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EFFECT OF DIFFERENT RATES OF ~~PHOSPHORU~~S FERTILIZER ON SOYBEAN {GLYCINE MAX (L.) MERRIL} INOCULATED WITH RHIZOBIUM

Abstract

The experiment was conducted during the 2 farming season at the University for Development Studies, Nyankpala in the Northern Region of Ghana. The objective of the study was to determine the influence of rhizobium inoculants and phosphorus (P) at different application rates on yield and yield components of soybean. Two levels of inoculation regimes (un-inoculated (-In) and inoculated (+In)) were combined with four application rates of phosphorus (0 kg P/ha, 15 kg P/ha, 30 kg P/ha and 45 kg P/ha). The experiment was laid in a Randomized Complete Block design with three replications rameters measured were crop emergence, plant height, leaf area, canopy spread, number and weight of nodules, number of pods and total grain yield. The results c ned indicated significance differences in all the parameters measured. Phosphorus application at 45 kg P/ha plus Rhizobium inoculants recorded significantly higher growth and yield parameters than the rest of the treatments. The economic analysis of the treatments also showed that inoculation of soybean seeds or a combination of inoculation with 45 kg P/ha was more profitable than the application of 45 kg P/ha without inoculation. The study recommends inoculation of soybean seeds with Rhizobium inoculants in association with application of phosphorus fertilizer at 45 kg P/ha.

Keywords: Inoculation, phosphorus rates, Yaralegume, Grain yield

1.0 Introduction

Soybean {*Glycine max* L. (Merrill)} is one of the important oil grain legume crops in the world. In the international world trade market, soybean is ranked number one in the world among the major oil crops such as rapeseed, groundnut, cotton seed, sunflower, linseed, sesame and safflower [1]. Soybean is the cheapest protein source as compared to other protein sources such as egg, fish and meat. With an average protein content of 20% on dry matter basis, soybean has the highest protein content of all field crops and is second only to groundnut in terms of oil content among the food legumes [2] and [3]. Besides, its rich nutrition source for human and animals, it fixes atmospheric nitrogen which is a major boost

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to African farmers where the soils are low in nitrogen and commercial fertilizers are expensive to afford. It is also an important source of income to farmers [4].

In spite of its great potential, its production is still limited by low yields resulting in a wide gap between current production levels and the crop's potential. In order to improve production levels, areas to address include development of high yielding varieties, disease and pest control and development of improved cultural practices [5]. In northern Ghana, where the largest production occurs in the country, the average yield is about 2.5 tonnes/ha [6] as compared to that of USA which is 4.6 tonnes/ha [7]. The low yields can be attributed to soils which are inherently poor and deficient in organic matter and other vital nutrients such as phosphorus and nitrogen. Studies in parts of Northern, Upper West and Upper East Regions showed that available phosphorus is very low of about 6.0 mg/kg soil [8]. Phosphorus deficiency can limit nodulation by legumes and phosphorus fertilizer application can overcome the deficiency [9]. Availability of phosphorus in the soil influences the efficiency of *Rhizobium* that fixes atmospheric nitrogen in association with nodulating legumes as it is directly involved in biological nitrogen fixation via legume-*Rhizobium* symbiosis. This is attracting considerable research attention world-wide due to it economic viability for resource poor farmers and environmental friendliness [10], [11] and [12]. The objective of this study was to assess the impact of phosphorus at different application rates and Rhizobium inoculants on the growth and yield of soybean.

2.0 Methodology

2.1 Site description

The experiment was conducted on the experimental field of the Faculty of Agriculture of the University for Development Studies, Nyankpala in the Northern Guinea savannah ecological zone during the 2012 cropping season. The area experiences a unimodal annual rainfall of 1000 mm-1200 mm from April to November. The temperature distribution is uniform with mean monthly minimum and maximum values of 21^0C and 34.1^oC, respectively and a relative humidity of 53%-80% [13].

2.2 Experimental design

A 2 x 4 factorial experiment consisting of two inoculation regimes and four application rates of phosphorus fertilizer were laid out in a Randomized Complete Block design with four

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replications. The P treatments were 0 kg P/ha, 15 kg P/ha, 30 kg P/ha and 45 kg P/ha from Yaralegume fertilizer with or without inoculants. The plot size was 2.5 m x 2.5 m. Yaralegume fertilizer is composed of 0%N-18%P_2O_5 13%K_2O+31%Ca+4%S+2%Mg+0.6%Zn. This was applied two weeks after sowing by burying it in a trench dug about 5 cm away from the plant hill.

