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BIOFORTIFICATION OF *AMARANTHUS GANGETICUS* USING *SPIRULINA PLATENSIS* AS MICROBIAL INOCULANT TO ENHANCE IRON LEVELS

KALPANA P[1], SAI BRAMARI G[2] & L. ANITHA[3]

[1,2]Research Scholars, Department of Microbiology and Food Science & Technology, Institute of Science,
GITAM University, Visakhapatnam, Andhra Pradesh, India

[3]Associate Professor, Department of Microbiology and Food Science & Technology, Institute of Science,
GITAM University, Visakhapatnam, Andhra Pradesh, India

ABSTRACT

Biofortification of crops with cyanobacteria to enhance vitamins and minerals is a novel practice employed widely all over the world. *Spirulina platensis* is a unicellular blue green algae which is a nutrient dense (iron content 20.88mg/100g) and also used as microbial inoculant to enhance the nutrient status of the plant. In the present study, *Spirulina platensis* has been used as biofortifying agent to enhance the iron status in *Amaranthus gangeticus* plant. Iron is an important dietary component and the bioavailable iron form should be high to overcome iron deficiency anemia which is more prevalent in India (Pregnant women 87% and Children75%). Different methods like Soaking of seeds at different time intervals (1, 2, 3, 4, 5 hours and overnight), in different concentrations of *S.platensis* (5, 10, 15, 20, 25 and 30g) in 200ml of water and other methods like, *S.platensis* in combination with Biofertilizer, Vermicompost, Organic fertilizer and Chemical fertilizer, in different proportions (25:75, 50:50, 75:25) and Spray method (25, 50, 75 and 100g of *Spirulina* in 5Liters of water) were used.

Estimation of iron is carried out in the yield after the germination and growth of plant. The iron content after experimental treatments was found to be high in 2hours- 18.35mg/g for soaking the seeds in different time intervals, high in 30g-20.88mg/100g for the experimental set up of using different concentrations of *S.platensis*. In the set up of *S.platensis* in combination with biofertilizer, vermicompost, organic manure and chemical fertilizer, the iron content was recorded highest in 75:25 ratio (44.85mg/100g), 25:75ratio (43.99mg/100g), 50:50 ratio (4.2 mg/100g) and 50:50 ratio (15.5mg/100g) respectively. In the set up of *S.platensis* spray method, the iron content was recorded less when compared with control for all the variations. The results obtained were analyzed statistically and it was found that there is significant increase in iron levels of *Amaranthus* plants by using *S.platensis* as microbial inoculant when compared with control except Spray method.

KEYWORDS: *Spirulina platensis*, *Amaranthus gangeticus*, Iron, Dietary Supplements and Microbial Inoculants

INTRODUCTION

The most important plants for nutrition of humans and mammals are the highly evolved flowering plants (angiosperms). Iron (Fe) is found in all plant parts, which include roots, leaves, flowers, fruits and seeds, storage organs like tubers. Under natural conditions, all Fe of living organisms, ultimately enter the nutrition chain via plant roots. The Fe reaches leaves mainly in complexed form with citrate through the xylem, which is a plant conductive tissue for water and mineral long- distance transport (http://www.intechopen.com).

S. platensis also called as *Arthrospira* is a microscopic and filamentous cyanobacterium (Blue green algae) that has a long history of usage as food. Its name is derived from the spiral or helical nature of its filaments (Becker, 1993). *S. platensis* has been used as food for centuries by different populations and only rediscovered in recent years. It grows naturally in the alkaline waters of lakes in warm regions. Measuring about 0.1mm across, it generally takes the form of tiny green filaments, coiled in spirals of varying tightness and number, depending on the strain (Adbulquader & Tredici, 2000). *S.platensis* is cultivated worldwide, used as a dietary supplement as well as whole food and is available in the forms of cakes, tablets, powder. It is also used as a food supplement in the aqua culture, aquarium and poultry industries (Vonshok, 2001; Geitler, 1982).

