International Journal of Recycling of Organic Waste in Agriculture (2023)12: 155-164
Doi: 10.30486/IJROWA.2022.1941081.1344

ORIGINAL RESEARCH

Nutrient content of super liquid fertilizer (SLF) from dairy sludge waste and the potential as a biopesticide

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Received: 09 October 2021 / Accepted: 11 May 2022 / Published online: 15 July 2022

Abstract

Purpose The current use of pesticides and chemical fertilizers harms the environment, alternative substitutions using organic materials are needed. One of them is utilizing the dairy industry sludge waste to become a *Super Liquid Fertilizer* (SLF). This study aimed to analyze the nutrient quality of SLF (a dairy industry sludge waste that was added to some the microbial activators).

Method The types of microbes and the number of nutrients in pure sludge were identified, followed by composed SLF and applied to the larvae of *Spodoptera litura* to see the pathogenicity. Data were analyzed using SPSS.26 software.

Results The types of microbes that exist in the pure sludge waste of the dairy industry are dominated by *Pseudomonas bacteria*, as much as 50%. The nutrient content of SLF is appropriate to Indonesia's national standard for liquid organic fertilizer. Analysis of varian (ANOVA) analysis shows that the addition of activator microbe at P3 is the best. The number of microbes in SLF is significantly different ($P < 0.05$) at each treatment, where P3 is the best treatment. The pathogenicity of SLF is also good, as evidenced by the motility of the *Spodoptera litura* reaching 98% in the P5 treatment for 96 hours.

Conclusion This research has succeeded in producing SLF with the nutritional value of nutrients that are close to the Indonesian Ministry of Agriculture. The number of bacterial colonies in SLF is more than 10^6 CFU/mL, especially in the P3 treatment. SLF can kill *Spodoptera litura* caterpillars in a time period of 46 hours.

Keywords Organic fertilizer, Super liquid fertilizer (SLF), Dairy sludge waste, Biopesticide

Introduction

The development of the dairy industry in Indonesia has risen along with the increasing public demand for dairy products. The level of national milk consumption has increased by 1.6% each year (Mintarsih 2006). This growth was followed by the increase of the dairy industry wastewater called liquid sludge and the precipitate called solid sludge. The average liquid sludge volume is 2.5 liters for each liter of processed liquid milk or two liters for each kg of powdered milk (Mintarsih 2006; Keoleian and Spitzley 1999). Liquid sludge contains

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600-2000 mg/L of *Biochemical Oxygen Demand* (BOD), 800-4500 mg/L of *Chemical Oxygen Demand* (COD), 20-230 mg/L of total nitrogen (N), 20-100 mg/L of total phosphorus (P), 6.78 mg/L of potassium (K), 80-250 mg/L of fat, 1-2 mL/L of sediment, and pH 6-11 (Lutze and Engelhar 2020). Bittman et al. (2012) stated that the effectiveness of liquid sludge and solid sludge as compost showed an increase of conductivity value, sodium (Na), K, and P content, while the addition of 160 m^3/ha of sludge waste in acidic soils has been shown the increasing of crop yields. Wheat (*Lolium multiflorum*) was compared with the addition of NPK (15-15-15) treatment of 675 kg/ha. The results of the study by Lopez-Mosquera et al. (2000) reported that the use of sludge of 160 m^3/ha as fertilizer in grazing pasture areas gave the same results on the growth and productivity of grass fertilized with NPK (15-15-15) of 675 kg/ha, ammonium nitrate (20.5% N) of 120 kg/ha, and potassium sulfate (50% K_2O) of 120 kg/ha. It can be concluded that the application of sludge waste of 160 m^3/ha is effective as fertilizer in grazing areas.