2.3 Planting materials and sowing

The improved soybean variety, ~~Jenguma which was used was obtained from the CSIR-Savanna Agricultural Research Institute (SARI) located at Nyankpala~~. For the inoculated treatments, the seeds of Jenguma were inoculated with commercial Rhizobium inoculants, *Legumefix* (containing 1 x 10^9 cells/g) at the rate of 1 kg seed per 5 g of inoculants [14] before sowing. The sowing was done early in the morning to avoid exposing the inoculants to the direct rays of the sun which might affect the quality of the inoculants. Sowing was done at three seeds per hill at a distance of 50 cm between ridges and 10 cm within ridges. In order to avoid contamination of the un-inoculated treatments with the inoculants that might get stuck to the hand during sowing, the un-inoculated seeds were sown before the inoculated ones. The plants were allowed to grow to maturity under rain-fed conditions while the necessary management practices like weeding were observed.

2.4 Data collection

Data were collected on percent crop establishment, plant height, leaf area, canopy spread, shoot biomass, number of nodules, nodule biomass, number and dry weight of pods at maturity and grain yield. Partial budget analysis for the treatments was also carried out.

Percent crop establishment was determined three weeks after sowing by counting the emerged plants in the four inner ridges and dividing the number by the expected number of plants (i.e. number of seeds sown per the four hills) multiplied by 100. Plant height, leaf area and canopy spread were measured in the third, sixth and ninth weeks after sowing (3 WAP, 6 WAP and 9 WAP). A meter rule was used to take the height of each of the eight plants randomly selected from each of the four inner ridges (two plants per ridge), respectively and tagged by measuring from the base of the plant (ground level) to the top of the main stem. ~~For leaf area, one leaf each was selected from the lower, middle and upper parts of each plant and the breadth and length were measured with a meter rule. The product of these two parameters constitutes the area of the lea~~he means of the areas of the three leaves on each

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plant were taken as the leaf area of that plant while the mean leaf area of the eight sampled plants on each plot (treatment) was calculated and recorded as the leaf area of that plot. Canopy spread was measured from the last leaf on one side of each of the eight plants to the last leaf on the other side of the same plant using a measuring tape.

For the assessment of shoot biomass and nodulation, ~~the~~ eight plants which had been tagged for plant height measurements were sampled at full pod stage (R4 stage) by gently pulling them out of the ground after carefully loosening the soil around the plants with a cutlass before pulling them out gently with the hand making sure to collect the few nodules that got detached from the roots in the process. The shoots from each plot were then ~~severed~~ from the root system, bulked and weighed on a digital scale after which they were air-dried for one day followed by oven-drying at 80°C for 48 hours (to a constant weight) and the weights recorded. The nodules were detached from the roots, counted, weighed and then oven-dried at 70°C for 48 hours and the weights recorded.

At harvest, all the pods from the plants of the two innermost ridges of each plot were harvested manually and counted after which they were sufficiently sun-dried (~~to about 11-13% moisture content~~) on a concrete platform and weighed on a digital scale. The dried pods were threshed and winnowed to separate the grains from the husk and the former weighed to obtain the grain yield.

2.5 Data analysis

Data ~~collected~~ were subjected to a ~~one-way~~ analyses of variance (ANOVA) using the computer statistical package Genstat (2008 Edition) and treatment means compared using the least significance difference (LSD) at 5% probability level.

3.0 Results and Discussion

3.1 Percent crop establishment

~~Crop establishment was seen to be significantly better in inoculated plots than in un-inoculated plots and this was observed irrespective of inoculation regime though the effects due to higher P application rates were more pronounced in inoculated treatments (Figure 1).~~ There was therefore an interaction effect between P fertilizer application rate and Rhizobium inoculation resulting in the highest percent crop establishment being observed in treatments with joint applications of 45 kg P/ha and Rhizobium inoculants. This observation confirms findings by [15] who intimated that increased plant establishment from seeds appears to be a

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result of improved soil productivity due to bacterial activity and available nutrients. Increases in whole plant growth in response to phosphorus supply have been reported in soybean [16], cowpea [17] and groundnut [18]. With Rhizobium inoculation showing a better crop establishment than without inoculation in the 0 kg P/ha treatment, the improved establishment could be attributed to rhizobial activity and perhaps also to a vigorous germination and growth of the seeds used.