S. plantensis is rich in protein, vitamins, minerals and carotenoids, antioxidants that can help to protect cells from damage. It contains nutrients including B-complex vitamins, beta carotene, vitamin-E, manganese, zinc, copper, iron, selenium and gamma linolenic acid an essential fatty acid (Blinkal *et al*., 2000). Blue green algae can be helpful in agriculture as they have the capability to fix atmospheric nitrogen to soil and this nitrogen is helpful to the crops. Blue green algae is used as a bio-fertilizer. A biofertilizer is a substance that contains living microorganisms which, when applied to seeds, plant surfaces or soil, colonizes the Rhizosphere as the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey, 2003). As biofertilizers contain living-organisms their performance also depends on environmental surroundings (Lillian, 1992).

Amaranthus gangeticus shows a wide variety of morphological diversity among and even within certain species. Although the family is distinctive, the genus has few distinguishing characters among the 70 species included (Juan *et al*., 1988). *Amaranthus gangeticus* leaves are good source of dietary minerals including calcium, iron, magnesium, phosphorous, zinc, copper and manganese (Tucker, 1986). Vegetables, especially leafy vegetables are important in the diet as they are micro-nutrient dense foods, rich in carotene, and minerals such as calcium, iron and Phosphorous (Ali & Tsmscs, 1922). In 100g of eatable portion of *Amaranthus gangeticus* it contains Moisture: 85.7g, Protein: 4.0g, Fat: 0.5g, Minerals: 2.7g, Crude fiber: 1.0g, Carbohydrates: 6.1g, Calcium: 397mg, Phosphorous: 83mg, Iron: 3.49mg (Allen & Myers, 2003).

MATERIALS AND METHODS

The methodology adopted for the present study was presented in table 1. The *Amaranthus* seeds were coated with *Spirulina* hydrolysate for different soaking intervals and concentrations. The experimentation was also carried out with different combinations of *Spirulina* and fertilizers (Organic, Vermicompost, Chemical, and Biofertilizer) in different ratios. The last method followed was spray method where *Spirulina* along with water was sprayed on the leaves once in every week till the harvest. Iron content of the yield from different set-ups was estimated by Wong's method (1928). The results were tabulated and presented in the tables 2-7. The results thus obtained were subjected to statistical analysis by using Mini tab version-16.

Table 1: Field Experimental Set ups

S. No	Name of Set up	Variations						
1	Time period Soaking (5g of *Spirulina* in 100ml of sterile water)	1hour	2hours	3hours	4hours	5hours	Over night	Control
2	Soaking in different concentrations (In 100ml sterile water)	5g	10g	15g	20g	25g	30g	Control
3	*Spirulina*+Biofertilizer (S:B)	25:75	50:50	75:25	-	-	-	Control

Table 1: Contd.,

4	*Spirulina*+Vermicompost (S:V)	25:75	50:50	75:25	-	-	-	Control
5	*Spirulina*+Organic manure (S:O)	25:75	50:50	75:25	-	-	-	Control
6	*Spirulina*+Chemical fertilizer (S:C)	25:75	50:50	75:25	-	-	-	Control
7	Spray method (g/L)	25/5	50/5	75/5	100/5	-	-	Control

RESULTS AND DISCUSSIONS

Plants are essential sources of iron in the human and animal diet and that often iron concentration in plants is not enough to meet the daily dietary recommendations. In many parts of the developing world, large segments of the human population do not have access to animal sources of iron due to elevated costs and most people being vegetarians. In these cases, a commonly used strategy is iron fortification. However, iron fortification of plant foods is not always practical or economically feasible and many times this fortified iron is not highly bioavailable. Therefore a more sustainable approach, that is believed relevant to both urban and rural population, is to enhance the iron content of plant foods through Biofortification (Beard, 2001).

