Growth and Yield Response of Inoculated Soybean (*Bradyrhizobium japonicum*) to inorganic fertilizer (soil and foliar application) in the Northern Savannah zone of Ghana.

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Abstract

The need for a balanced nutrient supply for soybean cultivation at various growth stages cannot be underestimated. Reason being that research has confirmed that despite the ability of legumes to fix atmospheric nitrogen, it requires sole level of nutrient to start up with at the initial vegetative stage when the roots are yet to be well established, especially in low soil fertility areas. More so at certain growth stage soybean leaves experience senesces and nodule rupture. Therefore, there is need to supply balanced nutrients so has to sustain soybean growth at those critical periods of it's development. Hence, on-station trials (Nyankpala) were conducted at the experimental fields of CSIR-Savannah Agricultural Research Institute (SARI), to ascertain the effectiveness of some commercial fertilizers (soil applied inorganic (NPK), foliar applied inorganic (Boost-xtra) and bio-fertilizer (Bradyrhizobium japonicum) for improvement of soybean productivity in the Northern savannah zone of Ghana. Eight treatments were used for each study site; T₁=Boost xtra (BX), T₂=BX+Bradyrhizobium japonicum (INO), T₃=25 and T₈=Control which were laid out in a Randomized Complete Block Design (RCBD) replicated four times. Experimental plots measured 7 m x 7 m in 2012 and 3 m × 4.5 m in 2013. A consistent significant (P<0.05) response of soybean nodule number, nodule dry weight, shoot biomass yield, pod number, pod weight and grain yield increase (101 and 90%) in 2012 and (76 and 66%) 2013 to the combined application of INO+NPK and INO+NPK+BX respectively, over the sole application of each of the fertilizers and the Control, and in some cases INO+BX is an indication that indeed the use of combine fertilizers applied to soybean at different growth stages and different application mediums (soil, foliar and seed) are paramount in soybean cultivation in the study area. Nevertheless, the application of foliar fertilizer gave the best yield in 2012 which was 103% as compare to the control.

KEY NOTE: Inorganic fertilizer: Soil and Foliar applications, Bio-fertilizer, Soybean, Growth and Yield.

Introduction

Plants, like other living things, need food (both macro and micro nutrients) for effective growth and development. Hence, for optimum plant growth, nutrients must be balanced or in other words the soil must have nutrients that are needed for plants (Chen, 2006). The availability and uptake of nutrients are therefore very important especially in depleted soils of sub - Saharan Africa of which Ghana is no exception. The need to improve and increase grain yield of soybean cannot be overemphasized because

soybean possess lots of benefits to human, animal and soil fertility replenishment programmes. It can regulate the process which usually under low soil nitrogen conditions triggers nodule formation through a high energy demanding process; a process reported by Albareda et al., (2009) as being cheaper and environmental friendly. Unfortunately, the effectiveness of the microbes (symbiosis) according to Abaidoo et al. (2007) and Chalk et al. (2010) is specific and depends upon the soybean genotype and rhizobia strains under various edaphic and climatic conditions. Hence,

despite the known ability of legumes to fix nitrogen atmospheric in symbiotic association with rhizobia, it has been demonstrated that supplementary lead fertilization can to improved performance of these crops (Mallarino, 2005). It has been suggested that when a nutrient such as nitrogen is deficient in soil. it is more energy efficient for the plant to take up from available soil nitrogen sources, matter, manure organic or fertilizer application (Matt, 2009). Apart from soil application, foliar spray of nutrients has also proofed to be a practical means of replenishing the reservoir of nutrients in the leaves of legumes during pod development. since the efficiency of nutrient uptake by roots as well as symbiotic fixation activities are known to decline at this stage (Ashour and Thalooth, 1983). Furthermore, to reduce application to fertilizer soil. formulations of foliar fertilizer (micro - or macro - nutrients or both) are available worldwide and could be more effective than soil applied fertilizer in reducing effect of nutrient deficiencies on BNF (Zahran, 1999). Arief et al. (2006) confirm that based on soil properties, foliar spraying could be effective 6 to 20 times as compared to soil application. Therefore, for a legume (soybean) to be efficient in growth and yield improvement, production sustainability measures, aimed at maintaining soil health and sustaining crop production becomes paramount.