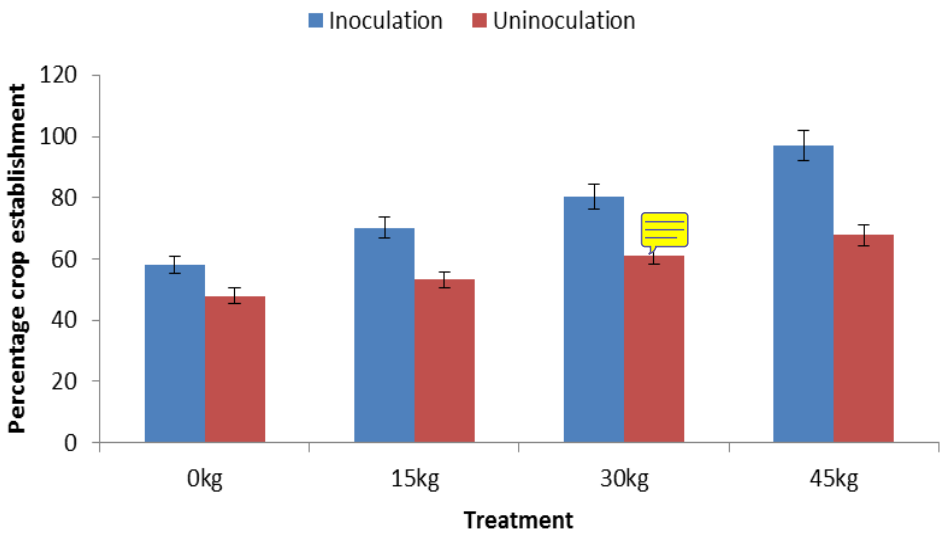

Figure 1: Influence of Rhizobium inoculation and phosphorus fertilizer application rates on establishment of soybean

3.2 Plant height

The application of ~~phosphorus~~ fertilizer and Rhizobium inoculants impacted on the growth of soybean when compared to the non-phosphorus and non-Rhizobium treatments (Figure 2). As expected, the plants grew taller with time within a fertilizer application regime, especially when inoculated (Figure 2). Irrespective of growth stage, plants fertilized with 45 kg P/ha and inoculated grew ~~tallest~~ (51.8 cm). The current study confirms other research findings by [19] and [20] that increased levels of phosphorus significantly increased plant height. ~~Also, [21] observed that Rhizobium inoculation increased plant height. [22] reported incidences of increased plant height due to interactions between phosphorus fertilizer application and~~

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~~Rhizobium inoculants whereas [23] observed that over-supply of phosphorus resulted in decreases in plant growth, nodulation and acetylene reduction in soybean.~~

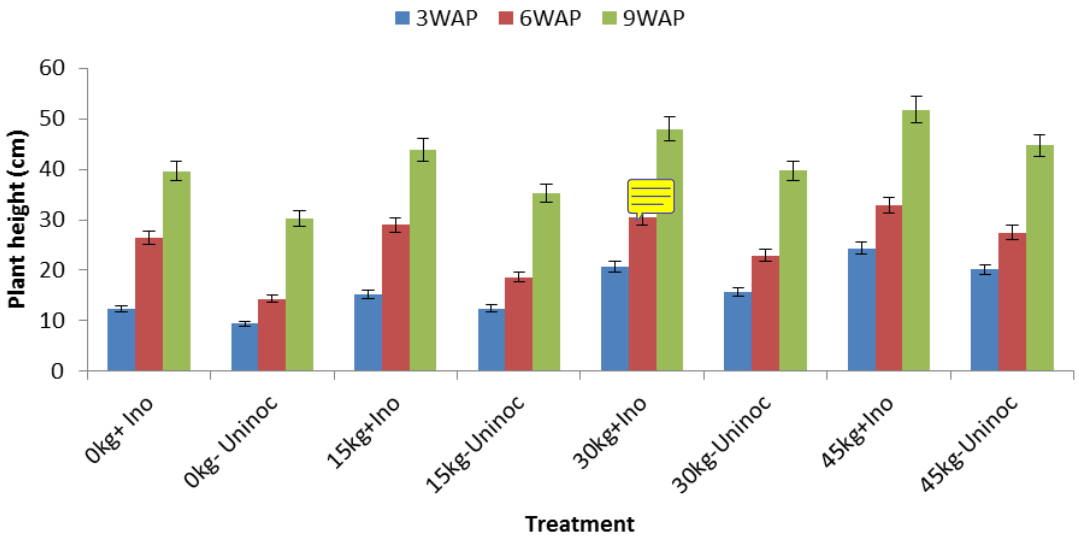

Figure 2: Influence of Rhizobium inoculation and phosphorus fertilizer application rate on plant height of soybean at different growth stages.

