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The Use of Sludge from Cow Manure Biodigester as Fertilizer and Carrier of *Cordyceps* sp. for White Grub Pest Control

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Abstract: Processing cow manure into biogas is a solution to reduce air pollutant, because it reduces the smell of the manure up to 70%. Besides producing biogas, the sludge of biodigester can also serve as solid and liquid fertilizer. The solid fertilizer can be used as carrier of entomopathogenic fungi to control the pest which lives underground. The research aimed to investigate the benefits of biodigester sludge as fertilizer and carrier of entomopathogenic fungi (*Cordyceps* sp.) to control white grub pest. The use of organic fertilizer and pesticide in the cultivation can minimize the environmental hazard. The method used in the study was completely randomized design with four treatments, which were the addition of *Cordyceps* sp. corn media as much as 0, 10, 20 and 30 g/kg of sludge. Every addition was repeated three times to corn planted in a tub. To examine the effect of the treatments, the data were analyzed with ANOVA and Duncan test was applied when differences occurred between treatments. The results of the research showed that the nutrients contained in the manure which had been processed into biogas were not lost. The plants cultivated in the planting medium supplemented with sludge grew healthier, bigger, taller and had stronger and longer roots. On the contrary, the plants cultivated without sludge addition were vulnerable to white grub attack. The *Cordyceps* sp., which was added into the sludge, was effective to control the white grub. The concentration of *Cordyceps* sp. as much as 20 g/kg of sludge in corn planting medium was the most effective concentration to control white grub pest.

Key words: Sludge, pollution, fertilizer, organic pesticide, *Cordyceps* sp..

1. Introduction

If left untreated, the accumulation of livestock waste can pollute the environment. One of the ways to reduce this pollutant is to process the solid wastes from livestock into compost, liquid organic fertilizer, bio-charcoal and biogas. The process of biogas production by anaerobic fermentation of livestock waste also produces sludge sediment from the substrate as the byproduct. The sludge of cow manure has undergone the final stage of anaerobic decomposition to produce methane. The sludge can make a source of carbon for the microbes [1], and the presence of microbes in soil can improve soil fertility. Therefore, the sludge as the byproduct of biodigester can be used as solid or liquid fertilizer.

Sludge originated from biogas plant can make a very good carrier, since it contains a variety of elements needed for the growth of microbes, such as P, Mg, Ca, K, Cu and Zn [2]. As potential organic fertilizer, the existence of N, P, K, Ca, protein and essential amino acids in the sludge can provide a fertile medium for the microbes. Sludge has the same nutrient content as ready-to-use organic fertilizers, like compost. Therefore, sludge can be directly used to fertilize crops. Liquid sludge can be used as liquid organic fertilizer, while solid sludge can be processed into compost by drying and packing it, so that it can be stored for a long time.

While the organic matter in the sludge can serve as

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nutrient source for plants, and the addition of biodigester's sludge into soil can increase its aggregate stability and infiltration rate, thereby reducing the surface runoff. Erosion can be prevented, because the organic material acts as a binding agent between soil particles that can increase soil aggregate and porosity. It can improve soil water holding capacity and cation-exchange capacity that will increase soil fertility [3].

Another benefit of the addition of sludge to soil is that it can be used for plant protection against pest. It is encouraged that pests are controlled with the means and ways that do not disrupt or threaten human's health and safety, nor cause disruption and damage to natural resources or environment. One of the microorganisms that act as biological control agents is fungi, including *Cordyceps* sp.. The group of fungi that infect insects is called entomopathogenic fungi. The entomopathogenic fungi can kill the adult and pre-adult insects (eggs, larvae, pupae) pests. Even if the pupae can live, the imago (adult insect) will have defects, since its wings are not perfectly developed. The solid fertilizer made of sludge can be used as carrier of entomopathogenic fungi to control the pest which lives underground. The objective of this work was to study the benefits of using sludge as fertilizer and carrier of *Cordyceps* sp. to control white grub pests.

2. Materials and Methods

2.1 Research Materials

The main material for this study was sludge from a biodigester which used cow manure obtained from Department of Animal Husbandry and Animal Health, Central Java, with a total N content of 1.28%, total P of 0.16% and total K of 0.87%. The planting media that contained farmland's soil and sand in the ratio of 1:1 was also prepared. The isolate of *Cordyceps* sp. fungus, obtained from Pests and Diseases Observation Laboratory, Temanggung, Central Java, and white grub were also used.

2.2 Research Procedure

2.2.1 Composting

Using a bucket, the sludge from biodigester output was put into a plastic bag, so that the water was reduced. After the water was all gone, the sludge was wind dried on the composting floor, and should not be dried in the sun so that the nutrient contents do not much evaporate along with the water contained in the cow manure.

