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Research article

Effects of Rearing Black Soldier Fly Larvae (*Hermetia Illucens*) from Organic Wastes

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Abstract: Organic waste is one of the major environmental problems, and the black soldier fly (*Hermetia illucens*) larvae can help decompose organic waste. Therefore, this research is interested in studying organic fertilizers' growth, nutritional value, and nutrient contents from the residue of black soldier fly larvae. Four experimental sets of plastic buckets containing organic wastes, i.e., pineapple peels, banana peels, durian peels, and vegetable scraps, were used to trap the black soldier flies and observe the spawning and the number of egg clusters. After that, the 4-day-old black soldier fly larvae were incubated in plastic boxes using different foods for the same duration. Three replicates of the experiment were performed, recording the growth period and size of the soldier fly larvae. The study found that the life cycle of the black soldier fly was divided into 5 stages: egg, larval, prepupal, pupal, and adult stages, in 47–67 days. The black soldier fly larvae fed pineapple peels both in the larval and prepupal stages were the largest, while the larvae fed with durian peels entered the larval stage the fastest. Subsequently, 100 g of fresh black soldier fly larvae samples from four experimental sets were analyzed for nutritional value. It was found that fresh black soldier fly larvae had protein contents of 9.25–10.69 g/100 g and a fat content of 3.29–15.90 g/100 g, which were suitable for use as an ingredient in animal feed to reduce production costs. Then, 100 g of the frass of the black soldier fly larvae fed with durian peels was collected for analysis of the essential nutrients for plants, i.e., nitrogen, phosphorus, and potassium, at about 2.46%, 2.77%, and 3.14%, respectively, which are suitable for use as organic fertilizers for soil amendments.

Keywords: Black soldier fly larvae; Organic waste; Alternative protein source

1. Introduction

Solid waste, especially organic waste, is an important problem that is increasing in volume yearly due to the increasing population, economic expansion, and changes in people's consumption behavior. However, proper waste management by local administrative organizations is still insufficient to cope with the amount of waste that tends to increase yearly [1]. Moreover, unclean waste management results in various problems, such as being a source of germs, disturbing odors, leachate contaminating surfaces and groundwater, and environmental impacts [2]. If people separate and reuse solid waste, especially organic waste such as vegetable scraps and various types of fruit peels, such as banana peels, pineapple peels, and durian peels, etc., which are

agricultural waste from consumption and processing industries that are in large quantities in the three southern border provinces. This will reduce the amount of solid waste and pollution in various areas and promote and support the use of natural resources as new raw materials [3].

The black soldier fly has the scientific name of *Hermetia illucens*. It can decompose animal manure, food scraps, and leftover parts from various processing operations with a low risk of causing harm to animals and humans. Additionally, the larvae have high protein and fat benefits compared to current animal feed raw materials [4, 5]. Currently, small animal farmers often face problems with the expensive cost of ready-made feed, which affects their livelihood. Therefore, farmers must find alternative protein sources for animal feed, such as soybean meal, fish meal, etc. [5, 6]. Hence, the black soldier fly larvae can be used as a high-quality protein source for animals, especially economic animals such as carp fish, ornamental fish, grouper fish, giant freshwater prawns, lobsters, organic chickens, fighting cocks, etc. In the United States, black soldier fly larvae are also used to raise trout, salmon, Nile Tilapia fish, and shrimp [7]. Furthermore, the black soldier fly larvae frass can make compost or organic fertilizer [2].

Organic waste management at the source with the black soldier flies larvae is a biotechnological method that is simple, safe, environmentally friendly and doesn't take long. The black soldier fly larvae can digest food scraps faster than composting them. It can also reduce contamination by pathogenic microorganisms from the decay of organic waste, help prevent being a source of breeding vectors for bluebottle flies, house flies, and fruit flies, as well as reduce the transmission of disturbing odors in the area [8]. This has led to using organic wastes to create various benefits, such as organic fertilizer and a source of high-protein raw materials. The black soldier fly larvae can digest food scraps up to 20 times their body weight, or 40 kilograms per square meter, within 24 hours, they can digest organic waste 5 times faster than earthworms, which reduces greenhouse gas emissions and low levels of ammonia. This may be because microorganisms within the intestinal tract of the black soldier fly larvae decompose quickly. In addition, it can add value to animal manure, reduce animal manure pollution by more than 60%, be used to produce compost for growing plants and nourishing the soil, reduce bad odors, and the movement of larvae helps inhibit the growth of bacteria that produce bad smell gas [9, 10].

