Middle East Journal of Agriculture Research
Volume: 15 | Issue: 02| April – June| 2026

EISSN: 2706-7955 ISSN: 2077-4605
DOI: 10.36632/mejar/2026.15.2.5
Journal homepage: www.curresweb.com
Pages: 90-95

Assessment of Locally Available Substrates for Enhancing Sustainable *Moina macrocopa* Production

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Received: 13 April 2026 **Accepted:** 30 April 2026 **Published:** 15 May 2026

ABSTRACT
Artemia is commonly used to feed fish larvae, but its high cost, specific hatching conditions, and limited local availability make it less accessible in developing countries. *Moina macrocopa*, a nutritionally rich and widely distributed zooplankton, offers a promising low-cost alternative, though knowledge on its large-scale production and feeding techniques remains limited. The present study was designed to evaluate the effect of different animal manures and food waste as substrates in the mass culture of *Moina macrocopa*, to identify the most effective and sustainable method for maximizing its production in terms of both quality and quantity. To assess the efficacy of various locally accessible substrates for the sustainable mass culture of *Moina macrocopa*, three laboratory experiments were carried out. Four treatments soy milk plus poultry manure, rice bran plus fish meal, cow dung plus yeast, and cow dung alone were applied to a pure culture of M. macrocopa that was fed with Chlorella sp. Moina population counts were taken every day for seven days throughout each experiment, which was conducted under controlled circumstances with constant aeration. The combination of yeast and cow dung produced the greatest average population (228.16 individuals per 600 ml) among the treatments, suggesting that this substrate is the most efficient and sustainable choice for producing *Moina macrocopa* on a wide scale.

Keywords: Organic substrates, *Moina macrocopa*, zooplankton.

1. Introduction
Sri Lanka, predominantly agricultural, has a considerable amount of its population living in rural areas- more than 60%- depending on agriculture for their livelihoods. With this in mind, fishing is very important, because, apart from contributing to food security by offering cheap protein sources, it is also an alternative to red meat in a sustainable way. Fish are appreciated for their high biological value as they provide essential amino acids, vitamins A and D, and key minerals such as phosphorus, iodine, iron, and potassium (Kasagala, 2008; Athauda *et al*., 2019; Athauda and Chandraratna, 2020).

Aquaculture levels its successes, especially during early fish development, depending on how well feeding strategies are installed. Generally speaking, these live feeds are regarded as being required for proper larval growth, survival, and development (Sales, 2011; Samat *et al*., 2020; Vu & Huynh, 2020). Many research indicate that lack of live feed during initial larval stages inhibits growth and survival (Teixeira, 2004; Kalaiselvan *et al*., 2024; Treanor *et al*., 2024). Zooplankton, including Moina sp., Daphnia sp., Brachionus sp., and Artemia sp., are common live feeds used in aquaculture, with *Moina macrocopa* being popular for its high protein content and also for ease of culturing and adaptability to different environmental conditions (Fermin 1991; Nakamoto *et al*. 2012; Rahman *et al*. 2023; Bhosle *et al*., 2020; Khan and Rahman 2025).

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The *Moina macrocopa*-type freshwater cladoceran is very much suitable for usage in such tropical fisheries-aquaculture systems by virtue of having a somewhat swift rate of reproduction, tolerating oxygen levels lower than usual, and being able to survive in environments rich in organic matter (Rottmann *et al*., 2003; Yuslan *et al*., 2021). It is widely accepted to serve as an important feed for larvae and post-larvae of finfishes as well as crustaceans and is cheaper to produce than the more costly and imported *Artemia* (Bhosle *et al*., 2020). However, at the commercial scale, large-scale production of Moina remains majorly prevented by the food availability and the cost of the food source. As a matter of fact, conventional feeding inputs such as commercial algal cultures (e.g., *Chlorella vulgaris*) and processed protein supplements can raise the high production costs and may not always be available in rural areas or those settings limited in resources (Yuslan *et al*., 2021; Khan and Rahman 2025).

