Formulating feed for fish by culturing Bloodworms *(Chironomus sp.)* in different concentrations of Rice Washing Water (RWW) and Fermented Citrus Peel (FCP). Mumbai (Maharashtra)

ABSTRACT

Bloodworms are a well-receiving and significant food source for freshwater fish, and they are also used as lures for fishing. The aim of this study was to estimate the finer growth of Bloodworms that were fed with different concentrations of Rice Washing Water (RWW) and Fermented Citrus Peel (FCP). Rice washing water accommodates important soluble nutrients. Fermented Citrus Peel is also a magnificent source of nutrients. On a daily basis, we let go of numerous wastes that can be utilised for cost-effective culturing. A possible solution is to use (RWW) & (FCP) waste as nourishing sources for Bloodworms. The method for analysis obtained was testing with five tests and three readings. C1 = 0% rice wash water, 100% fermented citrus peel; C2 = 25% rice wash water, 75% fermented citrus peel; C3 = 50% rice wash water, 50% fermented citrus peel; C4 = 75% rice wash water, 25% fermented citrus peel; and C5 = 100% rice wash water, 0% fermented citrus peel. Analysis of variance and least significance difference were used as data analytics. According to the results, treatment C3 showed the highest population density and growth rate. Altogether, the water quality lies in a temperature range of 23–28 °C, and a pH value of 6.5-8 implies an ideal environment for the growth of bloodworms.

Keywords: Bloodworm *(Chironomus sp.)*, Rice Washing Water, Fermented Citrus Peel, Formulating Fish Feed

INTRODUCTION

Feeding is one of the elements that substantially support aquaculture business activities. (Marhaendro Santoso et; al., 2023). Bloodworm are most common and organic fish feed in aquaculture for delivering an adequate amount of natural feed to fish larvae. (Bambang Sulistiyarto et; al., 2023) Chironomus larvae, aquatic insects belonging to the Diptera: Chironomidae family, can serve as a potential natural food source. (Bogut, Irella et; al, 2007), (A. Sahragard, et; al., 1385), and (D, 2016) Accessibility and quality of live food are challenging, especially in fish larval culture close to the spawning site. (Agung, et; al., 2020), and (Kumar.D et; al., 2014)

There is extensive research on use of household wastes as a sustainable waste management. Rice washing water is the water obtained after rice is washed before cooking to remove dust and grime. (Nabayi Abba et; al, 2021) It is one of the potential feeding sources to cultivate aquatic life. (Agung, et;

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Commented [R9]: Please use this format; Marhaendro et al., 2023 al., 2020) Citrus peel waste is a vital source of probiotics (Alessia Tropea et; al., 2021). In prior work, the yeast *Saccharomyces* has successfully degraded citrus peel waste to ethanol by means of enzymes. (Mark R. Wilkins et; al., 2007) Fermented citrus peels yields a nutrient-rich liquid. (Imraotun Nadila et; al., 2023) Numerous studies have been conducted on the growth of chironomus cultures in various diet mediums. (Dubey, Monika et; al., 2016)

The study affirms that using household waste as a natural feed for aquatic organism culture can promote sustainable waste management. (Imraotun Nadila et; al., 2023) Natural feeds provide comprehensive nutritional benefits. (Bambang Sulistiyarto et; al., 2023) It also does not contaminate water as industrial food does because of its high dry matter content. (Bogut, Irella et; al, 2007) The purpose of this analysis is to look at how the nutrient supplement in rice washing water and fermented citrus peel affects Bloodworm sp. population density, specific growth rate, and water quality indicators.

MATERIAL AND METHODS

Research Execute Phase

Bloodworm sp. growth study was execute under proper covering and indirect sunlight at the Bhavans Autonomous College Zoology Laboratory at Mumbai University. Two methods were used to obtain bloodworm larvae: first, from scratch, and second, by preserving eggs from scratch setup in plastic containers filled with sterile water. From the laboratory cultured bloodworms eggs were extracted. Three essays and five tests were used in the completely impartial study design. The treatment involved giving the bloodworms five different types of feed, each with varying percentages of RWW and FCP, and constant percentage of cow dung, fish waste from fish culture tanks. Fish waste was filtered through a cloth after it was sucked and precipitated. Meals were added to the growth medium at a rate of 1.5 g/l.

Preparations

Preparations of Rice Washing Water (RWW): Rice preparation is a daily routine, and this is where the idea for the rice washing water arose. Aquatic organisms are now fed by washing water, which is typically discarded. A 1:1 mixture of yogurt and rice washing water is combined. This exact proportion is used to guarantee a perfect and well-balanced mixture that supports the growth and well-being of aquatic life, upholding a peaceful coexistence with the aquatic environment.

