Journal of Natural Sciences
December 2015, Vol. 3, No. 2, pp. 17-32
ISSN 2334-2943 (Print) 2334-2951 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/jns.v3n2a2
URL: http://dx.doi.org/10.15640/jns.v3n2a2

Quality Enhancement Studies of Growth and Nutrient Content in *Phaseolus aureus, Spinacia oleracea and Ocimum sanctum*using Local Bio-Fertilizers

Dr. K.Vijaya Rachel[1], Dr.P. Kiranmayi[2] & G. V. D. Sirisha[3]

Abstract

A pot culture experiment was conducted on *Phaseolus aureus, Spinacia oleracea and Ocimum sanctum*to study the effect of two bio-fertilizers viz, and Annapurna and Navajeevan.Annapurna contains *Azotobacter, Azospirillum*, Neem, Phosphate solubilizing bacteria, Castor and *Trichoderma*. Navajeevan contains bio-extract, organic fertilizer, sea-weed amino acids. The endophytic nature of Azospirillumholds promise, as nitrogen fixed by these bacteria is more available to the plant than in the rhizosphere associations.Production of hormones by Azotobacter enhances growth. Trichoderma and Phosphate Solubilizing Bacteria solubilize phosphates, micronutrients, increases the number of deeproots, increasing the plant's ability to resistdrought.It also helps in bioremediation of soil from xenobiotics. The ability of microorganisms to fix atmospheric nitrogen and transform native soil nutrients like phosphorus, zinc, copper, iron and sulfur from the non-usable to usable form and decompose organic wastes which can be easily assimilatedresulted in enhanced growth and metabolism.The atomic absorption spectrum of the plant tissues at different time intervals exhibited an increase of micronutrients viz Zn, Fe, Mn and Cu. This study concludes that Annapurna bio-fertilizer enhanced germination, plant growth, shoot biomass and mineral nutrient content, food quality and sustaining environmental quality from the ecological and agricultural perspectives.

Keywords: Bio-fertilizer; *Phaseolus aureus; Spinacia oleracea; Ocimum sanctum*; Biochemical content; mineral nutrients.

[1] Assistant Professor, Department of Biochemistry& Bioinformatics, Institute of Science,GITAM University,Visakhapatnam - 530 045, India. Email ID: rachelrkumar@yahoo.co.in
[2] Assistant Professor, Department of Biotechnology, Institute of Science, GITAM University, Visakhapatnam - 530 045, India. Email ID: kiranmayi.patnala@gmail.com
[3] Research Scholar, Department of Biochemistry/Bioinformatics, Institute of Science, GITAM University, Visakhapatnam-530045, India. Email ID: gvd.divya@gmail.com

1. Introduction

Plants are the source of raw materials for the food, clothing and shelter.These green plants have also provided all the medicaments and health care needs to man and his domestic animals ever since the advent of civilization. India is one of the ancient countries in the world growing wide range of pulse crops as prime source of protein. Further, India is the leading country in pulse cultivation area and contributes 25, 27% of the world production and consumption respectively but also the largest importer of pulses with the contribution of 34% of the global food use (FAOSTAT, 2008).India shares 70% of the total world black gram and green gram (*Vignaradiata*) production (FAOSTAT, 2008, Elzebroek et al., 2008)

Over the last four decades the country is struggling to increase the area, production and most importantly the productivity, despite rapid growth in cereals (Ministry of Agriculture, 2008). Since the beginning of green revolution, greater importance was given to cereals which led to rapid growth in production and productivity of cereals and almost quite reverse in pulse crops. As a result, the national average pulse productivity (638 kg haG1) declined far below than the global average of 857 kg haG1. Consequently, per capita availability of pulses declined from 60.7 to 29.4 g dayG1 during 1951 to 2007 (Ministry of Agriculture, 2008)against world health organization (WHO) recommended level of 80 g dayG1 (FAO, 2007). International Plant Nutrition Institute has confirmed that Indian soils are under nutrient crisis and also concluded that in the absence of nutrient recycling "it is likely that the nutrient balance sheet of Indian agriculture will continue to be negative" (Tamil Nadu Agricultural University, 2009).This situation urges the need to improve soil nutrients and pulse productivity.

Kalmegh, Ashwagandha and Tulsi are important medicinal plants mentioned in ancient Ayurvedic literature. These medicinal plants are used in various drugs in curing fever to acute jaundice. The irregular and low germination is the main problem in the propagation of many medicinal plants (Koppad et al., 2006). This can be corrected and enhanced by application of bio-fertilizers.Bio-fertilizers application in medicinal plants production in sustainable agriculture with aim of remove or reduce the chemical input in order to reach to quality increasing and sustainability of yield is very important(Sharma et al., 2002). Usage of correct nutrition by bio-fertilizers, will obtain quantitative and qualitative yield maximum from medicinal plants such as basil.

