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Influence of *Rhizobium* Inoculation and Phosphate Rock Fertilizer Application on Growth and Yield Components of Green gram (*Vigna radiata*) in Tharaka-Nithi County, Kenya

F. K. Mbaka[1*], G. O. Oloo-abucheli[1], P. K. Njoroge[1] and H. O. Ndukhu[1]

[1]Department of Plant Sciences, Chuka University, P. O. Box 109-60400, Kenya.

Authors' contributions

This work was carried out in collaboration among all authors. Author FKM identified the research gap and objectives, wrote the first draft of the manuscript. Authors HON and GOOA designed the study and performed statistical analysis. Author PKN assisted in search of literature and formatting of the work. All authors read and approved the final manuscript.

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ABSTRACT

Green gram is an important drought tolerant grain legume crop in Kenya. In Tharaka Nithi County green gram is as a major source of food security. Hence, there is need for a cheap and sustainable system of agricultural production is needed to increase its production. The legume/*Rhizobium* symbiosis in combination with phosphate rock offers a natural system for improving green gram nitrogen fixation. Therefore, this study was aimed at determining the effect of *Rhizobium* and phosphate rock fertilizer application on growth and yield attributes of N26 and KS20 varieties. The study was carried out at Chuka University Horticultural Research Farm for two seasons, November 2019 to January 2020 and February to April 2020. Factorial experiment of 2x2x2 was laid out in a Randomized Complete Block Design (RCBD). There were three factors, variety (N26 and KS20), phosphate rock (0 and 30 kg P ha^{-1}) and *Rhizobium MEA* 716 (0 and 100 g ha^{-1}). The experiment contained eight treatments which were replicated three times. The data was collected fortnightly on four randomly selected plants on parameters such as plant height, number of leaves, branches,

**Corresponding author: E-mail: mbakafelister3@gmail.com, mbakafelister@gmail.com;*

pods, total dry biomass, shoot and root dry weight and grain yield. These data was analysed using Statistical Analysis Software (SAS). Significant means are separated using Least Significant Difference (LSD) at probability level of 5%. Results for both seasons indicated that combined application of *Rhizobium MEA* 716 *Rhizobium* 100 g ha^{-1} and phosphate rock 30 Kg ha^{-1} phosphate under variety in treatment (R1P1V2) showed significantly (P<0.05) higher plant height (76.07 cm), number of branches (14.08 $plant^{-1}$), shoot dry biomass (52.01 g $plant^{-1}$), root dry biomass (7.60 g $plant^{-1}$), total dry biomass (146.4 g $plant^{-1}$), number of pods (84 $plant^{-1}$) and yield (2158 kg ha^{-1}) compared to variety N26 in treatment (R1P1V1). Therefore, combination of *Rhizobium* MEA 716 100 g ha^{-1} and phosphate rock 30 Kg ha^{-1} under variety KS20 led to improved growth attributes equally yield components of KS20 variety over N26. From these findings, application of *Rhizobium* MEA716 at 100 g $ha^{-1,}$ and 30 Kg ha^{-1} phosphate rock under variety KS20 was recommended for improved and a sustainable green gram production in the study area.

Keywords: Low green-gram production; Rhizobium inoculation; phosphate rock application; green gram yield improvement.

1. INTRODUCTION

Green-gram (*Vigna radiata*) is the most important pulses in the world for its nutritive values as well as used for soil fertility improvement [1]. It is consumed as green pods and dry seeds as well as young shoots. Green-gram is majorly cultivated in the warmer regions of the world due to its drought tolerant characteristics and low inputs requirements [2]. It is currently cultivated on an area of about six million hectares worldwide, most of which are located in Asian countries and to a small extent in some parts of Africa, USA, and Australia [3]. Ninety per cent of the world's green gram production is Asia with India contributing around 50% [4]. Green gram can approximately fix 30-251 kg N /ha/year [5]. Nitrogen fixing symbionts productivity of green-gram is often limited by N deficiency [6]. Hence, its production was increased by the application of commercial N fertilizers. The rate of N-fixation varies considerably depending on the type of legume cultivar, presence of appropriate *Rhizobia* and other soil variables [7]. In particular symbiotic N-fixation demands high phosphorous which consumes large amounts of energy [8] generating metabolism strongly depends upon availability of P [9].

Africa contributes to 0.4 – 1% of world's green gram production. The main producers included; Nigeria, South Africa, and Zimbabwe. In Eastern Kenya region green gram the major production areas being in order of importance found in Kitui, Makueni, Tharaka Nithi, Machakos and Mbeere are the leading areas cultivating green gram on an average of 260,000 ha. National production ranges from 61,000 to 121,000 MT/Ha. Over the same period, national consumption ranges from 58.000 to 127,000 MT/Ha and the deficit is bridged through imports. Yields average 0.15 MT/Ha [10]. Studies that were done between 2011 to 2014 showed that Kenya's green gram consumption was mainly from imports [11].

