Density of Infusoria on The Substrate of Banana Leaves, Ketapang Leaves, Rice Straw and Corn Husk

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Abstract – *One of the natural foods used by fish breeders is infusoria, which is a small group of single-celled organisms. The infusoria plant substrates that can be used include banana leaves, ketapang leaves, rice straw, and corn husk. In practice, infusoria cultivators do not know the type and mass of the substrate that has the maximum density output of the infusori cultivated. The study aims to evaluate the influence of the type and mass of the substrate on the infurosia density. This study is an experimental study of the Complete Random Design (CRD) two-factor treatment of the substrate type (banana leaves, ketapang leaves, rice straw, and corn husk) and the mass of the substratum (1 g and 5 g) with 5 repetitions. The research phase includes sample preparation and sample analysis. Sample analysis includes measurement of pH, temperature, density. Data analysis using Two Way ANOVA and Tukey tests at a significance level of 5%. The results of the study showed that the type and mass of the substrate had a significant influence (P sig. <0.05) on the infusory density and there was no interaction between the two variables. The corn husk has the lowest density and differs significantly from the substrates of banana leaves, ketapang leaves and rice straw. The mass of the substrate 5 g has the highest density compared to the mass of 1 g.*

Keywords: *Infusoria, Density, Mass, and Substrate*

INTRODUCTION

Cultivation in the fisheries sector can be hindered, one of which is the availability of feed, especially for fish larvae. The availability of feed with suitable quality and protein content is crucial for producing high-quality fish seeds (Renita et al., 2016). One suitable type of feed for fish larvae is natural feed consisting of phytoplankton, zooplankton, and benthos. One type of natural feed widely developed by cultivators is infusoria. Infusoria are single-celled organisms, generally consisting of ciliate and flagellate groups. Infusoria are small, soft-bodied organisms with high nutritional value, making them suitable as natural feed for fish, especially at the larval stage (Mukay et al., 2017). Infusoria can be seen with the naked eye when clustered with a white color on the water surface. Infusoria reproduce by cell division and conjugation. Their habitat can be found in shaded freshwater areas with abundant aquatic vegetation because they do not thrive in sunlight (Fitriani et al., 2019).

Infusoria are widely used by cultivators as natural feed due to their numerous advantages. Apart from having high protein content (approximately 36.82%), infusoria also have dense cells, are non-toxic, and reproduce rapidly (Fitriani et al., 2019). The presence of vitamins in them also promotes rapid growth and health in fish larvae. Infusoria are also suitable as natural feed, especially for fish larvae measuring <1.1-1.5 cm (Akbar, 2016). Several species of fish larvae commonly utilizing infusoria as feed include Peres fish (*Osteochilus sp.*) (Akhyar et al., 2016), guppy (*Poecilia reticulata*) (Maarti, 2021), and betta (*Betta sp.*) (Awaludin et al., 2019).

Typically, infusoria cultivators use readily available materials from the surrounding environment as substrates for infusoria growth. Commonly used materials include organic substances containing nutrients that enhance infusoria population growth. Some materials tested for infusoria cultivation include water spinach, cabbage, papaya, banana stems, and kipahit leaves as growth substrates (Darmanto et al., 2000 in Sambode et al., 2013). Other commonly used materials by infusoria cultivators include banana leaves (*Musa paradisiaca*), ketapang leaves (*Terminalia catappa*), rice straw (*Oryza sativa*), and corn husk (*Zea mays*). These materials are known to have relatively high organic content, making them suitable substrates for infusoria growth. The organic materials contained in these substrates are utilized by bacteria for growth and development. Bacteria are then utilized as a food source, and the substrate decomposition products, detritus, are also utilized by infusoria for growth (Fitria et al., 2018). Moreover, these materials are easily obtainable in abundance and are underutilized.

However, in practice, infusoria cultivators are unaware of the types and mass of substrates that yield maximal infusoria density from the cultivated results. Cultivators only utilize substrate types available in the surrounding environment and use an uncertain substrate mass. Based on this description, researchers became interested in examining the density of infusoria on each type of substrate used.

