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# Effect of Supplementation of *Spirulina platensis* to Enhance the Zinc Status in Plants of *Amaranthus gangeticus*, *Phaseolus aureus* and Tomato

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## Abstract

**Trace elements or micronutrients play a major role in the metabolism of both plants and humans. Zinc has a major specific role in metabolism when compared with other elements. The microbial biofertilizers are applied in the form of seaweed liquid extracts, microbial inoculants, biostimulators and biofortification agents. All these categories of microbial biofertilizers are involved in the enhancement of plant nutrient uptake. In the present study, *Spirulina platensis* is used as a biofortification agent to enhance zinc levels in cultivars of *Amaranthus gangeticus*, *Phaseolus aureus* and Tomato. Different experimental methods were followed including soaking seeds in different concentrations of *Spirulina* (5, 10, 15, 20, 25 and 30 g in 100 ml of water); soaking seeds in *Spirulina* hydrolysate at different time intervals (1, 2, 3, 4, 5 hrs and overnight); *Spirulina* in combination with biofertilizers, chemical fertilizer, organic fertilizer and vermicompost in various proportions (25:75; 50:50; 75:25) and foliar spray with different concentrations of *Spirulina* (25, 50, 75, and 100g in 5 litres of water) respectively. The zinc content of the yield of the cultivars was estimated and the study results indicated that there was a significant increase in zinc levels with p-value 0.015, 0.003 and 0.035 for *Amaranthus gangeticus*, *Phaseolus aureus* and Tomato respectively when compared with the control and between the set-ups, with biofortification of *Spirulina platensis.* The obtained results emphasize the application of *Spirulina platensis* to enhance the mineral nutrient in plants which are non-polluting, inexpensive, utilizable renewable resource to maintain the soil fertility.**

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## Keywords

**Zinc, Biofortification, Biofertilizers, *Spirulina platensis***

## 1. Introduction

Plants are autotrophic organisms that can synthesize the essential components for their growth and development but require macro and micro nutrients from external source to carry out their physiological activities and metabolism effectively. The unavailability of macro or micro elements may obstruct the plants' growth and development [1]. The macro and micro elements are very essential elements in plant metabolism as they are involved in all major physiological activities like cellular organization, protein and nucleic acid metabolisms etc. Among the micro nutrients, Zinc is a vital nutrient required for plants, animals and humans. Zinc performs specific roles in plant metabolism in cellular organization, antioxidative defense, protein synthesis, carbohydrate metabolism, auxin metabolism and genetic stability [2]. Thus the micro nutrient Zinc plays an important role in plants and its deficiency may cause stress in plants and decrease nutritional quality in food crops [3].

The yield of agricultural crops depends on balanced nutrition. Micro elements' distribution within plants influences the growth and development of plant. The trace element Zinc is directly involved in hormone regulation and pigment synthesis in plants. The influence of various concentrations of Zinc uptake by plants has been studied by many researchers [4]. Zinc deficiency in food crops is widespread and almost 50% of productive agricultural soils are deficient in Zinc [5] and about 50% of world human population is also Zinc deficient [6].

The methods of application of zinc have a great influence on yield and grain zinc concentration. Soil and seed application of zinc improves the shoot growth. In case of immobility of zinc in soil, the zinc fertilizers can be directly sprayed on to the leaves of growing crops [7]. The foliar application of zinc found to fill the grains directly resulting in high grain zinc concentration. The soil + foliar application results in much more grain yield and high grain zinc concentrations [8].

Several types of zinc fertilizers are available in the form of chelated zinc that is relatively mobile in the soil. The inorganic fertilizers like zinc oxides, sulphate and nitrates are widely used but are highly expensive [9]. The low cost biofertilizers had shown good effects in increasing the zinc concentration in soil and crops. It has been suggested that the integrated use of organic and inorganic fertilizers hugely benefits the sustainable agriculture in the form of obtaining great yields and good quality grains [10]. To enhance the Zinc levels in the foods biofortification is the potential approach. Enrichment of Zinc in seeds of wheat, pulses and vegetables is helpful in meeting the Zinc requirement. Biofortification is done through effective fertilization and selection of crops that have potential to efficient absorption of Zinc from soil and ability to translocate the Zinc to various plant parts.

