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Bio fertilizers and their role in sustainable Aquaculture with particular reference to Azolla & Spirulina

N.R.Chattopadhyay[1] & P.P.Ghorai[2]

1Visiting Research Professor, Dept. of Biotechnology, Govt. of India
2 Department of Zoology, Vidyasagar University, West Bengal, India

Corresponding author: nrchatterjee40@gmail.com

Abstract – *Bio fertilizers are natural fertilizers that are microbial inoculants of bacteria, algae and fungi (separately or in combination), which may help biological nitrogen fixation for the benefit of plants. They help build up the soil micro-flora and there by the soil health. Bio fertilizer also includes organic fertilizers (manure, etc.) Use of bio-fertilizer is recommended for improving the soil fertility in organic farming.* ***Bio fertilizers*** *are the substance that contains microorganism's living or latent cells.* ***A bio fertilizer increases*** *the nutrients of host plants when applied to their seeds, plant surface or soil by colonizing the rhizosphere of the plant.* ***Bio fertilizers*** *are more cost-effective as compared to chemical fertilizers.*

Key words: **Bio fertilizers, Azolla** , **'symbiotic relationship, Organic fertilizers**

2. Introduction

Bio fertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants' uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil. There are five bio fertilizers viz. Rhizobium, Azotobacter, Azospirillum and blue green algae (BGA), traditionally used as **Bio fertilizers**. **Bio fertilizers** play an important role in improving soil fertility and boosting crop yields. Besides several microorganisms such as algae and various inorganic compound fixing bacteria, **Azolla** is also used as bio fertilizer in temperate as well as tropical rice growing areas. Azolla is able to do this because it has a unique mutually beneficial **'symbiotic relationship'** with a cyanobacterium (blue-green alga) called Anabaena. Each partner gives something to the other in this Perfect Marriage. The Azolla - Anabaena **symbiosis**. Each partner gives something to the other. *Azolla* is unique because it is one of the fastest growing plants on the planet – yet it does not need any soil to grow.

Unlike almost all other plants, Azolla is able to get its nitrogen fertilizer directly from the atmosphere. That means that it is able to produce bio fertilizer, livestock feed , food and bio fuel exactly where they are needed and, at the same time, draw down large amounts of CO2 from the atmosphere, thus helping to reduce the threat of climate change. Azolla is able to do this because it has a unique mutually beneficial 'symbiotic relationship' with a cyanobacterium (blue-green alga) called Anabaena. Azolla provides an enclosed environment for Anabaena within its leaves. In return, Anabaena sequesters nitrogen directly from the atmosphere which then becomes available for Azolla's growth, freeing it from the soil that is needed by most other land plants for their nitrogen fertilization.

Spirulina is a multicellular spiral shaped chain of cells. It is a blue-green alga belonging to the family oscillatoriaceae. It consists of 6-8 µm diameter cylindrical cells in unbranched helicoid trichomes. The filaments show movement, gliding along their axis. The trichomes elongate by intercalary cell divisions. They do not have heterocysts. Spirulina

can grow in widely differing environments such as soils, marshes, brackish and sea waters and thermal springs. It can even grow in waters whose alkalinity is so high (upto pH-11). In this alkalinity, other microorganisms cannot exist (John Jothi, 2006). There are two species *Arthrospira platensis* and Arthrospira maxima which is most widely distributed and is mainly found in Africa and Asia. Mant species of spirulina are cultivated for rich protein and - carotene they have. Spirulina can be used as a conservator to reclaim alkaline and saline soil (Venkataraman , 2005)

3. Categorization of Bio fertilizers

- Symbiotic nitrogen fixers, Rhizobium spp.;
- Non-symbiotic, free-living nitrogen fixers (Azotobacter, Azospirillum, etc.);
- Algal bio fertilizers (blue-green algae or blue-green algae in association with Azolla);
- Phosphate-solubilising bacteria;
- Mycorrhizae;
- Organic fertilizers.

4. Working principle of bio fertilizers

Bio fertilizers trap atmospheric nitrogen to the soil and convert them into plant usable forms. They also convert the insoluble phosphate forms into plant available forms. They stimulate root growth by producing some hormones and anti metabolites.

5. Azolla as Bio fertilizer

Azolla is a floating pteridophyte, which contains as endosymbiont the nitrogen-fixing cyanobacterium Anabaena **azollae** (Nostocaceae family). Widely cultivated in the Asian regions, **Azolla** is either incorporated into the soil before rice transplanting or grown as a dual crop along with rice (Alexander, M. (1974).

