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Short Communications

Effectiveness of Liquid Organic Fertilizer Byproduct of Black Soldier Fly Maggot to the Growth of Mustard Plant (*Brassica juncea* L.)

Laras Nur Janah[1], Anisa Azmi Nurrisky Apriliani[1], Afifah Nur Hidayah[1], Vania Adhelia[1], Sukirno Sukirno[2]*, Budi Setiadi Daryono[3]

1)Undergraduate Students, Faculty of Biology, Universitas Gadjah Mada, Indonesia, 55281
2)Entomology Laboratory, Faculty of Biology, Universitas Gadjah Mada, Indonesia, 55281
3)Genetics and Breeding Laboratory, Faculty of Biology, Universitas Gadjah Mada, Indonesia, 55281
* Corresponding author, email: sukirnobiougm@ugm.ac.id

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ABSTRACT

The need for consumption of mustard plant (*Brassica juncea* L.) has increased every year. One of the efforts to increase its production and quality by applications of inorganic and organic fertilizer. This study aimed to evaluate the productivity of mustard plants treated with liquid organic fertilizer, a by-product of black soldier fly (BSF), and inorganic fertilizer (NPK). Mustard plants were grown at Karanggayam Research Station, Caturtunggal, Depok, Sleman, Yogyakarta were treated with water as a control, NPK fertilizer, DoctoRS organic fertilizer at 0.05%, organic fertilizer A, and B at 0.1, 0.15, 0.20, and 0.25%. The effects of treatments to the phenotypic and the chlorophyll of the mustards were done after 2 weeks of treatments. The results showed that there were significant different on the stem height, number of leaves, leaf width, leaf length, and wet weight. These were in line with the total chlorophyll. Liquid organic fertilizer content analysis showed that DoctoRS liquid organic fertilizer and liquid organic fertilizer A were the most in accordance with the national standards for organic fertilizers on the parameters of pH, Mg, Ca, and TPC.

Indonesia is known as agricultural country. One of crops which mostly cultivated by the community is mustard. Mustard plant (*Brassica juncea* L.) is a crucifer originating from China and East Asia. It was cultivated or 2500 years ago in China then spread widely to the Philippines and Taiwan (Rukmana 2007). The consumption of mustard is increasing with the increase of human population. It has benefit for health because contains protein, fat, carbohydrates, Ca, P, Fe, vitamin A, vitamin B, and vitamin C (Fahrudin 2009). The production of mustard plants decreased by 5.08% to 117.68 quintals/ha from 123.98 quintals/ha in 2013 and the average productivity of mustard plants continues to decline every year in line with the decrease in land area (BPS 2020). Thus, we need to increase the quality and quantity of mustard plant production.

One of the efforts to increase mustard production is by fertilizer applications. There are two types of fertilizers mostly used, namely inorganic fertilizers or chemical fertilizers and organic fertilizers (Zamriyetti

et al. 2019). Farmers mostly depend on the use of inorganic fertilizers for their crops production. In the long term, the use of inorganic fertilizers can cause the decrease of organic matter levels in the soil. It also may damage the soil structure, and increase the environmental pollution. This fertilizer is also quite expensive for farmers (Dewanto et al. 2017). In contrast, organic fertilizers can be used by farmers and are very beneficial for increasing the productivity both in quality and quantity. It also has a role in reducing the pollution as well as improve land quality in a sustainable manner. The use of organic fertilizers for a long term is also able to increase soil fertility and to prevent land degradation (Prasetyo 2014).

Susilo (2009) revealed that pakcoy plant (*Brassica rapa* L.) with the treatment of liquid organic fertilizer (LOF) and watering interval has significant effect on the yields. The fertilizers at 20 ml/L and 6 days interval was the best treatment which produce weight of 190.67 g or equal to 47.5 tons/ha. One of the organic fertilizers that can be used by farmers on horticultural crops is organic fertilizer made from organic household waste. The organic waste can ferment by using BSF to produce liquid organic fertilizer which has been widely developed by rural communities, for example the village community at Gejayan, Condongcatur, Sleman, Yogyakarta, Indonesia. The use of BSF larvae as organic waste processor is very promising, because it accelerates organic waste break down, and the BSF also can be harvested as animal feed alternatives (Firmansyah & Noor 2020).