The raw materials used for water quality control include dispersants, flocculants, coagulants, dispersants, and precipitates. These useful chemicals in a wide variety of raw materials in water treatment include: dispersants, flocculants, coagulants, dispersants, and precipitates. Sponge and microbial purifiers are the natural replacement sources. This paper reviews some aspects of the treatment of surface waters with synthetic colloidal dispersing agents or coagulants. Composite opposite are compatible with environmentally used on commercial dispersed systems with some obvious advantage (Tacon 1988; Rottman *et al*., 2003).

During the development of *Moina macrocopa*, production in rice bran, poultry manure, cow dung, and other local wastelands features on inexpensive substrates. Use of cheap substrates could lead to reduced production costs. Organic substrates are used to promote microbial and algal communities that are food sources of *Moina*. This is the formation of mass culture media rich in nutrients (Tacon 1988; Rottmann *et al*., 2003).

Although organic materials are promising for live feed production, there remains little experimental material comparing the efficiency of alternative local materials, for bringing about the sustainable cultivation of *Moina macrocopa*. This is vital for lubricating the path of cheaper and ecological ways of practicing aquaculture in Sri Lanka and similar situations (Yakupitiyage *et al*., 1988; Rottmann *et al*., 2003; Patil and Nirmale, 2010; Faria *et al*., 2012; Kabery *et al*., 2019).

Hence, this study has been undertaken to evaluate the performance of *Moina macrocopa* cultured by different locally produced organic substrates. The study, therefore, aims to identify the most sustainable, nutritionally efficient substrate capable of supporting Moina production on a large scale and at low cost. By trying out poultry manure, rice bran, cow dung, yeast, and soya milk under controlled conditions, the study endeavors to contribute towards practical, low-input strategies to boost live feed supply in rural aquaculture systems.

2. Materials and Methods

Moina macrocopa samples were gathered at the Ornamental Fish Breeding and Training Center's wet laboratory in Rambodagalle. The Aquaculture Development Center in Muruthawela and the Fish Nutrition and Testing Laboratory at the Carp Breeding Center in Udawalawa provided additional materials and laboratory equipment. To allow for acclimatisation and multiplication, the obtained samples were kept in a lab setting for around a month. *Moina* was first isolated using a fine dropper and a microscope. Isolated individuals were transferred into new culture media to create purified subcultures. By separating *Moina* from mixed zooplankton populations and keeping it on *Chlorella sp.* feed with constant aeration for optimal growth, a pure *Moina macrocopa* stock culture was created.

2.1. Culture of *Chlorella* sp.

A 50 mL sample of *Chlorella* pure culture was placed into beakers. Subsequently, 0.6 mL of betel manure extract was added, and the culture was aerated continuously for three days. Following initial growth, the culture volume was increased by adding 150 mL of distilled water to enhance algal biomass for use as feed in the *Moina* cultures.

2.2. Experimental design

Six locally available ingredients were selected for media preparation: soya milk, poultry manure, rice bran, fish meal, cow manure, and yeast. The experimental setup included 12 plastic bottles, each pre-filled with 600 mL of dechlorinated water, were used in the experiment. Seventy-five *Moina* individuals from the stock culture were used to inoculate each bottle. Throughout the experiment,

EISSN: 2706-7955 ISSN: 2077-4605 DOI: 10.36632/mejar/2026.15.2.5

sufficient light was supplied and daily measurements of the water quality parameters (pH, ammonia, and temperature) were made. The experiment consisted of four distinct treatments designed to evaluate the effects of various nutrient combinations on the target variable. Treatment 1 (T1) utilized a combination of soya milk at a concentration of 50 mL/L and poultry manure at 200 g/L. Treatment 2 (T2) consisted of 100 g/kg of rice bran supplemented with 50 g/kg of fish meal. Treatment 3 (T3) involved the application of yeast at 5 g/L alongside cow manure at a concentration of 150 g/L. Finally, Treatment 4 (T4) served as the negative control group, receiving only cow manure at a concentration of 200 g/L to provide a standard for assessing the incremental impact of the supplemental ingredients used in the other test groups. All concentrations were maintained precisely throughout the duration of the study to ensure experimental consistency and reproducibility and it was duplicate three times.