A. Azolla as Fish Feed

The use of algae as **fish feed** additives may be limited to the commercial production of high value **fish**. Laboratory feeding trials on the use of fresh or dried **Azolla** as a complete diet for **fish** show inconclusive results.

B. Importance of Azolla as Bio fertilizer in sustainable Aquaculture

a. What is Azolla

Azolla a dichotomously branched free floating aquatic fern is naturally available mostly on moist soils, ditches marshy ponds and is widely distributed in tropical belt of India. The shape of Indian Species is typically triangular measuring about 1.5 to 3.0 cm in length 1 to 2 cm in breadth. Roots emanating from growing branches remained suspended in water. The dorsal lobe., which remains exposed to air, is having specific cavity containing its symbiotic cavity contain its symbolic partner, a Blue Green Algae (BGA), Anabaena Azolae. The fern is capable of fixing atmosphere nitrogen in the soil in the form of NH_4 and becomes available as soluble nitrogen for the rice crop. The symbiosis Azolla Anabaena is outstanding due to its high productivity combined with its ability to fix nitrogen at high rates. Because of this, in recent decades, countless studies have been conducted on this association, but with insufficient synthesis and coordination (Yatazawa, M. Et.et. al., 1980).

Because of the growing concern about conservation of the environment and the need for deploying renewable, sustainable resources; the application of Azolla as a bio fertilizer on agricultural crops (Anand Titus Pereira, 1984), in order to provide a natural source of the crucial nutrient nitrogen, can be very beneficial to the future of our planet. Besides the environmental appropriateness of the use of Azolla, for multitudes of farmer's in many parts of the world who cannot afford chemical fertilizers, Azolla application can enhance their economic status, increasing yields while minimizing costs. Due to the fact that rice paddy fields form an ideal environment for Azolla, one of its most suitable applications is on rice. Besides its utilization as a bio fertilizer on a

variety of crops, Azolla can be used as an animal feed, a human food, a medicine, and a water purifier. It may also be used for the production of hydrogen fuel, the production of biogas, the control of weeds, the control of mosquitoes, and the reduction of ammonia volatilization which accompanies the application of chemical nitrogen fertilizer.

Fig.1. Azolla Fronds freely floating on water surface

Azolla partners blue green algae inside its lobes and is capable of harvesting atmospheric nitrogen. Due to this invisible partnership the fern multiplies very fast. The symbiotic association of the algae aids in the creation of a huge amount of biomass on the surface of the water. It is then harvested, dried and used as biofertilizer to supplement the needs of nitrogen in coffee farms. Since Azolla species are commonly found worldwide in coffee producing countries, it could provide the coffee farmers with an in expensive way of supplementing part of their nitrogen requirement, in an eco-friendly manner, without polluting the environment.

Table .1 . Elemental composition of Azolla

Elements	Percentage (%)
Nitrogen	4.5
Phosphorus	5.0
Patassium	0.5
Calcium	2.0-4.5
Magnesium	0.1-1.0
Manganese	0.65
Iron	0.16
Crude fat	3.0-3.5
Sugar	3.0-3.3
Starch	3.4-3.5
Chlorophill	6.5
CHLOROPHYLL-A	0.25-0.50
Ash	9.0-9.3%
crude lipid	6.0 to 6.7%
crude fiber	9.2-11.3%
crude protein content	20.3-31.2%

b. Classification (Taxonomy)

- Class : Pteridophyta
- Order : Salvinales
- Family : Azollaceae/ Salvinaceae
- Genus : Azolla
- Sub Genus : Eu-Azolla

c. Geographical range

Native range: Africa and Madagascar, India, South Asia, China and japan, Malaya and the Philippines, the New Guinea mainland and Australia.

Known introduced range: Papua New Guinea, Australia, China, Japan, New Zealand, Vietnam, US.

d. The Elemental / Nutritive composition for *Azolla filiculoides* on a dry weight basis

Nutrient composition of Azolia Azolla filiculoides cultured in the secondary treated effluent showed a high crude protein content (20.3-31.2%), and comparatively low contents of crude fiber (9.2-11.3%) and ash (9.0-9.3%) expressed on a dry weight basis. The crude lipid content ranged from 6.0 to 6.7% and the nitrogen-free extract content ranged from 35.1 to 46.2%.

