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EFFECTS OF INDIAN ALMOND LEAF (*Terminalia catappa*) INFUSION-BASED AND LIVESTOCK MANURE-BASED CULTURE MEDIA ON THE PRODUCTIVITY OF *Daphnia* sp

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ABSTRACT

Zooplankton play an important role in aquatic ecosystems by contributing to secondary production and serving as natural food sources for fish larvae. *Daphnia* sp. is widely used in aquaculture because of its high nutritional value, high digestibility, and rapid reproductive rate. However, its growth and productivity are strongly influenced by the nutrient content and availability of natural food in the culture medium. This study aimed to evaluate the effects of culture media based on Indian almond leaf (*Terminalia catappa*) infusion and livestock manure on the biomass and secondary production of *Daphnia* sp. The experiment was conducted under laboratory conditions using two culture medium treatments, with three replicates per treatment. We initially introduced ten individuals into each culture unit and maintained them for 10 days. The observed parameters included temperature, dissolved oxygen, pH, biomass, and secondary production rate. Water temperature ranged from 25.7 to 26.4 °C, dissolved oxygen concentrations ranged from 5.7 to 8.0 mg L^{-1}, and pH ranged from 6.3 to 7.7. *Daphnia* sp. biomass increased throughout the culture period in both treatments. The livestock manure-based medium produced a mean secondary production rate of 17.4931 ± 3.6678 mg L^{-1} day^{-1}, whereas the Indian almond leaf infusion-based medium produced 10.5246 ± 4.1927 mg L^{-1} day^{-1}. However, one-way analysis of variance showed that culture medium type did not significantly affect the secondary production rate of *Daphnia* sp. ($F_{1,4} = 4.6946$; $p = 0.0962$). These findings indicate that both culture media supported *Daphnia* sp. biomass development and secondary production under the experimental conditions. Although the livestock manure-based medium produced a higher secondary production rate descriptively, the difference between the two treatments was not statistically significant.

Keywords: Biomass, Water Quality, Natural Feed, Secondary Productivity, Zooplankton.

1. INTRODUCTION

Zooplankton are an important component of aquatic ecosystems because they contribute to secondary production and serve as a natural food source for fish larvae and various other aquatic organisms. Zooplankton not only influence energy flow within aquatic food webs but can also indicate ecosystem condition, as these organisms are relatively sensitive to environmental changes[1].

One zooplankton group widely used in aquaculture is *Daphnia* sp. This organism has a high nutritional value and can reproduce rapidly through parthenogenesis, allowing its population to increase

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substantially within a relatively short period. Its body size fits the mouth opening of fish larvae, and its high digestibility makes *Daphnia* sp. an effective and efficient natural feed source[2].

To support its growth and reproduction, *Daphnia* sp. requires an adequate food supply. Its primary food source is phytoplankton, particularly small-sized microalgae that can be filtered from the water column. In addition to phytoplankton, *Daphnia* sp. can utilize bacteria, protozoa, detritus, and suspended organic particles as supplementary food sources. Therefore, culture media that support the growth of phytoplankton and other food microorganisms can directly influence the abundance, reproductive rate, and productivity of *Daphnia* sp.

The growth and productivity of *Daphnia* sp. are strongly influenced by culture medium characteristics. Media containing adequate nutrient concentrations can stimulate the growth of phytoplankton and bacteria, which are subsequently utilized as natural food by *Daphnia* sp. [3]. Several studies have shown that culture media enriched with fermented rice bran water, livestock manure, or probiotics can significantly increase the population density and growth of *Daphnia* sp.[4].

Ketapang water was selected because Ketapang leaves contain various organic compounds and secondary metabolites, such as tannins, that can influence the chemical characteristics of the culture medium. The release of these compounds into the water may alter the pH and create relatively stable conditions for aquatic organisms. Bioactive compounds in ketapang leaves are also known to inhibit the growth of certain pathogenic microorganisms, thereby helping maintain the quality of the culture environment[5].

Nevertheless, the effectiveness of ketapang water-based culture media in supporting the productivity of *Daphnia* sp. depends on their ability to provide or promote the development of food resources. The decomposition of organic matter from ketapang leaves may release nutrients and organic particles that bacteria and other microorganisms can use. These microorganisms may subsequently serve as supplementary food sources for *Daphnia* sp. However, when phytoplankton availability in the culture medium is low, the growth and reproduction of *Daphnia* sp. may also become limited.

Livestock manure was selected because it contains organic matter and nutrients, particularly nitrogen and phosphorus, that are required to support phytoplankton growth. Increased availability of phytoplankton and bacteria in the culture medium can provide a more abundant natural food supply for *Daphnia* sp., thereby potentially increasing its population density and productivity[6].

