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EVALUATION OF BANANA PEEL EXTRACT AND HYDROLYSATE AS ALTERNATIVE MEDIA FOR *Daphnia magna* CULTURE

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ABSTRACT

The high cost and technical requirements of live-food production limit its large-scale use. Although daphnia (Daphnia magna) is a promising live feed for fish larvae, microalgal feeds commonly used for its cultivation are costly. This study aimed to assess the performance of banana peel extract (BPE) and banana peel hydrolysate (BPH) to be used as cheap alternative culture media for D. magna. D. magna was cultured for 18 days where six experimental treatments namely, T_1 (D. magna cultured with live Chlorella sp., control), T_2 (1% BPE), T_3 (5% BPE), T_4 (1% BPH), T_5 (5% BPH) and T_6 (1% BPE + 1% BPH) were designed with three replications each. The results indicated that population density (510.64 ± 11.07 ind. L^{-1}) at the peak and offspring number at the first reproduction (9.50 ± 0.26 ind. L^{-1}) in T_4 were similar to those in control T_1; however, significantly higher ($p<0.05$) than in the other treatments. Moreover, a significantly lower ($p<0.05$) age at first reproduction (5.47 ± 0.25 days) was recorded in T_4 compared with the other treatments. Therefore, the findings of this study revealed that a 1% banana peel hydrolysate medium may provide a low-cost substrate option for D. magna culture.

KEYWORDS: Age at first reproduction; agricultural waste; live food culture; low-cost media

INTRODUCTION

Live food, such as rotifers and artemia, is commonly used in aquaculture because of their small size and nutritional profiles (Kamrunnahar *et al.,* 2019). However, in developing countries, artemia and rotifers are quite expensive and may not be locally available. In the search for substitutes for these conventional live foods, locally available live food species and their cost-effective, simple production techniques may play a key role in aquaculture (Tine *et al.,* 2022). Among the live foods, zooplankton is the most suitable first food for cultured fish, supporting their growth and survival (Das *et al.,* 2012). Daphnia (*Daphnia magna*) is an excellent live feed candidate for fish larvae and fry. Its suitable prey size, high digestibility, and nutritionally rich composition have made it especially beneficial for very early larval stages (Bogut *et al.,* 2010). *D. magna* provides around 47.7% protein and essential lipids. It is equipped with endogenous digestive enzymes, such as proteases, amylase, lipase, and cellulase, which help fish larvae to utilize nutrients (Roozbehfar *et al.,* 2013). For industrial production of *D. magna,* microalgae are mainly used as media (Haydarov *et al*., 2020). However, the use of microalgae relies on expensive pure chemical media, which is a major challenge for large-scale cultivation (Mtaki *et al.,* 2021). Therefore, researchers have recently focused on using natural materials, such as manure and animal waste, as culture media. For example, Damle & Chari (2011) found that livestock waste, especially poultry manure, is very effective for the cultivation of *D. magna*. While animal waste has the potential to support *D. magna* growth, several problems are associated with it, including the risk of pathogen contamination and unpredictable yields. Therefore, recent research has focused on exploring agricultural and household wastes as safe and reliable culture media. In this context, fruit-based substrates have gained attention as potential culture media (Peerakietkhajorn *et al*., 2020).

As a major component of household waste, banana peel has the potential to be a culture medium for *D. magna.* Banana peel extracts and hydrolysates have a high content of soluble carbohydrates, proteins, minerals, and bioactive compounds. Microorganisms utilize these components for their growth

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and reproduction, on which *D. magna* survives (Alvarado *et al.,* 2024; Fatmawati *et al*., 2025).

Previous studies have investigated the use of fruit-derived substrates (Peerakietkhajorn *et al*., 2020), fermented organic wastes such as bread and tofu (Herawati *et al*., 2018), and fermented cabbage and banana peels (Damayanti *et al*., 2023) as culture media for *D. magna*. Although fermented banana peels have been explored as a culture substrate, information on the effectiveness of banana peel extract (BPE) and banana peel hydrolysate (BPH) in promoting the growth and reproduction of *D. magna* remains limited. Given the nutritional potential of banana peels as an inexpensive organic resource for zooplankton culture, this study evaluated the effects of BPE and BPH as low-cost alternatives to microalgae for the culture of *D. magna*.

