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Cultivation of *Spirulina*: An innovative approach to boost up agricultural productivity

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Abstract
Limited consumption of natural food stuff in the 21st century has led to deficiency of vitamins and other important minerals in the human body. Due to excess and repetitive use of chemical fertilizers, the crop productivity is declining day by day. *Spirulina* is multi-cellular and filamentous blue-green algae biomass which belongs to the class of cyanobacteria discovered by non-referenced Mexicans in the 16th century can be a viable solution of all these problems. In recent years, *Spirulina* has gained enormous attention from research point of view as well as industries as a flourishing source of nutraceutical and pharmaceuticals. It has a very high content of macro as well as micronutrients, essential amino acids, proteins, lipids, vitamins, minerals and anti-oxidants. It is among the most nutritious, concentrated whole food sources that exists in nature, and is known as a superfood. *Spirulina* exhibits anticancer, antidiabetic, anti-inflammatory, immunomodulatory and many other properties. *Spirulina* has the potential of reducing the negative impacts of wastewater discharge through bioremediation. Two major technologies are being considered for the cultivation of *Spirulina*: closed photobioreactors (PBR) and open raceway ponds. In developing countries like India where malnutrition is a renowned social challenge and it can be defeated by the supplementation of *Spirulina* products in the diet. The commercial cultivation of *Spirulina* and converting it to consumable forms (tablets or powder) can be a new way of agribusiness. Therefore, *Spirulina* is emerging as a cost-effective means of improving livestock and crop productivity in a sustainable manner to ensure food and nutritional security.

Keywords: Biofortification, Biostimulator, Blue green algae

Introduction
Spirulina is a ubiquitous spiral-shaped blue-green algae (Cyanobacteria). Blue-green algae are the evolutionary bridge between green plants and bacteria. *Spirulina* is Earth's oldest living plant approximately 3.6 billion years ago and considered to be the ancestor from which the higher plants evolved. It is the first photoautotrophic organism that directly transforms sunlight into compound metabolic pathways (Supramaniyan and Bai, 1992) [45]. The name 'Spirulina' is derived from a Latin word which means helix or spiral. It is most commonly found in seawater and brackish water. The blue-green colour of the organism is due to the presence of several photosynthetic pigments such as chlorophyll, carotenoids, phycocyanin and phycoerythrin. Phycocyanin is responsible for the blue color of the organism. According to World Health Organization (WHO) *Spirulina* is an interesting food rich in iron and protein and declared it as best food for future.

History of *Spirulina*
It was first discovered by Spanish scientist Hernando Cortez and Conquistadors in 1519. Wild *Spirulina* was cultivated in the alkaline lakes of Mexico and on the African continent, although it is now commercially grown and harvested all over the world. In the sixteenth century, the Aztecs living in the Valley of Mexico harvested it from Lake Texcoco (Sasson, 1997) [38]. They collected it with the help of net and making a blue-green cake from it. During 1964-65, Jean Léonard, reported that a greenish, edible cakes were being sold in local markets of Fort-Lamy in Chad, Africa.

Morphology of *Spirulina*
Spirulina consisted multicellular, filamentous, unbranched trichomes. The filaments were called 'trichome'. Motile structure like flagella and heterocysts which are generally present in many blue green algae were absent. The cells are cylindrical and the spiral are loose. The presence of gas-filled vacuoles in the cells along with the helical shape of the filaments results

in floating mats. The cells exhibit active rotary movements. The helical shape of the trichome is characteristic of the genus but the helical parameters varied with the species even within the same species (Somasekaran, 1987) [41]. The trichomes are surrounded by a thin sheath. They show more or less slightly noticeable constrictions at cross-walls and have apices either slightly or not at all reduced. The width of the trichomes, composed of cylindrical shorter than broad cells varies from about 6 to 12µm (16µm) in a variety of forms.

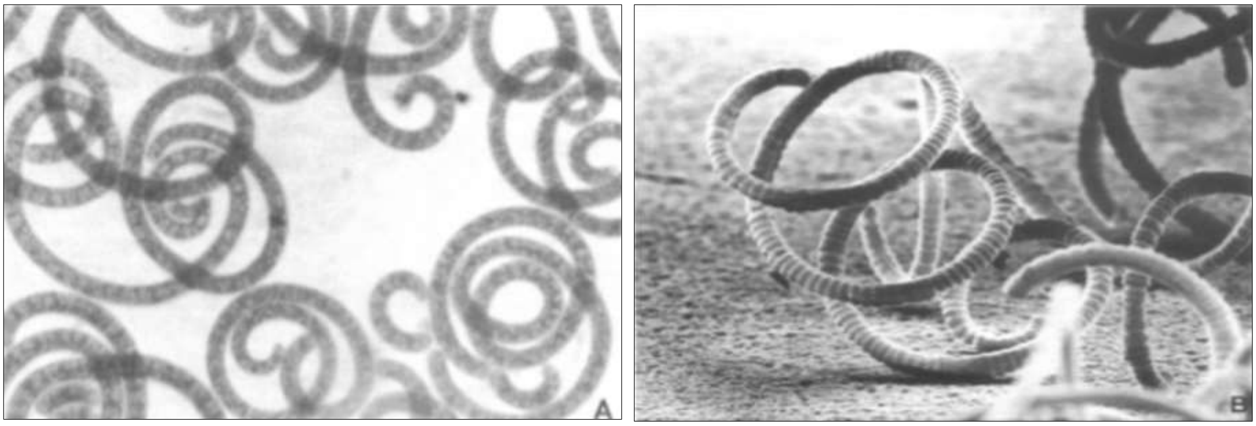

Fig 1: Morphology of *Spirulina.* (A) Optical microscopy of axenic *S. platensis*. (B) Scanning electron micrograph of a trichome of axenic *S. platensis*. (Ali *et al*., 2012) [1]

Life Cycle of *Spirulina*

There are four fundamental stages: Trichomes fragmentation, hormogonia cells enlargement and maturation processes, and trichome elongation as shown in Fig.2. Then this mature trichomes get divided into filaments or hormogonia cells in the hormogonias gets increased by binary fission, grows lengthwise and takes their helical form (Balachandran *et al*., 2006) [3].

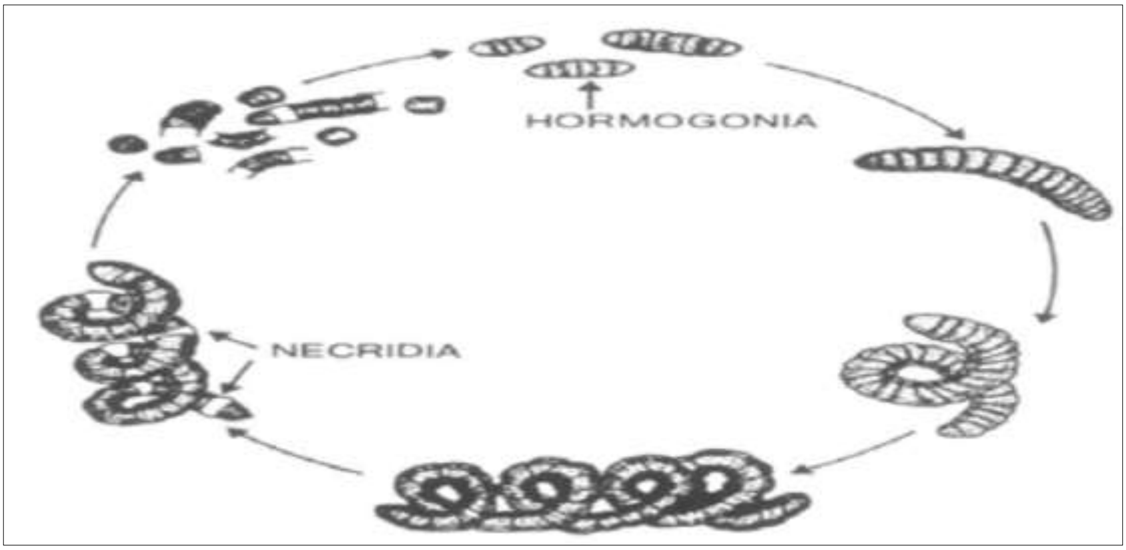

Fig 2: Lifecycle of *Spirulina* (Ali *et al*., 2012) [1]