Every day, newborn Moina were counted using a Sedgewick-Rafter cell counter and a microscope. Each original *Moina's* total number of offspring was noted, and the individuals' development was tracked throughout the trial.

2.3. Analysis of data

To find significant changes ($p < 0.05$) in the reproductive production of *Moina macrocopa* across treatments, one-way ANOVA in SPSS was used to analyse the obtained data. The effects of various substrate treatments on the average number of *Moina macrocopa* individuals produced over the study period were compared using a one-way ANOVA. Treatment means were compared post-hoc using Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

3.1. Water Quality Parameters

Table 1 summarizes the water quality parameters recorded across the different treatments during the study period. The pH levels ranged from 6.87 in the cow manure (control) treatment to 8.76 in the soya milk + poultry manure treatment. While pH fluctuations were observed, these differences were not statistically significant ($p > 0.05$). The pH remained within acceptable limits for *M. macrocopa* culture, as reported by Rottmann *et al*. (2003).

Temperature varied slightly across treatments, with values ranging between 25.14°C and 25.31°C. A significant temperature difference ($p < 0.05$) was observed among treatments, although these differences were biologically minor and remained within optimal ranges for *Moina* culture. The highest dissolved oxygen (DO) levels were recorded in the rice bran + fish meal treatment (6.61 mg/L), whereas the cow manure control recorded the lowest DO (5.78 mg/L). However, DO levels did not differ significantly among treatments ($p > 0.05$).

Table 1: Water quality parameters (mean) for the *M. macrcopa* culture at different treatments

Treatment	Average number of *M. macrcopa*	pH	Temperature	DO
Soya milk + Poultry manure	128.63^{a}	8.757	25.138	5.841
Rice bran + Fish meal	150.35^{b}	6.916	25.184	6.610
Cow dung+ Yeast	228.16^{b}	6.890	25.240	5.849
Cow dung	183.25^{c}	6.870	25.306	5.781

3.2. *M. macrocopa* Production in Different Substrates

The average production of *M. macrocopa* differed significantly across treatments (Table 1; Fifure 1). The cow dung + yeast treatment yielded the highest mean count (228.16 individuals), significantly outperforming all other treatments ($p < 0.05$). The cow dung control followed with 183.25 individuals. Soya milk + poultry manure (128.63) and rice bran + fish meal (150.35) produced comparatively lower counts, with no significant difference between them ($p > 0.05$). The trend remained consistent: cow dung + yeast supported significantly higher *M. macrocopa* production. No major differences were observed among treatments during the initial days of culture, but substantial increases in *M. macrocopa* counts became evident, particularly in the cow dung + yeast treatment over the course of the study period. These findings highlight that combining cow dung with yeast creates a nutrient-rich environment that supports enhanced *M. macrocopa* growth. According to earlier research, zooplankton proliferation is facilitated by the ideal combination of organic matter and microbial populations that cow manure and yeast provide (Rottmann *et al*., 2003; Siddque *et al*., 2004). The results of the analysis showed that the four treatments' production of *M. macrocopa* differed significantly ($p < 0.05$).

