The Journal of Agricultural Sciences - Sri Lanka
Vol. 17, No 3, September 2022. Pp 370-378
http://doi.org/10.4038/jas.v17i3.9918

Can Biofilm Biofertilizer Cut Down Chemical Fertilizers in Leafy Vegetable Cultivation? A Case Study with *Centella asiatica* (*Gotukola*)

K. M. S. M. Gangthilaka[1], M. Premarathna[1], H. M. S. P. Madawala[2] and G. Senviratne[1*]

Received:24th November 2021 / Accepted: 13th June 2022

ABSTRACT

Purpose: *In Sri Lanka, an excessive usage of chemical fertilizers (CF) has been observed in agriculture which affects negatively for health and wellbeing of consumers as well as environment. Biofilm biofertilizer (BFBF) is a novel technology which is being practiced in many crops to enhance the productivity while improving soil quality. However, the BFBF has not been applied for leafy vegetables thus far. This study was conducted to introduce BFBF to C. asiatica (Gotukola) cultivation as a case study, in order to reduce the usage of CF.*

Research Method: *Firstly, a field experiment was carried out to evaluate the minimum CF rate that would not hamper the yield of C. asiatica cultivation. Then, different combinations of reduced rates of the minimum CF level and BFBF (Biofilm-veg™) were applied with a control of no amendments, and yield was recorded to explore the possibility of CF reduction with the BFBF application. One-way ANOVA, Tukey's HSD test, and cost-benefit analysis were used to compare the treatments.*

Findings: *The study revealed that even with only 60% of the farmers' CF rate, there was no observable yield compromise. Thus, the optimum CF requirement for the crop was identified as 60% of the farmers' current CF rate. By coupling of BFBF the amount of CF could be brought further down to 48% of farmers' current CF rate. Furthermore, the application of 48%CF+BFBF produced a plant growth comparable to that with 100% CF.*

Research Limitations: *There were some practical limitations such as long dry spells and also finding suitable fields for the experiment, due to farmers' hesitation on possible yield reduction.*

Originality/ Value: *Application of BFBF is an effective method to cut down CF by half in C. asiatica cultivation, thus minimizing the adverse effects of CF. Several studies have been conducted thus far to analyze the possibility of biofertilization in cutting down CF in agriculture, but they have only been limited to inoculation of isolated soil microbial mixtures. Present study is the first attempt to evaluate the capability of developed microbial biofilm-based BFBF application in reducing CF use in leafy vegetable cultivation.*

Keywords: *Agroecosystem, Biofilm biofertilizer, Centella asiatica, Chemical fertilizer*

INTRODUCTION

Leafy vegetables are consumed as a main supporting dish with the staple food in many Sri Lankan households, and they have been identified as a cheap as well as a good source of mineral nutrients, vitamins and dietary fiber for children, pregnant women, breast-feeding mothers, people with nutrient deficiency and elderly people (Okafor, 1983). Phytochemical components of leafy vegetables act as supplements for food and enhance the consumer health standard while the fiber component improves gastrointestinal

[1*] *Microbial Biotechnology Unit, National Institute of Fundamental Studies, Kandy, Sri Lanka.*
gamini.se@nifs.ac.lk
[2] *Department of Botany, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka.*
https://orcid.org/0000-0003-1562-4097

function and reduces metabolic diseases (Mensah *et al.,* 2008). One of the most popular leafy vegetables among Sri Lankans is *Centella asiatica* (Gotukola) which is a tender aromatic herb, and it is consumed frequently as a freshly prepared salad or porridge.

Commercial leafy vegetable cultivation is done in large scale in North Western province, and soil nutrients are removed as farmers continuously harvest the crop from the system under intensive cropping (Dawe *et al.,* 2000). The sustainable crop production needs replacing those nutrients through biological processes or through the addition of fertilizers (Vitousek *et al.,* 2009). On the other hand, excessive application of chemical fertilizers (CF) has resulted in many negative effects such as leaching and pollution of water resources, destruction of soil microbiome, crop susceptibility to disease infestations and acidification or alkalization of the soil, thus degrading soil fertility further. Moreover, CF are potential sources of radionuclide and toxic heavy metals (Savci, 2012). Once these harmful substances enter into the soil-plant systems, they tend to bio accumulate in the food chain. These negative effects have caused a permanent damage to the overall agricultural ecosystem (Chen, 2006). Thus, for the optimum growth of plants, a balanced nutrient supply is needed.

