Vol-1,Pg-44-57,Yr-2019-AJABTR

A Comparative study on the impact of bio fertilizer and chemical fertilizer in the biosynthesis of secondary metabolites in Okra (*Abelmoschus esculentus* L.)

[1]Susmita Mukherjee and [2]Pratik Talukder[*]

[1,2]Department of Biotechnology, University of Engineering and Management, Kolkata University Area, Plot, Street Number 03, Action Area III, B/5, Newtown, Kolkata, West Bengal 700156

[*]Corresponding- pratiktalukder@gmail.com

Abstract:

Vegetables are the integral part of the balanced diet of human since time immemorial. Globally, the role of vegetables has been recognized in solving the problem of food and nutritional security. Okra (*Abelmoschus esculentus* L.) is an important vegetable crop of Malvaceae family, which supplies higher nutrition. The present study was intended at determining the importance of using organic bio fertilizers instead of harmful chemical fertilizers in Okra (*Abelmoschus esculentus*). The requirements of fertilizers in Okra are important for the early growth and total production. Integrated use of organic bio fertilizers can improve crop productivity. The modern system of farming, it is increasingly felt, is becoming unsustainable as evidenced by declining crop productivities, damage to environment, chemical contaminations, etc. The necessity of having an alternative agriculture method which can function in a friendly eco-system while sustaining and increasing the crop productivity is realized now. The objective of the study was to assess the comparative effect of organic bio fertilizers and chemical fertilizers in terms of growth, nutrition value and secondary metabolite production. The result of this study clearly indicates the nutritional benefits of consuming vegetables grown by using bio fertilizers and also states how enhanced accumulation of natural antioxidants such as polyphenols are additionally adding to our health benefits.

Keywords: *Abelmoschus esculentus*, Antioxidant, Okra, Plant secondary metabolism, Polyphenol

1. Introduction:

Plants and the metals in the soil have had a long and intimate evolutionary association that has resulted in many complex interactions mediated by specialized plant metabolites like phenolics. Plants are capable of producing different secondary metabolites which have various important roles in plant's biotic and abiotic stress resistance. Class phenolics constitutes of phenolic acids, flavonoids, lignans, stilbenes and tannins, which is structurally diverse, ranging from monomeric phenolic acids to complex polymers like tannins [1]. Phenolics in fruits and vegetables represent the major class of antioxidant supplements in our diet [2]. There are several defense mechanisms evolved in plants to diminish the negative impact of environmental and soil associated hazards including the production of natural antioxidants such as polyphenols [3].

Food habit in human has evolved over the course of human evolution and human civilization. One of the main ingredients of our diet is vegetables. This study was conducted on Okra (*Abelmoschus esculentus*). It is important vegetable plant of plant of the Malvaceae family. It is widely cultivated in the sub tropical regions in India. Okra is a rich source of nutrients [4]. It is not only nutritious but also a good reservoir of plant secondary metabolites mainly polyphenolic. In India okra (*Abelmoschus esculentus* L.) is an important part of our daily diet as it is a rich source of macro and micro nutrients [5]. It belongs to the malvaceae family. Not only in India but also it is consumed worldwide. Several studies showed that apart from being a rich source of protein, carbohydrate and microelements it also serves as a potent producer of secondary metabolites such as polyphenols and flavonoids [6]. Being a fast growing developing nation with such a huge population it is of utmost importance to increase the production of agricultural products including vegetables such as okra. Due to rapid urbanization and industrialization the amount of agriculture land is decreasing day by day. Farmers are now growing vegetables in small hamlets nearer to the suburban areas and using higher amount of chemical fertilizers to maximize the production. These chemical fertilizers are not only affecting the health of the vegetable plants but also get accumulated in the soil and water thus causing huge negative impact on our environment. Unscrupulous use of pesticides has become a practice. Although this contributes to higher yield to some extent but contributes to nutrient imbalance, production of reactive oxygen and nitrogen species (ROS and RNS) and eventually brings about deterioration of crop and soil quality [7]. One the approaches which can be adopted to enhance the vegetable productivity without harming the plant health and also minimizing the ill effect on the people who are consuming these