Another observation by Suryani and Bahri (2015) shows that liquid sludge waste from the dairy processing industry is very good to use as irrigation water for rice plants because it can increase rice crop productivity by up to 30%. Rice plants require food for growth and development. Plants need sixteen basic elements, which are carbon (C), hydrogen (H), and oxygen (O) that are obtained from the atmosphere and groundwater. The remaining thirteen other basic elements are nitrogen (N), P, K, calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), boron (B), molybdenum (Mo), and chlorine (Cl) that are obtained from soil minerals (Suryani and Bahri 2015). Sludge waste will be very good for rice plants, but the influence of pests on the rice plants cannot be ruled out. The armyworm *Spodoptera litura* is one of the most important leaf-eating pests. Yield losses due to pest attacks can reach 80%, even puso, if not controlled. Pest control efforts at the farm level still rely on insecticides but are less effective (Sulistyono et al. 2020). The other host plants of armyworms are chili, soybeans, corn, tomatoes, sugarcane, beans, oranges, tobacco, shallots, eggplant, potatoes, and beans (soybeans, peanuts), kale, spinach, bananas, and ornamental plants (Sulistyono et al. 2020). With the number of problems and negative impacts caused by the use of chemical insecticides and chemical fertilizers, as well as the high level of industrial sludge waste disposal in the environment, it is necessary to convert this waste *into Super Liquid Fertilizer* (SLF) with the addition of activators of various types of bacteria, so that it can substitute the use of chemical pesticides and chemical fertilizers.

Materials and methods

Production of SLF from dairy industry liquid sludge waste

As much as 400 L of liquid sludge waste was prepared, then separated into four parts (P0, P1, P2, and P3) of 100 L for each treatment. Each treatment added with 100 mL of molasses, 100 mL of coconut water, and dairy sludge waste, then stirred until smooth. After that, three types of activating bacteria, *Bacillus subtilis*, *Bacillus thuringiensis*, and *Pseudomonas aeruginosa*, were added as much as the percentage of treatment that has been determined. Adding of the activator microbe composition are P0 (*Bacillus subtilis* 0 mL, *Bacillus thuringiensis* 0 mL, *Pseudomonas aeruginosa* 0 mL), P1 (*Bacillus subtilis* 50 mL, *Bacillus thuringiensis* 50 mL, *Pseudomonas aeruginosa* 50 mL), P2 (*Bacillus subtilis* 75 mL, *Bacillus thuringiensis* 75 mL, *Pseudomonas aeruginosa* 75 mL),

P3 (*Bacillus subtilis* 100 mL, *Bacillus thuringiensis* 100 mL, *Pseudomonas aeruginosa* 100 mL). Fermentation of the mixture of materials was conducted for one month under aerobic conditions.

Identification of microbes contained in pure liquid sludge waste

The test method refers to Komarawidjaja (2007) and Ishak et al. (2011). The identification stage of bacteria begins with isolation and characterization. In the isolation stage, the activated sludge waste sample was first diluted with sterile distilled water in stages 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. The bacteria were then cultured by inoculating the sludge waste bacteria from the dilution into *Plate Count Agar* (PCA) media. The incubation process was conducted under aerobic conditions for 24-48 hours. The single colonies formed were examined using Gram staining to see the characteristics of the cell wall and the shape of the bacteria cells. The next step is identification. This step was carried out based on the observations of colony morphology (results from characterization) and biochemical tests which included cell motility, catalase, and sugar tests. The results of the observations are then matched with the bacterial identification book.

Complete test of total nutrients contained in pure liquid sludge waste and SLF

The analysis of C-organic content using the method (Walkley and Black 1934) : spectrophotometer, pH value using the method 994.18 : pH Meter, N-Organic using the method : Kjeldahl; Titrimetry. Total P_2O_5 test using the method: wet xidation (HNO_3+ $HC1O_4$) spectrophotometer. Total test of potassium oxide (K_2O), Fe, Zn, Mn, Cu, Mo, and carbon monoxide (CO) using the method : wet oxidation (HNO_3+$HClO_4$) ; atomic absorption spectrophotometry (AAS) (García and Báezs 2012).