Due to these adverse effects of the CF, the world is now moving towards the eco-friendly organic agriculture. Options like biofertilizers are gaining popularity, because of their less bulkiness and cost effectiveness. A biofertilizer is a material, which contains live beneficial microorganisms such as Plant Growth-Promoting Rhizobacteria (PGPR) helping in restoring the natural nutrient cycling, thus releasing nutrients for plant uptake (Chen, 2006). Some PGPR have the ability in fixing nitrogen and they can improve the crop production by increasing both carbon and nitrogen nutrition (Nisha *et al.,* 2007). Moreover, they solubilize immobile phosphorus and make it available for plant uptake (Glick, 2012), increase the micronutrient uptake by crops (Rana *et al.,* 2012) and synthesize hormones such as Gibberellins and Auxins which stimulate several functions like seed germination, seedling vigour etc. (Cassan *et al.,* 2009). The PGPR microorganisms can make the crop plants more tolerant to abiotic and biotic stresses, and they can degrade toxic soil pollutants, destroy pathogenic microorganisms and reduce water stress creating a favorable environment for crop growth (Zhang *et al.,* 2019).

Another branch developed recently in the field of biofertilization is the biofilm biofertilizers (BFBF). Microbes attached on to biotic or abiotic surfaces are called biofilms (Rudrappa *et al.,* 2008). They consist of microbial cells encased in self-produced extracellular polymeric substances. Microorganisms in a biofilm have different physiological and biochemical activities compared to that of their individual free-living state. A significant up-regulation of genes can be seen in the biofilm cells over the planktonic cells (Seneviratne *et al.,* 2008). Using microbes, beneficial biofilms can be developed *in-vitro* and be used as biofertilizers, which are then called BFBF. They consist of a collection of several isolated beneficial bacterial strains such as PGPR and fungal strains (Seneviratne *et al.,* 2008). The role of the BFBF is to reinstate sustainability of degraded agro-ecosystems through the breaking of dormancy of the soil microbial seed bank developed under stress conditions, thus restoring biodiversity and ecosystem functioning (Seneviratne and Kulasooriya, 2013), mainly through biochemical signaling among plants, soil microbes and fauna (Seneviratne *et al.,* 2010). It has been reported that the conventional practice of biofertilization with mono and mixed cultures of effective microbes does not provide the highest microbial effect at ecosystem level, which can only be achieved by biofilm formation (Bandara *et al.,* 2006). As such, the concept of BFBF is not only biofertilization, but also a holistic ecosystem approach.

Currently a very high amount of CF is used along with pesticides and insecticides in the leafy vegetable cultivation depending only on visual plant growth. Therefore, in order to reduce the excessive use of CF, it is timely and important to introduce user friendly biological interventions like the BFBF. Thus, the aim of this study was to identify the minimum CF level that should be coupled with the BFBF to obtain a yield equivalent to that of 100% CF in *C. asiatica* cultivation as a case study.

MATERIALS AND METHODS

Field sites

This field experiment was done in *C. asiatica* fields in Chilaw, North Western Province, Sri Lanka (7^o35'N 79^o48'E). The area receives 1000mm to 1500mm rainfall with 24.0°C - 35.0°C average annual temperature, and contains Red yellow podzolic soil (Ministry of Agriculture, 2014).

Initial soil sampling and analysis

Soil pH was measured using a pH meter, and soil moisture (SM) was analyzed by using oven dry method (105^oC). Soil organic carbon (SOC) was determined using Walkley-Black colorimetric method (Baker, 1976). Soil total N (STN) and total P (STP) were measured by Kjeldahl method (Page *et al.,* 1982) and vanadomolybdate method (Pearson, 1970), respectively. Soil exchangeable potassium (SK) was analyzed by using atomic-absorption spectrophotometer (David, 1960).