However, the cultivation of black soldier fly larvae in households has not been widely studied. The foods used for growing the black soldier fly larvae are household leftovers, organic waste, and animal manure. The nutritional value and quality of the black soldier fly larvae depends on the nature and quality of the food used for raising them, which is a restriction on its use as a raw material in animal feed. Thus, if you choose to use food or formula suitable for the black soldier fly larvae, you will be able to control the quality of the larvae to a constant level. This includes expanding the results of raising black soldier fly larvae to be used as raw material to replace protein sources in the animal feed industry more efficiently [11]. This research is therefore interested in studying the management of organic waste using black soldier fly larvae. The objectives are (1) to study the life cycle and growth of black soldier fly larvae fed on different types of organic waste, including pineapple peels, banana peels, durian peels, and vegetable scraps; (2) to compare the nutritional value of the black soldier fly larvae; and (3) to analyze the amount of macronutrients in organic fertilizer from the frass of the black soldier fly larvae.

2. Materials and Methods

2.1 Trapping black soldiers fly to lay eggs

A completely randomized design (CRD) was applied to 4 experimental sets (4 types of organic waste), each consisting of fruit peels and vegetable scraps from fruit and vegetable shops. Then place 2 kilograms each of pineapple peels, banana peels, durian peels, and vegetable scraps into a 10-liter plastic bucket with a diameter of 25 centimeters and a height of 30 centimeters, with a tight lid and a triangular slit at the top edge and 10 centimeters on each side, so that the soldier fly can easily lay their eggs. The plastic buckets were placed 30 centimeters below the eaves in soft sunlight for 7 days. The egglaying and number of ovaries were observed and surveyed every day. The black soldier flies were found laying eggs around the edge of the inner tank. When the food ran out, 500 grams were added as new food at a time for every experimental set. Record the number of egg groups by counting the number of eggs per group and averaging the number of eggs in each group of each experimental set. Finally, collect the egg groups that have been raised for further cultivation.

2.2 Cultivation of black soldier fly larvae

Conducting research by adapting the process of breeding the black soldier fly larvae of the Department of Environmental Quality Promotion [8] by preparing a container for the nursery of the black soldier fly larvae, width 45 centimeters and height 16 centimeters, adding 500 grams of chicken feed, and laying eggs 10 grams of the black soldier fly larvae on the cardboard, 1 centimeter wide and 1 centimeter long, were placed above chicken feed. Then, observe the changes and hatching rate. After the black soldier fly larvae hatch from the eggs, 500 grams of food are added to the larvae until they have grown for 16 days, which is the stage when the larvae can be separated for use in test rearing. The experiment was planned to be completely randomized, with 4 experiment sets (4 organic waste types) and 3 replicates. In each experimental set, 100 grams of the black soldier fly larvae were placed in a rectangular box measuring 21.5 centimeters in width, 21.5 centimeters in length, and 18 centimeters in height. Afterward, the larvae are fed by adding fruit peels and vegetable scraps obtained from fruit and vegetable shops by chopping them into small pieces, including 100 grams of pineapple peel, banana peel, durian peel, and vegetable scraps. Each rectangular box was covered with a thin white cloth. Make observations every day, and food was added to all sets of black soldier fly larvae equally every 2 days or until food was gone. The new food was added 50 grams at a time to every experimental set. Record the growth period of the black soldier fly larvae from the egg stage to the adult stage, and the survival rate of the black soldier fly larvae in each experimental set.

2.3 Nutritional analysis

The samples of the prepupal stage of black soldier fly larvae were taken from 4 types of organic waste, including pineapple peels, banana peels, durian peels, and vegetable scraps, 100 grams per experimental set, placed in ziplock bags and refrigerated at a temperature of approximately 10°Celsius to prepare for nutritional analysis in the laboratory by proximate analysis according to the method of AOAC (2019) to analyze the values of moisture, ash, protein, fat, fiber, carbohydrates, and total energy. All data were analyzed for variance (Analysis of Variance: ANOVA), and the differences between the means were compared using Duncan's New Multiple Range Test (DMRT) at a confidence level of 95% ($P < 0.05$).