Furthermore, organic fertilizer has some advantages as cheaper, eco-friendly, and indirectly can help to reduce waste and to support sustainable agriculture (Hartatik et al. 2015). The used of maggot as a component for fertilizer productions are still new among farmers. This research is important to provide information on the effectiveness of the use of liquid organic fertilizer by product of BSF as a ZPT agent for plant, especially mustard plant production.

The research was conducted in September – October 2021 located at Karanggayam Research Station, Depok, Sleman, Yogyakarta Special Region, Indonesia. The LOF properties such as pH, Mg, S, Na, Ca, and bacteria contents were carried out at the Laboratory of Analysis at CV Chem-Mix Pratama, Yogyakarta in October 2021. The analysis of chlorophyll content of mustard leaves after grown under different fertilizer treatments were carried out at the Laboratory of Plant Physiology, Faculty of Biology, UGM. The 0.5 g of fresh leaves for every treatment was homogenized in a homogenizer with 10 mL of acetone 80%. The samples were centrifuged at 10,000 rpm for 15 minutes at 40°C. The 0.5 mL of supernatant was collected and mixed with 4.5 mL of acetone 80%. The mixture was analysed for chlorophyll-a content in a spectrophotometer (Parkin) at a wavelength of 663.2 nm (Sumanta et al. 2014). The chlorophyll content measurements were done triplicates.

There were several steps taken to produce liquid organic fertilizer. First, the rotten fruits or vegetables were periodically put in a digester (20 L volume). Then the BSF maggots were introduced in the digester. This will trigger faster microbial growth so that the produced volatile aroma will initiate wild BSF to lay eggs. Second, organic household waste can be added regularly. Third, after 4 weeks of fermentation, the leachate from the larvae's digestion was harvested then put into a clear bottle with a loosened cap. Fourth, the leachate in the bottle then where exposed under the sun light until it turned brown in colour and there no strong odour. The sunlight exposure of the leachate was done for 12 and 10 months for LOF A and B, respectively. These two LOFs were produced by Gejayan Condongcatur community, whereas, DoctoRS LOF was taken from DoctoRS organic fertilizer production house in Klaten,

Central Java.

Mustard seeds were sought at the Karanggayam Research Station, Depok, Sleman, Yogyakarta. The seeds were planted in a medium which was made from a mixture of soil: goat manure: husks in a ratio of 1:1:1. Planting medium was put on the pot tray for sowing mustard seeds for 14 days. After that, the seedlings were transferred into large polybags (15 cm x 15 cm) using the above-mentioned medium ratio. Mustard seedlings then placed in the field with full sunlight. LOF A and B at 5 different concentrations, namely 0.05, 0.1, 0.15, 0.2, and 0.25% (v/v), distilled H_2O as a control, NPK fertilizer (N:P:K = 16:16:16 Mutiara ®), and DoctoRS LOF at 0.05% as a positive control treatment for LOF were used to evaluate the LOFs quality. All the treatments were done for five replicates. The 14 days old mustards were treated with the weekly above-mentioned treatments up to 35 days after planting.

In this study, the plant height, number of leaves, leaf length, leaf width, plant gross weight, and chlorophyll content were observed after 35 days of planting. These phenotypic data were then analysed using One Way ANOVA which then continued with mean separation using Duncan Multiple Range Test (DMRT at α: 0.05). All the statistical procedures were done using SPSS version 24. The results of the average chemical content of macro and micro elements of DoctoRS, LOFs A, and B in the study were listed in Table 1.

In this study, synthetic fertilizers and organic fertilizer were used. The use of NPK, a synthetic fertilizer, was assumed to be a positive control. NPK fertilizers are commonly used by the community and are easy to find. DoctoRS, LOFs A, and B were made from household waste or fruit residue which is then fermented with the help of BSF larvae. BSF maggot consumes and degrades several organic materials contained in the waste up to 70% (Lalander et al. 2014). This fermentation not only produces liquid organic fertilizers (LOF) and compost but also maggots which can be used as animal feed and biofuels (Gao 2019).

