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Preliminary Studies on Low-Cost Laboratory Culture Method for *Daphnia*

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Abstract: *Daphnia* is one of the important members of the phylum Branchiopoda (also called Phyllopoda) and belongs to the suborder Cladocera which are small crustaceans. They also serve a crucial part in aquatic food chains in water as food for fish or a variety of invertebrate predators. It has been used as a model organism to study the effects of environmental stressors and toxicological intervention studies. The current work is preliminary study on laboratory culture method with the main focus on preparation of low-cost laboratory culture of *Daphnia* using field water, Dry yeast, *Spirulina*, Peanut meal and Jaggery.

Keywords: *Daphnia,* Dry yeast, *Spirulina,* Culture, Branchiopoda

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Introduction

Daphnia is one of the important members of the phylum Branchiopoda (also called Phyllopoda) and belongs to the suborder Cladocera which are small crustaceans. Their body remains enclosed in an uncalcified shell known as the carapace which encompasses the whole trunk of the body with the exclusion of head and the apical spine. The key important feature for taxonomic identification of *Daphnia* is the presence of flattened leaf-like legs necessary to produce a water current for the filtering apparatus and the carapace which is made up of polysaccharide chitin (Ebert, 2005).

More than 100 species of freshwater zooplankton organisms from all over the globe belonging to the genus *Daphnia* have been reported. With the exception of harsh environments like hot springs, they can be found in various types of standing freshwater habitat. All age groups of *Daphnia* have strong swimming abilities and are primarily pelagic, or remain

present in open water. Even though they are filter feeders by nature, some species can frequently be seen clinging to aquatic vegetation or even digging through the bottom sediments of small ponds. The size of an adult ranges from less than 1 mm to 5 mm, with the smaller species usually being found in water bodies where fish predation is more (Sed'a and Petrusek, 2011).

Daphnia is an important species in ponds and lakes, where it is the major consumer, filter-feeding tiny suspended particles, particularly unicellular algae. They also serve a crucial part in aquatic food chains in water as prey for fish or a variety of invertebrate predators (Ebert, 2022). It has been used as a model organism to study the effects of environmental stressors on zooplankton communities for several decades as they are an essential component of the trophic structure of aquatic ecosystems (Harris *et al*., 2012). Furthermore, they are engaged in critical biogeochemical processes related to ecosystem functioning and are sensitive to environmental changes. They have been extensively used in toxicological intervention studies to assess the toxicity of pharmaceutical products such as drugs and also to access the potentially toxic effects of chemical compounds (Kim *et al*., 2015; Tkaczyk *et al*., 2021; Ebert, 2022; Liu *et al*., 2022).

As it has great importance in laboratory studies, the culture method for them have not studied well till date. There are few reports available suggesting possible culture methods in larger tanks using different algal species such as *Selenastrum*, *Chlorella*, *Ankistrodesmus*, *Chlorobium* or *Chlamydomonas* (Taub and Dollar, 1968; Gophen, 1977; Hadas *et al*., 1982; Buchberger *et al*., 2019). The present work was carried out to prepare a culture medium for *Daphnia* in lab by using easily available cost effective materials for evaluation of toxicity of chemical compounds.

Materials and Methods

Study area:

Varkute lake:

It is located in Varkute khurd village of Indapur Tahsil of Maharashtra at $18^{0}03'29"$ N and $74^{0}55'05"$ E. This village is situated 125 km away from district headquarter of Pune and 15 km away from Indapur tehsildar office. The water of the lake is used mainly for agricultural purpose. Its total area is about 2 acre with depth of about 10 feet. The water in lake remains for a period of 8 months after the monsoon. The water gets accumulated in the lake from surrounding regions of small streams around lake.

Sample collection:

The water samples were collected from selected study area between January to March of 2023. The collected water samples were filtered by using plankton net no. 25. About 10 L of water was filtrated. The filtrate containing *Daphnia* was brought to laboratory for further culture study. 1 L of water sample without *Daphnia* was also collected in a separate polythene bottles for preparation of culture medium and renamed as "field water".