The decline of green gram exports can be made sustainably by biological nitrogen fixation in green gram to replenish soil fertility [12]. Commercial inoculant strains may evolve quickly in the soil [11]. There are even reports from Brazil of re-isolated *Rhizobia* that had become more competitive in their new environment while they maintained their nitrogen fixation ability [13]. Also phosphate rock has been recommended for sustainable agriculture [14]. Addition of phosphate rock fertilizer leads to increase in legume [15]. Therefore, this research study was aimed at to determine the interactive effects of *Rhizobium* inoculation and phosphate rock on two different varieties namely KS20 and N26.

2. MATERIALS AND METHODS

2.1 Site Description

The experiment was carried out at Chuka University Horticultural farm in Tharaka-Nithi County for two seasons (November 2019 to January 2020 and February to April 2020). The site was situated at latitude of about 0.3195° S, longitude of 37.6575° E with an altitude of 1401 m above mean sea level and in a midland zone [16]. The average annual temperature is 20.8°C and annual rainfall of 1599 mm distributed bi-modally March to May and October to December [17]. The soils were of volcanic origin in this region classified as nitisols with basic and ultra-basic igneous rocks which are highly leached [18]. The climate is favourable for cultivation of green-gram, tea, coffee, maize, cowpeas, pigeon pea, tobacco and livestock keeping [19].

2.2 Experimental Design

A factorial experiment of 2x2x2 was laid out in a randomized complete block design (RCBD. There were three factors, varieties (N26-nylon and KS20-uncle), phosphate rock (PR (0 and 30 kg P ha^{-1}) and MEA 716 *Rhizobium* rates (0 and 100 g ha^{-1}) making a total of eight treatments. Each plot measured 1.5 x 1.8 m with a spacing of 45 cm between rows and 15 cm within plants, accommodating plant population of 148,149 plants per hectare.

2.3 Material Purchase

The commercial inoculum for green gram MEA 716 was procured from MEA Company limited (Nairobi). The seeds of KS20 and N26 green gram varieties were purchased from Kenya Seed Company (Nairobi) and Phosphate rock fertilizer from local agrochemical shop.

2.4 Microbial Inoculation Process

The inoculum was applied as per the manufacturer's instructions (5 g of inoculant per 15 kg of seeds). Seeds were wetted with warm water then coated with a filter mud containing inoculum using gum Arabica (supplied with the inoculum). Inoculated seeds were air dried at room temperature for 15 minutes to avoid seed dehydration [20].

2.5 Land Preparation and Planting of Green gram

Land was prepared to a fine tilth condition. In the first season planting was done in November 2019 and second season February 2020. Treated seeds with inoculum were planted first to avoid contamination with uninoculated seeds. Phosphate rock was applied according to the treatment arrangement. Two seeds per hill were planted at a spacing of 45 cm between rows and 15 cm within rows at a depth of 3 cm and followed by light compaction to enhance seed-soil contact.

2.6 Data Collection

The data collection on growth variables was collected on four randomly selected and tagged plants from two middle rows starting from 28 DAS at an interval of 14 days upto full maturity. This is because nodulation and nitrogenase activity initiated after 10 - 11 days after emergence and can only supply the required nutrient demand after 25 days after emergence [21]. The data on growth and yield variables such as plant height, number of leaves, number of branches, shoot and root dry biomass, total dry biomass, number of pods and grain yield are measured based on weight basis. The number of pods was measured at physiological maturity of four tagged plants from two inner rows in each experimental plot counted and recorded according to the treatment. Ten plants were randomly selected from each experimental plot and grain yield determined by hand picking of pods from each plant separately. The dry weight of seeds harvested was recorded as seed yield per plant and extrapolated to yield in Kg ha^{-1}.

2.6.1 Measurements of growth and yield parameters

Plant height, number of leaves and branches was done at 28 days after planting on four randomly selected plants from middle two rows of each experimental plot. A graduated meter rule was used to measure plant height from the soil level to the terminal leaf of the green-gram plants. Height from each plant was recorded in centimeters.

Shoot and root dry biomass - four randomly selected plants were dug together with roots and shoots, but shoots were cut off then packed separately in labeled plastic bags and taken immediately to the laboratory for shoot and root dry weight determination. Shoot and root dry weights per plant were taken after the fresh plant samples were oven dried for 48 hours at 70°C as described by [22] and their dry weights were determined using an electronic balance and recorded separately in grams.

Total dry biomass -this was done at maturity on randomly selected four plants from two inner rows of each experimental unit. The selected plants were dug together with roots and placed in labeled plastic bags and taken immediately to the laboratory for biomass determination. The plants were oven dried at 60°C for 48 hours and dry weight biomass determined using an electronic balance. The weight of each plant was recorded in kilograms per hacter.

