EFFECT OF *RHIZOBIUM* INOCULATION AND PHOSPHATE ROCK FERTILIZER APPLICATION ON BIOMASS PRODUCTION, NUTRIENT USE EFFICIENCY AND YIELD PARAMETERS OF ~~GREEM~~GREEN GRAM (*VIGNA RADIATA)*

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ABSTRACT

The research was focused on an ecologically sound and highly production of legumes particularly green gram through the application of *Rhizobium* and rock phosphate fertilizer. Therefore, biomass production, nutrient use efficiency and yield parameters were determined for two growing seasons (November 2019 - January 2020 and February - April 2020), at Chuka university horticultural research farm. Biological nitrogen fixation (BNF) in green grams can improve sustainable soil fertility management and increased production. In Kenya, green gram is a major source of food security particularly in Tharaka Nithi County. However, green gram yields are usually low due to low phosphorous and nitrogen levels of the soil. A factorial experiment of 2 x 2 x 2 was laid out in a randomized complete block design. There were three factors, varieties (N26-nylon and KS20-uncle), phosphate rock (0 and 30 kg P ha^{-1}) and *Rhizobium MEA* 716 (0 and 100 g ha^{-1}) making a total of eight treatments which were replicated three times. Soil sampling and analyses were done before planting and after harvesting of green grams. Data on grain yield, total dry biomass, shoot and root dry weights. Phosphorus use efficiency (PUE) and nitrogen use efficiency (NUE) were done. Data was analysed using Statistical Analysis Software (SAS). Significant means were separated using Least Significant Difference (LSD) at probability level of 5 %. Results for both wet and dry seasons indicated that variety KS20 under *Rhizobium* inoculation and phosphate rock fertilizer (R1P1V2) showed significantly ($P<0.05$) higher increase in 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}). Also, there was significant ($P<0.05$) higher phosphorous use efficiency of 279.32 Kg/ha and nitrogen use efficiency of 1732 kg/ha in treatment R1P1V2 over other treatments. From these results it was concluded that variety KS20 (V2) performed better compared to N26 (V1) under combined application of *Rhizobium* and phosphate rock fertilizer. Hence, based on the findings, for sustainable and improved green gram production farmers in Tharaka Nithi County.

Key Words: *Green gram Varieties, Nutrient Use Efficiency, factorial experiment, sustainable production and Food Security*

1.0 INTRODUCTION

Legume cultivation in the tropics and sub-tropics mostly occurs on soils with low P content and this is mainly due to processes such as weathering, erosion and P fixation [1]. In most soils in arid and semi-arid lands (ASALs) including, parts of Tharaka Nithi County have a low extractable P [2]. This is because this region is dominated by nitisols which have phosphorous sorption. This therefore, makes low P availability one of the major limiting factors for legume production in these areas. In the recent times, there has been much focus on application of phosphate rock to soils due to its potential as alternative to water soluble phosphatic fertilizers such as single and triple super phosphates which have become much more expensive [3]. Phosphate rock has been recommended for sustainable agriculture [4]. Large increases in legume production due to addition of phosphate rock fertilizer have been reported [5]

Leguminous plants can establish symbiosis with rhizobia [6]. Inoculation of the soil with *Rhizobium* strains increased nodulation, nitrogen acquisition, and legume yield [7]. The process responsible for reducing molecular nitrogen into ammonia is referred as nitrogen fixation [8], and rhizobia play important roles in agriculture, performing biological nitrogen fixation (BNF). BNF is important agronomically because it reduces the need for chemical nitrogen fertilizers [9]. However, the efficiency of the biological process depends on several factors related to the host plant and bacteria, and edaphic factors such as soil acidity, low soil fertility, high temperatures, and drought often limit the contribution of nitrogen fixation [10]. *Rhizobium* inoculation is very effective at enhancing BFN and crop yields in most legumes, and this practice is adopted by most producers [11].

