Increasing Population of *Daphnia Magna* Natural Feed Using the Probiotic Effective Microorganisms-4 (EM_4)

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Abstract

This study aims to determine the effectiveness dose of EM_4 (Effective Microorganism-4) pro-biotic to increase the population of *Daphnia magna* natural feed. This study used a completely randomized design (CRD) with three treatments and three replications. The treatment in this study used pro-biotic EM_4 with a dose of 1 ml for treatment A, a dose of 3 ml for treatment B. 5 ml dose for treatment C and a dose of 7 ml for treatment D. The highest population growth rate was found in treatment A dose 1 ml. Followed by treatment B with a dose of 3 ml, treatment C at a dose of 5 ml and the lowest at treatment D at a dose of 7 ml with values of 0.29, 0.09, 0.05 and 0.03 respectively. ANOVA analysis showed that the provision of pro-biotic EM_4 had an effect on the population growth rate of *Daphnia magna*. The data from the further test of the least significant difference (LSD) showed that treatment A was significantly different from treatment B, C and D. Treatment B, C and D were not significantly different. The results of water quality measurements for temperatures ranging from 26-29 ° C, pH ranges from 5-6 and Dissolved Oxygen (DO) ranges from 4.4-4.9 mg / liter. This condition is the optimum range in *Daphnia magna* culture.

Keywords: *Daphnia magna*; EM_4 (Effective Microorganisme-4); population increase

I. Introduction

Until now, *Daphnia magna* cultivation techniques have been carried out in many studies on feed nutrients that are suitable for its growth, but there are still deficiencies (Mubarak et al., 2010). Using manure or vegetable waste will result in the medium becoming less hygienic because it will stimulate pathogenic microbes (Prastya et al., 2016).

The lack of hygiene of the *Daphnia magna* culture media can contaminate the fish culture media that will be fed with *Daphnia magna* natural feed. Media and food sources containing poor nutrition are one of the inhibiting factors for population growth of *Daphnia magna*. Population growth itself can be interpreted as an increase in the number of individuals at a certain time in a population. To achieve this, it is necessary to find other alternatives, so that quality and continuous supply of *Daphnia magna* can be obtained.

Apart from phytoplankton, feed for *Daphnia* can also be suspended organic particles and bacteria (Suwignyo, 1998). The *Daphnia* requires nutrients for its growth. These nutrients can be obtained from suspended organic matter, plankton, and bacteria obtained from feed added to the culture media (Prastya et al., 2016).

EM_4 (Effective Microorganism-4) technology is a mixed culture of several microorganisms that are beneficial for plant growth (Winedar et al., 2006). EM_4 contains 90% of *Lactobacillus* sp. (lactic acid producing bacteria) phosphate solvent, photosynthetic bacteria, *Streptomyces* sp, cellulose-decomposing fungi and yeast. EM_4 is an addition to optimize the use of food substances because the bacteria contained in EM_4 can digest cellulose, starch, sugar, protein and fat (Surung, 2008).

II. Research Methods

The tools used in this study consisted of a jar container, oxymeter, thermometer, lacquer paper, measuring cup, tablespoon, blower, aeration hose, aeration valve and aeration stone. The materials used were clean water, EM_4 (Effective Microorganism-4) and *Daphnia magna*.

Daphnia magna population counts are carried out directly using the sense of sight (eyes) every day in the morning. The jar was lighted and counted. At times the population can no longer be counted using the eye. Then cleaning is to be done and the counting is continued in a petri dish.

The population was tabulated in order to calculate the population growth rate. According to Kusumaryanto (1988) in Dina (2002) the population growth rate of *Daphnia* is calculated from the first day to the peak of the population using the formula.

The data of Daphnia magna population growth were tested using the one-way analysis of variance test (ANOVA) to determine the effect of each treatment. Treatments that had an effect were then further tested using the least significant difference (LSD) with an 99% confidence to determine the differences between treatments. Water quality data were analyzed descriptively (Putri, 2016). Quantitative data analysis was using the SPSS version 23 application.

III. Results and Discussion

3.1 Polpulation Increase of *Daphnia magna*

Daphnia magna with the addition of EM4 at different doses experienced increases and decreases in different populations. The graph of the increase in the *Daphnia magna* population can be seen in Figure 1 below.