The number of pods was measured at physiological maturity of four tagged plants from two inner rows in each experimental plot counted and recorded according to the treatment. Ten plants were randomly selected from each experimental plot and grain yield determined by hand picking of pods from each plant separately. The dry weight of seeds harvested was recorded as seed yield per plant and extrapolated to yield in Kg ha^{-1}.

Grain yield - at physiological maturity, 10 plants were randomly selected from each experimental plot and grain yield determined by hand picking of pods from each plant separately. The grains from each plant were air dried and dry weights determined using an electronic balance and recorded accordingly. The dry weight of seeds harvested was recorded as seed yield per plant and extrapolated to yield in Kg ha^{-1} by multiplying the yield per plant by the possible yield in Kg/ha [23].

2.7 Statistical Analysis

Data was collected and analysed by using Statistical Analysis Software (SAS) version 9.4 [24] and subjected to analysis of variance (ANOVA) as implemented in SAS. Significant means were separated using Least Significance Difference (LSD) at probability level of 5%.

3. RESULTS AND DISCUSSION

3.1 Interactive Effects of *Rhizobium* Inoculation and Phosphate Rock Fertilizer Application on Growth Attributes of KS20 and N26 Green Gram Varieties

3.1.1 Plant height of KS20 and N26 green grams varieties

Combined application of *Rhizobium* and phosphate rock shows its influence in increasing trend in means of plant height of green grams with respect to the day after sowing. However, treatment R1P1V2 recorded significantly ($P<0.05$) higher plant heights in the first season (14.99, 33.28, 58.26, 71.83 and 75.86 cm, respectively) compared to R1P1V1 (Table 1). In the second season significant ($P<0.05$) difference on plant height was observed between variety KS20 and N26 at 28, 42 and 56 DAS with R1P1V2 recording higher plant heights of 15.55, 36.21 and 64.07 cm, respectively (Table 1). These significant differences in plant height between the two varieties might be due to the growth habit and differences in soil adaptability with respect to environmental condition. These results were in agreement with [25] observed variations in plant height among soybean varieties after application of *Rhizobium* and phosphorous. On the other hand, single application of phosphate rock as a dose showed significant ($P<0.05$) differences between the two varieties in the second season for the treatment R0P1V2 recording higher plant heights (11.16, 32.85, 54.32, 66.08 and 69.90 cm) over R0P1V1 (Table 2). This is because phosphorous promotes the rate of root growth and extension of root hairs. Thereby, effective exploration of soil and interception of nutrients has been promoted by plant height [26].

The differences in plant height between KS20 and N26 varieties are related with the findings of [27] demonstrated significant differences in growth of five different soybean varieties. Similarly observation also findings of [28], showed that phosphorus application significantly increased mungbean height over controls. In addition, *Rhizobium* inoculation also recorded significantly ($P<0.05$) similar increase in plant height compared to controls between KS20 and N26 (Table 1). Similar observations have been observed in [29] and [30] which showed that, *Rhizobium* inoculation increased plant height over the controls. Treatments R0P0V1 and R0P0V2 recorded shortest plant heights which were significantly ($P<0.05$) similar throughout the growing periods. This might have been due to low P availability in soils which restricted deeper root development.

3.1.2. Influence of *Rhizobium* and phosphate rock application on number of leaves between KS20 and N26 green grams varieties

The interactive effects of phosphate rock and *Rhizobium* inoculation had a significant ($P<0.05$) influence on the number of fully mature leaves per plant between two varieties KS20 and N26 varieties over other treatments. In the first season treatment R1P1V1 recorded significantly ($P<0.05$) higher number of leaves at 70 and 84 DAS as 11.91 and 14.25 per plant respectively. Similar trends were observed in the second season at 70 and 84 DAS 12.50 and 14.75 per plant respectively (Table 2). This increase in number of leaves may be due to prolonged rains and nitrogen inoculation which promoted vegetative growth. Additionally, combined application of *Rhizobium* and phosphate rock leads to improved soil properties and increased microbial activity thus significant increase in number of leaves [31].