Green vegetables are a major source of iron and calcium for any diet. Green vegetables are rich in vitamins, and also improve immune function. They are useful in reducing the risk of cancer and heart disease since they are low in fat, high in dietary fiber, and rich in folic acid, vitamin C, potassium and magnesium, as well as containing a host of phytochemicals, such as lutein, β-cryptoxanthin, zeaxanthin and β-carotene.

Growthimprovement and quality improvement of green vegetables is very necessary because it is used for the treatment of many diseases. To avoid the residual toxicity of chemical fertilizers, it is always advisable to raise these vegetables through organic cultivation practices.

The use of bio-fertilizers has been reported to be beneficial for the cultivation of vegetable and cereals by many workers(Kapoor et al., 2004, Mehrotra et al., 1971, Mehrotra et al., 1979, Venkateswarlu et al., 1983, Hadas et al., 1987). The amounts of active principles are depending on total biomass yield which is further depends on the climatic feature, method of agro-techniques, water management and also fertilizer applications. So yield improvement can be achieved by standardizing the agronomy especially with respect to those parameters.

The use of organic amendments and its practices improves soil structure, physical properties and especially its water holding capacity (Abiven et al., 2009) which could be the great asset in tropics and for its sustainability. The present investigation was undertaken to study the effect of commercial bio-fertilizers on micronutrient content, morphological and biochemical parameters in three divergent groups of plants belonging to pulses, vegetable and medicinal categories.

2. Materials and Methods

2.1 Selected Bio-fertilizers and Plants

Two commercial Bio-fertilizersvia Annapurna and Navajeevan were used.
Bio-fertilizers-Annapurna: Contains *Azotobacter*, *Azospirillum*, Neem, phosphate solubilizing bacteria, castor and *Trichoderma*.

Bio-fertilizers-Navajeevan: Contains bio extract, organic fertilizer, and sea weed amino acids.

Three different plants were selected for the present study. Those are

1) A pulse plant-*Phaseolus aureus*
2) A leafy vegetable-*Spinaciaoleracea* and
3) A medicinal plant-*Ocimum sanctum*

2.2 Soil amendments: Earthen pots, 20-cm diameter and 30-cm depth, were filled with 5 kg soil. Soil was amended either with Annapurna bio-fertilizer or Navajeevan fertilizers. The soil mixture was prepared by combining the commercial bio-fertilizer and soil in a ratio of 1:1. Both the fertilizers were mixed 3days prior to sowing. All pots were irrigated with tap water and left for organic material decomposition.

2.3 Treatments and experimental design: There were three treatments, with three replicates, for each of the three soil amendment systems. These were

i) Control-soil without bio-fertilizer
ii) Soil with Annapurna bio-fertilizer and
iii) Soil with Navajeevan bio-fertilizer.

Pots were arranged on a bench in a wire-netting greenhouse under natural environmental conditions.

2.4 Harvesting: Observations were recorded after 1day, 15 days, 30 days, 45 days and 60days after sowing. Morphological parameters such as shoot length, root length and total leaf area were calculated. The number of seeds germinated in each concentration was counted on 7thday and the germination percentage was calculated by using the following formula

$$\text{Germination Percentage} = \frac{\text{No. of seeds germinated}}{\text{Total no. Of seeds sown}} \times 100$$

The leaf area was calculated by measuring the length and breadth of the leaf as described below

Leaf area (cm^2) = K × length × breadth
Where, K = Kemp's constant (for dicot leaves = 0.66).

2.5 Estimation of Protein:

Protein content was determined by the method of Lowry *et al.,* (Lowry et al., 1951) 0.5 g of plant sample (shoot) was homogenized in 10 ml of 0.1M phosphate buffer and the homogenate was centrifuged at 3000rpm for 10 minutes. 0.5 ml of the supernatant was taken in a test tube and volume is made upto 1ml with distilled water. Then 5 ml of reagent 'C' (protein reagent) was added.

This solution was mixed well and kept in dark for 10 minutes. Later, 0.5 ml of Folin- ciocalteau reagent was added and the mixture was kept in dark for 30 minutes. The sample was read at 660 nm in the colorimeter.

2.6 Estimation of Total Sugar Content:

The total sugar content was determined by the Anthrone reagent method (Hodge et al., 1962). 100mg of the sample was weighed and hydrolyzed by keeping in boiling water bath for 3 hours with 5ml of 2.5N HCl and cooled to room temperature. Later it was neutralized with solid sodium carbonate until the effervescence ceased. The volume was made upto 100ml and centrifuged. 0.5 and 1ml aliquots of the supernatant were taken for analysis.

