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Growth Optimization and Time-Dependent Population Dynamics of *Paramecium caudatum* Using Locally Available Organic Substrates in Lake-Water Microcosms

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ABSTRACT

This research investigated the growth and population dynamics of *Paramecium caudatum* in lake-water microcosms using seven locally available organic substrates: poultry manure, sheep manure, cow dung, corn leaf powder, barley straw, palm fibre powder, and yeast solution, compared to natural lake water as a control. Water samples were collected from Hajra Lake. Treatments were made by the addition of nutrients to 1 L of lake water and supplemented with *Chlorella* sp. The samples were distributed into five replicates in sterile 200 mL glass containers, and 2 mL of pure *P. caudatum* culture (initial density 180×10^3 cells/L per replicate) was added. Population counts were recorded over 44 days at 11 time points. The growth patterns varied significantly across treatments, with poultry manure proving to be the most effective, showing a peak population density of 1.4×10^7 cells/L at day 40. Other substrates exhibited varying growth trends, with specific peak populations observed on different days. Statistical analysis revealed significant differences among treatments ($p < 0.01$). The findings highlight the importance of substrate composition and nutrient quality in optimizing the culture of *P. caudatum*, emphasizing poultry manure as the most suitable nutrient source for promoting ciliate growth, with potential applications in aquaculture and water quality management.

1. INTRODUCTION

Aquatic ecosystems are not only becoming more known to be important components of global sustainability due to their extensive geographic coverage, but also due to their role in providing a great percentage of global biodiversity, particularly aquatic invertebrates. The rise in the development of aquaculture as one of the most rapidly developing food production sectors is evidence of the increasing global demand for aquatic protein. However, the sustainability can be attributed to the equilibrium among environmental, economic, and social aspects [1]. Fish, oysters, shrimp, and other aquatic life have caused more stress to natural food webs and trophic relationships that, in most instances, have led to an ecological imbalance [2, 3]. The intensive systems can reduce the resilience of the ecosystem and affect productivity in the long term because of nutrient enrichment, chemical pollution, and habitat degradation [4]. This highlights the need to adopt sustainable solutions, such as recirculating aquaculture and ecosystem-based management techniques, to enhance productivity and reduce environmental impacts [5]. In this respect, the microbial ecology of water systems has been of great interest. Aquatic food webs are built on the basis of microorganisms, including bacteria, unicellular algae, and protozoa, which play an indispensable role in the ecosystem [6, 7]. They form the core of nutrient cycling, the decomposition of organic matter, and natural water purification, thereby maintaining water quality and ecosystem balance [8]. For example, bacteria stimulate the mineralization of organic compounds, whereas protozoa can control bacterial communities and facilitate energy transfer to higher trophic levels.

A study has found that microbial loop interactions contribute significantly to the productivity and nutrient supply of water bodies [9]. Similarly, Sherr and Sherr [10] reported the importance of protozoan grazing in controlling bacterial populations and increasing nutrient recycling. Protozoa, and ciliated protozoa in particular, are considered to occupy a strategic ecological position between microbial producers and higher consumers. The most widespread of them is *Paramecium caudatum*, a model organism due to its adaptability and ecological versatility. This ciliate can grow in various water bodies, including freshwater, seawater, and slightly salty water, and it is highly tolerant of the environment [11, 12]. Bacterial grazing is one of the major ecological roles that involves it taking huge amounts of bacteria, which in effect controls the growth of the bacteria, hence averting excessive growth of bacteria [10]. This grazing activity not only stabilizes the microbial communities but also increases the effectiveness of the microbial food web. *Paramecium caudatum* is also essential in nutrient cycling besides bacterial control. It aids in recycling inorganic nutrients, such as

nitrogen and phosphorus, into the environment by consuming and digesting microbial biomass, which becomes accessible to primary producers [13]. This process supports continuous nutrient recycling and enhances primary productivity in aquatic environments [7]. In addition, *P. caudatum* participates in the decomposition of organic matter, including detritus and organic debris. Through its interactions with bacterial communities, it increases the rate at which complex organic substances are broken down into simpler substances, thereby enhancing nutrient fluxes at the ecosystem level [14, 15].

The present research aimed to determine how locally available substrates affect growth rate, maximum population density, and temporal dynamics of *P. caudatum* in lake water microcosms. Specifically, the study focused on identifying the suitability of different organic materials in the promotion of microorganism growth, to investigate the growth and population change dynamics of *P. caudatum* over a certain duration of time, and to identify possible cost-effective, sustainable sources of nutrients that will trigger the growth and maintenance of protozoans in water bodies. This study hypothesized that organic substrates promote the growth of bacteria and algae, thereby increasing *P. caudatum* populations and improving water quality through microbially mediated processes.