Since the fern has a desirable carbon nitrogen ratio, it decomposes rapidly can be used in a very short time. The fern also acts as a store house for potash accumulation and stores more than five times its requirement. The significance of Azolla as a cheap source of nitrogen was first observed in China. The art of feeding the people (Chih Min Tao Shu), a book on agricultural techniques written in 540 A.D. by JiaSsu Hsieh, describes the cultivation and use of Azolla in rice fields. At the beginning of the 17th century, there were many local records of Azolla use as manure both in China and Vietnam. Azolla plants have been described by the Chinese and Vietnamese as being miniature nitrogen fertilizer factories.

On hydrolysis Azolla is found to contain a considerable number of different amino acids.The amount of total amino acids is about 230 g kg- 1 dry weight. An important amino acid, lysine is comparatively more abundant than in the reported amino acid composition of other aquatic plants though the proline, methionine, and histidine levels are lower than those in commercial feed (Shiomi and Kitoh 1987b). As a feed with a higher protein content is considered to be suitable for Tilapia,

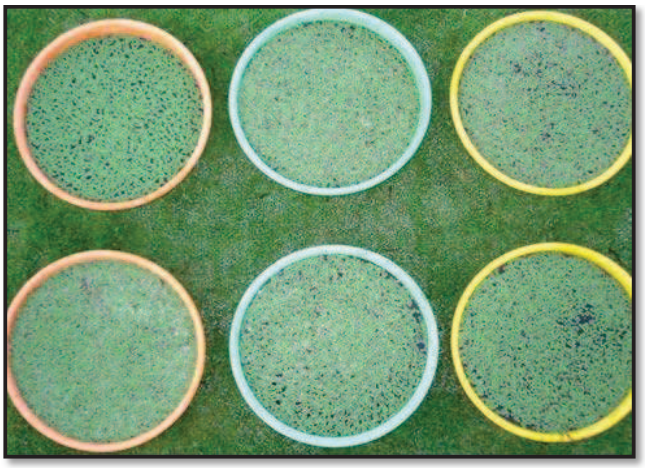

Fig. 2. Azolla isolates from varied coffee

e. Azolla – Anabaena Symbiotic Association

In the Azolla – Anabaena symbiosis, the fern is generally referred to as the macro symbiont and the blue green algae, namely anabaena is known as the micro symbiont. The two partners live in a very close relationship with one another. The fern provides the protection to the micro symbiont from oxygen damage from the external environment and the anabaena in turn provides the nitrogen to the fern for its growth and multiplication. Both the partners harvest solar energy via photosynthesis and the total nitrogen requirement can be supplied by the assimilation of nitrogen fixed by anabaena, the micro symbiont (Each leaf of azolla has the potential of harboring 75,000 Anabaena cells containing 3 to 3.5 % nitrogen). The beauty of this fern is that it is quite hardy and during favorable environmental conditions multiplies in geometric proportions. The algal symbiont is closely associated with all stages of the fern's development. The symbiont resides in the cavities formed in the dorsal lobe of the fern. Rapid multiplication of the fern takes place in summer months.World over there exist different species of Azolla. Azollacaroliniana, A. filiculoides, A. mexicana, A. microphylla, A. pinnata and A. nilotica. In India, A. pinnata is commonly observed. The algal symbiont belongs to the family Nostocaceae and is generally referred to as Anabaena Azollae.

