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Benefits of Rhizobial Inoculation and Phosphorus Application on Growth of Lucerne (*Medicago-sativa*) in Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Author MCM designed the study, wrote the protocol and wrote the first draft of the manuscript. Author JPGO reviewed the study design and all drafts of the manuscript. Author NKK managed the analyses of the study. Author MCM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Field experiments were conducted to study the effects of Phosphates on growth components of Lucerne during the long and short rain seasons of 2016-2017 at Kenyatta University farm in Kiambu County in Kenya. The experiments were laid out in split-plot arrangement in a Randomized Complete Block Design (RCBD) with four replicates. The treatment factors were: (i) Main plot factor – Lucerne, (ii) Sub-plot factor – inoculation and no inoculation and (iii) Sub-sub plot factor - different phosphorus forms, no phosphate, RP and TSP totalling to twelve treatment combinations which were replicated three times. Rows of 50cm apart were made, leaving 25 cm on the edge. The maximum number of branches (18) was recorded on plants under the TSP treatment, followed by RP (15) whereas the plants in the control had the lowest number of branches (11). Inoculation significantly reduced the number of nodules in Lucerne probably due to the high population of indigenous rhizobia already present in the soil which might have led to negative competition.

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1. INTRODUCTION

Lucerne (*Medicago-sativa*) also known as alfalfa, is the single most important fodder crop that a dairy farmer should always have on the farm. Lucerne grows in most parts of Kenya and especially in Kiambu County. Lucerne is a multi-purpose plant that can either be grazed *in situ* or conserved as hay/silage for use during times of the year when pasture quality or diet protein levels are low [1]. Lucerne has excellent stock acceptance and produces higher yields with high quality feed. The main constraint facing small scale dairy farmers in smallholder mixed farming, pastoral and agro-pastoral production systems across East Africa is the inability to provide sufficient quantity and quality feeds to their livestock consistently [2]. The fodder yields are still very low and inconsistent. This is partly attributed to low soil fertility, the low presence or inadequate, infectivity or efficacy of the native rhizobia and to the low symbiotic specificity between legumes and rhizobia that fail to supply wholly the nitrogen required for optimum yields and low soil fertility [3].

Phosphorus is one of the major fertilizer nutrients required for crops in Kenya, being second in importance to nitrogen [4]. Phosphorus is the most limiting nutrient in legume production in Africa [5]. The ability of Lucerne to fix atmospheric nitrogen is not always adequate for yield maximization due to different factors like temperature, soil physical and chemical characteristics such as pH of the soil, low phosphorus, presence of inefficient native rhizobia in the soil that compatible to the legume planted [6]. Most tropical soils are deficient in appropriate effective strains capable of fixing nitrogen and available phosphorus. There is a need to find appropriate rhizobia strain which will enhances nitrogen fixation attributes and yield of Lucerne under different phosphorus levels.

Low level of phosphorus and nitrogen may impair growth of most legumes. Furthermore, inadequate compatible rhizobial strain to particular legume plants result to poor plant growth including less chlorophyll formation and photosynthesis. There is therefore a need of supplying legumes with appropriate rhizobial inoculants with phosphorus for optimum productivity in legumes.

Phosphorus is essential for energy transfer to root nodules for N-fixation, enhancing root growth and is important for nodulation and increase of rhizobia density in the rhizosphere [7]. In legume production, phosphorus and inoculation with the appropriate *Rhizobium* strains have positive effects on nodulation, growth and yield parameters [8]. The absence of the required rhizobia species and optimal phosphorus levels limit legume production in different parts of the world. Inoculation with compatible and suitable rhizobia with optimum phosphorus levels may be essential where a low population of native rhizobial strains prevail and is one of the key components of which grain legume farmers can use to optimize yields.

Most tropical soils are deficient in appropriate effective strains capable of fixing nitrogen and available phosphorus. There is need to find appropriate rhizobia strain which enhances nitrogen fixation and growth of Lucerne under different phosphorus forms. Thus, the present study was conducted to determine the effect of phosphorus fertilizer forms on the growth components and of Lucerne in the Central region of Kenya.

2. MATERIALS AND METHODS

2.1 Site Description

The study was carried at the Kenyatta University farm, in Kiambu County about 20 km from Nairobi city along Nairobi-Thika road. The county has a warm climate with temperatures ranging between 12°C and 18.7°C. The geographical coordinates are 1° 10' 0" South, 36° 50' 0" East with an elevation of 1720 meters above sea level (ASL). The rainfall is bimodal with an average 989 mm during the long rains which occurs between mid-March and May while the short rains is between October and December. The experiment was done during the long rain season of May 2016 and short rains of November 2016 – 2017.