Preparation of culture medium:

The constituents required for preparation of culture medium are field water, *Spirulina*, Dry yeast, Peanut meal and Jaggery. The *Spirulina* pure powder for the study was purchased from Bioven Ingradients (Greater Noida, Uttar Pradesh, India). The yeast powder was purchased from local shop Anand Dryfruits and Garam Masala, Baramati. The Peanut meal and Jaggery were purchased from Phulchand Gandhi Kirana Store, Phaltan. The culture medium was prepared by addition according to Table 1.

Initially pure *Spirulina* and dry yeast each of 0.002 g along with 0.004 g of each peanut meal and Jaggery were mixed in 900 ml field water in glass beaker. Mixture was warmed for 2 min on burner gas followed by cooling for 20 min and kept for stabilization for about 5 min. The final volume of culture medium was filled with field water again till it becomes 1000 ml. The final medium was further used for culture of *Daphnia*.

Table 1: Composition of culture medium

S. No.	Constituent	Amount (Volume or gram)
1	Field Water	1000 ml
2	*Spirulina* (Pure)	0.002 g
3	Dry yeast	0.002 g
4	Peanut meal	0.004 g
5	Jaggery	0.004 g

Table 2: Composition of medium for assessing the effect of distilled water and field water on Growth and development of *Daphnia*

S. No.	Constituent	Volume or gram amount for distilled water	Volume or gram amount for field water
1	Water	1000 ml	1000 ml
2	*Spirulina* (Pure)	0.002 g	0.002 g
3	Dry yeast	0.002 g	0.002 g
4	Peanut meal	0.004 g	0.004 g
5	Jaggery	0.004 g	0.004 g

Validation of culture medium:

The finally prepared culture medium was validated with experimental setup using varying concentrations of yeast and *Spirulina*, and also by comparing the field water with distilled water for possible better results. For every set of experiment, 100 ml culture medium from stock culture media was taken into glass beaker followed by handpicking method for *Daphnia* from collected *Daphnia* containing water sample by using compound microscope and dropper. The identification of *Daphnia* was performed using standard identification keys. A total of 10 *Daphnia* were added in each of three beakers from collected samples by handpicking method. All beakers were exposed to sunlight for 2 h prior to transfer into incubator at 20°C.

The composition mentioned in Table 1 was again added in 1000 ml distilled water to compare the effect of distilled water and field water on growth and development of *Daphnia* (Table 2). All ingredients were added in distilled water and field water in separate 1000 ml glass beakers and named as stock culture medium of distilled and field water accordingly. Among the whole volume, 100 ml of each distilled water culture medium was then transferred to three 100 ml breakers and similar procedure was carried out for field water culture medium. Each of these beakers was added with 10 *Daphnia* each. All beakers were exposed firstly to sunlight for 2 h followed by incubation at 20°C in incubator.

The effect of change in concentration of yeast and *Spirulina* were assessed for two different concentrations of 0.002 gl^{-1} and 0.004 gl^{-1} of each *Spirulina* and yeast. The parameters taken into consideration were heart beats and death percentage of *Daphnia* (Table 3). All experiments were been conducted in triplicate. All sets of experiments were exposed to sunlight for 2 h followed by keeping at 20°C in incubator.

Results and Discussion

The culture medium prepared was further analyzed for efficacy of medium where triplicate trials were conducted with 2 h exposure sunlight and further for incubation at 20°C. The results were checked after 2 days. Initially 10 *Daphnia* were added in the 100 ml medium beaker. After two days, the total number of live *Daphnia* recorded was 20, 20 and 24 in trial- I, II and III, respectively (Table 4). The significant increased number of *Daphnia* showed that the culture medium was suitable for growth and development of *Daphnia*.

Further analysis on effect of water source such

Table 3: Composition of medium for assessing the effect of Yeast and *Spirulina* on Growth and development of *Daphnia*

Component	Set-A	Set-B	Set-C	Set-D
Field Water	1000 ml	1000 ml	1000 ml	1000 ml
Spirulina (Pure)	0.002 g	0.002 g	0.002 g	0.004 g
Dry yeast	0.002 g	0.004 g	0.002 g	0.002 g
Peanut meal	0.004 g	0.004 g	0.004 g	0.004 g
Jaggery	0.004 g	0.004 g	0.004 g	0.004 g

Table 4: Validation of culture medium by trial method

Trial number for validation of culture medium	No. of *Daphnia* added	Count of *Daphnia* after 2 days			Total alive *Daphnia* after 2 days
		Adult	Young stage	Dead	
I	10	7	13	3	20
II	10	9	11	1	20
III	10	8	16	2	24

