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Effect of Different Types of Vegetable on the Population Growth of Infusoria

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ABSTRACT

This study aimed to evaluate the effect of vegetable type and processing method on the population growth of infusoria species. To compare the suitability of different substrates for raising infusoria, a two-factor experiment was designed using 10-L plastic jars, each containing 8L of river water and 100g of vegetables (lettuce or cabbage) that had undergone different processing methods (chopped, unchopped, ground), corresponding to 6 treatments. Infusoria were first observed on day 3 of the experiment. There was an interaction of vegetables and vegetable processing method that had a very clear effect on population growth: processed vegetables tended to support higher population densities than unprocessed vegetables. Population density reached a maximum of 1,816 ind./mL with ground lettuce, while culture with ground cabbage yielded a maximum of 1,165 ind./mL. *Euplotes* sp. accounted for around 80% of the population composition of infusoria for both vegetables.

INTRODUCTION

Infusoria is the common name for single-celled microorganisms (ciliates, protist, amoebas, rotifers, blue flagellates, etc.) belonging to the protozoan kingdom. With a small size of 25–300µm **(Poon, 1996)**, infusoria have soft bodies and are rich in nutrients, making them an ideal initial food for early larval fish **(Das *et al*., 2012)**. **Côrrtes *et al*. (2013)** noted that ciliated protozoans of the genus *Euplotes* often appear to contaminate mass culture environments of rotifers, which also have the potential to be used as live food in fish larval culture. According to **Holt and Holt (2000)**, marine fish larvae in the wild often feed on large amounts of zooplankton, including protozoans. These organisms are the initial food for juvenile *Tilapia nilotica* **(Delupio, 1994)**, larval flounder *Paralichthys olivaceus*, and grouper *Epinephelus akaara* **(Yoo & Hur, 2002)**. Infusoria can be enriched with HUFA and vitamin C as food for *Clarias magur* catfish larvae **(Lal *et al*., 2022)**. Infusoria are present in water, although usually not enough to feed fish. Therefore, it is better to culture them in a separate tank. Infusoria can be

cultured with many different types of food, such as rice bran or any other available plant source. **Mendes *et al*. (2016)** showed that the density of infusoria grew quickly when cultured with baker's yeast (16,495 individuals per milliliter, ind./mL) but was low when fed with microalgae (72.2 ind./mL). **Mukai *et al*. (2016)** reported that density of *Euplotes* sp. can reach up to 400-500 ind./mL when cultured with vegeatbles (*Brassica pekinensis* and *Brassica chinensis*). However, the growth rate of infusoria is affected by vegatable type and dose and decomposition rate of culture media; the highest growth rate was recored when infusoria were cultured with cabbage at 10g/ L of culture medium **(Widjaja & Suwignyo, 1981)**.

Currently, the number of studies on raising infusoria biomass is very limited compared to that on rotifers and other zooplankton, although most relevant studies mention the importance of infusoria or protozoans as an initial larval food source. To obtain more information about raising infusoria using vegetables, this study investigated the type of food material that best stimulates infusoria population growth and determined the species composition of the resulting infusoria populations.

MATERIALS AND METHODS

Experimental design

Infusoria were provided with different types of vegetables as food to stimulate population growth. The experiment was carried out in 10-L plastic jars each containing 8L of Mekong River water (containing infusoria seed). Two types of vegetables, lettuce and cabbage, were used as a food source for the infusoria. These vegetables were a waste products from general market and were prepared in three ways: unchopped, chopped, and ground, corresponding to 6 treatments. Then, 100g of each type of vegetable was placed in each jar, and slight aeration was provided throughout the experiment. The study had a random design, with 3 replicates; the experimental setup is outlined in Table (1).

Table 1. Experimental setup for rearing infusoria with different types of vegetables

Treatment code	Lettuce type	Cabbage type
T1	Unchopped	
T2	Chopped	
T3	Ground	
T4		Unchopped
T5		Chopped
T6		Ground

Experimental management

The culture water was not changed during the experiment. The experiment ended once the population density was recorded with a 10% decrease compared to the maximum density in each jar.

