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Inoculated Soybean Yields Response to Nitrogen and Phosphorus Application

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Authors' contributions

This work was carried out in collaboration among all authors. Author ATP designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors EMM, JOO and RY managed the literature searches, data analyses, data arrangement and data synthesis. Author DK managed the field experiment, collected data and conducted the nodulation study. All authors read and approved the final manuscript.

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ABSTRACT

Inherent low soil nitrogen (N) and phosphorus (P) is one of the major hindrances of increased soybean productivity in Malawian soils. Although, inoculation of legumes with *rhizobia,* has been advocated for decades as a way of boosting leguminous crops' productivity through biological nitrogen fixation (BNF), the effectiveness of this strategy, has been low. An experiment was carried out to investigate the effect of the application of small doses of N and P to inoculated soybean. It was laid out in a complete block design (CBD) replicated three times and the treatments included: 1. Soybean only, 2. Inoculated soybean, 3. Inoculated soybean + 30 kg N ha^{-1}, 4. Inoculated soybean + 30 kg N ha^{-1} + 25 kg P ha^{-1}, 5. Inoculated soybean + 30 kg N ha^{-1} + 35 kg P ha^{-1}. The soybean in all treatment plots except for treatment 1 was inoculated with 30 kg N ha^{-1} applied as urea to treatment plots 3, 4 and 5. Phosphorus as TSP was applied to treatment 4 and 5 at the rate of 25 and 35 kg P ha^{-1}. Data collected included; selected soil physical and chemical properties,

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biomass and pod yields. Data obtained were subjected to Analysis of Variance (ANOVA) using the GenStat statistical package and treatment effects tested for significance using the F-test at 5% level of significance. Means were separated using the least significant difference ($P<0.05$). Results indicate that inoculating soybean with *rhizobia* and inoculating soybean with *rhizobia* plus applying 30 kg N ha^{-1} did not significantly increase biomass yields. However, inoculating soybean plus the combined application of 30 kg N ha^{-1} and, 25 kg P ha^{-1} or / and 35 kg P ha^{-1} increased the soybean biomass yields by 54% and 70% respectively above control. The lack of significant response when N was applied without P points to the significant role played by phosphorus in root development and energy transfer processes within the plant. Effective nodulation, however, was significantly higher ($p<0.05$) above the control where inoculation was combined with the application of 30 kg N ha^{-1} and 35 kg P ha^{-1}, hence underlining the role played by phosphorus in nodule development and the role of starter N in soils low in N.

Keywords: Soybean; inoculation; biomass; biological nitrogen fixation.

1. INTRODUCTION

Soybean is a very important leguminous crop in Malawi. The country is the third largest producer of the crop in SADC (73,000 tones) after South Africa (588,000 tones) and Zambia (112,000 tones), though having more land under the crop (75,000 ha) than Zambia (62,000 ha) [1]. The crop is cultivated under diverse climatic conditions, on well drained soils. It has high content of protein (40%) and no-cholesterol oil (20%) [2], qualifying it as appropriate for human consumption as well as livestock feed. Its nitrogen fixing ability positions the crop as being suitable for a wide range of cropping systems in the country. Soybean production in the country is dominated by smallholder farmers [1].

