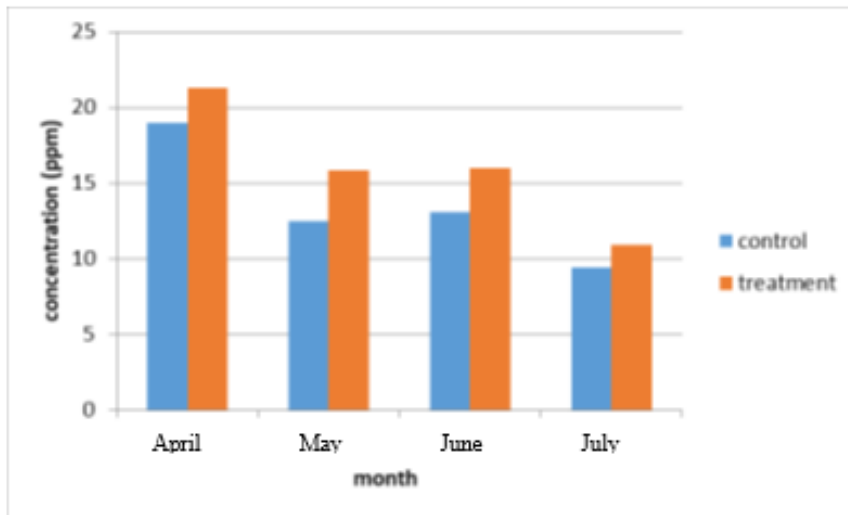
### 3.2. Influence of bionutrient-C213 on chlorophyll and nitrogen level in leaf

**Chlorophyll level.** Analysis of the chlorophyll level was carried out to compare the level of chlorophyll in leaves of treatment and in the control group. We analyzed the content both in the form of chlorophyll a and in chlorophyll b. The sum of the content level of chlorophyll a and chlorophyll b are summarized in Fig. 3.

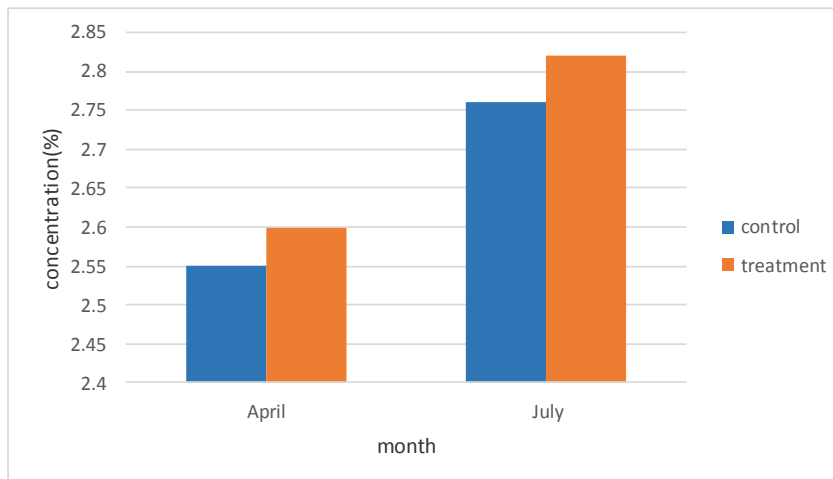
Figure 3 shows the comparison of chlorophyll level in leaves of coffee plant control and treatment. The tree in the treatment group had higher chlorophyll levels compared to control trees. There is a noticeable increase in the chlorophyll level as the result of treatment using Bionutrient-C213. The phenomenon was supposedly caused by the high content of nitrogen in Bionutrient-C213 which was able to increase the synthesis of chlorophyll in the leave of the coffee plant.

**Total Nitrogen Level.** An analysis of the level of N in leaves of the coffee plant was done to compare the level of nitrogen in the tree control and treatment. The result of the analysis of the nitrogen level in the tree control and treatment is presented in Fig. 4.

Figure 4 shows a comparison chart of the nitrogen level in the treatment and control group. The trees in the treatment group have greater nitrogen content compared to that in the control group.