MATERIALS AND METHODS

Preparation of Banana Peel Extract (BPE)

The method described by Semangoen *et al.* (2024) was slightly modified for preparing banana peel extract (BPE). Fresh banana peels were thoroughly washed, chopped into small pieces, and oven-dried at 60 °C for 72 hours. The dried peels were then ground into a fine powder using a blender. A 10% stock solution was prepared by soaking 100 g of the dried banana peel powder in 1 L of distilled water. The mixture was kept at room temperature for 48 hours to allow nutrients to be released into the solution. After this period, the mixture was filtered using a vacuum filter. The resulting filtrate was stored at 4 °C as BPE stock solution until further use. Working solutions (culture media) of 1% and 5% BPE were subsequently prepared from this stock for experimental treatments.

Preparation of Banana Peel Hydrolysate (BPH)

Initially, 10% banana peel hydrolysate (BPH) was prepared following the method of Fatmawati *et al.* (2025), with slight modifications. Fresh banana peels were chopped, oven-dried, and ground into a fine powder. Then, 100 g of powder was subjected to alkaline pretreatment by mixing it with a 2% (w/v) sodium hydroxide (NaOH) solution at a solid-to-liquid ratio of 1:10 (w/v), and the mixture was heated at 80 °C for 1 hour. Following pretreatment, the mixture was filtered to separate the liquid fraction from the solid residue. The residue was washed with distilled water until the wash water reached near-neutral pH. For enzymatic hydrolysis, the pretreated powder was suspended in citrate buffer solution (pH 4.8), and a commercially available enzyme preparation (Acmezyme Vet®) was added at a dosage of 1.2 mL per 100 g of initial dry powder. The enzyme primarily contained cellulolytic and hemicellulolytic components (e.g., cellulase, hemicellulase, α-galactosidase, pectinase, amylase), to facilitate the breakdown of structural polysaccharides present in banana peel. The mixture was incubated at 50 °C with continuous shaking at 150 rpm for 3 days. Enzyme activity was terminated by heating the mixture at 90 °C for 10 minutes. The hydrolysate was filtered to remove solids and stored at 4 °C as banana peel hydrolysate (BPH) stock solution. For experimental treatments, BPH at 1% and 5% concentrations was prepared from this stock solution. The proximate composition of powdered banana peel is presented in Table 1. The composition of the raw banana peel powder is presented to provide baseline information on the source material used for preparing both BPE and BPH, although the chemical compositions of the final extract and hydrolysate were not determined in this study.

Collection, Isolation, and Stock Culture of *D. magna*

Zooplankton samples were collected from a depth of 10-15 cm below the water surface of the freshwater pond located at Gazipur Agricultural University (GAU) using a plankton net (120 μm). From the collected zooplankton samples, *D. magna* was identi-

Table 1. Proximate composition of powdered banana peel (Mean ± SD)

Banana Peel			
Proximate Composition (% d.w.)		Mineral Composition (mg g^{-1})	
Protein	3.97 ± 0.05	Potassium (K)	83.17 ± 6.58
Fiber	14.63 ± 0.08	Calcium (Ca)	18.05 ± 0.00
Carbohydrate	58.47 ± 0.32	Magnesium (Mg)	2.68 ± 0.01
Ash	9.11 ± 0.06	Iron (Fe)	0.61 ± 0.22
		Zinc (Zn)	8.14 ± 0.01

Note: Dry weight (d.w.).

fied based on morphological characteristics, as described in standard manuals (Bledzki, 2023). Before the experimental culture was initiated, a starter culture consisting of approximately 100 adult *D. magna* was maintained in a 200 mL conical flask containing filtered pond water. The culture was subsequently transferred to a 20 L container with gentle aeration to establish the mother stock for subsequent experiments.

Experimental Design

The experiment was conducted in 1 L culture containers for 18 days. Six treatments were evaluated: T_1 (*D. magna* grown on live *Chlorella* sp., control), T_2 (1% BPE), T_3 (5% BPE), T_4 (1% BPH), T_5 (5% BPH) and T_6 (1% BPE + 1% BPH), each with three replications. The *Chlorella* sp. culture used in the control treatment was maintained in the Department of Aquaculture, Gazipur Agricultural University, Bangladesh, using Bold's Basal Medium (BBM). The concentration of *Chlorella* in the control was maintained at a baseline of 1.0×10^6 cells/mL, and this value was checked and adjusted every 2 days. The 1% and 5% treatment concentrations were prepared by adding 10 mL and 50 mL of the 10% stock solution (BPE or BPH), respectively, to 1 L of culture medium. Twenty individuals of *D. magna* (neonates < 24 hours old) were stocked in each container.

