Spirulina culture trial for better resilience to COVID-19 in Toamasina

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Abstract

A first experimental study on the production of spirulina (Arthrospira platensis) has been carried out at the Multifunctional Laboratory of the ISSEDD-University of Toamasina. The purpose is to verify the feasibility of such a crop in an area where climatic parameters could be a limiting factor in the production of spirulina. With the aim of monitoring the growth of this alga under laboratory conditions in muros and in a controlled greenhouse extra muros, this manuscript starts from the hypothesis that it would be possible to practice spirulina cultivation in Toamasina. Cultivation was carried out successively from a 1.5-liter inoculum, then in 30-liter containers, before transfer to a large 3 m³ extra muros greenhouse container. Periodic checks of the temperature, turbidity and salinity of the culture medium, as well as regular monitoring of the growth and productivity of the algae were carried out over a period of 180 days (a). The algae are growing at an average rate of 2.073 g.m⁻².d⁻¹, or the equivalent of 5.01 mg.j⁻¹.d⁻¹. A harvest of 4.31 g.m⁻².d⁻¹, with a specific growth rate of 0.0028 h⁻¹ and a generation time of 251.73 h were recorded during the experiment. Compared with the values obtained in Tulear, one of the spirulina-producing areas in southwestern Madagascar, these values turn out to be low but promising, given the climatic conditions of Toamasina where the sky is often overcast, with less brightness, more humid air and a rainy climate. For better growth and sustained productivity, controlling climatic parameters, coupled with the recovery of local materials are recommended in the case of extra mural cultivation. This trial constitutes an interesting avenue for the fight against COVID-19 insofar as spirulina is known for its immune-stimulatory and antiviral actions. Improving the nutritional quality of a predominantly vulnerable population of Toamasina via this alga will thus contribute to increasing its social resilience in the face of this pandemic.

Keywords: Spirulina culture; Toamasina; COVID-19; Resilience; Technical feasibility

Résumé

Une première étude expérimentale sur la production de spiruline (Arthrospira platensis) a été menée au sein du Laboratoire Multifonctionnel de l’ISSEDD-Université de Toamasina. Elle a pour objet de vérifier la faisabilité d’une telle culture dans une zone où les paramètres climatiques pourraient constituer un facteur limitant dans la production de spiruline. Ayant pour objectif de suivre la croissance de cette algue dans des conditions de laboratoire in muros et d’une serre contrôlée extra muros, ce manuscrit part d’une hypothèse selon laquelle il serait possible de pratiquer la spiruliniculture à Toamasina. La culture s’est faite successivement à partir d’un inoculum de 1,5 litre, puis dans des bacs de 30 litres, avant transfert dans un grand récipient sous serre extra muros de 3 m³. Des contrôles périodiques de la température, de la turbidité et de la salinité du milieu de culture, tout comme le suivi régulier de la croissance et de la productivité des algues ont été effectués durant 180 jours (j). Les algues s’accroissent au rythme moyen de 2,073 g.m⁻².j⁻¹, soit l’équivalent de 5,01 mg.j⁻¹.d⁻¹. Une récolte de 4,31 g.m⁻².j⁻¹, avec un taux de croissance spécifique de 0,0028 h⁻¹ et un temps de génération de 251,73 h ont été enregistrés durant l’expérimentation. Comparées avec les valeurs obtenues à Tulear, une des zones productrices de spiruline dans le Sud-Ouest de Madagascar, ces valeurs s’avèrent faibles mais prometteuses, vu les conditions climatiques de Toamasina où le ciel est souvent couvert, avec une luminosité moins prononcée, un air plus humide et un temps plus pluvieux. Pour une meilleure croissance et une productivité soutenue, la maîtrise des paramètres climatiques, couplée à la valorisation des matériaux locaux sont recommandées en cas de culture extra muros. Cet essai constitue une piste intéressante dans la lutte contre la Covid-19 dans la mesure où la spiruline est connue pour ses actions immunostimulante et antivirale. L’amélioration de la qualité nutritionnelle d’une population majoritairement vulnérable à Toamasina via cette algue contribuera ainsi à l’augmentation de sa résilience sociale face à cette pandémie.