A myriad of production constraints however, have kept the productivity of the crop far below the yield potential of the many varieties currently being grown in the country. At present, the mean grain yield under smallholder farmers' conditions is pegged at 892 kg ha^{-1} against a yield potential of over 4,500 kg ha^{-1} for most of the varieties currently being grown by the farmers [3]. Low crop productivity has been attributed chiefly to poor soil fertility status of Malawian soils, which are inherently low in nitrogen (N) and phosphorus (P) [4,5]. This limits the productivity of the crop. Being a legume, soybean fixes atmospheric N through symbiotic relationship with nodule dwelling *rhizobium* bacteria [6], a process called Biological Nitrogen Fixation (BNF). Both N and P are requisite in the growth and development of the crop. Root development and subsequent nodule formation after root infection by the N fixing bacteria requires energy derived from the phosphorus containing ATP molecule [7]. Furthermore, nitrogen is required for the general vegetative growth of the legumes. The element is part of leaf protein which is vital for chroloplast formation and photosynthesis [8]. This entails that where the element is limiting photosynthesis is crippled hence crop development and productivity is limited. This is most critical in the early stages of the development of leguminous crops before N fixation by the nodule dwelling bacteria commences. It is worthwhile to note that only 25 to 60% of N in soybean dry matter is derived from symbiotic N fixation, the rest is absorbed from the soil [9]. However a balance must be struck; since if too much soil N is available, nodulation is constrained as the plant will prioritize soil N over fixed N and no nitrogen fixation will take place. Therefore, it follows that where soil N and P are limiting, judicious starter doses of the nutrients coupled to inoculation is essential for crop establishment prior to nodulation and subsequent atmospheric fixation of nitrogen. A review of soybean research by [10] indicated that in experiments where soybean was treated with mineral N fertilizer or not treated with mineral fertilizer with or without inoculation with *Bradyrhizobium japonicum*, N fertilization with or without inoculation significantly increased grain yields compared with untreated experimental plots. On the other hand, in northern Nigeria, [11] conducted a study on smallholder farms in 2011 and 2012 on understanding variability in soybean yields and response to P-fertilizer and rhizobium inoculants on farmers' fields using four treatments: no inputs (control); single super phosphate (SSP) fertilizer (P); inoculants (I) and SSP plus inoculants (P + I). A strong response to both P and I, was observed which significantly increased grain yields by 452 and 447 kg ha^{-1} respectively. The combined effect of P + I (777 kg ha^{-1}) resulted in the best average yields.

For the case of Malawi, inoculating legumes with *rhizobia,* particularly the soybean, has been advocated for decades as a way of boosting productivity through BNF. But, the effectiveness of the strategy however, has been constrained by the low N and P status of Malawian soils. This has been compounded by the deficiency of some micronutrients in the soil. The strategy has been crippled further by the low inoculants production capacity in the country, which has resulted into commercialization of the production of the product through the private sector. Production though and availability of inoculants remain low. This implies that many smallholder farmers grow the crop without inoculating with effective *rhizobia* capable of fixing atmospheric nitrogen and without applying starter doses of inorganic fertilizer. Resultantly, soybean productivity is compromised. The need therefore, to establish nutrient rates combined with inoculation for increased soybean production on smallholder farmers' fields in the country is apparent. To contribute to this knowledge, a study, at Bvumbwe Agricultural Research Station in Malawi was conducted to assess the effect of applying N and P starter doses to inoculated soybean on soybean yields.

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted on experimental fields of Bvumbwe Agricultural Research Station, (150º 55' S 350º 04' E). The Station is at 1174 to 1228 m above sea level off the Limbe-Thyolo road in Malawi on the Shire Highlands. The average of annual rainfall is 1,219 mm most of which falls in December to April. Frequent mist and drizzles (Chiperoni), and occasional frosts occur during the cool months of May to August are a special feature. The study was conducted off the 2013/2014 cropping season under irrigation from June to August, 2014.

2.2 Materials

Materials used during the study included the following; soybean (Nasoko) which matures in 3 months, soybean inoculants produced at Chitedze Agricultural Research Station, triple super phosphate (TSP) ($Ca(H_2PO_4)_2.H_2O$) as a source of P and urea ($CO(NH_2)_2$) as a source of N.

2.3 Soil Sampling and Preparation for Laboratory Analysis

The sampling area was 0.093 acres. Nine sampling points were randomly selected in a zig zag pattern. The soil auger was used to collect soil samples at depths of 0 – 20 cm and 20 –40 cm. A Composite soil sample was made for each soil depth and thereafter soil was placed in well labeled bags [12]. The soil samples were air dried, crushed in pestle and mortar and thereafter sieved using 2 mm sieve. The soil samples were therefore ready for analysis of physical and chemical properties at Bvumbwe Soils Laboratory, at Bvumbwe Agricultural Research Station, in Southern Malawi.

