
Response of Soybean (*Glycine max* L.) to Rhizobia Inoculation and Molybdenum Application in the Northern Savannah Zones of Ghana

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Abstract: Compatible rhizobia populations are seldom available in soils where soybean has not been grown before. Inoculating soybean seeds with superior rhizobia strains is necessary for nodulation and nitrogen fixation. Ironically, many commercial agricultural products (biological and chemical) claim increases in crop productivity but their efficacy cannot be guaranteed. Thus, three separate on-station trials (Manga, Kpongungu and Nyankpala) were conducted at the experimental fields of CSIR-Savannah Agricultural Research Institute (SARI), to ascertain the effectiveness of some commercial microbial inoculant and micronutrient fertilizer for improvement of soybean productivity in the Northern savannah zones of Ghana. Four treatments were used for each study site; Control, Teprosyn Mo, Legumefix and Teprosyn Mo+Legumefix which were laid out in a Randomised Complete Block Design (RCBD) with three replications. Experimental plots measured 4.5 m x 4.5 m. A significant ($P < 0.05$) response of soybean nodule dry weight to Legumefix was observed in Kpongungu and Manga but not Nyankpala. At harvest, Teprosyn Mo+Legumefix, Legumefix and Teprosyn Mo treatments increased soybean grain yield by 205.62%, 135.54% and 110.24% respectively over the control in Manga. In Nyankpala, the application of Legumefix and Teprosyn Mo+Legumefix increased soybean grain yield significantly by 22.43% and 42.10% respectively relative to the control while no significant response was observed in grain yield among treatments at Kpongungu. The combined application of Teprosyn Mo+Legumefix was the most economically viable among the treatments (VCR = 2.65).

Keywords: Economically Viable, Microbial Inoculants, Micronutrient, Nodules, Rhizobia, Soybean, Symbiosis, Yield

1. Introduction

In Ghana, soybean is widely cultivated in the Northern, Upper East, Upper West, Central and Volta regions where the crop is well adapted. However, production is faced with a number of constraints including low soil fertility [34], irregular rainfall patterns, drought, inadequate access to certified seed and poor agronomic practices which result in poor yields especially in the Northern savannah zones of Ghana. Although there is a growing need for chemical fertilizers to enhance crop yields [66], about 60% of African smallholder farmers are unable to afford the high prices of chemical fertilizers [68]. Moreover, the production and intensive application of chemical fertilizers in agriculture has led to a series of environmental problems [48, 69] and damage to the ecological state of agricultural systems [60]. In

order to increase food production in Africa, efforts are now geared towards approaches of internal and renewable resources and the use of effective management practices [58, 62] to increase food production without compromising on sustainable agriculture. This has led to the promotion of commercial biological and chemical products intended to restore or enhance the fertility and organic matter content of soils in an eco-friendly manner [33]. [16] reported that, the presence of microorganisms in the soil is critical to the maintenance of soil function, in both natural and managed agricultural soils. The microbes are involved in key processes such as soil structure formation, decomposition of organic matter, toxin removal, and the cycling of elements - carbon, nitrogen, phosphorus, potassium, and sulfur [2]. It is also

clear that beneficial microorganisms play key roles in suppressing soil-borne plant diseases and in promoting plant growth and changing the vegetation [21].

Biological nitrogen fixation (BNF) is seen as a cheap way to get renewable nitrogen in agriculture as it uses photosynthetically produced energy and is environmentally cleaner [3]. Many research experiments have clearly justified the positive effect of inoculation in enhancing BNF [30, 55, 62]. Although most commercial microbial and micronutrient products claim to increase BNF and consequently crop yield, their true benefits cannot be vouched for. Preliminary trials conducted in Kenya, Nigeria and Ethiopia to test some commercial microbial inoculants showed varied responses on growth and yield parameters measured. Furthermore, information regarding the use of microbial and micronutrient products in improving soybean production in Ghana is scanty.

The objective of this study was therefore to assess the effectiveness of some commercial microbial inoculant and micronutrient fertilizer in improving soybean production in the Northern savannah zones of Ghana.