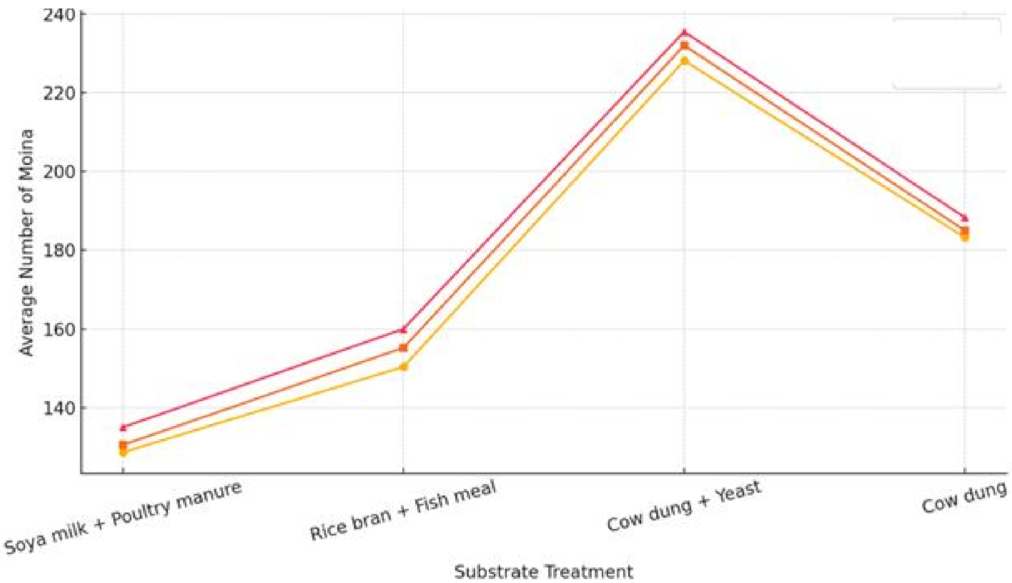

Fig. 1: The production of *M. macrocopa* across treatments

4. Discussion

The higher effectiveness of the cow dung + yeast substrate is due to the complimentary nature of both ingredients in enhancing the nutritional environment for *Moina macrocopa*. Yeast (like *Saccharomyces cerevisiae*) contains readily digested protein, B vitamins, and micronutrients that directly boost *M. macrocopa* development and reproductive success (Lavens & Sorgeloos, 1996; Azam *et al*., 2018). Additionally, yeast cells stimulate the growth of heterotrophic bacteria and improve the microbial diversity in the culture media (Rottmann *et al*., 2003). These microbial populations provide as a highly nutrient-dense food supply for *M. macrocopa*, which are known to graze efficiently on bacteria, yeast cells, and detritus (Santhosh & Singh, 2007).

In order to support the growth of phytoplankton, bacteria, and protozoa the fundamental parts of the Moina food web cow dung provides organic matter and vital nutrients like nitrogen, phosphate, and trace elements (Gupta *et al*., 2015; Chakrabarti *et al*., 2006). The ability of cow dung to maintain a healthy micro-ecosystem, creating a nutrient-rich environment that permits natural food creatures to continue flourishing, is another well-known advantage (Kibria *et al*., 1997). Particularly in semi-intensive and low-input aquaculture systems, the breakdown of organic matter in cow dung releases ammonia and other compounds that can increase primary productivity (Edwards *et al*., 1988).

This synergy supports better reproduction, quicker development rates, and increased survival by giving *Moina* access to a diverse and abundant food supply (Chakrabarti *et al*., 2006; Lavens & Sorgeloos, 1996). Cow dung promotes primary productivity, and yeast raises the microbial load. However, this balanced contribution to primary and microbial production may not be provided by substrates like soy milk or poultry dung, which would restrict their capacity to support sustainable *Moina macrocopa* growth.

Furthermore, use yeast and cow dung as a substrate is consistent with sustainable aquaculture principles since both resources are inexpensive, easily accessible locally, and can reduce environmental impact while lowering reliance on costly commercial feeds (Hasan & Chakrabarti, 2009). Rice bran + fish meal showed intermediate productivity due to the high protein and fat content of rice bran (Faria *et al*., 2012; Murtaza *et al*., 2011). However, this combination was still less effective than cow dung + yeast, possibly due to differences in nutritional availability or microbial stimulation. The relatively low production of the soy milk + poultry manure treatment suggests that, despite its nutrient richness, it may not have created the ideal conditions for microbial proliferation or may have exacerbated the degradation of water quality.

All things considered, these findings support the use of readily available cow dung and yeast as a cost-effective and sustainable substrate for *M. macrocopa* production in Sri Lanka. The results are in line with earlier research that recommended mass *Moina* culture utilising yeast and cow manure (Rottmann *et al*., 2003; Siddque *et al*., 2004).

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