Mots clés: Spiruliniculture; Toamasina; Covid-19; Résilience; Faisabilité technique

1. Introduction

Spirulina is known for its richness in proteins.
minerals, iron and vitamins, as well as its content of vitamins A and B12; it strengthens man’s immune system in a powerful way (Michka, 2005). Depending on its origin, it contains 55 to 70% of excellent quality proteins (Vermorel et al., 1975; Falquet, 2000). It also contains an interesting quantity of unsaturated fatty acid of the omega 6 family, chlorophyll (which has a positive influence on the manufacture of red blood cells and purifies the blood), minerals and trace elements (Michka, 2005). Studies also show the antiviral action of spirulina in vitro and in mice, especially in the case of influenza A (Hayashi et al., 1996). Spirulina plays a role in the inhibition of virus penetration and inhibition of the replication phase of viruses. A study published in the journal Nature (Chen et al., 2016) shows the effectiveness of a spirulina extract to stop the spread of the influenza virus. Spirulina extract prevents the formation of viral plaques and thus stops the infection that could result. Chen et al (2016) confirm that spirulina is most effective in containing the virus when it is used in the early stages of infection. These claims have been supported by McCarty & DiNicolantonio (in press), who argue that apart from the anti-inflammatory and antioxidant properties of blue-green algae such as spirulina, it would contribute to the reduction in the mortality rate of mice infected with RNA viruses, including influenza and coronavirus.

Given these nutritional and medical virtues of spirulina, it is interesting to turn to this alga in the fight against the coronavirus, especially in Toamasina, a Malagasy city where the COVID-19 pandemic has been rife since March 2020, and where over 75% of the population live below the poverty line defined by the World Bank (Banque Mondiale, 2019; Solofoniaina et al., 2020) . According to official figures as of August 7, 2020, Madagascar, including Toamasina, has been recording since March 2020, 12,526 positive cases for the novel coronavirus, with 134 deaths (WHO, 2020a). In addition to the criteria of vulnerability to COVID-19 linked to smoking (WHO, 2020b; Vardavas & Nikitara, 2020; Cai, 2020; Guan et al., 2020), age (CDC COVID-19 Response Team, 2020) and co morbidity (Fang et al., 2020), it appears that more than 3 out of 4 positive individuals are socio-economically vulnerable to this pandemic (Solofoniaina et al., 2020).

Affecting respectively 42% and 51% of Malagasy children under 5 years old, the deficiencies in vitamin A and iron, as well as the importance of the ratio of vulnerable people / coronavirus positive cases in Toamasina (Solofoniaina et al., 2020), as well as the increase of the malnutrition rate to 50.1% (MEN, 2015), justify the promotion of spirulina production in the city. Strengthening the physical resilience of the population through a supply of vitamins and protein via this alga would help reduce the vulnerability of the most disadvantaged groups. Thus, the local production of spirulina not only makes this alternative a reality, but also facilitates access to this product, which has so far been imported from other producing localities such as Tulear and Majunga.

Therefore, this manuscript is based on a hypothesis according to which spirulina culture would be technically feasible in Toamasina, with the possibility of having a good yield. Its purpose is to verify the feasibility of such a culture in an area where climatic parameters could be a limiting factor in the production of spirulina. Its objective is to evaluate the growth of algae under in vitro laboratory conditions and in an extra muros controlled greenhouse. The article thus relates the results of a first experimental test of spirulina culture
carried out within the Multifunctional Laboratory of the Higher Institute of Sciences, Environment and Sustainable Development (ISSEDD – University of Toamasina) in Toamasina, with a view to future popularization of such a practice in strengthening the resilience of the population to various viral diseases such as COVID-19.

2. Materials and methods

2.1. Study zone

This first trial of spirulina culture took place in the Multifunctional Laboratory of ISSEDD located at the University of Toamasina - Madagascar (18°07’56.38”S; 49°22’43.50”E; 74 m of altitude). The city of Toamasina is subject to a permanent Trade wind regime where a hot and humid tropical climate prevails. It records an annual average rainfall of 2968.2 mm for the year 2017, with an annual average temperature of 24.39 °C, a total annual sunshine of 1946 h, or 162 h per month (Infoclimat, 2017). During the period 2000 to 2020, the area has an annual average temperature of 24.06 °C, with a minimum average of 20.02 °C and a maximum average of 28.47 °C. Toamasina does not register any dry months in the year; still according to Infoclimat (2017), the coolest months are found in July - August (16.1 to 18.3 °C for 2017), while the hottest period of the year is between December and February (30.9 °C to 31.9 °C in 2017).

