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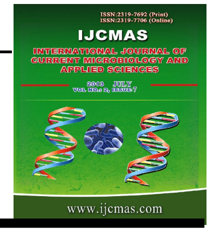

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Original Research Article

An assessment for the need of soybean inoculation with *Bradyrhizobium japonicum* in some sites of Kasungu district, Central Malawi

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Keywords

Inoculation; Biological nitrogen fixation; *Brady - rhizobium japonicum;* Nodulation; Soil fertility.

A B S T R A C T

Soybean is one of the most important legume crops in Malawi. Over the years, yield of soybean has mostly fallen below an optimum level which is attributed to a number of factors including poor nodulation with the native rhizobia available in the soil. An on-farm study was done to assess the need for soybean inoculation with *Bradyrhizobium japonicum* in five sites of Mkanakhothi Extension Planning Area (EPA) of Kasungu district, Central Malawi. The trials included inoculated soybean plot, un-inoculated soybean plot and un-fertilized maize plot that was used in the estimation of biological nitrogen fixation using the nitrogen difference method. The treatments were arranged in a randomized complete block design. Results showed significant differences ($p < 0.05$) due to both inoculation and site effects. Inoculation of soybean significantly ($p < 0.05$) increased nodule numbers per plant and nodule dry weight in all sites by ranges of 75 to 90% and 25 to 53% respectively. Biological nitrogen fixation (BNF) increased by 74 to 87% (26 to 56 kg N ha^{-1}) in inoculated soybean as compared to the un-inoculated soybean (5 to 9 kg N ha^{-1}). Grain yield was also significantly ($p < 0.05$) higher in inoculated plots as compared to un-inoculated plots with ranges of 524 to 1868 kg ha^{-1} for inoculated soybean and 246 to 905 kg ha^{-1} for un-inoculated soybean. From this study it can be concluded that although soybean has commonly been grown in the EPA inoculation is still needed to all the sites. Application of different starter doses of N, different rates of P fertilizer and soil liming should be considered for further research in Mkanakhothi EPA of Kasungu district and similar agro-ecological zones in the country in order to improve BNF and yields of soybean.

Introduction

Soybean (*Glycine max* (L.) Merill) is an annual legume that belongs to the legume family *Fabaceae* (Tefera, 2011). It is a crop with so many benefits including human and animal nutrition, source of income from sales and soil fertility improvement through biological nitrogen fixation (BNF). It is also reported to have medicinal value (Dixit *et al*., 2011). In terms of food components, soybean contains 35 - 40% protein, 19% oil, 35% carbohydrate (17% of which is a dietary fibre), 5% minerals and several other components including vitamins (Liu, 1997). In Malawi, soybean is commonly grown for food, sale and soil fertility improvement. It is processed into number products including flour for porridge, cooking oil, soy meat, soy milk and soy coffee (Opperman, 2011). It is also reported that Malawi is a significant consumer and regional exporter of maize-soy blend flour and market demand for soybean in sub-Saharan Africa is expected to more than double in the next decade (Opperman, 2011). Soybean is very important in soil fertility improvement because of its ability to fix nitrogen biologically in its root nodules through a symbiotic association with rhizobial micro-symbionts commonly *Bradyrhizobium japonicum* (Giller, 2001). Soybean can biologically fix nitrogen in the range of 85 - 154 kg N ha^{-1} (Giller, 2001).

However, despite Malawi being one of the countries known to produce substantial quantities of soybean in Africa (ranked 5^{th} in Africa based on three year data, 2006 - 2008), the average grain yield of the crop is low (700 kg ha^{-1} based on 2006 - 2008 data) (Tefera, 2011). This average yield is far below the potential soybean grain yield of around 4000 kg ha^{-1} (MoAIFS, 2005; Roy et al., 2006). Challenges that face soybean production in Malawi and other Southern Africa countries include seed viability, poor nodulation with native rhizobia available in the soil, low availability and awareness of inoculants, lack of some essential nutrients in the soil, for example phosphorus (P), and Sulphur (S), land constraints and lack of farmer market power and information (Opperman, 2011). Though yield limitation is a function of many factors, it is noted that most farmers in Malawi usually grow soybean without applying inoculants. The objective of this study was to assess the need for soybean inoculation with *Bradyrhizobium japonicum* in some sites of northern Kasungu district, Central Malawi.