It is therefore important that an enabling by supplying is created environment balanced nutrients required at the right time, in the required quantity, coupled with the application of rhizobium inoculant strain, especially when the soil has low indigenous rhizobia population and fertility due to many years of soil degradation (Hansen et al., 1995). Although the required nutrients for soil fertility replenishment are available in the market for farmers, however the efficacy of these products has often not been broadly substantial investigated. lack Farmers knowledge on the benefits of agro inputs in soybean markets especially for production. Because, they assumed that legumes (soybean) do not require fertilizer at all, based on their experience in crop rotation year in year out. Moreso the use of foliar spray is a new method for crop feeding, which nutrients in form of liquid are used into leaves (Nasiri et al; 2010). It is thus important that these commercial products are evaluated before being recommended for farmers use in areas where the knowledge of their use is minimal.

Objective

To determine the influence of inorganic fertilizers (NPK, applied through the soil) and (boost xtra, applied through the leaves (foliar fertilizer)), and bio-fertilizer (*Bradyrhizobium japonicum* inoculation) on soybean nodulation, growth and yield.

Materials and Methods

This experiment was carried out at the experimental field of CSIR-Savanna Agricultural Research Institute (Nyampala), located about 16 km west of Tamale, and lies on latitude 09° 23'22.4" N and longitude 01° 00' 12.1" W, at an elevation of 195 m above mean sea level of the interior Guinea Savanna agro - ecological zone of Ghana. The rainfall is mono - modal (April / May - October), and a dry season with severe harmmattan wind occurring between December and January. The total annual rainfall ranges from about 1,000 to 1,200 mm (SARI, 1996) and the annual temperature ranges from a minimum of 13 °C to a maximum of 40 °C, with a mean of 28 °C. The experimental field used in 2012 had been previously cultivated to hot pepper for three consecutive years, while the field used in 2013 has been cultivated to maize in the previous years. The soil of the study area is Tingoli series classified as Ferric Luvisol (FAO/UNESCO, 1988).

Soil sampling and preparation

Soil samples for laboratory analyses were taken from (0 - 20 cm depth) the experimental sites prior land preparation. The samples were taken randomly across each field using a soil augur following a 'W' design per replicate block. Samples were then thoroughly mixed, air-dried and composite

samples taken for chemical and physical analyses and biological assays using standard protocols

Determination of soil chemical and physical properties

Soils collected from the experimental field were analyzed for pH in a 1:2.5 suspension of soil to distilled water (soil:water) ratio using the electrometric method (Page et al., 1982), organic carbon content by the modified Walkley and Black procedure (Nelson and Sommers. 1982), for total N the macro Kjeldahl method involving digestion and distillation procedure according to Bremner and Mulvaney, (1982) was used, available phosphorus by Bray 1 (Bray and and Kurtz, 1954) and potassium using flame photometry as described by Helmke and Sparks (1996). Copper, iron and manganese in the soil were determined using the diethylenetriamine pentaacetic extraction method. Ten (10) grammes air dried soil was weighed into separate plastic bottles for Cu, Fe and Mn after which hundred milliliters DPTA extract was added to each. It was shaken for two hours and filtered with Whatman No. 42 filter paper. Their values were all read on an Atomic Absorption Spectrophotometer using the appropriate standards. Particle distribution was determined the hydrometer method (Day, 1953).

Estimation of Indigenous Rhizobia Population

The most-probable-number (MPN) method [65] was used to determine the population of native rhizobia by the most probable number enumeration system (MPNES).

Land preparation and layout

The land was ploughed, harrowed and ridges were constructed mechanically in the two years. Plots measuring 7×7 m² were demarcated for planting in 2012 and 3×4.5 m² in 2013. An alley of 2 m between plots and 3 m between blocks were constructed.

Inoculation

Soybean seeds (var. Jenguma) were inoculated prior to planting with a peat based inoculum of *Bradyrhizobium japonicum* at the rate of 5 g kg⁻¹ seed using

the slurry method as described by Woomer et al. (1990).

Planting

Soybean seeds were planted at two seeds per hill on ridges made at 0.05 m within rows and 0.75 m between rows and covered with soil and thinned to one seed per hill two weeks later. Planting was done in June 2012 and 2013 starting with the un-inoculated plots followed by the inoculated plots to avoid contamination.

Treatments

The treatments used for the study were: T_1 =Boost xtra (BX), T_2 =BX + Bradyrhizobium japonicum, T_3 =25 kg N ha⁻¹, T_4 =25 kg N ha⁻¹ + B. japonicum (INO), T_5 =B. japonicum + 25 kg N ha⁻¹ + BX, T_6 =25 kg N ha⁻¹ + BX, T_7 = INO and T_8 =Control.