Monitoring of Culture Conditions

Population density of *D. magna* was monitored at three-day intervals by microscopic counting using a Sedgwick–Rafter counting chamber. To determine the age at first reproduction and the number of offspring produced at first reproduction, 10 individual adult females were randomly collected from each replicate culture container and transferred separately into 4-mL chambers containing the corresponding culture medium. Thus, the ten chambers represented subsamples from each replicate culture rather than independent experimental units. Each chamber contained a single female, which was monitored daily until the first reproductive event. The age at first reproduction and the number of offspring produced by each female at first reproduction were recorded. For statistical analysis, observations from the 10 females in each replicate culture were averaged, and the resulting mean for each replicate container was used in ANOVA. Water temperature, pH, and dissolved oxygen were measured every three days. The *D. magna* cultures were maintained at an ambient temperature of 26.0–27.5 °C under a natural photoperiod (approximately 13 h light:11 h dark). Light intensity was not measured. Gentle, continuous aeration was provided using an air stone throughout the culture period. The ranges of DO and pH were from 5.0 to 6.3 $mg.L^{-1}$ and 6.8-8.4, respectively (Khan *et al.,* 2020).

The culture medium was not renewed, except for the control treatment, in which fresh *Chlorella* suspension was supplied every two days to maintain the desired feeding level. The bottoms of the culture containers were cleaned daily to remove settled debris and dead *D, manga*.

Sampling and Analysis

D. magna was harvested by siphoning through a fine plankton net (120 ìm) at the end of the 18-day experimental period. Further assessment was done using the following formula:

$$\text{Population density (Ind.L}^{-1}) = \frac{a \times C \times 1000}{L}$$

Where, *a* is average number of *D. magna* counted in one small counting cell; C = volume of concentrate in mL; L = Volume of water filtered in liters.

Statistical Analysis

Data were recorded throughout the experiment in a computer spreadsheet. Normality and homogeneity of variance were tested using the Shapiro-Wilk test and Levene's test, respectively. One-way ANOVA was used to analyze the data, complemented with Tukey's HSD for multiple comparisons. Data were expressed as mean ± SD and analyzed using Statistix 10 software at the significance level $p < 0.05$.

RESULTS AND DISCUSSION

Population density (ind. L^{-1}) of *D. magna* under different culture media is shown in Figure 1. The population density of *D. magna* in the control and in T_4 (1% BPH) was not significantly different (>0.05). On day 12, the population density in T_4 (1% BPH) was 510.64 ± 11.07 ind. $L^{-1,}$ which was 4.4 times higher than that in T_2 (1% BPE), 8.6 times higher than T_3 (5% BPE), 2 times higher than T_5 (5% BPH), and 3.8 times higher than T_6 (1% BPE + 1% BPH). After reaching its peak on day 12, the population density gradually declined.

The higher population density observed in the control and T_4 treatments may be associated with favorable culture conditions and nutrient availability reported in previous studies of *D. magna* culture (Choi *et al.*, 2014). Onyango *et al.* (2024) identified nutrients as an important factor influencing *D. magna* population, reporting that optimal nutrient levels can stimulate their growth. However, excessive nutrients may reduce *D. magna* growth due to nutri-

ent imbalance and unfavourable culture conditions. However, the present study did not directly measure nutrient uptake; therefore, the underlying mechanisms require further investigation. Rapid reproduction of *D. magna* via parthenogenesis also contributed to their population growth. Differences in population density across treatments may be associated with variation in nutrient composition and quality of the culture conditions (Choi *et al.,* 2014). Nevertheless, the subsequent decline after reaching peak density could be attributed to the large number of *D. magna* neonates that had not yet matured for reproduction (Adoteye *et al.,* 2015). The declining trend after the population reached its peak may be attributed to density-dependent factors, including limited space, increased intraspecific interactions and chemical signaling within the culture medium. High population density is known to suppress population growth and reproduction under laboratory conditions. A similar decline following peak population density was reported by Kabery *et al.* (2019). Although the observed decline is consistent with density-dependent regulation, changes in water quality or microbial composition during the culture period cannot be excluded and may also have contributed to the reduction in population density.