Materials and Methods

Site and experimental layout description

Researcher designed on-farm trials were done in the 2008/09 cropping season in Mkanakhothi Extension Planning Area (EPA) (12^{o} 35′S, 33^{o} 31′ E), Northern Kasungu district, Central Malawi. The experiment was conducted in five sub-sites (villages) within the EPA (Chaguma, Chisazima, Kaunda, Ndaya and Tchezo). The dominant soils in the area are Ultisols (Lowole M. W., Bunda College – Personal communication). The area receives an annual average rainfall of 680 mm (Phiri *et al.*, 2010). A randomized complete block design experiment was laid out in five sub-sites of the EPA on 15 farmers' fields. In each sub-site the experiment was conducted on three farmers' fields with each farmer's field representing a replicate.

The treatments included plots of soybean inoculated, soybean un-inoculated and an un-fertilized maize plot that was included for the estimation of biological nitrogen fixation using the nitrogen difference method. Plot sizes for all treatments were 10 m x 10 m, with 12 ridges spaced 75 cm apart. Soybean was planted 2 seeds per planting station at a distance of 5 cm along the ridge. The ridge spacing and plant population of soybean was done according to recommended procedures (MoAIFS, 2005). The variety of soybean seed used in the study was solitaire, an indeterminate, relatively new variety obtained from Seed Co Limited.

Inoculation method

The inoculant containing *Bradyrhizobium japonicum* was acquired from Chitedze Research Station in Lilongwe. The inoculation process included mixing 50 g inoculant into a 5% sugar solution (this amount is enough for 25 kg of soybean seed) to form a slurry. The slurry was then mixed with the seed until it was evenly coated. The coated seed was left to dry in the shed for 30 minutes and planting was done with 24 hours of inoculation. All the procedures followed as described in this section were based on current recommendation (MoAIFS, 2005).

Soil and plant sampling and rainfall data collection

Soil sampling was done two times from each farmer's fields, before planting and after harvesting. In the first sampling, soils were collected randomly from five points on each farmer's field at depth ranges of 0 – 15 cm and 15 – 30 cm. Soils from each depth range were thoroughly mixed and 500 g composite sample taken. These samples were stored in plastic bags. Second sampling was done at harvesting from each of the treatment plots from all the fields involved. This time samples were collected from only three points in each plot at the same depth ranges of 0 – 15 cm and 15 – 30 cm. The same procedure described above was followed in order to obtain composite samples. Each time all samples were air dried, passed through a 2 mm sieve and then kept in plastic bags ready for laboratory analysis.

Plant sampling was done using systematic random sampling from within the net plot of eight ridges. Eight maize plants were sampled at tasseling stage and 16 soybean plants at podding stage. Sampling stages were based on the stage at which these crops attain highest dry matter and N content whereas the number of plants sampled differed because these crops were planted with different plant population. Samples were oven dried at 70^{o} C for 48 hours and ground to powder and kept in plastic bottles ready for laboratory analysis. Rainfall data were collected from two strategic sub-sites, Kaunda and Ndaya (sites situated in middle of two opposite ends) of the whole study area. Rain gauges were installed in these sites and well trained community agricultural workers collected the daily rainfall data which were later processed by the researcher.

Soil and plant analysis

A number of soil and plant analyses were done following standard procedures as presented in Table 1.

Evaluation of nodulation and nodule effectiveness

Destructive sampling for nodule counts and nodule effectiveness were done at flowering according to standard procedures (Peoples *et al*., 1989). Sixteen plants, two from each ridge (one planting station) were dug up, and uprooted from a net plot of 8 ridges, nodules counted and an average number of nodules per plant was computed. A maximum of ten nodules were stripped off the crown and main roots of each sampled plant and sliced into halves to expose the centre where specific nodule colours were observed. Effective nodules were determined by red, pink and brown colours in the centre whereas yellow, green and other colours depicted ineffectiveness (Sylvia *et al.,* 2005). The percentages of effective nodules for each treatment were calculated and the data was transformed by Arc sine transformation