Data collection and calculations

Temperature and pH were measured twice a day, at 7:00 in the morning and 14:00, with a temperature and pH meter (Hanna HI 98128) to get the lowest and the highest temperature values in a day, which may have negative effect on the growth rate of infusoria.

Water samples were collected every 3 days at 8:00 and were analyzed for total ammonia nitrogen (TAN) and nitrite (NO_2^-) using a Sera test kit (Germany).

To calculate infusoria density (ind./mL), samples were collected daily from day 2 until the end of the experiment. Aliquot samples from each culture were collected with 200µL of water to determine population density under a magnifying glass with 4X magnification. Density was calculated according to the following formula:

Density (individuals/mL) = Number of animals counted in the sample × 5

The population growth rate of infusoria was determined according to the formula:

$$\mathbf{K = (\ln N_t - \ln N_0)/t}$$

Where:

K: Population growth rate of infusoria

N_t: Infection density at time t (individuals/mL)

N_0: Initial density of infusoria

t: Culture time (days)

To determine the species composition of the infusoria, a 200-mL aliquot was collected from each culture jar on the day that the infusoria population reached maximum density in that jar. The samples were then fixed with formalin (5%) to determine species composition under a microscope with 4X magnification. Infusoria were identified and classified based on their external morphology and comparison with the taxonomic description by **Shirota (1966)**.

Statistical analysis

The collected data were preprocessed in the Excel program and statistically processed with Statistica 7.0 software, using the two-factor analysis of variance (ANOVA) method to compare errors. Significant differences between treatments at $P<0.05$ were detected using Tukey's test.

RESULTS AND DISCUSSION

High or low water pH values affect the growth of infusoria populations. For example, *Paramecium caudatum* species tolerate a wide pH range, but they die off when

suddenly immersed in pH 4 and above pH 11 **(Heydarnejad, 2008)**. The pH values in the current study did not differ greatly between the culture jars, with pH ranging from 8.1 to 8.2 (Table 2), within the suitable range for infusoria growth.

Similarly, the temperature of the culture did not fluctuate significantly ($P \geq 0.05$) during the experiment (Table 2), ranging from 26.1 to 30.1°C, which is suitable for the development of infusoria **(Nakaoka & Oosawa, 1977)**.

The TAN content in the culture water ranged from 0.6 to 4.7mg/ L, and was significantly higher ($P<0.05$) in the lettuce treatments than that in the cabbage treatments. The highest TAN was recorded in the ground lettuce treatment (4.7mg/ L) and the lowest in the ground cabbage treatment (0.6mg/ L). In the initial few days of the experiment, the TAN in the culture water was high because bacteria decomposed the lettuce and cabbage, causing the organic matter content in the water to increase. The TAN gradually decreased in the following days of culture as the infusoria population grew, likely because the infusoria were using this organic matter to grow and develop **(Kamiyama, 1994)**. Meanwhile, the NO_2^- content in the experimental treatments ranged from 0.3 to 4.9mg/ L, with the cabbage treatment being significantly higher ($P<0.05$) than the treatment with lettuce. The lowest NO_2^- content was recorded in the chopped lettuce treatment (0.3mg/ L), and the highest was recorded in the ground cabbage treatment (4.9mg/ L).

Table 2. Overview of experimental culture-water parameters. Values are mean ± standard deviation (n = 3)

Treatment code	pH		Temperature (°C)		TAN	NO_2^-
	7:00	14:00	7:00	14:00	(mg/L)	(mg/L)
T1	8.1±0.1	8.1±0.2	26.1±0.2	29.8±0.8	$3.0±0.4^b$	$1.1±0.3^b$
T2	8.2±0.1	8.2±0.2	26.1±0.2	30.1±0.2	$3.3±0.4^b$	$0.3±0.1^a$
T3	8.2±0.2	8.2±0.1	26.2±0.2	30.1±0.3	$4.7±0.6^c$	$0.5±0.1^a$
T4	8.2±0.1	8.2±0.1	26.1±0.1	29.9±0.2	$1.2±0.2^a$	$4.6±0.3^c$
T5	8.1±0.3	8.1±0.1	26.1±0.1	29.8±0.2	$0.9±0.2^a$	$4.9±0.3^c$
T6	8.1±0.4	8.1±0.1	23.3±0.1	30.1±0.2	$0.6±0.2^a$	$4.7±0.2^c$