2. MATERIALS AND METHODS

2.1 Water sample collection

Water samples were collected from the stagnant waters of Hajra Lake near the city of Sabha. The lake is used as a place to oxidize and filter the treated municipal wastewater. The sampling was done by means of a 1 L bottle fixed to a scrubber, with five replicates obtained from each point. The samples were immediately transported to the laboratory and transferred to a permanent culture system consisting of a $30 \times 30 \times 60$ cm^3 glass tank. Then, the samples were allowed to acclimate under laboratory conditions for one week. After acclimation, the initial abundance of *Paramecium caudatum* in the field samples was recorded, and a control sample was maintained for comparison.

2.2 Evaluation of substrate effects on the growth of microbes and protozoa

To evaluate the effect of different substrates on microbial and protozoan growth, seven environmentally available materials: poultry manure, sheep manure, cow manure, corn leaf powder, barley straw, palm fibre, and yeast solution were tested as treatments. The concentrations of substrates were as follows: poultry manure, sheep manure, and cow manure at 5 g dry weight/L; corn leaf powder, barley straw, and palm fibre at 3 g dry weight/L; and yeast solution at 1 mL/L. Each treatment was applied in five replicates using sterile 200 mL glass containers. After recording the initial *P. caudatum* count, 2 mL of a pure laboratory-prepared inoculum (1.8×10^3 cells/mL) was added to the substrate. The cultures were then monitored over time to assess growth, adaptation, and suitability of each substrate.

2.3 Preparation of cultures and nutrient treatment setup

To isolate pure *Paramecium caudatum* cultures, 1 L of lake water was used as the original sample. The sample was filtered using sterile filter paper. Sterile droppers were used to transfer a 1 mL aliquot to the filtered medium until the cell density matched that of the original sample. This standardized culture was used as the source of stock in subsequent experiments. The rest of the original sample was also filtered to obtain a growth medium whose physicochemical properties and nutrient composition were of a uniform nature. Five replicates of each treatment were prepared in 1 L glass beakers that were sterilized. The predetermined concentrations of the following substrates were then added: poultry manure, sheep manure, cow manure, yeast solution, corn leaf powder, barley straw, palm fibre, and the control, where no nutrient was added. Growth rates were determined with the help of these treatments, as well as the comparison of *P. caudatum* performance upon various nutrient regimes, following the standard protocols of media preparation and inoculation [8]. In addition, cultures were supplemented with *Chlorella* sp. as a primary food source to support bacterial and protozoan growth, ensuring a stable nutrient base for *Paramecium caudatum*.

2.4 Enumeration of physicochemical and biological parameters

The temperature of the water was measured after three days of employing a mercury thermometer, and the laboratory temperature was kept at 24-25 ℃. A Piccolo Plus pH meter (Hanna Instruments, Romania) was used to measure the pH of the control and all seven treatments, and was constant at 7.18 over the course of the experiment. Water was daily replaced through evaporation, with the amount of water lost in each treatment replenished by replenishing the remaining filtered medium. The results were measured after every four days during the experiment. The experiments were conducted in a constant laboratory environment in terms of lighting and temperature. Appropriate labelling was followed by the random mixing of samples. The number of *Paramecium caudatum* was counted with a hemocytometer under an Olympus CH20 compound microscope (Olympus Corporation, Japan; 10× magnification). The remaining slide was subdivided into longitudinal and transverse directions, and the number of protozoa was counted following the fixation of 10% formalin. Following the addition of water, slides were allowed to sit for 30 minutes to immobilize the organisms. Counts were recorded a total of eleven times over 44 days.

2.5 Statistical analysis

Differences among treatments were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test at a significance level of $\alpha = 0.01$. Additionally, simple correlation coefficients were calculated to assess relationships among variables. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, and results were presented as mean ± standard error (SE) [16].

3. RESULTS AND DISCUSSION

The growth rates of *P. caudatum* under different nutritional regimes are presented in Tables 1 and 2 and Figures 1-8. Growth responses varied across treatments, ranging from moderate to very high. All treatments containing organic

substrates, including animal manures and plant fibres, supported significantly higher growth rates of *Paramecium caudatum* compared to the control ($p \leq 0.001$).

A comparison of *Paramecium caudatum* growth and reproduction among the different treatments showed a highly significant effect of medium composition on population dynamics ($p = 4.26 \times 10^{-39}$), as shown in Table 2. The results showed that optimal growth conditions varied among substrates.

