

Evaluation of biological commercial inoculants on soybean production in Bungoma county, Kenya

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Abstract Nitrogen (N) and Phosphorus (P) are the major nutrients limiting crop production in sub-Saharan Africa (SSA) leading to food insecurity. Replenishing these soil nutrients is a major capital investment, which partly answers the question of sustainable crop production. Commercial inoculants (e.g. rhizobial and mycorrhizal inoculants) enhance the productivity of specific legumes (soybean). On-farm demonstration trials were conducted during the 2010 short rains to evaluate the effects of these commercial inoculants on soybean production in Bungoma county, western Kenya. Inoculated soybean seeds with rhizobia (Legumefix) and one of the mycorrhizal inoculants were planted in plots of 4.5 m x 5.0 m with treatments applied in RCBD per farm at seven sites. Each farm was a replicate in a multi locational design. Analysis of Variance was done to determine the effects of the products on soybean nodulation and grain yields. Results showed that there were significant ($P < 0.05$) differences in nodulation at 50% podding. Treatments Legumefix + Myco Tea (1.3 g plant⁻¹), Legumefix + Myco Apply soluble Maxx (1.2 g plant⁻¹) and Legumefix + Endorize (1.17 g plant⁻¹) had significantly higher nodulation compared to the control (0.44 g plant⁻¹). At harvest, grain yields differed significantly $p < 0.05$ among the treatments with Rhizatech + Legumefix giving (0.65 t ha⁻¹) and Legumefix + P (0.62 t ha⁻¹) as compared to control which (0.26 t ha⁻¹). These results shows that Legumefix contributes significantly to soybean production and a positive effect is realised when combined with either inorganic P or other commercial inoculants. Use of agricultural commercial inoculants is an environmental friendly and economically sound means of improving soil fertility.

Key words: Biological nitrogen fixation, commercial inoculants, *Glycine max* L. (Merrille), Mycorrhiza, nodulation, Rhizobia

Introduction

Nitrogen (N) and phosphorus (P) are widely deficient in SSA resulting in increased food insecurity in the region. Technologies to overcome these nutrient deficiencies have been developed and include the use of inorganic and organic fertilisers and their combinations (Okalebo *et al.*, 2006). The high prices of fertilisers and demand for food have further constrained food production while attempts to restore N and P to their original levels in soils have not been successful especially for the resource poor smallholder farmers (Buresh *et al.*, 1997, Giller *et al.*, 1997). Soybean [*Glycine Max* L. (Merrille)] is one of the most popular legumes in the world. It is a multipurpose crop grown for industrial oil production, human food, livestock feed, soil fertility improvement, income generation for small holder farmers, and more recently, as a source of bio-energy (Myaka *et al.*, 2005). Unlike most other legumes that contain about 20% protein, soybean contains 40% (Greenberg & Hartung, 1998). Soybean varieties currently grown in Kenya produce low yields, commonly 0.2 t ha⁻¹ (Nekesa, 2007). The low production particularly in western Kenya is due to low soil fertility, especially N and P deficiencies, minimum use of inputs because of high fertiliser prices and lack of interest by farmers due to limited knowledge on soybean utilisation. Soybean production in Kenya can be improved by use of chemical, organic

and biofertilisers (commercial inoculants). The biofertilisers are relatively simple and installation cost is very low as compared to chemical and inorganic fertilisers. Biofertilisers contain microorganisms which play a major role in increasing nutrient availability for high yield and reducing production cost by limited chemical fertiliser application. Phosphorus biofertilisers could help increase the availability of phosphate for plant growth by solubilisation, promoting the plant growth through production of plant growth-promoting substances, and by increasing the efficiency of biological nitrogen fixation (Kucey *et al.*, 1989). Rhizobia, also a biofertilisers, boosts the natural population of beneficial N-fixing bacteria to form effective nodules that are responsible for effective Biological Nitrogen Fixation (BNF) process. Although the role of P fertilisers in nodulation, N fixation, nutrient-use efficiency and growth of soybean has been reported (Israel, 1987; Tsvetkova & Georgiev, 2003; Israel, 2006; Miao *et al.*, 2007), information is scanty regarding the interactions of N-fixing bacteria and P Solubilising Bacteria (PSB) or Arbuscular Mycorrhizal Fungi (AMF) in P bioavailability on improving soybean production in western Kenya.

