Wolffia globosa

Ultimate Cultivation Guide

The white paper offers a crucial manual for the successful development of Wolffia globosa, the tiniest angiosperm, in indoor farming settings. We have covered the ecological factors that significantly affect produce quality as well as the physiological and chemical factors that control Wolffia globosa growth. Additionally, we showed how to successfully produce Wolffia globosa indoors over an extended period so that the seeds might be used to sustain future human and animal populations.

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4. Introduction

Progressive simplification of *Spirodela* to *Lemna* to *Wolffia*

The aquatic plant *Wolffia globosa* belongs to the Lemnaceae family, also known as duckweeds, which is the smallest and fastest-growing

family of angiosperms, or seed plants, in the world[1]. The main method of reproduction for the protein-rich *Wolffia* is "budding off," as it does not have a stem, leaf, or root[2].Duckweed is classified into five genera: *Landoltia*, *Spirodela*, *Lemna*, *Wolffiella*, and *Wolffia*. The more derived and reduced species of *Wolffia* are the result of the simplification and evolution of the ancient Spirodella species that produces adventitious roots (Figure 1) has been simplified and evolved into the more derived and reduced species of *Wolffia*[3]. A portion of these two plant species have a diameter of less than 0.5 mm and proliferate quickly by budding[4].

Figure 1. Life stages of Spirodela.

The Genus Wolffia

The genus *Wolffia* has no stem or roots and its smallest specie has a diameter of 1 mm which minimise the need for any non-photosynthetic organs in the plant body [4]. Due to its miniature size, Wolffia has been considered as world's smallest angiosperm plant [5]. Based on morphological features, there are 11 species of Wolffia, namely W. *angusta* or W. *microscopica*, W. *arrhiza*, W. *cylindracea*, W. *australiana*, W. *globosa*, W. *neglecta*, W. *borealis*, W.*brasiliensis*, W. *columbiana*andW.*elongata*[6]. The phylogenetic analysis of different species belonging to the duckweed (Araceae) family revealed

closeness of W. *globosa* and W. *arrhiza* as related sister species [5].The W. arrhiza is also the largest among all the species and the W. globosa is the smallest specie [7]. The *Wolffia* has single photosynthetic unit called frond or thallus which are globosa ovoid shape. During budding, the daughter fronds are formed from the meristematic cells within a pocket of the mother frond. This process of vegetative reproduction in *Wolffia* maintains the genetic uniformity which has relevance in industrial applications[8].The growth rate of *Wolffia* is remarkable 0.155- 0.559 (per day) and varies from with the species and clones [8]. In other words, *Wolffia* doubles its biomass within 2-3 days under its optimal temperature of 20-30˚C at pH range of 5-7 [5]. Similar to *Spirodela*, *Wolffia* also produce regenerative bodies called turions when the temperatures drop below 15˚C or under nutrient stress conditions [8]. *The detailed morphological identification key to the various genera and species of the duckweed family is provided in* [9].

Occurrence and Habitat of Wolffia

Wolffia is a cosmopolitan plant found in the lentic ecosystems of Earth, except Antarctic and Arctic regions [8]. Different species of Wolffia are native to different regions such as *W. arrhiza* is the only native species of Europe[10]. In recent times, the *W. globosa* which is native to Southeast Asia has become invasive to Europe [11].

In India, *W. globosa* is found in Eastern states such as West Bengal where it is found growing in un-disturbed freshwater lakes (Figure 2).

Figure 2. One of the lakes where *W. globosa* was found in abundance in the month of March.

5. Duckweed cultivation systems

The duckweeds are easy to grow plants. In indoor cultivation, species of duckweed are grown in different types of vessels. The smallest is the transparent bucket with surface area as small as 0.067 m^2 (Figure 3) and opaque rectangular trays with surface area of 0.08 m^2 (Figure 4).

Figure 3.Smallest cultivation vessel for growing duckweeds. The surface area is 0.067 m^2. Left is the top view and on right is the lateral view.

Figure 4. Opaque cultivation tray for growing *Spirodela* has surface area of 0.08m^2.

