Utilization of Soil Microbial Diversity for Crop Production in Sri Lanka

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Abstract

Although microorganisms represent a very small fraction in soil, they play vital roles in the maintenance of soil health and provision of nutrients to plants in a sustainable manner. In these studies eubacterial, cyanobacterial and fungal taxa were isolated from different soils regularly used for short term crop cultivation. The isolates included heterotrophic eubacteria, symbiotic rhizobia from legume root nodules, photoautotrophic cyanobacteria and free living and rhizospheric fungi. Screened and selected isolates were lab and field tested for their ability to associate intimately with targeted crop plants, form efficient symbiotic root nodules with legume crops and the best candidates were used to prepare solid and liquid biofertilizer inoculants. Two main groups of biofertilizers: rhizobial biofertilizers and biofilm-biofertilizers were prepared and tested in farmers' fields in several different locations. Performances of the inoculants were always tested in comparison to mineral fertilizer levels recommended to the different crops. Results showed that rhizobial inoculants can replace the application of N-fertilizer (urea) completely and biofilm-biofertilizers can replace at least 50% of all three N, P & K fertilizers without any reduction and sometimes with increase in crop yields.

Keywords: biofertilizers, rhizobiology, biofilm-biofertilizer, minimizing chemical fertilizer, eco-friendly agriculture.

Introduction, scope and main objectives

Application of chemical fertilizer though capable of producing high yields in short term crops, the yields level off with time and continuous use of chemicals becomesecologically detrimental in the long run. It has been demonstrated that chemical fertilizer additions result in the depletion of soil microorganisms which maintain soil health (Seneviratne *et al.,* 2011) and increasing crop yields on such 'dead soils' is a tremendous task. Moreover, nutrient absorption from chemical fertilizers is highly inefficient and 60 to 70% of the added nutrientsare not taken up by crop plants. The residual chemicals are liable to all types of losses resulting in environmental pollution. Losses of highly labile nitrogen fertilizers have been shown to be largely responsible for environmentally related health problems. Such consequences led to the decision to reduce chemical N-fertilizer use by at least 20% by the year 2030 as a sustainable development goal.

In Sri Lanka excessive application of chemical fertilizers had been suspected as a major contributory factor responsible for chronic kidney disease of uncertain etiology (Jayatilake *et al.,* 2013) and cancer and 'blue baby syndrome' particularly in Northern Sri Lanka (Jeyakumaran, 2013).Environmental pollution especially from P-fertilizer losses have led to toxigenic cyanobacterial bloom formation in fresh water reservoirs of Sri Lanka (Kulasooriya, 2017).

The present studies were therefore undertaken to develop biofertilizers that could minimize the application of chemical fertilizers in crop production.

Methodology

Rhizobial and Biofilm biofertilizers

Isolation and purification of rhizobia followed procedures described by Somasegaran and Hoben (1994). Initial isolations from root nodules of legumnous crops were done on Congo Red Yeast Mannitol Agar to detect contaminants that appear pink on this selective medium. Both split (for large nodules) and crush (for small nodules) methods were used. After several successive sub-culturing isolated pure cultures were maitained on Yeast Mannitol Agar (YMA). These isolates were authenticated using the original host plants and screened in a greenhouse for effective nodulation and plant growth. The best strains were then semi-mass cultured in 20L aspirator bottles under sterile conditions in YMbroth. Once a culture reached a density around 10^8 cells/ml 15ml of the broth culture diluted to 150ml with sterile water was injected into 250g of autoclaved, modified coir dust packeted in a polypropylene bag (Seneviratne,Van Holm and Ekanayake, 1999). These bags were incubated in the dark, under room temperature (25°C to 33°C) for a week to mature. Such carrier-based inoculants are supplied for use by the farmers. Agronomic scale field trials were conducted with soybean (*Glycine max*), mung bean (*Vigna radiata*), vegetable bean (*Phaseolus vulgaris*), groundnut (*Arachys hypogea*) and the forage legume clover (*Trifolium repens*) to compare the yields obtained with the recommended levels of urea with those obtained with inoculation in the absence of urea.

Biofilm-biofertilizers are multi-microbial inoculants (bacteria and fungi) growing in a common matrix. Preparation of biofilm-biofertilizers followed the procedure described in Seneviratne *et al* (2011). Studies with biofilm-biofertilizer were designed to compare plant and endophytic microbial parameters under farmers'practicesof chemical fertilizer application with biofilm-biofertilizerinoculation using rice (*Oryza sativa* L.) as the test crop. Field trials were conducted in 37 representative farmers' fields with diverse soil types spreading over thousands of hectares in Ampara (n=3), Kurunegala (n=14), Hambantota (n=8) and Polonnaruwa (n=12) districts. Two consecutive, uniformly managed paddy fields were used to apply the treatments separately. The treatments were (i) biofilm-biofertilizer practice (1000 ml of biofilm-biofertilizer with 225 kg NPK/ha) and (ii) Farmers' practices (425 kg NPK/ha). Paddy was broadcasted and irrigation water was managed separately in the two fields, without mixing from surrounding fields. The two consecutive treatment plots were taken as a block design in each site. Thirty-seven field locations acted as replicates.

