Indian J. Dryland Agric. Res. & Dev. 2014 29(2) : 104-107 DOI 10.5958/2231-6701.2014.01224.X

Improving Phytochemical and Nutritional Quality of Spinach (*Spinacia oleracea)* through Phosphate Solubilizing Bacteria

K. Sreedevi Shankar[1], K. Suma[1], K. Bhuvana Chary[1], A. Prem kumar[1], Minakshi Grover[1], Shankar Mekala[2], Maruthi Sankar[1], M. Vanaja[1] and K.L. Sharma[1]

[1]Central Research Institute for Dryland Agriculture, Hyderabad-500 059, Telangana
[2]Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad-500 030, Telangana

Email: sreedevikobaku@gmail.com

ABSTRACT: A field study was conducted to find out the effect of phosphate solubilizing bacteria (PSB) on physico-chemical quality of organically grown spinach. Spinach is low in calories and is a good source of vitamin C, vitamin A, and minerals like iron. In order to deliver enhanced nutrition within a food-based system, it is necessary to increase the nutritional value of the food. By enhancing nutrient dense crops, severe deficiencies can be eliminated in developing countries. Hence, one of the ways by which this goal could be achieved is through natural method, possibly with organic farming and biofortification. The treatments tested in the study were: farmyard manure (FYM), phosphate solubilizing bacteria **(**PSB), FYM + PSB, FYM + PSB + Citrate, 100% recommended dosage of fertilizer (RDF) and no chemical. After 6 weeks of cultivation, spinach crop was harvested and the crop cut data were analysed to assess the effect of treatments on physical and phytochemical parameters *viz.,* yield weight, vitamin C, β-carotene, minerals and antinutritional factors. The results of analysis of variance (ANOVA) showed that, the treatments were significant on phyto-chemical parameters. However, the treatments FYM, FYM + PSB and FYM + PSB + Citrate fared better compared to 100% RDF.

Key words: Spinach, biofortication, PSB, minerals, vitamins, hidden hunger

Spinach (Spinacia *oleracea)* is a power packed green leafy vegetable, inexpensive and easily accessible source of vegetable with highly essential micronutrients. The leaves are bright green in colour, lustrous, fleshy and its broader leaves with tender stems are highly accepted by the varied groups of population. The crop can be harvested 6-7 times, with application of nitrogen after each harvest. Application of phosphate solubilizing bacteria can help in reducing the input of chemical fertilizer as well as in maintaining better soil health. Bacteria belonging to *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Aereobacter, Flavobacterium* and *Erwinia* are reported as efficient phosphate solubilizers. Microbial strains with multiple plant growth promoting properties are always beneficial as compared to strains with single plant growth promoting trait. Mineral malnutrition also known as hidden hunger presents a significant global challenge. In addition to the traditional interventions of supplementation and fortification of foods, several agronomic options are available to increase the food minerals. The ultimate solution is a dietary diversification, but this is not immediately practical. Therefore, biofortification of edible crops is advocated through either mineral fertilization and or plant breeding. Both these strategies could increase mineral concentrations in edible portions and improve yields of crops grown on infertile soils. However, biofortication might provide a more sustainable and cost-effective solution in the long run, delivering minerals and vitamins to the entire population. Keeping in view of immense need to enable increased mineral and vitamin concentrations in edible portions of crop plants without affecting the yield or quality, a pilot study has been taken up with an objective to address the micronutrient malnutrition in the rural community and the findings are discussed in this paper.

Materials and Methods

A field experiment was conducted at Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad. For the present study, certified seeds of spinach were obtained from Agricultural Research Institute, Prof. Jaya Shankar Telangana Agricultural University, Hyderabad. The present biofortification study was carried out, to analyse with standard methods to compare the nutrient profile minerals (Dhyan Singh, 2005), vitamin C (Dichlorophenol-Indophenol visual titration method, (Anonymus, 1966)), total carotenes and β-carotene (Zakaria *et al.,*1979), tannins (Sadasivam and Manickam, 2004), oxalates and phytic acid (Wheeler and Ferrel, 1971). The physical characteristics of organically grown spinach crop were also recorded.