Nitrogen was applied as ammonium sulphate (25 kg N ha⁻¹) seven days after planting with triple super phosphate and muriate of potash (30 kg ha⁻¹) (applied as basal). Boost Xtra (foliar fertilizer) was applied at two weeks intervals (4 L ha⁻¹) from 50% flowering growth stage to advanced podding stage corresponding to 20% NPK, 1.5% MgO, 0.15%, 0.075% Mn, Fe and Zn, 0.0012%, Co and Mo.

Experimental design

Fertilizer application

The plots were laid in Randomized Completely Block Design (RCBD), with the treatments replicated four times.

Data collection Nodulation

Ten soybean plants at 50% flowering growth stage from each net plot were carefully uprooted from each experimental plot by digging around the plant using a spade and washed with clean tap water to remove all attached soil from the roots and the nodules were carefully picked. The detached nodules from the roots were counted and oven – dried at 70 °C for 48 hours. The dry weights of the nodules were then recorded.

Determination of shoot dry weight

The shoots of the ten plants used for the nodule sampling were separated from the roots. They were then dried in the oven at 70

°C for 72 hours. After the measurement of the shoot dry weight, the shoot was separated into leaves and stems and the two organs were grounded separately. One volume of milled leaves and two volumes stems were mixed for the measurement of the shoot N and phosphorus (P) content (IITA, 1982).

Determination of grain yield

Harvesting of matured soybean was done according to the plot size at physiological maturity stage, air-dried, threshed and winnowed. The grains were then dried in an oven at 60 °C for 72 hours and the dry weight recorded. One hundred (100) seed weight was recorded after drying at 60 °C for 72 h. The grain yield was then estimated from the dry weight of the grains as suggested by Okogun *et al.* (2005).

Statistical analytical procedure

The collected data were subjected to Analysis of Variance (ANOVA) using the GENSTAT version 9 (2007). Means comparison of treatments showing significant effect were separated using the least significant

difference (LSD) at 95% confidence level. Count data were transformed (Log) before running the analysis.

Results

The soils of the experimental sites were slightly acidic and low in all the soil nutrients measured (Table 1). The organic carbon (< 20 g kg⁻¹), total nitrogen (< 1 g kg⁻¹), exchangeable cations (< 5 cmol (+) kg-1), effective cation exchange capacity (< 5 cmol $^{(+)}$ kg⁻¹) and extractable P (< 10 mg kg⁻¹) were low. The MPN count of indigenous rhizobia population were estimated as 5.12×10^1 and 6.3×10^{1} cells g⁻¹ soil in 2012 and 2013 respectively. The rainfall trend during the planting season shows that in 2012 the rainfall commenced as early as May and was consistent till sometimes in July, then there was a reduction in August after which the rain picked up again till soybean maturity. While in 2013, erratic drop in rainfall was experienced and lasted for three months from August till soybean maturity (Fig.1).

Table 1. Physical and chemical properties and MPN counts of the experimental sites

Soil properties	Values	its of the experimental sit
compagnes of Best property for	2012	2013
pH (1:2.5 H ₂ O)	5.5	5.5
Organic carbon (%)	0.9	0.7
Total N (g kg ⁻¹)	0.5	0.3
Extractable P (mg kg ⁻¹)	5.7	7.4
Ca (cmol ⁽⁺⁾ kg ⁻¹)	2.30	
$Mg (cmol^{(+)}kg^{-1})$	0.71	2.92
K (cmol ⁽⁺⁾ kg ⁻¹)	0.06	0.65
Na (cmol ⁽⁺⁾ kg ⁻¹)	0.08	0.09
Mn (mg kg ⁻¹)	4.09	0.05
Cu (mg kg ⁻¹)	9.02	5.00
Fe (mg kg ⁻¹)	19.00	4.02
Exchangeable acidity (cmol ⁽⁺⁾ kg ⁻¹)		10.00
Sand (%)	0.73 68	0.58
Silt (%)		70
Clay (%)	24 8	20
Texture	Sandy loam	10 Sandy loam
MPN (cell g ⁻¹ soil)	5.12×10^{1}	6.3×10 ¹

MPN = Population estimate for indigenious soil rhizobia population

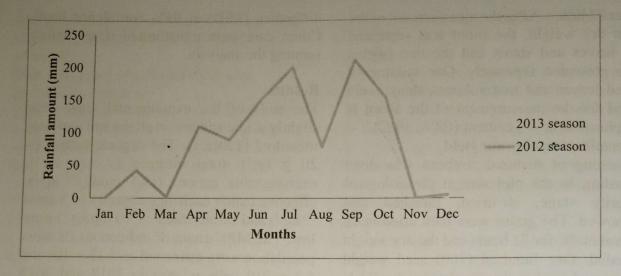


Fig. 1: Rainfall pattern in the study sites.