When iron supply is not sufficient to plant, supplementation through the *S.platensis* can be done. Increasing iron levels through supplementation of a biofertilizer or microbial inoculants is the most widespread iron-acquistion mechanism in plants strategy (Mara schuler, 2012). *Amaranthus* plants are biofortified by the supplementation with *S. platensis* to enhance the concentration of iron in the plants. Normally plants uptake iron from soil by two ways. One is by increased root surface (Kramer *et al*., 1980; Moog & Bruggemann, 1994) and other method by transporting from roots to leaves through xylem (Tiffin, 1966; Brown & Chaney, 1971).

In the experimental set up 1 the seeds were soaked for different time periods in the water along with the *Spirulina* before sowing. From the table 2 it is evident that the iron content estimated was higher than the control. The sample with two hours of soaking has recorded a high of (18.35±0.03 mg/g^{-1}) of iron content. All the variations showed the iron content higher than the standard value of NIN (National Institute of Nutrition, Hyderabad) i.e., 3.49mg/100g except the variation of four hours of soaking where there is no difference. The increased iron content in the *Amaranthus* with supplementation of *Spirulina* may be attributed due to the seeping of *Spirulina* in to the seed through the seed coat and finally into the cotyledons where the action of growth hormones gets triggered. Since the surface area of the seeds is less the seeds were unable to take *Spirulina platensis* from the medium due to saturation point by 2hrs of soaking. Hence there was decrease in iron content after 2hrs of soaking.

In the experimental set up 2 the seeds were soaked in different concentrations of *Spirulina* and from the table 3 results it is evident that the iron content estimated was higher than the control. The sample with 30g concentration of *Spirulina* showed an increase in the iron content (20.88±0.48 mg/g^{-1}) followed by the 20g concentration of *Spirulina* (17.70±0.58 mg/g^{-1}). All the variations showed an enhancement of iron content except the sample with 25g concentration of *Spirulina* showed a less iron content. There are fluctuations in the iron content of Amaranthus for second set up due to multiple actions from sowing till the harvest of the green leafy vegetable. Typical sink organs like immature organs receive Fe via the phloem pathway, which represents the conductive tissue for assimilation and signaling (Jeong & Guerinot, 2009). Iron is distributed in all parts of the plant, including cellular compartments, this distribution is carried out by a membrane bound metal transport proteins (Curie et al., 2002).

The iron in the soil mainly exists as Fe^{3+}, often bound as iron hydroxides in mineral soil particles. To meet their demand for iron, plants need to mobilize Fe in the soil by rendering it more soluble before the plants are able to take up into their roots (Jeong & Guerinot, 2009). The phytosiderophores complexes of the mugenic acid family which are Fe3+ chelating methionine derivatives that are systhesis and secreted by the plants. These plants are monocotyledonous grasses which come under strategies-II plants. The phytosiderophores complexs substance will take $Fe3^+$ in the experimental set up 3 *Spirulina* & biofertilizer were mixed in different ratios with *Amaranthus* seeds and the iron content was estimated. From the table 4 results it is evident that the ratio of *Spirulina*: biofertilizer i.e., (75:25) showed a highest increase in the iron content (44.85±0.78 mg/g^{-1}) when compared with other variations and control. This may be attributed to the biofertilizer and *Spirulina* combined effect which symbiotically associate with plant roots. This colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the plant (Vessey, 2003). Since in the biofertilizer the microbial density is more and apart from Spirulina platensis supplementation will evidently increase the iron status by strategy I transportation of the mineral.

The *Amaranthus* seeds of set up-4 were mixed with different ratios of *Spirulina* & vermicompost and the iron content was estimated in the yield. From the table 5 results it is evident that the first ratio of *Spirulina* & vermicompost i.e., (25:75) recorded a high in the iron content (43.99 ±0.77 mg/g^{-1}) when compared to the control and reference standard. However the remaining 2 ratios of *Spirulina* & vermicompost show a decrease in their iron content. Increasing the amount of soil organic matter may significantly increase the cation exchange capacity. With increasing the amount of compost amount in the soil, the amount of nutrients will be increased (Edward, 2000). Combined application of vermicompost and Spirulina can improve the micronutrient availability for plants which is evident from our study. Hellal, (2007) reported that compost increases the amount of soluble, exchangeable, acid-soluble iron in the soil and iron is linked with organic matter.