Table 1. Means of Plant height (cm) under different treatments at different growth days

Seasons	Treatments	28DAS	42DAS	56DAS	70DAS	84DAS
I	R0P0V2	8.050^{*cd}	15.97a	26.15^{a}	48.12^{a}	58.96^{a}
	R0P1V2	10.675^{fg}	30.36^{de}	50.43^{cd}	60.84^{c}	68.95^{de}
	R1P0V2	8.850^{cde}	27.08^{bc}	35.26^{b}	60.84^{c}	65.28^{bc}
	R1P1V2	14.992^{i}	33.28^{ef}	58.26^{f}	71.83^{fg}	75.86^{g}
	R0P0V1	6.717^{b}	13.71^{a}	23.88^{a}	45.29^{a}	56.89^{a}
	R0P1V1	9.583^{ef}	29.45^{cd}	49.51^{c}	60.20^{bc}	67.68^{cd}
	R1P0V1	7.992^{c}	25.23^{b}	35.26^{b}	55.39^{b}	66.90^{bcd}
	R1P1V1	11.408^{gh}	29.72^{cd}	52.31^{cde}	67.73^{ef}	72.04^{ef}
II	R0P0V2	5.850^{a}	16.13^{a}	26.38^{a}	48.17^{a}	59.71^{a}
	R0P1V2	11.167^{gh}	32.85^{e}	54.32^{def}	66.08^{de}	69.16^{de}
	R1P0V2	8.917^{cde}	27.33^{bc}	36.58^{b}	63.17^{cde}	65.28^{bc}
	R1P1V2	15.558^{i}	36.22^{f}	64.08^{g}	75.13^{g}	76.07^{g}
	R0P0V1	5.317^{a}	13.55^{a}	24.14^{a}	46.48^{a}	57.10^{a}
	R0P1V1	9.242^{de}	30.77^{de}	51.69^{cde}	63.96^{cde}	67.78^{cd}
	R1P0V1	8.475^{cde}	25.66^{b}	35.55^{b}	62.55^{cd}	64.50^{b}
	R1P1V1	11.933^{h}	32.31^{de}	56.19^{ef}	72.58^{fg}	74.26^{fg}
	C.V (%)	8.5	7.6	7.4	5.6	3.1
	$LSD_{0.05}$	1.3251	3.212	5.089	5.460	3.391

*Legend: *Means followed by the same letter in the same column are not significantly different from each other at (P<0.05) level of significance. R0P0V2- Rhizobium 0g/ha X phosphate rock 0kg/ha x KS20, R0P1V2 - Rhizobium 0g/ha X phosphate rock 30kg/ha x KS20, R1P0V2 - Rhizobium 100g/ha phosphate rock 0kg/ha x KS20 , R1P1V2 - Rhizobium 100g/ha X phosphate rock 30kg/ha x KS20, R0P0V1, Rhizobium 0g/ha X phosphate rock 0kg/ha x KS20 , R0P1V1 -Rhizobium 0g/ha X phosphate rock 30kg/ha x N26, R1P0V1(N26)-Rhizobium 100g/ha X phosphate rock 0kg/ha x N26, Rhizobium 100g/ha X Phosphate rock 30kg/ha x N26, C.V- Coefficient of Variations, LSD- Least Significant Difference*

Table 2. Means of Number of leaves per plant under different treatments at different growth days after sowing of KS20 and N26 green grams varieties

seasons	Treatments	28DAS	42DAS	56DAS	70DAS	84DAS
I	R0P0V2	0.417^{*a}	1.500^{a}	4.167^{a}	5.583^{a}	7.33^{a}
	R0P1V2	1.833^{bcd}	4.667^{b}	6.333^{cd}	8.667^{bc}	9.83^{de}
	R1P0V2	1.917^{bcd}	4.583^{b}	7.000^{d}	9.083^{bcd}	10.50^{e}
	R1P1V2	3.250^{ef}	7.500^{c}	9.583^{fg}	10.667^{e}	13.92^{g}
	R0P0V1	0.333^{a}	2.083^{a}	5.00^{ab}	6.333^{a}	8.00^{abc}
	R0P1V1	1.583^{bc}	4.33^{b}	6.750^{cd}	9.250^{bcd}	10.25^{e}
	R1P0V1	2.083^{cd}	4.917^{b}	7.300^{d}	9.333^{cd}	10.83ef
	R1P1V1	2.667^{de}	7.333^{c}	9.833^{g}	11.917^{ef}	14.25^{gh}
II	R0P0V2	1.00a	4.417^{b}	5.667^{bc}	6.500^{a}	7.17^{a}
	R0P1V2	2.167^{cd}	6.500^{c}	7.083^{d}	8.333^{bc}	8.67^{bcd}
	R1P0V2	2.667^{de}	7.500^{c}	7.417^{de}	9.667^{d}	10.00^{e}
	R1P1V2	4.250^{g}	9.250^{d}	10.25^{g}	10.250^{d}	12.33^{f}
	R0P0V1	0.583^{a}	4.471^{b}	4.917^{ab}	6.00^{a}	7.50^{ab}
	R0P1V1	2.000^{cd}	5.083^{b}	6.417^{cd}	8.00^{b}	8.75^{cd}
	R1P0V1	2.750^{de}	7.583^{c}	8.417^{ef}	9.50^{cd}	10.95^{f}
	R1P1V1	3.750^{fg}	9.671^{d}	10.750^{g}	12.50^{e}	14.75^{g}
	C.V (%)	30.3	15.8	12.1	9.6	8.0
	$LSD_{0.05}$	1.0500	1.4476	1.4210	1.395	1.32608