The city is located on the east coast of Madagascar, a country where more than 75% of the population earns less than USD 1.9 per day (Banque Mondiale, 2019). Socio-economically, the majority of the population can be described as “vulnerable” due to its low income, limited access to basic medical care (prevention and treatment) and education, as well as poor nutritional quality, negatively impacting its standard of living and socio-economic well-being (Solofoninaia et al., 2020).

2.2 Experimental protocol

The small cultivated aquatic being was an unbranched, spirally coiled, filamentous prokaryote 0.3 mm long, consisting of juxtaposed cells (Jourdan, 2006; Fox, 1999; Fox, 1996). Having the shape of a tiny coil spring, the species Arthospira platensis object of the present protocol, has an average of 7 turns with a filament diameter of about 10µm (Ravelo, 2001). However, its morphology and length could vary according to the conditions of the culture medium, including light intensity, temperature, mineral content, etc.

The starting inoculum was a strain of Arthospira platensis from Tulear (South-West of Madagascar). The test took place between mid-August 2017 and mid-February 2018. Cultivated usually in vitro in a 1.5 liter balloon, the culture was carried out over time, by contribution of ‘new medium’, in 5 liter containers after 30 days (D30) of culture, then in 10 liter containers after 60 days (D60). Transfer to 30-liter B1, B2 et B3 tanks took place between the 60th (D60) and the 90th day (D90) of cultivation. Finally, controlled greenhouse production in a large 3 m3 tank (45 cm high and 2.9 m in diameter) began at D90, until the end of the trial (D180) in February 2018. It should be noted that the renewal of the medium consists of a supply of culture water (subjected to room temperature) rich in N, P and K, with sodium bicarbonate dosed (NaHCO3) at 8 g.l-1, sea salt (NaCl) at 5 g.l-1, urea (CO(NH2)2) at 0.1 g.l-1, phosphoric acid (H3PO4) at 1 ml.l-1, sea water at 16 ml.l-1, and iron (Fe2(SO4)3) dosed at 0.2 ml.l-1.

Daily checks of the temperature, turbidity and salinity of the culture medium, as well as regular monitoring of the growth and productivity of the algae were carried...
out during the experimentation period. The essential physicochemical parameters (temperature, salinity...) were measured using a portable water analysis kit WTW Multi 3430 SET F IDS. Other parameters such as turbidity and concentration of the medium were measured using the Secchi disc. The shape and number of filaments were assessed by microscopic observations and counts. The development of the culture was assessed from the Specific Growth Rate (SGR = $\mu$) and the splitting time or regeneration time $G$ (Vonshak, 1991); these parameters are given by the following formulas:

$$
\mu = \frac{\ln xf - \ln xi}{tf - ti}
$$

$$
G = \frac{\ln 2}{\mu} = \frac{0,693}{\mu}
$$

Productivity, which is the increase in biomass per unit volume or area per unit time, was also calculated to measure dry weight per liter per day and dry weight per m$^2$ per day. The formulas used were as follows (Rambolarimanana, 2006):

$$
P = \frac{1}{dt} \times [Bf - Bi]
$$

$$
P = \frac{1}{dt} \times [Bf - Bi] \times V \times \frac{1}{S}
$$

\[P_{\text{production}} \text{ in g of dry weight per liter per day}
\]

\[dt: \text{Time interval between two measures in day}
\]

\[Bf \text{ and } Bi: \text{Final and initial biomass (dry weight in g.l$^{-1}$)}
\]

\[V: \text{Volume of the liquid in the tank in liter}
\]

\[S: \text{Area of the tank in m$^2$}
\]

3. Results

3.1. Variable physico-chemical parameters but kept acceptable

Subjected to the same culture conditions, the three 30 liter tanks $B_1$, $B_2$ and $B_3$ recorded temperatures between 27.1 °C and 21.3 °C during the first 2-3 months (Figure 1). Between $D_{90}$ and $D_{90}$, the values given by the Secchi disc vary between 3 and 7 cm, which are inversely proportional to the concentration of spirulina. As for the salinity, it oscillates between 9.8 to 18.7‰.

Between $D_{30}$ and $D_{180}$, the temperatures of the culture medium hover around 25.2 and 29.5 °C in a controlled greenhouse extra muros. Contrary to the case of the 30 liter tanks, the salinity in the large 3 m$^3$ container decreases, oscillating between 9.4 and 11.5‰ (Figure 2).