In an attempt to overcome the problem there is need for co-inoculation of legume seed (soybean) to enhance the efficiency of BNF process. The International Institute of Tropical Agriculture (IITA) Nigeria developed soybean genotypes designated TGX (Tropical Glycine Cross),

which have been tested in many African countries without N fertilizer. Results from the trials have indicated that indigenous rhizobium inoculants do not meet N demand of the tested TGX genotypes (Sanginga *et al.*, 1996). This technology, which involves use of bio inoculants, is the most untapped and poorly understood resource for nutrient acquisition especially N and P in Agriculture, (Johnston *et al.*, 1991). The progress in utilising this resource is incomplete, (Sanginga & Woomer, 2009), and the fundamental reason underlying this slow progress is lack of suitable methods for identifying and evaluating these products under field's conditions, thus need for research study. Many commercial inoculants exist in the market in SSA, which claim increases in crop productivity. They appear to have benefits for wide range of legumes and to substantially increase yield and quality produce. The inoculants need to be tested independently to ascertain their efficiency in different areas like Western Kenya and under different soil types.

A study was conducted to enhance the understanding of using the commercial inoculants in relation to yields. The specific objectives were; co-inoculation of soybeans using rhizobia inoculant (Legumefix) and any of the PSB or AMF inoculants, which improve soil N and P status in the region and the overall food production, through the use of promiscuous soybean variety that nodulate and fix N₂ with inoculated Rhizobial strain, PSB and Mycorrhizal fungi in acid soils of western Kenya.

Materials and Methodology

On-farm demonstration trials were conducted during the 2010 short rains (October - December) to evaluate the effects of commercial inoculants on soybean production in Bungoma county, western Kenya. The annual average rainfall ranges from 1250 to 1800mm and the average altitude is 1,200 m above the sea level. The mean annual temperatures vary between 18°C to 25°C, (Republic of Kenya, 1997). Initial soil characterization was done to analyze the soil pH, % organic carbon, available P (Olsen) and particle size distribution in the soil (depth: 0 – 15 cm) before application of inoculants. Co-inoculated soybean (TGX 1740-2F) seeds with Rhizobia inoculant (Legumefix) and 26 kg P ha⁻¹ or either of the PSB or AMF inoculants (Table 1), were planted in plots of 4.5 m × 5 m and compared against a control (without inoculation). The treatments were arranged in RCBD per farm at seven sites with each farm serving as a replicate in the multi-location trial.

Soybean TGX 1740-2F exhibits promiscuous nodulation, early to medium maturity and grown for grain yield rather than for biomass production.

Nodulation and yield assessment. An area of 0.75m² was identified within the net plot (12m²) and soybeans within the area cut at the ground surface. The roots of the cut plants at 50% podding were carefully removed and washed to separate the nodules from the roots. Above ground fresh weight biomass, total number of nodules, fresh weight nodules and fresh weight roots were recorded. The

nodules were then taken to Nairobi immediately for further analysis.

Analysis of variance was done to determine the effect of the products on soybean nodulation grain yield at harvest. The data were analyzed using statistical analysis system (SAS) and significance assessed at 95% confidence level.

Results and Discussion

Soil analysis (Okalebo *et al.*, 2002) results for the seven farms prior to the application of treatments are presented in Table 2. Only means are recorded, soil pH was 5.38 indicating moderate acidity according to the classification carried out by Kanyanjua *et al.* (2002). Soils from these sites had P deficiency with a mean value of 8.54 mg P kg⁻¹. This value is below the critical value of 10 mg P kg⁻¹ by the Olsen method and the soils would thus respond to P fertilisation. At 1.35%, soil organic carbon content was also low, suggesting that crop N availability was problematic.