Determination of the minimum CF requirement for C. asiatica cultivation

Field preparation

Three representative evenly managed *C. asiatica* fields, each of about 6 m x 17 m were used in order to minimize the heterogeneity of land. In each field, fifteen plots, each of 2 m x 3.4 m were constructed, and irrigation water was managed separately without mixing between plots. The plots were arranged in a randomized block design for five treatments with three replicates. Altogether there were 9 replicates for each treatment in the three *C. asiatica* fields.

Treatment application

Four different percentages of farmers' CF practice i.e.100% CF (Urea 70 kg/ha + YaraMila™ complex 40 kg/ha) (T1), 75% CF (T2), 50% CF (T3), 25% CF (T4) were applied in splits in the second and fourth week between two consecutive harvests of six week intervals, with a control without any amendments (T5). Preliminary discussions were conducted with farmers and it was realized that majority of them applied a fairly similar rate of CF and to be realistic, the same rate was used as 100%CF treatment. The CF was broadcasted to each plot followed by the water application through sprinkler irrigation.

Sample collection

C. asiatica yield (fresh weight) in a plot was measured using a quadrate of 1 m x 1 min six-week intervals. This study was done within a period of 6 months, and four harvests were collected during the period.

Evaluating the potential of BFBF in further reduction of minimum CF requirement

Field preparation

Two representative *C. asiatica* fields, each 7m x 14m were used for the study. In each field, twenty-one plots each of about 2.3m x 2m were constructed and irrigation water was managed separately without mixing between plots. The plots were arranged in a randomized block design with three replicates. Altogether there were 6 replicates for each treatment in the two *C. asiatica* fields.

Treatment application

The minimum level of CF that can be applied without hampering yield, denoted as X was reduced further with the application of BFBF (Biofilm-veg™, exact composition of BFBF cannot be revealed due to intellectual property right reasons). Six levels of CF were used as treatments with a control having no amendments, whereas four CF treatments out of those were coupled with BFBF. Accordingly, the treatments were; 100% farmers' CF practice (Urea 70 kg/

ha + YaraMila™ complex 40 kg/ha) (T1), X (T2), X + BFBF (T3), 80% X + BFBF (T4), 60% X + BFBF (T5), 40% X + BFBF (T6) and the control with no amendments (T7). Generally, the BFBF alone application is not recommended, because they are fungal-bacterial biofertilizers, which may absorb a significant fraction of plant available soil nutrients to the microbial biomass, thus decreasing plant growth (Seneviratne *et al.,* 2011). At each application, CF particles were sprinkled to the plots prior to the BFBF application. After that BFBF was mixed with sand (BFBF:Sand, 1.25 l/ha:12 kg/ha) and broadcasted to the specific treatment plots and water was added through sprinkle irrigation.

Sample collection

Plant parameters such as yield, shoot length, root length, and width of the leaf blade were analyzed. Yield was analyzed by performing 1m x1m crop cuts in each plot four times within a period of six months. Plant samples were taken from each plot (n=3). Plants were carefully uprooted without causing damages to the root system and root length, shoot length and width of the leaf blade were measured using a ruler.

Data analysis

One-way ANOVA and Tukey's HSD tests were conducted using Minitab 17 software.

Benefit:Cost ratio

Treatments which showed significantly higher yields were used for the benefit-cost analysis. The Benefit:cost ratio (BCR) was calculated using the data of costs and revenues per hectare. (Nurudeen *et al.,* 2015). The total cost was calculated with reference to fertilizer treatment (cost with government subsidy in 2019), irrigation, labor and harvesting.

BCR = NR / TC

NR = Net revenue

TC = Total cost

RESULTS AND DISCUSSION

Initial soil analysis

The initial soil data were not significantly different between the *C. asiatica* field locations (P>0.05), and hence they were pooled (Table 01). The soil pH and moisture were relatively low in the experimental locations. The initial discussions with farmers revealed that they have been continuously applying CF for more than 15 years for these *C. asiatica* fields. Long term usage of CF increases soil acidity and depletes soil organic matter which leads to lower soil moisture. There are evidences for acidification of soil which occur due to the prolonged usage of nitrogen fertilizers (Savci, 2012).