*Legend: *Means followed by the same letter in the same column are not significantly different from each other at (P<0.05) level of significance. R0P0V2- Rhizobium 0g/ha X phosphate rock 0kg/ha x KS20, R0P1V2 - Rhizobium 0g/ha X phosphate rock 30kg/ha x KS20, R1P0V2 - Rhizobium 100g/ha phosphate rock 0kg/ha x KS20 , R1P1V2 - Rhizobium 100g/ha X phosphate rock 30kg/ha x KS20, R0P0V1, Rhizobium 0g/ha X phosphate rock 0kg/ha x KS20 , R0P1V1 -Rhizobium 0g/ha X phosphate rock 30kg/ha x N26, R1P0V1(N26)-Rhizobium 100g/ha X phosphate rock 0kg/ha x N26, Rhizobium 100g/ha X Phosphate rock 30kg/ha x N26, C.V- Coefficient of Variations, LSD- Least Significant Difference*

Moreover leaves in KS20 variety appeared larger and darker in colour compared to N26 variety which resulted in higher uptake of N and P and their use efficiency. A field observations study conducted by [32] concluded that phosphorus in combination with *Rhizobia* inoculation enhanced dark green colour of leaves showing that *Rhizobia* inoculation enhances P uptake leading to the development of a larger photosynthetic area. *Rhizobium* inoculated treatments in the second season recorded significantly (P<0.05) higher number of leaves at 70 and 84 DAS with treatment R1P0V1 (Table 2). This is because *Rhizobium* inoculation increases nitrogen levels in soils and plant uptake which promoted vegetative growth. Biofertilizers application increased growth parameters such as number of leaves [26]. Moreover, treatment with phosphate rock application alone resulted to a significant (P<0.05) difference in number of leaves at 42 and 56 DAS with R0P1V1 treatment recording higher number of leaves over R0P1V2 (Table 2). Treatments without phosphate rock and *Rhizobium* R0P0V1 and R0P0V2 recorded lowest number of leaves for both seasons which had insignificant differences between KS20 and N26 for both seasons (Table 2). This shows clearly nitrogen and phosphorous plays a key role in root development nutrient uptake from soil pools number of leaves.

3.1.3 Influence of *Rhizobium* and phosphate rock application on number of branches of green grams varieties N26 and KS20

A significant effect of combined application of *Rhizobium* and phosphate rock on number of branches was observed at 70 and 84 DAS with R1P1V2 treatment recording highest number branches of 9.00 and 10.83 per plant respectively compared to R1P1V1 of N26 variety with 8.16 and 9.91 plant^{-1} (Table 3). In the second season R1P1V2 recorded significantly (P<0.05) higher number of branches throughout the growing period over R1P1V1 treatment (Table 3). The highest number of branches increases the surface area for production. Increase in number of branches in treatment R1P1V2 can be attributed to higher uptake of P and its use efficiency. These results corroborates with the findings of [33] stated that P fertilizer applied at planting can enhance root development leading to increased nutrient uptake thus promoting branch development. There is also significant increase in number of branches was observed in R0P1V2 treatment with application of phosphate rock alone at 70 and 84 DAS 8.83 and 9.50 per plant respectively in second season (Table 3). *Rhizobium* inoculation alone had no significant (P<0.05) differences in number of branches plant^{-1} between N26 and KS20 varieties for both seasons (Table 3). This shows that phosphorus plays a vital role in growth, physiological and developmental process in plant [34].

3.2 Interactive Effects of Treatments on Biomass and Yield Components of two Green gram Varieties

3.2.1 Effect of *Rhizobium* and phosphate rock on shoot, root and total dry biomass weight of KS20 and N26 green gram varieties

Interaction effect of *Rhizobium* and phosphate rock significantly (P<0.05) affected shoot and root biomass of KS20 and N26 varieties. However, treatment R1P1V2 recorded the highest shoot and root dry biomass of 49.91 and 52.01 g per plant, 142.3 and 143.01 g per plant respectively for the two seasons (Table 4). This is because combined application of PR and *Rhizobium* resulted in optimum nutrient supply particularly N and P which favored high rate of photosynthesis. This was in agreement with [35] who observed that N and P affected the capacity of climbing beans to produce higher shoot biomass and the increase in root dry weight can be attributed to P increase in nutrients which promoted development. Higher root dry matter of soybean variety was influenced by P application and *Rhizobium* inoculation [29]. Similar findings are also reported by [35] and [36].