Nitrogen is important for maintaining and improving crop growth and yield. However, the long-term excessive use of chemical fertilizers in agriculture has unanticipated environmental impacts [12], including soil fertility degradation, soil organic matter deterioration, and decreased water and nutrient holding capacities and nutrient use efficiency [13]. An alternative to N fertilizer is effective, efficient rhizobial N-fixing bacteria alone or together. *Rhizobium* inoculants are relatively inexpensive for leguminous crop production [14]. Therefore, the use of efficient inoculants can be considered an important strategy for sustainable management and reduction of environmental problems by decreasing the use of chemical fertilizers [15].

There is a growing need to improve food production to meet the requirements of the increasing world population. This may be done in either of two ways: increasing the area under cultivation or enhancing the yield per unit area. The former is not possible in many countries of the world due to a number of restrictions including the availability of water or soil resources, climate change, drought, and soil salinization [16]. On the other hand, one of the ways to increase the yield per unit area is to improve the nutritional properties of the soil. As an essential plant nutrient, P is required for carbon metabolism, energy generation, energy transfer, enzyme activation, membrane formation, and nitrogen (N_2) fixation [17]. P also forms key biological molecules like ATP, nucleic acids, and phospholipids [18]. P deficiency is a significant limiting factor for the growth and yield of crops that affects approximately 50% of all agricultural ecosystems around the world [19]. To address this issue, there has been an enormous worldwide increase in the use of P fertilizers. The high agricultural P demand has put the sustainability of P mining for fertilizer production into question [20]. P fertilizers often lead to the addition of a large excess of P in agricultural soils. Unfortunately, >80% of the P fertilizers applied to the soil is lost due to adsorption and fixation processes [21] or it is transformed into organic forms, which represent 40–80% of total soil P [22], with phytases as the most common form [23]. Therefore, the availability of this added P to plants is limited (about 0.1% of the total P). The theoretical increase in plant growth efficiency from adding chemical P fertilizers has peaked so that additional chemical P fertilization cannot be expected to significantly increase plant yield [24]. Twenty-two million tons of P (3-4% of the total P demand) are annually extracted from natural sources (i.e., non-renewable phosphate rocks), according to the US geological survey [25], which puts the natural P sources in risk of depletion [27]. Therefore, a more efficient use of P is needed, including maximizing P acquisition and utilization efficiencies [28]

Green-gram is majorly cultivated in the warmer regions of the world due to its drought tolerant characteristics and low inputs requirements [29]. It is currently cultivated on about six million hectares worldwide most of which are located in Asian countries and to a small extent also in some parts of Africa, USA, and Australia [30]. Ninety per cent of the world's production of green-grams is in Asia with India taking around 50% of the species World's production [31]. Green-gram (*Vigna radiata*) also called mungbean is one of the most important pulses in the world. It is valuable for humans and animals because of its nutritive values as well as used for soil fertility improvement [32] It is consumed as green pods and dry seeds as well as young shoots [33].

1.2 Objective

To determine the effect of *Rhizobium* inoculation and phosphate rock fertilizer application on Biomass Production, yield parameters and nutrient use efficiency (P and N) of KS20 and N26 green-gram varieties

2.0 METHODOLOGY

2.1 Site Selection

The study was carried out at Chuka University Horticultural research and demonstration field, Meru South Sub-County in Tharaka-Nithi County for two cultivations, November 2019 - January 2020 and February - April 2020. The site is situated at latitude 0.3195^o S, longitude 37.6575^o E with an altitude of 1401 m above local sea level and in a midland zone [34]. The average annual temperature is 20.8^{o}C. Annual rainfall is 1599 mm distributed bi-modally with the months of March to May being long rains season and October to December short rains season [35]. The Soils are classified as nitisols of volcano origin with basic and ultra-basic igneous rocks which are highly leached [36]. The climate is favourable for cultivation of green-grams, tea, coffee, maize, cowpeas, pigeon peas, tobacco, and variety of other food crops and livestock keeping.

2.2 Experimental Design

A factorial experiment of 2 x 2 x 2 was laid out in a randomized complete block design (RCBD). There were three factors; varieties (N26-nylon and KS20-uncle), Minjingu phosphate rock (MPR) application rates (0 and 30 kg P ha-1) and *Rhizobium* inoculation rates (0 and 100 g ha-1) making a total of eight treatments. The eight treatments were replicated three times making 24 experimental plots. Each plot measured 1.5 by 1.8 m with spacing of 45 cm between rows and 15 cm within plants, making a population of 148,149 plants per hectare. There were four rows each with 10 plants translating to 40 plants per experimental plot. The guard rows measured 0.5 m. Factor levels were combined to constitute the treatments. Watering was done uniformly to supplement rainfall, pest and diseases were controlled using appropriate measures.