SLF bacterial colony total test using total plate count (TPC) method

The sample was measured aseptically as much as 25 mL, and 225 mL of Butterfield's Phosphate Buffered solution was added, then homogenized for two minutes. The homogenate is a 10^{-1} dilution solution. As much as 1 mL of the homogenate was taken using a sterile pipette and was put into a bottle containing 9 mL of Butterfield's Phosphate Buffered solution. A sample with a dilution of 10^{-2} was obtained. Each dilution was shaken at least 25 times. The sample calculations, It was conducted for the dilutions 10^{-3}, 10^{-4}, 10^{-5}, and so on, according to the sample conditions. Furthermore for the pour plate method, 1 mL of each dilution was pipetted and put into a sterile petri dish in duplicate using a sterile pipette. As much as 12-15 mL of PCA media, which has been stored to 45^0C was added to each cup containing the sample. After the agar solidified, the petri dish containing the agar and the sample solution was put into the incubator in an inverted position for 48 hours, 35^{o}C. The number of bacterial colonies was counted in the petri dish. The number of bacterial colonies calculated was a petri dish with bacterial colonies between 25 - 250 colonies.

SLF pathogenicity test against *Spodoptera litura* larvae

Pathogenicity test refers to Salaki and Tarore (2018) with the following stages : providing bacterial culture, followed by multiplying the test larvae. The pathogenicity test was carried out by leaf dipping (*Leaf Dipped Method*) Hamilton and Attia (1977) by using five pieces

of rice leaves with a length of 5 cm, the leaves were immersed in a solution of SLF (5 mL, 10 mL, 15 mL, 20 mL, and 25 mL) for ten minutes, then air-dried. It was put into a clock bottle (6 cm diameter) that had been sterilized and previously filled with *Spodoptera litura* larvae (9 birds per bottle) fasted for eight hours. For each volume, five replicates (bottles) were used. As a control, leaves dipped in sterile Ringer's solution were used. Symptoms of illness and larval behavior were observed at six hour intervals, while larval mortality was calculated after 24, 48, 72, and 96 hours of incubation. The killing power of each isolate was expressed by the percent mortality.

Results and discussion

Microbes contained in pure sludge waste

According to the analysis of microbes contained in the sludge waste of the dairy industry, it was found that there were 12 types of microbes, including (*Comamonas-Pseudomonas*, *Alcaligenes*, *Pseudomonas (fluorescent group)*, *Paracoccus*, Unidentified (gram-negative rods), *Aeromonas*, *Flavobacterium-Cytophaga*, *Bacillus*, *Micrococcus*, *Coryneform*, *Arthrobacter*, and Aureobacterium-*Microbacterium*. There are three types of microbes (*Pseudomonas* 50%, *Aeromonas* 1.9%, and *Paracoccus* 11.5%) that are good for soil fertility and one type of bacteria that can function as a biopesticide (*Bacillus* 1.9%) (Table 1).

Bacteria is one of the microorganisms that are widely found in nature which has some harmful properties, but many are also beneficial because they have antagonistic properties. This antagonistic nature can be utilized in biological control efforts because it can inhibit the development of pathogens (Rasdiana 2016). Organisms that are antagonistic to plant pathogens are said to be ideal if these organisms meet the criteria, namely a) able to live and reproduce in the rhizosphere or near the resting structure of the pathogen, b) able to form broad-spectrum antibiotics, but do not inhibit other antagonists that do not cause plant damage, c) antagonistic agents can adapt to conditions to be produced on a large scale, d) antagonist agents are more adaptive than pathogens to environmental factors,e) antagonist spores germinate faster than pathogens (Kim et al. 1997).

Table 1 Test results for pure sludge waste microbes before being processed into SLF

Types of bacteria	Total % isolate
Comamonas-Pseudomonas[a]	50.0
Alcaligenes	5.8
Pseudomonas (fluorescent group)	1.9
Paracoccus	11.5
Unidentified (gram-negative rods)	1.9
Aeromonas	1.9
Flavobacterium-Cytophaga	13.5
Bacillus	1.9
Micrococcus	1.9
Coryneform	5.8
Arthrobacter	1.9
Aureobacterium-Microbacterium	1.9

[a]The most dominant type of microbe.