Preparations of Fermented Citrus Peel (FCP): The local juice vendor provided the citrus peels in bulk also some of these peels were accumulated from home waste that were used. After being cleaned and chopped into square pieces, the citrus peels were weighed up to 300 g and put in a clear plastic bag for aeration. After 30 minutes of steaming, 1.35 g of yeast saccharomyces were added to the citrus peel once it had cooled. Anaerobic fermentation is created and allowed to sit at room temperature in a jar for 8 days. 300 g of fermented citrus peel and 1000 mL of water are combined to create the fermented citrus peel solution of different concentrations. After fermenting citrus peel and water, the mixture is then homogenized and filtered and put to use.

Research Methods

There were three readings and five tests in the truly unbiased study design. The following are the research solutions: C1 is equivalent to 100% fermented citrus peel (FCP) and 0% rice washing water; C2 is equivalent to 25% rice washing water (RWW) and 75% fermented citrus peel (FCP); C3 is equivalent to 50% rice washing water and 50% fermented citrus peel; C5 is equivalent to 75% rice washing water and 25% fermented citrus peel; and C5 is equivalent to 100% rice washing water and 0% fermented citrus peel.

Population Density:

The population density of bloodworms was calculated 2 times (1st & 8th day). Observations were made during morning time. For calculating population density, first 40 ml of sample media was taken in a petri dish, the bloodworms were counted respectively and computed by using cell counting method "Sedgwick Rafter" (Dubey, Monika et; al., 2016)

Population Density of bloodworm was computed as;

$$a = b \times (p/q)$$

Where:

a = No. of individual bloodworm sp. in the culture medium (ind.L-1),

b = average number of bloodworm sp. from repeated calculations (Ind),

p = volume of culture media (l),

q = volume of sample culture media (converted 40ml to 1l)

Counting was done in three tests with respect to number of individuals and the results were average.

Population Growth Rate:

Growth rate of bloodworm was calculated in the form of initial and final days.

$$G = \frac{In\,Nt - In\,No}{In\,No} \times 100\%$$

Where: G = Population growth rate (%);

No = initial no. of bloodworms during the study;

Nt = final no. of bloodworms during the study. (Widanarni, D.D. et; al., 2006)

Survival Rate:

The survival rate was obtained by counting the number of bloodworms that remained alive from initial to final day after the bloodworms were extracted on eight day.

Physio-chemical Analysis:

The physical and chemical parameters including, pH (power of hydrogen) and temperature, were examined and maintained during 8 days maintenance period.

~~Research~~Data Analysis

The statistical data gathered in the form of a population density of Bloodworms sp. is presented as a population growth graph. The whole data for the mean value was evaluated at the highest level of confidence using ANOVA (Analysis of Variance). The LSD (Least Significant Difference) test was used to examine the impact of dietary items on the rate of bloodworm growth, mostly if the data showed some

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significant differences. Analysis were done using statistical analysis tools Microsoft Office Excel 2013 software. (Imraotun Nadila et; al., 2023) & (Agung, et; al., 2020). And lastly data on water quality parameters were studied descriptively.

RESULT AND DISCUSSION

Results based on the study, the tests of fermented citrus peel solution was observed to be effective in case of bloodworm population growth.

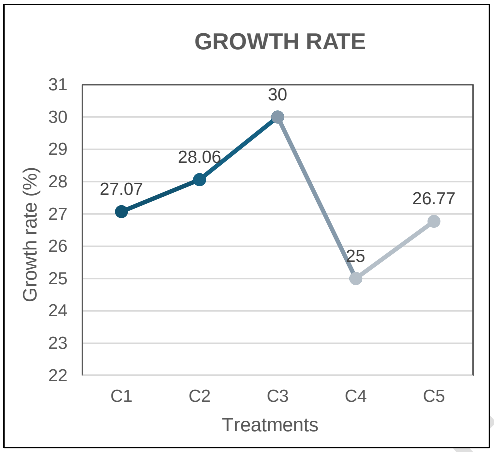

Fig 1: Population growth rate of bloodworm sp. in different culture ~~medium~~media.