The anthrone reagent was prepared right before analysis by dissolving 0.2 g of anthrone (0.2%) in concentrated sulfuric acid, protected from light and used within 12 h. Anthrone reagent (4.0 ml) was added cautiously to each tube containing 1.0 ml of standard solutions of glucose (10 – 100 µg/ml) and test solution. Tubes were then placed at 5^0C for 10 min. Subsequently, tubes were boiled for 5 min on constant boiling water bath. After heating, the tubes were allowed to cool at room temperature for 15 min. Later absorbance was measured at 620 nm in colorimeter against reagent blank.

2.7 Estimation of fat:

Fat content was determined by chloroform-methanol method. 0.2gm of sample was weighed into screw capped tubes. 10ml of chloroform-methanol (2:1) was added and vortex for 3mins (extracts the fat into organic layer). The tubes were kept in a cold room overnight. The organic layer is taken into another empty weighed beaker. The beakers are incubated at 60°C, overnight and weighed after incubation. Fat content was estimated using the difference in the weight of the beaker before and after solvent extraction.

2.8 Nitrogen Analysis:

Total Nitrogen in plants is estimated by the Kjeldahl method (Jackson, 1956,). In plants, N is present in protein form, and digestion of the sample was carried out with H_2SO_4 containing digestion mixture (10 parts potassium sulphate and 1 part copper sulphate). Sample size of 0.5-1.0 g depending on the type of crop and the plant part was taken. The procedure involves sample digestion, distillation and estimation of Nitrogen.

2.9 Analytical Methods

2.9.1 Dry Ashing

High-temperature oxidation destroys the organic matter. The plant sample is ashed at 500–600 °C by placing 3.0 g of the sample in a silica crucible and heated in a muffle furnace for 4–6 hours. The ash residue was dissolved in dilute HCl, filtered through acid-washed filter paper, and the volume is made up to 100 ml in a volumetric flask. The estimation of micronutrients was carried out in the dry-ashed sample solution by Atomic Absorption Spectrophotometer (AAS GBC-932plus). Dry ashing is a preferred method for the analysis of Fe, Zn, Cu, Mn and other trace elements.

2.9.2 Polyacrylamide Gel Electrophoresis (PAGE):

The PAGE gel was carried out according to the modified method of Laemmli (Laemmli, 1970). This discontinuous gel system used a 15.0 cm x 14.0 cm gel containing 12% separating gel and 4% stacking gel. 20% (v/v) of sample buffer (0.5 M Tris-HCl pH 6.8, 20% (v/v) glycerol, 0.05% (w/v) bromophenol blue was added to the leaf extracts.

A total volume of 20µl protein extract solution was loaded into each well and electrophoresis was carried out at 100V until the bromophenol blue dye reaches the bottom of the gel. After the electrophoretic separation, the gel was stained with Coomassie brilliant blue and bands are observed.

3. Results and Discussion

In this investigation two commercial bio-fertilizerswere used to see the effect on the morphological parameters of growth and yield of green gram, spinach and tulsi plants. The results so obtained are described as under. From 10 randomly selected plants average data has been obtained. The treated plants have been found to have highest increase in all the parameters, than that with the control. Plants treated with Annapurna bio-fertilizer have been shown to have fastest growth rate as compared to other treatment and control plants.

3.1. Germination: The germination and seedling parameters of plants tested were furnished in Table 1. There were significant differences in germination per cent.

Table 1: Germination Percentage (%)

S. No.	Name of the plant	Soil	Soil + Annapurna	Soil + Navajeevan
1.	Green gram	60.15%	71.72%	70.38%
2.	Spinach	70.1%	80.16%	70.38%
3.	Basil	50.34%	60.13%	60.21%

The Spinach recorded higher germination percentage (80.16) followed by green gram (71.72 %) and the least was in tulsi (60.13 %) in Annapurna treated plants. Germination in Navajeevan treated plants showed that Spinach and green gram were on par with each other followed by tulsi. Significant differences were noticed in germination due to bio-fertilizers.

The germination per cent did not vary much due to the interaction between medicinal plants and bio-fertilizers. However, in tulsi Navajeevan treated soil (60.21%) recorded germination on par with Annapurna (60.13%) and the least was in control (50.34%).

The probable reason is the relative enhancement of germination might be attributed to the role of phosphorous solubilizing bacteria; N_2 and Azospirillum enhance the metabolic activity in germinating seeds thus, resulted in early and higher seed germination.This may be due to its better adaptation to acidic soil conditions and higher N fixing as well as phytohormone producing ability. Hence, it will be advantageous to use this type of bio-fertilizer for large-scale production of pulses and vegetables. Present investigation findings are in conformity with results of Vijayakumari,Janardhananin *Ceibapentandra*(VijayaKumari and Janardhanan, 2003)and Koppad,Umarbhadshain *Ocimum sanctum* (Koppad and Umarbhadsha, 2006a).

3.2. Shoot and Root length: The shoot and root lengths were measured at 15 days' time intervals for green gram, spinach and tulsi and are presented in tables 2 and 3. A gradual increase in these growth parameters was observed.