The first infusoria were detected on day 3 of the experiment, with density ranging from 3 to 79 ind./mL (Table 3). The density in the lettuce treatment was significantly higher ($P<0.05$) compared to that in the cabbage treatment, except for treatment 1 (unchopped lettuce), in which the density was not significantly higher than that in the cabbage treatments. The highest population density (79 ind./mL) was detected in the culture containing ground lettuce, while the lowest (3 ind./mL) was recorded in that containing unchopped cabbage. The density continued to increase gradually over subsequent culture days. By day 6 of culture, the density of infusoria in the ground lettuce continued to increase and reached a peak at 1,816 ind./mL, nearly 33 times higher than the density on day 3; this was statistically significantly greater ($P<0.05$) than the peak

densities of the remaining treatments. Although the unchopped cabbage had a peak population density of 133 ind./mL—an increase of over 44 times compared to the 3rd day of culture—it remained the culture with the lowest density. This was statistically significantly lower ($P<0.05$) compared to all other treatments, except for the unchopped lettuce (451 ind./mL), compared to which no significant difference ($P\geq0.05$) in density was detected.

On day 7, the density of infusoria in the ground lettuce treatment decreased slightly after reaching the maximum density, from 1,816 to 1,281 ind./mL; despite this, this treatment still had the highest density. Meanwhile, the density in the remaining treatments increased to over 1,000 ind./mL except for the culture using unchopped cabbage; that is, the density of infusoria in the unchopped and chopped lettuce and chopped and ground cabbage treatments increased, the density of these treatemts were still lower than that in the ground lettuce treatment, although the difference was not statistically significant ($P\geq0.05$). Although the density of infusoria in chopped cabbage increased 5-fold on day 7 compared to that on day 6, reaching 620 ind./mL, it was still the lowest density detected among all the treatments. This difference was statistically significant ($P<0.05$). From day 7 to the last day of the experiment, the density of infusoria decreased simultaneously, exept for the un-chopped cabbage treatment, the density of infusoria increase on day 8 and then decreased.

The growth of infusoria is dependent on food type. According to **Côrrtes *et al.* (2013)**, *Euplotes* sp. population density increased rapidly, reaching 15,848 ind./mL, when provided with baker's yeast over 6 days of culture. In their study, this exceeded the density obtained through culturing with Selco 3000 (INVE, Belgium) (11,287 ind./mL). However, the density of *Euplotes* was low (3 ind./mL) when provided with *Nannochloropsis* sp. for the same number of days in culture.

In general, the interaction of vegetable type and vegetable processing method on the growth of infusoria was very clear (Table 3), particularly from day 3 to day 6. These factors were found to be highly significantly different ($P<0.001$). On day 7, this interaction still remained but showed signs of decreasing significance compared to the previous day of culture. The difference in vegetable type and processing method, and the effect of the interaction between these factors on population growth, decreased to a significant level ($P<0.01$). However, the difference between the effects of vegetable processing methods on infusoria growth was very clear and highly significant ($P<0.001$). On day 8 and day 9 (the last 2 days of culture), the effect of the vegetable type and of the interaction of both experimental factors on population growth became less clear. In this case, however, the vegetable processing method still had a clear and significant effect ($P<0.05$) on infusoria population growth.

The growth of infusoria populations differed among the cultures, tending to be high in the initial days of culture and then gradually decreasing (Table 4). On day 4, the population growth rate under provision of both types of chopped and ground vegetables

was higher than that of the unchopped vegetables, ranging from 0.11–2.16%. The cabbage treatments had statistically significantly higher density ($P<0.05$) compared to the lettuce treatment, except for chopped lettuce (1.04%) and ground lettuce (1.26%), the density of which did not differ significantly ($P\geq0.05$) compared to that of the cabbage cultures. The chopped cabbage treatment had the highest population growth (2.16%), while the lowest population growth (0.11%) was observed in the unchopped lettuce treatment.