Table.1 Soil and plant parameters and the methods used for analysis

Parameter	Method	References
Soil texture	Hydrometer method	Anderson and Ingram, 1989
Soil pH	Potentiometric determination	Wendt, 1996
Soil organic carbon (%SOC)	Walkely-Black method (in water, 1: 2.5)**	Anderson and Ingram, 1993
Total soil nitrogen (%N)	Modified Kjeldahl method	Anderson and Ingram, 1989
Available soil P, copper (Cu), iron (Fe), Zinc (Zn)	Mehlich-3 extraction procedures	Anderson and Ingram, 1989
Available soil boron	Hot water/0.02 M $CaCl_2$ extraction procedures	Anderson and Ingram, 1989
Mineral soil N (NO_3^-; NH_4^+)***	Colorimetric determination	Anderson and Ingram, 1993
Total plant N (%)	Modified Kjeldahl method	Anderson and Ingram, 1989

**Soil organic matter (SOM) was obtained by multiplying SOC by 1.72 (SOM = % SOC x 1.72); ***Only mineral N (NO_3-N and NH_4-N) was determined in the postharvest soil samples for modifying the N-Difference method of estimating BNF

method in order to subject them to analysis of variance (Gomez and Gomez, 1984). The sampled nodules were later dried at 70^o C for 48 hours and nodule weights determined in order to compare nodule dry weights of legumes for different cropping systems.

Estimation of biological nitrogen fixation

Biological nitrogen fixation was estimated using the modified Nitrogen-Difference method or technique (Peoples *et al*., 1989). In the N-difference method, the difference between total plant nitrogen of an N_2-fixing legume and a control crop (non-N_2-fixing) is considered to be nitrogen that has been fixed biologically. This basic principle is modified to improve accuracy of measurements when a non-nodulating isoline is not available and the legume and a control being used are not well matched (Evans and Taylor, 1987). Therefore the modified Nitrogen-Difference technique was used in this study because the control crop used was maize which is not well matched with the soybean. The quantity (Q) of biologically fixed N in a modified technique was calculated as follows:

$$Q = [\text{N yield (legume)} - \text{N yield (control)}] + [\text{N soil (legume)} - \text{N soil (control)}]$$

Where:

Q (kg ha^{-1}) = Quantity of the biologically fixed nitrogen

N yield [legume] (kg ha^{-1}) = Nitrogen yield of a legume

N yield [control] (kg ha^{-1}) = Nitrogen yield of a non-fixing plant

N soil (kg ha^{-1}) = Post-harvest soil nitrogen in legume and control plot

Harvesting and yields determination

A net plot of 8 m x 8 m was demarcated leaving a 1 m border from all the sides. Soybean plants were pulled up/uprooted and roots removed to have a consistent measure of weights of above ground biomass (total above ground biomass) and fresh weights were determined. Soybean pods were unshelled while on the stalks and fresh weights of net plot grain determined. 100 seeds were sampled by following a quartering procedure (Kanyama-Phiri, Bunda College – Personal communication). Their fresh weights were measured and later taken to the laboratory and oven dried at 70^{o} C for 48 hours. The moisture content of the seed was determined as follows:

$$\text{Seed moisture content (\%)} = (W_1 - W_2) \times 100/W_1$$

where

W_1 = weight of seed before oven drying
W_2 = weight of seed after oven drying

Net plot grain yield was determined at a predetermined moisture percentage of 10% by using standard procedures (Mloza-Banda, 1994). The other fresh weights measured were those of stover and shells and their samples were collected, 500 g for haulms and 200 g for shells. These samples were taken to the laboratory, oven dried at 70^{o} C for 48 hours and dry weights measured. The total dry matter yield (total biomass yield) was determined by adding dry weights of grain, shells, stover and senesced leaves. The senesced leaves were estimated per plot by systematically collecting them from the 3 furrows per net plot, find the average weight for 1 furrow and multiply weight of senesced leaves for 1 furrow by 7 (number of furrows per net plot).

Data analysis

Data collected were subjected to two-way Analysis of Variance (ANOVA) using GenStat edition 14. Separation of treatment means were done using least significant differences (LSD) at $p = 0.05$.

Results and Discussion

Rainfall amounts

The total annual rainfall for the study area was 716 mm. Specifically, the strategic sites of Kaunda and Ndaya received total annual rainfall of 713 mm and 719 mm respectively (Figure 1). The rainfall amounts were in the range of rainfall received in the area in the previous 7 years (Phiri *et al*., 2010).