2.2 Experimental Design, Treatments and Data Collection

The field experiments were laid out in a Randomized Complete Block Design (RCBD) with split split-plot arrangement. The main plot constituted of the legume species Lucerne while the sub-plot was made of, Rhizobium inoculation and no inoculation. (with and without inoculation) and Sub-sub plot had different phosphorus forms (no phosphate, RP and TSP) which gave a total of twelve treatment combinations. The treatments were replicated three times. Dry Lucerne seeds of the inoculation treatment were inoculated with a charcoal-based culture of *Bradyhizobium japonicum* USDA 110C inoculum, licensed by University of Nairobi and produced by MEA Ltd branded BIOFIX®. The inoculant was mixed with water and sticker and applied to the seeds as slurry at the rate of 200 g for 50 kg seeds. The slurry was thoroughly mixed with seeds under the shade until the all seeds were fully coated and thereafter sown immediately and covered with soil to avoid exposure to direct sunlight and dehydration. The fertilizer rates were applied according to their treatment arrangement at planting to the side of the furrows to avoid direct contact with the seeds and seeds were planted into the moist soil. Rows of 50 cm apart were made, leaving 25 cm at the edge. Plants were thinned to 10 cm apart giving a population of 20 plants per row and a total of 100 plants per plot.

Routine field management practices such as weeding, watering and spraying against pests and diseases were performed uniformly when necessary using recommended fungicides and pesticides respectively.

The representative data were collected from the two middle rows of each plot in the experiment which comprised a net plot size of 2.5 m x 3.0 m. The parameters were either calculated or measured. The total above ground biomass and root biomass was determined using electronic weighing balance at harvest maturity. Number of branches, leaves and nodules per plant were counted and recorded at physiological maturity. Plant height and root length were measured using a standard ruler at harvest maturity.

2.3 Data Analysis

Data collected on yield components were subjected to analysis of variance using GenStat statistical software version 15.1 edition. The mean separation for treatments was done using Fischer's Protected Least Significance Difference (L.S.D) test at 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Phosphorus Effect on Total Number of Branches per Plant

The number of branches per plant in response to phosphorus application was found to be significant in the two seasons (Fig. 1). Number of branches per plant of Lucerne trifecta increased significantly ($P<0.05$) due to the applied phosphorous treatments. The maximum number of branches (18) was observed on plants that TSP, followed by those under RP with 15 branches whereas plants in the control produced the lowest number of branches (11).

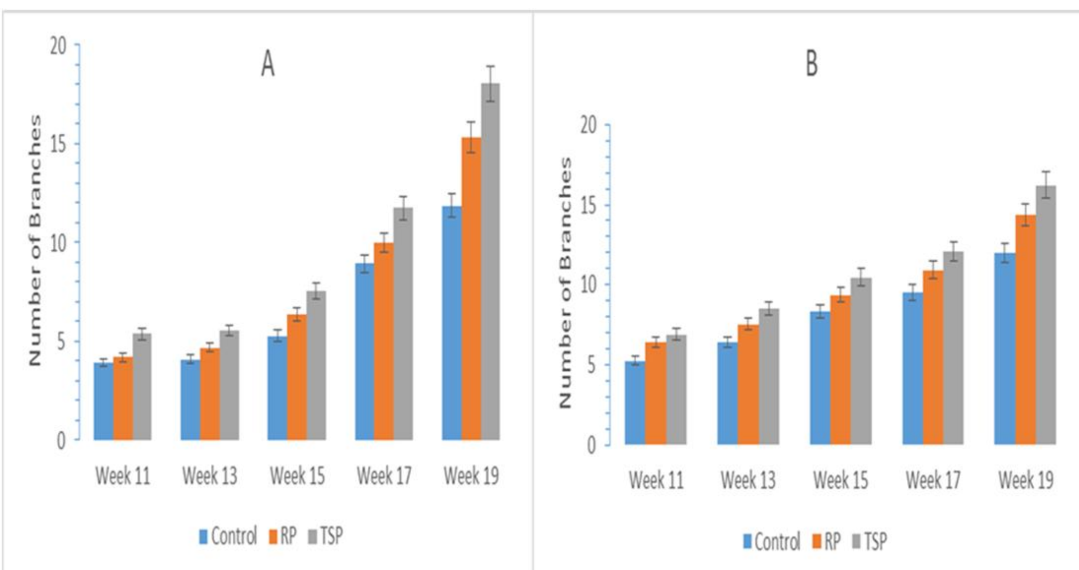

Fig. 1. Number of branches as affected by phosphorous forms on Lucerne trifecta during season 1 (A) and season 2 (B)

The numerical increase in the number of branches was probably due to the enhanced metabolic processes due to optimum phosphorus and lack of reduced metabolic activity. Phosphorus is the second most vital plant nutrient but for legumes, it presumes primary significance, which plays an important role in root proliferation and thereby atmospheric nitrogen fixation. Especially in the early stages of plant development, adequate supply of P is required for development of the reproductive parts which includes branches and also root growth, early maturity, and reduced disease incidence. Similar results were observed by [9] who reported that the yield of legumes is greatly influenced by application of phosphorus and biofertilizers. Studies done by [10,8] provide proof that increased phosphorus application enhances plant growth significantly. It is widely reported that phosphorus stress may lead to reduced growth, and yield in field crops including legumes such as Lucerne.

3.2 Effect of Phosphorus on Root Length

Phosphorus application led to significant differences ($p<0.05$) on root length of Lucerne for the two seasons (Table 1).