Table 2: Shoot length after sowing seeds at an interval of 15 days (in cm)

Sample	1 day	15 days	30days	45 days	60 days
Soil green gram	0	3.1	6.2	12.2	20
Soil + Annapurna green gram	0	5.2	8.5	15	25
Soil + Navajeevan green gram	0	3.5	6.5	13	21
Soil spinach	0	4	8.9	15.6	25
Soil + Annapurna spinach	0	5.3	9	17	31
Soil + Navajeevan spinach	0	4	8.4	17	28
Soil Basil	0	1.8	8.2	15	23.5
Soil + Annapurna Basil	0	3	8.5	16	28
Soil + Navajeevan Basil	0	2	8.2	15.7	25

The higher shoot and root lengths were recorded in Annapurna soil mixture of the three plants. Spinach recorded higher shoot length of 31.26 cm followed by tulsi(28.1cm) and then green gram(25.2cm).These results are corroborate with present findings of Ponnuswamy (Ponnuswamy, 1993) and Vanagamudi*et al.,* in Neem(Vanangamudi et al., 1993).

Table 3: Root length after sowing seeds at an interval of 15 days (in cm)

Sample	1 day	15 days	30days	45 days	60 days
Soil green gram	0	1.5	2.2	5.7	8.0
Soil + Annapurna green gram	0	1.9	2.2	6.3	8.2
Soil + Navajeevan green gram	0	1.7	2.3	7.0	8.3
Soil spinach	0	1.2	1.5	2.0	2.5
Soil + Annapurna spinach	0	1.5	1.8	2.1	2.9
Soil + Navajeevan spinach	0	1.5	1.7	2.1	2.7
Soil Basil	0	2.5	5.1	7.2	8.5
Soil + Annapurna Basil	0	2.8	5.7	7.6	9.0
Soil + Navajeevan Basil	0	2.8	5.5	7.1	8.5

There was significant difference in root length of Annapurna treated Tulsi (9.1cms) when compared to other treatments. Treatments *viz*., Navajeevan treated soil and control are on par with each other (8.2cms). The improvement of root length due to combined bio-fertilizer inoculation in tree legumes (Delacruzet al., 1988) is in agreement with the results of the present study. The same findings were reported due to effluent treatment in green gram by Kumar and Bhargava (Kumar and Bhargava, 1998). The root length did not show any significant difference among the plants. The application of bio-fertilizers had no significant variation in all the treatments.

3.3. Leaf area: The leaf area was measured at 15 days' time interval for green gram, spinach and tulsi, parameters was represented in Table 4.

Table 4: Leaf area after sowing seeds at an interval of 15 days (in cm^2)

Sample	1day	15 days	30 days	45 days	60 days
Soil green gram	0	0.99	2.97	3.3	4.62
Soil + Annapurna green gram	0	1.32	3.15	4.35	5.77
Soil + Navajeevan green gram	0	1.18	2.64	.3.96	4.95
Soil spinach	0	1.32	10.56	27.72	36.96
Soil + Annapurna spinach	0	3.66	19.8	36.96	52.8
Soil + Navajeevan spinach	0	3.66	16.5	32.34	47.52
Soil Basil	0	0	0.99	1.32	4.62
Soil + Annapurna Basil	0	0	1.98	3.96	5.28
Soil + Navajeevan Basil	0	0	1.32	2.64	4.62

Annapurna fertilizer treated plants have been found to have highest leaf size. The average increase in leaf size than that of control in case of spinach plants is found to be equal to 42.8 %, whereas average increase in case of tulsi plants having treatment (Annapurna)have been shown to increase by50%.

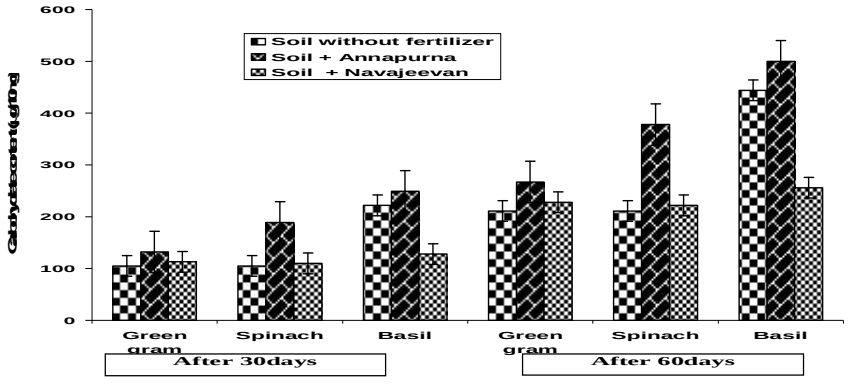
An increase of 24.9% was observed in green gram. Greater increase in leaf area increases the chances of yield of active principles in case of medicinal plants and a close increase in spinach gives better yield of foliage.