3.3 Leaf area

~~The response of leaf area of soybean to Rhizobium inoculation and rate of P fertilizer (Figure 3) showed a similar trend as that of plant height (Figure 2). Inoculation increased leaf area at all the three stages of sampling irrespective of the rate of P applied (Figure 3) and whether plants were inoculated or not, leaf area increased with plant age becoming more significant at 9 WAP in uninoculated plants. However, leaf area was highest in all inoculated plants fertilized with 45 kg P/ha compared with other treatments. The results of the current study confirm results of a similar study conducted by [24] who reported that primary whole plant response to phosphorus addition was an enhancement in shoot growth caused by increases in leaf area expansion. In a similar study on common bean, the observed increase in cell division due to phosphorus application resulted in increasing amounts of ATP at growth centers [25] which affected many plant characteristics such as leaf area.~~

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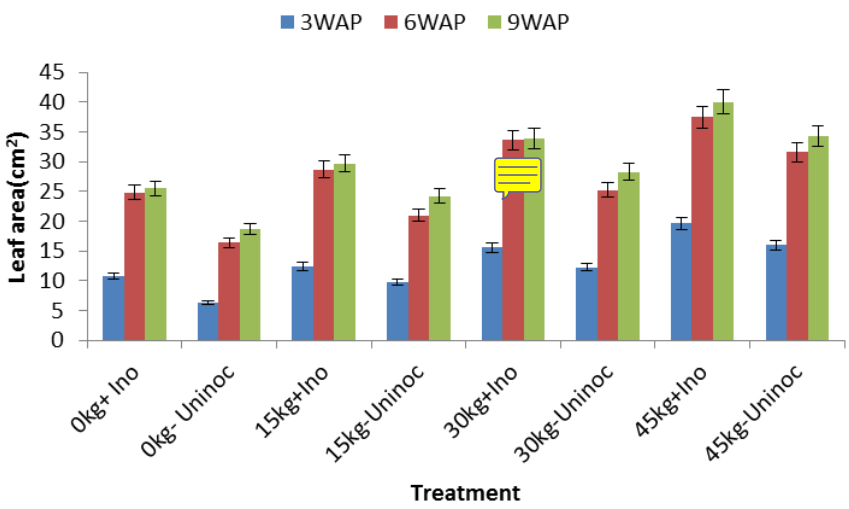

Figure 3: Influence of Rhizobium inoculation and phosphorus fertilizer application rate on leaf area of soybean at different growth stages.

3.4 Canopy spread

Phosphorus application rate, Rhizobium inoculation and their interaction significantly affected canopy spread of soybean plants (Figure 4). Phosphorus application rate of 45 kg P/ha with Rhizobium inoculation recorded the highest canopy spread at 3, 6 and 9 WAP compared to the spreads at 15 kg P/ha and 30 kg P/ha 9 WAP. The above results are in line with the findings of [26] that application of phosphorus significantly increased most of the soybean growth parameters.

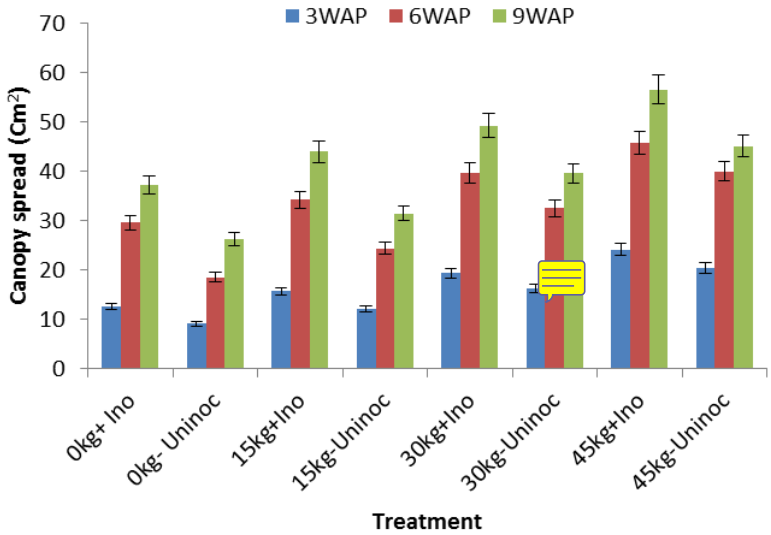

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Figure 4: Influence of Rhizobium inoculation and phosphorus fertilizer application rate on canopy spread in soybean at different growth stages.