Although both types of culture media have been used in aquaculture, studies directly comparing the effects of ketapang water-based and livestock manure-based culture media on Daphnia sp. productivity remain limited. Most previous studies have evaluated only one type of culture medium or used specific combinations of organic materials[7]. This limitation indicates the need for further investigation to determine which culture medium best provides suitable environmental conditions and food resources to support the growth and reproduction of *Daphnia* sp.

Based on these considerations, this study aimed to compare the productivity of *Daphnia* sp. cultured in two media: ketapang water-based and livestock manure-based. The results are expected to identify a more effective culture medium to increase Daphnia sp. production as natural feed for fish larvae and improve the efficiency of secondary production in artificial aquatic ecosystems.

2. RESEARCH METHOD

Time and Place

This study was conducted from February to March 2026 at the Secondary Productivity Laboratory, IPB University.

Method

This study employed an experimental design with two culture media treatments and three replicates per treatment. The treatments were as follows:

A : Indian almond leaf-infused water-based culture medium.
B : Manure-based culture medium

Procedures

Preparation of Culture Media

The culture media used in this study consisted of an Indian almond leaf-infused water-based medium and a manure-based medium. We prepared each medium before introducing *Daphnia* sp. stock cultures.

Preparation of the Indian Almond Leaf-Infused Water Medium

The dried Indian almond leaves were cleaned to remove adhering impurities and then soaked in freshwater until the water turned brownish. The resulting solution was subsequently filtered to remove leaf residues and coarse particles. The filtered water was then used as the culture medium for Treatment A.

Preparation of the Manure-Based Medium

Animal manure was mixed with freshwater. The mixture was stirred thoroughly and then allowed to stand to facilitate decomposition of organic matter and release nutrients into the water. The solution was then filtered to remove coarse particles. The filtered water was used as the culture medium for Treatment B.

Stocking and Maintenance of *Daphnia* sp.

The *Daphnia* sp. individuals used as the initial stock were selected based on their healthy condition, active movement, and relatively uniform body size. Before stocking, the organisms were gradually acclimated to the respective treatment media to minimize stress from environmental changes. We introduced 10 Daphnia sp. individuals into each culture container. Stocking was done carefully to prevent physical damage and physiological stress. Following stocking, the cultures were maintained under controlled laboratory conditions for 10 days. During the maintenance period, the culture medium volume was kept constant. Freshwater was added when the medium volume decreased because of evaporation. The culture containers were also regularly inspected to prevent the introduction of other organisms that could interfere with *Daphnia* sp. growth.

Data Collection

Water Quality

Water-quality measurements were conducted to determine the environmental conditions of each culture medium. The measured parameters included temperature, pH, and dissolved oxygen concentration[8].

Abundance of *Daphnia* sp.

We determined the abundance of Daphnia sp. by collecting a 100 mL water sample from each culture unit. Before sampling, the culture medium was gently homogenized to ensure even distribution of organisms. The sample was then collected using a pipette, transferred to a Petri dish, and examined under a microscope. The number of observed *Daphnia* sp. individuals was recorded and converted to individuals per liter. Counting was performed three times for each sample to obtain more representative results[9].

$$D = \frac{N \cdot DV}{SV \cdot TV}$$

where:

D	=	zooplankton density individuals (L^{-1})
N	=	number of individuals counted in the subsamples
DV	=	diluted sample volume (mL)
SV	=	total subsample volume counted (mL)
TV	=	total water volume sampled (L)

Biovolume of *Daphnia sp.*

Biovolume measurements were performed to estimate the body size and biomass of *Daphnia* sp. in the culture medium. Three individuals were randomly collected from each culture unit and examined under a microscope equipped with a body-length measurement device. The body length and width of each individual were measured, and biovolume was subsequently calculated using a geometric approximation adapted from Hessen[10]. For calculation purposes, the body of Daphnia sp. was assumed to approximate a right circular cylinder, with body width treated as the cylinder diameter and body length treated as the cylinder height. Individual biovolume was calculated using the following equation:

$$V = \frac{\pi}{4} \cdot\ d^2 \cdot h$$

where:

V = biovolume (mm^3)
d = diameter (mm)
h = body thickness (mm)