Why *Spirulina* is known as Superfood

Spirulina called a superfood because of its nutrient profile is more potent than any other food, plant, grain or herb (Fathima and Salma, 2001; Dillon, 2014) [12, 10]. These nutrients make *Spirulina* a whole food alternative to isolated vitamin supplements. The United Nations world at food conference declared that *Spirulina* as the best food for the future, and its popularity is increasing nowadays among the wide population (Pulz & Gross, 2004) [30]. *Spirulina* is one of nature's near-perfect foods. It helps to boost our immune system, and is a good immediate energy source. It is a natural detoxifier as it helps detoxification of toxins and impurities present in our body. Due to the several health benefits, *Spirulina* is gaining more and more interest, especially in the sector of food supplements, where it is either used as a powder (Fig.3), or consumed in the form of capsules or tablets (Fig.4).

Fig 3: *Spirulina* Powder

Fig 4: *Spirulina* Tablet/Capsule

Table 1: Nutritional profile of *Spirulina* powder (composition by 100 g)

	Nutritional Value
Calories	373Kcal
Total fat	4.3g
Total carbohydrate	17.8g
Essential amino acids	21.9g
Non-essential amino acids	35.4g

Minerals	**Nutritional Value**
Calcium	468 mg
Iron	87.4 mg
Phosphorus	961 mg
Iodine	142 mcg
Magnesium	319 mg
Zinc	1.45 mg
Selenium	25.5 mcg
Copper	0.47 mg
Manganese	3.26 mg
Potassium	1660 mg
Sodium	641 mg
Vitamins	**Nutritional Value**
Vitamin A (as β-carotene)	352.0 IU
Vitamin K	1090 mcg
Thiamine HCL (Vitamin B1)	0.5 mg
Rivoflavin (Vitamin B2)	4.53 mg
Niacin (Vitamin B3)	14.9 mg
Pyridox HCl(Vitamin B6)	0.96 mg
Vitamin B12	162 mcg
Phyto-nutrients	**Nutritional Value**
Phycocyanin (mean)	17.2%
Chlorophyll (mean)	1.2%
Superoxide dismutase (SOD)	531,000 IU

Gamma linolenic acid (GLA)	1080 mg
Total carotenoids (mean)	504 mg
β-carotene (mean)	211 mg
Zeaxanthin	101 mg

Source: Salmeán *et al*., 2015 [36]; mcg = microgram, mg = milligram, IU = international unit

Table 2: Nutrient profile of *Spirulina* vs other foods (Moorhead *et al*. 2005) [22]

Components	
Protein	6 times higher than egg, 3 times higher than beef or pork, 2 times than soyabean
Fibre	4 times higher than flour or corn and oat
Vitamin B12	3-4 times higher than animal liver
Beta Carotene	40 times higher than spinach, 2 times higher than carrots
chlorophyll	20 times higher than wheatgrass
Calcium	14 times higher than milk, 2 times higher than yogurt
Iron	9 times higher than spinach, 5 times than soyabean
Potassium	4 times higher than banana

Pharmacological actions of *Spirulina*

- **Anti-inflammatory and immunomodulatory effect-** *Spirulina* comprises a pigment known as phycocyanobilin, which is a powerful inhibitor of an enzyme that is necessary for causing an inflammation (Soni *et al.*, 2015) [42]. *Spirulina* enhances immunity by producing a huge amount of antibodies, interferon-gamma, and cytosine.
- **Effect on diabetes and obesity-** *Spirulina* fraction was discovered to be efficient in reducing the amount of serum glucose at fasting, while water-insoluble fraction suppressed glucose levels at glucose load and also reduced cholesterol, triglycerides, LDL cholesterol in blood and thus act as anti-hyperlipidemic agent in clinical pathways that could be protective against atherosclerosis and euglycemia (Metwally *et al.*, 2015) [21].
- **As Nutraceuticals and cosmetics-** *Spirulina* whitening face masks, which are protein-rich cosmetics, thus enhances the beauty by removing dead skins, and reduces wrinkles and thus exhibiting anti-aging property (Mourelle *et al.*, 2017) [23] as well as in skin toning, healing of dark circles, skin purification and promote hair growth by dandruff treatment (Joshi *et al.*, 2018) [17].
- **Anti-anemic effect-** *Spirulina* supplementation may be of great potential importance during pregnancy and lactation as it includes all the hematopoietic nutrients that will eventually benefit both mother and fetus (Poormoosavi *et al.*, 2019) [28].
- ***Spirulina* in biofuel production-** *Spirulina platensis* is a precious candidate for biodiesel production because of its elevated growth rate of 2.23g/Ld, adequate lipid content, desiring an easy and cheap crop medium and generating other precious by-products that would reduce the global price bio-diesel production (Behera *et al*., 2015) [4].
- ***Spirulina* as therapeutic agent-** Apart from being a health food, *Spirulina* has invaluable medical applications. Dietary supplementation of this organism showed protective effect towards food allergy. *Spirulina* has two types of water-soluble polysaccharides that are calcium spirulan and Immunila. These polysaccharides show inhibitory effects against some viruses like HIV and they help in activating the immune system during cancer chemotherapy. *Spirulina* extracts prevents the formation of tumors and shows hypocholesterolemic and anti-diabetic properties.

Table 3: Summary of some studied biological effects of *Spirulina* microalgae (Hoseini *et al*., 2013) [15]

Biological Properties	Specific Effects	Bioactive Component
Anticancer	Repairing of damaged DNA	Polysaccharides
	Selective Inhibition of Cyclooxygenase-2	C-phycocyanin
	Induction of G1 cell cycle arrest, mitochondria mediated apoptosis in MCF-7 human breast carcinoma	Se-enriched *Spirulina*
Antiviral	Blocking virus adsorption and penetration into vero cells	Calcium spirulan (sulfated polysaccharide)
	Inhibition of the DNA polymerase activity	Sulfolipids
	Inhibition of enterovirus 71-induced cytophtic effect, viral plaque formation, and viral-induced apoptosis	Protein-bound pigment allophycocyanin
Metalloprotective	Inhibiting lipid peroxidation, scavenging free radicals, enhancement of the activity of GSH peroxidase and superoxide dismutase	Antioxidant components
Antioxidant	Metal-chelating activity, free radical scavenging activity	Carotenoids, vitamin E, Phycocyanin, and chlorophyll

Cultivation of *Spirulina*

Spirulina is one among various algal species discovered growing in natural freshwaters. These are observed in natural habitats such as soil marshes, seawater, and brackish waters where alkaline waters subsist. In natural habitats, their growth cycles depends mainly on the supply of nutrients. Today, there are more than 22 countries: Benin, Brazil, Burkina Faso, Chad, Chile, China, Costa Rica, Côte d'Ivoire, Cuba, Ecuador, France, India, Madagascar, Mexico, Myanmar, Peru, Israel, Spain, Thailand, Togo, United States of America and Viet Nam, that cultivate *Spirulina* commercially on a large-scale (Ravi *et al*., 2010) [32]. Shimamatsu (2004) [40] observed that the total industrial production of *Spirulina* is almost 3000 tonnes a year. Presently, two major technologies are being considered for the cultivation of *Spirulina* that are closed photobioreactors (PBR) and the other one is through open pond method. Both approaches are used commercially to produce high value products. Fig.5 illustrates the flowchart for *Spirulina* cultivation phases starting from strain selection to pellets formation.

Species Selection

It is a very crucial stage in *Spirulina* cultivation. Among the various species, *S. platensis* and *S. maxima* are the only two used due to its valuable components, positive effects, and being a supplement, which is nontoxic for human well-being.