2.4 Analysis of macronutrient contents from black soldier fly larvae

At the end of the organic fertilizer process, the frass of the black soldier fly larvae obtained from the decomposition of organic waste in all four experimental sets observed that the fertilizer had the appearance of fine, crumbly pellets, was blackish-brown, and was lightweight. Therefore, samples of the black soldier fly larvae frass were collected in each experimental set. The fertilizer from the black soldier fly larvae frass was mixed evenly throughout the pile and spread out. Then, 100 grams of fertilizer samples were collected from each experimental set, and the samples were stored in separate plastic bags for each experimental set to analyze the amount of essential nutrients for plants, including nitrogen, phosphorus, and potassium.

3. Results and Discussion

3.1 Study of the life cycle and growth period of black soldier fly fed with different types of organic waste

From the study of the egg-laying behavior of the black soldier flies, it was found that the black soldier fly hovers around 30 centimeters above the food source and then approaches the food source. It takes approximately 5-10 minutes to lay eggs. The newly laid egg clusters are soft white and have a sticky consistency that makes the eggs stick together in groups and stick well to the material. The groups of eggs will be found under the surface of the food, which is dry, and on the inside edges of food containers. The counting the number of eggs study laid by five adult black soldier flies found that each one laid only one group of eggs, which had an average number of eggs of 483.00 ± 134.30 eggs per group. The experiment set fed with pineapple peels had the highest mean number of eggs, which was 517 ± 110.15 eggs per group, and the experiment set fed with banana peels had the lowest, 383 ± 175.13 eggs per group. This is consistent with a previous study [12], which documented that female black soldier flies prefer to lay their eggs over organic waste in dry areas, small openings, and sheltered areas. There are 400 to 800 eggs per egg group. The male and female breeders will die after mating and laying eggs. Furthermore, a study of the culture of the black soldier fly by using organic wastes as food found that the life cycle of the black soldier fly can be divided into 5 stages (Figure 1),

including the egg stage, in which the eggs are oval-shaped, approximately 0.5-1 millimeters in length, and challenging to observe with the naked eye. During the first 1-2 days, they will be so cloudy white that the eggs will hatch into larvae and crawl into the culture medium for approximately 3-4 days. The average hatching rate from 4 experimental sets is 98.5 ± 0.58%. In addition, the study results found that the larval stage is where they eat a lot of organic waste as food. Therefore, it is an appropriate time to dispose of organic waste.

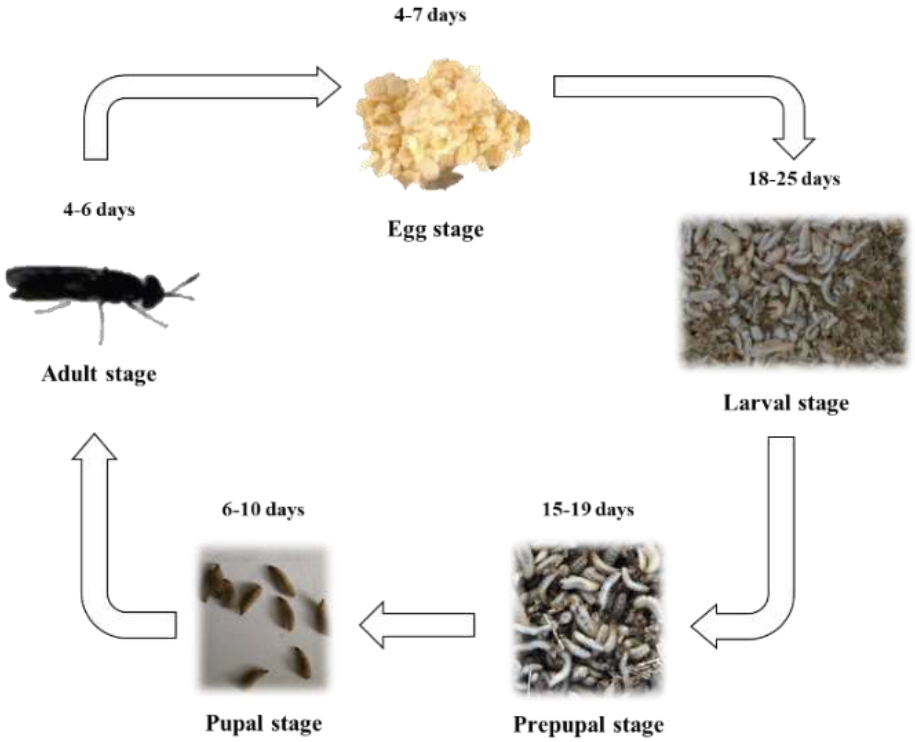

Figure 1. The life cycle of the black soldier flies.