Table 5: Effect of water source of culture medium on survival of *Daphnia* after 2 days. I, II and III are trials during triplicate analysis

	Medium with distilled Water			Medium with field Water		
Trial no.	I	II	III	I	II	III
No. of *Daphnia* added	10	10	10	10	10	10
No. of alive *Daphnia*	0	0	0	21	22	20
No. of dead *Daphnia*	10	10	10	0	0	0

as distilled and field water was analyzed in triplicate trials. The significant difference was found in all *Daphnia* from distilled water culture medium which were found to be dead after 2 days in all three beakers. On the other hand, field water culture medium was found to be beneficial to *Daphnia* as the number was found to be increased up to 21, 22 and 20 in three beakers. It clearly indicates that the change in water is also detrimental to survival of *Daphnia* and is sensitive to small change in water (Table 5). Furthermore this set reported that while preparing culture medium, the field water should be used in laboratory.

Analysis of effect of dry yeast and *Spirulina* each of 0.002 gl^{-1} and 0.004 gl^{-1} indicated that the number of alive *Daphnia* was increased significantly in beakers with 0.002 gl^{-1} of both dry yeast and *Spirulina*. The increased numbers were again twice than primarily added *Daphnia*. A 21, 24 and 20 *Daphnia* were been recorded in the same set. While in other sets consisting of 0.002 gl^{-1} dry yeast with 0.004 gl^{-1} of *Spirulina*, 0.004 gl^{-1} dry yeast with 0.002 gl^{-1} *Spirulina* and 0.004 gl^{-1} dry yeast with 0.004 gl^{-1} *Spirulina* were recorded the death of all *Daphnia*. A set with 0.002 gl^{-1} dry yeast and 0.004 gl^{-1} of *Spirulina* caused the toxicity. The excess *Spirulina* leads to the death of all *Daphnia*. A set with 0.004 gl^{-1} dry yeast and 0.002 gl^{-1} of *Spirulina* caused the toxicity of excess dry yeast leads to the death of all *Daphnia*. A set with 0.004 gl^{-1} dry yeast and 0.004 gl^{-1} of *Spirulina* caused the toxicity, excess of both dry yeast and *Spirulina* leads to the death of all *Daphnia*. These results clearly showed that a small increase in concentration of dry yeast and *Spirulina* is lethal to survival of *Daphnia*. A 0.002 gl^{-1} is the best concentration for successful culture (Table 6).

There are few studies which reported that *Daphnia* shows its high growth at temperature

Table 6: Effect of different concentrations of dry yeast and *Spirulina* on total count and survival of *Daphnia* after 2 days. All sets have been added with 10 *Daphnia* each. I, II and III are trials during triplicate analysis (*Toxicity due to dry yeast, **Toxicity due to *Spirulina* & ***Toxicity due to both dry yeast and *Spirulina*)

Component of medium		Dry yeast (0.002 gl^{-1})			Dry yeast (0.004 g l^{-1})		
		I	II	III	I	II	III
Spirulina (0.002 gl^{-1})	I	21	-	-	0*	-	-
	II	-	24	-	-	0*	-
	III	-	-	20	-	-	0*
Spirulina (0.004 gl^{-1})	I	0**	-	-	0***	-	-
	II	-	0**	-	-	0***	-
	III	-	-	0**	-	-	0***

between 16^0C to 22^0C. The best temperature range for culture is between 18°C to 22°C (Khan and Khan, 2008; Müller *et al*., 2018; Seefeldt and Ebert, 2019; Adamczuk, 2020).

Conclusion

The culture medium prepared by using field water and 0.002 gl^{-1} culture media of each dry yeast and *Spirulina* was found to be effective for culture at laboratory level. The culture medium containing distilled water instead of field water was found to be detrimental to *Daphnia*. Also the dry yeast and *Spirulina* concentration of 0.004 gl^{-1} culture medium was found to be the cause for death of *Daphnia*. The current culture method is cost effective which requires less expenses as well as is well suitable for various toxicological studies. Although the incubation temperature was kept as 20^0C, the optimum temperature for current culture method is needed to verify with more experimental set-ups. The work on the same plan is ongoing in our lab.

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