Around 30% of the world's human population has diets deficient in zinc. Zinc deficiency in humans affects physical growth, the functioning of the immune system, reproductive health and neuro behavioral development. Therefore the zinc content of foods is of major importance. There is a rapidly developing field of research on the biofortification of plant foods with zinc. This involves both the breeding of new varieties of crops with the genetic potential to accumulate a high density of zinc in cereal grains (genetic biofortification) and the use of zinc fertilizers to increase zinc density (agronomic biofortification). Although the plant breeding route is likely to be the most cost-efficient approach in the long run, for the time being, the use of fertilizers is necessary to improve the zinc density in diets while the plant breeding programmes are being carried out. Hence, in addition to ensuring that crop yields are not restricted by deficiency, zinc fertilizers will also be used, where necessary, to increase the zinc density of foods. However, it will be necessary to monitor both the zinc concentrations in the cultivars and also the soil to ensure that the enrichment of the foods occurs without the accumulation of zinc in soils to possibly harmful levels [10]. In this context in the present study *Spirulina platensis*, Blue green algae which is nutrient has been used as agronomy biofortification agent to enhance the mineral nutrient status in selected plants.

Zn fertilizers increase both the yield and quality of several crops, including wheat [9] [11], rice [12], and peas [13]. Proper management of Zn fertilization can increase the concentrations of Zn in plants edible parts. Low Zn in plant tissues is a reflection of both genetic- and soil-related factors. A basic knowledge of the dynamics of Zn in soils, understanding of the uptake and transport of Zn in plant systems and characterizing the response of plants to Zn deficiency are essential steps in achieving sustainable solutions to the problem of Zn deficiency in plants and humans [10]. This is of paramount importance for adequate levels of this nutrient in the human diet. Most of the Blue green algalization research till recent times has focused on rice and hence there is scanty research on other cultivars. In this context the present research of biofortification of *Spirulina platensis* will give an input to the Agriculture and Nutrition fraternity to establish potential benefit of using blue green algae to enhance the zinc status in plants.

## 2. Methodology

In the modern era, importance was given to diet and nutrition which focuses on the role of macro and micro nutrients and their metabolisms. The nutrient analysis of *Spirulina* done earlier in the laboratory and in pot studies conducted on *Amaranthus gangeticus* using *Spirulina* as a Biofortification agent has given sufficient encouragement to conduct investigation at field level. Field experiment was started by land selection. Land was selected at Sabbavaram village and the land is constituted of light clay soil. Total area of the selected land was 1 acre.

The field experiment was carried out using *Amaranthus gangeticus*, *Phaseolus aureus* and Tomato plants which comes under GLV, whole pulse and vegetable category. Also the yield of these crops can be harvested within 60 - 90 days for *Amaranthus gangeticus* and *Phaseolus aureus* and 100 - 120 days for Tomato. The seeds of *Amaranthus*, *Phaseolus aureus* and Tomato were procured from local market. Local Variety of *Amaranthus gangeticus* seeds, *Phaseolus aureus* variety ML-267 and Tomato variety-PKM1 were procured.

The total land was divided into eight blocks of width- 4356 Square Feet (SFT) and randomized block design was followed. The experimental design for the present study contains seven set ups (**Table 1**) comprising of three plants *i.e. Amaranthus gangeticus*, *Phaseolus aureus* and Tomato. The first set up comprised of 6 variations of the seed coating by *Spirulina* hydrolysate for different time intervals. The second set up consists of 6 variations of the seed coating in different concentrations of *Spirulina* and water. The setups from three to six comprises of using *Spirulina* as Bio-fortification agent in combination with different fertilizers which are mostly used by the farmer community. The combinations were 25:75, 50:50, and 75:25 of *Spirulina* vs vermicompost, Organic, Chemical, and Bio-fertilizer respectively. The last method was spray method. Test Controls without *Spirulina* were maintained for all the set ups. The experiment was carryout with duplicate set ups. For each experimental set up, 100 numbers of seeds of each variety of plant were sown and the germination percentage was calculated.

*Amaranthus gangeticus*, *Phaseolus aureus* and Tomato cultivar samples yield were taken, dried and powdered. Zinc estimation was carried out by Atomic Absorption Spectrophotometer (AAS) at regional agricultural research station, Anakapalle, Visakhapatnam District, Andhra Pradesh, India working under ANGRAU (Acharya N.G. Ranga Agricultural University, Hyderabad).