The main biofortificant inoculated to the crop is phosphate solubilizing bacteria (PSB) and also farmyard manure (FYM) was used. The bacterial strain PSB-1 was chosen for this study based on its ability to solubilize inorganic forms of phosphate (Ca_3 $(PO_4)_2$) and zinc (ZnO and $ZnCO_3$) and to produce siderophores (Fe^{3+} chelating compounds) *in vitro* and also to promote traits like IAA production, antagonism against plant pathogens, hydrogen cyanide and ammonia production *in vitro* (Richardson, 1994., Dey, 1988., Rodriguez, 1999 and Benarjee *et al.,* 2010).

Spinach crop is grown in northern India as kharif (June-July) as well as rabi crop (September-October) while in southern India, during October-December. This crop can also be grown in cool hilly areas during April-June. The variety of spinach crop selected for the study was all green, seed rate was 10-12 kgs/ acre, spacing 20 × 10 × 4 cm and fertilizer dosage was as per recommendations of ANGRAU, Vyavasaya Panchangam, 2012.

The experimental design used was RBD with six different treatments in 3 replications each. The plot size was 2 m × 3 m. The treatments tested in the study were: T_1 = farmyard manure alone, T_2 = combination of farmyard manure and phosphate solubilizing bacteria (FYM + PSB), T_3 = combination of farmyard manure, phosphate solubilizing bacteria and citrate (FYM + PSB + C), T_4 = phosphate solubilizing bacteria alone (PSB), T_5 = no chemicals and T_6 = 100% recommended dose of fertilizers (100% RDF). Application of organic manure (FYM), biofortificant (PSB) and chemical fertilizers in addition to seed sowing of spinach was taken up as per recommendations of ANGRAU [Vyavasaya Panchangam, 2012]. The phosphate solubilizing bacterial strain PSB-1 used in the present investigation was isolated from arid ecosystem. The samples were analysed in triplicates and results obtained were on dry weight basis. Analysis of variance (ANOVA) test was used as suggested by Snedecor and Cochran (1989).

Results and Discussion

The data on the effects of treatments on different quality parameters *viz.,* Zinc, Iron, Calcium, Magnesium, vitamin-C, total carotenes, β-carotenes, and antinutrients such as, phytates, tannins and oxalates were collected and analysed. The results are presented in Tables 1-3 and Figures 1-3.

Effect of treatments on physical parameters

The results of the study showed that spinach crop grown with farmyard manure had significantly well developed shoot and root systems compared to other treatments. The total produce of each treatment obtained from the field was significantly higher in PSB inoculated plots when compared to other treatment plots. On individual basis, the produce was significantly higher in FYM + PSB + Citrate treated plots. This is because the phosphate solubilizing microbes have the potential to increase the availability of soluble phosphate and to enhance the plant growth by improving biological nitrogen fixation (Kucey *et al.,* 1989).

Table 1 : Effect of PSB on produce of biofortified spinach crop in different treatments

Treatment	Fresh weights (kg) (Mean ± S D)
FYM	1.27 ± 0.23
FYM + PSB	1.96 ± 0.15
FYM + PSB + Citrate	2.09 ± 0.07
PSB	1.53 ± 0.53
No chemical	0.75 ± 0.25
RDF	1.54 ± 0.14
CD (P=0.05)	0.48

Effect of treatments on phyto-chemical (nutrient) parameters

Based on the results of ANOVA test, the zinc content in spinach leaves was significantly higher at 1% probability level in PSB alone (1.38 mg/100g) treatment, followed by FYM + PSB + Citrate treated plot (1.32 mg/100g), compared to other treatments. While the results of analysis indicated that spinach leaves had significantly higher iron content in FYM + PSB plot (6.76 mg/100g), followed by FYM treated plot (4.03/mg/100g), compared to control i.e., RDF plot (3.33 mg/100g) (Figure 1).