2.2.2 *Cordyceps* sp. Cultivation

The corn rice was cleaned by washing it with water, then steamed for 10 min and let it cool down. Approximately 100 g of half-cooked corn was put in a heat-resistant plastic bag and sterilized with autoclave. After being sterilized and cold, the corn was inoculated with *Cordyceps* sp. isolates in a sterile room and incubated at temperature of 27 °C. The mycelia of *Cordyceps* will grow evenly in the corn after 7-21 d.

2.2.3 Application of *Cordyceps* sp.

The planting media was a mixture between soil and sand with 1:1 ratio. The fungi *Cordyceps* sp. in the corn media should be destroyed by squeezing them, so that the spores were separated from each other and scattered evenly in the corn media. The corn was then weighed, and 10, 20 and 30 g of the corn should be dissolved in water with the addition of 2 g of sugar/L of water. Then 1 kg of sludge compost was mixed with water that has been given *Cordyceps* sp. and then should be mixed with the planting media and put into the planting tubs. After that, the corn seeds were planted into the planting media and the white grubs were released into it. The amount of white grubs should be released was 10 per planting tub. The media, with the corn and white grub in it, should be observed every week.

2.3 Research Variables

The dependent variables used in this study were the number of dead white grubs infected, the number of damaged plants, the corn plant height and the trunk girth of the corn plants. The independent variable was

the weight of *Cordyceps* sp. in the corn media which was added to the sludge. They were 0, 10, 20 and 30 g per kg of sludge.

2.4 Data Analysis

The method used in this study was the experimental method of completely randomized design with four treatments: control (SC0, without sludge and *Cordyceps* sp.), and the mixture of sludge and *Cordyceps* sp. in the corn media with 10 g/kg (SC1), 20 g/kg (SC2) and 30 g/kg (SC3) sludge. Each treatment was repeated three times. To determine the effect of the treatment, the data were analyzed with ANOVA using SPSS. Duncan test would be conducted if there were differences.

3. Results and Discussion

3.1 The Girth and Height of Corn Plants

There were differences between the growth of the corn plants in media with sludge addition compared to the one without sludge addition. The evidence could be observed in the girth and the height of corn plants in the age of four weeks, as shown in Table 1 and Table 2, respectively.

It was showed that SC0 treatment (without sludge addition) had very different result when compared to the other three treatments with sludge addition. The availability of nutrients, such as N, P and K, in the sludge made the plants grow better compared to the ones growing without sludge.

The corn plants' height in SC1, SC2 and SC3 treatment was also different from the one under SC0 treatment (without the sludge addition) which has the lowest girth (1.85 cm) and height (3.87 cm). It was highly possible that the macro elements contained in the sludge made the plants grow healthier and stronger compared to the one growing in the media without the sludge addition, like the dwarf one in SC0. The existence of those elements in the sludge made the plants grow healthier and stronger compared to the one growing in the media without the sludge addition.

The data also showed that there were differences in height and girth of the corn plants in SC1, SC2 and SC3 treatment. However, the ANOVA results indicated that the data had no big difference ($P < 0.05$). This might occur because of the interaction between abiotic and biotic factors in the field. The plant growth was not only influenced by the availability of nutrients alone. The environmental factors like sunlight, humidity, and temperature also affected their growth.

3.2 The Influence of Cordyceps sp. Concentration on White Grubs Mortality

Cordyceps sp. can infect many orders of insects, such as Orthoptera, Isoptera, Hemiptera, Coleoptera, Diptera and especially the larvae and pupae of the Lepidopteraorder. *Cordyceps* sp. contains cordycein acid which is a toxic material if injected into insects [4]. This fungus can disrupt the central nervous system. The influence of *Cordyceps* sp. concentration on white grubs mortality was presented in Table 3.

Table 3 showed that in SC0 treatment, where the planting medium was not given the addition of sludge and *Cordyceps* sp., the white grubs mortality rate was 0%, i.e., no white grub was dead. They can stay alive by eating the roots of the corn plant and stay healthy,

Table 1 Average girth of corn plants in planting media with sludge and *Cordyceps* sp..

No.	Treatment	Girth (cm)
1	SC0	1.85[a]
2	SC1	3.95[b]
3	SC2	3.81[b]
4	SC3	3.60[b]

There were no big differences between the numbers in the same column followed by the same letter at the test level of 5%.

Table 2 Average height of corn plants in planting media with sludge and *Cordyceps* sp..

No.	Treatment	Height (cm)
1	SC0	3.87[a]
2	SC1	81.00[b]
3	SC2	77.30[b]
4	SC3	76.60[b]

There were no big differences between the numbers in the same column followed by the same letter at the test level of 5%.

Table 3 Average mortality of white grub in planting media with sludge and *Cordyceps* sp..