## 3. Results and Discussion

Many plant species are affected by zinc deficiency on a wide range of soil types in most agricultural regions of the world. The major staple cereal crops: rice, wheat and maize are all affected by zinc deficiency, together with many different fruit, vegetable and other types of crops. The soil conditions which most commonly give rise to

**Table 1.** Field experimental set-ups for *Amaranthus gangeticus*, *Phaseolus aureus* and Tomato.

| S No | Name of set up | Variations | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1. | Time period soaking (5 g of *Spirulina* in 100 ml of sterile water) (w/v) | 1 h | 2 h | 3 h | 4 h | 5 h | Over night | *Control |
| 2. | Seed soaking in different concentrations (g/100 ml sterile water) (w/v) | 5 g | 10 g | 15 g | 20 g | 25 g | 30 g | C |
| 3. | *Spirulina* + Biofertilizer (S:B) (w/w) | 25:75 | 50:50 | 75:25 | - | - | - | C |
| 4. | *Spirulina* + Vermicompost (S:B) (w/w) | 25:75 | 50:50 | 75:25 | - | - | - | C |
| 5. | *Spirulina* + Organic matter (S:B) (w/w) | 25:75 | 50:50 | 75:25 | - | - | - | C |
| 6. | *Spirulina* + Chemical fertilizer (S:B) (w/w) | 25:75 | 50:50 | 75:25 | - | - | - | C |
| 7. | Spray method (g/L of water) | 25/5 | 50/5 | 75/5 | 100/5 | - | - | C |

deficiencies of zinc in crops can include one or more of the factors: low total zinc concentrations (such as sandy soils); low pH, highly weathered parent materials with low total zinc contents (e.g. tropical soils); high calcium carbonate content (calcareous soils); neutral or alkaline pH (as in heavily limed soils or calcareous soils); high salt concentrations (saline soils); peat and muck (organic soils); high phosphate status; prolonged waterlogging or flooding (paddy rice soils); high magnesium and/or bicarbonate concentrations in soils or irrigation water. India is a country with wide spread zinc deficiency problems and soils with one or more of the above mentioned factors [10].

*Spirulina* has been used as a biofortification agent to enhance micronutrient status in *Amaranthus gangeticus*, *Phaseolus aureus* and Tomato plants. Significant effect of *Spirulina* was evident in all three plants from **Table 2** though with some variations. *Spirulina* increased the zinc level in three plants which was distinct when compared with control. In *Amaranthus gangeticus*, the combination of *Spirulina* and biofertilizer in the ratio of 75:25 had shown the zinc levels highest when compared with all the set-ups and cultivars *i.e.*, 77.23 ± 0.02 ppm. The difference between the variations in the set-ups and between the set-ups is statistically significant with p-value 0.015 at 5% level. This indicates that the difference is due to effect of biofortification of *Spirulina*. The *Spirulina* + biofertilizer combination used for the supplementation of nutrients in the crops had shown the positive effect. The mutual association between the plant roots and the *Spirulina* + biofertilizer combination had induced high amounts of phytosiderophores into the soil. These phytosiderophores uptake the chelated zinc ions from soil and transport them to the plants. Thus, increase in the zinc levels at 75:25 proportions can be attributed to the phytosiderophores concentration that transported zinc efficiently. The low levels of zinc at other proportions might not sufficiently stimulate the suitable phytosiderophore concentration in the soil [14].

The zinc uptake by *Phaseolus aureus* (**Table 2**) was observed high in *Spirulina* + organic manure in 50:50 proportions (54.4 ± 1.69 ppm). The difference between the variations in the set-ups and between the set-ups is statistically significant with p-value 0.003 at 5% level. This indicates that the difference is due to effect of biofortification of *Spirulina*. The increase in zinc value can be attributed to the increase in the iron content in *Phaseolus aureus* plants [15]. 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 [16]. 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. The interactions between soil phosphorus, copper and zinc had shown the effect on zinc uptake by soybeans (*Glycine max*). The microbial intervention and application of fertilizers enrich the crops with sufficient zinc levels [17].

The increase in zinc values were observed in experimental treatment of soaking of seeds at 2 hours of time period (5.28 ± 0.09 ppm) in Tomato cultivar (**Table 2**) which is statistically significant at 5% level of p-value of 0.003. It has been suggested that zinc influences the auxin levels in the crops. The auxins promote the shoot growth and nutrient uptake by the plants. The increase in the auxin levels and shoot length can be attributed to increase in the zinc levels. *Spirulina* rich in zinc initiated the Tomato seeds to uptake the zinc there by promoting the auxin levels in the plants and resulting in overall growth [18].

