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MAKING LIQUID ORGANIC FERTILIZER FROM BLACK SOLDIER FLY LARVA LEACHATE USING A VARIETY OF CANTEEN WASTE

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ABSTRACT

This research aims to develop a POC using BSF larvae leachate and bioactivators as a preliminary study for producing POC using a fermenter in the form of a biodigester. The POC production process involves inserting BSF waste larvae (kasgot) and water into the biodigester at a 1:1 ratio and adding the EM-4 bioactivator; in other variations, only local microorganisms found in kasgot are used. BSF larvae were fed in two variations: vegetable waste and IT Del canteen mixed waste. POC was produced in a biodigester for 28 days. This research analysed pH, temperature, and NPK levels. The results show that POC meeting SNI quality standards was obtained from vegetable waste samples, with a final pH of 5.02 and N content of 0.2315%, P of 0.4031%, and K of 0.4929%. The bioactivator used during fermentation of BSF larval leachate influenced the quality of the resulting liquid organic fertiliser. The EM-4 + molasses + leachate treatment yielded the best quality, achieving the highest total NPK content (0.66%) after 15 days of fermentation. The best treatment for plant growth was the liquid organic fertiliser produced after 12 days of EM-4 fermentation, which produced an average plant height of 11.9 cm.

INTRODUCTION

Indonesia is an agrarian country, where most Indonesians depend on agriculture to meet their daily needs. During cultivation, plants also require fertilization. Fertilization is the process of providing nutrients to plants to ensure their survival. However, agricultural yields decline every year, making the current state of Indonesian agriculture quite concerning.

During fertiliser scarcity, farmers face many challenges and must use expensive non-subsidised fertilizers, which increases operational costs and reduces income. To address this issue, researchers are developing innovations that can help alleviate the scarcity of subsidised fertilizers. One innovation is creating a POC using inexpensive, readily available raw materials (Syarif et al., 2022).

POC offers several advantages over chemical inorganic fertilizers, including easier absorption by plant roots, higher crop yields, better crop product quality, reduced use of inorganic fertilisers, and the ability to address nutrient imbalances more quickly. Additionally, POC can be distributed more evenly and adjusted more easily to meet plant needs. However, because the community has a poor understanding of the benefits and process of making organic fertilizer, they are reluctant to use it (Junaidi et al., 2022).

New raw materials have potential for the community to use to create POC. This potential raw material can be obtained by utilising black soldier fly (BSF), commonly called BSF larvae. One study by Suwirmen et al. (2022) showed that BSF larvae can break down organic waste by extracting energy and nutrients from it. Black Soldier Flies are a group of insects with a high growth rate, short life cycle, and high reproductive capacity. These flies live for 5 to 8 days and are approximately 15-20 mm long (Monita et al., 2017).

BSF can reduce organic waste by 47.75%, from 68,321 kg to 32,623 kg in 36 days (Suwirmen et al., 2022). When larvae reach their maximum size, they have a protein content of 36–48% and a fat content of 33%. BSF larvae develop into adults in approximately 14 days (Holmes, L., Vanlaerhoben, S., & Tomberlin, 2012). Waste can also be used as organic fertilizer for plants because of its high nutrient content and environmental friendliness. This does not affect crop productivity or quality, making it an ideal substitute for inorganic fertilizers. Using BSF larval feed leachate is one alternative that reduces environmental pollution and farmers' need for fertilizer. BSF larval feed leachate comes from the solution of organic matter decomposed by BSF. BSF larval feed leachate contains natural nutrients that have been broken down, making it easier for plants to absorb and increasing their growth (Junaidi et al., 2022).

The combination of liquid organic degradation products and processed BSF larvae can affect chilli plant growth and yield. Combining soil and compost with 10% POC can increase vegetative growth and chilli production more than using NPK at the optimal dose. Based on the above description, research is needed on raw materials for making POC that are neither detrimental nor damaging to the environment. BSF larvae can potentially be used as a raw material for POC, and research is needed on using BSF larvae, with their leachate as POC. This study aims to determine the best feeding regimen for producing BSF larvae leachate as POC and to understand the effect of adding the bioactivator EM-4 on the resulting POC. In addition, this study also identified the microorganisms contained in the produced POC.