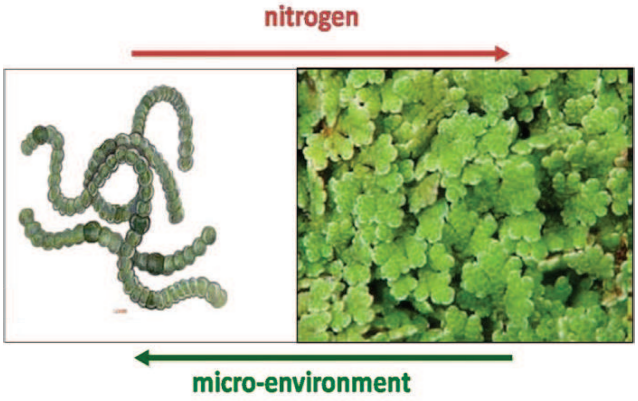

Fig.3. Azolla – Anabena symbiotic association

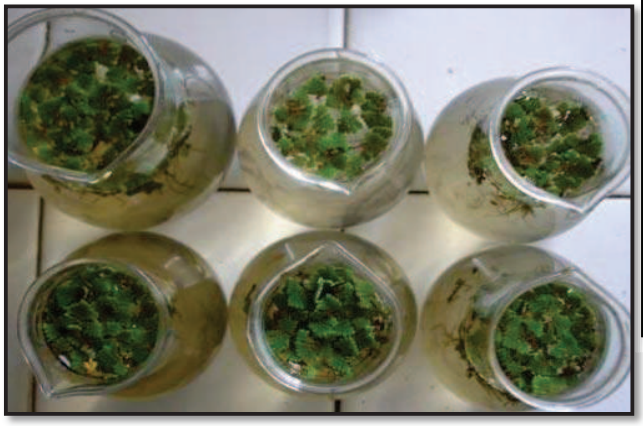

Fig. 4. Artificial Culture of Azolla

The amount of nitrogen, phosphorus (Anand Titus Pereira et. al., 1987) and potassium content in Azolla varies with variety, environment and field management. Different Varieties of Azolla species occur with the desired characteristics. The field requirement of Azolla and its nitrogen fixing symbiont, Anabaena azollae, for growth and nitrogen fixation are complex and strongly related to the biochemistry of each of the components of the association. For the survival of the partners, under adverse environment conditions, the life cycle of both Azolla as well as that of the algae is important.

f. MORPHOLOGY

The Azolla fern is a freely floating fern with multi branches and long roots. Each leaf has two lobes, the ventral and dorsal lobes. The dorsal lobes are chlorophyllous and house the algal symbiont. The nitrogen fixing symbiont is present during all development stages of the fern. More importantly, the micro symbiont exhibits host specificity because no other algal species is found in the leaf cavities. The fern appears lush green in color but turns orange red under stress conditions. This color comes from anthocyanin pigments ((Anand Titus Pereira et. al., 1987).

Fig. 5. **Underwater Root system of** Azolla

g. PHYSIOLOGICAL REQUIREMENT

Air, light, water and mineral nutrients are important factors in determining the growth and development of the fern. A few scientists have worked on a variety of inorganic media to accelerate growth. Phosphorus and potassium are the major elements along with iron, molybdenum, cobalt.

i. pH

Our studies point out to the fact that most of the strains collected from coffee growing regions grow well in natural pH. The optimum hydrogen ion concentration for Azolla species found in Joe's sustainable farm is around neutral pH. Azolla species are also sensitive to high temperature and the optimum temperature is between 25 and 30 degree centigrade.

ii. LIGHT INTENSITY

Azolla grows well under semi shaded conditions. However, strong sunlight for a period of two to three hours is not detrimental for the growth and multiplication of the fern.

iii. TEMPERATURE

The macro and micro symbiont exhibit growth over a relatively wide temperature range but the optimum range appears to be between 25 and 30 degree centigrade. Very high temperatures are unfavorable for vegetative growth of the fern.

iv. PHOTOSYNTHESIS

The macro and the micro symbiont consist of photo synthetically active pigments. The light harvesting pigments of the individual partners are complementary. The Azolla contains chlorophylls a and b while the alga contains chlorophyll a and phycobilins. Both partners contain carotenoids..

v. NITROGEN FIXATION

The atmospheric nitrogen is harvested by the algal symbiont. Heterocysts are the sites of nitrogen fixation. Heterocyst frequency increase along the stem from apex to base in the successive leaves.

vi. SURVIVAL

The sporocarps produced by azolla can be endure long periods of desiccation. Under natural conditions proliferation of azolla is entirely through vegetative reproduction. However, Sexual reproduction, which is essential to the survival of the population during temporary adverse conditions, also occurs. A combination of favorable environmental conditions favors the germination of spores.

vii. WATER TURBULENCE

Plant density and turbulence of the water surface markedly influences growth rate and nitrogenous activity of the fronds.

viii. DOUBLING TIME

Under favorable conditions the fern multiplies in geometric proportions and doubles in three to four days with an average nitrogen content of 3%.

ix. EFFECT OF HERBICIDES &PESTICIDES

Observations point out to the fact that the presence of Azolla is more markedly reduced in intensive crop management than in traditional zones where sustainable agriculture is practiced. Herbicides and pesticides have a deleterious effect on the occurrence of Azolla (Anand Titus Pereira et. al., 1987).