The results of chemicals analysis on the three organic fertilizers showed the average of pH ranging from acidic to alkaline, respectively, namely LOFs A > LOFs DoctoRS > LOFs B. It showed that LOFs A has a higher pH than LOFs DoctoRS and LOFs B. Based on the Ministry of Agriculture Decree No.28/Permentan/SR.130/5/2009 about the organic fertilizer, biological fertilizer and soil rehabilitation, the standard pH for LOF is 4-9. It suggested that that the pH value in LOFs B exceeds the standard and it does not meet the requirements, but both LOFs DoctoRS and LOFs A have met the standard. At the beginning of the fermentation process, the acidity will convert organic matter into organic acids, after that, the changes that occur during the fermentation process will produce nitrogen and ammonia so that it will cause an increase in the pH value (Rukmayanti 2020). The longer the fermentation time does not mean the

Table 1. The content of macro and micro elements of DoctoRS, A, and B organic liquid fertilizers.

No	Parameters	Results		
		DoctoRS	A	B
1	pH	8.74 ± 0.005^{b}	5.3 ± 0.001^{a}	$9.29 \pm 0{,}005^{b}$
2	Magnesium (%)	0.641 ± 0.024^{c}	0.495 ± 0.021^{a}	0.514 ± 0.014^{b}
3	Sulphur (%)	1.133 ± 0.007^{a}	1.274 ± 0.042^{b}	1.574 ± 0.040^{c}
4	Potassium (%)	0.673 ± 0.190^{b}	0.553 ± 0.032^{a}	0.927 ± 0.010^{c}
5	Calcium (%)	1.370 ± 0.006^{a}	1.406 ± 0.027^{b}	1.313 ± 0.006^{a}
6	Bacteria (10^3 CFU/mL)	14 ± 1.00^{a}	20 ± 2.00^{b}	34 ± 1.00^{c}

Note: Numbers (mean ±SE) followed by different letters in the same row mean significant different at α:0.05.

pH value is also increased, because the fermentation process is directly related to micro-organisms (Kusumadewi et al. 2019). The high pH on LOFs B can occur due to various environmental factors that affect the growth of microorganisms such as unstable temperatures, nutrients, or the medium in which bacteria grow (Meriatna et al. 2019).

Magnesium (Mg) in the form of magnesium oxide (MgO) is one of the main minerals in the process for the formation of plant's chlorophyll. Magnesium plays an important role in the process of exchanging phosphate substances, participating in influencing the respiratory process and activating the enzymes transphosphorylase, dehydrogenase, and carboxylase (Amelia et al. 2017). The results showed that on average the content of MgO from the largest to the smallest was LOFs DoctoRS > LOFs A > LOFs B. Based on Ministry of Agriculture Decree, the standard value Mg in organic fertilizers is < 0.63%, which mean that the Mg content in the three liquid organic fertilizers meet the standard. The sulphur content showed that the average sulphur content of the three organic fertilizers from the largest to the smallest was LOFs B > LOFs A > LOFs DoctoRS. Sulphur that is applied to the soil will be converted into H_2SO_4 by microorganisms. S element is an important part of ferredoxin, which is a complex of Fe and S in chloroplasts that is used in carbohydrate catabolism for optimal photosynthate. Furthermore, photosynthate will be translocated to all parts of the plant (Hanifah et al. 2021).

In the potassium analysis, the result showed that the average potassium content from the largest to the smallest was LOFs B > LOFs DoctoRS > LOFs A. Based on the Ministry of Agriculture decree, the standard K_2O value in the organic fertilizer is 3-6%. It revealed that the K_2O content in all liquid organic fertilizers in this study has not met the standard. Potassium is one of the macro nutrients other than P- and C- which is needed by plants. Potassium is needed in increasing plant resistance in the process of osmotic regulation, enzyme catalysis of cellular pH regulation, and ion regulation in cells. During the optimal period of fermentation, generally, the potassium content in liquid fertilizer will increase, due to the activity of microorganisms in the decomposition of organic matter. The activity of microorganisms in the degradation process results in the breaking of the carbon chain in organic matter to be simpler so that there is an increase in the element of potassium in fertilizers. Bacteria produce potassium compounds and use K+ ions in fertilizer raw materials for the benefit of their metabolism so that potassium levels will increase along with the growing bacteria (Widyabudiningsih et al. 2021). It is possible that the decrease in potassium levels occurs due to the cleavage activity of microorganisms. When the microorganisms have reached the equilibrium phase, the longer the fermentation time does not mean the potassium content is also increasing. If the fermentation is continued, the microorganisms will die because the nutrients from the microbes have been reduced, so that in this phase the activity of microorganisms in breaking down organic compounds will decrease and the results will be less potassium (K) levels (Kusumadewi et al. 2019). The result indicated that fermentation period of the used fertilizers was not optimal enough to support the activity of microorganisms in the decomposition of organic compounds into potassium.