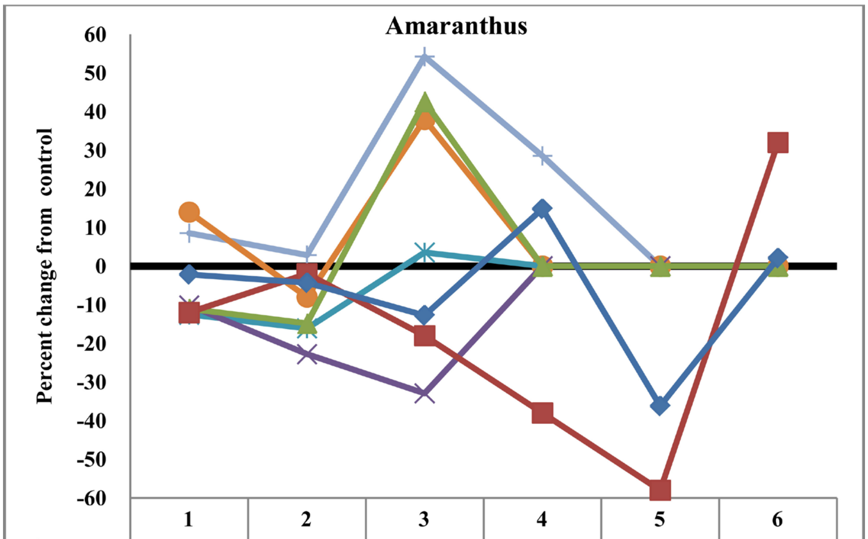
It is evident from **Table 2** and **Figures 1(a)-(c)** and **Figures 2(a)-(c)**, that the zinc levels are enhanced in all three plants. The zinc levels were increased in all experimental treatments with some variations, than the test controls and with NIN standard values (National Institute of Nutrition, 1980). This may be due to high levels of zinc present in *Spirulina* as well as the fertilizers that had shown the mutualistic action to increase the nutrient status. From **Figure 1(a)** it is evident that both positive and negative percent change has been observed in the zinc status in *Amaranthus gangeticus* when the setups were compared with their respective controls. In the figure the spray method has shown the positive change which is according to results of the previous study [19] [20]. In *Phaseolus aureous* the trend is more towards positive percent change in the experimental variations of all setups and as the concentration of *Spirulina* increased there is an increase in zinc status of the yield. The results are according to the studies done by Liu Shiming and Liang, Shizong (1998) [21], and El-Nahas and Abd El-Azeem (1999) [22] on pulses (**Figure 1(b)**). The same trends of results are obtained for Tomato cultivar also (**Figure 1(c)**).

When the zinc status was compared with the standard values of NIN (*Amaranthus gangeticus*—1.8 ppm, *Phaseolus aureus*—30 ppm and Tomato—4.1 ppm) a 10 - 20 fold positive percent change was observed in *Amaranthus gangeticus* (**Figure 2(a)**). In *Phaseolus aureus* and Tomato plants both positive and negative percent change from reference value can be observed (**Figure 2(a)** and **Figure 2(c)**). Though there are variations in the percent change it can be observed that mostly as there is an increase in *Spirulina* concentration in application methods the zinc level in the yield is increased. This increase might be attributed to the combined effect of light clay soil and *Spirulina* where the zinc can be holded with in the soil and made available to plant and since leaf tissue is the storage part of the plant [10]. However bioavailability studies, form of zinc, and transportation studies at

**Table 2.** Effect of *Spirulina* supplementation on zinc content (Mean ± SD ppm) of *Amaranthus gangeticus*, *Phaseolus aureus* and Tomato plants.