The transparent plastic buckets encourage the growth of blue green filamentous and green colonial microalgae. Microalgae proliferate throughout the summer, when sunlight is at its strongest, covering the entire bottom of the vessel with filamentous blue-green microalgae. Other cultivation characteristics like inoculation density, light, temperature, nutrition content, and quality of the starting fronds also affect the level of contamination.

Stainless steel tanks of different capacities are used to increase biomass and prevent microalgae contaminations. (Figure 5).

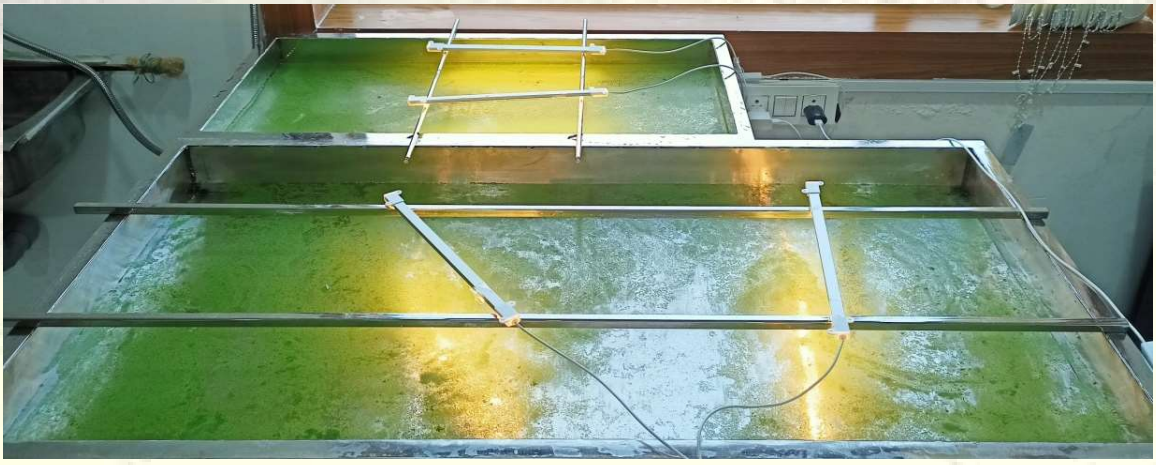

Figure 5. Stainless steel tanks for mass indoor cultivation of *W. globosa*.

6. Step by Step Guide to Use Wolffia 'seeds' after transportation

1. **Prepare a clean 3 to 5 liter plastic rectangular tub\tank*, sterilized spatula and minimum two cleaned/sterilized beakers filled with deionized or RO water (Figure 6).**

Figure 6. Wolffia seed cleaned in beakers followed by inoculation into a small transparent bucket of area 0.067 m^2.

2. **Prepare the sterilized\autoclaved hydroponics solutions using RO water complying with following physio-chemical conditions (as close as possible):**
    i. **pH- 6.5-7**
    ii. **Temperature- 25-30°C**
    iii. **TDS- 150 ppm**
    iv. **NO3- <100 ppm**
    v. **PO4- <20 ppm**
    vi. **NH4- <3**
3. **Put 3 liter of RO water in a 5 Liter tank/tub and add the sterilized hydroponic solutions. Keep it aside.**
4. **Open the Wolffia package and remove the container containing the plant sample.**
5. **Put whole sample in to the first cleaned beaker containing RO water and give a gentle stir with the autoclaved spatula. Let it settle. (If fronds are sticking to the edges, use wash bottle with RO water to suspend it back into the beaker.)**
6. **Using the spatula, move the fronds from the top layer of the above beaker into the second beaker with RO water. Repeat step 5 at least twice to clean the fronds before using it as 'seed' for the growth tank. After seeding, place the tanks in dim light and preferably do**

the transfers in the evenings and place it near the North or West facing windows for available sunlight.