Soil characterization

The means of total nitrogen in the topsoil ranged from 0.07% to 0.10% with Ndaya indicating a relatively higher value than the rest of the sites (Table 2). A similar trend appeared in the subsoil with a total nitrogen range of 0.05% to 0.09%. Both overall means of total soil nitrogen from the topsoil and subsoil were below the critical value of 0.1%. The ranges of values for available phosphorus (Mehlich-3 P) in the topsoil and subsoil were both below the critical value of 25 ppm with Chisazima and Kaunda indicating significantly higher values of P than the rest of the sites. The overall means of the hot water extractable boron (B) in the topsoil and subsoil were both below the critical value of 1.1 ppm with Chaguma, Ndaya and Tchezo indicating significantly ($p < 0.05$) higher values as compared with Chisazima and Kaunda (Table 2). There were no significant differences in all sites for extractable iron (Fe) and zinc (Zn).

The mean values of zinc fell above the critical value of zinc (2.0 ppm). The ranges of Zn, Fe, Cu and B fell within the range of those reported for Malawi in a global study on micronutrient and nutrient status of soils (Sillanpaa, 1982). All the study sites had moderately acid to acid soils with the overall mean pH of 5.4 and 5.2 for the topsoil and subsoil respectively. The top-soil textures ranged from loamy sandy (coarse-textured) to light sand clay loam (moderately fine-textured). The subsoil textures ranged from loamy sandy to sandy clay loam (fine-textured). On average the topsoils are coarse textured whereas the subsoils are comparatively fine textured (Table 2). Kaunda and Ndaya showed slightly higher SOM than the other three sites. From the results summarized in Table 2 it can generally be stated that the soils under study were low in nutrients and organic matter, and are acidic.

Effect of inoculation and site on nodulation, BNF and yields of soybean

From the results it is noted that there was a similar trend in most parameters measured such as nodulation (nodule numbers, nodule weights and nodule effectives), BNF, grain yield and total dry matter yield in that they were all higher with inoculation than without inoculation. Table 3 shows that inoculation significantly ($p < 0.05$) increased nodule numbers per plant by 75 to 90%. There were no significant differences in nodule numbers as influenced by site though Kaunda site showed a slightly higher number than other sites. There were significant differences ($p < 0.05$) in nodule dry weights (Table 4) due to both inoculation and site (Table 3). The nodule dry weights were higher in inoculated soybean with a range of 25 % (Chaguma) to 53% (Ndaya). However, there were no significant differences in nodule effectiveness by site though Kaunda and Ndaya showed slightly higher percentages (Figure 2).

There were significant differences ($p<0.05$) in amount of biologically fixed nitrogen as influenced by both inoculation and site (Figure 3). Inoculation led to BNF increase by a range of 74% to 87% as compared with non-inoculated soybean. Kaunda site produced BNF amount that was significantly ($p < 0.05$) higher than other sites with a range from 67% (Ndaya having the second highest amount of biologically fixed N) to 119% (Chaguma having the lowest amount of biologically fixed N).

Figure 4 and Figure 5 show that both inoculation and site led to significantly ($p < 0.05$) high grain and total dry matter yields respectively. Grain yield was higher for inoculated soybean by a range of 50 to 63% whereas total dry matter yield was higher by a range of 9 to 55% than in un-inoculated soybean. For total dry matter yield, site differences were significantly ($p < 0.05$) higher for inoculated soybean which showed interaction between effects of inoculation and site.

These observations are in agreement with other similar studies done in other countries. Research shows that compared with other legumes soybean needs special types of rhizobia in such that even promiscuous varieties have shown response to inoculation (Muhammad, 2010). A study done in Nigeria's Southern Guinea savanna Alfisol, an early maturing promiscuous soybean cultivar (TGX 1485) responded with an increased number of nodules, grain yield and dry shoot biomass (Muhammad, 2010).