g. HOW TO GROW AZOLLA

- In low land, field is ploughed; leveled and small bunds of 50cm width are made to make small ponds of 3 x 2 a 1 M size.
- Only 10-15 cm standing water is allowed in the ponds.
- The green azolla @ 50-200 g/sqm + PO through SSP @ 20 kg/ha along with Furadan 1 g/kg of Azolla is mixed and released in the pond maintaining a 10-15cm of water level, for further growth and multiplication of Azolla.
- Azolla multiplies rapidly and form a green mat like a carpet on water surface of ponds in just two weeks. This green Azolla is harvested in bamboo basket and transferred and released in the transplanted rice field for further multiplication, as dual cropping with rice for fixing nitrogen to rice crop.
- Harvested green Azolla could be converted in to compost by pounding in pits for a month which is then used like FYM for other crops grown under upland situation.
- During summer, green Azolla is harvested at an interval of 15-20 days but during winter growth of Azolla becomes slow due to moisture stress and low winter temperature, hence Azolla can be harvested at 25-30 days interval during winter.

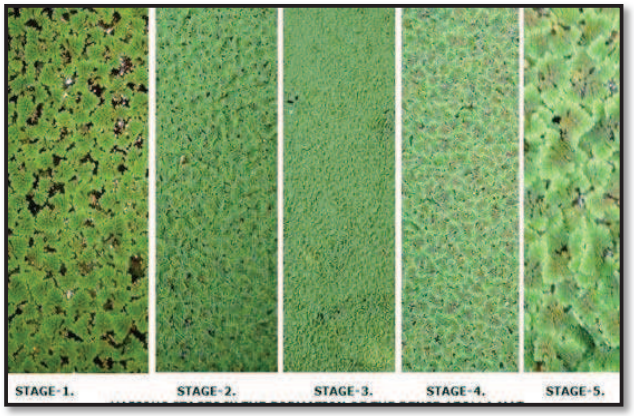

Fig. 6 Formation of Dense Azolla Mat in Successive Stages

h. HOW AZOLLA FIXES ATMOSPHERE NITROGEN

The remarkable feature of Azolla is that its symbiotic relationship with Cyanobacterium (*Anabaena azollae*) which remained on the dorsal leaf cavity of Azolla.Thre fern provides protein substances to Anabaena (BGA). The BGA then absorbed the atmospheric nitrogen and decomposes it through enzymic activity and converted in to soluble ammonia (NH+) (Anand Titus Pereira & Gowda, T.K.S. (1987) .

i. Contribution of Azolla in Aquaculture

- Azolla reduces evaporation from water surface and increases water use efficiency in rice (Watanabe, I., et. al., 1977).
- Dry Azolla flakes can be used as poultry feed and green Azolla is also a good feed for fishes.
- **Effect of azolla.** Four fish species (grass carp, tilapia, crucian carp, and lotus carp) are raised with azolla for 110-112 days. The fish species best suited to the rice – azolla – fish system are grass carp and tilapia. Both like to eat azolla and adapt easily to the rice field environment. The omnivorous crucian carp and lotus carp (which are benthic and planktivorous feeders) can be raised in the rice field in lower numbers. Grass carp and tilapia eat over 60% of their body weight in azolla each day; whereas, crucian crap and lotus carp eat about 8% of their body weight in Azolla (Anand Titus Pereira & Gowda, T.K.S. (1991).

j. MULTIPLE USES OF AZOLLA

- Basal application on green Azolla manure @ 10-12 t/ha increase soil nitrogen by 50-60 kg/ha and reduces 30-35 kg of nitrogenous fertilizer requirement of rice crop ((Watanabe, I., et. al., 1977).
- Release of green Azolla twice as dual cropping in rice crop @ 500 kg/ha enriches soil nitrogen 50 kg/ha and reduces N requirement by 20-30 kg/ha.
- Use of Azolla increase rice yield by 20 to 30%.
- Rice varieties like DR-92, RCPL-1-87-8, Mendri, H-2850 and Manipuri produced more than 30 q / ha rice yield when grown with Azolla as dual cropping under natural soil fertility (Paul, E.A. and Clark, F.E., 1996) .
- Under low land condition a thick Azolla mat does not allow the weeds to grow in rice field thus, Azolla suppresses the weed growth and creates congenial condition for rice production (Michelle and Jude Fanton., 1990)

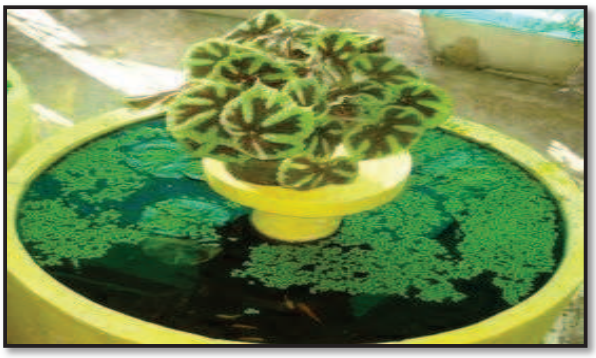

Fig. 7. Formation of dense Azolla Mat

The potential uses of Azolla are numerous. Azolla is a nutrient rich fern and has traditionally been used throughout Asia and parts of Africa as feed for livestock, poultry and fishes. Azolla contains very

high levels of protein and fat and promote the development of both grain production and stock breeding. In some countries the fern is used for ornamental purposes. At times Azolla is also used as human food.