Table 1. Population density of *Paramecium caudatum* under different nutritional treatments ($\times 10^3$ cells/L)

Treatment	Initial Count	Days of Incubation										
		4	8	12	16	20	24	28	32	36	40	44
Control	180	195	266	378	410	50	45	20	35	50	55	55
PM	180	288	680	875	3970	4350	6100	7156	9932	12910	14000	8030
SM	180	228	320	500	826	4100	2100	1044	800	460	470	490
CM	180	350	428	810	944	1400	804	592	390	192	154	100
CLP	180	560	810	910	1068	2450	2324	3170	1540	2110	2140	1120
BS	180	281	406	436	562	1237	1157	1625	764	1054	1089	2230
PF	180	250	300	430	650	820	1340	1150	354	330	2520	1498
YS	180	700	876	2128	2174	2880	3500	3034	4900	5770	9000	4000

Note: PM: Poultry manure; SM: Sheep manure; CM: Cow manure; CLP: Corn leaf powder; BS: Barley straw; PF: Palm fibre powder; YS: Yeast solution

Table 2. Analysis of variance (ANOVA) with degrees of freedom (df)

Source	Degree of Freedom	Sum of Squares (SS)	Mean Squares (MS)	F-Value	p-Value
Treatment	7	140170700	20024385.71	1657.32	4.26×10^{-39}**
Error	32	386636.6	12082.39	-	-
Total	39	140557336.6	-	-	-

Note: Treatments differ very significantly at the 0.01 level of probability; MS: mean square

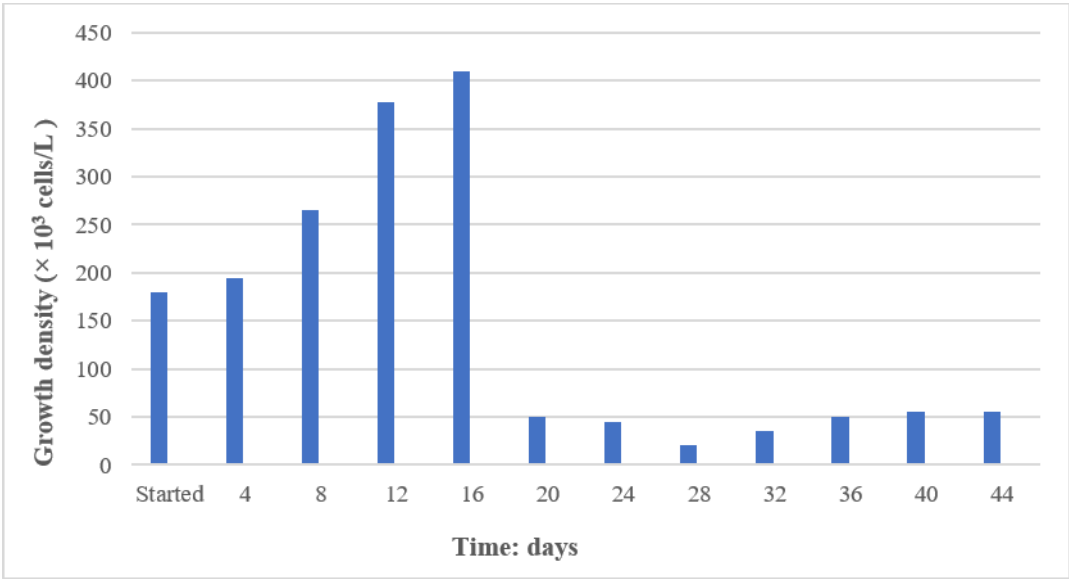

Figure 1. Growth of *Paramecium caudatum* as the control experiment after 44 days of incubation

3.1 Growth of *Paramecium caudatum* as a control

In the control treatment, *P. caudatum* density increased from an initial count of 180×10^3 cells/L to 195×10^3 cells/L and 266×10^3 cells/L by days 4 and 8, respectively, reaching a peak on day 16 (410×10^3 cells/L). This was followed by a sharp decline to 50×10^3 cells/L by day 20 and a further decrease to 20×10^3 cells/L by day 28. A moderate recovery was observed, with counts rising to 55×10^3 cells/L on days 40 and 44 (Table 1 and Figure 1). Low dissolved oxygen (hypoxia), exhaustion of food sources that could have been available, possible contamination, or uneven sampling may explain the significant reduction in *P. caudatum* abundance, which occurred in the control group, between 410×10^3 cells/L and 50×10^3 cells/L between days 16 and 20. These dynamics indicate that the highest growth performance in the control medium occurred between days 12 and 16, after which growth was constrained by the limited availability of algae and naturally occurring bacteria as food resources. As the food supply became insufficient to sustain high *P. caudatum* numbers, the population declined, consistent with patterns of nutrient-limited growth observed in protistan cultures under similar conditions [17-19].

3.2 Effect of poultry manure treatment on the growth of *Paramecium caudatum*

Poultry manure treatment on *P. caudatum* had a highly

significant difference ($p < 0.01$) in growth compared to the control (Table 1 and Figure 2). Growth was initially slow, reached its peak on day 40, and then declined on day 44. An increase in *P. caudatum* density of 288 × 10^3 cells/L on day 4 to 680 × 10^3 cells/L and 875 × 10^3 cells/L on days 8 and 12, respectively, showed a significant increase in population. Peak values were observed on days 36 and 40, with values of about 1.3 × 10^7 and 1.4 × 10^7 cells/L, respectively, then a further decline to 8 × 10^6 cells/L on day 44. Days 20–24 are the period of significant rapid growth, while days 36–40 are the optimal harvest period. The extended high densities suggest that this medium offered better nutrient conditions and environmental factors for growth and survival over other treatments and the control. Various factors, such as long-term growth periods in nutrient-saturated environments, are in agreement with other recent research that has highlighted how resource enrichment and substrate quality can significantly alter protozoan population dynamics, as well as the carrying capacity of aquatic microcosms [20, 21].