Table.2 Mean baseline soil properties in the 0 - 15 cm and 15 - 30 cm depth ranges for the five study villages/sites in Mkanakhothi EPA, Kasungu district, Central Malawi

Soil property	Soil depth	Critical values	Study sites and statistics						
			Chisazima	Tchezo	Kaunda	Ndaya	Chaguma	Grand mean	Std. Dev
Total N (%)	0-15	0.1	0.07	0.08	0.08	0.10	0.09	0.08	0.02
	15-30		0.05	0.08	0.07	0.09	0.08	0.07	0.02
Mehlich-3 P (ppm)	0-15	25	23.2	10.3	20.9	11.9	4.10	14.1	8.20
	15-30		21.4	8.52	11.6	7.30	2.20	10.2	7.60
B (ppm)	0-15	1.1	0.07	0.57	0.02	0.62	0.67	0.40	0.07
	15-30		0.09	0.72	0.16	0.49	0.54	0.30	0.06
Zn (ppm)	0-15	2.0	3.56	2.25	2.64	3.03	3.31	2.96	0.98
	15-30		2.70	2.60	1.90	2.23	2.60	2.43	0.82
Fe (ppm)	0-15	-	93.4	77.9	91.1	92.4	71.7	85.3	15.8
	15-30		101	83.8	89.0	83.2	90.2	89.5	11.5
Cu (ppm)	0-15	-	0.99	1.14	2.68	2.47	1.87	1.83	0.6
	15-30		0.71	2.89	2.74	1.85	1.73	1.98	0.4
SOM (%)	0-15	1.5	1.20	1.50	1.70	2.10	1.70	1.60	0.5
	15-30		0.90	0.90	1.20	1.10	1.00	1.00	0.2
pH (1:2.5 H_20)	0-15	-	5.50	5.20	5.60	5.30	5.30	5.40	0.2
	15-30	-	5.20	5.00	5.50	5.20	5.10	5.20	0.2
Sand (%)	0-15	-	86.0	80.2	80.4	74.4	70.3	78.3	6.1
	15-30	-	84.6	77.1	70.7	63.8	65.0	78.2	8.7
Clay (%)	0-15	-	13.0	19.4	16.0	21.6	25.7	19.1	4.9
	15-30	-	12.3	19.3	23.7	28.3	24.3	21.6	6.1
Textural class	0-15	-	Loamy sand	Sandy loam	Sandy loam	Sandy clay loam	Sandy clay loam	-	-
	15-30	-	Loamy sand	Sandy loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	-	-

Key: SOM = Soil organic matter (soil organic C x 1.72); Critical value = A value below which implies low amount; Std. Dev = Standard deviation; - = value not obtained.

Table.3 Effect of inoculation and site on soybean nodule numbers per plant

Treatment	Site				
	Chaguma	**Chisazima**	**Kaunda**	**Ndaya**	**Tchezo**
Inoculated soybean	6±1.23[a]	8±2.61[a]	11±2.45[a]	8±3.73[a]	9±1.88[a]
Un-inoculated soybean	1±0.01[b]	1±0.34[b]	1±0.02[b]	2±1.01[b]	1±0.21[b]
F pr. (inoculation)	< 0. 02		LSD $_{(0.05)}$ (inoculation)		3.89
F pr. (site)	0.66		LSD $_{(0.05)}$ (site)		8.55
F pr. (inoculation*site)	0.91		CV (%)		27

Means with different letters in the same column are significantly different; No statistical differences are shown for site effect

Table.4 Effect of inoculation and site on soybean nodule dry weight (mg/nodule)

Treatment	Site				
	Chaguma	**Chisazima**	**Kaunda**	**Ndaya**	**Tchezo**
Inoculated soybean	1.31±0.29 [aB]	1.65±0.32 [aA]	1.54±0.1 [aA]	1.49±0.26 [aA]	1.05±0.56 [aC]
Un-inoculated soybean	1.04±0.01 [bA]	1.18±0.17 [bA]	1.15±0.002 [bA]	0.97±0.43 [bB]	0.72±0.11 [bC]
F pr. (inoculation)	< 0. 001		LSD $_{(0.05)}$ (inoculation)		0.12
F pr. (site)	< 0.044		LSD $_{(0.05)}$ (site)		0.18
F pr. (inoculation*site)	< 0.035		CV (%)		29

Means with different small letters (in the same column) and means with different capital letters (in the same row) are significantly different

Nodulation, N_2 fixation and growth of soybean also increased in both promiscuous variety (TGX 1740-2F) and non-promiscuous variety (Nyala) in Kenya with inoculation (Thuita *et al*., 2012). The higher value in the measured parameters with inoculation shows the need for inoculation in the area. Though soybean has been grown for some time in Malawi and in the study area, in most cases yields are low. It is noted that inoculants are rarely available to smallholder farmers in Southern Africa and there is also little awareness on their importance among smallholder farmers (Opperman, 2011).