The aim of this research is to evaluate the influence of substrate type and mass on infusoria density. It is hoped that this research can be applied in fisheries cultivation, especially in natural feed cultivation, and can serve as a reference for further research.

MATERIALS AND METHODS

This research was conducted from September to October 2021 at the Laboratory of Ecology and Environment (AU 11), Faculty of Biology, Satya Wacana Christian University, Salatiga..

1. Materials

The tools used in this research included 1.5 L plastic bottles used as infusoria culture containers, scissors, Lutron pH-201 pH meter, thermometer, analytical balance, microscope, glass slides, oven, and camera. The materials used included distilled water, fish pond water as the medium for infusoria growth, dried banana leaves, dried ketapang leaves, dried rice straw, and dried corn husk as substrates for infusoria growth.

2. Research Design

This research was an experimental study with a Completely Randomized Design (CRD) involving two treatment factors: four types of substrates, namely banana leaves, ketapang leaves, rice straw, and corn husk, and two substrate masses, namely 1 g and 5 g (Insanni, et al. 2022). Each type of dried substrate was washed with clean water, then dried in an oven for 2 hours at 80°C to standardize the moisture content and weighed to obtain 1 g and 5 g masses. Each substrate was cut into small pieces and placed in 1.5 L bottles with 1 liter of pond water added. The filled bottles were then placed in a shaded area away from direct sunlight.

3. Parameters

The parameters measured were infusoria density, pH, and temperature on days 2, 4, 6, 8, 10, 12, and 14. Infusoria density was determined based on calculation results and identification using a microscope. The density was calculated by total count on each sample using the swipe method on glass slides, expressed as individuals per liter. Mathematically, infusoria density was determined using the formula (Dewiyanti et al., 2015):

$$N = \frac{q}{f \times v}$$

N = Infusoria density per liter (ind/L)

q = Infusoria abundance

f = Fraction taken (sub-sample volume per sample volume)

v = Filtered water volume

pH measurement was conducted using a pH meter according to the procedure outlined by Zamaruddin (2018), by inserting the pH meter electrode into the water sample until a stable reading was obtained, previously calibrated with distilled water. Temperature was measured using a thermometer by immersing it in the sample for several minutes until a constant temperature was reached, then recording the obtained temperature (Mukarromah, 2016)..

4. Data Analysis

Data on infusoria density were analyzed using SPSS version 22, including tests for homogeneity and normality, followed by Two-Way ANOVA and mean analysis using Tukey's test at a significance level of 5%.

RESULT AND DISCUSSION

1. Infusoria Density

The use of different substrates resulted in variations in infusoria population density, depending on the mass of each substrate. Substrates with a mass of 5 g had a higher population density compared to substrates with a mass of 1 g for all types of substrates used. Further data can be seen in Figure 1 and Figure 2.

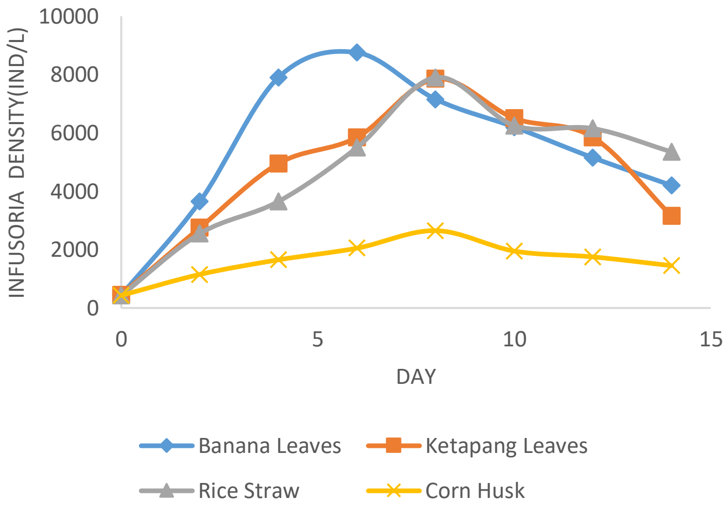

Figure 1. Infusoria Density of Each Substrate Type with a Mass of 1 g.