Secondary Productivity

Secondary productivity was determined by changes in the abundance and biomass of *Daphnia* sp. over the observation period. Data on abundance, body size, biovolume, and biomass were used to estimate *Daphnia* sp. production in each culture medium. Observations were conducted at the beginning and end of each observation interval. Productivity was expressed as biomass per unit volume per unit time. The productivity values obtained from the Indian almond leaf-infused water-based medium and the manure-based medium were compared to determine which culture medium was more effective in supporting the growth and production of *Daphnia* sp. Secondary production of *Daphnia* sp. was estimated using the instantaneous growth method described by Gillespie and Benke[11].

$$P = g\ .\ B$$

where:

P = secondary production rate (mg/L/d)
g = instantaneous specific growth rate per day
B = biomass (mg/L)

Data Analysis

Data on water quality, abundance, biovolume, biomass, and secondary productivity were presented as means and standard deviations of three replicates. The data were also presented in tables and figures to illustrate changes in each parameter throughout the culture period. Differences in the secondary production rates of *Daphnia* sp. between the Indian almond leaf infusion-based medium and the livestock manure-based medium were

3. RESULT AND DISCUSSION

Water Quality

The results of temperature, dissolved oxygen (DO), and pH measurements in the manure-based culture medium are presented in Table 1.

The temperature of the manure-based culture medium during the study ranged from 25.9 to 26.4 °C. Temperature values remained relatively stable, with the lowest mean of 26.00 °C recorded on Day 2 and the highest on Days 8 and 10 at 26.30 °C. Dissolved oxygen (DO) concentrations ranged from 6.4 to 6.9 mg L^{-1}. The mean DO concentration decreased from 6.90 mg L^{-1} on Day 0 to 6.53 mg L^{-1} on Day 10. Meanwhile, pH values ranged from 6.3 to 7.7 and increased throughout the maintenance period. The mean pH increased from 6.53 on Day 0 to 7.70 on Day 10.

The results of water-quality measurements in the culture medium prepared using Indian almond leaf-infused water are presented in Table 2.

The temperature of the Indian almond leaf-infused water-based medium during the study ranged from 25.7 to 26.4 °C. Temperature values remained relatively stable, with the lowest mean of 25.90 °C on Day 4 and the highest of 26.40 °C on Day 10. Dissolved oxygen (DO) concentrations ranged from 5.7 to 8.0 mg L^{-1}. The mean DO concentration decreased from 8.00 mg L^{-1} on Day 0 to 5.70 mg L^{-1} on Day 10. The pH values ranged from 6.5 to 7.4 and increased throughout the maintenance period, rising from a mean of 6.57 on Day 0 to 7.40 on Day 10. Overall, the temperature in both culture media remained relatively stable throughout the study. Dissolved oxygen concentrations in both treatments decreased, with a greater decline in the Indian almond leaf-infused water-based medium. In contrast, the pH values of both culture media increased during the maintenance period.

Table 1. Water quality parameters of the manure-based culture medium

Day	Temperature (°C)			DO (mg/L)			pH		
	U1	U2	U3	U1	U2	U3	U1	U2	U3
0	26,1	26,3	26,1	6,9	6,9	6,9	7	6,3	6,3
2	26,1	26,0	25,9	6,8	6,8	6,8	6,8	6,5	6,8
4	26,2	26,1	25,9	6,8	6,8	6,8	6,8	6,7	6,8
6	26,2	26,2	26,0	6,7	6,8	6,7	7,1	7	7,1
8	26,4	26,3	26,2	6,7	6,6	6,7	7,4	7,4	7,4
10	26,4	26,3	26,2	6,4	6,6	6,6	7,7	7,7	7,7

Table 2. Water quality parameters of the Indian almond leaf-infused water-based culture medium

Day	Temperature (°C)			DO (mg/L)			pH		
	U1	U2	U3	U1	U2	U3	U1	U2	U3
0	26,0	26,0	26,0	8,0	8,0	8,0	6,5	6,7	6,5
2	26,1	26,0	25,9	6,7	6,8	6,7	6,6	6,5	6,6
4	26,1	25,7	25,9	6,8	7,0	6,7	6,8	6,7	6,8
6	26,3	26,2	26,1	6,6	6,7	6,6	7,2	7,1	7,2
8	26,3	26,3	26,3	6,2	6,1	6,1	7,3	7,3	7,3
10	26,4	26,4	26,4	5,7	5,7	5,7	7,4	7,4	7,4

Changes in *Daphnia* sp. Biomass

Based on Figure 1, the biomass of *Daphnia* sp. in the manure-based culture medium changed throughout the maintenance period. The magnitude of biomass change varied among replicates, indicating differences in population growth among the culture units.