This study also found that black soldier fly larvae are plump, flat, and creamy white (Figure 2). Nevertheless, if the larvae have a brown body, they will eat less food to prepare before entering the pupal stage. Food should not be added at this stage because the larvae will molt for the last time to pupate [8].

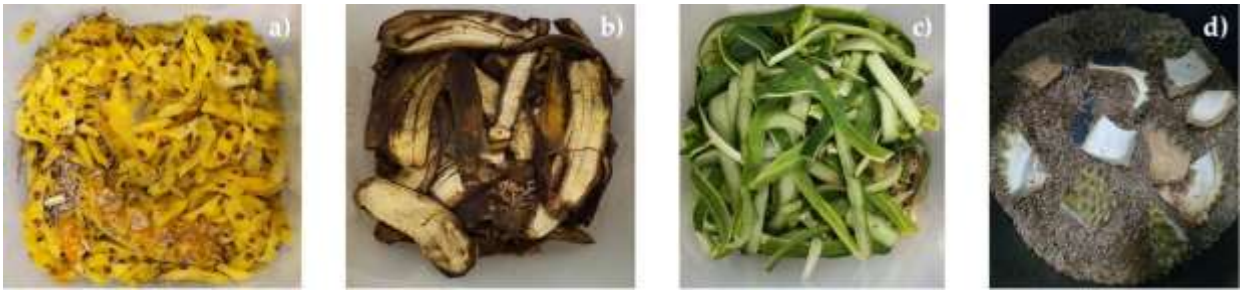

Figure 2. The black soldier flies larvae fed on different types of organic waste: a) pineapple peels, b) banana peels, c) vegetable scraps, and d) durian peels.

It was found that the type of food used to feed the black soldier fly larvae affects their growth and survival rate [10, 13]. The black soldier fly larvae fed with pineapple peels were the largest, with an average size of approximately 10.6 ± 0.2 millimeters. In contrast, the black soldier fly larvae fed with durian peels were the smallest, with an average size of roughly 7.3 ± 0.3 millimeters (Table 1). The moisture content and type of food waste that is the medium of larval growth significantly affect larval development [14]. The study by Muenmee and Prasertboonyai [15] found that durian peel has a moisture content of 78.64 ± 0.38%, while

pineapple peel has a moisture content of 85.09 ± 0.07%. This could also explain why the larvae that consumed durian peels had the lowest size. In the prepupal stage, the larvae were light brown to dark brown, and their body size ranged from 13 to 20 millimeters in length. The prepupal larvae can climb up from the rearing area at an angle of 40-65°Celsius and move as far as 100 meters. The black soldier fly larvae fed with pineapple peels were the largest, with an average size of approximately 15.2 ± 0.4 millimeters. In contrast, the black soldier fly larvae fed with durian peels were the smallest, with an average size of approximately 11.4 ± 0.1 millimeters (Table 1). Moreover, it was found that the black soldier fly larvae fed with durian peels could enter the pupal stage the fastest, at 15 days, followed by the larvae fed with pineapple peels and banana peels. As for the larvae fed with vegetable scraps were able to enter the pupal stage the slowest, at 19 days, with an average survival rate of 85.5 ± 1.15%. For the pupal stage, when the larvae have a dark black body and dry, hard skin. It is about 7 days old and has a complete pupation rate of 30.5 ± 0.18% because most of the pupae atrophy, but the rate of hatching into adults is 90.04 ± 0.29%. In the adult stage, the survival rate from egg to adult was 25.25 ± 0.11%. The black soldier fly larvae fed with pineapple peel had a higher rate of hatching eggs, the prepupal stage, and hatching into adults, but the pupation rate was low.

Table 1. The size of the black soldier fly larvae fed on different types of organic waste.