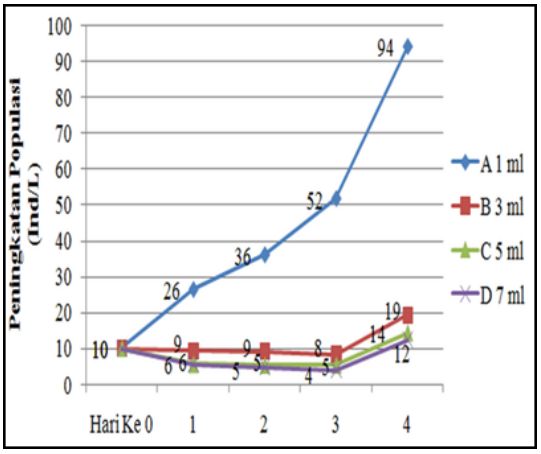

Figure 1. Population increase of *Daphnia magna*

Based on Figure 1 above, the highest population increase after the first day to the fourth day is the addition of 1 ml of EM_4 dose, with a population of 94 individuals / liter at the end of the observation. This is influenced by the presence of organic matter and bacteria contained in the culture media that can be used directly by *Daphnia magna* as a food source, resulting in an increase in the number of new individuals.

According to Darmanto et al., (2000) eating habits of *Daphnia*, by making a flow in the media, namely by moving an additional device in the mouth, so that the feed enters his mouth. Types of feed that are good for *Daphnia* growth are bacteria, phytoplankton and detritus. This statement is confirmed by Mokoginta (2003). *Daphnia* feeds on a variety of bacteria, yeast, single-celled algae, and detritus. Bacteria and fungi top the list of nutritional value. While the main feed for *Daphnia* is algae and protozoa. *Daphnia* takes its food by filtering food or "filter feeding".

Delbare and Dhert (1996) state that only small feed can be consumed. *Daphnia* sp. is a group of small crustaceans that are non-selective filter feeders, easy to culture, fast harvest time and can be enriched with certain ingredients. Further reinforced by the statements of Priyambodo and Wahyuningsih (2001), *Daphnia* sp. is a non-selective filter feeder, which eats whatever size corresponds to the opening of its mouth. *Daphnia*

sp. are bacteria, phytoplankton, algae, diatomes, protozoa and detritus.

In treatment B, C and D with the addition of EM_4 doses of 3, 5 and 7 ml, on the first day showed a decrease in population until the third day and after the third day each culture media showed an increase in population numbers that were not much different, namely 19, 14 and 12 individuals / liter. The decrease in the population of *Daphnia magna* during culture may be influenced by the over dose of EM_4. EM4 concentrations at doses of 3.5 and 7 ml are thought to be able to inhibit oxygen diffusion in *Daphnia magna*, so that from the beginning of the culture, many adult that were stocked had died. This can be seen in the water color of the culture medium to become thick. The concentration of this culture medium may be influenced by substances contained in EM_4 probiotics that are given excessively or called lethal doses.

According to Rahayu and Piranti (2011), the availability of dissolved oxygen is one of the triggers for the decline in environmental conditions / culture media of *Daphnia*, which has the potential to result in a decrease in population. Sanyoto (2000) states that one of the causes of the decline in the *Daphnia* population is the death of several adult *Daphnia* who are unable to adapt to the new environment.

Dina (2002) argues that a very high concentration of fertilization using organic matter can contaminate the culture media due to organic matter which cannot be oxidized and can produce CO2 gas. In an environment that contains excessive organic matter it can be anaerobic which can inhibit the diffusion of dissolved oxygen by *Daphnia*. As a result, *Daphnia* lacks oxygen and experiences death.

3.2 Population Growth Rate of *Daphnia magna*

The data obtained from the population of *Daphnia magna* are then tabulated to calculate the population growth rate as can be seen in Table 1 below:

Table 1. Population growth rate of *Daphnia magna*

Repetition	EM_4 Treatments			
	Dose 1 ml	Dose 3 ml	Dose 5 ml	Dose 7 ml
1	0,40	0,11	0,06	0,03
2	0,17	0,09	0,07	0,05
3	0,31	0,08	0,03	0,01
Mean	**0,29**	**0,09**	**0,05**	**0,03**

The growth rate is the increase in number of individuals at a certain time in a population. The highest population growth rate was found in treatment A using EM_4 at a dose of 1 ml. Whereas for treatment B at a dose of 3 ml, C at a dose of 5 ml and D at a dose of 7 ml, the growth of *Daphnia magna* was inhibited because of death.

The data from the calculation of the population growth rate (Table 1) above, clarifies the results of the observations in Figure 1 that the highest *Daphnia magna* population growth rate is at a low dose of 1 ml / l of water. While the EM_4 doses of 3, 5 and 7 ml the average growth of *Daphnia magna* was the lowest. This is thought to be in addition to the concentration of the culture medium, namely *Daphnia magna* at higher doses, requiring a longer adaptation process. It is possible that after 4 days of adaptation, *Daphnia magna* can grow rapidly. Because of the availability of food sources that are abundant and untapped.