Table 2: Effect of inorganic fertilizer (soil and foliar) on inoculated soybean nodulation

Treatments	Nod. Number	Nod. Number plant-1		Nod. dry weight kg ha ⁻¹	
and the state of t	2012	2013	2012	2013	
BX	1.9	2.1	12.70	11.67	
INO+BX	2.3	2.5	21.23	17.00	
NPK	2.0	2.2	15.20	15.00	
INO+NPK	2.3	2.6	21.23	22.00	
INO+NPK+BX	2.3	2.4	23.10	23.00	
NPK+BX	1.8	2.4	13.60	19.67	
INO	2.2	2.4	21.23	15.00	
CONTROL	1.9	2.1	16.17	11.67	
CV	10	8.1	31.20	30.04	
LSD	0.37	0.33	10.51	11.93	

Soybean number of nodule at 50% flowering presented in Table 2 shows that the use of INO+BX, INO+NPK, and INO+NPK+BX resulted in the same and the highest nodule number (2.3 plant⁻¹) in the year 2012. Which were significantly (P<0.05) different from those obtained from sole application of the treatments (except inoculum) and the control leading to 21% increase in number of nodules over sole fertilizer application and control. Likewise in the year 2013, INO+NPK gave the highest number of nodule (2.6 plant⁻¹) followed by INO+BX (2.5 plant-1) which were 24 and 19% significantly higher than the control respectively, also

INO+NPK+BX, NPK+BX and sole INO (2.4 plant⁻¹ each) recorded 9%, 14% and 14% increase in number of nodule over sole application of NPK, BX and Control respectively. In both years the application of INO+NPK+BX recorded the highest nodule weight which resulted in 43 and 97% increase over the control in 2012 and 2013 respectively. Also INO+NPK, INO+BX and sole INO resulted in more than 30% nodule weight increase over the control in 2012, while the same combination of treatments gave 46% increase as compare to control in 2013.

Table 3: Effect of inorganic fertilizer (soil and foliar) on inoculated soybean biomass yield

	Biomass yield kg ha ⁻¹		
BX	2012	2013	
INO+BX	2196	1266	
NPK	2418	1607	
INO+NPK ·	3190	1476	
INO+NPK+BX	3618	2541	
NPK+BX	4580	2843	
INO	3666	1574	
CONTROL	1866	1378	
CV	1449	1218	
LSD	13.3	35.5	
	1082	1092	

The combination of inorganic fertilizer (soil and foliar) and inoculation (INO+NPK+BX) highest gave soybean biomass accumulation at the two locations in both years. The combination of INO+NPK+BX resulted in 2.7 and 12% biomass accumulation increase over the use of INO+NPK in the year 2012 and 2013 respectively. Also it resulted in 89 and 79% increase over INO+BX in the year 2012 and 2013 respectively. However. combinations of INO+NPK+BX, NPK+BX and INO+NPK were at par and were significantly higher than the other treatments used and the control in 2012. Likewise

INO+NPK+BX and INO+NPK were at par and were significantly (P<0.05) higher than the biomass yield from other treatments and the control in 2013. The treated plots with soil and foliar fertilizer (NPK+BX) performed better than INO only and produced 66 and 29% and above biomass increase as compared to control in 2012 and 2013 respectively. So also the biomass value recorded from plots treated with sole fertilizers were all lower than those from the combine fertilizer plots, while the control had the least biomass value in the two years (1449 and 1218 kg ha⁻¹) respectively.

