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A Brief Insight on the *In vitro* Growth of Lesser Duckweed – *Lemna minor* L. under varying light conditions

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ABSTRACT

Exhaustive investigation in plant physiology, plant biochemistry and ecological toxicity especially in aquatic environment requires a promising aquatic model plant for which *L.minor* L. is best suited. It is considered to be a rich source of proteins and can replace traditional source of proteins both for food and feed. Its optimal growth and biomass accumulation under laboratory condition requires balance among the different macro and micro elements of the composition of the culture medium along with optimal spectral distribution. The focus of the present review is to study the *in vitro* growth of *L.minor* L. under different culture parameters maintained with normal and modulated physico-chemical conditions including varying photoperiod regime along with different spectral distribution.

Key words*: Nitrogen, RGR, L.minor L., Phosphorus, Quantum yield, G.I.*

Introduction

Duck weeds are aquatic plants under the family Araceae, Sub family- Lemnoideae, mainly confined to the surface of stagnant water bodies and found to occur throughout the world. They are represented by five genera namely *Wolffia sp, Lemna* sp, *Spirodela* sp, *Landoltia* sp and *Wolfiella* sp (Landolt, 1988) . The free floating aquatic plants are known to proliferate in eutrophic and hypereutrophic water bodies and can concentrate Nitrogen (N) and Phosphorus (P) in their body. Following Quasi Exponential Growth they are known to double their biomass within 24 hours (Khondkar, 1988). Waters accumulated from municipal wastes and industrial effluents are best suited for their natural growth (Ansari *et al*., 2009). The high starch content of duckweeds makes them a potential source of biofuel (Lui *et al*., 2014). According to Aslam (2017), they are also a good source of fooder for domesticated herbivores owing to its minimum fibre content along with maximum protein. Cultivation of Duckweeds is quite profitable as compared to other fooder crops because of its minimum fertilizer requirement and also do not compete with other crops and gathers nutrients from the surrounding waters.

L. minor L. commonly known as lesser duck weed, is quite commonly found to occur in the surface of eutrophic stagnant waters. This free floating aquatic macrophyte, distributed in Asia, Africa and Europe, bears a succulent thallus with smooth margins. The plant bears a single rootlet with a length of 3-5 mm covered with a sheath that remains immersed under water. The fronds attains a length of about 2.5 mm that remains floating above the surface of water. The length of roots and fronds are

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known to vary according to the concentration of Nitrogen (both Nitrate and Ammonia) and Phosphorus. (Appenroth, 2015). It mainly reproduces vegetatively by producing clones, sexual reproduction is rare. Flowers bear a single androecium with which two anthers are attached. (Landolt, 1988), fruits bear only one seed. Leminids like *Spirodela* sp and *Lemna* sp are a potential source for obtaining pharmaceuticals and vetenary medicine (Rajbhandary *et al.,* 2000). Alpha interferon can be obtained from *Lemna sp.* cultured in bioreactors added with fermenters (Gaskada, 2015). The plant has got antibacterial and antifungal potentialities along with profound anti oxidant property. The aim of this review is to gather knowledge regarding the growth response of *L.minor* L. in normal and modulated *in vitro* growth contitions.

Growth Response under *in vitro* conditions in Different Culture media of *L. minor* L.