MATERIALS AND METHODS

Material

The main equipment for making POC is a biodigester-shaped fermentor measuring 38 cm high and 22 cm in diameter, with a total capacity of 5 L, as shown in Figure 1.

Figure 1. Biodigester used for fermentation

Methods

Preparation of BSF Larvae and Leachate

First, BSF larvae were placed in two containers. Then, the BSF larvae were fed different types of feed. In the first container, vegetable waste from the canteen was provided, and in the second container, leftover food waste from the IT Del canteen was provided. 1 kg of feed was given once every 3 days for 30 days. After that, BSF larval manure was collected at the end of the harvesting period to be used as leachate water for POC.

Preparation of BSF Larvae and Leachate

EM-4, which was prepared previously, was taken in an amount of 20 mL and added to the fermentation container (biodigester) containing BSF larvae manure and water in a 1:1 ratio. The mixture was then stirred until the EM-4, water, and BSF larvae manure were homogeneous. The fermentation process was then carried out for 28 days under non-obligate anaerobic conditions, with a total volume of 3 litres.

Fermentation Process without using EM-4

BSF larvae manure and water in a 1:1 ratio were put into the biodigester. Then, the water and manure were mixed, and the mixture was fermented for 28 days to a total volume of 2.5 litres.

Identification of Microbial Count

The BSF larvae leachate fermented for 28 days was first filtered and then identified by counting bacteria. The bacterial count was performed using the pour plate method, where 1 mL of the sample to be tested was transferred with a sterile pipette into a 9 mL solution of distilled water to achieve a 10-1 dilution. The same procedure was repeated for dilutions of 10-2, 10-3, and 10-4. Finally, 1 mL (culture medium) from each dilution was suspended and inoculated onto Petri dishes containing previously prepared NA agar medium.

Identification of Microbial Species

The isolated bacteria were identified through morphological tests. Gram staining was performed in the morphological tests, while catalase, motility, and carbohydrate fermentation tests were conducted in the biochemical tests. At this stage, the type of isolated microorganism can be determined.

Sample Testing

Temperature Measurement

Temperature measurements were taken for each fermentation variation using a mercury thermometer. Measurements were taken twice a day, at 8 am and 4 pm. The thermometer was inserted into the middle of the POC sample, and the device displayed the fermentation temperature. The temperature measurement results were used as information to determine the condition of the fermentation process.

pH Measurement

pH measurements were taken for each compost variation using a pH meter. Measurements were taken once a day in the morning. The pH meter was inserted into the POC sample, and the pH result was displayed on the monitor.

Addition of NaOH

2% NaOH was added to each POC sample as a precautionary step in case the 28-day fermentation resulted in a pH that did not meet SNI standards. Initially, take a sufficient amount of POC sample and measure its pH using a pH meter. Then, slowly add 2% NaOH solution to the POC while stirring continuously. Then, add the solution gradually and measure the pH after each addition. Repeat step 3 until the POC reaches the desired pH. Once the pH is reached, homogenise the solution by stirring thoroughly.

RESULTS AND DISCUSSION

Kasgot Results

In this study, maggots were fed for 30 days until they reached the pupal stage. The type of feed given to the maggots had two variations: mixed feed (student mixed waste) and vegetable waste (Table 1).

Table 1. Final Weight of Kasgot

Container + Mixed Waste Compost	Container + Vegetable Waste Compost
1095,8 gram	778,9 gram

Based on Table 1, the final weight data for the harvested cassava peels have been obtained. The weight produced by each feed variation is as follows: for mixed waste, 1095.8 grams, and for vegetable waste, 778.9 grams.

Measurement of POC pH levels during the Fermentation process

This research examined the pH conditions of mixed waste POC and vegetable waste, with and without EM-4, during the fermentation process. The fermentation period was 28 days.