K. RECOMENDATIONS FOR RESEARCH

- Establishment of Azolla germplasm collection in coffee growing regions.
- Characterization of various Azolla isolates should be undertaken so as to determine their nitrogen fixing efficiency.
- Package of practices for Azolla production should be standardized.
- Development of techniques for the germination of Azolla spores.
- Since various Azolla cultures are present in various agro climate coffee regions, we need to develop techniques for the differentiation of Anabaena azollae strains.

Fig.8. Azolla Mother Culture

6. Spirulina as Bio fertilizer

a. What exactly spirulina is

Spirulina is one of the oldest life forms on Earth. In fact, this blue-green microalgae is partly responsible for producing the oxygen in the planet's atmosphere that billions of years ago allowed the planet's originating life forms to develop. Spirulina is the world's first super food, and one of the most nutrient-rich foods on Earth. Spirulina has between 55 and 70% protein (more than beef, chicken, and soybeans), 9 essential and 10 non-essential amino acids, as well as high levels of gamma - linolenic acid (GLA), beta-carotene, linoleic acid, arachidonic acid, vitamin B12, iron, calcium, phosphorus, nucleic acids RNA & DNA, chlorophyll, and phycocyanin, a pigment-protein complex that is found only in blue - green algae.

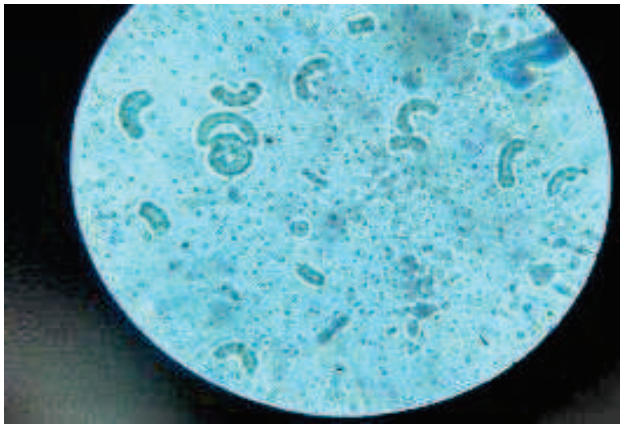

Fig.9. Microscopic view of *Spirulina platensis* in 40X magnification

b. Benefits of Spirulina

Spirulina provides a wide range of health benefits almost immediately upon ingestion. It provides a near-instantaneous boost to one's energy, while helping to improve endurance and reduce fatigue. It helps improve
the immune system, and provides exceptional support for the heart, liver, and kidneys. Spirulina is also a

natural detoxifier, oxygenating the blood, and helping cleanse the body of toxins and other impurities that may be causing illnesses or other health complications. Spirulina is also a natural appetite suppressant, and it helps to improve the body's digestive system. It also has very powerful antioxidant properties and it helps to balance the body's pH, thereby reducing inflammation throughout the body in a safe and chemical-free way.

c. Sources of Spirulina

Spirulina is a spiral-shaped microalga that grows naturally in the wild in warm, fresh water lakes. Its deep blue-green color is what gives the water its greenish hue. Spirulina is also cultivated and harvested in man-made reservoirs like those used by Nutrex Hawaii, on the Kona coast of Hawaii. This particular type of spirulina is the only one of its kind to be cultured in a Bio Secure Zone that is free of pesticides, herbicides, and GMOs. Available in both powder and tablet forms, Nutrex Hawaii's 100% vegan Hawaiian Spirulina Pacifica® is a unique, superior strain of Spirulina, with the highest known nutritional content in the world.

d. Side Effects of Spirulina

Spirulina is a safe and effective super food that is highly digestible, with no side effects. It does, however, contain iodine, so those allergic or sensitive to iodine should avoid taking it.