vegetables is to switch to the use of organic bio fertilizers. Studies showed [8] that organic bio fertilizers can contribute to the betterment of the soil quality as well as plant health and yield by fixing higher amount of nitrogen in soil and by enhancing secondary metabolite production in soil [9]. Plants produce secondary metabolites mainly via Phenylpropanoid pathway. The first step of this pathway is accomplished by Phenylalanine ammonia Lyase (PAL). Bio fertilizer also fix appreciable amount of atmospheric nitrogen in soil, enhance plant growth by production of organic acid and growth substances, and make available the complex phosphorus to the plant, which may cause an appreciable reduction in consumption of inorganic fertilizers. The main should be to increase the use of bio fertilizers keeping in mind that the yield of the plant should not be compromised. This study documents the comparative analysis on the impact of bio fertilizers and organic fertilizers on nutritional values, accumulation of secondary metabolites in okra (*Abelmoschus esculentus* L.). The results of this study would enable us to understand the secondary metabolite composition of okra and its mode of modification due to the effect of use of exogenous fertilizers.

2. Materials and methods:

2.1 Plant materials and treatments

Okra (*Abelmoschus esculentus*) [Variety- BCO- 1, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India] seeds were used for this study.

2.2 Composition of organic bio fertilizer used in this study

Compost, mustard cake, neem cake, bone dust was mixed in 5:1:1:2 ratio and the mixture was administered in the field.

2.3 Composition of organic Chemical fertilizer used in this study

NPK fertilizer (10:10:10), magnesium sulphate, ferrous sulphate, zinc sulphate mixture was used as chemical fertilizers in 5:1:2:2:2 ratio

Three different fields were prepared.

In Field- 1 neither organic bio fertilizers nor chemical fertilizers were used (Control group- F1)

In Field- 2 only organic bio fertilizer was used (Organic bio fertilizer group-F2).

In Field- 3 only chemical fertilizers was used (Chemical fertilizer group- F3).

Fifteen plants were maintained in each field.

2.4 Determination of stress

2.4.1 Alteration of seedling height

Root and shoot length of two month old plants were calculated and expressed as mean ± standard error of mean (SEM).

2.4.2 Estimation of chlorophyll and carotenoids

Amount of Chlorophyll was measured according to the method of Lichtenthaler (1987) [10] with little modifications.

2.4.3 Estimation of proline

Total proline content was measured according to Bates et al. (1973) [11] with few modifications [12]. Total proline content (mg/g fresh weight of tissue [FW]) was calculated from a standard curve of proline which was prepared with a range of known concentrations of proline.

2.4.4 Lipid peroxidation

The level of lipid peroxidation can be determined by measuring the amount of malondialdehyde (MDA). Method of Heath and Packer (1968) [13] with little modifications [12] was used to measure the extent of lipid peroxidation. Result was expressed as MDA equivalents in µM/g fresh weight of tissue.

2.5 Preparation of plant extract

Plant extracts were prepared according to Brolis et al. (1998) [14] with little modifications [15]. 300 mg of fresh tissue was homogenized in 50% ethanol (Merck, Germany) and then sonicated for 40 minutes. The homogenate was centrifuged at 10000 g for 5 minutes. Supernatant was collected and used as plant extract.

2.6 Estimation of phenolics

2.6.1 Determination of total polyphenol content

Total polyphenol content was estimated according to Singleton et al. 1999 [16]; with few modifications [15]. A standard curve of gallic acid (Sigma Aldrich, St. Louis, MO, USA) was prepared. Total polyphenol content was expressed as µg Gallic Acid Equivalent (GAE)/g of fresh weight (FW) of tissue by comparing with that standard curve.

2.6.2 Determination of total flavonoid content

Total flavonoid content was determined by method described by Lin and Tang 2007 [17], with few modifications [15]. Total flavonoid content was calculated as µg Rutin Equivalent (RE)/g of FW of tissue by comparing with a standard curve of rutin.

2.6.3 Determination of different specific phenolics by HPLC analysis

HPLC analysis was carried out according to Talukder et al. 2015 [15] to isolate and measure the amount of different polyphenols present.

Standard	Absorption maxima	Retention time	Reference
Gallic acid	270 nm	3.7 min	[18]
Chlorogenic acid	325nm	7.0 min	[19]
Coumaric acid	340 nm	12.8 min	[20]
Caffeic acid	321 nm	9.8 min	[21]
Cinnamic acid	270 nm	18.6 min	[22]
Rutin	350 nm	11.8 min	[23]
Quercetin	370 nm	16.5 min	[23]

Table 1: Retention time and absorption maxima of the phenolic compounds studied

Concentrations of different phenolic constituents were calculated using their respective standard curves.