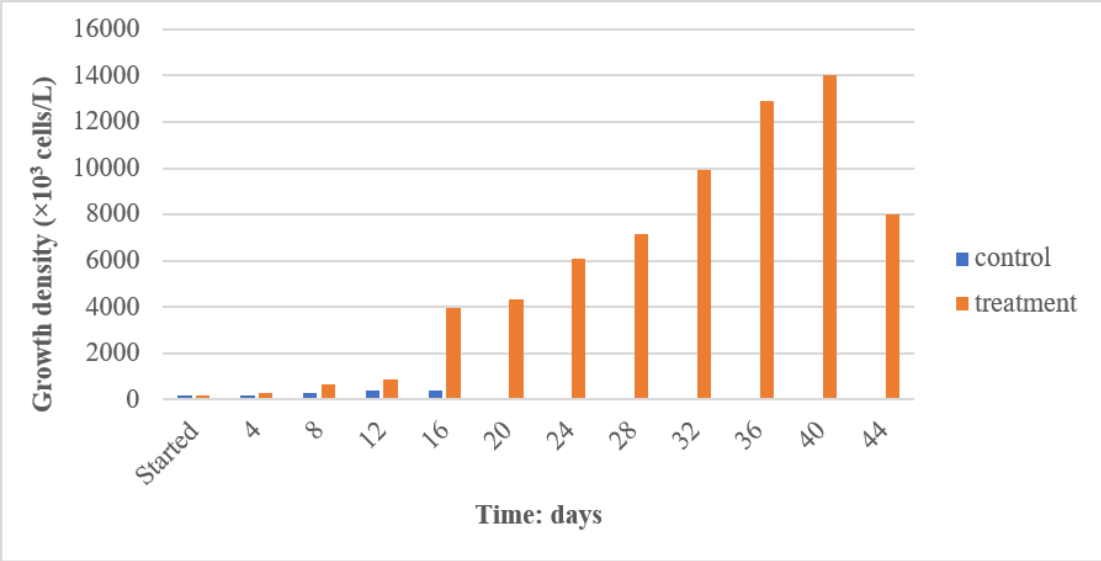

Figure 2. Growth of *Paramecium caudatum* treated with poultry manure after 44 days of incubation

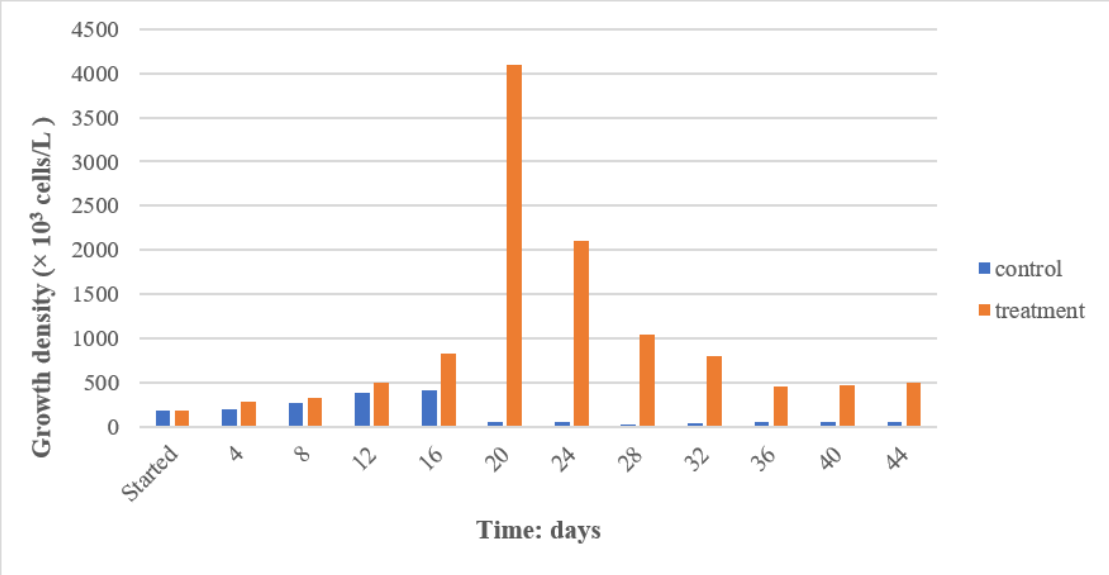

Figure 3. *Paramecium caudatum* growth in the presence of sheep manure after a 44-day incubation period