Open raceway pond system

Open ponds can be categorized into two types that are natural waters as lakes, lagoons, ponds and artificial ponds or containers. The most commonly used systems are shallow big ponds, circular ponds, tanks and raceway ponds. Open systems are easy to construct and operate, resulting in low production as well as operating cost (Ugwu *et al*., 2008) [47]. The cultivation is usually carried out in two ways: a) concrete ponds and, b) pits lined with PVC or other plastic sheets. The major drawback in open ponds includes poor light use efficiency by the cells, losses due to evaporation, diffusion of carbon dioxide to the atmosphere, and requirement of large areas of land. Also, due to reduced aeration in open cultivation systems, their mass transfer rates are very poor leading to reduced biomass productivity. The growth also depends on location, season, temperature, pH level, nutrient and carbon dioxide supply (Cuaresma *et al*., 2011) [8]. The other major drawback of open pond system is that it is susceptible to contamination by fauna and other fast-growing heterotrophs.

Photobioreactors

Photobioreactor refers to closed systems that have no direct exchange of gases as well as contaminants with the environment. It results in high productivity of algae. Photobioreactors facilitate better control of culture environments such as carbon dioxide supply, water supply, optimal temperature, efficient light intensity, culture density, pH levels, gas exchange, aeration and culture density. Algal culture systems can be illuminated by using artificial or natural light or by both. In order to deal with the problems with open ponds, much attention should be on the development of suitable closed systems such as flat-plate, tubular, vertical column and internally-illuminated photobioreactor. Generally, laboratory-scale photobioreactors are illuminated artificially internally or externally by fluorescent lamps or other light sources. Some photobioreactors can be easily tempered. Large scale outdoor systems mainly tubular photobioreactors cannot be easily modified without high technical support. Efforts have been taken in designing temperature controlled photobioreactors, such as double-walled internally lighted photobioreactor with both heating as well as cooling water circuit (Chetsumon *et al*., 1998) [7].

Hybrid system

A hybrid type of photobioreactor is most widely used to utilize the advantage associated with the two different types of reactor and overcome the disadvantage of other. Hybrid systems have the features of both open ponds as well as photobioreactors (Hoekema *et al*., 2002) [14]. First can be covered open pond this concept reduces the possibility of contamination, evaporative losses, and CO_2 desorption. The other type is Cleaning process is difficult. Harvesting cost is high but productivity is high. Another drawback is large area is required. Integrated airlift system and external tubular loop placed horizontally in a thermostatic pond of water have been reported (Zittelli *et al*., 2013) [50]. The external loop acts like the light-harvesting unit and gives high surface area to volume ratio and controls the temperature of the culture. The airlift system acts as a degassing system where probes can also be integrated in order to regulate the other culture variables. It has the advantage of better control over culture variables, enabling higher productivities and reducing power consumption (Cuaresma *et al*., 2011 [8]; Singh and Sharma 2012 [44]; Ugwu *et al*., 2008) [47]. A partially filled tubular design widened and inflated to approximate an open pond; this design is mainly aimed at reducing costs (Hoekema *et al*., 2002 [14]; Olguín *et al*., 2003 [26]; Tredici & Materassi, 1992) [46].

Growth parameters

A. Media

Spirulina flourishes in alkaline brackish water. The culture medium should provide all essentials to nourish *Spirulina* in a suitable environment. It should be composed of sodium carbonate and other suitable medium which provides nitrogen, phosphorus, iron and other trace metals (Raoof *et al.*, 2006) [31]. The makeup media should also consist of urea. It can grow on either nitrate or urea alone but using both at the same time is more advantageous. The water used should be clean or filtered to avoid the growth of other algae during cultivation. The media preparations should be arranged in such a way that it meets the local growing conditions for *Spirulina*. The most commonly used is Zarrouks media (Pragya *et al.*, 2013) [29]. The cost of nutrients accounts for about 15-25% of the total production cost (Selvendran, 2015) [39].

B. Mother culture

Fully grown concentrated *Spirulina* culture is required For Inoculums preparation and culture maintenance. The chosen *Spirulina* strain must have a high proportion of coiled filaments (<25% straight filaments, or none), and at least 1% of gamma-linolenic acid (GLA) based on dry weight. Concentrated *Spirulina* seed culture can be obtained either from the floating layer of a composed culture, or by diluting a freshly filtered biomass. Colour of the culture should be clearly green. The growth rate is about 30%/day when the temperature and other climatic conditions are adequate (Pal *et al*., 2011) [27]. As the growth is proportional to the area of the culture exposed to light, it is recommended to maximize this area at all times. It is reported that minimum cell population is necessary to initiate and sustain *Spirulina* cultures.

C. Mixing and aeration

Agitation of the culture is necessary to homogenize and ensure a good distribution of lighting among all the filaments of *Spirulina*. Mixing plays an important role in the productivity of ultrahigh density cultures. Aeration is very necessary for getting good quality and better yields of *Spirulina* species. It can be achieved by rotators, which maintain the cells in suspension by gentle agitation of growing cells. The *Spirulina* species produces high biomass yield when the growth medium is aerated (bubbling with air). It also helps to distribute carbon dioxide concentration uniformly and removes inhibitory substances as oxygen (Dubey, 2006; Richmond & Vonshak, 1978) [11, 33]. Aeration is, therefore, essential for the cultivation of the *Spirulina* filaments such as *Spirulina platensis*. Adequate and turbulent mixing is essential for higher biomass productivity. Mixing of raceway pond is affected by means of a paddle wheel. Low velocities result in dead zones around corners while high velocities incur high energy cost, and may result in shear stress that damages the algae. It also noted that continuous mixing of the culture medium is required to prevent cell sinking and thermal stratification. It is also required to maintain even nutrient distribution, and to remove excess oxygen. When aeration is not adequate, the efficiency of energy utilization and biomass production will be low. Similarly, if growth medium is not aerated, the cell on the surface of the medium float to the surface due to the presence of air-filled vacuoles. These cells suffer photoinhibition, resulting in low growth or low biomass production.

D. Temperature and pH

Spirulina can develop at 20°C - 37°C but the ideal temperature for *Spirulina* for high growth with high protein content is between 29°C - 35°C (Kumar *et al*., 2011) [19]. The variation in atmospheric temperature is the main factor affecting the biomass production rates in *Spirulina* cultivation. The bleaching of cultures may take place when temperatures are above 35°C while it cannot withstand in temperatures less than 20°C (Kumar *et al*., 2011) [19]. It is reported that the effect of pH on the algal growth, pigment production and protein content of *Spirulina* species has the direct effect on the antioxidant system (Ogbonda *et al*., 2007) [24].

The pH maintenance of the media over 9.0 is obligatory in *Spirulina* cultures in order to avoid contamination by other algae. The pH adjustment is made by increasing the carbon dioxide level by the addition of carbonate salts into the culture (Selvendran, 2015) [39]. When pH is between 9 and 11, it indicates a healthy culture. The effect of pH on the algal growth, pigment production and protein content of *Spirulina* species has the direct effect on the antioxidant system (Ogbonda *et al*., 2007) [24].

E. Light intensity

All photoautotrophic organisms including photosynthetic bacteria, cyano-bacteria, and higher plants, transform light energy into chemical energy through photosynthesis. In open-air cultivation system, natural light or solar radiation is the sole source of light for the autotrophs. The light has a direct effect on *Spirulina* production for its protein content, growth rate, and pigment synthesis (Saeid & Chojnacka, 2015) [33]. The optical density of the culture is directly proportional to the light intensity. Higher the optical density higher is the requirement of light and lower is the optical density, lower is the requirement of light (Samuel *et al.*, 2010) [37]. Light is an important factor but direct sunlight is not recommended, 30% of full sunlight is actually better, except that more may be required to quickly heat up the culture in the morning (Saeid & Chojnacka, 2015) [33]. Growth takes place only in the light, but illumination 24 h a day is also not recommended. During dark periods, chemical reactions take place within *Spirulina*, like a synthesis of proteins and respiration.