2.7 Determination of antioxidant activity

Total antioxidant assay [24] was carried out with minor modifications [15] to examine the total antioxidant activity of phenolics. The antioxidant activity was expressed as µg ascorbic acid equivalents (AAE)/g FW of tissue by comparing with the ascorbic acid standard curve.

3. Results

3.1 Alterations in phenotype

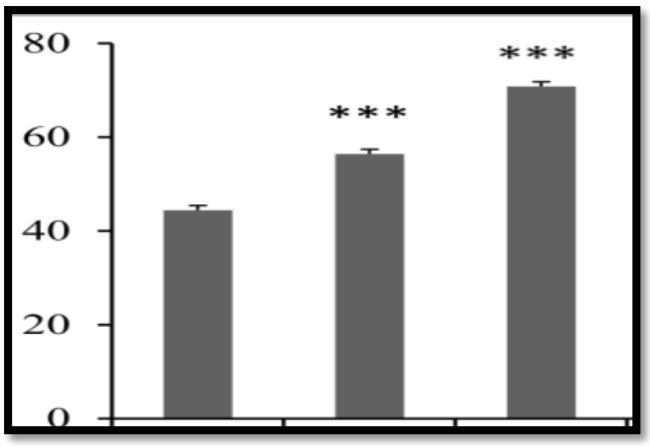

Fig. 1: Reduction in shoot length of seedlings of *Abelmoschus esculentus* grown without any fertilizer (control group), Organic bio fertilizer group- BF, Chemical fertilizer group- CF. Error bars indicate the standard error of the mean (SEM). Level of significance: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$(***)

Fertilizer treatment showed a significant impact on growth of the seedlings especially on root length. The highest length of shoot was observed in plants grown in soil treated with organic fertilizer. When chemical fertilizer is present in the soil, Okra plants exhibited reduced shoot growth. Based on the mean of the fifteen replicates, plants treated with chemical fertilizer had significantly ($p<0.001$) smaller shoots compared to that of the plants treated with organic bio fertilizer (Fig 1).

3.2 Chlorophyll content

Total Chlorophyll content was measured in both plants treated with chemical fertilizer and organic bio fertilizer in order to get an idea of the impact on plant health as changes in pigment content are associated with plant illness. In case of bio fertilizer treated plants chlorophyll content was significantly higher lesser than that of chemical treated plants (Fig 2).

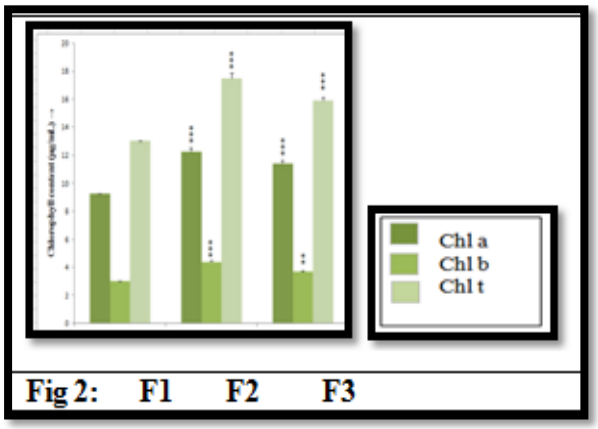

Fig. 2: Changes in total chlorophyll content in *Abelmoschus esculentus* grown without any fertilizer (control group) - F1, Organic bio fertilizer group- F2, Chemical fertilizer group- F3.. Data are represented as mean ± standard error. Level of significance: p <0.05 (*); p < 0.01 (**); p <0.001(***)

3.3 Total proline content

Proline gets accumulated due to oxidative stress and ROS production; it is a very important parameter in measuring stress response. Total free proline content was measured in both plants treated with chemical fertilizer and organic bio fertilizer. Accumulation of proline was found to be highest in plants belonging to Chemical fertilizer group. Almost a two fold increase in proline production was recorded in plants treated with chemical fertilizer compared to that of plants treated with organic bio fertilizer (Fig 3).