Treatments with *Rhizobium* inoculation for R1P0V1 and R1P0V2 had no significant (P<0.05) difference in shoot dry biomass between R1P0V2 and R1P0V1 for both Seasons (Table 4).The increase in shoot dry biomass due to *Rhizobium* inoculation could be attributed to the increased N uptake by root of inoculated plots and soil nutrient availability leading to a higher shoot biomass production [37]. Application of phosphate rock treatment R0P1V2 recorded significant (P<0.05) higher shoot dry biomass of (39.43 and 41.00 g plant^{-1}) over R0P1V1 with 29.01 and 33.08 g per plant for the two seasons (Table 4). This increased in shoot dry biomass due application demonstrated the positive impact of P fertilizer in shoot dry biomass. Phosphorous is important in the activation of metabolic processes necessary for vegetative growth resulting in high shoot dry matter accumulation [37].

There was significant interaction between phosphate rock and green gram varieties on root biomass matter yield. However, R0P1V2 recorded slight significant ($P<0.05$) higher root dry biomass of 4.28 and 4.99 g per plant over R0P1V1 with 3.48 and 4.46 g per plant suggesting differences in P use efficiency between KS20 and N26 varieties. The results suggested that P fertilization plays a vital role in the performance of the green gram varieties depending on the available soil nutrients. The increase in root dry weight with P application was in agreement with the findings of [38] who reported that application of P alone significantly increased root length and root dry biomass. Treatment with *Rhizobium inoculation* alone had insignificant ($P<0.05$) difference in shoot and root dry biomass, although the results were slightly higher compared to treatments without PR or *Rhizobium.* This finding agrees with [39] who observed a significant increase in shoot and root dry biomass of inoculated soybean over uninoculated. This was contrary, to the works done by [40] who observed that, commercial *Rhizobia* did not give positive results of root d weight.

Significant ($P<0.05$) increase in total dry biomass of KS20 and N26 varieties are observed with combined application of *Rhizobium* and phosphate rock over other treatments. However, R1P1V2 treatment recorded significantly ($P<0.05$) higher total dry biomass of 145.539 and 146.32 g per plant compared to R1P1V1 treatment (Table 4). This is because in legume biomass increases exponentially at vegetative stages when the plants uptake optimum nutrient where P becomes an essential element for energy transfer processes in plants [41]. Also research has revealed that biomass yield is dependent on legume variety [42]. Treatments with *Rhizobium i*noculation alone recorded a significant ($P<0.05$) slightly higher TDB in treatment R1P0V2 (135.3 And 137.4 g per plant) over R1P0V1treatment. Phosphate rock application resulted to a significant ($P<0.05$) increase of total dry biomass in R0P1V2 of 122.2 and 115.7 g per plant for the first and second season respectively (Table 4). This reconciles with the findings of [43] reported that P supply stimulated vegetative growth of soybean resulting into more dry biomass.

Table 3. Means of Number of branches per plant under different treatments at different growth days after sowing of green grams

Seasons	Treatments	42DAS	56DAS	70DAS	84DAS
I	R0P0V2	0.583^{*ab}	1.500^{a}	$3.583a^{b}$	5.500^{a}
	R0P1V2	1.333^{bcd}	2.750^{bc}	6.417^{fg}	8.167^{c}
	R1P0V2	0.917^{abc}	2.500^{abc}	6.010^{efg}	7.917^{bc}
	R1P1V2	3.167^{f}	6.667^{g}	9.00^{i}	10.833^{e}
	R0P0V1	0.333^{a}	1.417^{a}	3.00^{a}	5.167^{a}
	R0P1V1	0.971^{abc}	1.833^{ab}	4.833^{cd}	7.833^{bc}
	R1P0V1	0.833^{abc}	2.33^{abc}	5.750^{def}	7.583^{b}
	R1P1V1	2.833^{ef}	6.270^{f}	8.167^{hi}	9.917^{de}
II	R0P0V2	1.750^{cd}	1.833^{ab}	5.167^{cde}	5.750^{a}
	R0P1V2	3.333^{f}	5.417^{ef}	8.833i	9.500^{d}
	R1P0V2	2.167^{de}	4.583^{de}	5.917^{def}	7.417^{bc}
	R1P1V2	4.917g	8.250^{h}	11.083^{j}	14.083^{f}
	R0P0V1	1.167^{abc}	2.00^{abc}	4.333^{bc}	5.083^{a}
	R0P1V1	2.750^{cd}	3.000^{c}	7.083^{gh}	8.333^{c}
	R1P0V1	1.650^{cd}	4.167^{d}	$6.00e^{fg}$	6.917^{b}
	R1P1V1	3.583^{f}	7.333^{g}	9.167^{i}	10.417^{de}
	C.V (%)	22.9	19.7	12.0	9.0
	$LSD_{0.05}$	1.0753	1.2274	1.2603	1.1889