e. Pigments: Spirulina contains distinctive natural pigments, including carotenoids and C-phycocyanin (C-PC). Phycocyanin is a pigment-protein complex from the light-harvesting phycobiliprotein family, along with allophycocyanin and phycoerythrin. It is an accessory pigment to chlorophyll. All phycobiliproteins are water-soluble, so they cannot exist within the membrane like carotenoids can. It is a blue pigment that is important in the **photosynthesis** (food **production**) of cyanobacteria. It assists the function of chlorophyll especially during low light conditions. Spirulina is reported to contain diverse type of pigments as well as essential fatty acids as below -

Table. 2. Nutritive and Medicinal importance of Spirulina

SL No.	Minerals	Quantity (mg / Kg)		SL No.	Vitamins	Quantity (mg / Kg)
1.	Calcium	10,000		1.	Beta Carotene	1400
2.	Phosphorus	8,000		2.	Vitamin E	100
3.	Magnesium	4,000		3.	Thiamine	35
4.	Iron	1,500		4.	Reboflavin (B-2)	40
5.	Zinc	30		5.	Niacin (B-3)	140
6.	Copper	12		6.	Vitamin B-6	8.0
7.	Manganese	50		7.	Vitamin B-12	3.2
8.	Chromium	2.8		8.	Folic Acid	0.1
9.	Sodium	9,000		9.	Biotene	0.05
10	Potassium	1,400		10.	Pantothenic Acid	1.0

Phycocyanin --

C-phycocyanin (C-PC) - 29.7 to 86.1 mg g $^{-1}$.
allophycocyanin (A-PC) - 2.53 to 6.11%
R-phycocyanin (R-PC) - 5.75 to 12.35%
Chlorophyll - 1.1042 - 3.3004 mg/ml
Total Carrotinoids -

beta-carotene	49.6 to 319.5 µg g-1
lutein	0.06 to17.21 µg g-1
astaxanthin	6.61 to 160.27 µg g-1
zeaxanthin	1.25 to 18.55 µg g-1
cryptoxanthin	1.41 to 20.13 µg g-1

f. Essential Fatty Acids (gm/Kg)

Linolinic Acid	8.0
α Linolinic Acid	10.0

g. Application Areas

i. Food Supplement –

The World Health Organisation (WHO) has found Spirulina to be an excellent food for human consumption and Spirulina has the approval of the Food & Drugs Authority of the United States for being sold as a natural food. In Japan and in the United States, business executives take Spirulina tablets to combat stress. Athletes and joggers take Spirulina for quick energy synthesis.

ii. Health &Medicine

Non insulin dependent diabetes; Cholesterol control; Vitamin 'A' deficiency & malnutrition; Adjunct to cancer patients undergoing chemotherapy; Formulations with other natural products as a general health supplement; Liver corrective for liver disorder; Burns therapy, skin grafting; Control obesity; Lactating agent for mothers.

iii. Cosmetics

Spirulina is used as pimple lotions, Facial asks, Hair oil, Shampoo, Mineral bath, Skin cleaner, Tooth paste.

iv. In Pisciculture

Speciality feed for aquarium fish, Colour enhancement feed for Gold fish, Formulation with existing feeds for augmentation of vitamins, High protein feed for table variety fishes (fresh water), and Special feed for shrimp farming.

v. In Poultry

As a feed supplement for enhancing production of broilers / Table birds.

vi. Extraction from Spirulina

Beta-carotene for medicinal & laboratory use, C-phycocyanin colouring agent in food, microbiological areas cosmetics, C-phycocyanin - colouring agent in food, cosmetics, etc. Chlorophyll -colouring agent, Essential amino acids - for microbiological & chemical essays mulberry consuming silk worm, Specialty feed for breeding.

vii. Process of Cultivation

Spirulina Cultivation essentially consists of four major steps which are --

1. Development of inoculum
2. Culturing the Spirulina in the production ponds
3. Separation and washing of the bio-mass from the growth medium
4. Drying or dehydration of bio-mass

7. CONCLUSION

From time immemorial, farmers all over the world have used compost, green manures and other organic residues as major sources of nitrogen to promote plant growth and increase crop production. In recent year's commercial fertilizer have supplied the bulk of the coffee industry's fertilizer needs. Both economic and environmental considerations strongly favor biological nitrogen fixation as the process of choice in the future. Azolla has the potential of supplying part of the nitrogen requirement of coffee through biological means. In approximately 75 days, a hectare of azolla can produce three layers of green manure. The value of this amount to 25 kg nitrogen per hectare. The azolla can be harvested and either incorporated into soil or used in the preparation of compost.