Past researches have shown that a balance between nutrient composition and other growth parameters is responsible for fast *in vitro* propagation of *L.minor* L. Depending on the media composition and concentration of macro and micro elements, different invitro growth measuring parameters are known to vary accordingly for *L.minor* L. One of such important parameter is the growth index which is calculated as per Khellaf *et al.* (2010) as the ratio of fresh weight on the days of experiment, also tend to vary along with changed growth media. Similarly Doubling time (DT) and Relative growth rate (RGR), measured by counting the fronds on different days of experiment (Zeigler *et al.,* 2015), also shows remarkable variation in different culture medium. RGR gives an idea about the biomass accumulation in plants (Xu *et al.,* 2015) where as Doubling time and linear growth rate indicates vigorous growth in vitro. *In vitro* proliferation of *L.minor* L. is known to be supported by many basic growth medium (Stomp, 2005). Such basic medium includes Algal Assay Procedure (AAP) along with Murashige and Skoog (MS). The most important basic medium for invitro proliferation of *L.minor* L. as per previous investigation till date is Schenk and Hildebrandt (SH). In other *Lemna* species (*L.gibba*), AAP growth medium was reported to be best suited. As reported by Devlamyck (2020), synthetic medium (SN) containing essential macro and micro elements (Sree *et al.,* 2015), the biomass accumulation (5.8/day) and Relative Growth Rate (0.14/day) was found relatively higher. Growth Index, Doubling time and linear growth rate of *L.minor* L. was also reported follow the same trend. According to Appenroth (2015), there is a great variation in the major and minor nutrients in the SH and MS medium, with concentration being higher in SH as compared to MS. It is evident from the study of Ghanev *et al*. (2015) that the Growth Index and Linear Growth Rate of *L. minor* L. after 21 days of growth was 2.7 times higher than in AAP and in MS medium it ceased to grow. In other growth medium, the values decreased after 14 days. Table 1 shows the variations in the growth parameters in in vitro growth of *L. minor* L. in different culture medium after 14 days of *in vitro* growth, followed by histogram representation of the same.

***In-vitro* Growth Response of *L. minor* L. in different nutrient media by varying concentration of the nutrients and supplements**

According to Jamshidi *et al*., 2016 in Plant Biotechnology, predominantly in plant tissue culture research, plentiful experiments have been performed for optimization of nutrients for better biomass yield and deriving important secondary metabolites. Relationship study between expansion of *in-vitro* proliferation and nutrient medium may lead to the design more effective medium for *in-vitro* proliferation of plantlets (Appenroth, 2015). Based on organic substance concentration, plant growth can be regu-

Table 1. Comparison between three Growth parameters of *L.minor* L. propagated in different growth medium (After Devlamyck *et al*., 2020)

Culture	RGR		DT		GI	
Medium	After 14 days	After 21 days	After 14 days	After 21 days	After 14 days	After 21 days
MS	0.09	0.12	12.2	00	72	00
SH	0.18	0.28	3.8	3.1	389	1096
AAP	0.16	0.18	4.9	5.7	129	431
KP	0.12	0.15	6.3	7.1	102	337
GM	0.15	0.20	6.9	8.9	112	320
SN	0.10	0.21	5.1	6.7	223	489

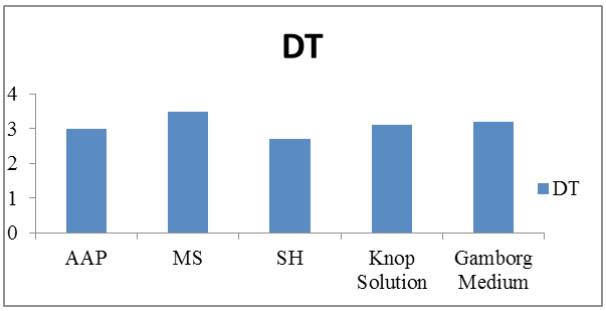

Fig. 1. This histogram represents the (DT) in five different Growth media after 14 days of *in vitro* culture

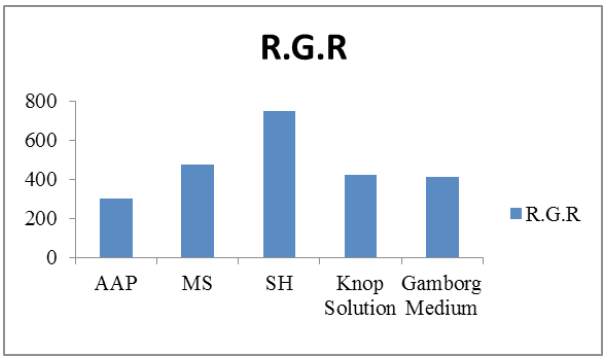

Fig. 2. This histogram represents the RGR in five different Growth media after 14 days of *in vitro* culture