F. Growth Rate & Productivity

Salinity or nutrient concentration affects the growth rate of algae. The highest growth was achieved at the lowest salinity ratio for studies performed with various concentrations of $NaHCO_3$ and NaCl salts. The use of certain nutrients can alter production costs and affect growth or biomass composition. Annual biomass production of *Spirulina* in PBRs is 3000 tonnes which are maximum when compared to other microalgae species (Bharathiraja *et al*., 2015 [5]; Jayati *et al*., 2015) [16]. Maximum biomass yield of *Spirulina* reported in the large open pond is lower than other species. *Spirulina* biomass yield of 35 tonnes/hectare/year has been reported in a commercial open mass cultivation pond at Siam Algae, Bangkok (Habib *et al*., 2008) [13]. Productivity rates between 20 and 30 g/m^2/day in the range of usual open raceway performance (Bharathiraja *et al*., 2015) [5]. In commercial *Spirulina* farming, it is needed to recreate the culture medium where water is the main source medium for *Spirulina* to grow naturally. It should have all the essential and required resources of nutrition for the healthy growth of *Spirulina* (Bharathiraja *et al.*, 2015) [5]. The water level in tanks should be controlled which is important for the photosynthesis process to take place in *Spirulina*. A minimum shallow level of 20 cm is ideal water level height. The deeper the water level, sunlight penetration will be reduced, which will affect algae growth.

Harvesting system

The concentration of algae in the production unit (pond) will be the determining factor for harvesting. In general, the *Spirulina* will be ready for harvest after five days once the seeding process is done. The most suitable time for harvesting is early morning because the percentage of proteins in the *Spirulina* is highest during the morning. Besides, the cool temperature makes the work easier and more sunshine hours (during day-time) will be available to dry the product. The harvesting of *Spirulina* is carried out in two steps:

Centrifugation

It is a method to separate *Spirulina* algae from the media. It is generally carried out by a centrifuge is equipment, driven by a motor that puts an object in rotation around a fixed axis, applying a force perpendicular to the axis. This method is reasonably efficient but sensitive algal cells may be damaged by pelleting against the rotor wall.

Filtration

During commercial production processes filtration devices are used for harvesting. These are of two types, i.e., inclined and vibrating screens. The inclined screens are 380-500 mesh with a filtration area of 2-4 m^2 per unit and are capable of harvesting nearly about 10-18 m^3 of *Spirulina* culture per hour (Ogbonna *et al.*, 1999) [25]. It is considered as most suitable method for harvesting *Spirulina*. The vibrating screens filter the same volume per unit time as the inclined screens but require one-third of the area. The next step is the washing of excess salts from the biomass. The washed cake is frequently homogenized before being dried.

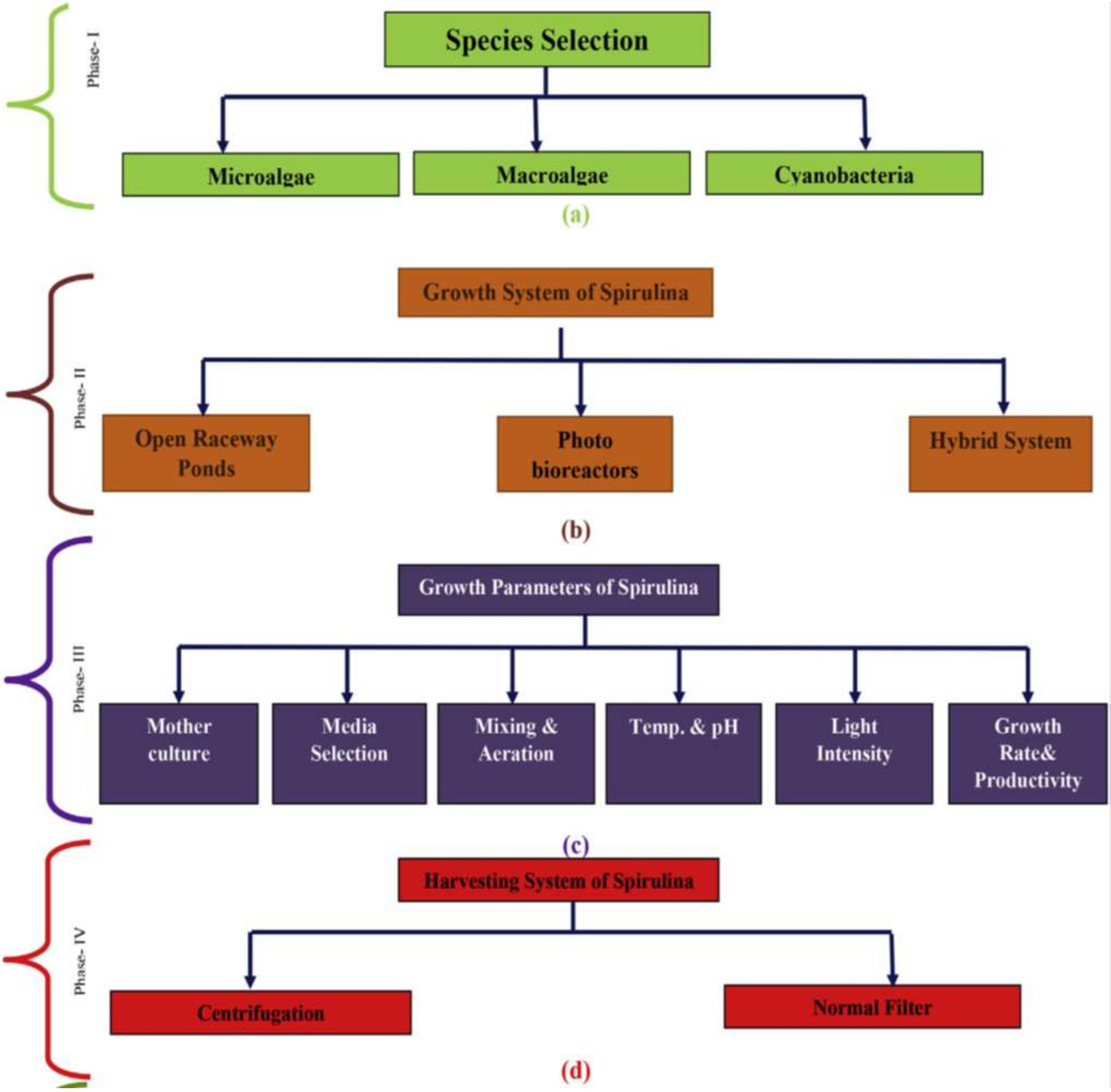

Fig 5: Different phases of *Spirulina* cultivation system (a) strain selection (b) Growth systems (c) growth parameters (d) Harvesting system (Soni *et al*., 2017) [43]

***Spirulina* as a bioremediation agent for wastewater**

This experiment was conducted in 100% tilapia aquaculture wastewater supplemented with 25% Zarrouk medium (ZM). The ZM composition was $NaHCO_3$ (16.8 g/L); $NaNO_3$ (2.5 g/L); NaCl (1.0 g/L); K_2HPO_4 (0.5 g/L); K_2SO_4 (1.0 g/L); $MgSO_4$, $7H_2O$ (0.2 g/L); $CaCl_2$ (0.031 g/L); EDTA (0.08 g/L); $FeSO_4$, $7H_2O$ (0.01 g/L). Then, *Spirulina* was inoculated to that. The aquaculture wastewater chemical characterization showed no detectable change in the concentrations of lead (Pb), cadmium (Cd), nickel (Ni), copper (Cu), manganese (Mn), zinc (Zn), and Fluoride (F) as shown in Table-4. However, *Spirulina* showed highest removal efficiency of Bromine (Br) in the residual water (96.77%). *Spirulina* in wastewater removed nitrate by 72.11% and nitrite by 79.28%. Phosphate, Sulfate and Chemical oxygen demand (COD) removal efficiency 93.84, 94 and 90% respectively. So, *Spirulina* showed high nutrient removal efficiencies from wastewater, becoming a sustainable alternative to improve the environmental conditions of aquaculture practice as well as reducing the impacts of wastewater discharge.