The study revealed that the application of even 60% of farmers' CF rate (i.e. X) produces a yield similar to that of the yield under 100% CF rate (Figure 01). As such, farmers have applied an excessive amount of CF thus far. In general, beneficial soil microbial community is suppressed by higher rates of CF, and therefore, reduction of CF enhances the soil fertility which lead to improved crop production and plant parameters (Seneviratne *et al.,* 2011).

Table 01: Initial soil physico-chemical parameters of the fields. Determination of minimum CF requirement for *C. asiatica* cultivation

pH (n=3)	SM (%) (n=3)	SOC (%) (n = 3)	STN (%) (n=3)	STP (%) (n=3)	SK (mg/kg) (n=3)
5.31±0.17	13.15±1.25	0.7±0.06	0.17±0.02	0.094±0.08	14.59±2.31

Mean +/- SE values in each column - Soil pH, moisture (SM), organic carbon (SOC), total nitrogen (STN), total phosphorous (STP) and exchangeable potassium (SK)

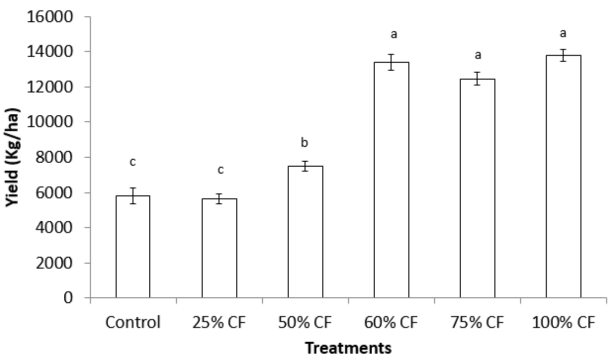

Figure 01: Yield obtained under four different percentages of CF used in the farmers' CF practice.

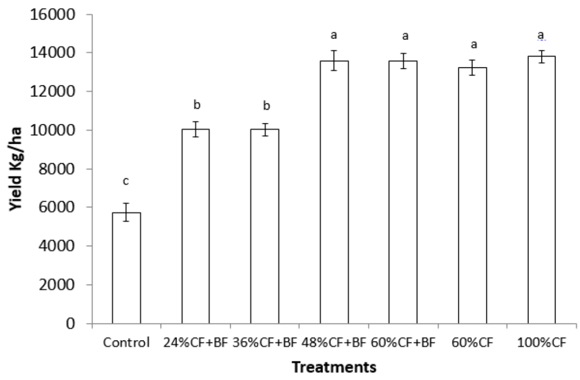

Figure 02: Yield obtained under different percentages of CF used in the farmers' CF practice coupled with BFBF.

Identifying the possibility of CF reduction in *C. asiatica* cultivation by coupling with BFBF

Application of farmers' different CF percentages coupled with BFBF showed that similar yields are produced under 80%X CF (i.e. 48% CF+ BFBF) (X CF = Urea 42 kg/ha + YaraMila™ complex 24 kg/ha) treatment and 100% CF treatment (Figure 02). Furthermore, in the presence of BFBF, even a small increase in CF produced a higher yield than applying CF alone (Figure 03). This indicates that the BFBF can be considered as a key contributor to increase the CF use efficiency. In addition, the increased yield could also be attributed to supplemental nitrogen provided by effective nitrogen fixation of PGPR when they are in biofilm mode (Bandara *et al.,* 2006). The PGPR in biofilm mode perform an important role in agriculture which promotes plant growth to produce a high yield (Seneviratne *et al.,* 2010).

Improved stability of the structure, and hence successful survival of BFBF help to enhance the crop productivity in acidic soils even with reduced CF usage (Seneviratne *et al.,* 2011). These findings can be applied internationally to reduce the CF application, since urea is used as the main nitrogen supplier for leafy vegetable cultivation in many areas of the world (Luyen, 2004; Chen, 2004). Similar research has been conducted to analyze the effect of coupling CF with other organic fertilizers such as organic manure and cow dung (Olarewaju, 2018). The present study is the first attempt to evaluate the effect of combined application of BFBF with CF for leafy vegetable cultivation.