2.3 Data Collection

Data collection was done on four randomly selected from two middle rows at an interval of 14 days upto full maturity. Data on yield parameters started once the crop reached physiological maturity and data collected included, shoot dry biomass, root dry biomass, total dry biomass, number of pods and grain yield, Phosphorus and nitrogen use efficiency.

Figure 1: Data collection and Recording

Shoot and root dry weight - this was done at flowering on randomly selected four plants from two inner rows of each experimental plot. The selected plants were dug together with roots and shoots and were cut off then packed separately in llabelled 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 Jones (2001) 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 labelled plastic bags and taken immediately to the laboratory for biomass determination. The plants were oven dried at 60^{o}C for 48 hours and dry weight biomass determined using an electronic balance. The weight of each plant was recorded in kilograms per hacter.

Number of pods - was done at physiological maturity on the four tagged plants from two inner rows in each experimental plot. Number of pods per plant were counted and recorded accordingly.

Determination of 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 pods underwent further air drying then threshed manually and winnowed to separate the seeds from the debris. 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}.

Nitrogen and phosphorous use efficiency - the nitrogen use efficiency of phosphate rock (PUE) and nitrogen use efficiency (NUE) were evaluated as described by [37]
NUE per Kg = Yield of inoculated plots/*Rhizobium* inoculation applied.
PUE per Kg = yield of P applied plots/phosphorous applied.

2.4 Data Analysis

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

3.0 RESULTS AND DISCUSSION

3.1 Interactive Effects of *Rhizobium* and phosphate rock fertilizer on Shoot and Root Dry Weights and Total Dry Biomass Weight of two Green Gram Varieties (N26 and KS20)

3.1.1 Shoot Dry Weight of two Green Gram Varieties (N26 and KS20)

Combination application of both of *Rhizobium* and phosphate rock (PR) significantly ($P<0.05$) affected shoot dry weight of KS20 and N26 varieties. Treatment R1P1V2 recorded the highest shoot dry weight of 49.91 and 52.01 g plant^{-1} compared to R1P1V1 with 46.78 and 47.87 g plant^{-1} for the two cultivations, respectively (Table 1). This is because combined application of PR and *Rhizobium* resulted to optimum nutrient supply particularly N and P which favoured high rate of photosynthesis and sufficient cell expansion thus, shoot growth rate. This was in agreement with [38] who observed that N and P affected the capacity of climbing beans to produce higher shoot biomass. Treatments with *Rhizobium* inoculation alone had no significant ($P<0.05$) difference in shoot dry weight between R1P0V2 and R1P0V1 for both cultivations (Table 1).

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. In sole application of phosphate rock treatment R0P1V2 recorded significant ($P<0.05$) higher shoot dry weight of (39.43 and 41.00 g plant^{-1}) over R0P1V1 with 29. 01and 33.08 g plant^{-1} for the two cultivations (Table 1). This increase in shoot dry weight due application demonstrated the positive benefits of P fertilizer in shoot dry weight. [39], stated that P is important in the activation of metabolic processes necessary for vegetative growth resulting in high shoot dry matter accumulation. The treatments with no PR or *Rhizobium* recorded least shoot dry weight for both cultivations (Table 1).

Table 1 Shoot, Root dry Weight and Total Dry Biomass and Grain Yields of Green-grams varieties under different treatments.