The differences due to site can be attributed to some slight differences in soil parameters. Soil physico-chemical factors such as pH, P, N and micronutrient levels are also important in nodulation, BNF and growth of soybean (Mohammadi *et al*., 2012; Giller, 2001). In this study it is shown that N, P, and B were below the critical values in all the sites (Table 2) and could be one of the contributing factors for the relatively low numbers in most of the parameters measured irrespective of inoculation or no inoculation.

Phosphorus is very important in the overall crop development, nodulation and nitrogen fixation (Brady and Weil, 2008). Non optimum P levels were reported to affect N_2 fixation and yields of soybean by decreasing the nodule mass and nitrogenase activity (Tsvetkova and Georgiev, 2003). Nitrogen fixation under P deficiency is reported to affect several physiological characteristics of the nodule such as N_2-dependent growth, nodule respiration and control of oxygen diffusion

(Serraj, 2004). On the other hand B is also reported to be important in cell division and root nodule development (Bolanos *et al*., 1996).

It is also observed that for most of the parameters measured, values/amounts were either significantly higher or slightly higher for Kaunda followed by Ndaya than other sites. The relatively higher values in most parameters for Kaunda and Ndaya sites could be attributed to slightly higher SOM levels as compared to the other sites and P and pH for Kaunda was also slightly higher than the other sites. Soil organic matter has so many benefits to the soil including keeping nutrient reserves for both microbial activities and plant growth, improving soil buffering capacity, water holding capacity and enhancing chelation and bioavailability of micronutrient elements to both plants and soil microorganisms (Sylvia *et al*., 2005). Chisazima site showed slightly higher P values than other sites but nitrogen fixation and yields were low. This can be attributed to nutrient imbalance as other nutrients were very low for instance it had the lowest N levels. Athough very high available N is known to suppress nitrogen fixation (Havlin *et al*., 2005), very low N levels also affect plant development and nitrogen fixation (Giller, 2001; Ajeigbe *et al*., 2010).

There are two main sources of N for soybean, which are soil and BNF and where the crop fails to fix enough, N becomes the main limiting factor of soybean yield (Salvagiotti *et al*., 2008).

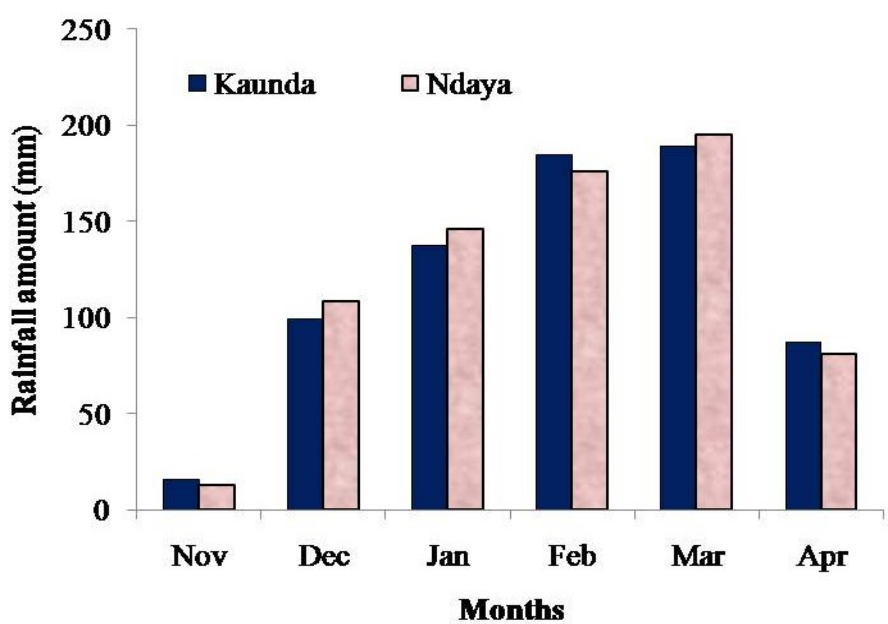

Figure 1 Monthly rainfall for the two strategic study sites of Kaunda and Ndaya in the 2008/09 cropping season