**Fig. 3. Comparison between chlorophyll level in coffee plant leaf taken from the treatment and control group.**



**Fig. 4. Total nitrogen level both in treatment and control coffee plant.**

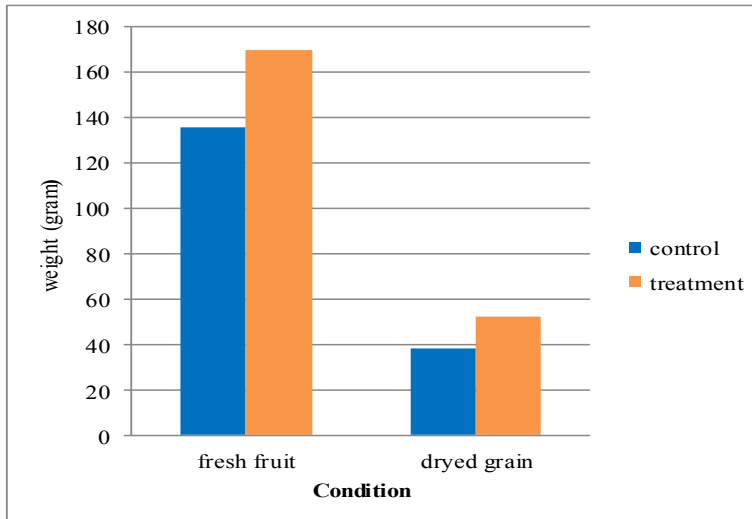
The rate of photosynthesis increase, which is indicated by the level of chlorophyll in the treatment group is greater than that in the control group. The use of chlorophyll for photosynthesis causes chlorophyll to decrease from the beginning of spraying until harvesting season. The phenomenon could be explained that at the time of the development of fruit, photostat in the plant was used for ripening the fruit. In that period, the plant could not be able to form any new shoots.

### 3.3. Influence of bionutrient-C213 on productivity

Harvesting was done four times in 2 weeks. The mass of harvested fruits is presented in Table 1, and the average mass is shown as Fig. 5.

**Table 1. Mass of 100 fresh fruits and mass of 100 dried coffee beans for the experiment group.**

Number of weighing	Mass of fresh fruit (gram)		Mass of dried bean (gram)	
	control	experiment	control	experiment
1	127.3831	170.0937	33.6925	52.4191
2	141.566	168.0285	41.5821	46.9984
3	135.698	176.2616	38.8849	65.4862
4	138.094	164.3247	40.1709	44.8473

**Fig. 5. Mass average of fresh fruit and dried coffee beans in the experiment group.**

As can be seen in Fig. 5, the mass average of fresh fruit and the dried seed of the experiment group is higher than that of control. Based on the result reported in Table 1, Bionutrient-C213 may increase the mass average of fresh fruit of coffee for every 100 grain, namely 169.88 grams  $\pm$  4.99, while that in control is about 135.68  $\pm$  6.03 grams. In addition, the Bionurient-C213 application was able to increase the average mass of dried seed per 100 coffee bean, namely 52.43  $\pm$  9.26 grams, while that in control is 38.58  $\pm$  3.44 grams. This fact could be addressed to the photosynthesis processes. We might suppose that the photosynthesis occurs in such a way to produce carbohydrates (photostat) to be distributed into the fruit. The result is in good agreement with the research of Vaast, et al. [14] that the competition in up-taking carbohydrates may influence the fruit and seed size of coffee.

### 3.4. Influence of bionutrient-C213 on glucose level

Examination of glucose level using the DNS method indicates that the experiment group has as much as 5.95%, while the control is 5.6%. It can be inferred that Bionutrient-C213 was able to increase the content of glucose in coffee by 0.35% compared with the control group.



The taste of coffee is affected by the content of sugars, organic acid and various aromatic compounds. Increase of pH on ripe fruit followed by a decrease in sugar level. The maturity of the fruit can be inferred from the pH level, the more the maturity the lower the pH level. In this research, we found that the pH level of the experiment group is lower than that of the control, evidently 4.53 for the experiment group and 5.82 for the control group.

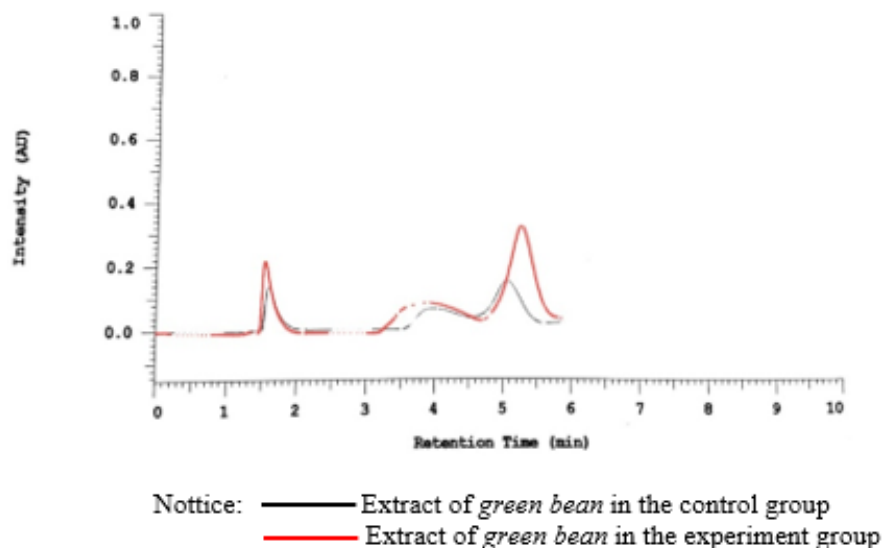
### 3.5. Influence of bionutrient-C213 on caffeine level