Cultivations	Treatments	Shoot dry weight g plant^{-1}	Root dry weight g plant^{-1}	Total Dry Biomass g plant^{-1}
I	R0P0V2	15.87^{*a}	0.965^{a}	94.2^{b}
	R0P1V2	41.00^{c}	4.280^{de}	122.2^{d}
	R1P0V2	42.18^{c}	3.780^{bcd}	135.3^{fg}
	R1P1V2	49.91^{de}	6.560^{h}	145.5^{ij}
	R0P0V1	14.86^{a}	0.806^{a}	89.0^{a}
	R0P1V1	29.01^{b}	3.482^{bc}	115.7^{c}
	R1P0V1	41.23^{c}	3.966^{cde}	131.3^{e}
	R1P1V1	46.78^{d}	5.189^{g}	142.3^{h}
II	R0P0V2	15.71^{a}	0.937^{a}	94.0^{b}
	R0P1V2	39.43^{c}	4.997^{fg}	126.4^{e}
	R1P0V2	40.70^{c}	3.656^{bc}	137.4^{g}
	R1P1V2	52.01^{e}	7.607^{i}	146.4^{j}
	R0P0V1	15.15^{a}	0.757^{a}	84.8^{a}
	R0P1V1	33.08^{b}	4.465^{ef}	122.3^{d}
	R1P0V1	42.25^{c}	3.213^{b}	135.1^{fg}
	R1P1V1	47.87^{de}	7.009^{h}	143.01^{h}
	C.V (%)	8.3	10.4	1.5
	Mean	35.44	3.789	123.29
	$LSD_{0.05}$	4.768	0.6374	3.087

*Legend: *Means followed by the same letter in the same column are not significantly different from each other at ($P<0.05$) level of significant. 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 X 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 N26, R0P1V1- Rhizobium 0g/ha X phosphate rock 30kg/ha X N26, R1P0V1-Rhizobium 100g/ha X phosphate rock 0kg/haXN26, Rhizobium 100g/ha X Phosphate rock 30kg/ha X N26, C.V- Coefficient of Variations, LSD- Least Significant Difference*

This might be due to low the N in these treatments leading to fewer and small number of leaves hence low shoot dry weight. Phosphate rock application has been reported to increase soybean shoot and leaf growth and other leguminous crops [38]. Findings by [39] showed that inoculation promoted growth factors, such as, production of larger leaves. Inoculation of soybean increases nutrients, nitrogen fixation potential and other growth factors for the enhancement of shoots as reported by [40].

3.1.2 Root Dry Weight of Green Gram Varieties (N26 and KS20)

Root dry weight was significantly ($P<0.05$) high in the two varieties with application of both *Rhizobium* inoculation and phosphate rock compared to other treatments. However, treatment R1P1V2 produced the highest root dry weight of 6.56 and 7.60 g plant^{-1} over R1P1V1 with 5.18

and 7.0 g plant^{-1} (Table 1). This increase in root dry weight can be attributed to increase in nutrients especially P which promoted development of more roots. [41] reported that, higher root dry matter of soybean variety was influenced by P application and *Rhizobium* inoculation. Similar findings were also reported by [42]. The stimulation of root hairs and root growth is one key factor of plant growth promotion by rhizobacteria. The *Rhizobium* has a close association with plant roots and can enhance plant growth and root parameters, such as, lateral root number, total root length and root dry matter [43]. In a comparison of root growth for soybean treatments, rhizobia and phosphorus 48 fertilizer produced the highest root dry matter [44] which results from an increase in cell dry matter accumulation with well-developed tap roots during plant growth. This finding agrees with [45] who observed a significant increase in root dry matter yield of inoculated improved soybean over un-inoculated soybean.

There was significant interaction between phosphate rock and green gram varieties on root dry matter yield. However, R0P1V2 recorded slight significant ($P<0.05$) higher root dry weight of (4.28 and 4.99 g plant^{-1}) over R0P1V1 with 3.48 and 4.46 g plant^{-1} suggesting differences in P use efficiency between KS20 and N26 varieties. Results suggest that P fertilization played a very big 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 [, who reported that, application of P alone significantly increased root length and root dry weight. Treatments with *Rhizobium inoculation* alone had insignificant ($P<0.05$) difference in root dry weight, although the results were slightly higher compared to treatments with no PR or *Rhizobium*. This finding agrees with [46] who observed, a significant increase in root dry matter of inoculated soybean over un-inoculated. This was contrary, to the works done by [47] who observed that, commercial *Rhizobia* did not give positive results of root dry weight.