3.5 Shoot biomass

Shoot biomass per plot was significantly influenced by Rhizobium inoculations and increasing fertilizer rates (Figure 5). The highest shoot biomass was obtained in inoculated plants fertilized with 45 kg P/ha whilst plants that received no P application with or without inoculation produced the lowest shoots biomass. Across the P application rates, inoculation increased soybean shoot dry weight by a significant average of 30% over the uninoculated plants varied phosphorus rates and their interactions (Figure 5). Shoot biomass increased with increasing application of phosphorus + Rhizobium inoculants. Phosphorus at 45 kg P/ha + Rhizobium inoculant recorded the highest shoot biomass per plot as compared to 15 kg P/ha + Rhizobium inoculants which recorded the least with regards to phosphorus and Rhizobium inoculants. With the sole fertilizer treatments, shoot biomass increased with increasing phosphorus rates with 45 kg P/ha recording the highest. At low phosphorus application, the straws yield was lower than treatments that received the highest amount. In an earlier work, the authors reported of similar increases in shoot biomass of soybean as a result of increased rates of P from different fertilizer sources [27]. ~~The significant increase in shoot biomass due to increased P application rates in the current study also agrees with [28] who reported of a 20.7% increase in biomass yield due to phosphorus application.~~

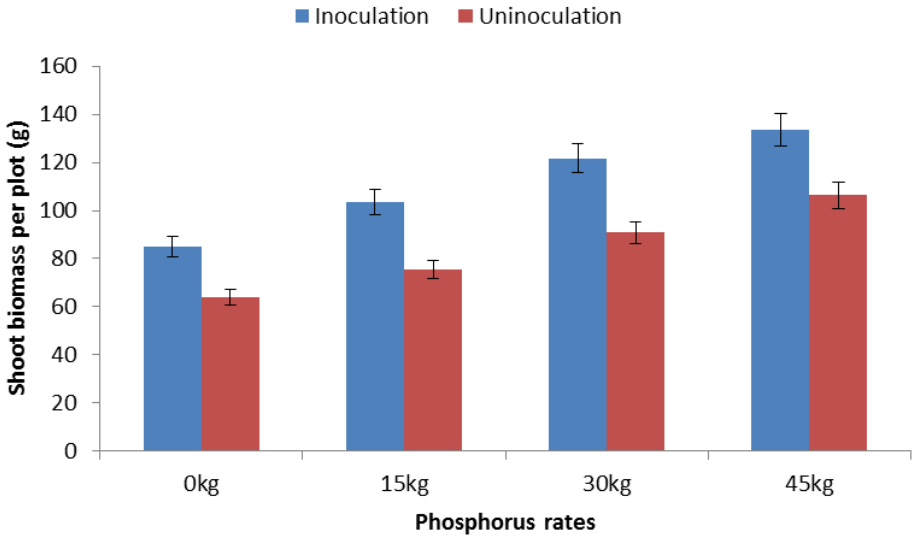

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Figure 5: Influence of Rhizobium inoculation and phosphorus fertilizer application rate on shoot dry biomass of soybean at full pod stage.

3.6 Nodulation

~~Nodule number~~ was significantly higher in inoculated plants and increased with P application rate whether plants were inoculated or not (Figure 6). Nodule formation in inoculated plants increased by 28%, 39% and 56% by applying 15, 30 and 45 kg P/ha, respectively compared to nodulation in the unfertilized plants whereas inoculation effect was greatest (80%) in the unfertilized treatments compared to 64%, 32% and 30% increases in the 15, 30 and 45 kg P/ha treatments, respectively (Figure 6). This suggests that Rhizobium inoculation influenced nodulation more positively than P fertilizer. [29] demonstrated from pot studies that nodulation and N_2 fixation of promiscuous soybean may be increased by inoculation with effective *Bradyrhizobia.* [30] also attributed the increase in nodulation to the competitive ability of *Bradyrhizobium* used, a result which was confirmed by [31].

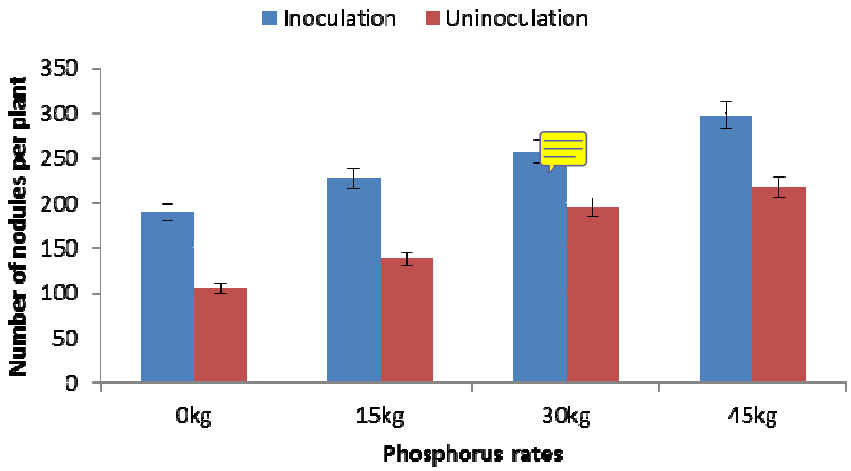

Figure 6: Influence of Rhizobium inoculation and phosphorus fertilizer application rate on number of nodules per plant at full pod stage of soybean.