Amaranthus seeds were treated with different ratios of *Spirulina* & organic manure in set up-6. The results (table 6) indicate that the ratio of *Spirulina* & organic manure (50:50) recorded a high in the iron content (4.2±0.21 mg/g^{-1}) when compared to the control value (2.43± 0.16 mg/g^{-1}). However all the variations showed an enhancement in the iron content, when compared to the control sample. The majority of nitrogen supplying organic fertilizers contains insoluble nitrogen and act as slow releasing fertilizers. *Spirulina* when mixed with the organic matter re-emphasize the role of humus and other components of soil. It mobilizes the existing soil nutrients by less nutrient densities with less wastage. It helps to prevent top soil erosion (responsible for desertification) and retains soil moisture leading to the availability of minerals in a most accurate manner to the plants (Hewett, 2012; Linna *et al.*, 2012).

The experiment was carried out with combination of *Spirulina* and chemical fertilizer in the ratios followed for the other combination. The ratio (50:50) *Spirulina*: chemical fertilizer showed highest iron content (15.5±7.28 mg/g^{-1}). These chemical fertilizers when mixed with the *Spirulina* in appropriate ratios increases soil fertility by altering nutrient, water, heat and aerated conditions. The combinations of chemical fertilizer & Spirulina also encourage a more economical consumption of H_2O and nutrients by plants.

In the experimental set up 7 the water is mixed in different concentrations of *Spirulina* and sprayed to the *Amaranthus* leaves. From the table 8 it is evident that the *Spirulina* & water spray method has not shown any increase in the iron content of the leaves when compared to the control value. However the ratio of *Spirulina* & water i.e., (25: 5L) showed a slight increase in the iron content (4.14 ±0.36 mg/g^{-1}) when compared to the standard values of NIN i.e.,3.79mg/100g. Normally deficiency of iron in soil occurs due to high soil pH, in calcareous soils and in medium soil. The iron deficiency in crops appears as pale yellow interveinal chlorosis on younger leaves at the base of the plant. Phosphorus levels in soils lead to decreased iron levels in soil. The iron solubilizes and its translocation in crop improves by decreased Phosphorus values (upendra *et al*., 2003). Since Spirulina platensis contains high phosphorous and iron dynamics is through root system there is no increase in the iron content by spray method.

Micronutrient deficiency affects more than one-half of the world’s population, especially women and preschool children (SCN 2004). Reaching the Millennium Development Goals to reduce the under-5 child mortality ratio by two-thirds and the maternal mortality ratio by three-quarters between 1990 and 2015 will require additional technologies and approaches to improving nutritional status, which is an important determinant of these mortalities. Biofortification of food crops is a new public health approach to control vitamin A, iron, and zinc deficiencies in poor countries. The biofortified crop system is highly sustainable. Biofortification provides a feasible means of reaching undernourished populations in relatively remote rural areas, delivering naturally fortified foods to people with limited access to commercially marketed fortified foods that are more readily available in urban areas (Haines, 2004). The study clearly indicates that the biofortification of Amaranthus plant with *Spirulina platensis* increased the iron content in the plant in all the treated plants, however the bioavailable studies require to ascertain the results.

CONCLUSIONS

Spirulina platensis treated plants have shown increase in iron content when compared to the control and reference value. The present study reveals that the combination of Spirulina and biofertilizer has maximum effect in increasing the iron nutrient status in the Amaranthus plant. Thus it is concluded that *Spirulina platensis* which is a blue green algae can be helpful in agriculture as an enhancer of plant growth as it has the capability to promote mineral content in terms of iron dynamics to the plants. However further bioavailable and molecular studies are needed to substantiate the results.