3.1.3 Total Dry Biomass (TDB) of Green-grams Varieties (N26 and KS20)

There was significant ($P<0.05$) increase in total dry biomass of KS20 and N26 varieties were observed with combined application of *Rhizobium* and phosphate rock over other treatments. However, treatment R1P1V2 recorded significantly ($P<0.05$) higher TDB of 145.539 and 146.32 g plant^{-1} compared to R1P1V1 with 142.3 and 143.01 g plant^{-1} for both cultivations (Table 1). This because in legume biomass increases exponentially at vegetative stages when the plants get optimum nutrients and especially P which is an essential element for energy transfer processes in plants [48]. Phosphorus fertilization and *Rhizobia* inoculation was shown to record the highest dry matter residues to up to 56 days after planting [49]. Also research has revealed biomass yield is dependent on legume variety [50].

Treatments with *Rhizobium i*noculation alone recorded a significant ($P<0.05$) slightly higher TDB in treatment R1P0V2 (135.3 And 137.4 g plant^{-1}) over R1P0V1 with 131.3 and 135.1 g plant^{-1} (Table 1). Also single application of phosphate rock resulted to a significant ($P<0.05$) increase of TDB in R0P1V2 of 122.2 and 115.7 g plant^{-1} for the first and second cultivation, respectively (Table 1). This reconciles with the findings of [51] who reported that, P supply stimulated vegetative growth of soybean resulting into high plant dries biomass.

3.2 Interactive Effects of Treatments on Number of Pods and Grain Yield of Green-gram Varieties (N26 and KS20)

3.2.1 Number of Pods of Green-gram Varieties (N26 and KS20)

Number of Pods plant^{-1} of KS20 and N26 were significantly ($P<0.05$) influenced by combined application of *Rhizobium* and phosphate rock. However, treatment R1P1V2 recorded significantly ($P<0.05$) more number of pods 83.25 and 84.41 pods plant^{-1} which also appeared to be quite heavy comparable to R1P1V1 with 72.67 and 73.75 pods plant^{-1} for the first and second cultivations, respectively (Table 2). This increase in number of pods can be attributed to the increased nutrient uptake especially P which promoted development of reproductive structures such as pods. 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 number of pods. This was in agreement with [52], who reported that P application with *Rhizobium significantly* increased pod number in cowpea. [53] reported contrasting results about potential negative response on the number of pods from inoculation in chick pea

Likewise, treatments with phosphate rock alone increased pod numbers plant^{-1} which were significantly ($P<0.05$) higher in R0P1V2 (67.50 and 71.0 pods plant^{-1}) over R0P1V1 with 66.5 and 68.50 pods plant^{-1}. 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, resulting in increased photosynthetic rate, growth and yield [54]. These results were in agreement with [55] who demonstrated that, the number of pods and pod weight significantly increased by phosphorus.

Rhizobium inoculation alone showed insignificant difference in number of pods between R1P0V2 and R1P0V1 for both cultivations (Table 2). [56] reported that inoculated soybean produced more pods per plant than uninoculated treatments. These results were similar with reports of [57], who concluded that there was a significant increase of pod number of Mung bean and soybean by *Bradyrhizobium* in Ghana. Also treatments with no PR or *Rhizobium* recorded lowest number of pods plant^{-1} which appeared to be small in size. This might be due to the low soil nutrients and uptake which led to poor growth and development of green gram varieties.

3.2.2 Effect of *Rhizobium* inoculation and Phosphate rock fertilizer application on Grain Yield Kgha^{-1} of Green Grams Varieties (N26 and KS20)

Phosphate rock and *Rhizobium* inoculation on grain yield of green-grams. Significantly ($P<0.05$) higher grain yield were recorded in treatment R1P1V2 (2122.68 and 2158.26 Kg ha^{-1}) over R1P1V1 which registered yield of 1842.83 and 1899 Kg ha^{-1} for the first and second cultivation, respectively (Table 2). The higher grain-yield in treatment R1P1V2 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 dry weight, flowers and pods. In addition, combined application of P and *Rhizobium* inoculation increased nitrogenease activity, growth, and grain yield of cowpea as well as improved soil fertility [58]. Also [59] reported significant yield variations among soybean varieties with *Rhizobium inoculation* and phosphorous fertilizer.