Fresh weight nodules as affected by the application of the treatments. At 50% podding, the highest mass of nodules were observed in treatments T4 or Legumefix + Myco Tea (1.3 g plant⁻¹), T9 or Legumefix + Myco Apply soluble Maxx (1.2 g plant⁻¹) and T7 or Legumefix + Endorize (1.17 g plant⁻¹). All other treatments which were Legumefix(0), Legumefix(P), Mycotea +Legumefix, PHC Biopak + Legumefix and Endorize + Legumefix had nodule fresh weights per plant ranging from 0.9 g to 1.0 g/plant. The negative control gave the least fresh weight nodules in grams per plant (0.44 g/plant), as illustrated in Figure 1.

Grain yields. Highest grain yield of 0.65 and 0.62 t ha⁻¹ were obtained from T3 or Legumefix + Rhizatech and T10 or Legumefix + 26 kg P ha⁻¹, respectively (Fig. 2). These posted significantly (P<0.05) higher grain yields than for the control (T8), which had 0.26 t ha⁻¹ (Fig. 2). However, there were no significant (P>0.05) between the other treatments with respect to grain yield.

Discussion

Legumefix is a rhizobia inoculant which boosts the natural population of beneficial nitrogen-fixing bacteria to form effective nodules that are responsible for effective BNF process (Jen-Hshuan Chen, 2008). This explains the over 100% increase in fresh weight nodules obtained in all the treatments in which the inoculants were applied compared to the control plot (Fig. 1). This shows the importance of rhizobia, PSB and AMF in the process of nodule formation and N fixation through phosphorus bioavailability in the root zone.

With right climatic and soils conditions, soybean can yield relatively well. Rienke & Joke (2002) reported 1.5 to 2.5 t ha⁻¹ in Nigeria and South America, respectively. Particularly in Brazil yields as high as 4 t ha⁻¹ have been reported (R.J. Okalebo, pers com.). In this work, the highest

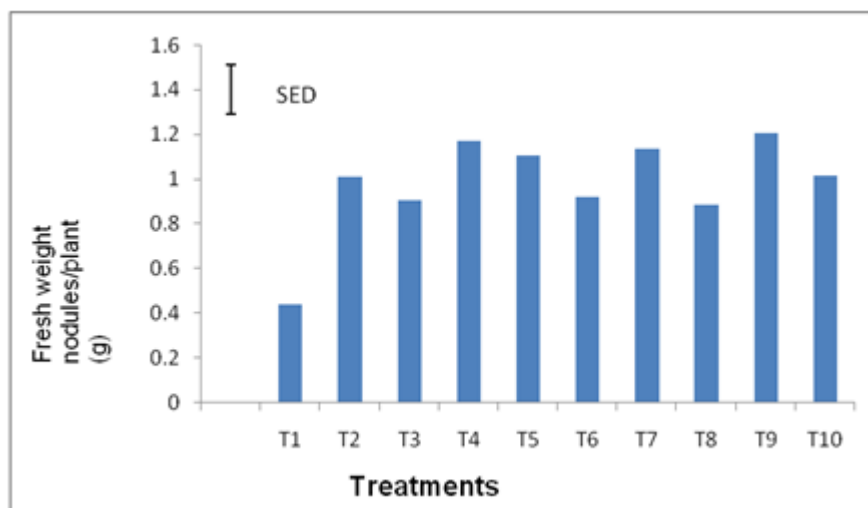
Table 1. Bio inoculants used and there active ingredients installed in short rains 2010 in Bungoma.