3.3 Sheep manure treatment and its effect on the growth of *Paramecium caudatum*

As shown in Table 1 and Figure 3, the initial population of *P. caudatum* was 180 × 10^3 cells/L, and it grew to 228 × 10^3 cells/L on day 4. Table 1 shows that the sheep manure's day 8 concentration is 320 × 10^3 cells/L, and the day 12 concentration is 500 × 10^3 cells/L, with a peak of about 4100 × 10^3 cells/L on day 20. Following this maximum, the density dropped to 2100 × 10^3 cells/L on day 24 and then proceeded to decline until the end of the experimental time. According to these time dynamics, the best period for growing the culture and harvesting it was between days 16 and 24 under test conditions. The identified trend of increased growth and then decreasing is not the first in numerous studies that have shown that nutrient enrichment and quality of substrates make a significant impact on the population trend of protozoan and microzooplanktons by modulating the availability of resources and their carrying capacity in aquatic microcosms [22].

3.4 Effect of cow manure treatment on the growth of *Paramecium caudatum*

Paramecium caudatum density increased from an initial value of 1.8×10^5 cells/L to a maximum of 1.4×10^6 cells/L on day 20, followed by a steady decrease in density to about 1.0×10^5 cells/L on day 44, as highlighted in Table 1 and Figure 4. These interaction patterns indicate that the most suitable days in terms of population growth and harvesting with this kind of treatment were 16-24 days. These findings also show that the overall survival and productivity of the culture were relative, with the highest densities of up to 1.4×10^6 cells/L. A potential reason for this trend is that the introduction of cow manure increased ammonia and other nitrogenous materials in the water-based medium. This could affect microbial activities and organism functions because the availability and toxicity of nitrogen would be altered [23]. Moreover, the eating activity of ciliates on organic debris can decrease the amount of available food, which also adds to the fact that population density can decrease over time. These results are consistent with more recent studies that indicate that alterations in nitrogen inputs may cause substantial changes in microbial community structure and growth success in aquatic ecosystems [24].

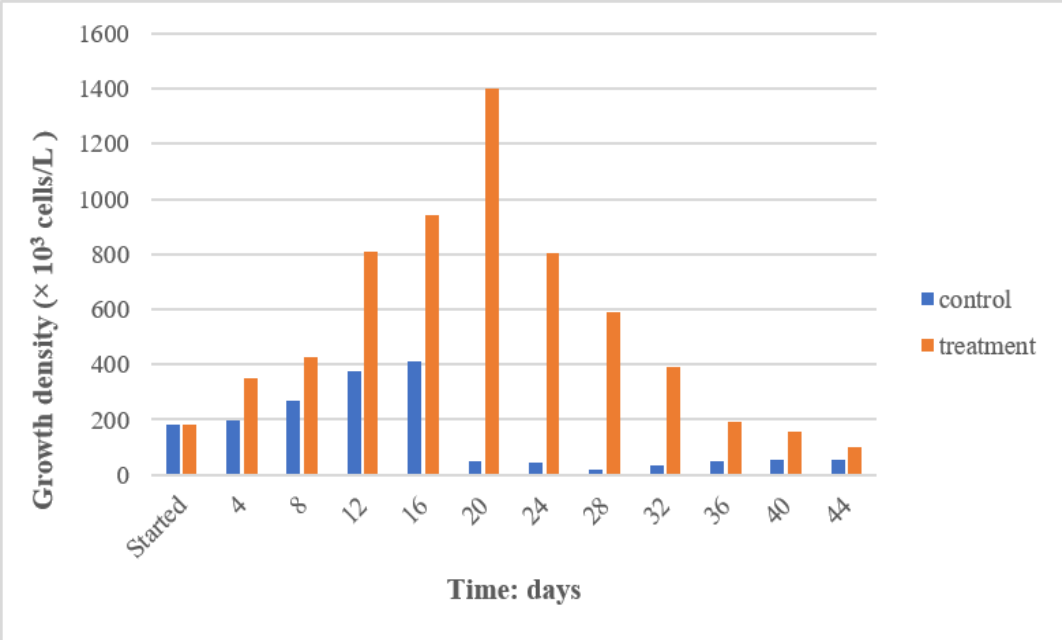

Figure 4. The *Paramecium caudatum* was grown in the presence of cow manure after 44 days of incubation