*Legend: *Means followed by the same letter in the same column are not significantly different from each other at (P<0.05) level of significance. R0P0V2- Rhizobium 0g/ha X phosphate rock 0kg/ha x KS20, R0P1V2 - Rhizobium 0g/ha X phosphate rock 30kg/ha x KS20, R1P0V2 - Rhizobium 100g/ha phosphate rock 0kg/ha x KS20 , R1P1V2 - Rhizobium 100g/ha X phosphate rock 30kg/ha x KS20, R0P0V1, Rhizobium 0g/ha X phosphate rock 0kg/ha x KS20 , R0P1V1 -Rhizobium 0g/ha X phosphate rock 30kg/ha x N26, R1P0V1(N26)-Rhizobium 100g/ha X phosphate rock 0kg/ha x N26, Rhizobium 100g/ha X Phosphate rock 30kg/ha x N26, C.V- Coefficient of Variations, LSD- Least Significant Difference*

Table 4. Means of Shoot root dry weight and total dry biomass, number of pods and grain yields of KS20 and N26 green grams varieties under different treatments

Seasons	Treatments	Shoot dry biomass g plant^{-1}	Root dry biomass g plant^{-1}	Total Dry Biomass g plant^{-1}	Number of pods plant^{-1}	Yield kgha^{-1}
I	R0P0V2	15.87*^{a}	0.965^{a}	94.2^{b}	31.17^{a}	529^{b}
	R0P1V2	41.00^{c}	4.280^{de}	122.2^{d}	67.50^{bcd}	1472^{gh}
	R1P0V2	42.18^{c}	3.780^{bcd}	135.3^{fg}	66.33^{cd}	1404^{fg}
	R1P1V2	49.91^{de}	6.560^{h}	145.5^{ij}	83.25^{g}	2123^{j}
	R0P0V1	14.86^{a}	0.806^{a}	89.0^{a}	31.67^{a}	370^{a}
	R0P1V1	29.01^{b}	3.482^{bc}	115.7^{c}	66.50^{b}	1230^{d}
	R1P0V1	41.23^{c}	3.966^{cde}	131.3^{e}	66.17^{bc}	1143^{c}
	R1P1V1	46.78^{d}	5.189^{g}	142.3^{h}	72.67^{ef}	1843^{i}
II	R0P0V2	15.71^{a}	0.937^{a}	94.0^{b}	32.25^{a}	487^{b}
	R0P1V2	39.43^{c}	4.997^{fg}	126.4^{e}	71.00^{e}	1514^{h}
	R1P0V2	40.70^{c}	3.656^{bc}	137.4^{g}	67.27^{cd}	1446^{gh}
	R1P1V2	52.01^{e}	7.607^{i}	146.4^{j}	84.42^{g}	2158^{j}
	R0P0V1	15.15^{a}	0.757^{a}	84.8^{a}	30.83^{a}	325^{a}
	R0P1V1	33.08^{b}	4.465^{ef}	122.3^{d}	68.50^{d}	1338^{ef}
	R1P0V1	42.25^{c}	3.213^{b}	135.1^{fg}	65.67^{bc}	1284de
	R1P1V1	47.87^{de}	7.009^{h}	143.01^{h}	73.75^{f}	1900^{i}
	C.V (%)	8.3	10.4	1.5	2.3	6.3
	$LSD_{0.05}$	4.768	0.6374	3.087	2.253	129.37

*Legend: *Means followed by the same letter in the same column are not significantly different from each other at (P<0.05) level of significance. R0P0V2- Rhizobium 0g/ha X phosphate rock 0kg/ha x KS20, R0P1V2 - Rhizobium 0g/ha X phosphate rock 30kg/ha x KS20, R1P0V2 - Rhizobium 100g/ha phosphate rock 0kg/ha x KS20 , R1P1V2 - Rhizobium 100g/ha X phosphate rock 30kg/ha x KS20, R0P0V1, Rhizobium 0g/ha X phosphate rock 0kg/ha x KS20 , R0P1V1 -Rhizobium 0g/ha X phosphate rock 30kg/ha x N26, R1P0V1(N26)-Rhizobium 100g/ha X phosphate rock 0kg/ha x N26, Rhizobium 100g/ha X Phosphate rock 30kg/ha x N26, C.V- Coefficient of Variations, LSD- Least Significant Difference*

3.2.2 Influence of *Rhizobium* and rock phosphate fertilizer on number of pods of KS20 and N26 green-gram varieties

Number of Pods per plant of KS20 and N26 were significantly (P<0.05) influenced by combined application of *Rhizobium* and phosphate rock. However, R1P1V2 treatment recorded significantly (P<0.05) more number of pods namely 83.25 and 84.41 pods per plant compared to R1P1V1 treatment with 72.67 and 73.75 pods plant^{-1} for the first and second seasons, respectively (Table 4). This increase in number of pods can be attributed to increased nutrient uptake especially P which promoted pod development. In addition, the differences in morphology of KS20 and N26 varieties might be due the variation in nutrient uptake and moisture from the soil contributing to the differences in the number of pods. This was in agreement with [44], who reported that P application with *Rhizobium significantly* increased pod number in cowpea. Contrasting results about potential negative response on the number of pods from inoculation in chick pea [45].