Daphnia magna population growth rate data (Table 1), in each treatment then tabulated into a table to perform a statistical test of Analysis of Variance (ANOVA). The data obtained from the analysis are presented in Table 2 below:

Table 2. ANOVA analysis

Sumber Keragaman	derajat bebas	Jumlah Kuadrat	Kuadrat Tengah	F_{hitung}		F_{tabel} 5%	F_{tabel} 1%
Perlakuan	3	0,13	0,04	12,57	* *	4,07	7,59
Galat	8	0,03	0,00				
Total	11	0,16					

The two star sign (**) in Table 2 above indicates that the daily population growth of *Daphnia magna* with stocking densities of 5, 10 and 15 individuals / liter has a very significant effect at the 1% level. So a further test was carried out using the Least Significant Difference (LSD) test. This is intended to see the extent of the differences in each treatment. The results of the LSD test showed that treatment B, C and D were not significantly different and treatment A was very significantly different with treatment B, C and D. Data on each treatment showed that the numbers followed by different letters meant significantly different at the level of α 1%.

According to Zahidah et al., (2012) the growth of *Daphnia* consists of an adaptation phase, an exponential phase, a stationary phase and a death phase. The adaptation phase is the stage for *Daphnia magna* to adapt to the new culture container.

The adaptation phase shows the same results between treatments B, C and D, the adaptation phase lasts on day 0 to day 3 and treatment A does not show any adaptation stage. Treatment A showed *Daphnia magna* quickly adjusted to the new culture container. This is because the administration of 1 ml of EM_4 is optimal for the growth of *Daphnia magna*. The exponential phase in treatment A occurred from day 0 to day 4. For treatment B, C and D occurred on the same day, namely the 4th day. This is presumably due to the high dose of EM_4 given so that *Daphnia magna* requires a longer adaptation process.

The high population growth rate in treatment A is supported by the ability of *Daphnia magna* which is able to optimize feed and water quality parameters in the culture container and also the EM_4 dose is still within the tolerance range of *Daphnia magna*. This means that the addition of a 1 ml dose of EM_4 can increase the population growth rate of *Daphnia magna*. The contents and nutrients contained therein can be used by *Daphnia magna* to grow and reproduce. This is in accordance with the research of Ruslan et al., (2009) in Izzah et al., (2014). The results of his research using 1 ml of EM_4 were the optimum results for the growth of *Daphnia*.

According to Hardianto (2004) EM*4* is a solution consisting of a mixed culture of useful microbes and functions as a bioinoculant. The main organisms contained in EM*4* cultures include: photosynthetic bacteria, lactic acid bacteria, yeast, actinomycetes, and fermented fungi. Kendali et al., (2015) added that EM_4 contains nutrients in the form of C-organic and nitrogen.

Djarijah (1995) stated that *Daphnia*'s main diet consists of micro plants (phytoplankton), remains of crushed organic matter, and microorganisms (zooplankton). This statement is reinforced by Rahmawati (2008) that *Daphnia magna* is a filter animal. Feeder that is filtering water to get feed in the form of various kinds of bacteria, yeast, single-celled algae, detritus and dissolved organic matter.

Fertilizers that are often used in *Daphnia* culture are organic fertilizers derived from livestock manure, the type often used is chicken manure. The process of decomposition of organic fertilizers will grow bacteria which in turn will be used as feed for Daphnia (Zahidah et al., 2012). Djarijah (1995) states that the amount of fertilizer 2-5 grams of dry chicken manure and 0.2 grams of coconut meal flour / liter of water can be used for *Daphnia* culture.

3.3 Water Qualities

Results of water quality measurements carried out during the study can be seen in Table 3.

Table 3. Water quality measurements

Treatments	Parameters	Day of measure	
		Begin	End
A	DO (mg/l)	4,4	3,6
	Temp (^{0}C)	25	29

	pH	7	7
B	DO (mg/l)	3,4	3,4
	Temp (^{O}C)	29	29
	pH	7	7
C	DO (mg/l)	3,2	3,4
	Temp (^{O}C)	29	29
	pH	6	6
D	DO (mg/l)	3,0	3,2
	Temp (^{O}C)	30	29
	pH	6	6

The water quality data above (Table 3) shows that pH and temperature are the optimum ranges for *Daphnia magna* cultivation. However, the DO range of water in treatment B, C and D is below the optimum limit, thus inhibiting the growth of *Daphnia magna*. Based on the research results of Prastya et al., (2016) dissolved oxygen ranges from 3.93-4.23 mg / l, pH 8.7-92 and a temperature of 28.1-28.4 are the optimum ranges for Daphnia magna cultures. It is confirmed by Delbare and Dhert (1996) that, temperature 22-32ºC, DO> 3.5 mg / l, pH 6-8 are optimum for the growth of *Daphnia*.

IV. Conclusion and Suggestion

Probiotic EM4 (Effective Microorganism-4) gives an effect on the population growth rate of *Daphnia magna*. The highest population growth rate was treatment A with a dose of EM4 1 ml. Further research is needed with a longer observation time.

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