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Article

Laboratory culture of *Brachionus plicatilis* rotifer with two different concentrations of dried cow feces

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ABSTRACT
The current study aims to determine the optimum concentrations of organic matter (dried cow dung) used in the culture of *Brachionus plicatilis* rotifer and to calculate the total numerical density that can be obtained under laboratory conditions. *B. plicatilis* was cultured for ten days and under laboratory conditions at (21°C). They were fed organic matter (cow dung) at two concentrations (1 and 2g/ L). The highest density of rotifers was (29000 and 8000 individuals/L) at concentrations of (2 and 1 g/L), respectively. The growth rate (K) was (1.02 and 0.89), and the doubling times (D) were (0.77 and 0.67/day), respectively. Completely randomized statistical analysis (CRD) was used, and significant differences were found between the treatments.
Keywords: culturing, organic matter, rotifer.

INTRODUCTION

The problem of food shortage, especially animal protein, is general in the world, and in many countries, including the Arab countries, where the per capita share of fish is low. The large increase in the population on the one hand as well as the large and increasing shortage in the quantity of fish caught on the other hand This led to a shortage of fish, which pushed people towards aquaculture[1]. Based on the recent study of the Food and Agriculture Organization (FAO) of the United Nations on fisheries and aquaculture, the latter witnessed rapid growth and increased its contribution in the field of supplies of fish, carnivores and crustaceans[2,3]. Since the introduction of B. plicatilis for marine fish fry production in Japan, great advances have been made in techniques aimed at mass selection and conservation, the genus Brachionus is one of the indispensable zooplankton species in aquaculture systems, and has been used as a primary live food for marine fish larvae since the 1960s, furthermore, this group is a suitable model for studying different aspects of ecology, a series of studies related to aquaculture and basic sciences have led to significant advances in our understanding of the evolution of life history, studies in these two fields are closely linked, providing readers with comprehensive information on how rotifers are employed now in biological investigations[4,5]. Due to the expansion of culture in general, which requires the provision of food for the early stages of the life of fish and crustaceans, researchers have developed techniques for cultivating this species and it has become worldwide widespread[6]. Also, most farmers and breeders aim to preserve fingerlings for fish, and rotifers are important foods[7]. It should be noted that the environment that is suitable for the genus Brachionus is water that contains high amounts of organic matter[8]. Saltwater rotifers *B. plicatilis* are widely used in aquaculture as prey for first-time feeding fish[9]. [10]recorded a population density of 145 individuals/ml in the culturing of B. plicatilis using sheep manure. In a local genetic study of the strains and their comparison with the reference strains, *B. plicatilis* was the second most common species, accounting for 29.69 of the total[11]. [12] concluded that B. plicatilis is most common in the south of the Shatt al-Arab. There are very few local studies on the culture of rotifers, including the studies of[13, 14 and 10]. The current study aims to determine the concentrations of organic matter (dried cow dung) and to calculate the total numerical density that can be obtained in laboratory conditions

MATERIAL AND METHODS

Rotifer samples were collected from ponds in Al-Toba and Al-Nakhilah area in the Basra governorate (30º32′51.9″N and 47º43′55.8″E) (Figure 1) using a zooplankton net with a mesh size of (53µm) and a diameter of (20cm). The rotifer samples were transferred to the laboratory and the *B. plicatilis* (S type is the preferred of fish feed) specimens were isolated (Figure 2). The individuals were left to acclimatize in the laboratory thermal control at a temperature of (21ºC) and supplied with oxygen at a constant salinity rate of (4p.p.t.) After the animals start producing new individuals, individuals of the same age are taken and placed in plastic aquariums with a capacity of (5 liters) containing chlorine-free water with an initial density of (2 individuals/L). Two concentrations of cow dung powder (1 and 2g/L) were used, sterilized by oven at (60°C) for a whole day, and placed in a piece of cloth and left in the basin to allow the fertilizer to decompose, and the continuous and gradual release of nutrients, thus stimulating the growth of phytoplankton on which the rotifers feed, the growth rate (K) and doubling time (D) of the *B. plicatilis* were calculated using[15]:

$K= \ln N1 - \ln N0/ T$ ………………………………………. Equation (1)

$D=\log e^2 / K$ ………………………………………. Equation (2)

where:

K = growth rate

N1 = final number of cultured rotifers

N0 = the starting number of the cultured rotifers

T = time

D = doubling time /day

Completely randomized statistical analysis (CRD) was used to find significant differences between treatments.