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APPENDICES

Table 2: Iron Mean ± S.D of the Samples of Treated and Untreated Plants of Different Soaking Periods

S. No	Sample Code	Control (Mean±S.D)	Time Period Soaking	Iron mg/100g (Mean ± S.D)	P Value	Cor Relation
1	A1	A7 0.67±5.66	1h	4.76±0.01	0.00	1.00
2	A2		2h	18.35±0.03	0.00*	-1.00
3	A3		3h	6.46±0.02	0.00	-1.00
4	A4		4h	3.44±0.02	0.01	-1.00
5	A5		5h	6.02±0.04	0.01	-1.00
6	A6		OVERNIGHT	7.39±0.23	0.02	-1.00

Table 3: Iron Content of Treated and Untreated Plants of Soaking in Different Concentrations of *Spirulina*

S. No	Sample Code	Control (Mean±S.D)	Soaking in Different Concentration	Iron mg/100g (Mean ± S.D)	P Value	Cor Relation
1	A8	A14 2.35±0.03	5g	17.03±0.98	0.03*	1.00
2	A9		10g	6.0±0.71	0.06*	1.00
3	A10		15g	3.77±0.16	0.04	1.00
4	A11		20g	17.70±0.59	0.02*	-1.00
5	A12		25g	2.49±0.13	0.30	1.00
6	A13		30g	20.88±0.48	0.01*	-1.00

Table 4: Iron Content of Different Ratios of *Spirulina* vs Bio-Fertilizer Treated Plants

S. No	Sample Code	Control (Mean±S.D)	Bio Fertilizer	Iron mg/100g (Mean ± S.D)	P Value	Cor Relation
1	A15	A18 17.92±0.06	S:B(25:75)	31.80±2.17	0.07	-1.00
2	A16		S:B(50:50)	21.65±0.66	0.09	-1.00
3	A17		S:B(75:25)	44.85±0.78	0.01*	1.00

*****(S:B) Spirulina: Biofertilizer

Table 5: Iron Content of Different Ratios of *Spirulina* vs Vermicompost Treated Plants

S. No	Sample Code	Control (Mean±S.D)	Vermi-compost	Iron mg/100g (Mean ± S.D)	P Value	Cor Relation
1	A19	A22 2.35±0.32	S:V(25:75)	43.99±0.77	0.01*	-1.00
2	A20		S:V(50:50)	2.76±.0.01	0.31	1.00
3	A21		S:V(75:25)	2.35±0.03	0.28	1.00

*****(S:V) Spirulina: Vermin compost

Table 6: Iron Content of Different Ratios of *Spirulina* vs Organic Matter Treated Plants

S. No	Sample Code	Control (Mean±S.D)	Organic Manure	Iron mg/100g (Mean ± S.D)	P Value	Cor Relation
1	A23	A26 2.43±0.16	S:O(25:75)	4.1±0.70	0.22	-1.00
2	A24		S:O(50:50)	4.2±0.21	0.09	-1.00
3	A25		S:O(75:25)	3.39±0.22	0.17	-1.00

*****(S:B)Spirulina: Organic matter

Table 7: Iron Content of Different Ratios of *Spirulina* vs Chemical Fertilizer Treated Plants

S. No	Sample Code	Control (Mean±S.D)	Chemical Fertilizer	Iron mg/100g (Mean ± S.D)	P Value	Cor Relation
1	A27	A30 4.36±0.54	S:C(25:75)	4.59±0.53	0.03	1.00
2	A28		S:C(50:50)	15.5±7.28	0.02*	1.00
3	A29		S:C(75:25)	4.36±3.85	0.01*	1.00

*****(S: B) Spirulina: Chemical fertilizer

Table 8: Iron Content of Treated and Untreated Plants of Spray Method with *Spirulina*

S. No	Sample Code	Control (Mean±S.D)	Spray Method	Iron Mg/100g (Mean ± S.D)	P Value	Cor Relation
1	A31	A35 17.16±0.03	S:W(25:5L)	4.14.±0.36	0.01*	1.00
2	A32		S:W(50:5L)	3.23±0.01	0.00*	-1.00
3	A33		S:W(75:5L)	2.78±0.04	0.00*	-1.00
4	A34		S:W(100:5L)	3.01±0.60	0.02*	1.00

***(**S:B)Spirulina: Water in different ratios with distilled water