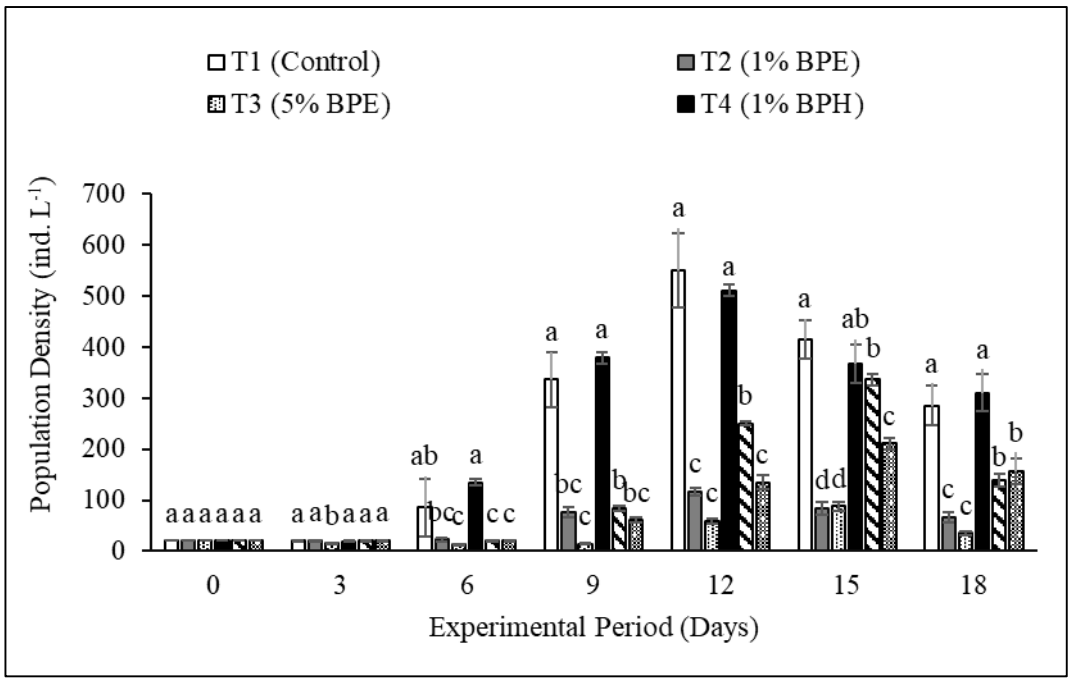

Figure 1. Population density (ind. L^{-1}) of *D. magna* cultured in different media over an 18-day experimental period. Values are presented as mean ± SD (n = 3). Different letters indicate significant differences ($p < 0.05$) among treatments within the same sampling day.

The results showed that extract-based treatments yielded poor growth and reproductive performance compared to the control and hydrolysate-based treatments. Komes *et al*. (2011) demonstrated that extraction time and hydrolysis greatly affect the phenolic profiles.

Naturally occurring plant compounds may influence the growth and reproductive performance of *D. magna* (Griffiths *et al*., 2021). Banana peel contains various bioactive compounds, including alkaloids, terpenoids, and phytosterols (Kibria *et al*., 2019), which may affect nutrient utilization and reproductive performance. Since these compounds were not quantified in the present study, their specific effects on *D. magna* remain speculative and require further investigation.

The age at first reproduction of *D. magna* differed across the different culture media (Figure 2). Individuals reached reproductive maturity most rapidly in the control and T_4 treatments with averages of 5.93 ± 0.57 days and 5.47 ± 0.25 days, respectively. There was no significant difference ($p > 0.05$) between these two treatments, indicating that *D. magna* in both treatments reached reproductive maturity in the shortest time. In contrast, reproductive maturity was substantially delayed in T_2 (1% BPE) and T_3 (5% BPE), suggesting that these treatments were less favorable for early reproduction. Banana peel is an organic substance that can increase the number of bacteria and organic particles if it is used as a culture medium. The bacterial decomposition can further increase the nutrient supply in the culture environment (Elissen *et al*., 2015). The present study revealed that