Nevertheless, the results of this study were in contrast with the findings of [60] who reported that, yield components and grain yield in soybean were not significantly influenced by P application. Sole application of phosphate rock treatments recorded significant ($P<0.05$) higher grain yield in treatment R0P1V2 (1472 and 1514 Kg ha^{-1}) over R0P1V1 with 1230 and 1338 Kg ha^{-1} for the two cultivations, respectively (Table 2). This could be due to the positive benefits of

P fertilizer which is important on the activation of metabolic processes necessary for branches and seed production and resulting in surface are for production high dry matter accumulation and therefore giving rise to high yield [61].

Furthermore, *Rhizobium* inoculation alone recorded significant ($P<0.05$) higher grain yield in treatment R1P0V2 (1404 and 1446 Kg ha^{-1}). [62] reported that inoculation of soybean increases nutrients, nitrogen fixation potential and other growth factors which lead to increased yields. Treatment without PR nor *Rhizobium* inoculation recorded least grain yields which were significantly ($P<0.05$) different with R0P0V2 recording slightly higher grain yield of (529 and 487 Kg ha^{-1}) over R0P0V1 with 370 and 325 Kg ha^{-1} (Table 2). There was a decline of grain yield in the second cultivation. This drop in grain might have resulted from the lack of specific *Rhizobia* nodulating green-grams and low P levels in the study area [63] hence suggesting for inoculation and P application in the study area. Also these plots were much affected with pests and diseases contributing to poor growth and yield. The yield increase measured with inoculation therefore indicates that experimental area did not have sufficient populations or competitive *Rhizobia* strains for green gram. In addition, the inoculated plots were not much affected with diseases and pests since nitrogen-fixing *Rhizobium* triggers enzyme-mediated induced resistance reactions [64] which might have led to production of defensive compounds against diseases and pests [65].

Table 2: Number of Pods, Grain Yields of Green Grams Varieties under Different treatments.

Cultivations	Treatments	Number of pods $plant^{-1}$	Yield $kgha^{-1}$
	R0P0V2	31.17^{a}	529^{b}
I	R0P1V2	67.50^{bcd}	1472^{gh}
	R1P0V2	66.33^{cd}	1404^{fg}
	R1P1V2	83.25^{g}	2123^{j}
	R0P0V1	31.67^{a}	370^{a}
	R0P1V1	66.50^{b}	1230^{d}
	R1P0V1	66.17^{bc}	1143^{c}
	R1P1V1	72.67^{ef}	1843^{i}
	R0P0V2	32.25^{a}	487^{b}
II	R0P1V2	71.00^{e}	1514^{h}
	R1P0V2	67.27^{cd}	1446^{gh}
	R1P1V2	84.42^{g}	2158^{j}
	R0P0V1	30.83^{a}	325^{a}
	R0P1V1	68.50^{d}	1338^{ef}
	R1P0V1	65.67^{bc}	1284^{de}
	R1P1V1	73.75^{f}	1900^{i}
	C.V (%)	2.3	6.3
	Mean	61.06	1285.3
	$LSD_{0.05}$	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 significant. 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 X 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 N26, R0P1V1- Rhizobium 0g/ha X phosphate rock 30kg/ha X N26, R1P0V1-Rhizobium 100g/ha X phosphate rock 0kg/haXN26, Rhizobium 100g/ha X Phosphate rock 30kg/ha X N26, C.V- Coefficient of Variations, LSD- Least Significant Difference*

That, for high seed nitrogen content in bean plants proper combination of *Rhizobium has* to be identified to enhance more effective nitrogen fixation, P and N concentration, as well as their uptake in grain. However, contrary results were reported by [66] who stated that, inoculation in chick pea did not result on significant increase in N and P uptake.