3.7 Nodule biomass

Nodule biomass production was enhanced significantly by inoculating soybean with Rhizobium inoculants whether the crop was fertilized with phosphorus or not (Figure 7). The effect of inoculation was, however, greater in the presence of P and also when higher rates of

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P were applied but the highest percent increase (105%) in biomass due to inoculation occurred with P rate of 30 kg/ha. The other increases were 43%, 78% and 88% for 0 kg P, 15 kg P and 45 kg P per hectare, respectively. Figure 7 reveals that in un-inoculated plants the highest biomass of 1.6 g/plant was obtained at 45 kg P/ha application rate but a co-application of inoculants and only 15 kg P/ha was able to produce the same nodulation effect. This means that with respect to nodule biomass (which is an index of biological ~~N fixation if all nodules are acti~~ application of 250 g of inoculants (inoculation rate used in this study was equivalent to 250 g inoculants/ha) could make savings equivalent to 30 kg P (i. e. 381.7 kg Yaralegume) in this study. The positive effect of Rhizobium inoculation on nodulation has been confirmed by [32] who recorded a significant increase in nodulation by native soil *Rhizobium* population in single inoculations conducted on a legume. Phosphorus is also known to initiate nodules formation, increase the number of nodule primordial and is essential for the development and functioning of formed nodules [33] and [34]. Several researches have reported that the supply of phosphorus plays important roles in the establishment, growth and function of nodules [26], [35] and [36]. Both figures 6 and 7 show that the interactive application of phosphorus and Rhizobium inoculants had a greater impact on nodulation than either treatment alone.

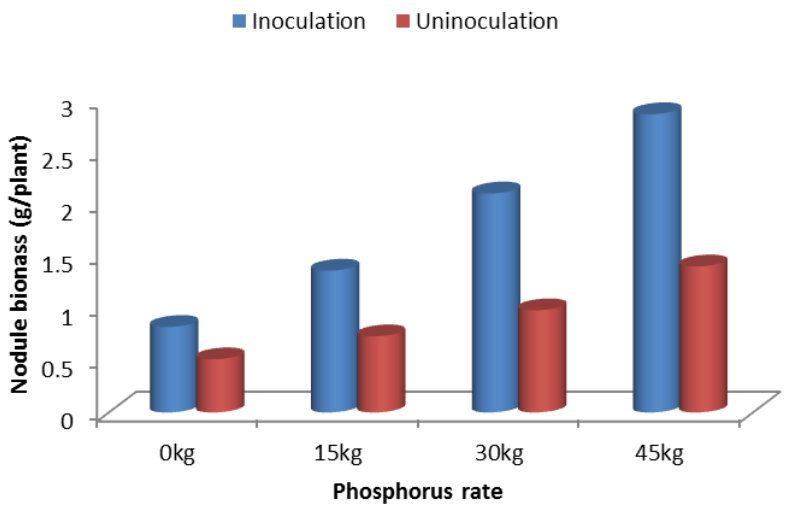

Figure 7: Influence of Rhizobium inoculation and phosphorus fertilizer application rate on nodule biomass of soybean at full pod stage.

3.8 Pod production

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The number and weight of pods produced by soybean in response to applications with P fertilizer and Rhizobium inoculants are shown in figures 8 and 9, respectively. Rhizobium inoculation remarkably increased both pod number and pod weight but whereas the increases due to inoculation were observed at all the P application rates in the former (Figure 8), there was no effect of inoculation on pod weight at 15 kg P/ha rate (Figure 9). Also, whether plants were inoculated or not, pod production generally increased with increasing rates of P application (Fig. 8 & 9) which agrees with [37] and [20] who reported high numbers of pods per plant in response to high doses of phosphorus. [38] also reported that a mixed inoculation improved the productivity of soybean when compared to the control treatment. Increased numbers of pods per plant in response to inoculation were also reported by [39] and [21]. The increases in pod weight at the different increasing rates of P plus Rhizobium inoculation may have been due to enhanced stimulation of pod filling in such treatments as confirmed by [21] when they reported of significant high numbers of seeds per pod when 100 kg P was applied in contrast to minimum numbers of seeds where no P was applied