All the phosphorus forms produced significantly higher ($P<0.05$) root length compared to the control. Generally, higher root length was recorded in treatments with TSP fertilizer, followed by PR and where no P was applied had the shortest. The longest root lengths were observed during season 2. The increase in root length was probably due to the positive influence of phosphorus in initiation and growth of roots that in turn sped up and increased the uptake of essential elements and moisture from the soil. Phosphorus plays a vital function in increasing plant tip and root growth, decreasing the time needed for developing nodules to become active and of benefit to the host legume. Phosphorus has important effects on photosynthesis, root development, fruiting and improvement of crop quality. Similar results have indicated that phosphorus enhanced root system which provides greater root-soil contact and eventually higher uptake of phosphorus and other important and low mobility nutrients and absorption of higher concentration of mineral nutrients [11]. Also, [12] reported that application of P alone significantly increased root length and root dry weight by 33 and 64% over the control. Root improvement, stalk and stem vigor, flower and seed formation, crop production, crop maturity and resistance to plant pests and diseases are the attributes associated with phosphorus availability. Furthermore, phosphorus has important effects on photosynthesis, root development, fruiting and improvement of crop quality [13]. Phosphorus enhances the ability of catalyzing stress in the symbiotic relation between root bacteria and legume plants [14]. Inadequate P restricts root growth, photosynthesis, translocation of sugars, and other such functions, which directly or indirectly influence nitrogen fixation by legume plants [15].

3.3 Effect of Inoculation on Total Number of Nodule

As evident from the results presented in (Table 2), inoculated Lucerne led to significantly ($P<0.05$) low number of nodules in both seasons. The low nodulation in Lucerne may be associated with lack of specificity between host plant and rhizobia, since the strains inoculated were isolated.

Poor nodulation were obtained in some treatments in both soils. The poor performances could be due to the development in the soil of endogenous Bradyrhizobium strains which sometimes could possess incompatible effects on new strains introduced.

Low occupancy is the consequence of the inability of inoculant strains to compete with indigenous rhizobia for nodulation on the legume host [16]. This shows the presence of native rhizobia in the soil which are capable of nodulating. The native rhizobia are more persistent and well adapted to local conditions and this gives them an added advantage of competing successfully at the expense of introduced strains for nodulation [17]. The presence of nodules in uninoculated treatments during both seasons might be the result of existing indigenous soil rhizobium in the soil. Lack of response to inoculation can be attributed to intrinsic characteristics of both the host plant and the bacteria, as well as the great sensitivity of the symbiosis to environmental stresses, such as high temperatures, soil dryness and low soil fertility [18,19]. To reduce the production cost with mineral fertilizers and provide protection to the environment, more legume production could be achieved through seed inoculation with beneficial Rhizobium bacteria [20], which are known to influence nodulation, symbiotic nitrogen fixation, growth and yield of legumes.

Table 1. Root length as affected by phosphorous forms during season 1 and 2 from week 11 to 19

Lucerne	Season 1					Season 2				
Treatment	**Week 11**	**Week 13**	**Week 15**	**Week 17**	**Week 19**	**Week 11**	**Week 13**	**Week 15**	**Week 17**	**Week 19**
Control	11.45c	13.87c	17.11c	18.00b	22.60c	12.98c	15.85c	23.04c	27.95c	30.64c
RP	14.87b	16.67b	18.78b	20.17a	26.78b	17.61b	20.48b	26.54b	30.50b	33.54b
TSP	17.42a	19.56a	20.50a	21.78a	29.98a	20.31a	23.72a	28.26a	32.56a	37.19a
P Value	<.001	<.001	<.001	0.002	<.001	<.001	<.001	<.001	<.001	<.001
LSD	1.037	1.213	1.246	1.643	1.299	0.652	0.3716	0.4058	0.606	0.762

Means followed by the same letter(s) within a column are not significantly different at P = 0.05 (Fischer's Protected LSD Test)

Table 2. Total number of nodules as affected by inoculation during season 1 and 2 from week 11 to 19

Lucerne	Season 1					Season 2				
Treatment	**Week 11**	**Week 13**	**Week 15**	**Week 17**	**Week 19**	**Week 11**	**Week 13**	**Week 15**	**Week 17**	**Week 19**
Inoculated	0.93a	1.95a	2.12a	2.40a	2.63a	0.93a	1.85a	1.99a	1.83a	2.01a
Non Inoculated	2.46b	3.69b	4.54b	5.88b	7.80b	3.03b	3.46b	4.01b	4.69b	6.43b
P Value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
LSD	0.418	0.59	0.69	0.31	0.63	0.37	0.42	0.37	0.29	3.00

Means followed by the same letter(s) within a column are not significantly different at P = 0.05 (Fischer's Protected LSD Test)

4. CONCLUSION

Rhizobial inoculation and phosphorus supplements affected positively the growth of Lucerne in both seasons. Significant influence was observed in all the treatments with the use of TSP, followed by RP and the lowest in the control. The absence or presence of compatible rhizobia populations in the soil is one of the determinants of the success of inoculation. Therefore, when proper strains are used, rhizobium inoculation can be used to boost Lucerne productivity among smallholder farmers in Kenya.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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