2. Materials and Methods

2.1. Study Sites and Soil Characteristics

The trial was conducted at the experimental fields of the CSIR-Savannah Agricultural Research Institute at Kpong (Latitude 09° 59' 34.0" N and Longitude 002° 31' 30.3" W with an elevation of 315 m above sea level) in the Upper West region; Nyankpala (Latitudes 09° 36' 31.3" N and Longitude 001° 02' 14.1" W with an elevation of 195 m above sea level) in the Northern region and Manga (Latitude 11° - 01' N and Longitude 00° - 16° W with an elevation of 249 m above sea level) in the Upper East region during the 2013 major cropping season. Rainfall distribution in these study sites is unimodal, with an average annual rainfall of about 1000 - 1200 mm annually [49] and mean temperatures between 26 °C and 30 °C, with little variation throughout the year.

Initial soil sampling was done by collecting at least 8 cores from a depth of 0 - 15 cm with a soil auger following a 'W' design per replicate block. Soil samples were thoroughly mixed, bulked, air-dried and composite samples taken for physico-chemical analyses and biological assays using standard protocols. Soil pH was determined according to the electrometric method described by [45] in a suspension 1:2.5 soil to distilled water (soil:water) ratio. The modified Walkley and Black procedure as described by [38] was used to determine organic carbon content in soil sample. The macro Kjeldahl method involving digestion and distillation as described by [54] was used in the determination of total nitrogen. The readily acid-soluble forms of phosphorus were extracted with Bray No. 1 solution (HCl:NH₄F mixture) [9, 44]. Particle size distribution was determined by the hydrometer method [18].

2.2. Field Layout and Experimental Treatments

All the experimental fields were weeded, ploughed and

harrowed after which the layouts were done. The plot sizes measured 4.5 × 4.5 m each with an alley of 1 m between plots. Four (4) treatments (T₁-Control, T₂-Teprosyn Mo, T₃-Legumefix and T₄-Teprosyn Mo+Legumefix) were arranged in a Randomized Complete Block Design (RCBD) with three replications in all the experimental sites. A promiscuous medium-maturing soybean variety "Jenguma" was used as test crop. Soybean seeds were treated with Legumefix at a rate of 4.0 g kg⁻¹ seed and 20 mL (kg seed)⁻¹ of Teprosyn Mo planted at three seeds per hole of about 5 - 7 cm deep at a spacing of 50 cm × 5 cm. At about 2 - 3 weeks after planting (WAP), plants were thinned to two seedlings per hill.

2.3. 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).

2.4. Data Collection and Statistical Analysis

At 50% podding, ten (10) soybean plants were randomly collected within the net plot of each plot. The plants were carefully uprooted by digging 15 cm around the plant using a spade. The plants were separated into shoots and roots. The roots were washed gently with clean water to remove all attached soil from the roots and the nodules. The nodules were counted and oven-dried at 60 °C for 48 hours to determine their dry weights. The shoots were also oven-dried at 60 °C for 72 hours and dry weights recorded for each sample. Seed yield of soybean plants were harvested at physiological maturity stage, air-dried, threshed and winnowed. The grains were oven-dried at 60 °C for 72 hours. The dry weights for each plot were then determined and used to estimate the grain yield (per hectare) [42]. One volume of milled leaves and two volumes of stems were mixed for measurement of shoot N and P content.

The collected data were subjected to Analysis of Variance (ANOVA) using the GENSTAT version 12 [24]. Means comparison of treatments showing significant effect were separated using the least significant difference (LSD) at 95% confidence level.

Partial budgeting and profitability analysis were estimated using value cost ratio as explained by [40]. The costs of Teprosyn Mo and Legumefix per hectare were USD 4.25 and USD 10.50 respectively. The farm-gate price of soybean grain was USD 40 per 100 kg bag in all the study locations. Prices were collected in local currencies and converted to US dollars at the prevailing exchange rates (GH¢ 1 to USD 2). Profitability was estimated by the value cost ratio (VCR).