2.4 Laboratory Soil Analysis

Laboratory soil analysis of the composite soil samples was done in order to characterize the soil. The composite soil samples were analyzed for organic carbon (OC), total nitrogen (N), available phosphorus (P), exchangeable potassium (K), magnesium (Mg), calcium (Ca) and soil pH (H_2O). Soil pH was measured in water (1:2.5) using the pH meter [12]. Soil analysis for P, exchangeable K, Mg and Ca was done using Mehlich 3 extraction procedures [13] while OC was determined using the Wakley black (colorimetric) method [14] and total N was determined by Kjeldahl method [15].

2.5 Preparation of the Seed Bed

The field was demarcated into three blocks, with each block divided into five, 5 m x 4 m plots for planting the experiment. Ridges were made and spaced at 75 cm apart. The experiment was laid in a Complete Block Design (CBD). The field where the experiment was conducted was largely homogeneous in terms of topography and fertility.

2.6 Inoculation of Soybean

In order to inoculate soybean 30 g of sugar was added to 200 ml of clean lukewarm water in a container and was shaken well until all the sugar dissolved. Later 10 kg of soybean seeds and one packet of soybean inoculant were added to the solution in the container. Thorough mixing of the soybean seeds and the inoculant was done until all the seeds were uniformly covered with the inoculant. The process was done at the field and the inoculated soybean seeds were dried under a under shade and planted thereafter under moist conditions.

2.7 The Field Experiment

The experiment was laid out in a CBD replicated three times. The treatments were as follows:

1. Soybean only, 2. Inoculated soybean, 3. Inoculated soybean + 30 kg N ha^{-1}, 4. Inoculated soybean + 30 kg N ha^{-1} + 25 kg P ha^{-1}, 5. Inoculated soybean + 30 kg N ha^{-1} + 35 kg P ha^{-1}. The soybean in all treatment plots except for treatment 1 was inoculated with 30 kg N ha^{-1} applied as urea to treatment plot 3, 4 and 5. Phosphorus as TSP was applied to treatment 4 and 5 at the rate of 25 and 35 kg P ha^{-1}. Adequate water was supplied to the crop through irrigation thrice a week using sprinkler irrigation. Yields data that included pod and biomass yields were collected at harvest time. Poor seed yields were registered as the crop was attacked by soybean rust. The poor seed yields were not weighed.

2.8 Biomass and Pod Yields Assessment for the Soybean

Soybean biomass yields at harvest were assessed as described by [16]. Biomass and pods were harvested from a 3 m x 2.25 m net plot. Shelling of the pods was done, but most of the pods were not filled with seed. The biomass was oven dried for 72 hours at 70ºC to constant weights.

2.9 Statistical Analysis

All the soil and biomass data were analyzed using Genstat statistical package and were subjected to analysis of variance at 95% level of confidence. Means were separated by the least significant difference (P<0.05).

3. RESULTS

3.1 Level of Soil Nutrients at the Site

Tables 1 summarize the physical and chemical properties of soils for the experiment site before planting. The laboratory results indicate that soil texture was predominantly sandy clay (SC) at 0-20 cm to 20-40 cm. The soil reaction was moderately acid at both soil depths (pH= 6.0) (17). The total N content at 0-20 cm and 20-40 cm depths was low (≤ 0.12%) according to [17]. The soil organic carbon (OC) content was medium (≥ 0.88%) [17] between 0-20 cm. Plant available P was adequate (≥ 30 mg P kg^{-1}) at both soil depths due to use of poultry manure by other researchers at the site. The field had adequate (> 0.105 cmol kg^{-1}) exchangeable potassium (K) between 0-20 cm (0.39 cmol kg^{-1}) and between 20-40 cm (0.62 cmol kg^{-1}) while calcium and Mg was high (≥ 2 cmol kg^{-1}; ≥ 2.04 cmol kg^{-1}) [17] at both soil depths.