| S. NO | TREATMENTS | *AMARANTHUS* | GREEN GRAM | TOMATO |
|---|---|---|---|---|
| TIME PERIOD SOAKING | 1 h | 45.0 ± 0.64 | 42.80 ± 0.12 | 4.932 ± 0.06 |
| | 2 h | 44.56 ± 0.02 | 43.84 ± 0.71 | **5.28 ± 0.09** |
| | 3 h | 40.99 ± 1.08 | **50.82 ± 0.69** | 4.14 ± 0.03 |
| | 4 h | **54.48 ± 0.71** | 50.15 ± 0.74 | 4.95 ± 0.77 |
| | 5 h | 30.17 ± 0.07 | 46.70 ± 1.69 | 2.21 ± 0.31 |
| | Over Night | 48.54 ± 1.71 | 46.84 ± 0.67 | 3.0 ± 0.14 |
| | Control | 46.98 ± 2.31 | 42.75 ± 0.70 | 3.75 ± 0.70 |
| SOAKING IN DIFFERENT CONCENTRATIONS | 5 g | 44.00 ± 2.82 | 34.35 ± 3.39 | 3.06 ± 0.01 |
| | 10 g | 48.75 ± 0.33 | **40.03 ± 0.52** | 3.75 ± 0.21 |
| | 15 g | 40.85 ± 0.74 | 37.26 ± 2.12 | 3.30 ± 0.28 |
| | 20 g | 30.67 ± 2.17 | 37.63 ± 0.04 | **4.55 ± 0.03** |
| | 25 g | 21.19 ± 1.35 | 37.24 ± 2.09 | 4.05 ± 0.04 |
| | 30 g | **66.36 ± 0.50** | 34.83 ± 1.34 | 3.12 ± 0.04 |
| | Control | 50.11 ± 0.54 | 30.29 ± 0.71 | 3.23 ± 0.02 |
| BIOFERTILIZER (S:B*) | (25:75) | 47.65 ± 0.72 | 31.64 ± 1.70 | 3.3 ± 0.28 |
| | (50:50) | 45.82 ± 1.18 | 27.43 ± 2.80 | 2.8 ± 0.14 |
| | (75:25) | **77.23 ± 0.02** | **43..66 ± 1.25** | **3.58 ± 0.01** |
| | Control | 54.4 ± 0.07 | 35.47 ± 0.02 | 3.57 ± 0.01 |
| VERMICOMPOST (S:V*) | (25:75) | **71.25 ± 0.77** | **41.46 ± 0.66** | 2.8 ± 0.14 |
| | (50:50) | 60.51 ± 1.61 | 37 ± 0.33 | **4.35 ± 0.63** |
| | (75:25) | 53.0 ± 3.59 | 39.78 ± 0.79 | 3.00 ± 0.56 |
| | Control | 50.94 ± 0.05 | 41.37 ± 0.01 | 3.26 ± 0.01 |
| ORGANIC MANURE (S:O*) | (25:75) | 49.36 ± 0.77 | 27.14 ± 0.68 | 2.74 ± 0.30 |
| | (50:50) | 47.34 ± 0.61 | **54.4 ± 1.69** | 2.34 ± 0.31 |
| | (75:25) | **58.36 ± 2.17** | 38.90 ± 1.40 | 2.56 ± 0.07 |
| | Control | 55.95 ± 0.04 | 45.73 ± 0.01 | **2.76 ± 0.01** |
| CHEMICAL FERTILIZER (S:C*) | (25:75) | 57.36 ± 2.27 | 37.73 ± 1.66 | 2.33 ± 0.16 |
| | (50:50) | 45.82 ± 4.47 | 28.33 ± 1.42 | 2.59 ± 0.11 |
| | (75:25) | **69.29 ± 0.67** | **43.72 ± 1.44** | **4.44 ± 0.31** |
| | Control | 50.24 ± 0.01 | 33.2 ± 1.55 | 3.77 ± 0.02 |
| SPRAY METHOD (S/W*) | (25/5L) | 37.9 ± 0.14 | 38.35 ± 2.15 | 2.23 ± 0.02 |
| | (50/5L) | 36.32 ± 0.00 | 30.28 ± 0.02 | **3.71 ± 0.05** |
| | (75/5L) | **54.08 ± 2.07** | **40.98 ± 0.01** | 2.19 ± 0.07 |
| | (100/5L) | 44.6 ± 1.35 | 30.38 ± 0.02 | 2.34 ± 0.04 |
| | Control | 34.70 ± 0.02 | 29.87 ± 0.60 | 2.16 ± 0.02 |
| p-value | | **0.015** | **0.003** | **0.035** |

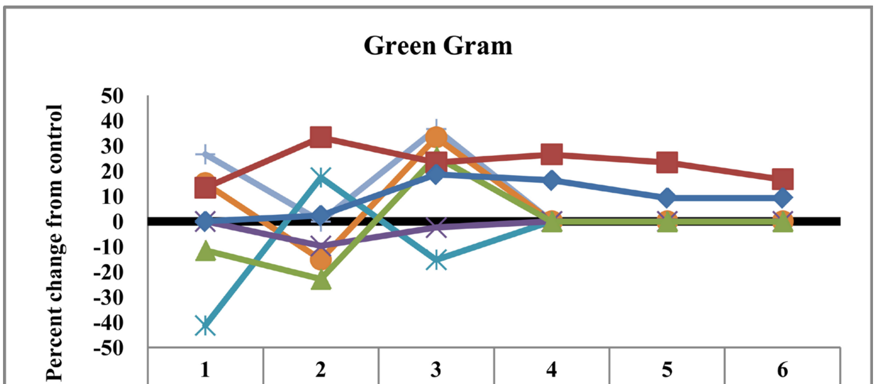
*S:B—*Spirulina*:Biofertilizer; S:V—*Spirulina*:Vermicompost; S:O—*Spirulina*:Organic Manure; S:C—*Spirulina*:Chemical Fertilizer; S/W—*Spirulina*/Water.




| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| G | 8.571 | 2.8571 | 54.28 | 28.57 | | |
| F | 14 | -8 | 38 | | | |
| E | -12.5 | -16.07 | 3.571 | | | |
| D | -10.12 | -22.78 | -32.91 | | | |
| C | -11 | -14.81 | 42.59 | | | |
| B | -12 | -2 | -18 | -38 | -58 | 32 |
| A | -2.127 | -4.255 | -12.765 | 14.893 | -36.17 | 2.127 |

A—Time period soaking, B—Soaking in different concentrations of *Spirulina*, C—*Spirulina*:Biofertilizer, D—*Spirulina*: Vermicompost, E—*Spirulina*:Organic Manure, F—*Spirulina*:Chemical Fertilizer, G—*Spirulina*/Water.