It was observed that there were comparable root lengths in both 100% CF and 48% CF + BFBF treatments (Figure 04). The similar root growth even with the reduced rate of CF may have been attributed to the action of plant growth promoting hormones supplied by the BFBF (Seneviratne *et al.,* 2010).

It was also observed that the shoot lengths under 48% CF + BFBF and 100% CF were comparable. Herath *et al.* (2013) reported similar results with BFBF in lettuce, caused by the action of several bioactive compounds secreted by microorganisms in the biofilm. Moreover, the leaf blade widths under 60%X CF (i.e. 36% CF + BFBF) and 100% CF were also similar. The developed root system with the application of BFBF has contributed to the successful crop growth with high shoot lengths and wide leaf blades (Figures 05 & 06).

Benefit:cost analysis

Higher benefit:cost ratio indicates a high net return (Nurudeen *et al.,* 2015). The analysis clearly denotes that treatments namely, 60% CF, and 48% CF +BFBF have a high Benefit:cost ratio indicating a high net return (Table 02). Therefore, 48% CF + BFBF treatment is more environmentally beneficial as it provides both economic advantage as well as environmental security with reduced amount of CF.

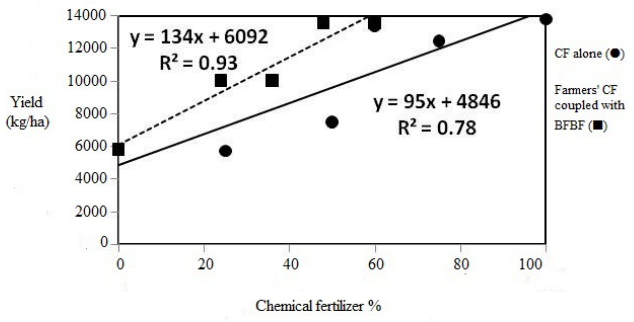

Figure 03: **Yield of Centella asiatica grown under CF alone (●), and farmers' CF coupled with BFBF (■).**

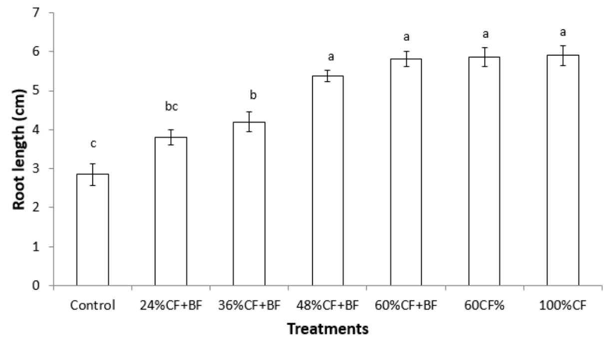

Figure 04: **Root length of the plants under different percentages of CF used in the farmers' CF practice coupled with BFBF.**

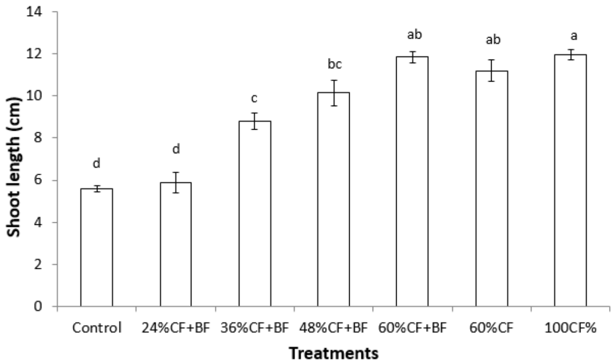

Figure 05: **Shoot length of the plants under different percentages of CF used in the farmers' CF practice coupled with BFBF.**