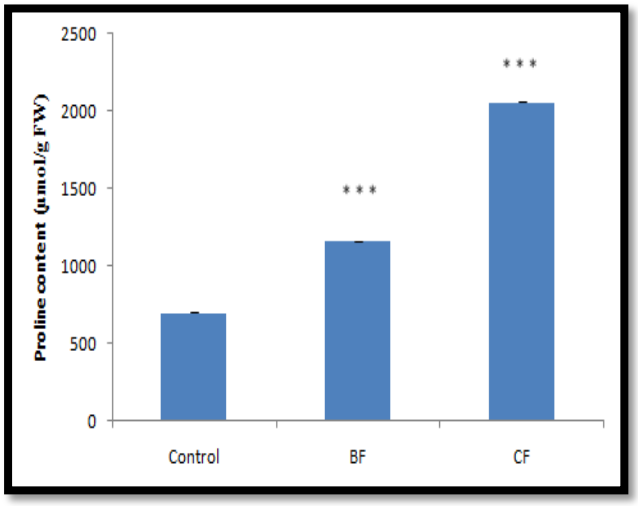

Fig. 3: Changes in total proline content in plants grown without any fertilizer (control group), Organic bio fertilizer group- BF, and chemical fertilizer group- CF. Data are represented as mean ± standard error. Level of significance: p <0.05 (*); p < 0.01 (**); p <0.001(***)

3.4 Lipid peroxidation

Increased ROS production causes Lipid peroxidation which eventually leads to the cellular damage. Lipid peroxidation leads to the formation of MDA; Higher MDA production means the cell is in more stressed condition. In this study, it was observed that MDA content was significantly higher in the plants of Chemical fertilizer group (Fig 4).

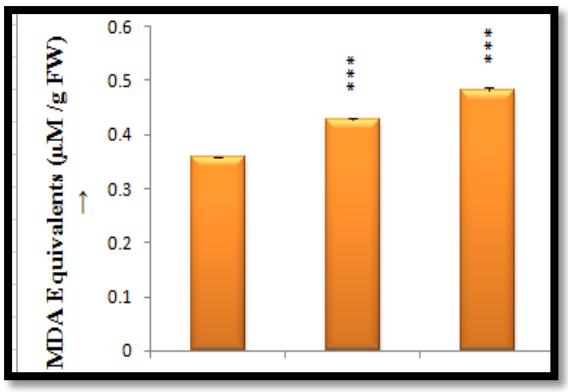

Fig. 4: Lipid peroxidation assay of *Abelmoschus esculentus* in plants grown without any fertilizer (control group- F1, Organic bio fertilizer group- F2, Chemical fertilizer group- F3. Level of significance: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$(***)

3.5 Total polyphenol content

The total polyphenol content in the samples were quantitated from the gallic acid standard curve. A gallic acid standard curve was prepared with a linear regression equation of Y= 0.004X and a correlation coefficient of R^2= 0.995. Polyphenol content in untreated control was 1432.66 µg GAE/g FW but a sudden increase in polyphenol content was observed in plants treated with chemical fertilizers which was 2216.67 µg GAE/g FW (Fig 5). Highest amount of polyphenol production (2546.67 µg GAE/g FW) was found in plants treated with bio fertilizers. It shows that organic bio fertilizers do play significant role in inducing the production of plant secondary metabolites including polyphenols.

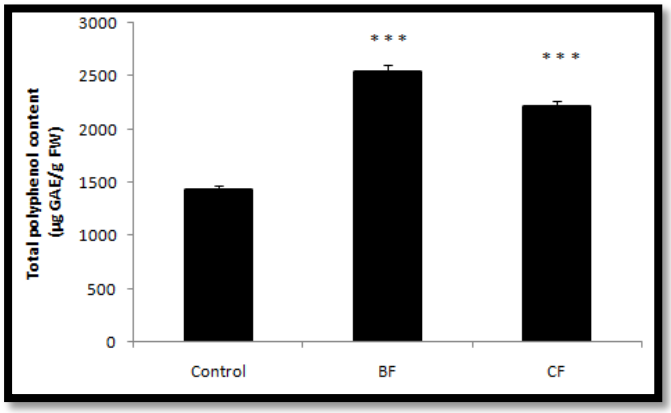

Fig. 5: Total polyphenol content in *Abelmoschus esculentus* in plants grown without any fertilizer (control group), Organic bio fertilizer group- BF, Chemical fertilizer group- CF. Level of significance: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$(***)

3.6 Total flavonoid content

The total flavonoid content in the plants was quantitated from the rutin standard curve. Flavonoid content in untreated control was 476.04 µg RE/g FW but a sudden increase in favonoid content was observed in plants treated with chemical fertilizers which was 1465.95 µg RE/g FW (Fig 6). Highest amount of flavonoid accumulation (2151.02µg RE/g FW) was found in plants treated with bio fertilizers. It shows that organic bio fertilizers do play significant role in inducing the production of plant secondary metabolites including flavonoids.