3.3 Effect of *Rhizobium* inoculation and Phosphate rock fertilizer application on Phosphorous Use Efficiency (PUE) and Nitrogen Use Efficiency (NUE) of Green-Gram Varieties

Interactive effects of combined application of *Rhizobium* and phosphate rock significantly (P<0.05) improved PUE and NUE of KS20 and N26 varieties greatly for both cultivations. Although, R1P1V2 recorded slightly higher increase in PUE (227.39 and 279.32 Kgha^{-1}) and NUE (1791 and 1732 Kgha^{-1}) compared to R1PV1 for the two cultivations, respectively (Table 3). This was attributed to the increase of P and N in soils and also differences between the two varieties was due to differences in ability of nutrient acquisition from soil. These results were in agreement with those of [67] who stated that use efficiency of P and N in soybean was affected by phosphorus application and *Rhizobium* inoculation. This variation in use efficiency between KS20 and N26 was justified from different studies which showed wide differences in P and N acquisition among many legume varieties [68].

In sole application of phosphate rock, R0P1V2 recorded significantly (P<0.05) higher PUE of (157.72 and 193.37 Kgha^{-1}) compared to R0P1V1 and controls (Table 3). This also collaborates with [69], who indicated that PUE indices increased with increase in P fertilizer concentration. [70] collaborated this finding by reporting that P application increased PUE in soybean in the moist savanna zone of West Africa. [71] also found that PUE was higher in soybean treatments at 30 kg P ha^{-1}. Also *Rhizobium* alone had greater NUE in R1P0V2 (1154 and 1185 Kgha^{-1}) compared to R1P0V1. Treatments with no PR or *Rhizobium* recorded PUE and NUE of zero (0) this is because *Rhizobium* and PR use efficiency dependent on amount that was applied. Furthermore, phosphorus requirement has been shown by other researchers to vary among legume varieties with the degree of use efficiency reported to depend on varieties [72]. These results demonstrated that PUE and NUE are the major agronomic indices that can be used to describe fertilizer use efficiency. It is therefore, pertinent to place more emphasis on P use efficiency other than increased application of P and N.

Table 3: Nitrogen and Phosphorous Uptake, phosphorous use efficiency (PUE) and Nitrogen use efficiency (NUE) under different treatments

Cultivations	Treatments	NUE kg/ha	PUE kg/ha
	R0P0V2	0^{a}	0^{a}
I	R0P1V2	0^{a}	157.72^{c}
	R1P0V2	1154^{c}	0^{a}
	R1P1V2	1791^{e}	227.39^{e}
	R0P0V1	0^{a}	0^{a}
	R0P1V1	0^{a}	131.72^{b}
	R1P0V1	9643^{b}	0^{a}
	R1P1V1	1559^{d}	197.41^{d}
1I	R0P0V2	0^{a}	0^{a}
	R0P1V2	0^{a}	193.37^{d}
	R1P0V2	1185^{c}	0^{a}
	R1P1V2	1732^{e}	279.32^{f}
	R0P0V1	0^{a}	0^{a}
	R0P1V1	0^{a}	146.06^{bc}
	R1P0V1	9764^{b}	0a
	R1P1V1	1546^{d}	270.21^{f}
	C.V (%)	14.0	19.1
	Mean	6866	98.26
	$LSD_{0.05}$	1542.9	30.14

*Legend: *Means followed by the same letter in the same column are not significantly different from each other at (P<0.05) level of significant. 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 X 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 N26, R0P1V1- Rhizobium 0g/ha X phosphate rock 30kg/ha X N26, R1P0V1-Rhizobium 100g/ha X phosphate rock 0kg/haXN26, Rhizobium 100g/ha X Phosphate rock 30kg/ha X N26, C.V- Coefficient of Variations, LSD- Least Significant Difference*

CONCLUSION

Results from this research showed positive interaction effects of *Rhizobium* and phosphate rock with green-gram varieties compared with single application of the treatments and controls. Which led to significantly higher biomass production, yield parameters and nutrient use efficiency. Higher shoot, root, total dry weights, number of pods and total grain yield per hectare were recorded in combined application of *Rhizobium* 100g ha^{-1} and 30 kg P ha $^{-1}$ phosphate rock with variety KS20. Hence, KS20 was superior to N26 in all the tested variables and this confirmed the existence of variation in response to inoculation between KS20 and N26 green-gram varieties. Hence, 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* 100g ha^{-1} and 30 kg P ha^{-1} phosphate rock with KS20 variety will give positive results in Chuka area - Tharaka Nithi County.

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COMPETING INTERESTS

There is no competing interests exist.

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