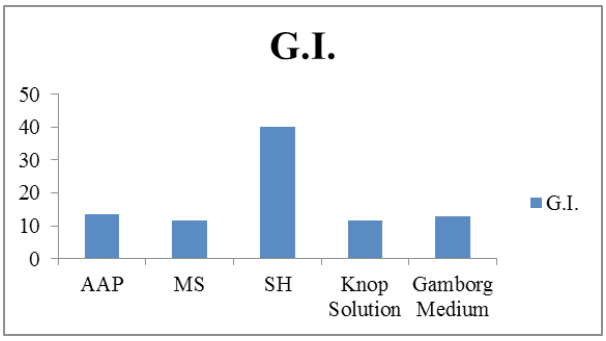

Fig. 3. This histogramre presents the GI in five different Growth mediaafter 14 days of in vitro culture

lated but also can cause osmotic stress and down-regulation of photosynthesis (Desjardins, 1994; Balen *et al*., 2012). There has been indication of considerable variation of biomass population, dry weight, total soluble protein by varying the concentration of nutrients for duckweed *L. minor* L. (Akter *et al*., 2011). On doubling the quantity of nutrients over the basal requirements, there is an upward surge of total dry weight, biomass and other growth measuring parameters. Biomass of *L. minor* L. can be increased up to 72.8% as compared to the control by addition of carbohydrates like sucrose or fructose in the basal media of HA.

Increased TSP production (in mg) was visible with separate addition of L-glycine and L-glutamine and also in combination. 41 % biomass increase was also noticed in the growth medium supplemented with Thiamine, Folic acid, Pyridoxine and Ascorbic acid. Four times biomass increase was noticed in *L. minor* L. in all organic supplements over basal medium. Another significant change was visible in *L. minor* L. by modulation of the concentration of inorganic nitrogen in different growth medium. Nitrate is the significant source of inorganic nitrogen and an imbalance among nitrate uptake and its assimilation may guide nitrate accumulation in *L. minor* L. (Marynard *et al*., 1976). It has been reported that with the amplification of inorganic nitrogen (N), RGR and biomass production of *L. minor* L. increases showing a positive correlation between them.

In-vitro growth response of duckweeds under different Light conditions and Sucrose supplements

According to Wang, (1990) cultivation of *L. minor* L. was done in different nutrient media such as Bristolls media, Hunters media, Hoagland and Schenk and Hilderbrandt media. Stimulation of *L. minor* L. growth can be done with organic compounds which according to their concentration (Zhang *et al.*, 2014). There are differences in intensity and spectral characteristics for artificial and natural light. CW, i.e. Cool white light have blue-green and yellow light energy of the spectrum whereas Gro lux light (GL) bears light energy of the blue and the light regions of the visible spectrum (Balen, 2012). Several previous experiments revealed maximum Growth Rate (0.3/day) under Cool white light (CW) and Gro lux light (GL) photosynthetic flux density of 50 µmol photon (m^2/s) supplemented with sucrose (10g/l) (Zelika *et al.*, 2013). According to ISO and OECD protocols for *L. minor* L. Doubling time (DT) is 2.5 days (ISO 20079, 2004, OECD 221, 2006). Growth of *L. minor* L. in Cool white light (CWL) and Gro lux light (GL) with sucrose supplement in Pirson – Seidel (PS) nutrient solution exhibits direct relationship with Doubling time, Growth rate and photosynthetic efficiency (Pirson and Seidel, 1950). Maximum variation can be found between Quantum yield of PSII (F_v/F_m) and Photosynthetic flux Density (PPFD) (Maxwell and Johnson, 2000). Productivity and optimal growth of *L.minor* L. is directly co-related with spectral diversity have been