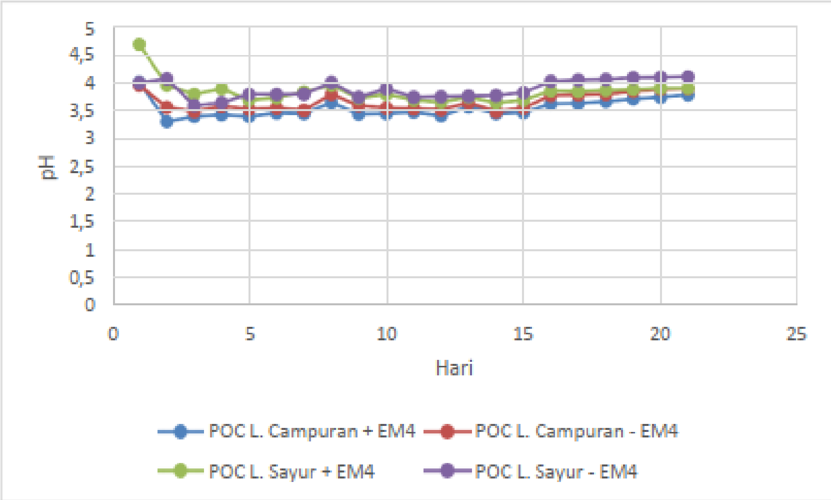

Figure 2. Graph of the POC pH levels during the fermentation process

Based on Figure 2, we obtained data on the pH content in POC during the fermentation process. The pH measurement results for POC during fermentation showed an initial decrease. The graph indicates that the pH of POC at the beginning of fermentation (day 1) ranged from 4.01 to 4.68, and on day 21, it ranged from 3.79 to 4.11.

Measurement of POC pH level with NaOH addition

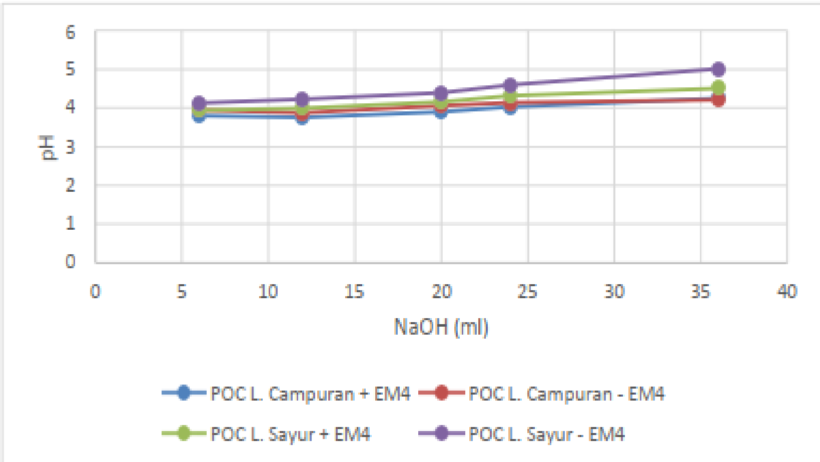

Figure 3. Graph of the POC pH levels with NaOH addition

This research examined the subsequent pH conditions of mixed waste and vegetable waste with and without EM-4. Before adding 2% NaOH, the results showed that the desired standard pH was not achieved. The amount of NaOH added varied at 6 mL, 12 mL, 20 mL, 24 mL, and 36 mL, added on day 28.

Based on Figure 3, adding 6 mL of NaOH produced a pH range of 3.8 to 4.13. With the addition of 12 mL, it ranged from 3.76 to 4.23. With the addition of 20 mL of NaOH, it ranged from 3.9 to 4.4. With the addition of 24 mL, it ranged from 4.03 to 4.06, and with the addition of 36 mL, it ranged from 4.25 to 5.02. This pH value has already met the SNI standard.

Identification of microbial count in POC

This research was conducted to determine the number of microorganisms contained in each variation of POC with different types and treatments, and also to compare the number of POC microorganisms using EM-4 and those without EM-4 isolated onto NA media.