On day 5, population growth in all treatments was at a high level, and there was no difference ($P\geq0.05$) between cultures with lettuce compared to cultures with cabbage. The population growth rate of infusoria in the cultures ranged from 1.09–1.64%, with the highest (1.64%) detected in the culture with ground cabbage and the lowest (1.09%) in the culture with chopped lettuce. On day 6 and day 7, the population growth rates in the cabbage treatments were not clearly different from those in the lettuce treatments. However, the population growth rate was highest in ground cabbage (1.47%) and unchopped cabbage (1.32%), and lowest in the chopped and ground lettuce (at 0.93% and 0.70%, respectively) on these days.

On day 8 and day 9, the population growth rates in the cultures began to decrease. Despite this, the population growth was significantly higher ($P<0.05$) in the cabbage treatment than in that using lettuce. The highest population growth rate (from 1.12–0.83% on days 8–9) occurred in the unchopped cabbage treatment, while the lowest (from 0.51–0.33% on days 8–9) was observed in the ground cabbage treatment.

The rate of decomposition of vegatables affected the growth of infusoria **(Witdjaja & Suwignyo, 1981)**. The results in the present study again comfirmed that the rate of decomposition of culture media has an effect on the population growth of infusoria. Infusorria grew rapidly in all cultures where chopped and gorund vegetables were used as the culture media.

Infusoria species composition

The recorded infusoria species composition was not diverse, with only 3 to 4 species detected in each treatment (Table 5). The treatment with lettuce had a higher species composition than the treatment with cabbage. Among them, *Euplotes* sp. and *Paramecium* sp. had the largest average values, with 76.2 and 21.5%, respectively. *Moina* sp. was also detected in the population, but in very low numbers, averaging 2.3% of the total species composition. In addition, two individuals of *Arcella* sp. were recorded for the unchopped lettuce treatment, equivalent to 0.2% of the species composition in the population, but *Arcella* sp. was not detected in the rest treatments. Although they had the same water source, but *Arcella* only appeared in the unchopped cabbage treatment. This may also be due to other treatments, the density of other infusoria grew quite quickly and was dominated by the species, preventing *Arcella* from growing. According to **Widjaja and Suwignyo (1981)**, protozoa appeared in the culture on day 2, while Rotifer grew in the second week for banana peel, cabbage and lettuce media. *Euplotes* sp. and

Paramecium sp. have previously been identified as the initial fresh food sources for the larvae of some fish species **(Mitchell, 1991; Lahnsteiner & Kletzl, 2018)**.

Table 5. Infusoria species composition by percentage. Values are mean ± standard deviation (n = 3). Different superscript letters in the same column denote significant differences (P<0.05).

Species	Percentage (%) of species composition					
	T1	T2	T3	T4	T5	T6
Euplotes sp.	80.3±9.8^{c}	71.2±8.7^{c}	74.9±7.6^{c}	86.8±5.2^{c}	67.5±10.7^{c}	76.4±5.3^{c}
Paramecium sp.	15.7±7.5^{b}	26.8±7.8^{b}	22.6±6.8^{b}	11.3±4.4^{b}	30.9±10.2^{b}	21.6±5.0^{b}
Moina sp.	3.8±2.4^{a}	2.0±1.6^{a}	2.5±1.1^{a}	1.9±0.9^{a}	1.6±0.6^{a}	2.0±0.6^{a}
Arcella sp.	0.2±0.1^{a}	0	0	0	0	0

Table 3. Combined effect of vegetable type and processing method on infusoria density (ind./mL). Values are mean ± standard deviation (n = 3). Different superscript letters in the same column denote significant differences ($P<0.05$). ***: denotes $P<0.001$; **: $P<0.01$; and *: $P<0.05$