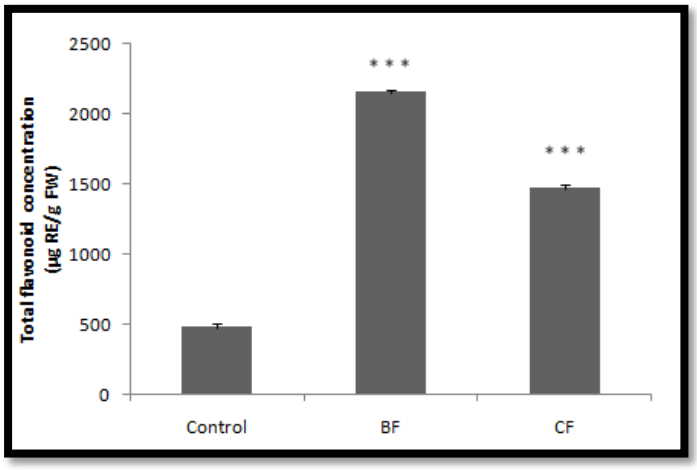

Fig. 6: Total flavonoid content in *Abelmoschus esculentus* in plants grown without any fertilizer (control group), Organic bio fertilizer group- BF, Chemical fertilizer group- CF. Level of significance: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$(***)

3.7 Quantitation of specific phenolics by HPLC

Specific phenolic compoundswere identified and quantitated by HPLC analysis, It was done by the retention time (Rt) and absorption maxima of samples with that of standards at particular wavelengths. The amount of polyphenols was measured from the peak area by comparing with the standard curves. It was observed that gallic acid is the main polyphenol present. Among the other polyphenols studied chlorogenic acid, caffeic acid and coumaric acid showed a similar pattern of accumulation but to a significantly lower concentration. Production of phenolic acids was quite lesser in control plants and as well as in plants treated with chemical fertilizers where as a significant increase was observed in plants grown in soil administered with organic bio fertilizers. Among the flavonoids rutin and quercetin were the predominant ones. The detailed observation is given in the following table:

Treatment	Polyphenol composition in µg/g FW of shoot tissue						
	Gallic acid Rt-3.7 min	Chlorogenic acid Rt-7.1 Min	Caffeic acid Rt-9.8 min	Coumaric acid Rt-12.8 min	Cinnamic acid Rt-18.6 min	Rutin Rt-11.8 min	Quercetin Rt-16.5 min
Control	345.2±6.7	36.7±2.6	29.1±2.1	15.1±1.2	11.2±3.1	186.8±4.7	74.3±3.4
CF	447.6±5.1	56.4±3.2	30.1±2.6	18.4±1.9	23.1±1.2	297.6±10.7	161.9±3.5
BF	613.5±10.7	93.3±.6.7	64.5±2.7	59.2±3.8	33.7±2.3	477.5±14.1	231.1±8.7

Data are represented as mean ± standard error. The results indicated $p<0.05$ and considered as statistically significant

Table 2: Content of different phenolic compounds present in plants grown without any fertilizer (control group), Organic bio fertilizer group- BF, and Chemical fertilizer group- CF.

3.8 Total antioxidant assay

Total antioxidant activity was calculated with the help of the Ascorbic acid standard curve and expressed as ascorbic acid equivalent (AAE)/g FW of tissue by comparing with the calibration curve of standard ascorbic acid. The linear regression equation for the ascorbic acid standard curve was $Y= 0.004X$ with an R^2 value of 0.992. Total antioxidant activity in untreated control was 1306.20 µg AAE/g FW but an increase was observed in plants treated with chemical fertilizers which was 1719.22 µg AAE/g FW. It increased significantly and the highest amount of 1832.36 AAE/g FW was observed in plants treated with bio fertilizers. It shows that organic bio fertilizers do play significant role in inducing the production of plant secondary metabolites such as polyphenols and flavonoids which are natural antioxidants and hence total antioxidant activity has also increased (Fig 7).