Table 4: Chemical composition and nutrient removal efficiency of treatment with 100% aquaculture wastewater supplemented with 25% Zarrouk culture medium before and after the *Spirulina* (Cardoso *et al*., 2020) [6]

No.	Component	Before cultivation (mg/L)	After cultivation (mg/L)	Removal Efficiency (%)
1	Lead (Pb)	< 0.03	< 0.03	-
2	Cadmium (Cd)	< 0.02	< 0.02	-
3	Nickel (Ni)	< 0.01	< 0.01	-
4	Copper (Cu)	< 0.25	< 0.25	-
5	Manganese (Mn)	< 0.01	< 0.01	-

6	Zinc (Zn)	< 0.01	< 0.01	-
7	Fluoride (F)	< 0.25	< 0.25	-
8	Nitrogen nitrate (NO^{-3})	18.65 ± 0.01a	5.20 ± 0.01b	72.11
9	Nitrogen nitrite (NO^{-2})	10.33 ± 0.05a	2.14 ± 0.03b	79.28
10	Dissolved organic carbon (COD)	300 ± 0.03a	30 ± 0.12a	90.00
11	Sulfates (SO^{-2})	27.54 ± 0.01b	1.65 ± 0.04a	94.00
12	Phosphate (PO^{-3})	11.37 ± 0.02b	0.70 ± 0.02a	93.84
13	Bromine (Br)	0.31 ± 0.01b	< 0.01a	96.77
14	Ammonia Nitrogen (NH^{-3})	< 0.25	< 0.25	-
15	Total Dissolved Solids (TDS)	0.11 ± 0.01b	0.03 ± 0.06a	72.72

[Mean ± standard deviation. Equivalent letters (a, b) in the same line indicate that there was no significant difference between the values at 95% confidence level].

***Spirulina platensis* as a complementary ingredient to reduce dietary fish meal**

Dietary treatments with graded levels of fish meal (FM) replacement with *Spirulina*: control, 2% FMR (13.2 g/kg *Spirulina* in diet), 4% FMR (26.4 g/kg *Spirulina* in diet), 6% FMR (39.6 g/kg *Spirulina* in diet) and 8% FMR (52.8 g/kg *Spirulina* in diet). The growth performance of caspian brown trout juvenile fed diets containing different levels of *Spirulina* is presented in Table 5. According to the results, fish fed diet supplemented with 6% FMS and 8% FMS had a significantly higher weight gain rate (239.51% and 231.27%) and specific growth rate (1.74% bw per day and 1.71% bw per day) compared with those fed the control diet (18.18 g and 1.37% bw per day). Furthermore, 6% FMS and 8% FMS treatments had statistically higher protein efficiency (0.76 and 0.78), lipid efficiency (1.89 and 1.94) and statistically lower feed conversion ratio (2.47 and 2.41) compared with other treatments ($p < 0.05$). Overall, the optimum growth performance was obtained at 6% FMS and 8% FMS treatments, whereas the fish fed control diet had the lowest growth performances. The present results indicated that *Spirulina* supplementation might be a good method to improve fish fillet quality and 8% FMS was advantageous in this context. *Spirulina* could be a strategic option to spare fish meal, given its price availability.

Table 5: Production performance of juvenile caspian brown trout fed different levels of *Spirulina* (Roohani *et al*., 2018) [34]

Growth performance	Control	2% FMS	4% FMS	6% FMS	8% FMS
Initial weight (g)	11.18 ± 1.23	11.91 ± 1.04	11.16 ± 0.56	10.91 ± 1.61	11.19 ± 0.93
Weight gain rate (%)	162.61 ± 3.3 b	188.49 ± 3.33 ab	206.09 ± 3.14 ab	239.51 ± 4.1 a	231.27 ± 3.92 a
SGR (%/day)	1.37 ± 0.13 a	1.55 ± 0.10 ab	1.60 ± 0.12 ab	1.74 ± 0.16 c	1.71 ± 0.26 bc
FCR	3.33 ± 0.34 a	3.35 ± 0.29 a	3.25 ± 0.36 a	2.47 ± 0.49 b	2.41 ± 0.16 b
Protein efficiency	0.56 ± 0.10 b	0.56 ± 0.07 b	0.58 ± 0.08 b	0.76 ± 0.11 a	0.78 ± 0.10 a
Lipid efficiency	1.41 ± 0.20 b	1.42 ± 0.15 b	1.44 ± 0.22 b	1.89 ± 0.18 a	1.94 ± 0.11 a

*Weight gain rate (%) = 100× (final weight − initial weight/initial weight)

SGR: Specific growth ratio = ((Ln final weight − Ln initial weight)/experiment period) × 100

FCR: Feed conversion ratio = amount on consumed feed (g)/increased of body weight (g)

PE/LE: Protein or Lipid efficiency = body weight gain/consumed protein or lipid

Means marked without letter are not significantly different. Letters a, b and c indicate significant differences among treatments, according to the Duncan multiple range test ($p < 0.05$).

***Spirulina* as an animal feed supplement**

Two parallel groups of Lithuanian black and white cattle, of similar productivity and body condition scoring, estimated in their dry period (30 days before calving), were formed (I–control and II–experimental). In both groups cows received 15 kg silage and haylage, 2 kg of hay and an additional 350 g of combined fodder per 1 litre of milked milk after calving.

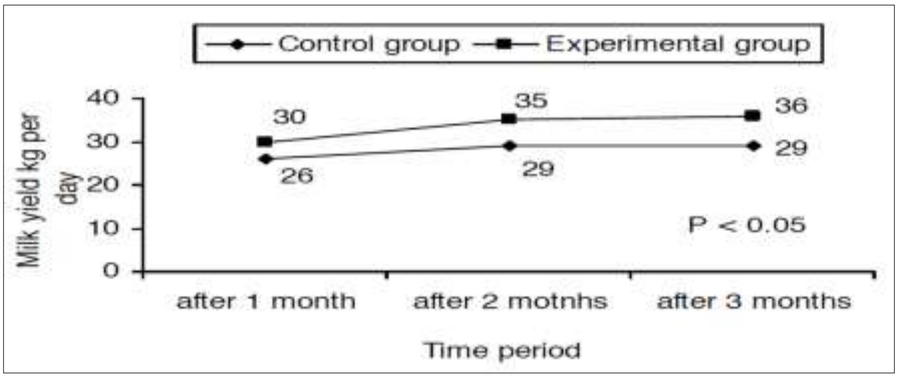

Fig 6: Influence of fodder additive *Spirulina platensis* on milk productivity (Kulpys *et al*., 2009)

Each cow of the experimental group additionally received 200g of '*Spirulina platensis*' additive dry biomass (UAB 'Vingrune'), which was mixed manually into combined fodder. The milk yields of experimental cows increased progressively (Fig.6): during the first month their milk yield average was by 4 kg or 15% more, and during the second and the third months, respectively, by 6 kg (21%) and 7 kg (24%) more than from the control group (P < 0.05). During the entire treatment period, the average milk yield of experimental cows was by 6 kg or 21% larger than that of the controlled herd. The positive influence on productivity of the experimental group of cows could be a subsequence of *Spirulina platensis* additives chemical composition and biological activity on the microorganic activity of the cattle's fore-stomachs. *Spirulina platensis* consists of alkaline elements and other substances which can change the cattle forestomachs' reaction to the alkaline side. Lactose is the main carbohydrate in milk which influences milk secretion, therefore the concentration of lactose in the milk is one of the most important factors of milk productivity (Vattio, 2004). Analysis of the concentration of lactose established that the concentration of lactose increased constantly during the experiment in milk of both groups of cows, and was within the mark. However, the lactose concentration in the milk of the experimental group was slightly higher (Fig.7). *Spirulina platensis* had a positive influence on increase in milk productivity and can be used as a food supplement.