At the beginning of the observation period (t0), the initial biomass values among the replicates were relatively similar. The biomass values recorded for the first, second, and third replicates were 50.30 mg, 58.41 mg, and 59.05 mg, respectively. At the end of the observation period (t5), greater variation was observed among the replicates. The biomass values for the first, second, and third replicates were 190.59 mg, 337.02 mg, and 303.39 mg, respectively.

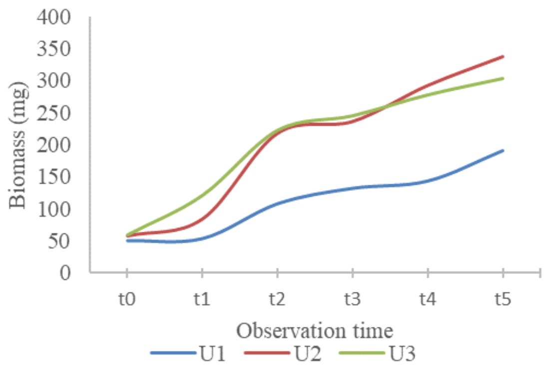

Figure 1. Changes in *Daphnia* sp. biomass in the manure-based culture medium

Based on Figure 2, the biomass of *Daphnia* sp. in the Indian almond leaf-infused water-based culture medium also changed throughout the maintenance period. The biomass change pattern observed in each replicate reflected the response of the *Daphnia* sp. population to the culture medium conditions.

At the beginning of the observation period (t0), the initial biomass values among the replicates were relatively similar. The biomass values recorded for the first, second, and third replicates were 51.67 mg, 59.88 mg, and 59.05 mg, respectively. At the end of the observation period (t5), biomass varied among replicates. The biomass values for the first, second, and third replicates were 95.24 mg, 105.51 mg, and 96.49 mg, respectively.

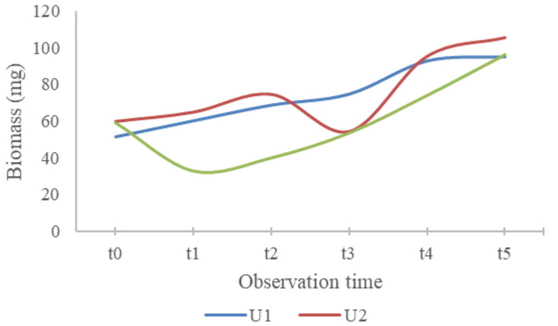

Figure 2. Changes in *Daphnia* sp. biomass in the Indian almond leaf-infused water-based culture medium

Based on Figure 3, the highest secondary production rate was recorded in the third replicate of the livestock manure-based culture medium, reaching 21.5702 mg L^{-1} day^{-1}. The secondary production rates varied among replicates in both culture-media treatments.

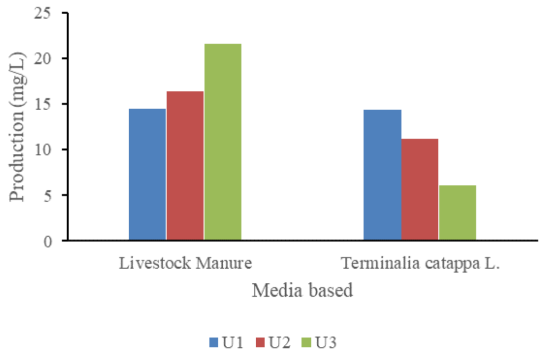

Figure 3. Comparison of total production between treatments

A one-way analysis of variance (ANOVA) was performed to determine the effect of culture-medium type on the total production of *Daphnia* sp. The results showed that the calculated F-value (4.6946) was lower than the critical F-value (7.7086). This indicates that the manure-based medium and the Indian almond leaf-infused water-based medium did not have a statistically significant effect on the total production of *Daphnia* sp.

The growth and biomass of *Daphnia* sp. are influenced by water quality conditions and the availability of food resources in the culture media. During the study, water temperature in both treatments ranged from 25.7 to 26.4 °C, *dissolved oxygen* concentrations ranged from 5.7 to 8.0 mg L^{-1}, and pH ranged from 6.3 to 7.7. Water temperature remained relatively stable in both culture media, whereas dissolved oxygen concentrations decreased and pH values increased throughout the culture period. These conditions allowed *Daphnia* sp. to survive, grow, and reproduce in the culture media. Stable temperature and sufficient dissolved oxygen are important for supporting the metabolic activity, growth, and reproduction of zooplankton (Boersma & Meunier, 2020). In addition to directly affecting the physiological activities of *Daphnia* sp., the characteristics of the culture media also determine the availability of phytoplankton, bacteria, and organic particles that can be utilized as food. *Daphnia* sp. is a filter-feeding organism that obtains food by filtering small particles from the water column. Therefore, culture media that support the development of phytoplankton and microorganisms may provide a more abundant food supply for *Daphnia* sp.