In the calcium oxide analysis, the results showed that the average of calcium oxide content in the three organic fertilizers from the largest to the smallest was LOFs A > LOFs DoctoRS > LOFs B. Based on Ministry of Agriculture decree, the standard value of Ca is < 25.49% so that the Ca content in the liquid organic fertilizers studied has met the requirements. Calcium oxide or lime is known as a source of ameliorant material used in improving soil fertility. Calcium oxide is a contributor to

Ca^{2+} which is an indispensable nutrient in plant growth as well as its function in neutralizing acidic compounds (Fauzi et al. 2020). Plants absorb calcium in the form of Ca^{2+}. Ca^{2+} plays a role in the formation of the structure and permeability of cell membranes. Deficiency of this element cause disruption of plant growth at the root growth points and storage networks (Tehubijuluw et al. 2014).

The presence of pathogenic bacteria in fertilizers also needs careful observation. This study used the total plate count (TPC) methods to detect the number of microbes in liquid organic fertilizer. Based on Ministry of Agriculture decree, the standard number of bacteria contained in LOFs is $< 10^2$ MPN/mL, while in this study, the number of bacteria found exceeds that standard so that this LOFs does not meet the standard. The high bacteria content can cause microbial foodborne disease. Therefore, it is necessary to carry out further assessment of these risk factor to identify the possible impact of contamination in the production area (Sarmiento et al. 2014).

The results in Table 2 and Figure 1 showed that the application of inorganic fertilizers and liquid organic fertilizers with different concentrations had significant effects on stem length, number of leaves, leaf length, leaf width, and plant wet weight. It gave different growth responses because plants obtained different amounts of macronutrients and micronutrients. In stem length, it was known that treatment with 0.25% LOF A had the best value compared to other treatments. In the number of leaves, it was known that treatment with LOF A at the concentration of 0.15% has the best value compared to other treatments. Meanwhile, on leaf length, leaf width, and wet weight, it was known that treatment with NPK fertilizer had the best value compared to other treatments (Makmur & Magfirah 2018).

The length and width of the leaves influence the metabolism of mustard plants, especially in the process of photosynthesis. The more levels of fertilizer the more stimulation of the metabolic processes of cells, especially in the meristematic tissue at the point of leaf growth. Components in fertilizers such as P, K, and N may stimulate the plant growth (Oviyanti et al. 2016). In addition, the wet weight data obtained in the NPK fertilizer treatment showed that it has the highest. The rapid

Table 2. The effects of organic liquid fertilizers to the growth of mustard plants.

Treatment	Stem Length (cm)	Number of Leaves (cm)	Leaf length (cm)	Leaf Width (cm)	Wet Weight (g)
Control	3.2 ± 0.25^{abc}	7.6 ± 0.50^{a}	11.8 ± 0.90^{ab}	7.72 ± 1.93^{a}	31.8 ± 1.77^{abc}
NPK fertilizer	2.7 ± 0.30^{ab}	9.6 ± 0.40^{bc}	13.1 ± 0.30^{b}	10.2 ± 0.33^{b}	44.2 ± 3.0^{abc}
DoctoRS	2.4 ± 0.36^{a}	9.0 ± 0.44^{abc}	10.92 ± 0.62^{a}	8.22 ± 0.50^{ab}	35.0 ± 3.98^{abc}
0.05% OLF A	3.9 ± 0.33^{c}	8.6 ± 0.67^{abc}	12.0 ± 0.41^{ab}	9.9 ± 0.30^{ab}	36.8 ± 3.67^{bc}
0.1% OLF A	2.9 ± 0.18^{abc}	10.0 ± 0.77^{c}	11.02 ± 0.40^{a}	8.86 ± 0.10^{ab}	34.2 ± 1.71^{abc}
0.15% OLF A	3.2 ± 0.43^{abc}	10.2 ± 0.48^{c}	11.64 ± 0.59^{ab}	9.24 ± 0.27^{ab}	38.0 ± 3.96^{c}
0.20% OLF A	3.02 ± 0.15^{abc}	10.0 ± 0.44^{c}	12.26 ± 0.67^{ab}	9.84 ± 0.49^{ab}	34.8 ± 1.46^{abc}
0.25% OLF A	3.9 ± 0.29^{c}	9.2 ± 0.37^{abc}	12.16 ± 0.68^{ab}	9.2 ± 0.38^{ab}	33.0 ± 2.19^{abc}
0.05% OLF B	3.3 ± 0.30^{abc}	7.8 ± 0.48^{ab}	11.1 ± 0.48^{a}	8.34 ± 0.49^{ab}	26.4 ± 2.89^{a}
0.1% OLF B	3.5 ± 0.44^{bc}	8.6 ± 0.50^{abc}	10.5 ± 0.45^{a}	8.4 ± 0.29^{ab}	31.0 ± 1.78^{abc}
0.15% OLF B	3.2 ± 0.25^{abc}	9.8 ± 0.66^{c}	10.84 ± 0.66^{a}	9.36 ± 0.46^{ab}	28.0 ± 3.56^{ab}
0.20% OLF B	3.3 ± 0.12^{abc}	9.2 ± 0.58^{abc}	10.5 ± 0.14^{a}	8.1 ± 0.13^{ab}	29.0 ± 1.30^{abc}
0.25% OLF B	3.1 ± 0.36^{abc}	10.0 ± 0.83^{c}	10.82 ± 0.49^{a}	9.06 ± 0.66^{ab}	31.4 ± 2.15^{abc}