Trit code	Product	Treatment description	Quantity applied
T1	Legumefix	<i>Bradyrhizobium japonicum</i>	400 g/100 kg of soybean seed
T2	Legumefix + Myco ApplyEndo	Endo:Glomus intraradices, Glomus Mosseae, Glomus aggregatum and Glomus etunicatum; Soluble Endo:Glomus intraradices, Glomus Mosseae, Glomus aggregatum and Glomus etunicatum T7Endo Plus: Glomus intT8raradices, Glomus mosseae, Glomus aggregatum and Glomus etunicatum (30 propagules/cc <i>Trichoderma konigii</i> and <i>Trichoderma harzianum</i>)	2.25 kg/ha
T3	Legumefix + Rhizatech	Spores and mycelial fragments of AMF (50 propagules/cm ³)	60 kg/ha
T4	Legumefix + Myco tea	<i>Trichoderma lignorum</i> , <i>Chaetomium globosum</i> , <i>Verticillium lecanii</i> , <i>Paecilomyces lilacinus</i> , <i>Penicillium chrysogenum</i> , <i>Azotobacter chroococcum</i> , <i>Bacillus polymyxa</i> and <i>Saccharomyces cerevisiae</i>	1 kg/ha
T5	Legumefix + PHC Biopak	<i>Bacillus azotofixans</i> , <i>Bacillus licheniformis</i> , <i>Bacillus megaterium</i> , <i>Bacillus polymyxa</i> , <i>Bacillus subtilis</i> , <i>Bacillus thuringiensis</i> (7.5 x 10 ⁹ CFU/g each)	2 kg/ha
T6	Legumefix + Subtilex	<i>Bacillus subtilis</i> , MBI 600 strain (5.5 x 10 ¹⁰ spores/mL, 2.75%)	10.5 g/45 kg of soybean seed
T7	Legumefix + Endorize premium	Arbuscular Mycorrhizal Fungi, <i>Glomus spp.</i>	2 Kg/ha
T8	None		
T9	Legumefix + Myco Apply soluble MAXX	Endomycorrhiza(4 <i>Glomus spp</i>)Ectomycorrhizal (7 <i>Rhizopogon spp</i>)	448 g dissolve into 600litres of water (Apply 1.9 L /planting hole)
T10	Legumefix + inorganic P	<i>Bradyrhizobium japonicum</i>	400 g/100 Kg of soybean seed

Table 2. Mean antecedent soil physical and chemical characteristics (0-15 cm) from farmers' fields in Bungoma county, western Kenya, 2010.

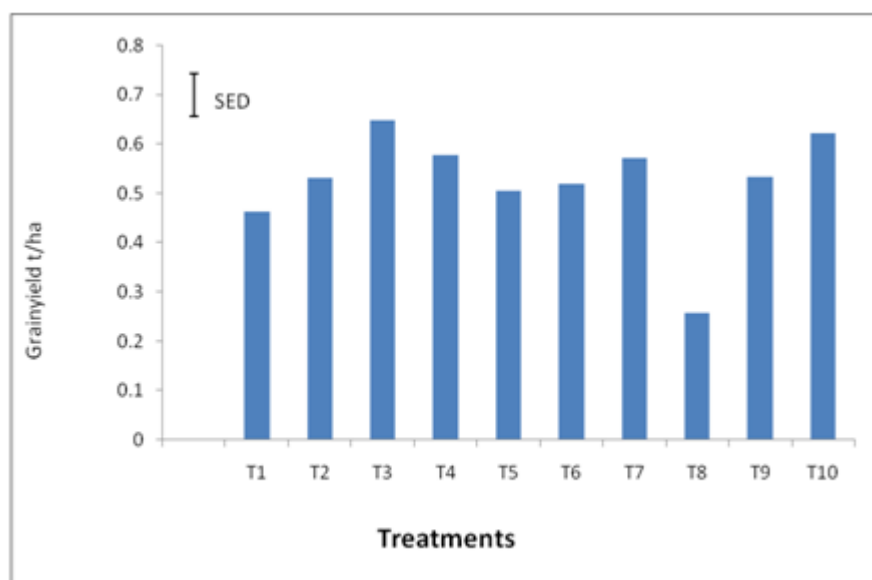
Soil property	Mean value
Textural class	Four farms had Sandy Clay Loam while 3 farms had Sandy Loam
pH (1:2.5 soil : water)	5.38
% Organic carbon	1.35
Olsen P (mg/kg)	8.54