Treatment code	Infusoria density (ind./mL)							
	Days 1–2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
T1	-	15 ± 5^{ab}	17 ± 5^{a}	153 ± 30^{ab}	451 ± 20^{ab}	$1{,}139\pm162^{b}$	807 ± 67^{a}	492 ± 24^{a}
T2	-	36 ± 10^{b}	102 ± 23^{b}	315 ± 72^{b}	568 ± 12^{b}	$1{,}065\pm112^{b}$	885 ± 117^{a}	451 ± 38^{a}
T3	-	79 ± 18^{c}	274 ± 32^{c}	876 ± 151^{c}	$1{,}816\pm289^{d}$	$1{,}281\pm132^{b}$	981 ± 86^{a}	580 ± 17^{a}
T4	-	3 ± 2^{a}	18 ± 3^{a}	61 ± 19^{a}	133 ± 45^{a}	620 ± 64^{a}	841 ± 17^{a}	452 ± 28^{a}
T5	-	9 ± 3^{a}	74 ± 21^{b}	159 ± 43^{ab}	667 ± 84^{bc}	$1{,}089\pm55^{b}$	852 ± 18^{a}	578 ± 104^{a}
T6	-	12 ± 4^{a}	76 ± 15^{b}	308 ± 14^{b}	946 ± 21^{c}	$1{,}165\pm83^{b}$	913 ± 28^{a}	557 ± 33^{a}
ANOVA: P-value								
Vegetable type (1)	-	0.000002***	0.000003***	0.000004***	0.000048***	0.001750**	0.491564	0.376279
Processing method (2)	-	0.000036***	0.000000***	0.000000***	0.000000***	0.000511***	0.023856*	0.019046*
Interaction (1) X (2)	-	0.000381***	0.000002***	0.000210***	0.000082***	0.002581**	0.426755	0.025396*

Table 4. Infusoria population growth rate. Values are mean ± standard deviation (n = 3). Different superscript letters in the same column denote significant differences (P <0.05).

Treatment code	Population growth rate (%/day)						
	Days 1–3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
T1	-	0.11 ± 0.54^{a}	1.16 ± 0.29^{ab}	1.15 ± 0.11^{ab}	1.09 ± 0.09^{bc}	0.80 ± 0.07^{b}	0.59 ± 0.06^{b}
T2	-	1.04 ± 0.40^{ab}	1.09 ± 0.11^{a}	0.93 ± 0.08^{a}	0.85 ± 0.07^{ab}	0.64 ± 0.05^{a}	0.42 ± 0.03^{a}
T3	-	1.26 ± 0.15^{bc}	1.21 ± 0.14^{ab}	1.05 ± 0.07^{ab}	0.70 ± 0.06^{a}	0.51 ± 0.05^{a}	0.33 ± 0.04^{a}
T4	-	0.75 ± 0.50^{bc}	1.47 ± 0.30^{ab}	1.24 ± 0.24^{ab}	1.32 ± 0.12^{c}	1.12 ± 0.10^{c}	0.83 ± 0.07^{c}
T5	-	2.16 ± 0.07^{c}	1.46 ± 0.04^{ab}	1.46 ± 0.08^{b}	1.22 ± 0.09^{c}	0.93 ± 0.07^{c}	0.71 ± 0.07^{b}
T6	-	1.88 ± 0.51^{bc}	1.64 ± 0.16^{b}	1.47 ± 0.12^{b}	1.15 ± 0.09^{bc}	0.87 ± 0.07^{b}	0.65 ± 0.07^{b}

CONCLUSION

The type of vegetable and the method by which it was processed showed a clear interactive effect on the population growth of infusoria. Moreover, infusoria grew faster with lettuce than with cabbage. The chopped or ground vegetables of both types supported higher infusoria populations than the unchopped vegetables.

Infusoria density peaked at 1,816 ind./mL on day 6 of the experiment when cultured with lettuce, while a peak of 1,165 ind./mL was attained on day 7 for the populations cultured with cabbage.

Euplotes sp. was found to account for about 67.5–86.8% of the population under treatment with both types of vegetables.

The results indicated that infusoria has the ability to grow quickly in density when reared with lettuce and cabbage, especially chopped and ground vegetables produced higher infusoria than un-chopped vegetable. Therefore, when used to produce infusoria as food for larval aquatic animals, it is necessary to use easily decomposable vegetables as culture media to obtain higher infusoria density.