Note: Numbers (mean ±SE) followed by different letters in the same column mean significant different at α:0.05.

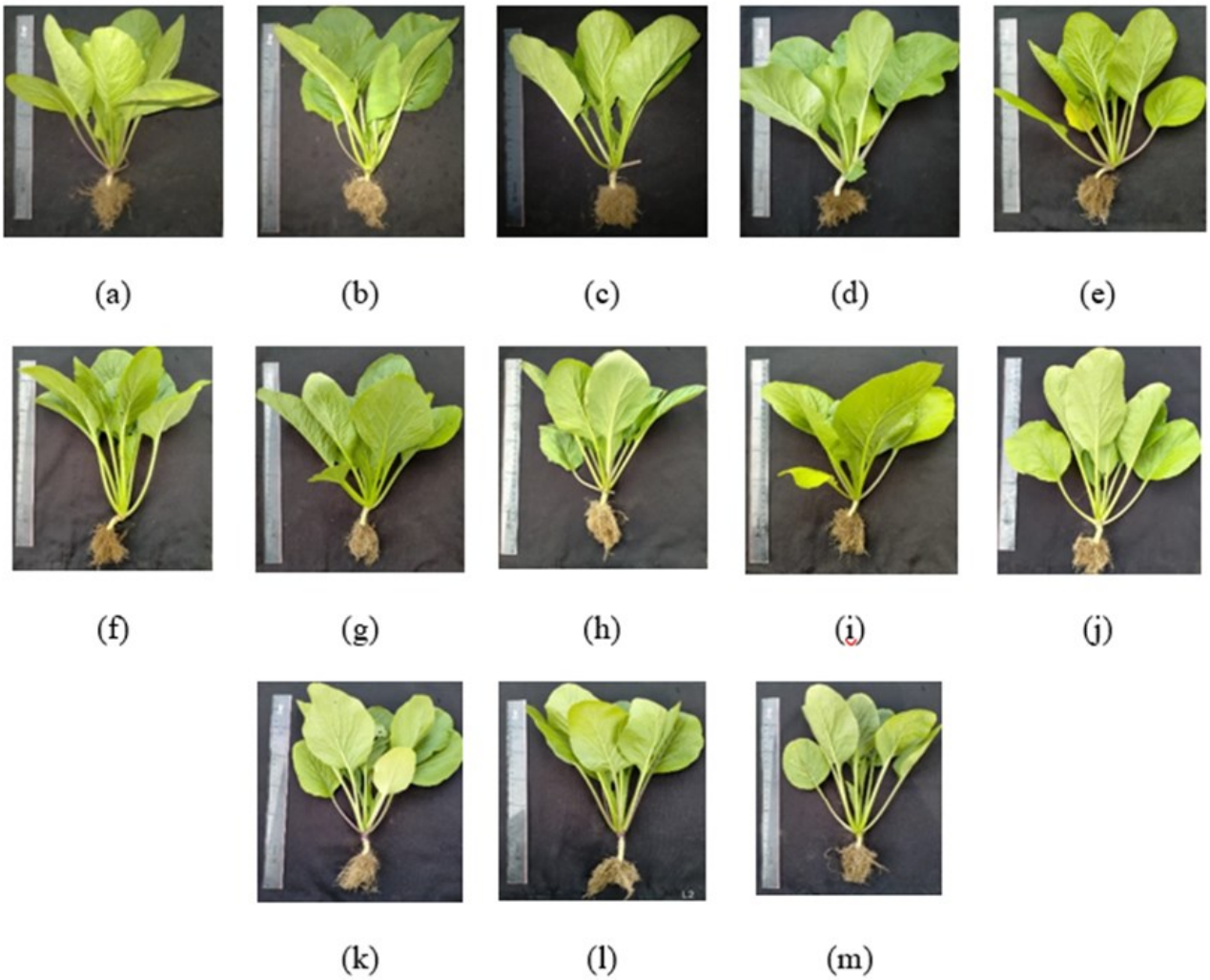

Figure 1. The phenotypes of mustard plants on treatments: (a) control; (b) NPK fertilizer; (c) DoctoRS; (d) 0.05% OLF A; (e) 0.1% OLF A; (f) 0.15% OLF A; (g) 0.20% OLF A; (h) 0.25% OLF A; (i) 0.05% OLF B; (j) 0.1% OLF B; (k) 0.15% OLF B; (l) 0.20% OLF B; (m) 0.25% OLF B.

growth of roots and leaves causes optimal absorption of nutrients, water, and light for a more optimal photosynthesis process. It resulting in high assimilate, thus showing fast growth. In addition, the higher plant height and leaf area, the higher the fresh weight of the plant (Prasetya 2009).

Figure 2 showed that the highest chlorophyll content of mustard plant to the lowest in a row, namely plants with a treatment of NPK fertilizer >0.25% LOF A >0.15% LOF A > DoctoRS LOF >control >0.05% LOF A >0.15% LOF B >0.05% LOF A >0.20% LOF A > 0.1% LOF B > 0.20% LOF B > 0.1% LOF A >0.25% LOF B. There were four treatments which had greater chlorophyll content (NPK, 0.15% LOF A, 0.25% LOF A, and DoctoRS LOF) than control. This is in line with the morphological data obtained, showing that these treatments had significant effect on the plant growth. The treatment of NPK fertilizer has the highest chlorophyll content of 18.46 mg/L. Furthermore, fertilizer treatment is thought to have an influence on the content of chlorophyll as a bio booster or growing regulatory substance or phytohormones. Other factors, that may affect the chlorophyll content are availability of nutrients in the media (Manurung et al. 2020). Photosynthesis occurs in chloroplasts where there is a chlorophyll pigment, especially in the thylakoid section. Photosynthesis occurs in 2 stages, namely light reactions and dark reactions (Urry et al. 2017). The process of photosynthesis can be influenced by several factors, both internal factors, such as chlorophyll