3. Results

3.1. Soil Characteristics and Indigenous Rhizobia Populations

Initial physico-chemical characteristics from the study sites are presented in Table 1. Soil pH values of the study sites ranged from acidic to moderately acidic (4.12 - 5.53)

with generally low fertility status. The textural classes at the three study sites were loamy sand in Kpongu and Manga, and sandy loam in Nyankpala. The organic carbon (OC) levels at all the study sites were very low; 0.66% in Kpongu, 0.40% in Manga and 0.44% in Nyankpala. Total N at all the study sites were generally low ranging from 0.02 - 0.06%. Results for available phosphorus (P) were 1.96 mg kg⁻¹, 1.24 mg kg⁻¹ and 2.70 mg kg⁻¹ for Kpongu, Manga and Nyankpala respectively which is below the critical range (10.0 - 14.0 mg kg⁻¹). The populations of rhizobia in the study soils were 3.19 × 10¹, 2.79 × 10¹ and 4.36 × 10¹ cells g soil⁻¹ of soil for Kpongu, Manga and Nyankpala respectively.

Table 1. Physico-chemical and biological properties of the study sites

Parameter	Kpongu	Manga	Nyankpala
pH (1:2.5 H ₂ O)	5.53	4.12	5.37
Organic Carbon (%)	0.66	0.44	0.40
Total Nitrogen (%)	0.06	0.02	0.04
Available Phosphorus (mg kg ⁻¹)	1.96	1.24	2.70
Soil texture	Loamy sand	Loamy sand	Sandy loam
IRP (cells g ⁻¹ of soil)	3.19 × 10 ¹	2.79 × 10 ¹	4.36 × 10 ¹

*IRP – Indigenous Rhizobia Population

3.2. Shoot Biomass as Affected by the Application of Treatments

Teprosyn Mo, Teprosyn Mo+Legumefix and Legumefix increased shoot biomass yield by 15.48%, 14.91% and 13.60% in Kpongu respectively over the control (Figure 1). In Manga, the highest shoot biomass was recorded in Legumefix (1338 kg ha⁻¹) whilst the control recorded the least (790 kg ha⁻¹) (Figure 1). In Nyankpala, percentage increases in shoot biomass over the control were 22.42%, 18.17% and 6.91% for Legumefix, Teprosyn Mo+Legumefix and Teprosyn Mo respectively (Figure 1).

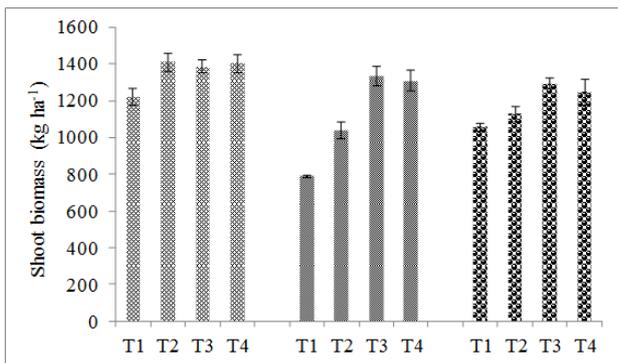


Figure 1. Soybean shoot biomass in the three study sites as influenced by the application of rhizobia inoculant and Teprosyn Mo.

Key: T₁ = Control, T₂ = Teprosyn Mo, T₃ = Legumefix, T₄ = Teprosyn Mo+Legumefix. Error bars represent the mean ± SED.

3.3. Nodule Dry Weight

Rhizobia inoculation (Legumefix) increased significantly nodule dry weight in Kpongu and Manga (Figure 2). Legumefix and Legumefix+Teprosyn Mo increased nodule

dry weight significantly by 130% and 83.49% respectively over the control in Kpongu. In Manga, Legumefix was significantly (P<0.05) effective in eliciting increased nodulation response (371 mg plt⁻¹) over the control. Furthermore, nodule dry weight increased by 63.44%, 43.17% and 31.28% for Legumefix, Teprosyn Mo+Legumefix and Teprosyn Mo respectively over the control. In Nyankpala, no significant (P>0.05) differences were observed among treatments (Figure 2).

3.4. Grain Yield

Figure 3 shows the effects of the treatments on grain yield in the 3 study sites. None of the treatments had significant (P>0.05) effect on soybean grain yield in Kpongu. However, at Manga, significant (P 0.05) differences existed among the treatments with Teprosyn Mo+Legumefix producing the highest grain yield (1522 kg ha⁻¹) while the control recorded the lowest grain yield (498 kg ha⁻¹). In Nyankpala, the application of Teprosyn Mo+Legumefix increased grain yield significantly (P<0.05) by 42.10% while Legumefix recorded an increase of 22.43% but was not significant (P>0.05) relative to the control. Teprosyn Mo however, resulted in a 2.08% decline in soybean grain yield.