7. Duckweed cultivation conditions

The important factors that need to be optimised for obtaining dense and cleaner crop of *Wolffia* are light, nutrients, pH, temperature and seeding. We have been performing indoor cultivation of *Wolffia* using sunlight as source of light by keeping the cultivation tanks closer to the windows. Experiments done with extra LED lights showed detrimental effects on growth and vigour of *Wolffia*. The fronds growing beneath the LEDs were darker in colour and showed high algae contamination (Figure 7). In addition, enhanced bubbling or increased oxygen production was observed when the LED lights were placed within 10 cm to the surface of the cultivation tanks (Figure 8). *W. globosa* can be grown in an economical and user-friendly medium using the typical hydroponics nutrient solution, which includes both macro and micronutrients. The usual hydroponic nutrition solutions include large amounts of nutrients, so it is necessary to optimize the hydroponics solution's strength. Both the macro and micronutrient stock solutions, A and B, make up the typical hydroponics solutions that are sold on the market. It is best to evaluate several dilutions of the hydroponics nutrient solutions because they may include high concentrations of phosphate and nitrate. The recommended dosage of the typical hydroponics solutions was tested at several dilutions (1:3, 1:6, and 1:10) (Table 1).

Figure 7. Wolffia tank equipped with LED lights showed patchy growth of fronds.

Figure 8. Enhanced bubbling and algae contamination in LED equipped Wolffia cultivation tanks.

Test parameters	Hydroponic solution dilutions	
	1:6	1:3
pH	**6.2**	**6.0**
Total dissolved solids (TDS)	**207**	**339**
Electrical conductivity	**414**	**678**
NO^-_3(ppm)	**154**	**362**
NO^-_3- N (ppm)	**35**	**82**

PO^{-}_{4} (ppm)	36	74
P (ppm)	12	24
P_2O_5 (ppm)	28	55
NH_4 (ppm)	3.4	5.2
NH_3 (ppm)	3.2	4.9
NH_3-N (ppm)	2.6	4.0

Table 1. Physiochemical parameters of the duckweed growth medium at two different dilutions.

8. Biological diversity in Wolffia tanks

Mass cultivation of duckweed does not occur in ecological isolation. The most common contaminants or symbionts of duckweed are filamentous colonial microalgae, rotifers, larvae (Figure 9), and occasionally mold. Mold and fungal infestation in duckweed cultivation is exposure to moisture. High humidity will lead to mold growth. Another factor that promotes mold is the presence of organic compounds such as acetates and sugars in the cultivation medium.