Similarly, application of phosphate rock alone increased pod numbers per plant which were significantly (P<0.05) higher in R0P1V2 (67.50 and 71.0 pods per plant) over R0P1V1treatment with 66.5 and 68.50 pods per plant. This might be due to adequate supply of phosphorus which in turn increased the carboxylation efficiency and increased the ribulose-1-5-diphosphate carboxylase activity, which results in increased photosynthetic rate, growth and yield [46]. These results coincide with [47] who demonstrated that, the number of pods and pod weight significantly increased by phosphorus application as rock phosphate. *Rhizobium* inoculation alone showed insignificant difference in number of pods between R1P0V2 and R1P0V1 for both seasons (Table 4). Inoculated soybean produced more pods per plant than uninoculated treatments [48]. These results were similar with reports of [49] and Bhuiyan [50], who concluded that there was a significant increase of pod number of Mung bean and soybean by *BradyRhizobium* in Ghana. Treatments without rock phosphate or *Rhizobium* recorded lowest number of pods plant^{-1} which appeared to be small in size. This might be due to low soil nutrient uptake which led to poor growth and development of green gram varieties.

3.2.3 Influence of *Rhizobium* and rock phosphate fertilizer on grain yield kgha^{-1} of KS20 and N26 green gram varieties

There was an interactive effect ($P<0.05$) of phosphate rock and *Rhizobium* inoculation on grain yield of green grams. Significantly ($P<0.05$) higher grain yield are recorded in R1P1V2 treatment (2122.68 and 2158.26 Kg ha^{-1}) over R1P1V1 treatment which registered yield of 1842.83 and 1899 Kg ha^{-1} for the first and second season, respectively (Table 4). The higher grain-yield in R1P1V2 treatment can be attributed to the improved N and P use efficiency and uptake which in turn promoted growth and development of yield components such as number of Pods and yield. In addition, combined application of P and *Rhizobium* inoculation increased nitrogenase activity, growth, and grain yield of cowpea as well as improved soil fertility [51]. Significant yield variations among soybean varieties with *Rhizobium inoculation* and phosphorous fertilizer were observed [52]. Nevertheless, the results of this study are in contrast with the findings of [53], who reported that grain yield in soybean was not significantly influenced by P application. Phosphate rock application recorded significant ($P<0.05$) higher grain yield in treatment R0P1V2 (1472 and 1514 Kg ha^{-1}) over R0P1V1 respectively for the two seasons (Table 4). This may be due to the positive P fertilizer application on metabolic activity resulting in surface area for high dry matter production and therefore giving rise to high yield [53,54].

Furthermore, *Rhizobium* inoculation recorded significant ($P<0.05$) higher grain yield in treatment R1P0V2 (1404 and 1446 Kg ha^{-1}). Inoculation of soybean increases nitrogen fixation potential and other growth factors which lead to increased yield [55]. Treatment without phosphate rock application and *Rhizobium* inoculation recorded least grain yield which are significantly ($P<0.05$) different with R0P0V2 recording slightly higher grain yield of (529 and 487 Kg ha^{-1}) respectively over R0P0V1 (Table 4). The decreases in grain yield have resulted due to lack of specific *Rhizobia* nodulating green gram and low P levels in the study area [56]. Hence, suggesting for inoculation and P application in the study area. These plots are much affected with pests and diseases thereby contributing to poor growth and yield. The inoculated plots were not much affected with diseases and pests since nitrogen-fixing *Rhizobium* triggers enzyme-mediated induced resistance reactions [57] which might have led to production of defensive compounds against diseases and pests [58].

4. CONCLUSION

Growth and yield attributes of KS20 and N26 green gram varieties are greatly influenced with the combined application of *Rhizobium* and phosphate rock when compared to other treatments. Increase in plant height, number of branches, leaves, dry weights, number of pods and total grain yield per hectare were recorded with combined application of *Rhizobium* 100g ha^{-1} and 30 kg P ha^{-1} phosphate rock under variety KS20. It may be due to increased nutrient use efficiency of *Rhizobium* and phosphate rock application compared to KS20. Hence, KS20 was superior to N26 in growth and yield components. There is need in selection of efficient green gram variety that is responsive to inoculant *Rhizobia* strain(s) form the basis of enhanced BNF in green-grams. Therefore, combined application of *Rhizobium* application at the rate of 100g ha^{-1} and 30 kg P ha^{-1} phosphate rock with KS20 variety will lead to increased productivity of green gram Chuka area Tharaka- Nithi County.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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