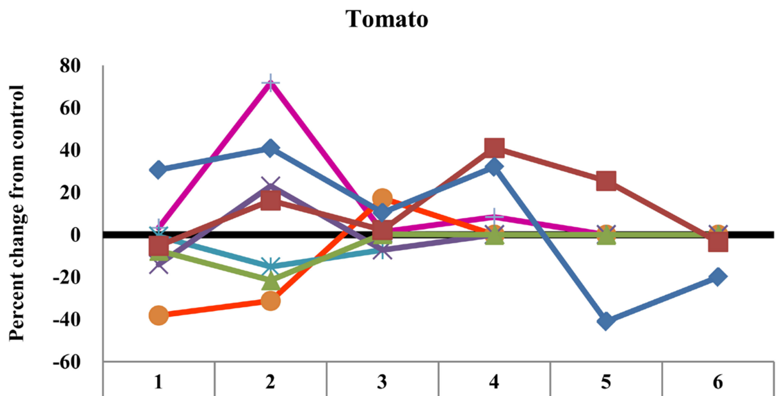
(a)




| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| G | 26.66 | 0 | 36.66 | 0 | | |
| F | 15.15 | -15.15 | 33.33 | | | |
| E | -41.3 | 17.39 | -15.21 | | | |
| D | 0 | -9.75 | -2.439 | | | |
| C | -11.42 | -22.85 | 25.71 | | | |
| B | 13.33 | 33.33 | 23.33 | 26.55 | 23.33 | 16.66 |
| A | 0 | 2.32 | 18.6 | 16.27 | 9.3 | 9.3 |

A—Time period soaking, B—Soaking in different concentrations of *Spirulina*, C—*Spirulina*:Biofertilizer, D—*Spirulina*: Vermicompost, E—*Spirulina*:Organic Manure, F—*Spirulina*:Chemical Fertilizer, G—*Spirulina*/Water.

(b)

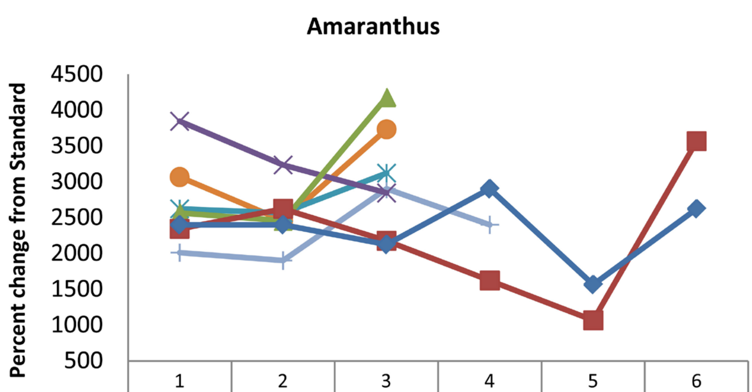



| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| G | 3.24 | 71.75 | 1.38 | 8.33 | | |
| F | -38.19 | -31.29 | 17.17 | | | |
| E | -0.72 | -15.21 | -7.24 | | | |
| D | -14.11 | 23 | -7.2 | | | |
| C | -7.56 | -21.56 | 0.28 | | | |
| B | -5.26 | 16.09 | 2.167 | 40.86 | 25.38 | -3.4 |
| A | 30.6 | 40.8 | 10.4 | 32 | -41 | -20 |

A—Time period soaking, B—Soaking in different concentrations of *Spirulina*, C—*Spirulina*:Biofertilizer, D—*Spirulina*: Vermicompost, E—*Spirulina*:Organic Manure, F—*Spirulina*:Chemical Fertilizer, G—*Spirulina*/Water.