Types of organic waste	The average size of the larval stage of the black soldier fly larvae (mm.)	The average size of the prepupal stage of the black soldier fly larvae (mm.)
Pineapple peels	10.6 ± 0.2	15.2 ± 0.4
Durian peels	7.3 ± 0.3	11.4 ± 0.1
Banana peels	9.4 ± 0.3	13.3 ± 0.2
Vegetable scraps	9.1 ± 0.1	12.1 ± 0.2

3.2 Study of the nutritional value of black soldier fly larvae fed with different types of organic waste

The prepupal stage black soldier fly larvae samples were taken from 4 different types of organic waste, i.e., pineapple peels, banana peels, durian peels, and vegetable scraps, to be analyzed for chemical composition by estimation methods in the laboratory. It was found that the total protein, carbohydrate, and total energy contents in every experimental set were not statistically different. The black soldier fly larvae fed with durian peels had the highest amount of fiber, ash, and moisture, which were 10.69%, 0.32%, 2.54%, and 82.21%, respectively. In contrast, the black soldier flies larvae fed banana peels had the highest amount of fat, which was 15.90%, as in Table 2, because banana peels are high in fat [16]. It could be stated that the type of food affects the nutritional value of the black soldier fly larvae, especially their protein and fat content [17].

Table 2. The nutritional value of fresh black soldier fly larvae.

Types of organic waste	nutritional value (%)						
	Protein	Fat	Fiber	Ash	Moisture	Carbohydrate	Total energy
Pineapple peels	9.25	7.20a	0.08a	2.18a	79.65a	1.72	108.63
Durian peels	10.69	3.29b	0.32ab	2.54b	82.21b	1.28	77.49
Banana peels	10.10	15.90c	0.10b	1.83a	71.92a	0.25	184.54
Vegetable scraps	10.50	8.02d	0.12c	2.24c	79.11c	0.13	114.71
F-test	ns	*	*	*	*	ns	ns

Calculate the protein value by using a protein conversion factor of 6.25 for the black soldier fly larvae.
Means with the same letters in the same column are not statistically different at the 95% confidence level, compared using Duncan's multiple range test (DMRT).
* = There was a statistically significant difference ($P < 0.05$)
ns = There was no statistical difference ($P > 0.05$)

In a previous study [18], pineapple peels contained soluble carbohydrates in the form of reducing sugars, including sucrose (70%), glucose (20%), and fructose (10%), which microorganisms living in animal

stomachs can use for fermentation as an important source of energy, and this energy is transferred to herbivorous animals. The black soldier fly larvae are highly nutritious, and the nutrients that accumulate in the bodies of adults and pupal stages contain 44.96-50.56% protein, 16.84-28.89% fat, and 5,520-6,480 kilocalories of energy per kilogram [19]. In the larval and pupal stages of the black soldier fly, protein and fat accumulate within the body when eating food. This is beneficial to adults when mating and laying eggs [20]. A previous study of the use of black soldier fly larvae fed with soy waste powder in a broiler diet [21] showed that the black soldier fly larvae powder had 94.28% dry matter, 45.68% protein, 34.53% fat, 1.22% calcium, 0.8% phosphorus, and a total energy of 6,235 kilocalories per kilogram. Furthermore, testing of the nutritional value of black soldier fly larvae fed on general food scraps and vegetable wastes from the market found that fresh black soldier fly larvae, dried black soldier fly larvae, pupa casings, and black soldier fly carcasses had protein values of 15.23, 33.38, 45.04, and 59.07 grams per 100 grams, respectively, as well as lipid values of 17.64, 39.08, 19.32, and 4.75 grams per 100 grams, respectively [10]. Thus, the black soldier fly larvae can be an alternative source of protein that can be used as a dietary supplement to feed local chickens, organic chickens, fighting cocks, carp fish, ornamental fish, grouper fish, trout, salmon, Nile Tilapia fish, giant freshwater prawns, lobster, pigs, and frogs [7, 19, 22]. Subsequently, it is suggested that agricultural waste has the potential to be used as an ingredient in food for growing black soldier fly larvae.