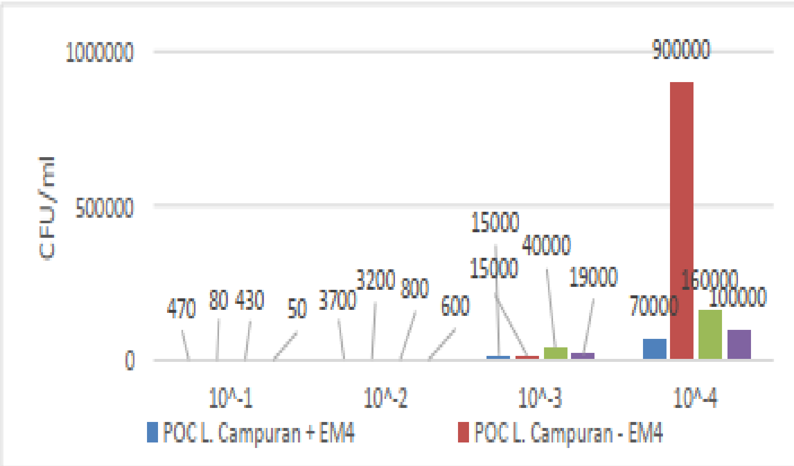

Figure 4. Graph of the number of microbial count in POC sample colonies

Based on Figure 4, it can be observed that the highest cell counts at dilutions of 10-1 and 10-2 were obtained in POC produced using mixed waste as BSF larval feed and with the addition of EM-4, while at a dilution of 10-3, the highest cell count was observed in POC produced using vegetable waste as feed with the addition of EM-4, and at a dilution of 10-4, the highest cell count was observed in POC produced using mixed waste without the addition of EM-4.

Measurement of POC Temperature in the Fermentation Process

Temperature measurements were taken for each fermentation variation using a mercury thermometer. The temperature measurement results were used as information to determine the condition of the fermentation process. Based on the research conducted, the following data were obtained.

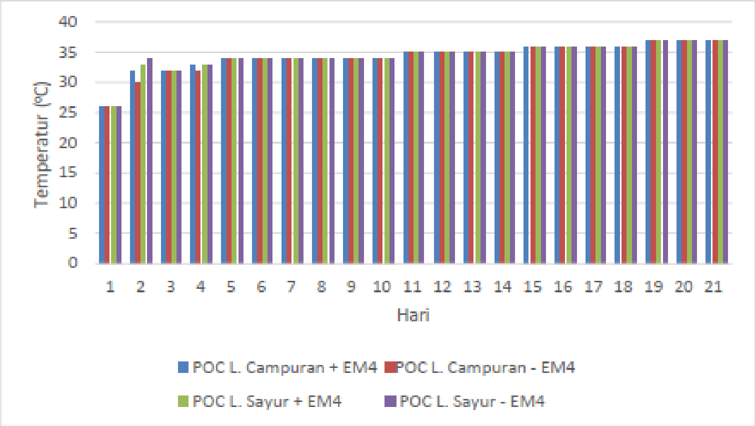

Figure 5. Graph of POC temperature during fermentation process

Based on Figure 5, we obtained the POC temperature observation results over 21 days of fermentation. Based on the data, the fermentation temperature of POC over 21 days ranged from 30°C to 37°C.

Identification of Microbial Species in POC

Gram Staining

Based on the Gram staining results, the POC sample was dominated by rod-shaped, Gram-negative bacteria that appeared pink. At the same time, other samples also contained Gram-positive bacteria characterised by small, blue or purple spheres. The following are the Gram staining results obtained.

Table 2. Gram Staining Results

Sample Name	**Gram Positive (+) or Gram Negative (-)**
EM-4 + Mixed Waste	+ and -
Mixed Waste	+ and -
EM 4 + Vegetable Waste	-
Vegetable Waste	-

Motility Test

The researchers inoculated bacteria into semi-solid media, such as Sulfide Indole Motility (SIM) medium. SIM medium contains peptone, agar, mineral salts, a pH indicator, and reagents for the indole and hydrogen sulfide tests. A positive result is indicated by the spread of microbes throughout the medium. A negative result is indicated by bacterial growth only in the puncture area. Here are the results of the motility test conducted.

Table 3. Motility Test Results

Sample Name	Result
EM-4 + Mixed Waste	+
Mixed Waste	-
EM 4 + Vegetable Waste	+
Vegetable Waste	+

Carbohydrate Fermatation

In the fermentation test, microbial enzymes dissolve the medium containing simple carbohydrate compounds (sugars), producing organic acids that lower pH and may produce CO2 gas. A positive result in this study is indicated by air bubbles in the Durham tube. Here are the results of the carbohydrate fermentation research.