(c)

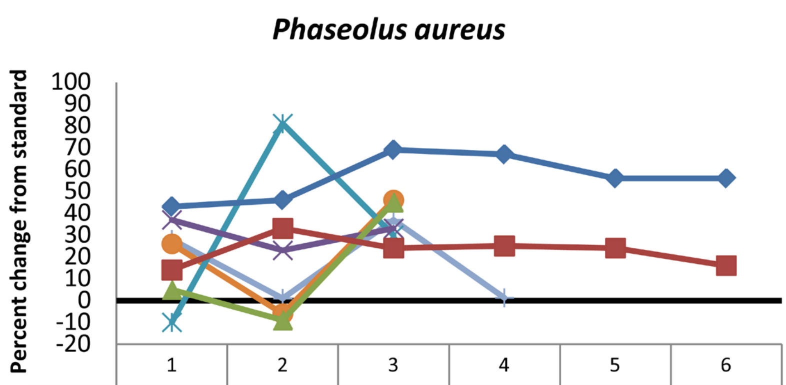
**Figure 1.** (a) Effect of *Spirulina* on zinc status of *Amaranthus* yield when compared with control; (b) Effect of *Spirulina* on zinc status of *Phaseolus aureus* yield when compared with control; (c) Effect of *Spirulina* on zinc status of Tomato yield when compared with control.




| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| G | 2011 | 1900 | 2900 | 2400 | | |
| F | 3066 | 2455 | 3733 | | | |
| E | 2622 | 2566 | 3122 | | | |
| D | 3844 | 3233 | 2844 | | | |
| C | 2566 | 2455 | 4177 | | | |
| B | 2344 | 2622 | 2177 | 1622 | 1066 | 3566 |
| A | 2400 | 2400 | 2122 | 2900 | 1566 | 2622 |

A—Time period soaking, B—Soaking in different concentrations of *Spirulina*, C—*Spirulina*:Biofertilizer, D—*Spirulina*: Vermicompost, E—*Spirulina*:Organic Manure, F—*Spirulina*:Chemical Fertilizer, G—*Spirulina*/Water.

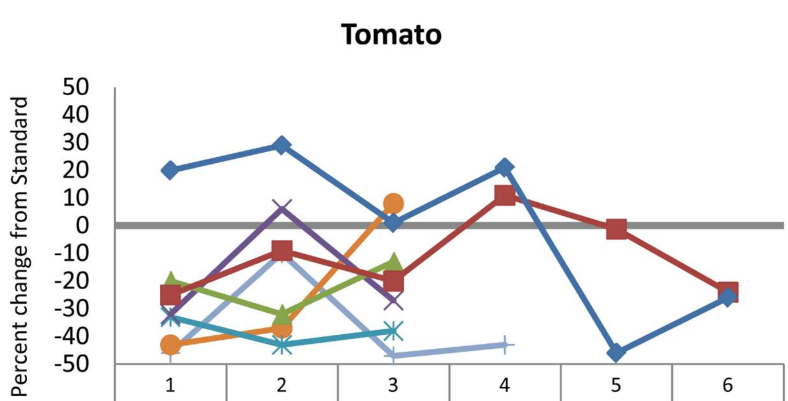
(a)




| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| G | 28 | 0.93 | 37 | 1.26 | | |
| F | 26 | -6 | 46 | | | |
| E | -10 | 81 | 30 | | | |
| D | 37 | 23 | 33 | | | |
| C | 5 | -9 | 45 | | | |
| B | 14 | 33 | 24 | 25 | 24 | 16 |
| A | 43 | 46 | 69 | 67 | 56 | 56 |

A—Time period soaking, B—Soaking in different concentrations of *Spirulina*, C—*Spirulina*:Biofertilizer, D—*Spirulina*: Vermicompost, E—*Spirulina*:Organic Manure, F—*Spirulina*:Chemical Fertilizer, G—*Spirulina*/Water.

(b)




| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| G | -46 | -10 | -47 | -43 | | |
| F | -43 | -37 | 8 | | | |
| E | -33 | -43 | -38 | | | |
| D | -32 | 6 | -27 | | | |
| C | -20 | -32 | -13 | | | |
| B | -25 | -9 | -20 | 11 | -1.2 | -24 |
| A | 20 | 29 | 1 | 21 | -46 | -26 |

A—Time period soaking, B—Soaking in different concentrations of *Spirulina*, C—*Spirulina*:Biofertilizer, D—*Spirulina*: Vermicompost, E—*Spirulina*:Organic Manure, F—*Spirulina*:Chemical Fertilizer, G—*Spirulina*/Water.

(c)

**Figure 2.** (a) Effect of *Spirulina* on zinc status of *Amaranthus* yield when compared with standard; (b) Effect of *Spirulina* on zinc status of *Phaseolus aureus* yield when compared with standard; (c) Effect of *Spirulina* on zinc status of Tomato yield when compared with standard.

molecular level are further to be investigated to substantiate the results. Kiekens (1995) [23] stated that there appeared to be two different mechanisms involved in the adsorption of zinc by clays and organic matter. One mechanism operates mainly in acid conditions and is closely related to cation exchange, and the other mechanism operates in alkaline conditions and mainly involves chemisorption and complexation by organic ligands.