yields were only 0.65 t ha⁻¹ where Legumefix + Rhizatech were applied. The generally low yields could be attributed to hailstones that severely affected the soybean earlier in the season and inadequate rainfall during grain filling phase later in the season. Low yields especially in the treatments where no P was applied could be attributed to low initial P of the sites. The element P is required for nodulation and consequently N fixation, and thus its low concentrations in the soil could have contributed to the observed low yields.



Key: T1– Legumefix(0), T2– Legumefix(P), T3– Rhizatech + Legumefix, T4– Myco Tea + Legumefix, T5–PHC Biopak + Legumefix, T6– Subtilex + Legumefix, T7– Endorize Premium + legumefix, T8 - No inoculants, T9– Myco Apply soluble MAXX, T10- Myco Apply Endo + Legumefix, P – 26 Kg P/ha.

Figure 1. Higher nodulation at 50% podding obtained after using commercial inoculants.



Key: T1– Legumefix(0), T2– Legumefix(P), T3– Rhizatech + Legumefix, T4– Myco Tea + Legumefix, T5– PHC Biopak + Legumefix, T6– Subtilex + Legumefix, T7– Endorize Premium + legumefix, T8 - No inoculants, T9– Myco Apply soluble MAXX, T10- Myco Apply Endo + Legumefix, P – 26 Kg P/ha.

Figure 2. Soybean grain yield (t/ha) at harvest after using biological commercial inoculants.

Better nodule formation resulting to high yields in some of the plots like the one applied with Legumefix + Rhizatech was probably due to the mycorrhizal fungi in Rhizatech that form a network of fungal hyphae around the feeder roots of the soybean plants thereby, increasing the surface area for absorption and translocation of essential plant nutrients (Jen-Hshuan Chen, 2008). PSB inoculants (Mycotea, Subtilex and PHC Biopak) co-inoculated with Legumefix gave yield increases of 92% - 123% compared to the control plot. When compared to the plot applied with sole Legumefix over 76% yield increases were realized. Bungoma soils range from moderate to highly acidic, and under such soil conditions large amounts of P are fixed in the soil thus unavailable to plants. PSB inoculants contain free-living bacteria that break down inorganic soil phosphates to simpler forms that enable P uptake (Sundara *et al.*, 2002). This explains the significant increase in yield as compared to control experiment plot after using PSB inoculants.

Treatments with AMF inoculants (Rhizatech, Endorize Premium, Myco Apply soluble MAXX and Myco Apply Endo) combined with Legumefix, gave relatively higher soybean grain yield. Yield increase of over 100% and 40% when compared to the negative control and that with Legumefix alone, respectively was observed. The control plot had the least yield of 0.26 t ha⁻¹ due to deficiency of macro nutrient elements especially N and P in the soil.

Conclusions

Use of rhizobia inoculant on soybean production resulted to 40% increase in grain yield when compared to the control. Higher yields were further obtained after co-inoculating the AMF inoculants with the rhizobia inoculant. Therefore, farmers should be taught on the use of these inoculants, especially AMF inoculants co-inoculated with rhizobia inoculant to increase soybean production in Bungoma county. This will reduce low soybean yield and will also be a source of income to poor smallholder farmers in the region.