Table 4: Effect of inorganic fertilizer (soil and foliar) on inoculated soybean pod

formation	SECTION AND LINES. HE			R CARLON MARKET	
Treatments	Pod num	Pod number plant ⁻¹		Pod weight kg ha-1	
	2012	2013	2012	2013	
BX	66	47	6178	5565	
INO+BX	85	45	6926	5005	
NPK	74	37	5924	4962	
INO+NPK	57	57	4645	5004	
INO+NPK+BX	61	60	5408	6602	
NPK+BX	58	43	5991	5088	
INO	65	57	4893	3228	
	50	36	3162	2523	
CONTROL	25.8	27.1	30.7	45.7	
CV		31.66	2895	2001	
LSD	29.19	31.00			

Soybean pod number was influenced by the treatments applied in 2012 and 2013. In the year 2012, INO+BX recorded the highest pod number (85 plant⁻¹) while, sole NPK and BX had 74 and 66 pods plant-1 respectively, the combination of INO+NPK+BX also recorded 22% increase in pod number as compare to control which produced the least pod number (50 plant⁻¹) in all. In the year 2013, the combination of INO+NPK+BX gave the highest number of pods (60 plant⁻¹) while INO+NPK and sole INO resulted in 57 pods plant⁻¹ each, which resulted in 67, 58 and 58% increase in pod number over control respectively. The pod gotten from the plots treated with INO+BX was weightier,

followed by sole BX in the year 2012, however they resulted in pod weights which were at par with the other treated plots while the control recorded the lowest pod weight (50 kg ha⁻¹). Likewise, in the year 2013 the pod weight trend was similar where plots treated with INO+NPK+BX, BX, NPK+BX, INO+BX and INO+NPK recorded pod weights of 6602, 5565, 5088, 5005, and 5004 kg ha⁻¹ respectively. The use of sole or combined fertilizer resulted in an increase in pod weight over the control in both locations and years hence, the control plot recorded the lowest pod weight (3162 and 2523 kg ha⁻¹) in both years respectively (Table 4).

Table 5: Effect of inorganic fertilizer (soil and foliar) on inoculated soybean grain yield

the limits of the orange of the same and	Seed yield kg ha ⁻¹		
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BX	2969	1113	
INO+BX	2729	999	
NPK	2486	1277	
INO+NPK	2940	2013	
INO+NPK+BX	2772	1559	
NPK+BX	1853	1432	
INO	1979	1129	
CONTROL	1462	940	
CV	20.4	30.4	
LSD	901.8	696.4	

The application of sole Boost xtra (foliar fertilizer) gave the highest yield in 2012 followed by INO+NPK which were (103 and 101%) significantly higher than control respectively. However, was at par with INO+NPK+BX, BX+INO and NPK which resulted in 90, 87 and 70% increase over control respectively. Likewise the combine use of the treatments resulted in 18 and 49% increase for INO+NPK as against sole NPK and sole INO. Also, in 2013 plots treated with INO+NPK recorded the highest seed yield (2013 kg ha-1) in all and was 78% significantly higher than the grain yield from other treated plots and the control, except INO+NPK+BX and NPK+BX produced 66 and 52% more grain yield over the control, the control remained the lowest

producing plot (940 kg ha⁻¹). INO+NPK gave 58 and 78% increase in grain yield than sole NPK and sole INO in the two locations and years respectively.

DISCUSSION:

The soil fertility status of the study area was generally low (Table 1). As confirmed by Buri et al., (2009) who reported that soil nutrient levels in the Savannah zones of Ghana are low with high pH value, low organic matter, nitrogen and available P levels. The most probable number count of rhizobia was moderate in the study locations according to the rating given by Thies (1991). The number and weight of nodule are commonly used as the criteria for effective complementary interaction between macro-

and - micro symbionts; thereby correlate on the whole with the rate of atmospheric fixation (Datsenko et al, 1997). However, the use of only inoculant (Bradyrhizobium japonicum) did not significantly improve nodule number relative to the control, suggesting that the rhizobial populations' nodulating soybean were moderately adequate in the study site (Table 1), yet are ineffective these indicates Bradyrhizobium japonicum inoculum alone was not enough to satisfy the vegetative take off requirement of soybean at the initial growth stage when (Chris et al. 1999) the root system is still poorly developed and the process of nodule initiation and N2 fixation are yet to be completed. Therefore requires supplements such as soil and foliar fertilizers depending on the plant growth stage at the point of application. The plant therefore depends on the applied fertilizer especially nitrogen for its survival at this stage, this is supported by Singleton et al. (1999) report; that a starter N response will occur when the host plant shows poor nodulation or when ineffective rhizobial are present as was the case in the study site. Hence, soybean nodule number significantly enhanced with combination of INO+NPK, INO+BX and INO+NPK+BX over the sole use of the various fertilizers used and most especially over the control in the two years (Table 2), The finding in this study on nodule number response to the use of fertilizer combination lends credence to the use of starter N for soybean production efficiency under low soil fertility status in the study area (Table 1).