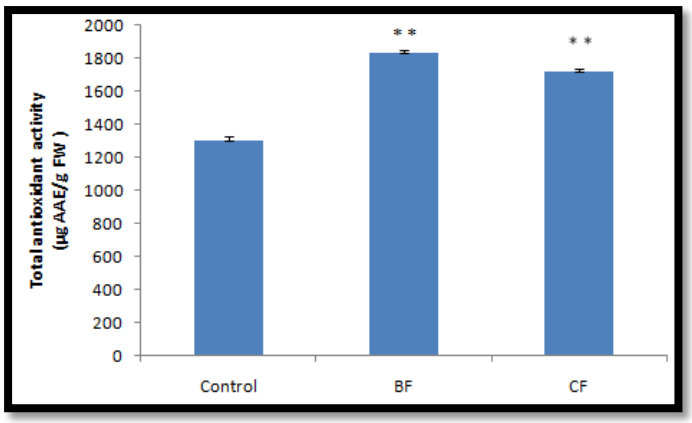

Fig. 7: Total antioxidant activity in *Abelmoschus esculentus* in plants grown without any fertilizer (control group), Organic bio fertilizer group- BF, Chemical fertilizer group- CF. Level of significance: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$(***)

Discussion

Phenolic substances are one of the most diverse groups of secondary metabolites produced primarily by phenylpropanoid pathway. Some important stress markers such as seedling height, chlorophyll content, proline content and lipid peroxidation were studied to assess the magnitude of copper toxicity on *Okra*. Proline content was measured as it is an indicator of oxidative stress. According to a study by Agarwal and Pandey (2004) [25], proline content increased with increasing salt (NaCl) concentration in *Cassia angustifolia*. The findings of our study also showed a significantly high proline accumulation when plants were grown in chemical fertilizer treated soil. Significant reduction in total chlorophyll content was not observed in plants grown in chemical fertilizer treated soil compared to that of plants which were grown in soil treated with bio fertilizers as well as in the control group. It suggests that organic bio fertilizers are beneficial for the growth and development of okra where as chemical fertilizers have negative impact on the plant health. Another indirect marker of stress is malonaldehyde (MDA), the product of lipid peroxidation. In this study it was observed that MDA production was enhanced in plants exposed to chemical feetilizers. In a recent study [26] it was shown that polyphenols being natural antioxidants possess the potential of restricting either initial or other subsequent steps of lipid peroxidation and hence reduces MDA production. Results suggest chemical fertilizers strongly increased MDA amounts in okra. The low lipid peroxidation level in plants treated with organic bio fertilizers can be correlated with the high phenolic and flavonoid content in those plants because both of these compounds are known to be natural antioxidants which may have contributed to the inhibition of lipid peroxidation by exerting the antioxidant activity. Altogether these findings

and the results of this study portray the adaptive response of plants exposed to different types of fertilizers by higher production of polyphenols and flavonoids. The major findings of this study indicate that organic bio fertilizers are contributing to the enhancement in the production of natural antioxidant such as polyphenolic compounds. As the okra grown in the fields administered with organic bio fertilizers are synthesizing higher polyphenols which are not only natural antioxidants but also have immense medicinal value it is highly beneficial for us to use bio fertilizers instead of chemical fertilizers which not only causes harm to the plant but also accumulates in the vegetables and hence negatively impacts those who consume these vegetables.

Conclusion

Altogether these findings suggest that organic bio fertilizers are not only contributing to the enhancement of natural antioxidants such as secondary metabolites but also increasing the nutritional value of the vegetables; as we are consuming these vegetables on daily basis this study strongly advocates the use of bio fertilizers instead of the chemical fertilizers. The results not only clearly indicates the nutritional benefits of consuming vegetables grown by using bio fertilizers but also states how enhanced accumulation of natural antioxidants such as polyphenols are additionally adding to our health benefit.

Acknowledgement

The authors sincerely acknowledge University of Engineering and Management, Kolkata for providing funding for this research work.

Conflict of interest

The authors do not have any conflict of interest.

Reference

1. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem. 99:191-203. 2006.
2. Liang N, Kitts DD. Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions. Nutrients 8:16. 2016.
3. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7.9: 405- 410. 2002.