Fig 7: Influence of fodder additive *Spirulina platensis* on The concentration of lactose in milk, % (Kulpys *et al*., 2009)

Differential response of cotton growth, yield and fiber quality to urea fertilizer and foliar application of *Spirulina platensis*

In case of cotton vegetative and reproductive growth proceed simultaneously, this work targeted assessment of whether soil application of a N fertilizer along with foliar application of destructed cells of the cyanobacterium *Spirulina platensis* can assist cotton growth. Urea (46% N) at 96, 144 or 192 kg N/ha was applied in two equal doses as main plot treatments. *Spirulina platensis* was grown and total proteins, contents of 16 amino acids and Fe, K, Mn and P of dried and destructed cells were estimated and used for foliar application at the rate of 6, 12 or 18 g cells per hectare, used as sub-plot treatments. Results showed concomitant increases in total chlorophyll with application of the N fertilizer and destructed cells of *Spirulina* (Table 6). Maximum concentrations of these photosynthetic pigments were obtained by application of 192 kg N/ha along with 18 g of destructed *Spirulina* cells. At least 12 g/ha of *Spirulina along with* 192 kg N/ha was needed for scoring the highest number of open bolls/plant. The highest cotton seed yield value (4.320 ton/ha) was obtained by using the 192 kg fertilizer-N/ha along with foliar application of 6 g destructive *Spirulina* cells. The fineness did not show statistically significant differences. Conclusively, both the urea and the destructive *Spirulina* cells acted synergically for enhanced plant growth and performance.

Table 6: Effect of N fertilizer doses and foliar spray of destructed cells of *Spirulina platensis* on Plant height, open bolls/plant, seed cotton yield, fineness in the cotton (*Gossypium barbadense*) (Yanni *et al*., 2020) [48]

N- doses (kg N/ha)	Destructed cells of *Spirulina platensis* (g/ha)	Total chlorophyll	No. of open bolls/plant	Seed cotton yield (t/ha)	Fineness (Micronaire)
96	Control	4.18 g	30.14 g	3.780 d	4.0 c
	6	4.58 g	31.23 f	3.855 c	4.2 b
	12	4.61 g	31.54 f	3.861 c	4.0 c
	18	5.32 f	32.32 ef	3.899 bc	4.1b
144	Control	6.22 e	33.50 e	3.932 bc	4.1 b
	6	6.61d e	33.87 e	3.945 bc	4.0 c
	12	6.84 cd	34.00 d	3.969 bc	4.0 c
	18	7.13 bc	34.02 d	4.069 b	4.2 b
192	Control	7.30 bc	34.19 c	4.158 ab	4.1 b
	6	7.33 bc	35.12 b	4.320 a	4.1 b
	12	7.68 b	36.64 a	4.107 b	4.3 a
	18	8.30 a	36.51 a	4.082 b	4.2 b

Application of *Spirulina* as a source of nutrient

This study evaluated the growth and yield of brinjal grown under different foliar fertilizer treatments with *Spirulina platensis*. The treatments consisted of four fertilizer concentrations applied at four phenological stages at 10 days after transplanting (DAT) followed by five-day application interval: M1 (10, 15, 25, and 35 g/L), M2 (15, 20, 30, and 40 g/L), M3 (20, 25, 35, and 45 g/L), and M4 (control plants, water spraying only). Foliar application of *Spirulina* at low concentrations (M1) increased brinjal fruit production as compared to control (M4). High fertilizer concentrations (M3) increased vegetative which which intercepts the solar radiation used in photosynthesis for biomass accumulation, triggering intraspecific competition for water, light, and nutrients that may adversely affect fruit yield of brinjal (Fig.8).

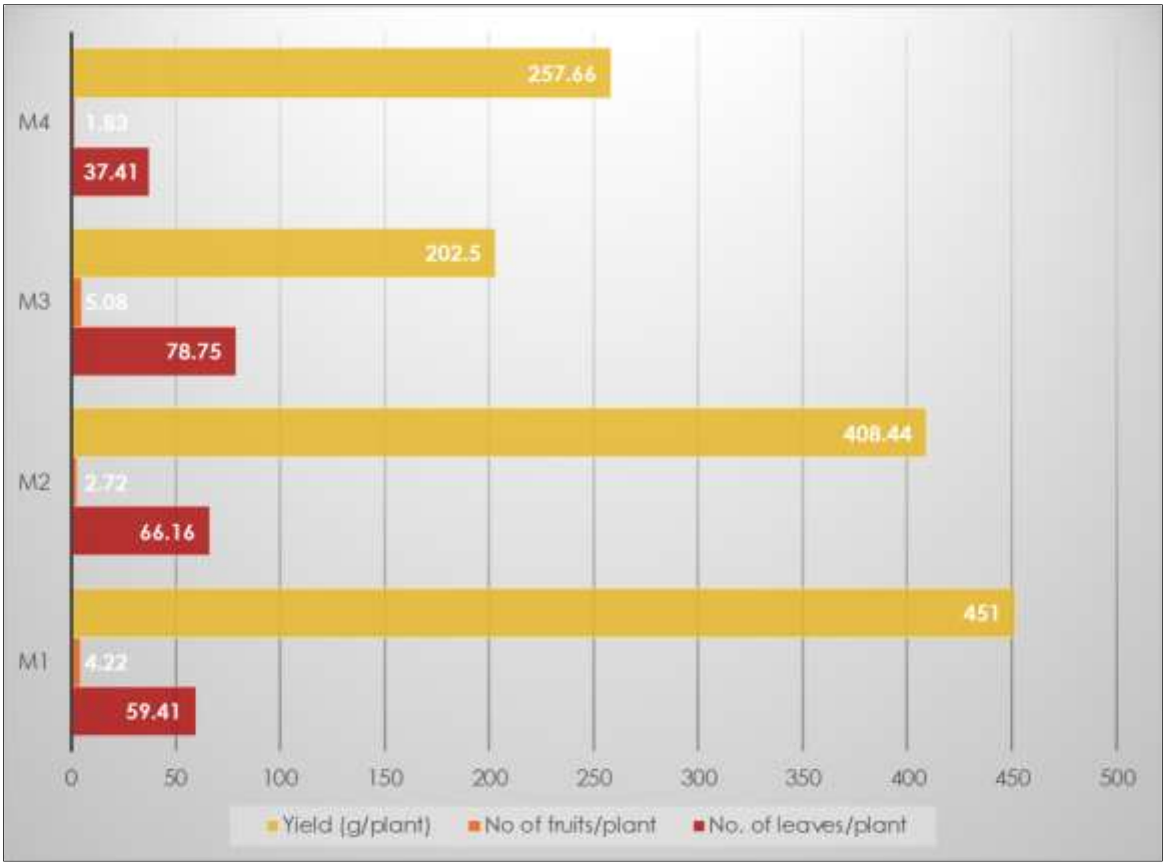

Fig 8: Growth and yield of eggplant produced under different foliar treatments of *Spirulina platensis* treatments (Dias *et al*., 2016) [9]

This experiment was conducted in Indian mustard. Before sowing, the seeds were soaked in different concentration of *Spirulina* suspension, T1 (1g/L), T2 (2g/L), T3 (3g/L), T4 (4g/L), T5 (5g/L) and the control in the pure water for 1 hour. The highest value of mean plant height of Indian mustard was found in T3 and followed by T4, T5, T2 and T1. The mean plant height of Indian mustard was not further raised at 56 days after sowing (Fig.9).