Table 5. Results of Carbohydrate Fermentation Test

Sample Name	Lactose Test	Glucose Test	Sucrose Test
EM-4 + Mixed Waste	-	+	+
Mixed Waste	-	-	-
EM 4 + Vegetable Waste	-	+	-
Vegetable Waste	-	-	+

NPK Test

To analyse the NPK content, we sent the liquid organic fertiliser sample to PT Sucofindo. The NPK analysis results are listed in the following table.

Table 6. NPK Test Results

Name	Sample	Result Parameter
EM-4 + Mixed Waste	N	0,2760 %
	K_2O	0,3311 %
	P_2O_5	0,1459 %
Mixed Waste	N	0,4357 %
	K_2O	0,3648 %
	P_2O_5	0,2125%
EM 4 + Vegetable Waste	N	0,1401%
	K_2O	0,4603%
	P_2O_5	0,3490%
Vegetable Waste	N	0,2315%
	K_2O	0,4929%
	P_2O_5	0,4031%

Kasgot Results

Based on the data obtained, the greater mass of kasgot indicates that BSF larvae consumed more organic matter from the mixed waste. Therefore, liquid organic fertilizer made from kasgot from mixed waste will contain more nutrients needed by plants (Putri, 2020). The greater mass of kasgot indicates that BSF larvae consumed more organic matter from the mixed waste. Kasgot from mixed waste has a higher nitrogen content than kasgot from vegetable waste, as shown in the NPK test results (Table 6). At harvest, the yield of BSF casgot from vegetable waste was lower than that from mixed waste (28.9%). This occurred because BSF larvae did not have enough energy to process vegetables due to their larger size compared with mixed waste, which contained smaller pieces of rice (Agustin et al., 2023).

Measurement of pH Levels in POC

The research results on pH measurement of POC during the fermentation process showed a decrease on the first day of fermentation. This is caused by microorganisms that produce organic acids during fermentation. Over time, the pH of POC gradually increased. On day 21, the pH of POC ranged from 3.79 to 4.11. This increase in pH is caused by the decomposition of organic acids by other microorganisms. According to Rukmayanti (2017), the pH of the first POC initially decreases during fermentation before gradually rising to a stable level. Acid-producing microbes, such as lactic acid and acetic acid, cause the pH to drop at the beginning of fermentation. These organic acids lower the pH of the fermentation medium. Other factors that cause the pH of POC to decrease include the addition of EM-4.

Measurement of POC pH with NaOH addition

Fermentation for 21 days is a crucial step in producing nutrient-rich liquid organic fertilizer (POC). However, after fermentation is complete, the pH does not reach the standard SNI-compliant range for POC (4-6), so adding NaOH plays an important role in raising the pH to the required level. According to Nuraini et al. (2018), the addition of NaOH to POC can also improve the quality and stability of POC because the addition of NaOH can increase nutrient content, microbial activity, stability, and plant absorption capacity. However, excessive NaOH can negatively affect POC quality. Therefore, it is important to determine the appropriate NaOH concentration for each type of POC.

Based on the research conducted, adding NaOH significantly increased the pH of mixed waste POC + EM-4, mixed waste POC – EM-4, and vegetable waste POC + EM-4. After adding 20 mL of NaOH, the pH of POC became 4. Adding 24 mL and 36 mL of NaOH was appropriate to meet the POC pH according to government standards. At a volume of 24 mL, the pH obtained was 4.03-4.06, and at an addition of 36 mL, the pH obtained was 4.25 – 5.02. Microbes involved in the fermentation process can be harmed if the pH is too low, and POC becomes useless if the pH is too high (Evanuarini, 2017).

Identification of Microbial Count in POC

Based on the data obtained, it can be seen that the number of colonies obtained from POC (mixture + EM-4) is the highest, namely 470 colonies at a dilution of 10-1, 3700 colonies at a dilution of 10-2, 15000 colonies at a dilution of 10-3, and 70000 colonies at a dilution of 10-4. This high number of colonies indicates that POC has very high microbial activity. According to research conducted by Simatupang (2018), the number of bacterial colonies in POC made from Cymodocea serrulata litter with the EM-4 bioactivator ranged from 3.7 x 105 CFU/mL to 7.2 x 105 CFU/mL.