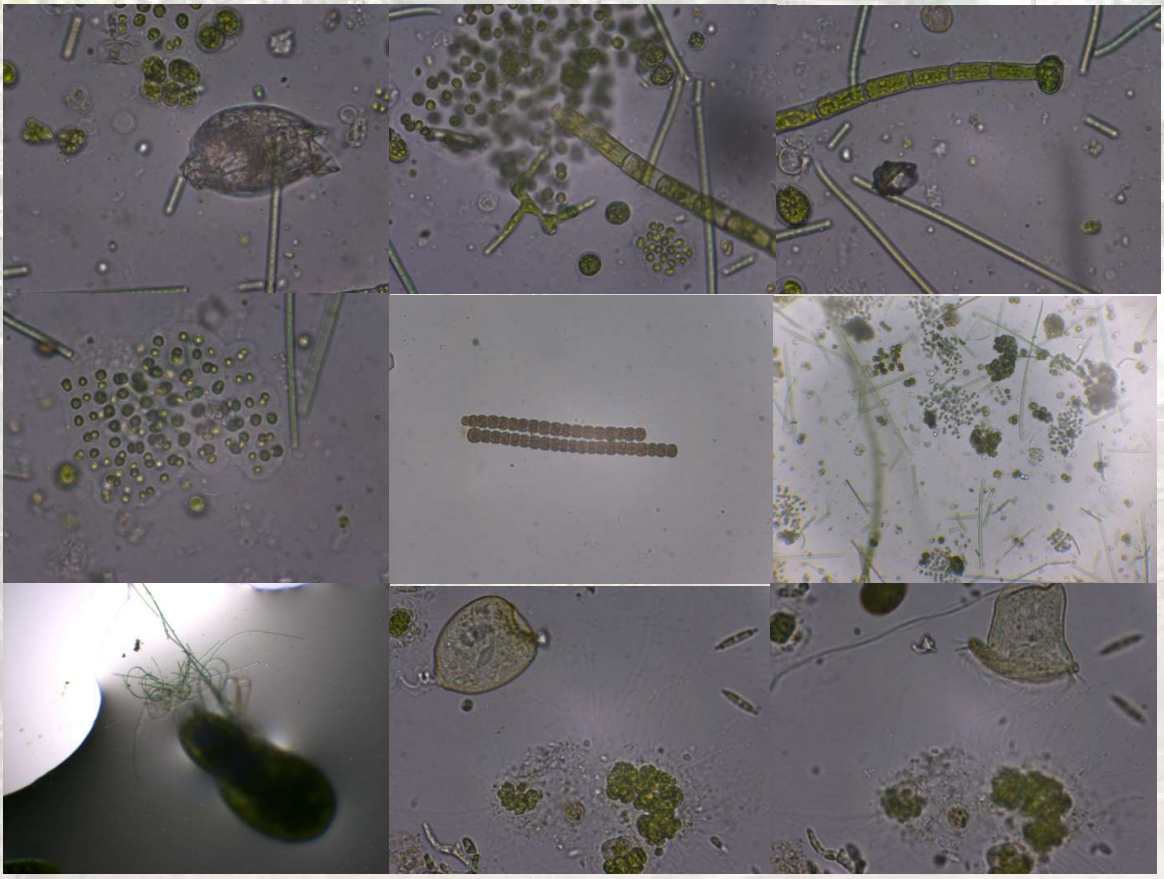

Figure 9. Types of biological contaminants found in *Wolffia* tanks.

The high nutrient concentrations of the duckweed growth medium promote growth of algae which is further aggravated in presence of high light. The following are the recommendations to minimise or avoid the growth of algae in *Wolffia* growth tanks.

- **Increasing the inoculation density such that the whole surface area of the cultivation tank or bucket is full covered with Wolffia.**
- **Use lower dilutions of the growth medium**
- **Avoid brighter LED lights. LED lights put too closed (Figure 5) to the growth tank promote algae growth which causes the excess bubbling in the growth tank; thereby causing the production of reactive oxygen species and irreversible damage to the *Wolffia* fronds**
- **Use steel tanks for growing *Wolffia***
- **Use cleaner fronds to seed the cultivation tank**

9.Duckweed sterilization protocol

The mold and filamentous algae can be treated in duckweed using potassium permanganate. The optimised protocol is given below.

1. **Reagent- 0.1% potassium per manganate ($KMnO_4$). Prepare fresh 0.1% $KMnO_4$ solution in distilled/reverse osmosis (RO) water.**
2. **Transfer the fronds/duckweed plants in a tub containing 0.1% $KMnO_4$ and keep it in this solution for 10 minutes.**
3. **After 10 minutes, strain out the solution and put the fronds/duckweed in clean RO water and keep it here for 5 minutes.**
4. **Strain out this water and transfer the fronds/duckweed into fresh RO water in a tub. Repeat the washings until you do not see any purple color in the water to ensure complete removal of $KMnO_4$.**
5. **After washings, transfer the fronds/duckweeds in freshly prepared growth medium in the tub.**

Notes

This is an effective treatment for killing any fungus/mold/filamentous algae contamination.

After the $KMnO_4$ treatment, the white mold/algae filaments are killed and get settled to the bottom. Therefore, instead of straining or removing the water, you can also transfer the fronds from the surface using a sieve.

10.Harvesting and processing of the biomass

The matured crop of Wolffia is harvested from the tank using stainless steel dust pan (Figure 10) and is then sieved through the nylon mesh strainer or net. The harvest crop is washed repeatedly with reverse osmosis water to remove contaminants such as green microalgae, small ciliates and rotifers. When the crop is sold as freshly produced biomass, no further processing is required. Once the crop is harvested, it is kept in the refrigerator to avoid any decomposition.

Figure 10. Steps involved in harvest of mature crop of Wolffia from tank to the packaged product.

11.Conclusions

This white paper serves as a user's guide for those who are new to cultivating Wolffia globosa indoors. The data presented in this white paper is the result of over a year's worth of intensive study and investigation on duckweeds, in particular the Wolffia globosa. We provide Wolffia globosa seeds at a reasonable cost, taking into account that this plant is difficult to locate. Producing pure Wolffia globosa seeds has never been simple, but with the help of this guidance, cultivators will be able to successfully cultivate clean Wolffia globosa crops in much less time than a year.

12. Selected references

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