Data of population density is computed in the form of growth rate graph (Fig. 1), through observation it can be stated that there is variation in population density as there are variations in results of population growth rate. Population density is measured in ind.L-1 (individuals per litre of water) (Agung, et; al., 2020). From the start of the investigation until it reached its climax, the population increase of Bloodworm sp. was monitored. (Fig. 1) contains information on the study's population growth rates. Treatment C3, which consists of 50% RWW and 50% FCP, had the quickest population growth rate among all treatments, with an average growth rate of 30%, according to the graph. At an average growth rate of 28.06%, treatment C2 also showed a high rate. Similar growth rates, ranging from 25% to 27.07% on average, were seen in treatments C1, C4, and C5. Based on these findings, C3 appears to offer the best circumstances for bloodworm growth.

Table 1: Population density & growth rate of bloodworm sp. in different ~~medium~~culture media.

TREATMENT	INITIAL				FINAL				GROWTH RATE
	Repeat			***Mean***	***Repeat***			***Mean***	
C1	19	16	13	**400**	20	18	23	**508.3**	**27.07%**
C2	21	19	17	**475**	24	25	24	**608.3**	**28.06%**
C3	20	19	21	**500**	27	25	26	**650**	**30%**
C4	15	19	14	**400**	19	20	21	**500**	**25%**
C5	16	17	15	**407.5**	19	21	22	**516.6**	**26.77%**

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C1, C2 and C3 these treatments have different levels of population density. Among all treatments C3 results into highest average density, with approximate average value of 500 ind.L-1 in initial and 650 ind.L-1 on final day. C1 and C2 also show high densities ranging from 400 & 475 ind.L-1 initially and 608.3 & 508.3 ind.L-1 on final day .

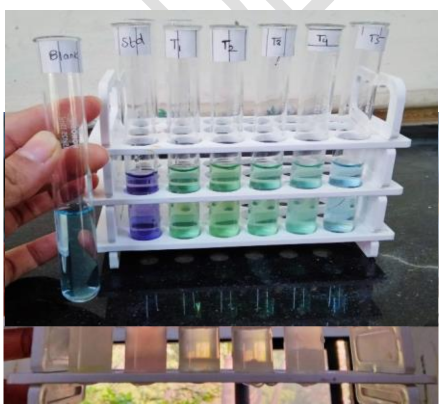
TEST	DIFF.	T.STAT	LSD	TEST	DIFF.	T.STAT	LSD
C1-C2	-75	-1.75	*NOTSIG*	**C1-C3**	-100	2.33	*SIG*
C2-C3	-25	-0.58	*SIG*	**C1-C4**	0	0	*NOTSIG*
C3-C4	100	2.33	*SIG*	**C2-C4**	75	1.75	*SIG*
C4-C5	0	0	*NOTSIG*	**C2-C5**	75	1.75	*SIG*
C5-C1	0	0	*NOTSIG*	**C3-C5**	100	2.33	*SIG*

(Table: 1). In comparison to rest of the treatment if RWW density is increased it may affect the growth of bloodworms

Table 2: LSD (Least Significance Difference) for Population density of bloodworm sp. in different medium

It is clear that treatment C3 (50% RWW, 50% FCP) has a considerably greater population density than the other treatments based on the findings of post hoc analyses employing the Least Significant Difference (LSD) test (Table: 2) (Imraotun Nadila et; al., 2023). This combination probably offers a more ideal environment for bloodworm proliferation (BNT, $p<0.05$). The treatments C1 with C3, C2 with C3, C4, C5, and C3 with C4, C5 showed notable changes in this investigation.

Fig. 2: Protein & lipid estimation of RWW & FCP



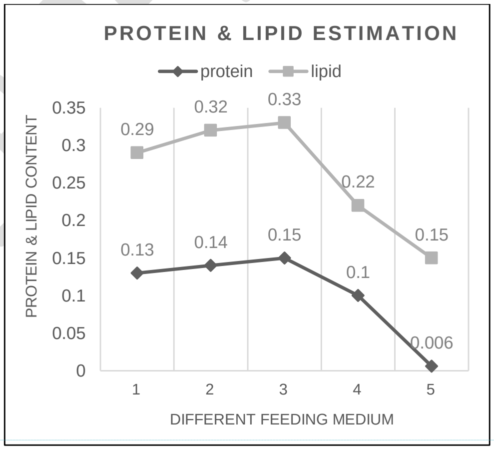


Fig 3: Nutritional content of (RWW & FCP) feedstock

Based on tests there are many impact on Bloodworm sp. population growth (Bogut, Irella et; al, 2007). Nutrients amount in RWW and FCP is one of these variables (Rahul podder et; al. , 2018). According to (Fig: 3) test C3 consist of highest amount of nutrients i.e. 0.33 mg/ml of protein and 0.15 mg/ml of lipid. RWW contains extra nutrients that bloodworms