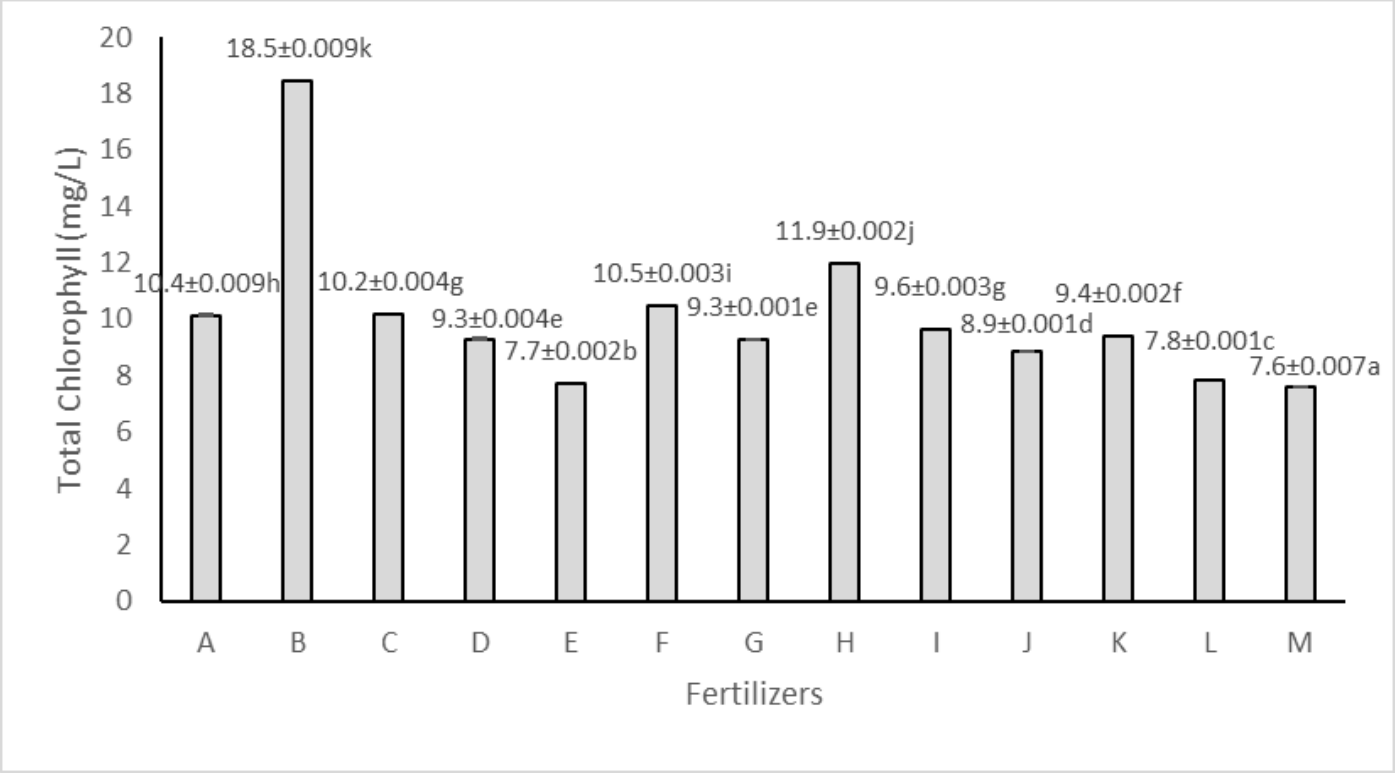

Figure 2. Chlorophyll content of mustard plant with treatment (a) control; (B) NPK fertilizer; (C) DoctoRS LOF; (D) (D) 0.05% LOF A; (E) 0.1% LOF A; (F) 0.15% LOF A; (G) 0.20% LOF A; (H) 0.25% LOF A; (I) 0.05% LOF B; (J) 0.1% LOF B; (K) 0.15% LOF B; (L) 0.20% LOF B; (M) 0.25% LOF B. (Numbers (mean ± SE) followed by different letters mean significant different at α:0.05).

contents and number of stomata, and external factors, such as temperature (Ferdous et al. 2017). In addition, the nitrogen is one of the main components of chlorophyll, which is about 60%. Nitrogen is one of the components in protein molecules, purines, pyrimidines, and porphyrins. Porphyrins are important in the formation of chlorophyll. Nitrogen is catalysed by the enzyme glutamine synthetase into glutamic acid which functions as a porphyrin ring precursor for the formation of chlorophyll (Fadilah et al. 2020).

The results of the study proved that the application of a combination of organic and inorganic fertilizers can improve physical, chemical, and biological properties. Madusari et al. (2021) revealed that the administration of LOF fermented maggot showed a positive increase in the growth of oil palm seedlings, especially in plant height and root length. But, it did not show significant differences on the morphological and physiological of oil palm plants. Although the application of LOF has potential to increase crop production, the application of inorganic fertilizers (N, P, and K) sometimes still needed (Wasito & Tedjasarwana 2003; Hanifah et al. 2021) to support the growth and production. These results conclusively showing that in order to have high crop productivity we should have balanced fertilizers application.

AUTHORS CONTRIBUTION

L.N.J. design the research and supervised the processes, A.A.N.A analysed the research data, A.N.H collected the data samples, V.A wrote the initial manuscript. S.S. manage the research funding, design and supervise the experiment analysis. SS and B.S.D. critically reviewed, revised, and proofread the final manuscript.

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CONFLICT OF INTEREST

There is no conflict of interest regarding the research or the research funding.

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