3.3 Analysis of macronutrient contents of organic fertilizer from the frass of black soldier fly larvae fed with different types of organic waste

At the end of the organic fertilizer process, the black soldier flies larvae frass was obtained from the decomposed organic waste in 4 experimental sets: pineapple peels, banana peels, durian peels, and vegetable scraps. The result of the study revealed that the black soldier fly larvae were always eating food. When organic waste or food scraps are decomposed, they can be turned into organic fertilizer. So, the black soldier fly larvae frass was analyzed for physical and chemical properties to compare with the standard values of organic fertilizer (Table 3). It was found that pineapple peels, banana peels, and vegetable scraps contained a lot of water because these foods have a high moisture content. As a result, the remains of the black soldier fly larvae frass dissolve in the remaining food scraps into a liquid that cannot be used to test the macronutrient contents. Nevertheless, the frass of black soldier fly larvae obtained from the decomposition of durian peels has a moisture content, total nitrogen, total phosphorus, and total potassium that meet the standards for organic fertilizers set by the Department of Agriculture [23], which can be used as a soil amendment material.

Table 3. The quality of the frass of the black soldier fly larvae obtained from the decomposition of organic wastes.

The quality of the frass of the black soldier fly larvae	Durian peels	Criteria*
Moisture content	39.41	Not more than 35%
Total nitrogen	2.46	Not less than 1%
Total phosphorus	2.77	Not less than 0.5%
Total potassium	3.14	Not less than 0.5%

*Announcement of the Department of Agriculture: Organic Fertilizer Standard B.E. 2548 [23]

In addition, it can be used in various ways, such as covering the base of durian trees or making compost, which is the proper management of agricultural residues and helps improve soil, add organic matter, and create a balance of nutrients in the soil. This may be because durian peels are high in potassium and an easily decomposed organic material. The element potassium helps promote the growth of strong roots and stems, promote flowering in plants, and increase quality production [24]. Durian peels are a material that easily decomposes and contains carbon elements. Hence, it is a source of organic matter and plant nutrients, including 1.70% nitrogen, 0.44% phosphorus, and 2.13% potassium [25]. Moreover, a study by the Department of Science Service found that durian peels contain nutrients that plants need, especially potassium and phosphorus, and when mixed with soil in vegetable plots, they can help improve and restore soil health very well, as well as store nutrients and absorb some toxins [26]. Our study is consistent with the previous study [10] that the black soldier flies larvae frass contains essential nutrients for plants, including nitrogen, phosphorus, and potassium, with values equal to 6%, 3.9%, and 5.9%, respectively. This result suggests that

the frass of the black soldier fly larva is suitable for use as organic fertilizer and is a soil amendment for growing vegetables in the kitchen garden.

Based on these findings, evaluating the nutritional components of the black soldier fly fed on other types of organic waste at different life stages is necessary. This will help create a database of suitable substrates for producing black soldier fly larvae and improve the economic level of inclusion in animal feeds [27]. Additionally, the black soldier fly could also serve as a model biorefinery for valorizing various organic resources into biodiesel and other high-value products such as chitin and its derivative (chitosan), a binder (in dyes), edible films, industrial membranes, biodegradable surgical thread, a fining agent (in winemaking), protein hydrolysates, bioplastic, natural pigments (e.g., melanin and ommochromes), a potential source of industrially important enzymes (e.g., trypsin, chymotrypsin, ligninase, and cellulase), and antimicrobial peptides [28, 29].

4. Conclusions

The results of this study indicate that the life cycle of the black soldier fly can be divided into 5 stages: egg stage, larval stage, prepupal stage, pupal stage, and adult stage. When the prepupal stage of black soldier fly larvae fed with different types of organic waste was analyzed for nutritional value, it was found that fresh black soldier fly larvae had protein and fat contents in the range of 9.25-10.69 grams per 100 grams and 3.29-15.90 grams per 100 grams, respectively. This revealed that agricultural waste has the potential to be used as an ingredient in food for raising black soldier fly larvae and as an ingredient in animal feed to reduce the cost of producing animal feed. The frass of the black soldier fly larvae obtained from the decomposition of durian peels contains macronutrient contents for plants, including nitrogen, phosphorus, and potassium, with values equal to 2.46%, 2.77%, and 3.14%, respectively. The quality met the organic fertilizer standard BE 2548 of the Department of Agriculture, Thailand. Accordingly, it can be concluded that the black soldier fly larvae frass can be used as a soil improvement material and for other agricultural purposes. This will help reduce the problem of organic waste management in the community, reduce costs in purchasing fertilizer and animal feed, add value to agricultural wastes, increase income for people in the community and farmers in the area, and be a way to use natural resources with maximum sustainable efficiency.

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