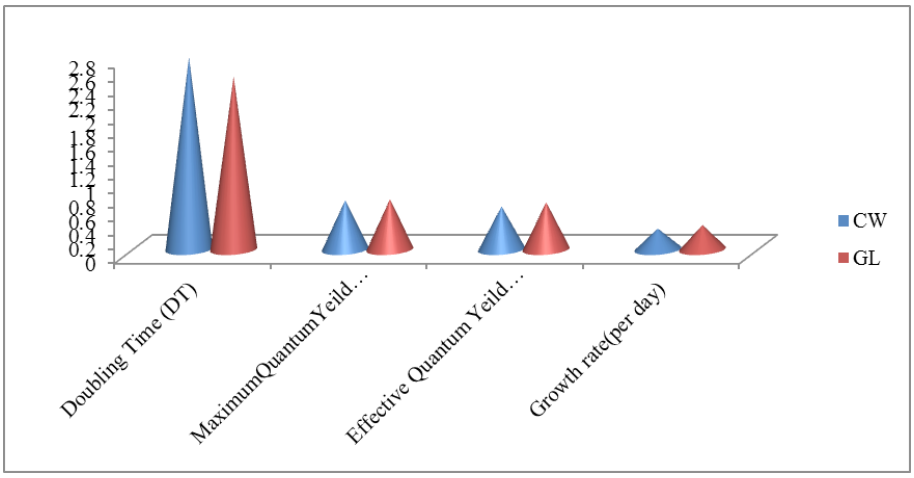

Fig. 4. This figure describe Doubling time (T), Maximum Quantum Yield, Effective Quantum Yield and Growth rate of *L. minor* L. following 16 days of cultivation on PS nutrient medium supplied with sucrose (10g/l) under diverse light sources.

proved by many experiments. Initially both CW and GL light show same F_v/F_M,but after 7 days of constant exposure to CW and GL. GW light showed added successful stimulatory effect. After 16 days of incubation of overall observation using 16:8 photoperiod and with sucrose supplement given below.

Optimal growth of *L.minor* L. was in MS medium was also recorded in 16:8 photoperiod at 65 µ mol/ m^2/sec (Frick H 1994) while mediocre growth was reported when *L. minor* L. was grown in MS medium in 12:12 photoperiod and 65 µ mol/ m^2/sec yielding lower photosynthetic flux density and effective flux density.

Conclusion

Like other duckweeds, *L. minor* L. prefers nutrient rich water under natural conditions. The present review aims in gathering knowledge regarding the balanced nutrient solution for plant tissue culture and optimization of the growth parameters of *L. minor.* L. Several basic axenic culture mediums are well known for *in-vitro* proliferation of *L. minor* L., but modulating their nutrient composition can result in increase or decrease of biomass yield. SH medium was found to be most effective with respect to all the growth measuring parameters for *L. minor* L., followed by AAP and MS. For maintaining Stock cultures of *L. minor* L. MS or Knops solution can be used However as the previous researches have suggested, modulating the nutrient components, particularly inorganic nitrogen (N) and phosphate (P), there is manifold increase in the biomass of *L. minor* L. Increasing the sucrose supplement in the growth media also can enhance the Growth rate over normal. The influence of light spectral distribution and intensity on plant growth rate and photosynthesis was proved a long time ago. It is well known that the growth and photosynthetic rate of plants are greatly influenced by intensity and spectral distribution of light. Optimum light intensity with proper spectral distribution is essential for achieving adequate growth in plants. So use of different light sources such as GL or CWL have shown different effective Quantum yield ($Ö_{PSII}$) and maximum Quantum yield (F_v/F_m). Previous researches have indicated that GL light was quite effective in *in-vitro* growth over CW light in 16/8 hr photoperiod. However in sucrose supplemented growth media even lights with low spectral distribution can promote optimal growth for *L. minor* L. So, from the above review it may be concluded that in vitro of *L. minor* L. depends on growth media composition and photoperiod, whose modulation, either singly or in combination can vary it's in vitro response.

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