Figure 1. Map of the sample collection site.

Figure 2. *B. plicatilis* rotifer (S-type).

RESULTS

Table (1) shows the main environmental factors in which B. plicatilis was cultured. The temperature was (21°C), salinity was (4g/L), pH (7.6), and dissolved oxygen was (7.4 mg/L). Table (2) shows the initial and final number of rotifers that were cultured under (21°C). The final number reached after ten days at a concentration of (2g/L), which amounted to (29000 individuals/ liter), while at the (1g/L) it reached (8000 individuals/ liter) for the same period (Figure 3) The results of the statistical analysis showed that there were significant ($P \leq 0.05$) differences in the densities between the two treatments (Table 2). A growth rate

(K) achieved (0.89) with a doubling time (D) (0.77 days) for the rotifers in the first concentration, while the second concentration was the growth rate (1.02) and the doubling time was (0.67 days) (Figures 4 and 5).

The environmental factor	The value ± SD
Water temperature (°C)	21 ± 0.03
Salinity (p.p.t.)	04 ± 0.30
pH	7.6 ± 0.21
Dissolved oxygen (mg/L)	7.4 ± 0.23

Table 1. The values of the environmental factors during the culture of *B. plicatilis* rotifer

Concentration of Organic matter (g/L)	Initial number (individuals /L)	Final number (individuals/L) ± SD	Growth rate (K) ± SD	Doubling time (D) day ± SD
1	2	8000 ± 37.31 a	0.89 ± 0.003	0.77 ± 0.002
2	2	29000 ± 32.53 b	1.02 ± 0.004	0.67 ± 0.003

Table 2. The initial and final number, growth rate (K), and doubling time (D) for *B. plicatilis* cultured at (21°C) for a period of (10 days) using (1 and 2g/L) of organic matter a, b: significant differences were found between the treatments.

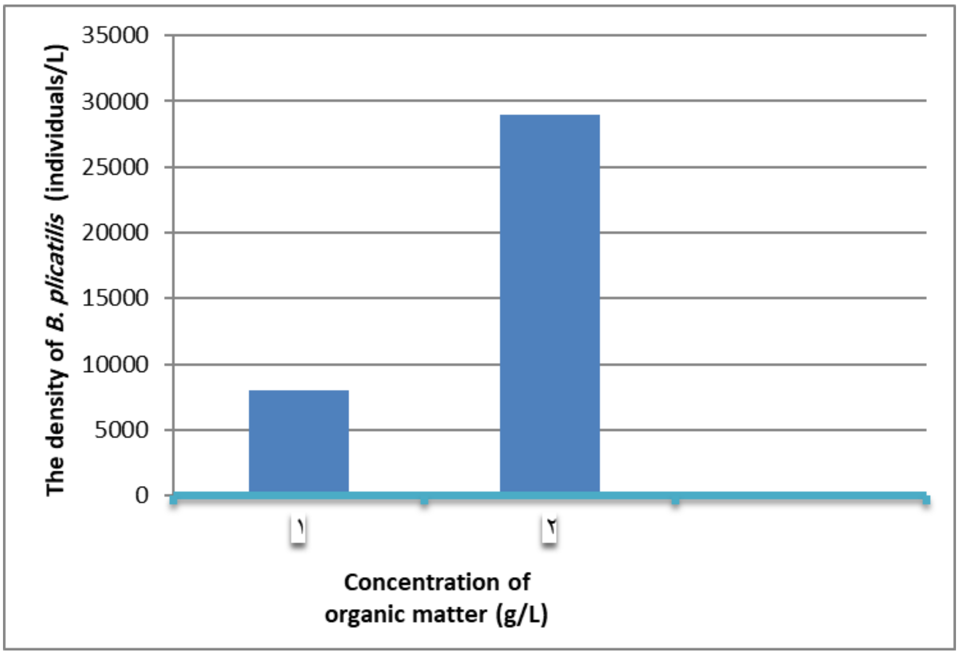

Figure 3. The density of *B. plicatilis* cultured at (21°C) for a period of (10 days) using (1 and 2 g/L) of organic matter.