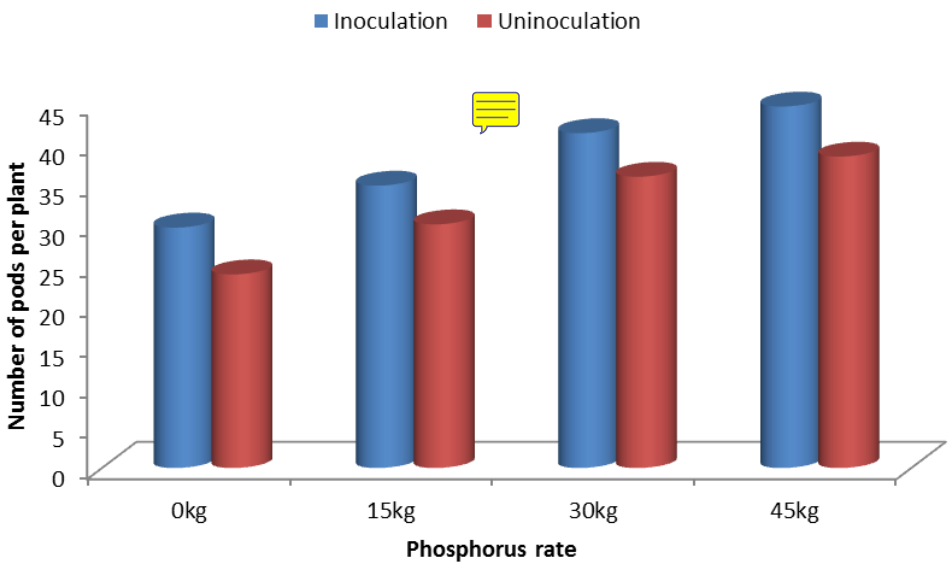

Figure 8: Influence of Rhizobium inoculation and phosphorus fertilizer application rate on number of pods of soybean at full pod stage.

3.9 Pod weight

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There were significant difference ($p<0.05$) among varied phosphorus rate, Rhizobium inoculants and the interaction between phosphorus rates and *Rhizobium* inoculants on pod weight (figure 9). Different rates of phosphorus fertilizer plus *Rhizobium* inoculant stimulated pod filling and thereby improved the pod weight as the phosphorus level progressively increased. Phosphorus at 45 kg P ha^{-1}+ *Rhizobium* inoculant recorded the highest pod weight as compared to phosphorus at 15 kg P/ha which recorded the least. The current study agrees with the findings of [40] and [21] who also significantly recorded the highest number of seeds per pod produced when 100 kg P ha^{-} was applied and minimum number was recorded where no phosphorus was applied. Similar results were obtained by [41] who reported of significant effects of phosphorus rates on harvest index of soybeans.

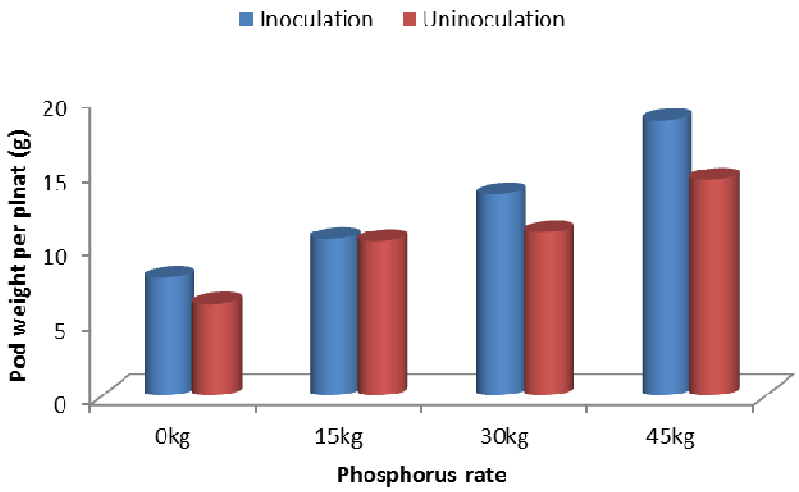

Figure 9: Influence of Rhizobium inoculation and phosphorus fertilizer application rate on pod weight of soybean at full pod stage.

3.10 Total grain yield

Similar to pod production, grain yield of soybean was significantly influenced by varied phosphorus rates, Rhizobium inoculation and their interaction (Figure 1The grain yield increased significantly at every higher rate of P application from 0 to 45 kg/ha with or without inoculation. Phosphorus application at 45 kg P/ha plus Rhizobium inoculant recorded the highest grain yield of 1955 kg/ha compared to 897 kg grain per hectare in the unfertilized inoculated treatment which constitutes a yield increase of 118% (Figure 10). However, though inoculation enhanced grain yield at every P application rate, within the P application

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regimes Rhizobium inoculation caused the highest percent grain yield increase (25%) at 15 kg P/ha. Also, these results are in agreement with [42] who concluded that phosphorus and inoculation induced a pronounced effect on grain yield. [39] also reported significant increases in grain yield with Rhizobium inoculation.