The increase in shoot length, root length, and total leaf area were recorded in Annapurnabio-fertilizer soil mixture. It may be the presence of microorganisms like phosphate solubilizing bacteria, fungal inoculants in the bio-fertilizer. Soil organism tends to increase the oxygen concentration. The soil becomes looser and opens the pores of the soil causing more aeration and increasing the growth of the plant.

3.4. Biochemical parameters: The plants grown in Annapurna bio-fertilizer treated soil recorded higher protein yield compared to other individual treatments. This result emphasizes N_2 nutrition for increased protein yield (Dhage et al., 1984). There was an increase of 54.8% protein in 60d old green gram plants as against the control.

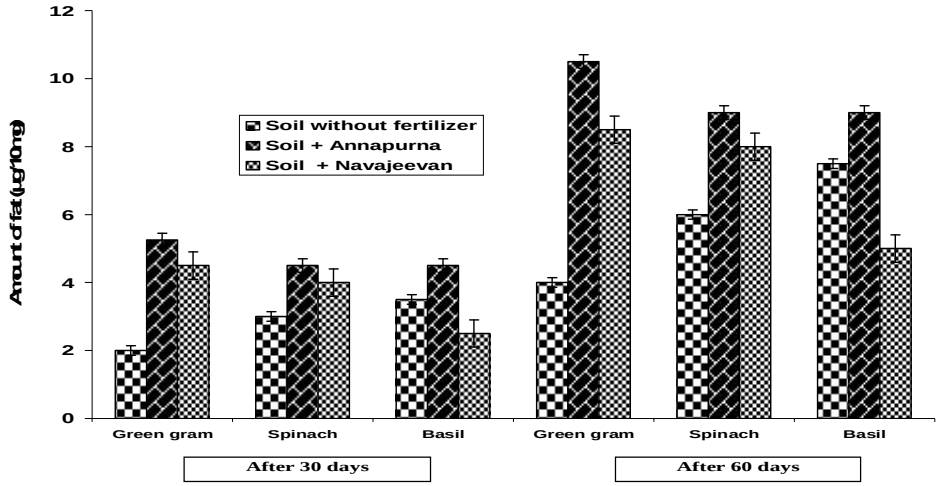
The highest yield could be of P and N respectively andattributed to enhance supply of N and P, production of phytohormones and better portioning of dry matter into the economic (Dhage et al., 1984, Rudresh et al., 2005). Selvakumar*et al.,*(Selvakumar et al., 2009) concluded that N and P nutrition is vital and the trend of variation in protein content was similar to that of N and P. Similar reports of increase in yield attributes, grain yield, total plant dry matter weight (TDM) and protein content were reported when bio- fertilizers and organic manures applied together(Selvakumar et al., 2009, Uyanöz, 2007, Gomma, 2007). Similar results were obtained for carbohydrate and fat analysis as shown in Figure1 & 2.

Figure 1: Effect of fertilizer on carbohydrate content



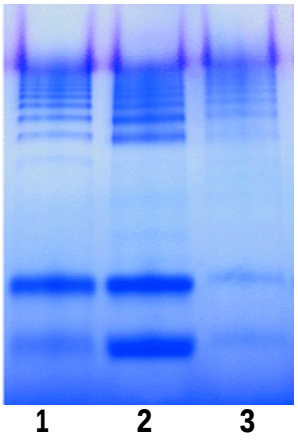
Carbohydrate content was estimated using Anthrone method after 30 days and 60 days of sowing seeds.

Figure 2: Effect of fertilizer on fat content



As shown in Fig 3 it is clearly evident that the Annapurna bio- fertilizer is enhancing the protein content in green gram when compared with soil without fertilizer.

Figure 3: Polyacrylamide gel electrophoresis

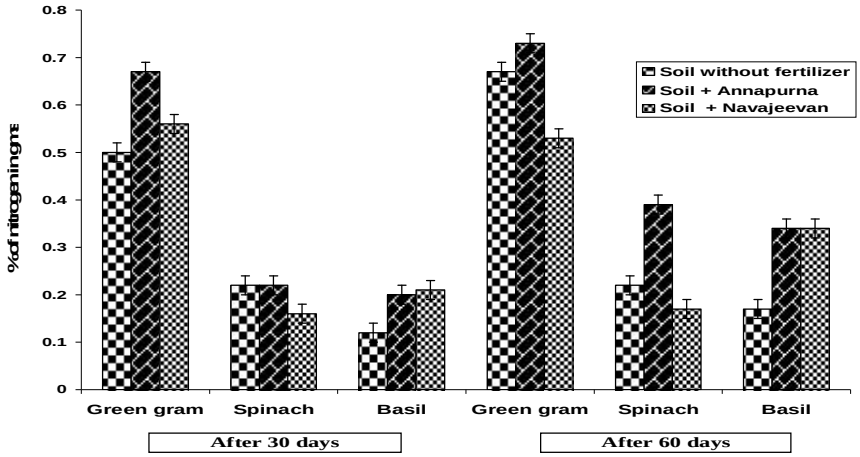


1. Navajeevan green gram 2. Annapurna green gram 3. Soil green gram

Even on protein profiling, the result showed that Annapurna samples have high protein content. The results (Fig 4) showed that the amount of Nitrogen content increased irrespective of treatments.