Four random rice hills with rhizosphere soil were uprooted carefully at 50% flowering stage from each paddy field. Soil was carefully removed, and the root system was washed without damaging it. Roots and shoots were separated and oven dried at 65°C for constant weight, and then root dry weight (RDW) and shoot dry weight (SDW) were recorded. Seed samples were collected from four random hills at physiological maturity stage. Yield was analysed by performing five 1 m x 1m crop cuts in each plot and thousand grain weight (TGW) was measured.

Endophytic diazotrophs (ED) and non-diazotrophs (END) in plant leaves were enumerated by culturing them at 10^{-6} dilution in combined carbon medium (CCM) (Rennie, 1981) and modified CCM medium (CCM + NH_4NO_3), respectively. Colony counts were taken after 48 hours.

Means and correlations of all the variables underbiofilm-biofertilizer practice and farmers' CF practice were calculated. T-test was done for mean comparison after confirmation of normal distribution of data using normality test. All data were analysed statistically using Minitab 17 version.

Results

In all the trials with legume crops the yields obtained with inoculation were either equal or marginally above those obtained with urea application. A fertilizer N-response curve obtained with soybean showed that the effect of inoculation superseded that of the highest level of urea application (Fig 1).

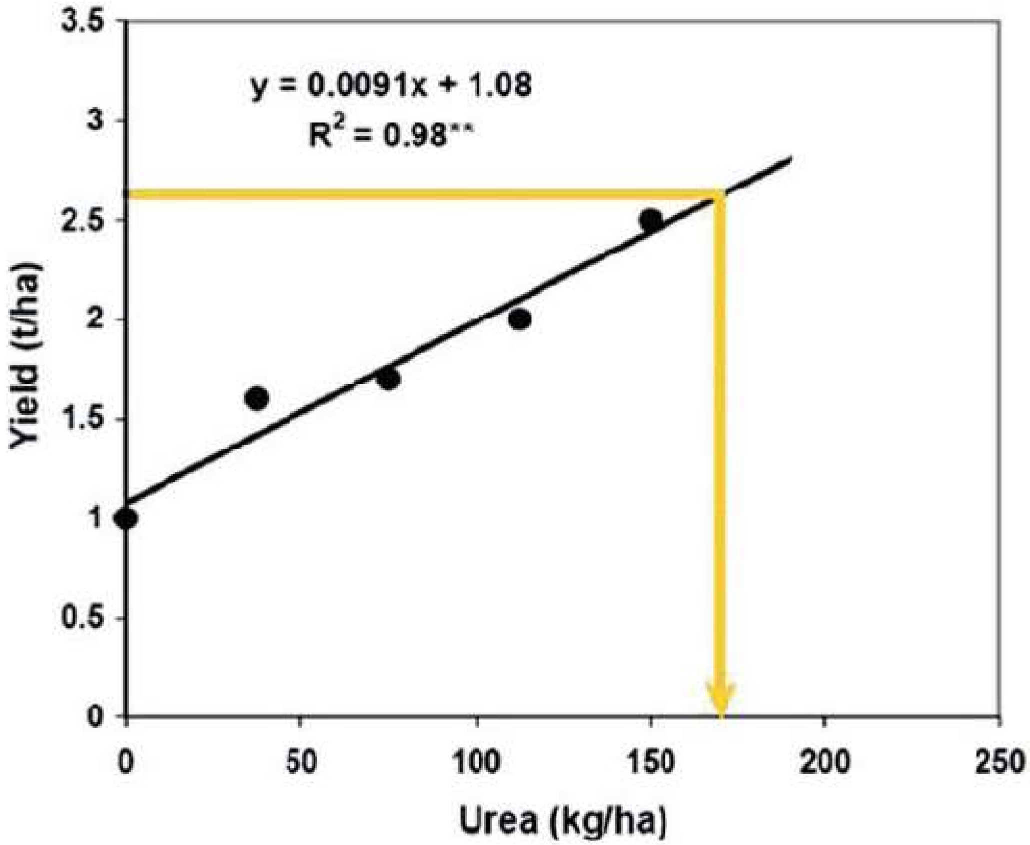

Figure 1 Yield response of soybean to different levels of urea fertilizer. The yellow arrow indicates the yield obtained with inoculation which marginally superseded that obtained with the highest level of urea. (Reproduced from Kulasooriya *et al.*, 2017)

A similar yield response curve was also obtained with vegetable bean (*Phaseolus vulgaris*). In this experiment measurement of weed biomass under the different treatments showed a 60% reduction in weed growth under inoculation in comparison to the full N-fertilizer application. This is understandable because nitrogen fixed by root nodules is largely available to the host legume while broadcast urea is freely available to all plants including the more aggressive weeds.