Pseudomonas carry out to dissolve phosphate from a form that plants cannot absorb. In addition, *Pseudomonas* can help in the process of decomposition of organic matter. *Pseudomonas* produces a decomposing enzyme called lignocellulose which also breaks down phosphate complexes in the soil (Mohan et al. 2003; Pranata 2010). Some phosphate solubilizing bacteria also act as biocontrols that can improve the root health and plant growth by protecting against disease. It can prevents plants from

yeast pathogens originating from the soil and has the potential to use as a biocontrol agent in the greenhouses and the field (Raj et al. 2012). Meanwhile, Huang et al. (2016) stated that *P. fluorescens* bacteria have a beneficial effect on the plant development and growth, namely "*Plant Growth Promoting Rhizobacteria*" (PGPR). Bacteria also produce antibiotics that can inhibit the growth of pathogens, especially soil-borne pathogens, and can colonize plant roots. Bacteria interact with pathogens in the form of nutrient competitors, producing antibiotics, siderophores, and cyanide (Kwon et al. 2003).

Complete test of total nutrients contained in pure liquid sludge waste and SLF

Milk processing waste can be obtained in every area of the dairy industry which is spread in almost every province on the Java and Sumatra islands in Indonesia, this is a potential for the utilization of waste, one of which is sludge waste which is produced from the final processing of dairy industry waste as an alternative substitution for organic fertilizer. The results of the nutrient analysis (Table 2) show ~~a~~ good results where the N-total, C/N ratio, P_2O_5, K_2O, and total Fe content are close to the standard for organic fertilizer nutrient content as stipulated in the Regulation of the Indonesian Ministry of Agriculture No. 01 of 2019 (Indonesian Ministry of Agriculture 2019).

According to Ashekuzzaman et al. (2019) that the production of milk processing waste is estimated to reach 1000-2000 kg every processing 450,000 kg of milk/day. The average liquid sludge volume is 2.5 liters per liter of processed liquid milk or 2 liters per kg of powdered milk (Yachya 2017). Liquid sludge contains BOD of 600-2000 mg/L, COD content of 800-4500 mg/L, total nitrogen content of 20-230 mg/L, total phosphorus content of 20-100 mg/L, potassium content of 6.78 mg/L, fat substance 80-250 mg/L, sediment content 1-2 mL/L, and pH 6-11 (Sari et al. 2018; Porwal et al. 2015). For every 2000 grams of milk waste (slurry), 250 grams of milk slurry can be obtained. The nutritional value is quite high as a protein source, namely the content of crude protein 34.98%, lactose 4.42%, crude fiber 9.77%, crude fat 11.04 %, Ca 2.33%, P 1.05%, and Mg 0.4% based on dry matter (Ashekuzzaman et al. 2019).

Table 2 Complete test results of total nutrients of pure sludge waste before being processed into SLF

Parameter	Score	Unit	Method
Total N	6.22 ± 1.12	%	Kjeldahl, titrimetry
C/N	6.29 ± 1.59	-	Calculation
P_2O_5	1.75 ± 0.40	%	Wet Oxidation (HNO_3 + $HClO_4$) molibdovanadat, spectrophotometry
K_2O	0.26 ± 0.07	%	Wet Oxidation (HNO_3 + $HClO_4$), AAS
Total Fe	5913.83 ± 50.12	ppm	Wet Oxidation (HNO_3 + $HClO_4$), AAS

The quality of organic fertilizers is largely determined by their nutrient content. According to the Regulation of the Indonesian Minister of Agriculture No.01 of 2019 (Indonesian Ministry of Agriculture 2019) states that the nutrient content of liquid fertilizer in the form of C-Organic is > 10%, pH 4-9, N-Organic > 0.5%, P_2O_5 2-6%, K_2O 2-6%, Fe available 250-5000 ppm, Zn 125-2500 ppm, Mn 250-5000 ppm, Cu 250-5000 ppm, Mo