The magnitude of such changes, however; varied with treatments, being highest Nitrogen content (7.3 g kg-1) in the Annapurna treated plants which was closely followed by the Navajeevan. Such increase in Nitrogen content might be due to Azotobacter, PSB and Azospirillum causing relatively greater utilization of available Nitrogen by plants.

Figure 4: Percent Nitrogen content



Percent of nitrogen content was estimated by kjeldahl method after 30 days and 60 days of sowing seeds.

3.5 Micronutrients content in plants:

Figure 5: Micronutrient content after 30days of sowing

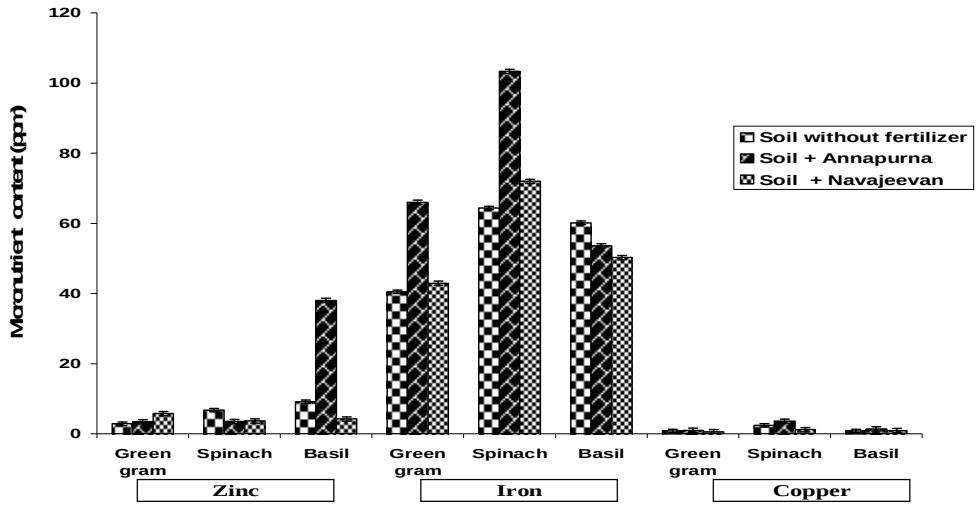
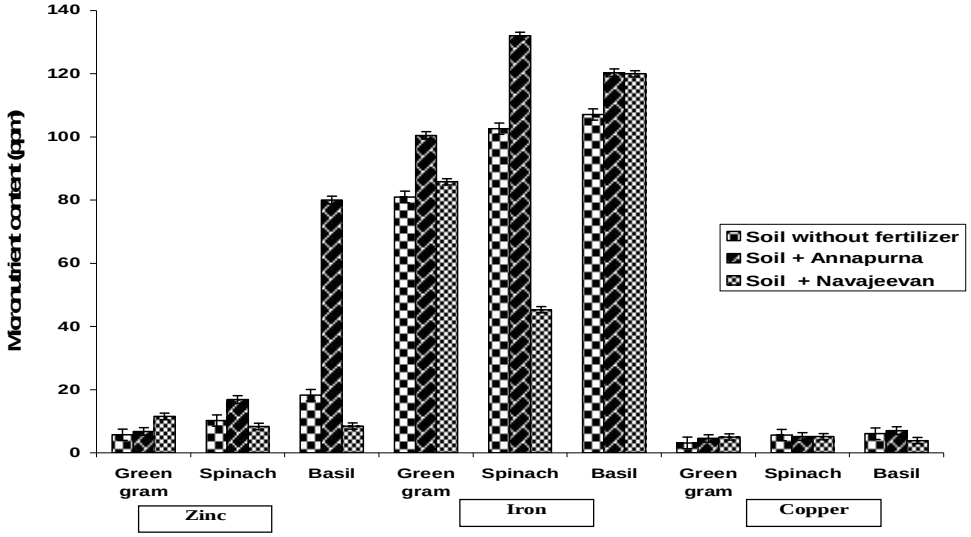


Figure 6: Micronutrient content after 60days of sowing



The results (Fig 5, 6) show that basil has recorded highest Zn content which is followed by spinach and then green gram. The concentration of Zn was highest (80ppm) in basil leaves, which is in correlation with findings of Bhowmik*et al.,* (Bhowmiket al., 2008). Zinc plays a vital role in human health so the higher concentrations of zinc enhance medicinal value for which it is known. Similarly spinach exhibited high concentration of Fe content mainly in the Annapurna sample which is followed by the green gram and basil. There was an increase of ~ 30% of iron content in spinach which shows an increase of micronutrient content of this leafy vegetable. Cu content is mostly present in traces and no drastic increase of metal ion by the addition of bio- fertilizers.