4. Siemonsma JS, Kouame C. Vegetable. Plant Resource of Tropical Africa 2. PROTA Foundation, Wageningen, Nettherlands. 21–29. 2004.
5. Lyngdoh, Y. A., Mulge, R. and Shadap, A. Heterosis and combining ability studies in near homozygous lines of chilli [*Abelmoschus esculentus* (L.) Monech] for growth parameters. The Bioscan. 8(4): 1275-1279. 2013.
6. Yang, Y.; Jin, Z.; Mao, P.; Jin, J.; Huang, J.; Yang, M. Study on anti-fatigue effect of chilli extracts. Chin. J. Mod. Appl. Pharm. 29, 4. 2012.
7. Mal, B., Mahapatra, P., Mohanty, S. and Mishra, N. Growth and yield parameters of chilli (Abelmoschus esculentus) influenced by Diazotrophs and chemical fertilizers. J. Crop and Weed. 9(2): 109-112. 2013.
8. Gayathri, K. and Syam Sundar Reddy, P. Effect of integrated nutrient management growth and yield of chilli (Abelmoschus esculentus (L). Moench) cv. Arka Anamika. Veg. Sci. 40(2): 246-248. 2013
9. Das Ipsita and Singh, A. P. Effect of PGPR and organic manures on soil properties of organically cultivated mungbean. The Bioscan. 9(1): 27-29. 2014.
10. Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosyn-theticbiomembranes. Methods Enzymol. 148: 350–382. 1987.
11. Bates L, Waldren RP , Teare ID. Rapid determination of free proline for water-stress studies . Plant and Soil 39: 205– 207. 1973.
12. Ghoshal N, Talapatra S, Talukder P, Sengupta M, Ray SK, Chakraborty A, Sen Raychaudhuri S. Cross-adaptation to cadmium stress in *Plantago ovata* by pre-exposure to low dose of gamma rays: Effects on metallothionein and metal content. International Journal of Radiation Biology 91(8):611-23. 2015
13. Heath RL and Packer L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys125:189–198. 1968.
14. Brolis M, Gabetta B, Fuzzati N, Pace R., Panzeri F, Peterlongo F. Identification by high-performance liquid chromatography–diode array detection–mass spectrometry and quantification by high performance liquid chromatography–UV absorbance detection of active constituents of *Hypericum perforatum.* Journal of Chromatography 825:9–16. 1998.
15. Talukder P, Talapatra S, Ghoshal N, Sen Raychaudhuri S. Antioxidant activity and HPLC analysis of phenolic compounds during *in vitro* callus culture of *Plantago ovata* Forsk and effect of exogenous additives on accumulation of phenolic compounds. Journal of the Science of Food and Agriculture 96(1): 232–244. 2016

16. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol 299:152–178. 1999.
17. Lin J Y, Tang C Y. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation.FoodChem101:140–147. 2007.
18. Deshmukh H, Prabhu PJ. Development of RP-HPLC method for qualitative analysis of active ingredient (gallic acid) from stem bark of *Dendrophthoe falcate* Linn. International Journal of Pharmaceutical Sciences and Drug Research 3(2):146-149. 2011.
19. Dao L, Friedman M. Chlorogenic acid content of fresh and processed potatoes determined by ultraviolet spectrophotometry. Journal of Agricultural and Food Chemistry 40:2152-2156. 1992.
20. Hussein SZ, Yusoff KM, Makpol S, Mohd, Yusof YA. Antioxidant capacities and total phenolic contents increase with gamma irradiation in two types of Malaysian honey. Molecules 16:6378-6395. 2011.
21. Wang L, Hsu K, Hsu F, Lin S. Simultaneous determination of caffeic acid, ferulic acid and isoferulic acid in rabbit plasma by high performance liquid chromatography. Journal of Food and Drug Analysis16:34-40. 2008.
22. Lin I, Cham T, Wu S. Simultaneous determination of hesperidin, ferulic acid, cinnamic acid and cinnamaldehyde in chinese tonic wine by high performance liquid chromatography. Journal of the Chinese Chemical Society57:429-435. 2010.
23. Cvetković D, Marković D, Cvetković D, Radovanović B. Effects of continuous UV-irradiation on the antioxidant activities of quercetin and rutin in solution in the presence of lecithin as the protective target. Journal of the Serbian Chemical Society76(7):973-985. 2011.
24. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem 269: 337–341. 1999.
25. Agarwal S, Pandey V. Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biologia Plantarum 48:555-560. 2004.
26. Olajire AA, Azeez L.Total antioxidant activity, phenolic, flavonoid and ascorbic acid contents of Nigerian vegetables. African Journal of Food Science and Technology 2: 22-29. 2011