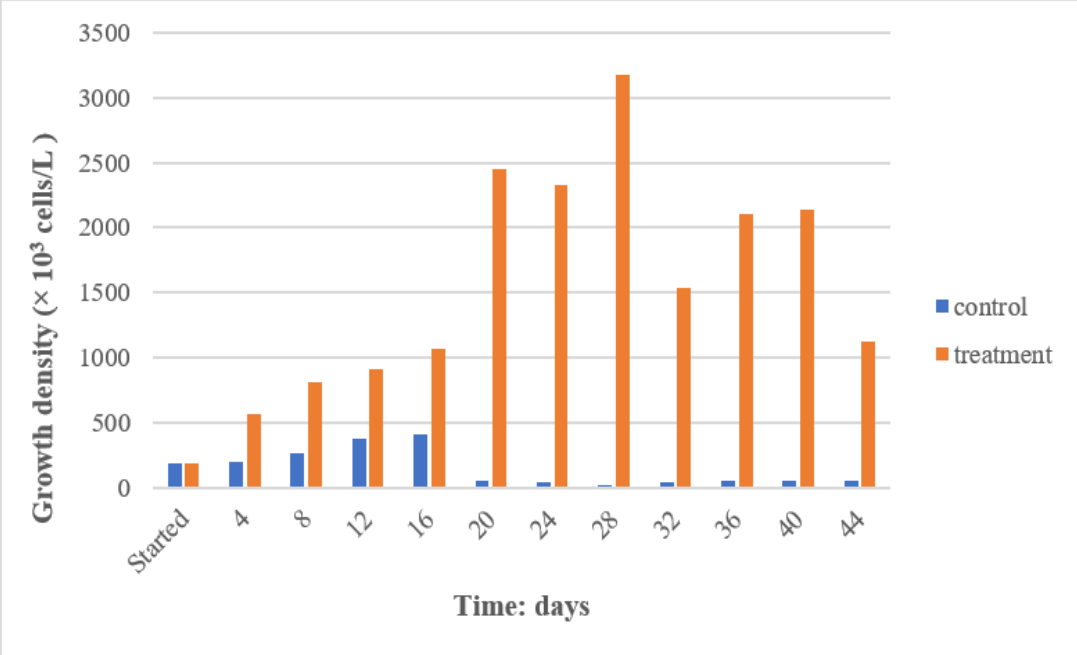

Figure 5. Growth of *Paramecium caudatum* treated with corn leaf powder after 44 days of incubation

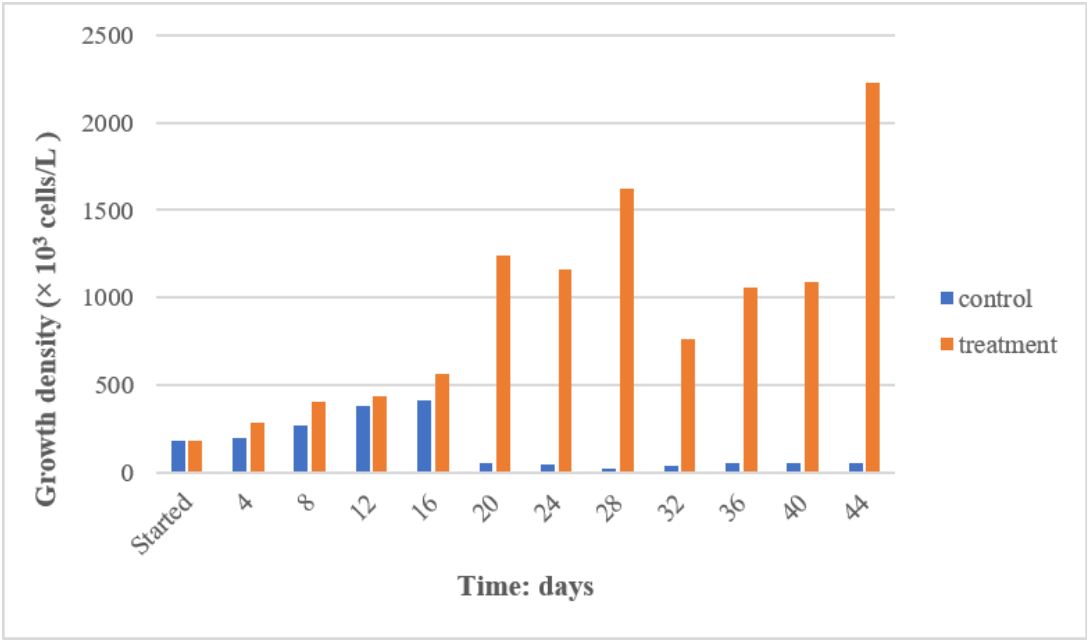

Figure 6. Growth of *Paramecium caudatum* treated with barley straw after 44 days of incubation

3.5 Influence of corn leaf powder treatment on the growth of *Paramecium caudatum*

The growth of *P. caudatum* started slowly and then suddenly peaked at 3.17 × 10^6 cells/L on day 28, then fluctuated with a high of 1.12 × 10^6 cells/L on day 44 (Table 1 and Figure 5). The prolonged high population of *P. caudatum* was probably because the nutrients in the corn leaf powder were not released instantaneously but gradually. Corn leaf powder, being a plant-based product, is made up of plant cellulose, which releases nutrients slowly with time to give a prolonged supply of nutrients. The slowness of this degradation enables *P. caudatum* to persist longer in feeding on the accessible nutrients and still sustain high population levels. Due to the continuous release of organic matter, the protozoa have a continuous supply of food, resulting in prolonged growth even after a peak, a typical feature of substrates that sustain long-term nutrient supply to microbial and protozoan cultures. These findings show that the growth under this treatment was intermittent; the best time for the first harvest was between days 20 and 28, and a second harvest between days 36 and 40. The treatment had a high level of difference ($p < 0.01$) compared to the control. The long-term sustainability of the system over a long period can probably be explained by the gradual breakdown of the substrate particles, e.g., plant cellulose, which gives a long-term source of nutrients and habitat to *P. caudatum*, as has been proposed earlier [25].