Furthermore, it was noted that the variation in the value of the three essential parameters (temperature, turbidity and salinity) of the culture medium occurs after each addition of liquid. The evolution of the physico-chemical parameters also follows that of the season during which the test was carried out: the transition to the hottest months of the year (from mid-August to mid-October to mid-November-February, respectively $D_{30} - D_{90}$ to $D_{90} - D_{180}$) is marked by exposure of extra muros devices to higher temperatures, therefore to more marked evaporation, resulting in an
increase in the salinity of the culture medium and a decrease in the value of the Secchi disc. Despite these various parametric variations, the measured values are acceptable in the absence of significant mortality (<10-15%) of algae cultivated both in vitro and in a controlled greenhouse.

3.2. Promising growth parameters

With an average Specific Growth Rate of \( \mu = 0.0012 \text{ h}^{-1} \), the 30 liters tanks recorded a SGR between 0.0010 and 0.0013 \text{ h}^{-1} and a dry weight production of 0.11 \text{ g.m}^{-2}.\text{d}^{-1}, that is 0.00029 \text{ g.l}^{-1}.\text{d}^{-1} after 60 to 90 days of culture which has a generation time oscillating between 519.47 and 667.18 h, with an average G of 600.02 h. Between D0 and D180, the mean values reach \( \mu = 0.0028 \text{ h}^{-1} \) and G = 251.73 h, with a dry weight production of 2.073 \text{ g.m}^{-2}.\text{d}^{-1}, that is 0.0051 \text{ g.l}^{-1}.\text{d}^{-1}. At the end of the trial, that is to say on D180, the harvest at the level of the large extra-muros container of 3 m³ resulted in a yield of 4.31 \text{ g.m}^{-2}.\text{d}^{-1} of dry spirulina, that is the equivalent by fresh weight of 889.18 g for the whole of a container of 6.67 m² of surface, or else 215.93 g in dry weight for a desiccation rate of 23.63%.

**Figure 1:** Daily evolution (days D<sub>60</sub> to D<sub>90</sub>) of the temperature in °C, of the turbidity in centimeters and of the salinity in %o in the 30 liter tanks

**Figure 2:** Daily evolution (days D<sub>60</sub> to D<sub>180</sub>) of the temperature in °C, of the turbidity in centimeters and of the salinity in %o in the 3 m³ container

After counting and microscopic observations of the drops taken from the culture medium, here are the main morphological characteristics of the filaments:

- Average number of turns per filament at D<sub>60</sub>: 4 to 7; 5 for 75% of the total size;
- Number of filaments for each 30 liters tank at D<sub>60</sub>: 3400 x 10<sup>6</sup> to 3825 x 10<sup>6</sup> (the equivalent of 120,400 ± 7083 filaments.ml<sup>-1</sup>) with an average size of 3612 x 10<sup>6</sup> and a linear rhythm of increase (figure 3);
- Visual color of the filaments: olive green

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Regarding the growth rate of the culture, interpreted through the values of the Secchi disc obtained, it turns out that between D₉₀ and D₁₈₀, the algae grow at the rate of 1.91 cm.d⁻¹ ± 0.36 (95% confidence interval), compared to 0.56 cm.d⁻¹ ± 0.37 (95 % confidence interval) at the time the culture entered its first 60-90 days. Student’s statistical test shows that the two speeds of growth differ significantly at the probability threshold of 5% (tₐₐₜ = 1.36 > t₀.₀₅ ; Sd = 0.36).

4. Discussions and recommendations

4.1. Unfavorable abiotic factors but possibilities for improvement with promising results

The luminous intensity of solar origin, one of the parameters which are involved in the success of a spirulina culture (Niangoran, 2017), being less provided in Toamasina where one registers 162 hours of sunshine per month, that is to say 5.4 hours per day, against 3600 hours per year in Tulear, or 10 hours per day (Infoclimat, 2017), one of the potential producing sites of spirulina in Madagascar. Containing chlorophyll, spirulina needs light to develop. Average annual temperature is around 24.4 °C in Toamasina, it rises to 25.5 °C in Tulear where the air is drier (<60-65% average annual humidity against >75-80% in Toamasina) (Infoclimat, 2017). Compared to results obtained by Rambolarimanana (2006) and Noniarimalala (2007) in Tulear, Toamasina presents:

- a SGR 2 times lower than that of Tulear;
- a G 2 times lower than that of Tulear;
- a number of filaments per millilitre 2 times lower than that of Tulear;
- a daily yield in dry weight per liter 3.8 times lower than that of Tulear;
- a daily yield in dry weight per area 1.2 times lower than that of Tulear.