Livestock manure-based culture media contain organic matter and nutrients, particularly nitrogen and phosphorus. The decomposition of organic matter can release dissolved nutrients that support the growth of phytoplankton and bacteria. These organisms can subsequently be utilized as natural food by *Daphnia* sp., thereby supporting population growth and biomass production. The relationship between increased nutrient availability, phytoplankton growth, and zooplankton biomass has also been reported in previous studies[12].

Descriptively, the highest secondary production rate was recorded in the third replicate of the livestock manure-based treatment, reaching 21.5702 mg L^{-1} day^{-1}. This value demonstrates the variability in biomass among the culture units and indicates that the livestock manure-based medium can support *Daphnia* sp. biomass production under certain conditions. The availability of phytoplankton, bacteria, and organic particles in the culture medium may have provided energy sources for individual growth and reproduction. An adequate supply of natural food has been reported to promote the growth and biomass production of *Daphnia* sp. in culture systems.

The Ketapang water-based medium also supported the survival and growth of *Daphnia* sp. However, the biomass recorded in several culture units was descriptively lower than that observed in the livestock manure-based medium. Ketapang leaves contain organic matter, tannins, and various phenolic compounds that can affect the chemical characteristics of the culture medium. These bioactive compounds may inhibit the growth of certain pathogenic microorganisms and help stabilize aquatic conditions. However, the Ketapang water-based medium may not provide nitrogen and phosphorus at concentrations comparable to those supplied by livestock manure. This condition may restrict the development of phytoplankton and other microorganisms that serve as food sources for *Daphnia* sp.

The effectiveness of the Ketapang water-based medium also depends on leaf decomposition and the release of organic matter into the water. Bacteria and other microorganisms that develop during decomposition can be utilized as supplementary food sources. However, tannins and phenolic compounds may also affect the microbial community within the medium. Therefore, the effect of Ketapang water on *Daphnia* sp. biomass is determined not only by water quality but also by the amount of decomposed organic matter and the availability of food organisms.

The ANOVA results showed that the type of culture medium did not have a significant effect on *Daphnia* sp. biomass ($p = 0.0962$). Therefore, although the livestock manure-based medium produced a descriptively higher biomass, the difference was not statistically significant compared with the Ketapang water-based medium.

The absence of a statistically significant difference between the two treatments may be due to variability in biomass across replicates. This variability may have been influenced by differences in individual body size, acclimatization success, organism distribution within the culture vessels, food availability, and microenvironmental conditions in each culture unit. *Daphnia* sp. can reproduce rapidly; therefore, small changes in culture conditions may produce substantial differences in population density and biomass among culture vessels. In addition, using only three replicates per treatment may have limited the statistical power to detect differences when within-treatment variability was relatively high.

The decline in dissolved oxygen concentrations during the culture period may have been associated with increased respiration by the cultured organisms and microbial activity during organic matter decomposition. The decrease in dissolved oxygen was more pronounced in the Ketapang water-based medium, where the concentration declined from 8.0 to 5.7 mg L^{-1}. In the livestock manure-based medium, dissolved oxygen decreased from 6.9 to an average of 6.53 mg L^{-1}. Nevertheless, the *dissolved oxygen* concentrations recorded at the end of the study still supported the survival of *Daphnia* sp. The increase in pH observed in both treatments may have been associated with changes in biological activity, organic matter decomposition, and phytoplankton carbon dioxide uptake.

Overall, both livestock manure-based and Ketapang water-based culture media supported *Daphnia* sp. under laboratory conditions. The highest biomass was recorded in one replicate of the livestock manure-based treatment; however, the ANOVA results showed no significant difference in biomass between the two-culture media. Therefore, the present study does not provide sufficient statistical evidence to conclude that one culture medium was superior to the other. Further studies with a larger number of replicates, standardized material concentrations, phytoplankton abundance measurements, and nutrient analyses, particularly nitrogen and phosphorus, are required to clarify the mechanisms through which each culture medium affects the biomass and productivity of *Daphnia* sp.

4. CONCLUSION

Based on the results, the Ketapang water-based and livestock manure-based culture media did not significantly affect *Daphnia* sp. biomass ($p = 0.0962$). However, descriptively, the livestock manure-based medium produced the highest biomass, with 21.5702 mg L^{-1} day^{-1}.

Further studies are recommended to evaluate different concentrations of ketapang water and livestock manure to determine the optimal culture medium for *Daphnia* sp. growth

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