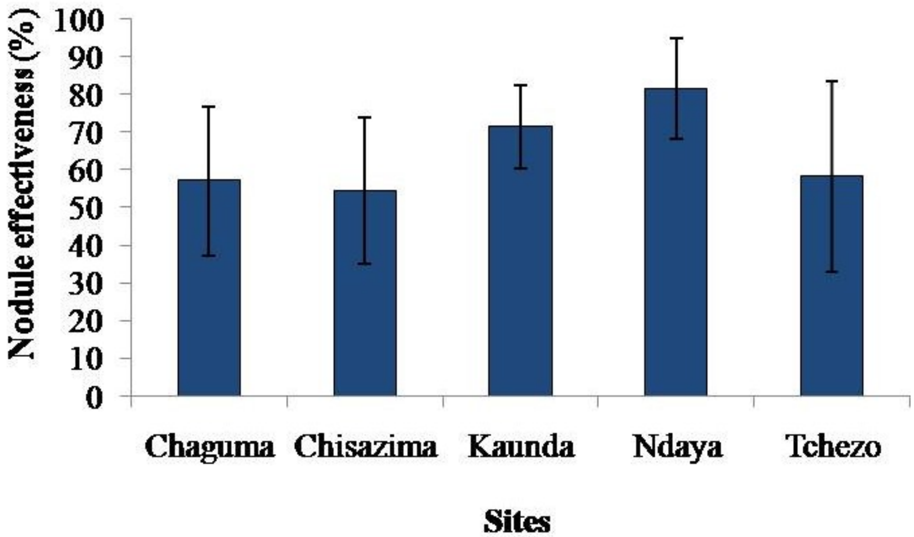

Figure 2 Soybean nodule effectiveness as influenced by sites. Error bars indicate standard errors of the means

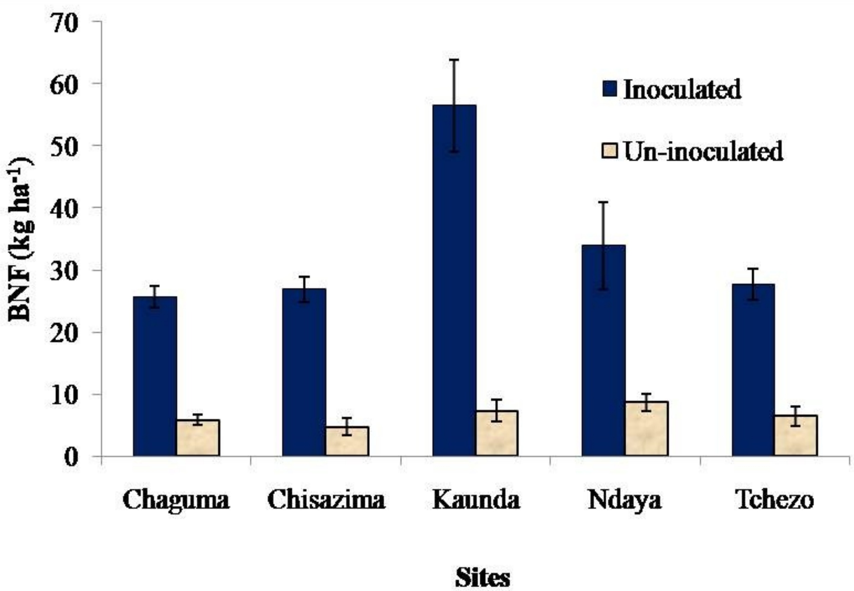

Figure 3 Biological nitrogen fixation by soybean as influenced by inoculation and site. Error bars indicate standard errors of the means