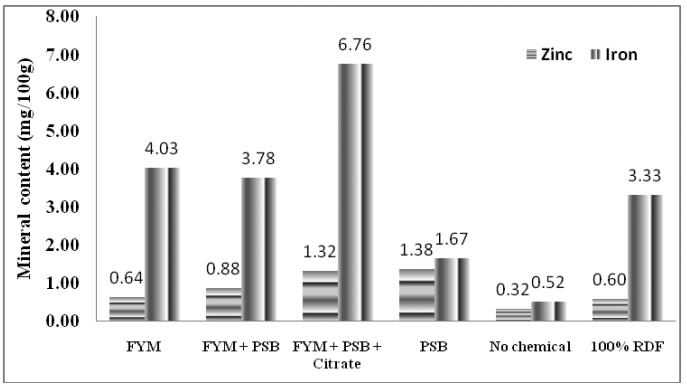

Fig. 1 : Zinc and iron content of biofortified spinach leaves grown under different treatments

The calcium content in leaves of biospinach, grown with FYM + PSB + Citrate (4.08 mg/100g) was significantly higher at 1% probability level compared to 100% RDF, FYM alone, and PSB alone treatments. FYM treated spinach crop was significantly higher (5% probability level) in calcium compared to 100% RDF was also reported by Sreedevi *et al.* (2008). PSB alone treated spinach crop was also significantly higher at 1% probability level in calcium content compared to 100% RDF. Magnesium content of biofortified spinach leaves was observed to be significantly higher in FYM, FYM + PSB, FYM + PSB + Citrate and PSB alone treated crop compared to 100% RDF. FYM + PSB + Citrate treatment was significantly higher at 5% probability level compared to PSB grown spinach (Figure 2).

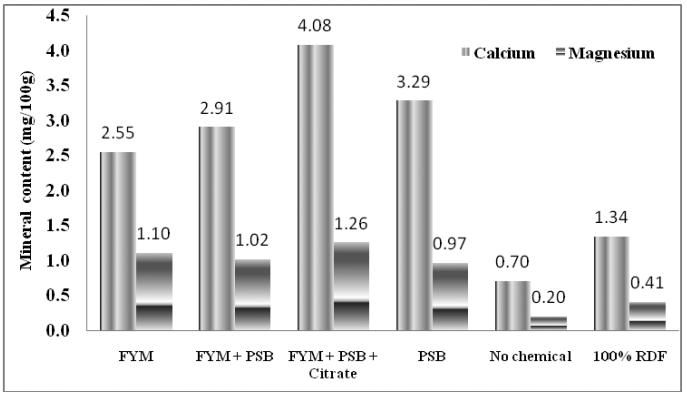

Fig. 2 : Calcium and magnesium content of biofortified spinach leaves grown under different treatments

The Vitamin-C values ranged from 8 mg/100g (under no chemical treatment) to 16 mg/100g (under FYM). Further, it was observed that the increase in Vitamin C over control ranged from 66.67% under FYM + PSB + Citrate treatment to 100% under FYM treatment. Based on the results of ANOVA test, it was found that both organic and inorganic fertilizer treatments had a significant effect on the values of Vitamin C. The results showed that total carotenes were significantly higher (15.81%) in spinach leaves under FYM plot compared to 100% RDF treatment (Figure 3).

The total carotenes content was found to be 15.83% higher under FYM treatment compared to RDF. Based on ANOVA, the organic and inorganic fertilizer treatments had a significant effect on the values of total carotenes and these results are in confirmation with the findings reported by Sreedevi *et al.* (2011) with organic treatment. The results also indicated that the content of total carotenes was significantly higher in the FYM sole treated spinach leaves compared to the plots treated with FYM + PSB, FYM + PSB + Citrate, PSB, No chemical and 100% RDF (Figure 3).

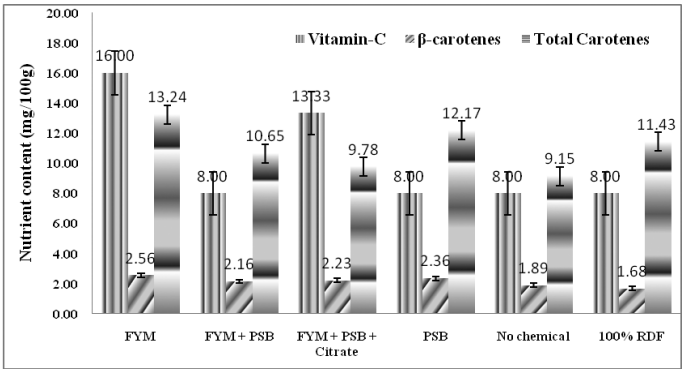

Fig. 3 : Phytochemical content of biofortified spinach leaves grown under different treatments

Effect of treatments on antinutritional parameters

Table 2 showed that the effect of FYM, FYM + PSB and FYM + PSB + Citrate treatments had significantly lower on phytate content in spinach leaves compared to non-chemical treatment. The phytate content in FYM, FYM + PSB and FYM + PSB + Citrate treatments were 117.38 mg/100g, 130.13 mg/100g and 133.13 mg/100g respectively, compared to 100% RDF (159 mg/100g).