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References

- Buresh, R.J., Smithson, P.C. & Hellums, D.T. 1997. Building soil P in sub Saharan Africa.
- Giller, K.E., Beare, H.M., Lavelle, P., Izac, A.M.N. & Swift, M.J. 1997. Agricultural intensification, soil biodiversity and ecosystem function. *Applied Soil Ecology* **6**, 3-16.
- Greenberg, P. & Hartung, H.N. 1998. *The Whole Soy Cookbook: 175 Delicious, Nutritious, Easy-to-Prepare Recipes Featuring Tofu, Tempeh, and Various Forms of Nature's Healthiest Bean*. Three Rivers Press, New York.
- Kanyanyua, S.K., Ireri L., Wambua, S. & Nandwa, S.M. 2002. Acidic soils in Kenya: constraints and remedial options. Kenya Agricultural Research Institute (KARIA) Technical Note No.11..KARI Headquarters, Nairobi, Kenya.
- Israel, D.W. 1987. Investigation of the role of phosphorus in symbiotic dinitrogen fixation. *Plant Physiology* **84**(3), 835-840.
- Israel, D.W. 2006. Symbiotic dinitrogen fixation and host-plant growth during development of and recovery from phosphorus deficiency. *Physiologia Plantarum* **88**(2), 294-300.
- Jen-Hshuan Chen. 2008. The combined use of chemical and organic fertilizers and/or biofertilisers for crop growth and soil fertility. Paper presented at the International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use 16 – 20 October 2006. Land Development Department, Bangkok 10900 Thailand.
- Jonhson, N.C., Pfleger, F.L., Crookston, R.K. Simmons, S.R. & Copeland, P.J. 1991. Vesicular Mycorrhiza response to corn and soybean cropping history. *New Phytologist* **117**(4), 657-663.
- Kucey, R.M.N., Janzen H.H. & Leggett, M.E. 1989. Microbially mediated increases in plant-available phosphorus. *Advances in Agronomy* **42**, 199-228.
- Miao, S.J., Qiao, Y.F., Han, X.Z. & An, M. 2007. Nodule formation and development in soybeans (*Glycine max* L.) in response to phosphorus supply in solution culture. *Pedosphere* **17**, 36-43.
- Myaka, F.A, Kirenga, G. & Malema, B. (eds.). 2005. *Proceedings of the First National Soybean Stakeholders Workshop*, 10-11 November 2005, Morogoro, Tanzania.
- Nekesa, O.A. 2007. Effect of Mijingu Phosphate Rock and Agricultural Lime in Relation to Maize, Groundnuts and Soybean Yields on Acid Soils of Western Kenya. Mphil Thesis, Department of Soil Science, Moi University, Eldoret, Kenya.
- Okalebo, J.R., Gathua, K.W. & Woomer, P.L. 2002. *Laboratory Methods of Soil and Plant Analysis: A Working Manual*, 2nd Edition. TSBF-CIAT and SACRED Africa, Nairobi, Kenya.
- Okalebo, J.R., Othieno, C.O., Woomer, P.L., Karanja, N.K., Semoka, J.R.M., Bekunda, M.A., Mugendi, D.M., Muasya, R.M., Bationo, A. & Mukhwana, E.J. 2006. Available technologies to replenish soil fertility in East Africa. *Nutrient Cycling in Agroecosystems* **76**, 153-170.
- Sanginga, N. & Woomer, P.L.(eds.). 2009. *Integrated Soil Fertility Management in Africa: Principles, Practices and Developmental Process*. TSBF-CIAT, Nairobi, Kenya.
- Republic of Kenya 1997. *Uasin Gishu District Development Plan (1997-2001)*. Office of the Vice President and Planning and National Development. Government Printer, Nairobi, Kenya.

- Rienke, N. & Joke, N. 2002. Cultivation of soya and other legumes. First Edition Agromisa Foundation, Wageningen. 69p.
- Tsvetkova, G.E. & Georgiev, G.I. 2003. Effect of phosphorus nutrition on the nodulation, nitrogen fixation and nutrient use efficiency of *Bradyrhizobium japonicum*-soybean (*Glycine max* L. Merr.) symbiosis. Bulgarian Journal of Plant Physiology (Special Issue). pp. 331–335.
- Sundara, B., Natarajan, V. & Hari, K. 2002. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields. Field Crops Research **77**, 43-49.