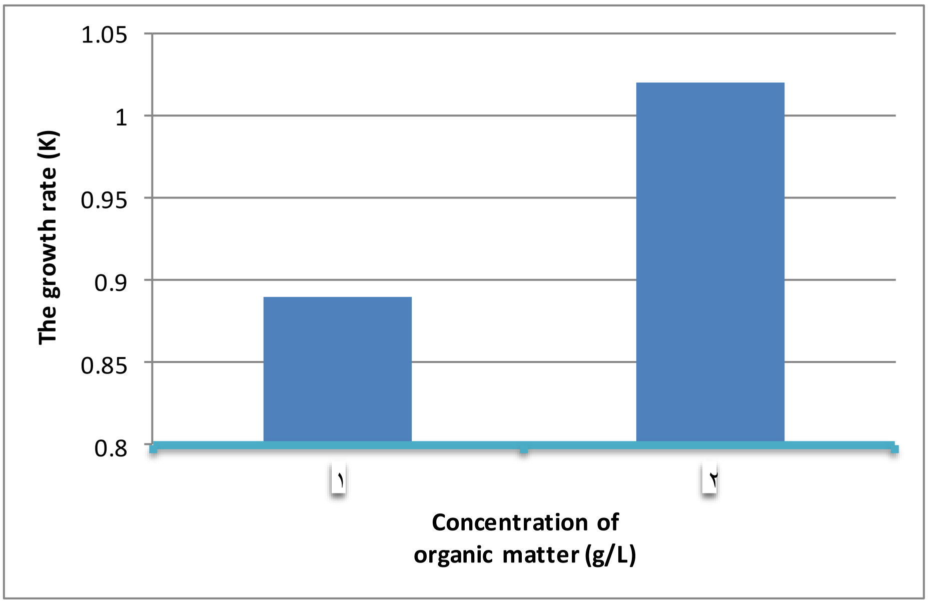

Figure 4. The growth rate (K) of *B. plicatilis* cultured at (21°C) for a period of (10 days) using (1 and 2 g/L) of organic matter.