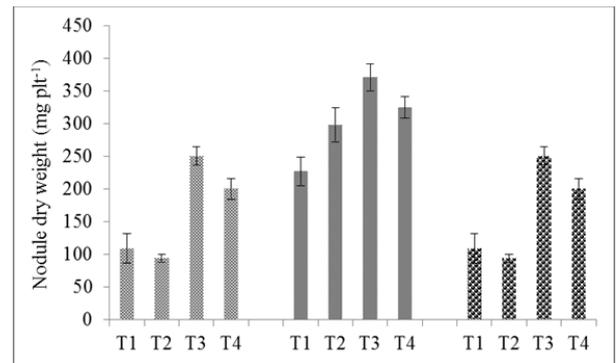


Figure 2. Soybean nodule dry matter in the three study sites as influenced by the application of rhizobia inoculant and Teprosyn Mo.

Key: T₁ = Control, T₂ = Teprosyn Mo, T₃ = Legumefix, T₄ = Teprosyn Mo+Legumefix. Error bars represent the mean ± SED.

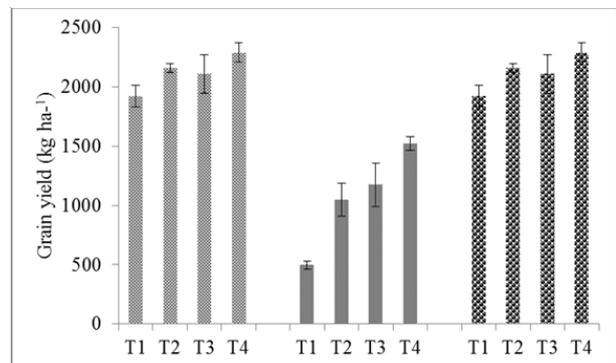


Figure 3. Soybean grain yield in the three study sites as influenced by the application of rhizobia inoculant and Teprosyn Mo.

Key: T₁ = Control, T₂ = Teprosyn Mo, T₃ = Legumefix, T₄ = Teprosyn Mo + Legumefix. Error bars represent the mean ± SED.

3.5. Economic Assessment of Products Tested

The economic analysis showed that additional benefits were achieved when Teprosyn Mo, Legumefix and Teprosyn Mo + Legumefix were applied except for Teprosyn Mo in Nyankpala. The value cost ratio analysis also showed that all the treatments had a VCR below 2 except the combined application of Teprosyn Mo + Legumefix in Manga (VCR = 2.65).

All the treatments had a VCR below 2 except Teprosyn Mo+Legumefix in Manga (VCR = 2.65).

4. Discussion

The soil fertility status at Kpongu, Manga and Nyankpala was generally low (Table 1). This is in line with [11], who reported that soil nutrient levels in the Savannah zones of Ghana are particularly low with high pH values, low organic matter, nitrogen and available P levels. Soils of Nyankpala had relatively higher indigenous rhizobia population than Kpongu and Manga (Table 1). Nonetheless, the soils of the study sites had a low number of rhizobia population (< 50 cells g^{-1} soil) according to [53].

No significant differences in shoot biomass dry weight were observed among treatments tested in all the study sites (Figure 1). This is in agreement with [42, 43] who reported that inoculation of soybean variety (TGx1448-2E) did not significantly increase shoot yield. A temporary drought, low and poorly distributed rainfall during crop growth in all study sites could be contributory factors. Plants whose roots have been subjected to stress deficient in soil water cause at least in part a change in the amount and kind of growth regulators supplied from the roots which result in reduced shoot growth [17].

Legumefix increased nodule dry weight significantly over the control and Teprosyn Mo in Kpongu (Figure 2). This is in agreement with [31], who observed that rhizobia inoculation significantly increased nodule dry weight of soybean over control. The results also indicated that Teprosyn Mo treated plots produced the lowest nodule dry weight. This observation was however, in contrast with those of [32] and [39] who indicated that nodule number and dry weight of cowpea were increased by Mo application. This observation can also be attributed to the acidic nature of the soil (pH 5.53) in the study area. In the soil solution, Mo exists as an anion at soil pH above 4 [36] but becomes deficient under pH levels below 6.0 [47]. In Manga, Legumefix recorded the highest nodule dry weight (Figure 2). Soils in Manga recorded the lowest indigenous rhizobia population (2.79×10^1) and as such responded significantly to rhizobia inoculation. According to [53], where there are low (<50 rhizobium bacteria g^{-1} soil) naturalised populations of rhizobia specific to a target legume, the introduction of new strains by seed inoculation is normally successful. Therefore the introduced strain provided enough viable and effective rhizobia to participate in the infection process [13] for higher nodulation. Nodule dry weight in the sole Teprosyn Mo and