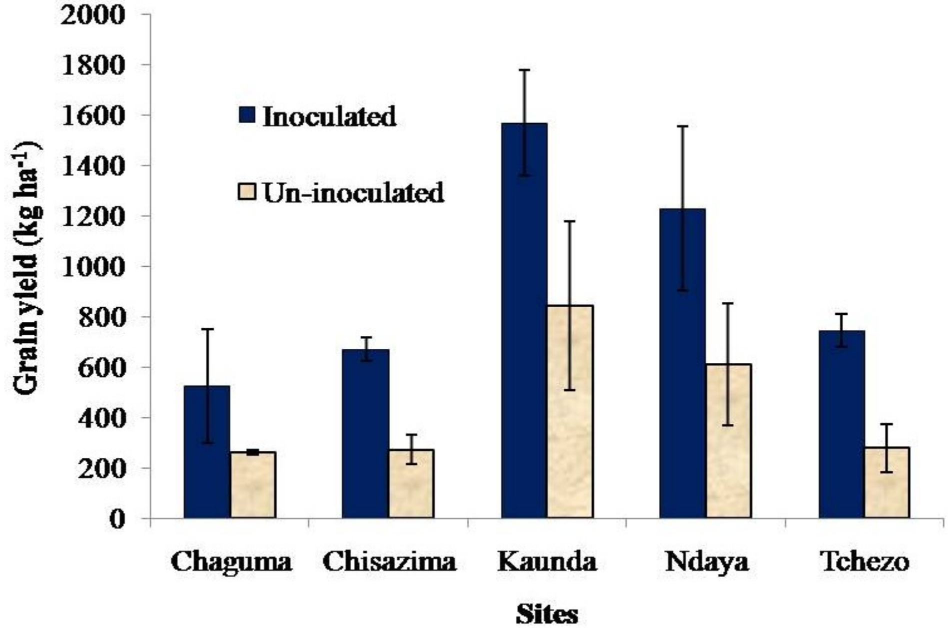

Figure 4 Grain yield of soybean as influenced by inoculation and site. Error bars indicate standard errors of the means

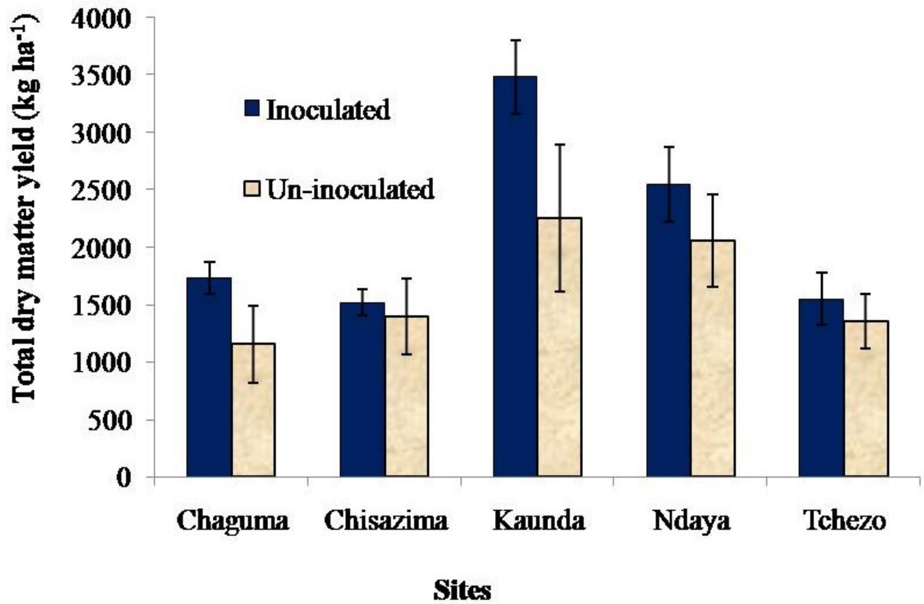

Figure 5 Total dry matter yield of soybean as influenced by inoculation and site. Error bars indicate standard errors of the means

This is because there is reduced N efficiency in soybean due to higher investment of energy per unit of grain that is needed for higher accumulation of N, protein and oil in soybean seed (Amthor *et al*., 1994).

From this study it can be concluded that although soybean has commonly been grown in the area inoculation is still much needed in order to improve yields of the crop. Despite some sites (Kaunda and Ndaya) showing significantly higher BNF and yields than others, all sites responded positively to inoculation and therefore should be considered for inoculation when soybean is grown. It is also recommended that studies need to be done in more areas with different varieties of soybean. Application of different starter doses of N, different rates of P fertilizer and soil liming should be considered for further research in Mkanakhothi EPA of Kasungu district and similar agro-ecological zones in the country in order to improve BNF and yields of soybean.

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