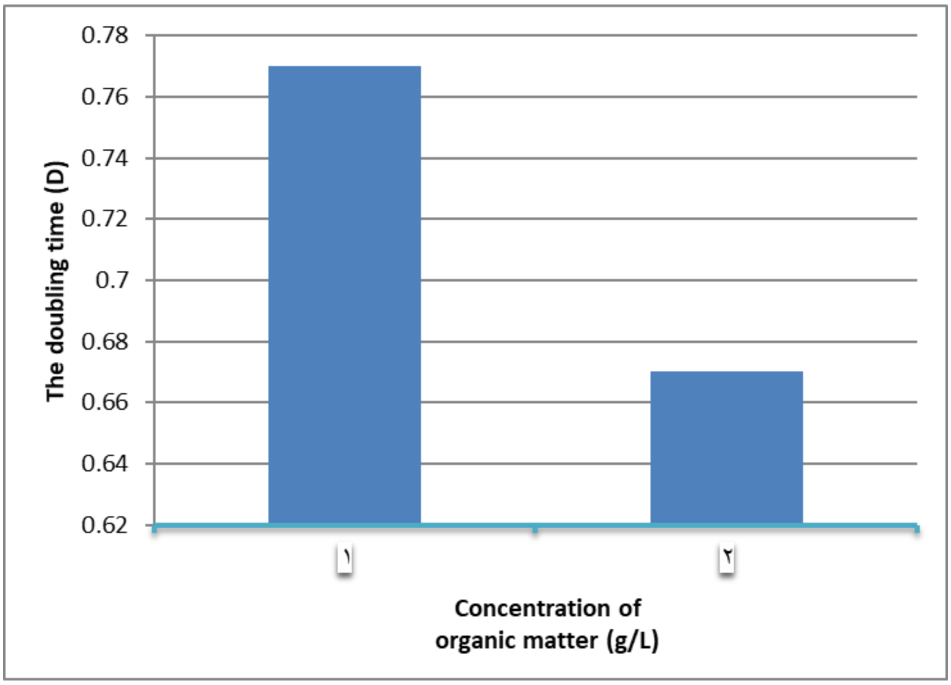

Figure 5. The doubling time (D) of *B. plicatilis* cultured at (21°C) for a period of (10 days) using (1 and 2g/L) of organic matter.

DISCUSSION

The choice of temperature is important in the cultivation of rotifers, as it depends on the required temperature, because there are two types of rotifers that depend on temperature, the first S- type that is produced at high temperatures, and the second L- type that is produced at low temperatures, because the temperature rise is within the ideal range it produces generations of rotifers that are characterized by their small size compared to the rotifers cultured at low temperatures[16]. What was also observed during the current study and this application is also of special importance in aquaculture in order to find rotifers that are commensurate with the size of the mouth opening of the fish larvae that feed on them, because the rotifers of the L-type in size do not match the size of the mouth opening of fish and crustacean larvae in the early stages, when using rotifers of the S-type for the relatively large larvae, this leads to the disbursement of energy by the larva, due to its need for large quantities of food in order to reach the saturation point, and this means increasing movement and swimming, and thus spending additional energy[16]. As for salinity, this type is characterized by its high salt tolerance and can range between (1 to 97 p.p.t.), and it was seen in the current study in (100 p.p.t.), but the ideal range for this type is (4-20 p.p.t.)[17]. The density of rotifers that the current study reached is low compared to[14] at the highest concentration, while it is very low depending on the lowest concentration in his study on B. calyciforus, his density recorded (396 individuals/ mL) and at a temperature of (26°C), Studies have shown that the effect of a temperature of (26°C) results in an increase in the density of the rotifer and it decreases with a decrease in temperature to (21°C). The highest density of the cultured rotifer was recorded to be (29000 individuals/ L) in the high concentration of organic matter (2g/ L), the growth rate was (1.02) and the doubling time was (0.67), while its density in the lowest concentration of organic matter (1g/ L) was (8000 individuals/ L), the growth rate is (0.89) and the doubling time is (0.77). The obvious increase in the density and the high growth rate recorded in the high concentration of organic matter can be attributed to the fact that the rotifer got its needs of nutrients and elements that directly affect the growth processes and enhance the animal's performance to carry out various vital activities[18]. There is no doubt that the fertilizers contain good amounts of nutrients, and therefore it is possible to benefit from the cow droppings to obtain necessary and cheap nutrients for use in the field of aquaculture[19]. One of the studies showed that B. plicatilis had the highest average density and growth rate, as it was able to live in laboratory conditions as it was fed on yeast and animal manure[14]. Survival rates of (100%) were achieved for the common carp and (98%) for the grass carp of the larvae fed on rotifers of the genus Brachionus, compared to other live foods Artemia and Cladocera[14]. In a study conducted to feed larvae of Barbus xamthopterus fish, survival rates of up to (85%) were achieved when feeding on rotifers, while the survival rate reached (79 and 65%) when feeding on phytoplankton and industrial food respectively[20]. The differences in densities between the current study and the study of[10] can be explained by the difference in the temperature used and the difference in the quality of animal manure.

CONCLUSIONS

We conclude that the use of cow dung as a cheap organic material for the purpose of breeding and producing B. plicatilis rotifer, and this information is useful to many aquaculture growers, such as fish.

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Authors' Contributions

All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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Data Availability

All datasets obtained or studied during this study are incorporated in the manuscript.

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