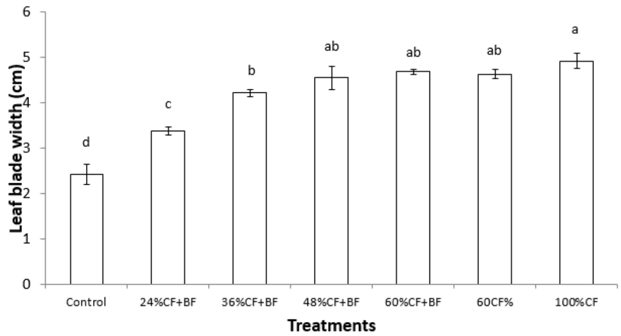

Figure 06: **Leaf blade width of the plants under different percentages of CF used in the farmers' CF practice coupled with BFBF.**

Table 02: **Benefit: cost ratios for different treatments.**

Treatment	Net revenue (SLR)	Total cost (SLR)	Benefit:cost ratio
100% CF	689,825	13,336	51.73
75% CF	622,700	10,002	62.26
60% CF	669,350	8,002	83.65
60% CF + BFBF	699,825	10,002	69.96
48% CF + BFBF	699,675	8,402	83.27

CONCLUSIONS

In Sri Lanka, farmers have used an overdose of CF for *C. asiatica* cultivation which can permanently damage the soil system making it acidic and lifeless. It was identified that the minimum CF requirement is 60% of the farmers' CF practice. Blending of BFBF with CF could further decrease the amount of required CF down to 48%. Application of BFBF coupled with reduced rates of CF advances the root system of plants enhancing their water and nutrient absorption capacity. This leads to the increased yield due to improved structural quality of the plant. Therefore, BFBF can be used as a holistic approach in enhancing the crop yield and quality. This study benefits the worldwide leafy vegetable cultivation as it proposes a method of reducing current chemical fertilizer usage.

ACKNOWLEDGMENT

Sincere gratitude is expressed to all the members of Microbial Biotechnology unit for their support and collaboration. Thanks also go to all the farmers who helped in preparing fields and data collection while sharing their practical knowledge in order to make this project a success.

Declaration of Conflict of Interest

Authors declare that they have no conflict of interest.

REFERENCES

Baker, J.M. (Eds.) (1976). Marine Ecology and OilPollution. Applied Science Publishers Limited, Essex England, 483–536.

Bandara, W.M.M.S. and Seneviratne, G, Kulasooriya, S.A. (2006). Interactions among endophytic bacteria and fungi: effects and potentials, *Journal of Biosciences*.31:645–650.

Cassan, F., Perrig, D., Sgroy, V., Masciarelli, O., Penna, C. and Luna, V. (2009). *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.), *European Journal of Soil Biology*. 45(1):28–35.

Chen, J.H. (2006). The combined use of chemical and organic fertilizers and/or Biofertilizer for crop growth and soil fertility. In: International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use. Land Development Department, Bangkok, Thailand. 1-11.

Chen, Q., Zhang, X., Zhang, H., Christie, P., Li, X., Horlacher, D. and Liebig, H.P. (2004). Evaluation of current fertilizer practice and soil fertility in vegetable production in the Beijing region. Nutrient Cycling in Agroecosystems. 69(1), pp.51-58.

David, D. (1960). The determination of exchangeable sodium, potassium, calcium and magnesium in soils by atomic-absorption spectrophotometry. Analyst. 85(1012), 495-503.

Dawe, D., Dobermann, A., Moya, P., Abdulracman, S., Bijay Singh, Lal, P., Li, S.Y., Lin, B., Panaullah, G., Sariam, O., Singh, Y., Swarup, A., Tan, P.S. and Zhen, Q.X. (2000). How extensive are yield declines in long-term rice experimentals in Asia? Field Crops Research. 66.

Glick, B.R. (2012). Plant growth-promoting bacteria: mechanisms and applications, Scientifica. 2012:1-15. https://doi.org/10.6064/2012/ 963401.

Herath, H.M.L.I., Senanayeke, D.M.N., Seneviratne. G. and Bandara, D.C. (2013). Variation of biochemical expressions of developed fungal-bacterial biofilms over their monocultures and its effect on plant growth. *Tropical Agricultural Research* 24: 186-192.

Luyen, L.T. and Preston, T.R. (2004). Effect of level of urea fertilizer on biomass production of water spinach (*Ipomoea aquatica*) grown in soil and in water, Livestock Research for Rural Development. 16(10), pp.67-73.