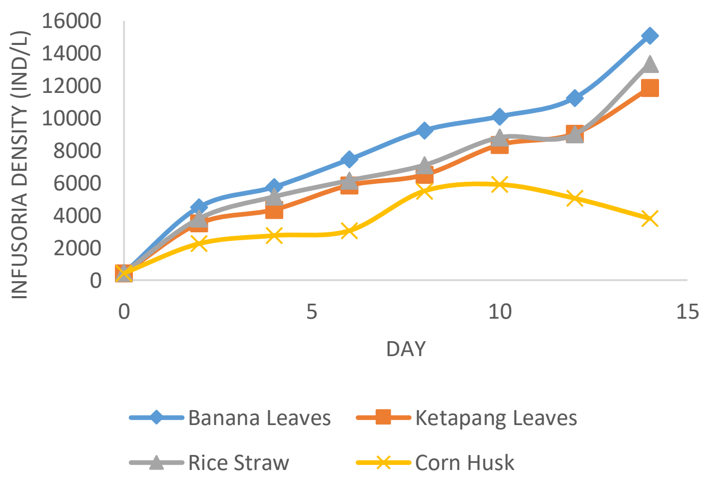

Figure 2. Infusoria Density of Each Substrate Type with a Mass of 5 g.

The density patterns of infusoria in each treatment show distinct variations, especially in the highest infusoria density. The use of different substrate types has a significant effect on infusoria density, as indicated by the ANOVA test with a P value of (0.003) < 0.05. Treatment with banana leaves substrate had the highest density, while the use of corn husk substrate resulted in the lowest infusoria density and was considered less suitable as infusoria growth substrate. This may be due to the significant difference in cellulose content between the two. Banana leaves have a cellulose content of 10.85% (Mayun, 2007 in Suparti et al., 2018), while corn husk has a cellulose content of 31-39% (Mendes et al., 2014). Cellulose is insoluble in water and the longer its chains, the stronger and more resistant it is to the effects of chemicals, light, and microorganisms due to its strong intra- and intermolecular hydrogen bonds in its structure (Mulyadi, 2019). This makes it difficult for organic matter decomposing bacteria to break down the substrate into detritus, which would be used as a food source for infusoria. Further data on the influence of each substrate type on infusoria density can be seen in Table 1.

Table 1. Average Infusoria Density on Each Substrate Type from Day 0-14.

Substrate Type	Population Density (Individuals/Liter)
Banana Leaves	6700.43 ± 0.82[a]
Ketapang Leaves	5720.37±0.83[a]
Rice Straw	5640.81 ± 0.85[a]
Corn Husk	2800.06 ± 0.83[b]

Note: Different superscripts within the same column indicate significantly different results (P < 0.05). The displayed data represent Mean ± SE (n = 5)

Based on post-ANOVA tests, it was found that corn husk substrate significantly differed in mean compared to other substrate types, while banana leaves, ketapang leaves, and straw substrates did not show significant differences in density. The observed variations in population density among treatments are presumed to be due to differences in organic elements present in each substrate. Banana leaves contain 47.8% C, 6.02% H, 44.95% O, and 1.75% N (Lokahita et al., 2016). Ketapang leaves contain 43.219% C, 5.77% H, 39.312% O, and 0.648% N (Shah and Ghodke, 2017). Rice straw contains 45.2% C, 6.2% H, 44.1% O, and 0.8% N (Wannapeera et al., 2008). Meanwhile, corn husk contains 31.06% C, 3.63% H, 32.63% O, and 1.09% N (Maj et al., 2019). It is evident that each substrate contains different organic elements. Banana leaves have the highest organic content, while corn husk has the lowest percentage of organic elements. This disparity influences the availability of organic elements for infusoria growth across different substrate types. Additionally, differences in detritus availability and bacterial quantities in the culture media are possible, relating to bacterial metabolism in decomposing organic matter into detritus on each substrate.

Figures 1 and 2 also illustrate that the decline in population density varies among treatments on different days. For treatments with a mass of 5 g, a decrease in infusoria population density is not evident except for corn husk substrate treatment. By the end of the observation period (day 14), treatments with a substrate mass of 5 g for banana leaves, ketapang leaves, and rice straw still exhibited high infusoria population density. This suggests that nutrient availability in substrate form may still support infusoria growth.