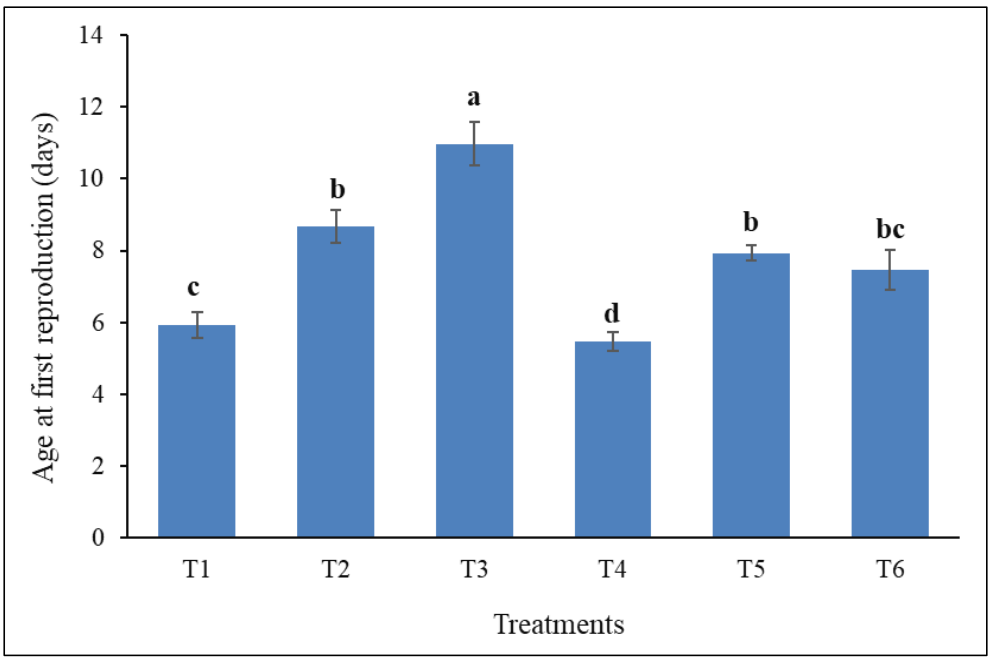

Figure 2. Age at first reproduction (day) of *D. magna* under different culture media. Bars represent mean ± SD (n = 3). Different letters indicate significant differences among (Tukey's HSD, $p < 0.05$). T_1 = Control (*Chlorella* sp.), T_2 = 1% BPE, T_3 = 5% BPE, T_4 = 1% BPH, T_5 = 5% BPH, and T_6 = 1% BPE + 1% BPH.

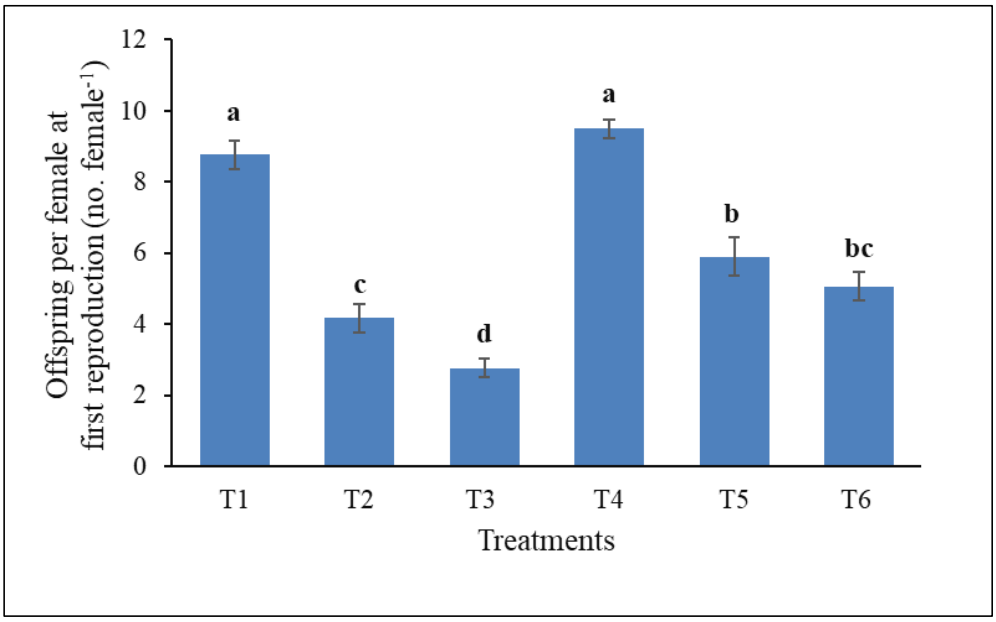

Figure 3. Number of offspring per female at first reproduction of *D. magna* under different culture media. Bars represent mean ± SD (n = 3). Different letters indicate significant differences among (Tukey's HSD, $p < 0.05$). T_1 = Control (Chlorella sp.), T_2 = 1% BPE, T_3 = 5% BPE, T_4 = 1% BPH, T_5 = 5% BPH, and T_6 = 1% BPE + 1% BPH.

banana peel hydrolysate markedly accelerated reproductive maturation in *D. magna*. Hydrolyzed plant materials serve as food for bacteria and other microbes, providing an environment that supports the reproduction of *D. magna*. Hydrolysates are rich in simpler nutrients, including soluble sugars (glucose, fructose), free amino acids, short peptides, and mineral ions. Hydrolysis yields these compounds by breaking down complex components such as cellulose, hemicellulose, and pectin (Fatmawati *et al.,* 2025; Glud *et al.,* 2015). Hydrolyzed plant materials may stimulate the growth of bacteria and other microorgan-

isms, which subsequently serve as food resources for *D. magna*. The enhanced reproductive performance observed in the hydrolysate treatments may therefore be linked to improved microbial production within the culture medium. (Chakri *et al.,* 2014).