Azotobacteriaone of the ingredients of Annapurna bio-fertilizer synthesizes auxins, cytokinins, and GA–like substances, and these growth materials are the primary substances controlling the enhanced growth. These hormonal substances, which originate from the rhizosphere or root surface, affect the growth of associated plants.

In order to guarantee the high effectiveness of inoculants and microbiological fertilizers it is necessary to find the compatible partners, i.e. a particular plant genotype and a particular Azotobacter strain that will form a good association. The strain used in this fertilizer is compatible with spinach followed by green gram and basil.Soil-dwelling diazotrophs such as Azotobacter are especially useful in gauging the health and virility of the ground.

Azospirillum can positively influence plant growth, crop yields and N-content of the plant. This plant stimulatory effect exerted by Azospirillum has been attributed to several mechanisms, including biological nitrogen fixation and auxin production.Azospirillum inoculation alters root morphology, which has been ascribed to the bacterial production of plant growth regulating substances (Oda and Jos, 2000). An increased number of lateral roots and root hairs enlarges the root surface available for nutrients. This results in a higher nutrient uptake by inoculated roots and an improved water status of the plant, which in turn could be the main factor enhancing plant growth.

Trichoderma sps. are free-living fungi that are common in soil and root ecosystems. Some are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi. At least some strains establish robust and long-lasting colonizations of root surfaces and penetrate into the epidermis and a few cells below this level. They produce or release a variety of compounds that induce localized or systemic resistance responses. These root–microorganism associations cause substantial changes to the plant proteome and metabolism (Gary et al., 2004). Root colonization by Trichoderma spp. also frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients.Trichoderma strains playan important role in the bioremediation of soil thatare contaminated with pesticides and herbicides.They have the ability to degrade a wide range ofinsecticides: organochlorines, organophosphatesand carbonates.

This was clearly evidenced by better growth parameters and nutrient content in Annapurna fed plants which contains trichoderma as one of the ingredient.

4. Conclusion

The various observations and results obtained from the above study of the effect of two different commercial bio-fertilizers on the morphological, biochemical and nutrient parameters is beneficial and increased the yields. Howeverbest results were obtained in the treatment of Annapurna bio-fertilizer in all plants under study i.e., green gram, spinach and basil (pulse crop, vegetable and medicinal plant respectively). This may be due to co-operative microbial activities in the root and soil environment benefiting the plant growth and health and also soil quality.Azotobacter, azospirrillum, phosphate solubilizing bacteria and trichoderma present in the Annapurna biofertilizerplayed a vital role in enhancing germination, vegetative growth parameters, mineral content and soil quality which are not supplemented in navajeevan bio-fertilizer. Soil microorganisms play an important role in increasing the food quality and sustaining environmental quality from the ecological and agricultural perspectives.

Acknowledgements

Authors are grateful to Regional sugar cane research institute, ANGRAU, Anakapalle (AP), for kind cooperation and help in providing AAS for analyzing plant samples. We are also thankful to GITAM Institute of Science, GITAM University for providing necessary laboratory facilities to carry out this work.

References

Abiven, S.,Menasseri, S.,Chenu, C. (2009).Theeffects of organic inputs over time on soil aggregate stability – A literature analysis, Soil Biology and Biochemistry. 41, 1–12.

Bhowmik,S., Chowdhury, S.D.,Kabir,M.H., Ali, M.A. (2008). Chemical composition of some medicinal plant products of indigenous origin, Bangladesh Veterinarian. 25, 32-39.

Delacruz, R.E., Manalo, M.Q.,Aggangan,N.S., Ambalo, J.D.T. (1988) Growth of three legumes trees inoculated with VAM fungi and Rhizobium, Plant Soil. 108, 111-15.

Dewan,G.I., Rao, N.S.S. (1979). Seed inoculation with Azospirillum brasilense and Azotobacter chroococcum on the root biomass of rice (*Oryza sativa*), Plant Soil. 53, 295-300.

Dhage, A.R.,Patil,N.D., Kadam, S.S. (1984). Effects of N and P fertilization on yield and composition of black gram (*Vignamungo* L. Hepper) seeds, Plant Soil. 81, 441-444.

Elzebroek,T., Wind,K. Guide to Cultivated Plants. (2008). CAB International, United Kingdom, 262.

FAO, (2007) Protein and amino acid requirements in human nutrition, Report of a joint WHO/FAO/UNU expert consultation, (WHO) technical report series, Geneva, Switzerland, pp. 935.