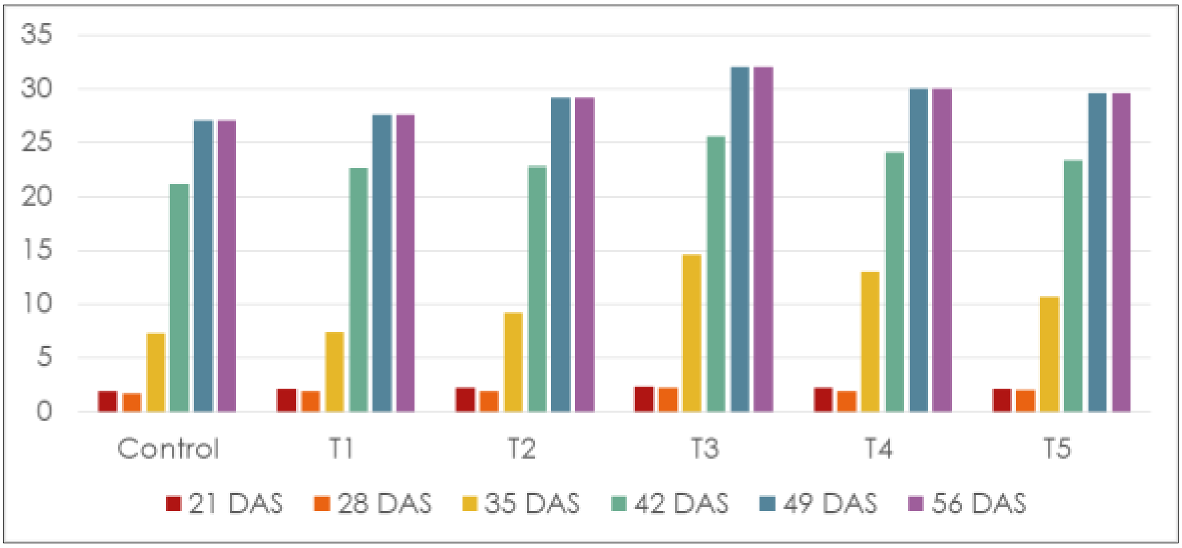

Fig 9: Effect of *Spirulina* suspension on mean plant height (cm) of Indian mustard (*Brassica campestris*) (Yee *et al*.,2020)

The highest of fruit number per plant of T3 and followed by T5, T4, T2, T1 and control on 56 days after sowing (Fig. 10). It was ranged 35.0 - 45.4 but control was 20.6. The highest of seed number per plant was found at T3 and followed by T4, T5, T2, T1 and control. It was ranged 74.4 - 80.2 but control was 60.4. Therefore, the *Spirulina* suspension when applied to seeds promotes growth without unpleasant side effects.

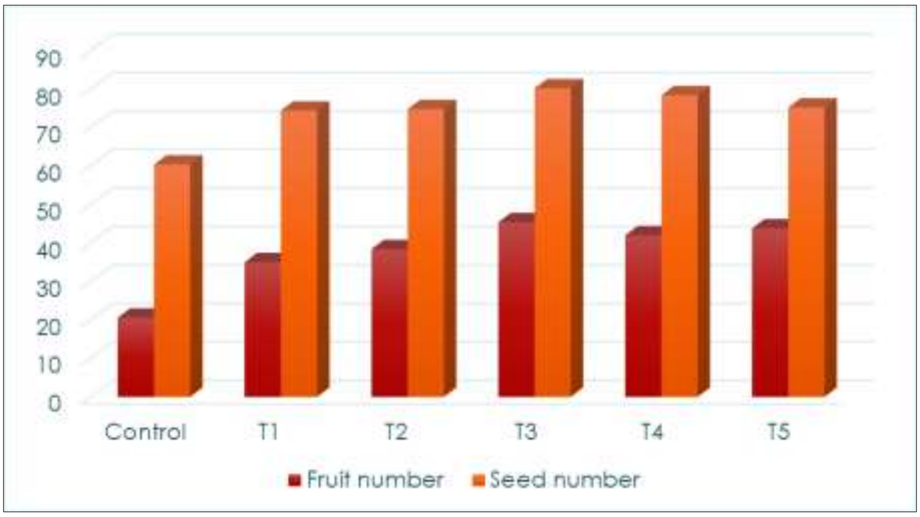

Fig 10: Effect of *Spirulina* suspension on yield and yield components in harvest of Indian mustard (*Brassica campestris*) (Yee *et al*., 2020)

***Spirulina platensis* as bio-stimulator for organic farming**

The yield of pepper was gathered 4 times as shown in the Fig. 11. The first collection of pepper was higher in the presence of *Spirulina platensis* compared to the other two treatments which reached 9, 6 and 7 Kg for compost and NPK, respectively. This result could due to high content of the free amino acids of the *Spirulina* product, as well as its content of macro and micro elements. In addition to the presence of some growth promoting substances directly absorbed by the leaves. These substances are absorbed rather faster than the nutrients from the soil by using mineral fertilization or compost. Also, the second collection after two weeks behaved the same trend. On the other hand, the third week revealed that the yields due to both NPK and *Spirulina platensis* fertilization were nearly the same (30 and 28 kg) respectively. The NPK treatment showed the highest yield in the fourth collection, compared to the compost and *Spirulina platensis* respectively. This could due to the effect of NPK addition after every collection of the yield, which was more effective. It could be concluded that the yield of pepper inoculated with bio-stimulator is recommend than that of NPK, using organic farming system, as the decrease in the total yield could be recovered by the high price of crops produced by organic farming as well as it is more safe from the health point of view.

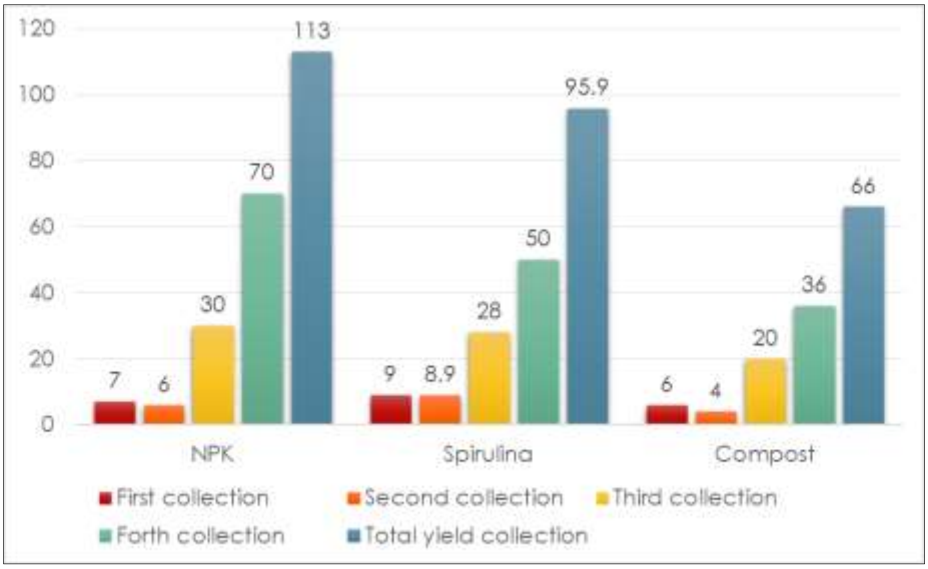

Fig 11: The crop yield of pepper (Kg/60 m^2) as affected by different fertilization treatments (Aly M. S *et al*., 2008)

***Spirulina platensis* as a biofortification agent**

Trace elements or micronutrients play a major role in the metabolism of both plants and humans. Firstly, treatments were comprised of 6 variations of the seed soaking for different time intervals i.e. 1, 2, 3, 4, 5 hours, overnight and control by 5 g of *Spirulina* in 100 ml of sterile water (Fig. 12). The increase in zinc values were observed in experimental treatment of soaking of seeds at 3 hours in greengram.