combined Teprosyn Mo+Legumefix treatments did not differ significantly from the control in Manga (Figure 2). This could be explained by the high acidic level of the soil as stated by [47] that, in acidic soils (pH $<$ 5.5), Mo availability decreases as anion adsorption to soil oxides increase. Due to the saline or acidic sources of micronutrients, seed treatment with Mo can damage rhizobia, drastically affecting the survival of inoculated bacteria on the seeds, thus resulting in reduced nodulation and N_2 fixation [4, 22].

No significant differences in nodule dry weight were observed among the treatments in Nyankpala (Figure 2). This agrees with the findings of [42] and [14] who reported no significant increase in nodulation following rhizobia inoculation. Soils in this study site recorded the highest indigenous rhizobia population of 4.36×10^1 . High population density of indigenous and naturalized rhizobia population of 1×10^2 rhizobia cell g^{-1} soil has been reported to prevent nodulation and displace applied inoculums [10, 27, 52]. This suggests that failure of this soil to respond to rhizobia inoculation was primarily due to the presence of sufficient number of indigenous rhizobia population to adequately compete with the introduced strain for nodule occupancy [5]. Since the indigenous rhizobia are present through the soil while the introduced rhizobia are only present on the seed coat, there is the competitive advantage of the native rhizobia over the introduced strain (Denton *et al.*, 2002). Several reports have also indicated no improvement or toxicity from Mo seed-coating through suppressive effects of salts used as Mo sources on *Bradyrhizobium* affecting bacterial survivability and nodulation [4, 12].

Treatment effects on grain yield in Kpongu were not significantly different from the control (Figure 3). A study by [25] also confirms that soybean yield did not differ between inoculant products and the controls. The lack of significant grain yield response in the study sites could be attributed to the temporary drought experienced during plant growth and mineral nutrient deficiencies (N, P) which are the major constraints limiting legume N_2 fixation and yield [41]. [50] suggested a potential negative yield response from inoculation under extreme drought conditions occurring during pod fill due to an increased vegetative sink.

In Manga, significant differences in grain yield were observed among treatments with Teprosyn Mo+Legumefix recording the highest (1522 kg ha^{-1}) (Figure 3). This is in confirmation with a multi-locational trial conducted by [29] at farmers' fields in Bangladesh, which showed that, the efficiency of seed treatment with Mo may be further enhanced by adding rhizobium as yield increases were 37% - 90% over the untreated control. According to [12], Mo in legumes serves as an additional function to help root nodule bacteria to fix atmospheric N resulting in increased yield which account for over 200% increase in grain yield. Legumefix, a rhizobia inoculant helps to boost the natural population of beneficial nitrogen-fixing bacteria to form effective nodules that are responsible for effective BNF [15] and explains the over 100% increase in grain yield compared

to the control. Furthermore, a series of field experiments in DR-Congo and Nigeria resulted in significant yield increases of 80 – 300% with inoculation [6, 46].

Significant differences were observed among treatments in grain yield at Nyankpala (Figure 3). Teprosyn Mo+Legumefix and Legumefix increased grain yield by 42% and 22% respectively over the control while Teprosyn Mo recorded the lowest grain yield. This is in line with a study by [8] who observed that addition of molybdenum alone gave a lower yield in soybean than its addition with rhizobium inoculant.

5. Conclusion

The application of Legumefix resulted in over 50% increase in soybean yield relative to the control in all the study sites and was further enhanced when combined with Teprosyn Mo with a relative increase of 205.62% in Manga. The co-application of Teprosyn Mo+Legumefix was the most profitable treatment (VCR = 2.65) in Manga. Farmers in Manga can therefore obtain greater economic benefit from the combined application of Teprosyn Mo and Legumefix for soybean production.

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