No.	Treatment	Mortality of white grub (%)
1	SC0	0.0[a]
2	SC1	16.7[b]
3	SC2	20.0[b]
4	SC3	16.7[b]

There were no big differences between the numbers in the same column followed by the same letter at the test level of 5%.

because they were uninfected by *Cordyceps* sp. fungi. On the contrary, in each treatment using *Cordyceps* sp., some white grubs were found dead, allegedly being infected since they had hardened body like mummies and did not rot. If the dead white grubs were not infected by *Cordyceps* sp., the size of the body would remain the same and they gradually decayed.

Infection mechanism started with the attachment of conidia on the larval cuticle, and then the conidia entered the haemolymph by penetrating into the cuticle. In the haemolymph, the fungi grew and secreted enzymes and toxin which will damage the body tissues of the larvae and reduce their activity. The insects infected by entomopathogenic fungi can be determined from the lack of mobility and ability to eat [4]. The pathogenic fungi *Cordyceps* sp. penetrated directly into their bodies through the skin or integument. After the fungi's conidia had entered the bodies of the white grubs, they reproduced themselves through the formation of hyphae in the network of epicuticle, epidermis, hemocoel and other tissues. In the end, all of the networks are filled with the fungal mycelia. The infection mechanism in enzymatic and chemical ways will cause the rise in blood pH level, blood clotting and cessation of blood circulation in the body of the insects, which in turn will cause death and the dead larvae will be overgrown by mycelia [5].

Duncan range test showed that there was big difference between the result of control treatment (SC0) and the treatments with *Cordyceps* sp. application. However, this was not the case for the three treatments with different concentration levels of *Cordyceps* sp.. The results were only slightly different. This was presumably due to the failure of the germination of conidia *Cordyceps* sp. within the condition that had less moisture and high exposure to sunlight. The germination of conidia requires relative humidity above 90% and the optimum temperature ranges from 20 °C to 30 °C [6]. The conidia *Cordyceps* sp. on the leaves which are exposed to direct sunlight will lose their viability and virulence against the host by 50% to 100% within 24 h to several days [7]. The effectiveness of entomopathogenic fungi is determined by environmental conditions, such as rainfall and sunlight, especially ultraviolet rays that can damage the fungal conidia [8].

Conidia are one of the infective organs (propagule) of fungi that can cause infections in the integument of insects and lead them to death. The high dose of entomopathogenic fungi given to the targeted insects can increase the contact between the fungi and insects [9]. This in turn will speed up the dying process of the infected larvae.

3.3 The Effect of Cordyceps sp. on Plant Damage

The number of conidia will determine the effectiveness of entomopathogenic fungi in controlling the tested insects [8]. At a low conidial density, the fungi are not able to break down the chitin and fatty layers of the insects' cuticle, so that the penetration and infection do not occur.

In the control without the application of *Cordyceps* sp. (SC0), there were the highest (20%) damaged plants. It was because that the plants were less healthy without the additional fertilizer from the sludge, so the roots were not strong. Moreover, the white grubs which lived in the growing media were in healthy condition, since they were not infected by *Cordyceps* sp.. They could move freely and eat the roots of the corn plants to survive.

As presented in Table 4, in the treatments SC2 and SC3, the level of plant damage was 0%. It was presumably

Table 4 Average damage of corn plants in planting media with sludge and *Cordyceps* sp..

No.	Treatment	Damaged plants (%)
1	SC0	20.0[b]
2	SC1	3.3[a]
3	SC2	0.0[a]
4	SC3	0.0[a]

There were no big differences between the numbers in the same column followed by the same letter at the test level of 5%.

because the white grubs were already infected by the fungus *Cordyceps* sp. with the higher concentration (20 g and 30 g), so that their activity started to decrease, their movement began to slow down, their eating activity began to decline, they did not move around much and their bodies were bent like letter C. The concentration of *Cordyceps* sp. as much as 20 g/kg of sludge in corn planting medium was the most effective concentration to control white grub pest.

4. Conclusions and Recommendation

The result showed that sludge, the byproduct of manure biogas, was a good carrier of fungus *Cordyceps* sp. without reducing the nutrients contained in the sludge. And the plant growing with the mixture of sludge and *Cordyceps* sp. in its planting medium could grow better when compared to the one growing without it, which can be determined from the big differences in the plant's height and size of girth.

Entomopathogenic fungus *Cordyceps* sp. made a good biopesticide to control destructive insect pest, like white grubs. The mortality level of white grubs treated with *Cordyceps* sp. was much higher than the one without *Cordyceps* sp. Thus, the sludge from cow manure biodigester could be used as carrier of entomopathogenic fungus to eradicate the pests that lived in the soil.

It often fails to detect early the white grub attacks, therefore, it is recommended to add *Cordyceps* sp. into the basic fertilizer when preparing the land for growing crops, so that the growth of the pests can be controlled from the very beginning.

Although the concentration is low, the addition of sludge from cow manure biodigester can support the plants with essential nutrients. It is recommended to investigate further the addition of other organic materials that can enhance the nutrient content in the sludge in order to reduce the need for inorganic fertilizers that are not environmentally friendly because it leaves residue.

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