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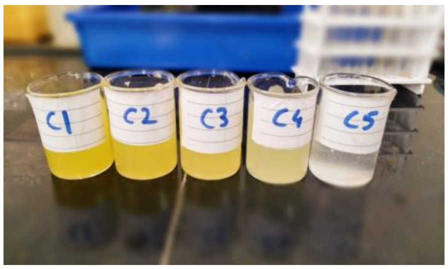
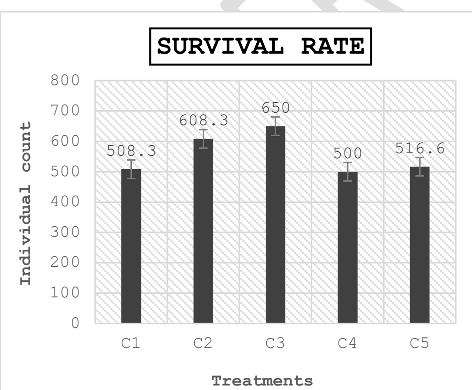


et; al., 1385) this can be interpreted in the context of this study as the feed's evenly distributed composition across treatments providing bloodworms enough nourishment.(Fig. 2)

Fig. 4: Different concentration of RWW & FCP sol.

Fig 5: Survival rate of bloodworm sp. in different medium.

Nutritional concentrations are crucial for survival, development, and reproduction, as indicated by earlier parameters. Monitoring the initial and final populations of Bloodworms sp. allowed the analyses



to be made appropriately. (Bambang Sulistiyarto et; al., 2023), & (Widanarni, D.D. et; al., 2006). The final result shows that test C3, that consist of (50% of RWW and FCP) has the highest rate of survival, followed by treatments C1 and C2, respectively. While treatments C4 and C5 have the lowest rates of survival (Fig: 5), the variations in the concentration and densities of RWW and FCP solutions (Fig. 4) used for bloodworm culture account for the discrepancies in these results respectively.

Table 3: Water quality parameters of bloodworms culture.

TEST	pH		Temperature (°C)	
	Initial	**Final**	**Initial**	**Final**
C1	5.4	5.3	30	30.1
C2	6.1	6.0	29	29
C3	6.8	6.5	27.8	28
C4	7.0	7.0	28	28
C5	7.1	7.0	28.3	28.5

Variations in water pH and temperature can have a significant impact on bloodworms, according to the results (Table: 3) of physicochemical parameter assessments of bloodworm sp. rearing water media gathered during the study, pH and temperature fluctuations might affect the nutritional availability of the water, which is critical for their development and reproduction. An increase in pH and temperature can reduce the quality of the food sources available to these organisms (Imraotun Nadila et; al., 2023).

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Second, pH fluctuations can influence a chemical compound's solubility (Widanarni, D.D. et; al., 2006). For example, heavy metals may become more soluble at higher pH levels. Thus tests with higher pH values, such as C4 and C5, may increase the concentration of harmful compounds in the water, lastly, pH fluctuations can affect the overall chemical stability of the aquatic ecosystem, including dissolved oxygen availability and bloodworms' ecological connections with other species (Bambang Sulistiyarto et; al., 2023).

CONCLUSION

According to this study, the population density of Bloodworm sp. in test C3 developed on day eight, and environmental parameters such as food supplies and water pH/temperature influenced the optimum density. The population density graph evolved, indicating the typical trend of population growth for Bloodworm sp. Furthermore, the C3 variant showed the highest population growth rate for Bloodworm sp., demonstrating that organic supplies are more helpful to small organisms than commercial feedstock. Despite having a brief life span, they are mostly utilized as live fish feed in aquaculture and fish feed. Bloodworms can also provide vital nutrients for aquaculture feed, making them an invaluable source. Environmental pollution is a result of improper management of organic waste. These negative consequences can be mitigated and a circular economy built by diverting organic waste from landfills and repurposing it in aquaculture. Plant decomposition and other organic waste can serve as a great substrate for bloodworms. In conclusion, organic matter has more nutritional value than commercial or industrial feedstock, it should be employed in a range of applications to offer natural feed for small aquatic organisms such as bloodworms. Bloodworms are a valuable source of protein and essential nutrients for aquaculture feed. Organic waste, such as decaying plant matter and sewage sludge, can serve as an ideal substrate for bloodworm cultivation. This not only reduces waste but also provides a sustainable feed source for fish farming. Incorporating organic waste into fish diets can enhance their nutritional value and reduce reliance on conventional feeds, such as fishmeal.

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