Secondly, the treatment combinations were 25:75, 50:50, and 75:25 of *Spirulina* with organic manure (Fig. 13). The zinc uptake by green gram was observed high in *Spirulina* + organic manure in 50:50 proportions respectively. The increase in zinc value can be attributed to the increase in the iron content in green gram. This is because, a cross talk between iron and zinc uptake mechanism is observed in dicot plant generally. Hence, increase in iron content results in increase of zinc content in the plants. Moreover, the organic manures are enriched with micro flora that are capable of inducing the crops to uptake the micronutrients and the synergistic action of *Spirulina* along with organic manures promoted the zinc levels in the plant. Deficiency of zinc is a major risk factor to the global agriculture and human health. About 2 billion people are currently suffering with zinc deficiency around the globe. To address the problem, a more beneficial approach is biofortification through *Spirulina*.

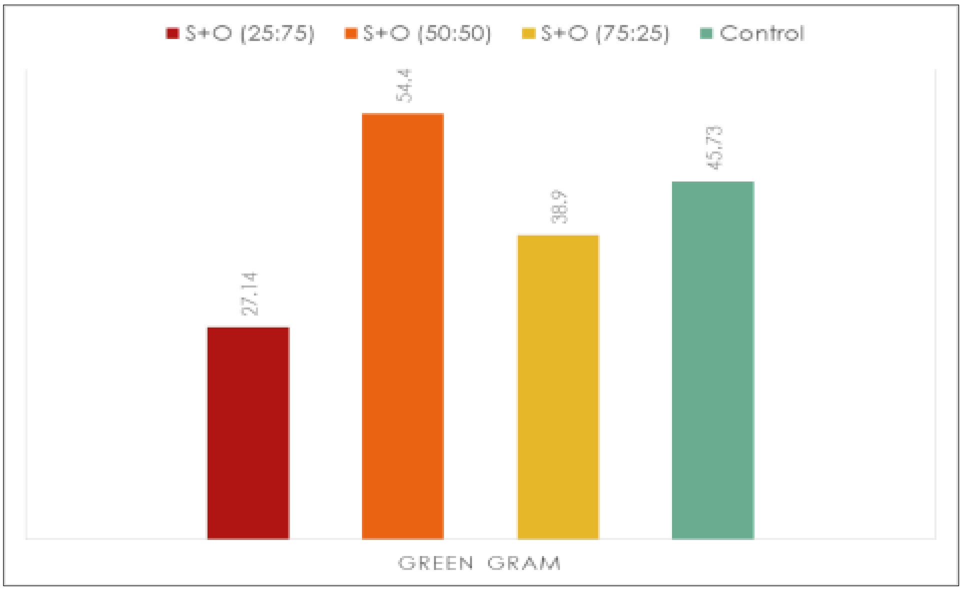

Fig 12: Effect of *Spirulina* supplementation on zinc content (ppm) through different time period soaking of green gram (Layam *et al*., 2016) [20]

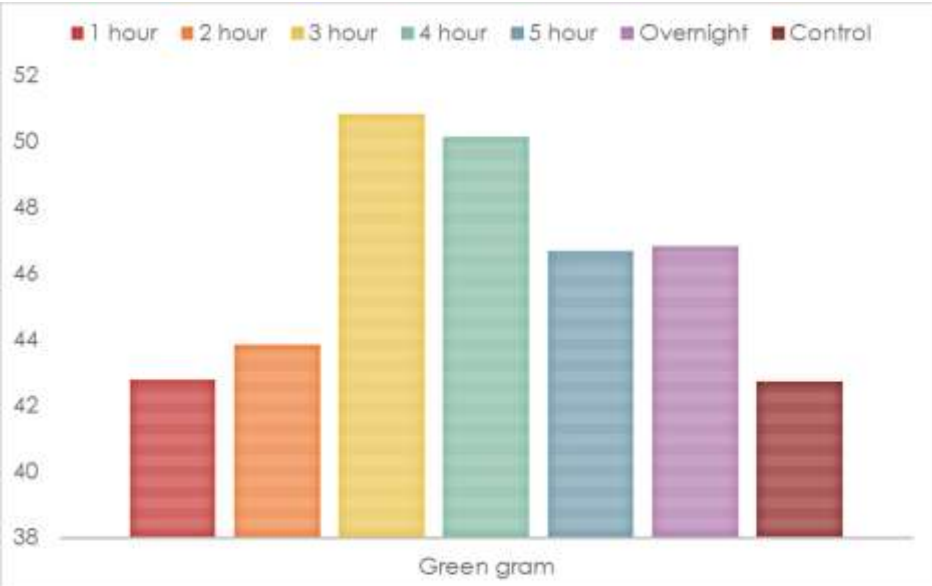

Fig 13: Effect of *Spirulina* supplementation in combination with organic manure on zinc content (ppm) of green gram (Anitha *et al*., 2016)

Cultivation of *Spirulina*: An Agribusiness outlook

This economics of *Spirulina* farming is adopted from DST, 2019, which means to provide a general overview of investment and revenues and the figures mentioned are not actual but for an agribusiness understanding. The pond should be constructed of size 10'×20' and required about 20 such ponds. Each pond will generate on an average about 2 kg wet culture per day. It should be noted that 1 kg wet culture will give 100 grams of dry powder only. Based on this, on an average, a 20 tank *Spirulina* farming business will generate 4-5 kg of dry *Spirulina* powder on a daily basis. The production of *Spirulina* in a month will be around 120 to 150 kg per month. Dry *Spirulina* powder in the market will fetch about Rs. 600 per kg. A farmer can earn about Rs. 40000-45000 per month. A farmer can make more profits by increasing tanks made with low-cost, durable materials apart from concrete ponds by utilizing maximum space available in the land, which will reduce labour and investment with more profit returns. According to Credence Research Market Analysis, the global market for algae products, especially used for nutraceuticals, pharmaceuticals as well as food and feed supplements, will register an annual growth rate of 5.8% in the period 2017-2026.

Table 7: Production Economics of *Spirulina* farming

Capital Investment	
Particulars	**Cost (Rs.)**
Pond Construction (20 @ 50,000/-)	10,00,000.00
Plant Machinery	15,000.00
Laboratory Equipment	5,000.00
Water Treatment Plant	1,50,000.00
Piping Work	25,000.00
Electrical Works	15,000.00
Drying Screens	10,000.00
Harvesting Screens	5,000.00
Total Capital Investment	12,25,000.00
Operational cost (monthly basis)	
Particulars	**Cost (Rs.)**
Packing Materials	2,500.00
Chemicals	2,000.00
Labour	18,000.00
Miscellaneous	2,500.00
Total operational cost	25,000.00
Total cost	12,50,000.00
Economics of Farming	
Sale of *Spirulina* Powder (@ Rs. 600 per kg)	72,000.00
Income per month (Rs.) (Sale - operational cost)	47,000.00

Conclusion

Spirulina is one of the natural and powerful supplements blessed by nature with negligible side effects. *Spirulina* has the potential for being a 'wonder food supplement'. It established as a flourishing source of nutraceutical and pharmaceuticals. In developing countries like India can be very suitably utilized as a diet supplement to overcome the challenge of social malnutrition. At present, *Spirulina* production is restricted to some countries (i.e. United States of America and China). In India, there should be a considerable expansion of it, especially in the areas of coastal and alkaline areas where traditional agriculture struggles, especially under the increasing influence of salination and more vulnerable to natural calamities. Then youths can able to earn profits by taking *Spirulina* as an Agri-entrepreneurship. It has good turnover with low capital investment, and also providing employment opportunities to others. *Spirulina* has been used as human food supplement for over 20 years, but its use as an animal feed supplement is relatively recent. *Spirulina* is emerging as a cost-effective means of improving animal productivity for a sustainable and viable food security future. *Spirulina* can be a strategic option to spare fish meal also. *Spirulina* can also be used as biofortification. Proper designing of cultivation system, growth efficient techniques and use of organic fertilizer may be adopted to maximize the protein content of *Spirulina*. It can be used as biostimulator in organic farming system but more research and farmers should be aware about *Spirulina* as a successful biofertilizers instead of chemical fertilizer for growth and yield of different crops.

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