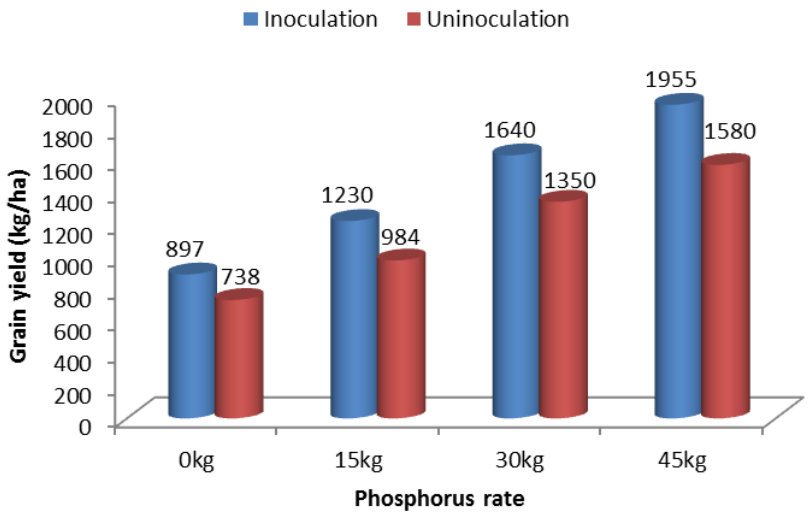

Figure 10: Influence of Rhizobium inoculation and phosphorus fertilizer application rates on grain yield of soybean.

Economic analysis

Information on costs and benefits of treatments is a prerequisite for adoption of technical innovation by farmers [43]. The studies assess the economic benefits of the treatments to help develop recommendation from the agronomic data. This enhances selection of the right combination of resources by farmers in the study area. The results in this study indicated that the inoculated treatments resulted in higher net benefits than the uninoculated treatments for all P fertilizer treatments (Table 1). The implications are that farmers will be better off inoculating soybeans seeds before planting as it will improve incomes of farmers through increased grain yields. The partial budget analysis showed that the application of 45 kg P/ha for both inoculated and uninoculated treatments yielded higher net benefits than all the treatments. The control (no fertilizer) treatment also produced the lowest net benefit for both the inoculated and un-inoculated treatments. This implies that farmers would be better off inoculating their soybean in combination with application of 45 kg P/ha as these increase soybean yields and thus increase farmers' income.

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Table 1: Partial budget for inoculated and un-inoculated soybean seeds under four different treatments

ITEM	TREATMENT							
	Inoculated				**Un-inoculated**			
	15 kg P/ha	30 kg P/ha	45kg P/ha	0 kg P/ha	15 kg P/ha	30 kg P/ha	45kg P/ha	0 kg P/ha
Average yield (kg/ha)	1230	1640	1955	897	984	1350	1580	738
Adjusted yield (kg/ha)[1]	1107	1476	1759	807	885	1215	1422	664
Gross field benefit (₵)[2]	2656	3542	4222	1937	2125	2916	3412	1594
Cost of Yaralegume (₵/ha)	16	33	48	0	16	33	48	0
Cost of inoculation (₵/ha)	15	15	15	15	0	0	0	0
Total cost that vary (₵)	31	48	63	15	16	33	48	0
Net benefit (₵)	2625	3494	4159	1922	2109	2883	3364	1594

[1] Average yield adjusted 10% downwards; Farm gate price of soybean as at December 2012= GH ₵2.4 per kg; Price of 50 kg of Yaralegume as at December, 2012 = GH ₵50.00 and price of inoculants = GH ₵15.00/ha.

Conclusion and Recommendation

Growth and development parameters of the soybean measured increased with increasing rates of phosphorus fertilizer coupled with Rhizobium inoculation. Generally, plants that received phosphorus fertilizer performed better than plants that were not fertilized as well as than plants that received inoculation only. However, the positive effects of P fertilizer application on growth and grain yield of soybean in this study were significantly boosted in the presence of Rhizobium inoculants. The growth and grain yield enhancements observed in this investigation may be attributed to an increased symbiotic relationship of rhizobia (bacteria)

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with the roots of leguminous crops which reflected in increased nodulation resulting in the fixation of atmospheric nitrogen into the roots of soybean which was favored by increased P nutrition.

From the results of the economic analysis conducted, this study therefore recommends that farmers inoculate their soybean seeds with *Rhizobium* and apply phosphorus fertilizer at 45 kg P/ha to maximize their grain yield and their farm income.

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