3.2 Effective Nodules, Soybean Biomass, Number and Weight of Soybean Pods

The effective nodules, number of pods ha^{-1}; yields of pods in kg ha^{-1} and soybean biomass in kg ha^{-1} were as presented in Figs. 1, 2, 3 and 4. As indicated earlier, soybean seed yields were not reported because the yields were extremely low across the treatments, due to the attack by soybean rust [19]. However statistical analysis of the collected data indicated significant differences (p<0.05) for the number of effective nodules, number of pods, the yields of pods and biomass yields across the treatments as indicated in Figs. 1, 2, 3 and 4.

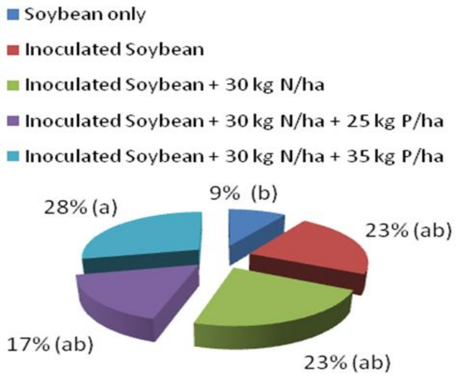

Fig. 1. Effective nodules

4. DISCUSSION

The pods and biomass yields in Figs. 3 and 4 indicates that inoculating soybean with *rhizobia* and inoculating soybean with *rhizobia* plus applying 30 kg N ha^{-1} did not affect nodulation, the effectiveness of the nodules (Fig. 1) nor increase biomass yields. However, inoculating soybean plus the combined application of N at 30 kg N ha^{-1} and P at 25 and 35 kg P ha^{-1} increased the soybean biomass yields by 54% and 70% respectively above control. The lack of significant response when N was applied without P points to the significant role played by P in root development and energy transfer processes within the plant [20]. P also plays a role in initial nodule formation and development [21]. Other researchers [22,23] found no significant increase in soybean biomass yields using different commercial inoculants but up to 50% increased seed yields while [24] found 9 to 55% increase in seed and biomass yields respectively.

Table 1. The soils' physical and chemical properties at the study site before the experiment

Parameter	Depth		Depth		Range	Reference
	0-20 cm	Rating	20-40 cm	Rating		
% sand	48	-	44	-	-	-
% clay	40	-	42	-	-	-
% silt	12	-	14	-	-	-
Texture class	SC	-	SC	-	-	[18]
pH_{H2O}	6.0	Low	5.76	Low	≤ 6.0	[17]
Soil reaction		Moderately acid		Moderately acid	5.5-5.7	"
% OC	1.02	Medium	0.99	Medium	0.88-1.5%	"
Total N (%)	0.09	Low	0.09	Low	≤ 0.12%	"
P mg kg^{-1}	42.2	Adequate	41.6	Adequate	≥30 mg P kg^{-1}	"
Ex. K cmol kg^{-1}	0.62	Adequate	0.32	Adequate	>0.11-4.0 cmol kg^{-1}	"
Ex. Mg cmol kg^{-1}	2.75	High	2.24	High	>2.0 cmol kg^{-1}	"
Ex. Ca cmol kg^{-1}	10.4	High	8.23	High	>2.04 cmol kg^{-1}	"