REFERENCES

Côrrtes, G. F.; Tsuzuki, M. Y. and Melo, E. M. C. (2013). Monoculture of the ciliate protozoan *Euplotes* sp. *(Ciliophora; Hypotrichia)* fed with different diets. *Centro de Ciências Agrárias, Departamento de Aquicultura*, Universidade Federal de Santa Catarina, Rod. Doi: 10.4025/actascibiolsci.v35i1.11795.

Das, P.; Mandal, S. C.; Bhagabati, S. K.; Akhtar, M. S. and Singh, S. K. (2012). Important live food organisms and their role in aquaculture. *Frontiers in aquaculture*, *5*(4), 69-86.

Delupio, A. S. (1994). The effects of infusoria and earthworm as cultured fish-food on the growth of *Tilapia nilotica*. *Retrieved from* *https://animorepository.dlsu.edu.ph/etd_bachelors/1189*

Heydarnejad, M.S. (2008). Survival of *Paramecium caudatum* at various pH values and under normoxic and hypoxic conditions. *Pak. J. Biol. Sci. 11*(3):392-397. doi: 10.3923/pjbs.2008.392.397.

Holt, J. and Holt, S. A. (2000). Vertical distribution and role of physical processes in the feeding dynamics of two larval *sciaenids S. ocellatus and C. nebulosus*. *Marine Ecology Progress Series*, v. 193, p. 181-190.

Kamiyama, T. (1994). The impact of grazing by microzooplankton in northern Hiroshima Bay, the Seto Inland Sea, Japan. *Marine Biology*, 119 (1), 77-88.

Lahnsteiner, F. and Kletzl, M. (2018). A method for rearing perch, *Perca fluviatilis*, larvae using *Pramecium caudatum*, followed by wild zooplankton and formulated dry feed in combination with adequate tank systems. *Journal of*

Agricultural Science Published by Canadian center of Science and Education. Doi: 10.5539/jas.v10n8p26.

Lal, J.; Kumar, P.; Rai, S.; Srivastava, P. P.; Kumar, S.; Ram, R. K. and Rai, S. C. (2022). Effect of HUFA- and vitamin C-enriched live food, infusoria on growth and survival of *Clarias Magur* (Hamilton, 1822) larvae. *Aquaculture research* 53(17),5865-5874

Mendes, C.; Chambel, J.; Lopes, J.; Calado, R. and Maranhão, P. (2016). Effect of different diets on growth of the ciliate protozoan *Euplotes* sp. *International Meeting on Marine Research 2016*. doi: 10.3389/ conf.FMARS.2016.04.00032.

Mitchell, S.A. (1991). A technique for the intensive culture of *Paramecium* (group *caudatum*, Para - meciidae) as a first food for ornamental fish fry. *Aquaculture*, 95: 189-192.

Mukai, Y.; Sani, M.Z.; Mohammad-Noor, N. and Kadowaki, S. (2016). Effective method to culture infusoria, a highly potential starter feed for marine finfish larvae. *International Journal of Fisheries and Aquatic Studies, 4*(3): 124-127

Nakaoka, Y. and Oosawa, F. (1977). Temperature - sensitive behavior of *Paramecium caudatum. J. Protozol., 24(4), 575–580.* doi:10.1111/j.1550-7408.1977.tb01018.x

Poon, R. (1996). *Infusoria and Paramecium Cultures*. Calgary aquarium society. https://www.calgaryaquariumsociety.com/infusoria-and-paramecium-cultures/

Shirota, A. (1966). *The plankton of South Vietnam: Fresh water and marine plankton.* Japan: Overseas Technical Cooperation Agency.

Widjaja, F.T. and Suwignyo, S. (1981). Infusoria as an indicator of autoeutrophication, its culture for fish larvae. *SIL Proceedings, 1922-2010, 21(*3), 1503–1506. doi:10.1080/03680770.1980.1189722

Yoo, J. H. and Hur, S. B. (2002). Evaluation of ciliate *Euplotes* sp. as a live food for marine fish larvae. *J. Korean Fish. Soc.* 35(5), 542-544.