Rice grain yield increased by 24% (Table 1)with the biofilm-biofertilizer practice over farmers' CF alone practice (P = 0.002) due to improved grain filling as reflected from increased TGW (P = 0.063). Enhanced plant growth with the biofilm-biofertilizer application may have contributed to this, as shown by increased SDW (P = 0.000) and RDW (P = 0.017). Increased END more than ED with the biofilm-biofertilizer practice over farmers' CF alone practice (P = 0.082) seem to be responsible for this process as END's role in plant growth is well known (Hardoim, van Overbeek and van Elsas, 2008). Generally, EDs' role is to fuel communities of ENDs by supplying biologically fixed N_2 for their growth and functions.

Table 1: Plant and endophytic microbial parameters of the biofilm-biofertilizer practice and farmers' CF practice of rice cultivations. Plant samples were collected at 50% flowering whereas yield parameters were obtained at physiological maturity.

Parameter	Biofilm-biofertilizer practice (n = 37)	Farmers' CF alone practice (n = 37)	Difference*
Grain yield (kg/ha)	5860 ± 243	4733 ± 173	1127 (0.002)
Thousand grain weight (g)	20.92 ± 0.65	17.73 ± 0.72	3.19 (0.063)
Shoot dry weight (g/m^2)	3293 ± 245	2150 ± 135	1143 (0.000)
Root dry weight (g/m^2)	1130 ± 100	733 ± 128	397 (0.017)
Endophytic diazotrophs (colony count per plate)	191 ± 38	106 ± 30	85 (0.121)
Endophytic non-diazotrophs (colony count per plate)	56 ± 5	43 ± 5	13 (0.082)

Mean ± SE values in each column. *Values within parentheses are probability levels at which differences are significant.

Discussion

Results obtained with all the legume food crops tested in farmers' fields in different locations in Sri Lanka have confirmed that rhizobial inoculants have the capacity to completely replace urea application without any reduction in crop yields and sometimes with marginal increases.Currently our project annually supplies rhizobial inoculants to about 10,000 acres of legume crop cultivations and the demand for inoculants by farmers show an increasing trend.

Application of biofilm-biofertilizer increases ENDs among other things, which contribute to hormone production, bio-control of pathogens etc., thus supporting the increase of plant growth and yield. Under large scale rice cultivations in farmers' fields, biofilm-biofertilizer can replace at least 50% of all three N, P & K fertilizers without any reduction of the harvest and sometimes with increase in crop yields. This biofertilizer practice has now been applied to thousands of acres of the rice cultivation in the country. Limited field trials conducted with biofilm-biofertilizer have also shown that at least a 50% reduction of N, P & K chemical fertilizer application to corn, several vegetable and fruit crops as well as plantation crops like tea can be achieved without any reduction in crop yields.

Conclusions

Application of biofertilizer inoculants has a tremendous potential to enhance eco-friendly agriculture. This is bound to receive a boost as the new Government of Sri Lanka had decided to embark upon an ambitious program from this year to minimize the application of chemical fertilizer in crop cultivation by adopting alternative non-chemical inputs.

References

Hardoim, P.R., van Overbeek. L.S. & van Elsas, J.D. 2008. Properties of bacterial endophytes and their proposed role in plant growth. *Trends in Microbiology*,16: 463-471.

Jayatilake, N., Mendis, S., Maheepala, P. & Mehta, F.R. 2013. Chronic kidney disease of uncertain aetiology: prevalence and causative factors in a developing country. *BMC Nephrology*, 14(1): 1-13

Jeyakumaran, N. 2013. Priorities for cancer prevention in northern region of Sri Lanka. In: *Voice for Change, Jaffna Manager's Forum,17*: Agrarian Services Lane, Nallur, Jaffna: 105 – 115.

Kulasooriya, S. A.2017. Toxin producing freshwater cyanobacteria of Sri Lanka. *Ceylon Journal of Science (Biological Science)*, 46: 3 – 16.http://doi.org/10.4038/cjs.v46i1.7413

Kulasooriya, S. A.,**Seneviratne Gamini & Ekanayake E. M. H. G. S.** 2017. Soil Microbial Diversity and Its Utilization in Agriculture in Sri Lanka. Chapter 9. In: J. K. Patra et al, eds. *Microbial Biotechnology*, pp. 203- 224. Springer Nature, Singapore Pte Ltd. https://doi.org/10.1007/978-981-10-6847-8_9.

Rennie, R.J. 1981. A single medium for the isolation of acetylene-reducing (dinitrogen-fixing) bacteria from soils. *Canadian Journal of Microbiology*, 27: 8-14.

Somasegaran, P. & Hoben, H. J. 1994.*The Handbook for Rhizobia: Methods in Legume-Rhizobium technology.* New York, Springer-Verlag.

Seneviratne, G., Van Holm L. H. J. & Ekanayake, E. M. H. G. S. 1999. Effect of peat and coir-dust based rhizobial inoculants on the nodulation, plant growth and yield of soybean (*Glycine max L*. Mettil CV PB1). *Trop Agric Research & Extension,* 2: 136 - 139.

Seneviratne, G., Jayasekera, A. P. D. A., De Silva, M. S. D. L. & Abeysekera, U. P. 2011. Developed microbial biofilms can restore deteriorated conventional agricultural soils. *Soil Biology & Biochemistry*,43: 1059 – 1062.