On the contrary; Zhang et al. (2000) reported that mineral N affects several steps of the nodulation process by specifically reducing isoflavonoid concentration of soybean root systems. Possibly this could explain the reason why the freatments applied did not significantly influence nodule dry weight despite the nodule number advantage it had over the control. Likewise Nyoki and Ndakidemi, (2014) reported that soil pH (5.5) might not favour efficient nodule

development. However, more than 30% increase in nodule weight was recorded in the treated plots in both years relative to their controls (Table 2).

Generally, parameters recorded in 2012 were higher compared to the values recorded in 2013, this could be attributed to the erratic rain fall pattern experienced in the year 2013 (Fig.1). The positive effect due to the timely application of the various fertilizers applied at different growth stages cannot be underestimated. The combine application of bio-fertilizer, soil and foliar fertilizers reveals effective complementary stimulated amongst the different types of fertilizers used. Therefore, accumulation of soybean biomass which is the fundamental basis for effective photosynthesis was supported by the combine treatments, over sole application (Table 3) hence growth and yield were enhanced. The inability of inoculant to promote shoot biomass relative to the control could be complemented with Aliyu et al. (2013) report that the tendency of the native rhizobia to compete with the introduced strain cannot be underestimated. The findings of Bekere et al. (2012) also showed no positive relationship of seed inoculation and shoot dry weight as was the case in this study. The trend observed in the biomass yield was reflected in the pod number whereby highest pod number was recorded in 2012 as compared to 2013 (Table 4). However, soybean pod number and weight (Table 4) from all treated plots performed better than those from the untreated plots which implies that the amendments were necessary to argument soybean performance in the study area. The outstanding performance of treatments supplemented with the use of boost xtra (foliar fertilizer) shows that indeed the top dressing of soybean at mid-vegetative growth stage with macro and micronutrients aided vegetative growth which nodule destruction and plant senescence would have hindered at that growth stage. Moreso transformation into pod development in preparation for seed formation, which is the essence of cultivation was also enhanced. The importance of the use

of soil fertilizer at the onset of the experiment could be attributed to the ability of starter N in averting the N hunger stage, therefore inducing effective photosynthesis especially at the early stage of the vegetative growth, an indication that nitrogen is indeed a major component of proteins and protoplasm and plays a vital role in achieving increased biomass development. SeifiNadergholi et al. (2011) reported that foliar application could be an advantage for growth. The foliar fertilization of the plant also gives room for quick mobilization and assimilation of the various nutrients in the boost xtra used for top dressing which translocate through the leaves to the other parts of the plant thereby stimulating the development of the plant reproductive parts resulting into well filled pod formation and grain, sustaining and enhancing the plant growth after nodule destruction. Vahedi, (2011) report that soybean high yield and quality as well as its oil could be obtained by foliar spray of nutrient elements.

The use of Boost xtra resulted in substantial grain yield increase over the control, which is in line with the reports by Odeleye et al. (2007) and Hanway (1980) that the use of foliar fertilizer during seed filling increased soybean yield. Foliar fertilization provides more rapid utilization of nutrients and permits the correction of observed efficiency symptoms in less time than would be required by soil application (Fageria et al., 2009).

Conclusion: It can be concluded from this study that the response of soybean to japonicum Bradyrhizobium attain optimum growth and development was enhanced by the use of minimum level of fertilizer 25 kg N, 30 kg P₂O₅ and 30 kg K ha⁻ 1 (soil). Also it can be deduced that the top dressing (4 L ha-1) at mid-vegetative stage to early podding stage with boost xtra sustained inoculated soybean development to maturity. However the response of soybean to the applied foliar fertilizer (Boost xtra) demands better understanding. So as to correlate the appropriate plant growth stage to the time of application at which the various nutrient contents of boost xtra can be maximize to

ascertain the actual response of soybean to foliar fertilizer. However, the complementary application of soil and or foliar fertilizer to japonicum inoculated Brayrhizobium soybean proofed the best in the study area as compare to the sole application of fertilizers and should therefore be encouraged amongst soybean cultivating farmers in the study area.

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