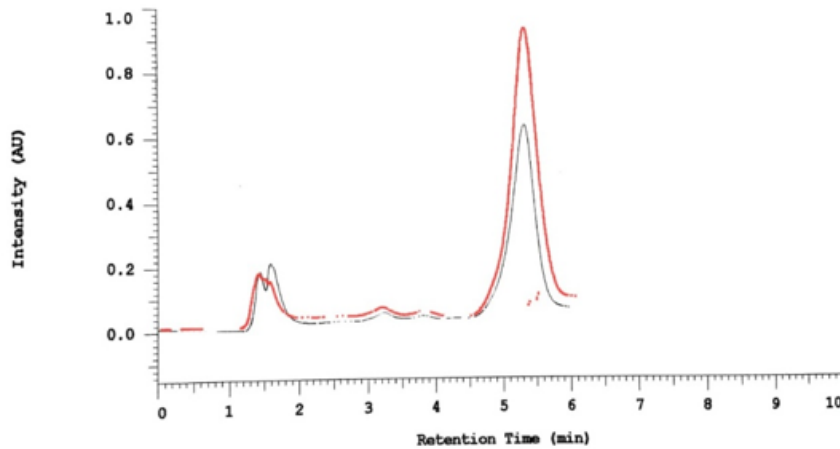
The examination of caffeine level was done on green beans coffee and roasted coffee beans both for the experiment and control group. Caffeine level analysis was performed using the HPLC instrument. The HPLC chromatogram for the green beans is presented in Fig. 6, while the roasted bean is represented in Fig. 7.

It is clear in Fig. 6 that the chromatogram of the extract of coffee green bean in the experiment group is higher than that of in control group. The peak of the chromatogram for the green bean sample on the experiment group exhibited 4059764 while that for the control group is 2033554. A similar result has occurred in roasted coffee beans, as shown in Fig. 7.

Based on the chromatogram in Fig. 7, the extract of roasted coffee bean from the experiment group has a higher chromatogram peak compared to the control group. The peak of chromatogram produced from the roasted seeds in the experiment group is 21930345, while the control is 7682117.

In addition, the level of caffeine in coffee beans resulted from the experiment group is higher than that of the control group. The caffeine level of roasted coffee beans in the experiment group is 3.94%, while the control is 2.79%. The caffeine level of coffee beans for green bean treatment is 2.96%, while the control is 1.46%. The caffeine level in the green bean of the experiment group increase by 102.74%. We may compare the result to the work of Vinecky et al. [15] that showed the increase exhibit 15% after the addition of NPK fertilizer.



**Fig. 6. HPLC Chromatogram of green bean extract.**

Notice: ——— Extract of roasted coffee bean in the control group  
 ——— Extract of roasted coffee bean in the experiment group

**Fig. 7. HPLC Chromatogram of the extract of roasted coffee bean.**

#### 4. Conclusion

It might be concluded that the optimum dose for application the Bionutrient-C213 to the coffee plant is 5 mL/l. The Bionutrient-C213 application on Arabica coffee plant is able to increase the content of chlorophyll and total nitrogen level, increase the mass average of fruit per 100, i.e., for experiment group is  $4.99 \pm 169.88$  grams while for the control is  $135.68 \pm 6.03$  grams, increase the mass average of dried seed per 100, i.e., for experiment is  $52.43 \pm 9.26$  grams while for the control is  $38.58 \pm 3.44$  grams, accelerate the fruit ripening, increased level of glucose as much as 6.25%, and increase level of caffeine, where the caffeine level of roasted coffee beans experiment groups is 3.94% and control is 2.79%, while the caffeine level of green beans coffee in experiment group is 2.96% and control group is 1.46%.

#### Acknowledgment

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