2-10 ppm, and CO < 1.0%. The results of the SLF nutrients produced (Table 3) are good because the average of each treatment showed results that were close to the standards of the Indonesian Ministry of Agriculture in 2019. Based on statistical tests, the treatment had a significant effect of P < 0.05% on the nutritional value of SLF nutrients. From each treatment, the P3 treatment had the best nutrient value when compared to the P2, P1, and P0 treatments. Nutrient values in P3 treatment were C-Organic 41.1%, pH 7.5, N-organic 1.1%, P_2O_5 1.9%, K_2O 1.9%, Fe 103.3 ppm, and Zn 191.66 ppm.

The use of 100% liquid organic fertilizer will dissolve by root and all parts of a plant thereby it can overcome nutrient deficiencies and have no problems with nutrient leaching and also able to provide nutrients for plants rapidly (Ardiyanto and Jazilah 2019). Organic fertilizers can stimulate and increase the microbial population in the soil, much more than just applying chemical fertilizers. Organic fertilizers can also able to improve the structure and fertility of the soil. Organic fertilizers can prevent soil erosion because the nitrogen content and nutrient content released by organic matter will slowly undergo a mineralization process.

Table 3 Results of complete analysis of the amount of SLF nutrients from various treatments

	Observation parameter						
Treatment	**C-Organic % (W/V)**	**pH**	**N- Organic % (W/V)**	**P_2O_5 % (W/V)**	**K_2O % (W/V)**	**Fe ppm**	**Zn ppm**
PO	26.0 ± 1.00	7.7 ± 0.05	0.5 ± 0.05	0.4 ± 0.05	0.4 ± 0.05	366.0 ± 50.40	117.6 ± 2.50
P1	23.0 ± 1.00	7.4 ± 0.05	0.2 ± 0.01	1.7 ± 0.15	1.8 ± 0.11	212.0 ± 10.50	160.0 ± 17.30
P2	23.6 ± 0.50	7.6 ± 0.15	0.8 ± 0.05	1.8 ± 0.11	1.8 ± 0.06	123.3 ± 25.16	173.33 ± 15.27
P3	41.3 ± 3.20	7.5 ± 0.15	1.1 ± 0.11	1.9 ± 0.20	1.9 ± 0.20	103.3 ± 5.77	191.66 ± 12.58

Note: Adding of the activator microbe composition = **PO** (*Bacillus subtilis* 0 mL, *Bacillus thuringiensis* 0 mL, *Pseudomonas aeruginosa* 0 mL), **P1** (*Bacillus subtilis* 50 mL, *Bacillus thuringiensis* 50 mL, *Pseudomonas aeruginosa* 50 mL), **P2** (*Bacillus subtilis* 75 mL, *Bacillus thuringiensis* 75 mL, *Pseudomonas aeruginosa* 75 mL), **P3** (*Bacillus subtilis* 100 mL, *Bacillus thuringiensis* 100 mL, *Pseudomonas aeruginosa* 100 mL).

Total SLF bacterial colonies

Based on the results of the TPC test, the total microbes were significantly different from each treatment. The test results show that the highest number of bacterial colonies was obtained in P3 (Table 4), which are *Bacillus subtilis* 7.7 x 10^6 CFU/mL, *Bacillus thuringiensis* 5.1 x 10^6 CFU/mL and *Pseudomonas aeruginosa* 2.9 x 10^7 CFU/mL. From the number of colonies obtained, the SLF meets the bacterial colony standards in the Regulation of the Indonesian Minister of Agriculture No.01 of 2019 (Indonesian Ministry of Agriculture 2019). The number of microbial populations is largely determined by nutrition, the environment and the number of initial colonies. *Bacillus subtilis* is one bacteria that can be used as a biological agent to control soil-borne diseases and can reduce sprouting disease in sugar beets. *Bacillus subtilis* has the potential to be developed in controlling anthracnose caused by *C. capsici* (Lucy et al. 2019). *Bacillus thuringiensis* has two growth phases, namely the germination phase (vegetative growth) and the sporula-

tion phase. The germination phase occurs when the bacteria are in a nutrient-rich environment. In this phase, cells will multiply by dividing. The sporulation phase occurs when the nutrients in the environment are depleted or pressure from environmental conditions on the growth of *Bacillus thuringiensis*.