The number of offspring produced per female at first reproduction is shown in Figure 3. The results indicated that the highest number of offspring (9.50 ± 0.26 ind. L^{-1}) was observed in females of T_4 (1% BPH). Offspring number in T_4 was significantly higher (pÂ 0.05) than those in the T_2, T_3, T_5, and T_6. However, there were no significant differences ($p>0.05$) in the outcome between T_1 and T_4 (1% BPH). In contrast, T_2 (1% BPE) and T_3 (5% BPE) yielded the worst results among all the treatments.

Better growth and reproductive performance of *D. magna* in this study may be attributed to the soluble sugars present in BPH. A similar result was demonstrated by Peerakietkhajorn *et al.* (2020). Their study revealed that the oligosaccharide derived from dragon fruit can significantly enhance the reproductive performance of *D. magna*.

However, another finding of our study was that the mixed diet containing both 1% BPE and 1% BPH did not produce the best results for the growth and reproduction of *D. magna*. This may have resulted from the nutrient imbalance and the inhibitory substances from the combination of the banana peel extract and the hydrolysate. In contrast, Khan *et al.* (2020) reported that *D. magna* tends to consume a mixed diet rather than a single diet, which clearly differs from the findings of our study. In their study, different combinations of green water, yeast, powdered green pea, and brown flour were used as feed ingredients to prepare different types of mixed diets.

The current study revealed that higher concentrations of hydrolysate did not enhance the performance of *D. magna*. One possible explanation is the presence of phenolic compounds reported in banana peel by previous studies. Since phenolic content was not measured in the present study, this interpretation should be considered speculative and warrants further investigation. Banana peel contains higher levels of phenolic compounds compared to other fruit peels, such as watermelon, melon, avocado, pineapple, papaya, passion fruit, etc. Approximately 907 mg per 100g dry weight phenolic content is present in banana peel (Ansari *et al*., 2023). This may have interfered with nutrient digestibility, thereby slowing growth and reproductive maturity in *D. magna*. Phenolic compounds are considered anti-nutritional factors that may inhibit digestive enzymes such as amylases, proteases, and lipases, thereby slowing the digestion process. This can reduce the absorption of nutrients, including sugars, amino acids, and fatty acids (Melzig, 2023). These compounds can also form complexes with proteins via hydrogen bonding and hydrophobic interactions, thereby reducing protein availability (Tazeddinova *et al.,* 2022). Studies have also reported that phenolic compounds can induce physiological stress and adverse effects in *D. magna* (Lehun *et al.,* 2020), which may contribute to reduced growth and reproductive performance under higher phenolic exposure.

The superior growth and reproductive performance of *D. magna* in a 1% banana peel hydrolysate may be associated with readily digestible nutrients generated during hydrolysis, which enhance growth and reproduction. Although the present study demonstrated the potential of banana peel extract and hydrolysate as alternative culture media for *D. magna*, several aspects warrant further investigation. The study was conducted under laboratory conditions over an 18-day culture period; therefore, the long-term stability and scalability of these culture media require further validation. In addition, the biochemical composition and microbial safety of the harvested *D. magna* were not evaluated. Future studies should also investigate the nutritional quality of the cultured Daphnia and assess their performance as live feed for fish larvae under practical aquaculture conditions.

CONCLUSIONS

The findings of this study demonstrate that banana peel can be used effectively as a low-cost, sustainable culture medium for *Daphnia magna*. The results highlight that a hydrolysate derived from banana peels yields better population and reproductive performance than banana peel extract and provides performance comparable to that of live *Chlorella* sp. In particular, among the tested treatments, the 1% banana peel hydrolysate produces the highest population and reproductive performance of *D. magna*. The results demonstrate that banana peel hydrolysate can reduce dependence on expensive commercial microalgae and serve as a substitute for *Chlorella*-based media. Further field-level studies are recommended to evaluate its application under large-scale conditions.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest regarding this research and manuscript.

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