We have interviewed a number of second generation and third generation farmers from coffee growing regions and they are of the opinion that the farming communities in the early 1930's and 1960's were familiar with the use of Azolla as a green manure. However, with the discovery of synthetic fertilizers, the fern slowly lost its prominence. They also point out to its present disappearance from its natural environs due to the indiscriminate use of herbicides, pesticides and weedicides. Unfortunately, today, the fern is present in only a few pockets and need to be carefully nurtured like an endangered species. We are confident that in the coming years, the combined efforts of scientists and planners will stimulate the interest of coffee farmers, in the use of azolla as an ecofriendly bio fertilizer in supplying the nitrogen needs of coffee.

Spirulina represents a biomass of cyanobacteria that can be consumed by humans and other animals. The two species are *Arthrospira platensis* and A. maxima. Cultivated worldwide, Arthrospira is used as a dietary supplement or whole food. Spirulina Plankton (*Spirulina platensis*) is a blue-green vegetable micro-algae found in the highly alkaline lakes of Africa and Mexico. The natives of these places have been using Spirulina as part of their diet for centuries. Today, Spirulina cultivation is becoming a world-wide phenomena owing to its extra-ordinary nutritional qualities. The various considerations that highlight the importance of Spirulina under present day context are: The only single, natural source providing the highest amount of protein ever known to man is Spirulina which contains 71% protein. The protein content in Spirulina is three times that of soybean, five times which of meat and the protein quality is among the best with a good degree of amino gram. The protein yield per unit area per year is the highest compared to other protein yielding crops. Like all other microbial cells, Spirulina contains all natural vitamins including the 'B'complex range, minerals and growth factors including gram-linolenic acid (highest after milk).

It contains the highest amount of β-carotene a precursor of Vitamin 'A'. It is the only vegetable source of vitamin 'B-12' containing two and half times that of liver. The concentration of nucleic acids is among the lowest recorded for microbial cells considered as food or feed. The other micro organisms including those pathogenic to humans and other animals are eliminated in the production process of Spirulina due to its requirement of a very high alkaline growth medium. The only single, natural source providing the highest amount of protein ever known to man is Spirulina which contains 71% protein. The protein content in Spirulina is three times that of soybean, five times that of meat, and the protein quality is among the best with a good degree of amino gram. The protein yield per unit area per year is the highest compared to other protein yielding crops. Like all other microbial cells, Spirulina contains all natural vitamins including the 'B' complex range, minerals and growth factors including gram-linolenic acid (highest after milk and 'evening prime rose oil'). It contains the highest amount of b - carotene a precursor of Vitamin 'A'. It is the only vegetable source of vitamin 'B12' containing two and half times that of liver. The concentration of nucleic acids is among the lowest recorded for microbial cells considered as food or feed. The other micro organisms including those pathogenic to humans and other animals are eliminated in the production

process of Spirulina due to its requirement of a very high alkaline growth medium. Spirulina's preference for tropic and sub tropic climatic conditions offers a best land use in arid areas.

Experimental use of Azolla as Tilapia feed indicates highly satisfactory results when fed for 3 weeks with diets containing Azolla as filler. Four dietic. Component (A,B,C,D) Diet/composition (%) were tested. The feeding results are expressed as the percentage of the weight gain of the fish. The control diet (A) containing 68.9% flour as filler exerted a slightly unfavourable effect on the percentage of weight gain (56.4%) in Tilapia for 3 weeks, compared with the diet containing a-starch and cellulose powder (Shiomi and Kitoh 1987b). Other diets with increasing concentrations of Azolla were not suitable. Diet B which contained 20.7% Azolla, displayed the same effect as the control for 2 weeks, then induced a 5.2% decrease in Tilapia weight after 3 weeks. Diets C and D exerted the same effect on the growth of Tilapia after 3 weeks of feeding with a 17% decrease of growth compared to the control. Diet A consisting of only flour as a filler would be ideal. Hence, the fact that the diet containing 20.7% of Azolla (B) induced the same effect on growth as that of the control is highly significant. Attempts were made to improve the food of Tilapia using various mixtures. It is envisaged that about 10% of commercial fish meal can be replaced by soybean refuse. The results indicate that Azolla can replace about 20% of Tilapia feed, which indicates the beneficial effect of the use of aquatic plants. Though Azolla could become a potential source of fish feed, factors such as nutritional value of various species or changes with aging, edibility, stable yield of Azolla, and storage techniques require further studies.

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