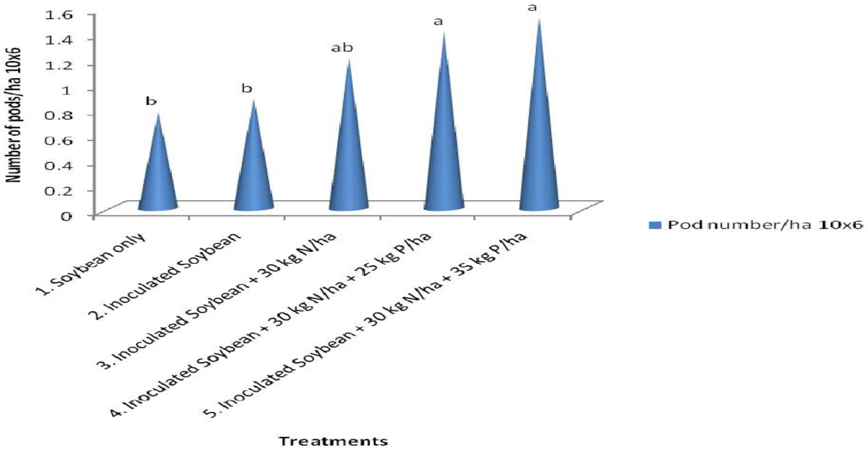

Fig. 2. Number of pods ha-^{1}

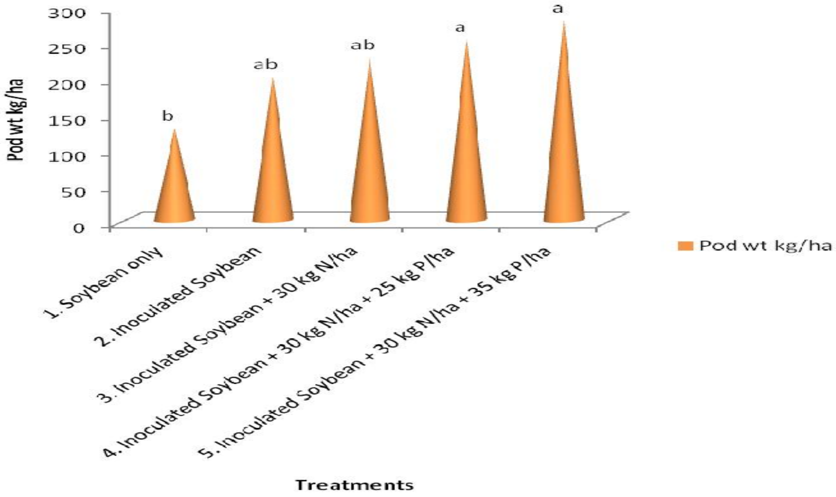

Fig. 3. Weight of pods kg ha^{-1}

Under the conditions of the study, where soybean was inoculated and N applied the biomass yields were statistically the same with the treatments to which inoculation of soybean was complemented with the application of both N and P. Thus indicates that the response to P was statistically insignificant. This may be due to the initial adequate P amounts (42.2 mg kg^{-1}) in the soil attributed to previous use of poultry manure at the site (Table 1). Effective nodulation however was significantly higher above the control where inoculation was combined with the application of N (30 kg ha^{-1}) and a higher rate of P (35 kg ha^{-1}), hence underlining the role played by P in nodule development and effectiveness [21].

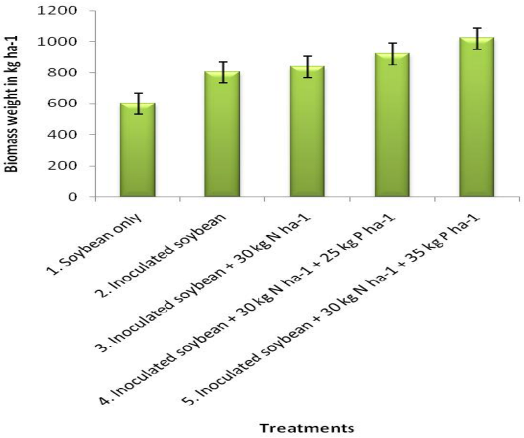

Fig. 4. Biomass yields for the soybean according to treatments

5. CONCLUSION

Under the conditions of the study it can be concluded that inoculation of soy bean without fertilizers generate yields similar to the non inoculated soy bean while inoculation of soy bean together with application of 30 kg N ha^{-1} and 35 kg P ha^{-1} fertilizer increases yields relative to that of non-inoculated soy bean. Further studies with different nutrient levels including micronutrients may give further insight into the best nutrient combinations for increasing soybean yields in Malawi.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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