Table 2 : Phytates content in biofortified spinach leaves of different treatments

Treatment	Phytates (mg/100g) (Mean ± SD)
FYM	117.375* ± 09.113
FYM + PSB	130.125* ± 45.929
FYM + PSB + Citrate	133.125* ± 30.847
PSB	160.500 ± 12.609
No chemical	206.250 ± 05.123
RDF	159.000 ± 18.289
CD (P =0.05)	55.532*

Tannin content was also found to be significantly lower in FYM + PSB (33.75 mg/100g) treatment compared to 100% RDF (70.00 mg/100g) and the tannin values ranged from 33.75 mg/100g to 58.75 mg/100g in organic treatments (Table 3). The content of oxalates was found to be lower and non-significant with PSB application.

Table 3 : Tannins content in spinach leaves of different treatments

Treatment	Tannins (mg/100g) (Mean ± SD)
FYM	56.250 ± 13.149
FYM + PSB	33.750 ± 02.500*
FYM + PSB + Citrate	58.750 ± 02.500
PSB	87.500 ± 18.484
No chemical	62.500 ± 08.660
RDF	70.000 ± 05.773
CD (P= 0.05)	23.126

Conclusions

Malnutrition in the rural communities is of major concern and can be addressed through application of PSB. Results have clearly shown that, application of PSB not only improves yield but also nutritional quality. The application of PSB in combination with FYM treatments is recommended which helps in lowing phytates, tannins and oxalates content in spinach leaves.

References

Anonymus. 1966. Methods of vitamin assay, the association of vitamin chemists, inter science publishers, New York, Third edition. 287p.

Banerjee S, Palit R, Sengupta C and Standing D. 2010. Stress induced phosphate solubilization by Arthrobacter sp. and Bacillus sp. isolated from tomato rhizosphere. Australian J. Crop Sciences, (6):378-383 ISSN: 1835-2707.

Dey KB. 1988. Phosphate solubilizing organisms in improving fertility status. In: Sen SP, Palit P, editors. Biofertilizers: Potentialities and Problems. Calcutta: Plant Physiology Forum, Naya Prokash, pp 237-48.

Dhyan Singh, Chhonkar PK, and Pandey R N. 2005. Manual on soil, plant and water analysis. IARI, New Delhi.

Kucey. RMN, Janzen H H and Logget ME.1989. Microbial mediated increases in plant available phosphorous. Adv. Agron, 42:199-228.

Richardson AE. 1994. Soil microorganisms and phosphorus availability. In: Pankhurst CE, Doube BM, Grupta VVSR, Grace PR, editors. Soil Biota, Management in Sustainable Farming Systems. Melbourne, Australia: CSIRO, 1994, pp 50-62.

Rodriguez H, and Fraga R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv, 17:319-339.

Sadasivam and Manickam. 2004. Biochemical methods, TNAU, New age international (P) Limited, Publishers, New Delhi.

Snedecor WG and Cochran WG. 1989. Statistical Methods. Iowa State University press. Ames, Iowa, U.S.A.

Sreedevi Shankar K, Sumathi S and Shankar M. 2008.Minerals and Microbiological Quality of organically and conventionally grown vegetables. Indian J. Dryland Agric. Res. & Dev., 23(1): 87-95.

Sreedevi Shankar K, Anjani Devi Ch and Ramachandra CSV. 2011. Phytoremediation of Heavy metals with Amaranthus dubius in Semi-arid soils of Patancheru, Andhra Pradesh. Indian Journal of Dryland Agriculture and Development, 26 (2): 71-76.

Vyavasaya panchangam. 2012. Acharya N.G. Ranga Agricultural University, University press, Hyderabad.

Wheeler EL and Ferrel RE. 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chem, 48: 312-320.

Zakaria M, Simpson K, Brown PR and Kostulovic A. 1979. Use of reversed phase HPLC analysis for the determination of provitamin A carotenes in tomatoes. Journal of Chromatography, 176: 109.

Received: October 2014; Accepted: December 2014