The increase in zinc levels may be due to increase in phytosiderophore concentrations in the soil, crosstalk between iron and zinc uptake mechanisms by the crops and the increase in the auxin levels [9]. The phytosiderophores not only influence the mobility of Zinc but also promotes the translocation of Zinc from root to shoot of the plant. The molecular manipulation of phytosiderophores biosynthesis and release by inefficient crops to improve their efficiency was observed in case of barley and durum wheat [24].

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. The deficiency of zinc is the fifth most important factor to be addressed in women and children and it is an alarming situation for developing countries. To address the problem, a more beneficial approach, biofortification of food to enhance the mineral nutrient content in staple food crops has been suggested [25]. The degree of zinc deficiency accumulated and hence make evident that zinc fortification has been jointly recommended by WHO and FAO [26]. Application of zinc fertilizers in the form of organic manures or vermicompost or biofertilizers or inorganic chemical fertilizers is found to be useful and quick approach for improving the zinc concentration in the food crops [9].

From the last 10 - 15 years a rapid progress has been conducted in determining the molecular mechanisms of metal transport across cell membranes, in order to assess the translocation and uptake of the $Zn^{2+}$ mechanisms in the plant species. The genetic and the molecular techniques are applied in a wide range of gene families in order to identify the heavy metals transported in the plants [27]. The *Arabidopsis thaliana* is the first plant in which a Zn transporter gene sequences were identified and belongs to the family ZIPI-4 are uninfluenced and down regulated by Zn fertilization [28] [29].

The molecular mechanisms of zinc uptake by plants have not been elaborately understood but it was thought that the increased secretions of phytosiderophores enable the plants to uptake the zinc. Several ZIP proteins have been reported from many monocot plants suggesting that this protein family is involved in the zinc uptake by crops [1]. Transgenic plants can be produced by inducing the ZIP proteins into the crops to sustain in low zinc soils. The gene expression control for zinc transport from soil to plant is still unknown but it is suggested that transformation or over expression of zinc transporter proteins may enhance the zinc uptake by plants [1].

A new zinc transporter protein expressing gene (NAC gene) was recently identified from wheat crops that accelerates senescence and improves the zinc mobilization from leaves to grains [30]. It has been reported that there is a possibility of cross-talk between iron and zinc transport pathways. This can be known from over expression of iron reductases and iron transporters in transgenic or mutant plants had shown increased zinc levels [16].

Zinc is responsible for membrane integrity, synthesis of cytochromes, nucleotides, chlorophyll and auxin. Zinc is integral part of many enzymes such as Carbonic Anhydrase (CA), alcohol dehydrogenase, carboxy peptidase [31]. So, zinc has a major role in the formation of leaf area and leaf weight because zinc is involved in the metabolism of acting as a coenzyme with the above mentioned enzymes. The mechanism of stomatal response to high Zn concentration, seem to be related to changes in CA activity, also the stomatal opening is also influenced by the Zn, as it is a possible constituent of CA [19]. Zinc is the part of auxin synthesis thus mediates the leaf formation. With these positive effects, zinc exhibits a significant impact on the morphology and physiology of a plant [32]. The facts that have been explained by researchers in different studies might explain the high zinc content in the present study.

High phosphorous levels in soil result in reduced zinc levels. However in the present study though the phosphorus levels are high and towards alkaline pH of soil, the zinc status has been increased in the yield of the cultivars. The possible mechanism has to be substantiated with further studies. The zinc levels are low in saline soils. High zinc levels in soils could be toxic to crops [33]. In some cases the water with solubilized phosphorus or organic manure with low zinc levels can reduce the zinc toxicity on crops [34]. The zinc concentrations varied from 0.023 to 0.04 g/day in dry grains of 16 different mung bean lines [35] and increase in Zinc content in seeds of common bean by 50% was observed [36].

## 4. Conclusion

In the present study, the zinc levels were found to be increased after supplementation of *Spirulina* in different combinations and application methods. The increase was observed when compared with control as well as NIN standard values. The positive and negative percent change in zinc nutrient status which was evident from the present study in the cultivars of *Amaranthus gangeticus*, *Phaseolus aureus* and Tomato can be attributed due to the biofortification of *Spirulina platensis*. The results are statistically significant at 0.015, 0.003 and 0.035 for *Amaranthus gangeticus*, *Phaseolus aureus* and Tomato cultivars respectively. Further studies have to be carried out at molecular level to establish the zinc transport mechanism and bioavailability with the positive results from present study.

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