According to Desmawati et al. (2020), optimal nutrient conditions lead to rapid growth and high environmental carrying capacity, whereas low nutrient conditions and declining environmental conditions inhibit reproduction, resulting in predation and population decline. This observation is further supported by the ANOVA test results for substrate mass, with a P value of (0.01) < 0.05, indicating significant density differences between mass 1 g and 5 g substrates.

2. Temperature and pH

Temperature and pH measurements were used to indicate the environmental conditions of the culture media. The temperature and pH measurement ranges of the infusoria culture media were relatively consistent. Further details can be seen in Tables 2 and 3.

Table 2. Temperature of Infusoria Culture Media for Each Treatment.

Treatment	Temperature (°C) Day							
	0	2	4	6	8	10	12	14
Banana Leaves 1 gram	25.6	25.6	25.8	26	26	26.5	26.5	26.6
Banana Leaves 5 Gram	26.1	26.1	26.3	26.5	26.6	26.6	26.8	26.9
Ketapang Leaves 1 gram	25.3	25.3	25.3	25.5	25.8	26.2	26.2	26.2
Ketapang Leaves 5 gram	25.8	26	26	26.2	26.4	26.5	26.5	27
Rice Straw 1 gram	25.8	26	26.1	26.3	26.3	26.5	26.6	26.7
Rice Straw 5 gram	26	26	26.2	26.5	26.7	27	27	27
Corn Husk 1 gram	25	25	25.1	25.3	25.6	25.8	26	26.2
Corn Husk 5 gram	25.1	25.2	25.5	25.8	26.1	26.3	26.5	26.5

Table 3. pH of Infusoria Culture Media for Each Treatment

Treatment	pH Day							
	0	2	4	6	8	10	12	14
Banana Leaves 1 gram	7.15	6.95	6.85	6.65	6.55	6.45	6.45	6.45
Banana Leaves 5 Gram	7.15	7.10	7.00	6.95	6.65	6.30	6.30	6.30
Ketapang Leaves 1 gram	7.35	7.30	7.15	6.90	6.75	6.65	6.55	6.50
Ketapang Leaves 5 gram	7.20	7.05	6.90	6.75	6.65	6.55	6.35	6.35
Rice Straw 1 gram	7.25	7.20	7.00	6.65	6.45	6.35	6.35	6.30
Rice Straw 5 gram	7.20	7.10	6.95	6.75	6.65	6.65	6.45	6.40
Corn Husk 1 gram	7.05	7.00	6.95	6.95	6.85	6.65	6.55	6.35
Corn Husk 5 gram	7.10	7.05	7.00	6.95	6.75	6.55	6.35	6.30

In each treatment, both temperature and pH did not exhibit significant differences. Temperature was observed from the beginning to the end, ranging from 25°C to 27°C, while pH ranged between 6 to 7.35. From the research findings, it can be inferred that both temperature and pH parameters remain within the optimal range for infusoria growth. According to Akbar (2016), the optimal temperature for infusoria growth is between 25°C to 27°C. Meanwhile, the optimal pH for infusoria growth ranges from 6 to 7.9 (Fitria et al., 2018). Additionally, temperature and pH are crucial factors for growth, alongside nutrition, as they play a vital role in determining environmental quality. Adequate environmental conditions in the culture media promote optimal infusoria growth. Based on both tables, it can be observed that with prolonged observation, temperature increases while pH decreases. This could be attributed to the decomposition process by bacteria, where one of the by-products is ethanol, leading to a decrease in pH and an increase in media culture temperature. Bacterial metabolism results in the production of acidic products such as lactic acid and other organic acids. The high concentration of H+ ions from these acids, as detected by the pH meter, leads to a decrease in pH value (Mal, 2013).

CONCLUSION

Based on the conducted research, it can be concluded that both the type and mass of substrate significantly influence infusoria density. Corn husk substrate exhibited the lowest density, significantly differing from banana leaves, ketapang leaves, and rice straw substrates. A substrate mass of 5 g resulted in higher density compared to 1 g mass. Banana leaves, ketapang leaves, and rice straw substrates are recommended for use as growth substrates in infusoria cultivation.

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