The number of colonies is lower than the number of colonies obtained from Mixed POC + EM-4, Mixed POC - EM-4, and Vegetable POC + EM-4, but higher than Vegetable POC - EM-4, indicating that microbial activity and its potential application as a strong biofertilizer are found in vegetable waste POC without EM-4. In Edy's (2019) research, POC from cassava peels at concentrations of 50% and 100% produced a high number of bacterial colonies (108 CFU/ml) and significantly improved the growth of corn plants compared to the control without POC.

The results indicate a positive correlation between the number of microorganisms and POC quality. More colonies of microorganisms indicate higher nutrient and plant hormone content in the POC, which significantly improves plant growth and production compared to controls without POC.

Measurement of POC Temperature in the Fermentation Process

Based on the data obtained, the anaerobic fermentation temperature of POC for 21 days ranged from 30°C to 37°C. This temperature falls within the mesophilic range, which is optimal for the growth of anaerobic microorganisms that produce POC. The research results are supported by a study by Darnah (2023), which found that the optimal temperature for anaerobic fermentation of POC from livestock liquid waste is 37°C. At this temperature, the best-quality POC was obtained: greenish-yellow in colour, with a characteristic fermentation odour and white spots.

Temperature plays a very important role during anaerobic fermentation. At 30°C, anaerobic microorganisms are inhibited, resulting in slower fermentation and reduced POC productivity. At 40°C or higher, anaerobic microorganisms can die, causing the fermentation process to stop and POC quality to decrease (Rahmawati, 2017).

Identification of Microbial Species

Gram Staining

Mixed waste samples with and without EM-4 showed Gram-negative and Gram-positive bacteria. Vegetable waste samples, with and without EM-4, showed only Gram-negative bacteria. These results are supported by Dewi (2016), who identified the most common Gram-negative and Gram-positive bacteria in food waste, and by Mitta (2014), who identified the most common Gram-negative bacteria in vegetable waste. Based on the Gram staining results and comparison with other studies, it can be concluded that the most common bacteria found in food and vegetable waste are Gram-negative and Gram-positive.

Motility Test

The EM-4 sample + mixed waste showed a positive result (+). This indicates that the bacteria in this sample have flagella, organelles that allow them to move, suggesting that the bacteria are motile. EM-4 can increase the motility of Bacillus subtilis because EM-4 contains plant hormones and micronutrients that can help bacteria grow and develop. The mixed waste sample showed a positive result (+). This result is consistent with other research showing that mixed waste can contain various types of bacteria, including motile bacteria. A study conducted by Arifin et al. (2018) found that mixed waste from the palm oil processing industry contains various types of bacteria. This may be due to the high amount of organic matter in the mixed waste, which can serve as food for bacteria. The EM-4 + vegetable waste sample showed a positive result (+).

Other studies have shown that EM-4 can increase bacterial motility in vegetable waste. Sari et al. (2019) found that using EM-4 can significantly improve the ability of E. coli to absorb vegetable waste. This may be because EM-4 contains plant hormones and micronutrients that can support bacterial growth and development. The vegetable waste sample showed a positive result (+). Other research shows that vegetable waste can host many types of bacteria, including motile bacteria.

Catalase Test

The EM-4 compost sample + mixed waste showed positive results. The mixed waste compost sample showed negative results. The EM-4 compost sample + vegetable waste showed positive results. The vegetable waste compost sample showed positive results. A positive result indicates that the waste contains the catalase enzyme, which can break down hydrogen peroxide (H2O2) into water (H2O) and oxygen (O2). This ability is useful for reducing H2O2 levels in waste, which can be toxic to microorganisms. Negative test results indicate the absence of catalase. This means that H2O2 cannot be naturally decomposed in mixed waste. This could indicate that the microorganism cannot protect itself from damage caused by H2O2.