Table 4 The number of bacteria in SLF

Treatment	Parameter	Number of bacterial colonies (CFU/mL)	Method
PO	*Bacillus subtilis*	2.3×10^4	TPC
	Bacillus thuringiensis	4.4×10^5	TPC
	Pseudomonas aeruginosa	8.3×10^5	TPC
P1	*Bacillus subtilis*	5.6×10^5	TPC
	Bacillus thuringiensis	7.3×10^6	TPC
	Pseudomonas aeruginosa	1.1×10^6	TPC
P2	*Bacillus subtilis*	3.3×10^6	TPC
	Bacillus thuringiensis	8.6×10^6	TPC
	Pseudomonas aeruginosa	2.6×10^7	TPC
P3	*Bacillus subtilis*	7.7×10^6	TPC
	Bacillus thuringiensis	5.1×10^6	TPC
	Pseudomonas aeruginosa	2.9×10^7	TPC

Note: Adding of the activator microbe composition = **PO** (*Bacillus subtilis* 0 mL, *Bacillus thuringiensis* 0 mL, *Pseudomonas aeruginosa* 0 mL), **P1** (*Bacillus subtilis* 50 mL, *Bacillus thuringiensis* 50 mL, *Pseudomonas aeruginosa* 50 mL), **P2** (*Bacillus subtilis* 75 mL, *Bacillus thuringiensis* 75 mL, *Pseudomonas aeruginosa* 75 mL), **P3** (*Bacillus subtilis* 100 mL, *Bacillus thuringiensis* 100 mL, *Pseudomonas aeruginosa* 100 mL), **TPC** (Total Plate Count).

Pseudomonas has the property of luminescence, and grows at 4°C. They can hydrolase arginine, and some can hydrolyze gelatin, and carry out the denitrification process (Galitskaya et al. 2016). *Pseudomonas* functions to dissolve phosphate from a form that cannot be absorbed by plants. In addition, *Pseudomonas* can help in the process of decomposition of organic matter. *Pseudomonas* produces a decomposing enzyme called lignocellulase (Pranata 2010) which also breaks down phosphate complexes in the soil (Mohan et al. 2003).

Pathogenicity of SLF against larvae *Spodoptera litura*

It was found that the percentage of motility of *Spodoptera litura* caterpillars given SLF was significantly different, $P< 0.05$. The highest motility results were obtained in P5 treatment (98%) using 25 mL SLF and 96 hours of observation (Fig. 1). The SLF biopesticide works in poisoning the larvae of *Spodoptera litura.* There is a physical disturbance on the insect's outer body (cuticle), namely washing the waxy coating that protects the insect's body which causes death because the insect will lose a lot of body fluids. How the biopesticide compounds trapped in SLF enter the insect's body is as a stomach poison that enters through the digestive tract of food or the stomach.

Insect toxic compounds will penetrate the intestinal wall which in turn will disrupt the insect's metabolism and cause a lack of energy needed for life activities, seizures, and eventually cause death.