3.6 Effect of barley straw treatment on the growth of *Paramecium caudatum*

The growth of *P. caudatum* slowly rose to its peak on day 28 (1.625 × 10^6 cells/L), as illustrated in Table 1 and Figure 6, and the harvesting period was found to be from day 20 to day 28. Growth in this treatment was very significant compared to the control ($p < 0.01$). The findings also indicate that the medium enhanced waving but steady growth, which kept the population at par with time. These results indicate that barley straw has an appropriate substrate that can be used to develop multiple ciliates and small crustaceans, which is probably attributed to slow decomposition and the release of nutrients. Statistical comparisons with the control consistently showed highly significant differences across all measurement points ($p < 0.01$) [26].

3.7 Effect of palm fibre treatment on the growth of *Paramecium caudatum*

The population of *P. caudatum* increased slowly to a maximum of 1.3 × 10^6 cells/L on day 24 and 1.15 × 10^6 cells/L on day 28, then sharply decreased to 3.5 × 10^5 cells/L and 3.3 × 10^5 cells/L on days 32 and 36, respectively (Figure 7). An increment was noted on day 40, which reached the highest level of 2.5 × 10^6 cells/L and then decreased to 1.5 × 10^6 cells/L on day 44. These differences were highly significant ($p < 0.01$) compared to the control. The palm fibre treatment of *P. caudatum* displayed the first peak of population at days 24-28 with a subsequent decrease, and the second peak at day 40. The first peak was attributed to the nutrient content in the palm fibre that facilitated quick growth. But, with the loss of nutrient levels and the decreasing algae growth due to light attenuation by the palm fibre pigments, the population decreased. The second peak was when the slow-release nutrient of the palm fibre and organic matter recycling supported a recovery phase, so that the renewed growth and population resurgence were made possible. These observations are in agreement with earlier reports that show that substrate composition and light attenuation by pigments may greatly contribute to ciliate growth dynamics [27].

3.8 Effect of yeast-treated medium on the growth of *Paramecium caudatum*

The growth of *P. caudatum* gradually increased in the yeast-treated medium until it reached the peak on day 40, amounting to 9.0 × 10^6 cells/L, and then declined to 4.0 × 10^6 cells/L on day 44 (Figure 8). Compared to the control, the results show a highly significant difference ($p < 0.01$). Therefore, the best harvest period was on days 32 to 40. However, when

compared to the poultry manure treatment, it was observed that the latter recorded 1.4×10^7 cells/L. The corn leaf powder medium produced values close to those of the yeast medium, reaching 3.17×10^6 cells/L. These results demonstrate the ability of naturally available media to compete, and achieving favourable growth across all treatments was very highly significant (Table 3).

Table 3. Tukey's Honestly Significant Difference (HSD) statistical analysis

Group 1	Group 2						
	BS	Control	CLP	CM	PF	PM	SM
Control	-1613.91**						
CLP	943.45**	2557.36**					
CM	-1195.27**	418.64**	-2138.73**				
PF	-879.09**	734.82**	-1822.35**	316.18**			
PM	4455.00**	6068.91**	3511.55**	5650.27**	5334**		
SM	-594.00**	1019.91**	-1537.45**	601.27**	285.09**	-5049.00**	
YS	1786.36**	3460.27**	842.91**	2981.64**	2665.45**	-2668.64**	2380.36**

Note: BS: Barley straw; PM: Poultry manure; SM: Sheep manure; CM: Cow manure; CLP: Corn leaf powder; PF: Palm fibre powder; YS: Yeast solution; These mean differences are statistically significant at $p < 0.01$