Mensah, J.K., Okoli, R.I., Ohaju-Obodo, J.O. and Eifediyi, K. (2008). Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria, *African Journal of Biotechnology*. 7(14): 2304-2309.

Ministry of Agriculture. (2014). Maps. Retrieved from http://www.agrimin.gov.lk/web/index.php/en/downloads/maps

Nisha, R. Kaushik, A. and Kaushik, C.P. (2007). Effect of indigenous cyanobacterial application on structural stability and productivity of an organically poor semi-arid soil, Geoderma. 138:49–56.

Nurudeen, A. R., Quansah, G. W., Tetteh, F. M., Fosu, M. and Osuman, A. S. (2015). Improving maize yield on ferric lixisol by NPK fertilizer use.

Okafor, J.C. (1983). Horticulturally promising indigenous wild plant species of the Nigerian forest zone, *Acta Horticulturae*. 123: 165-177.

Olarewaju, O.A., Alashi, A.M., Taiwo, K.A., Oyedele, D., Adebooye, O.C. and Aluko, R.E. (2018). Influence of nitrogen fertilizer micro-dosing on phenolic content, antioxidant, and anticholinesterase properties of aqueous extracts of three tropical leafy vegetables. *Journal of Food Biochemistry*. 42(4), 12566.

Page, A.L., Miller, R.H. and Keeney, D.R. (1982). Methods of soil analysis. Second Edition. *American Society of Agronomy*, Madison, Wisconsin, USA. 595-622.

Pearson, D. (1970). Determination of phosphate by the vanadomolybdate colorimetric method. In The Chemical Analysis of Foods - sixthedition. London. 23–24.

Rana, A., Saharan, B., Nain, L., Prasanna, R. and Shivay, Y.S. (2012). Enhancing micronutrient uptake and yield of wheat through bacterial PGPR consortia, *Journal of Soil Science and Plant Nutrition*. 58(5):573–582.

Rudrappa, T., Biedrzycki, M.L. and Bais, H.P. (2008). Causes and consequences of plant-associated biofilms, *FEMS Microbiology. Ecology*. 64:153–166.

Savci, S. (2012). An Agricultural Pollutant: Chemical Fertilizer, *International Journal of Environmental Science and Development*. 3(1): 77-79.

Seneviratne, G. and Kulasooriya, S.A. (2013). Reinstating soil microbial diversity in agroecosystems the need of the hour for sustainability and health, Agriculture, Ecoystems and Environment. 164:181-18.

Seneviratne, G., Jayasekara, A.P.D.A., Silva, M.S.D.L. and Abeysekara, U.P. (2011). Developed microbial biofilms can restore deteriorated conventional agricultural soils, Soil biology and biochemistry. 43(5):1059-1062.

Seneviratne, G., Weerasekara, M.L.M.A.W., Seneviratne, K.A.C.N., Zavahir, J.S., Kecskés, M.L. and Kennedy, I.R. (2010). Importance of biofilm formation in plant growth promoting Rhizobacterial action, Plant growth and health promoting bacteria.18:81-95.

Seneviratne, G., Zavahir, J.S. and Bandara, W. (2008).Fungal-bacterial biofilms: their development for novel biotechnological applications, *World Journal of Microbiology and Biotechnology*. 24:6(739).

Vitousek, P.M., Naylor, R., Crews, T., David, M.B., Drinkwater, L.E., Holland, E., Johnes, P.J., Katzenberger, J., Martinelli, L.A., Matson, P.A., Nziguheba, G., Ojima, D., Palm, C.A., Robertson, G.P., Sanchez, P.A. and Townsend, A.R. (2009). Nutrient Imbalances in Agricultural Development, Science. 324 (5934)1519-20.

Zhang, L.N., Wang, D.C., Hu, Q., Dai, X.Q., Xie, Y.S., Li, Q., Liu. H.M. and Guo, J.H. (2019). Consortium of plant growth-promoting Rhizobacteria strains suppresses sweet pepper disease by altering the rhizosphere microbiota, *Frontiers and Microbiology*. 10:1668.