Niangoran (2017) confirms that if we manage to control the other parameters such as the quality of the culture medium and the temperature (ideally 28 ° C), a lighting time of 17 h per day would allow good performance in vitro, in the order of 72 mg.l⁻¹.d⁻¹. Thus the low yield of 5.1 mg.l⁻¹.d⁻¹ recorded in Toamasina is explained, among other things, by the lack of lighting during the extra muros test, as well as the coolness of the temperature linked to the almost daily rainfall.

The challenge is therefore to control the limiting effects of climatic parameters on the crop by using an adjustable greenhouse (in...
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Requiring a moisture content of less than 9% (Jourdan, 1999), the crop should be stored and protected from prolonged exposure to the open air. Rambolarimanana (2006), in its prefeasibility study on spirulina cultivation in Toliara, demonstrates that the valorization of local materials and production techniques is a promising socio-economic orientation. For example, product drying devices, such as greenhouses, lighting systems and production bins, can easily be made locally, making the crop inexpensive, easily duplicated and reproducible among a wide range of sufficiently trained populations. However, the supervision of researchers, the proper conservation and control of strain production are essential in the context of the action research to be undertaken. With such technical arrangements, spirulina culture in Toamasina could be even more promising than that carried out in the present trial.

4.2. Sustained production of spirulina with socio-economic and health stakes of COVID-19 in Toamasina

As part of the fight against COVID-19 in Toamasina, the stakes and challenges related to spirulina cultivation consist of profitable and less expensive production, with products accessible to a wide range of affluent populations. Increasing the nutritional and health resilience of vulnerable groups should be a priority strategy for dealing with the pandemic.

In addition to the economic poverty and vulnerability criteria put forward by the World Bank (Banque Mondiale, 2019) and Solofoniaina et al., 2020, other medical criteria such as being diabetic, suffering from renal failure, or hypertensive, in short those qualified as comorbid factors (Fang et al., 2020), are also important medical issues in the fight against COVID-19. The value of producing spirulina accessible to vulnerable people lies, among other things, in the fact that phycocyanin and phycocyanobilin, obtained from this alga protect against diabetic nephropathy and renal failure; they have an inhibitory power against oxidative stress (Zheng et al., 2013; Baynes, 1991). In addition, spirulina strengthens the human immune system, thanks to polysaccharides; it could also exert a certain antiviral activity, which is linked to the sulfoquinovosyldiacylglycerol rich in sulfolipids (Falquet & Hurni, 2006; Girardin-Andréani, 2005).

Rabe (2017) demonstrates in his prefeasibility study on the production of spirulina in 5 basins of 75 m² in Toamasina, that such a culture would have an Internal Rate of Return (IRR) of 24.37% and a Profitability Index of 1.12. Sold on the market at a price of 200 Ariary per gram, spirulina from other localities in Madagascar remains difficult to access for a vulnerable household in Toamasina with 5-6 people in charge (DREPA, 2017), whose daily income does not exceed 1.9 USD (Solofoniaina et al., 2020, Banque Mondiale, 2019). By producing spirulina locally (see also recommendations above), a product 1.2 times cheaper Rabe (2017) could be marketed. This price reduction would contribute to facilitating access to spirulina in the context of nutritional and immune improvement of vulnerable populations. To be effective, a policy of popularization and promotion of this...
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Conflicts of Interest

The authors have no conflict of interest to declare. Although a member of the Board of the Journal, the 2nd author did not participate in the review process of this manuscript.

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5. Conclusion

This paper tried to show to what extent spirulina could be technically feasible in a coastal zone with (a priori) unfavorable climatic parameters such as Toamasina. Its interest lies in the fact that such a practice could be adapted, not only to local ecological conditions, but it could also be reconciled with local socio-economic, nutritional and health needs, in particular to deal with COVID-19. Also, for a sustained production of spirulina in Toamasina, the popularization of techniques compatible with local socio-ecological realities, as well as the promotion of a Research-Action approach, allows to better meet the spirulina needs of vulnerable people to face the COVID-19 challenges.

the production of spirulina must be carried out in parallel with the Research-Action undertaken by the technicians. In this sense, spirulina should not be presented as a drug, but a simple nutritional contribution essential to strengthening the human body in the face of various diseases such as COVID-19.

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