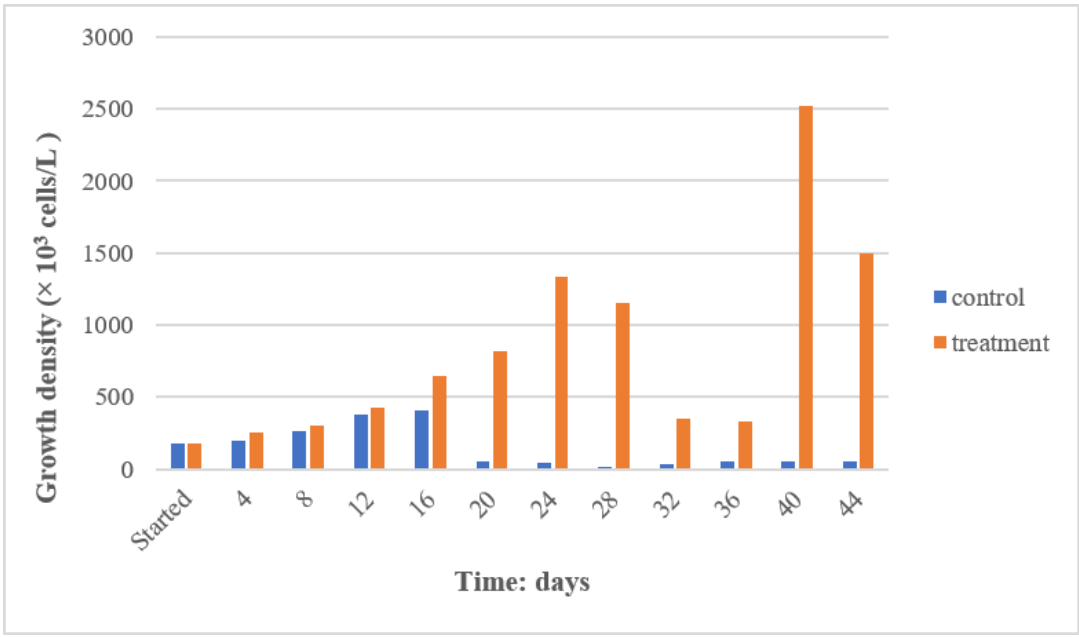

Figure 7. Growth of *Paramecium caudatum* treated with palm fibre after 44 days of incubation

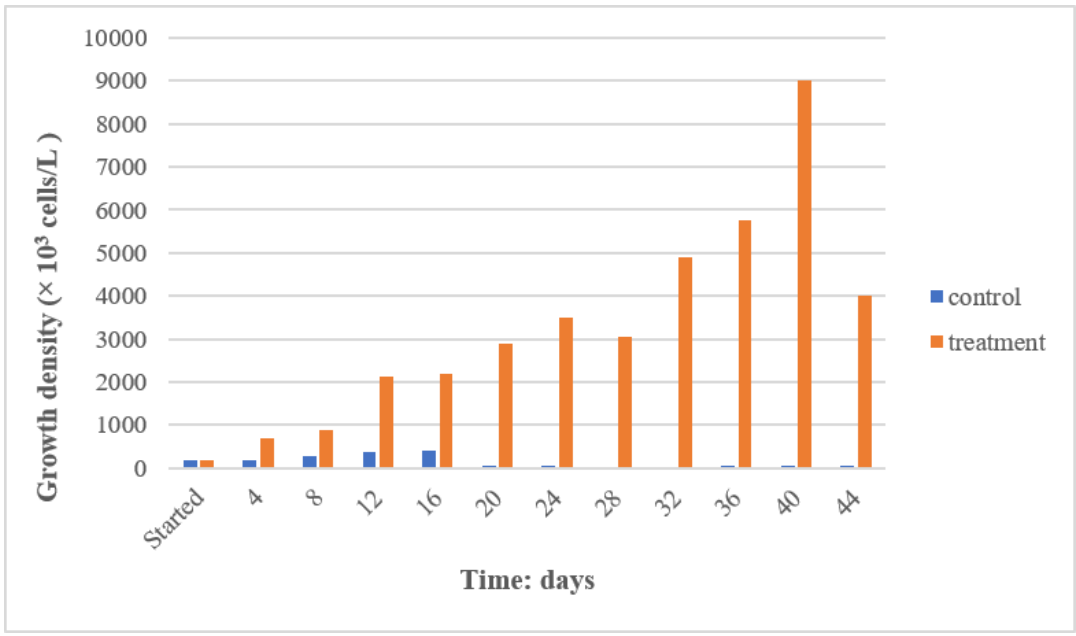

Figure 8. Growth of *Paramecium caudatum* in yeast-treated medium after 44 days of incubation

4. CONCLUSION

The findings of the current study demonstrated that all treatments had a significant effect on the growth of *Paramecium caudatum* compared with the control ($p < 0.01$). Poultry manure was the optimal substrate, having the highest number of cells (1.3×10^7 cells/L - 1.4×10^7 cells/L) at optimal harvest times (days 36 - 40). The growth of the various substrates produced a specific growth pattern, with the best harvest time depending on the substrate. The additives had effects on the bacterial and microalgal populations, which form the major source of food to *P. caudatum*, and other additives, like palm fibre, discharged pigments that inhibited algal growth. The majority of treatments raised the media pH to slightly alkaline conditions, which adds to long-term ciliate viability. These results underline the importance of the substrate composition and nutrient quality in the maximization of protozoan culture productivity.

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