FAOSTAT (2008). Food and Agriculture Organization of United Nations (FAO), http:// faostat.fao.org/ site/339/ default.aspx.

Gary Harman,E., Charles Howell,R., Ada Viterbo, Ilan Chet and Matteo Lorito (2004).Trichoderma species — opportunistic, avirulent plant symbionts, Nature Reviews Microbiology. 2, 43-56

Gomma,A.M., Mohamed, M.H. (2007). Application of bio-organic agriculture and its effect on Guar (*Cyamopsistetragonoloba* L.) root nodules, forage, seed yield and yield quality, World Journal of Agriculture Science. 3,91-96.

Hadas,R., Okon,Y. (1987). Effect of Azospirillum brasilense inoculation on root morphology and respiration of tomato seedlings, Biological Fertility of Soil. 5, 241-47.

Hodge, J.E. and Hofreiter, B.T. (1962). In: Methods in Carbohydrate Chemistry, (Eds. Whistler, RL, Be Miller JN), Academic Press, New York.

Jackson,M. L.(1956) Soil Chemical Analysis, Prentice-Hall, Inc.

Kapoor, R., Giri,B., Mukerji,K.G.(2004). Improved growth and essential oil yield and quality in Foeniculumvulgare Mill on mycorrhizal inoculation supplemented with P-fertilizer, Bioresource Technology. 93, 307-311.

Koppad,A.G., UmarbhadshaN.K. (2006). Effect of Pre-sowing treatments on seed germination of Babehi and Senna in nursery, Karnataka Journal of Agriculture Science. 19, 441 – 442.

Koppad,A.G., Umarbhadsha, N.K. (2006a) Effect of Pre-sowing treatments on seed germination of Laving Tulsi and White Tulsi in nursery beds, Karnataka Journal of Agriculture Science. 19,443–444.

Kumar, R., Bhargava, A.K. (1998). Effect of sugar mill effluent on the vegetative growth and yield of *Triticumaestivum*, Advances in Plant Sciences. 11, 221-227.

Laemmli, U.K.(1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227, 680–685.

Lowry,O.H., Rosebrough,N.J., Farr,A.F., Randall, R.J. (1951). Protein measurement with the Folin- phenol reagent, Journal of Biological chemistry. 193, 265-275.

Mehrotra,C.I., Lehri, L.K.(1971). Effect of Azotobacter inoculation on crop yields, Journal of Indian Society Soil Science. 19, 243-48.

Ministry of Agriculture, Government of India, Department of Agricultural and Cooperation, Directorate of Economics and Statistics: Agricultural statistics at a glance (2008) (http:// dacnet.nic.in/ eands/ latest_2006.html).

OdaSteenhoudt, Jos Vanderleyden(2000).Azospirillum, a free-living nitrogen-fixing bacterium closelyassociated with grasses: genetic, biochemical and ecological aspects, FEMS Microbiology Reviews. 24, 487- 506.

Ponnuswamy, A.S. (1993).Seed technological studies in Neem. M.Sc. Thesis, Tamil Nadu Agriculture University, Coimbotore (India).

Rudresh, D.L.,Shivaprakash, M.K., Prasad, R.D.(2005). Effect of combined application of Rhizobium, phosphate solubilizing bacterium and Trichoderma spp. on growth, nutrient uptake and yield of chickpea (*Ciceraritenium* L.), Applied Soil Ecology 28, 139-146.

Selvakumar,G., Lenin,M., Thamizhiniyan,P., Ravimycin,T. (2009). Response of biofertilizers on the growth and yield of black gram, Recent Research Science Technology. 1, 169-175.

Sharma,A.K. (2002a)Biofertilizers for Sustainable Agriculture, Agrobios, India, , 407.

Tamil Nadu Agricultural University, (2009). Department of agricultural meteorology (http:// www.tnau.ac.in/scms/Agmet/weather.html).

Uyanöz, R. (2007). The effects of different bio-organic, chemical fertilizers and their combination on yield, macro and micro nutrition content of dry bean (*Phaseolus vulgaris* L.), International Journal of Agriculture Research. 2, 115-125.

Vanangamudi, K.,Padmavathi, S.,Manonmani,V., Surendran, C. (1993) Effect of pelleting with biofertilizers, biocids and nutrients on the viability and vigour of Neem seeds. Paper presented In: International World Neem conference, Bangalore, , Feb. 24–28, p.64.

Venkateswarlu,B., Rao, A.V. (1983). Response of pearl millet to inoculation with different strains of Azospirillum brasilense, Plant Soil. 74, 379-86.

VijayaKumari,B., Janardhanan, K.(2003). Effect of biofertilizer on seed germination, seedling growth and biochemical changes in silk cotton (*Ceibapentandra*), Crop Research. 25, 328-332.