The results indicate that EM-4 can affect catalase enzyme activity in the sample. Adding EM-4 to mixed waste and vegetable waste samples can increase catalase enzyme activity, while adding EM-4 to POC can increase overall catalase enzyme activity, as found in the study by Singh et al. (2017), who discovered that adding EM-4 to compost can increase catalase enzyme activity. POC containing EM-4 can enhance catalase enzyme activity in plants grown in contaminated soil (Wang et al., 2018). The catalase test is an effective method for measuring catalase activity in various samples. The results indicate that EM-4 can affect catalase activity. In mixed waste, vegetable waste, and compost samples.

Carbohydrate Fermentation

Carbohydrate fermentation tests showed that microorganisms in the EM-4 + mixed waste, EM-4 + vegetable waste, and vegetable waste samples had different abilities to ferment sugars. Microorganisms in EM-4 + mixed waste could ferment glucose and sucrose but could not ferment lactose. Microorganisms in EM-4 + vegetable waste were only able to ferment glucose, and microorganisms in vegetable waste were only able to ferment sucrose. In the study by Dewi Widyabudiningsih et al. (2021), the influence of EM-4 concentration and the type of fruit peel waste (banana, mango, and orange) on liquid organic fertiliser (LOF) production was evaluated. Results indicate that all POC samples, with and without EM-4, fermented glucose and sucrose but could not ferment lactose. This indicates that the microorganisms in fruit peel waste have sucrase and glucosidase enzymes needed to break down glucose and sucrose, but lack the lactase enzyme required to break down lactose.

The results also align with the study by Nainggolan et al. (2018), which investigated the effect of EM-4 activator volume on POC production from tofu wastewater and water hyacinth. The results showed that all POC samples, with different EM-4 volume variations, fermented glucose and sucrose but could not ferment lactose. This indicates that the microorganisms in tofu wastewater and water hyacinth have sucrase and glucosidase enzymes but lack lactase enzymes. Mixed waste contains a variety of compounds, including phenolic compounds, tannins, and natural antibacterial substances from plants. These compounds can inhibit the growth of lactose bacteria and disrupt their enzyme activity.

NPK Test

Based on the table of NPK POC test results using kasgot as a base, the nitrogen (N) content in all kasgot POC samples ranged from 0.1401% to 0.4357%. The highest N content was found in kasgot POC from mixed waste + EM-4 (0.4357%), while the lowest N content was found in kasgot POC from vegetable waste (0.1401%). The potassium (K2O) content in all kasgot POC samples ranged from 0.3311% to 0.4929%. The highest K2O content was found in kasgot POC from vegetable waste + EM-4 (0.4929%), while the lowest K2O content was found in kasgot POC from waste (0.3311%). The phosphorus (P2O5) content in all kasgot POC samples ranged from 0.1459% to 0.4031%.

The highest P2O5 content was found in kasgot POC from vegetable waste + EM-4 (0.4031%), while the lowest P2O5 content was found in kasgot POC from waste (0.1459%). Some other studies on the NPK content of kasgot POC have shown varied results, depending on several factors, such as the type of kasgot, raw materials, processing methods, and fermentation duration. As in the research, Fadilah et al. (2020) showed that the NPK content of kasgot POC with the addition of EM-4 was 0.42%, 0.25%, and 0.12% for N, P2O5 and K2O, respectively, and Wulandari et al. (2021) showed that the NPK content of kasgot POC with the addition of brown sugar was 0.37%, 0.21%, and 0.15% for N, P2O5, and K2O, respectively.

Based on the NPK POC test results table using kasgot as a base, as discussed previously, the NPK content in kasgot POC is indeed considered low compared to the SNI standard, which requires a minimum of 2% for each element. This is supported by the fact that the NPK content in Achatina fulica kasgot ranges from 0.42% N, 0.27% P2O5, and 0.22% K2O, while in Helix aspersa kasgot it ranges from 0.68% N, 0.31% P2O5, and 0.25% K2O. Nevertheless, kasgot POC still has several other benefits for plants and can be used as a complementary organic fertilizer.

CONCLUSION

The research showed that adding a bioactivator during the fermentation of BSF larva leachate influenced the quality of the resulting liquid organic fertiliser. The EM-4 + molasses + leachate treatment yielded the best quality, achieving the highest total NPK content (0.66%) after 15 days of fermentation. The best treatment for plant growth was the liquid organic fertiliser from 12 days of EM-4 fermentation, which produced an average plant height of 11.9 cm.

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