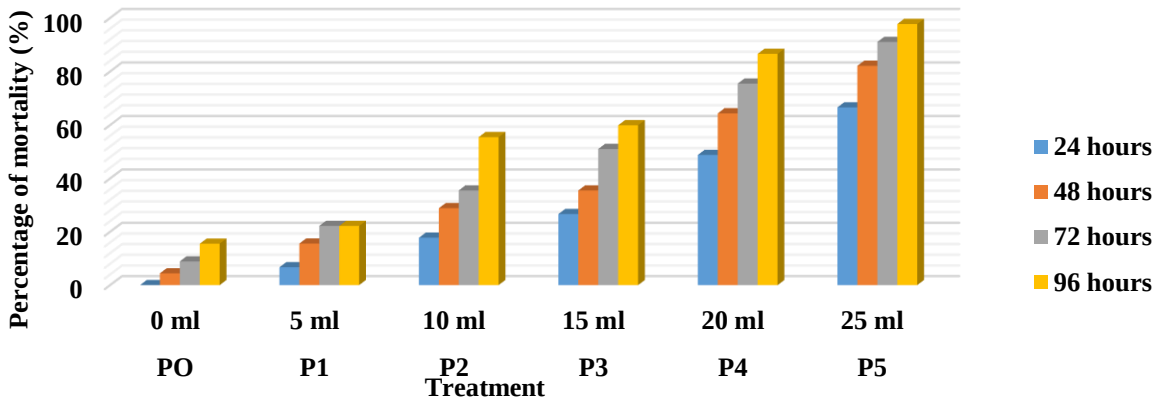

Fig. 1 Percentage of mortality of *Spodoptera litura* larvae at each level of administration

The picture A is the condition of the caterpillar without SLF that was still alive after several times with a mortality of < 20% (Fig. 2). The picture B indicates that the caterpillar were died during the treatment by adding SLF with a mortality close to 100% (Fig. 2). And picture C shows that there was a change of color to black on the caterpillar (Fig. 2).

A B C

Fig. 2 The caterpillar with different conditions after treatment

The death of *Spodoptera litura* is due to the presence of pathogenic microbes. According to Vanittanakom et al. (1986) *Bacillus subtilis* can produce antibiotics such as baclysin and fengymycillo which are antifungal and some isolates produce antibiotics that have a wide range of activity, both against bacteria and fungi. *Bacillus thuringiensis* also produces three kinds of other substances that are toxic to insects, namely: (a) *α-exotoxin* which is an enzyme produced by developing bacteria in the form of phospholipase C or lacretinase C, this substance functions to break down essential phospholipids in insect body tissues, (b) *β-exotoxin* (fly-factor or heatable exotoxin) which is a bacterial cell secretion in the surrounding medium that is wáter - soluble, heat resistant, and

can cause the death of insect because it inhibits RNA synthesis, (c) *γ-exotoxin* is a toxin whose identification has not been identified, however it is suspected that it functions to break down phospholipids into fatty acids (Griego and Spence 1978; Wu et al. 1994). Among the toxins produced by *Bacillus thuringiensis*, the most common and markedly toxic effects on insects are *α-exotoxin* and *γ -exotoxin*. *α-exotoxin* in the insect body will inhibit protein synthesis, disrupting the molting process. Protein crystals are the main toxin that causes the death of larvae of various insects, including *Spodoptera litura* and *Lepidoptera* larvae because they can cause damage to the intestinal wall epithelial cells, causing paralysis of the insect planning system (Griego and Spence 1978; Wu et al. 1994).

Conclusion

This study concluded that there are twelve types of microbe dominated by *Pseudomonas* by 50%. Several treatments of SLF nutritional value is close to the national standard set by the Ministry of Agriculture of the Republic of Indonesia in 2019. The P3 treatment (*Bacillus subtilis*, *Bacillus thuringiensis*, and *Pseudomonas aeruginosa* 100 mL) was the best treatment. P3 treatment had C-Organic 41.1%, pH 7.5, N-organic 1.1%, P_2O_5 1.9%, K_2O 1.9%, Fe 103.3 ppm, and Zn 191.66 ppm. P3 had total microbes of *Bacillus subtilis* 7.7 x 10^6 (CFU/mL), *Bacillus thuringiensis* 5.1 x 10^6 (CFU/mL), and *Pseudomonas aeruginosa* 2.9 x 10^7 (CFU/mL). The best result of pathogenicity of P3 is obtained by adding 25 mL of SLF with the ability to kill 98% of caterpillars for 96 hours.

Acknowledgements The present scientific research was financially afforded by the Center for Agricultural Education Research Fund, 2021, Ministry of Agriculture, Republic of Indonesia.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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